STUDIES OF SOME CYTOTOXIC PLANT EXTRACTS

A Thesis presented for the degree of Doctor of Philosophy

By

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Declaration

Experimental Work described in this thesis was carried out by the author of this thesis in the Department of Chemistry, University of Dhaka, Dhaka, Bangladesh and partly at the HEJ Research Institute of Chemistry, University of Karachi, Karachi, Pakistan under a research collaboration. This work has not been presented, and is not being presented for any other degree.

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Dadicated To The

Sacred Memory of My Beloved Parents

Who Lit A Candle in Me Many Before

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Ismet Ara Jahan

STUDIES OF SOME CYTOTOXIC PLANT EXTRACTS

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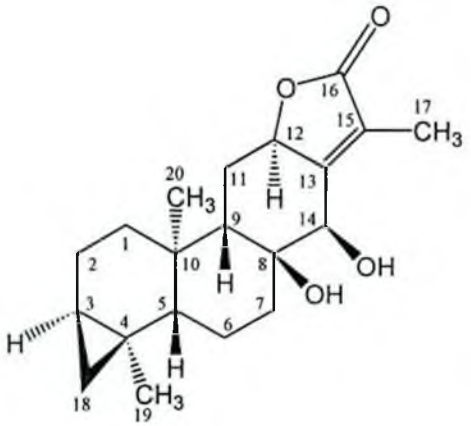
SUMMARY

SUMMARY

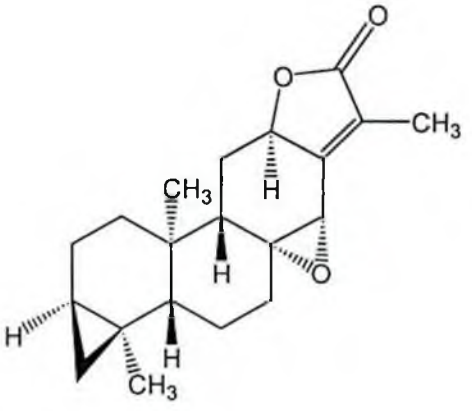
This thesis describes the phytochemical investigations on three medicinal plants, *Suregada multiflora* (Juss) Bail (Syn. *Gelonium multiflorum*, Euphorbiaceae), *Iris germanica* Linn (Iridaceae) and *Ficus racemosa* Linn (Moraceae). The part A (Page 18-116) of the thesis describes the bioassay-guided phytochemical investigations on a Bangladeshi plant *Suregada multiflora*. Pat-B (Page 117-160) describes the bioassay-guided isolation and characterization of phytochemicals from *Iris germanica* Linn, a plant from Pakistan. The third part or Part-C (Page 161-196) deals with the biological and chemical investigations on another Bangladeshi plant *Ficus racemosa*.

Part-A

The CH_2Cl_2-MeOH extract of the bark of *S. multiflora* was found to possess selective and significant cytotoxicity in different 60-cell line tumor panels at the National Cancer Institute (NCI), USA. The dichloromethane soluble part of the parent extract also showed significant growth inhibitory activity in mechanism-based yeast bioassays conducted at the HEJ Research Institute of Chemistry. A mechanism-based yeast bioassay guided phytochemical investigation on the same extract led to the isolation of ten compounds. Four compounds were identified as members of a new class of diterpenes and were named as suregadolide A (**105**), suregadolide B (**106**), suregadolide C (**107**) and suregadolide D (**108**) (compounds **1**- **4**).

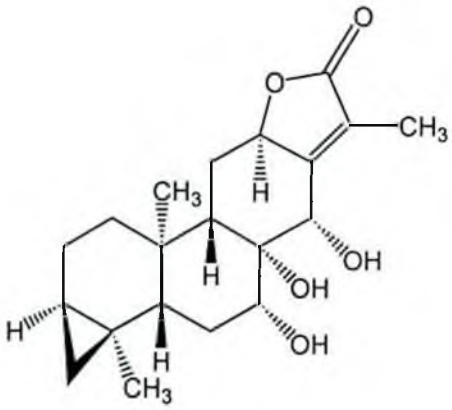


Suregadolide A (**105**)

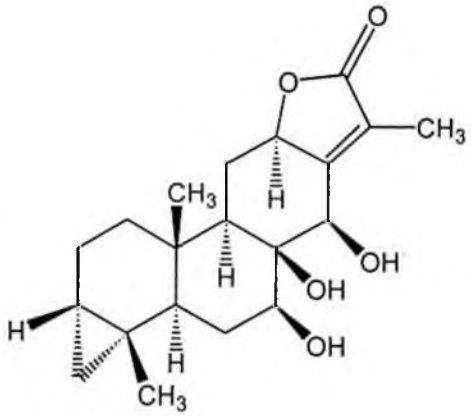


Suregadolide B (**106**)

These compounds contain a trisubstituted cyclopropane ring bridging C-3 and C-4 positions of ring A of the abietane framework. Suregadolides A-C (**105-107**) and suregadolide D (**108**) were identified as compounds of two antipodal series of the basic abietane skeleton.

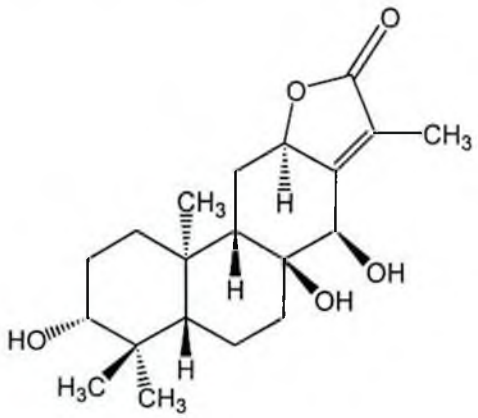


Suregadolide C (**107**)

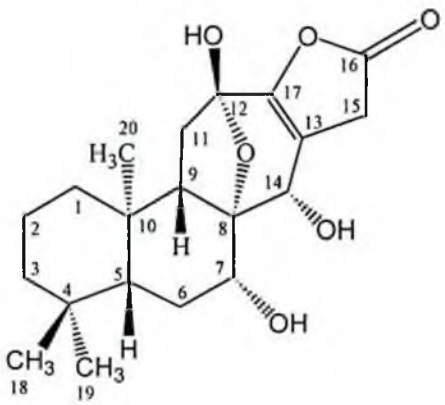


Suregadolide D (**108**)

Suremulide A (**111**), compound **5** was found to be a new abietene diterpene lactone. Compound **6** was identified as member of another new class of diterpene lactone, which possesses a novel carbon skeleton, and contains a seven-member ring, formed by the rearrangement of exocycilc C-17 in ring C of *ent*-pimarane framework which was named as bannaringaolide A (**110**).

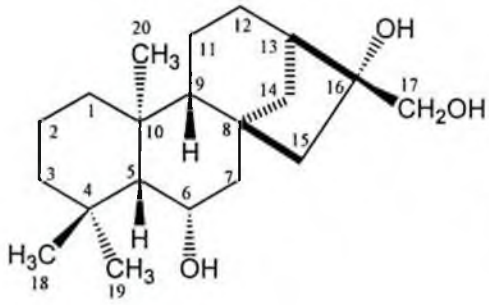


Suremulide A (**109**)

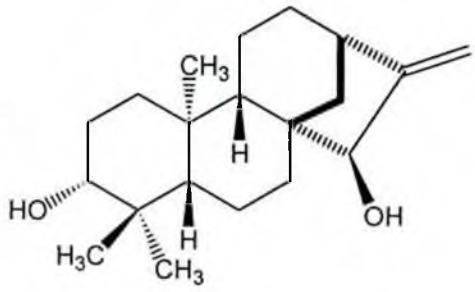


Bannaringaolide A (**110**)

A kaurane triol, suremulol A (**111**) (compound 7), and one kaurane diol suremulol B (**112**) (compound **8**) were identified as new diterpene metabolites.

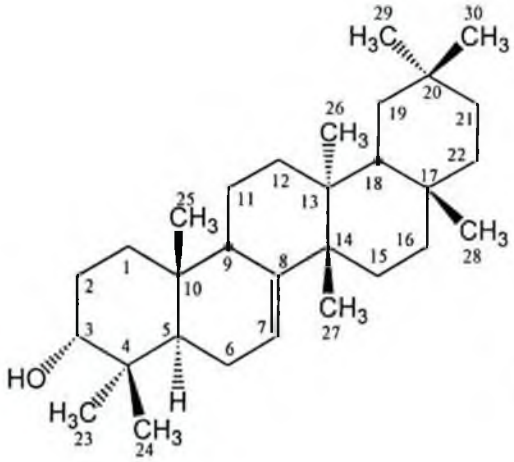


Suremulol A (**111**)

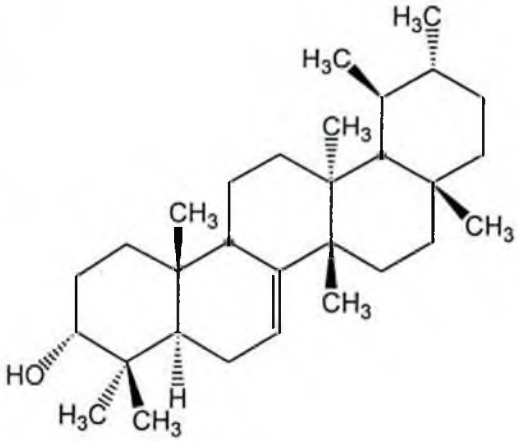


Suremulol B **(112)**

Two more compounds were characterized as known triterpenes named as multiflorenol (**44**) and baurenol (**45**) (compounds **9** and **10**).



Multiflorenol (**44**)



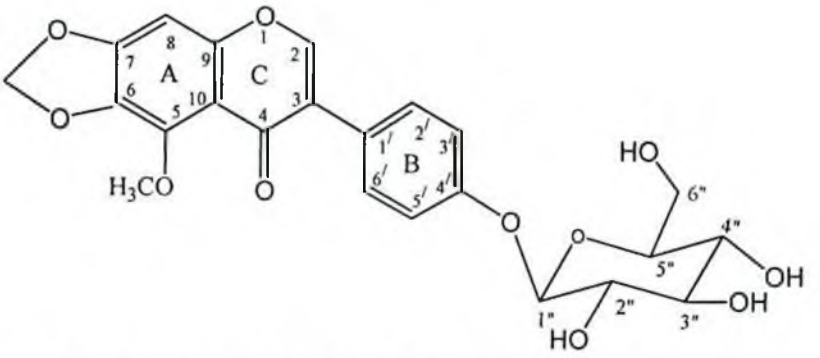
Baurenol (**45**)

Compounds **1**, **3** and **7** exhibited growth inhibitory activity with IC_{12} values of 35 and 70 μg/mL, 23 and 50 μg/mL, 53 and 100 μg/mL towards the RAD 52 and RAD^{+} mutant yeast strains, respectively in mechanism-based yeast bioassay.

Part-B

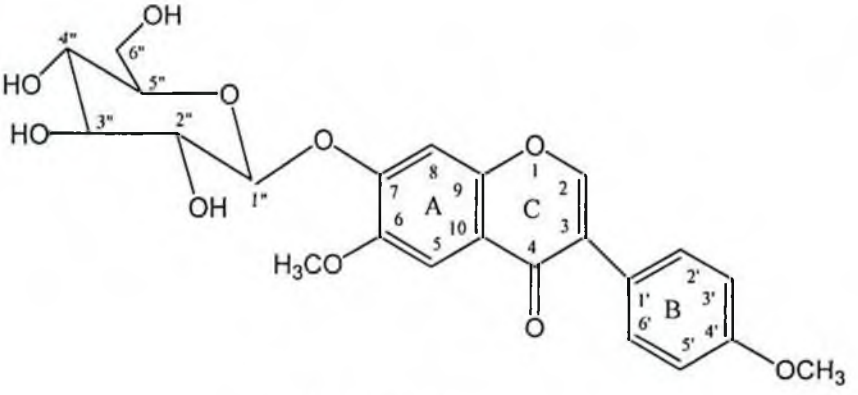
The dichloromethane and ethyl acetate soluble parts of the parent MeOH extract of the rhizomes of *Iris germanica* showed significant growth inhibitory activity in mechanism-based yeast bioassay. The bioassay guided fractionation on dichloromethane and ethyl acetate soluble part of the parent extract yielded eight pure compounds (compounds **11-18**). Two compounds were identified as isoflavone glycosides, five as isoflavone type compounds and one steroidal compound.

Compound **11**, nigricin 4′-*O*-β-D-glucoside (**171**) was identified as a new isoflavone glycoside.



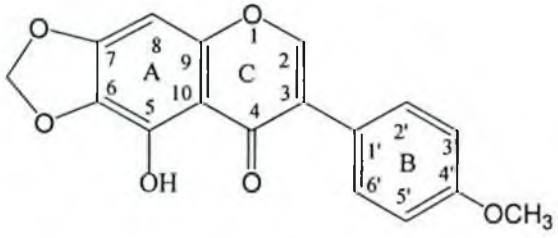
Nigricin 4′-*O*-β-D-glucoside (**171**)

Compound **12** was characterized as a known isoflavone glycoside, wistin (**172**).

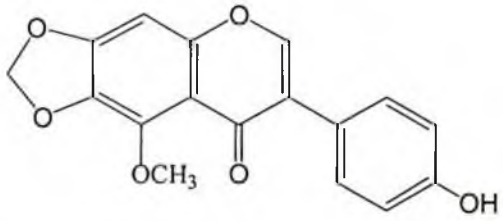


Wistin (**172**)

Compounds **13, 14** and **17** were characterized as known 5-Hydroxy-4′-methoxy-6,7-methylenedioxy-isoflavone (**173**), irisolone (**131**) and irilone (**128**) respectively. These three compounds contain methylene dioxy group in ring A.

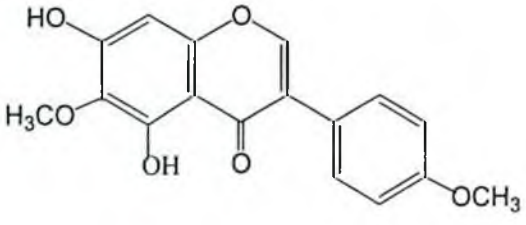


5-Hydroxy-4′-methoxy-6, 7-methylenedioxy-isoflavone (**173**)

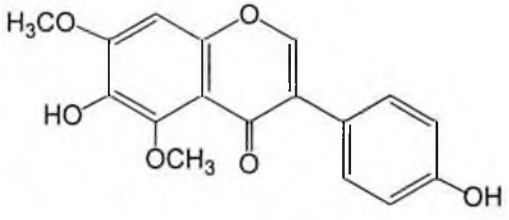


Irisolone (**131**)

Another two compounds **15** and **16** were also characterized as known isoflavones, irisolidone (**132**) and muningin (**174**), respectively. The compound **18** was β-sitosterol glucoside (**175**).



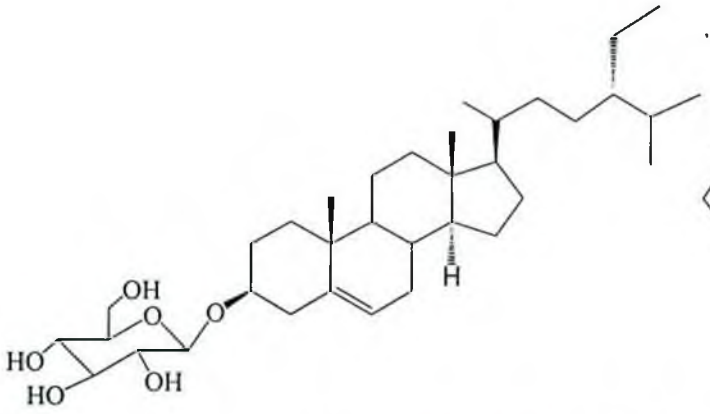
Irisolidones (**132**)



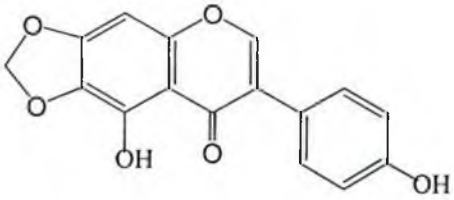
Muningin (**174**)

Compounds **12, 13, 16** and **18** have been isolated for the first time from this plant. Compounds **11, 13, 14** and **17** showed growth inhibition with IC_{12} values of 73 and 92

μg/mL, 43 and 100 μg/mL, 45 and 100 μg/mL, and 60 and 82 μg/mL towards the RAD 52 and RAD^+ mutant yeast strains, respectively in mechanism-based yeast bioassay.



β-Sitosterol glucoside (**175**)

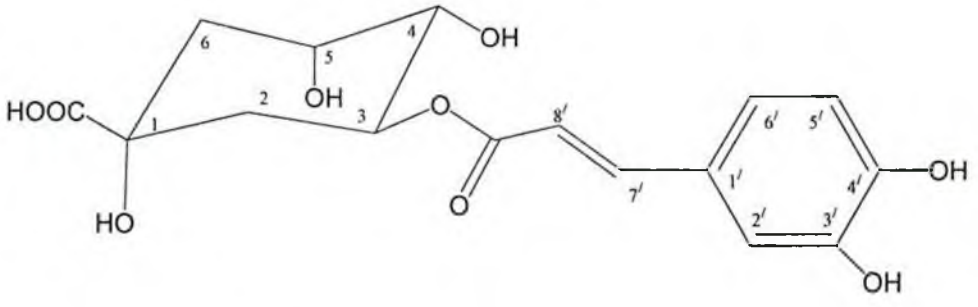


Irilone (**128**)

Part-C

The aqueous 80% EtOH extract of *Ficus racemosa* fruits exhibited significant hypoglycemic activity on healthy (non diabetic) and IDDM model rats when the extract was tested on healthy, NIDDM and IDDM diabetic model rats at BIRDEM (Bangladesh Institute of Research and Rehabilitation in Diabetis, Endocrine and Metabolic Disorders). The 1-BuOH soluble part of the parent extract also showed significant free radical scavenging (antioxidant) activity, when assayed at the HEJ Research Institute of Chemistry.

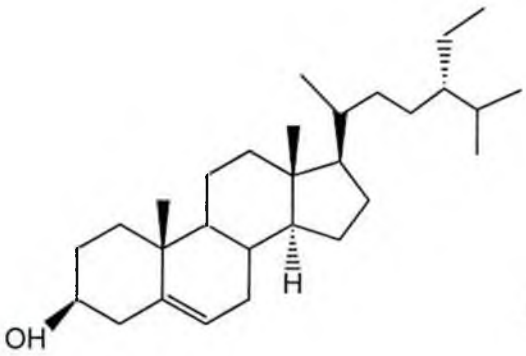
The activity-guided isolation on 1-BuOH soluble part yielded compound 3-*O*-(*E*)-caffeoyl quinate (**176**) (compound **19**).



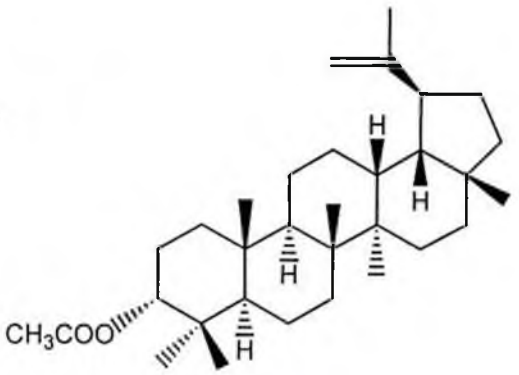
3-*O* (*E*)-Caffeoyl quinate (**176**)

The compound **19** have been isolated for the first time from this plant and exhibited significant free radical scavenging (antioxidant) activity.

Another five compounds were isolatd from the CH_2Cl_2 soluble part of the parent extract of *Ficus racemosa* fruits. Two compounds were characterized as β-sitosterol (compound **20**) and β-sitosterol glucoside (compound **21**).

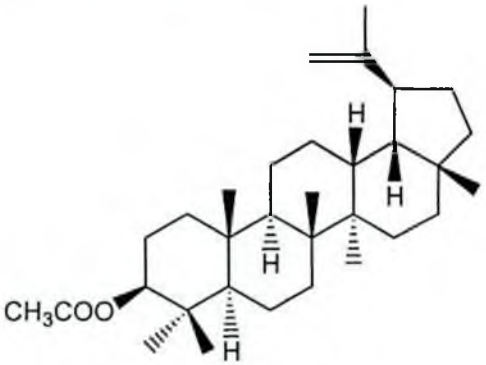


β- Sitosterol (**177**)

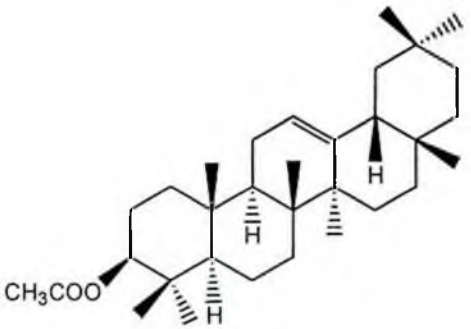


Epilupeol acetate (**178**)

Epilupeol acetate (**178**) (compound **22**), lupeol acetate (**179**) (compound **23**) and β-amyrin acetate (**180**) (compound **24**) were three known triterpens isolated from the CH_2Cl_2 soluble part of *Ficus racemosa* fruits. Epilupeol acetate (**178**) was isolated for the first time from this plant.



Lupeol acetate (**179**)



β-Amyrin acetate (**180**)

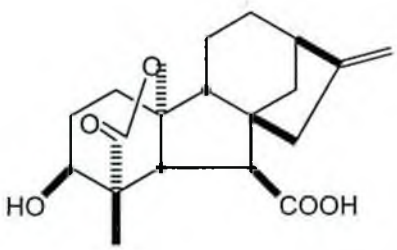
1. INTRODUCTION

01. Introduction

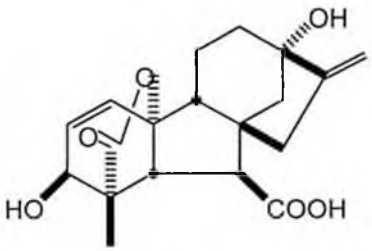
1.1 General

Plants by using different metabolic pathways synthesize a wide variety of organic compounds starting with photosynthesis. Carbohydrates, proteins, fats and nucleic acids are the primary metabolites which every plant produces. In contrast to the primary metabolic pathways, there exists another area of metabolism concerning with compounds, which have a limited distribution in nature. These compounds are the secondary metabolites and are known as natural plant products. The current interest in secondary metabolites of plant origin is mainly based on the fact that many of them are pharmacologically active.

A diverse variety of secondary metabolites are produced by plants. This may be due to many factors and needs such as, random synthesis by an organism to arrive at a useful compound for a certain physiological function (Pridham et al 1967), an alternative strategy to switching off metabolic-pathways temporarily (Dhar et al., 1971; Ponsinet et al., 1968), defense chemicals against predators, parasites, mechanical injury etc and ecological interactions (Sondheimer et al., 1970) [eg. allelopathy (Rice 1974)].



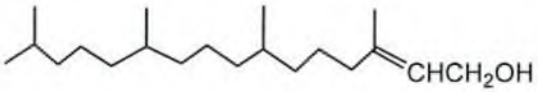
Gibberellin-A_4 (**1**)



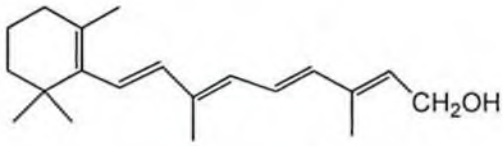
Gibberellic acid (**2**)

Specially, in case of diterpenoids, a clear physiological role for gibberellins (**1** and **2**) (MacMillan et al., 1973) as endogenous plant growth regulators has been established. Gibberellins promote cell elongation, induce parthenocarpy and induce new RNA and protein synthesis (Devlin et al., 1969). Phytol (**3**) is an integral part of chlorophyll and this

lipophilic side chain is nesserary for the biological activity. Retinol (**4**) is a vitamin (A) essential for mammalian growth. Retinol (**4**) and related compounds are involved in the visual function (Abrahamson et al., 1975) of not only mammals, but also insects and fish.

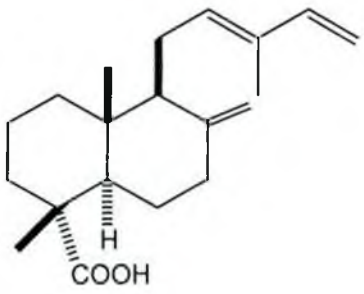


Phytol (**3**)

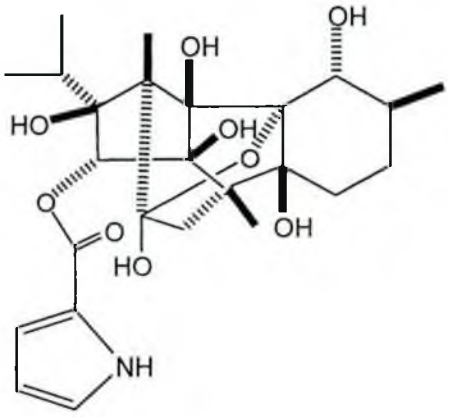


Retinol (**4**)

A number of compounds with insect growth regulatory activity (Fujita et al., 1976; Isogai et al., 1976) and insect antifeedent (Kubo et al., 1979)) or insecticidal activity (Casida 1976) have been isolated from higher plants: *trans*-ozic acid (**5**) (Stipanovic et al., 1979) and ryanodine (**6**) (Casida 1976) are the examples. While clerodin (**7**) (Hosozawa et al., 1974) is a representative of many similar antifeedent.



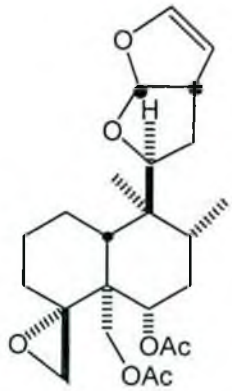
trans-Ozic acid (**5**)



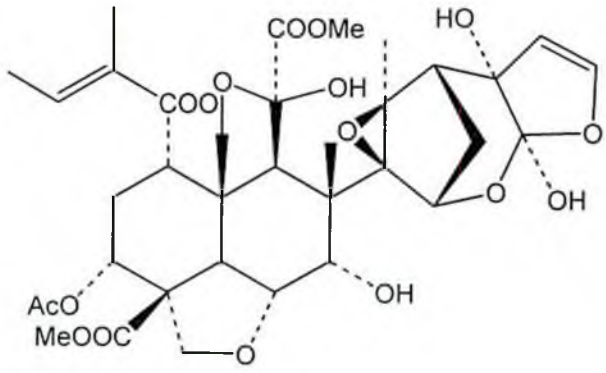
Ryanodine (**6**)

Azadiractin (**8**) the most interesting constituent of *Azadiracta indica* (neem tree) is a very potent insect antifeedent as well as an insect growth-regulating agent (Kraus et al., 1987). Insect chemistry has revealed the importance of several diterpenoids as pheromone e.g.

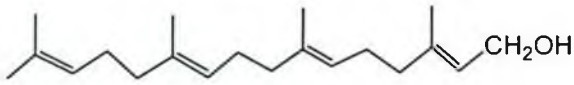
trans-geranylcitronellol (**9**) (Kullenberg et al., 1970) or defence chemicals, eg, sarcophine (**10**) from a soft-bodied coral (Bernstain et al., 1974).



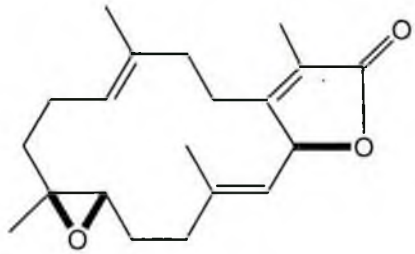
Clerodin (**7**)



Azadiractin (**8**)



Trans-Geranylcitronellol (**9**)

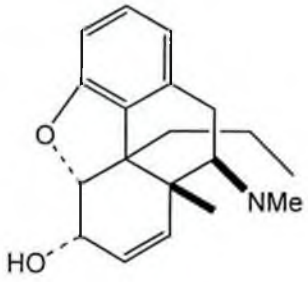


Sarcophine (**10**)

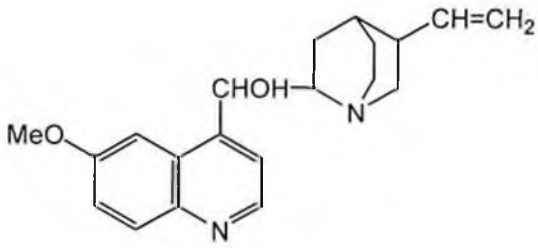
With the induction of newer and more effective seperation techniques, and powerful spectroscopic methods for structural analysis (especially nuclear magnetic resonance spectroscopy), coupled with recent advances in organic chemistry, and in our knowledge of the biosynthesis of naturally occurring molecules, the natural products have gained increasing importance in various aspects of human endeavors (Newman et al., 2000).

The primitive people used extracts of different plants for the releief of pain, and alleviation of the symptoms of diseases, as poisons for use in warfare and hunting and as stimulants to relieve fatigue and hunger etc. Many bioactive compounds were isolated and some are still commercially using as drug for the treatment of various diseases. Some

morphine (**11**), isolated from the latex of *Papaver somniferum* (Opium) in 1804 and quinine (**12**), isolated from *Cinchona spp* in 1820 are the examples (Ghani, 1991).

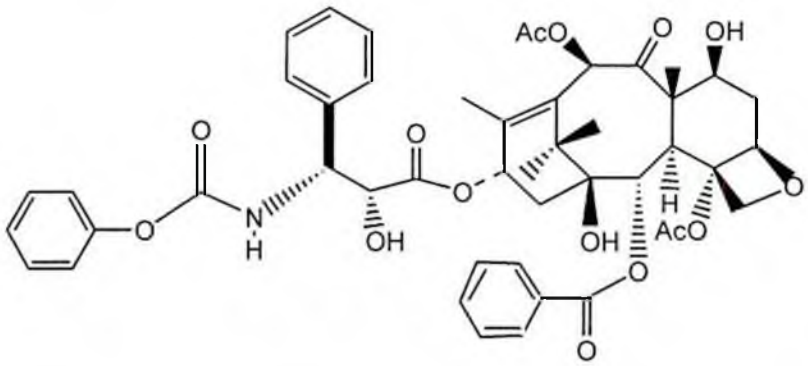


Morphine (**11**)



Quinine (**12**)

Recently, researchers are focusing their efforts on traditional medications of many cultures, and are screening a large number of both terrestrial and marine organisms for new bioactive compounds.



Taxol (**13**)

This led to the isolation of taxol (**13**), a chemical constituent from the stem bark of pacific Yew *Taxus brevifolia*, which has now been used as an important anticancer drug (Kingston et al., 1993). In 1992, the Food and Drug Administration (FDA) USA approved the use of taxol for the treatment of overian cancer and in 1994 it was permitted for the treatment of metastatic breast cancer (Rowinsky et al., 1993; Rowinsky and

Donehower, 1995). Some other useful anticancer natural productcs and their derivatives are exemplified as the compounds **14-26.**

Cancer is a disease, which has no geographic, national or ethnic boundaries. It accounts for a vast number of deaths and human sufferings. Solutions to the cancer problem involve both prevention and treatment, and advances on these two fronts require the continued development of novel and improved chemopreventive and chemotherapeutic agents.

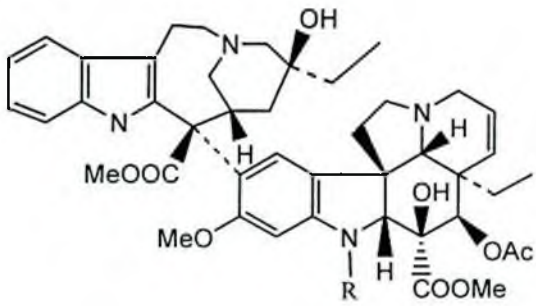
An essential step in an efficient natural product drug discovery program is the judicious selection of an initial screening strategy. The *in vitro* methods available for anticancer drug discovery can be grouped into two major categories. These are the disease-oriented approach and the mechanism-based approach. The disease-oriented approach involves cellular assays in which one looks for general cytotoxic agent or agents, which inhibit growth of cancer cells. Tumor cell lines utilized by the USA National Cancer Institute (NCI) belong to this category. Such bioassays are most powerful when they are used, as in the NCI program (Boyd et al., 1995). The disease-oriented panel of human tumor cell lines requires a major investment in personnel and facilities. The use of mechanism-based screen is a second approach to drug discovery. Mechanism-based approach for anticancer natural product drug discovery utilizes DNA repair or recombination-deficient mutants of the yeast *Sacharomyces cerevisiae* (Gunatilaka et al., 1992).

The phytochemical studies on medicinal plants serve the dual purpose of bringing up new therapeutic agents and provide useful leads for further studies directed towards the synthesis of new drugs, modeled on the basis of chemical structures of the natural products. Moreover, they promote studies in the correlation of chemical structures and physiological activity through functional variations in the chemical structures of the plant materials (Cordell, 2000).

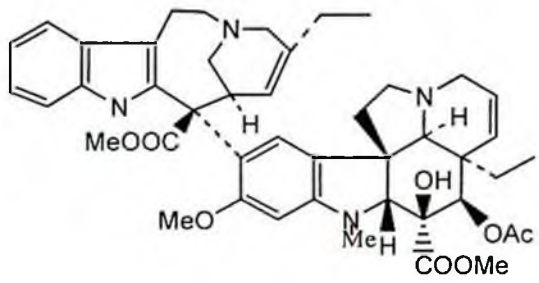
1.2 Anticancer Natural Products and their Derivatives

Traditionally, anticancer drugs were discovered through large-scale screenings of synthetic chemicals and natural products against animal tumor systems, primarily murine leukemias. The agents discovered in 1950 to 1980 for cancer chemotherapy largely interacted with DNA or its precursors, inhibiting the synthesis of new genetic material or causing irreparable damage to DNA itself. In recent years, the discovery of new agents has extended from the more conventional natural products, such as paclitaxel (TAXOL) (**13**) and semi synthetics such as etoposide (**18**), both of which target the proliferation of process, to entirely new fields of investigation that represent the harvest of new knowledge about cancer biology. The successful application of this knowledge includes diverse range of drugs.

Vincrinstine (**14**), vinblastine (**15**), vinorelbine **(16)** were discovered from *Catharanthus roseus* Linn. Vinblastine and vincrinstine are important clinical agents for the treatment of lymphomas, and testicular cancer. Vincristine and vinblastine, as well as the analog vinorelbine, have potent and selective antitumor effects, although their actions on normal tissue differ significantly.



R = CHO; Vincristine (**14**)
R = CH_3; Vinblastine (**15**)

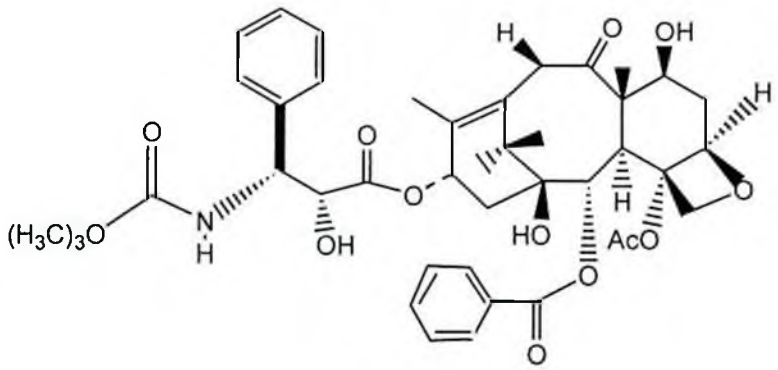


Vinorelbine (**16**)

Another Vinca compound, vinorelbine is active against lung and breast cancer. Vincristine is a standard component of regimens for treating pediatric leukemias and solid

tumors and is frequently used in adult lymphoma treatment. Vinblastine is employed primarily in treating testicular carcinomas and lymphomas and as second-line therapy for various solid tumors. Vinorelbine has impressive single-agent activity against non-small cell lung and breast cancers, and its range of usefulness is expanding as new trials completing (Budman 1997; Williams and Enhorn 1985; Fumoleau et al., 1993).

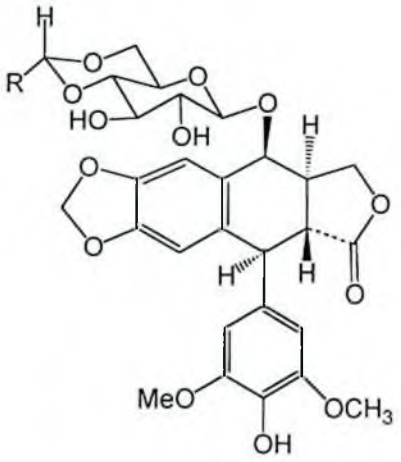
Paclitaxel (**13**) was first isolated from the bark of the 'Western Yew tree' *Taxus brevifolia* in 1971 (Wani et al., 1971). It exhibits unique pharmacological actions as an inhibitor of mitosis, differing from the vinca alkaloids and colonicine derivatives since they promote rather than inhibit the microtubule formation.

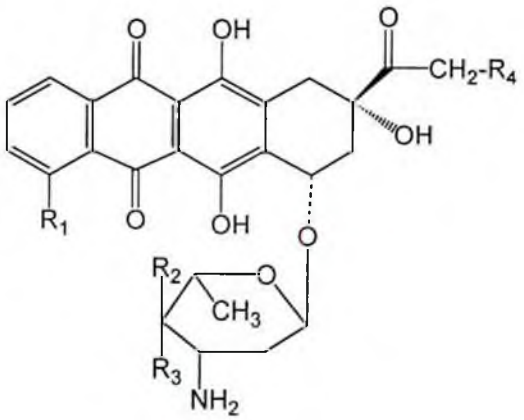


Docitaxel (**17**)

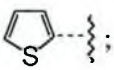
Following its introduction into clinical trials, the drug was approved for the treatment of cisplastin-refractory ovarian cancer in 1992 (Rowinsky et al., 1993; Rowinsky and Donehower, 1995) and has promising activity against cancers of the breast, lung, esophagus, and head and neck. Paclitaxel (TAXOL) is a diterpene that contains a complex taxane skeleton (**13**). The side chain linked to the taxane ring at C-13 is essential for its antitumor activity. Modification of the side chain has led to discovery of a more potent analog, docitaxel (TAXOTERE) (**17**), which has clinical activity against both breast and ovarian cancers. It also has been successfully synthesized (Nicolau et al., 1994) from simple off-the-shelf reagents in a complex series of reactions.

Podophylotoxins, extracted from the *Podophyllum peltatum* was used as a folk remedy by the American Indians and early colonists for its emetic, cathartic, and anthelmintic effects. Etoposide (VP-16-213) (**18**) and teniposide (VM-26) (**19**) are the two semisynthetic glycosides of the active principle, podophyllotoxin. Etoposide is primarily used for the treatment of testicular tumors, in combination with bleomycin and cisplastin, and in combination with cisplastin for small cell carcinoma of the lung (Nimati et al., 2000).





R = Me; Etoposide (**18**)

R =  ; Teniposide (**19**)

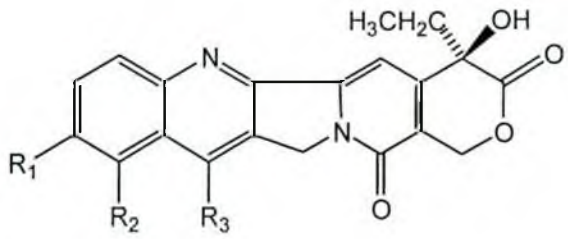
R_1	R_2	R_3	R_4	
OCH_3	H	OH	OH	Doxorubicin (**20**)
OCH_3	H	OH	H	Daunorubicin (**21**)
H	H	OH	H	Idarubicin (**22**)

It is also active against non-Hodgkin's lymphomas, acute nonlymphocytic leukemia, carcinoma of the breast, and Kaposi's sarcoma associated with acquired immunodeficiency syndrome (AIDS) (Chao et al., 2000; Tung et al., 2000). Teniposide is available for the treatment of refractory acute lymphoblastic leukemia in children (Odom and Gordon, 1984; Postmus et al., 1995; Boogerd et al., 1999).

Doxorubicin (**20**), daunorubicin (**21**), and idarubicin (**22**) are the anthracycline antibiotics. These compounds along with their derivatives are among the most important antitumor agents. They are produced by the fungus *Strep. peucetius* var. *caesius*. Although they differ only slightly in chemical structures, daunorubicin and idarubicin have been used

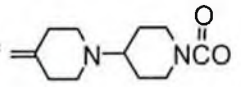
primarily in the acute leukemias, whereas doxorubicin displays a broader activity against human neoplasms, including a variety of solid tumors. The clinical value of these agents is limited by a usual cardiomyopathy, the occurance of which is related to the total dose of the drug; it is often irreversible. In a search for agents with high antitumor activity but reduced cardiac toxicity, hundreds of anthracycline derivatives and related compounds have been synthesized and screened. (Arlin et al., 1990; Feldman et al., 1993; Berman et al., 1991; Wiernik et al., 1992; Launchbury and Habboubi 1993)

The camptothecins are a promising new class of antineoplastic agents that target the enzyme topoisomerase 1. Camptothecin (**23**) was first isolated from the *Camptotheca acuminata* in 1966 (Wall et al, 1966). Toptican (**24**) and irinotecan (**25**) are currently the most widely used camtothecin analogs in the clinical settings, with established activity in colorectal, ovarian, and small cell lung cancer (Takimoto and Arbuck et al., 2001).



Camptothecin (**23**) $R_1 = R_2 = R_3 = H$;

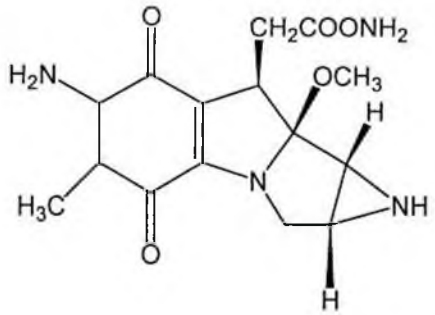
Toptican (**24**) R_1 = OH, $R_2 = CH_2N(CH_3)_2$, $R_3 = H$;

Irinotecan (**25**) $R_1 =$  R_2 = H, $R_3 = CH_2CH_3$

Toptican (**24**) is more active in previously treated patients with ovarian or small cell lung cancer. Its promosing antitumor activity also has been observed in hematological malignancies, particularly in chronic myelomonocytic leukemia and in myelodysplastic syndromes (ten Bokkel Huinink et al., 1997; von Powel et al., 1999).

Irinotecan (**25**) has significant clinical activity in patients with advanced colorectal cancer. It is now used for advanced colorectal cancer and may have an increasing role in the treatment of other solid tumors, including small cell and non-small cell lung cancer, cervical cancer, ovarian cancer and gastric cancer (Douillard et al., 2000; Cunningham et al., 1998)

Mitomycin (**26**) is an antibiotic isolated from *Strep. Caespitosus* by Wakaki and associates in 1958. Mitomycins have aziridine and a quinin groups in their structure, as well as a mitosane ring.



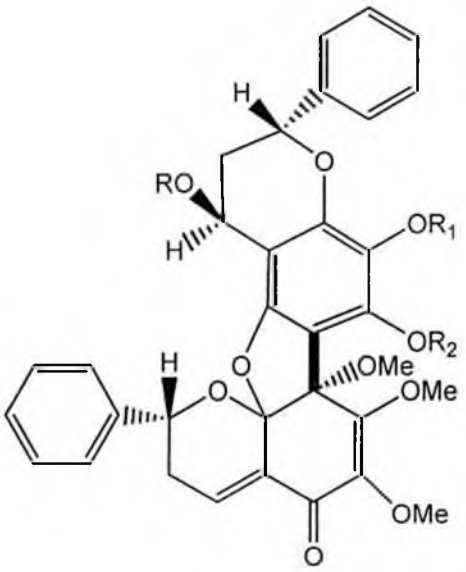
Mitomycin (**26**)

Each of these participates in the alkylation reaction with DNA. The mitomycin inhibits DNA synthesis. Mitomycin is primarily used in combination with 5-FU, *cis*-platin, or doxorubicin in carcinomous of the cervix, colon, rectum, breast, bladder, head and neck, and lungs (Boccardo et al., 1994).

1.3 Plant Derived Cytotoxic Agents

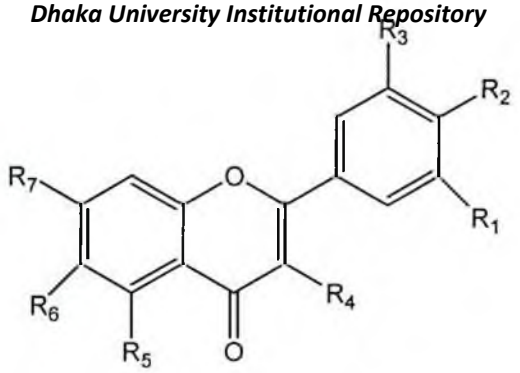
In addition to the above-mentioned natural compounds, a number of plant derived natural products are found as promising for future use in cancer chemotherapy.

Some important anticancer active constituents calycopterone (**27**), isocalycopterone (**28**) and 4-demethyl calycopterone (**29**), were discovered from *Calycopteris floribunda* Lamk. These have novel cytotoxicity in a diverse panel of human tumor cell line (Wall et al., 1994).



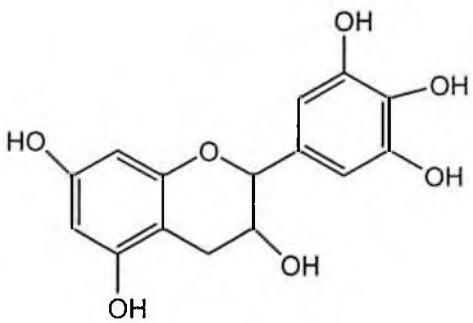
	R	R_1	R_2	
Calycopterone	Me	H	Me	(**27**)
Isocalycopterone	Me	Me	H	(**28**)
4-Demethyl calycopterone	H	H	Me	(**29**)

Quercetin (**30**) inhibited generation and effect or function of alloantigen-specific cytotoxic T lymphocytes. It also supressd the promoting effect of teleocidin on 7,12-dimethylbenzo [α] anthracene-induced skin tumors in mice. Two isomeric flavonols, 3,3′-dimethylquercetin (**31**) and 3,7-dimethyl quercetin (**32**) showed cytotoxic activity against P-388 cells *in vitro*. 4′,5,6,7-Tetramethoxyflavone (**33**) was found to be cytotoxic towards various strains of carcinoma cells (Pathak et al., 1991).

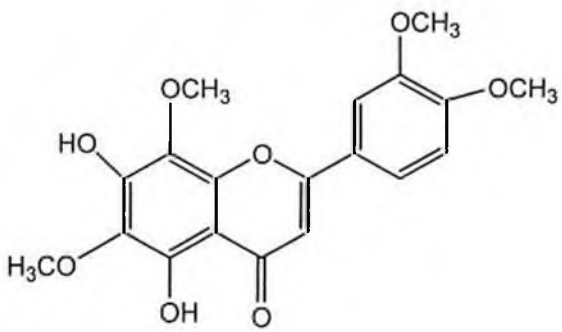


	R_1	R_2	R_3	R_4	R_5	R_6	R_7	
Quercetin	H	OH	OH	OH	OH	H	OH	**(30)**
3,3′-Dimethyl quercetin	H	OH	CH_3	CH_3	OH	H	OH	**(31)**
3,7-Dimethyl quercetin	H	OH	OH	CH_3	OH	H	CH_3	**(32)**
4′,5,6,7-Tetra-methoxy flavone	H	CH_3O	H	H	CH_3O	CH_3O	CH_3O	**(33)**
Myricetin	OH	OH	OH	H	OH	H	OH	**(34)**

Highly toxic myricetin (**34**) inhibited predominantly the uptake of thymidine, and uptake of uridine was inhibited by (-)-epigallocatechin (**35**).



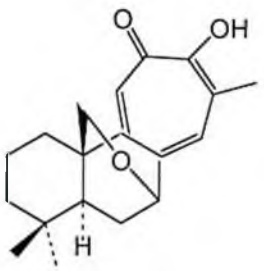
(-)-Epigallocatechin (**35**)



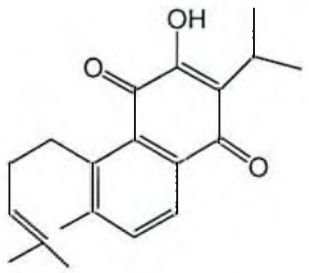
Hymenoxin (**36**)

Hymenoxin (**36**) isolated from *Scoparia dulcis* Linn was found to exhibit cytotoxicity against human cultured cells (Pathak et al., 1991).

A tropolone, militropolone (**37**) with potent cytotoxic activity, has also been isolated (Haro et al., 1990) from freshly collected roots of *Salvia miltiorrhiza*. The cytotoxic quinone sapriparaquinone (**38**) was discovered from the medicinal plant *S. prionitis* (Lin et al., 1990).

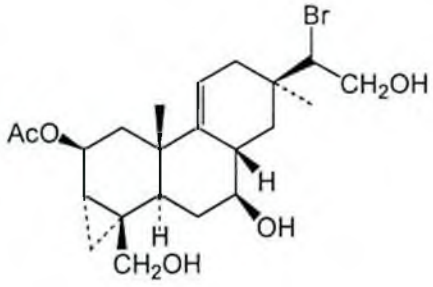


Militropolone (**37**)

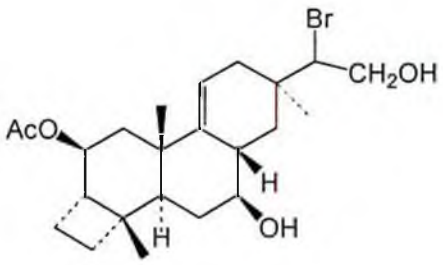


Sapriparaquinone (**38**)

Cytotoxic brominated diterpenoids e.g. perguerol (**39**) and isoperguerol (**40**) with a modified pimarane skeleton were reported from a specimen of sea hare, *Aplysia dactylomela* (Schmitz et al., 1982).



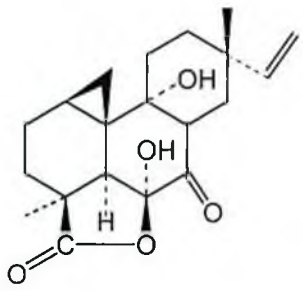
Perguerol (**39**)



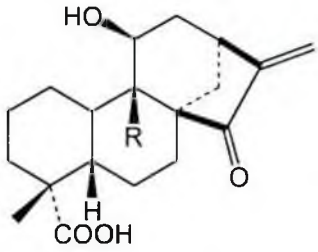
Isoperguerol (**40**)

Myrocin C (**41**), isolated from a species of *Myrothecium* (Hsu et al., 1987) was found as a tumor-inhibitory antibiotic. *Ent*-11α-hydroxy-6-oxokaur-16-en-19-oic acid (**42**) and the

corresponding *ent*-9α,11α-diol (**43**) isolated from *Adenostemma lavenia* showed cytotoxic activity against tumor cells (Shimizu et al., 1990).



Myrocin C (**41**)



Ent-11α-hydroxy-6- oxokaur-16-en-19-oic acid R = H; (**42**)

Ent-9α,11α-diol R = OH (**43**)

Some other plant based cytotoxic agents of different structural types have also been reported. Eupatilin (5, 7-dihydroxy-3,4,6-trimethoxyflavone) a pharmacologically active flavone derived from *Artemisia asiatica* Nakai (Asteraceae) has shown chemopreventive and cytotoxic effects in human promyelocytic leukemia cells (Seo et al., 2001). Solavetivone, isolated from *Solanum indicum* was found to be cytotoxic to OVCAR-3 cells (Syu et al., 2001). Four pyranocoumarins, dipaetaline alloxanthoxyletin, xanthoxyletin; and xanthyletin; and two lignans; sesamin and asarinin isolated from the northern Prickly ash, have been reported to inhibit the incorporation of tritiated thymedine into human leukaemia (HL-60) cells (Ju et al., 2001).

Seven new annonaceous acetogenins, muricins A-G, isolated from the seeds of *Annona muricata,* showed significantly selective *in vitro* cytotoxicities toward the human hepatoxia cell lines HEP G (**2**) and 2,2,15 (**11**) (Chang et al., 2001). *In vitro* cytotoxic activities have been reported for twenty four proton berberine alkaloids related to berberine which have been evaluated using a human cancer cell line panel coupled with a drug sensitivity data base. The highest level of activity was observed in 8-*O*-13-hexyl-substituted derivatives of berberine (Iwasa et al., 2001). Goniothsalamusin and two

mixtures, acetogenins A and B isolated from a petroleum ether extract of the stem bark of *Miliusa velutina* exhibited significant cytotoxic activities (Jumana et al., 2000).

A coumestan derivative, Psoralidin was found to be a cytotoxic principle of the seeds of *Psoraleacorylifolia* (Leguminosae) against the HT-29 (colon) and MCF-7 (breast) human cancer cell lines, respectively (Mar et al., 2001). The known (*S*)-17-hydroxy-9,11,13,15-octadecatetra- ynoic acid (minquartynoic acid) and two novel analogues, (*S*)-17, 18-dihydroxy-9-11,13,15-octadecatetraynoic acid and (*S*)-17-hydroxy-15-*E*-octadecan-9,11,13-triynoic acid were found to be significantly cytotoxic against a panel of human prostate tumor cell lines (Ito et al., 2001). A new prenylated naphthoquinane, lippsidoquinane isolated from ethanol extracts of *Lippia sidoicles* exhibited significiant activity against human Leukemia cell lines HL 60 and CEM (Costa et al., 2001).

Agehoustin A, isolated from the aerial part of the Asteraceae family of plants was found to be cytotoxic for both cells using LLC-MK 2 and C_6 glical cells *in vitro* (Sanchez et al., 2001). Triterpenoid acids, nigranoic acid, manwuweizic acid, schisandronic acid showed significant cytotoxic effect against human decidual cells and rat luteal cells *in vitro* (Chen et al., 2001). Skyrin, a anthraquinone isolated from *Ventilago leiocarpa* suppressed growth of Hela, Vero, K562, Raji, Wish, and Calu-1 tumor cell lines except in K562 cells (Lin et al., 2001).

1.4 Objective of the Present Work

Some natural products and their analogs e.g. compounds **13-26** are used as chemotherapeutic agents for cancer treatment. However, many of these agents have complex structure and would probably never emerge from a synthetic program alone or from a combinatorial approach. The approach to discover and develop new drugs from natural origin is therefore, still an attractive area along with complementary synthetic and biosynthetic approaches. The main objective of the present study was therefore to isolate new and novel bioactive secondary metabolites from the natural sources.

During the course of this study, the plant *Suregada multiflora* was collected on the basis of ethonomedical and folkloric reputation and was identified by the taxonomist. Extracts of the plant materials were screened in brine shrimp lethality assay. The toxic extracts were then further tested for their efficacy on human tumor 60-cell lines at the National Cancer Institute (NCI), USA. The CH_2Cl_2-MeOH extract of the bark of *Suregada multiflora* exhibited significant cytotoxic activity for non-small cell lung (NCI-H522), colon (COLO-205), (HCC 2998); renal (ACHN), (TK-10), (CAKIL); and breast (MDA-MB-235) on human tumor 60 cell panels (Section- 2.4.5). The same extract also showed significant growth inhibitory activity towards a DNA repair deficient yeast mutant in mechanism-based yeast bioassay (Gunatilaka et al., 1992).

Some of the *Iris* species are used for the treatment of cancer in the folkloric system of medicine and some cytotoxic compounds have also been isolated from some of the *Iris* species (Shawl et al., 1988). In the current study, the CH_2Cl_2 and EtOAc soluble parts of *Iris germanica* rhizomes were tested in the mechanism-based yeast bioassay and both the extracts showed significant growth inhibitory activity towards the DNA repair deficient yeast mutant strain RAD 52.

From literature survey *S. multiflora* has been identified as a rich source of diterpene and flavonoid type compounds (Das et al., 1994; Das, et al., 1993; Parveen et al., 1987; Talapatra et al., 1989; Talapatra, et al., 1998). *Iris germanica* was identified as a rich source of flavonoids and isoflavonoids (Ali et al., 1983; Ali et al., 1993; Dhar et al., 1971; Dhar et al., 1973; Kalla et al., 1978; Shawl et al., 1984). Many of the diterpenoids

(Shimizu et al., 1990; Schmitz et al., 1982), flavonoids (Pathak et al., 1991) and some isoflavoinds (Carman et al., 1985; Devon et al., 1975), isolated from different natural sources have been found to be cytotoxic and some of them are being used as anticancer agents e.g. taxol (Kingston et al., 1993; Rowinsky et al., 1993; Rowinsky and Donehower, 1995). A number of tumor-inhibitory diterpenoids have also been reported (Hsu et al., 1987).

The results of the bioassays on the extracts of *S. multiflora* bark and *I. germanica* rhizomes stimulated us to conduct a mechanism-based yeast bioassay guided isolation of bioactive constituents from *S. multiflora* bark and *I. germanica* rhizomes.

During the present study, the aqueous EtOH extract of *Ficus racemosa* fruits showed significant hypoglycemic activity when tested on healthy (non diabetic), NIDDM and IDDM diabetic model rats at BIRDEM (Ali et al., 1993). The 1-BuOH soluble part of the parent extract of *F. racemosa* fruits showed significant free radical scavenging (antioxidant) activity (DPPH) (Lamaison et al., 1990; Navarro et al; 1993), assayed at the HEJ Research Institute of Chemistry. An antioxidant can prevent the constant oxidative damage of DNA, which is one of the major causes for the development of cancer disease. Therefore a bioassay-guided investigation on this antioxidant active extracts of *Ficus racemosa* fruits was also carried out to isolate bioactive agents. The methodologies followed for the search of bioactive secondary metabolites include extraction, bioassay, bioactivity-guided fractionation, dereplication and structure elucidation.

2. PART A

Phytochemical Investigations On *Suregada multiflora* (Juss) Bail

2. Part

Phytochemical Investigations On *Suregada multiflora* (Juss) Bail

2.1 Introduction

The plant *Suregada multiflora* (Juss) Bail (Syn. *Gelonium multiflorum*) belongs to the family Euphorbiaceae and genus *Suregada* Roxb.

2.1.1 Family Euphorbiaceae

The family Euphorbiaceae consists of herbs, shrubs and trees with milky or colored juice. Leaves are alternate, opposite or sometimes whorled, usually simple, sometimes compound or divided at times very reduced, usually stipulate. This is one of the largest family consisting of 321 genera which contains 7950 species of both economic and decorative importance worldwide. Among the different types of compounds found in Euphorbieace family, the bioactive skin irritant polycyclic diterpenoids e.g. tigliane and ingenane type which are common in their milky latex. *Euphorbiaceae Kansui* are considered as a herbal remedy for edema, ascites and cancer in China. Some of the plants are used in folk medicine in order to cure some skin diseases, gonorrhea, migraine, and intestinal parasite (Evans et al., 1983; Singla et al., 1990).

2.1.2 The Genus *Suregada* Roxb

Suregada Roxb is a genus belonging to the family Euphorbiaceae. The genus consists of shurbs or small trees. Leaves are alternate, rarely opposite, entire or denate; stipule connate, sheathing, caducous. Flowers small, apetalous, shortly pedicelled in extra-auxiliary and leaf-opposed clusters or racemes. The Genus consists of 40 species from the old World tropics and distributed in the tropical and sub-tropical parts of Asia and Africa. Three species are

found in south East Asian region: *Suregada multiflora* (Juss) Bail, *Suregada lanceolata* (Willd) and *Suregada angustifolia* (Muell Arg).

2.1.3 *Suregada multiflora* (Juss) Bail

The species *Suregada multiflora* is a dioecious tree, 30-40 ft high, with thick grayish bark, bright green leaves. The tree is occasionally planted in gardens for its evergreen ornamental foliage. The wood is yellowish white, smooth, hard, heavy (wt. 47 lb/cu. ft), close-and even-grained. It has a waxy odor. It is used for rafters and posts and as fuel. The buds of the plant exude a yellow resin (*The Wealth of India* 1956)

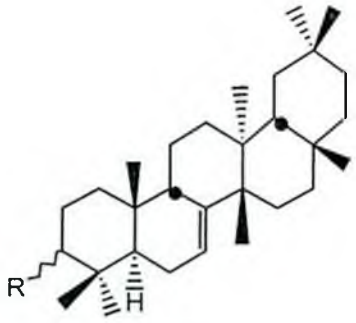
2.1.3.1 Medicinal Properties

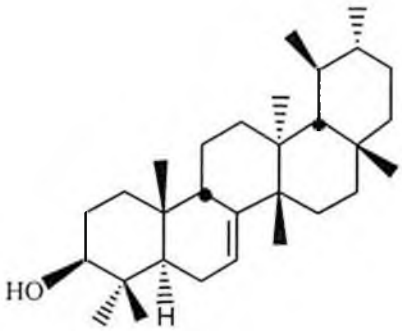
The plant *S. multiflora* is reported to be used for healthy gums and as a purgative in hepatic complaints in folkloric medicines (*The Wealth of India* 1956). The plant toxin gelonin (GAP 31), an extremely potent inhibitor of protein synthesis and similar in action to richin (Rosenblum et al., 1995) has been isolated from seeds of *S. multiflora*. GAP 31 is a new class of anti-HIV agent and inhibits HIV-1 infection and replication in a dose-dependent manner with little toxicity to target cells (Lee-Huang et al., 1995). It was also found to be effective against human breast cancer MDA-MB-231 *in vitro* and *in vivo* experiments (Lee-Huang et al., 2000). It is not toxic to human spermatozoa and may be useful in preventing the sexual transmission of human immunodeficiency virus type (Schreiber et al., 1999) and was found to be useful for the therapy of herpes simplex virus infections (Bourinbaiar et al., 1996).

2.1.3.2 Chemical Constituents

A brief literature survey on the genus *Suregada* Roxb showed that, chemical investigations on other species of the genus are not available. Sengupta and co-workers first reported a new triterpene alcohol 7-multifloren-3β-ol (**44**) and a known triterpene alcohol 7-baurenol-3β-ol (**45**) from the bark extract of *S. multiflora* (Sengupta et al., 1963). 7-multiflorene-3α-ol a

triterpene alcohol (**46**) was isolated by Raw. L. R. and co-workers from the bark extract of the same plant (Raw et al., 1969).

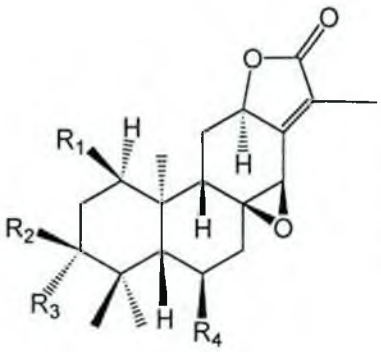




7-Multifloren-3β-ol (R = β OH) (**44**)
7-Multifloren-3α-ol (R = α OH) (**46**)

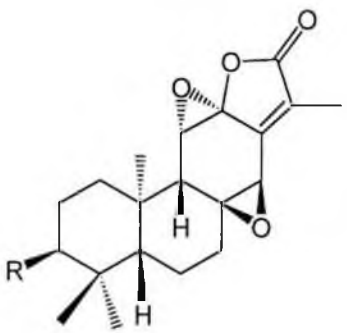
7-Baurenol-3β-ol (**45**)

Talapatra, S. K. and co-workers have reported six novel diterpene lactones gelomulide A-F (**47-52**) with *ent*-abietane skeletone from the leaf extract of *S. multiflora* (Talapatra et al., 1989).

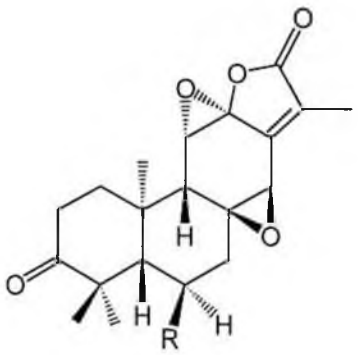


Gelomulide A ($R_1 = R_3 = R_4 = H$, $R_2 = OAc$) (**47**)
Gelomulide C ($R_1 = H$, $R_2+R_3 = O$, $R_4 = H$) (**49**)
Gelomulide F ($R_2 = OAc$, $R_2+R_3 = O$, $R_4 = H$) (**52**)
Gelomulide G ($R_1 = R_3 = H$, $R_2 = R_4 = OAc$) (**53**)

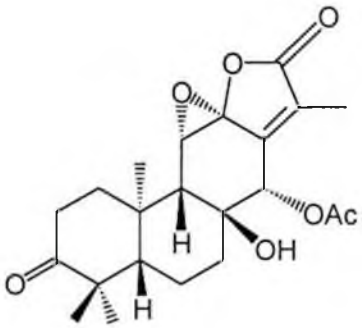
Reinvestigations of the leaves of *S. multiflora* led to the isolation of four more diterpne lactones gelomulides G-J (**53-56**) by Talapatra. B. and group) (Talapatra *et. al.*, 1998).



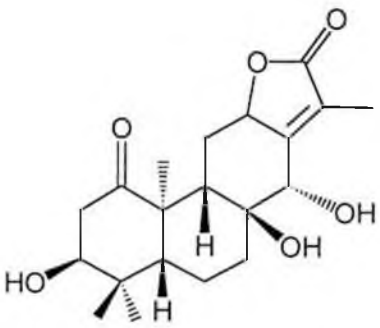
Gelomulide B (R = OAc) (**48**)
Gelomulide J (R = OH) (**56**)



Gelomulide D (R = H) (**50**)
Gelomulide E (R = OAc) (**51**)

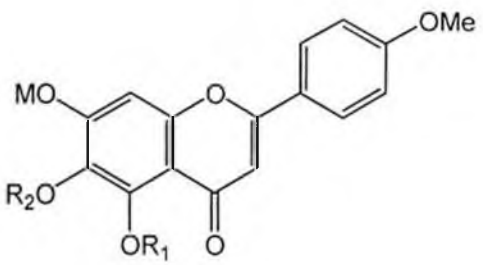


Gelomulide H (**54**)



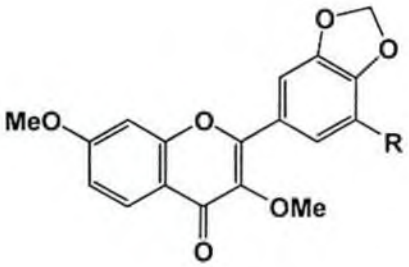
Gelomulide I (**55**)

Das, B. and co-workers reported two new flavone glycosides 7,4′-*O*-dimethoxyl-scutellarein 6-*O*-α-L-ramnopyranosyl (1→2)-β-D-glucopyranoside (**57**) and 6-*O*-β-D-xylopyranosyl (1→2)-D-glucopyranoside (**58**), along with a known flavone glycoside 7,4′-*O*-dimethoxylscutellarein 6-*O*-β-D-glucopyranoside (**59**) from seeds of *S. multiflora* (Das et al., 1993).

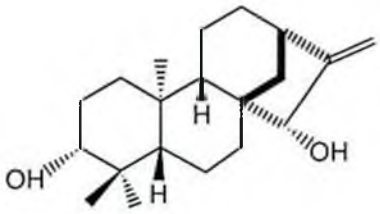


	R_1	R_2
7,4′-*O*-Dimethoxylscutellarein 6-*O*-α-L-rhamnopyranosyl -(1 →2)-β-D-glucopyranoside (**57**)	H	Glc^2-Rha
7,4′-*O*- Dimethoxylscutellarein 6-*O*-β-D-xylopyranosyl (1 →2)-D-glucopyranoside (**58**)	H	Glc^2-Xyl
7,4′-*O*- Dimethoxylscutellarein 6-*O*-β-D-glucopyranoside (**59**)	H	Glc

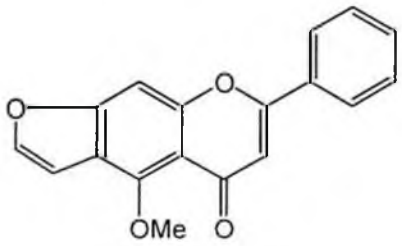
Das, B. and group reinvestigated the roots of *S. multiflora* in 1994 and reported a new diterpene *ent*-kaurane-3β,15β-diol (**60**) and 3 known flavones kanugin (**61**), dimethoxy panugin (**62**) and pinnatin (**63**) (Das et al., 1994).



