PART I: ISOLATION OF DIPENTYL 2-(4-(PENTAN-3-YL) PHENYL) MALONATE FROM SANGUISORBA OFFICINALIS LABILL
PART II: SYNTHESIS OF A NOVEL FAMILY OF ETHERS OF PODOCARPIC ACID

by

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ABSTRACT

The goal of part I of this thesis was to isolate pure anti cancer compounds from the Russian plant *Sanguisorba officinalis*. This plant was selected for investigation because it showed 100% activity against leukemia (L1210 mouse leukemia cells) during the preliminary screening of approximately 100 Russian plant extracts. This work has resulted in the isolation of novel compound 11 using traditional chromatography techniques. Compound 11 was characterized with spectroscopic techniques such as IR, ^{1}H NMR, ^{13}C NMR, DEPT, DQCOSY and MS. Compound 11 was assigned the structure dipentyl 2-(4-(pentan-3-yl) phenyl) malonate.

The goal of part II of this thesis was to synthesize novel ethers of podocarpic acid. Ethers are of great interest in biological studies and pharmaceutical applications because of their wide variety of uses in the treatment of various diseases. A novel family of ether derivatives was synthesized using podocarpic acid (a natural tricyclic diterpene derived from *podocarpus* species) as a template. Novel ether derivatives of podocarpic acid were synthesized from podocarpic acid in three steps. The first step involved methylation of podocarpic acid with dimethyl sulfate to form methyl-o-methyl podocarpate. The second step was iodination of methyl-o-methyl podocarpate with iodine in presence of a mercury catalyst to form 13-iodo methyl-o-methyl podocarpate. This was followed by formation of novel aliphatic ethers using a copper catalyst. Thus this research had led to the discovery of new methodology for synthesis of three novel aliphatic ether derivatives of podocarpic acid. These ethers will be tested for their biological activity against various types of cancer, tuberculosis by National Institutes of Health.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

^{0}C	Degrees Celsius
^{1}H NMR	Proton Nuclear Magnetic Resonance
^{13}C NMR	13-Carbon Nuclear Magnetic Resonance
DQCOSY	Double Quantum Correlation Spectroscopy
Fig.	Figure
equiv.	Equivalent
hr	Hour
HRMS	High Resolution Mass Spectrometry
IR	Infrared
$CHCl_3$	Chloroform
$CDCl_3$	Deuterated Chloroform
Hz	Hertz
g	Gram
m	Multiplet
d	Doublet
M^+	Molecular ion
mL	Milliliters
cm^{-1}	Reciprocal centimeters
GC	Gas chromatography
m.p	Melting point
DEPT	Distortion less Enhancement by Polarization Transfer

δ	Chemical Shift
ppm	Parts per million
q	Quartet (^{1}H NMR spectrum)
s	Singlet (^{1}H NMR spectrum)
t	Triplet (^{1}H NMR spectrum)
nm	Nanometers
UV	Ultraviolet
t_r	Retention time
NIH	National Institutes of Health

PART I: ISOLATION OF DIPENTYL 2-(4-(PENTAN-3-YL) PHENYL) MALONATE FROM SANGUISORBA OFFICINALIS LABILL

1. Introduction

1.1. Natural Products and Drug Discovery:

Modern medicine has had great success in discovering the new drugs from natural sources (animals, plants and microorganisms) as well as synthetic methods for the treatment of various infectious diseases like malaria, HIV, tuberculosis and cancer. Throughout the history of mankind there has been a rich tradition in the use of herbal medicine for the treatment of human ailments[1] with the first records dating from about 2600 BC in Mesopotamia[2]. This could possibly be because drugs obtained from plants may have fewer side effects and may reduce the possibility of microorganisms developing resistance against drugs. Today, plant-based medicines are being used by approximately eighty percent of the world's population for primary healthcare. Over 50% of the FDA approved anticancer drugs since 1960 have come from natural resources, mainly terrestrial plants[3]. Approximately one-third of the top-selling drugs in the world are natural products or their derivatives[4]. Approximately twenty five percent of the prescription drugs in the United States are the active constituents of higher plants[5]. Some plant based medicines are quinine (the anti malarial drug from Cinchona bark species) digitoxin (used for treatment of congestive heart failure and isolated from

Digitalis purpurea) taxol (the anti- tumor drug from Taxus brevifolia) and ephedrine,(the anti asthma drug from Ephedra sinica).

1.2. Etiology of Cancer and treatment:

Cancer is the second leading cause of death in the United States. Approximately thirty percent of the people develop cancer during their life time[6]. Cancer results from the uncontrollable abnormal growth of cells. Because of no differentiation between cancer and normal cells, cancer cells upon metastasis spreads to other tissues and organs. Hence cancer is a life threatening pandemic disease and must be treated. There are many anti cancer drugs, with a majority[7] of them derived from plant and animal sources. Anticancer drugs act by interfering with cancer cell growth and reproduction. There are many advanced methods for the treatment of cancer; the method to use depends on the decision made by a panel of doctors based on the type of cancer and its intricacies.

The most common types of cancer include colorectal cancer, breast cancer, prostate cancer, lung cancer, skin cancer and leukemia. Leukemia, the most common type of blood cancer affects about 30% of children in the United States. Crowley[8], the author of the book *Introduction to human diseases*, has explained the nature of the disorder leukemia. The term leukemia refers to a neoplasm of hematopoietic tissue. Leukemia is a malignant disease of bone marrow and blood, characterized by uncontrolled accumulation of white blood cells. There are two types of white blood cells called myeloids, which kill bacteria and lymphocytes, which have a significant role in the management of immune system. In contrast to solid tumors, which form nodular

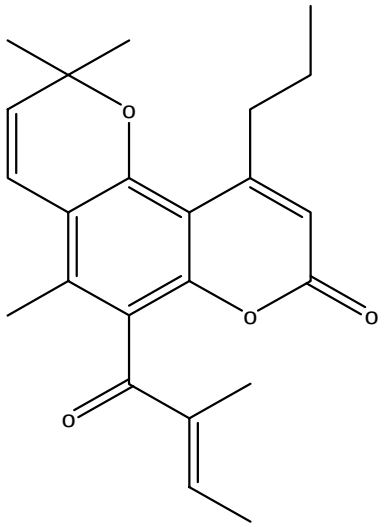
deposits, leukemic cells diffusely infiltrate the bone marrow and lymphoid tissues, spill over into the blood stream, and infiltrate through out the various organs of the body. There are more than dozen types leukemia, out of these, four[9] types of leukemia occur most frequently, including acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML). Leukemia is the most common cancer that affects children under the age of 19. About 78% of childhood leukemias[10] are of ALL type. ALL results when DNA of a cell in bone marrow has genetic injury. This involves accumulation of cells called lymphoblasts or leukemic blasts, which fail to function as normal red blood cells results in anemia, thrombocytopenia, and neutropenia in the blood. Myeloblastic leukemia or myelogenous leukemia (AML) develops when there is a defect in the cells in the bone marrow. AML occurs more often in adults. CML is known as myeloproliferative disorder, the disease in which bone marrow cells proliferate outside the bone marrow tissue. CLL results from gradual accumulation of lymphocytes. The leukemic cells may be mostly mature and can perform certain functions as in chronic lymphocytic or myelogenous leukemia or may be extremely primitive as in acute lymphocytic or myelogenous leukemia and can not perform any functions and must be treated immediately. The overproduction of white cells in leukemia may be reflected in the blood by a very high white blood cell count. In some cases of leukemia white cells multiplication mostly confined to bone marrow as in acute or chronic myelogenous leukemia.

Though treating leukemia is difficult, there is a hope to get success. Management of disease currently relies on a combination of early diagnosis either by bone marrow

biopsy or lumbar puncture and aggressive treatment, which may include one or more treatments such as surgery, radiation therapy, chemotherapy, immunotherapy and bone marrow transplantation available to treat leukemia. Researchers are focusing their studies on developing new effective methods or drugs to treat leukemia. Surgery involves removal of the whole tumor to avoid spreading of cancer to other parts of the body. Radiation therapy is done by passing high energy particles or waves, such as γ rays, x rays, electrons or protons to destroy or damage cancer cells. This works by breaking the strand of DNA inside the cancer cell, which prevents the growth and division of cancer cells. Immunotherapy (biological therapy) has proven useful in treating cancer within last few decades. This is the treatment done by using certain parts of immune system of the patient's body by stimulating the immune system or by using the man made immune system proteins. Nowadays three types of immune therapy are available immune cell administration, use of vaccines and antibody treatment. Immunotherapy[11] is the most promising treatment for leukemia and certain other cancers like kidney cancer, breast cancer, prostate cancer, colorectal cancer and lung cancer. Bone marrow transplantation is required for certain leukemia patients if their bone marrow has destroyed by disease, chemotherapy or radiation therapy. This is the best method available for some patients with acute leukemia and chronic granulocytic leukemia who have not responded to other conventional treatments[12]. The patient's own bone marrow is destroyed by large doses of anti cancer drugs and radiation. Then several hundred milliliters of bone marrow are aspirated from multiple sites in the pelvic bones of suitable donor, filtered to break up the clusters of marrow cells and form a homogeneous suspension of individual cells, and then inject to the patient. The

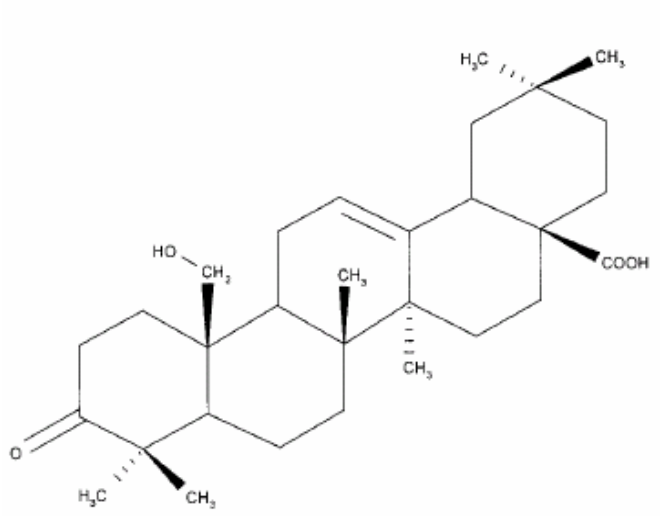
marrow cells circulate in the patient's blood but they become stable and grow in the marrow, produce new population of leukemia free marrow cells. As the bone marrow of donor is a foreign tissue, to avoid rejection, patient's immune system should be suppressed during transplantation. The last and most viable method for treating leukemia is Chemotherapy. Chemotherapy means taking medicines or drugs orally or intravenously to treat cancer. Cancer chemotherapy has been there to treat cancer since 1950[13]. Chemotherapy is helping cancer patients to have a prolonged happy life. Every method in use for the treatment of cancer involves chemotherapy at some stage during the treatment period.

A natural product that has been discovered recently by Shinya Kimura[13] et al for treatment of leukemia is GUT 70 (1), isolated from the stem bark of C*allophylum brasiliense*, which was collected from Brazil. GUT 70 (1) is a tricyclic coumarin named 5-methoxy, 2, 2-dimethyl-6- (2-methyl-1-oxo-2-butenyl) -10-propyl-2H, 8H-benzo (1,2-b; 3, 4-b) dipyran-8-one, which has shown antileukemic activity against five human leukemic cell lines (BV 173, K562, KALM 6, Hl-60 and SEM).



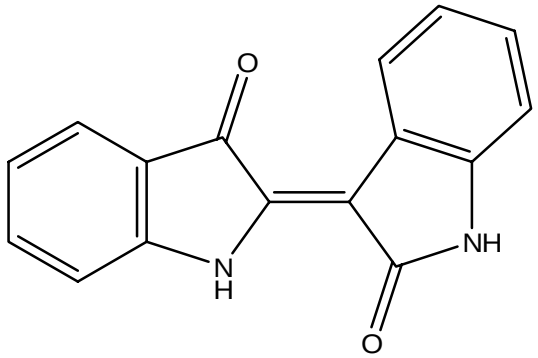
(1) GUT-70

Amooranin (2) is a novel plant triterpenoid that was isolated from *Amoora rohituka* by Chappail Ramachandran[14] et al. Compound 2 exhibited potent anti leukemic activity as was demonstrated by inducing G_2 + M phase arrest during cell cycle in leukemia and colorectal carcinoma.



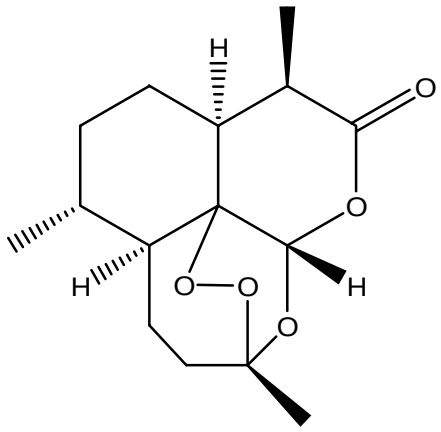
(2) Amooranin

Zhang J T [15] reported that Indirubin (3) was isolated from the leaves of *Indigo naturalis* which is a native Chinese plant. Compound 3 exhibited anti leukemic activity without bone marrow inhibition.



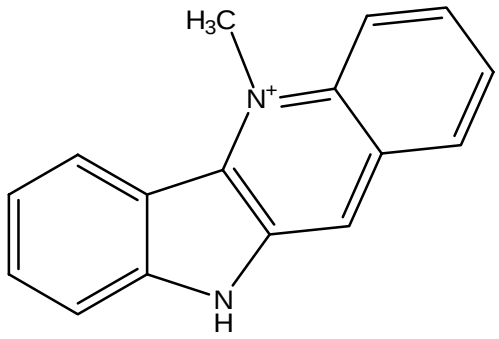
(3) Indirubin

Aryanti[16], Bintag and Maria reported that Artemisinin (4), (a sesquiterpene lactone), obtained from the transformed root of cultured *Artemisia cina that was* produced by infection of stems and leaves with agro bacterium rhizogenes. Compound 4 had a high inhibition activity on leukemic K562 cells with the IC_{50}=1ppm

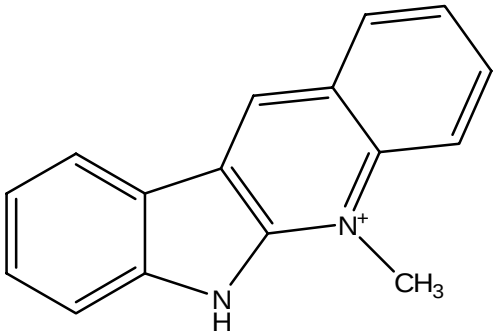


(4) Artemisinin

Two indole quinoline derivatives cryptolepine (5) and neocryptolepine (6) were isolated from the roots of theAfrican plant *Cryptolepis sanguinolenta.* Their cytotoxicity was tested on murine and human leukemia cells. Christian bailey et al[17] reported that cryptolepine (5), and to the lesser extent neocryptolepine (6), showed antileukemic activity by massive accumulation of p388 cells in G_2+M phase of cell cycle.

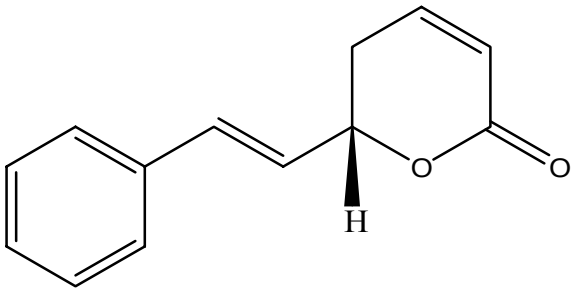


(5) Cryptolepine



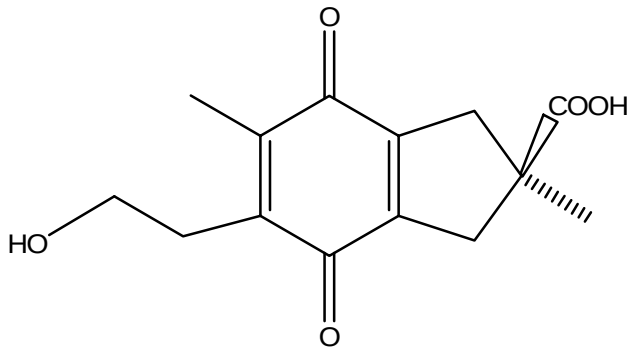
(6) Neocryptolepine

Goniothalamin (7)[18,19]was isolated from *Bryonopsis laciniosa* by Ali Abdul et al. The cytotoxicity of compound 7 was investigated on a T-lymphoblastic leukemia cell line. The results were compared to Etoposide anticancer activity, which indicated that Goniothalamin (7) had a potent apoptic activity. This was confirmed by observing the inter nucleosomal DNA fragmentation.



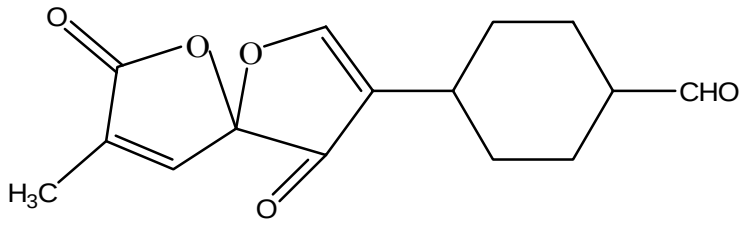
(7) Goniothalamin

The novel norilludalane sesquiterpene puraquinonic acid (8) was isolated by Becker and coworkers[20] from mycelial cultures of *Basediomycete mycenae Pura.* Puraquinonic acid (8) induces differentiation of 30-40% of HL-60 cells into granulocyte or monocyte/macrophage like cells at 380μm. U-937 cells were affected to a lesser extent at the same concentration.

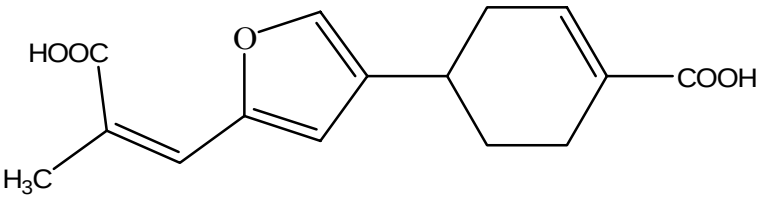


(8) Puraquinonic acid

Erkel and coworkers[21] isolated nidulal (9) novel inducer of differentiation of human HL-60 promyelocytic leukemia cells) from the fermentation of basidiomycete *Nidula candida*, together with small quantities of niduloic acid (10). Both are bisabolane sesquiterpenes.They reported that nidulal(9) activated transcription factor complex AP-1 mediated expression of secreted alkaline phosphatase in COS-7 cells.



(9) Nidulal



(10) Niduloic acid

Thus a variety of novel natural products have been isolated that may yield new drugs for the treatment of leukemia.

1.3. The Goal of This Research:

The goal of this work was to isolate pure compounds, which can inhibit the growth of cancer cells, from methylene chloride extract of Sanguisorba officinalis labill. This plant was selected for investigations as the preliminary screening of methylene chloride extract of this plant from Russia had shown hundred percent activity against leukemia and sixty seven percent anti thrombin activity[22].