Kanugin (**61**) R = OM e
Dimethoxy panugin (**62**) R =H



Ent-Kaurrene-3β,15β-diol (**60**)



Pinnatin (**63**)

2.2 Mechanism-Based Yeast-Bioassay

In the present study the parent bark extract of *S. multiflora* exhibited significant cytotoxic activity in different human tumor cell lines when tested at the National Cancer Institute, USA (Section-2.4.5, Page-32). The CH_2Cl_2 soluble part of the parent extract also showed significant growth inhibitory activity in mechanism-based yeast bioassay when conducted at the HEJ Research Institute of Chemistry (Section-2.46, Page-33). A bioassay-guided fractionation and isolation work was then carried out on the active CH_2Cl_2 soluble part of the parent extract of *S. multiflora* bark to isolate bioactive secondary metabolites. Therefore, a brief introduction about the mechanism-based yeast-bioassay is discussed in this Section.

Effective antitumor agents can act through many mechanisms. During this study we utilized a mechanism-based yeast bioassay (Gunatilaka et al., 1992) to guide the isolation work on CH_2Cl_2 soluble part of the parent bark extracts of the plant *S. multiflora*. The assay that we used is based on the differential response of DNA repair-deficient and repair-proficient yeast strains of *Saccharomyces cerevisiae* to the test sample. A genetically engineered yeast mutant strain RAD 52 which lacks the recombination pathway associated with repair of double-strand breaks and meiotic recombination was used in this assay (Gunatilaka et al., 1992).

DNA, the biochemical repository of the genetic inheritance of all cells, is subjected to damage by various chemicals and by radiations. An important and exploitable feature of most tumor cells is that they have defects on their ability to repair damaged DNA as compared to the normal cells. This internal deficiency was used in this work through the DNA repair-deficient (RAD 52) mutant strain combining with the repair-proficient (RAD^+) strains. The yeast mutants RAD 52 lack the DNA double strand break repair and meiotic recombination pathway cannot repair their damaged DNA as compared to the wild type yeast strain RAD^+. Thus the mutant yeast strain RAD 52 resembles the tumor cells, suggesting that agents with significant growth inhibitory activity towards DNA repair deficient yeast mutant RAD 52 might be a potential antitumor agents (Gunatilaka et al., 1992).

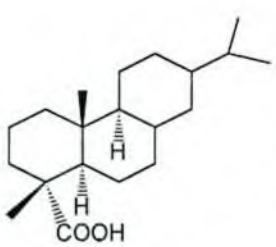
2.3 Diterpenoids

Suregada multiflora has been identified as a rich source of diterpenoids. A number of novel and new diterpenoids have been reported from different parts of the plant (Talapatra et al, 1989; Talapatra et al, 1998; Das et al., 1994). During the present study, we have also isolated several novel and new diterpenoids from the bark extract of *S. multiflora*.

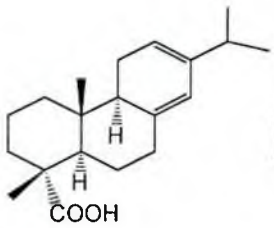
The diterpenoids are C-20 compounds comprising of four isoprene units and biogenetically derived from geranylgeranyl pyrophosphate. However, there are several naturally occurring compounds consisting of either fewer, or more carbon atoms than twenty, but because of their biosynthetic or historical relation with diterpenoids, they are classified in this category. The notable feature of diterpene structures is the fascinating variations encountered in their skeletons and the occurrence in nature of both normal and antipodal stereochemical series (Dev et al., 1986). Diterpenoids are widely distributed in the plant (MacMillan et al., 1973; Thomas et al., 1971) and animal kingdom, both terrestrial and marine (Fenical et al., 1978; Tursha et al., 1978; Birch et al., 1972; Prestwich et al., 1976; Kullenberg et l., 1970; Farrar et al. 1952) and have even been isolated from fossils also (Thomas et al., 1971; Streibl et al., 1969). Diterpenoids are also elaborated by a number of fungi (Turner et al., 1971), bryophyta and algae (Fenical et al., 1978; Wells et al., 1979).

The percentage and even nature of a diterpenoid may vary widely with the time of harvesting of the biological material. Different parts of the same plant may contain different diterpene constituents and one of the noted example was diterpenoids from *Trachylobium verrucosum* (Hugel et al., 1965). Co-occurance of di- and tri- terpenes in the same plant is rather rare, though some examples of co-occurances are on record: *Coffe coil* (Ponsient et al., 1968), *Cedrala toona* (Nagasampagi et al., 1967; *Gelonium multiflorum* (Sengupta et al., 1963; Raw et al., 1969; Talapatra et al, 1998; Das et al., 1994). In nature diterpenoids occur both in free and combined states. Hydrocarbons and most of the not highly functionalized diterpenes occur free. Acids invariably occur free though occurance of some methyl esters is known. Alcohols often occur both in the free state and as esters, in which the acid part is usually a simple aliphatic acid or less often an aromatic acid, though esters derived from more exotic acids have been encountered (Uemura et al., 1977).

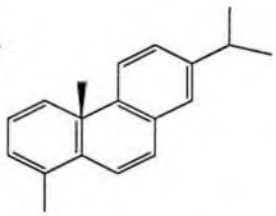
Abietic acid (**64**), the first diterpene was isolated from rosin. Rosin acids are a mixture of hardly separable diterpenoids and in fact abietic acid (**64**) was separated in an impure form from *Pinus palustris* and other *Pinus* spcies. For structure determination of these compounds, Ruzika suggested two important ways, dehydrogenation of abietic acid (**64**) and levopimaric acid (**65**) to retene (**66**) and secondly as according to isoprene rule the terpenoids are divisible to isoprene units, so the carbon skeleton (**67**) could be determined according to this rule (Ruzicka, 1924; Ruzicka, 1931). Then structure (**68**) of vitamin A was also determined by using isoprene rule (Karrer et al., 1931).



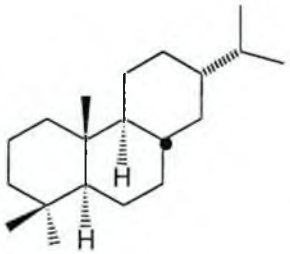
Abietic acid (**64**)



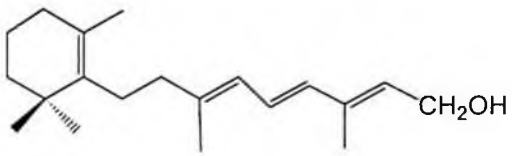
Levopimaric acid (**65**)



Retene (**66**)



Abietane (**67**)



Vitamin A (**68**)

2.3.1 Classification

Diterpenoids constitute a rich and diverse array of skeletal types. These skeleta are related to some main skeleton devided according to their number of rings as:

Acyclic and monocyclic: Phytane, retinane or 10,15- cyclophytane and cembrane;

Bicyclic: Labdane, clerodane and chettaphanane,

Tricyclic: Lathyrane, fujinane, pimarane, rosane, abietane, totarane, cassane and taxane; **Tetra-** and- **pentacyclic**: Tigliane, gibberellane, andromedane, kaurane, beyerane, atisane, trachylobane and aconane (Dev, 1986).

2.3.2 Biosynthesis

As mentioned earlier, the diterpenoids are invariably derived from isoprene. The biosynthesis of all isoperenoids begins from mevalonic acid which through various enzyme-catalyzed steps produce geranyl diphosphate (GPP) and isoperenyl diphosphate (IPP). For the synthesis of diterpenoids, geranyl geranyl diphosphate (GGPP) is the real precursor, which is derived by the condensation of geranyl diphosphate (GPP) with two units of isopentyl diphosphate (IPP) through the formation of farnesyl diphosphate (FPP). Farnesyl diphosphate and geranyl geranyl diphosphate "synthase" are the enzymes responsible for condensation steps (Fig. **1**). Cyclization reactions of GGPP mediated by carbocation formation, plus the potential for Wagner-Meerwein rearrangements, will allow many structural variants of diterpenoids to be produced. Biosynthesis of GGPP (**69**) and its classification according to the number of ring is given in Fig. 1 and 2.

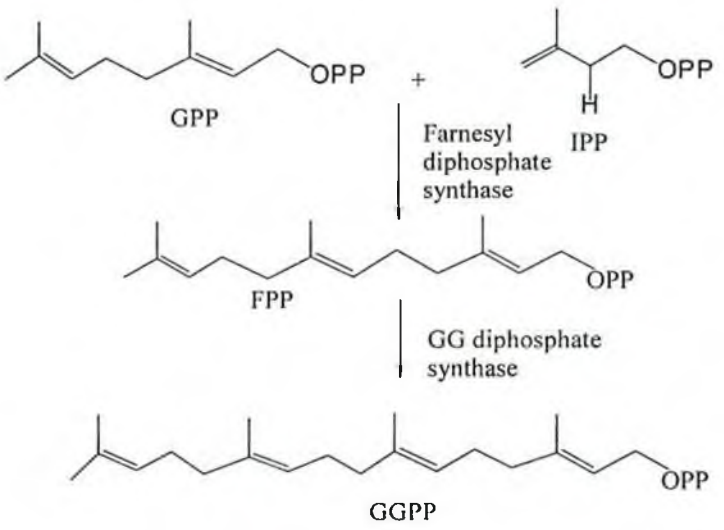


Fig. **1**. Biosynthesis of GGPP (**69**)

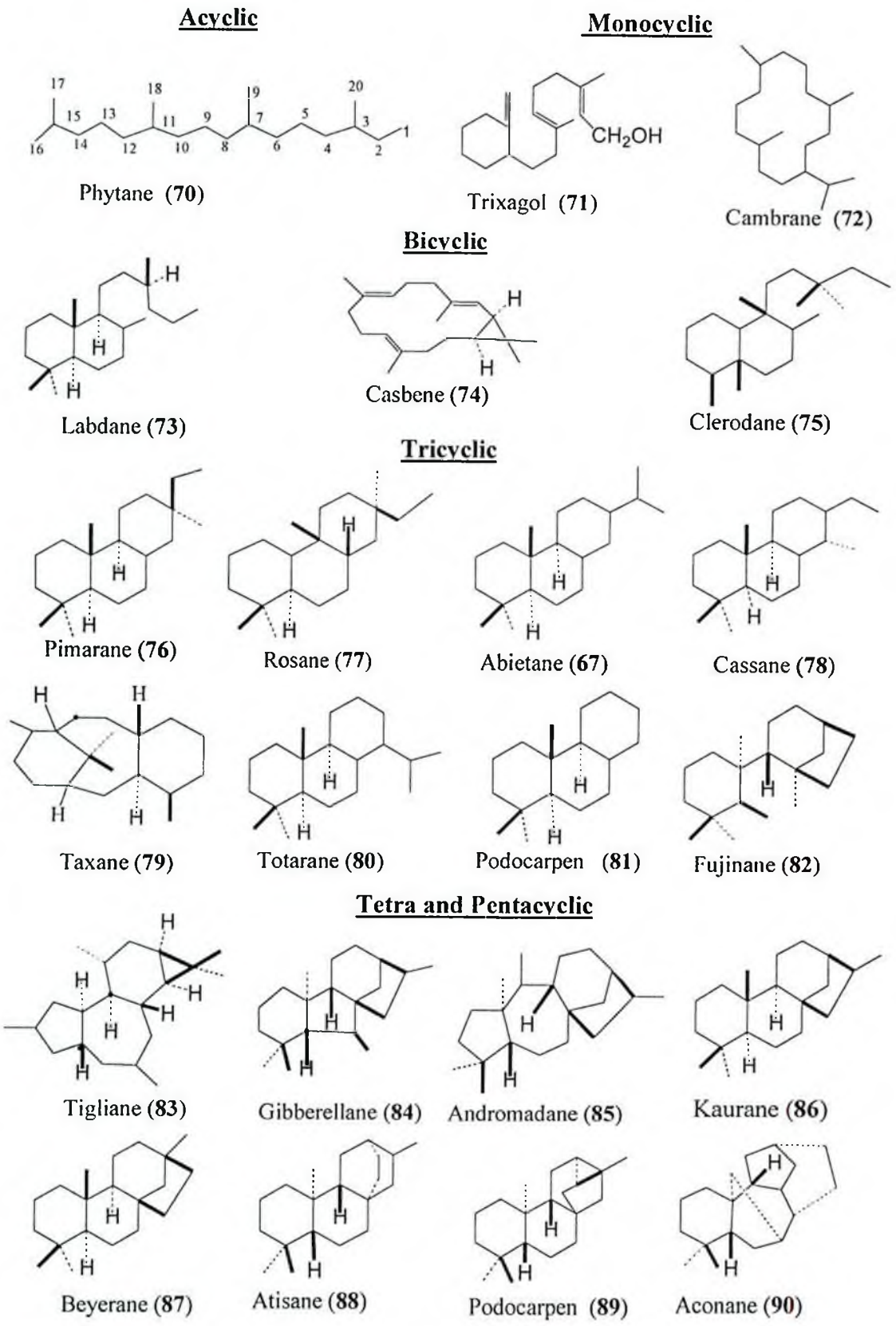


Fig. 2. Classification of main diterpene skeleta according to the number of rings.

Biogenesis of Cyclic Diterpenoids: The bicyclic cation structure copalyl PP (**91**) and the derived copalyl PP (**92**) play a central role in the biosynthesis of most of the bi-, tri-, tetra- and pentacyclic diterpenoids as indicated in summary, in Fig. 3. It may be noted that copalyl PP is an obligatory intermediate in going from GGPP to these tri- and tetracyclic diterpenoids.

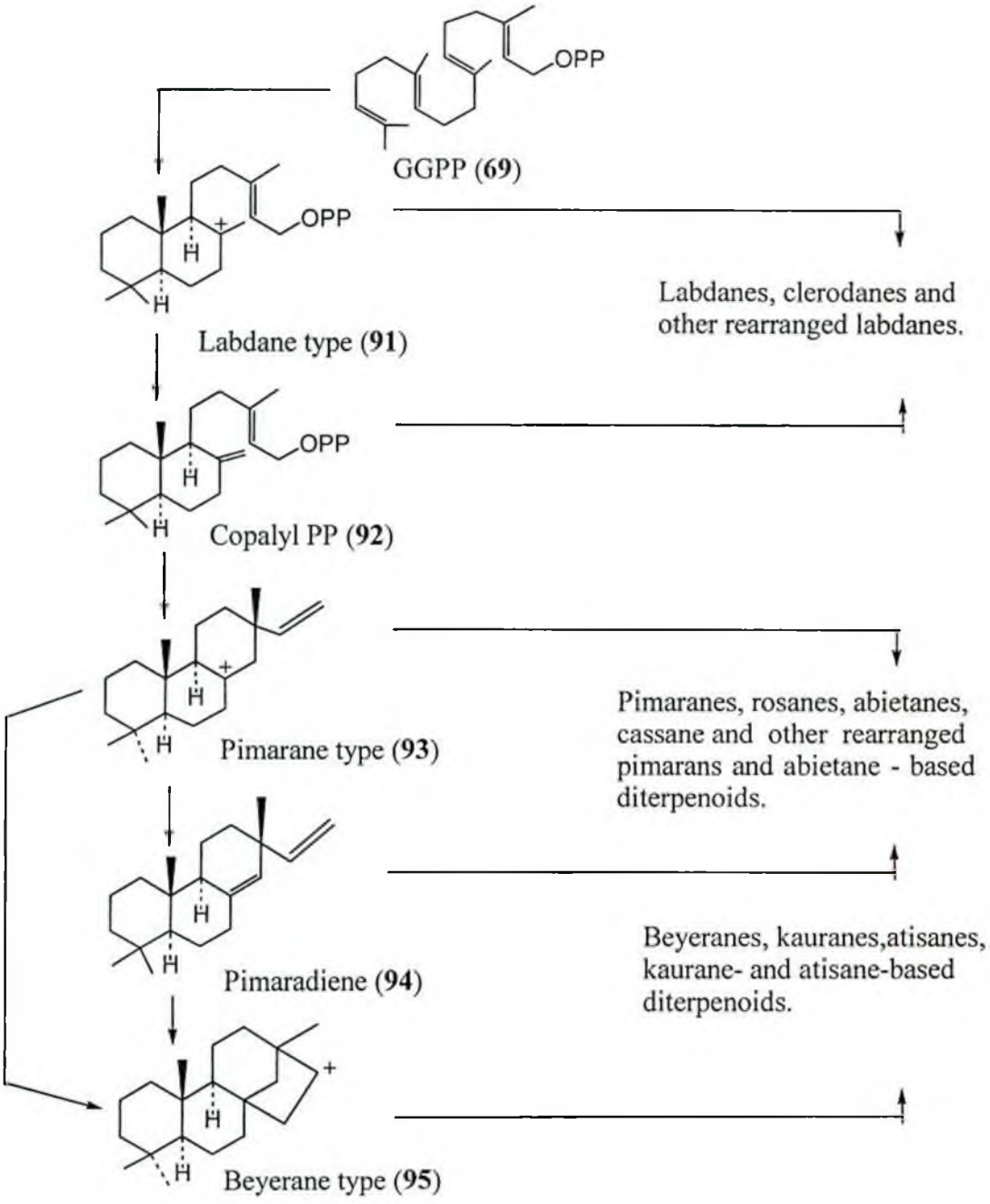
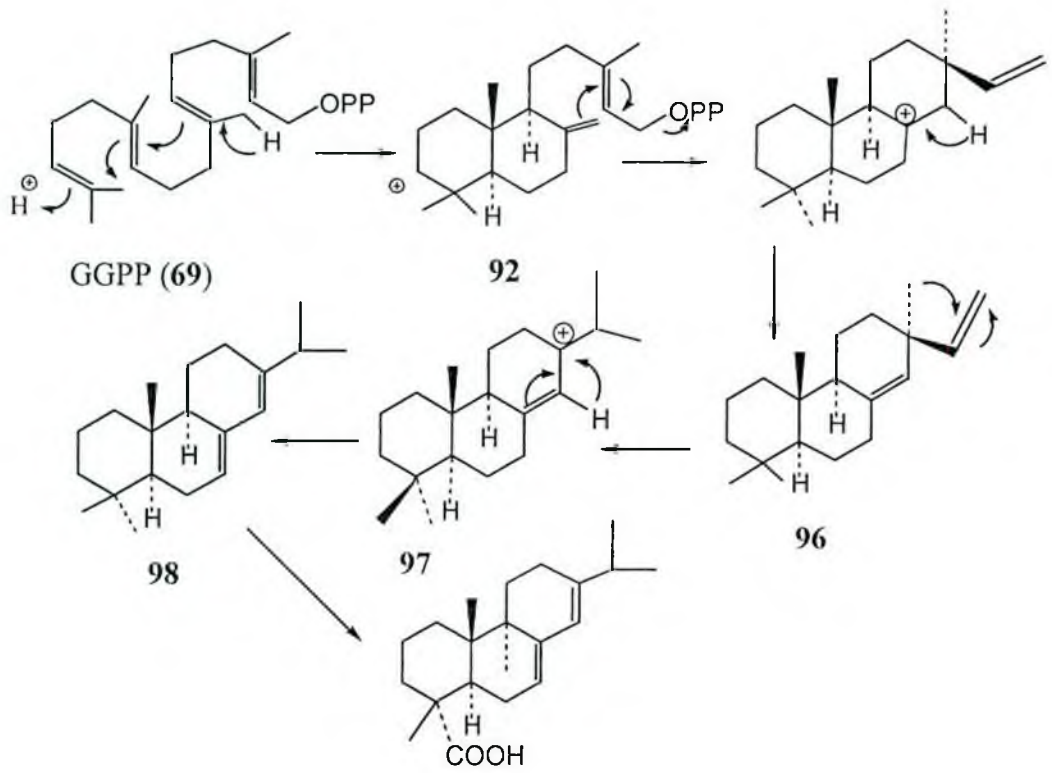


Fig. 3. Biogenesis of some cyclic diterpenoids

Biosynthesis of abietane diterpenoids: The biosynthesis of the abietane diterpene (**97**) is usually formulated as occuring via pimaradiene (**96**) (Fig. 4). Tomita et al. have proved the seterchemistry of the 1,2 methyl shift from C-13 to C-15 in the conversion of (**96**) to the abietane skeleta (**97**) (Tomita et al., 1987; Tomita et al., 1989). The tricyclic diterpene (-) abieta-7 (8), 13 (14)-diene (**98**) obtained from cyclization of GGPP (**69**) by the action of abietadiene synthase, is the first formed product on the pathway to (-) abietic acid (**99**) and related structures, through bicyclic diterpenes (Fig. 4). The single protein abietadiene synthase appears to catalyse the multistep cyclization (Dewick, 1997).



Abietic acid (**99**)

Fig. **4**. Bioisynthesis of abietane diterpenoids.

Biosynthesis of *ent*-kaurane diterpenes and gibberellic acid: The cyclization of GGPP to *ent*-kaurane ((**101**) is thought to be a multiple step process involving the byclic intermediate copalyl diphosphate (**100**) (Beale, 1991). The kaurane derivatives arc formed (**102**, **103**) as a result of a significant branching of the main gibberellin pathway in the formation of gibberellic acid (**104**) (Fig. 5)

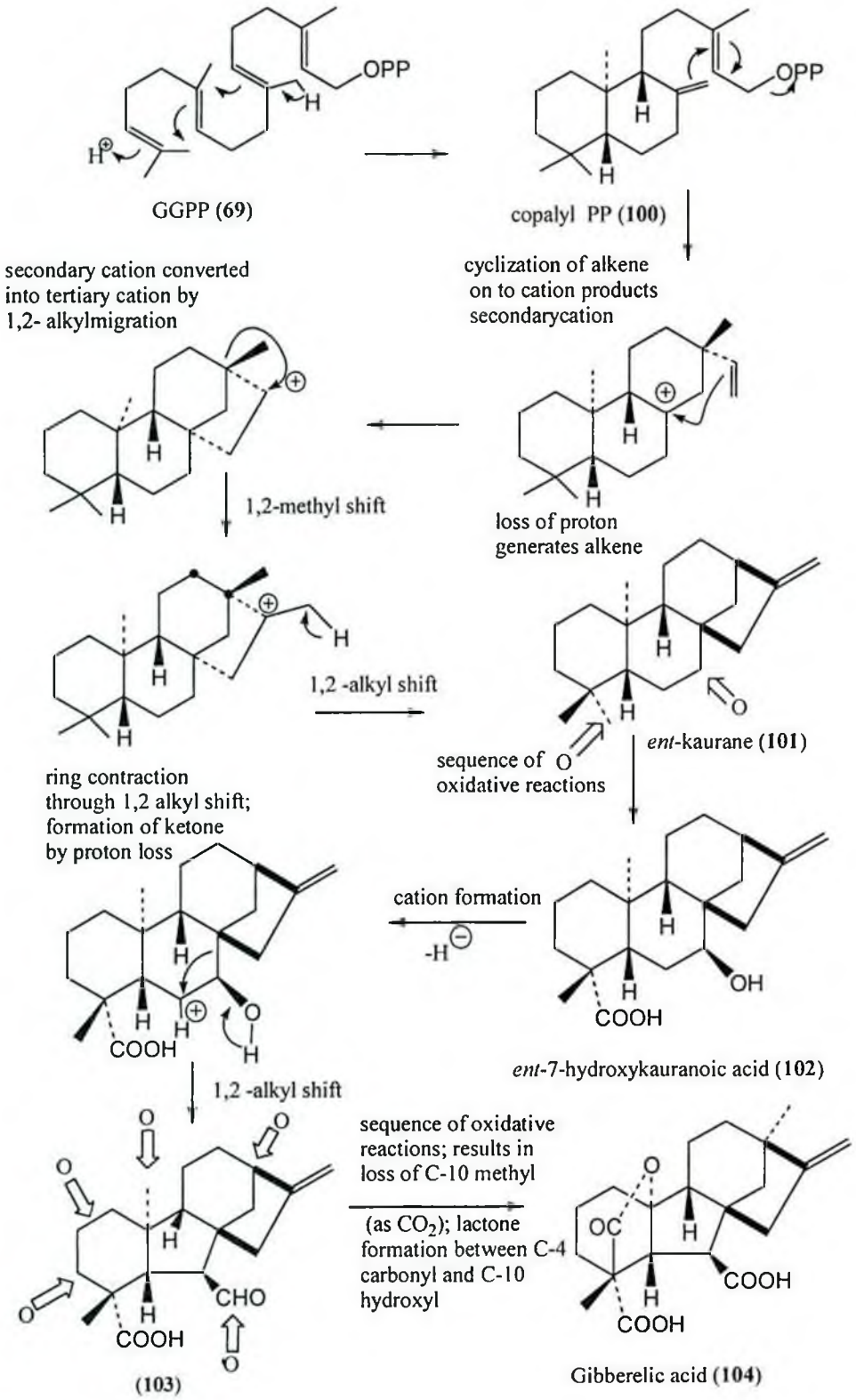


Fig. 5. Biosynthesis of *ent*-kaurane diterpene and gibberelic acid (**104**).

2.4 Experimental Section

2.4.1 General Experimental Condition

2.4.1.1 Physical Constants

Melting points (corrected and uncorrected) were determined in glass capillary tubes using Butchi 535 melting point apparatus, optical rotations were measured on Jasco Dip-360 digital polarimeter.

2.4.1.2 Spectsroscopic Techniques

The UV spectra were recorded in methanol on a Hitachi UV-3200 spectrophotometer. The IR (infrared) spectra were recorded on a Jasco A-302 spectrophotometer. The ^{1}H-NMR spectra were recorded in $CDCl_3$, CD_3OD, and C_5D_5N on Bruker AM-300, AM-400 and AMX 500 NMR spectrometers using a UNIX data system at 300, 400 and 500 MHz, respectively, while the ^{13}C-NMR spectra were recorded on the same instruments at 75, 100 and 125 MHz, respectively in the same solvents. The chemical shift (δ) values were reported in ppm and coupling constants (*J*) were measured in Hz.

The low-resolution electron impact mass spectra (LR-EIMS) were recorted on a Finnigan MAT 311 with MASPEC Data System. High-resolution and fast atom bombardment (HR-EIMS and FAB-MS) mass measurements were recorded on a Jeol JMS HX 110 mass spectrometer with the data system DA 500. FAB source using glycerol or thioglycerol as the matrix and cesium iodide (CsI) as internal standard of JMS H X 110 were used for the accurate mass measurements.

2.4.1.3 Chromatographic Techniques

Column chromatography was carried out on silica gel (E. Merck, G 60, 240–300 mesh). For preparative thin layer chromatography (TLC), precoated silica gel GF-254 aluminum sheets (20 × 20, 0.5 mm thick, E. Merck) was used. Purity of the samples was checked on precoated

silica gel (GF 284) aluminum plates (0.2 mm thick). TLC plates were viewed with ultraviolet light at 254 nm for fluorescence quenching spots and at 366 nm for fluorescent spots. Ceric sulphate, sulfuric acid and vaniline-sulfuric acid were used as spraying reagents to visualize the spots.

2.4.2 Plant Material

The fresh plant material (1.65 kg) was collected from the district of Cox's Bazar, Chunati Game Reserve, Harbang Beat by Lab Assistant of Organic Chemistry Lab, Dhaka University and Field Assistant of Bangladesh, National Herbarium (BNH) in April 1999. The plant was identified by Prof. M. Salar Khan, BNH, Dhaka. A herbarium specimen of this plant was deposited at BNH (Voucher No. DACB Accession 28004). The plant material was cleaned and air-dried.

2.4.3 Solvents and Chemicals

Analytical or Laboratory grade solvents and chemicals were used in all experiments and these were procured either from E. Merck or BDH. Commercial grade solvents were distilled prior to use for extraction and chromatographic separations.

2.4.4 Evaporation

All evaporations were carried out under reduced pressure using rotary vacuum evaporator at bath temperature not exceeding 40°C. Small volumes of non aqueous solvents were concentrated by flashing with nitrogen at room temperature.

2.4.5 Assay for Cytotoxicity on Human Tumor Cell Panels

The CH_2Cl_2-MeOH (parent) extracts of the bark of *Suregada multiflora* was tested for cytotioxicity at the National Cancer Institiute (NCI), USA on human tumor 60 cell panels.The extract exhibited significant cytotoxic activity for non-small cell lung (NCI-H522), colon (COLO-205), (HCC 2998); Renal (ACHN), (TK-10), (CAKIL); and breast (MDA-MB-235) cancer cell lines.

2.4.6 Assay for Mechanism-Based Yeast Bioassay

This assay was carried out by introducing the test sample (extract, fractions and pure compounds) dissolved in a 1:1 v/v mixture of methanol-dimethyl sulfoxide (DMSO) to a 100 μL well in agar plates, separately impregnated with normal 'wild-type' RAD^+ yeast cells and with mutant cells (RAD 52) and incubating the plates for 48 hours at 30° C. The test sample, which contained a DNA-damaging agent, inhibits the growth of mutant yeast strains producing a zone of inhibition i.e. a clear zone where growth of yeast cells had not occurred. The results are expressed as IC_{12} values, which represent the concentration of the test sample in μg/mL required to produce an inhibition zone of 12 mm diameter. The activity of sample in each plate is compared with a known anticancer drug as the positive (Fig. 6). Streptonigrin was used as the positive control. Table-9 shows the IC_{12} values for the standard and samples in our mechanism-based yeast bioassay.

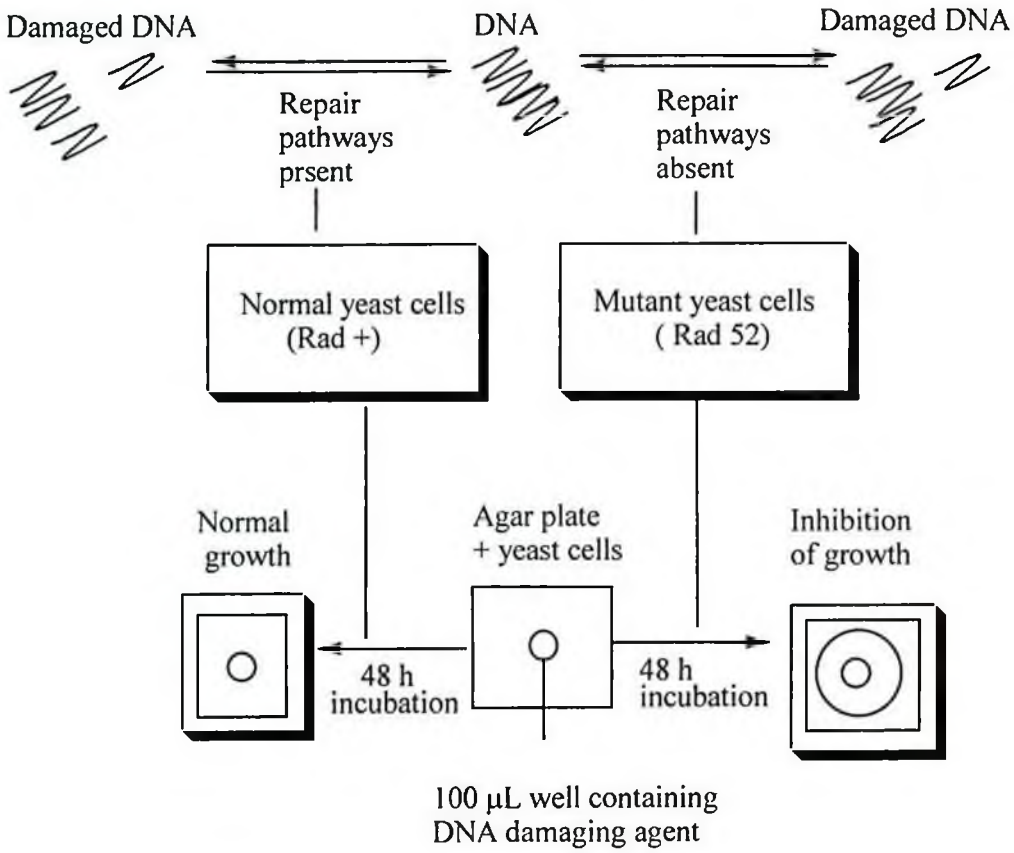


Fig. 6. Basis of mechanism-based yeast bioassay for the detection of DNA-damaging agents.

2.4.7 Extraction and Fractionation

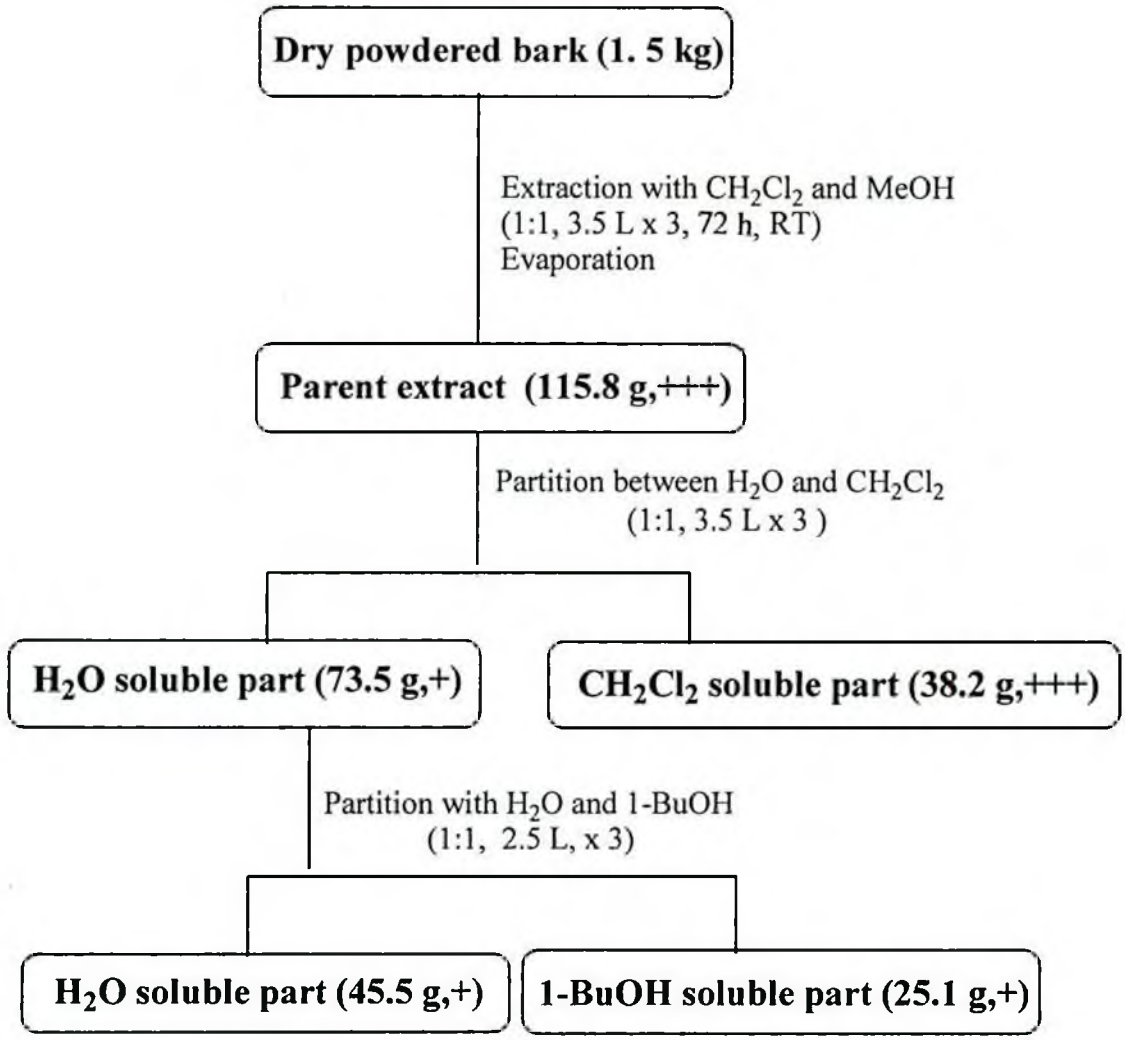
The air-dried bark (1.5 kg) of *Suregada multiflora* was pulverized into powder and was extracted 3 times with (3.5 L x 3, RT, 72 h) CH_2Cl_2-MeOH (1:1) by changing solvent every 24 hours. The resulting combined extract was concentrated to give a dark-brown syrupy residue (115.8 g, parent extract). The parent extract was tested at the NCI, USA and was found to possess significant cytotoxicity against different tumor cell lines (Section: 2.4.5). The parent extract was then partitioned between water and CH_2Cl_2 [1:1, 3.5 L x 3]. The CH_2Cl_2 soluble part was evaporated to solid mass (38.2 g). The remaining aqueous part was again partitioned with 1-BuOH [(1:1), 2.5 L x 3] and the 1-BuOH soluble part (25.1 g), was obtained (Scheme-1, Page-35). The parent (CH_2Cl_2-MeOH) extract and all the fractions were tested in mechanism-based yeast bioassay. The CH_2Cl_2 soluble part showed selective activity in yeast assay, while the other parts were not found to be selectively active (Scheme-1, Page-35; Table- 9, Page-115).

The active CH_2Cl_2 soluble extract was then subjected to VLC (vacuum liquid chromatography) over silica gel (600 g) and was eluted with gradients of solvents, hexane-CH_2Cl_2-MeOH (Scheme-2, Page-38). Eighty five fractions (fraction size 100 mL) were collected and these were pooled on the basis of their TLC profiles to yield 12 major fractions (SM-1- SM-12), which were tested by yeast bioassay. Out of the twelve, three fractions SM-3, SM-8 and SM-10 showed growth inhibitory activity in mutant yeast assay with IC_{12} values in the range of 100-200 µg/ mL (Table-9, Page-115).

2.4.8 Isolation of Compounds 1-10

A bioassay-guided isolation work on different active fractions of CH_2Cl_2 extract of *S. multiflora* bark by repeated column chromatography led to the isolation of 10 compounds **1-10** (Scheme 2-5). The percent yield of the compounds isolated are given on the basis of dry matter of the bark.

Scheme 1: Extraction and Fractionation of *Suregda multiflora* bark



+++ indicates selectively active, + indicates not selectively active in mutant yeast assay.

2.4.8.1 Compound 1- Suregadolide A (105)

The active fraction SM-8 (6.5 g, IC_{12} = 100 and 260 µg/ mL) eluted by CH_2Cl_2 and MeOH (9.5:0.5) from the first column was again subjected to column chromatography (silica gel, 60 g, 240-300 mesh) using gradients of hexane, EtOAc and MeOH, which yielded eight subfractions (SM-8-1 - SM-8-8). The active subfraction SM-8-5 (300 mg, IC_{12} = 60 and 120 µg/mL) eluted by hexane and EtOH (2:3) from the second column was subjected to further separation by repeated column chromatography (Scheme-2; Page-38) to afford four subfractions. Sub-fraction SM-8-5-3-3 (65 mg) was subjected to column chromatography by using the solvent system, hexane-acetone (4:1) which afforded the compound **1**, suregadolide A (**105**) (6.0 mg). The percent yield was 4×10^{-4} and the R_f value of the compound was found to be 0.11 (hexane-acetone, 4:1). Suregadolide A showed growth inhibitory activity with IC_{12} values 35 and 70 µg/ mL towards RAD 52 and RAD+ respectively, in mutant yeast assay.

Physical Characteristics of suregadolide A (105)

Appearance: White amorphous powder.

mp 233-234^0 C.

$[\alpha]_D^{25}$ -41 66^0 (*c*, $CHCl_3$, 0.08).

UV λ_{max} MeOH (log ε): 217.8 nm (2.23).

IR ν_{max} ($CDCl_3$): 3340 (OH), 3300 (OH), 2940 (C-H), 1760 (α, β-unsaturated γ-lactone C=O), 1450, 1410, 1110, 1060, and 1010 cm^{-1}.

^{1}H-NMR δ: Table **1**; Fig. **7**.

^{13}C-NMR δ: Table **1**. Fig. **8**.

EI-MS (*m/z,* relative intensity, %): 332 (30), 314 (12), 192 (63), 177 (89), 135 (68) and 55 (100).

HR-EI-MS (*m/z*): 332.4334 ($C_{20}H_{28}O_4$, calcd. 332.4339).

2.4.8.2 Compound 2- Suregadolide B (106)

Subfraction SM-8-5-3-2 (60 mg) obtained by repeated column chromatography from the CH_2Cl_2 soluble part (Scheme-2; Page-38) was further chromatographed on silica gel (240-300 mesh,) using the solvent system, hexane-acetone (4.1:0.9), which yielded compound **2**, suregadolide B (**106**) (10.0 mg). The percent yield was 6.7×10^{-4} and the R_f value of the compound was found to be 0.11 (hexane-acetone, 4:1). Suregadolide B was not active in mutant yeast assay.

Physical Characteristics of suregadolide B (106)

Appearance: White amorphous powder.

$[\alpha]_D^{25}$ -2.36^0 (*c*, MeOH 0.038).

UV λ_{max} MeOH: (log ε) 224 nm (1.82).

IR ν_{max} ($CDCl_3$): 2860 (C-H), 1740 (α,β-unsaturated γ-lactone C=O), 1440, 1380, 1025, 880 cm^{-1}.

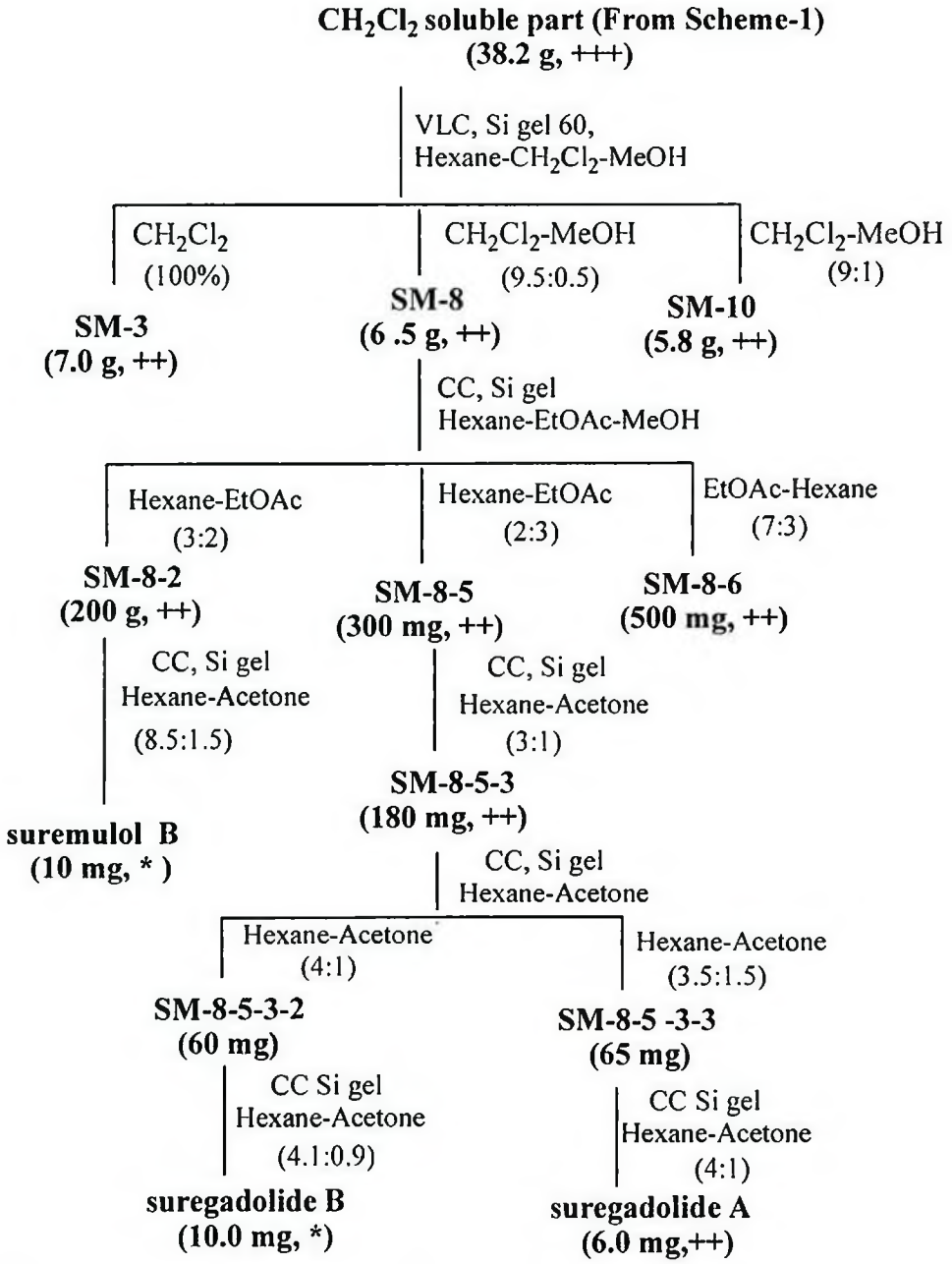
^{1}H-NMR δ: Table **2**; Fig. **11**.

^{13}C-NMR δ: Table **2**; Fig. **12**.

EI-MS (*m/z,* relative intensity, %): 314 (24), 272 (17), 203 (12), 161 (38), 93 (80) and 55 (100).

HR-EI- MS (*m/z*): 314.4180 ($C_{20}H_{26}O_3$, calcd. 314.4186).

Scheme 2: Bioassay Guided Isolation of Compounds 1, 2 and 8.



+++ Selectively active , ++ moderately active in mutant yeast bioassay, * not tested.

2.4.8.3 Compound 3- Suregadolide C (107)

The active sub-fraction SM-8-6 (500 mg, IC_{12}=60 and 100 µg/ mL) eluted by hexane-EtOH from the second column was further chromatographed on a silica gel column (240-300 mesh, 10 g) and eluted with a gradient of hexane-acetone, which yielded six subfractions (SM-8-6-1 – SM-8-6-6). The subfraction SM-8-6-1-2 (90 mg) was again chromatographed over silica gel column (Scheme-3; Page-41) and was eluted with acetone-hexane (3.1: 0.9), which afforded compound **3**, suregadolide C (**107**) (10 mg). The percent yield was 6.7×10^{-4} and the R_f value of compound **3** was found to be 0.48 (CH_2Cl_2-MeOH, 24:1). Suregadolide C showed IC_{12} values 23 and 50 µg/ mL towards RAD 52 and RAD+, respectively.

Physical Characteristics of suregadolide C (107)

Appearance: White amorphous powder (10.0 mg).

$[\alpha]_D^{25}$ -44.56^0 (*c*, 0.048, MeOH).

UV λ_{max} (MeOH): (log ε), 218.4 nm (3.44), 198.8 nm (3.53).

IR ν_{max} ($CHCl_3$): 3504 (OH), 2922, 2864, 2557 (C-H), 1740 (α,β-unsaturated γ-lactone, C=O), 1684, 1449, 1385, 1216,1133, 1059, 991 cm^{-1}.

^{1}H-NMR δ: Table **3**; Fig. **15**.

^{13}C-NMR δ: Table **3**. Fig. **16**.

EI-MS: (*m/z*, relative intensity, %): 348 (82), 218 (100), 193 (49), 135 (58.), 121 (97), 95.1 (66), 53 (17).

HR-EI- MS (*m/z*): 348.1905 ($C_{20}H_{28}O_5$, calcd. 348.1936).

2.4.8.4. Compound 4- Suregadolide D (108)

The subfraction F-8-6-3-2 (80 mg) obtained from the CH_2Cl_2 extract by repeated column chromatography (Scheme-3; Page-41) after rechromatography over silica gel (240-300 mesh) using the solvent system hexane-acetone (3:1) afforded compound **4**, suregadolide D (**108**) (8.0 mg). The percent yield was 5.3×10^{-4} and the R_f value of compound **4** was found to be 0.30 (CH_2Cl_2-MeOH, 24:1). Suregadolide D was not active in mutant yeast assay.

Physical Characteristics of suregadolide D (108)

Appearance: Light yellowish amorphous powder (8.0 mg).

$[\alpha]_D^{25}$ $+48.6^0$ (*c*, 0.038, MeOH).

UV λ_{max} (MeOH): (log ε) 224.8 nm (3.52), 198.8 nm (3.53);

IR ν_{max} ($CHCl_3$): 3326 (OH), 2925, 2864 (C-H), 1732 ((α,β-unsaturated γ-lactone, C=O), 15 17, 1446, 1381, 1283, 1092 1081, 983 cm^{-1}.

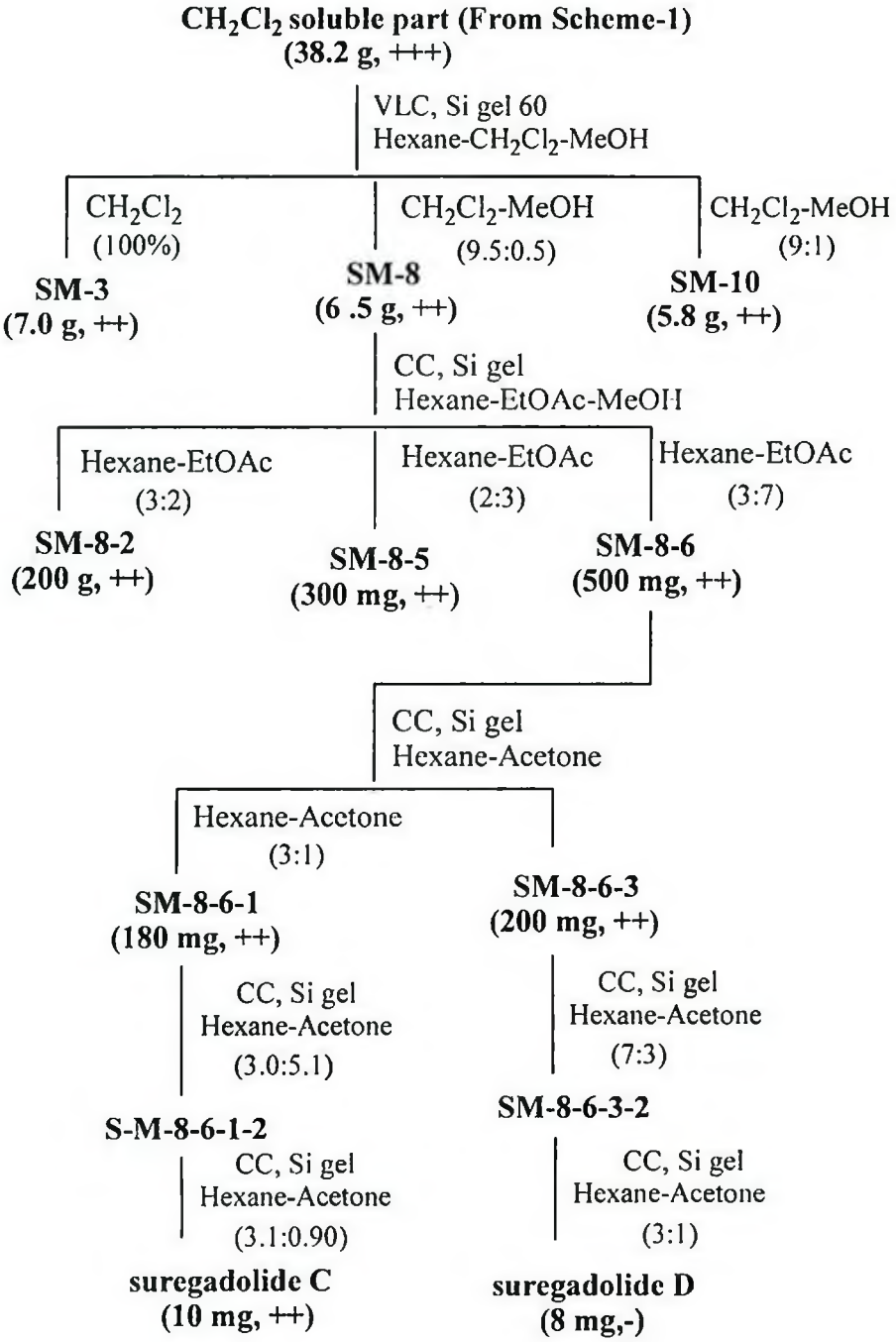
^{1}H-NMR δ: Table **4**; Fig. **19**.

^{13}C-NMR δ: Table **4**. Fig. **20**.

EI-MS: (*m/z*, relative intensity, %): 348 (69), 330 (8.8), 193 (38), 123 (68), 121 (75), 55 (100).

HR-EI- MS (*m/z*): 348.1930 ($C_{20}H_{28}O_5$, calcd. 348.1936).

Scheme 3: Bioassay Guided Isolation of Compounds 3 and 4



+++ Selectively active, ++ moderately active in mutantin yeast assay, -not active.

2.4.8.5 Compound 5- Suremulide A (109)

Fraction SM-10 (5.8 g, IC_{12} = 120 and 235 μg/mL in RAD 52 and RAD + respectively) obtained from the CH_2Cl_2 extract eluted by CH_2Cl_2-MeOH (9:1) (Scheme-4; Page- 45) from the first column was further fractionated into several fractions on silica gel (240-300) column using gradients of solvents, hexane-EtOAc-MeOH. All the fractions were tested for mutant yeast bioassay and only two fractions SM-10-4 (250 mg, IC_{12} = 73 and 155 μg/mL) eluted by EtOAc-hexane (9:1) and SM-10-6 (700, mg, IC_{12} = 65 and 100 μg/mL) eluted by EtOAc-MeOH (9:1) were found to be moderately active in yeast bioassay test. The fraction SM-10-4 (250 mg) was subjected to further fractionation on silica and a fraction SM-10-4-3 (50 mg) eluted by CH_2Cl_2-MeOH (9.9:0.1) was purified on silica gel using a solvent system CH_2Cl_2-MeOH (9.9:0.1) and was yielded the compound **5**, suremulide A (**109**) (4.0 mg), (Scheme-4, Page-45). The percent yield was found to be 2.6×10^{-4} (**5**). The R_f value of **5** was 0.5 (CH_2Cl_2- MeOH, 9.9:0.1). Suremulide A was not active in the mutant yeast assay.

Physical Characteristics of suremulide A (109)

Appearance: White amorphous powder.

$[\alpha]_D^{25}$ +33^0 (*c*, 0.048, MeOH).

UV λ_{max} (MeOH): (log ε), 219.6 nm (3.44).

IR ν_{max} ($CHCl_3$): 3415 (OH), 2925, 2857 (C-H), 1739 (α,β unsaturated γ-lactone, C=O), 1648, 1553, 1460, 1090, 1026, 770 cm^{-1}.

^{1}H-NMR δ: Table **5**. Fig. **23**.

^{13}C-NMR δ: Table **5**. Fig. **24**.

EI-MS: (*m/z*, relative intensity, %): 350 (40), 332 (14), 299 (9), 223 (19), 205 (11), 186 (16), 177 (54), 195 (68), 149 (50), 136 (60), 135 (73), 95 (61), 71 (57), 55 (100).

HR-EI-MS: (*m/z*), 350.2093 [$C_{20}H_{28}O_5$, requires 350.2090].

2.4.8.6 Compound 6- Bannaringaolide A (110)

The fraction SM-10-6 (700 mg, IC_{12} = 65 and 100 μg/mL) obtained from the CH_2Cl_2 extract by the repeated column chromatography (Scheme-4; Page-45) was subjected to further fractionation over silica gel column (240-300 mesh) using the solvent system CH_2Cl_2-MeOH. The subfraction SM-10-6-6 (50 mg) obtained by the solvent system CH_2Cl_2-MeOH (9:1) was purified on silica using the solvent system CH_2Cl_2-MeOH (9.2:0.8) which afforded the compound **6**, bannaringaolide A (**110**) (10 mg), (Scheme-4, Page- 45). The percent yield was 6.7×10^{-4} and the R_f value of compound **6** was found to be 0.34 (CH_2Cl_2-MeOH, 9:1). Bannaringaolide A was not active in mutant yeast assay.

Physical Characteristics of bannaringaolide A (110)

Appearance: White amorphous powder.

$[\alpha]_D^{25}$ $+10.8^0$ (*c*, 0.048, MeOH).

UV λ_{max} (MeOH): (log ε), 227 nm (3.31)

IR ν_{max} ($CHCl_3$): 3596, 3445 (OH), 2931, 2846 (C-H), 1732 (α,β-unsaturated γ-lactone, C=O), 1688, 1378, 1200, 1076, 1000, 930 cm^{-1}.

^{1}H-NMR δ: Table **6** and **6a**; Fig. **27**.

^{13}C-NMR δ: Table **6** and **6a**; Fig. **28**.

EI-MS: (m/*z*, relative intensity, %): Exact (M^+) was not found, 340.7 (8.78), 310 (19.7), 297 (21), 227 (25.57), 123 (51.48), 109 (34.59), 83 (35), 68.9 (100).

(-ve) HR-FAB-MS *m/z*: 364.4360.

2.4.8.7 Compound 7- Suremulol A (111)

The subfraction SM-10-6-4 (80 mg) obtained by the repeated column chromatography of CH_2Cl_2 extract (Scheme-4; Page-45) was purified on silica gel column (230-300 mesh) using the solvent system, CH_2Cl_2-MeOH (9.5:0.5), which yielded the compound **7**, suremulol A (**111**) (5.0 mg, IC_{12} = 53 and 100 µg/mL). The percent yield was 3.35×10^{-4} and the R_f value of compound **7** was found to be 0.43 (CH_2Cl_2-MeOH, 9:1). Suremulol A (**111**) showed IC_{12} values 53 and 100 µg/mL towards RAD 52 and RAD+, respectively.

Physical Characteristics of suremulol A (111)

Appearance: White amorphous powder.

$[\alpha]_D^{25}$ - 9^0 (*c*, 0.028, pyridine).

UV λ_{max} (MeOH): (log ε), 202.2 nm (2.03).

IR ν_{max} ($CHCl_3$): 3374 (OH), 2923, 2852 (C-H), 1739, 1462, 1368, 1036 cm^{-1}.

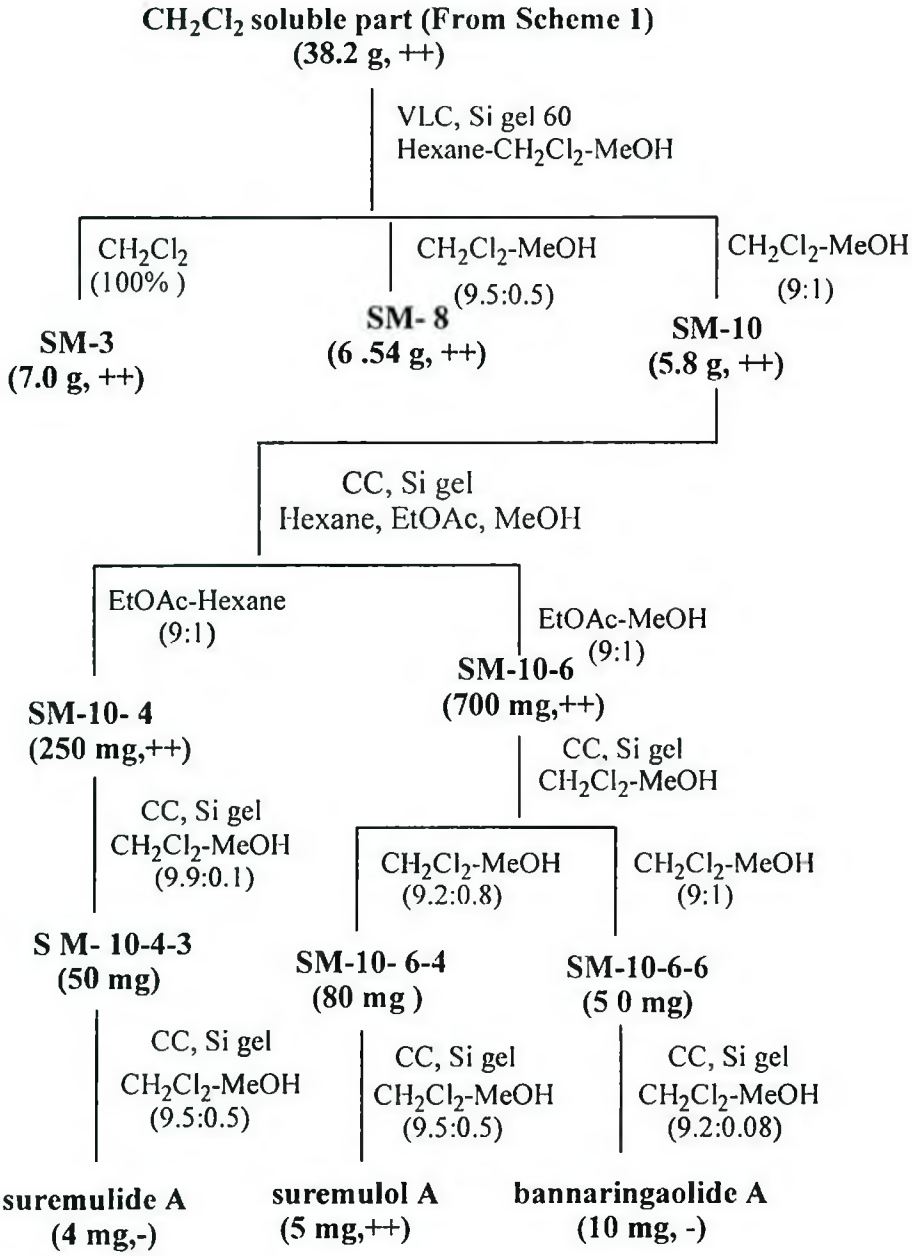
EI-MS (*m/z*, relative intensity, %): 322 (1.08), 304 (5), 291 (100), 230 (39), 177 (8), 161 (6), 109 (17), 95 (21), 69 (26), 55 (20).

^{1}H-NMR δ: Table **7**; Fig. **31**.

^{13}C-NMR δ: Table **7**; Fig. **32**.

HR-EI-MS: (*m/z*): 322.2090 [$C_{20}H_{34}O_3$, requires 322.2085].

Scheme 4: Bioassay Guided Isolation of Compounds 5, 6 & 7



+++ selectively active, ++ moderately active, - not active in mutant yeast assay.

2.4.8.8 Compound 8- Suremulol B (112)

Subfraction SM-8-2 (200 mg, IC_{12} = 70 and 135 μg/mL) obtained by the repeated column chromatography of CH_2Cl_2 extract was purified on silica gel column (240-300 mesh) using the solvent system, hexane-acetone (8.5:1.5), which afforded the compound **8**, suremulol B (**112**) (10 mg) (Scheme-2; Page-38). The percent yield was 6.7×10^{-4} and the R_f value of **8** was found to be 0.32 (CH_2Cl_2-MeOH, 10:0.5). Suremulol B (**112**) was not active in the mutant yeast assay.

Physical Characteristics of suremulol B (112)

Appearance: White amorphous powder.

$[\alpha]_D^{25}$ -14° (*c*, 0.0425, $CHCl_3$).

UV λ_{max} (MeOH): (log ε), 202.2 nm (2.03).

IR ν_{max} ($CHCl_3$): 3361 (OH), 2927 (C-H), 2867 (C-H), 1631, 1454, 1367, 1182, 1056, 1021, 996, 895, 756 cm^{-1}.

EI-MS (*m/z*, relative intensity, %): 304 (56), 286 (24), 271 (44), 253 (38), 135 (43), 121 (40), 107 (48), 105 (68), 91 (84), 55 (100).

^{1}**H-NMR** δ: Table **8**; Fig. **35**.

^{13}**C-NMR** δ: Table **8**; Fig. **36**.

HR-EI-MS: (*m/z*): 304.5290 [$C_{20}H_{32}O_2$, requires 322.2085].

2.4.8.9. Compound 9- Multiflorenol (44)

The fraction SM-3-3 (5.0 g) obtained by the repeated column chromatography of the CH_2Cl_2 extract (Scheme-5; Page-49) was subjected to column chromatography (240-300 mesh) for further fractionation. A fraction eluted by the solvent system, hexane-CH_2Cl_2 (1:1) afforded the compound **9,** multiflorenol (**44**) (40 mg). The percent yield was 26.8×10^{-4} and the R_f value of **9** was found to be 0.21 (hexane-CH_2Cl_2, 7:3).

Physical Characteristics of multiflorenol (44)

Appearance: White amorphous powder (20.0 mg).

$[\alpha]_D^{25}$ -27° (*c*, 0.028, $CHCl_3$).

UV λ_{max} (EtOH): (log ε), 207 nm (3.65).

IR ν_{max} (KBr): 3654 (OH), 2923 (C-H),, 2858 (C-H), 1462, 1388, 1056 cm^{-1}.

^{1}H-NMR δ ($CDCl_3$, 500 MHz): 3.28 (1-H, t, *J* = 3.0 Hz, H-3), 5.30 (1-H, m, H-7), 1.24, 1.12, 0.98, 0.89, 0.86, 0.8 0 and 0.74 (each 3-H, s).

EI-MS (*m/z,* relative intensity, %): 426 (100), 411 (60), 259 (46), 247 (68), 229 (30).

2.4.8.10 Compound 10- Baurenol (45)

Fraction SM-3 (7.0 g) obtained from the CH_2Cl_2 extract (Scheme-5; Page-49) was fractionated into several sub fractions by silica gel (240-300 mesh) column chromatographed using the solvent system hexane-CH_2Cl_2. A fraction SM-3-3 (5.0 g) eluted by hexane-CH_2Cl_2 (1:1) was further chromatographed on silica gel column (240-300 mesh). Fraction SM-3-3-2 (1.0 g) eluted by hexane-CH_2Cl_2 (3:2) was purified by the solvent system hexane-CH_2Cl_2 (7:3) by column chromatography, which yielded the compound **10**, baurenol (25 mg). The percent yield was $.75 \times 10^{-4}$ and the R_f value of compound **10** was found to be 0.22 (hexane-CH_2Cl_2, 4:1).

Physical Characteristics of baurenol (45)

Appearance: white amorphous powder.

$[\alpha]_D^{25}$ -25.5° (*c*, 0.74, $CHCl_3$).

m.p. 148-150° C.

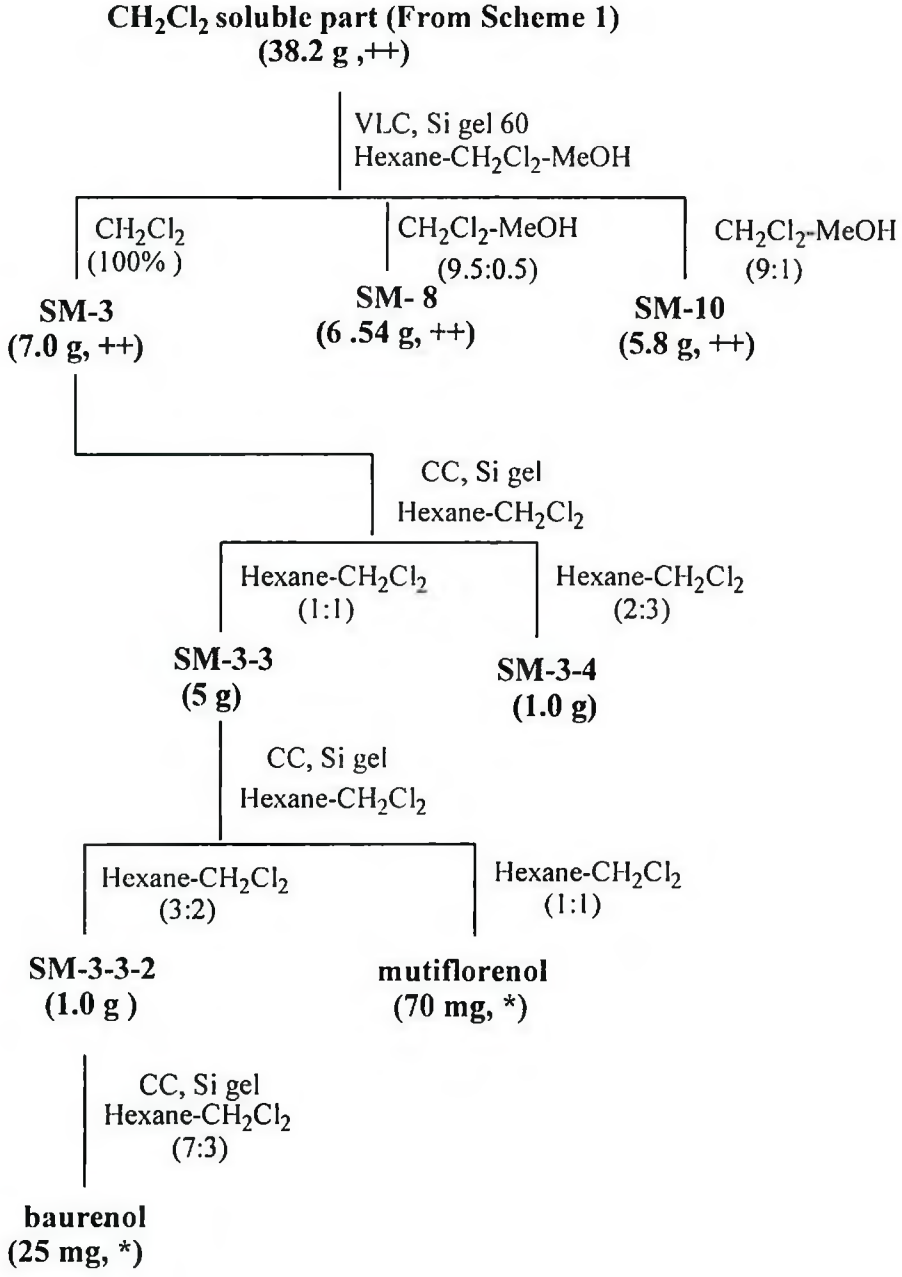
UV λ_{max} (EtOH): (log ε), 207 nm (3.65).