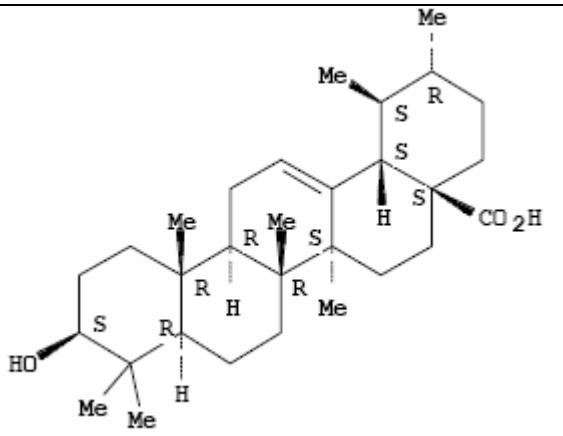
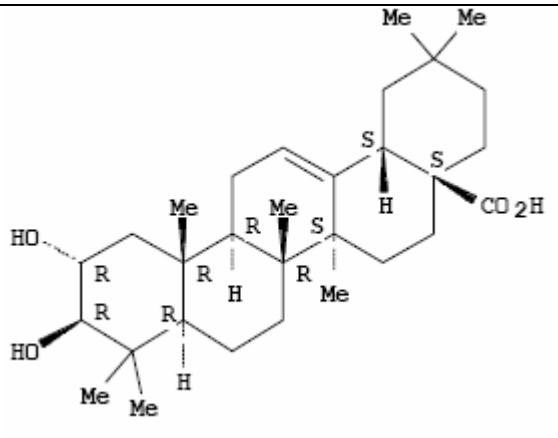
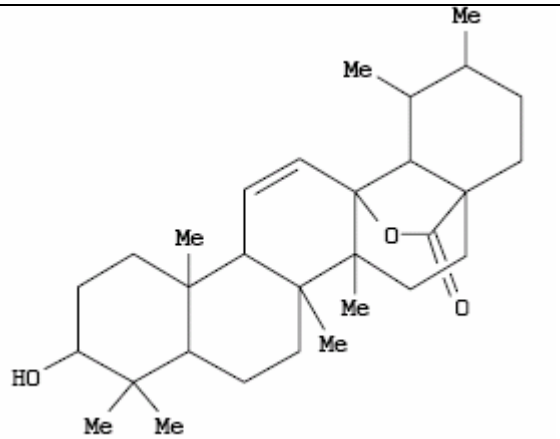
1.4. Sanguisorba Officinalis and its origin:

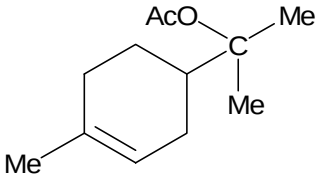
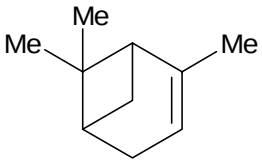
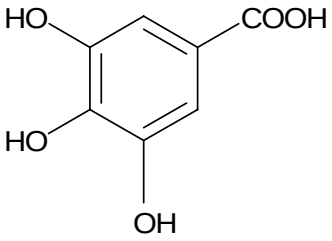
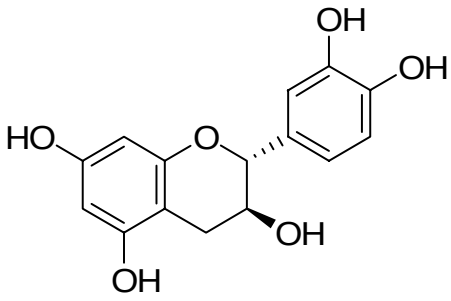
Although this plant material has collected from Russia, Sanguisorba is native to China belongs to family Rosaceae, a large family of 95 genera and 2800 species[23] *Sanguisorba officinalis labill* contains about 25 species. They grow in moist meadows and shady places, almost all over Europe. It grows in a wild from Maine to Minnesota in USA. The aborigines (native Chinese) have traditionally used sanguisorba to heal wounds and to treat fungal infections. Mainly they used in cure of heavy periods and hemorrhage. The extracts of Sanguisorba have been used as food additives, to make tea and in cosmetic formulations. Recently this plant has got much attention to investigate its medicinal uses. Research data has demonstrated that the extracts exhibit various biological activities such as antidiarrheal, antibacterial and antiemetic. It is a valuable astringent and is used for gastrointestinal problems, diarrhea, dysentery, ulcerative colitis and abdominal uterine bleeding. The crude extracts from *Sanguisorba officinalis* showed significant antimicrobial activity[24] against both Gram-positive and Gram-negative bacteria, when tested against five species of microorganisms (*Staphylococcus aureus, Pseudomonas aeruginosa, Escheria coli, Bacillus subtilis, Candida albicans*).

1.5. Previously isolated compounds from *Sanguisorba Officinalis*:

Previously several compounds have been isolated from Sanguisorba Officinalis, including ursolic acid[25], sanguisorbigenin, 2,4-dihydroxy-6-methoxy acetophenone, 3,3',4-tri (O-methyl) ellagic acid, oleanolic acid, masilinic acid[26], sanguisorbin, terpenyl acetate[27] and grandinin. The structures of these compounds shown in table 1. Ursolic acid is a triterpenoid that present in especially waxy coatings of leaves and in fruits, such as apples and pears. It can serve as insect repellant and anti microbial agent[28]. Although triterpenoids have limited medicinal use, recent studies indicate them as potential drug leads[29]. Ursolic acid has shown significant cytotoxicity in the lymphocytic leukemia cells P388 with ED_{50} 3.15mg/mL, L1210 with ED_{50} 4.00 mg/mL[30]. The anti tubercular activity of ursolic acid has also been reported[31].

Table 1 Structures of previously isolated compounds from *Sanguisorba officinalis*

General structure	Compound name
	Ursolic acid
	Maslinic acid
	11,12-Dehydro ursolic acid lactone

	Terpinyl acetate
	α-pinene
	Gallic acid
	Catechin

2. Results and Discussion

Sanguisorba officinalis was selected for this investigation because preliminary screening of the methylene chloride extract from this plant had shown hundred percent activity against leukemia when it was tested on mouse leukemia cells. Dried leaves of the plant material were collected from Russia and extracted with methylene chloride followed by methanol in a soxhlet apparatus for 12 hours. The solvent was removed in vacuum to obtain methylene chloride and methanol extracts. The methylene chloride extract was chromatographed on silica gel and then eluted with mixed solvents such as hexane, methylene chloride; chloroform and methanol in the order of increasing polarity.107 fractions were obtained. The eluent fractions were collected and monitored by TLC on silica gel with different solvent systems ranging from 25%:75% hexane: methylene chloride to 50%:50% hexane: methylene chloride and the results were observed under UV light. Based on these results fractions 1-27 were mixed and further chromatographed in a small liquid chromatography column to obtain twenty fractions. From the resultant fractions, a pure compound was isolated. This compound was a highly viscous liquid with pale yellow color and was proved to be dipentyl 2-(4-(pentan-3-yl) phenyl) malonate based on the spectroscopic data obtained. Normal phase open column chromatography results are shown in table 2 and table 3.

Table 2 Column Chromatography (I) of Sanguisorba Officinalis Labill

Fractions	Eluent	Weight (grams)
F 1-7	100% hexane	0.32
F 8-11	20% CH_2Cl_2 in hexane	1.69
F 12-20	30% CH_2Cl_2 in hexane	3.06
F 21-25	40% CH_2Cl_2 in hexane	1.34
F 26-30	50% CH_2Cl_2 in hexane	0.56
F 31-40	60% CH_2Cl_2 in hexane	2.78
F 41-50	70% CH_2Cl_2 in hexane	0.68
F 51-59	80% CH_2Cl_2 in hexane	3.57
F 60-74	90% CH_2Cl_2 in hexane	3.20
F 75-79	100% CH_2Cl_2	1.84
F 80-84	50% CH_2Cl_2 in $CHCl_3$	2.34
F 85-89	75% CH_2Cl_2 in $CHCl_3$	2.17
F 90-93	100% $CHCl_3$	1.98
F 94-99	50% $CHCl_3$ in CH_3OH	2.20
F 100-107	100% CH_3OH	3.12

Table 3 Column Chromatography (II) of Sanguisorba Officinalis Labill

Fractions	Eluent	Weight (grams)
F 1-2	100% hexane	0.34
F 3-4	20% CH_2Cl_2 in hexane	0.38
F 5-6	50% CH_2Cl_2 in hexane	0.28
F 7-9	75% CH_2Cl_2 in hexane	0.52
F 10	100% CH_2Cl_2	0.48
F 11-13	50% CH_2Cl_2 in $CHCl_3$	1.25
F 14-15	75% CH_2Cl_2 in $CHCl_3$	1.54
F 16	100% $CHCl_3$	0.87
F 17-18	50% $CHCl_3$ in CH_3OH	0.68
F 19	75% $CHCl_3$ in CH_3OH	0.43
F20	100% CH_3OH	0.62

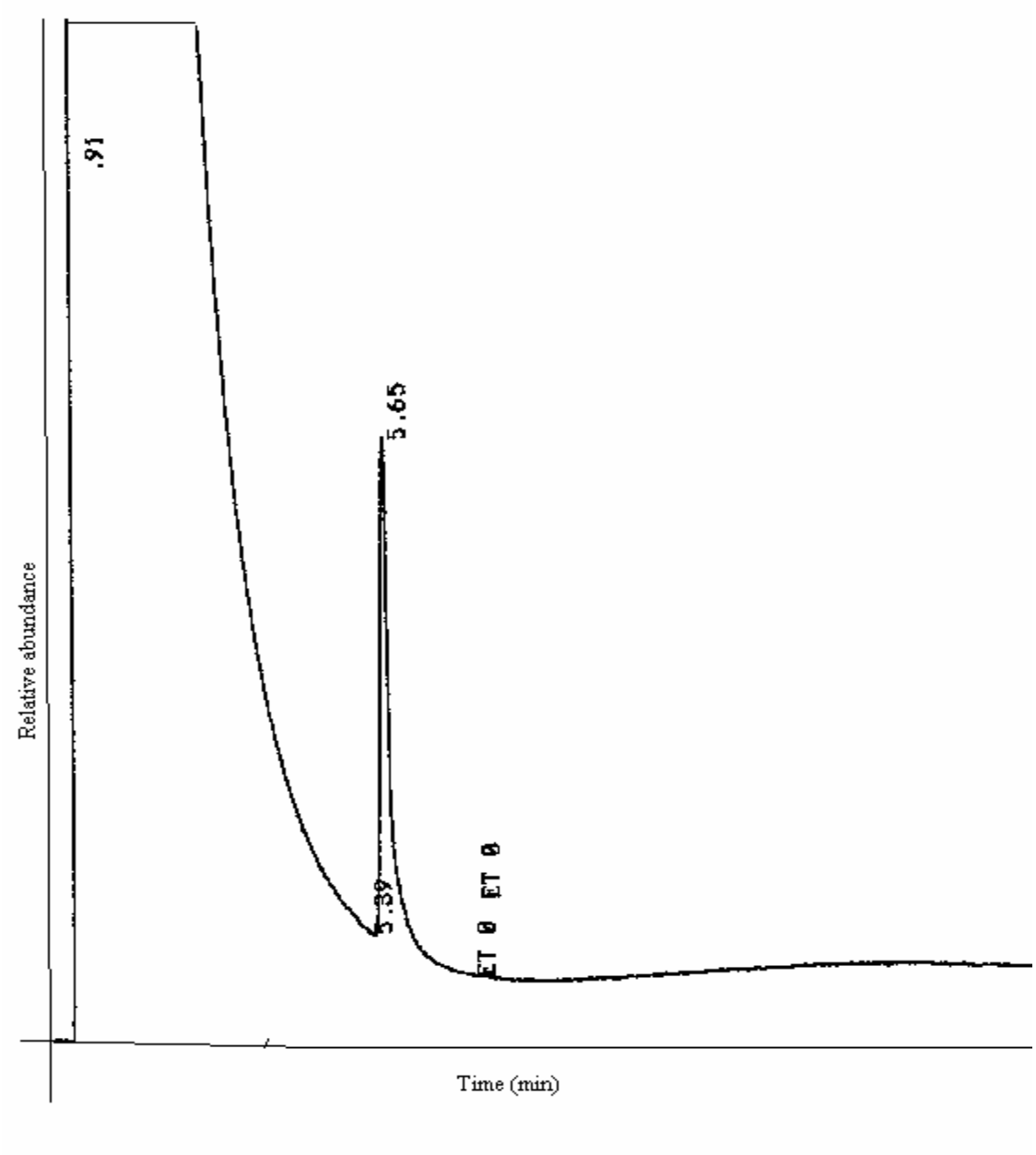


Figure 1 GC of dipentyl 2-(4-(pentan-3-yl) phenyl) malonate(11).

The purity of the isolated compound was evaluated with gas chromatography. The GC showed a single peak with retention time t_r = 5.68 min.

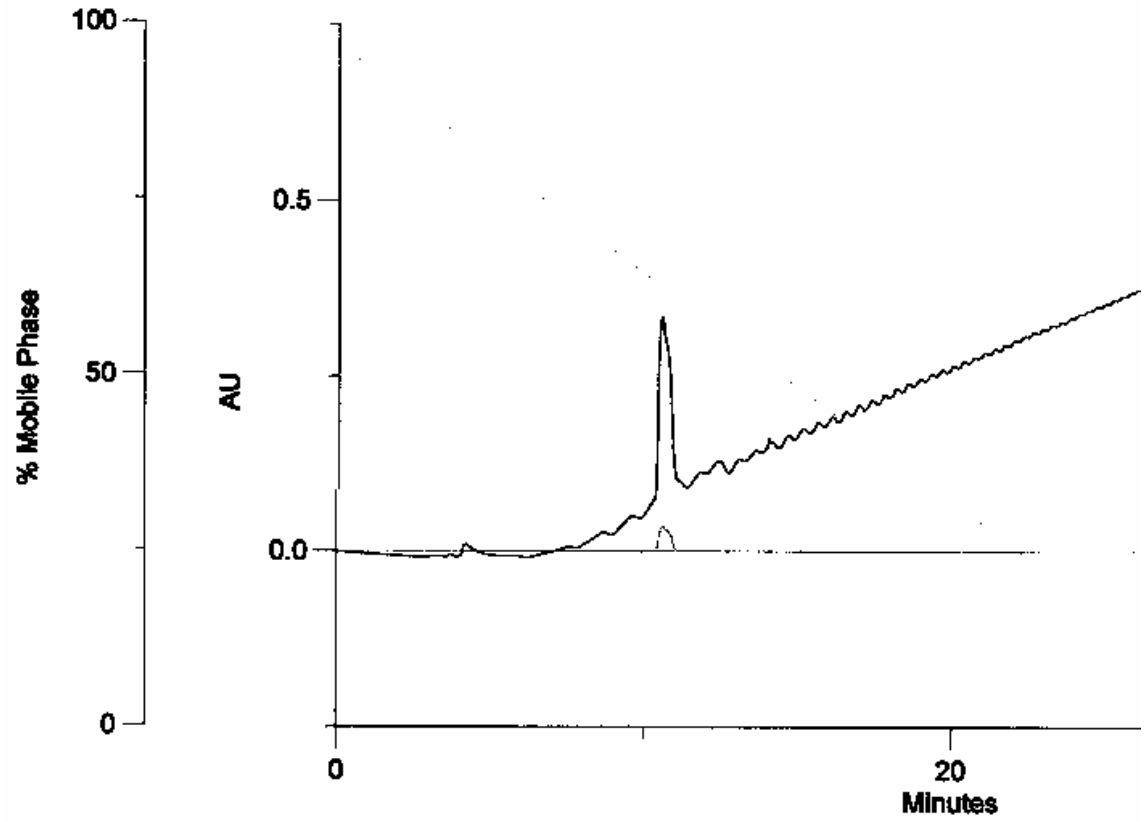


Figure 2 HPLC of dipentyl 2-(4-(pentan-3-yl) phenyl) malonate(11).

High performance liquid chromatography (HPLC) was used for separating and determining species in different materials. Normal phase HPLC was performed by using different compositions of several solvent systems that are normally used and the best results were obtained with hexane and methylene chloride (20 % to 80%).The chromatogram is shown in figure 4.

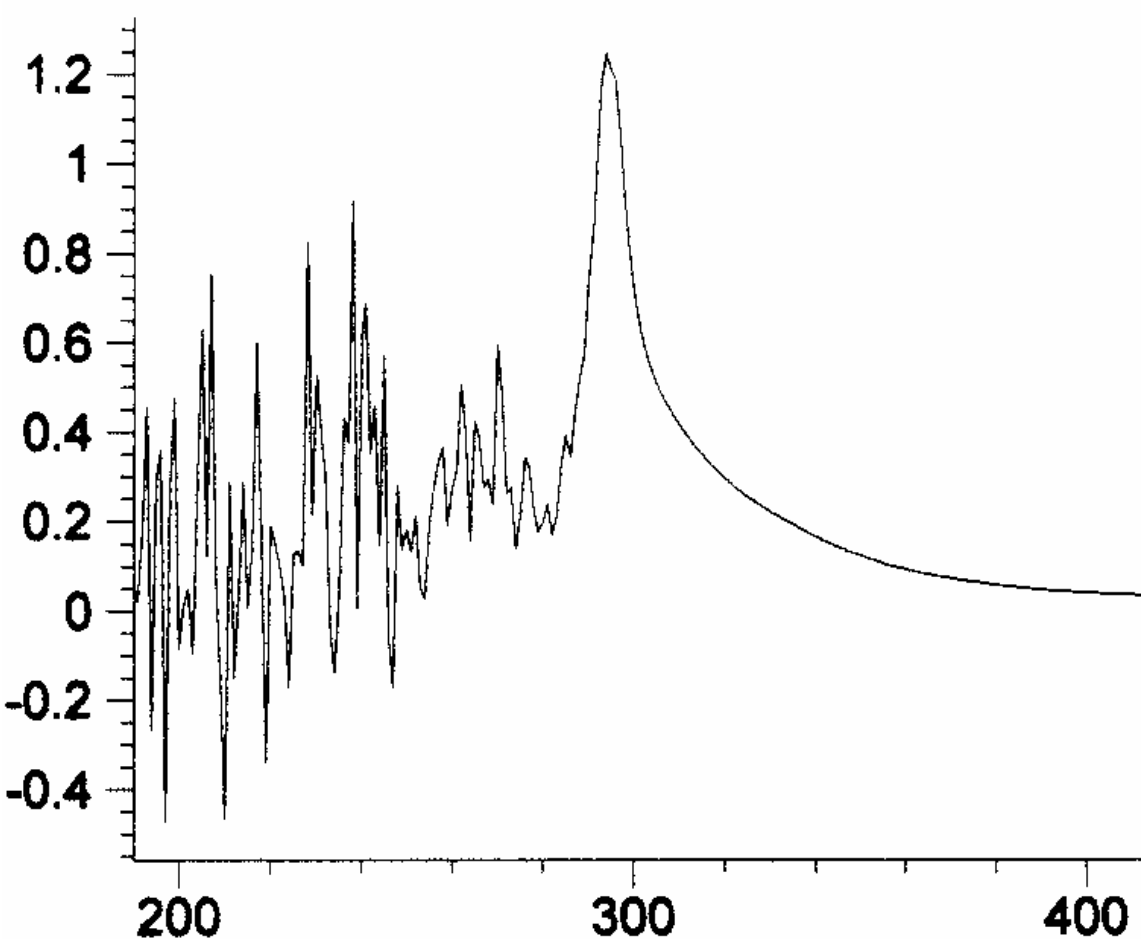


Figure 3 UV spectrum of dipentyl 2-(4-(pentan-3-yl) phenyl) malonate(11).