IR ν_{max} (KBr): 3380 (OH), 2974, 2868 (C-H), 1462, 1388, 1056 cm^{-1}.

^{1}H-NMR δ ($CDCl_3$, 500 MHz): 3.20 (1-H, dd, *J* = 11.0 Hz, *J* = 3.8 Hz, H-3), 5.30 (1-H, m, H-7), 1.04, 1.01, 0.98 and 0.95 (each 3H, s, 27, 28, 26, and 23 methyls), 0.94 (3H, bs, H-29), 0.91 (3H, d, *J* = 6.0 Hz, H-30), 0.86 and 0.75 (each 3H, s, C-24 and C-25 methyls).

EI-MS: (*m/z*, relative intensity, %): 426 (100), 411 (57), 259 (46), 247 (68), 229 (31).

Scheme 5: Bioassay Guided Isolation of Compounds 9&10



+++ selectively active, ++ moderately activite in mutant yeast assay, * not tested.

2.4.9 Derivatization of Compounds

2.4.9.1 Hydrolysis of Suregadolide B (106)

Compound **2,** suregadolide B (**106**) was dissolved in THF (Tetrahydrofuran, 10 ml, aqueous 80%) and conc. HCl (1% of the sample solution) was added to the solution drop wise. The solution was kept for 24 hours at room temperature with constant stirring. After completion of the reaction, the whole mixture was poured into EtOAc and was partitioned with H_2O. The organic layer was collected and dried to get 1.8 mg of product **2a**.

2.4.9.2 Diacetate of Suregadolide C (107)

Compound **3**, suregadolide C (**107**) (6.0 mg) was acetylated with Ac_2O in pyridine (3:1) at room temperature for 24 hours. Usual workup and preparative TLC (hexane- acetone, 4:1 twice) afforded **3a** as a solid amorphous powder {2.5 mg, R_f = 0.25, hexane-acetone (3.3:0.7); $[\alpha]_D^{25}$ - 27 (*c*, 0.095 $CHCl_3$)}.

^{1}H NMR ($CDCl_3$ + CD_3OD): δ 1.01 (s, CH_3-20), 0.94, (s, CH_3-19), 1.82 (d, J = 2.0 Hz, CH_3-17), 2.02 (s, Ac-7), 2.3 (s, Ac-14), 5.48 (m, H-12), 2.28 (dd, J = 13.0 Hz, 9 Hz; H-11b) and 1.32 (m, H-11a), 1.22, bd (J = 9.8 Hz, H-9), 1.38 (dd, J = 2.9 Hz, 13.6 Hz: H-5), .074 (dd, J = 5.4 Hz, 4.9 Hz; H-18a), 0.42 (dd, J = 9.3 Hz, J = 4.18 Hz; H-18b), 0.53 (m, $W_{1/2}$ = 9.5 Hz, H-3), 0.85 and 1.36 (m, H_2-1), 1.58 and 1.83 (m, H_2-2).

2.4.9.3 Diacetate of Suregadolide D (108)

Compound **4,** suregadolide D (**108**) (5.0 mg) was acetylated with Ac_2O in pyridine (3:1) at room temperature for 24 hours. Usual work up and preparative TLC (hexane-acetone, 7.5:2.5 twice) afforded **4a** as a solid amorphous powder {2.3 mg, R_f = 0.20 in hexane- acetone, (3.3:0.7); $[\alpha]_D^{25}$ + 31 (*c*, 0.035, $CHCl_3$)}.

^{1}H-NMR ($CDCl_3$ + CD_3OD): δ 0.88 (s, CH_3-20), 0.84, (s, CH_3-19), 1.71 (d, J = 1.8 Hz, CH_3-17), 1.95 (s, Ac-7), 2.04 (s, Ac-14), 4.55 (ddd, J = 11.0 Hz, 5.98 Hz, 1.8 Hz; H-12), 2.23 (m, H-11b), 1.25 (m, H-11a), 1.28 (dd, J = 13.4 Hz, J = 2.6 Hz; H-9), 1.30 (m, H-5), 0.06 (dd, J = 5.25 Hz, 4.75 Hz; H-18a), 0.34 (dd, J = 9.30 Hz, J = 4.2 Hz; H-18b), 0.45 (m, $W_{1/2}$ = 9.4 Hz, H-3), 0.58 and 1.35 (m, H_2-1), 1.49 and 1.76 (m, H_2-2).

2.4.9.4 Triacetate of Bannaringaolide A (110)

Compound **6**, bannaringaolide A **(110)** (5.0 mg) was acetylated with Ac_2O in pyridine (3:1) at room temperature for 72 hours. Usual workup and preparative TLC (CH_2Cl_2, thrice) afforded **6a** as a solid amorphous powder [2.5 mg, R_f = 0.20 CH_2Cl_2), $[\alpha]_D^{25}$ + 3.1 *c*, 0.035, $CHCl_3$.

^{1}H-NMR ($CDCl_3$ + CD_3OD): δ 0.87 (s, CH_3-20), 0.75 (s, CH_3-19), 0.71 (s, CH_3-18), 2.10 (s, Ac-14), 2.02 (s, Ac-12), 1.95 (s, Ac-7), 2.40 (H-15a) and 2.50 (H-15b). HREI MS *m/z*: 490.5447 [$C_{20}H_{34}O_9$, requires 490.5464].

2.5 Results and Discussions

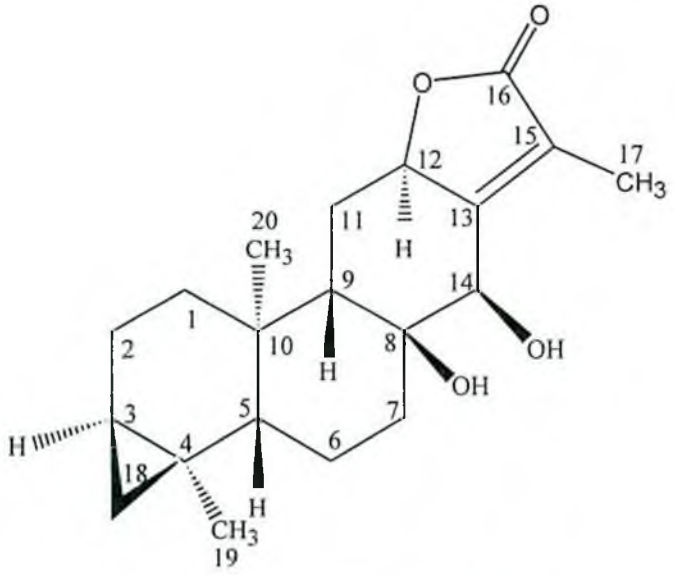
The search for new cytotoxic constituents from natural sources has led us to initiate a bioassay-guided phytochemical investigation on *Suregada multiflora* (Juss.) Bail (Syn. *Gelonium multiforum,* Euphorbiaceae) bark. The CH_2Cl_2-MeOH extract of the bark of *Suregada multiflora* exhibited significant cytotoxic activity for non-small cell lung (NCI-H522), colon (COLO-205), (HCC 2998); Renal (ACHN), (TK-10), (CAKIL); and breast (MDA-MB-235) human tumor cell lines in the National Cancer Institute 60-cell line tumor panels (Boyd et al., 1995). The dichloromethane soluble part of the parent extract of the plant also showed selective activity in mechanism based yeast bioassay (Gunatilaka et al., 1992). Ten compounds (**1-10**) (Scheme 1-5) were isolated from the same active extract by a mechanism based yeast-bioassay guided fractionation.

2.5.1 Diterpenoids

Out of ten compounds, eight has been characterized as diterpenoids. Four of them were identified as members of a new class of diterpenoids and were named as suregadolides A-D (**105-108**)) (compounds **1-4**). These compounds contain a trisubstituted cyclopropane ring bridging C-3 and C-4 positions of ring A of the abietane framework. Compound **5** was named as suremulide A (**109**) and was found to be a new abietene diterpene lactone. Compound **6** was identified as member of another new class of diterpene lactone which possesses a novel carbon skeleton and contains a seven-member ring, formed by the rearrangement of exocycilc C-17 in ring C of *ent*-pimarane framework and was named bannaringaolide A (**110**). A kaurane triol, suremulol A (**111**) (compound **7**), and one kaurane diol, suremulol B (**112**) (compound **8**) were also identified as new diterpene metabolites.

2.5.1.1 Suregadolide A (105)- A Novel Diterpene Lactone

Compound **1** was isolated as a white amorphous solid (Scheme-2; Page-38). The molecular formula of the compound was deduced as $C_{20}H_{28}O_4$ (*m/z* 332.4334) from HR-EIMS and ^{13}C-NMR data, along with the DEPT spectra. On the basis of 1D-NMR and IR spectral evidences, compound **1** was concluded to be a tetracyclic diterpene with an α,β-unsaturated γ-lactone chromophore (1760 cm^{-1}), a vinylic methyl (δ_H 1.78, δ_C 7.8), one secondary hydroxyl-bearing (δ_H 4.43, δ_C 72.2) and one tertiary hydroxyl-bearing (δ_C 74.2) carbons. Besides these, the presence of a cyclopropyl ring (δ_H -0.01 and 0.40; δ_C 21.8) and two more methyl groups (δ_H 0.93, δ_C 23.5; δ_H 1.11, δ_C 14.6) were also inferred. The complete structure was then deduced with the help of 2D-NMR studies (COSY 45°, HMQC, HMBC and NOED spectra) and from comparative studies with the reported diterpene lactones (Talapatra et al., 1989; Talapatra et al., 1998) and metasequic acids (Sakan et al., 1988).



Suregadolide A (**105**)

From ^{1}H-^{1}H COSY-45° and HMQC spectra (Table-1), it was inferred that H-12 (δ_H 5.15; δ_C 77.2) of the α,β-unsaturated lactone chromophore was coupled with two non-equivalent geminal methylene protons (H-11a, δ_H 1.50 m; H-11b, δ_H 2.42 dd, $J_{11a,\,11,b}$ = 13.3 Hz,

$J_{11b,12}$ = 6.6 Hz; δ_C 29.1) along with the vinylic C-17 methyl protons (δ_H 1.78, d, $J_{17,12}$ = 1.8 Hz; δ_C 7.8) (homoallylic coupling). Coupling of the C-9 methine proton (δ_H 1.39, d, $J_{9,11a}$ = 7.6 Hz; δ_C 52.7) with the H-11a methylene proton (δ_H 1.50, δ_C 29.1) indicated the presence of a CH-CH_2-CH-O- moiety in the molecule. Since H-9 could not be correlated with any other proton, except the C-11a methylene proton, it was concluded that this must have two adjacent quaternary carbons.

The ^{1}H- and ^{13}C- NMR spectra (Fig.-7 and -8; Page-58 and -59) of compound **1** clearly indicated the presence of a trisubstituted cyclopropane ring in the molecule. Two upfield signals of C-18 cyclopropane methylene protons [H-18 (*exo*), δ_H –0.01, dd, $J_{18\ exo,3}$ = 5.7 Hz, $J_{18\ exo,18\ endo}$ = 4.2 Hz and H-18 (*endo*), δ_H 0.40 (dd, $J_{18\ endo,3}$ = 9.2 Hz, $J_{18\ exo,18\ endo}$ = 4.0 Hz); δ_C 21.8], H-3 signal at δ_H 0.54 (ddd, $J_{3,2}$ = 9.0 Hz, $J_{3,18\ endo}$ = 8.5 Hz, $J_{3,18exo}$ = 6.0 Hz; δ_C 19.1) and one quaternary carbon signal (C-4, δ_C 16.1) confirmed unambiguously the presence of a trisubstituted cyclopropane ring. These values are also consistent with the values reported for the metasequic acids (Sakan et al., 1988), which contain a trisubstituted cyclopropyl substituent in ring A of a labdane skeleton. COSY-45°, and HMBC (Fig. 9) interactions further confirmed the position of cyclopropyl group in compound **1**.

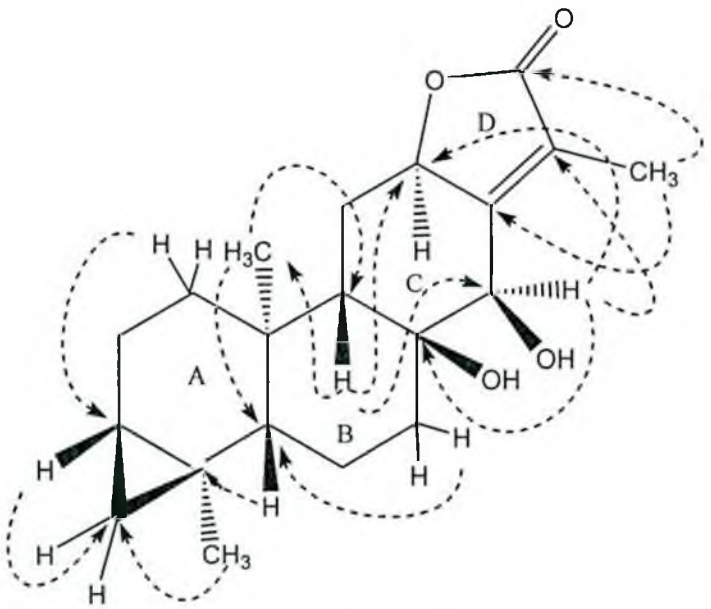


Fig. 9. Important HMBC interactions in suregadolide A (**105**).

The HMBC spectrum of compound **1** showed correlation of C–20 methyl protons (δ_H 1.11, δc 14.6) with C-9 (δ_C 52.7), whereas the C-19 and C-20 methyl protons (δ_H 0.93 & 1.11) and H-7b (δ_H 2.25) were correlated with the C-5 (δ_C 51.2). C-19 Methyl protons were also found to be correlated with C-4 (δ_C 16.1) and C-18 (δ_C 21.8), while; H-3 (δ_H 0.54) exhibited a correlations with C-18 (δ_C 21.8). H-5 (δ_H 1.05) showed interactions with C-3 (δ_C 19.1) and C-4 (δ_C 16.1) (Fig. 9).

In ^{1}H- and ^{13}C-NMR spectra ($CDCl_3$ + CD_3OD), the downfield signals of H-14 (δ_H 4.43 and δ_C 72.2) and the quaternary C-8 (δ_C 74.2) indicated the presence of one secondary hydroxyl group and one tertiary hydroxyl group in the molecule. The ^{1}H-NMR spectrum of **1**, when recorded in (C_5D_5N), showed signals for the two OH protons (δ_H 7.65 and 5.79), which disappeared when the ^{1}H-NMR spectrum was recorded in C_5D_5N with a few drops of D_2O. In HMBC spectra the H-14 signal was found to be correlated with C-8 (δ_C 74.2), C-13 (δ_C 162.7), C-15 (δ_C 122.5) and C-12 (δ_C 77.2). On the other hand, H-9 (δ_H 1.39) was correlated with C-8 and C-14 (Fig. 9; Table 1, Page-57).

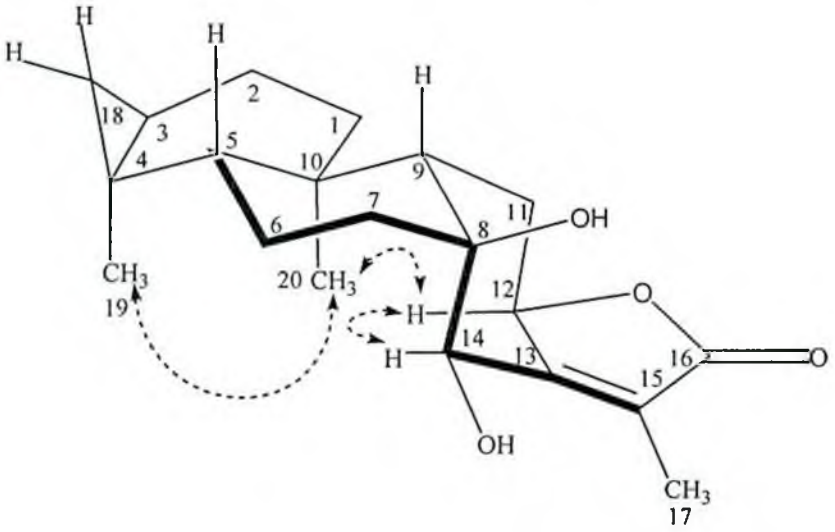


Fig. **10**. Important NOE interactions in suregadolide A (**105**).

The stereochemistry of the A/B ring junction was being inferred *trans* on biogenetic grounds (Talapatra et al., 1989). The stereochemistry of H-12 was determined as α (axial)

and H-9 as β (pseudoaxial) from the coupling constants and multiplicities of the H-9, H-11 and H-12 signals. H-12 (δ_H 5.15, ddd, $J_{12,\,11a}$ = 12.0 Hz, $J_{12,\,11b}$ = 6.4 Hz, $J_{12,\,17}$ = 1.6 Hz), resonance appeared as a doublet of doublet of doublets, while H-11b (δ_H 2.42, dd, $J_{11a,11b}$ = 13.3 Hz, $J_{12,\,11b}$ = 6.6 Hz) was a doublet of doublets and H-9 (δ_H, 1.39, d, $J_{9,11a}$ = 7.6 Hz) appeared as a doublet. These values were consistent with the values reported for gelomulide F, which contain a H-12 α (axial) and H-9β (Talapatra et al., 1989).

The NOE (Nuclear Overhauser Effect) difference experiments were carried out to find out the stereochemistry at different chiral centers of compound **1**. The effect on H_3-20 (δ_H 1.11) (16%) and H-12 (δ_H 5.15) (4%) by irradiation of H-14 (δ_H 4.43) indicated their spatial proximity. Since the H-12 methine has been found to bc α oriented in all other diterpene lactones reported from the same plant (Talapatra et al., 1989; Talapatra et al., 1998), it was therefore presumed that H-14 is α-oriented and thus C-14 OH β- oriented. The NOE effect on H_3-20 (27 %) by irradiation of H-12 signal and also the effect on H_3-19 (δ_H 0.93) (13 %) by irradiation of H_3-20 supported the α-orientation of the C-19 methyl and C-20 methyl groups and thus the β orientation of the cyclopropane ring in compound **1**. From the Drieding model of compound **1**, it was observed that these interactions were possible only when the C-8 hydroxyl group is β-oriented (Fig. **10**).

The structure of compound **1** was therefore elucidated as 3,4,18β-cyclopropa-8β,14β-dihydroxy-13,15-abieten-16,12-olide and was trivially named as suregadolide A (**105**).

Table 1. NMR spectral ($CDCl_3$+CD_3OD) data of suregadolide A (**105**)

C/H No.	^{1}H-NMR[a] δ_H (mult., J = Hz)	^{13}C-NMR[b] δ_C (mult.)*	COSY	HMBC	NOE
1a	0. 55 (m)	35.8 (t)	H-1b,H-2a,H-2b	C-3	
1b	1.55 (m)		H-1a, H-2a, H-2b	C-5	
2a	1.64 (m)	23.8 (t)	H-1a, H-1b, H-2b	C-1	
2b	1.84 (m)		H-1a, H-1b, H-2a, H-3	C-1	
3	0.54 (ddd, 9.0, 8.5, 6.0)	19.1 (d)	H-2b, H-18a, H-18b	C-2, C-18	H-18, *endo*
4		16.1 (s)			
5	1.05 (dd, 12.8, 3.2)	51.2 (d)	H-6a, H-6b	C-3, C-4, C-6	
6a	1.60 (m)	19.5 (t)	H-5, H-6b, H-7a, H-7b	C-5	
6b	1.82 (dt, 12.0, 5.5)		H-5, H-6a, H-7a, H-7b	C-10	H_3-19
7a	1.52 (m)	4 1.2 (t)	H-6a, H-6b, H-7b	C-8	
7b	2.25 (m)		H-6a, H-6b, H-7a	C-5, C-8	H-12, H-14
8		74.2 (s)			
9	1.39 (d, 7.6)	52.7 (d)	H-11a, H-11b	C-1, C-8, C-10, C-12, C-14, C-20	
10		37.4 (s)			
11a	1. 50 (m)	29.1 (t)	H-9, H-11b, H-12		
11b	2.42 (dd, 13.3, 6.4)		H-9, H-11a, H-12	C-8, C-12	H-12, H_3-20
12	5 .15 (ddd, 12.0, 6.4, 1.6)	77.2 (d)	H-11a, H-11b, H_3-17		H-14, H_3-20
13		162.7 (s)			
14	4.43 (s)	72.2 (d)		C-8, C-9, C-12, C-13, C-15	H_3-20, H-12
15		122.5 (s)			
16		175.7 (s)			
17	1.78 (d, 1.8)	7.8 (q,)	H-12	C-13, C-15, C-16	
18 *exo*	-0.01 (dd, 5.7, 4.2)	21.8 (t)	H-3, H-18b		
18 *endo*	0.40 (dd, 9.2, 4.0)		H-3, H-18a, H-3		H-5
19	0.93 (s)	23.5 (t)		C-3, C-4, C-5, C-18	H_3-20
20	1.11 (s)	14.6 (q)		C-1, C-5, C-9, C-10	H_3-19

[a] Data recorded at 500 MHz.
[b] Data recorded at 100 MHz.
*Multiplicity determined by DEPT and HMQC spectra.

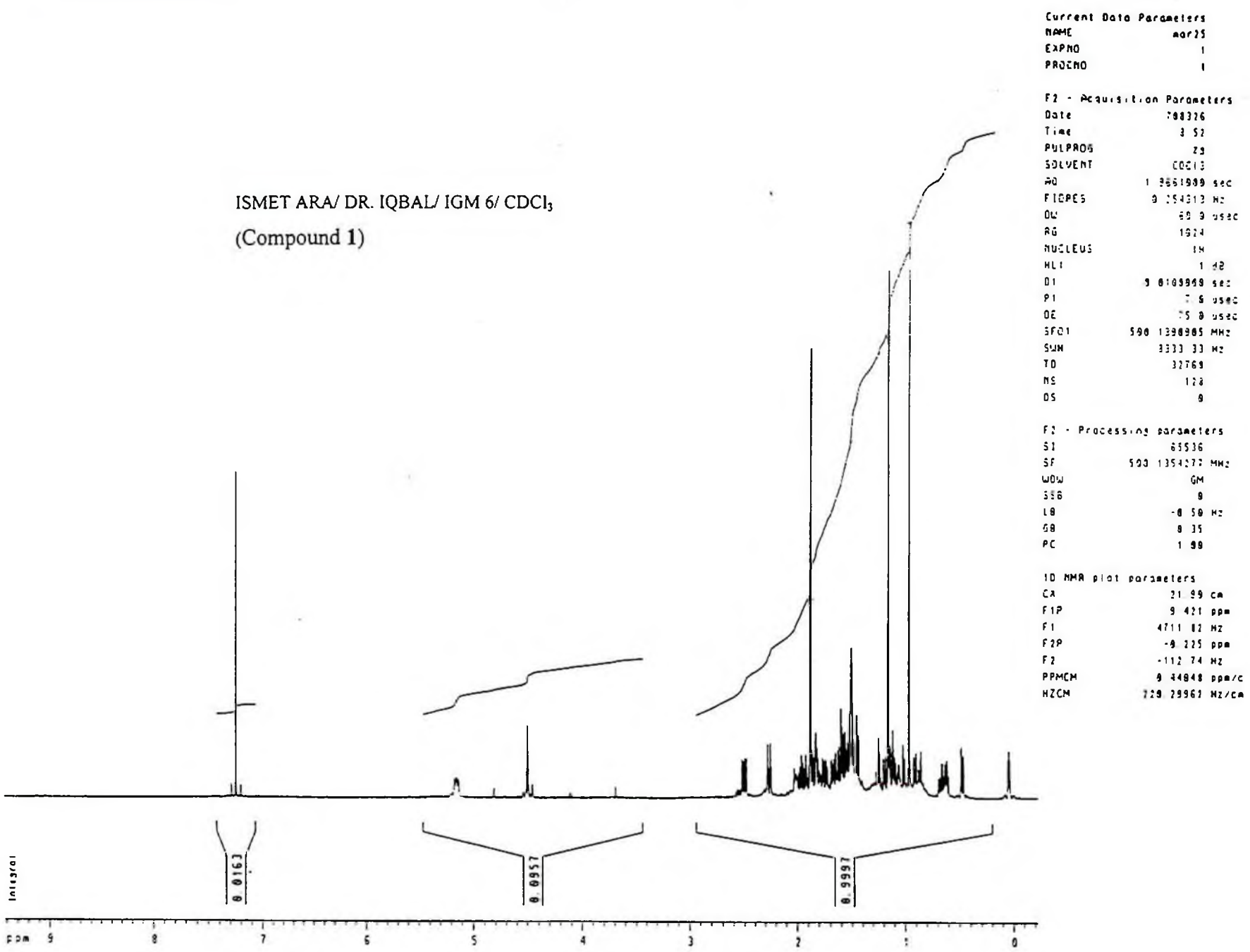


Fig. 7. ^{1}H-NMR Spectrum ($CDCl_3$, 500 M Hz) of Suregadolide A (**105**)

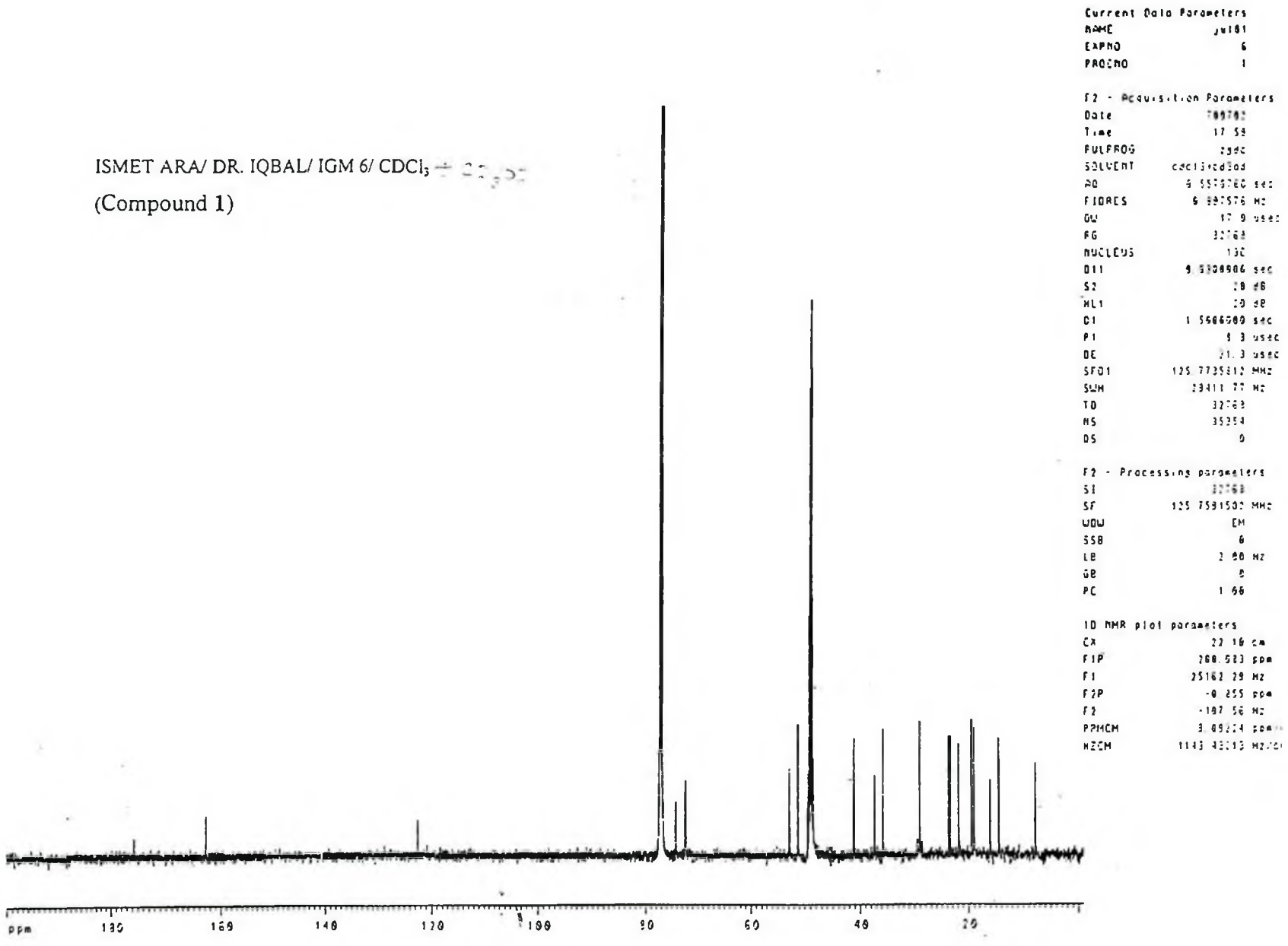


Fig. 8. ^{13}C- NMR Spectrum ($CDCl_3$, 100 M Hz)of Suregadolide A (105)

The complete structure of compound **2** was then deduced by comparing its NMR spectral data (Table-2; Page- 63) with those of suregadolide A and gelomulide A (Talapatra et al., 1989).

A comparative study of the NMR chemical shifts of C-8 (δ_C 61.5), C-14 (δ_C 55.5) and H-14 (δ_H 3.64) with gelomulide A (Talapatra et al., 1989) indicated the presence of a tri substituted oxirane group at C-8 and C-14 of compound **2**. The presence of the oxirane group was also determined by opening the ring of compound **2** by chemical reaction (Experimental Section). A comparative study of TLC of the reaction product **2a** with compound **2** showed some differences in R_f values. The R_f of compound **2** was found to be (0.412) while that of **2a** was found (0.247) in the same solvent system (hexane-acetone, 5: 1). The comparative study of R_f value indicated that the polarity of **2a** was increased due to the opening of oxirane ring and formation of hydroxy group in **2a**.

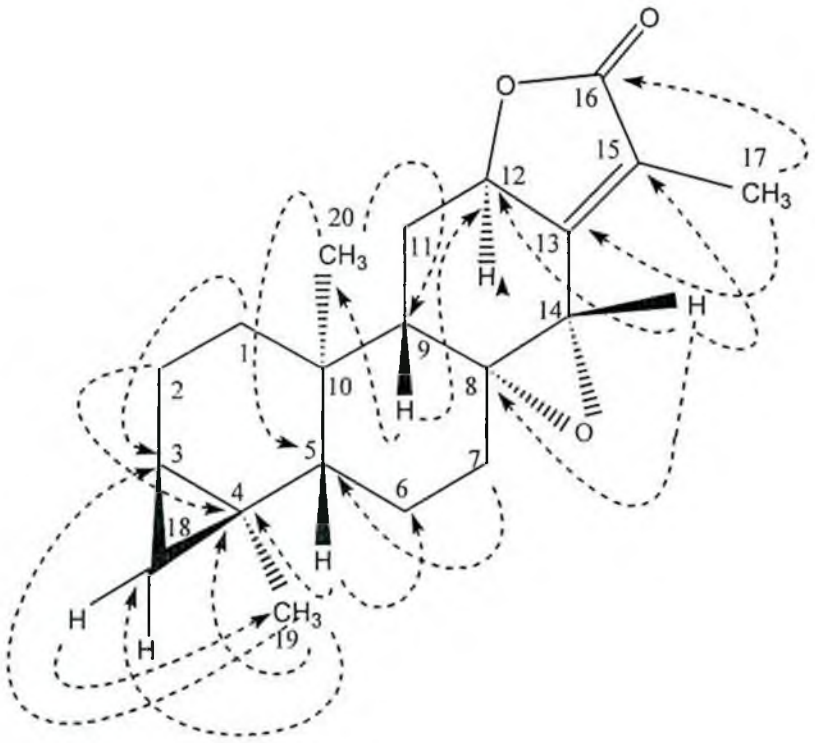


Fig. **13**. Important HMBC interactions in suregadolide B (**106**).

The stereochemistry of A/B ring junction was inferred to be *trans* on the biogenetic ground (Talapatra et al., 1989). The same stereochemistry was deduced for H-12 (α) and H-9 (β) as in compound **1**. The strong NOE interactions between H-12 (δ_H 4.18) and H_3-20 (δ_H 0.80) (35%) confirmed the orientation of CH_3-20 as α. No interaction was observed between H-12 and H-14 (δ_H 3.64) or between H_3-20 and H-14. On the other hand, H-14 showed a NOE interaction with H-9 (δ_H 1.67) (7%), which indicated its β-orientation. However, H-5 (δ_H 1.05) showed no NOE interaction with H_3-20 but exhibited interaction with H-18 (δ_H -0.09) (7%), which confirmed the β-orientation of the cyclopropane ring.

The structure of compound **2** was therefore, elucidated as 3,4,18β-cyclopropa-8,14 α -epoxy-13,15-abieten-16,12-olide and was named as suregadolide B (**106**).

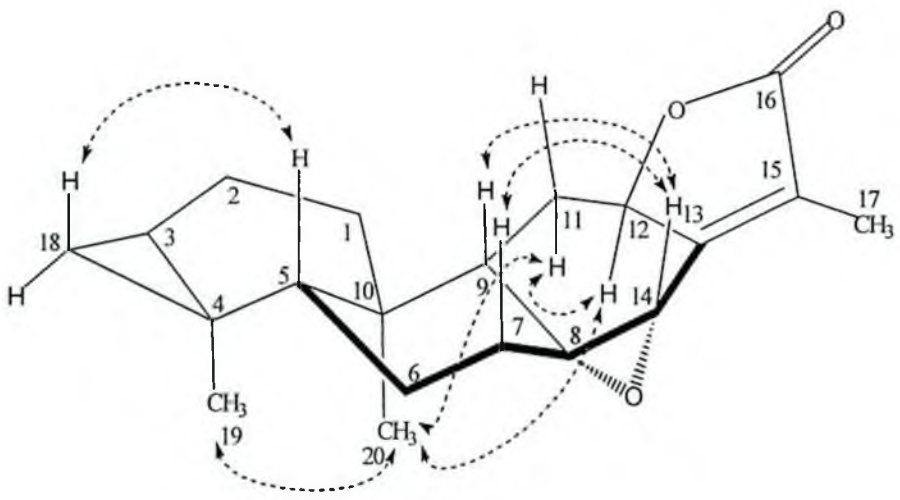


Fig. **14**. Important NOE interactions in suregadolide B (**106**).

Table 2. NMR spectral data ($CDCl_3$+CD_3OD) of suregadolide B (**106**)

C/H No.	^{1}H-NMR[a] δ_H (mul., J = Hz)	^{13}C-NMR[b] δ_C (mult.)*	COSY	HMBC	NOE
1a	0.66 (m)	33.2 (t)	H-1b,H-2a,H-2b	C-2, C-3	-
1b	1.52 (m)		H-1a, H-2a, H-2b	-	-
2a	1.64 (m)	23.8 (t)	H-1a, H-1b, H-2b	C-3, C-4	-
2b	1.79 (m)	-	H-1a, H-1b, H-2a, H-3	C-1	-
3	0.50 (ddd, 9.3, 9.2, 5.9)	16.4 (d)	H-2b,H-18a,H-18b,	C-1, C-2	-
4	-	14.2 (s)	-	-	-
5	1.09 (dd, 13.6,3.0)	50.9 (d)	H-6a, H-6b	C-4, C-6 C-10, C-19	18 *exo*
6a	1.45 (m)	20.8 (t)	H-5, H-6b, H-7a, H-7b	-	-
6b	1.70 (m)	-	H-5, H-6a, H-7a, H-7b	-	-
7a	1.38 (m)	34.4 (t)	H-6a, H-6b, H-7b	C-5, C-8, C-9	H-14
7b	1.8 0 (m)		H-6a, H-6b, H-7a	C-5, C-8	-
8		61.5 (s)	-	-	-
9	1.67 (bd, 6.8)	45.3 (d)	H-11a, H-11b	C-5, C-8, C-10, C-12, C-20	-
10	-	37.5 (s)	-	-	-
11a	1.25 (m)	29.3 (t)	H-9, H-11b, H-12	C-9, C-10, C-12	-
11b	2.1 (dd, 13.1, 5.7)	-	H-11a, H-12	C-12, C-13	H-12
12	4.18, (ddd, 10.3, 5.5, 2.3)	77.7 (d)	H-11a, H-11b, H-17		H-11b, H_3-20
13	-	157.5 (s)	-	-	-
14	3.64 (s)	55.5 (d)	-	C-8, C-12, C-15	H9
15	-	128.0 (s)	-	-	-
16	-	175.0 (s)	-		-
17	1.75 (d, 2.0)	8.0 (q)	H-12	C-13, C-15, C-16	-
18 *exo*	-0.09 (dd, 5.6, 4.6)	18.4 (d)	H-3, H-18b	-	-
18 *endo*	0.30 (dd, 9.3, 4.2)		H-3, H-18a	C-19	H-5
19	1.10 (s)	23.8 (q)	-	C-3, C-4, C-5, C-18	H-3, H_3-20
20	0.80 (s)	16.4 (q)	-	C-1, C-5, C-9, C-10	H-12, H-7a, H-2b, H_3-19

[a] Data recorded at 400 MHz.
[b] Data recorded at 100 MHz.
*Multiplicity determined by DEPT and HMQC spectra.

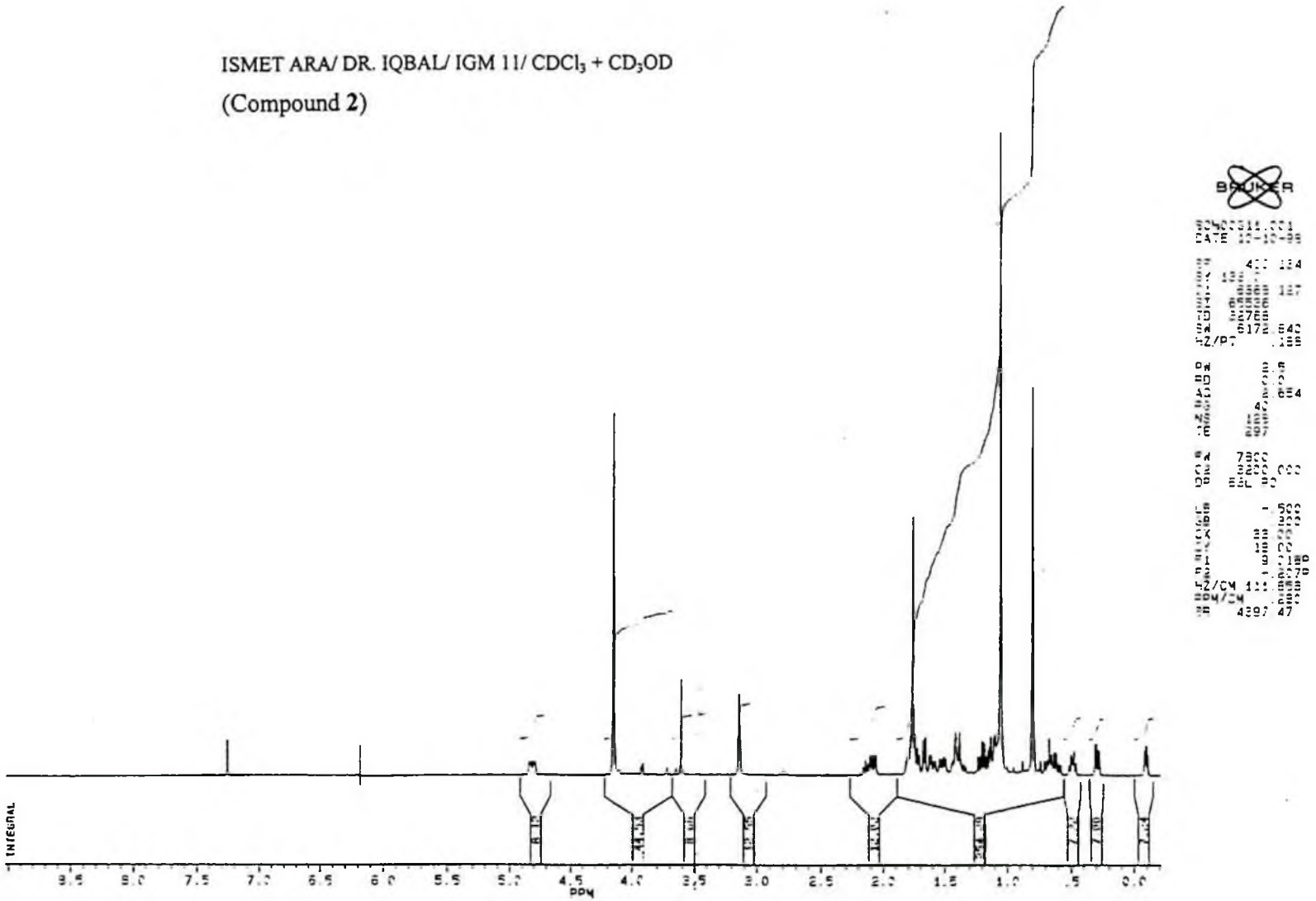


Fig. 11. ^{1}H-NMR Spectrum ($CDCl_3$ + CD_3OD, 400 M Hz) of Suregadolide B (**106**)

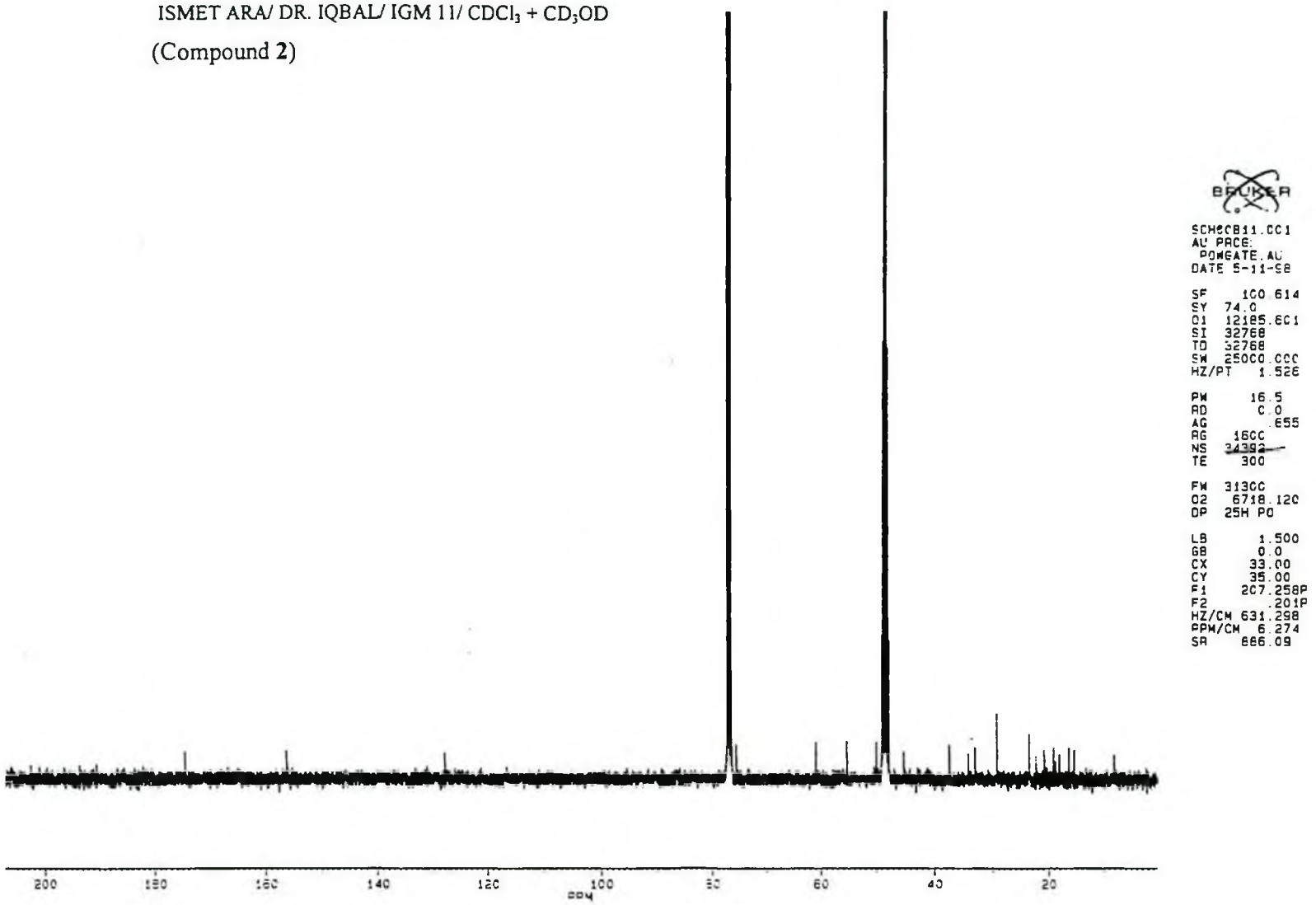
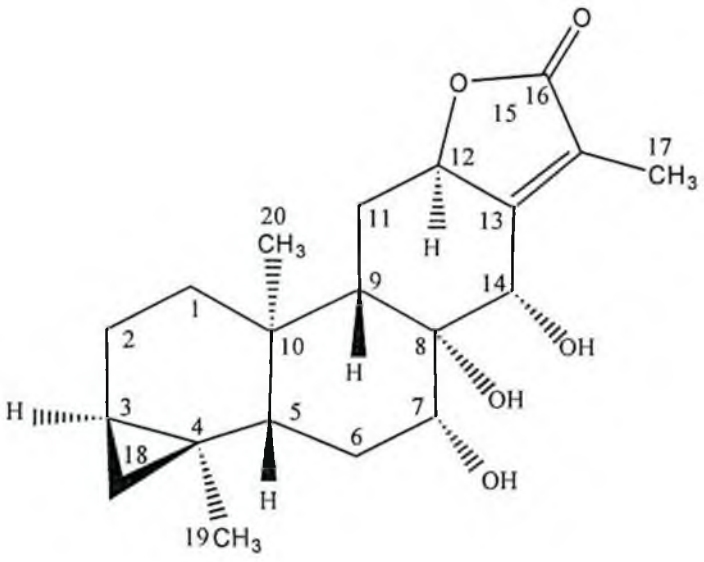


Fig. **12**. ^{13}C- NMR Spectrum ($CDCl_3$ + CD_3OD, 100 M Hz) of Suregadolide B (**106**)

2.5.1.3 Suregadolide C (107)- A Novel Diterpene Lactone

Compound **3,** $C_{20}H_{28}O_5$ was isolated as a white amorphous solid (Scheme-3; Page- 41). The IR spectrum of compound **3** showed absorption for hydroxy at 3504 cm^{-1} and α,β-unsaturated γ-lactone lactonic carbonyl absorption at 1740 cm^{-1}. The HR-EIMS (*m/z* 348.1905), ^1H- and ^{13}C- NMR and DEPT data of compound **3** revealed the presence of a rearranged abietane skeleton with signals of a vinylic methyl (δ_H 1.85, δ_C 8.5), two secondary hydroxyls containing methines (δ_H 3.8, δ_C 69.9 & δ_H 4.83, δ_C 64.9) and one tertiary hydroxyl bearing (δ_C 76.6) carbons. The next three signals at δ_H 0.05, 0.42 (δ_C 22.5) and δ_H 0.60 (δ_C 20.5) indicated the presence of a trisubstituted cyclopropyl substituent in the molecule, while the signals at δ_H 1.01/δ_C 24.9 & δ_H 1.14/δ_C 13.3 revealed the presence of two more methyl groups in the molecule.



Suregadolide C (**107**)

The above spectral data showed that the compound **3** must be a modified *ent*-abietane diterpene lactone, which contained a trisubstituted cyclopropyl group. These assumptions were further confirmed by analyzing the data (Table-3) of ^1H-, ^{13}C-, ^1H-^1H (COSY 45°), HMQC, HMBC and NOE spectra (Table-3, Page-70).

^{1}H-^{1}H (COSY-45°) and HMQC (Table-3, Page-70) spectra of compound **3** revealed that H-12 proton (δ_H 5.46, δ_C 79.4) of the α,β-unsaturated γ-lactone chromophore was coupled with two non-equivalent geminal protons of C-11 methylene (H-11a, δ_H 1.38,m, $W_{1/2}$ = 14.5 Hz. H-11b, δ_H 2.32, dd, $J_{11b\ 11a}$ = 13.0 Hz, $J_{11b,12}$ = 9.0 Hz; δ_C 27.9) and with the vinylic methyl protons CH_3-17 (δ_H 1.85, d, $J_{17,12}$ = 2.0 Hz; δ_C 8.5. The presence of a –CH-CH_2-CH-O- moiety in the molecule was supported from the correlation of H-11a methylene proton (δ_H 1.38) with C-9 methine proton (δ_H 1.20, bd, $J_{9,11}$ = 9.0 Hz, δ_C 42.5). It was therefore, concluded that C-9 must be attached to two adjacent quaternary carbons, since the C-9 (δ_H 1.20) could not be correlated with any other proton.

The presence of a trisubstituted cyclopropane ring in the molecule of compound **3** was clearly observed from its NMR spectra (Fig. 15, Page-71 and Fig. 16, Page 72). Two-upfield signals of methylene protons [H-18 (*endo*), δ_H 0.05, dd, $J_{18\ endo,3}$ = 5.8 Hz, $J_{18\ endo,\ 18\ exo}$= 3.8 Hz, and H-18 (*exo*), δ_H 0.42 (dd, $J_{18\ exo,3}$ = 9.3 Hz, $J_{18\ exo,18\ endo}$ = 4.0 Hz), δ_C 22.5], H-3 signal at δ_H 0.60 (ddd, $J_{3,2}$ = 9.0 Hz, $J_{3,18\ exo}$ = 8.8 Hz, $J_{3,18\ endo}$ = 6.1 Hz; δ_C 20.5) and one quaternary carbon (δ_C 16.9) signal, clearly indicated the presence of a trisubstituted cyclopropane ring in the molecule. These values were consistent with the values for suregadolides A and B. The position of cyclopropyl group in the molecule was confirmed from its COSY- 45° and HMBC (Fig 17) interactions (Table-3, Page-70).

HMBC spectra showed the correlations (Fig. 17; Table-3, Page-70) of C-20 methyl protons (δ_H 1.14,) and C-11 methylene protons (δ_H 1.38) with C-9 (δ_C 42.5). Whereas, H-18 *(endo)* methylene protons (δ_H 0.05), C-20 methyl protons (δ_H 1.14), H-7 methine proton (δ_H 3.8) and H-6b methylene proton (δ_H 2.08) were correlated with C-5 (δ_C 43.3). C-19 Methyl protons (δ_H 1.01) and H-5 methine proton (δ_H 1.63) were found to be correlated with C-4 (δ_C 16.9). H-5 (δ_H 1.63) was also correlated with C-20 (δ_C 13.3) and C-18 (δ_C 22.5). On the other hand H-18 (*exo*, δ_H 0.42) methylene proton and H-1b methylene protons (δ_H 1.40) were found to be correlated with C-3 (δ_C 20.5). H-18 (*endo*, (δ_H 0.05) was also correlated with C-19 (δ_C 24.9).

In ^{1}H- and ^{13}C-NMR spectra (CD_3OD+$CDCl_3$) of compound **3** (Fig. 15, Page-71; and Fig. 16, Page- 72), the downfield signals of H-7 (δ_H 3.80, δc 69.9), H-14 (δ_H 4.83, δ_C 64.9)

and quaternary C-8 (δ_C 76.6) indicated the presence of two secondary hydroxyls and one tertiary hydroxyl bearing carbons in the molecule. The ^{1}H- NMR (C_5D_5N) spectra showed the signals for three OH protons (δ_H 7.73, 6.54 and 5.53), which disappeared when the spectrum was recorded in C_5D_5N with the addition of a few drops of D_2O. Thus confirmed the presence of three hydroxyl groups in the molecule. Compound **3** on treatment with acetic anhydride in pyridine yielded diacetate derivative **3a**, which further confirmed the presence of two secondary hydroxyl groups in the molecule. These hydroxyl groups were attached at C-14, C-8 and C-7, confirmed from its HMQC, and HMBC interactions (Fig. **17**). The HMBC spectra showed the correlations of H-7 (δ_H 3.8), H-9 (1.20) and H-14 (δ_H 4.83) with C-8. H-9 was also found to be correlated with C-14. On the other hand, H-14 showed correlations with C-13 and C-15.

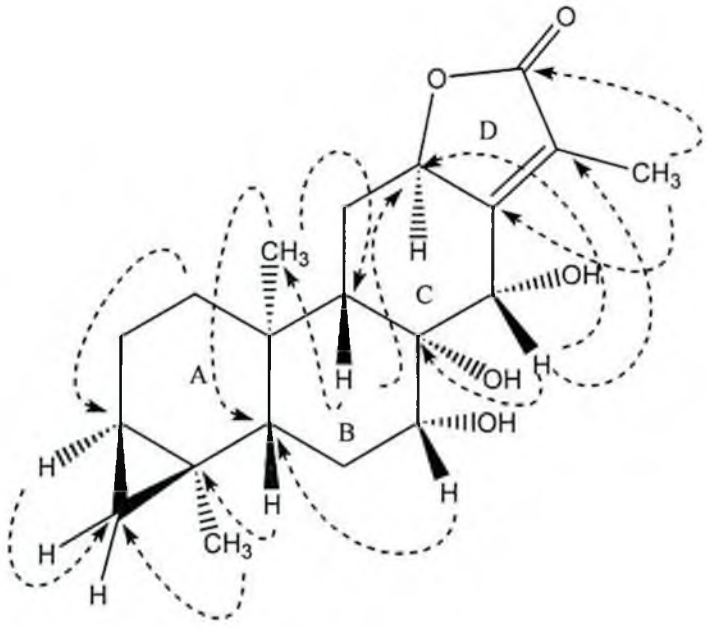


Fig. **17**. Important HMBC interactions in suregadolide C (**107**).

The relative stereochemistry at various chiral centers of compound **3** was deduced from the NOE difference measurements (Fig. 18), while the *trans* A/B ring junction was based on the biogenetic grounds (Talapatra et al., 1989). Since the H-12 (δ_H 5.46) was found to be α oriented in compounds **1** and **2** and in other *ent*-abietane diterpene lactones reported from the same plant (Talapatra et al., 1989; Talapatra et al., 1998) the H-12 of compound **3**

was also inferred as α (*pseudo* axial) and H-9 as β (*pseudo* axial) from the coupling constants and multiplicities of their signals (H-12, δ_H 5.46, ddd, $J_{12,11a}$ = 11.3 Hz, $J_{12,11b}$ = 7.6 Hz, $J_{12,17}$ = 1.8 Hz; and H-9, δ_H 1.2, bd, $J_{9,11b}$ = 9.0 Hz), (Table-**3**) .

The NOE between H-12 (δ_H 5.46) /H-11b (δ_H 2.32) (8%), CH_3-20 (δ_H 1.14) /H-11b (21%), CH_3-20/CH_3-19 (δ_H 1.01) (8%) and CH_3-19/H-3 (δ_H 0.60) (16%) indicated the α orientation of H-11b, CH_3-20, CH_3-19 and H-3, and thus the β-orientation of cyclopropane ring. NOE between H-18 *endo*/H-5 (11%) indicated β-orientation of H-5. The NOESY interaction between H-18 *endo* 0.05) /H-5 (δ_H 1.63), H-5/H-6a (δ_H 1.80), H-6a/H-7 (δ_H 3.80), H-7/H-14 (δ_H 4.83) and H-14/H-9 (δ_H 1.20) indicated the β- orientation of H-7, H-14 and H-9.

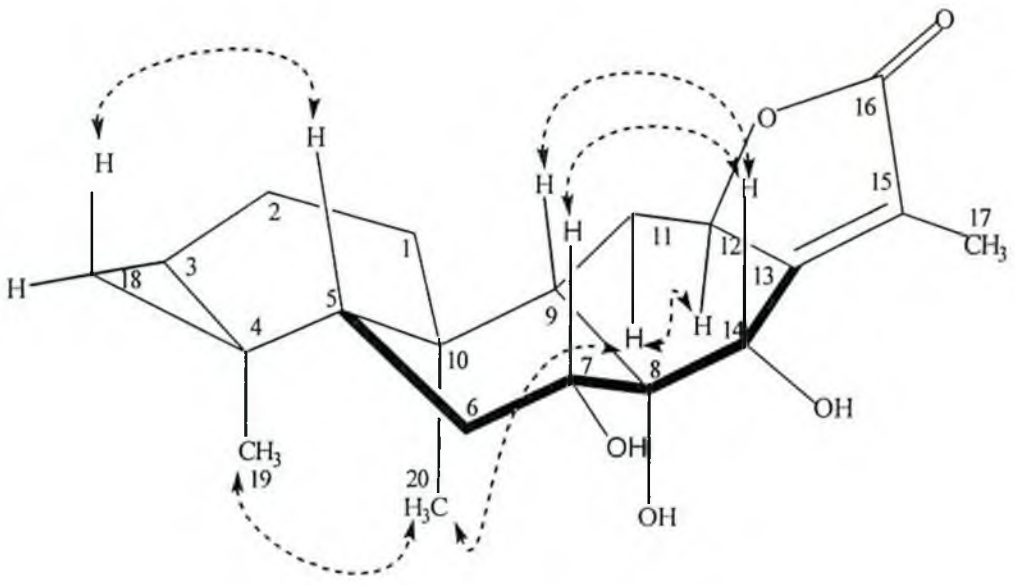


Fig. **18**. Important NOE interactions in suregadolide C (**107**).

From the Drieding model of compound **3**, it was observed that the NOE observed for H-7, H-9 and H-14 agree with an α- orientation of OH-8. The stereochemistry observed at H-5, CH_3-20 and H-9 of compound **3** further indicated the abietane diterpene lactone skeleton of *ent* (antipodal) series (Telapatra et al 1989; Dewick 1997).

The structure of compound **3** was elucidated as 3,4,18β-cyclopropa-9β-7α,8α,14α-trihydroxy-13,15-abieten-16,12-olide and was named as suregadolide C (**107**).

Table 3. NMR spectral ($CDCl_3$+CD_3OD) data of suregadolide C (**107**)

C/H No.	^{1}H-NMR δ_H (mul., J = Hz)	^{13}C-NMR δ_C (mult.)*	COSY	HMBC	NOE
1a	0.64 (m)	33.2 (t)	H-1b,H-2a,H-2b	C-2, C-9, C-10, C-20	-
1b	1.40 (m)	-	H-1a, H-2a, H-2b	C-2, C-3, C-9, C-10	H-11b
2a	1.60 (m)	19.8 (t)	H-1a, H-1b, H-2b	C-1, C-3, C-10	H-3
2b	1.80 (m)	-	H-1a, H-1b, H-2a, H-3	C-4, C-10	-
3	0.60 (ddd, 9.0, 8.8, 6.1)	20.5(d)	H-2b, H-18a, H-18b	C-1, C-5, C-18	H-2a, H-18, *endo*
4		16.9 (s)			
5	1.63 (dd, 13.7, 2.9)	43.3 (d)	H-6a, H-6b	C-1, C-3, C-4, C-10, C-18, C-20	H-18, *endo*
6a	1.80 (dt, 3.2)	29.6 (t)	H-5, H-6b, H-7	C-5, C-7, C-8	H_3-19, H_3-20
6b	2.08 (dt, 2.5)		H-5, H-6a, H-7	C-5, C-10	H-7
7	3.80 (t, 2.9)	69.9 (d)	H-6a, H-6b	C-6, C-8	H-6b, H-14
8		76.6			
9	1.20 (bd, 9.0)	42.5(d)	H-11a, H-11b	C-1, C-8, C-10, C-11, C-20, C-14	H-14
10	-	37.3 (d)	-	-	-
11a	1.38 (m, $W_{1/2}$=14.5)	27.9 (t)	H-9, H-11b, H-12	C-12, C-13	
11b	2 .32 (dd, 13.0, 9.0)		H-9, H-11a, H-12	C-9, C-12	H_3-20, H-12
12	5.46 (ddd, 11.3, 7.6, 1.8)	79.4 (d)	H-11a, H-11b, H_3-17	C-13, C-15	H-11b
13		164.0 (s)			
14	4.83 (bs)	64.9 (d)		C-8, C-13, C-15	H-7, H-9
15		126.0 (s)			
16		177.6 (s)			
17	1.85 (d, 2.0)	8.5 (q,)	H-12	C-13, C-15, C-16	
18*endo*	0.05 (dd, 5.8, 3.8)	22.5 (d)	H-3, H-18b	C-3	H-5
18*exo*	0.42 (dd, 9.3, 4.0)		H-3, H-18a	C-3	
19	1.01 (s)	24.9 (t)		C-3, C-4, C-5, C-18	H-3, H-6a
20	1.14 (s)	13.3 (q)		C-1, C-5, C-9, C-10	H-6b, H11b, H_3-19

[a] Data recorded at 500 MHz.
[b] Data recorded at 75 MHz.
*Multiplicity determined by DEPT and HMQC spectra.

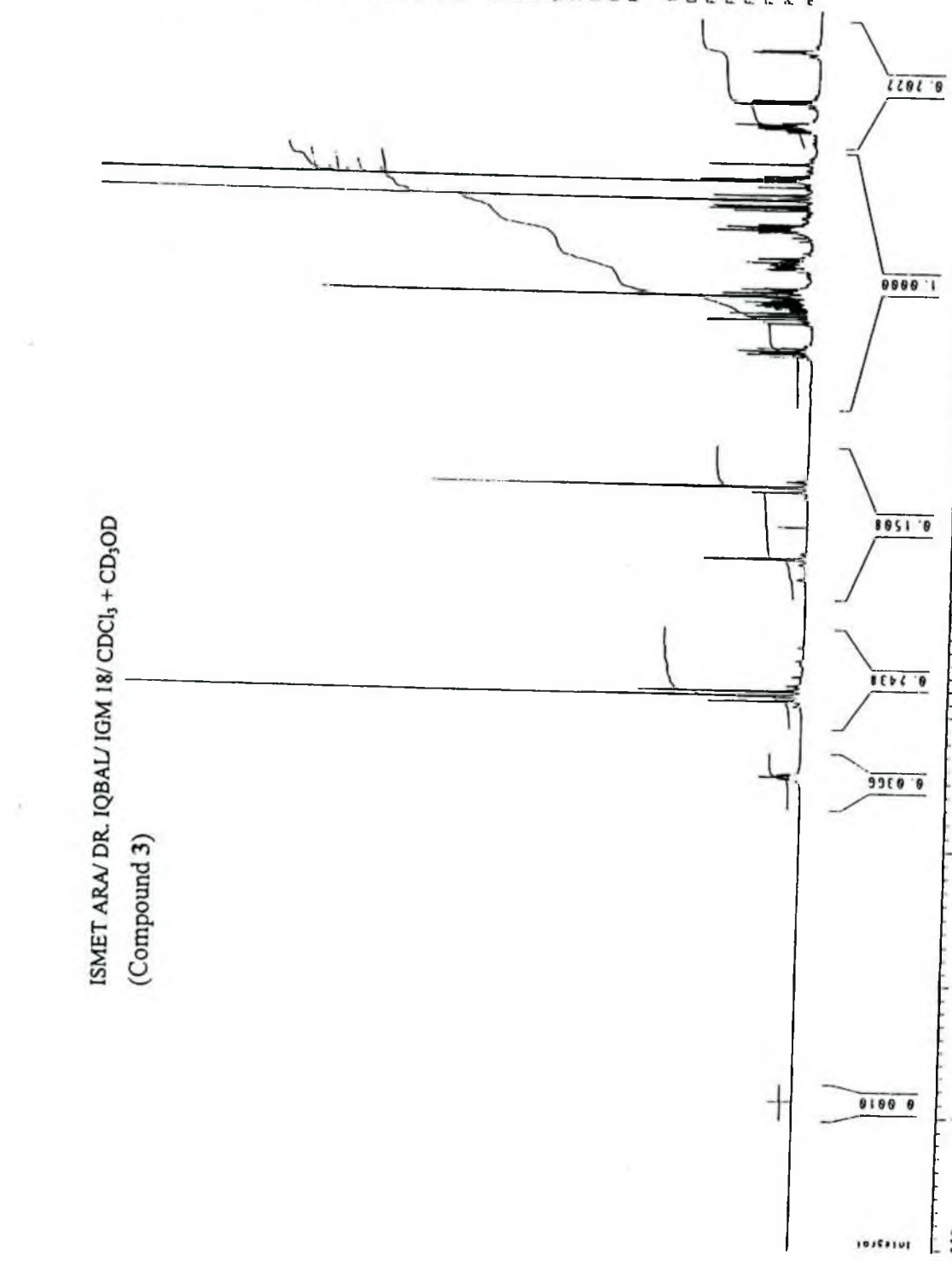


Fig. 15. ^{1}H-NMR Spectrum ($CDCl_3$ + CD_3OD, 500 MHz) of Suregadolide D (107)

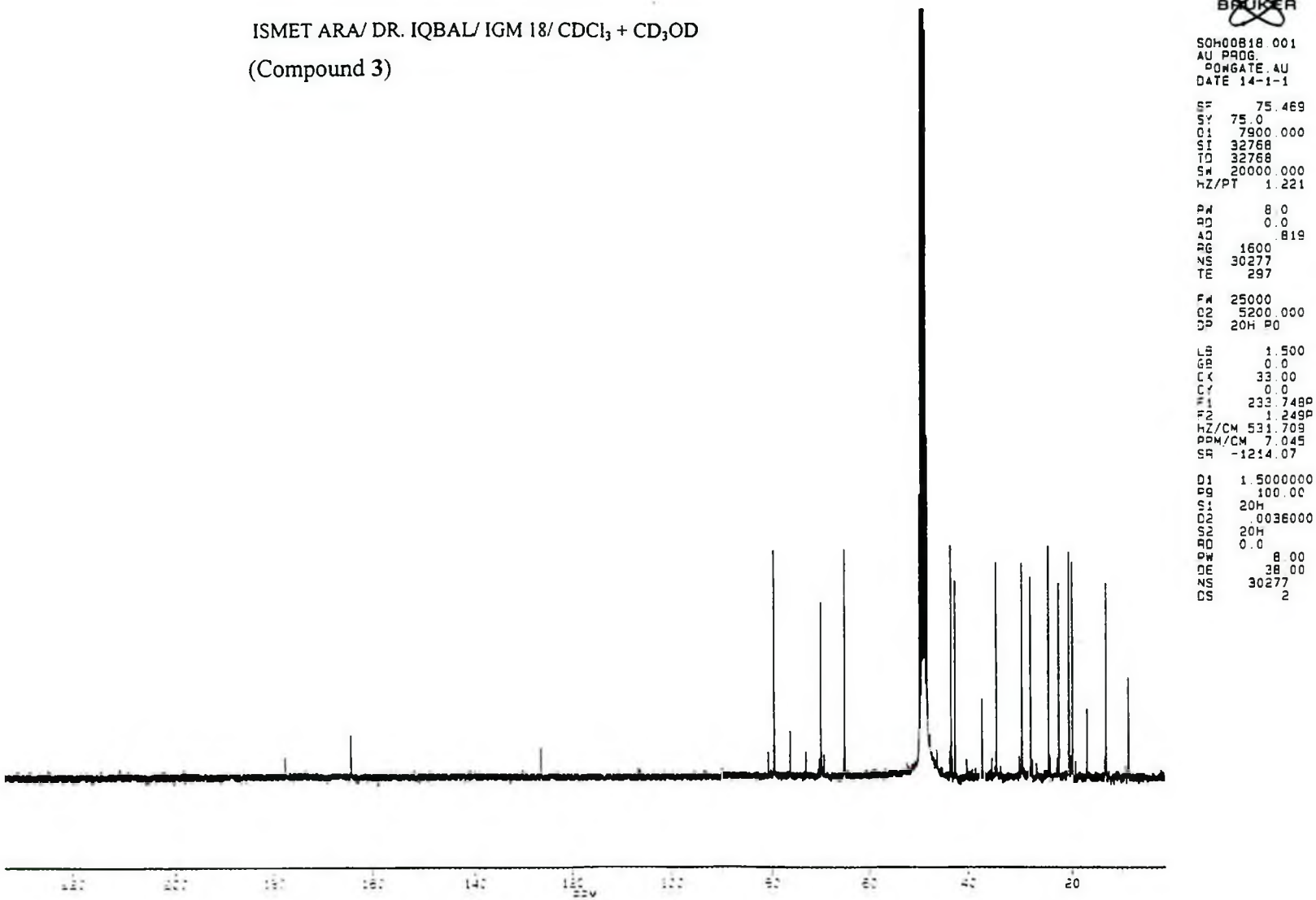
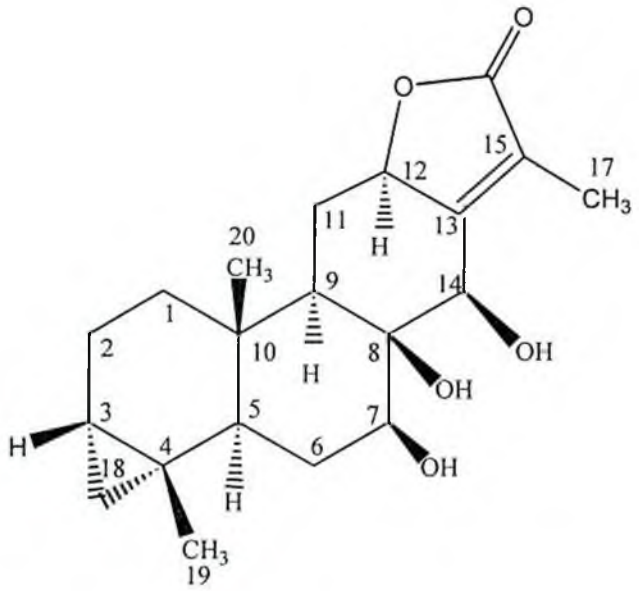


Fig. **16**. ^{13}C- NMR Spectrum ($CDCl_3$ + CD_3OD, 75 MHz) of Suregadolide C (**107**)

2.5.1.4 Suregadolide D (108)- A Novel Diterpene Lactone

Compound **4,** $C_{20}H_{28}O_5$ (*m/z* 347.8) was isolated as a light yellowish amorphous solid from the CH_2CL_2 soluble part (Scheme-3; Page- 41). The presence of α,β-unsaturated γ-lactone chromophore (1732 cm^{-1}) was observed in the IR spectra. From HR-EIMS *(m/z=* 348.1930) and ^{13}C-NMR and DEPT spectral data, compound **4** was also identified as a tetracyclic diterpene of rearranged abietane skeleton.

A vinylic methyl signal at δ_H 1.70/δ_C 8.2, two secondary hydroxyl methine signals at δ_H 3.62/δ_C 67.9 and δ_H 4.45/δ_C 71.4 and one tertiary hydroxyl-bearing carbon signal at δ_C 77.5 were observed in the NMR spectra (Fig. 19, Page-77; and Fig. 20, Page-78). The signals for cyclopropyl group (δ_H -0.17, 0.19/δ_C 21.7 and δ_H 0.34/δ_C 15.7 and δ_C 18.6) and two other signals for methyl groups (δ_H 0.72/δ_C 12.3 and δ_H 0.70/δ_C 23.1) were also inferred in the NMR spectra of compound **4**.



Suregadolide D (**108**)

The complete structure of compound **4** was then deduced with the help of ^{1}H-, ^{13}C- and 2D-NMR (Table-4, Page-76) studies, by chemical derivatization (Section- 2.4.8.2, Page-50) and from a comparative study with compound **3** and other reported compounds from

the same plant (Talapatra et al., 1989; Talapatra et al., 1998). An extensive comparative study of chemical shifts and multiplicities of compound **4** (Table-4, Page-76) with compound **3** (Table-3, Page-70) suggested that compound **4** was an stereoisomer of compound **3,** which was further confirmed by the NOE difference measurements. The NOE studies on compound **4** helped in the determination of relative stereochemistry at defferent stereogenic centers and indicated the normal abietane nature of the skeleton.

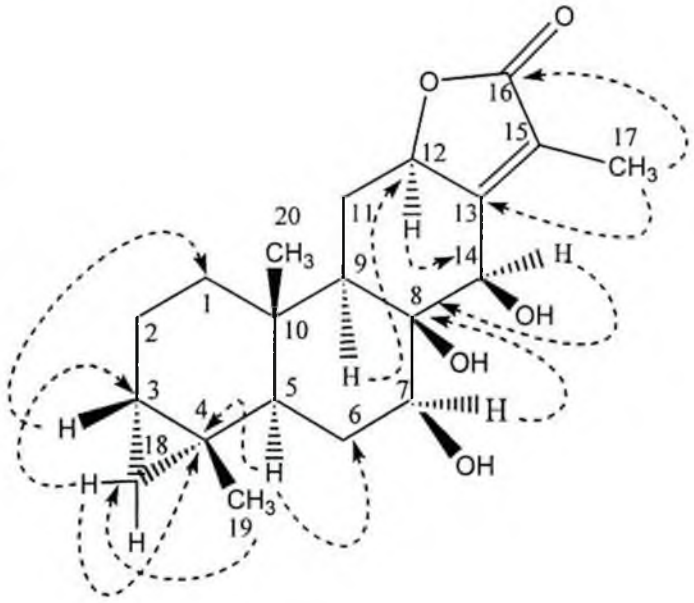


Fig. **21**. Important HMBC interactions in suregadolide D (**108**).

The stereochemistry of H-12 was determined to be as α (*pseudo* axial) from a comparative study of chemical shifts, multiplicities and coupling constants of H-12 (δ_H ddd, 4.54, $J_{12,11a}$ = 11.9 Hz, $J_{12,11b}$ = 6.0 Hz, $J_{12,17}$ = 2.0 Hz) (Table-4) with suregadolides A (δ_H 5.15, ddd, J = 12.0, 6.4, 1.6, Hz) and B (δ_H 4.18, ddd, J = 10.3, 5.5, 2.3 Hz) (Table-1 and -2) and also with three other compounds, gelomulides A (δ_H 4.99, ddd, J = 12.9, 5.3, 2.1 Hz), F (δ_H 4.84, ddd, J = 13.0, 5.4, 2.1 Hz) and H (δ_H 4.94, ddd, J = 12.3, 6.2, 2.0 Hz) (Talapatra et al., 1989; Talapatra et al., 1998) reported earlier from the same plant.

The NOE enhancement of H-9 signal (δ_H 1.10) (40%) by irradiation of H-12 signal, (δ_H (δ_H 4.54) suggested the α-orientation of H-9. The NOE observed between H-9 (δ_H 1.10) /H-5 (δ_H 1.35) (33%), H-9/H-7 (δ_H 3.62) (19%), H-9/H-12 (40%), H-9/H-14 (δ_H 4.45)

(33%) and H-9/H-11b (δ_H 2.02) (26%) suggested the α-orientation of H-5, H-7, H-11b and H-14, therefore indicated the β-orientation of OH-7 and OH-14. On the other hand, strong NOE was observed between C-20 methyl (δ_H 0.72) /H-11a (δ_H 1.24) (28%), which suggested the β-orientation of C-20 methyl. The strong NOE observed between H-18 *endo* (δ_H 0.17) /H-5 (δ_H 1.35) (40%) suggested the α-orientation of cyclopropane ring and thus the β-orientation of C-19 methyl. From the Drieding model studies, it is observed that the NOE between H-7 and H-9 agreed with a β- orientation of OH-8. The assigned stereochemistry of H-5, CH_3-20 and H-9 in compound **4** further indicated the presence of a normal abietane diterpene lactone skeleton (Dewick, 1997).

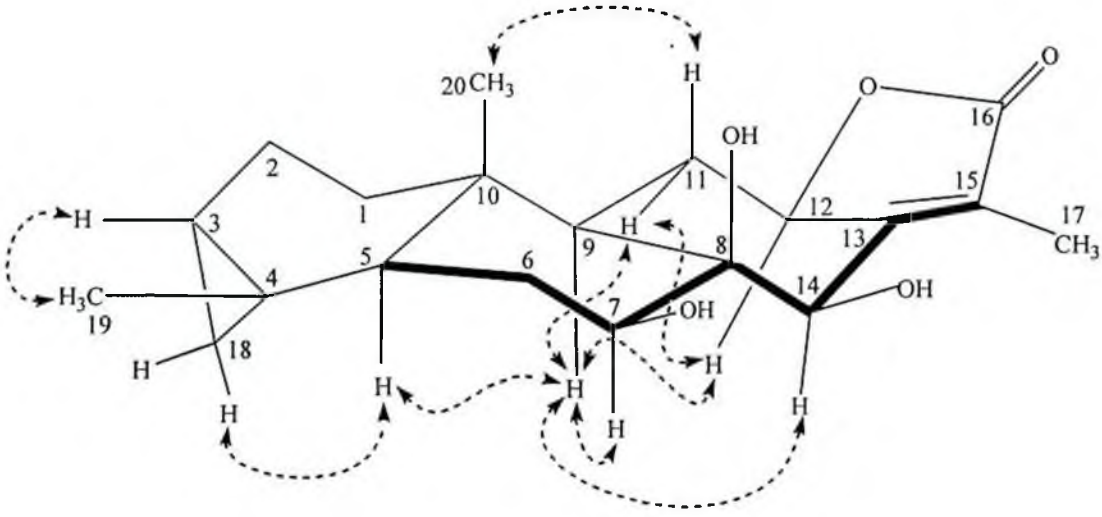


Fig. **22**. Important NOE interactions in suregadolide D (**108**).

The structure of compound **4** was thus elucidated as 3,4,18α-cyclopropa-9α-7β, 8β, 14β-trihydroxy-13, 15-abieten-16, 12-olide and was named as suregadolide D (**108**).

The presence of diterpenoids of normal (compound **4**) and antipodal (compound **3**) series in the same plant was an intriguing observation. However, the co-occurrence of diterpenoids of two antipodal series in the same species has been reported earlier from the plant *Oxystigma oxyphyllum* Harms. (Bevan, et al., 1987).

Table 4. NMR spectral data ($CDCl_3$+CD_3OD) of suregadolide D (**108**)

C/H No.	^{1}H-NMR[a] δ_H (mul., J = Hz)	^{13}C-NMR[b] δ_C (mult.)*	COSY	HMBC	NOE
1a	0.45 (m)	34.5 (t)	H-1b,H-2a,H-2b	C-2, C-9, C-10	
1b	1.30 (m)		H-1a, H-2a, H-2b	C-9, C-10	
2a	1.45 (m)	18.8 (t)	H-1a, H-1b, H-2b	C-1, C-3, C-4, C-10, C-18	H_3-19
2b	1.70 (m)		H-1a, H-1b, H-2a, H-3	C-1, C-3, C-10	
3	0.34 (ddd, 9.7, 7.0, 4.5)	15.7 (d)	H-2b, H-18a, H-18b	C-1, C-4	
4	-	18.6 (s)	-		
5	1. 35 (m)	42 .2 (d)	H-6a, H-6b	C-3, C-4, C-9, C-6, C-10, C-18, C-20	H-9, H-18, *endo*
6a	1.60 (m)	28.0 (t)	H-5, H-6b, H-7	C-10	H_3-19, H_3-20
6b	1.8 (dt, 3.5)	-	H-5, H-6b, H-7	C-5	
7	3.62 (dt, 13.0, 3.0)	67.9 (d)	H-6a, H-6b	C-5, C-8, C-9	H-9
8	-	77.5 (s)			
9	1.1 0 (dd, 13.0, 2.6)	41.1 (s)	H-11a, H-11b	C-12, C-13	H-5, H-7, H-11b, H-12, H-14
10		35.4 (d)			
11a	1.24 (m, $W/_{1/2}$=12.3)	28.5 (t)	H-9, H-11b, H-12	C-12	H_3-20
11b	2.02 (ddd, 1 2.0, 6.4, 2.8)		H-9,H-11a, H-12	C-9, C-12, C-13	H-9, H-12
12	4.54 (ddd, 11.9, 6.0, 2.0)	79.1 (d)	H-11a, H-9, H-12	C-14	H-9, H-11b
13		162.5 (s)			
14	4.45 (d, 1.8)	71.4 (d)	H_3-17	C-8	H-9
15		122.2 (s)			
16		176.0 (s)			
17	1.70 (d, 1.7)	8.2 (q)	H-12	C-13, C-15, C-16	
18 *exo*	0.19 (dd, 9.2, 4.6)	21.7 (t)	H-3, H-18b	C-19	
18 *endo*	-0.17 (dd, 5.8, 4.5)		H-3, H-18a	C-4	H-5, H-18a
19	0.70 (s)	23.1 (q)		C-5, C-18	H-3
20	0,72 (s)	12.3 (q)		C-1, C-5, C-9, C-10	H-11a

[a] Data recorded at 500 MHz.
[b] Data recorded at 125 MHz.
*Multiplicity determined by DEPT and HMQC spectra.

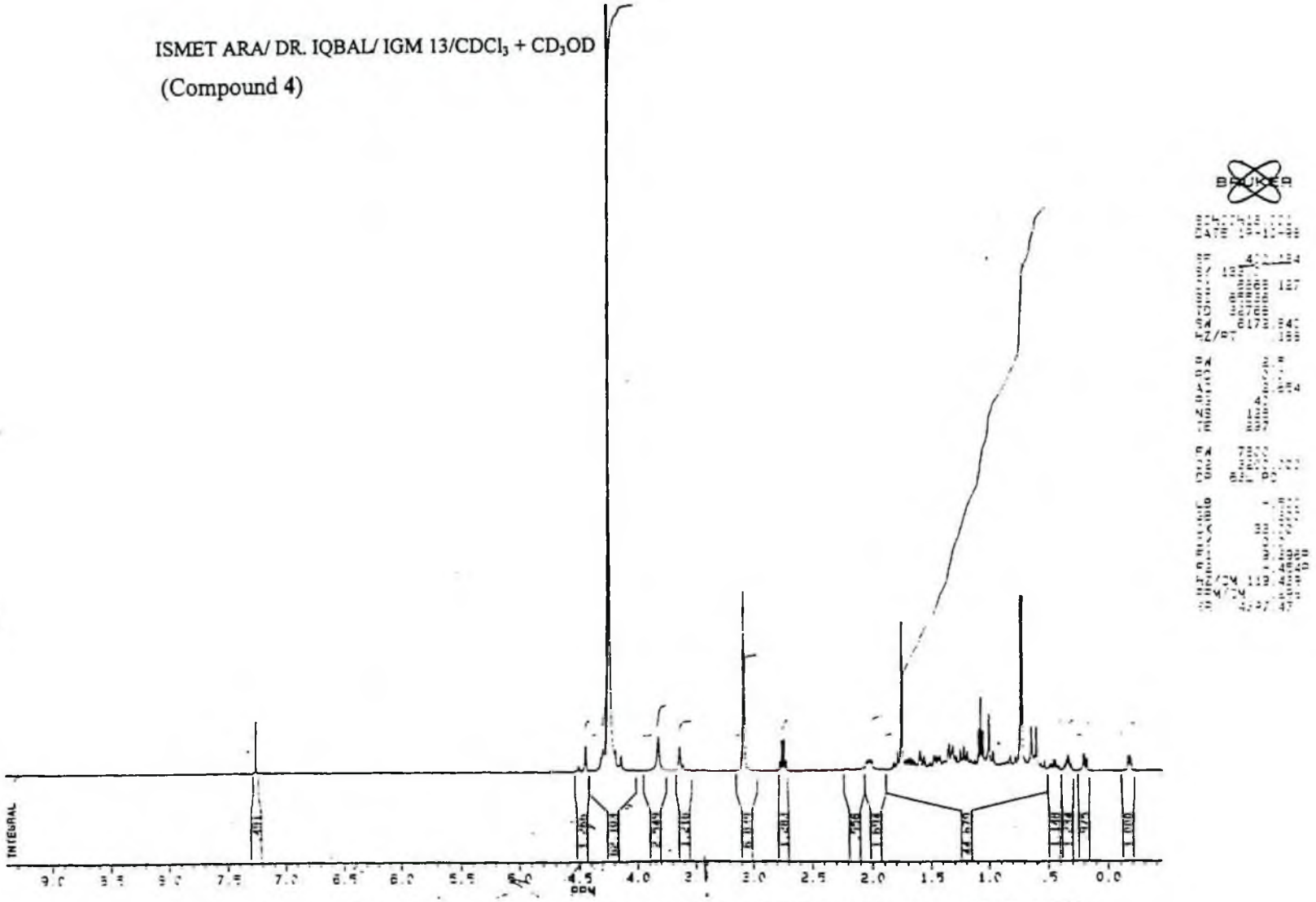


Fig.19. ^{1}H-NMR Spectrum ($CDCl_3$ + CD_3OD, 400 MHz) of Suregadolide D (**108**)

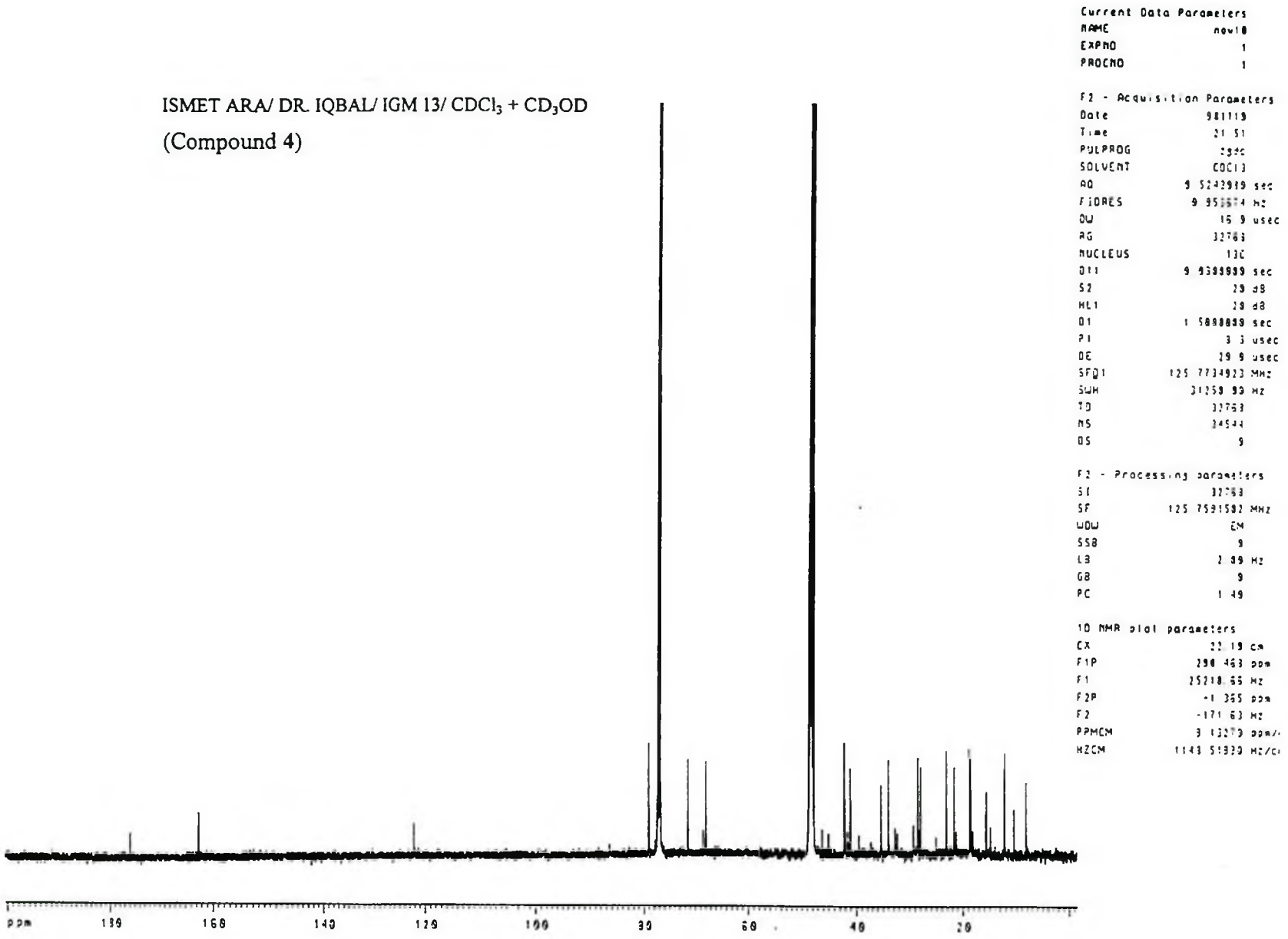
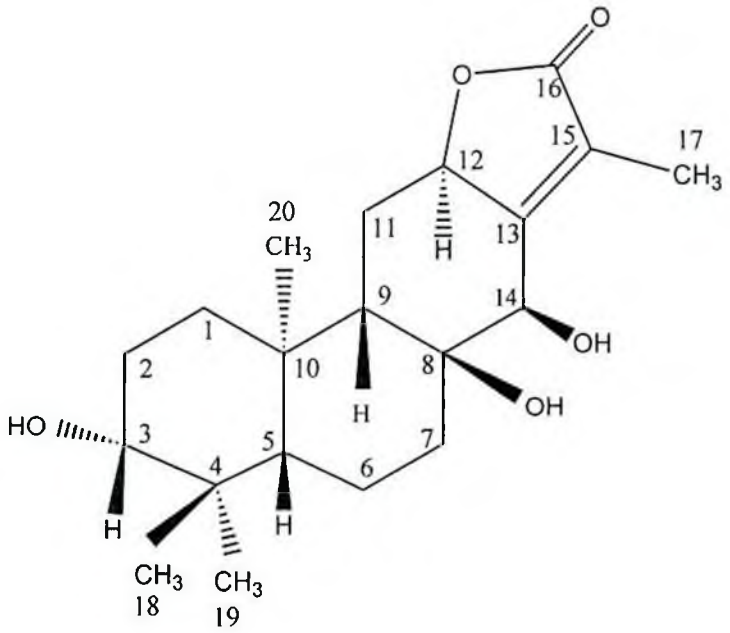


Fig.20. ^{13}C- NMR Spectrum ($CDCl_3$ + CD_3OD, 125 MHz) of Suregadolide D (108)

2.3.1.5 Suremulide A (109)- A New Diterpene Lactone

Compound **5** was isolated as a white amorphous solid (Scheme-4; Page- 44). The IR spectrum of **5** showed the presence of hydroxyls (3415 cm^{-1}) and a α,β-unsaturated γ-lactonic carbonyl (1739^{-1} cm). The molecular formula of **5** was determined from HR-EIMS (*m/z*, 350.2093) and confirmed by ^{13}C-NMR and DEPT data. Its ^{1}H- and ^{13}C- NMR spectra (Fig. 23 and 24) showed the presence of a vinylic methyl proton at δ_H 1.75 (d, J = 1.7), δ_C 7.5), two oxymethine protons at δ_H 3.12 (dd, $J_{3,2a}$ = 11.3 Hz, $J_{3,2e}$ 5.0 Hz, δ_C 78.3), δ_H 4.28 (s, δ_C 71.9) and an oxygen-bearing tertiary carbon at δ_C 74.0. The presence of other three methyl groups at δ_H 0.90/δ_C 28.5; δ_H 0.70/δ_C 15.4; δ_H 1.14/δ_C 16.0) in the molecule was also observed from its NMR data (Fig. 23, Page-83; Fig. 24, Page-84)



Suremulide A (**109**)

The preliminary observations suggested that the compound **5** might have an *ent*- abietane diterpene lactone skeleton.

The presence of three hydroxyls was confirmed by recording the ^{1}H-NMR spectrum in C_5D_5N, which showed three more downfield signals at δ_H 7.73, δ_H 6.54 and δ_H 5.53. These peaks disappeared, when the ^{1}H-NMR was recorded in C_5D_5N with a few drops of D_2O. NMR data of compound **5** (Table 5) particularly the chemical shifts, coupling constants and multiplicities of H-12 (δ_H 5.12, ddd, $J_{12,11a}$ = 10.3 Hz, $J_{12,11b}$ = 8.45 Hz, $J_{12,17}$ = 1.75 Hz and δ_C 77.3), H_2-11 (H-11a, δ_H 1.47,m; H-11b, δ_H 2.36, dd, $J_{11a,11b}$ = 12.2 Hz, $J_{11b,12}$ = 7.0 Hz and δ_C 28.4) and H-9 (δ_H 1.43, bd $J_{9,11b}$ = 7.9 Hz and δ_C 56.1) were similar to that of suregadolide A, and also to the reported compounds (Talapatra et al., 1989; Talapatra et al., 1998).

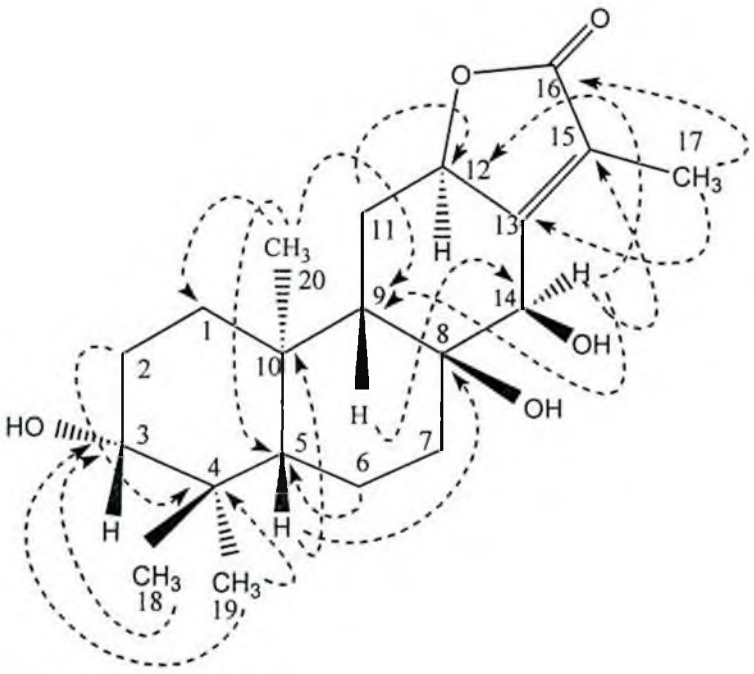


Fig. **25**. Important HMBC interactions in suremulide A (**109**).

An extensive analysis of NMR data [^{1}H-, ^{13}C-, ^{1}H-^{1}H (COSY - 45°), HMQC and HMBC (Fig. 25; Table-5, Page-82), showed that the values of compound **5** has distinct similarities to the reported compound gelomulide I (Talapatra et al., 1998).

The relative stereochemistry of all the chiral centers of compound **5** was deduced from its NOESY technique and Driedling model study as depicted in (Fig. **26**). The structure of the compound was therefore elucidated as 3α,8β,14β-trihydroxy-13,15-abiatene-16,12-olide and named as suremulide A (**109**).

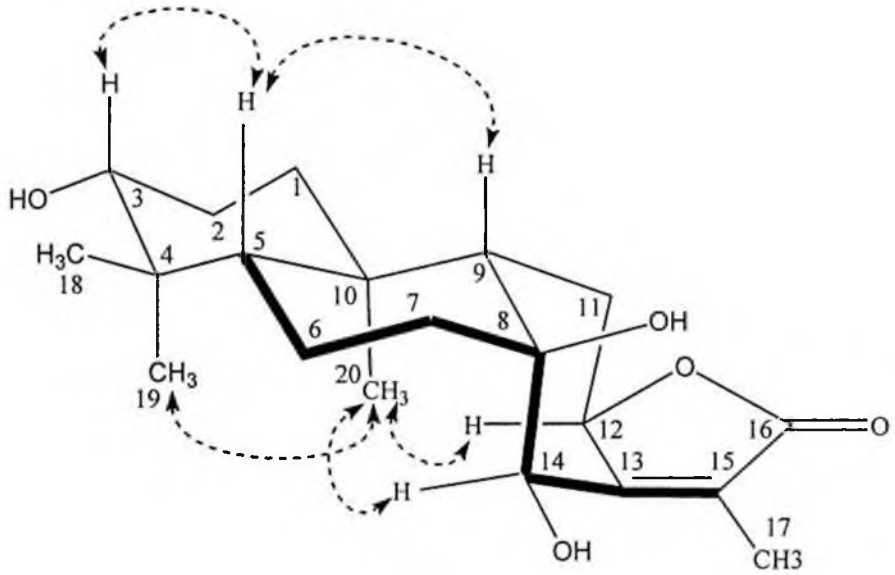


Fig. **26**. Important NOESY interactions in suremulide A (**109**).

Table 5. NMR ($CDCl_3$+CD_3OD) data of suremulide A (**109**)

C/H No.	^{1}H-NMR[a] δ_H (mult., J = Hz)	^{13}C-NMR[b] δ_C (mult.)*	COSY	HMBC	NOESY/ NOE
1a	0.90(m)	39.9 (t)	H-1b, H-2a	C-20	-
1b	1.82 (dt, 3.6)		H-1a, H-2a	C-3	-
2a	1.45 (m)	27.0 (t)	H-2b, H-3	C-1, C-3, C-4, C-10	-
2b	1.54 (m)		H-1a, H-1b, H-2a, H-3	C-3, C-4	
3	3.12 (dd, 11.3, 5.0)	7 8.3 (d)	H-2, H-18a, H-18b	C-18, C-19	H-5
4	-	38.5 (s)	-	-	-
5	0.82 (m)	5 4.2 (d)	H-6a, H-6b	C-3, C-6, C-7, C-10, C-19, C-20	H-3, H-9
6a	1.50 (m)	20.3 (t)	H-5, H-6b, H-7a, H-7b		-
6b	1.54 (m)	-	H-5, H-6b, H-7a, H-7b	C-8	-
7a	1.47 (m)	41.7 (t)	H-6a, H-6b, H-7b	C-8	H-9
7b	2.18 (dt, 2.7)	-	H-6a, H-6b, H-7a		H-14
8	-	74.0 (s)	-	-	-
9	1.43 (bd, 7.9)	56.1 (s)	H-11b	C-8, C-11, C-20, C-14	H-5, H-7a, H-11a,
10	-	38.5 (s)		-	
11a	1.47 (m)	28.4 (t)	H-11b, H-12	C-9, C-13	H-9
11b	2.36 (dd, 12.2, 7.0)		H-11a, H-12	C-12, C-13	H-12
12	5.12 (ddd, 10.3, 8.45, 1.75)	77.3 (d)	H-11a, H-11b, H_3-17	-	H-11b, H_3-20
13	-	163.0 (s)	-	-	-
14	4.28 (s)	71.9 (d)		C-8, C-12, C-13, C-15	H-7b, H_3-20
15	-	122.0 (.s)	-	-	-
16	-	176.0 (s)	-	-	-
17	1.75 (d, 1.7)	7.5 (q)	H-12	C-13, C-15, C-16	-
18	0.9 0(s)	28.5 (q)		C-4, C-18	
19	0.70 (s)	15.4 (q)	-	C-4, C-5, C-18	H_3-20
20	1.14 (s)	16.0 (q)		C-5, C-9	H_3-19, H-12

[a] Data recorded at 500 MHz.
[b] Data recorded at 100 MHz.
*Multiplicity determined by DEPT and HMQC spectra.

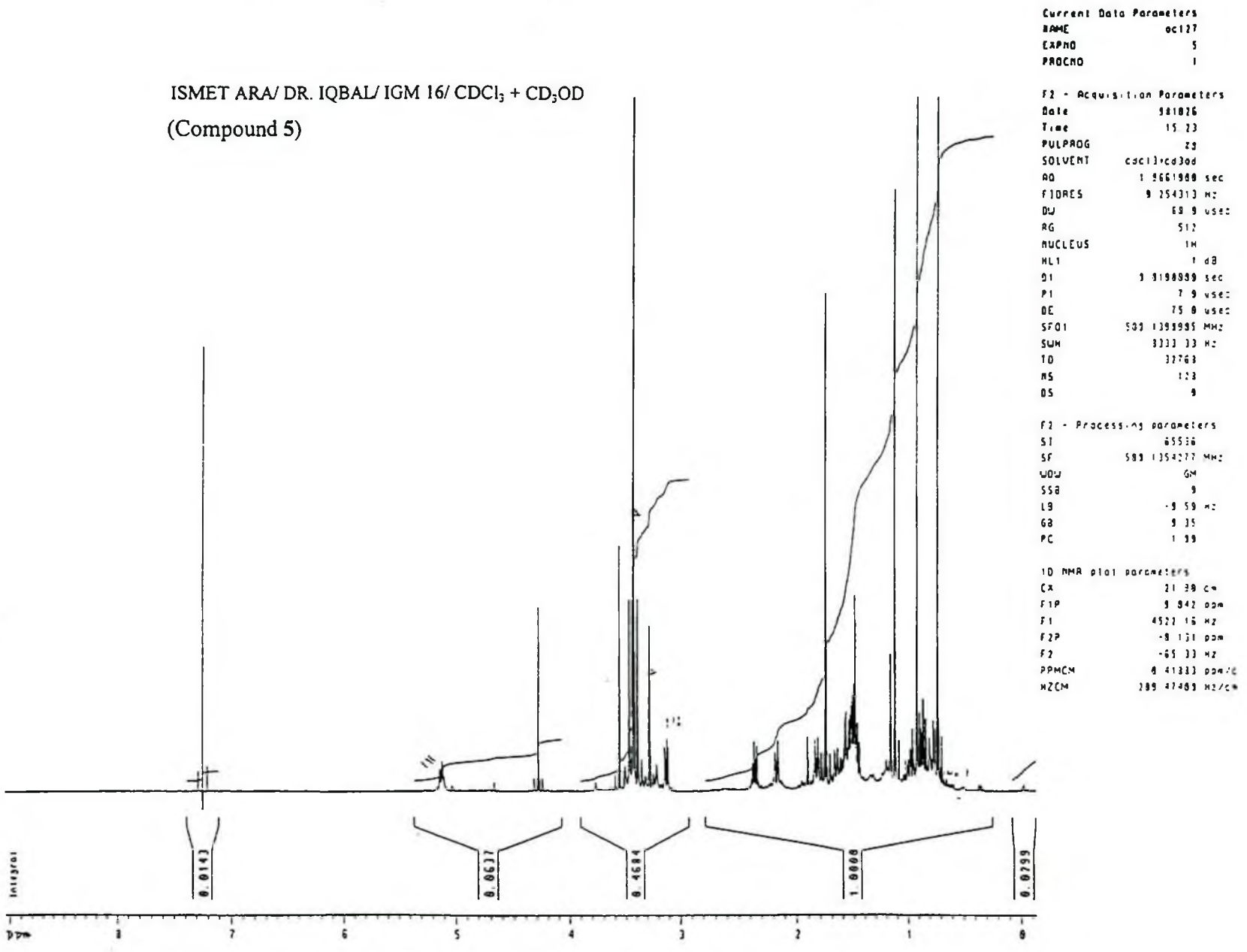
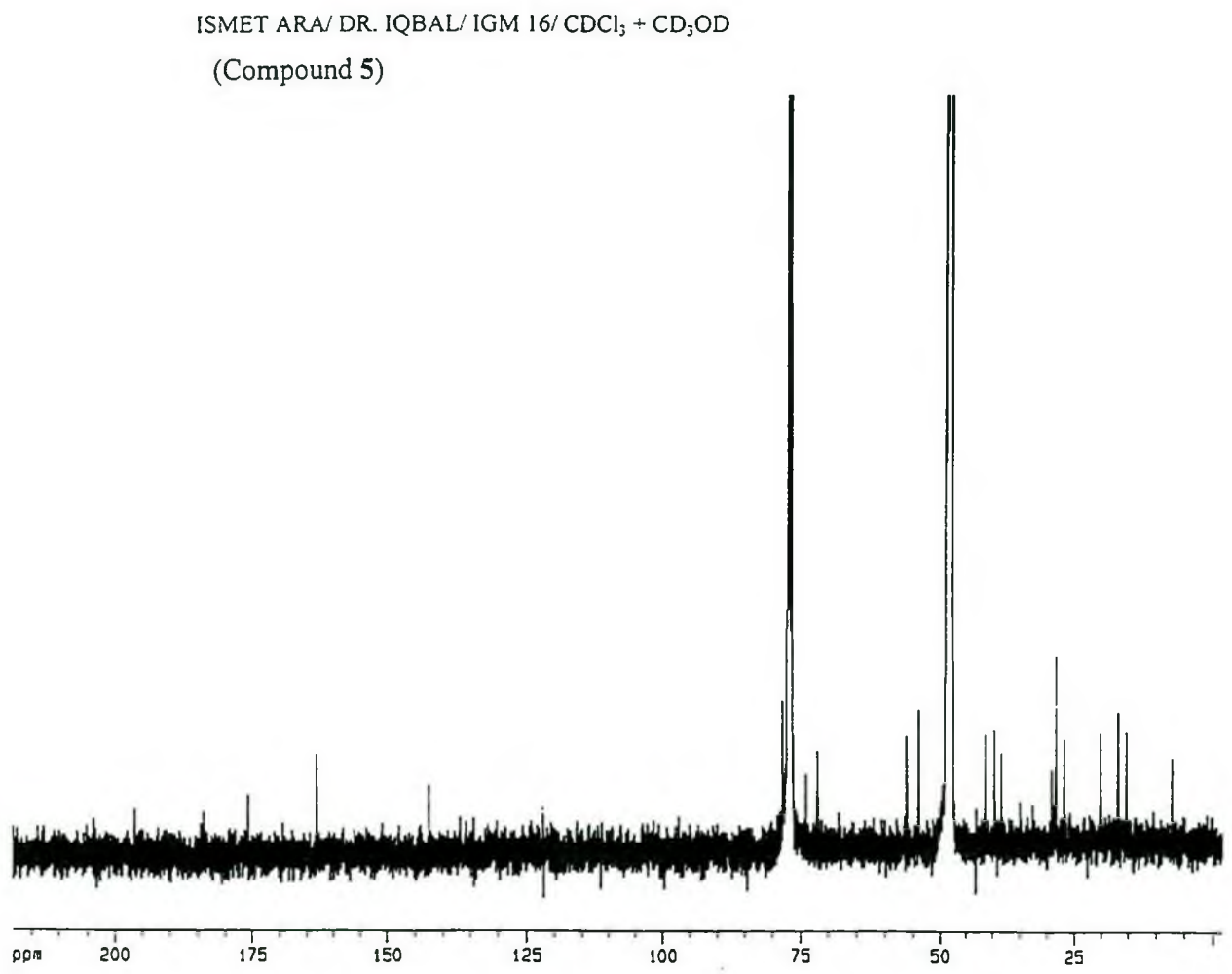


Fig.23 ^{1}H-NMR Spectrum ($CDCl_3$ + CD_3OD, 500 M Hz) of Suremulide A (**109**)

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PROCNO                    1

F2 - Acquisition Parameters
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PULPROG                zgpg
TD                    32768
SOLVENT          cdcl3+cd3od
NS                    54009
DS                        2
SWH               23640.662 Hz
FIDRES             0.721456 Hz
AQ                 0.6930932 sec
RG                    16384
DW                   21.150 usec
DE                   20.00 usec
TE                   300.0 K
D1                1.50000000 sec
D11               0.03000000 sec
D12               0.00002000 sec

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PL1                   0.00 dB
SFO1            100.6241289 MHz