The UV spectrum of dipentyl 2-(4-(pentan-3-yl) phenyl) malonate (11) showed the absorption maxima at λ_{max}: 200 nm, 268 nm, 298 nm, which suggested the presence of a conjugated system.

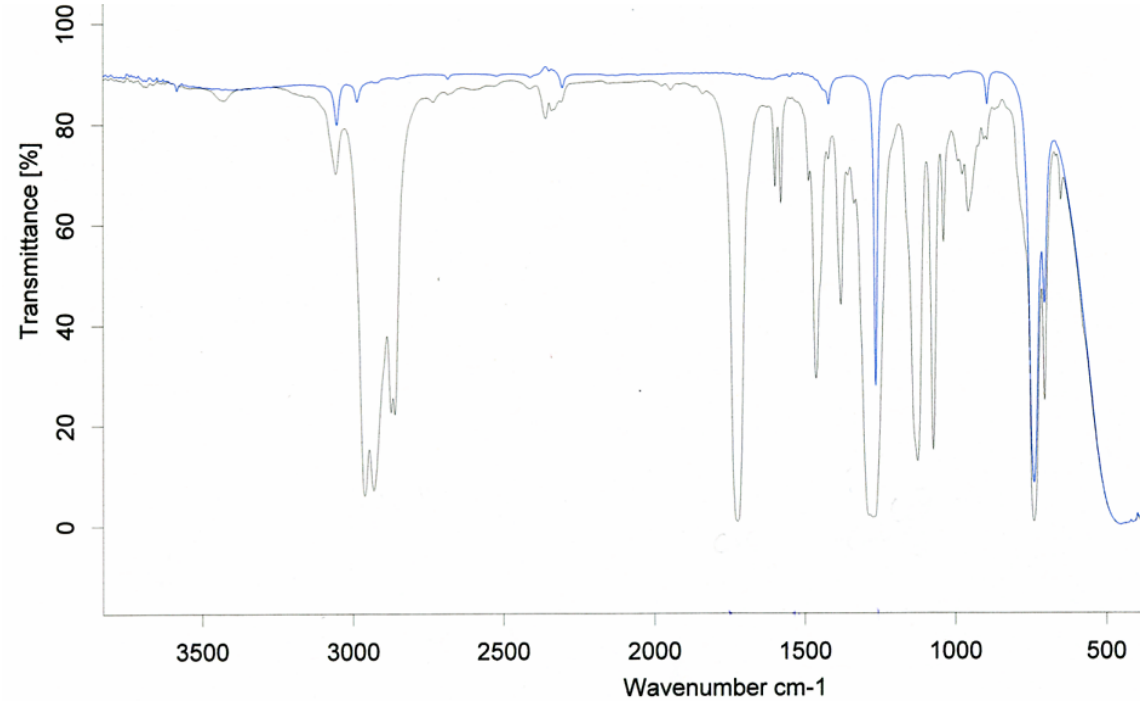


Figure 4 IR spectrum of dipentyl 2-(4-(pentan-3-yl) phenyl) malonate(11).

The IR spectrum has showed absorption maxima at λ_{max} 1740 cm^{-1} for an ester carbonyl group, 1121 cm^{-1} and 1267 cm^{-1} for C-O stretch and 1465 cm^{-1} and 1600 cm^{-1} for an aromatic system.

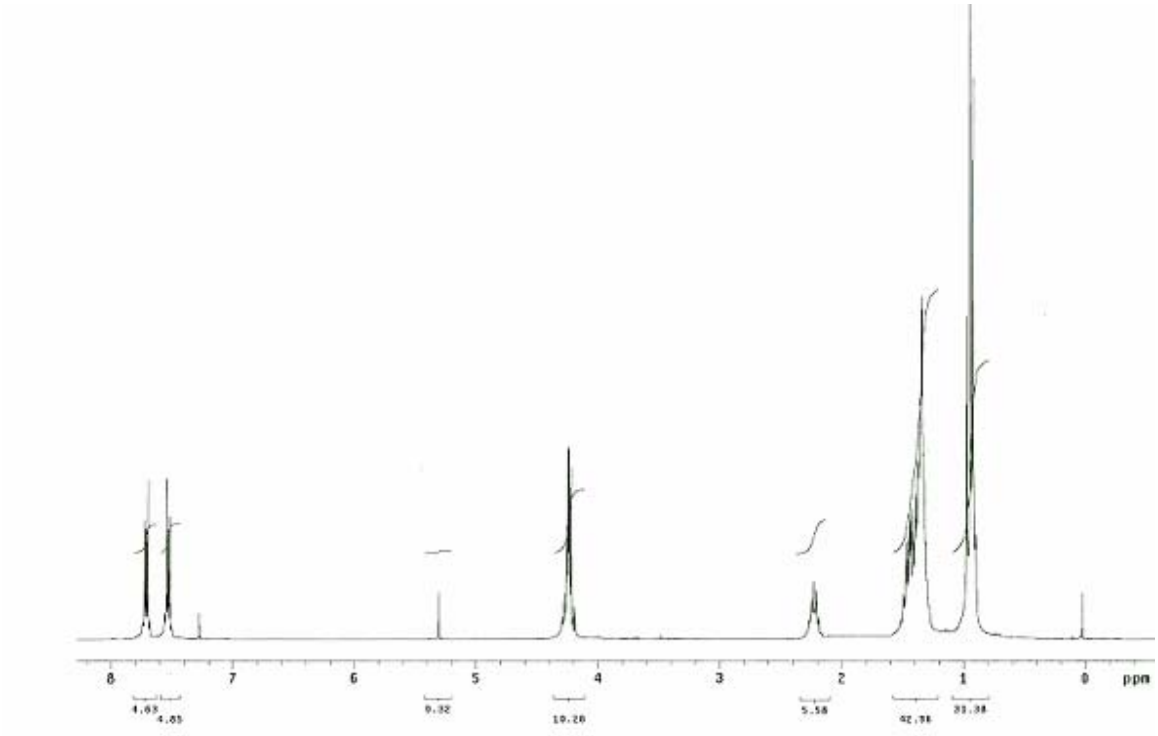


Figure 5 ^{1}H NMR spectrum of dipentyl 2-(4-(pentan-3-yl) phenyl) malonate(11).

^{1}H NMR, ^{13}C NMR, DEPT and DQCOSY data were obtained to complete the structure proof of compound 11. The spectra were run in deuterated chloroform and deuterated acetone. The ^{1}H NMR showed signals between 0.8 and 1.1 ppm for four methyl groups, between 1.2 and 1.6 ppm for eight methylene groups, between 2.2 and 2.8ppm for two methine groups and between 4.1 and 4.3 ppm for two methylene groups attached to oxygen. The signals at δ 7.5 and δ 7.7 correspond to aromatic protons. ^{1}H NMR is shown in Figure 5.

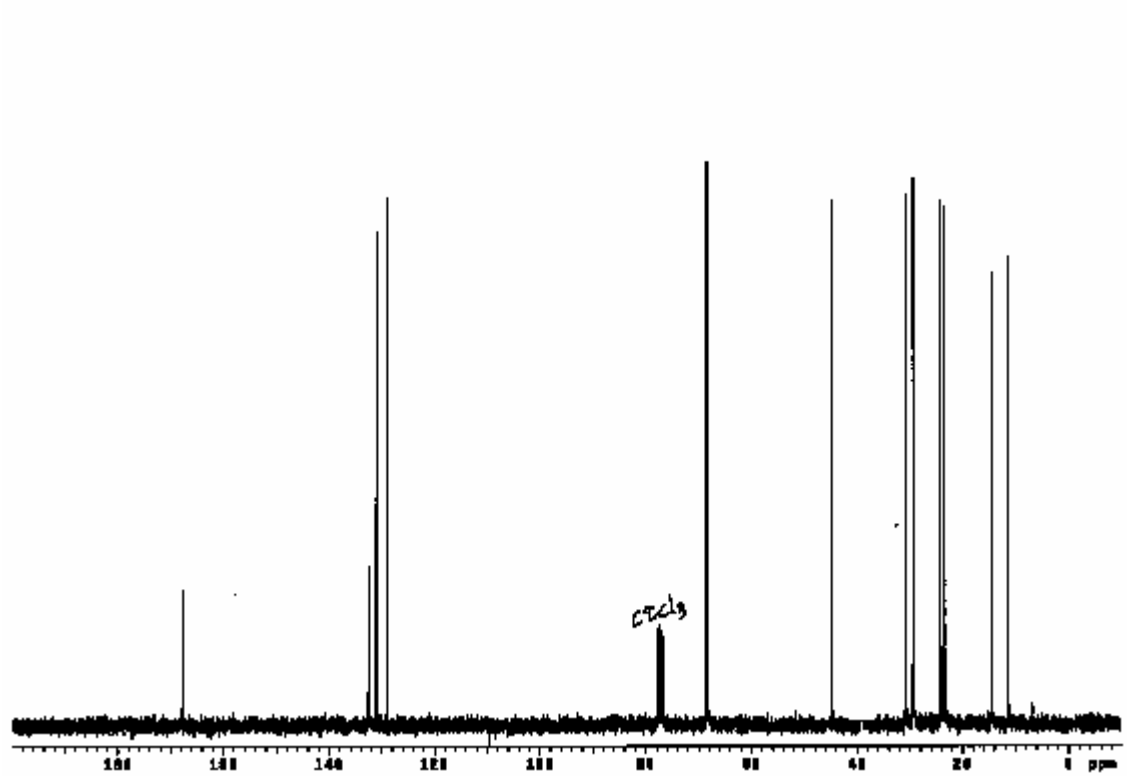


Figure 6 ^{13}C NMR spectrum of dipentyl 2-(4-(pentan-3-yl) phenyl) malonate(11).

The DEPT ^{13}C NMR spectrum (Figure 7) revealed four methyl carbons at δ 11.436 and δ 14.52. The methylene carbons appeared at δ 23.428, δ 24, δ 29.348 and δ 30.775, while the methine carbons appeared at δ 44.9, δ 128.9 and δ 130.9. The quaternary carbons appeared at δ 132.5 and the ester group carbon appeared at δ 167.7. The ^{13}C NMR spectrum is shown in Figure 6.

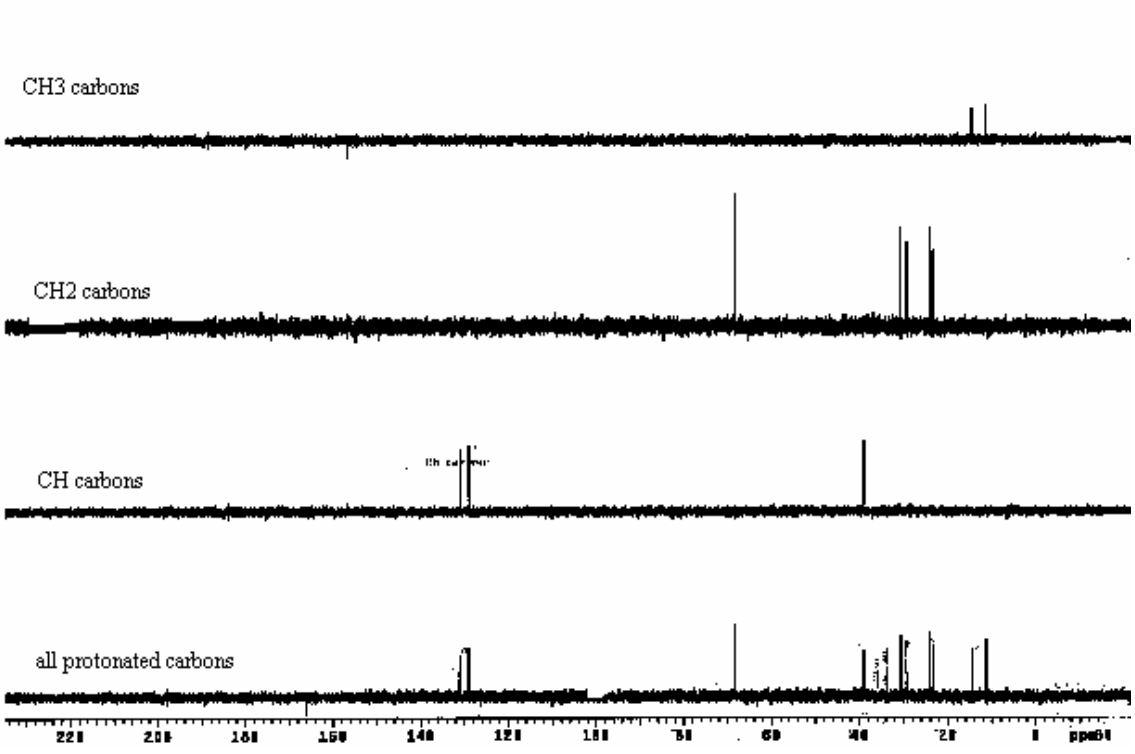


Figure 7 ^{13}C DEPT spectrum of dipentyl 2-(4-(pentan-3-yl) phenyl) malonate (11).

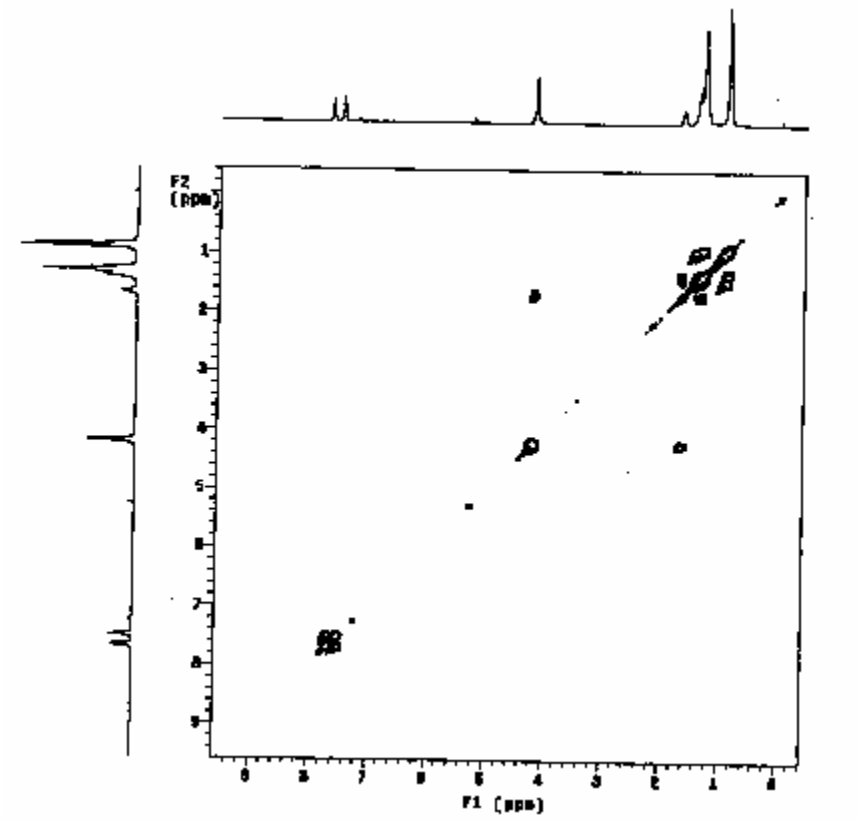


Figure 8 DQCOSY spectrum of dipentyl 2-(4-(pentan-3-yl) phenyl) malonate (11).

The DQCOSY showed that the methylene carbons attached to oxygen are attached to methylene groups and benzene is disubstituted. Some of methylene groups attached to methyl groups.

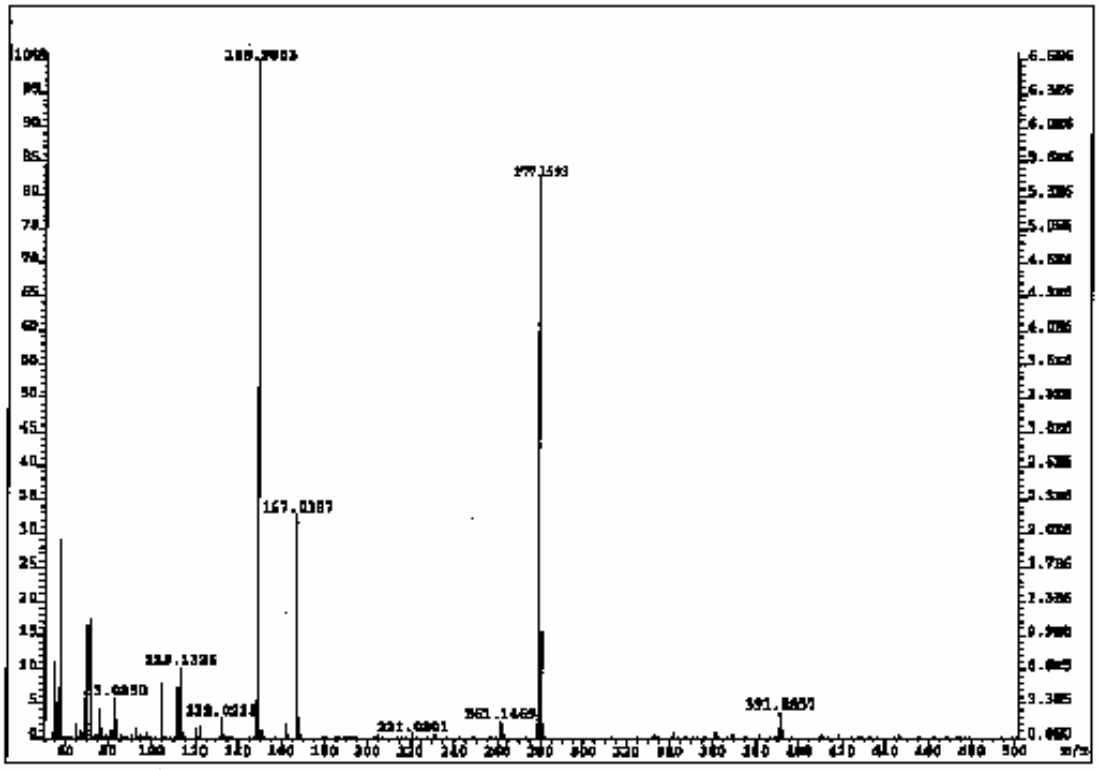


Figure 9 Mass spectrum of dipentyl 2-(4-(pentan-3-yl) phenyl) malonate (11).

The basic fragmentation pattern of proposed structure of dipentyl 2-(4-(pentan-3-yl) phenyl) malonate is illustrated in figure 10.