======== CHANNEL f2 ========
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PL12                  18.00 dB
PL13                  18.00 dB
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F2 - Processing parameters
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WDW                      EM
SSB                       0
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1D NMR plot parameters
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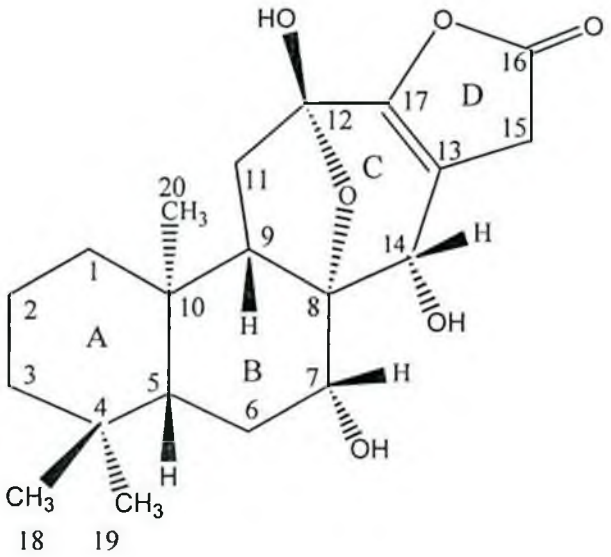
F1                21996.48 Hz
F2P                -1.656 ppm
F2                 -166.64 Hz
PPMCM             11.01403 ppm/cm
HZCM            1108.15601 Hz/cm
```

Fig.24 ^{13}C-NMR Spectrum ($CDCl_3$ + CD_3OD, 100 M Hz) of Suremudolide A (**109**)

2.5.1.6 Bannaringaolide A (110)-A Novel Diterpene Lactone

Compound **6** was isolated as a white amorphous powder from the CH_2Cl_2 soluble part. (Scheme-4, Page-45). The IR spectrum of compound **6** showed the presence of a lactonic C=O (1732 cm^{-1}) and hydroxyl (3596 cm^{-1} and 3445 cm^{-1}) groups. The molecular formula $C_{20}H_{28}O_6$ of compound **6** was inferred from the (– ve) HR-FAB-MS (*m/z* 364.4370) and DEPT data. The NMR data (C_5D_5N) revealed the presence of three methyls resonated at δ_H 0.76/δ_C 22.0, 0.87/δ_C 33.5) and 1.14/δ_C15.8), two oxygen-containing methines at δ_H 4.60/δ_C 69.7 and δ_H 5.94/72.7) and two tertiary oxygen-bearing carbons at δ_C 79.9 and 105.0. The presence of two quaternary carbon signals (δ_C 79.9 and 105.0), indicated the presence of an ether group. The lactonic carbonyl carbon resonated at δ_C 172.4. ^{13}C-NMR spectrum also showed the presence of three methyls, four methylenes, six methines and seven quaternary carbons in the molecule Fig 28, Page-92).



Bannaringaolide A (**110**)

The above spectral data indicated that the compound **6** has a diterpene lactone skeleton. The structure of the compound was then determined by analyzing ^{1}H- and ^{13}C- NMR, ^{1}H-^{1}H (COSY- 45°), HMQC, HMBC, TOCSY and NOESY spectra.

The ^1H-^1H (COSY- 45°) spectra (C_5D_5N) of compound **6** showed that H-11a (δ_H 2.30, m), and H-11b (δ_H 2.67, m) were coupled with methine H-9 (δ_H 2.40, bd, $J_{11b\ 9,11a,}$ = 13.0 Hz). H-5 (δ_H 1.99, bs) was found to be coupled with H_2-6 (H-6a, δ_H 1.80, dd, $J_{6a,6b}$ = 12.2 $J_{6a,5}$ = 3.0 Hz; Hz, H-6b, δ_H 2.08, m). H_2-6 was also coupled with the oxymethine proton (δ_H 4.60, bs). The non-equivalent C-15 methylene protons H_2-15 (δ_H 3.22, d, $J_{15a,15b}$ = 8.7 Hz; δ_H 3.56, d, $J_{15b,15a}$ = 8.6 Hz) exhibited only the geminal couplings. This suggested that C-15 methylene must be attached to two quaternary carbons; one of which may be an electron withdrawing lactone carbonyl. This was further confirmed by the observed ^1H-^1H (TOCSY-100 ms) and HMBC interactions (Fig. **29**).

The presence of three hydroxyl groups was confirmed from the signals at δ_{OH} 8.75, 6.40 and 4.80 were disappeared when the spectrum was recorded in C_5D_5N with a few drops of D_2O. In the COSY-45° spectrum the cross peaks between H-14 (δ_H 5.94, d, J = 5.4 Hz) with geminal OH-14 (δ_H 8.70, d, J = 5.8 Hz) and H-7 (δ_H 4.60, bs) with OH-7 (δ_H 6.40, d, J = 3.6 Hz) were observed, which confirmed the presence of two secondary OH in the molecule. The formation of a triacetate **6a** of compound **6** further confirmed the presence of three hydroxyl groups in the molecule.

The HMBC spectra (Fig. 29; Table-6, Page-89) showed the long-range couplings between carbons and protons, which helped in determining the position of different functionalities and chemical shifts of quaternary carbons in the molecule.

The HMBC spectra showed the interactions of C-20 methyl protons (δ_H 1.14) with C-1 (δ_C 40.0), C-5 (δ_C 47.0), C-10 (δ_C 38.0) and C-9 (δ_C 46.1). H-5 (δ_H 1.99) was found to be correlated with C-10 (δ_C 38.0) and C-6 (δ_C 26.8). The C-19 methyl protons (δ_H 0.76) and C-18 methyl protons (δ_H 0.87) were found to be correlated with C-3 (δ_C 42.0), C-4 (δ_C 33.8), and C-5 (δ_C 47.0). H-6a (δ_H 1.8) exhibited correlations with C-5 (δ_C 47.0), C-7 (δ_C 69.7), C-8 (δ_C 79.9) and C-10 (δ_C 38.0). The most downfield C-14 proton (δ_H 5.94) was found to be correlated with C-13. (δ_C 128.0) and C-17 (δ_C 162.8). On the other hand, H-15a (δ_H 3.22) and H-15b (δ_H 3.56) exhibited correlations with C-13 (δ_C 128.0) and C-17 (δ_C 162.8). H-15a also showed strong interaction with C-16 (δ_C 172.4). HMBC were also

found between H-11b (δ_H 2.67) and C-8 (δ_C 79.9), C-12 (δ_C 105.0) and C-17 (δ_C 162.8). While H-11a showed interaction with C-12.

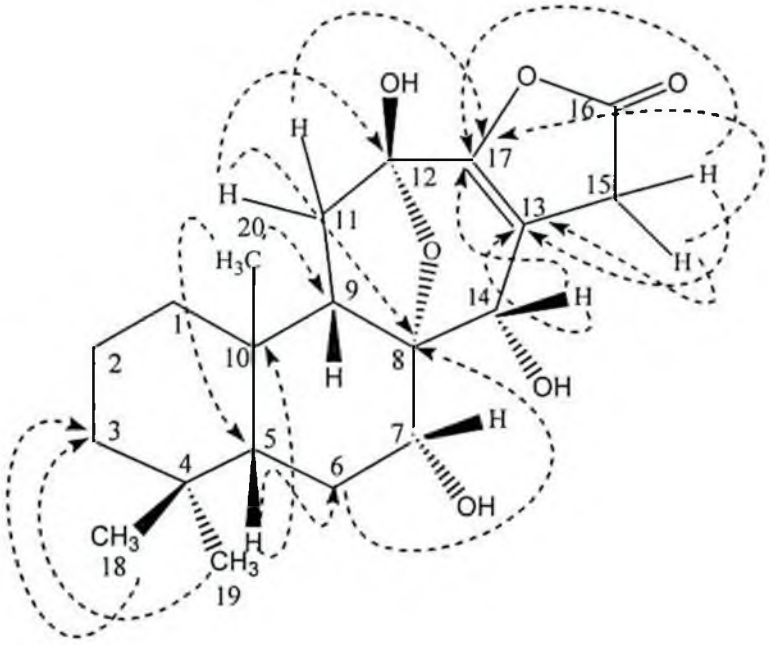


Fig. **29**. Important HMBC interactions in bannaringaolide A (**110**).

The presence of an ether bridge between two quaternary carbons *i.e.* C-12 (δ_C 105.0) and C-8 (δ_C 79.9) was predicted from the HMBC interactions (Fig. 29) and similar data has also been reported earlier in case of new furanoheliangolide derivative, "4,5-dihydroniveusin A" which contained an ether bridge in the molecule (Melek et al., 1985). These observations clearly identified the structure of compound **6** as a novel diterpene skeleton containing one lactone, three hydroxyls and one tetrahydrofuran ring.

Compound **6** was classified as a member of an *ent*-series of diterpenoid on biogenetic grounds and the stereochemistry of AB ring junction in compound **6** was inferred as *trans* (Talapatra et al., 1989). In *ent*-series of diterpene compounds, C-20 methyl is always found to be α- oriented (Dewick et al., 1997).

The relative configuration of compound **6** was determined from its NOESY spectrum ($CDCl_3 + CD_3OD$) as depicted in Fig. 30. The interaction of C-20 methyl (δ_H 0.72) with C-19 methyl (δ_H 0.68) suggested the α orientation of C-19 methyl and thus the β orientation of geminal C-18 methyl (δ_H 0.62).

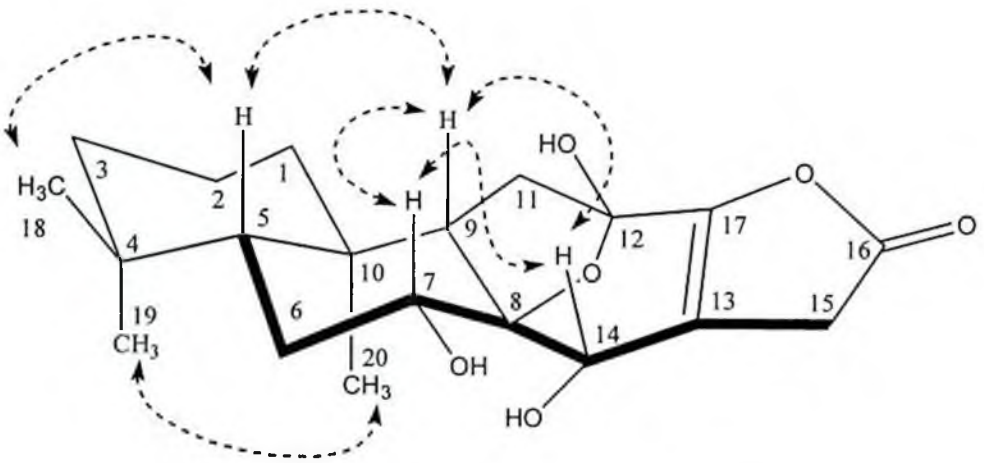


Fig. **30**. Important NOE interactions in bannaringaolide A (**110**).

The NOESY interaction between C-18 methyl with H-5 (δ_H 1.30) suggested the β orientation of H-5. NOESY interactions observed between H-9 (δ_H 1.48)/H-5 (δ_H 1.30), H-9/ H-14 (δ_H 4.67), H-9/H-7 (δ_H 3.73) and H-14/H-7 indicated the β orientations of H-9, H-14 and H-7. The NOE interactions between H-9/H-14 and H-7/H-14 in the NOESY spectrum of compound **6** supported the C-8α/C-12α ether link and thus a β- orientated OH-12.

The structure of compound **6** was therefore, determined to be as 7α,14α,12β-trihydroxy-8,12α-epoxy-13,17-pemarene-16,17-olide and was named as bannaringaolide (**110**).

Compound **6** represents a novel diterpene skeleton with a seven membered ring-C, a rare feature in tricyclic diterpenes. Some examples of previously known compounds with seven membered ring C include methyl verticoate (Harrison et al., 1988), strobic acid (Zinkel et al., 1973), hispanonic and hispaninic acids (Rodriguez et al., 1979).

Table 6. NMR (C_5D_5N) data of bannaringaolide A (**110**)

C/H No.	^{1}H-NMR[a] δ_H (mult., J = Hz)	^{13}C-NMR[b] δ_C (mult.)*	COSY	HMBC	NOESY/ NOE
1a	1.05 (dt, 11.2, 2.9)	40.0 (t)	H-1b, H-2a	C-20	-
1b	1.67 (bd, 12.4)		H-1a, H-2a	C-3	-
2a	1.34 (m)	18.8(t)	H-2b, H-3a.H-ia	C-1, C-3, C-4, C-10	-
2b	1.52 (m)		H-1a, H-1b, H-2b, H-3b	C-18, C-19, C-3, C-4	
3a	1.30 (m)	42.0 (t)	H-3b, H-2a	C-18, C-19	-
3b	1.40 (m)		H-3a, H-2a		
4	-	33.8 (s)	-	-	-
5	1.99 (bs)	47.0 (d)	H-6a, H-6b	C-3, C-6, C-10	H_3-18
6a	1.80 (dd, 12.2, 3.0)	26.8 (t)	H-5, H-6b, H-7a	C-8	
6b	2.08 (m)	-	H-5, H-6b, H-7a		
7a	4.60 (bs)	69.7 (d)	H-6a, H-6b, H-7b		H-6a, H-14a
7b	6.40, (OH, d, 3.65)	-	H-7a		-
8	-	79.9 (s)	-	-	-
9	2.40 (bd, 13.0)	46.1 (s)	H-11a, H-11b		H_{-5}, H-7, H-14
10	-	38.0 (s)	-	-	
11a	2.30 (m)	34.7 (t)	H-11b, H-9	C12	
11b	2.67 (m)		H-11a, H-9	C-8, C-12	
12	4.80 (OH, s)	105.0 (s)	-	-	
13	-	128.0 (s)	-	-	-
14a	5.94 (d, 5.4)	72.7 (d,)	H-14b	C-13, C-17,	H-7a, H-9
14b	8.70 (OH, 5.8)		H-14a	-	-
15a	3.22 (d, 8.7)	24.6 (t)	H-15b	C-13, C-16, C-17	-
15b	3.56 (d, 8.6)		H-15a	C-13, C-17	-
16	-	172.4 (s)	-	-	
17	-	162.8 (s)	-	-	
18	0.87 (s)	33.5 (q)	-	C-3	H-5
19	0.76 (s)	22.0 (q)	-	C-3	H_3-20
20	1.14 (s)	15.8 (q)	-	C-5, C-9	H_3-19, H-1b

[a] Data recorded at 400 MHz.
[b] Data recorded at 125 MHz.
*Multiplicity determined by DEPT and HMQC spectra.

Table 6a. NMR ($CDCl_3$-CD_3OD) data of bannaringaolide A (**110**)

C/H No.	^{1}H-NMR[a] δ_H (mult., J = Hz)	^{13}C-NMR[b] δ_C (mult.)*	NOESY/NOE
1a	0.75 (dt, 2.9,11.2)	39.3 (t)	-
1b	1.47 (bd, 12.4)		-
2a	1.20 (m)	18.3(t)	-
2b	1.50 (m)		
3a	1.22 (m)	41.7 ((t)	-
3b	1.28 (m)		
4	-	33.0 (s)	-
5	1.30 (bs)	46.6 (d)	H_3-18
6a	1.57 (dd, 3.0, 12.2)	25.3 (t)	
6b	1.68 (m)	-	
7	3.73 (bs)	68.5 (d)	H-14a
8	-	79.1 (s)	-
9	1.48 (bd, 13.0)	45.3 (s)	H_3-5, H-7, H-14
10	-	37.2 (s)	
11a	1.58 (t, m)	32.3 (t)	
11b	2.01 (m)		
12		104.1 (s)	
13	-	127.3 (s)	-
14	4.67 (d, 5.4)	71.8 (d)	H-7a, H-9
15a	2.60 (d, 8.7)	22.5 (s)	-
15b	2.45 (d, 8.6)		-
16	-	173.2 (s)	
17	-	162.3 (s)	
18	0.62 (s)	33.0 (q)	H-5
19	0.68 (s)	21.9 (q)	H_3-20
20	0.72 (s)	15.8 (q)	H_3-19, H-1b

[a] Data recorded at 400 MHz.
[b] Data recorded at 125 MHz.
*Multiplicity determined by DEPT and HMQC spectra.

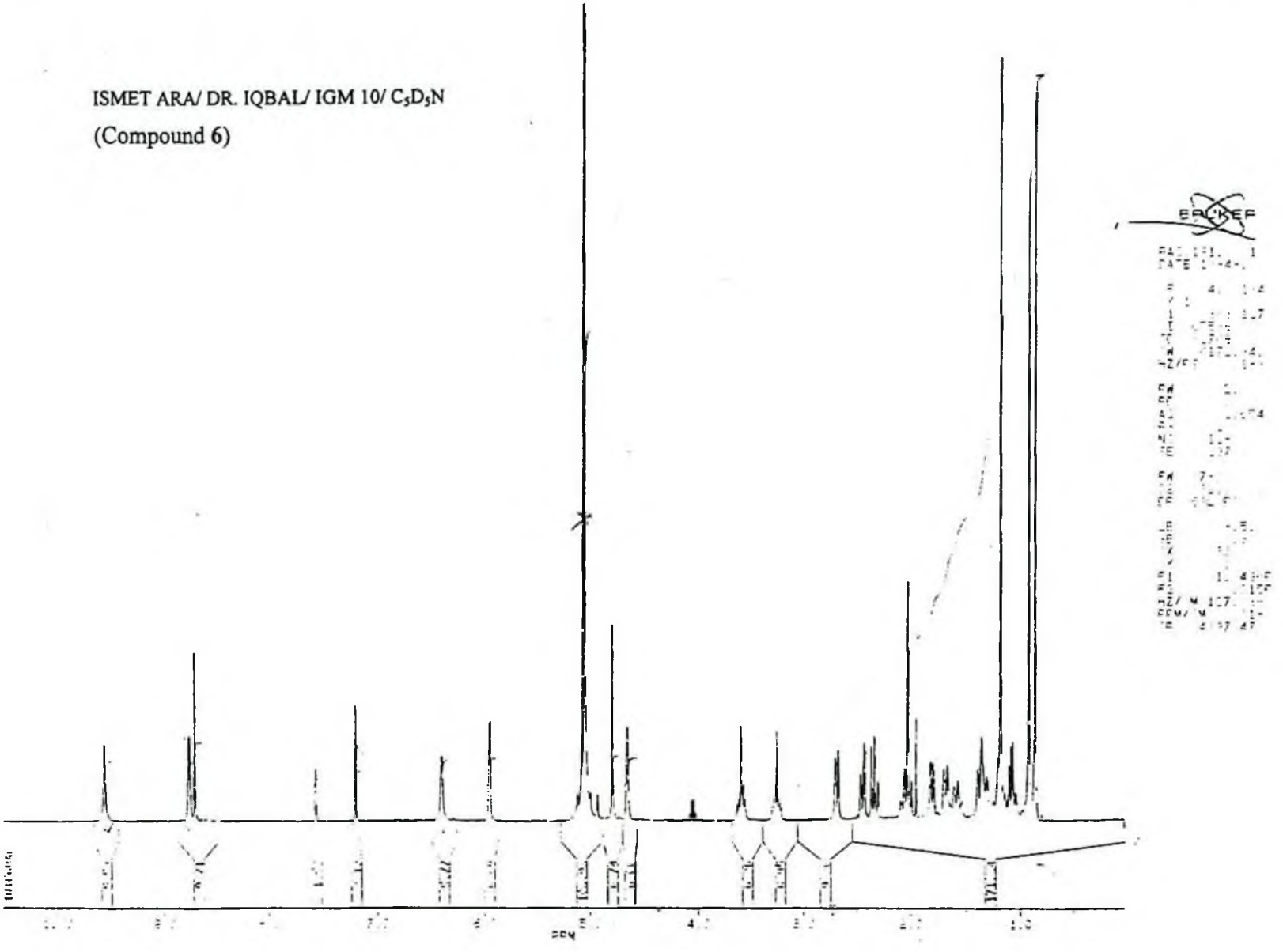


Fig. 27. ^{1}H-NMR Spectrum (C_5D_5N, 500 MHz) of Bannaringaolide A (**110**)

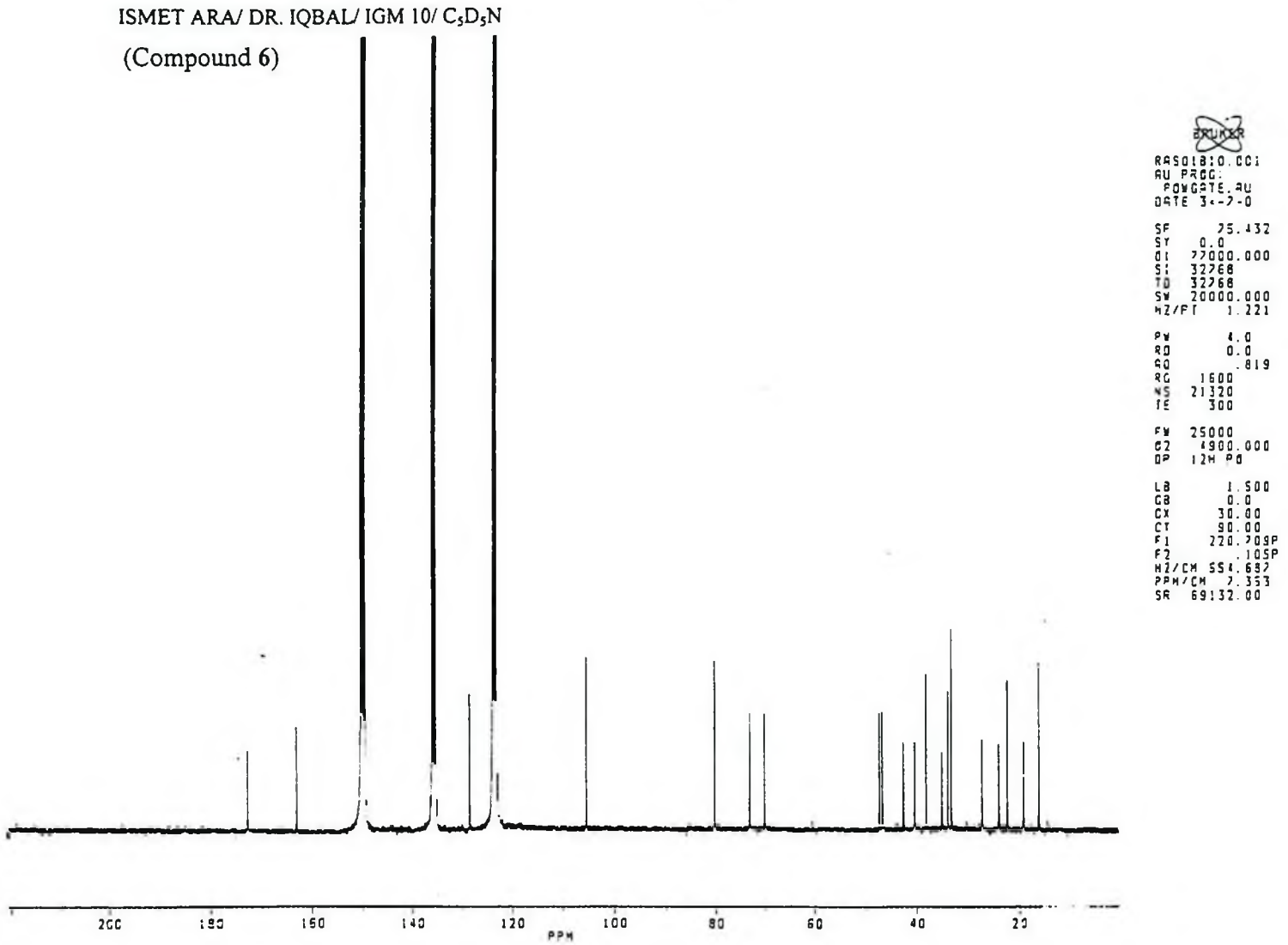
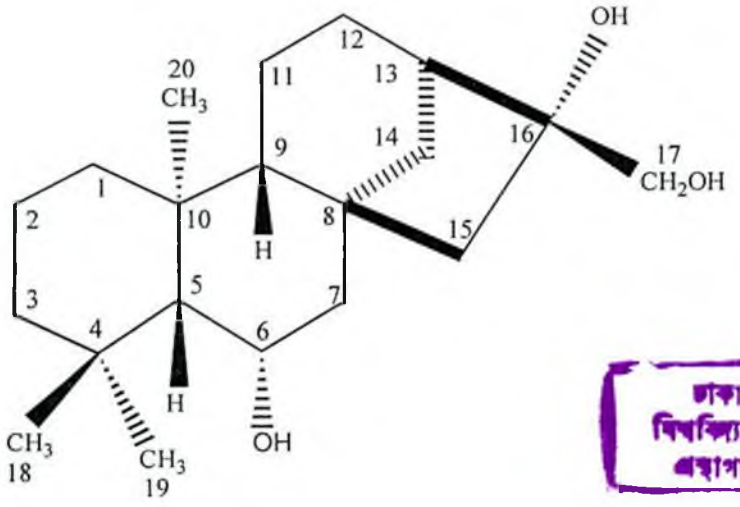


Fig. 28 ^{13}C- NMR Spectrum (C_5D_5N, 125 MHz) of Bannaringaolide A (**110**)

2.5.1.7 Suremulol A (111)- A New E*nt*-kaurane Triol

Compound **7** was isolated as an amorphous solid (Scheme-4, Page-44). The high resolution EIMS (*m/z* = 322.2090) of compound **7** together with the ^{13}C NMR and DEPT data supported the molecular formula as $C_{20}H_{34}O_3$. The IR spectrum of the compound showed the presence of hydroxyl group (3374 cm^{-1}). The presence of three methyl groups resonating at δ_H 1.14/δ_C 36.5, δ_H 0.98/δ_C 22.1, δ_H 1.04/δ_C 19.0, one primary hydroxyl-bearing carbon resonating at δ_C 65.9 and δ_H 3.56 (d, J = 11.25 Hz), and δ_H 3.59 (d, J = 11.25 Hz) and one secondary hydroxyl- bearing carbon at δ_C 69.9/δ_H 3.94 (m, $W_{1/2}$ = 6.15Hz) were inferred from the ^{1}H- and ^{13}C-NMR spectra (Fig. 31, Page-97; and Fig. 32, Page-98) of compound **7**.

The ^{13}C NMR spectrum of compound **7** (Table-7; Fig. 32, Page98) exhibited the presence of twenty skeletal carbons (three methyls, nine methylenes, four methine and four quaternary carbons). The preliminary observation suggested that the compound **7** might have an *ent*-kaurane diterpene skeleton. The structure of compound **7** was then determined by analyzing its IR, UV, ^{1}H- and ^{13}C NMR, ^{1}H-^{1}H COSY- 45°, HMQC, and HMBC spectra.



Suremulol A (**111**)

In COSY-45° spectrum of compound **7** it was observed that H-6 at δ_H 3.94 (m, $W_{1/2}$ = 6.15 Hz) was coupled with H-5 (δ_H 0.88, $W_{1/2}$ = 3.4 Hz,) and H_2-7 (δ_H 1.65, m; 1.86, m). H-9 (δ_H 0.75, dt, $J_{9,11a}$ = 12.5 Hz and $J_{9,14a}$ 3.7 Hz) showed interactions with H-11b (δ_H 1.35, m), H-14a (δ_H 1.64, m) and H-12b (δ_H 1.72, m).

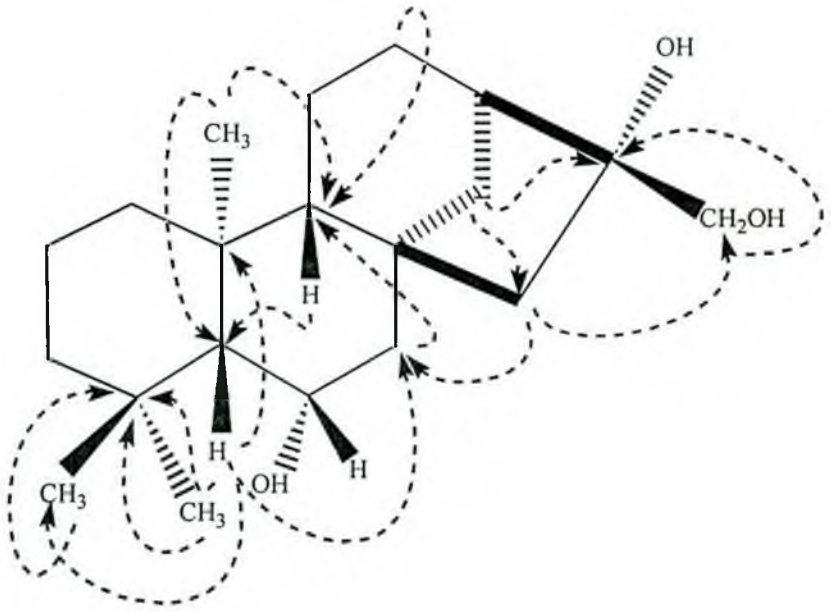


Fig. **33**. Important HMBC interactions in suremulol A (**111**).

In HMBC spectrum (Fig. 33) C-18 methyl protons (δ_H 1.14) and C-19 methyl protons (δ_H 1.04) were found to be correlated with C-4 (δ_C 33.5). H-5 (δ_H 0.88) was found to show interactions with C-18 (δ_C 36.5), C-4 (δ_C 33.5), C-6 (δ_C 69.9), C-7 (δ_C 52.1), C-10 (δ_C 40.9) and C-20 (δ_C 19.0). On the other hand, C-20 methyl protons (δ_H 1.04) were correlated with C-1 (δ_C 43.6), C-5 (δ_C 60.6), C-10 (δ_C 40.9) and C-9 (δ_C 56.1). H-7b methylene proton (δ_H 1.86) exhibited interactions with C-6 (δ_C 69.9), C-5 (δ_C 60.6 and C-9 (δ_C 56.1). H-12b (δ_H 1.72) methylene proton also showed interactions with C-9 (δ_C 56.1), and H-15a (δ_H 1.45) was found to be correlated with C-7 (δ_C 52.1), C-8 (δ_C 44.6 and C-16 (δ_C 81.0. On the other hand H-15b (δ_H 1.55) showed correlations with C-14 (δ_C 37.7) and C-17 (δ_C 65.9. H-17b (δ_H 3.59) showed interactions with C-16 (δ_C 81.0) while, H-17a (δ_H 3.56) was found to be correlated with C-15 (δ_C 53.0).

The above interactions and the observed spectral data of compound **7** (Fig. 33; Table-7, Page96) suggested the structure of the compound as *ent*-kaurane-6,16,17 triol, which is similar to the reported compound corymbol (*ent*-kaurane-6α,17β,16, triol), isolated from *Turbina corymbosa* (Perezamador et al., 1965) and *Calibrachoa Parviflora* (Elliger et al., 1992). But the optical rotation $[\alpha]_D$ –9° (c, 0.028, pyridine) of compound **7** was found to be substantially different from the reported value of corymbol [α]D – 38° (pyridine) (Elliger et al., 1992).

Compound **7** was found to be a member of *ent*-series where C-20 methyl was predicted to be α- oriented on the biogenetic grounds (Das et al., 1994). The relative stereochemistry of the important ciral centers of compound **7** was then deduced from its NOESY spectrum as depicted in Fig. 34. The cross peaks observed between C-20 (δ_H 1.04) and C- 19 (δ_H 0.98) methyls suggested the α- orientation of C-19 methyl and thus the β-orientation of C-18 methyl. The cross peaks between H-5 (δ_H 0.88) /C-18-methyl (δ_H 1.14) and H-5/H-6 (δ_H 3.94) in NOESY spectrum indicated the β- orientation of H-5, H-6 and thus the α orientation of OH-6.

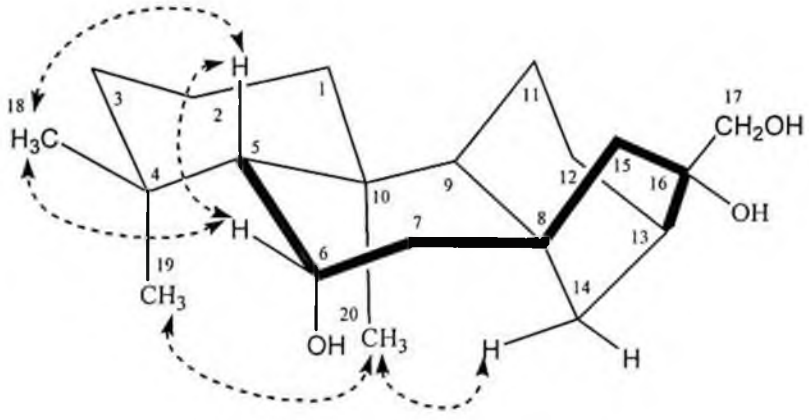


Fig. **34**. Important NOESY interactions in suremulol A (**111**).

The structure of compound **7** was therefore, elucidated to be as *ent*-kaurane-6β,17β,16, triol and was named as suremulol A (**111**).

Table 7. NMR ($CDCl_3$ + CD_3OD) data of suremulol A (**111**)

C/H No.	^{1}H-NMR[a] δ_H (mult., J = Hz)	^{13}C-NMR[b] δ_C (mult.)*	COSY	HMBC	NOESY
1a	1.24(m)	43.6 (t)	H-1b, H-2a, H-2b	-	
1b	1.35 (m)		H-1a, H-2a	-	
2a	1.45 (m)	18.2 (t)	H-1a, H- 2b, H-3b, H-1b	C-1	
2b	1.57 (m)	-	H-1b, H-2a, H-3a	C-1	
3a	1.58 (m)	40.3 (t)	H-2b, H-3b	C-1, C-5	
3b	1.70 (m)		H-2a, H-3a	C-1, C-18	
4	-	33.5 (s)	-	-	
5	0.88 (m, $W_{1/2}$ = 3.4)	60.6 (d)	H-6	C-4, C-6, C-10, C-7 C-20	H-9, H_3-18
6	3.94 (m, $W_{1/2}$ = 6.15	69.9 (t)	H-7a, H-5, H-7b	-	
	-	-	-	-	
7a	1.65 (m)	52.1 (t)	H-6, H-7b	C-5, C-6, C-8, C-14	
7b	1.86 (m)	-	H-6, H-7a	C-8	
8	-	44.6 (s)	-	-	
9	0.75 (dt, 12.5, 3.7)	56.1 (d)	H-11a, H-14a	-	H-5
10	-	40.9 (s)	-	-	
11a	1.2 5 (m)	18.4 (t)	H-9, H-11b, H-12a	-	
11b	1.35 (m)		H-11a, H-12a, H-12b	-	
12a	1.62 (m)	26.0 (t)	H-11a, H-11b, H-12b	-	
12b	1.72 (m)	-	H-11a, H-12a, H-13	-	
13	1.98 (bs)	45.2 (d)	H-12b, H-14a	C-15, C-16	
14a	1.64 (m)	37.7 (t)	H-12b, H-14b, H-15a	-	
14b	1.88 (m)	-	H-14a, H-15b	C-16, C-17	
15a	1.45 (m)	53.0(t)	H-14a, H-15b	C-8	
15b	1.55 (m)	-	H-15a, H-14b	C-14, C-17	
16	-	81.0 (s)	-	-	
17a	3.56 (d, 11.25)	65.9 (t)	H-17b	C-16	
17b	3.59 (d, 11.25)	-	H-17a	C-13, C-15	
18	1.14 (s)	36.5 (q)	-	C-4, C-19	H-5
19	0,98 (s)	22.1 (q)	-	C-4, C 18	H_3-20
20	1.04 (s)	19.0 (q)	-	C-1, C-9, C-10, C-5	H_3–19, C-9, C-7b

[a] Data recorded at 500 MHz.
[b] Data recorded at 125 MHz.
*Multiplicity determined by DEPT and HMQC spectra.

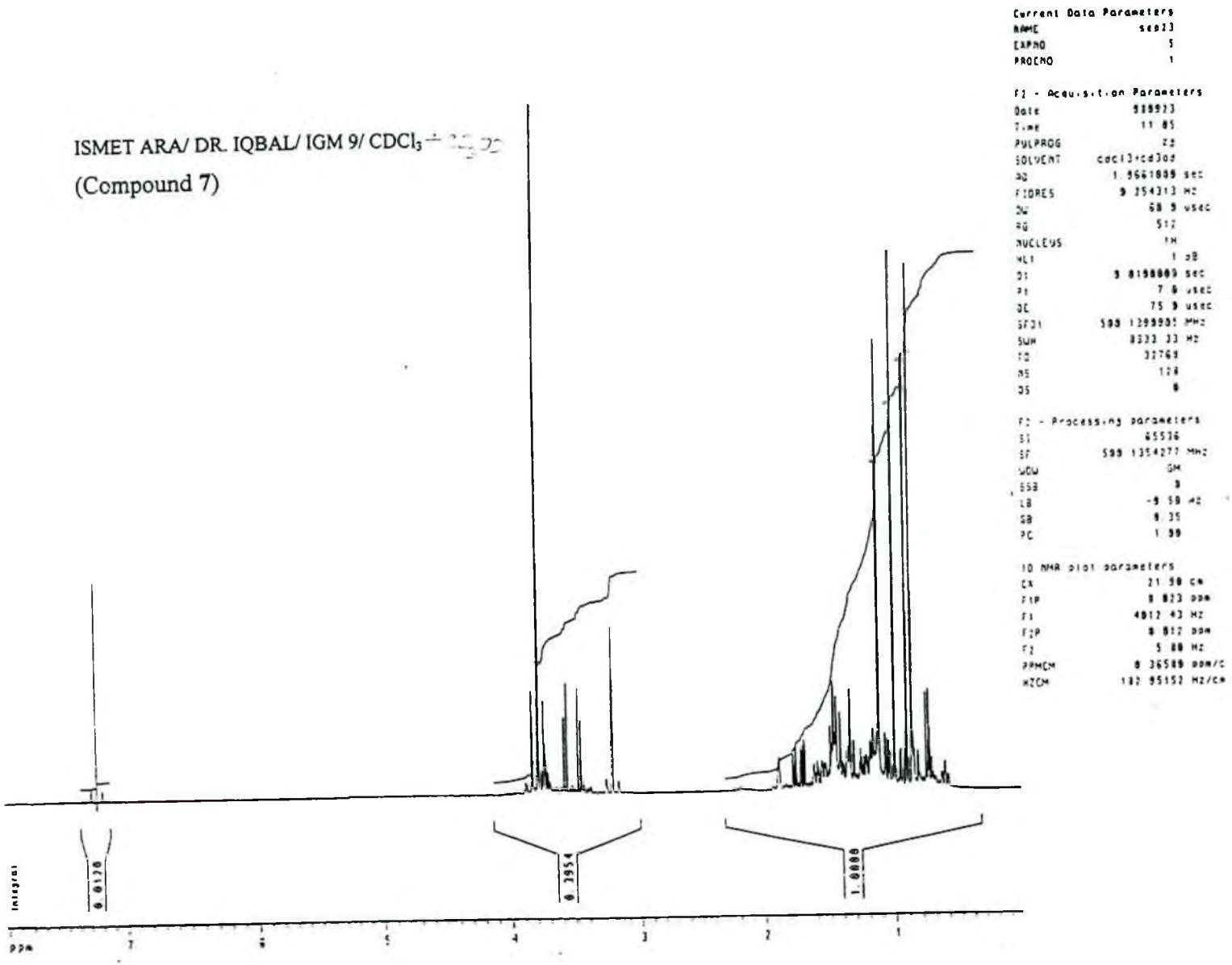


Fig.31 ^{1}H-NMR Spectrum ($CDCl_3$, 500 M Hz) of Suremulol A (**111**)

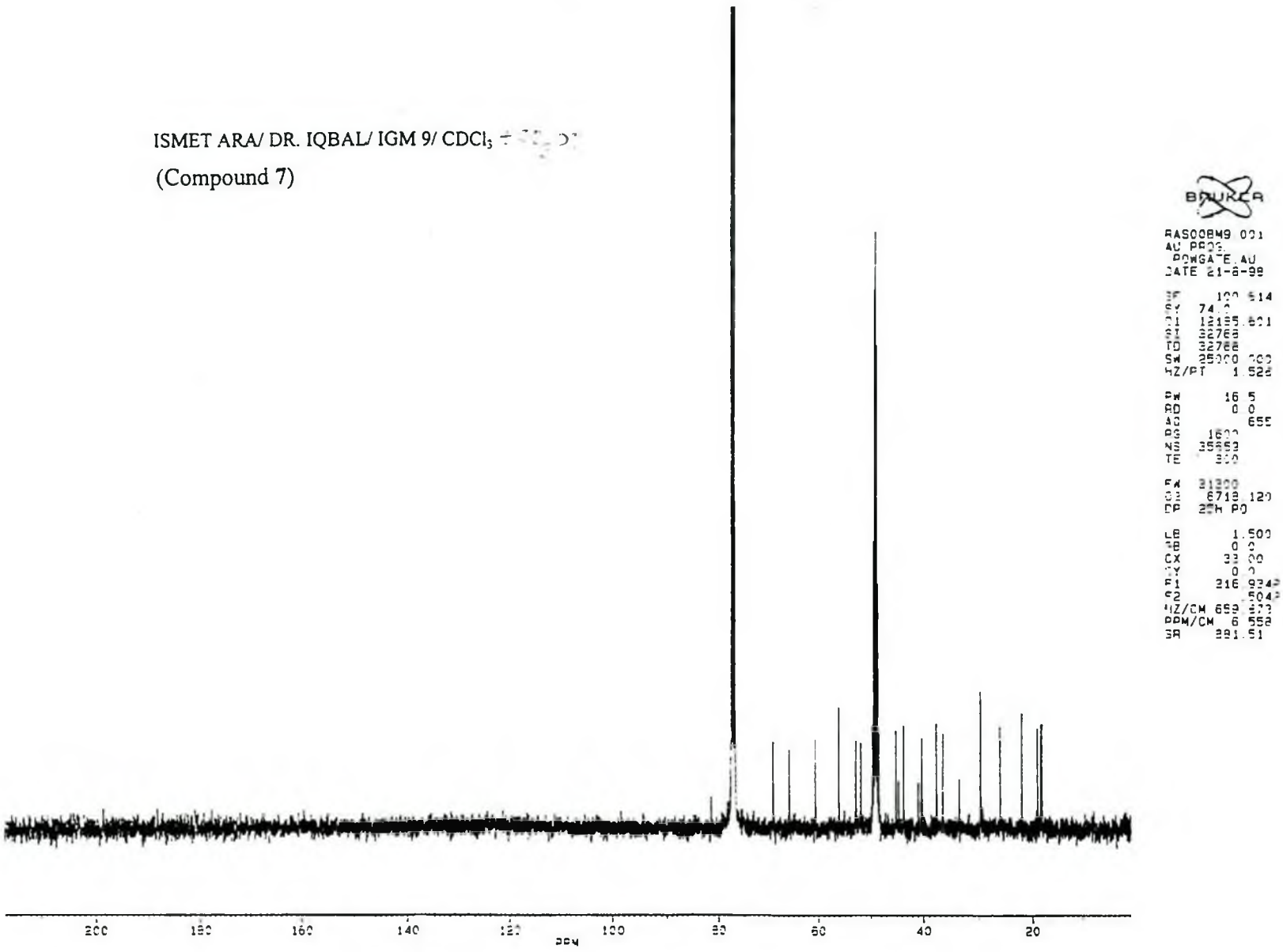
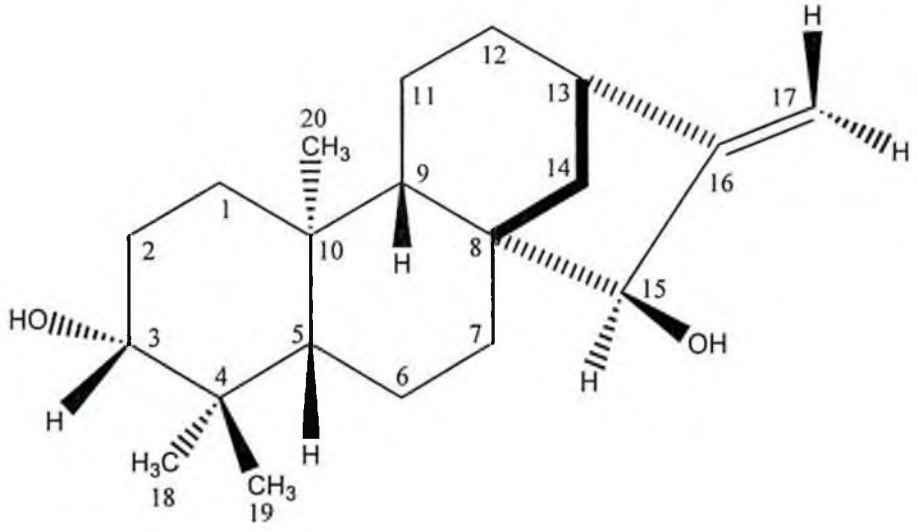


Fig. 32. ^{13}C- NMR Spectrum ($CDCl_3$, 125 MHz) of Suremulol A (**111**)

2.5.1.8 Suremulol B (112)- A New *Ent*-Kaurane Diol

Compound **8** was isolated as an amorphous solid (Scheme-2, Page-38). The molecular formula of the compound was determined as $C_{20}H_{32}O_2$ from the HR EIMS (*m/z* 304.5290) supported by its ^{13}C-NMR and DEPT spectra. The presence of hydroxyl group was inferred from the IR spectrum (3361cm^{-1}).



Suremulol B (**112**)

The ^{1}H and ^{13}C NMR spectra of compound **8** (Table- 8, Fig. 35, Page-104; and fig. 36, Page-105) displayed a double doublet resonating at δ_H 3.20 (dd, $J_{3,2a}$ = 11.5 Hz, $J_{3,2b}$ = 5.1 Hz, δ_C 79.0) and a broad singlet at δ_H 3.79 (δ_C 83.0). From the multiplicity of the signals, it was apparent that one of the hydroxyl groups was equatorial and flanked with two non-protonated carbons. The spectrum also showed the presence of an exocycilc methylene group resonating at δ_H 5.05 (bs) and 5.15 (m) (δ_C 108.4). The presence of three methyls (δ_H 1.01/δ_C 17.6, δ_H 0.97/δ_C 15.5 and δ_H 0.77/δ_C 28.1) were also inferred in the NMR spectra of compound **8**.

The ^{13}C NMR spectrum of compound **8** (Table-8, Page-105) exhibited the presence of 20 skeletal carbons (three were methyls, eight methylenes, five methines and four quaternary carbons). Two downfield signals at δ_C 108.0 and 160.0 represented two carbons of the

exocyclic methylene group and two signals resonating at δ_C 79.0 and 83.0 supported the presence of two hydroxyl-bearing carbons in the molecule.

On the basis of ^1H and ^{13}C NMR spectral evidences, compound **8** was concluded to be a tetracyclic diterpene with an *ent*-kaurane skeleton. The structure of the compound was then deduced with the help of ^1H and ^{13}C NMR, ^1H-^1H COSY- 45°, HMQC, HMBC and NOESY spectra.

The ^1H-^1H COSY and HMQC spectra (Table-8, Page-103) of compound **8** revealed that H-5 at δ_H 0.76 (dd, $J_{5,6a}$ = 12.6, $J_{5,6b}$ 4.5 Hz) was coupled with H_2-6 (δ_H 1.30, m and 1.40, m). H-9 at δ_H 0.85 (bd, $J_{9,11ab}$ = 12.5 Hz) was coupled with H_2-11 (δ_H 1.40, m and 1.55, m). H-15 (δ_H 3.79, bs) was coupled with the C-17 exocyclic methylene protons at δ_H 5.05 (bs) and 5.15 (m).

The HMBC spectrum (Fig. 37) of compound **8** showed the correlation of H-3 (δ_H 3.20) with C-2 (δ_C 27.7) and C-19 (δ_C 15.5). H-5 (δ_H 0.76) exhibited correlations with C-4 (δ_C 39.2), C-6 (δ_C 18.1), C-7 (δ_C 35.2), C-10 (δ_C 38.8), C-19 (δ_C 15.5) and C-20 (δ_C 19.4). C-20 methyl protons (δ_H 1.01) showed interactions with C-5 (δ_C 54.1) and C-9 (δ_C 55.0). On the other hand H-9 (δ_H 0.85) was found to be correlated with C-11 (δ_C 19.1) and C-10 (δ_C 38.8). H-14a (δ_H 1.30) showed interactions with C-15 (δ_C 83.0) and C-16 (δ_C 160.0). The downfield proton of exocyclic double bond H-17a, resonating at δ_H 5.05 was found to exhibit correlations with C-16 δ_C 16.0), C-15 (δ_C 83.0). While H-17b (δ_H 5.15) and H-12 a (δ_H 1.55) exhibited correlations with C-13 (δ_C 42.3) in the HMBC spectra of compound **8**.

The interactions observed in COSY (45°) and HMBC spectra of compound **8** (Table-8, Page-103) and a comparative study of NMR data with the reported value (Grande et al., 1991; Das et al., 1994) clearly demonstrated the structure of the compound as *ent*-kaurane 3,15 diol structurally related to *ent*-kaurane 3β, 15β-diol (Das et al., 1994), isolated from *Gelonium multiflorum* and its enantiomer (Grande et al., 1991) isolated from *Elaeoselinum tenuifolium*. The observed $[\alpha]_D^{25}$ -14° (c, 0.0425, $CHCl_3$) for compound **8** was substantially different from the reported values $[\alpha]_D^{25}$ -58.6° (*c*, 0.4, $CHCl_3$) (Das et al.,

1998) and $[\alpha]_D^{25}$ -61° (*c*,1.4,$CHCl_3$) (Grande et al., 1991). This suggested that compound **8** might be a stereoisomer of *ent*-kaurane 3β, 15β-diol or its enantiomer.

The C-20-methyl in structure of compound **8** was deduced to be α- oriented on biogenetic grounds (Das et al., 1994). The relative stereochemistry of compound **8** of the important chiral centers was then deduced from its NOESY spectrum as shown in Fig. 38.

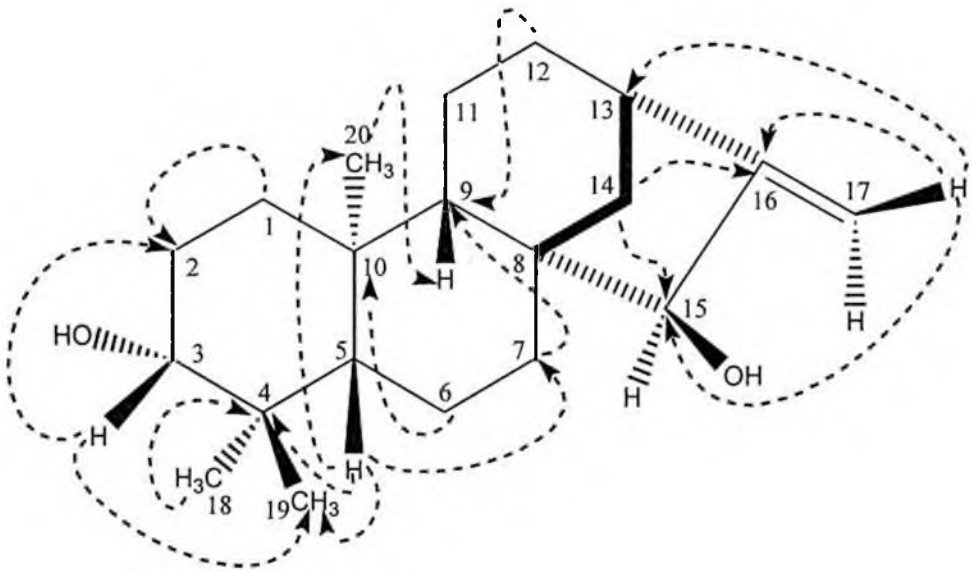


Fig. **37**. Important HMBC interactions in suremulol B (**112**).

The cross peaks observed between C-20 methyl (δ_H 1.o1)/ C-19 methyl (δ_H 0.97) in NOESY spectrum indicated the α orientation of C-19 methyl and thus the β orientation of C-18 methyl (δ_H 0.74). Interactions between C-18 methyl/H-3 (δ_H 3.20), H-3/ H-5 (δ_H 0.76) indicated the β orientation of H-3 and H-5. On the other hand cross peak between C-20 methyl and H-15 (δ_H 3.79) indicated the α- orientation of H-15 and thus the β-orientation of 15-OH and H-1b. The Driedling Model study and NOESY intractions found (Fig. 38) between H-15 and C-20 methyl agreed well with α-orientation of C-8/C-13 in ring D. In the reported structure (Grande et al., 1991) this fragment was found β-oriented.

The structure of compound **8** was elucidated as a stereoisomer of (*ent*-kaurane-3β,15α-diol) and was trivially named as suremulol B (**112**). Suremulols A and **8** were identified as *ent*-kaurane diterpenoids derived from two C-13 epimaric *ent*-pimarane compounds as shown in Scheme 10. Co-occurance of diterpenoids of C-13 epimar from the same plant has also been reported (Popa et al., 1963; Kimland et al., 1967).

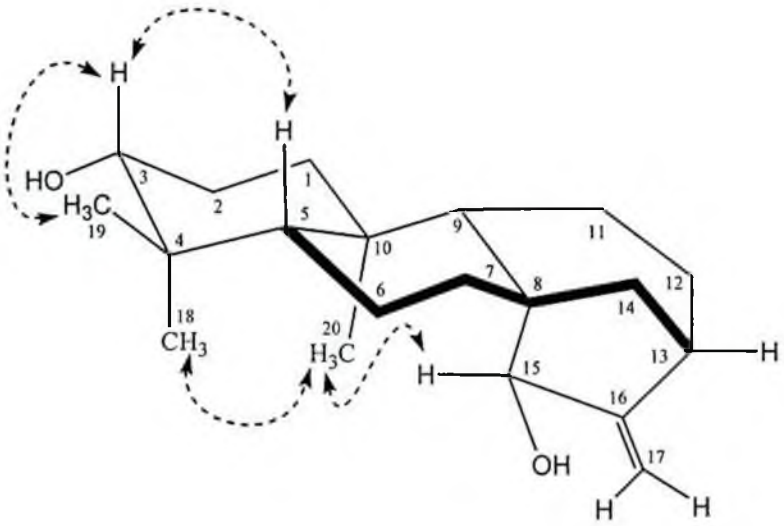


Fig. **38**. Important HMBC interactions in suremulol B (**112**).

Table 8. NMR ($CDCl_3$) data of suremulol B (**112**)

C/H No.	^{1}H-NMR[a] δ_H (mult., J = Hz)	^{13}C-NMR[b] δ_C (mult.)*	COSY	HMBC	NOE/ NSOESY
1a	0.85(m)	38.8 (t)	H-1b, H-2a, H-2b	C-2	, H_3-20
1b	1.85 (m)		H-1a, H-2a, H-2b		H-9
2a	1.62 (m)	27.7 (t)	H-1a, H-1b, H- 2b,		
2b	1.64 (m)		H-1a, H-1b, H-2a, H-3	C-18, C-19	
3	3.20 (dd, 11.5, 5.1)	79.0 (d)	H-2b, H-2a	C-2, C-19	H_3-19, H-5
4	-	39.2 (s)	-		
5	0.76 (dd, 12.6, 4.5)	54.1 (d)	H-6a, H-6b	C-4, C-6, C-7, C-9, C-10, C-19	
6a	1.30 (m)	18.1 (t)	H-5, H-6b, H-7a, H-7b		
6b	1.40 (m)	-	H-5, H-6a, H-7a, H-7b		
7a	1.45 (m)	35,2 (t)	H-6a, H-6b, H-7b	C-8	
7b	1.60 (m)	-	H-6a, H-6b, H-7a		
8	-	47.5 (s)	-	-	
9	0.85 (bd, 12.5)	55.0 (s)	H-11b, H-11a	C-10, C-11	H-1b
10		38.8 (s)			
11a	1.40 (m)	19.1 (t)	H-9, H-11b, H-12a		
11b	1.55 (m)		H-9, H-11a, H-12a, H-12b		
12a	1,55 (m)	32.7 (t)	H-11a, H-11b, H-12b		
12b	1.65 (m)		H-11a, H-11b, H-12a, H-13		
13	2.72 (bs)	42.3 (d)	H-12b, H-14a	-	
14a	1.30 (m)	36.2 (t)	H-13, H-14b	C-15, C-16	
14b	1.87 (m)		H-14a, H-15		
15	3.79 (bs)	83.0 (d)	H-14b, H-17	-	H_3-18, H_3-20, H-17b
16		160.0 (s)			
17a	5.05 (bs)	10 8.0 (t)	H-17a, H-17b, H-15	C-1 3, C-15, C-16	
17b	5.15 (m)		H-17a, H-17b, H-15		
18	0.74 (s)	28.1 (q)			H-1a, H-15, H_3-20
19	0.97 (s)	15.5 (q)			H-3, H-15
20	1.01 (s)	19.4 (q)		C-5, C-9	H_3-18, H-15

[a] Data recorded at 500 MHz.
[b] Data recorded at 125 MHz.
*Multiplicity determined by DEPT and HMQC spectra.

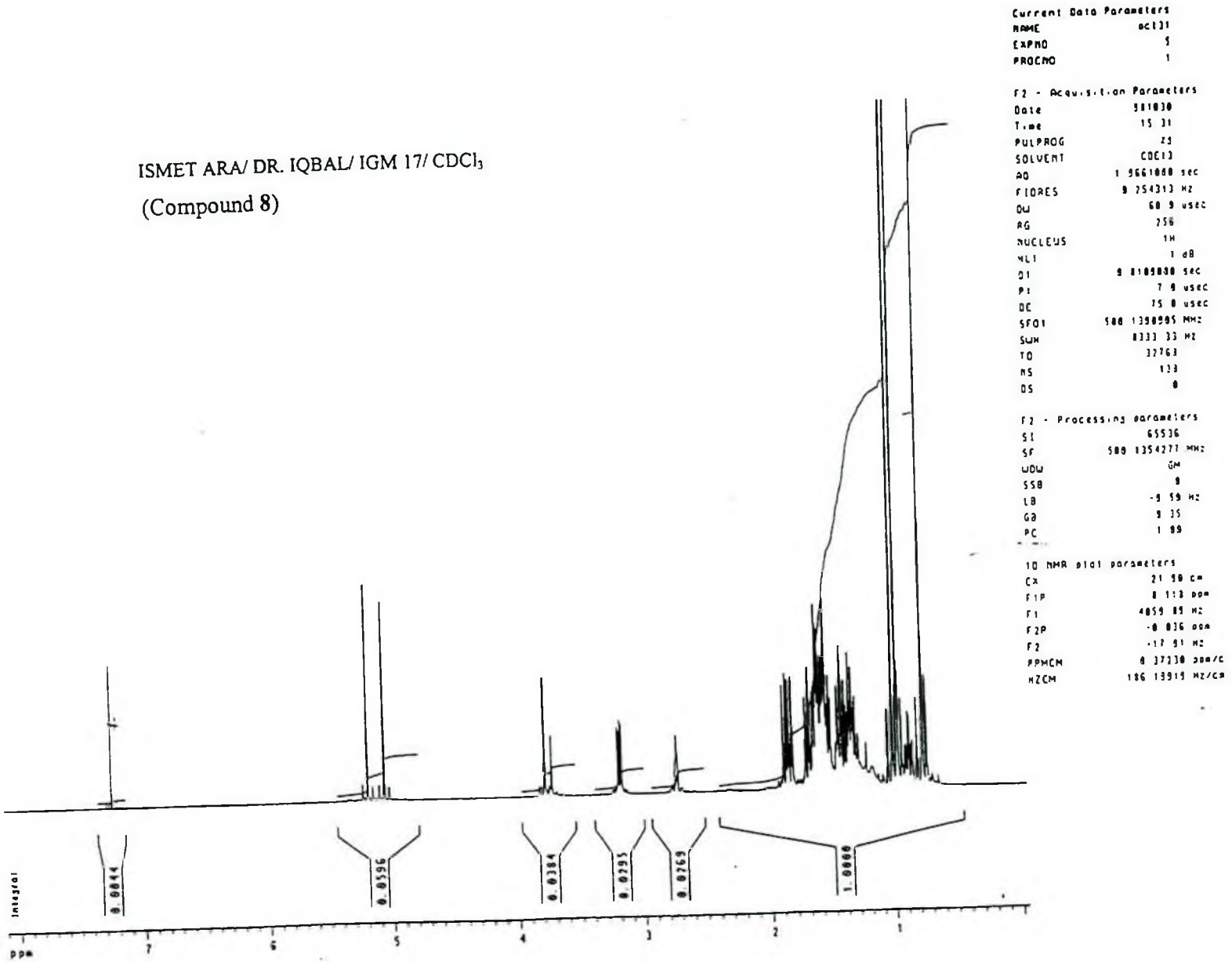


Fig. 35. ^{1}H-NMR Spectrum ($CDCl_3$, 500 M Hz) of Suremulol B (**112**)

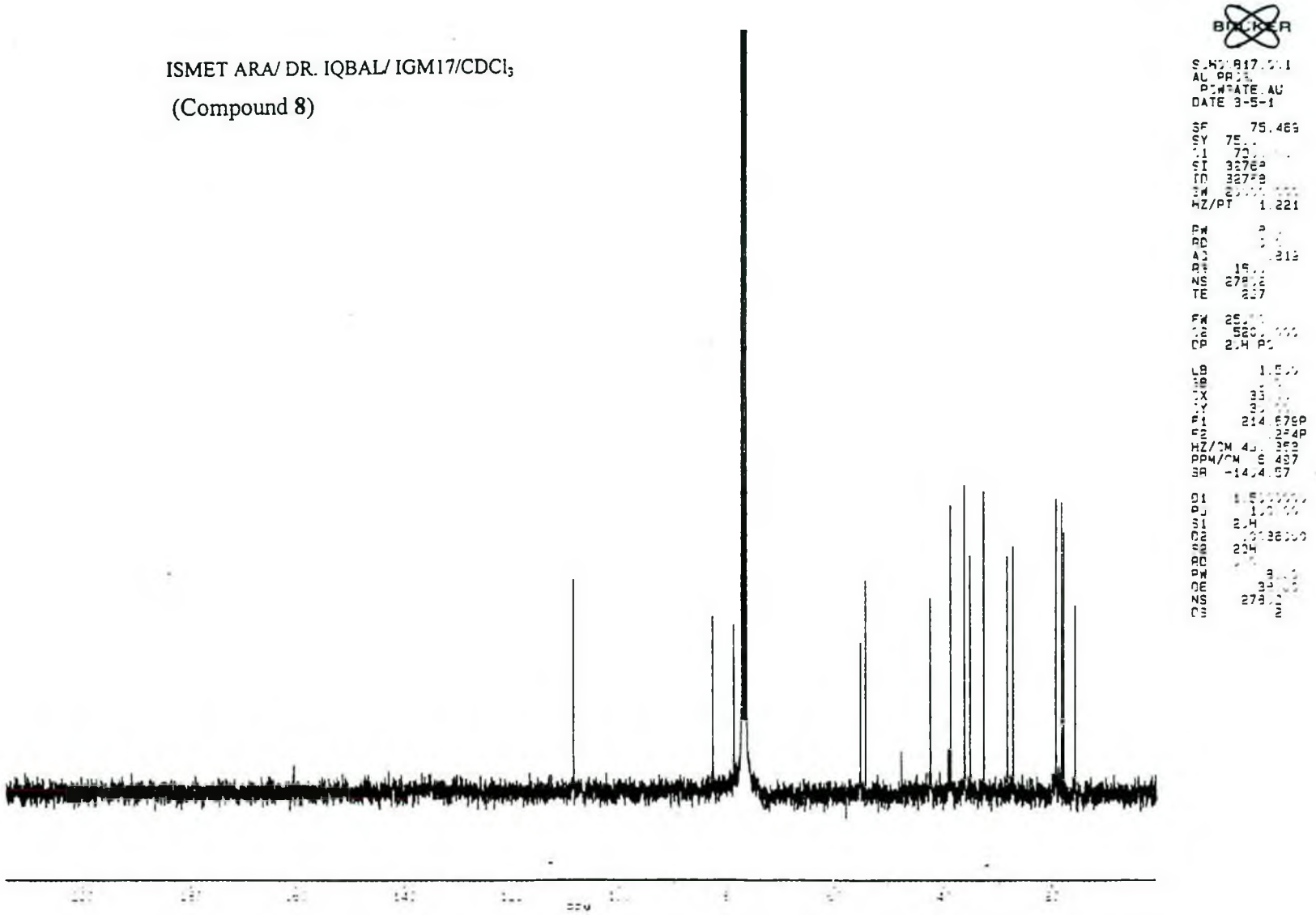
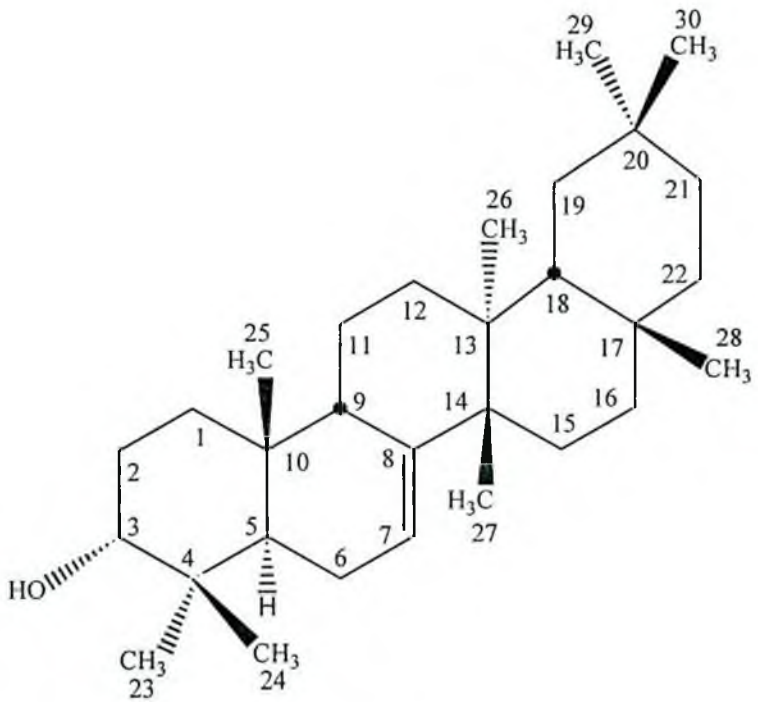


Fig.36. ^{13}C-NMR Spectrum ($CDCl_3$, 75 M Hz) of Suremulol B (**112**)

2.5.2 Triterpenoids: Two more compounds (**9** and **10**) were characterized as known triterpenes named as Multiflorenol and Burenol

2.5.2.1 Multiflorenol (44)- A Known Triterpene

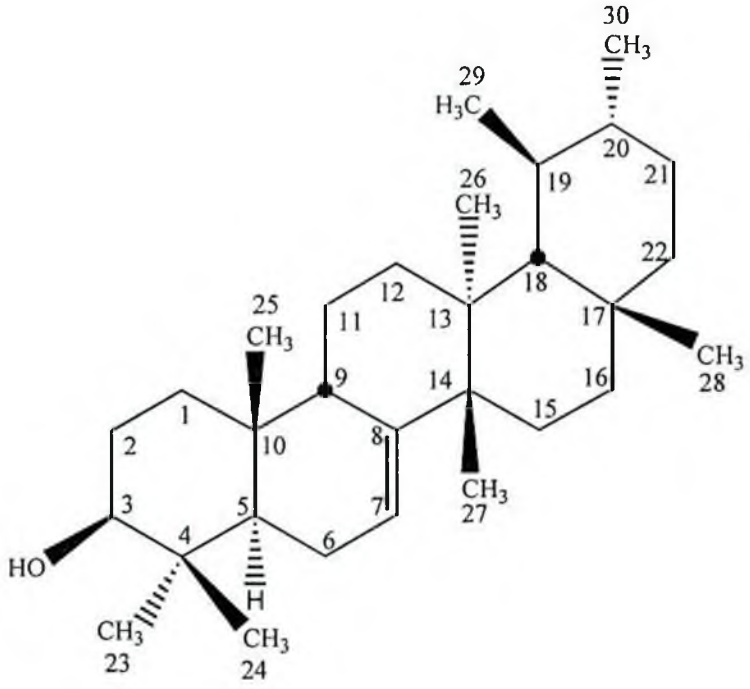
Compound **9** was isolated as a white amorphous solid (40 mg) from the CH_2Cl_2 soluble part of the parent extract of the same plant (Scheme-5, Page-49). The molecular formula of **9** was determined from the EIMS (*m/z* = 426). A comparative study of IR, UV and ^1H NMR data (Experimental Section) of compound **9** with the literature value of reported compound the structure of the compound was assigned as a known compound, 7-multiflorene 3β-ol (multiflorenol) (**44**) reported earlier from the same species (Sengupta et al., 1963).



Multiflorenol (**44**)

2.5.2.2 Baurenol (45)- A Known Triterpene

Compound **10** was isolated as a white amorphous solid (25 mg) from the same extract (Scheme-5, Page-49). The molecular formula of the compound was determined from its EIMS (*m/z* = 426) spectrum. A comparative study of IR, UV and ^{1}H NMR data (experimental section) of compound **10** with the reported compound confirmed the structure of compound as a known compound 7-baurene-3β-ol (baurenol, **45**), which was also isolated from the same plant (Sengupta et al., 1963).



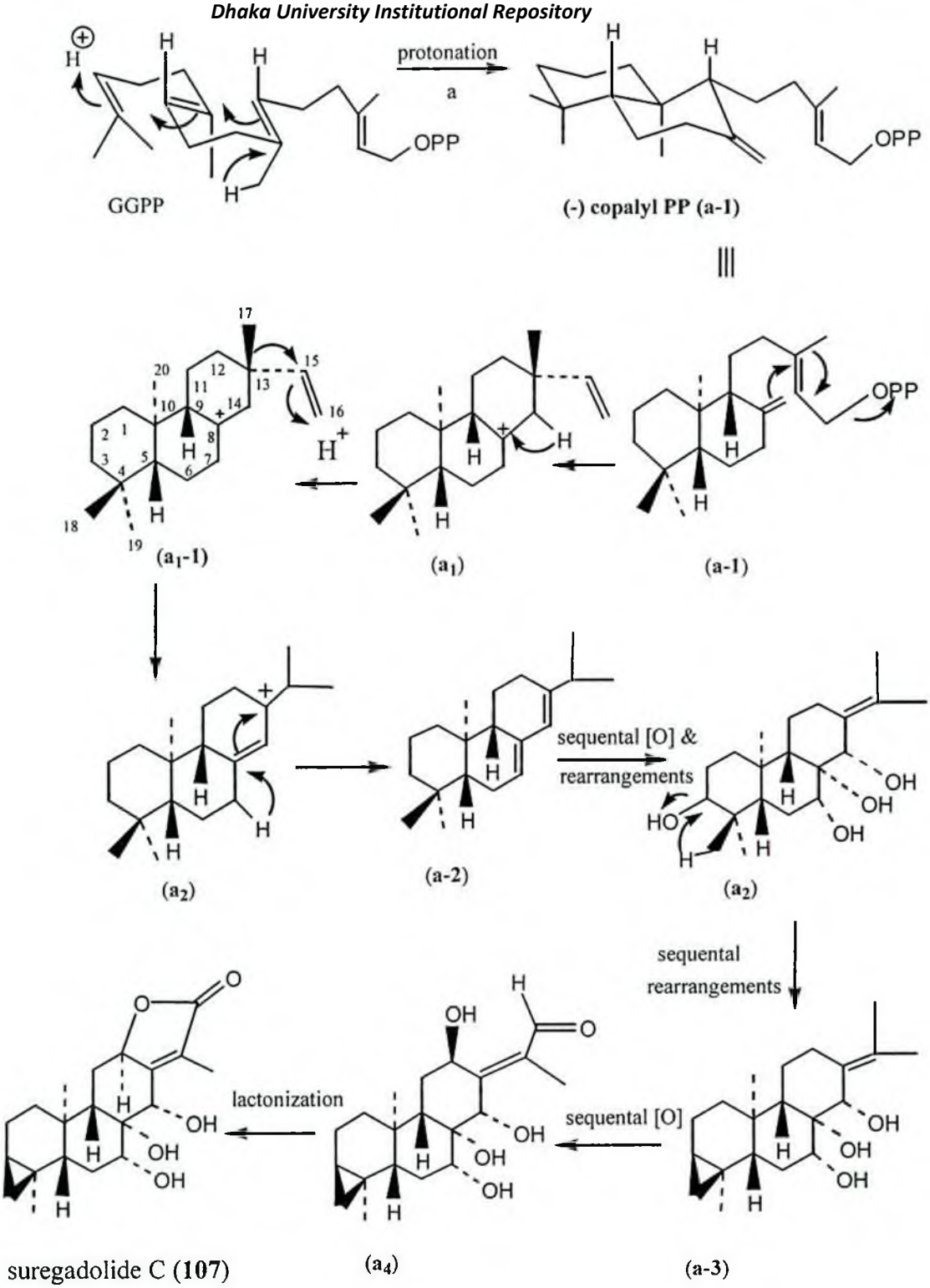
Baurenol (**45**)

2.5.3 Biogenesis of Compounds 1-8

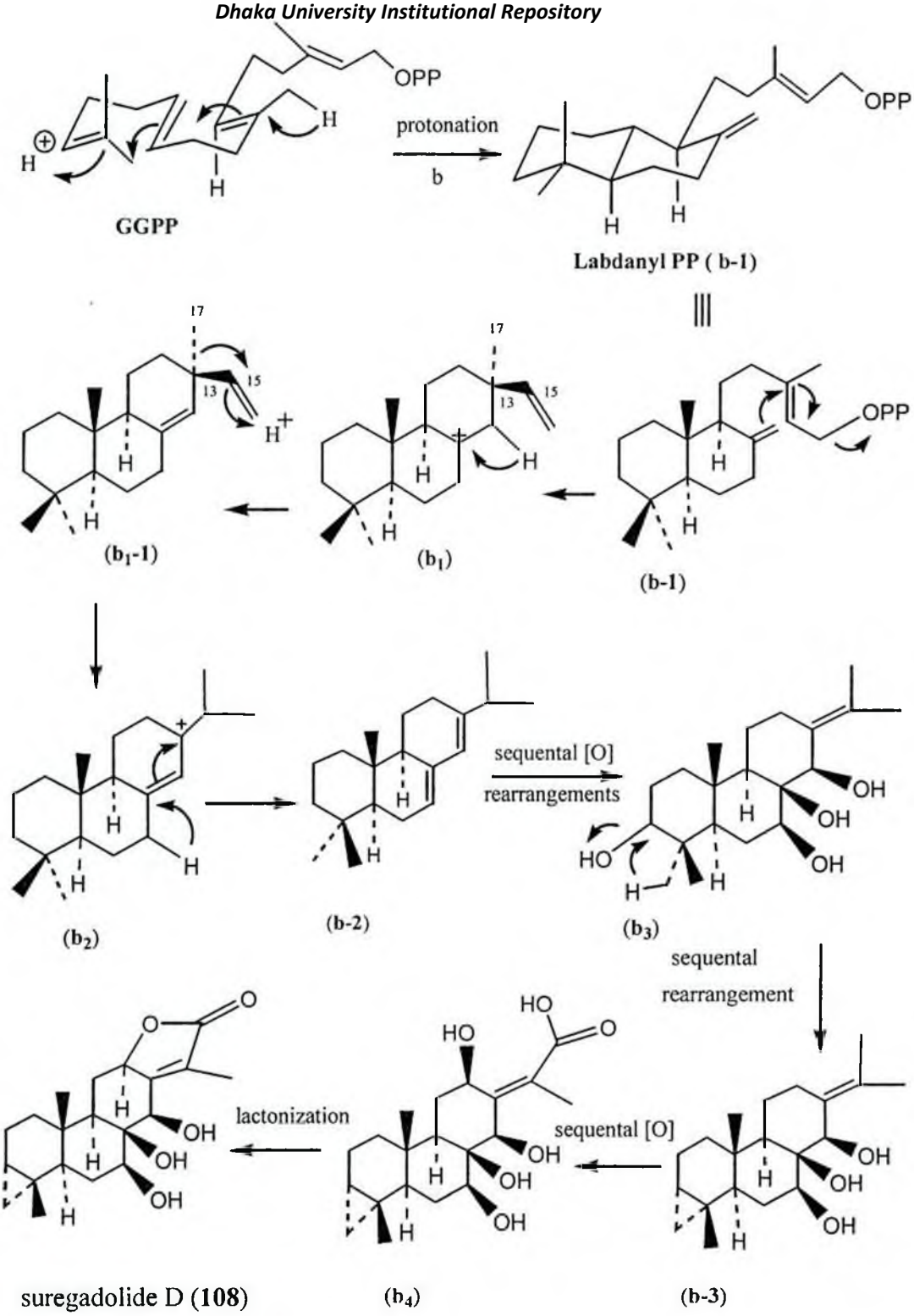
The proposed biogenesis of compounds **1-8** (Structure **105-112**) isolated from the bark extract of *Suregada multiflora* is presented in Schemes 6-10.

Schemes-6 and -7 proposes the biogenesis of antipodal suregadolide C (**107,** compound **3**,) and suregadolide D (**108,** compound **4**,) respectively starting from the protonation. GGPP, which trigger the cyclization sequence, terminated by a loss of H^+ from a methyl leading to all *trans* cyclization to form an *ent* AB system, which give rise to the product (-) copalyl PP (**a-1**). The stereochemistry in (**a-1**) is presumably controlled by the folding of the substrate on the enzyme (Scheme-6). On the other hand, an alternative folding can lead to an enantiomeric product labdadienyl PP [(+) copalyl PP] (**b-1**), having the opposite stereochemistry at all the chiral centers (Scheme **7**) (Dewick, 1997).

The elimination of pyrophosphate group from **a-1** and **b-1** facilitates further cyclization to form *ent*-pimarane skeleton ($\mathbf{a_1}$) and the pimarane ($\mathbf{b_1}$) and loss of proton from $\mathbf{a_1}$ and $\mathbf{b_1}$ generates the alkenes $\mathbf{a_1}$**-1** and $\mathbf{b_1}$**-1** respectively. Protonation of alkenes allows W-M 1-2 methyl shift of $\mathbf{a_1}$**-1** and $\mathbf{b_1}$**-1** to form the abietene skeletone $\mathbf{a_2}$ and $\mathbf{b_2}$. Loss of proton from $\mathbf{a_2}$ and $\mathbf{b_2}$ generates the abietadiene **a-2** and **b-2** respectively (Dewick, 1997). These intermediates on sequential oxidation and rearrangements through $\mathbf{a_3}$ and $\mathbf{b_3}$ may lead to cyclopropyl-containing intermediates **a-3** and **b-3** with opposite stereochemistry at all newly generated chiral centers (Schemes -**6** and -**7**). These intermediates on oxidations at C-16 and C-12 followed by lactonization with C-12 OH may form less strained lactone rings with *psudo*-axial H-12. This may lead to diterpene lactones, suregadolides C (**107**) (compound **3**) and D (**108**) (compound **4**) of two antipodal series. The similar sequence of biochemical reactions may also be responsible of the biogenesis of structurally related gelomulides (Talapatra et al., 1989; Talapatra et al., 1998).

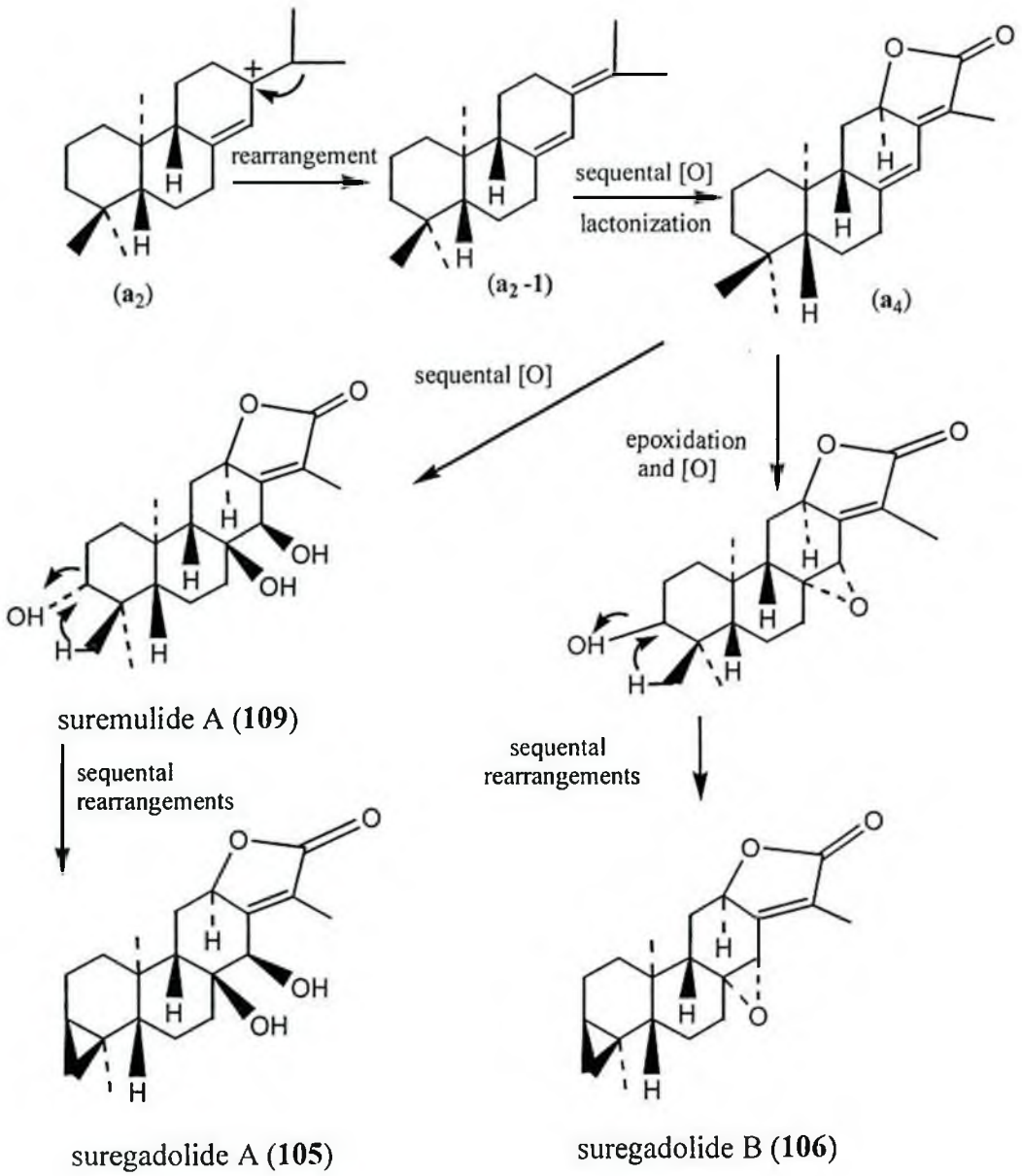


Scheme 6: Proposed biogenesis of suregadolide C.



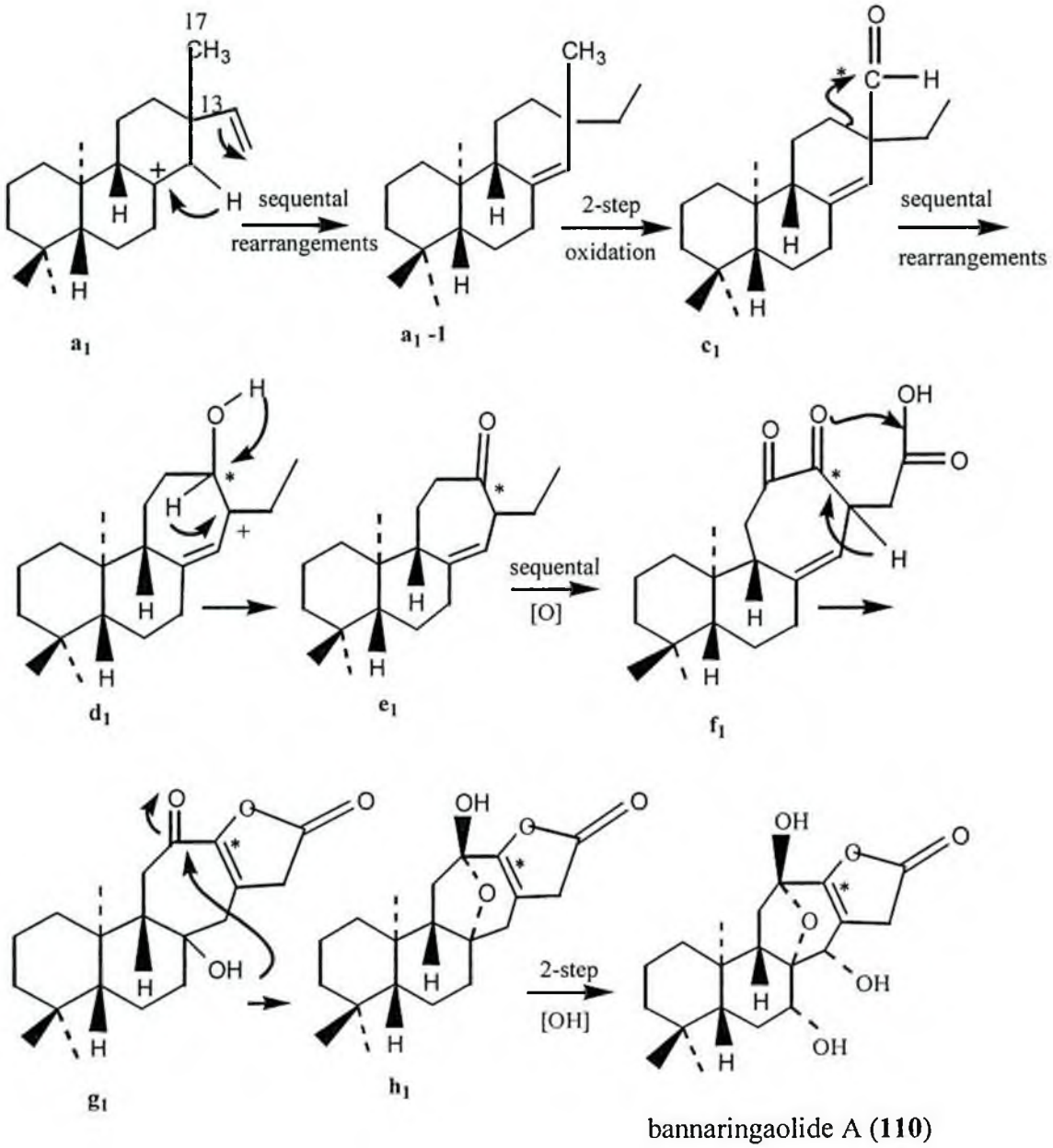
Scheme 7: Proposed biogenesis of suregadolide D.

The proposed intermediate $\mathbf{a_2}$ (Scheme-6) can give rise to compounds **5**, **1** and **2** (Scheme-8). The intermediate $\mathbf{a_2}$ on oxidation and rearrangement may yield δ-lactone bearing intermediate $\mathbf{a_4}$ which on further oxidation at the C-8/C-14 double bond$^{\nabla 8,13}$ from the less hindered β phase and at C-3 from α phase can yield compound **5,** suremulide A. On the other hand elimination of C-3 OH from compound **5** followed by rearrangement may lead to a rearranged abietene diterpene lactone suregadolide A, compound **1**. Compound **2,** suregadolide B might arise from the epoxidation of the C-8/C-14 double bond$^{\nabla 8,13}$ from the α phase and rearrangement (Scheme-8).



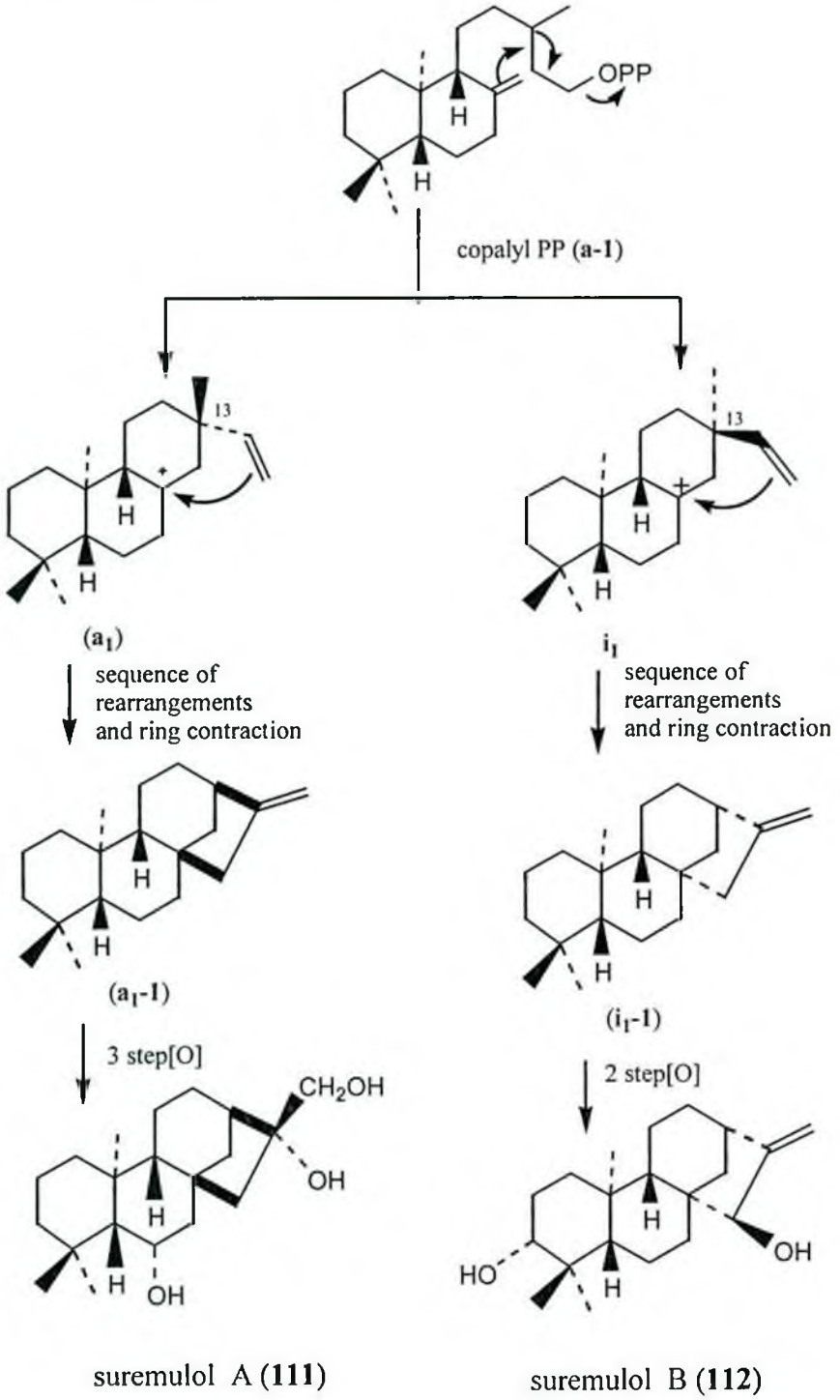
Scheme **8**: Proposed biogenesis of suregadolides A and B, and suremulide A.

The biogenesis of the novel diterpene lactone compound **6** may involve the pathway summarize in Scheme-**9**. The proposed intermediate (*ent*-pimarane skeleton) $\mathbf{a_1}$ (Scheme-**6**) undergoes a rearrangement to form $\mathbf{a_1}$-**1**. A two-step oxidation of $\mathbf{a_1}$-**1** lead to an aldehydic intermediate $\mathbf{c_1}$, which on sequential rearrangements may give rise a rearranged *ent*-pimarane skeleton $\mathbf{e_1}$. The $\mathbf{e_1}$ on further oxidation and lactonization form a diterpene lactone $\mathbf{g_1}$. The latter after rearrangement and two steps hydroxylation may yield the compound, bannaringaolide A (compound **6**) (Scheme-9).



Scheme **9**: Proposed biogenesis of bannaringaolide A.

Pimaranes with epimaric C-13 are abundantly found in nature. Cyclization of **a-1** may lead to the formation of two C-13 epimaric Pimaranes $\mathbf{a_1}$ and $\mathbf{i_1}$ The intermediates $\mathbf{a_1}$ and its epimer $\mathbf{i_1}$ (Scheme- 6 and -10) through cyclization, a sequence of rearrangements, ring contraction and oxidations may give rise to compounds **7** and **8** (suremulol A (**111**) and B (**112**) (Scheme-10).



Scheme **10**: Proposed biogenesis of suremulol A and suremulol B.

2.5.4 Results of Yeast Bioassay

All the extracts, fractions, sub-fractions and pure compound were screened for their potential anticancer activity utilizing the mechanism-based yeast bioassay employing genetically engineered yeast mutants to monitor the isolation of active compounds (Gunatilaka, et al., 1979; Johnson et al., 1986). Potential antitumor activity was presumed when the extract showed inhibition of the growth of the mutant strains RAD 52 and no inhibition of the growth of the wild type strain RAD+ or when it showed a lower IC_{12} value (defined as the dose that gives an inhibition zone of 12 mm using a 6 mm diameter, 100 μL well in an agar plate, Fig 6) (Gavindan, et al., 1995). The biological activity data for extract, fractions and pure compounds are given in Table- 9.

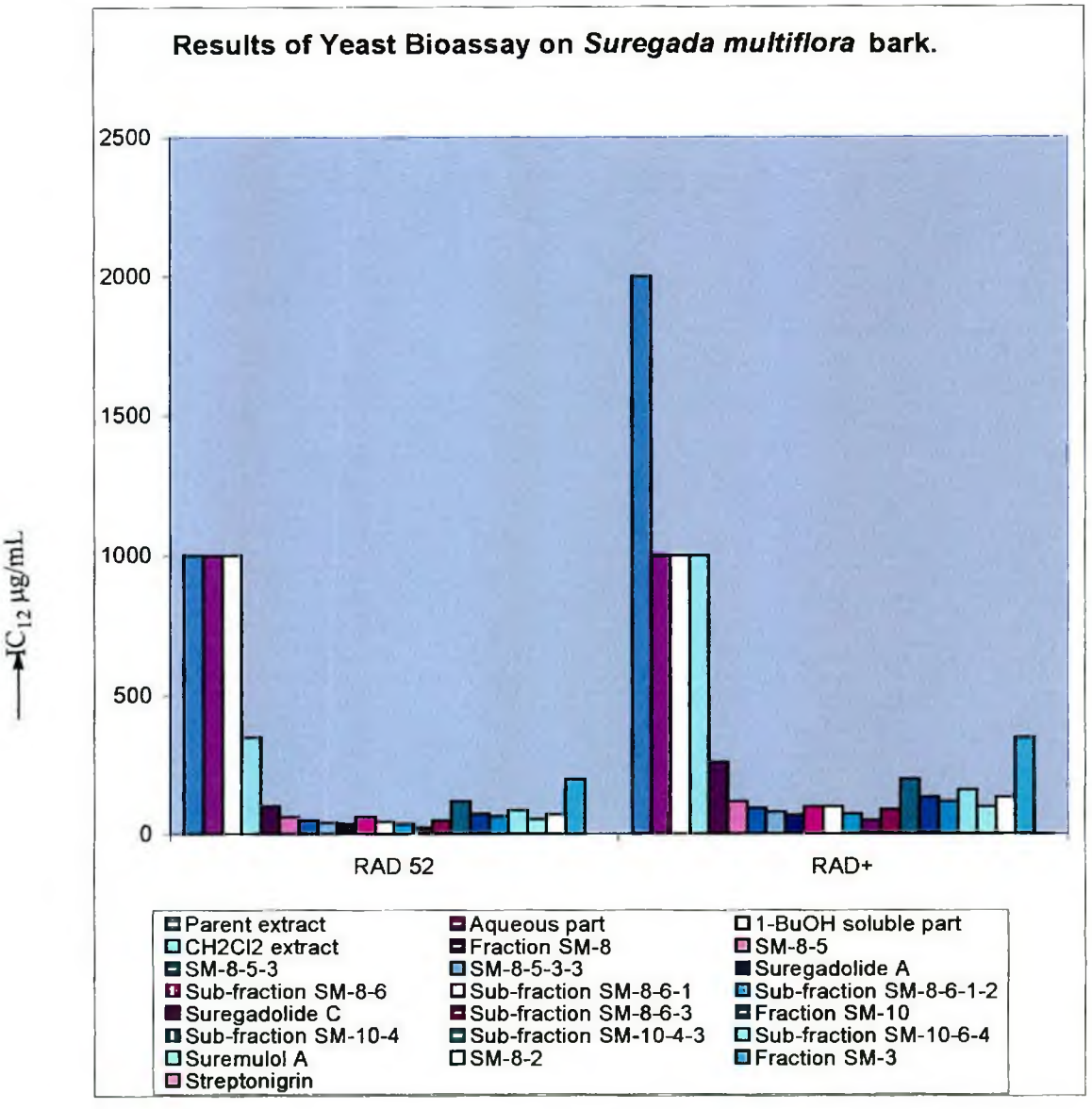
The CH_2Cl_2 soluble part of the parent bark extract showed significant activity with IC_{12} values 350 and 1000 μg/mL in RAD 52 and RAD^+ respectively. Whereas the aqueous and 1-BuOH soluble parts were not significantly active. Among twelve fractions of CH_2Cl_2 extract only three fractions SM-3 (IC_{12} = 200 and 350 μg/mL), SM-8 (IC_{12} = 100 and 260 μg/mL), and SM-10 (IC_{12} = 120 and 200 μg/mL) showed significant growth inhibition in mutant yeast bioassay. A bioassay- directed isolation work on these fractions (SM-3, SM-8 and SM-10) yielded 10 compounds (**1-10**) (Scheme: 1-5). Compound **1**, suregadolide A (**105**), compound **3**, suregadolide C, (**107**) and compound **7**, suremulol A (**111**) showed growth inhibition with IC_{12} values 35 and 70μg/mL, 23 and 50 μg/mL, 53 and 100 μg/mL, respectively towards RAD 52 and RAD+ in yeast bioassay.

Compounds **1**, **3** and **7** showed moderate activity against the repair deficient RAD 52 yeast strain, indicating that they act as DNA damaging agents (Heltzl, et al., 1993). The work demonstrates the ability of the yeast bioassay to lead to the isolation of compounds that may have significant activity in other established bioassays.

Table 9. Results of Yeast-Bioassay of *S.multiflora* bark.

Sample	Yeast strains	
	(RAD 52)[a]	(RAD^+)
Parent extract (CH_2Cl_2-MeOH)	1000	2000
Aqueous part	1000	1000
1-BuOH soluble part	1000	1000
CH_2Cl_2 extract	350	1000
Fraction SM-8 (From **CH_2Cl_2** extract)	100	260
SM-8-5	60	120
SM-8-5-3	48	97
SM-8-5-3-3	40	80
suregadolide A (From SM-8-5-3-3)	35	70
SM-8-5-3-2	-	-
suregadolide B (From SM-8-5-3-2)	Not active	-
Sub-fraction SM-8-6	60	100
Sub-fraction SM-8-6-1	45	100
Sub-fraction SM-8-6-1-2	35	75
suregadolide C (From sub-fraction SM-8-6-1-2)	23	50
Sub-fraction SM-8-6-3	48	90
Sub-fraction SM-8-6-3-2	-	-
suregadolide D (From sub-fraction SM-8-6-3-2)	Not active	-
Fraction SM-10	120	235
Sub-fraction SM-10-4	73	135
Sub-fraction SM-10-4-3	65	120
suremulide A (From sub-fraction SM-10-4-3)	Not active	-
Sub-fraction SM-10-6	-65	100
Sub-fraction SM-10-6-6	-	
bannaringaolide A (From sub-fraction SM-10-6-6)	Not active	-
Sub-fraction SM-10-6-4	85	160
suremulol A (From sub-fraction SM-10-6-4)	53	100
SM-8-2	70	135
suremulol B (From SM-8-2)	Not active	-
Fraction SM-3	200	350
Sub-fraction SM-3-3	Not tested	-
multiflorenol (From sub-fraction SM-3-3)	-	-
Sub-fraction SM-3-3-2	-	-
baurenol (From sub-fraction SM-3-3-2)	Not tested	-
[c] Streptonigrin (Control)	0.4	>1

[a] Results expressed as IC_{12} μg/mL., [c] reference compound.



Graph 1. Results on yeast-bioassay on *Suregada multiflora* bark.

3. PART B

Phytochemical Investigations on *Iris germanica* Linn

3. Part B

Phytochemical Investigations On *Iris germanica* Linn.

3.1 Introduction

Iris germanica Linn belongs to the family Iridaceae and genus *Iris* Linn.

3.1.1 Family Iridaceae

The plant family Iridaceae consists of 92 genera, which contains about 1800 species, occur nearly worldwide, but rare in tropical lowlands. The family consists of perennial herbs with various rootstock, narrow leaves, often distichous and equitant. Rhizomes are stimulant, emetic, and sudorific. In some cases the stigmas of the flowers are stimulant and emmenagoigue. Iridaceae is of considerable economic importance in horticulture and in the cut-flower industry, especially *Iris, Freesia* and *Gladiolus*. Several other genera (*Watsonia, Crocus* and *Dietes*) are cultivated in gardens of both tropical and temperate areas. *Moraea* and *Homeria* are poisonous and cause significant losses in cattle and sheep raising areas, especially in southern Africa. Some genera are of importance in traditional medicine, especially *Iris* and *Gladiolus*.

3.1.2 The Genus *Iris* Linn

The genus *Iris Linn* is well known to constitute about three hundred species. The plant genus *Iris* consists of perennial herbs with creeping or bulbous rootstock, leaves are equitant or ensiform, rhizome stimulant, catheric and diuretic. The genus comprise over 300 species. All of these plants are mainly known for their decorative value, as they form very beautiful colored flowers with durable scents. Many of the *Iris* species are used medicinally in Europe and Asia; *I. germanica* Linn, *I. ensata* Thumb, *I. faetidissima* Linn, *I. florentina* Linn, *I. pallida* Lam. etc. Orrisroot, a violet-scented

flavor used in dentifrice, perfumes and other products, is prepared from the powdered rhizomes.

3.1.3 *Iris germanica* Linn

Iris germanica Linn a speciec of the genus *Iris* has been found in Austria, Belgium, Denmark, Germany, Holland, Hungary, Italy, Japan, Portugal, Russia, Sweden, Switzerland, Turkey and Pakistan. The plant *I. germanica.* is a perinial herb. Roots of this plant is diuretic, alterative, aperient, and cathartic.

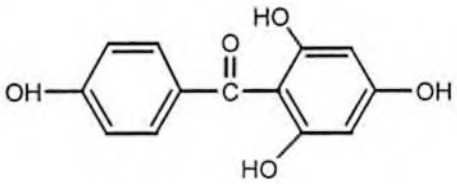
3.1.4 Medicinal Properties of *Iris* Linn

Iris species have an immense medicinal importance and are used in the treatment of cancers, inflammation bacterial and viral infections. The compounds isolated from these species were reported to have piscicidal, antineoplastic, antioxidant, antitumor, antiinflammatory, antiplasmodial and antituberculosis properties, (Ito t al., 1995; Miyake, et al., 1997; Bonfils et al., 2001; Seok, et al., 1995; Tan, et al., 2000; Hideyuki, et al., 1995) Some cytotoxic compounds have also been isolated from some of the *Iris* species (Shawl et al., 1988).

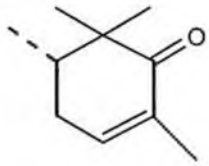
3.1.5 Chemical Constituents of *Iris* Linn

The phytochemical investigations on different species of *Iris* have resulted in the isolation of a variety of secondary metabolites including flavonoids, isoflavonoids, and their glycosides, triterpenes and stilbene glycosides (Kala, et al.; 1978; Choudhary, et al., 2001, Choudhary, et al., 2001; Attaur-ur-Rahman et al., 2000; Shawl et al., 1988; Shawl et al., 1988; Shawl et al., 1985; Reed 1963). Previous phytochemical investigations on different parts of *Iris germanica* have led to the isolation of flavonoids, isoflavonoids, triterpenoids along with a number of fatty acids and derivatives. *Iris germanica* has also been reported as a rich source of ascorbic acid and vitamins (Ali et al., 1993).

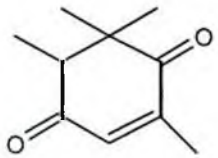
Maurer, B. and co-workers reported about the isolation of iriflophenone (**113**) along with a number of fatty acids and derivatives e.g., compounds (**114-127**) from the low polar fractions of *Iris germanica* rhizome (Maurer, et al., 1989).



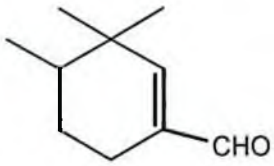
Iriflophenone (**113**)



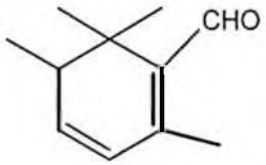
2,5,6,6-Tetramethyl-2-cyclohexene-1-one (**114**)



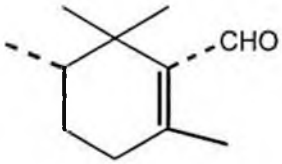
2,5,6,6-Tetramethyl-2-cyclohexene-1-4-dione (**115**)



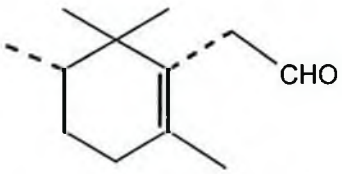
3,3,4-Trimethyl-1-cyclohexene-1-carboxaldehyde (**116**)



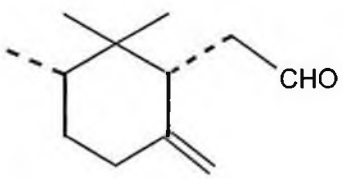
2,5,6,6-Tetramethyl-1-3-cyclohexadiene carboxaldehyde (**117**)



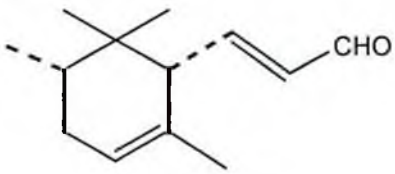
2,5,6,6-Tetramethyl -2- cyclohexene carboxaldehyde (**118**)



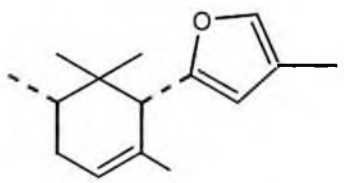
2,5,6,6-Tetramethyl -2- cyclohexene acetaldehyde (**119**)



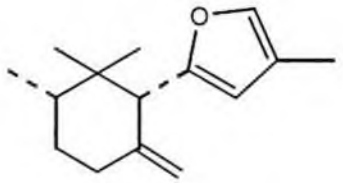
2,2,3-Trimethyl-6-methylenecyclohexane acetaldehyde (**120**)



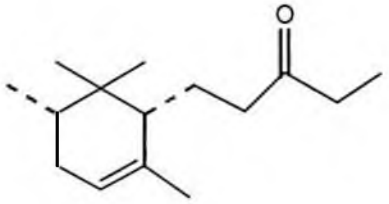
3-(2,5,6,6-Tetramethyl -2-cyclohexene-1-yl)-2-propanal (**121**)



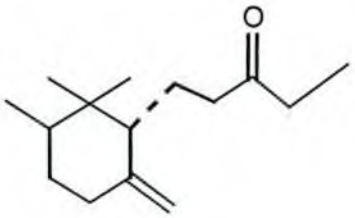
4-Methyl-(2,5,6,6-tetramethyl-2-cyclohexane-1-yl)-furan (**122**)



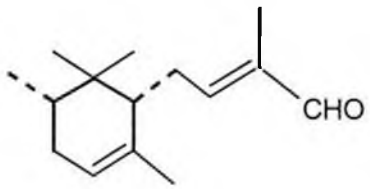
4-Methyl-2-(2,2,3-trimethyl-6-methylene-1-cyclohexyl)-furan (**123**)



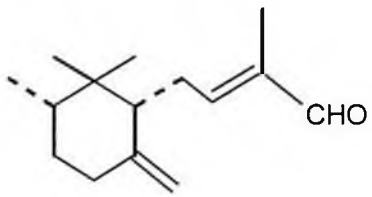
10-Methyl-α-irone (**124**)



10-Methyl-γ-irone (**125**)

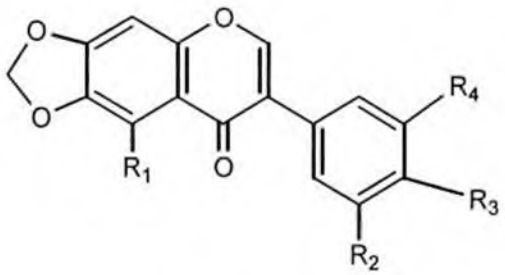


2-Methyl-4-(2,5,6,6-tetramethyl 2-cyclohexene-1-yl)-butenal (**126**)



2-Methyl-4-(2,2,3-trimethyl 6-methylene-1-cyclohexyl)-2-butenal (**127**)

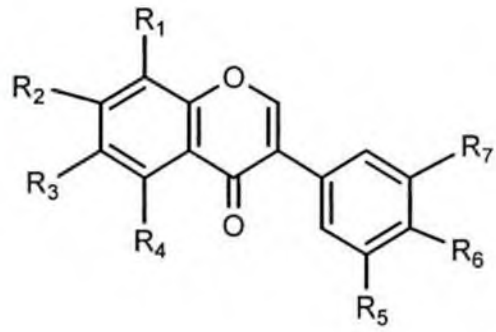
Dhar, K.I. and co-aurthers have reported irilone (**128**) a new isoflavone diol with methylene dioxy in A ring in 1973 from the rhizome of *I. germanica* (Dhar et al., 1973).



R_1	R_2	R_3	R_4	
OH	H	OH	H	(**128**)
OCH_3	H	OCH_3	OH	(**129**)
OCH_3	H	OCH_3	OCH_3	(**130**)
OCH_3	H	OH	H	(**131**)

Another isoflavone 6,7-methylenedioxy-5,3′,4′-trimethoxy-isoflavone (**129**) was reported from the plant *I. germanica* in 1973 (Pailer et al., 1973). Irishkumaonin (**130**), a

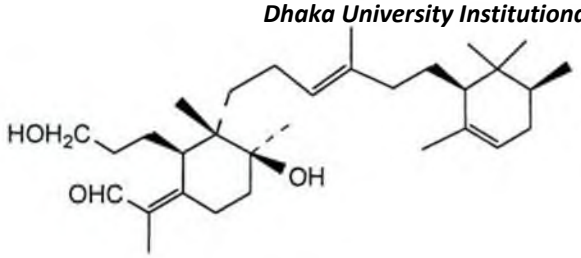
derivative of irilone have been isolated by Kala, A. K and his group from the same plant (Kalla et al., 1978). Shawl, A.S. and co-workers in 1984, reinvestigated on *I. germanica* rhizome and reported another new isoflavone irisolone (**131**) which also contain methylene dioxy group in A ring (Shawl et al., 1984).



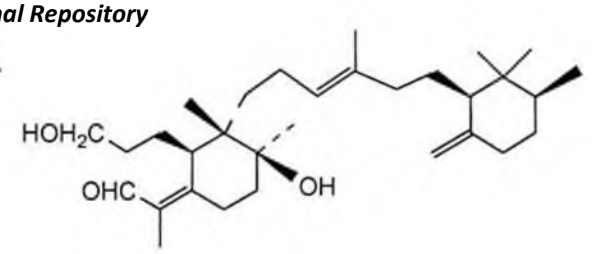
	R_1	R_2	R_3	R_4	R_5	R_6	R_7
Irisolidone (**132**)	H	OH	OCH_3	OH	H	OH	H
7-O-β-D-Glucopyranoside-5,5′-dihydroxy-3′,4′-dimethoxyisoflavone (**133**)	H	OGlc	H	OH	OH	OCH_3	OCH_3
7-O-β-D-Glucopyranoside-5-hydroxy-4′,8-dimethoxyisoflavone (**134**)	OCH_3	OGlc	H	OH	H	OCH_3	H

Irisolidone (**132**), an isoflavone and an isoflavone glycoside 7-*O*-β-D-glucopyranoside-5,5′-dihydroxy-3′,4′-dimethoxy isoflavone (**133**) was reported in 1983 (Pailer et al., 1983). Another isoflavone glycoside,7-*O*-β-D-glucopyranoside-21-hydroxyiridal-5-hydroxy-4′,8-dimethoxy-isoflavone (**134**) was found to be reported by Ali and coworkers, when they reinvestigated the same plant (Ali et al., 1992).

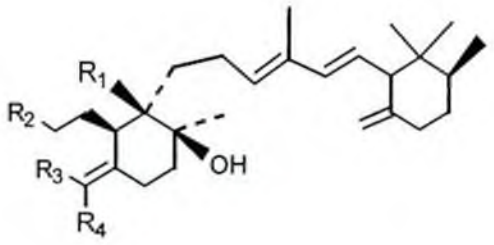
Marners, F-J. and group in 1982, reported two new triterpenes α-irigerminal (**135**) and γ-irigermanal (**136**) isolated from *I. germanica*. Maurer, B and co-workers reinvestigated the same plant in 1989 and isolated three more similar type of new triterpenes irisgermanical A-C (**137-139**) (Ito et al., 1995).

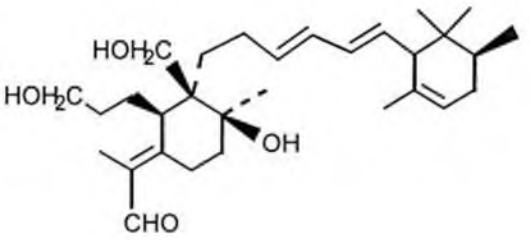


α-Irigerminal (**135**)



γ-Irigermanal (**136**)

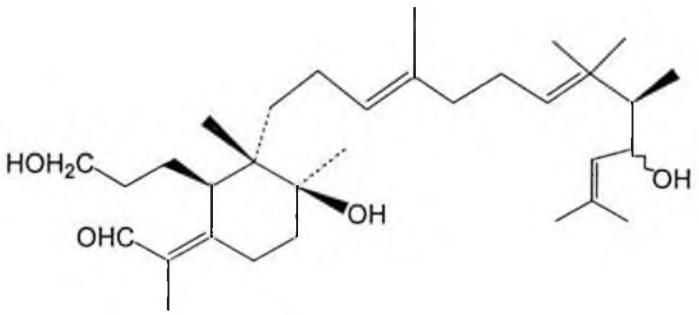




	R_1	R_2	R_3	R_4
Irisgermanical A (**137**)	CH_3	CH_2OH	CHO	CH_3
Irisgermanical B (**138**)	CH_2OH	CH_2OH	CH_3	CHO

Irisgermanical C (**139**)

Another new triterpene, iridogermanal-21-hydroxyiridal (**140**) have been isolated by Marner and co-workers in 1982 from the *I. germanica* (Marner et al., 1982).



Iridogermanal-21-hydroxyiridal (**140**)

3.2 Flavonoids

Various types of flavonoids have been reported from different species of *Iris*. The genus, however, is rich in isoflavonoid compounds (Shawl et al., 1988; Shawl et al., 1988; Shawl et al., 1985; Reed 1963). The plant *Iris germanica* has also been found as a rich source of isoflavonoids. During our current study, we have also isolated a number of isoflavonoids. Isoflavonoids have drawn considerable attention, because they have anti-mutagenic activity (Carman et al., 1985), and inhibitory effects on protein tyrosine kinase (Markham et al., 1970) and on pulmonary tumor promotion (Devon et al., 1975). The flavonoids, one of the most diverse and widespread groups of natural products occupies a prominent position among the natural phenols. These are biologically important plant ingradients and about 2% of all the primary carbon metabolites photosynthesized by plant are converted into flavonoids (Harbone, J. B., *et. al.*, 1988). Flavonoids are the coloring co-pigments of the plants (Zechmeister, L., et. al., 1957), which are also called anthroxanthins. The wide range of colors and shades in flowers are principally due to the presence of flavonoids (Monitto, 1981).

3.2.1 Classification

Flavonoids occur in a variety of structural forms. All contains fifteen carbon atoms in their parent nucleus and share the common structural feature of two phenyl rings linked by a three-carbon chain (biphenyl propane derivatives). The three main flavonoid types possessing a 1,3- diphenylpropane (**141**), 1,2- diphenylpropane (**142**), 1,1- diphenylpropane (**143**) skeleton.

The three carbon chain link two phenyl units may form a third ring through cyclization with the oxygen on one of the phenyl rings generating a tricyclic system. Four main skeletal types derived from 1,3-diphenylpropane systems are chalchonoids (**144**), auronoids (**145**), flavanoids (**146**) and flavonoids (**147**). The main skeletal types derived from 1,2-diphenylpropane systems are known as isoflavanoids (**148**), isoflavonoids (**149**), 3-phenylcoumarins (**150**) and petrocarpanoids (**151**). The compounds derived from 1,1-diphenyl system are neoflavonoids (**152**) (Fig. 39, Page-124).

Another flavonoids type is homoflavonoids, which contain an additional carbon in their skeleton, such as homoflavone (**153**) and homoisoflavones (**154**), homoisoflavanone, (**155**), homoisoflavan (**156**), rotenoid (**157**) and peltogynoid (**158**) (Fig. 40, Page-125).

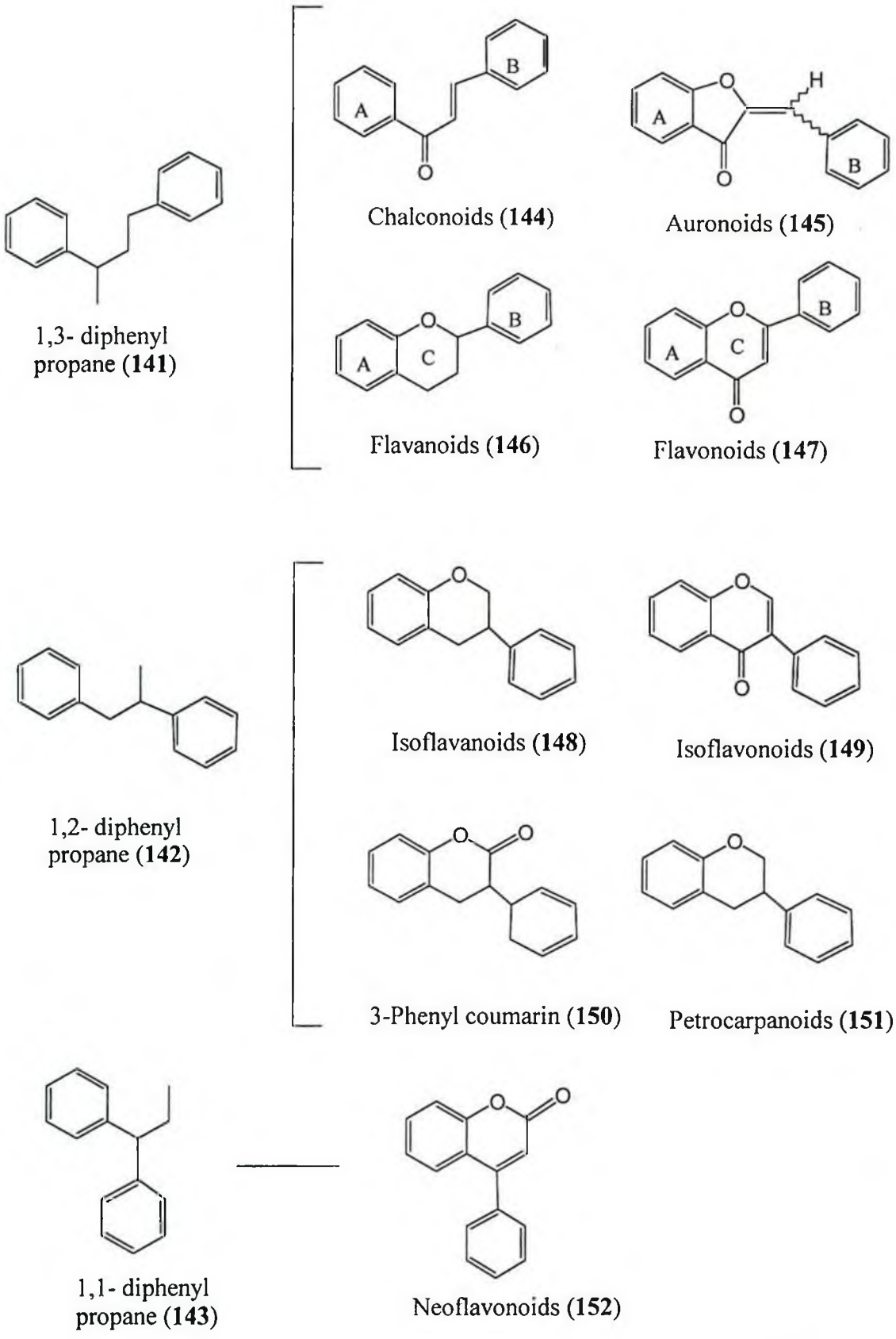
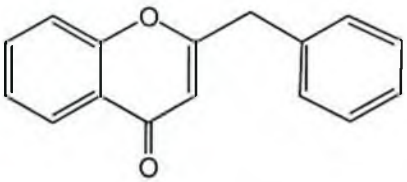
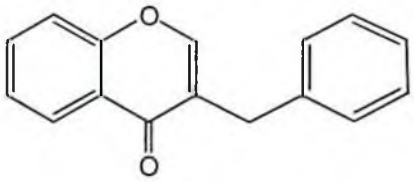


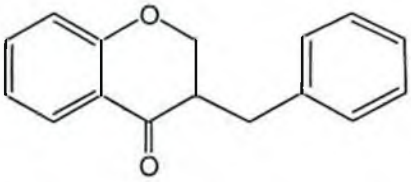
Fig. 39. Flavonoids having 1,3 - , 1,2 - and 1,1- diphenylpropane skeleton.



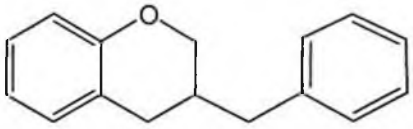
Homoflavonoids (**153**)



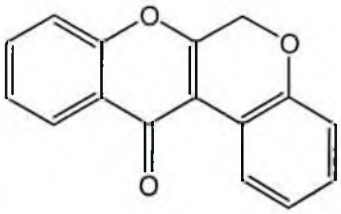
Homoisoflavonoids (**154**)



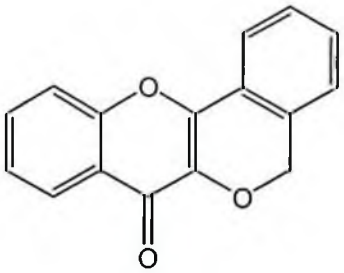
Homoisoflavanone (**155**)



Homoisoflavan (**156**)



Rotenoid (**157**)



Peltogynoid (**158**)

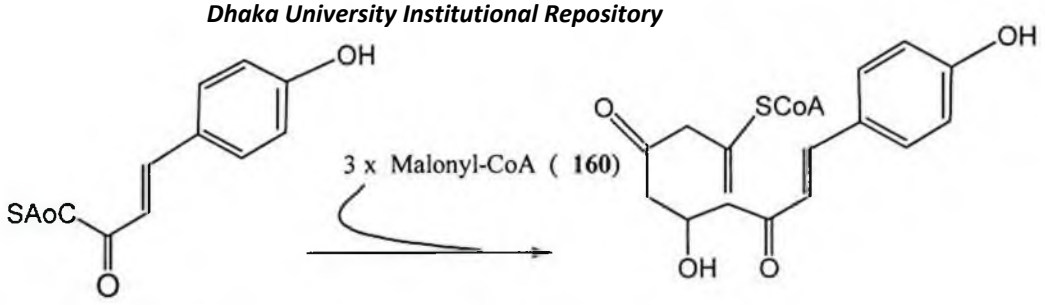
Fig. **40**. Main skeletons in homoflavonoids.

Natural flavonoids and isoflavonoids are usually polyoxygenateds in nature and bear hydroxyl and or methoxy substituents. A large number of flavonoids occur as *O*-glycosides in which one or more of the hydroxyl groups of the flavonoid nucleus are bound to a sugar or sugars via an acid labile hemiacetal bond. In flavonoid C-glycosides (Harbone, J. B., *et. al.*, 1975; Harbone, J. B., *et. al.*, 1982) the sugar is C- linked and this linkage is acid resistant. Glycosylation make the flavonoids less reactive and more water-soluble.

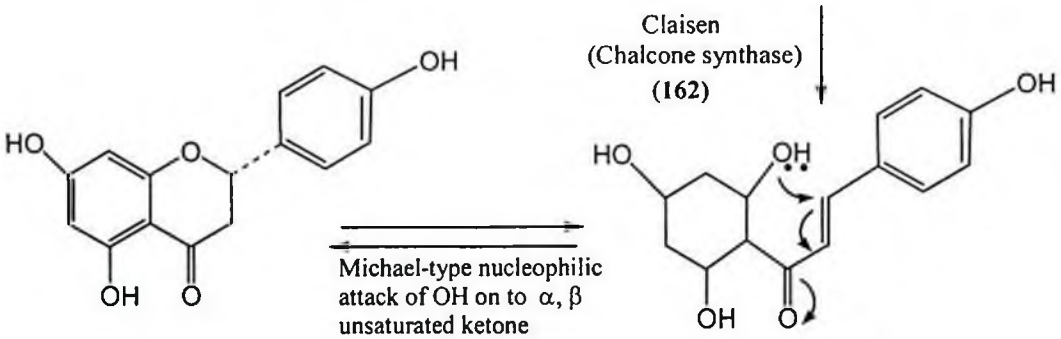
3.2.2 Biosynthesis

The biosynthesis of flavonoids involves the formation of a central C-15 itermediate chalcone. Chalcones act as precursors for a vast range of flavonoid derivatives found throughout the plant kingdom. A cinnamoyl-CoA unit (**159**) with three malonyl-CoA units (**160**), with the help of enzyme chalcone synthase (**162**) gives rise the chalcone (**163**) through the itermediate (**161**). The central role of chalcones in flavonoids biosynthesis has been confirmed in a number of investigations (Grisebach, et al., 1965; Wong, et. al., 1965). Most contain a six membered heterocyclic ring, formed by Michael-type nucleophilic attack of a phenol group on to the unsaturated ketone, giving a flavanone (**164**) (Fig. **41**). Engymetic conversion of flavanone then give rise to many varieties of this basic skeleton, e.g. flavones (**165**), flavonols, anthocyanides, catechins, isoflavones (**170**).

The isoflavonoids form quite distinct subclasses of flavonoid compound, being structural variants in which the shikimate derived aromatic ring migrate to the adjacent carbon of the heterocycle. This rearrangement process is brought about by a cytochrome P-450-dependent enzyme requiring NADPH (**166**) and O_2 cofactors (Hagmann, et al., 1984; Kochs, et al., 1986), which transforms the flavanones into the isoflavanones (**170**) through the intermediates (**167-169**) (Fig. 42). Hydroxylation (Hauteville, et al., 1979; Spribille, et al., 1984; Forkman, et al., 1986), methylation (Harbone et al., 1982; Jay et al., 1983; Jay et al., 1985), glycosylation (Kleinehollenhorst et al., 1982; Jourdan et al., 1982; Bajaj et al., 1983; Koster et al., 1981) in the two aromatic rings of flavones and isoflavones increasing the range of compounds enormously.

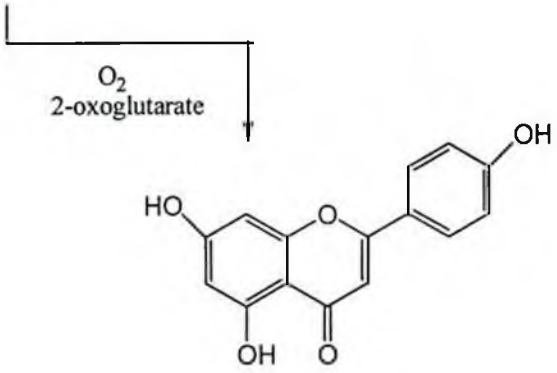


4-Hydroxycinnamoyl CoA (**159**) (**161**)



Naringenin (**164**)
(a flavone)

Naringenin chalcone (**163**)
(a chalcone)



Apigenin (**165**)
(a flavanone)

Fig. **41**. Biosynthesis of flavonoids.

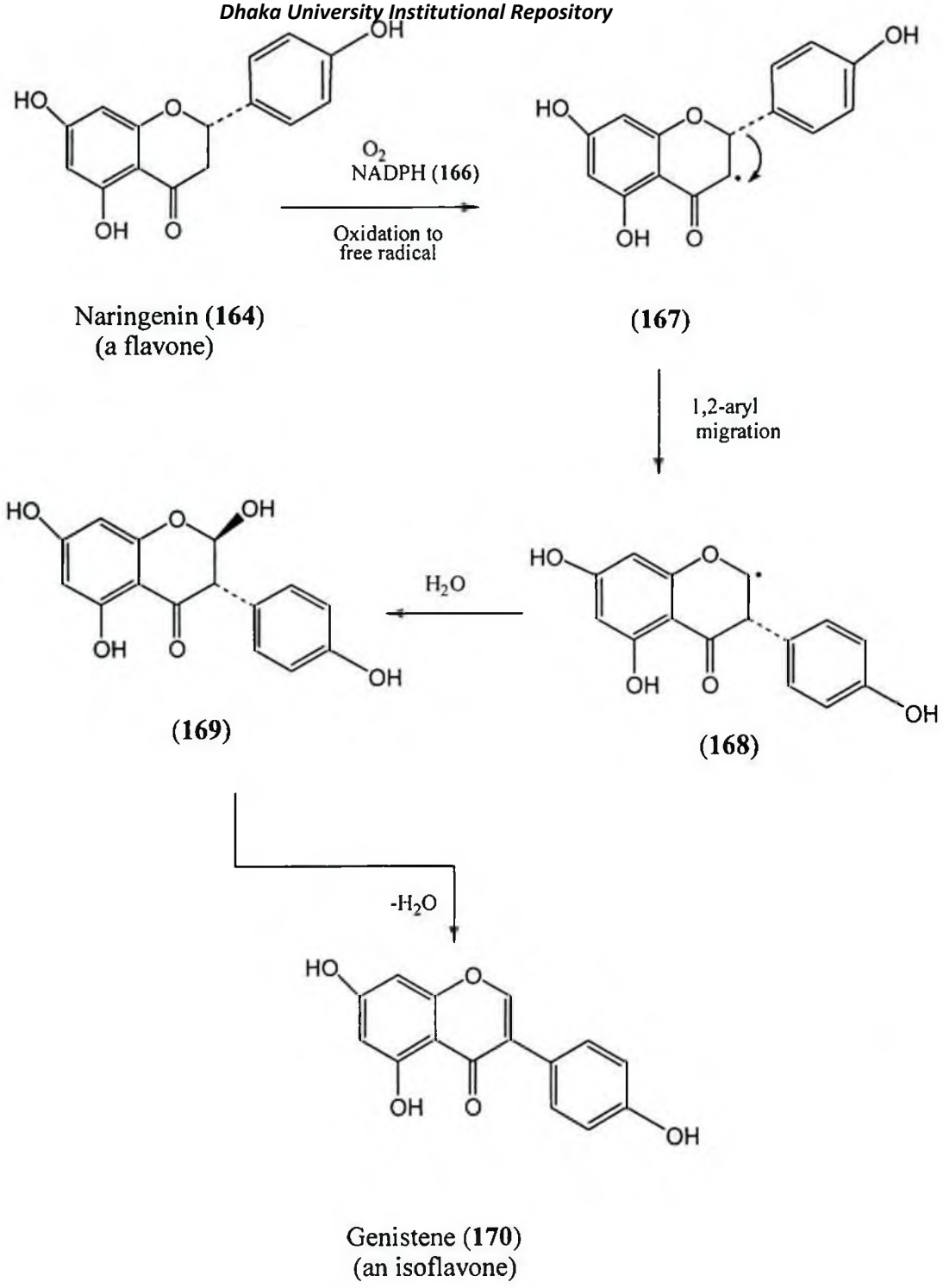


Fig. 42. Biosynthesis of isoflavones.

3.3 Experimental Section

3.3.1 General Experimental Conditions

The general experimental conditions, bioassay experimental and some other experimental procedures are same as described in part A (Page 31-33).

3.3.2 Plant material

Rhizomes of *Iris germanica* Linn was purchased from a herbal medicine shop of Jodia Bazar, Karachi, Pakistan, during the month of June 2000. Air-dried rhizomes of *Iris germanica* was (10 kg) powdered.

3.3.3 Extraction and Fractionation

Powdered rhizomes (10 kg) of *Iris germanica* was extracted with hexane (20 L), the extractive free residue was then extracted with MeOH (7 L x 3 72 h, RT). The extract was concentrated to give dark brown syrup of parent extract (500 g). This parent extract was tested for mechanism-based yeast-bioassay and was found to exhibit significant growth inhibitory activity. The parent extract was then suspended in water (7 L) and partitioned with CH_2Cl_2 (1:1, 7 L x 3, 72 h, RT). The resulting organic layer was evaporated under reduced pressure and a CH_2Cl_2 soluble part (150 g) was obtained. The aqueous part was concentrated, freeze dried and again suspended in water (5 L) and then partitioned with EtOAc (1:1, 5 L x 3) to obtain an EtOAc soluble fraction (50 g) (Scheme-11, Page-131). The aqueous part was concentrated under and freeze-dried and a water soluble part 200 g was obtained. CH_2Cl_2 and EtOAc soluble parts were found to show significant growth inhibitory activity in mechanism based mutant yeast bioassay (Table-11, Page-159).

The EtOAc extract (IG-3, 50 g, IC_{12} = 200 and 450 μg/mL) was subjected to silica gel column chromatography (G 60, 600 g) and was eluted by gradients of solvents hexane-

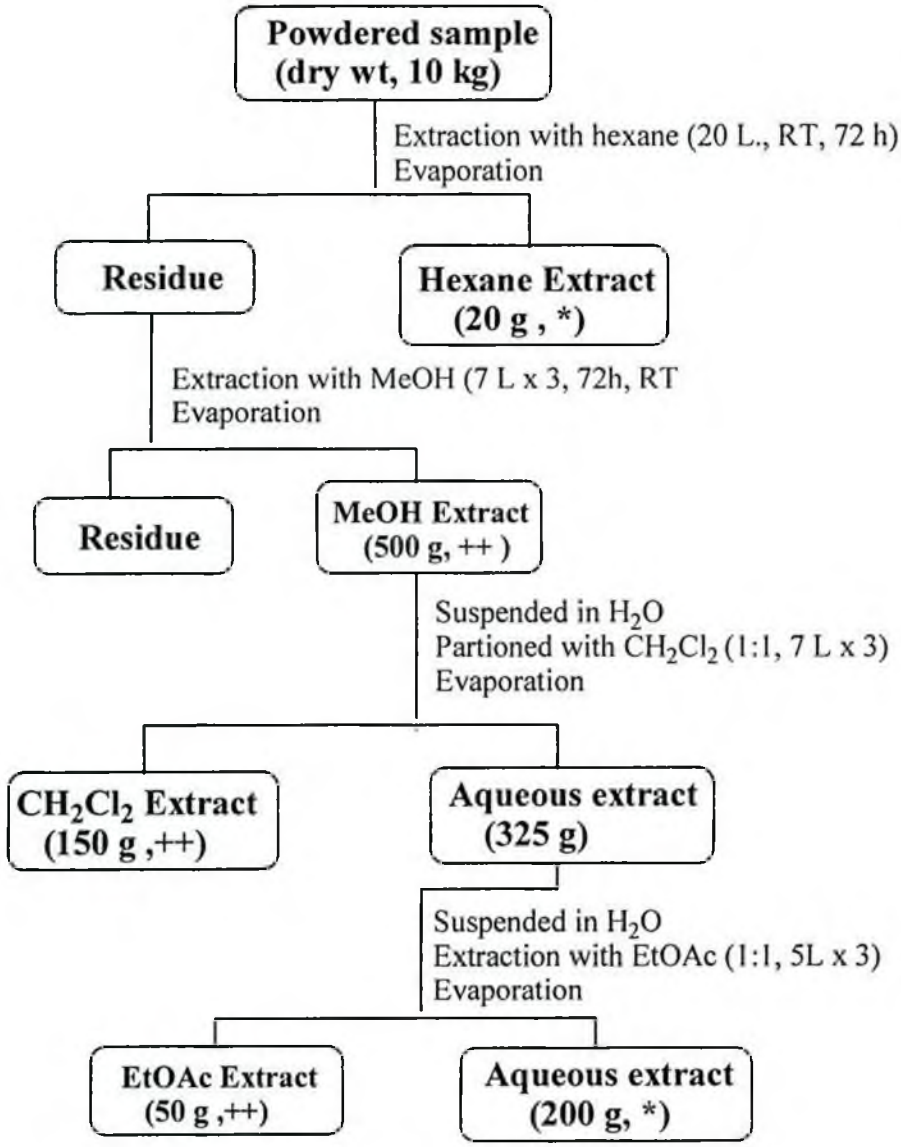
CH_2Cl_2- MeOH to yield 15 fractions (IG-3-1 – IG-3-15). Among the 15 fractions only 3 fractions, IG-3-3 (1.5 g, IC_{12} = 100 and 240 μg/mL), IG-3-5 (4.3 g, IC_{12} = 125 and 200 μg/mL) and IG-3-8 (4.5 g, IC_{12} = 145 and 350/ μg/mL) showed growth inhibitory activity in RAD 52 and RAD+, respectively in these assays. The fraction, IG-3-5 (4.3 g) eluted by CH_2Cl_2-MeOH (9:1) was subjected to further fractionation by silica gel column chromatography (60 g) and the elution was carried out by CH_2Cl_2 and MeOH to give 10 fractions (IG-3-5-1 - IG-3-5-10). Fractions IG-3-5-8 (150 mg, IC_{12} = 100 and 125 μg/mL) and IG-3-5-10 (200 mg, IC_{12} = 90 and 140 μg/mL) showed growth inhibition in yeast assays.

The CH_2Cl_2 soluble part (IG-2, 150 g, IC_{12} = 200 and 510 μ g/mL) was fractionated by VLC (vacuum liquid chromatography) over silica gel and was eluted with gradients of solvent mixture hexane-EtOAc-MeOH. Twenty fractions (IG-2-1–IG-2-20) were obtained based on their TLC profiles. All the fractions were tested for yeast assays. Out of twenty fractions only four fractions IG-2-3 (5.5 g, IC_{12} = 180 and 200 μg/mL), IG-2-5 (10.5 g, IC_{12} = 150 and 250 μg/mL), IG-2-8 (2.5 g, IC_{12} = 145 and 350 μg/mL) and IG-2-10 (2.5 g, IC_{12} = 145 and 300 μg/mL) showed growth inhibitory activity in the RAD 52 and RAD+, in yeast assays respectively.

3.3.4 **Isolation of Compounds 11-18**

A bioassay-guided isolation on CH_2Cl_2 and EtOAc soluble parts of the parent extract of *I. germanica* (rhizomes) by repeated column chromatography (CC) and thin layer chromatography (TLC) yielded eight pure compounds **11-18** (Scheme- 12-15)

Scheme 11: Extraction and Fractionation of *Iris germanica* Rhizomes



++ Moderately active in mutant yeast bioassay, * not active.

3.3.4.1 Compound 11- Nigricin 4′-*O*-β-D-glucoside (171)

The fraction IG-3-5-8 (150 mg, IC_{12} = 100 and 125 µg/mL) obtained from the EtOAc extract after repeated column chromatography (Scheme-12) was subjected to further fractionation on silica gel column using CH_2Cl_2 and MeOH as eluent. A fraction eluted by CH_2Cl_2-MeOH (9.5:0.5) yelded a pure fraction IIG 7, which afforded the compound **11,** nigricin 4′-O-β-*D*-glucoside (10 mg, IC_{12} = 75 and 92 µg/mL) (Scheme-12, Page-135). The percent yield was 1.01×10^{-4} and the R_f value of compound **11** was found to be 0.14 ($CHCl_3$-MeOH, twice; 92:8).

Physical Properties of nigricin 4′-*O*-β-D-glucoside (171)

Appearance: Light yellowish amorphous solid.

$[\alpha]_D^{24}$ + 50.2° (c, 0.57, MeOH)

UV λ_{max} MeOH (log ε) nm: 320 (3.808), 262 (3.287).

IR ν_{max} (KBr): 3345 (OH), 2924 (C-H), 1665 (C=O), 1565 (aromatic C=C), 935 (OCH_2O) cm^{-1}.

^{1}H NMR δ_H: Table **10**; Fig. **43**.

^{13}C NMR δ_C : Table **10**; Fig. **44**.

EI-MS (*m/z* relative intensity, %): 312 (M^+- one suger molecule, 89), 298 (100), 194 (20), 195 (26), 117 (15)

HR-FAB-MS (+ve) *m/z*: 475.1235 [$C_{23}H_{22}O_{11}$, requires 474.1240].

HR-FAB-MS (-ve) *m/z*: 473.1296 [$C_{23}H_{22}O_{11}$, requires 474.1240].

3.3.4.2 Compound 12- Wistin (172)

The fraction IG-3-5-10 (200 mg, IC_{12} = 90/140 µg/mL) obtained from the EtOAc extract was subjected to silica gel column chromatography and was eluted by CH_2Cl_2-MeOH. A fraction, eluted by CH_2Cl_2-MeOH (9.2: 0.8) yielded the compound **12** (10.0 mg), which is known as wistin (**172**) (Scheme-12, Page-135). The percent yield was 1.01×10^{-4} and the R_f value of **12** was found to be 0.38 (CH_2Cl_2-MeOH, 9.2: 0.8).

Physical Properties of wistin (172)

Appearance: Colourless needles.

mp: 210-211° C.

UV λ_{max} MeOH (log ε): 280, 238, 295 nm.

IR ν_{max} (KBr): 3350 (OH), 2925 (C-H), 1655 (C=O), 1482 (Ar C=C), 1110, 1050 cm^{-1}.

^{1}H-NMR (400 MHz, C_5D_5N): δ_H 8.13 (s, H-2), 7.20 (s, 2H, H-5/H-8), 7.10 (d, $J_{2',3'/6',5}$= 8.2 Hz, 2H, H-2′/6′), 7.80 (d, $J_{3',2'/5',6'}$ = 8.4 Hz, 2H, H-3′/5′), 5.85 (d, $J_{1'',2''}$ = 7.8 Hz, H-1″), 4.36 (H-2″), 4.22 (H-3″), 4.20 (H-4″), 4.30 (H-5″), 4.41 (dd, J =11.4 Hz, J = 2.0 Hz, H-6a″), 4.40 (d, J = 11.2 Hz, H-6b″), 3.68 (s, 2H, O-CH_2-O), 3.70 (s, 3H, OCH_3). 4.06 (s, 3H, OCH_3).

^{13}C-NMR (125 MHz, C_5D_5N): δ_C 153.3 (d, C-2), 125.0 (s, C-3), 175.0 (s, C-4), 105.0 (d, C-5), 147.2 (s, C-6), 154.0 (s, C-7), 103.5 (d, C-8), 153.4 (s, C-9), 118.3 (s, C-10), 124.0 (d, C-1′), 130.0 (d, C-2′/6′), 113.7 (d, C-3′/5′), 102.0 (d, C-1″), 73.2 (d, C-2″), 77.37 (d, C-3″), 69.8 (d, C-4″), 76.8 (d, C-5″), 60.7 (t, C-6″), 55.1 (s, OCH_3), 56.0 (s, OCH_3).

EI-MS (*m/z* relative intensity %): 298 (M^+- one suger moiety, 100), 283 (73), 157 (13), 271 (73), 157 (13), 69 (30), 57 (10).

HR-FAB-MS (-ve): 475.4108 [$C_{23}H_{22}O_{11}$, requires 476.4148].

3.3.4.3 Compound 13- 4′-Methoxy-5-hydroxy-6,7-methylenedioxy isoflavone (173)

Subfraction IG-3-3 (1.5 g, IC_{12} = 100 and 240 μg/mL), obtained from the EtOAc extract after rechromatography on silica gel column using CH_2Cl_2 and MeOH as eluent yielded eight fractions (IG-3-3-1 - IG-3-3-8). The subfraction IG-3-3-4 (100 mg, IC_{12} = 60 and 140 μg/mL) after further fractionation by silica gel column chromatography using CH_2Cl_2-MeOH (9.8: 0.2) as eluents gave a pure fraction IIG-2, which afforded the compound **13** (20 mg) (Scheme-12, Page-135). Another fraction, IG-2-8 (6.8 g, IC_{12} = 145 and 350 μg/mL) obtained from CH_2Cl_2 extract (Scheme-8) also yielded the compound **13** after repeated column chromatography on silica gel (Scheme-13, Page-137) (10 mg, IC_{12} = 43 and 100 μg mL) The percent yield was 3.03×10^{-4} and the R_f value of compound **13** was found to be 0.45 (CH_2Cl_2-MeOH, 9.9: 0.1)

Physical Properties of 4′-Methoxy-5-hydroxy-6,7-methylenedioxy-Isoflavone (173)

Appearance: Light yellowish amorphous powder.

IR ν_{max} (KBr): 3260 (OH), 2954 (C-H), 1645 (C=O), 1472 (Ar. C=C), 1257, 1104, 1054, 923, 832 cm^{-1}.

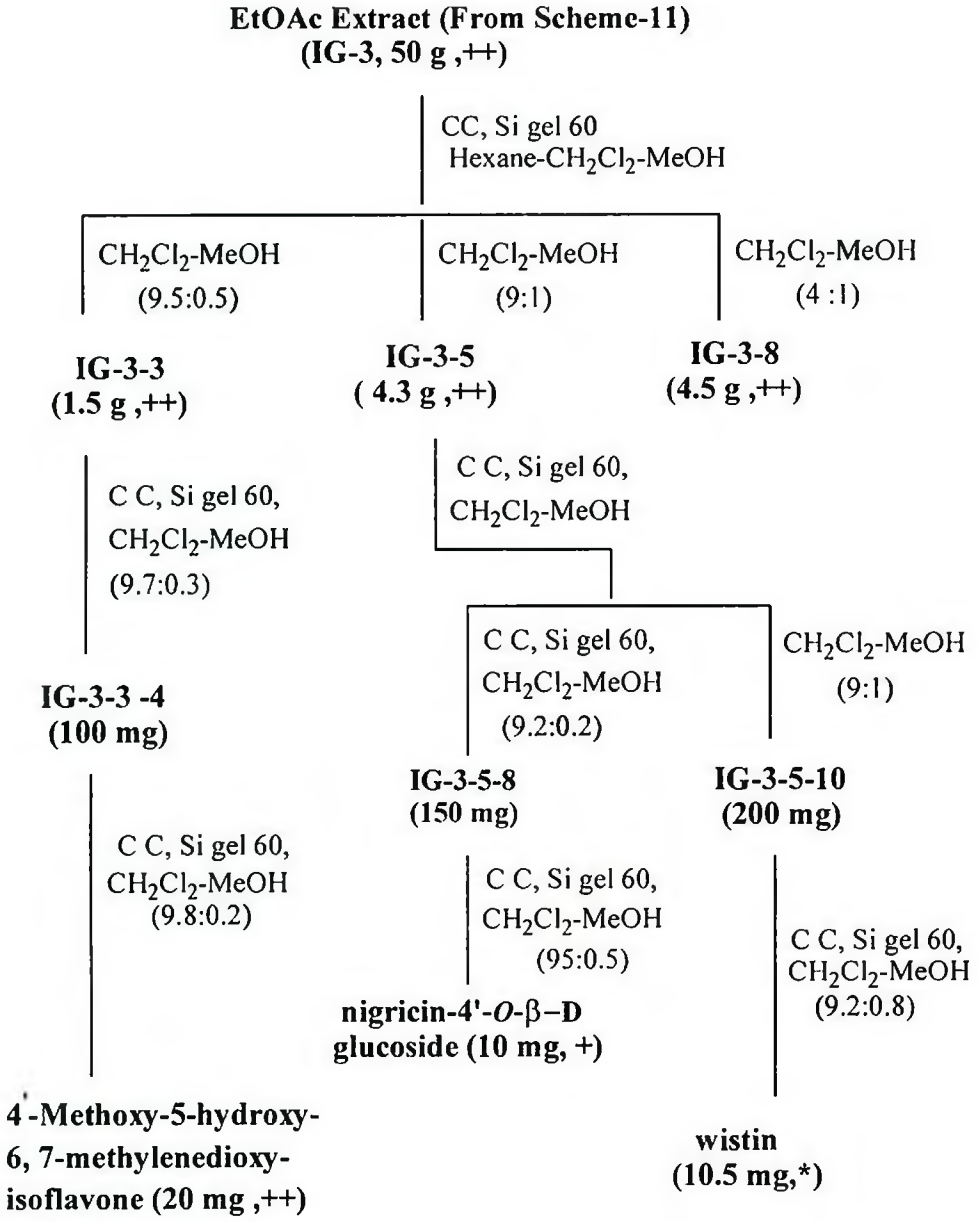
^{1}H-NMR (500 MHz, C_5D_5N): δ_H 8.10 (s, H-2), 6.70 (s, H-8), 7.20 (dd, $J_{2',3'/6',5}$= 8.70 Hz, $J_{2',6'/6',2'}$ = 2.1 Hz, 2H, H-2′/6′), 7.80 (dd, $J_{3',2'/5',6'}$ = 8.7 Hz, $J_{3',5'/5',3'}$ = 2.8 Hz, 2H, H-3′/5′), 5.85 (d, $J_{1'',2''}$ = 7.8 Hz, H-1″), 4.01 (s, 3H, OCH_3), 6.04 (s, 2H, O-CH_2-O).

^{13}C-NMR (125 MHz, C_5D_5N): δ_C 150.0 (d, C-2), 125.6 (s, C-3), 175.0 (s, C-4), 157.0 (s, C-5), 136.0 (s, C-6), 154.0 (s, C-7), 93.8 (d, C-8), 142.0 (s, C-9), 114.0 (s, C-10), 123.0 (d, C-1′), 131.0 (d, C-2′/6′), 116.0 (d, C-3′/5′), 153.0 (s, C-4′), 102.0 (s, O-CH_2-O), 61.2 (s, OCH_3).

EI-MS (*m/z* relative intensity %): 312 (97), 284 (92), 266 (72),195 (60), 166, 118 (55),

HR-EI-MS *m/z*: 312.2708 [$C_{17}H_{12}O_6$, requires 312.2735].

Scheme 12: BioassayGuided Isolation of Compounds 11-13



++ moderately active, + low active in mutant yeast bioassay,* not tested.

3.3.4.4 Compound 14- Irisolone (131)

Subfraction IG-2-8 (6.8 g, IC_{12} = 145 and 350 µg/mL) obtained from the CH_2Cl_2 extract (Scheme-11, Page-131) was subjected to further purification by column chromatography to afford twenty fractions (IG-2-8-1 – IG-2-8-20) on elution with gradients of CH_2Cl_2 - MeOH. The fraction IG-2-8-3 (3.0 g, 75 and 150 µg/mL) was found to be active in yeast assay and thus was subjected to further fractionation by silica gel column chromatography using CH_2Cl_2 and MeOH as eluents. The fraction IG-2-8-3-3 (2.0 g) was washed with MeOH, which yielded the pure compound **14** (1.5 g, IC_{12} = 45 and 100 µg/mL) (Scheme-13, Page-137). Another fraction IG-2-5 (10.5 g) was subjected to silica gel column chromatography and was eluted by hexane-EtOAc (3:2) (Scheme-14, Page-140). The fraction IG-2-5-8 (400 mg) was resubjected to silica gel column chromatography to obtain a pure fraction, which also yielded compound **14** (15.0 mg, CH_2Cl_2 100%), known as Irisolone (**131**). The percent yield was 1.65×10^{-1} and the R_f value of **14** was found to be 0.53 (CH_2Cl_2-MeOH, 9:9.01) IC_{12} = 45 and 100 µg/mL).

Physical Properties of irisolone (131)

Appearance: Light yellowish crystalline solid.

mp: 227-228° C.

IR ν_{max} (KBr): 3280 (OH), 2927 (CH), 1643 (C=O), 1520 (C=C), 1478, 908, 820 cm^{-1}.

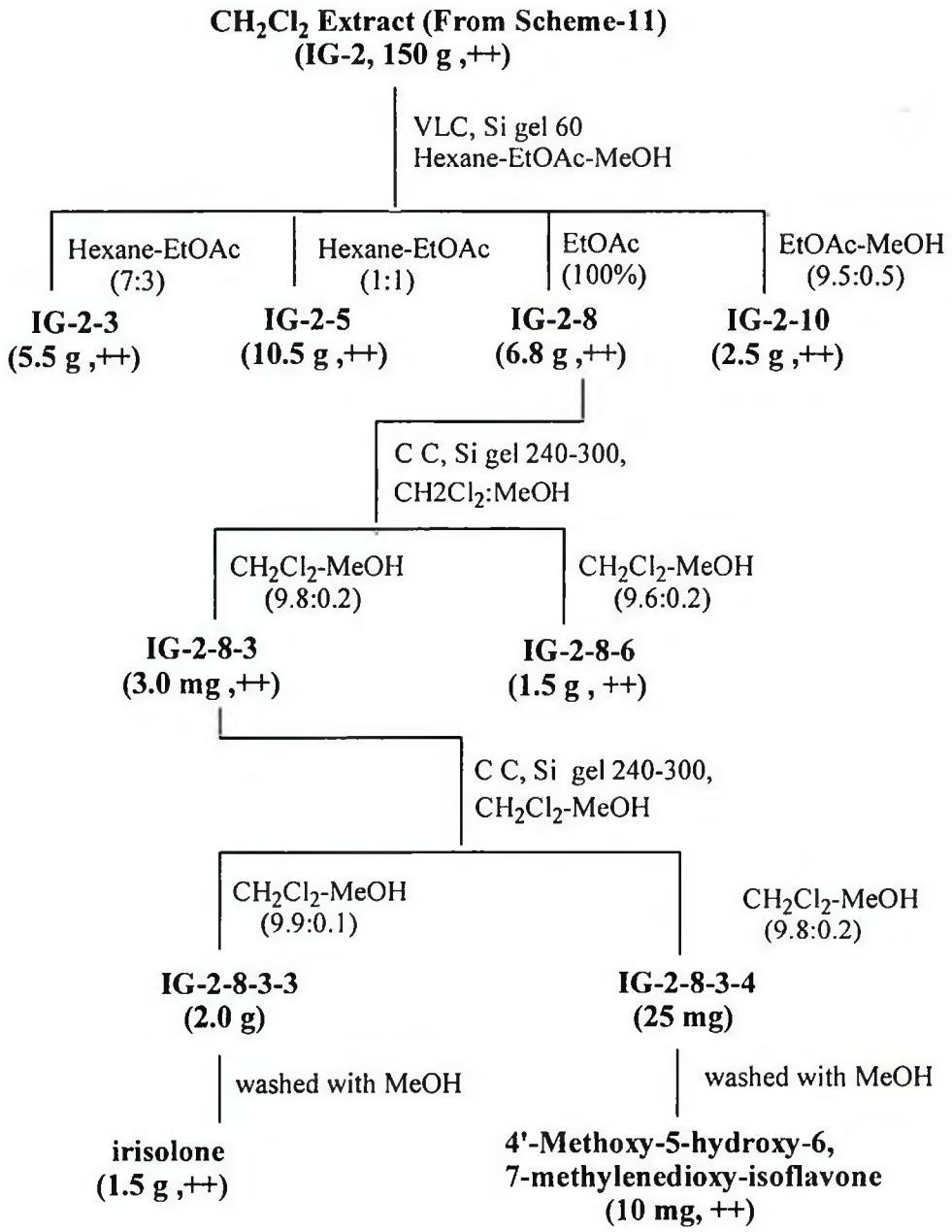
^{1}H-NMR (500 MHz, C_5D_5N): δ_H 8.00 (s, H-2), 6.80 (s, H-8), 7.20 (dd, $J_{2',3'/6',5}$= 9.0 Hz, $J_{2',6'/6',2'}$ = 2.5 Hz, 2H, H-2′/6′), 7.80 (dd, $J_{3',2'/5',6'}$ = 9.0 Hz, $J_{3',5'/5',3'}$ = 2.5 Hz, 2H, H-3′/5′), 5.85 (d, $J_{1'',2''}$ = 7.8 Hz, H-1″), 4.01 (s, 3H, OCH_3), 6.04 (s, 2H, O-CH_2-O), 11.7 (s, OH-5).

^{13}C-NMR (125 MHz, C_5D_5N): δ_C 150.7 (d, C-2), 125.6 (s, C-3), 175.0 (s, C-4), 153.0 (s, C-5), 136.0 (s, C-6), 154.2 (s, C-7), 93.0 (d, C-8), 141.0 (s, C-9), 114.5 (s, C-10), 123.5 (d, C-1′), 131.1 (d, C-2′/6′), 116.0 (d, C-3′/5′), 158.8 (s, C-4′), 102.0 (s, O-CH_2-O), 61.0 (s, OCH_3).

EI-MS (*m/z* relative intensity, %): 311.9 (97), 282.9 (100), 193.9 (25), 178.9 (17), 118 (60), 77 (38), 53 (52).

HR-EI-MS *m/z*: 312.2726 [$C_{17}H_{12}O_6$, requires 312.2736].

Scheme 13: Bioassay-Guided Isolation of Compounds 13 and 14



++ moderatly active in mutant yeast bioassay.

3.3.4.5 Compound 15- Irisolidone (132)

The subfraction IG-2-3 (5.5 g, IC_{12} = 150 and 200 µg/mL) eluted with hexane-EtOAc (7:3) from the CH_2Cl_2 extract (Scheme-14, Page-140) was rechromatographed on silica using solvent system hexane-EtOAc which yielded twelve fractions (IG-2-3–IG-2-3-12). The fraction IG-2-3-2 (500 mg) after further fractionation on column chromatography afforded the compound **15** (50 mg, hexane-EtOAc, 9.5: 0.5), known as Irisolidone (**132**). The percent yield was 5.05×10^{-4} and the R_f value of **15** was found to be 0.375 (hexane-EtOAc, 9:1).

Physical Properties of irisolidone (132)

Appearance: Light yellowish crystalline needle.

mp: 201-202° C.

IR ν_{max} (KBr): 3450 (OH), 2959 (CH), 2834 (CH), 1659 (C=O), 1575 (C=C), 1152, 981 cm-^{1}.

^{1}H-NMR (300 MHz, C_5D_5N): δ_H 7.86 (s, H-2), 11.5 (s, OH-5), 6.50 (s, H-8), 6.90 (dd, $J_{2',3'/6',5}$= 8.8 Hz, $J_{2',6'/6',2'}$ = 2.1 Hz, 2H, H-2′/6′), 7.40 (dd, $J_{3',2'/5',6'}$ = 8.8 Hz, $J_{3',5'/5',3'}$ = 2.1 Hz, 2H, H-3′/5′), 6.04 (s, 2H, O-CH_2-O), 4.00 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 11.70 (s, OH-5).

EI-MS (*m/z* relative intensity, %): 313.9 (100), 298.9 (93), 268 (14), 167 (12), 149 (23), 95 (15), 81 (13), 71 (11), 55 (15).

HR-EI-MS *m/z*: 314.0783 [$C_{17}H_{14}O_6$, requires 314.07901].

3.3.4.6 Compound 16- Muningin (174)

The subtraction IG-2-3-3 (350 mg, 145 and 200 μg/mL) obtained from the CH_2Cl_2 extract by repetitive column chromatography was subjected to further fractionation on a silica gel column. A fraction (Scheme-14, Page-140) obtained on elution with hexane-EtOAc (9:1) yielded the compound **16,** muningin (20 mg, hexane-EtOAc, 9.4:0.6). The percent yield was $2.02 \times 10^{-4}$and the R_f value of **16** was found to be 0.438 (hexane-EtOAc, 9:1).

Physical Properties of muningin (174)

Appearance: Yellow needle shaped crystals (20 mg).

mp: 185° C

UV λ_{max} MeOH (log ε): 268, 216, 199 nm.

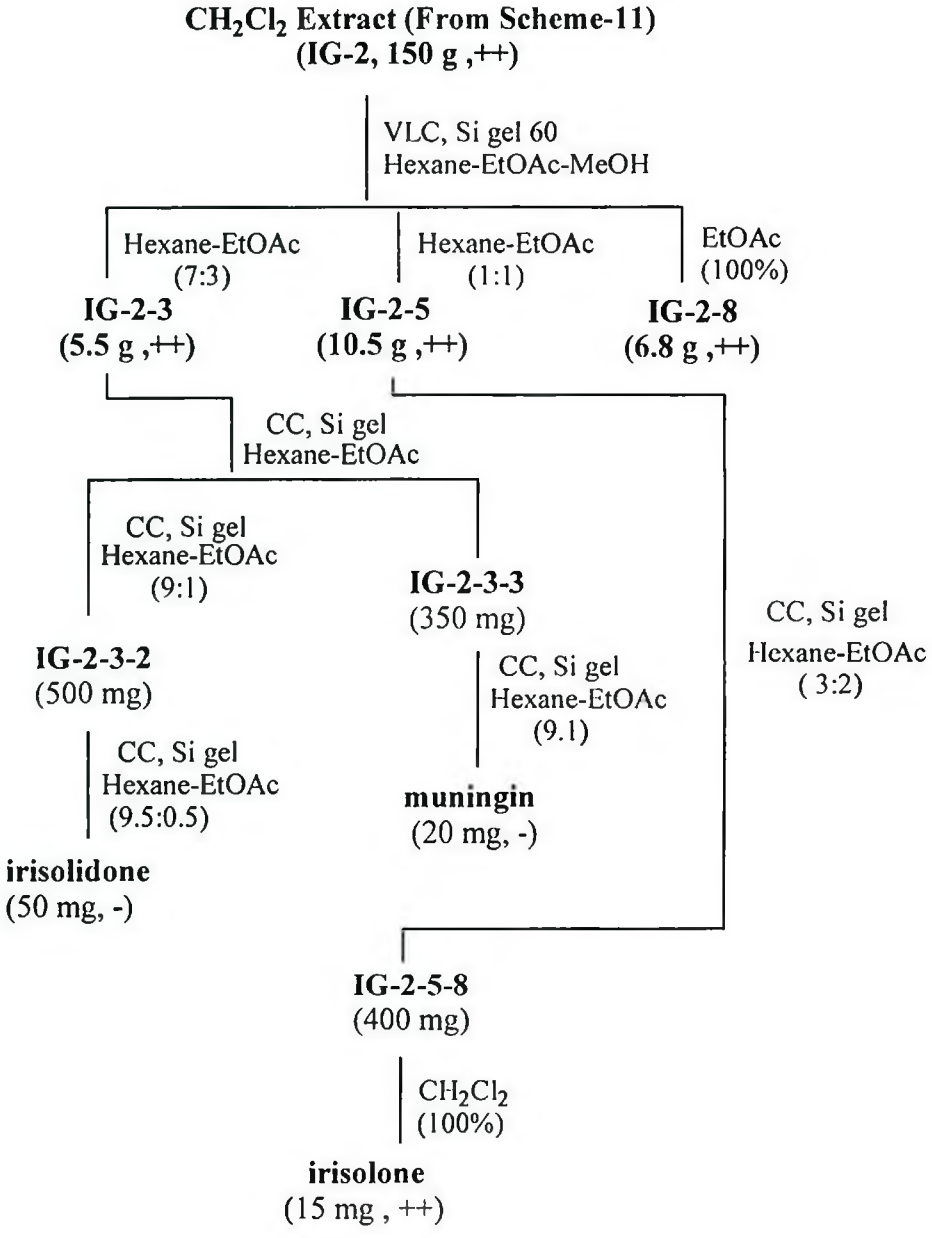
IR ν_{max} (KBr): 3454 (OH), 3360 (OH), 2959 (CH), 2832 (CH), 1623 (C=O), 1576 (C=C), 1152, 1036, 981, 934 cm^{-1}.

^{1}H-NMR (300 MHz, C_5D_5N): δ_H 7.80 (s, H-2), 6.50 (s, H-8), 7.45 (dd, $J_{2',3'/6',5}$= 8.8 Hz, $J_{2',6'/6',2'}$ = 2.2 Hz, 2H, H-2′/6′), 7.80 (dd, $J_{3',2'/5',6'}$ = 8.9 Hz, $J_{3',5'/5',3'}$ = 2.2 Hz, 2H, H-3′/5′), 4.10 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3).

EI-MS (*m/z* relative intensity, %): 314 (100), 298.9 (93), 268 (14), 167 (11), 149 (23), 95 (15), 81 (13), 71 (10), 55 (15).

HR-EI-MS *m/z*: 314.0408 [$C_{17}H_{14}O_6$, requires 314.0430].

Scheme 14: Bioassay-Guided Isolation of Compounds 14-16



++ moderately, - not active in mutant yeast bioassay

3.3.4.7 Compound 17- Irilone (128)

Fraction IG-2-10 (2.5 g, IC_{12} = 145 and 300 μg/mL) eluted by EtOAc-MeOH (9.5:0.5) from CH_2Cl_2 extract (Scheme-15, Page-143) was further fractionated into several fractions. The fraction IG-2-10-3 (50 mg) was purified on preparative silica gel plates (Merk, silica gel 60 F_{254}) using solvent system CH_2Cl_2-MeOH (9.8:0.2) to afford the compound **17,** Irilone (**128**). The percent yield was 1.5 x $10^{-4}$and the R_f value of compound **17** was found to be 0.20 (CH_2Cl_2, 100%). Compound **17** showed growth inhibition with IC_{12} values 60 and 85 μg/mL in RAD 52 and RAD+, respectively in yeast bioassay.

Physical Properties of irilone (128)

Appearance: Light yellowish crystalline solid.

mp: 230-231° C.

UV λ_{max} MeOH (log ε): 268, 215, 201, nm.

IR ν_{max} (KBr): 3455(OH), 3068 (CH), 2902 (CH), 1624 (C=O), 1529 (C=C), 1155, 1053, 922, cm^{-1}.

^{1}H-NMR (300 MHz, C_5D_5N): δ_H 7.30 (s, H-2), 6.00 (s, H-8), 6.74 (dd, $J_{2',3'/6',5}$= 8.7 Hz, $J_{2',6'/6',2'}$ = 2.1 Hz, 2H, H-2′/6′), 7.80 (dd, $J_{3',2'/5',6'}$ = 8.7 Hz, $J_{3',5'/5',3'}$ = 2.1 Hz, 2H, H-3′/5′), 6.74 (s, 2H, O-CH_2-O),.

EI-MS (*m/z* relative intensity, %): 297.9 (100), 239 (5), 180 (14), 149 (9), 118 (6), 57 (7).

HR-EI-MS *m/z*: 298.2463 [$C_{16}H_{10}O_{6,}$ required 298.24704.433).

3.3.4.8 Compound 18- β-Sitosterol glucoside (175)

The fraction IG-2-10-5 (90 mg, obtained from the CH_2Cl_2 extract by repeated column chromatography (Scheme 15, Page-143) was subjected to further column chromatography (silica gel) using solvent system CH_2Cl_2 and MeOH. A fraction eluted by CH_2Cl_2: MeOH (9.2:0.8) yielded the compound **18,** β-sitosterol glucoside (**175**) (40 mg). The percent yield was 4.04 x 10^{-4} and the R_f value of **18** was found to be 0.30 (CH_2Cl_2-MeOH, 9:1).

Physical Properties of β-sitosterol glucoside (175)

Appearance: Colorless needles from $CHCl_3$ and MeOH.

$[\alpha]_D^{25}$ -14.8° (*c*, 0.32, MeOH).

IR ν_{max} (KBr): 3450 (OH), 3040 (CH), 1645 (C=C) cm^{-1}.

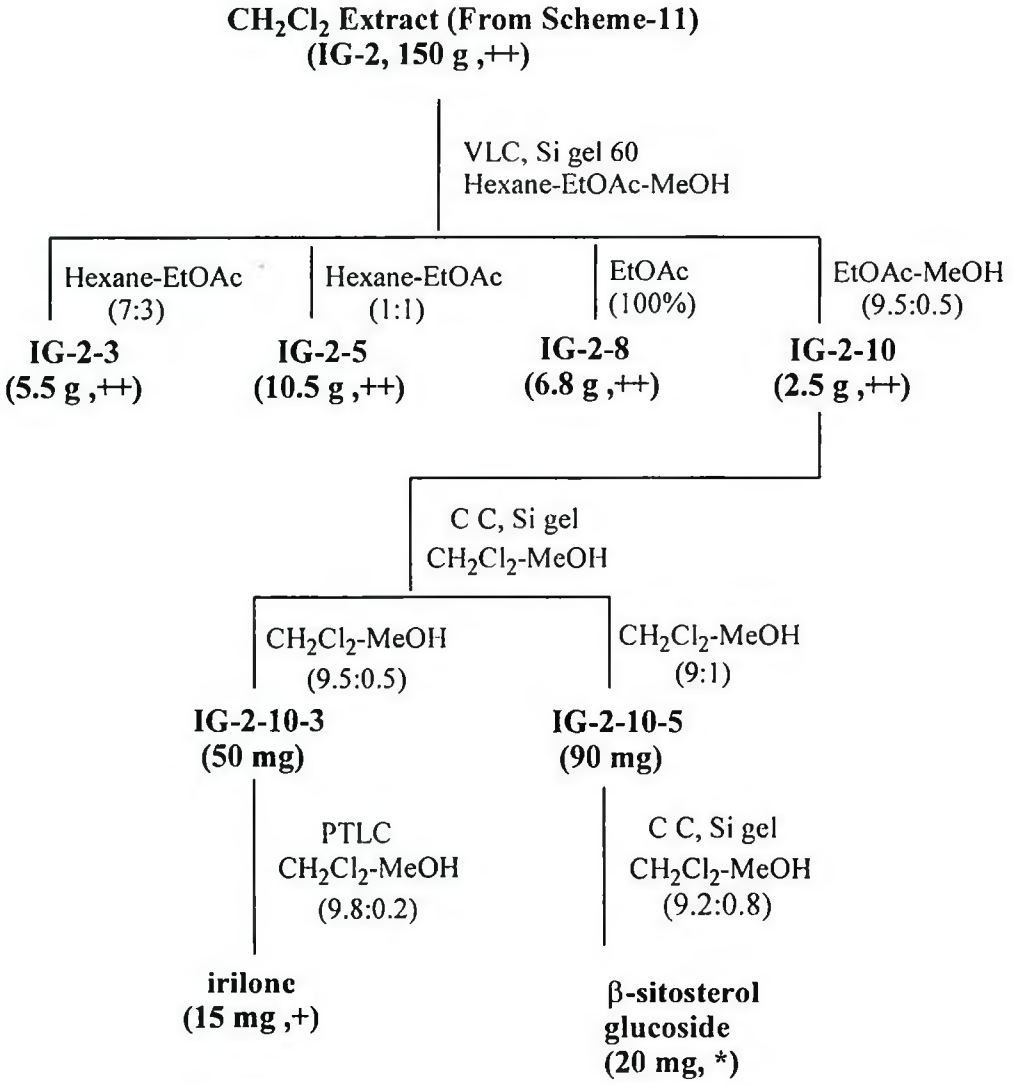
^{1}**H- NMR** (500 MHz, C_5D_5N): δ_H 5.3 (d, 7.2 Hz, H-1), 3.84 (m, H-3), 5.12 (bd, 5.4 Hz, H-6), 0.68 (s, H-18, 1.01 (s, 19-methyl), 0.92 (d, *J* = 6.2, 21- methyl),), 0.83 (d, *J* = 6.5), 0.81 (d, *J* = 6.5), 5.40 (d, 7.8 Hz, 1′′), 4.32 (H-2′′), 4.20 (H-3′′), 4.15 (H-4′′), 4.30 (H-5′′), 4.50 (dd, *J* =12.0 Hz, *J* = 2.0 Hz, H-6a′′), 4.40 (dd, *J* = 12.0 Hz, 5.5 Hz, H-6b′′).

^{13}**C-NMR** (125 MHz, C_5D_5N): δ_C 38.7 (t, C-1), 29.9 (t, C-2), 80.9 (d, C-3), 43.8 (t, C-4), 142.o (s), 122.1 (d, C-6), 33.0 (t, C-7), 32.9 (d, C-8), 50.4 (d, C-9), 37.1 (s, C-10), 21.5 (t, C-11), 40.8 (t, C-12), 43.0 (s, C-13), 56.9 (d, C-14), 25.8 (t, C-15), 29.7 (t, C-16), 56.6 (d, C-17), 12.2(q, C-18), 19.5 (q, C-19), 37.2 (d, C-20), 19.7 (q, C-21), 39.3 (t, C-22), 29.5 (t, C-23), 50.5 (d, C-24), 25.9 (d, C-25), 18.2 (q, C-26), 20.1 (q, C-27), 23.7 (t, C-28), 1.9 (q, C-29), 103.2 (d, C-1′), 74.1 (d, C-2′), 76.8 (d, C-3′), 70,2 (d, C-4′), 76.6 (d, C-5′), 61,9 (d, C-6′).

EI-MS (*m/z* relative intensity' %): 414 (M^+ -suger moiety, 18) 396 (25), 381 (70), 329 (20), 303(29), 275(10), 273 (22), 57 (100).

HR-EI-MS *m/z*, 576.4386 [$C_{35}H_{60}O_6$, required 576.4389].

Scheme 15: Bioassay-Guided Isolation of Compounds 17 and 18



++ moderately, + low activite in mutant yeast bioassay, *not tested.

3.4 Results and Discussion

3.4.1 Isoflavones and Isoflavone Glycosides

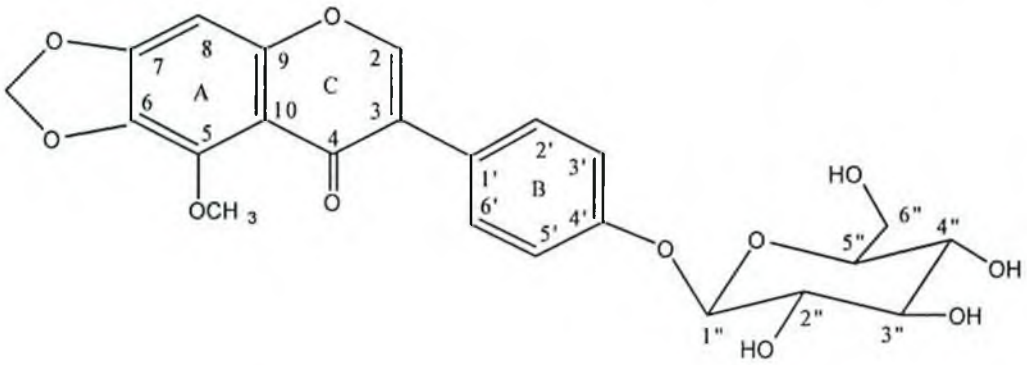
Rhizomes of *Iris germanica* (10 kg) were extracted with MeOH to obtain the parent extract (500 g). The parent extract was then suspended in H_2O and successively extracted with CH_2Cl_2 and EtOAc (1:1) to give a CH_2Cl_2 extract (150 g) and EtOAc extract (50 g) (Scheme 6, Page-). Both the extract was found to possess growth inhibitory activity in mechanism-based yeast bioassay. A bioassay guided fractionation on CH_2Cl_2 and EtOAc extracts were then carried out which yielded eight compounds (Experimental section, Scheme 6-10, Page-). A new isoflavone glycoside, one known isoflavone glycoside, five known isoflavones and one steroidal compound were isolated.

3.4.1.1. Nigricin 4′-*O*-β-D-glucoside (171) - A New Isoflavone Glycoside.

Compound **11** was isolated as a light yellowish solid from the EtOAc extract of *I. germanica rhizome* (Scheme-12, Page-135). The strong IR absorptions at 3345 (OH), 1665 (C=O), 1565 (aromatic C=C) cm^{-1} along with UV absorptions at 319, 262 nm suggested an flavone type skeletone in the molecule. This conclusion was further substrained by a ^{1}H-NMR signal at δ_H 7.96 (C_5D_5N), which is typical for the 2-hydrogen of an isoflavone (Harbone et al., 1975, Caballero et al., 1986; Tanaka et al., 1975).

The molecular ion (M^+) was not visible in the EI-MS of compound **11**. The molecular formula $C_{23}H_{22}O_{11}$ of compound **11** was determined by HR-FAB-MS (–ve), which showed pseudo M^+ peak at *m/z* 473.1296 (M^+ - H) (calcd. 474.1240) and HR-FAB-MS (+ve), showed M^+ at 475.1235 (M^+ + H) (calcd. 474.1240). The ions at *m/z* 312 (M^+ - one sugar molecule) indicated the presence of one sugar unit. The peak *at m/z*, 312 ($C_{17}H_{12}O_6$) in the EIMS was due to the loss of a sugar unit from M^+. The fragment at *m/z* 284 indicated the presence of *O*-methyl and methylenedioxy containing aromatic ring A, while the fragments at *m/z* 194 and 195 were resulted from the retro Diels-Alders cleavage of ring C.

The NMR spectra (C_5D_5N) of compound **11** showed a number of signals characteristic of sugar (anomeric proton at δ_H 5.66 (d, $J_{1'',2'}$ = 7.5 Hz /δ_C 10.2.0), one methylene dioxy (δ_H 6.02, s /δ_C 10.2.4) and *O*-methyl (δ_H 4.06, s /δ_C 61.1) and one carbonyl (δ_C 174.0) moieties. The broad band (BB) decoupled ^{13}C-NMR spectrum (C_5D_5N) of **11** showed resonances of twenty three-carbon atoms. The DEPT spectra revealed the presence of one more methylene, eleven methines and nine quaternary carbons in the molecule.



Nigricin 4′-*O*-β-D-glucoside **(171)**

From the above assumptions the structure of **11** was suggested to be an isoflavone compound with one *O*-methyl group, a methylenedioxy group and a sugar ring in the molecule. The complete structure of **11** was then determined by ^{1}H-, ^{13}C-NMR, COSY-45°, HMQC and HMBC spectral analysis (Table-10) and a comparative study with reported data (Nasr et al., 1980; Joao B F., et al., 1999).

From the ^{1}H-, ^{13}C-NMR and HMQC spectral analysis a one proton signal at δ_H 6.76 (s) / δ_C 93.8 was assigned for H-8 of ring A. Another one proton signal at δ_H 7.96 (s) / δ_C 151.4 was assigned to the H-2 in ring C. A pair of double doublets at δ_H 7.40 (d, $J_{2',3'/\ 6',5'}$ = 9.0 Hz/δ_C 130.0) and δ_H 7.64 (d, $J_{3',2'/5'6'}$ = 9.0 Hz / δ_C 116.0) each for two protons/carbons integration were assigned to C-2′, C-6′ and C-3′, C-5′ respectively. All the NMR assignments were confirmed by HMQC and HMBC spectral analysis (Table-**10**).
The HMBC spectra (Fig. 45) showed the long-range coupling between carbon and protons. H-2 at δ_H 7.96 was found to be correlated with C-3 (126.0), C-4 (δ_C 174.4) and

C-9 (δ_C 154.8). Strong interactions were found between H-2′/6′ (δ_H 7.40) of ring B with C-3 (δ_C 126.0), C-1′ (δ_C 125.0) and C-4′ (δ_C 158.0). On the other hand H-3′/5′ (δ_H 7.64) were correlated with C-4′ (δ_C 158.0), and C-1′ (δ_C 125.0).

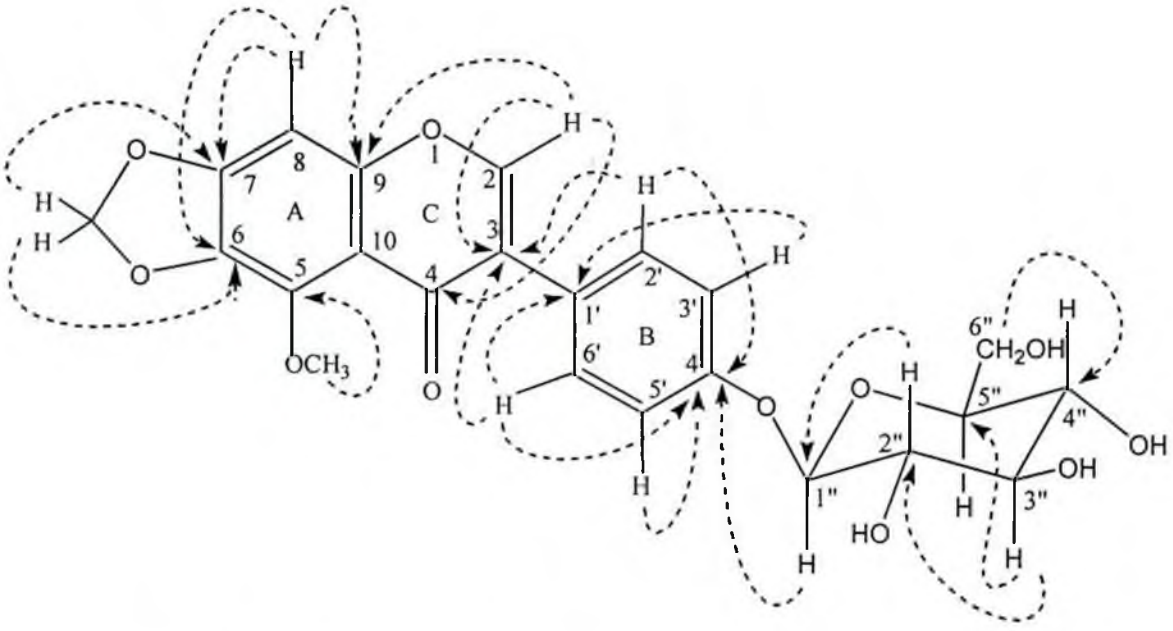


Fig. **45**. Important HMBC interactions in nigricin 4′-*O*-β-D-glucoside (**171**).

The *O*-methyl protons at δ_H 4.06 showed strong interactions with C-5 (δ_C 141.8) which confirmed its position at C-5 (δ_C 141.8) of ring A. Interactions of H-8 at δ_H 6.70 with C-9 (δ_C 154.8), C-7 (δ_C 153.0), C-6 (δ_C 136.0) and C-10 (δ_C 114.5) confirmed the position of H-8 in ring A at C-8 position. On the other hand interactions of $-O_2CH_2$ protons with C-6 (δ_C 136.0) and C-7 (δ_C 153.0) confirmed the position of methylenedioxy group at C-6 and C-7 position.

In ^{1}H-^{1}H Cosy-45° spectra of **11** it was found that the anomeric proton at δ_H 5.66 (d, $J_{1'',2''}$ = 7.5 Hz, δ_C 102.0) showed correlation with H-2″ at δ_H 4.32 (t, $J_{1'',2'',3''}$ = 9.0 Hz, δ_C 72.0). The H-2″ showed further interactions with H-3″ at δ_H 4.38 (t, $J_{2'',3'',4''}$ = 9.0 Hz, δ_C 78.0) and the latter also showed coupling with H-4″ resonated at δ_H 4.32 (t, $J_{3'',4'',5''}$ = 9.0 :Hz, δ_C 74.2). H-4″ was in turn coupled with H-5″ at δ_H 4.15 (m, δ_C 78.8). On the other hand H-5″ showed geminal couplings with H-6″ at δ_H 4.50 (dd, $J_{6''a,6''b}$ = 12.0 Hz,

$J_{6'a,5}$ = 2.0 Hz) and δ_H 4.40 (dd, $J_{6''b,6''a}$ = 12.0 Hz, $J_{6''b,5}$ = 5.5 Hz, δ_C 61.2). In HMBC spectra, H-3″ δ_H 4.38 was found to be correlated with C-5″ (δ_C 78.8) and C-2″ (δ_C 74.0). The correlation of H-2″ (δ_H 4.32) was also found with C-1″ (δ_C 102.0). The most important interactions of the anomeric H-1″ (δ_H 5.66) was found to be correlated with C-4′ (δ_C 158.0). The HMBC interactions confirmed the presence of a methylene dioxy at 6,7 position and a methoxy group at 5-positon of A ring. A comparative study of the chemical shifts with the reported data indicated that the aglycone in compound **11** is the known nigricin or irisolone (Gopinath et al., 1961; A-Khalil et al., 1994). The identity of the sugar as β-D- glucose was established through comparison of the NMR chemical shift of compound **11** with the reported data (Seo, et al 1978; Pfeffer, et al., 1979 and by comparative study of the R_f value after the hydrolysis of compound with the authentic samples (compound **131**). This confirmed the aglycone moiety of compound **11** as nigricin (Bedir et al., 2001) and the glycone moiety as glucose.

The structure of compound **11** therefore elucidated to be as, 5-methoxy-3-(4′-*O*-β-D-glucopyranosyl)-6,7-methylenedioxy-4H-1-benzo-pyran-4-one and was named as nigricin 4′-*O*-β-D-glucoside (**171**). The compound showed growth inhibition with IC_{12} values 75 and 125 μg/mL in RAD 52 and RAD+, in yeast assays respectively.

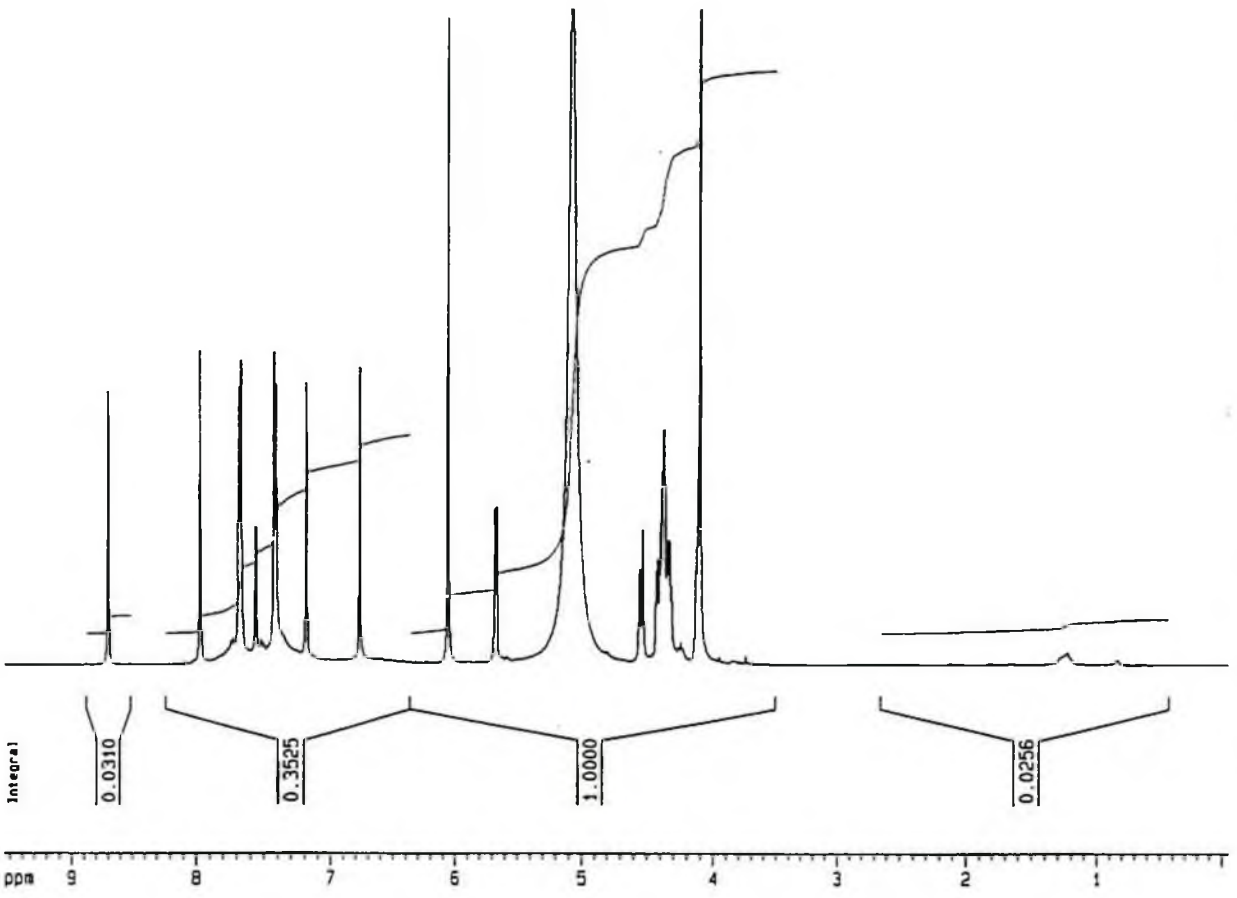
Table 10. NMR spectral data of nigricin 4′-*O*-β-D-glucoside (**171**).

	C_5D_5N		MeOH		C_5D_5N	
C/H No.	^{1}H-NMR[a] δ_H (mult., *J*=Hz)	^{13}C-NMR[b] δ_C (mult.)*	^{1}H-NMR[a] δ_H (mult. *J*=Hz)	^{13}C-NMR[b] δ_C (mult.	COSY	HMBC
2	7.96 (s)	151.4 (d)	8.00 (s)	153.2	-	C-3, C-4, C-9
3	-	126.0 (s)	-	126.3	-	-
4	-	174.4 (s)	-	177.5	-	-
5		141.8 (s)	-	142.4	-	-
6	-	136.0 (s)	-	136.6	-	-
7	-	153.0.0 (s)	-	155.4	-	-
8	6.76 (s)	93.8 (d)	6.77 (s)	94.0	-	C-9, C-7, C-6, C-10
9	-	154.8 (s)	-	156.4	-	-
10	-	114.8 (s)	-	114.4	-	-
1′		125.8 (s)	-	127.2	-	-
2′	7.40 (d, 9.0)	130.0 (d)	7.14 (d, 9.0)	131.5	H-2′, H-3′	C-1′, C-3, C-4′
3′	7.64 (d, 9.0)	116.0 (d)	7.43 (d, 9.0)	117.5	H-3′, H-2′	C-4′, C-1′
4′	-	158.0 (s)	-	158.9	-	-
5′	7.64 (d, 9.0)	116.0 (d)	7.43 (d, 9.0)	117.5	H-4′, H-6′	C-4′, C-1′
6′	7.40 (d, 9.0)	130.0 (d)	7.14 (d, 9.0)	131.5	H-6′, H-5′	C-1′, C-3, C-4′
1″	5.66 (d, 7.5)	102.0 (d)	4.90 (d, 7.5)	102.0	H-2″	C-4′
2″	4.32 (t, 9.0)	74.0 (d)	3.45 (m)	74.5	H-1″, H-3″	C-1″
3″	4.38 (t, 9.0)	78.0 (d)	3.46 (m)	78.1	H-2″, H-5″	C-2″, C-5″
4″	4.32 (t, 9.0)	74.2 (d)	3.41 (m)	74.9	H-3″, H-5″	-
5″	4.15 (m)	78.8 (d)	3.39 (m)	77.9	H-5″, H_2-6″	-
6a″	4.50 (dd, 12.0,2.0)	61.2 (t)	3.90 (dd, 12.0, 2.0)	62.5	H-6b″, H-5″	C-4″
6b″	4.40 (dd, 12.0, 5,5)	-	3.71 (dd, 120, 5.5)		H-6a″, H-3″	-
O-CH_2-O	6.02 (s)	102.0 (t)	6.10 (s)	104.0	-	C-6, C-7
OCH_3	4.06 (s)	61.1 (s)	4.03 (s)	61.3	-	C-5

[a] Data recorded at 500 MHz.
[b] Data recorded at 125 MHz.
*Multiplicity determined by DEPT NMR and HMQC spectra.

ISMET ARA/ DR. IQBAL/ IIG 7/C_5D_5N

(Compound 11)

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F2P                     -0.055 ppm
F2                    -27.52 Hz
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HZCM                240.14861 Hz/cm
```
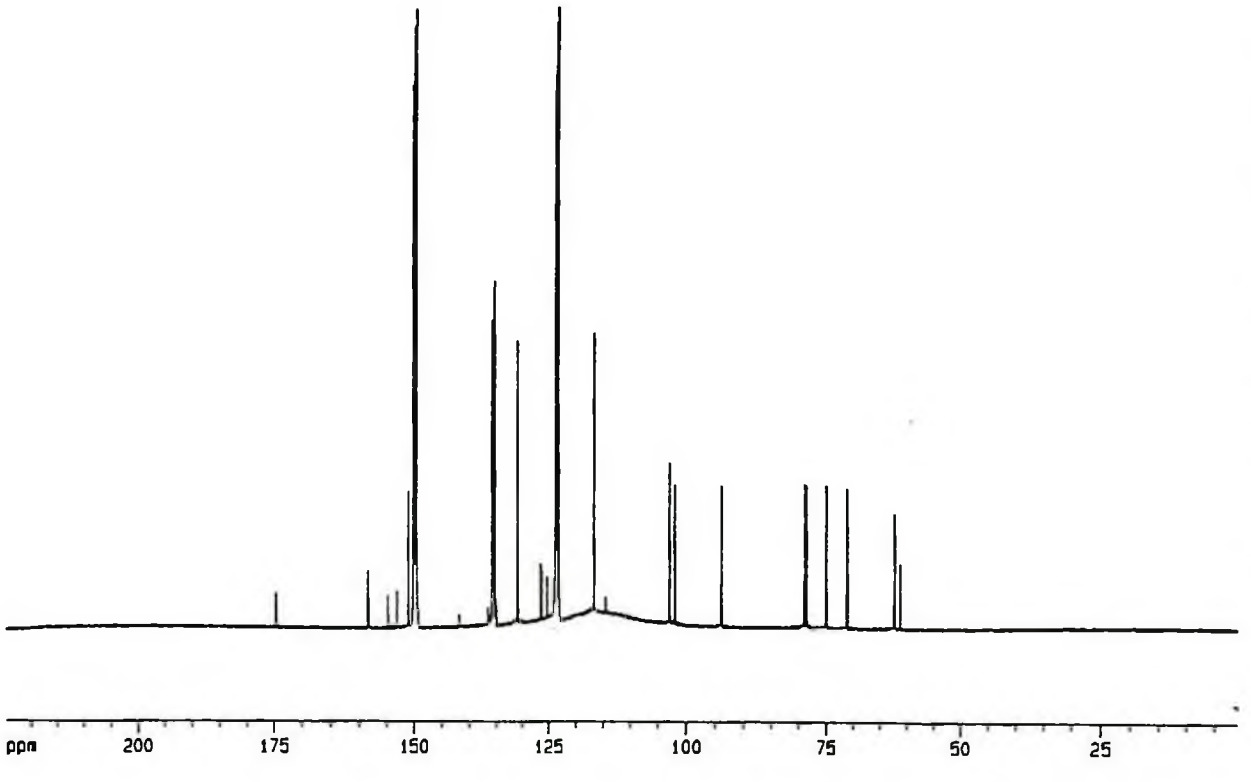