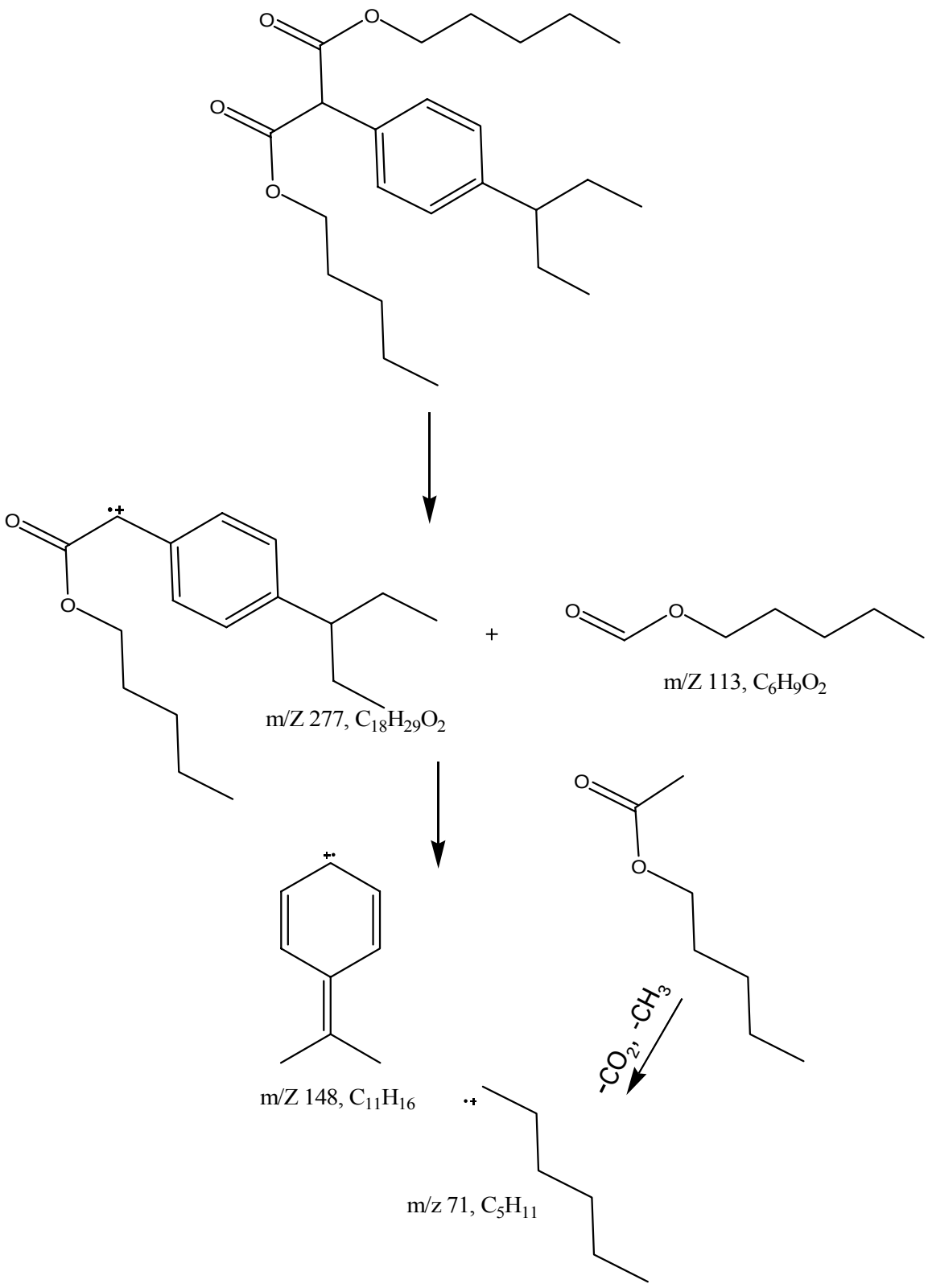
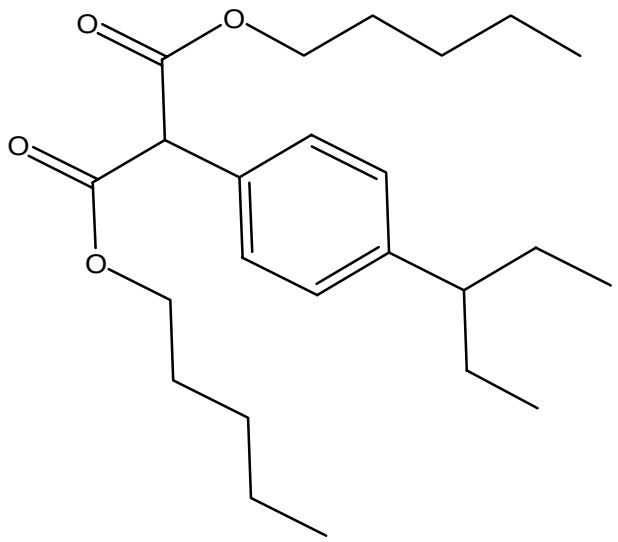


Figure 10 Scheme summarizing the mass fragmentation pattern of dipentyl 2-(4-pentan-3-yl) - phenyl) malonate (11).

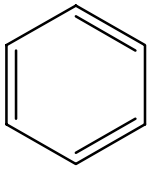
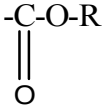
The UV, IR, 1H NMR, 13C NMR and MS data confirms the structure of compound 11 as dipentyl 2-(4-pentan-3-yl) phenyl) malonate.



(11) Dipentyl 2-(4-pentan-3-yl) phenyl) malonate.

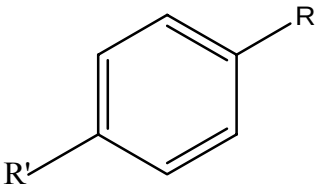
Figure 11 The proposed structure for compound 11, isolated from *Sanguisorba officinalis*

Structure elucidation of compound 11 was done as follows. The UV spectrum showed the absorption maxima at λ_{max}: 200 nm, 268 nm, 298 nm, which suggested the presence of a conjugated system. The IR spectrum has showed absorption maxima at λ_{max} 1740 cm^{-1} for an ester carbonyl group, 1121 cm^{-1} and 1267 cm^{-1} for C-O stretch and 1465 cm^{-1} and 1600 cm^{-1} for an aromatic system. Hence at this juncture, the presence of benzene ring and ester groups were confirmed.

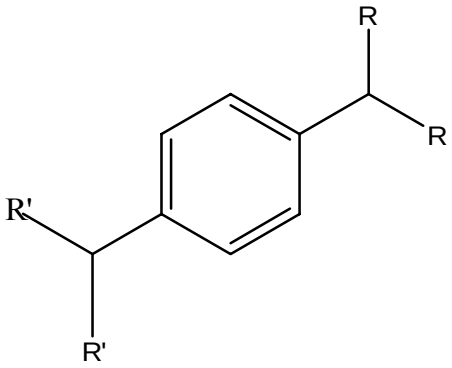
 and 

To know which R group was attached to ester group and benzene ring, the 1H NMR was performed. This showed the presence of Para disubstituted benzene ring by

showing doublet of doublets in between δ 6.0 ppm and δ 8.0ppm also showed the presence of 4 methyl groups, 8 methylene carbons, 2 methine carbons and 2 CH2-O moieties. At this point part of the compound 11 structure was as shown below.



R and R' groups are different. For further information about R and R' groups ^{13}C NMR and DEPT were performed. ^{13}C NMR showed 12 different signals indicated that compound 11 should be symmetric. The DEPT spectrum showed the presence of 2 types CH_3, 4 types CH_2, 1 type CH_2–O and 4types CH groups.



The DQCOSY showed that CH_2-O moieties are attached to CH_2 carbons. The HRMS gave the molecular formula for compound 11 as C24H38O4 and the fragmentation pattern was consistent with the mass spectrum obtained.

Hence from all the above information, by considering carbon-carbon connectivity's in DQCOSY spectrum and mass spectral fragmentation pattern the structure of compound 11 was confirmed as dipentyl 2-(4-pentan-3-yl) phenyl) malonate.

3. Experimental

3.1 General experimental procedure

UV spectrum was obtained in hexane with a Carry 500 scan UV-NIR spectrophotometer and the absorption maximum was given in nm. IR spectrum was recorded on a Perkin Elmer Spectrum One FT-IR spectrometer. The NMR spectra were obtained on a Varian 300 MHz and the solvents used were deuterated chloroform and deuterated acetone. GC-MS spectra were recorded on Trace GC/Trace DQC from Finnegan. A capillary column of RTX-5MS with a length of 15 m and 0.25 mm ID was used. The oven temperature was initially set up to 90C for 7 minutes then programmed to reach 250C. HPLC was run on Gilson equipment, using a silica gel column normal phase and the solvent used was a mixture of hexane and methylene chloride.

3.2 Extraction, isolation and structure determination

The plant material, *Sanguisorba officinalis*, was collected from Russia. The dried leaves (1.00 Kg) were extracted with methylene chloride followed by methanol for 12 hrs by using soxhlet apparatus. After extraction, the solvents were evaporated by using rotary evaporator and the residue from both extracts was collected. 97 g and 124 g of residues were obtained from methylene chloride and methanol extracts respectively. Then open column chromatography was carried out on silica gel (E.Merk, 70-230 mesh). 107 fractions were collected by using different solvent systems in the order of

increasing polarity. All fractions were analyzed by TLC. Fractions with same R_f value were mixed and again open column chromatography was carried out to isolate a pure compound. Pre-coated preparative silica gel GF-254 plates (20x20 cm, 0.5 mm thick, E.Merk) were used for thin layer chromatography.

PART II: SYNTHESIS OF A NOVEL FAMILY OF ETHERS OF PODOCARPICACID

1. Introduction

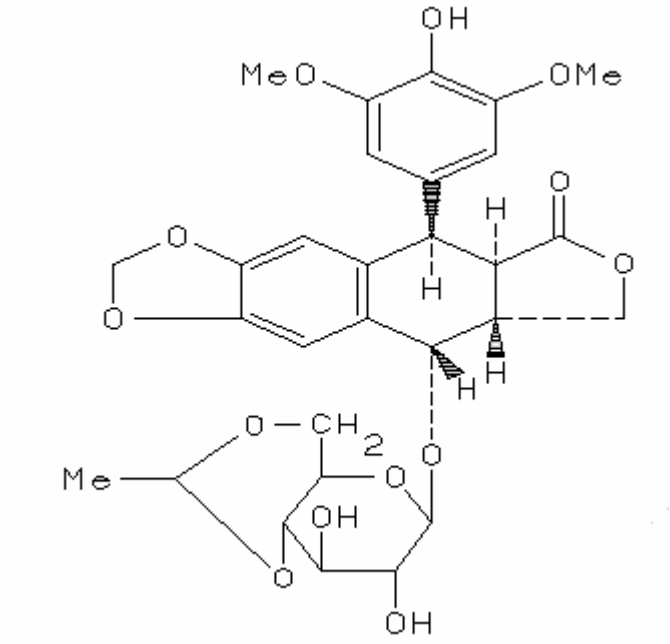
1.1 The Goal of this research:

The goal of this research was to synthesize a novel family of ethers of podocarpic acid by using podocarpic acid as a template. Previous research studies showed that podocarpic acid derivatives were biologically active as well as many compounds with ether functionality. Therefore the premise of this work was to synthesize a library of ethers of podocarpic acid so that their biological activity will be evaluated by the National Cancer Institute against 60 human cancer cell lines for their anti tumor activity and by National Institute of Allergy and Infections Diseases for their activity against tuberculosis.

1.2 Natural products and their anti tumor activity:

Natural products from plants, fungi and bacteria have been used as traditional medicine for the treatment of various diseases and cancers. According to annual report on medicinal chemistry from the year 1983 to 1994, 60% to 75% of the prescription drugs are natural products or their derivatives[32]. There is a huge supply of unexploited natural products with significant biological activity. Many of these compounds have antitumor activity[33]. As an example etoposide (12), antineoplastic agent, a semi synthetic

derivative of podophyllotoxin, extracted from root of *Podophyllum peltatum*, which was shown in figure 12.



(12) Etoposide

Figure 12 *Podophyllum peltatum*

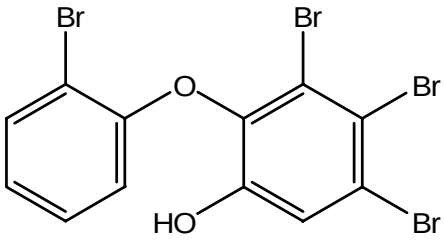
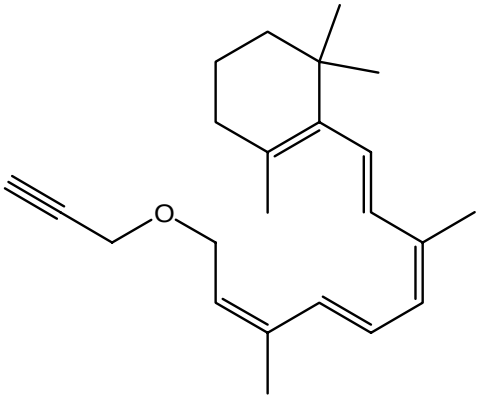
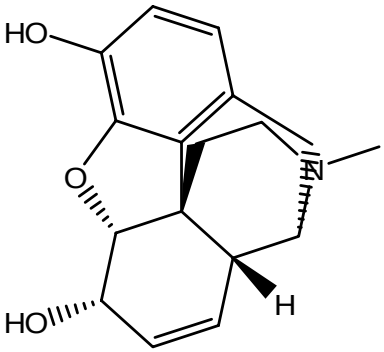
Boik John, the author of the book *Natural compounds in cancer therapy*[34], described about seven strategies to cure cancer. Those are: reducing genetic stability, inhibition of abnormal gene expression, inhibition of abnormal signal transduction, by encouraging normal cell to cell communication, tumor angiogenesis inhibition, inhibition of metastasis and increasing the immune response. Some anticancer drugs act by inhibiting cancer cells growth and thereby causing cell death or by arresting cancer cell proliferation because of molecular target interactions. In contrast, other drugs act by inhibiting cell multiplication indirectly by inducing some changes in the local environment, make it unfavorable for angiogenesis, invasion or metastasis.

Breast cancer is a significant health problem for women in the United States and through out the world. Although advances have made in the treatment of the disease, still breast cancer is the second leading cause of death for women in the United States. No vaccine or any other successful method not available for breast cancer treatment. Hormone therapy is very important treatment method for breast cancer since it is commonly stimulated by estrogens and sex hormones. So, this cancer is treated with anti cancer drugs that inactivate estrogens or limit the amount of estrogens in the body. The drug blocks steroid hormone action and stops cancer cell replication by altering hormone supplies. Tamoxifen is an estrogen antagonist, structurally related to the synthetic estrogen diethylstilbestrol. Tamoxifen acts by inhibiting estrogen action that is required to reduce the level of bioavailable estradiol that is necessary for breast cancer cell growth.

1.3 Some pharmaceutically important ethers:

Polybrominated di phenyl ether reported by Dian Handayani et al[54], which is antibacterial being active against *Bacillus subtilis* and anti fungal, being active against fungi *Cladosporium cucumerinum*. Retinyl-2-propynyl ether activity of suppressing mammary cancer reported by Fulmer shealy et al[55]. Morphine is a known analgesic.

Table 4 Structures of some bioactive ethers:

Structure of the compound	Name of the compound
	3, 4, 5-Tribromo-2-(2′-bromophenoxy) phenol
	Retinyl-2-propynyl ether
	Morphine

1.4 Podocarpic acid (13)

Podocarpic acid (13) is a natural conifer resin acid, which was isolated by Oudemans in 1873 from *Podocarpus Cupressinum.* It was extracted from 'kahikatea' tree *Podocarpus Dacrydioides* and from 'rimu' tree *Dacrydium Cupressinum.*

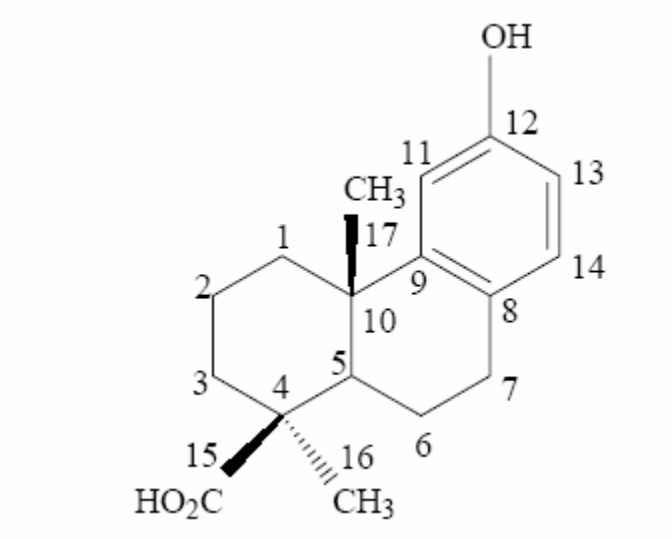


Figure 13 Structure of Podocarpic acid (13)

Figure 14 Podocarpus Cupressinum

Figure 15 Podocarpus Dacrydioides

Figure 16 Dacrydium Cupressinum

These are fast growing trees endemic in Newzealand, which is geographically shown in figure 16

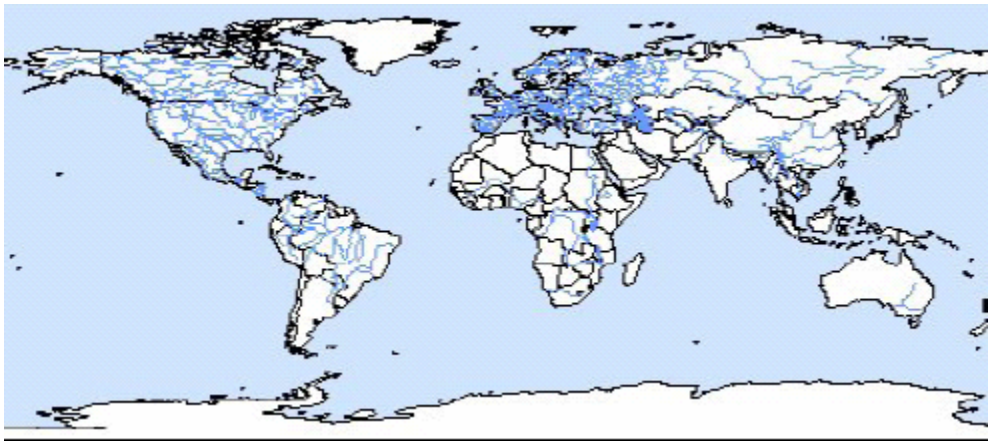


Figure 17 Distribution of Podocarpus (blue areas) in world map

Podocarpic acid (13) has extracted by methanol from heart wood of podocarpus species. Podocarpic acid has been used in Pharmaceutical industry[35] and in the preparation of soaps, paints and adhesives[36]. Podocarpic acid (13) is a white solid material with melting point 195C. It is a natural diterpenoid with tricyclic framework structure of phenanthrene. It has carboxylic acid and phenol functional groups. Because of steric hindrance and due to diaxial interaction with C-10 methyl group carboxylic acid moiety is unreactive[37, 38] as shown below.

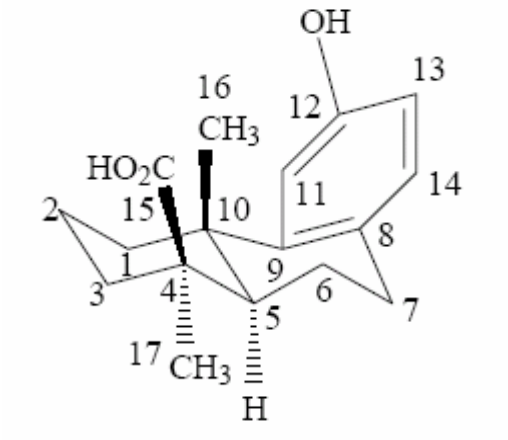
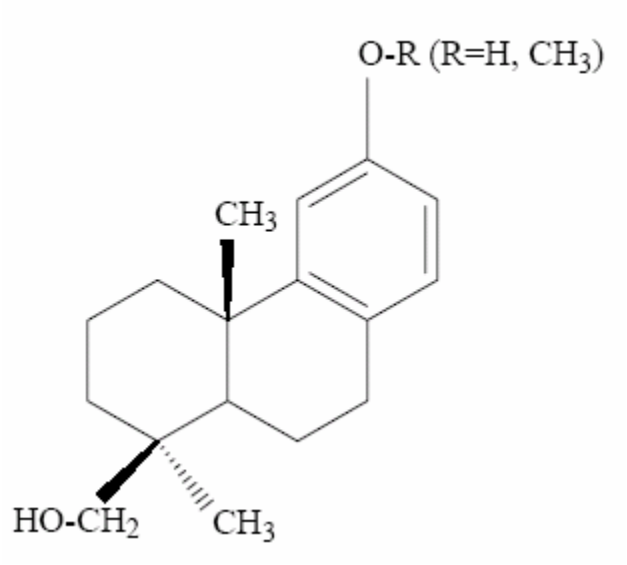


Figure 18 Podocarpic acid (13) conformation

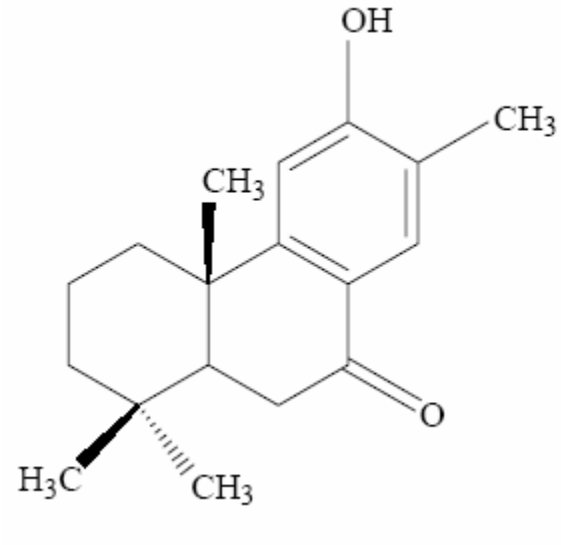
1.5 Previous studies done on podocarpic acid and its derivatives bioactivity:

Podocarpic acid (13) and its derivative podocarpinol's (14) oestrogenic activity was found back in 1948 by Brandt and Ross[39].

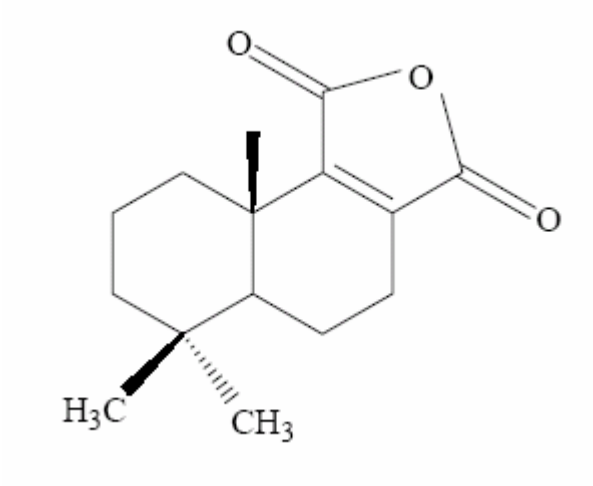


(14) Podocarpinol

Podocarpic acid (13) has been utilized in the synthesis of anti-inflammatory and antiviral agents[40, 41, 42]. Families of novel compounds containing lactones (cyclic esters) and lactams (cyclic amides) have been synthesized from podocarpic acid. Examples of derivatives of podocarpic acid are nimbiol (15) and winterin (16).

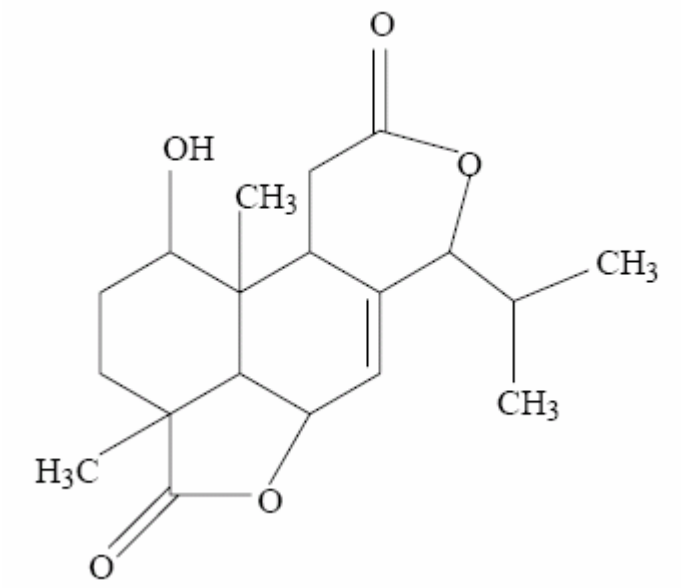


(15) Nimbiol



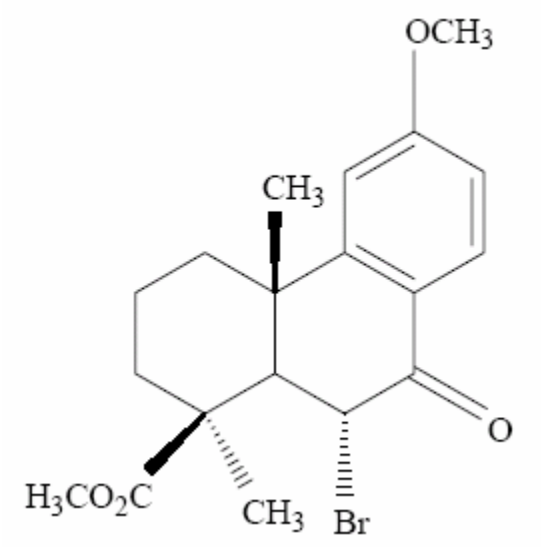
(16) Winterin

Hayashi et al[43] synthesized the biologically active dilactone, hydroxynagilactone (17) from podocarpic acid in 1982.



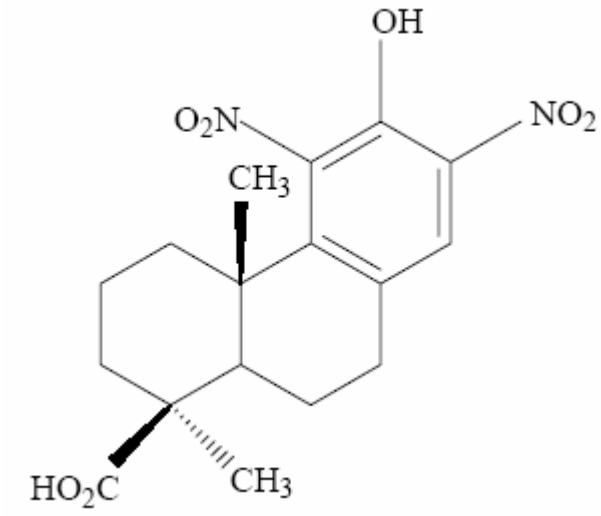
(17) Hydroxynagilactone

Parish and Miles[44] reported the antitumor activity of podocarpic acid derivatives such as methyl-6α-bromo-7-oxo-O-methyl podocarpate (18) in 1984. They investigated compound 18 activity against human epidermoid carcinoma of nasopharynx invitro.



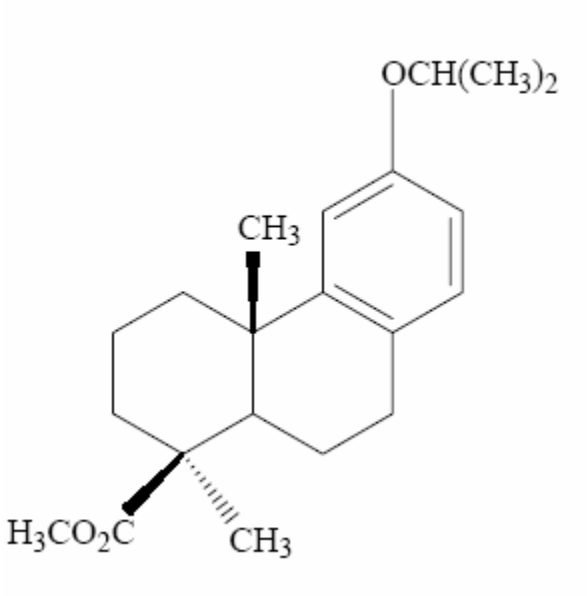
(18) methyl-6α-bromo-7-oxo-O-methyl podocarpate

In 1987, Parish[45] et al synthesized several podocarpic acid derivatives, which showed fungistatic activity. The most potent activity was shown by 11, 13-dinitropodocarpic acid (19).

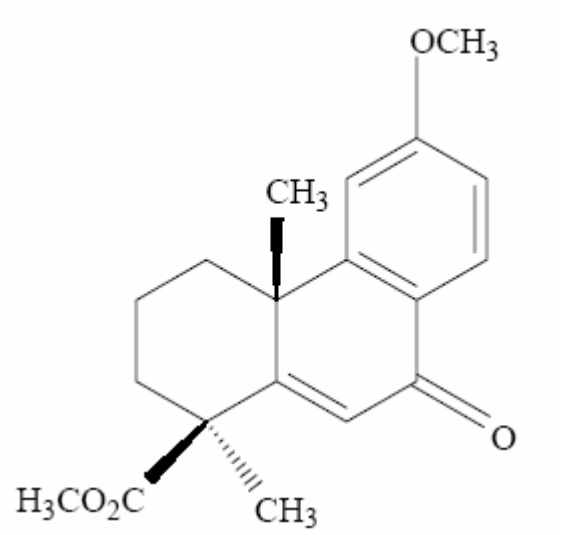


(19) 11, 13-dinitropodocarpic acid

In 1997, Eli lily and company conducted a study on the bioactivity of podocarpic acid derivatives for treatment of bacterial and viral infections. The most potent[46] drugs reported were isopropyl-O-methyl podocarpate (20) and methyl-O-6-en-7-oxo-methyl podocarpate (21).

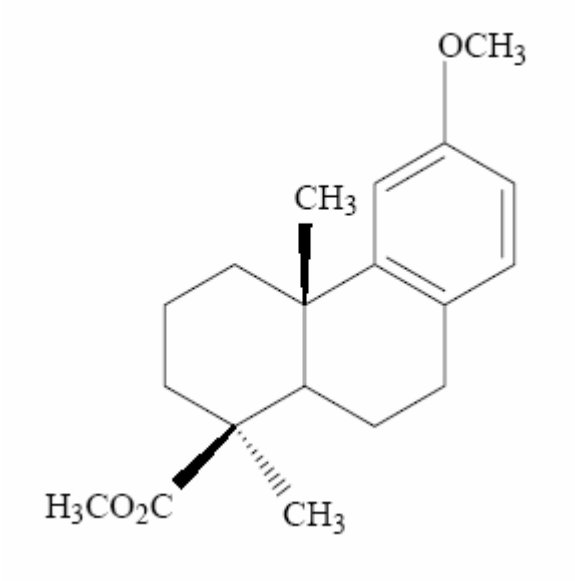


(20) Isopropyl-O-methyl podocarpate



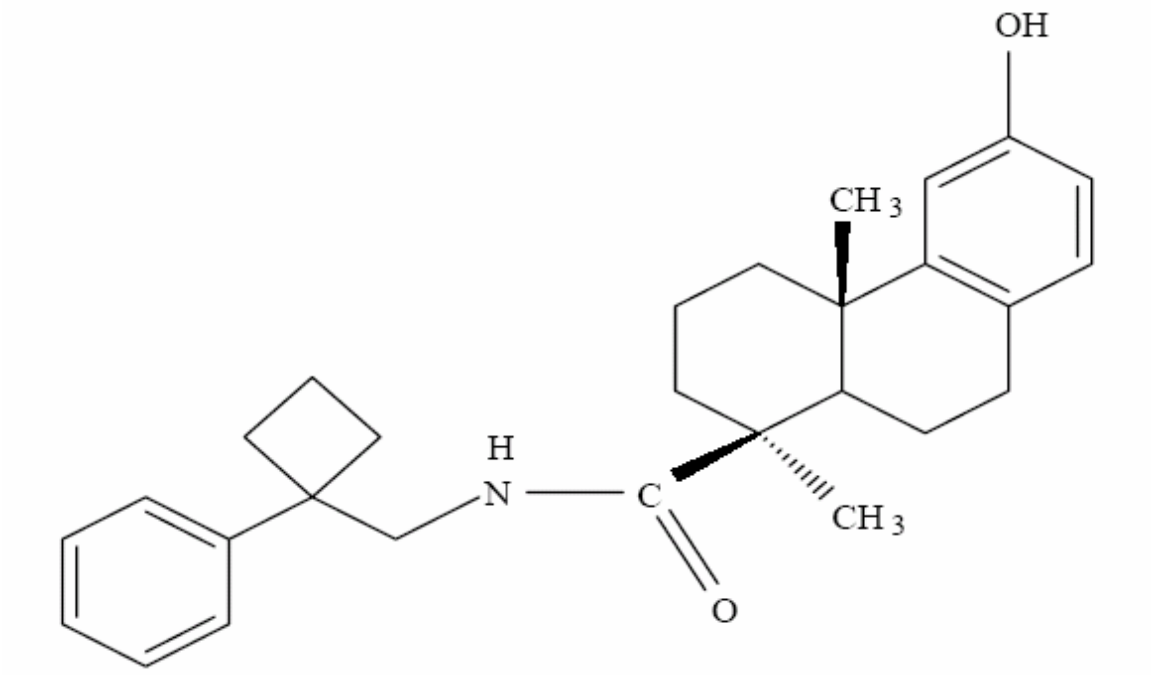
(21) Methyl-O-6-en-7-oxo-methyl podocarpate

In 1998, methyl-O-methyl podocarpate (22) was reported as antiviral agent. This compound 22 showed antiviral activity[47] by inhibiting replication of cells during protein synthesis of influenza A/ Kawasaki virus.



(22) methyl-O-methyl podocarpate

Adams[48] et al., in 2003, synthesized the podocarpic acid derivative 23 to use as an antihyperlipidemic agent (to treat conditions like depressed levels of HDL).



2. Results and Discussion

The goal of this research was to synthesize a family of novel ether derivatives of podocarpic acid (13) at the C-13 position of the aromatic ring as shown in figure 19.

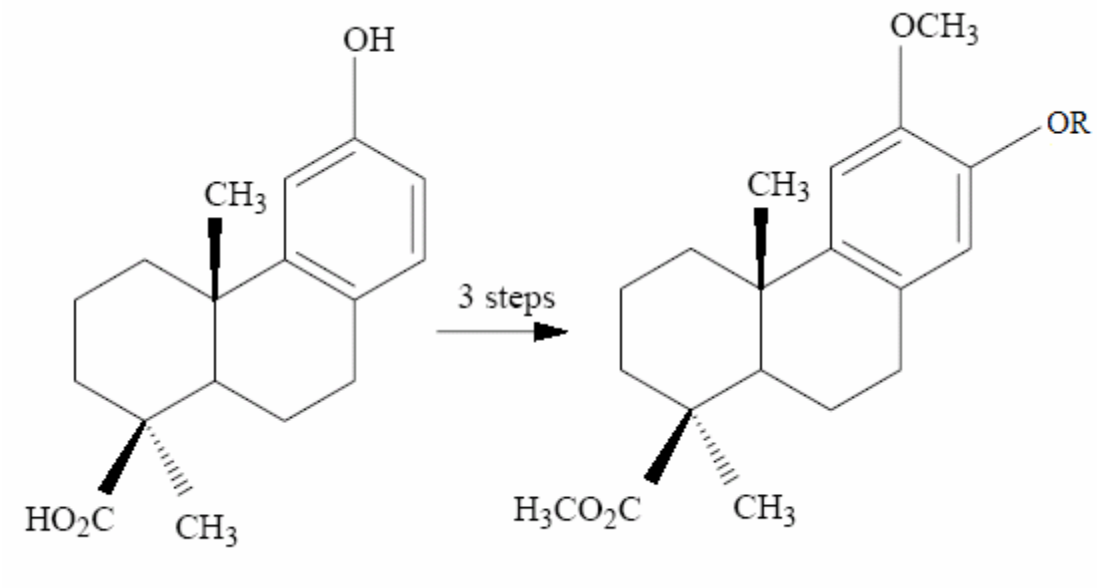


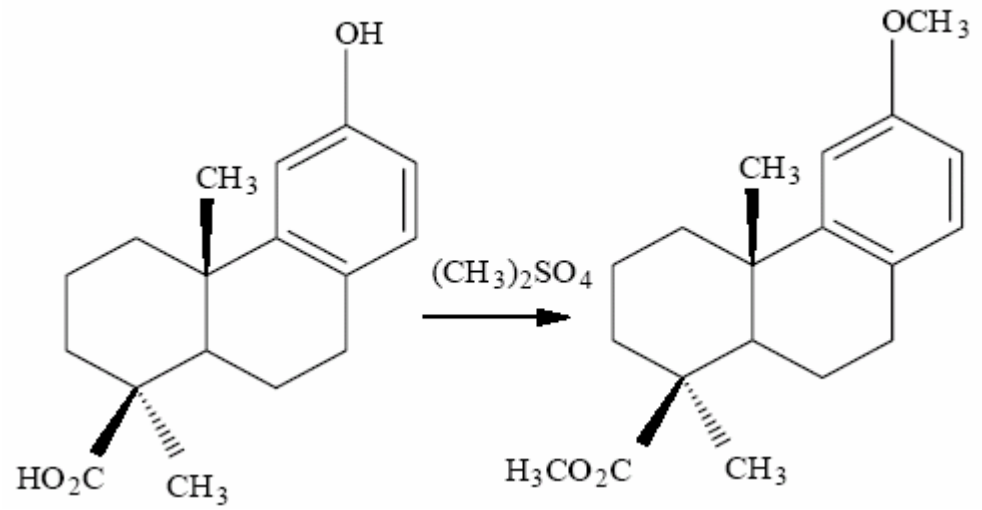
Figure 19 Synthesis of ether derivatives of podocarpic acid (13)

Ethers are of great interest in organic synthesis because of their wide variety of uses. It is known that aromatic amides can be prepared from aryl halides[49]. About hundred years ago in 1903 Ulmann[50] discovered the coupling of aryl bromide to form biaryl compound. In 1926, Goldberg studied the formation of ethers from aryl bromide by using copper catalyst. The reaction temperature of about 200C gave low yields of the products and separations were difficult.

Palladium has been known as a catalyst for formation of ethers at moderate temperatures with good yield. However palladium catalyst and phosphine ligands are expensive. As a result, the search for inexpensive copper catalyst[51, 52] was conducted for coupling of an aryl halide with an alcohol to make an ether.

The advantages of copper catalyst are that it is cheap and can be used in versatile conditions such as in the presence of moisture or oxygen. Hence a copper catalyst was used in the synthesis of novel ether derivatives of podocarpic acid.