Fig.43. ^{1}H-NMR Spectrum (C_5D_5N, 500 MHz) of Nigricin 4′-*O*-*β*-glucoside (171)

ISMET ARA/ DR. IQBAL/ IIG 7/C_5D_5N

(Compound **11**)



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D11 0.03000000 sec
D12 0.00002000 sec

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PL1 -6.00 dB
SFO1 100.6740915 MHz

======== CHANNEL f2 ========
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PCPD2 100.00 usec
PL2 120.00 dB
PL12 24.00 dB
PL13 24.00 dB
SFO2 400.3320000 MHz

F2 - Processing parameters
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WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

1D NMR plot parameters
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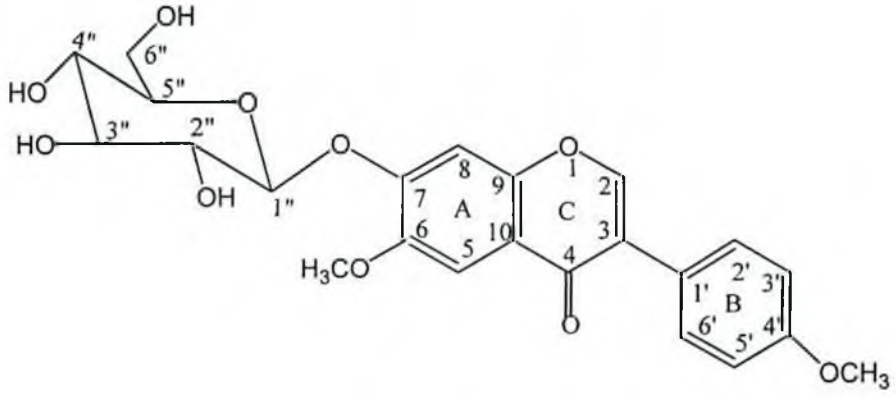
F1 22605.40 Hz
F2P 0.886 ppm
F2 70.30 Hz
PPMCM 11.19832 ppm/cm
HZCM 1126.75478 Hz/cm

Fig.**44.** ^{13}C- NMR Spectrum (C_5D_5N, 100 MHz) of Nigricin 4′-*O*-β-D-glucoside (**171**)

3.4.1.2 Wistin (172)- A Known Isoflavone Glycoside

The compound **12** was isolated as a white amorphous solid (10 mg) from the ethyl acetate extract (Scheme-12, Page-135) of *I. germanica*. The molecular formula of **12** was calculated as $C_{23}H_{24}O_{10}$ by combination of its HR-FAB-MS (-ve) *m/z* 459 $[M-H]^-$, EIMS *m/z*, 298 [M^+-one suger moiety], and ^{13}C NMR spectral data (Section: 3.3.4.2), which indicated the presence of one sugar moiety. The UV spectrum of compound **12** showed 320 nm (3.80), 262 nm (4.33) and its IR spectrum showed absorptions for OH (3350 cm^{-1}) and C=O (1730 cm^{-1}) (Section: 3.3.4.2).

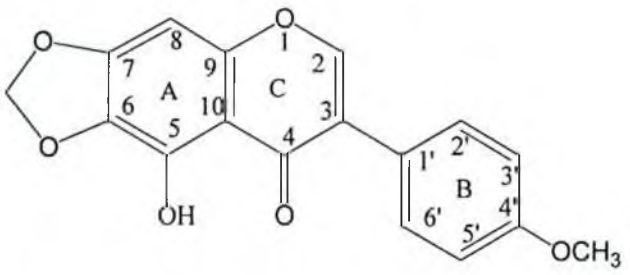


Wistin (**172**)

The above spectral data of **12** along with the signal for H-2 in ^1H-NMR at δ_H 8.13 (Harbone, J.B., et. al., 1975) and in ^{13}C-NMR the signal for C-2 at δ_C 153.3 and a quaternary carbon at δ_C 125.0 established that the compound **12** is an isoflavone glycoside. NMR spectra of **12** displayed in addition signals for two methoxyl groups at δ_H 3.70/δ_C 55.1 and δ_H 4.06/δ_C 56.0. A singlet at δ_H 7.20 for two protons integration was assigned for H-5 and H-8. Two doublets at δ_H 7.10 (d, J = 8.2 Hz)/δ_C 130.0 and δ_H 7.80 (d, J = 8.4 Hz)/δ_C 113.0 were assignable to H-2′/6′ and H-3′/5′ with the B ring oxygenated at 4′. A doublet at δ_H 5.85 (d, J = 7.8 Hz) was assigned for the glucose moiety of β linkage. The signal at δ_C 175.0 was attributed to the carbonyl carbon. A comparative study of IR, UV (Shibata et al., 1963; Harbone et al., 1963; Joao B.F., et al., 1999) and NMR data of compound **12** with the literature value (Joao B F., et al., 1999) fully supported the structure of **12** as afromosin-7-*O*-β-D-glucopyranoside, previously isolated from Bowdichia virgilioides named as wistin (**172**) (Velozo et al., 1999; Joao B F., et al., 1999).

3.4.1.3 5-Hydroxy-4′-methoxy-6,7-methylenedioxy-isoflavone-(173)- A known Isoflavone

Compound **13** (30 mg) was isolated as yellowish amorphous solid from the EtOAc extract of the same plant (Scheme-12 and-13, Page-135 and-137). A molecular formula of $C_{17}H_{12}O_6$ for compound **13** was determined by the molecular ion (*m/z* 312) and ^{13}C-NMR and DEPT data. The presence of a hydroxyl (3260 cm^{-1}) and a carbonyl group (1772 cm^{-1}) were indicated in its IR spectrum. This data together with the UV absorption maxima at 355 and 269 nm (Section: 3.3.4.3), suggested the presence of an isoflavone moiety and ^{1}H NMR signal at δ_H 8.00, is typical for the H-2 proton of an isoflavone.



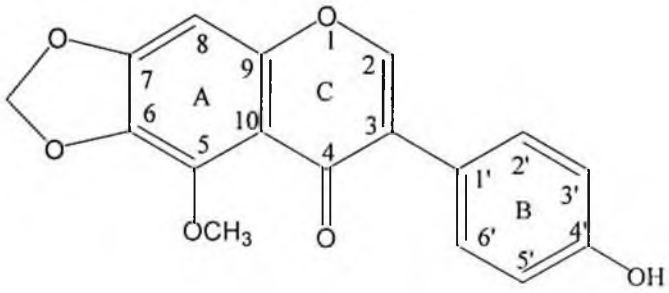
5-Hydroxy-4′-methoxy-6,7-methylenedioxy-isoflavone (**173**)

The presence of methylene dioxy moiety in **13** was supported by a sharp two protons singlet at δ_H 6.04/δ_C 102.0 in the ^{1}H and ^{13}C NMR spectra. A singlet of three protons intigration at δ_H 4.01/δ_C 61.2 revealed the presence of a methoxyl group in the molecule of **13**. The singlet at δ_H 6.80/δ_C 93.8 was attributed for H-8 of ring A. Another two signals each for two protons at δ_H 7.2 (dd, $J_{3',2'}$ = 9.0 Hz, and J $J_{3',5'}$ = 2.5 Hz)/ δ_C 131 and δ_H 7.8 (dd, $J_{2',3'}$ = 9.0 Hz, $J_{2',6'}$ = 2.0 Hz)/ δ_C 116 was assigned for H-3′/5′ and 2′/6′ respectively (Section 3.3.4.3). Which indicated the presence of an oxygenated group at 4′ position of B ring. A singlet at δ_H 11.05 confirmed the Presence of a OH group at C-5 position and thus confirmed the position of methoxy group at C-4′ position. The structure of compound **13** was then assigned as 5-Hydroxy-4′-methoxy-6,7-methelynedioxy-isoflavone (**173**) by a comparative study of its NMR data with the reported compound (Messanga, 1998). The compound **13** exhibited growth inhibitory activity IC_{12} = 43 and 100 µg and mL in RAD 52 and RAD+ respectively.

3.4.1.4 Irisolone (131)- A Known Isoflavone.

Compound **14** (1.5 g) was obtained from the EtOAc extract of the same plant by repeated column chromatography (Scheme-13 and -14, Page-137 and -140)). The molecular formula of **14** was deduced to be as $C_{17}H_{12}O_6$ from the EIMS (*m/z* 311) and DEPT spectra. Its IR spectra revealed the absorption for OH (3280 cm^{-1}) and C=O (1772 cm^{-1}).

The NMR spectra of **14** showed a number of signals for isoflavone (δ_H 8.00, s /δ_C 150.7); methylenedioxy (δ_H 6.04, s /δ_C 102.0) and a methoxy (δ_H 4.01, s /δ_C 61.0) group. A one proton signal at δ_H 6.80/δ_C 93.0 was assigned for H-8 of ring A. A comparative study of the NMR data of **14** (Section: 3.3.4.4) with the literature value of the reported compounds (Al-Khalil et al., 1994; Gopinath et. al., 1961) led to the structure of **14** as a known compound 4′-hydroxy-5-methoxy-6,7-methelyne dioxy-isoflavone, which was named as irisolone (**131**), previously isolated from *Iris nigricans (*Al-Khalil et al., 1994), *Iris nepalensis* (Gopinath et. al., 1961) and *Iris geremanica* (Dhar et al., 1972)) *etc.*

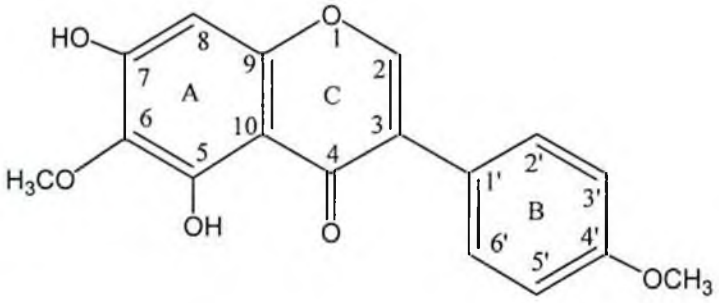


Irisolone (**131**)

The compound **14** showed growth inhibitory activity with IC_{12} values 45 and 100μg/mL.

3.4.1.5 Irisilidone (132)- A known Isoflavone

Compound **15** was isolated from the CH_2Cl_2 extract of the same plant (Scheme- 14, Page-140). The molecular formula for Compound **15** was determined as $C_{17}H_{14}O_6$ from the HR-EI-MS (*m/z* = 314.0783). The Presence of hydroxyl group (3450 cm^{-1}) and carbonyl group (1659 cm^{-1}) was assigned from its IR spectra.

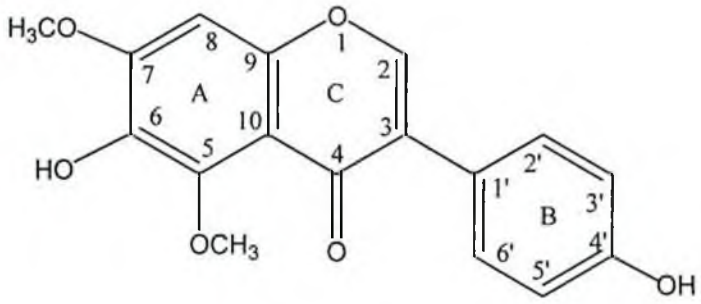


Irisolidone (**132**)

The ^1H NMR spectrum of compound **15** represents the characteristics signals for isoflavone moity (δ_H 7.80), two methoxyl groups δ_H 3.80 and 4.00. A singletat δ_H 11.70 confirmed the Presence of a OH group at C-5 position. The NMR data of **15** (Section: 3.3.4.5) with the reported values (Kaneko et al., 1988) assigned the structure of **15** as 5,7-Dihydrox-4',6-dimetyhoxy-isoflavone, a known compound, irisolidone (**132**), previously isolated from *Iris germanica* and also from other plants (Ali et al., 1983; Kaneko et al., 1988).

3.4.1.6 Muningin (174)- A Known Isoflavone

Compound **16** was also isolated from the EtOAc extract of the same plant (Scheme-14, Page-140). The molecular formula $C_{17}H_{14}O_6$ was assigned from the HREI MS (*m/z* 314.0408).

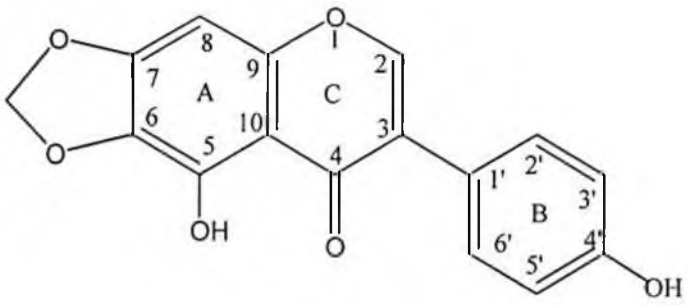


Muningin (**174**)

The ^{1}H NMR spectra of **16** (Section: 3.3.4.6) showed the signal for isoflavone (δ_H 7.80, s). A one proton signal at δ_H 6.50/ was assigned for H-8 of ring A. Two signals for three protons intrigation (δ_H 4.10 and 3.60) was assigned for two methoxyl groups. Another two signals each for two protons intigration at δ_H 7.45 (dd, $J_{2',3'/6',5}$= 8.8 Hz, $J_{2',6'/6',2'}$ = 2.2 Hz), and δ_H 7.80 (dd, $J_{3',2'/5',6'}$ = 8.9 Hz, $J_{3',5'/5',3'}$ = 2.2 Hz) was assigned for H- 2′/6′ and 3′/5′respectively. Which indicated the presence of a oxygenated group at 4′ position of B ring. The molecular formula of 16 agreed the presence of two hydroxyl group in the molecule. Absence of any downfield singlet as in compound **15** (δ_H 11.05) confirmed the position of one methoxyl group at C-5 position. A comparative study of the NMR data of **16** (Section: 3.3.4.6; Page-139) with compound **15** (Section: 3.3.4.5; Page-138) and with the literature value of the reported compound led to the structure of compound **16** as a known compound 4′,6-Dihydroxy-5,7-dimethoxy-isoflavone, previously isolated from *Podocapus angolensis* (King, 1952) and was named was muningin (**174**).

3.4.1.7 Irilone (128)- A known Isoflavone.

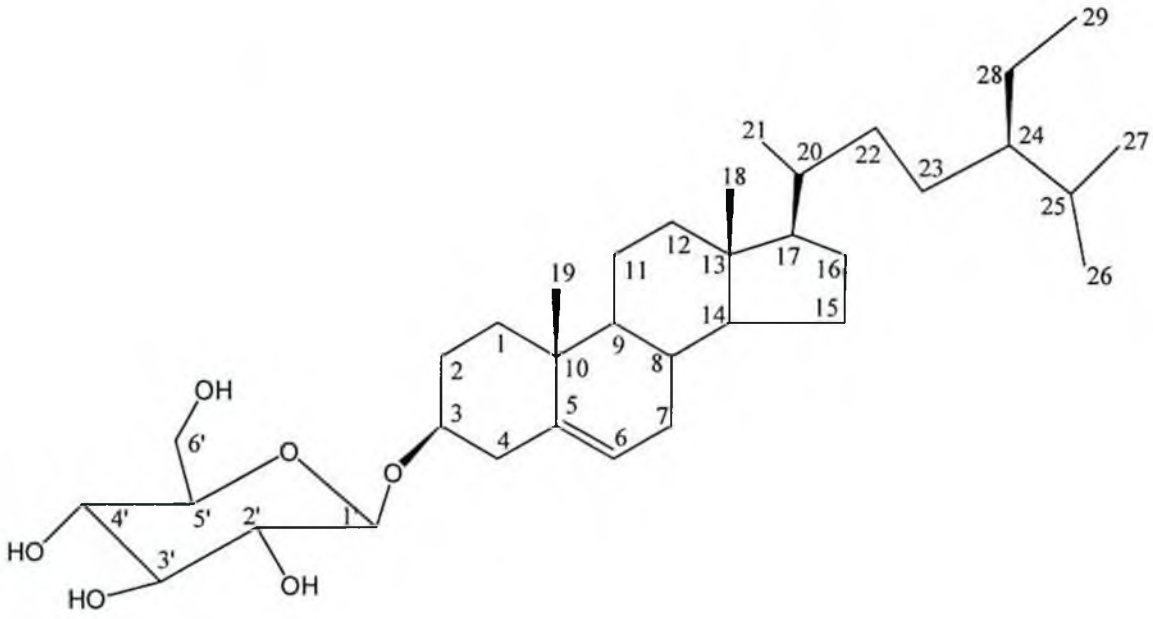
Compound **17** was isolated from the CH_2Cl_2 extract by repeated column chromatography (Scheme-15, Page-143). A molecular formula $C_{12}H_{10}O_6$ (**17**) was determined from HR-EI-MS (*m/z* 298.2463). Analysis of IR, UV, and ^{1}H-NMR data of **17** (Section: 3.3.4.7, Page-141) assigned the structure of **17** as a known compound 4′,5-Dihydroxy-6,7-methylenedioxy-isoflavone, earlier reported from the same plant and named as irilone (**128**) (Dhar et al., 1972). The compound was found to show growth inhibitory activity with IC_{12} values 60 and 80 µg/mL in RAD 52 and RAD+ respectively.



Irilone (**128**)

3.4.2 β-Sitosterol glucoside (175)-A Known Steroidal Glycoside.

Compound **18** was isolated as transparent needle shaped crystals from the CH_2Cl_2 extract of the same plant (Scheme-15, Page-143). A molecular formula $C_{35}H_{60}O_6$ was determined for compound **18** from its EI-MS (*m/z,* 414) and HR-FAB-MS. A comparative study of NMR data (Section: 3.3.4.8, Page-142) with the literature values (Daise et al., 1993; Jain et al., 1990; Kojo et al., 1989; Stone et al., 1966), the structure of compound **18** was determined as the known compound β-sitosterol glucoside (**175**), isolated for the first time from this plant.



β-Sitostereol glucoside (**175**)

3.4.3 Results of Yeast-Bioassay

The extracts, fractions, subfractions and pure compounds of *Iris germanica* rhizomes were tested in mechanism based yeast bioassay. The activity results is given in Table-11 and also shown in Graph-2.

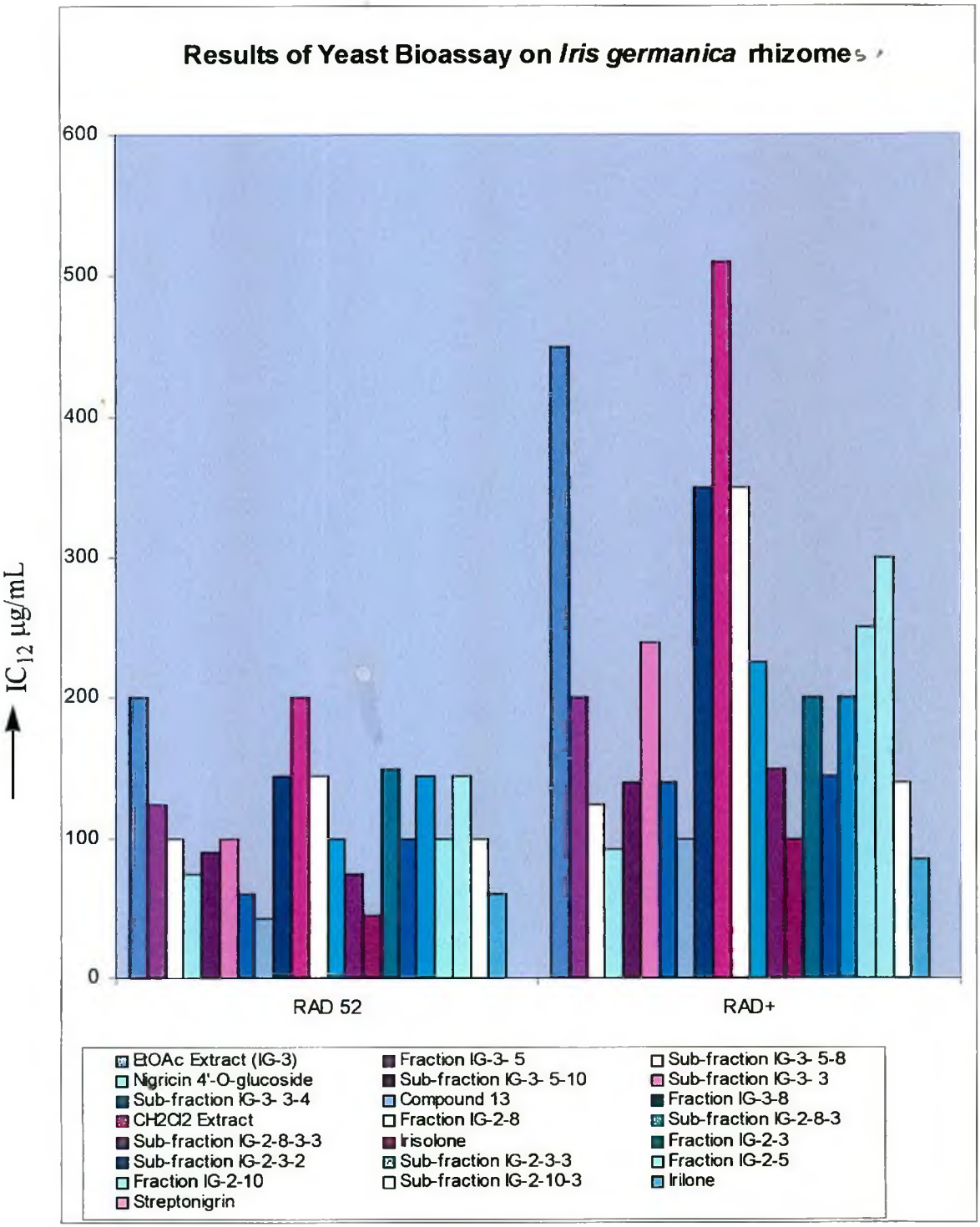
The CH_2Cl_2 and EtOAc soluble part of the parent MeOH extract of *Iris germanica* rhizome showed growth inhibition with IC_{12} values 200 and 510 μg/mL, and 200 and 450 μg/mL in RAD 52 and RAD+, respectively. A bioassay guided fractionation of the CH_2Cl_2 soluble part yielded twenty fractions. Out of twenty fractions only four fractions IG-2-3, IG-2-5, IG-2-8 and IG-2-10 showed growth inhibition with IC_{12} values 150 and 200 μg/mL, 100 and 250 μg/mL, 145 and 350 μg/mL, 145 and 300 μg/mL in RAD 52 and RAD+, respectively. Out of fifteen fractions of EtOAc extracts only three fractions showed growth inhibition IG-3-3, IG-3-5, IG-3-8 with IC_{12} values 100 and 240 μg/mL, 125 and 200 μg/mL, 145 and 350 μg/mL showed growth inhibition in RAD 52 and RAD+ , respectively.

A yeast-bioassay directed isolation work on these fractions yielded eight pure compounds (**11-18**) (Scheme- 11-15). Compounds **11, 13, 14,** and **17** showed growth inhibition with IC_{12} values 75 and 92μg/mL, 43 and 100 μg/mL, 45 and 100 μg/mL and 60 and 85 μg/mL respectively in RAD 52 and RAD+. Compounds **13** and **14** were moderately active whereas compounds **11** and **17** showed low activity in these assays.

Table 11. Results of mutant yeast bioassay on *Iris germanica* rhizomes.

Sample	Yeast strains	
	(RAD 52)[a]	(RAD^+)
EtOAc Extract (IG-3)	200	450
Fraction IG-3- 5	125	200
Subfraction IG-3- 5-8	100	125
Nigricin-4′-*O*-β-D-glucoside	75	92
Subfraction IG-3- 5-10	90	140
Wistin	Not tested	-
Subfraction IG-3- 3	100	240
Subfraction IG-3- 3-4	60	140
4′-Methoxy-5-hydroxy-6,7-methylenedioxy-isoflavone	43	100
Fraction IG-3-8	145	350
CH_2Cl_2 Extract	200	510
Fraction IG-2-8	145	350
Subfraction IG-2-8-3	100	225
Sub-fraction IG-2-8-3	75	150
Irisolone	45	100
Fraction IG-2-3	150	100
Subfraction IG-2-3-2	100	145
Irisolidone	-	-
Subfraction IG-2-3-3	145	200
Muningin	Not active	-
Fraction IG-2-5	100	250
Subfraction IG-2-5-8	60	-
Fraction IG-2-10	145	300
Subfraction IG-2-10-3	100	140
Irilone	60	85
[b] Streptonigrin	0.4	>1

[a] Results expressed as IC_{12} μg/mL, [b] Reference compound.



Graph-**2:** Results of Yeast Bioassay on *Iris. Germanica* rhizomes.

4. PART C

Phytochemical Investigations On *Ficus racemosa* Linn

4. Part C

Phytochemical Investigations On *Ficus racemosa* Linn

4.1 Introduction

Ficus racemosa Linn belongs to the family Moraceae and genus *Ficus*.

4.1.1 Family

Ficus is an important genus of Moraceae family (Kirtikar et al., 1984; Venkataraman et al., 1972) Corner's recent taxonomic treatment of the Moraceae has shown that the family constitutes a large taxa of over 50 genera and nearly 1400 species (Venkataraman et al., 1972).

4.1.2 The genus *Ficus*

Trees or shrubs of *Ficus* genera are sometimes scandent or epiphytic and juice is milky (Kirtikar et al., 1984). Different *Ficus* species have important medicinal value. The decoction of the roots of *Ficus gibbosa* acts as a powerful aperient.

The root-bark is stomachic and gently aperient. All parts of *Ficus bengalensis* are acrid, sweetish; astringent to the bowels; useful in ulcers, vomiting, vaginal complain, fever and inflammations. The milky juice of *Ficus benjamina* is used against whitening of the cornea by the Mundas of Chota Nagpur, India. All parts of *Ficus retusa* are pungent, bitter; tonic, aphrodisiac; useful in burning sensations, leucoderma, ulcers, leprosy, itching, biliousness and diseases of the blood. The bark of *Ficus religiosa* is used in gonorrhea. The juice of the root of *Ficus heterophylla* is internally administered in colic pains, and the juice of the leaves mixed with milk in dysentery. All the parts of *Ficus hispida* are used for ulcers, anaemia, piles, jaundice etc. *Ficus carica* has been reported to have hypoglycemic, hypotriglyceridaemic and hypocholesterolaemic effects in diabetic rats (Kirtikar et al., 1984).

4.1.3 *Ficus racemosa* Linn

Ficus racemosa is a moderate sized evergreen tree of Bangladesh, India and Ceylon (Kirtikar et al., 1984). Its another name is *Ficus glomerata* roxb. The local name is Jaga Dumur. The trees are generally 15-18 meter high with few short aerial roots (Kirtikar et al., 1984)

4.1.3.1 Chemical Constituents of *F. racemosa*

Ficus racemosa is an important medicinal plant. The roots, leaves and bark have been earlier investigated. Ceryl bethanate, lupeol, lupeol acetate, α-amyrin acetate have been isolated from the bark of *F. racemosa* (Baslas et al., 1985). Leucoanthoacyanins were reported from the ethanol extract of stem bark (Agrawal et al., 1977). Gluanol acetate, β-sitosterol, taraxasterol, β-amyrin acetate have been reported from leaves (Chandra et al., 1979). β-Sitosteryl-glucoside and friedelin were isolated from the petroleum ether extract of the bark of *F racemosa* (Baslas et al., 1985). Leucocyanidin 3-*O*-β-D-glucopyranoside, leucopelargonidin 3-*O*-α-L-rhamnopyranoside, β-amyrin acetate· ethyl cholester-5, 2L, 2*S*-trien-3β-ol have also been reported from *F. racemosa*.

4.1.3.2 Medicinal Properties of *F. racemosa*

Different parts of *F. racemosa* have been reported for wide variety of purposes. All parts of *F. racemosa* are cooling, sweet acrid, vulnerary, anti-dysenteric and useful in biliousness (Kirtikar et al., 1984). The leaves are astringent to the bowels and good for bronchitis and bilious affection (Benthal 1984). The root is useful in hydrophobia, dysentery and is used in diabetes (Yusuf et al., 1994). The fruits are considered astringent, stomachic and carminative and are useful in bronchitis, dry cough, diseases of kidney and spleen, inflammation and other urinary diseases (Kirtikar et al., 1984; Yusuf et al., 1994). The leaves of *F. racemosa* was found to have antifungal activity. The extract inhibited the growth of several plant pathogens. Psoralen was identified as the active compound and was shown to be biodegradable, having the potential to be developed as a fungicide against pathogens causing diseases on crops of economic importance (Deranjyagala et al.,

1988). The leaf extract of *F. racemosa* was found to show hepato-protective activity Mandal et al., 1998). The plant extract is used in African medicine for anti-inflamatory, analgesic and antipyretic activities (Forestieri et al., 1996). Anti-diabetic compound epicatechin and gymnemic acid have been reported from *F. racemosa*. It has been reported that, the bark of this plant has hypoglycemic activity (Chopra et al., 1956; Dhar et al., 1968; Shrotri et al., 1960).

Fruits of *Ficus racemosa* are being locally used for the management of diabetes. In the present study the fruits have been tested for hypoglycemic and antioxidant (free radical scavenging) activities. Therefore, a brief introduction about diabetes mellitus, oxidation and antioxidants, hypoglycemic and antioxidant (free radical scavenging) agents, their activities, and the testing methodologies are described in the introduction and Experimental Sections respectively.

4.2 Diabetes Mellitus and Hypoglycemic Agents

Diabetes is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in or deficient insulin secretory response. Along with hyperglycemia and abnormality in serum lipids (Settin et al., 1944; Brandy et al., 1950; Randle et al., 1963; Taskinen et al., 1993), diabetes is associated with microvuscular and macrovuscular complications, which are the major causes (Baynes et al., 1991) of morbidity and death in diabetic subjects. Diabetes mellitus is defined (Nussey et al., 2001) solely in terms of elevated blood glucose concentrations and because of the vital role of insulin in regulating glucose metabolism a reduction in insulin secretion and /or resistance is a common cause of diabetes mellitus. Thus effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs (Alberti et al., 1998). Diabetes mellitus is classified (Diabetes Atlas 2000) on the basis of etiology and chemical presentation of disorder into four types:

1. IDDM (Insulin dependent diabetes mellitus) or Type 1 diabetes
2. NIDDM (Non insulin dependent diabetes mellitus) or Type 2 diabetes
3. Gestational diabetes
4. Other specific types

Insulin, the most widely used modern drug in the treatment of diabetes mellitus (for Type 1 diabetes) and different groups of oral hypoglycemic agents for clinical use (for Type 2 diabetes), have characteristic profiles of side effects (Pickup et al., 1997; Holman et al., 1991; Williams et al., 1991; Kameswarw et al., 1997). Management of diabetes without any side effect is still a challenge to the medical system. These leads to increasing demands for natural products with hypoglycemic activity with less side effects. Although a number of compounds derived from different plant sources have been found to have hypoglycemic activity (Marles et al., 1995; Nahar et al., 2000) and none of the compounds yet been used as theraputic agents for diabetes. New molecules as an orally active natural product hypoglycemic agent, to stimulate endogenous insulin biosynthesis and secretion and to promote the insulin action with less or without any side effects are realistic possibilities.

4.3 Oxidation and Antioxidants

Most of the potentially harmful effects of oxygen are believed to be due to the formation and activity of reactive oxygen species (ROS) acting as oxidants, that is, compounds with a tendency to donate oxygen to other substances. Many ROS are free radicals. A free redical is any species that has one or more unpaired electrons. Many free radicals are unstable and highly reactive.

Human cells are constantly exposed to ROS such as superoxide (O_2^{+-}), hydroxyl (OH^+), lipid peroxyl (LOO^+) radicals and hydrogen peroxide (H_2O_2). These ROS are mostly produced from endogenous sources, such as electron transport chain, peroxisomes, and the cytochrome P-450 system. Other ROS and free radicals are generated by immune response, for example, macrophages or as a result of smoking, air pollution, or the influence of UV light.

Chronic exposure to ROS can damage DNA, membrane lipids, lipoproteins, and functional and structural proteins (Halliwell et al., 1997; Sohal et al., 1996). As constant oxidative stress has been linked to the development of chronic diseases such as cancer, cardiovascular disease, cataracts, (Buring et al., 1997; Block et al., 1994; Marx et al 1987) and dementia, (Lethem et al., 1997) its reductioin is seen as beneficial to public health.

The human body has eveloved antioxidant defence mechanism to minimize the potential for radical damage (Halliwell et al., 1995). The extremely reactive hydroxyl radical is particularly harmful, with a near diffusion-controlled rate constant of 10^9 to 10^{10} $M^{-1}s^{-1}$ enabling it to combine with virtually all models in living system (Anber et al., 1967). Intracellular antioxidant enzymes function as a first line of defense to neutralize ROS. The primary reaction is dismutation of superoxide radicals to hydrogen peroxide and oxygen by the enzyme superoxide dismutase, followed by removal of hydrogen peroxide that is catalyzed by catalase and glutathione peroxidase (Sies et al., 1993).

The endogenous enzymetic defenses against oxidative damage are not completely efficient, (Sohal et al., 1996, Jacob et al., 1996) and a range of endogenous and exogenous free radical scavenging antioxidants act as a second line of defence. Vitamin E, vitamin C and the carotenoids are well-recognized antioxidant nutrients (Sies et al., 1995; Olson et al., 1993; Frei et al., 1994). Some foods also contain other antioxidant substances and most of the antioxidants found in these foods are flavonoids and phenolic and polyphenolic compounds (Namiki et al., 1990; Okuda et al., 1993; Namiki et al., 1993; Pratt et al., 1992) and may contribute to oxidative damage. The antioxidant activity of flavonoids, phenols and polyphenols may be an important beneficial health properties and elucidation of their antioxidant potential is therefore of interest.

To be regarded as effective in biological sense, an antioxidant is a substance that when present at low concentrations relative to an oxidizable substrate (e.g., protein, or DNA molecule), can suppress, delay, or prevent oxidation of that substrate (Halliwell et al., 1990). Antioxidant potential is determined by chemical reactivity as an electron or hydrogen donor; the ability to delocalize and thus stabilize the unpaired electron; the reactivity with other antioxidant; and the reactivity with molecular oxygen (Rice-Evans et al., 1997). The chemical structures required for effective antioxidant activity of flavonoids and polyphenols have been identified recently (van Ackar et al., 1996; Ratty et al 1988; Bors et al., 1990; Mora et al., 1990; Limasser et al., 1993; Rice-Evans et al., 1996).

4.4 Experimental Sections

4.4.1 General Experimental Procedures

The general experimental procedures are similar as in part A. For polar fractions Sephadex LH-20, reversed phase RP-18 silica gel were used for chromatographic seperations. Purity of the polar compound and fractions were checked on RP-18 glass plates.

4.4.2 Plant Materials

Plant Materials (70 kg fruits) of *F. racemosa* was collected from Sonargaon, Dhaka by a laboratory assistant of the department of Chemistry, University of Dhaka during the month of July 1998. The plant material was identified by the exparts of National Harberium, Bangladesh.

4.4.3 Extraction and Fractionations

Fruits of *F. racemosa* were sliced and air-dried. The air dried fruits were then dried at 40° C in an electric oven and ground into powder. Powdered fruit materials (6 kg) was soacked into EtOH (80%) and extracted thrice (5L x 3, RT, 42 hrs.). The combined extract was evaporated to obtain a parent extract 500 g. The parent extract (FR-p) was suspended into H_2O and partitioned with CH_2Cl_2. A CH_2Cl_2 soluble part 120 g was obtained. The aqueous part was again partitioned with 1-BuOH (1:1, 2 L x 3) and a 1-BuOH soluble part (70 g) was obtained. The aqueous part was concentrated and freeze dried and an aqueous part (FR-2, 250 g) was obtained. The parent extract (FR-p) and the aqueous extract (FR-2) were tested for hypoglycemic activities and were found to show active on different model rats (Ali et al., 1993). 1-BuOH soluble part showed a significant antioxidant (free radical scavenging) activity (Dinis et al., 1994; Lamaison et al., 1990; Navarro et al; 1993).

4.4.4 Assay for Hypoglycemic Activity

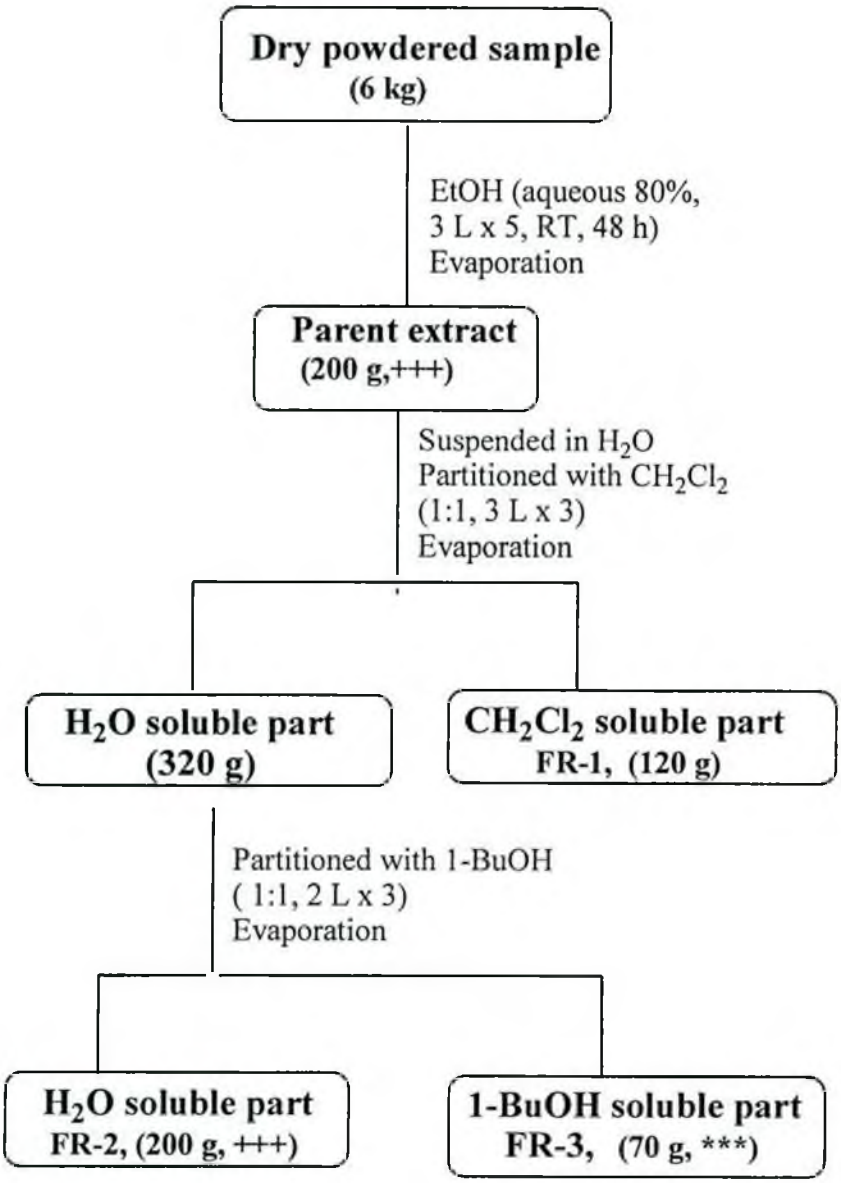
Plant materials and preparation of test sample: Fruits of *Ficus racemosa* was extracted and fractionated as shown in Scheme-16 (Page-168) and also described in Experimental Section (Section-4.4.3; Page-166). The parent extract (FR-p) and the aqueous extract (FR-2) were studied for their effects on serum glucose levels in nondiabetic and diabetic rat models at different parandial states.

Animal experiments: Long-Evans rats (180-220 g), bred at BIRDEM animal house, were used in this study. Diabetis simulating Type 1 was induced by a single intraperitoneal injection of streptozotocin (65 mg/kg body weight) to adult (3-4 months) rats. They were taken for experiments on the 7^{th} day after injection. Type 2 was induced by single intraperitoneal injection of streptozotocin (90 mg/ kg body weight) to 48 hr old pups as described (Bonner et al., 1981). Three months later they were taken for experiments when their weights were between 180-220 g. Solutions or suspensions of extracts (1.25 g/kg/10 ml water) with or without glucose were fed by metallic tubes to the rats fasted 12 hrs under mild ether anesthesia. Blood samples were collected by amputation of the tail tip under mild ether anesthesia. Effects on both fasting and postprandial blood glucose levels were studied by procedures standardized at BIRDEM. Glucose levels in the same serum samples were estimated on the same day by GOD-PAP (Boheringer-Mannheim GmbH) method. Statistical significances of differences among the groups were determined by an 'ANOVA' test (Duncan's new multiple-range test) (Katherine et al., 1992).

4.4.5 Antioxidant assay for DPPH Free Radical Scavenging Activity

The reaction mixture was prepared with 5 μL test samples (fractions and compound **19** was dissolved in DMSO) and 95 μL of DPPH in ethanol. Different concentrations of test samples were prepared while the concentrations of DPPH was 300 μM in the reaction mixture. These reaction mixtures were taken in 96-well plate microtitre plates (Molecular Devices, USA) and incubated at 37° C for 30 min; the absorbance was measured at 515 nm. Percent radical scavenging activiy by sample treatment was determined by control in comparison with a DMSO treated control group. Propyl gallate was used as a positive control. The activity results are given in Table-21.

Scheme 16: Extraction and Fractionations of *Ficus racemosa* Fruits



+++ Hypoglycemic active,*** Free radical scavenging active.

4.4.6 Isolation of Compounds

Fractionation and isolation work on CH_2Cl_2 and 1-BuOH soluble parts of *F. racemosa* fruits led to the isolation of six compounds (**19-23**).

4.4.6.1 Compound 19- 3-*O* (*E*)-caffeoyl quinate (176)

The 1-BuOH soluble extract (70 g, antioxidant active, 80%) was suspended into H_2O and then centrifuged and a H_2O soluble part 36 g was obtained. The H_2O soluble part was fractionated into 11 fractions by Sephadex LH-20 column using gradients of solvents, H_2O-MeOH-acetone. Out of 11 fractions (FR-3-1 – FR-3-11), 5 fractions were found to show free radical scavengening activity [FR-3-2, (92.5 %), FR-3-4 (94.88 %), FR-3-6 (92.12%), FR-3-7 (87.21%) and FR-3-8 (90.67%)]. The fraction FR-3-2 (15.0 g), eluted by H_2O: MeOH (4:1) was subjected to further fractionation by Sephadex LH-20, using gradients of solvents H_2O-MeOH-acetone. Out of 8 fractions only one fraction FR-3-2-4 (1.35 g) eluted with H_2O-MeOH (1:1) exhibited significant antioxidant activity (93.62%). The fraction FR-3-2-4 was then fractionated into 6 different fractions by RP-18 column using tbe solvent system H_2O-MeOH-acetone. A fraction FR-3-2-4-5, 300 mg, (94.8% antioxidant active) eluted by acetone: MeOH (1:1) was purified by Si gel column chromatography elution with EtOAc-MeOH (1:1). Which yielded the compound **19** (100 mg) (Scheme-17, Page-170). The percent yield was 16.75×10^{-4} and the R_f value of **19** was found to be 0.35 (EtOH-H_2O-Acetone, 3:1:1).

Physical Characteristics of 3-*O* (*E*)-caffeoyl quinate (176)

Appearance: White amorphous powder.

IR ν_{max} ($CHCl_3$): 3596 (OH), 3445 (OH), 2931 (CH), 2846 (CH), 1732 (α,β-unsaturated γ-lactone, C=O), 1688, 1378, 1200, 1076, 1000, 930 cm^{-1}.

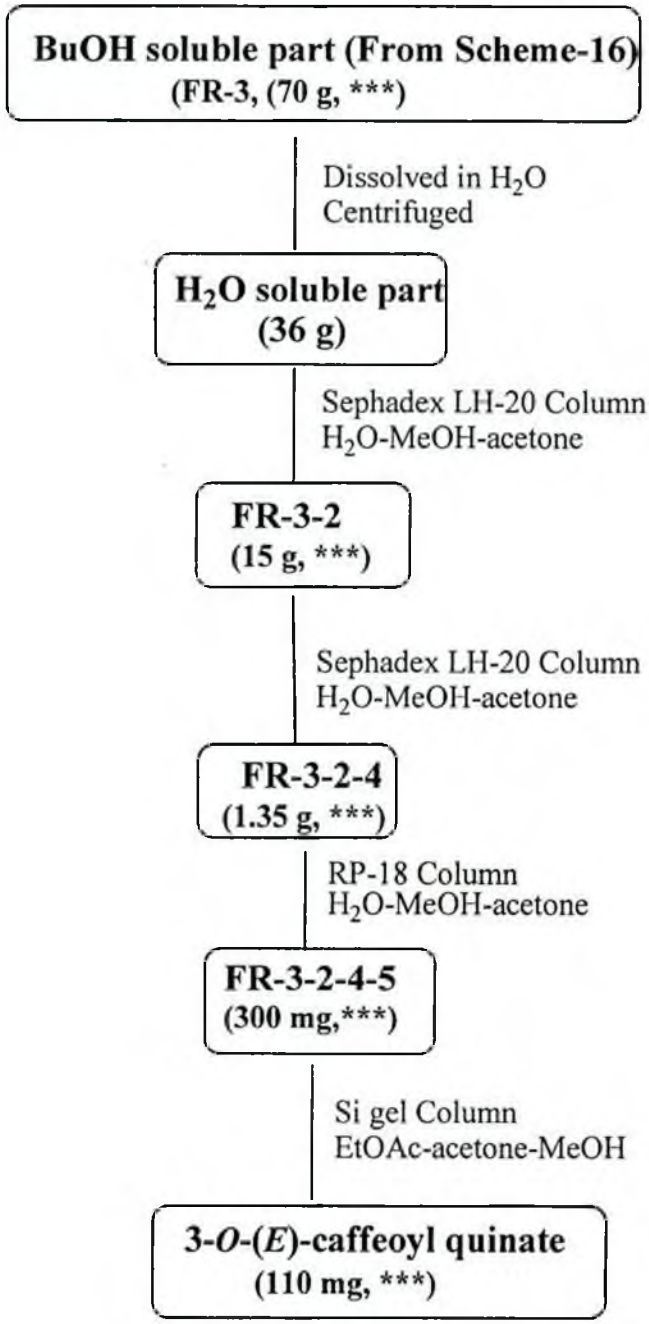
^{1}H-NMR δ_H: Table **12**.

^{13}C-NMR δc: Table **12**.

EI-MS: (*m/z*, relative intensity, %): 354 (3), 336 (11), 180 (50), 163 (100, 105 (42), 95 (12), 89 (21), 72 (34), 55 (25).

HREI MS: (*m/z*) = 354. 2998 [$C_{16}H_{18}O_9$ requires 354.2991].

Scheme 17: Bioassay-Guided Isolation of Compound 19



*** Free radical scavenging active

4.4.6.2 Compound 20- β-Sitosterol (177)

CH_2Cl_2 soluble part (FR-1, 100 g) (Scheme-16, Page-168) of F. *racemosa* fruits was subjected to silica gel column chromatography using the gradients of solvents hexane-CH_2Cl_2-EtOAc-MeOH. According to TLC profiles 12 fractions (FR-1-1 – FR-1-12) were obtained. Out of 12 fractions only four fractions were found good on TLC. A fraction FR-1-8 (1.0 g) eluted by CH_2Cl_2- EtOAc obtained from the CH_2Cl_2 extract (Scheme 18, Page-173) was further fractionated on Si-gel column chromatography using the solvent system hexane-CH_2Cl_2-EtOAc. A fraction eluted with 100% CH_2Cl_2 yielded the compound **20** (60 mg). The percentage yield was 1.0×10^{-3} and the R_f value of compound **20** was found to be 0.43 (CH_2Cl_2).

Physical Characteristics of the β-sitosterol (177)

Appearance: White needle shaped crystals.

IR ν_{max} (nuzle): 3410 (OH), 2814 (CH), 2742, 1618 (C= C), 1448, 1362, 1060 cm^{-1}.

^{1}H-NMR δ_H : Table **23**.

^{13}C-NMR δc: Table **23**.

EI-MS: (m/z, relative intensity, %): 414 (100), 412 (38), 303 (67), 231 (32), 213 (53), 159 (59), 133 (60), 95 (85), 55 (94).

4.4.6.3 Compound 21-β-Sitosterol glucoside (175)

A fraction FR-1-12 (1.5 g) obtained from CH_2Cl_2 fractionation of CH_2Cl_2 part was further fractionated by silica gel column chromatography using the solvent system EtOAc-MeOH. A fraction FR-1-12-4, 300 mg, eluted by EtOAc-MeOH (9:1) was purified on silica gel column using the solvent system CH_2Cl_2-MeOH (9:1), which yielded the compound **21** (120 mg) (Scheme-18, Page-173). The percent yield was 2.0×10^{-3} and the R_f value of the compound was found to be 0.50 (CH_2Cl_2-MeOH, 9:1).

Physical Characteristics of β-sitosterol glucoside (175)

Appearance: White crystalline solid.

IR ν_{max} ($CHCl_3$): 3596 (OH), 3445 (OH), 2931 (CH), 2846 (CH), 1732 (α,β-unsaturated γ-lactone, C=O), 1688, 1378, 1200, 1076, 1000, 930 cm^{-1}.

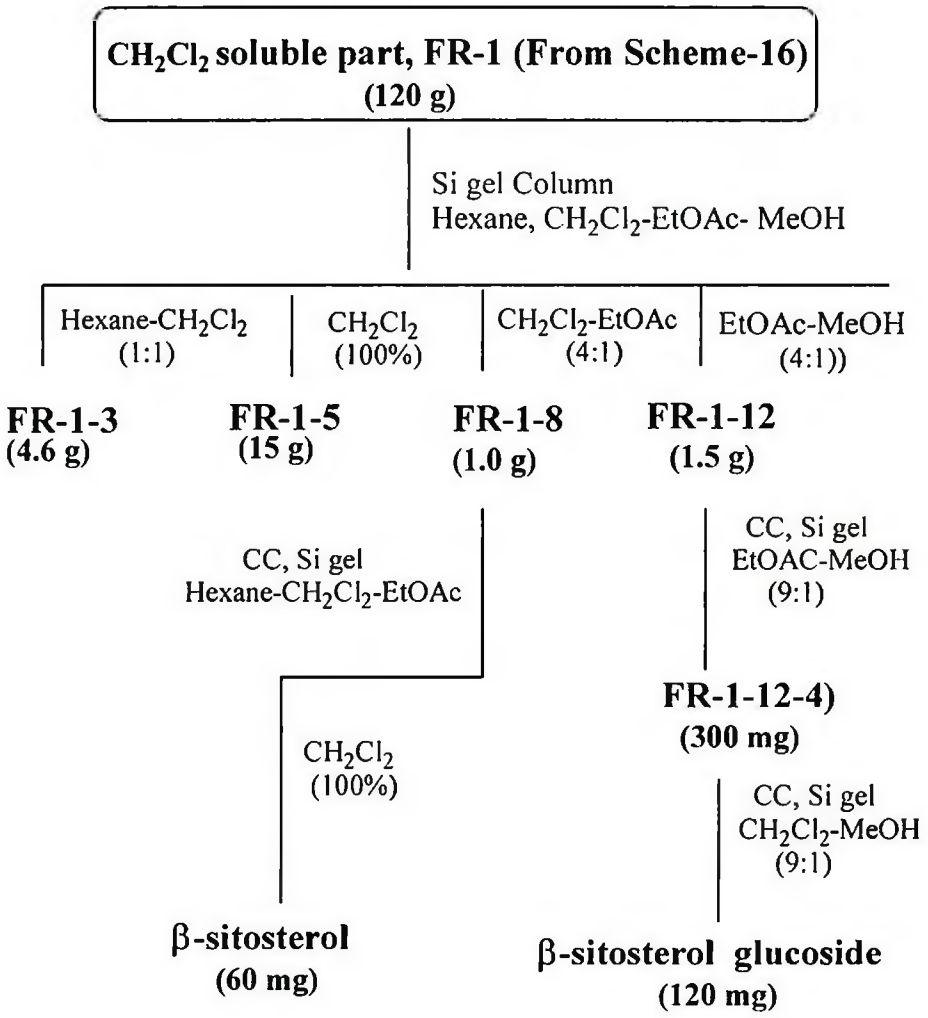
^{1}H-NMR δ_H: Table **14**

^{13}C-NMR δc: Table **1ssss4**

EI-MS (*m/z* relative intensity, %): 414 (M^+ -sugar moiety, 18) 396 (25), 381 (70), 329 (20), 303(29), 275(10), 273 (22), 57 (100).

HR-MS *m/z*, 576.4380 [$C_{35}H_{60}O_6$, required 576.4389].

Scheme 18: Isolation of Compounds 20 & 21



4.4.6.4 Compound 22- Epilupeol acetate (178)

Fraction FR-1-5 (1.5 g), eluted by 100% CH_2Cl_2 (Scheme 19, Page-177) was subjected to Si gel column chromatography using the solvent system hexane-CH_2Cl_2-MeOH. Eight different fractions (FR-1-5-1 – FR-1-5-8) fractions were obtained. Fraction FR-1-3 (4.6 g) eluted by hexane-CH_2Cl_2 (1:1) was again chromatographed on Si gel column by the solvent system hexane-CH_2Cl_2 (2:3) to give six fractions (FR-1-3-1 - FR-1-3-6). Fraction FR-1-3-4 (1.0g) was purified by Si gel column eluted by hexane-CH_2Cl_2 (1:4), which afforded the compound **22** (20 mg). The percent yield was 3.3×10^{-4} and the R_f value of compound **22** was found to be 0. 42 (hexane-CH_2Cl_2, 4:1).

Physical Characteristics of epilupeol acetate (178)

Appearance: White amorphous powder.

mp: 163-164° C.

$[\alpha]_D^{25}$: -7^0 (*c*, 0.048,$CHCl_3$).

IR ν_{max} (KBR): 3060 (CH), 2940 (CH), 2860, 1728, 1635, 1378, 1280 (C=O, acetate), 972, 878 cm^{-1}.

^{1}H-NMR δ_H: Table **15**.

^{13}C-NMR δc: Table **15**.

EI-MS: (m/*z*, relative intensity, %): 468 (58), 218 (100), 203 (26), 135 (13), 121 (76), 95 (88), 69 (81). 55 (53).

4.4.6.5 Compound 23- Lupeol acetate (179)

A Fraction (FR-1-4-3, 300 mg) eluted by hexane-CH_2Cl_2 (1:1) obtained from the CH_2Cl_2 part (Scheme-19, Page 177) was purified by Si-gel column chromatography using the solvent system hexane-CH_2Cl_2 (1:4) which, yielded the compound **22** (50 mg). The percent yield was 8.3×10^{-4} and the R_f value of **23** was found to be 0.40 (hexane- CH_2Cl_2, 4:1).

Physical Characteristics of lupeol acetate (179)

Appearance: White needle shaped crystal.

mp: 215-216° C.

$[\alpha]_D^{25}$ 35^0 (*c*, 0.055, $CHCl_3$).

IR ν_{max} (KBR): 3060 (CH), 1727, 1637, 1239 (C=O, acetate), 865 cm^{-1}.

^{1}H-NMR δ_H: Table **16**.

^{13}C-NMR δc: Table **16**.

EI-MS: (m/z, relative intensity, %): 468 (58), 218 (69), 203 (48), 135 (79), 121 (76), 95 (88), 69 (81). 55 (53).

4.4.6.6 Compound 24- β-Amyrin acetate (180)

A fraction (FR-1-4-6, 3.0 mg) eluted with hexane-CH_2Cl_2 (2:3) obtained by the repeated column chromatography of CH_2Cl_2 extract (Scheme 19, Page-177) was further purified by Si gel column chromatography using the solvent system hexane: CH_2Cl_2 (1:1), which afforded the compound **23** (200 mg). The percent yield was 3.4×10^{-3} and the R_f value of **23** was found to be 0.42 (hexane-CH_2Cl_2, 4:1).

Physical Characteristics of β-amyrin acetate (180)

Appearance: White crystalline solid.

mp: 241-242° C.

$[\alpha]_D^{25}$ 69^0 (

c, 0.094, $CHCl_3$).

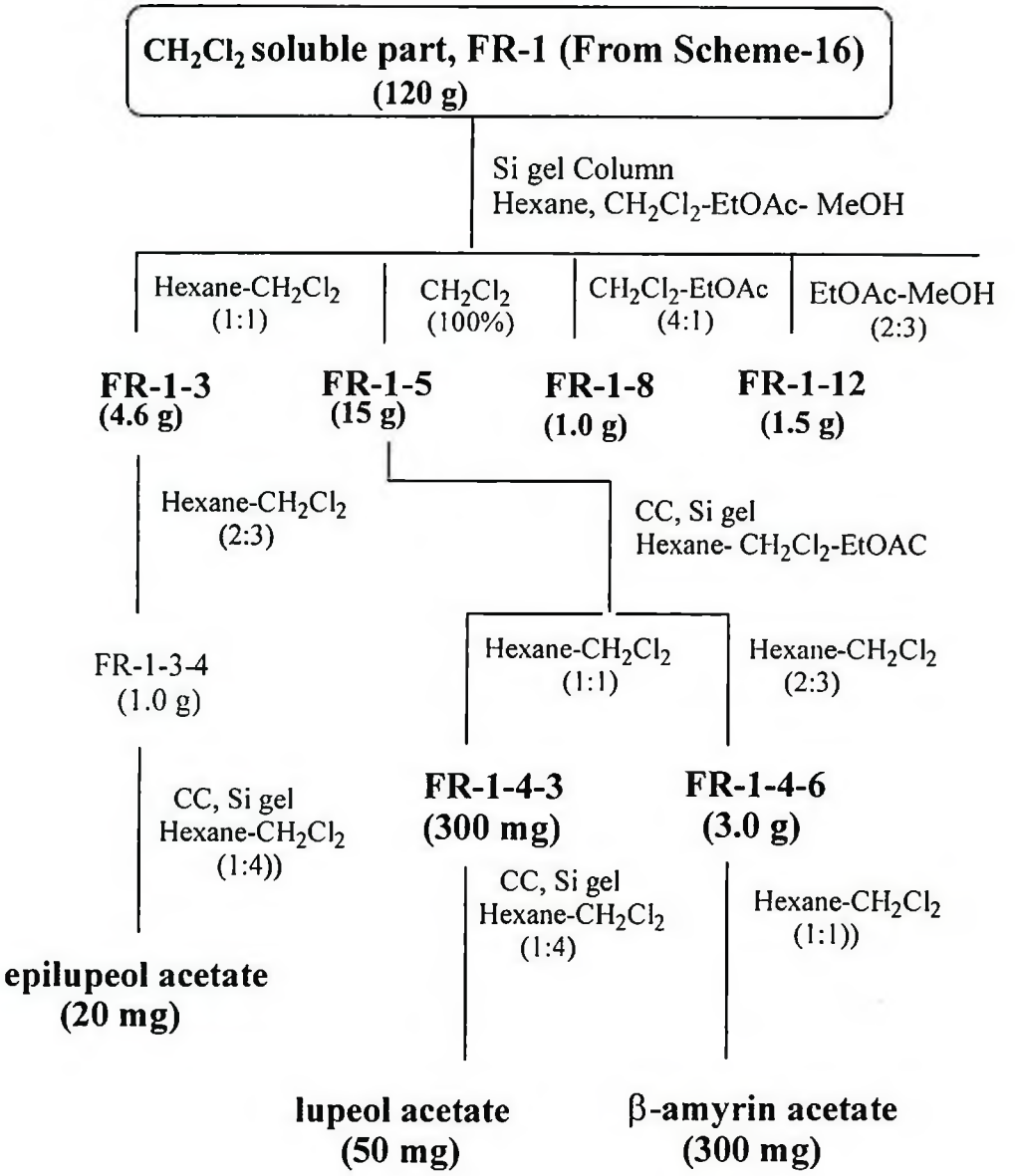
IR ν_{max} ($CHCl_3$): 1722, 1635 (C = CH), 1240 (C=O, acetate), 812 (C-OH) cm^{-1}.

^{1}H-NMR δ_H: Table **17**.

^{13}C-NMR δc: Table **17**.

EI-MS: (m/*z*, relative intensity, %): 468 (13), 218 (100), 203 (77), 175 (14), 147 (17), 135 (30), 119 (26), 107 (29), 95 (40), 69 (56), 55 (35).

Scheme 19: Isolation of Compounds 22-24

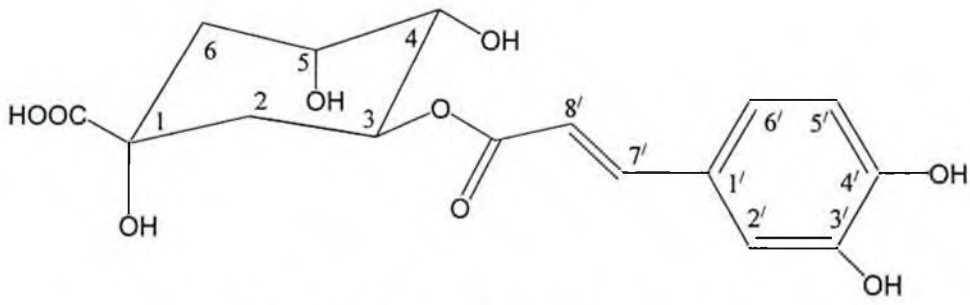


4.5 Results and Discussion

The aqueous 80% EtOH extracts (Scheme-16, Page-169) of *Ficus racemosa* fruits exhibited significant hypoglycemic activity tested on healthy, NIDDM and IDDM diabetic model rats at three different parandial states tested at BIRDEM. The 1-BuOH soluble part of the parent extract showed significant free radical scavenging activity (DPPH) when tested at the HEJ Research Institute of Chemistry. Free radicals scavenging activity-guided isolation on 1-BuOH soluble part yielded one compound (**19**). The other compounds **20-24** were isolated from the CH_2Cl_2 soluble part of the parent extract. Compound **19** exhibited significant free radicals scavenging activity.

4.5s.1 3-*O* (*E*)-caffeoyl quinate (176)- A known compound

Compound **19** was isolated as a crystalline powder (120 mg). The molecular formula of **19** was found as $C_{16}H_{18}O_9$ from EIMS (*m/z* 354) along with ^{13}C NMR and DEPT spectra. The IR spectrum showed the presence of hydroxyl groups at 3596 and 3445 and (α,β-unsaturated γ-lactone, C=O) at 1732 cm^{-1}.



3-*O*-*(E)*-caffeoyl quinate (**176**)

The structure of the compound was determined by analyzing the ^1H-, ^{13}C NMR, HMQC NMR spectral data of compound **19** and the interactions found in COSY 45^0 and HMBC spectra (Table.12, Fig. 46) and by comparing its data with the reported data (Pauli et al.,

1998). The stereochemistry of different stereogenic centers of compound **19** was determined from its coupling constants and from NOED spectra as depicted in Fig. 47.

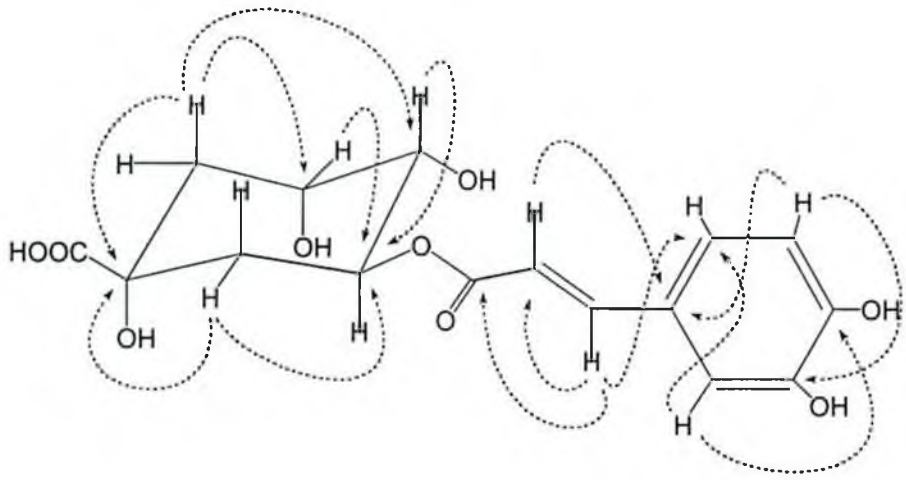


Fig. **46**. Important HMBC interactions in 3-*O-(E)*-caffeoyl quinate.

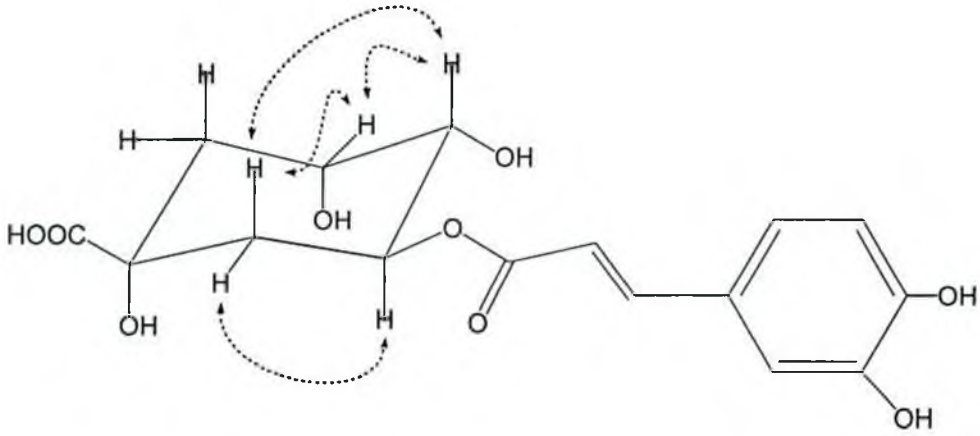


Fig. **47**. Important NOE interactions in 3-*0-(E)*-caffeoyl quinate.

The structure of **I9** was therefore, elucidatd as the 3-*0-E*-caffeoyl quinate (**176**) a known compound previously isolated from *Solidago canadensis* L., Asteraceae and *Hedera helix* L., Araliaceae (Trute and Nahrstedt, 1997; Trute et al, 1997) and from many other plants. The compound **19** showed significant free radical scavenging activity (Table-21, Page-196).

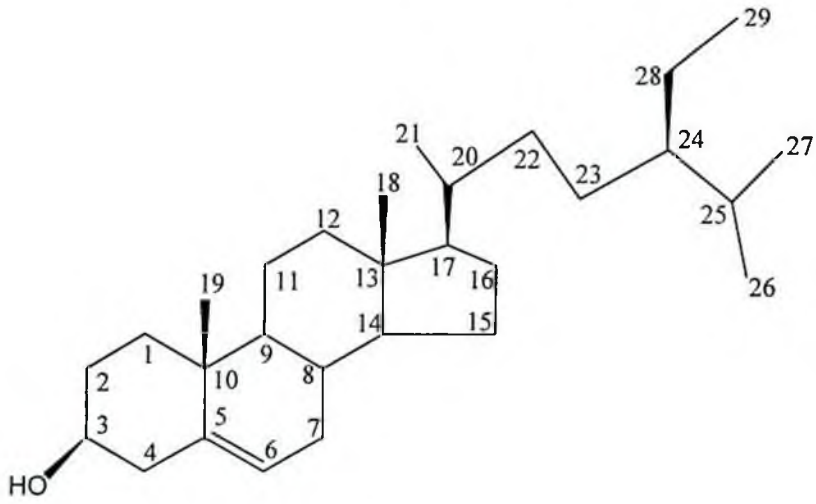
Table 12. NMR spectral data (CD_3OD) of 3-*O*-(*E*)-caffeoyl quinate (**176**)).

C/H No.	^{1}H-NMR[a] δ_H (mult., J = Hz)	^{13}C-NMR[b] δ_C (mult.)*	COSY	HMBC	NOE
1	-	177 .0 (s)	-	-	-
2eq	2.16 (m)	38.21 (t)	H-2ax, H-3,	C-3, C-4	-
2ax	2.23 (m)	-	H-eq, H-3	C-3, C-4	-
3	5.32 (ddd, 12.0, 9.1, 4.4)	72.0 (d)	H-2ax, H-4ax, H-2eq	C-2, C-4	H-4
4	3.72, (dd, 8.45, 3.15)	73.45 (d)	H-3, H-5	C-3, C-5	H-3, H-2ax, H-5
5	4.16 (m)	71.30 (d)	H-4, H6ax, H-6eq	C-4, C-6	H-2ax, H-4
6ax	2.01 (m)	38.2 (t)	H-2eq, H-5	C-5, C-4, C-1	-
6eq	2.06 (m)	-	H-2ax, H-5	C-5, C-4	-
1′	-	127.9 (s)	-	-	-
2′	7.03 (d, 2.03)	115.2 (d)	H-6′	C-4′, C-6′, C-7′	-
3′	-	147.0 (d)	-	-	-
4′	-	149.4 (d)	-	-	-
5′	6.76 (d, 8.2)	116.4 (d)	H-6′	C-4′, C-6′, C-3′	-
6′	6.95 (dd, 8.25,2.0)	123.0 (d)	H-5′, H-2′	C-5′, C-2′, C-4′, C-7′	-
7′	7.56 (d, 15.9)	146.5 (d)	H-8′	C-6′, C-8′, C-9′	-
8′	6.25 (d, 15.9)	115.2 (d)	H-7′	C-1′	-
9′	-	168.0 (d)	-	-	-

[a] Data recorded at 500 MHz.
[b] Data recorded at 100 MHz.
*Multiplicity determined by DEPT and HMQC spectra.

4.5.2 β-Sitosterol (177)- A known Compound

Compound **20** was also isolated from the CH_2Cl_2 soluble part of the parent extract of *F. racemosa* fruits as a white crystalline solid (120 mg) (Scheme-18). The molecular formula was obtained as $C_{29}H_{50}O$ from the EIMS (*m/z,* 414) together with the ^{13}C-NMR data of compound **20**. By analysing its IR. Mass (Experimental Section), ^{1}H-, ^{13}C-NMR spectral data (Table-13 and a comparative study of ^{13}C-NMR spectral data with the literature value of the reported compound (Wright et al., 1978), the structure of **20** was determined as the known compound β-sitostersol.



β-Sitosterol (**177**)

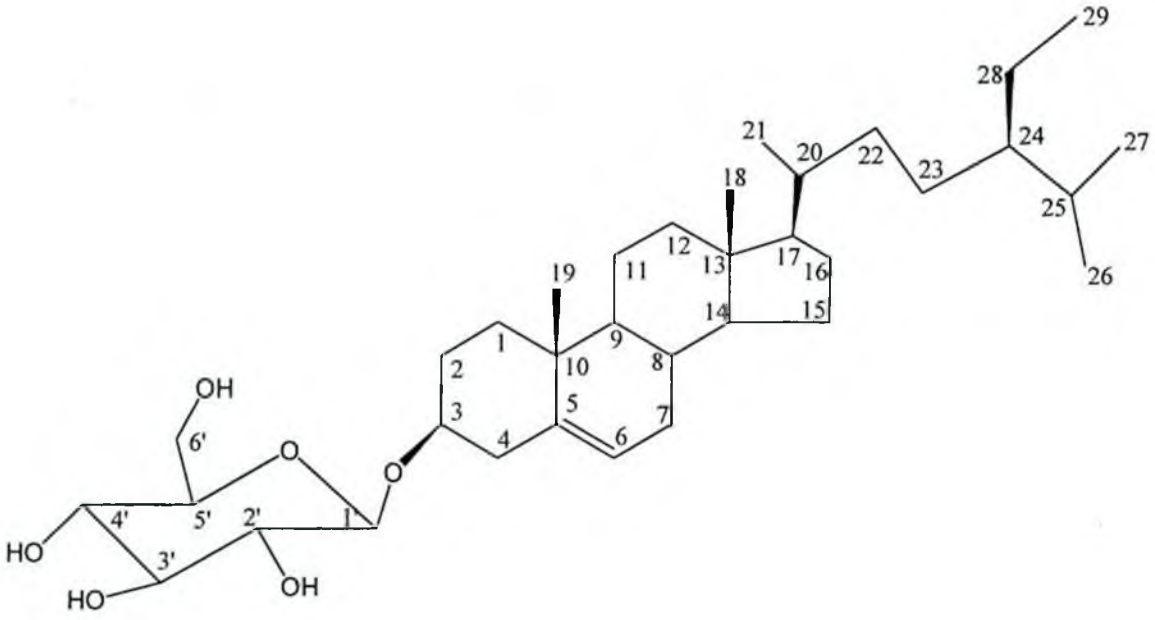
Table 13. NMR[a] spectral data ($CDCl_3$) of β-sitosterol (**177**).

C/H No.	^{1}H-NMR[a] δ_H mult., J = Hz	^{13}C-NMR[b] δ_C mult. *	C/H No.	^{1}H-NMR[a] δ_H mult., J = Hz	^{13}C-NMR[b] δ_C mult. *
1		38.7 (t)	16	-	29.7 (t)
2	-	29.9 (t)	17	-	56.6 (d)
3	3.52 (m)	80.9 (d)	18	0.70 (s)	12.2 (q)
4	-	43.8 (t)	19	1.01 (s)	19.5 (q)
5	-	142.0 (s)	20	-	37.2 (d)
6	5.35 (bd, 5.3)	122.1 (d)	21	0.92 (d, 6.2)	19.7 (q)
7	-	33.0 (t)	22	-	39.3 (t)
8	-	32.9 (d)	23	-	29.5 (t)
9	-	50.4 (d)	24	-	50.5 (d)
10	-	37.1 ((s)	25	-	25.9 (d)
11	-	21.5 (t)	26	0.83 (d, 6.5)	18.2 (q)
12	-	40.8 (t)	27	0.81 (d, 6.5)	20.1 (q)
13	-	43.0 (s)	28	-	23.7 (t)
14	-	56.9 (d)	29	0.84 (t, 7.0)	11.9 (q)
15	-	25.8 (t)	-	-	

[a] Data recorded at 500 MHz.
[b] Data recorded at 125 MHz.
*Multiplicity determined by DEPT NMR spectra.

4.5.3 β-Sitosterol glucoside (175)- A known steroidal glycoside

Compound **21** was isolated from the hexane part of *F. racemosa* fruits as white crystalline solid (120 mg) (Scheme-18, Page-173). The molecular formula was determined as $C_{35}H_{60}O_6$ from the HR-EIMS (*m/z,* 576.4376). By analysing the spectral data, IR, HR, EIMS (Section: 4.4.6.3), ^{1}H-, ^{13}C- NMR data (Table 14) of compound **21** and a comparative study with the compound 18 and also with the reported literature values of the reported compound (Daise et al., 1993; Jain et al., 1990; Kojo et al., 1989; Stone et al., 1966) the structure of compound **21** was assigned as the known compound, β-sitosterol glucoside isolated also from the same plant.



β-Sitosterol glucoside (**175**)

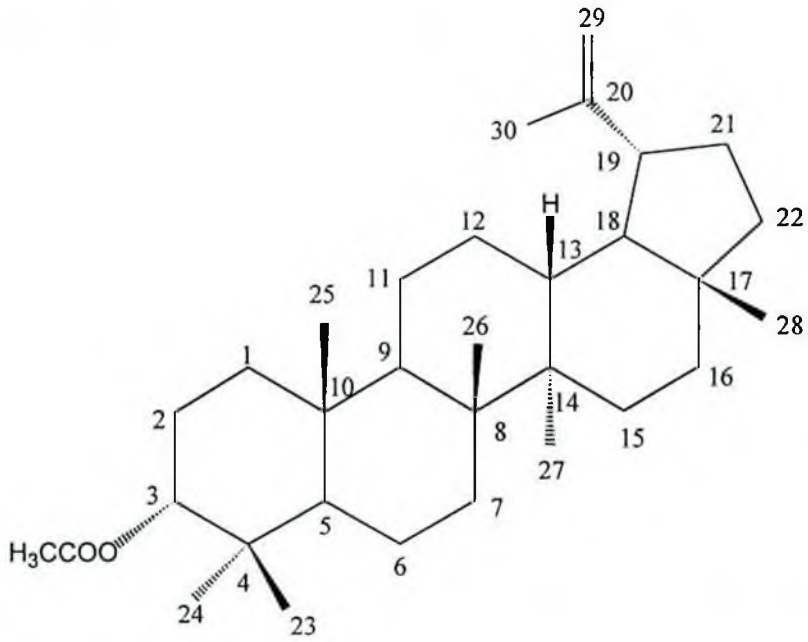
Table 14. NMR[a] spectral data (C_5D_5N) of β-sitosterol glucoside (**175**).

C/H No.	^{1}H-NMR[a] δ_H mult., J = Hz	^{13}C-NMR[b] δ_C	C/H No.	^{1}H-NMR[a] δ_H mult., J = Hz	^{13}C-NMR[b] δ_C
1		38.7	19	0.90 (s)	19.5
2	-	29.9	20	-	37.2
3	3.63 (m)	80.9	21	0.82 (d, 6.4)	19.7
4	-	43.8	22	-	39.3
5		142.0	23	-	29.5
6	5.26 (bd, 5.15)	122.1	24	-	50.5
7	-	33.0	25	-	25.9
8	-	32.9	26	0.70 (d, 7.0)	18.2
9	-	50.4	27	0.68 (d, 6.9)	20.1
10	-	37.1	28	-	23.7
11	-	21.5	29	0.84 (t, 7.0)	11.9
12	-	40.8	1′	5.60 (d, 7.5)	103.2
13	-	43.0	2′	4.32 (t, 9.0)	74.1
14	-	56.9	3′	4.40 (t, 9.0)	76.8
15	-	25.8	4′	4.35 (t, 9.0)	70.2
16	-	29.7	5′	4.25 (m)	76.6
17		56.6	6′	4.50 (dd, 12.0,2.0)	61.9
18	0.56 (s)	12.2		4.40 (dd, 12.0, 5,0)	

[a] Data recorded at 400 MHz.
[b] Data recorded at 125 MHz.
*Multiplicity determined by DEPT NMR spectra.

4.5.4 Epilupeol acetate (178)- A known Compound

Compound **22** was isolated as crystalline solid from the CH_2Cl_2 soluble part of the parent extract (Scheme 19). The molecular formula $C_{30}H_{54}O_2$ of **21** was determined from the EIMS (*m/z* 468) and ^{13}C-NMR and DEPT data. By analyzing its EIMS, IR, UV (Section: 4.4.6.4) and ^{1}H- and ^{13}C-NMR spectral data (Table 15) and a comparative study of compound **22** with the reported compound (Rahimtulla et al., 1966) assigned the structure of **22** as known compound epilupeol acetate (**178**) islated for the first time from this plant.



Epilupeol acetate (**178**)

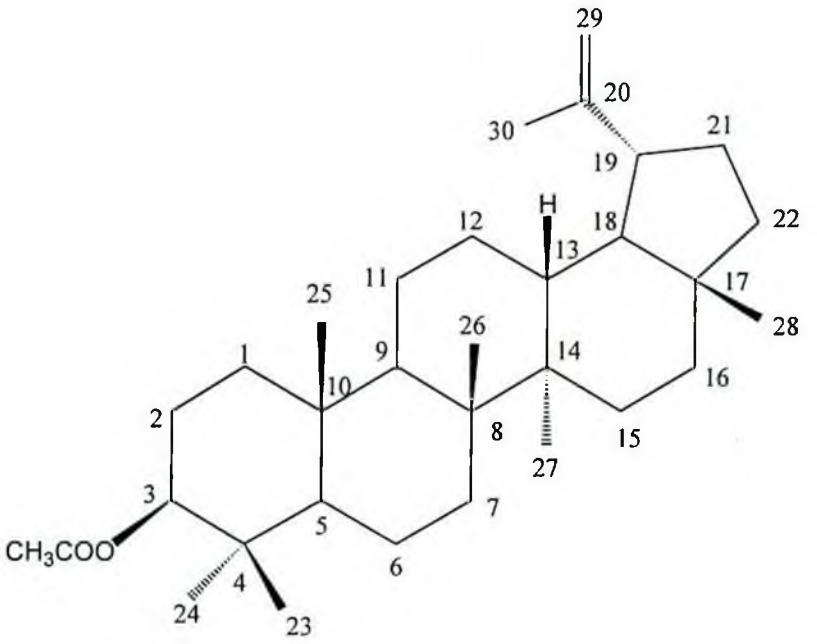
Table 15. NMR[a] spectral data (C_5D_5N) of epilupeol acetate (**178**).

C/H No.	^{1}H-NMR[a] δ_H mult., J = Hz	^{13}C-NMR[b] δ_C mult. *	C/H No.	^{1}H-NMR[a] δ_H mult., J = Hz	^{13}C-NMR[b] δ_C mult. *
1	-	35.7 (t)	18	-	48.4 (d)
2	-	24.0(t)	19	-	47.9 (d)
3	4.30 (m, *W/2* = 7.6)	78.8(d)	20	-	150.6 (s)
4	-	35.7 (s)	21	-	29.8 (t)
5	-	55.5 (d)	22	-	39.9 (t)
6	-	18.3 (t)	23	0.76	28.0 (q)
7	-	34.2 (t)	24	083	15.4 (q)
8	-	40.8 (s)	25	0.85	16.2 (q)
9	-	50.4 (d)	26	0.95	16.0 (q)
10	-	37.1 (s)	27	0.95	14.6 (q)
11	-	20.9 (t)	28	1.01	18.0 (q)
12	-	25.1 (t)	29a	4.67 (bd, 2.4)	109 (t)
13	-	38.0 (d)	29b	4.56 (q, 1.22)	
14	-	48.6 (s)	30	1.70	19.3 (q)
15	-	27.4 (t)	31	-	170.9 (s)
16	-	35.5 (t)	32	2.02 (s, acetate)	21.5 (q)
17	-	56.6 (s)	-	-	-

[a] Data recorded at 400 MHz.
[b] Data recorded at 100 MHz.
*Multiplicity determined by DEPT NMR spectra.

4.5.5 Lupeol acetate (179)- A known Triterpene

Compound **23** (60.0 g) was obtained from the CH_2Cl_2 soluble part of the of the parent extract by repeated column chromatography (Scheme-**19**). The molecular formula of compound **23** was deduced to be as $C_{30}H_{54}O_2$ from the EIMS (*m/z* 311) and DEPT spectra. Analysis of NMR (Table 16), IR and EIMS spectral data (Section: 4.4.6.5) of compound **23** and its comparative study with the reported compound led to the structure of compound **23** as a known compound lupeol acetate (**179**) (Mahato et al., 1994), which was isolated previously from the same plant.



Lupeol acetate (**179**)

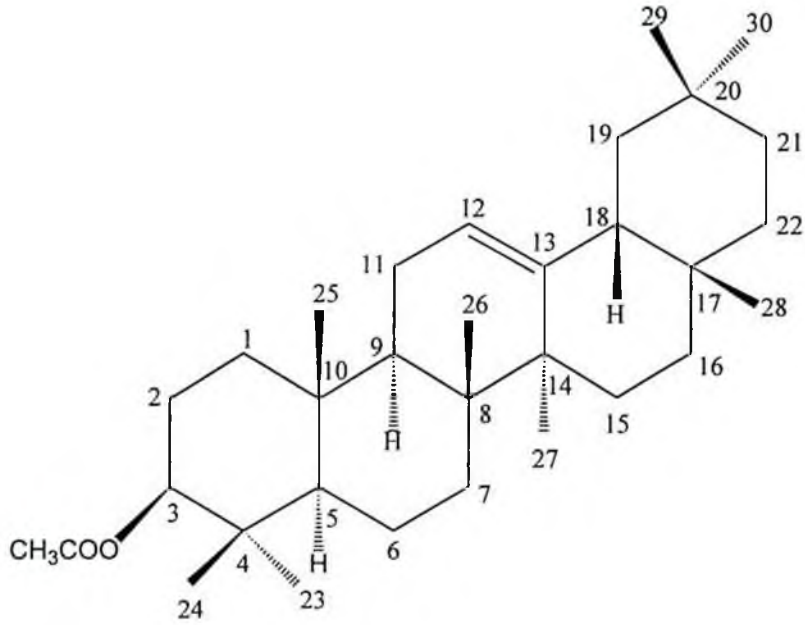
Table 16. NMR[a] spectral data ($CDCl_3$) of lupeol acetate (**179**).

C/H No.	^{1}H-NMR[a] δ_H mult., J = Hz	^{13}C-NMR[b] δ_C mult. *	C/H No.	^{1}H-NMR[a] δ_H mult., J = Hz	^{13}C-NMR[b] δ_C mult. *
1	-	38.3 (t)	18	-	48.2 (d)
2	-	23.7 (t)	19	-	48.0 (d)
3	4.47 (dd, 12.0, 6.0)	80.9 (d)	20	-	151.0 (s)
4	-	38.0 (s)	21	-	29.8 (t)
5	-	55.3 (d)	22	-	40.0 (t)
6	-	18.2 (t)	23	0.76	27..9 (q)
7	-	34.2 (t)	24	0.84	15.9 (q)
8	-	40.8 (s)	25	O.83	16.2 (q)
9	-	50.3 (d)	26	0.93	16.5 (q)
10	-	37.0 (s)	27	0.93	14.5 (q)
11	-	20.9 (t)	28	0.93	18.0 (q)
12	-	25.0 (t)	29a	4.67 (bd, 2.4)	109.3 (t)
13	-	37.8 (d)	29b	4.56 (q, 1.22)	-
14	-	42.8 (s)	30	1.68	19.3 (q)
15	-	27.4 (t)	31	-	170.6(s)
16	-	35.5 (t)	32	2.02 (s, acetate)	21.5 (q)
17	-	43.0 (s)	-	-	-

[a] Data recorded at 400 MHz.
[b] Data recorded at 100 MHz.
*Multiplicity determined by DEPT NMR spectra.

4.5.6 β-Amyrin acetate (180)- A known Triterpene

Compound **24** (1.0 g) was isolated as a crystalline solid (Scheme 19). The molecular formula $C_{30}H_{52}O_2$ was determined from the EIMS (*m/z* 368) and ^{13}C-NMR and DEPT spectra. Analysis of its IR (Section: 4.4.6.6), ^{1}H- and ^{13}C-NMR spectral data (Table-17) and by comparing its data with the literature values (Matsunaga et al., 1970; Chow et al., 1970) assigned the structure of compound **24** as a known compound β-amyrin acetate (**180**).



β-Amyrin acetate (**180**)

Table 17. NMR[a] spectral data ($CDCl_3$) of β-amyrin acetate (**180**).

C/H No.	^{1}H-NMR[a] δ_H mult., J = Hz	^{13}C-NMR[b] δ_C mult. *	C/H No.	^{1}H-NMR[a] δ_H mult., J = Hz	^{13}C-NMR[b] δ_C mult. *
1	-	38.4 (t)	18	0.82	47.4 (d)
2	-	23.8 (t)	19	-	46.9 (d)
3	4.5 (dd, 11.1, 6.0)	81.0 (d)	20	-	31.2(s)
4	-	37.8 (s)	21	-	34.8 (t)
5	-	55.4 (d)	22	-	37.2 (t)
6	-	18.4 (t)	23	0.8.6	28.1 (q)
7	-	32.6 (t)	24	0.86	16.9 (q)
8	-	39.9 (s)	25	O.99	15.6 (q)
9	-	47.7 (d)	26	0.97	15.6 (q)
10	-	36.9 (s)	27	1.15	26.0 (q)
11	-	23.6 (t)	28	0.93	27.0 (q)
12	5.21 (t, 3.5)	121.7 (d)	29	0.86	33.4 (t)
13	-	145.3 (s)	30	0.86	23.7 (q)
14	-	41.8 (s)	31	-	171.1 (s)
15	-	28.5 (t)	32	2.03 (s, acetate)	21.38 (q)
16	-	26.2 (t)	-	-	-
17	-	32.7. (s)	-	-	-

[a] Data recorded at 400 MHz.
[b] Data recorded at 75 MHz.
*Multiplicity determined by DEPT NMR spectra.

4.5.7 Results and Discussion of Hypoglycemic Activity

Results: Effect of *Ficus racemosa* fruits extract and fraction on fasting serum glucose levels of nondiabetic, Type 1 and Type 2 diabetic model rats has been presented in Table 18. It is evident from the Table that injection of Streptozotocin at higher doses to adult rats (producing Type 1 diabetic models) resulted in severe diabetes characterized by marked hyperglycemia (serum glucose level 26-28) mmol/L on the 7^{th} day after STZ injection. Fasting serum glucose levels were only slightly higher in Type 2 model rats (8.4-9.6 mmol/L) indicating the presence of functioning β-cells. The aqueous 80% EtOH extract (FR-p and its water soluble fraction FR-2 of *Ficus racemosa* fruits did not show any serum glucose lowering effect on non-diabetic and Type 2 diabetic rats at the fasting condition. However, FR-p showed significant hypoglycemic effect on Type 1 diabetic model rats at 120 min ($p < 0.01$).

Postparandial condition with simultaneously glucose load

In this experimental situation, FR-p significantly lowered serum glucose levels in nondiabetic rats at 30 and at 75 min (Table 19). Sum of the increments over the basal value was also significantly lowered serum glucose levels of non diabetic rats at 75 min.

In Type I diabetic model rats both FR-p and FR-2 showed significant antihyperglycemic effect at 30 ($p < 0.03 – 0.04$) and 75 min. Sum of the increments over the basal value was also significant for FR-2 ($p < 0.02$).

In Type 2 diabetic model rats in this situation FR-p lowered mean values for serum glucose levels at both the time points. Sum of the increments over the basal value was also significant for both the extracts.

Postparandial condition when the extracts were fed 30 minutes before glucose load:

None of the extracts showed any significant antihyperglycemic effect in any of the models under this condition.

Discussion: Extract FR-p had significant hypoglycemic effect at the fasting state of IDDM model rats. The probable mechanism may be that the extract enhances gluconeogenesis, which is characteristically activated at fasting state in diabetic animals. (Feling et al., 1990).

Both of the fractions were consistently active in both nondiabetic and Type 2 diabetic model rats fed simultaneously with glucose and were inactive when fed 30 minutes prior to oral glucose load, there seems to be a probability that the activities are mediated by inhibition of glucose absorption in the gut by various mechanism or enhanced disposal of glucose by increased insulin sensitivity.

Table-18. Effect of *Ficus racemosa* fruits extract and fraction on fasting serum glucose levels (M ± SD) of nondiabetic, Type I and Type 2 diabetic model rats.

	0 min	60 min	120 min
Non diabetic rat Group			
Control (n = 8)	7.85 ± 0.23 100	7.94 ± 0.40 101 ± 4	7.95 ± 0.49 101 ± 5
FR-p (n = 8) t/p value	8.39 ± 0.31 100 1.40/0.184	9.085 ± 0.19 100 ± 4 2.59/0.024	8.62 ± 0.44 102 ± 5 1.00/0.333
FR-2 (n = 8) t/p value	8.39 ± 0.31 100 1.38/0.189	9.08 ± 0.19 109 ± 4 2.59/0.022	8.59 ± 0.30 103 ± 5 1.11/0.287
Type 1 diabetic rat			
Control (n = 7)	26.79 ± 0.709 100	28.51 ± 2.06 106 ± 6	26.97 ± 1.58 100 ± 5
FR-p (n = 7) t/p value	25 00 ± 1.51 100 1.07/0.305	25.00 ± 1.68 88 ± 5 2.45/0.030* 2.27/0.042*	23.12 ± 1.64 80 ± 4 3.01/0.011* 3.33/0.006*
FR-2 (n = 8) t/p value	28.50 ± 1.074 100 1.36/0.201	25.85 ± 1.83 91 ± 7 0.95 ± 0.361	23.15 ± 0.88 82.4 2.02/0.069
Type 2 diabetic rat			
Control (n = 8)	8.45 ± 0.49 100	10.15 ± 1.13 119 ± 8	8.80 ± 0.98 103 ± 7
FR-p (n = 8) t/p val ue	9.51 ± 0.61 100 1.36/0.200	9.98 ± 0.39 106 ± 5 0.14/0.889	9.60 ± 0.40 103 ± 7 0.76/0.464
FR-2 (n = 8) t/p value	9.61 ± 0.4 100 1.83/0.092	11.25 ± 0.62 117 ± 4 -0.86/0.406	10.33 ± 0.51 107 ± 3 1.39/0.191

*Duncan test with significance level 0.05

Table 19. Effects of *Ficus racemosa* fruits extract and fraction on serum glucose levels (M ± SD) of nondiabetic, Type I and Type 2 diabetic model rats when the samples were fed simultaneously with glucose load.

	0 min	30 min	75 min	Δ
Non diabetic rat Group				
Control (n = 8)	7.86 ± 0.35 100	11.6 ± 0.68 149 ± 9	9.93 ± 0.36 128 ± 7	5.8 ± 0.97 77 ± 15
FR-p (n = 8) t/p value	7.90 ± 0.37 100 0.06/0.95	9.25 ± 0.59 118 ± 8 2.51/0.027*	8.62 ± 0.46 109± 3 2.30/0.040*	2.08 ± 0.68 27 ± 9 2.93/0.030*
FR-2 (n = 8) t/p value	7.70 ± 0.30 100 0.36/0.722	10.08 ± 0.69 132 ± 9 1.58/0.137	8.61 ± 0.20 113 ± 5 3.24/0.006*	3.3 ± 0.67 45 ± 11 2.12 ± 0.053
Type 1 diabetic rat				
Control (n = 7)	25.48 ± 2.00 100	36.92 ± 1.59 148 ± 7	33.00 ± 1.25 133 ± 9	18.96 ± 2.58 81± 16
FR-p (n = 7) t/p value	26.12 ± 1.48 100 0.26/0.801	32.22 ± 1.12 127 ± 11 2.41/0.033* 1.60/0.135	28.63 ± 1.64 113 ± 13 2.25/0.044* 1.28/0.225	8.61 ± 4.37 40 ± 24 2.04/0.064 1.46/0.171
FR-2 (n = 8) t/p value	25.00 ± 1.95 100 0.17/0.867	31.04 ± 1.79 126± 6 2.45/0.031*	28.42 ± 1.46 118 ± 10 2.38 /0.035*	9.46 ± 2.54 44 ± 15 2.62/0.022*
Type 2 diabetic rat				
Control (n = 8)	8.61 ± 0.42 100	22.24 ± 1.15 260 ± 12	22.13 ± 0.98 259 ± 14	27.14 ± 2.01 318 ± 26
FR-p (n = 8) t/p val ue	9.99 ± 0.64 100 1.80/0.097	17.10 ± 2.01 172 ± 20 2.17/0.051*	17.01 ± 1.96 175 ± 25 2.21/0.047*	14.3 ± 3.64 147 ± 44 3.13/0.009*
FR-2 (n = 8) t/p value	10.61 ± 0.46 100 3.14/0.008	21.35 ± 0.86 203 ± 8 0.60/0.561	19.43 ± 0.70 187 ± 15 1.95/0.73	19.55 ± 1.54 190 ± 22 3.04±/ 0.009*

* Duncan test with significance level 0.05

Δ Cumulative value (Sum of the increments over the basal value)

Table 20. Effect of *Ficus racemosa* fruits extract and fraction on serum glucose level (M ± SD) of nondiabetic, Type I and Type 2 diabetic model rats when the extracts were fed 30 minutes before glucose load.

Group	0 min	60 min	105 min	Δ
Nondiabetic rat				
Control (n = 7)	7.10 ± 0.36 100	10.93 ± 0.45 157 ± 11	9.16 ± 0.6 131 ± 10	5.8 9± 1.39 87 ± 21
FR-p (n = 7) t/p value	7.65± 0.32 100 1.15/0.272	10.88 ± 0.83 142 ± 8 0.05/0.963	10.64 ± 0.67 140± 8 1.58/0.141	6.23 ± 0.98 82 ± 13 0.20/0.847
FR-2 (n = 7) t/p value	7.65 ± 0.34 100 1.12/0.285	11.26 ± 0.78 148 ± 10 0.33/0.748	9.34 ± 0.41 113 ± 0.27/0.795	5.29 ± 1.13 71 ± 15 0.34± 0.741
Type 1 diabetic rat				
Control (n = 7)	25.56 ± 0.56 100	32.99± 2.73 128 ± 8	29.03± 1.58 113 ± 5	10.90 ± 2.72 42± 10
FR-p (n = 8) t/p value	24.29 ± 1.00 100 0.94/0.368	32.01 ± 2.02 132 ± 7 0.29/0.775	28.97 ± 2.03 120 ± 7 0.02/0.983	12.40 ± 3.60 52± 15 0.30/0.773
FR-2 (n = 7) t/p value	25.41 ± 1.14 100 0.01/0.921	32.41 ± 1.14 129± 5 0.23/0.825	27,41 ± 1.00 109 ± 4 0.91 /0.380	9.01 ± 2.02 38 ± 9 0.57/0.583
Type 2 diabetic rat				
Control (n = 7)	8.82 ± 0.627 100	21.91± 1.45 254± 19	19.82 ± 0.969 235 ± 23	24.10± 2.40 289 ± 39
FR-p (n = 8) t/p value	10.14 ± 0.807 100 1.30/0.219	20.24± 0.986 204 ± 17 0.83/0.422	16.44 ± 1.987 168 ± 29 1.72/0.114	16.39 ± 3.34 172 ± 45 1.92/0.081
FR-2 (n = 7) t/p value	9.77± 1.03 100 0.83/0.422	22.50± 0.581 242 ± 22 0.33/0.745	20.34± 0.845 224 ± 31 0.39/0.706	23.30 ± 2.51 266 ± 54 0.23/0.823

*Duncan test with significance level 0.05

Δ Cumulative value (Sum of the increments over the basal value)

4.5.8 Results of Antioxidant assay for DPPH Free Radical Scavenging Activity

Stable free radicals, which are used to evaluate antioxidants, include the radical cation of the compound 2,2'-azinobis-(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), the diphenyl-2-picrylhydrazyl radical (DPPH), and the galvinoxyl radical. In the current study DPPH have been used. DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Due to its odd electron, the ethanolic solution of DPPH shows a strong absorption band at 515 nm. DPPH radicals react with suitable reducing agents and then electrons become paired off and the solution loses color stiochiometrically with the number of electrons taken up (blios, 1958). Such reactivity has been widely used to test either the ability of compounds to act as free radical scavengers or the antioxidant activity of plant extracts (Dinis et al., 1994; Lamaison et al., 1990; Navarro et al; 1993).

Reduction of DPPH radicals is observed by the decrease in absorbance at 515 nm. 1-BuOH soluble part of the parent extract of *Ficus racemosa* fruits reduced DPPH radicals significantly. A bioassay-guided fractionation (Scheme-17) of the active 1-BuOH part led to the isolation of compound **19**, 3-*O* (*E*)-caffeoyl quinate. The antioxidant potentials of test samples were campared with standard Propyl gallate. The compound **19** showed significant free radical scavenging (antioxidant) activity against DPPH radicals significantly. The activity results are given in Table **21**.

Table 21. Free radical scavenging activities of 1-BuOH soluble part of *F. racemosa* fruits by DPPH reduction.

Samples *	DPPH Scavenging activity (% inhibition)
FR-3	80.00
FR-3-2	92.50
FR-3-4	94.88
FR-3-6	92.12
FR-3-7	87.21
FR-3-8	90.87
FR-3-2-4	93.62
FR-3-2-4-5	94.50
3-*O* (*E*)-caffeoyl quinate	95.10
Propyl gallate (Standard)	94.00

* Concentration 200μg/mL

5 CONCLUSION

5 Conclusion

Chemical and biological studies on three medicinal plants *Suregada multiflora, Iris germanica and Ficus racemosa* were carried out and our main objective was to isolate new and novel bioactive secondary metabolites from the natural sources. Extracts of *Suregada multiflora* bark showed significant cytotoxic activity on different human tumor cell line and on mechanism based yeast bioassay. Whereas the extracts of *Iris germanica* rhizomes exhibited growth inhibitory activity on mechanism based yeast bioassay. Extracts of *Ficus racemosa* fruits were found as hypoglycemic and antioxidant active. Therefore bioassay-guided investigations on the active extracts of these three plants were carried out. This led to the isolation of nine new (five with novel skeleton) and a number of known compounds.

Eight new diterpenoids and two known triterpenoids were isolated from the active extract of *Suregada multiflora* bark. Compounds suregadolides A-D were found as new compounds with novel skeletons having a trisubstituted cyclopropane ring bridging C-3, C-4 position on the basic abietane framework. Suregadolides A-C and suregadolide D were found as diterpenoids of *ent* and normal series respectively. Isolation of diterpenoids of both the antipodal series from the same plant was an intriguing observation. Compound suremulol A was a new diterpene lactone of *ent* series. Bannaringaolide A was another new class of diterpenoids. The beauty and the novelty of this compound was due to its seven membered ring C. Seven membered ring C in tri and tetra cyclic diterpenes is a rare feature in nature. Compounds suremulol A and B were found as *ent* kaurane diterpenoids derived from two C-13 epimaric *ent*-pimarane compounds. This supported the presence of C-13 epimar of *ent*-pimarane diterpenoids in the plant *Suregada multiflora*. The presence of two epimaric diterpenoids in the same plant is not very common in nature.

Seven isoflavonoids and one steroidal compound were isolated from the active extract of *Iris germanica* rhizomes. One compound nigricin 4′-*O*-β-D glucoside was a new compound having an ethylene dioxy group in ring A. Another three isoflavone Irisolone, 4′-Hydroxy-5-methoxy-6,7-methylenedioxy-isoflavone and irilone were also isolated as known compounds having ethylene dioxy group in ring A. Isoflavonoids having ethylene dioxy group in ring A are not many in number.

Three new diterpenoids suregadolide A & B, and suremulol A, from *S. multiflora* bark and two known compounds irisolone and 4′-Hydroxy-5-methoxy-6,7-methylenedioxy-isoflavone, isolated from *I. germanica* rhizomes showed growth inhibitory activity towards the repair deficient RAD 52 mutant yeast strain in mechanism-based yeast bioassay (Table 9 & 11 and graph 1 & 2 respectively). This result suggested that these compounds may show significant cytotoxic activity in other established bioassays. The other compounds which did not show activity towards the RAD 52 mutant yeast strain may also show activity towards the other mutant strains like RAD 3, RAD 6 of yeast *Saccharomyces cerevisiae* in yeast bioassay or in any other assays.

Six compounds were isolated from *Ficus racemosa* fruits extract. None of the compounds were found new. But the extract of *Ficus racemosa* fruits, its fractions and the compound 3-*O (E)*-caffeoyl quinate (a new source) showed significant antioxidant activity for DPPH free radical scavenging activity test (Table 21). A comparison study of the activity of these fractions and the pure compound 3-*O (E)*-caffeoyl quinate with a standard compound Propyl gallate showed that the activity of most of the fractions were very good and that of 3-*O (E)*-caffeoyl quinate (95%) was found more than the standard compound (94%). An antioxidant prevents the oxidative damage of DNA, protein etc Therefore the observed result suggested that the fruits of *Ficus racemosa* can be used as an antioxidant nutrient like the other well recognized antioxidant foods, contain Vitamin E, Vitamin B, Carotenoids etc. for the prevention of many chronic diseases like cancer, cardiovascular diseases, cataracts etc.

6 REFERENCES

7 PUBLICATIONS FROM THE PRESENT WORK

6. REFERENCES

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7 PUBLICATIONS FROM THE PRESENT WORK

1. Ismet Ara Jahan, Nilufar Nahar, M. Mosihuzzaman, Farzana Shaheen, Zeba, Parveen, Atta-ur-Rahman and M Iqbal Choudhary. Novel Diterpene Lactones from *Suregada multiflora*, *J. Nat Prod.* 55, 2002; 932-934.

2. Atta-ur-Rahman, Shama Nasreen, Irfan Beg, Ismet Ara Jahan, Bilge Sener, Ilkay Orhan and M Iqbal Choudhary. Isoflavone Glycosides from *Iris germanica*, *Chem. Pher. Bull.* 50, 2002; 1100-1102.

3. Ismet Ara Jahan, Nilufar Nahar, M Mosihuzzaman, Farzana Shaheen, Atta-ur-Rahman and M Iqbal Choudhary. *Six new diterpenoids from Suregada multiflora, J. Nat Prod.* (Accepted for publication), 2002.

4. Ismet Ara Jahan, Nilufar Nahar, M Mosihuzzaman, Rokeya Begum, Liaquot Ali, A K Azad Khan, Talat Makhmur and M Iqbal Choudhary. Chemical and Biological studies on *Ficus racemosa* fruits, Fitoterapia (manuscript).