The first step in this process was methylation of podocarpic acid (13) as described by Miles[53] to form methyl-O-methyl podocarpate (22) as shown in figure 20.

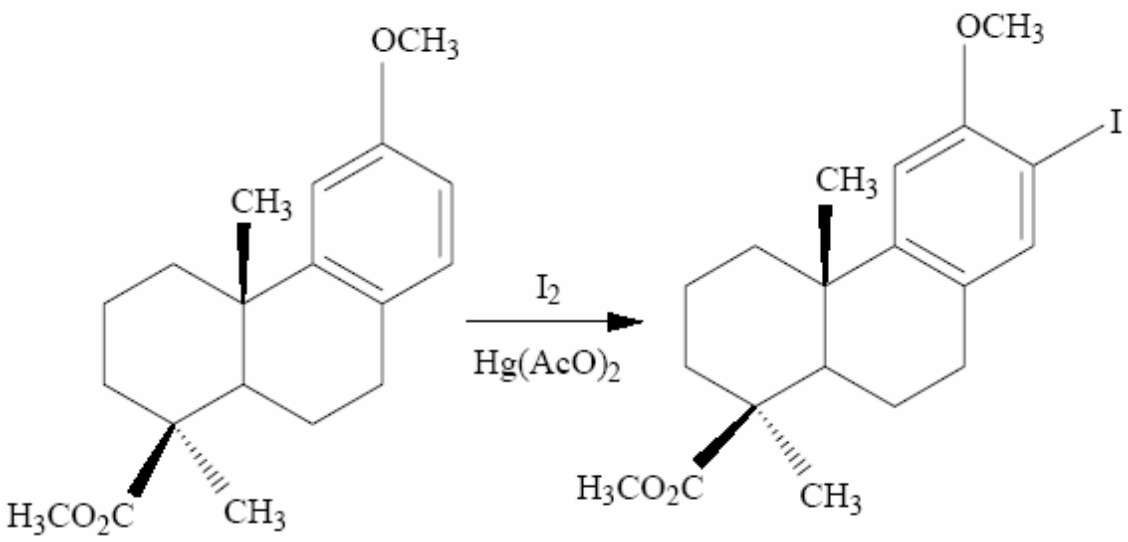


(13) (22)

Figure 20 Formation of methyl-O-methyl podocarpate (22)

Compound 22 was formed in 84% yield and melting point was 127C. Spectroscopic data such as IR, NMR and MS was consistent with the data shown[53].

Iodination of compound 22 was performed by reacting compound 22 with iodine to form 13-iodomethyl-O-methyl podocarpate (23) in 94% yield with a melting point of 149C. The spectroscopic data was consistent with the data shown.



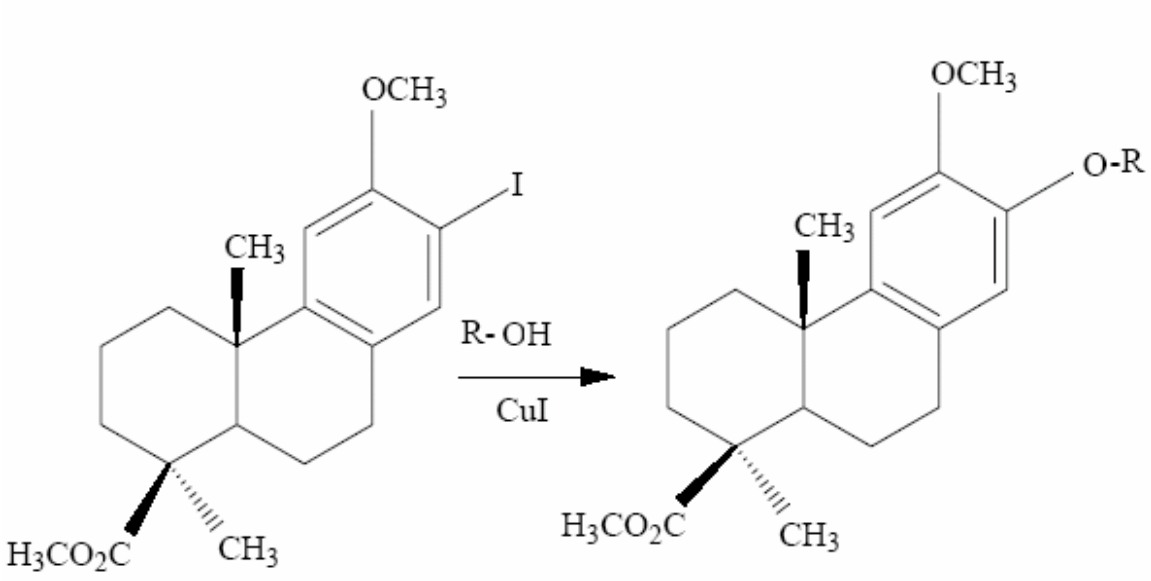
(22) (23)

Figure 21 Formation of 13-iodomethyl-O-methyl podocarpate (23)

Compound 23 was then reacted with a series of aliphatic alcohols (see Table 5) to form novel ethers at C-13 of podocarpic acid.

Table 5 Alcohols used in the synthesis of novel ethers from compound 23

Alcohol	Formula
Methanol	CH_3-OH
Ethanol	CH_3-CH_2-OH
Propanol	CH_3-CH_2-CH_2-OH



(23) (P)

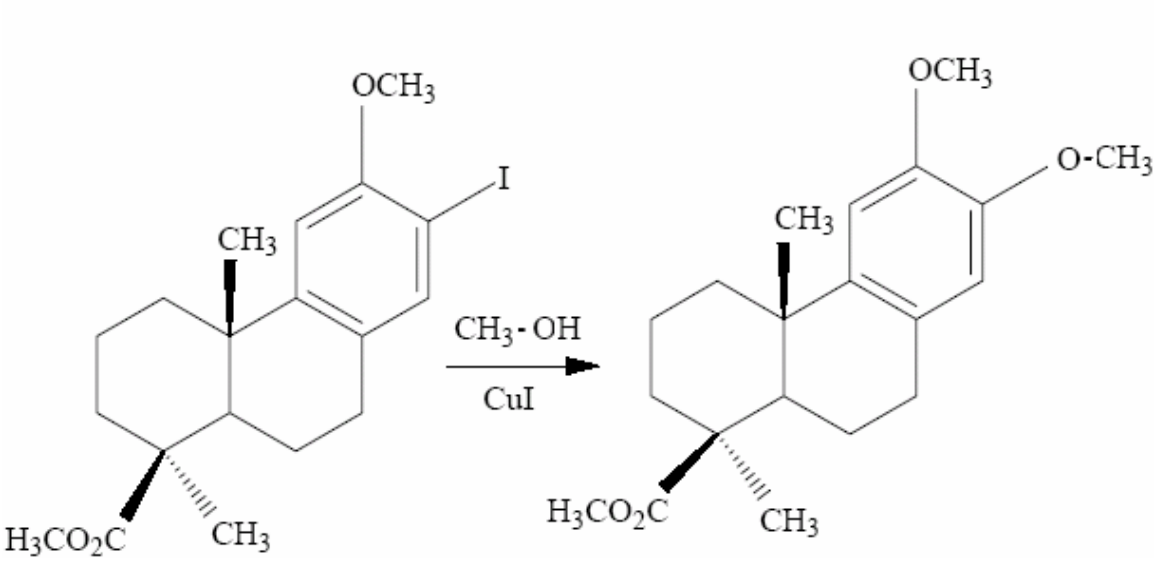
Figure 22: General reaction for formation of novel ethers of podocarpic acid

Table 6 Attempts in the synthesis of ethers with various R groups

R	$-CH_3$	$-C_2H_5$	$-C_3H_7$
Ether (P)	24	25	26

The general reaction involved mixing 1 molar equivalent of compound 23 with 1.5 molar equivalents of the corresponding alcohol and sodium alkoxide, 0.1 molar equivalent of copper catalyst and 2 molar equivalent of potassium carbonate in N, N- di methyl formamide at 110C and refluxing for four hours while monitoring the progress of reaction with TLC. When the reaction was completed, the reaction mixture was allowed to cool and the precipitate was obtained by vacuum filtration. Normal phase open column chromatography was carried out on silica gel 70-230 mesh to obtain the pure compound. The structure of each product was elucidated by IR, ^{1}H NMR, ^{13}C NMR and MS spectroscopy techniques.

The reaction with methanol formed a new compound 13-methoxy methyl-O-methyl podocarpate (24) in 84% yield with a melting point 143C. The IR, ^{1}H NMR, ^{13}C NMR and MS spectra of compound 24 are shown below.



(23) (24)

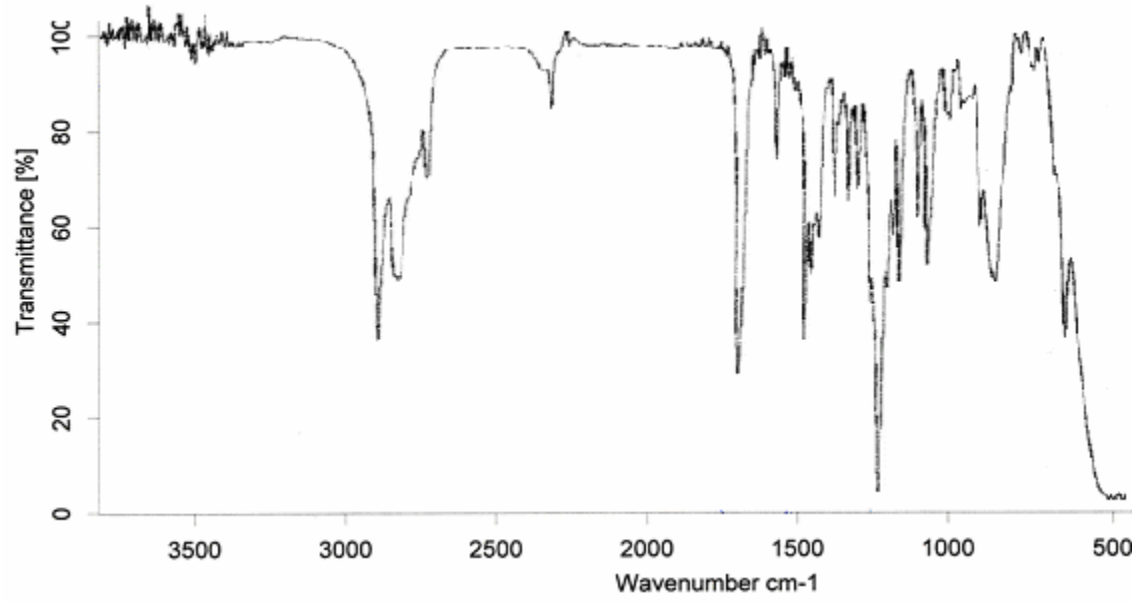
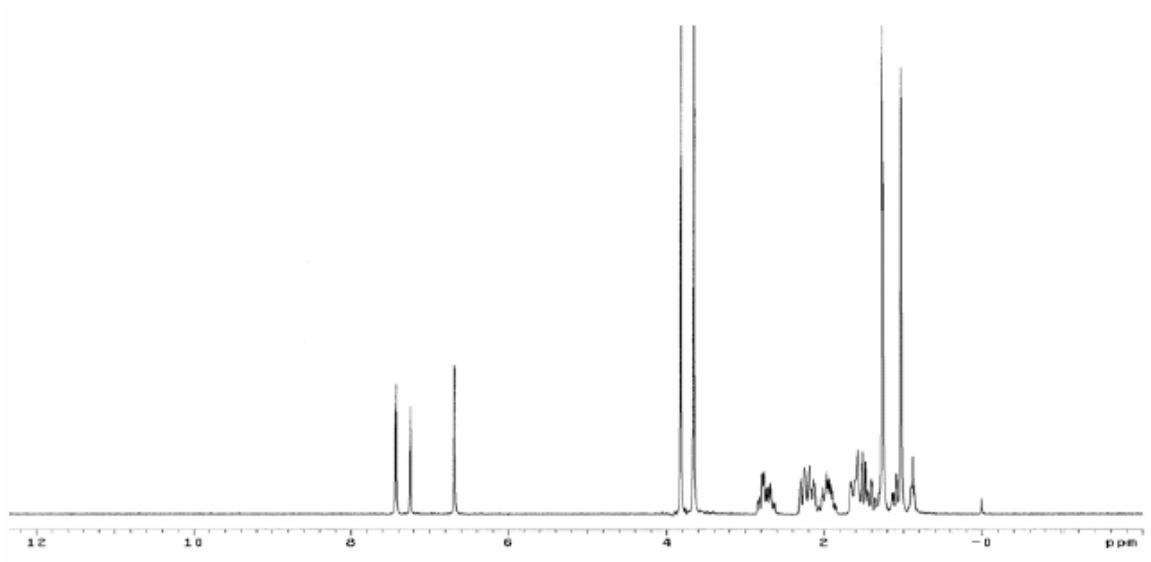


Figure 23 IR spectrum of compound 24

The IR spectrum of compound 24 gave a signal at 1730 cm^{-1} corresponding to carbonyl group of ester. The signals at 1450 cm^{-1}, 1490cm^{-1} and 1600cm^{-1} correspond to the aromatic ring. Strong absorption at 1200cm^{-1} corresponds to the C-O bond of ether and ester.



(23)

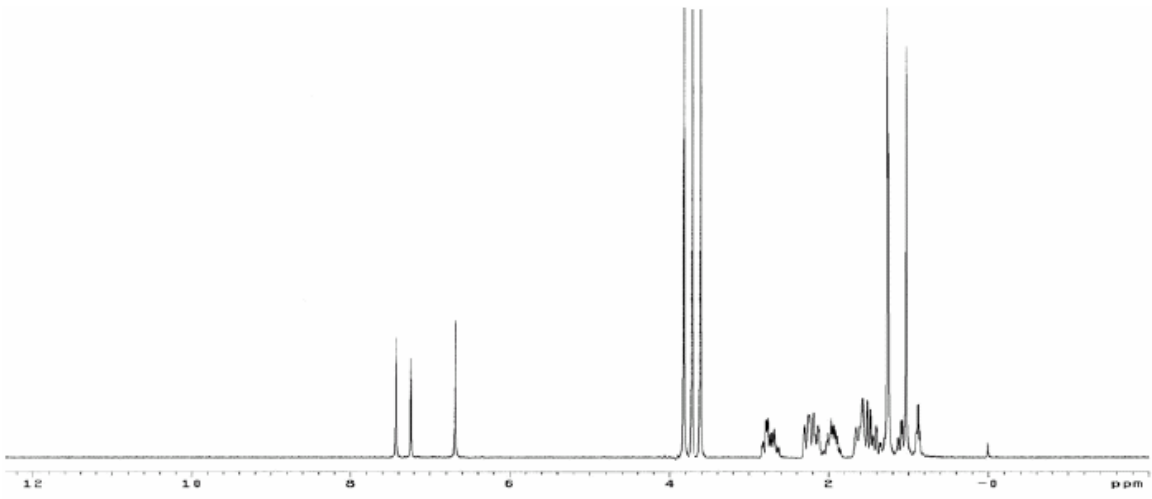
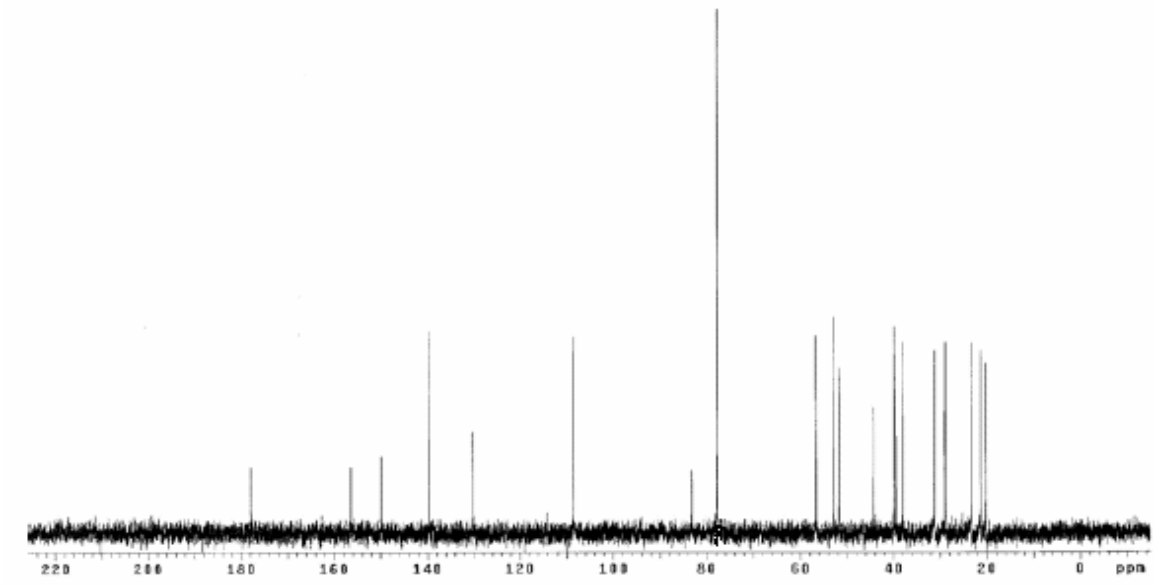


Figure 24 ^{1}H NMR spectrum of compound 24

The ^{1}H NMR spectrum of compound 24 showed an additional peak at δ 3.62 ppm(s) corresponding to methoxy group at C-13 position of podocarpic acid, in comparison to ^{1}H NMR spectrum of compound 23.



(23)

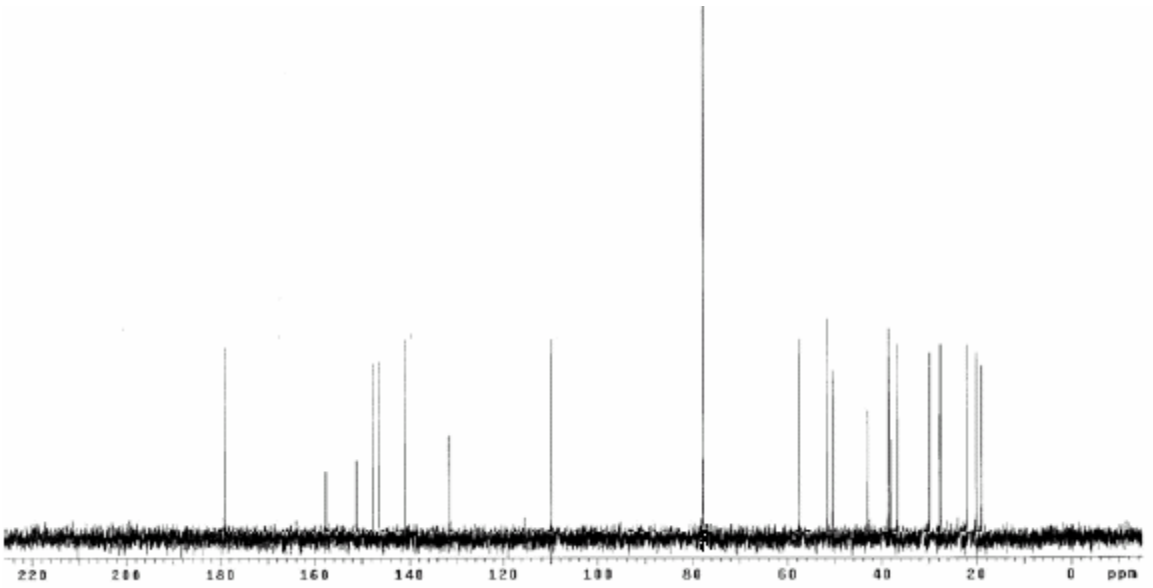


Figure 25 ^{13}C NMR spectrum of compound 24

The ^{13}C NMR spectrum of compound 24 gave peak at δ 57.2 ppm corresponding to two equivalent carbons of methoxy groups at C-13 and at C-12 of compound 24. The chemical shift of aromatic carbons was more downfield compared to compound 23.

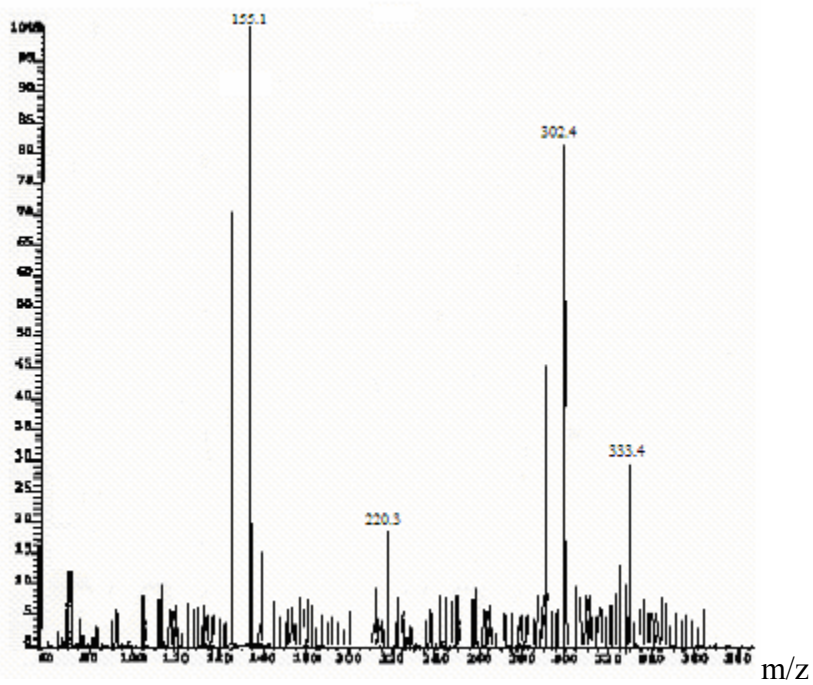


Figure 26 Mass spectrum of compound 24

The mass spectrum showed molecular ion m/z at 332.4 corresponding to molecular formula of $C_{20}H_{28}O_4$. The peak at m/z 302.1 represented methyl-O-methyl podocarpate formed because of cleavage of C-O bond at C-13 position of compound 24. The base peak was given at 154.1 corresponding to $[M-178]^+$. Basic fragmentation pattern for compound 24 is shown in figure 27.

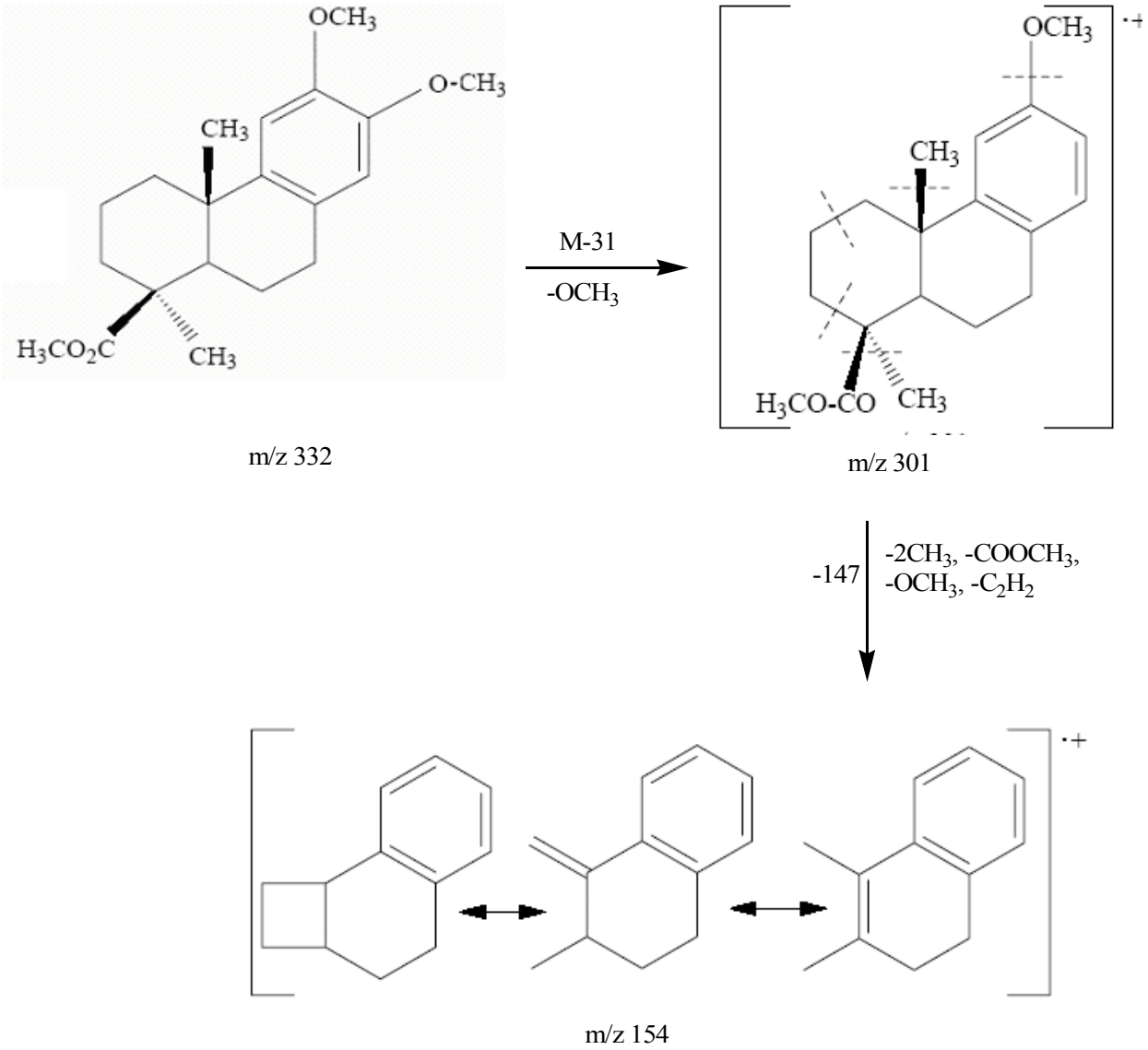


Figure 27 Fragmentation pattern of compound 24

Therefore from all the spectroscopic data obtained, compound 24 was found to be 13-methoxy methyl-O-methyl podocarpate. The structure of compound 24 is shown in figure 28.

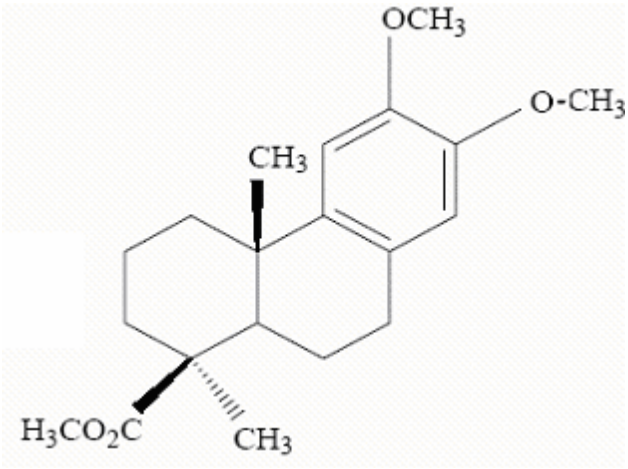


Figure 28 Structure of compound 24

Compound 25 was formed in 88% yield with a m.p of 146C. The IR, ^{1}H NMR, ^{13}C NMR, MS spectra of compound 25 are shown in figures 29, 30, 31 and 32 respectively.

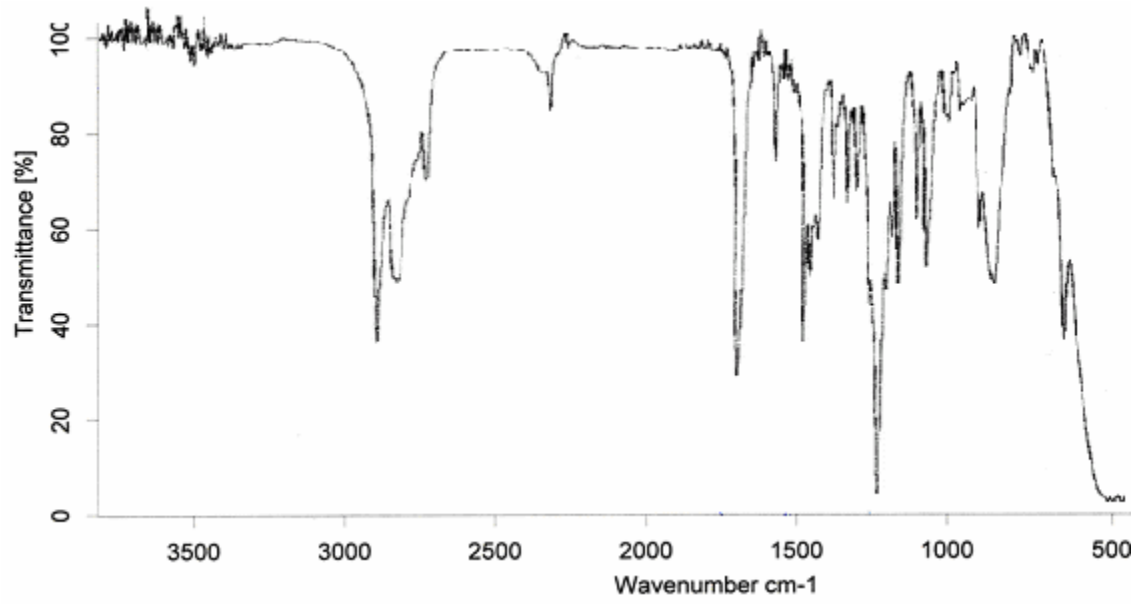
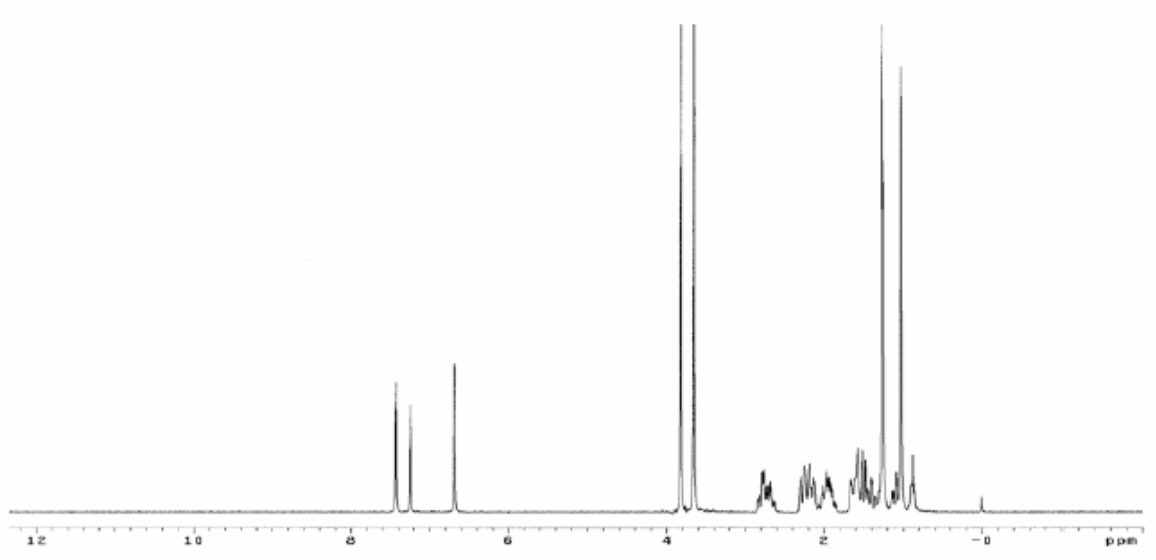


Figure 29 IR spectrum of compound 25

The IR spectrum of compound 25 gave a signal at 1730 cm^{-1} corresponding to carbonyl group of ester. The signals at 1450 cm^{-1}, 1490cm^{-1} and 1600cm^{-1} correspond to the aromatic ring. The strong absorption at 1200cm^{-1} corresponds to C-O bond of ether and ester.



(23)

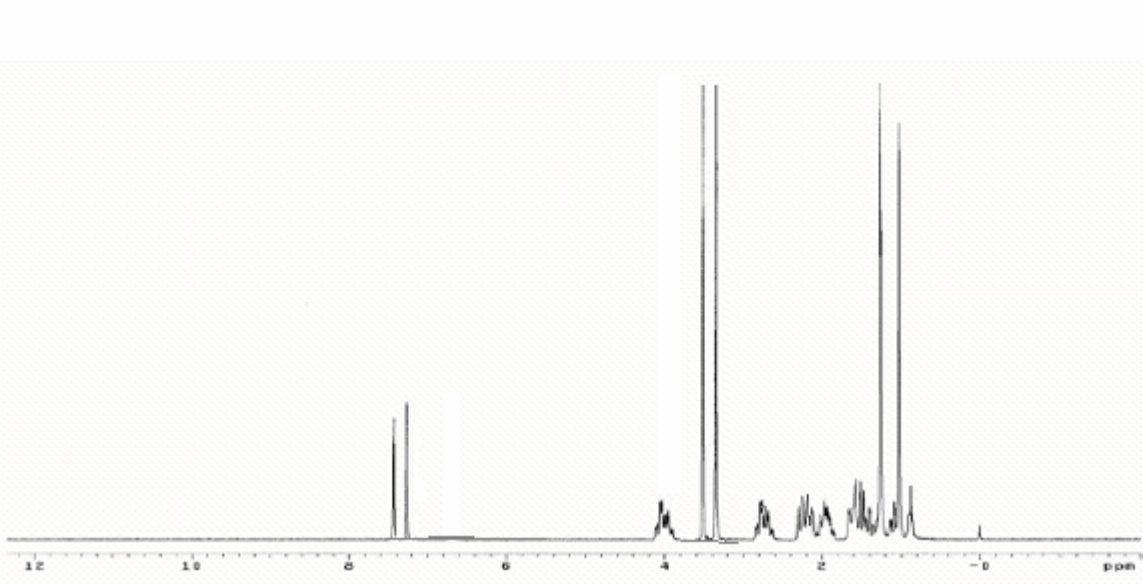
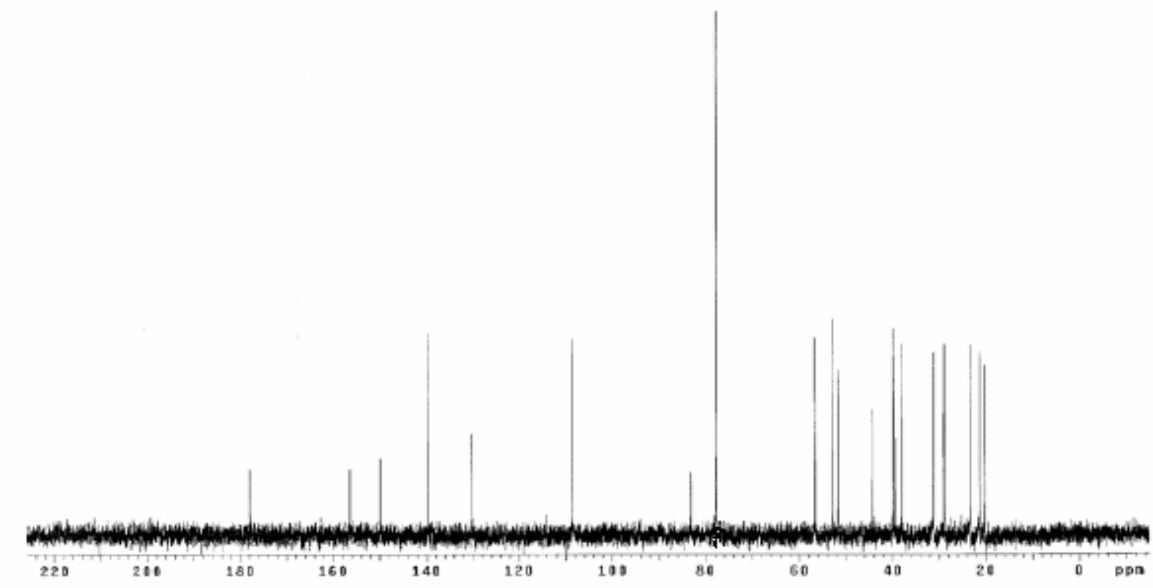


Figure 30 ^{1}H NMR spectrum of compound 25

The ^{1}H NMR spectrum gave new signal at δ 3.98 ppm (q) which could be assigned to the CH2-O moiety that is present in the compound 25. Methyl group attached to CH_2-O moiety gave signal at δ 1.33 ppm (t). Two methoxy groups from ether and ester gave signals at δ 3.85 (s) and δ 3.95 ppm (s) respectively.



(23)

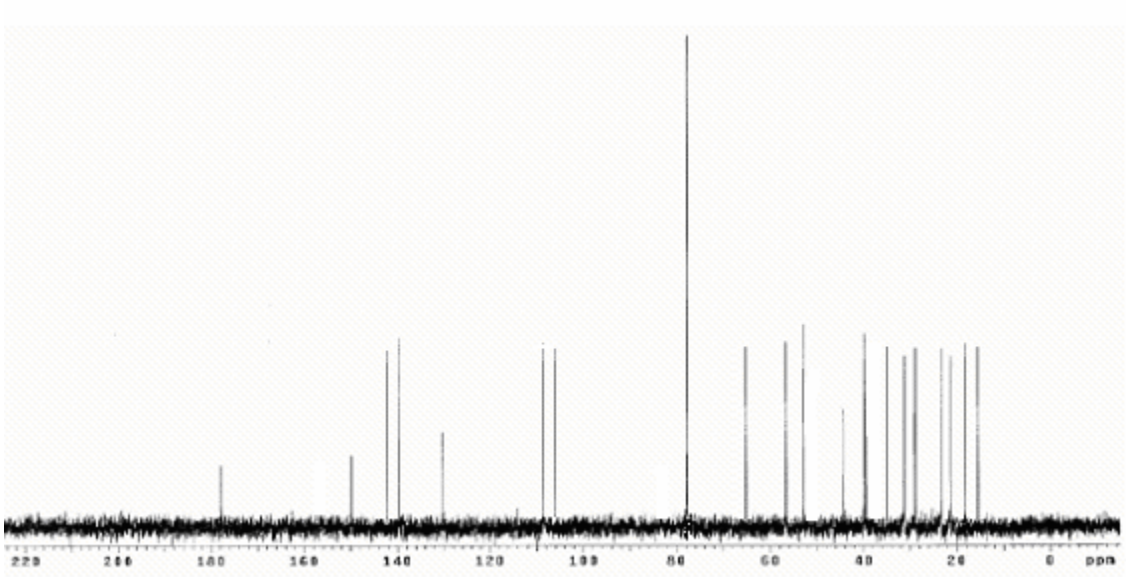


Figure 31 ^{13}C NMR spectrum of compound 25

The ^{13}C NMR of compound 25 showed new signals at δ 65 ppm and 14.8 ppm corresponding to CH_2-O and CH_3 respectively. Signals appeared down field compared to compound 23.

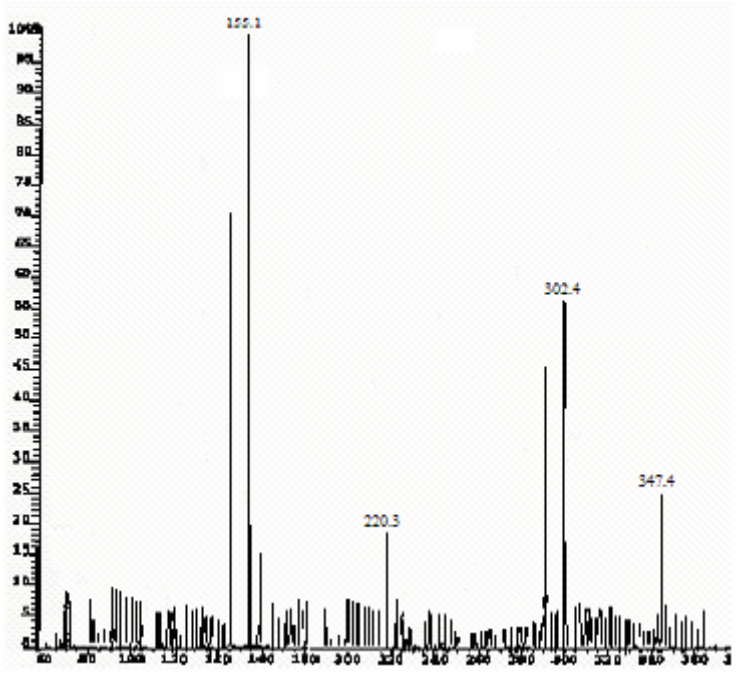


Figure 32 MS of compound 25

The mass spectrum of compound 25 showed a molecular ion at m/z 346.4, which was consistent with molecular formula $C_{21}H_{30}O_4$. The peak at m/z 302.4 corresponded to methyl-O-methyl podocarpate and could be the result of cleavage of the C-O bond at C-13 of compound 25. The basic fragmentation pattern for compound 25 is shown in figure 33.

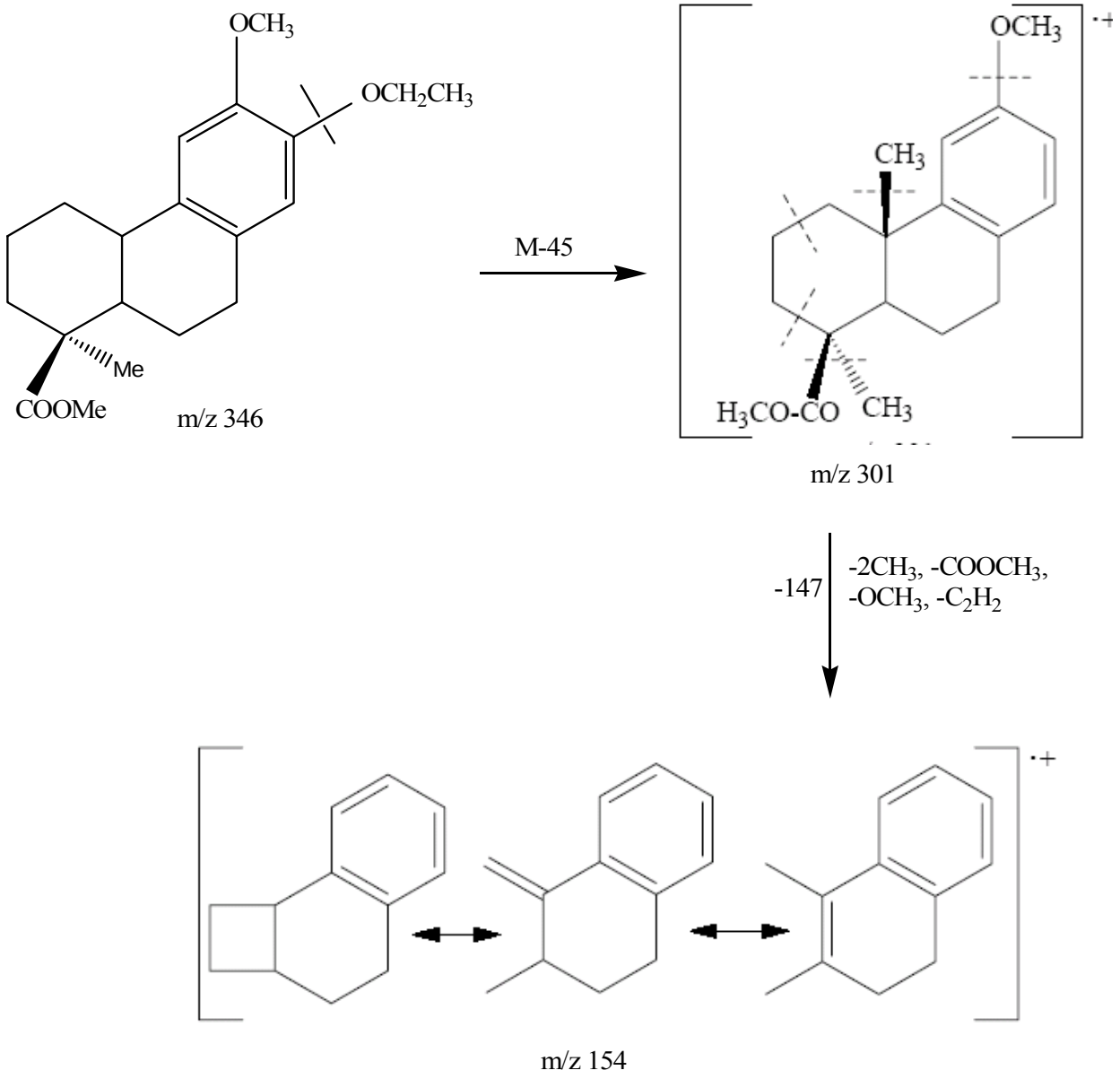


Figure 33 Basic fragmentation pattern for compound 25

Therefore compound 25 can be confirmed as 13-ethoxy methyl-O-methyl podocarpate. The structure of compound 25 is shown in figure 34.

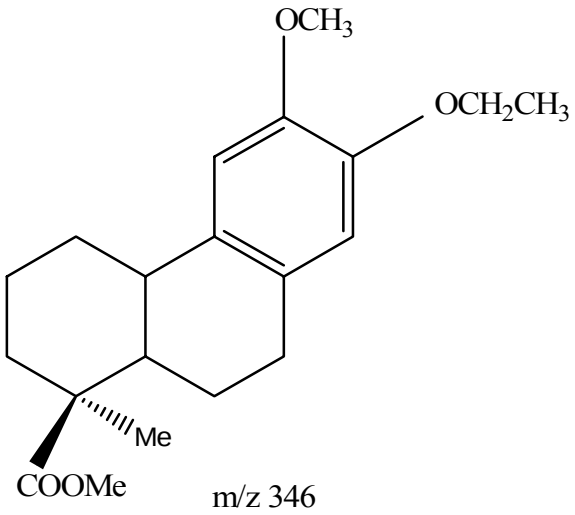


Figure 34 Structure of compound 25

Compound 26 was formed in 84% yield with a m.p of 148C. The IR, ^{1}H NMR, ^{13}C NMR and MS spectra were obtained and shown in figures 35, 36, 37 and 38 respectively.

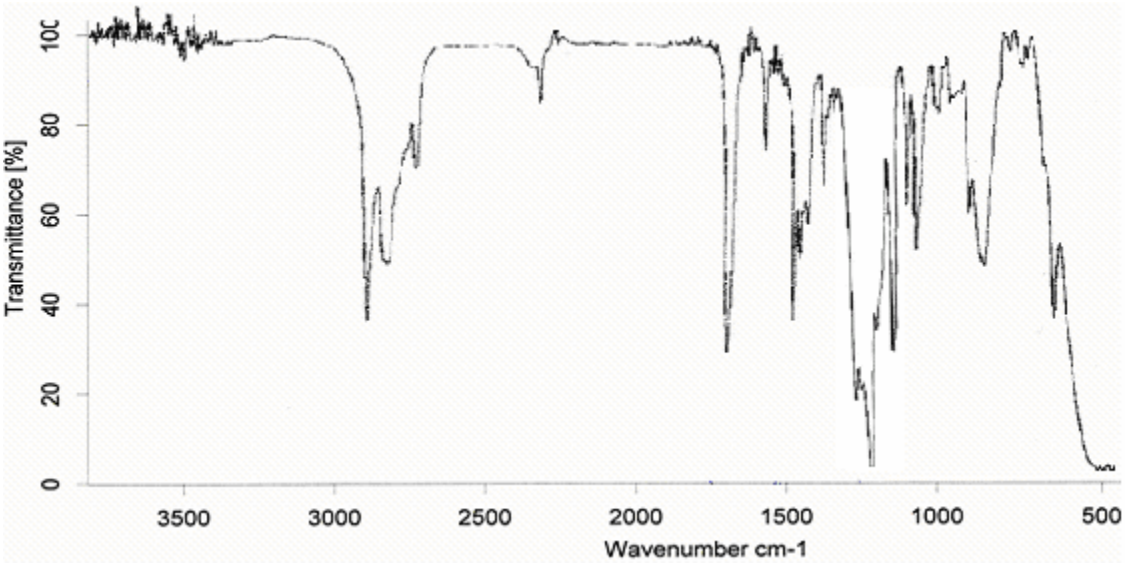
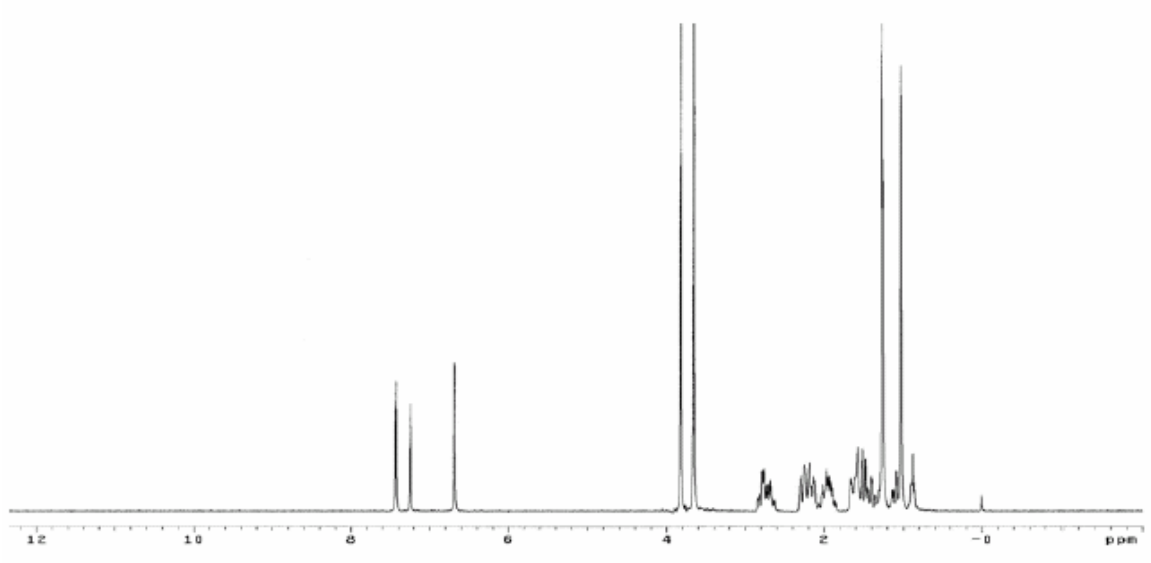


Figure 35 IR spectrum of compound 26

The IR spectrum of compound 26 gave a signal at 1220 cm^{-1}, corresponding to the C-O group of ether and ester that were present in compound 26. Absorptions for the aromatic ring were present at 1400 cm^{-1}, 1490cm^{-1} and 1600 cm^{-1}.



(23)

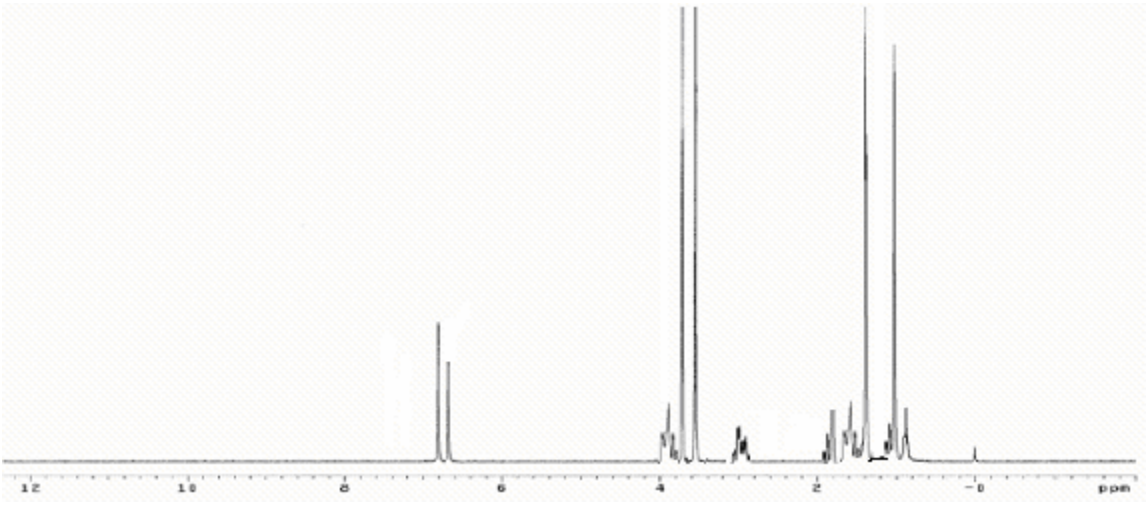
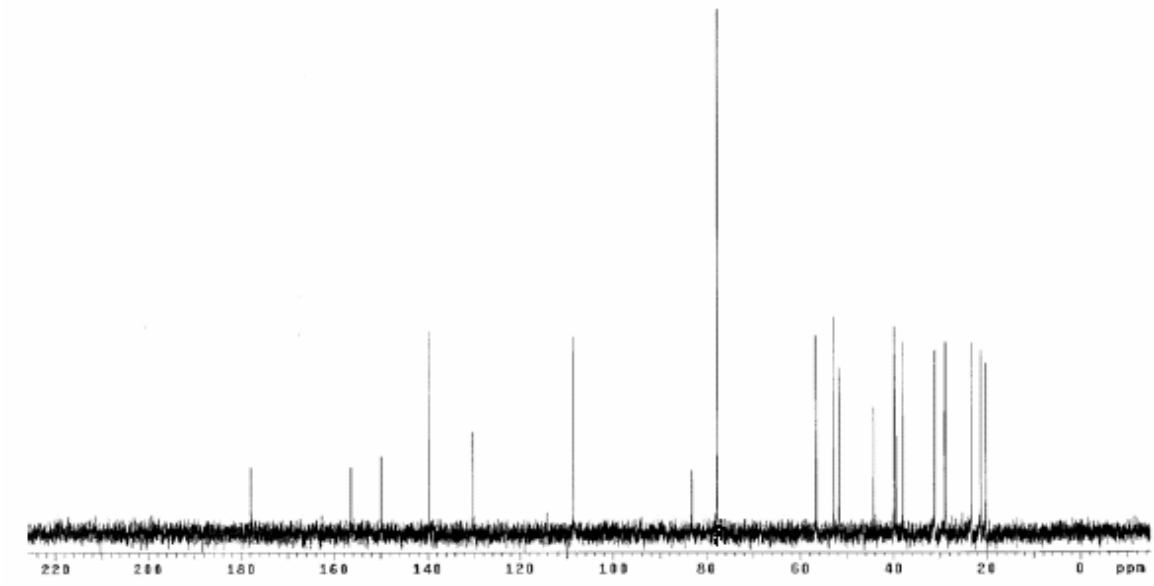


Figure 36 ^{1}H NMR spectrum of compound 26

The ^{1}H NMR spectrum of compound 26 showed two singlets at δ 6.62 ppm and δ 6.78 ppm for aromatic protons on benzene ring. The protons of methoxy groups appeared at

δ 3.65 and δ 3.78 ppm. The protons of propyl group appeared at, δ 3.95 ppm (t) for the methylene group attached to oxygen, δ 1.75 ppm (sextet) for methylene group and δ 1.10 ppm (t) for methyl group.



(23)

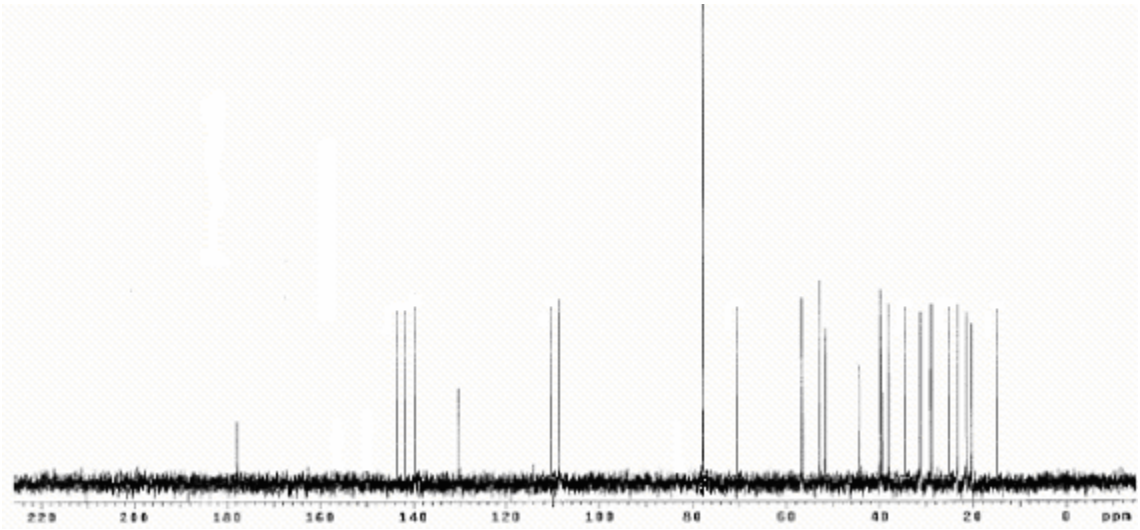


Figure 37 ^{13}C NMR spectrum of compound 26

^{13}C NMR of compound 26 showed twenty two signals for twenty two different carbons present in the compound 26. Seven carbons with the chemical shift down field from δ

100 ppm. The methylene attached to oxygen gave a signal at δ 73.5 ppm while a methyl group came at δ 14.2 ppm. Methylene group absorption was present at δ 22.7 ppm. A total fifteen carbons were present with the chemical shift up field from δ 100 ppm.

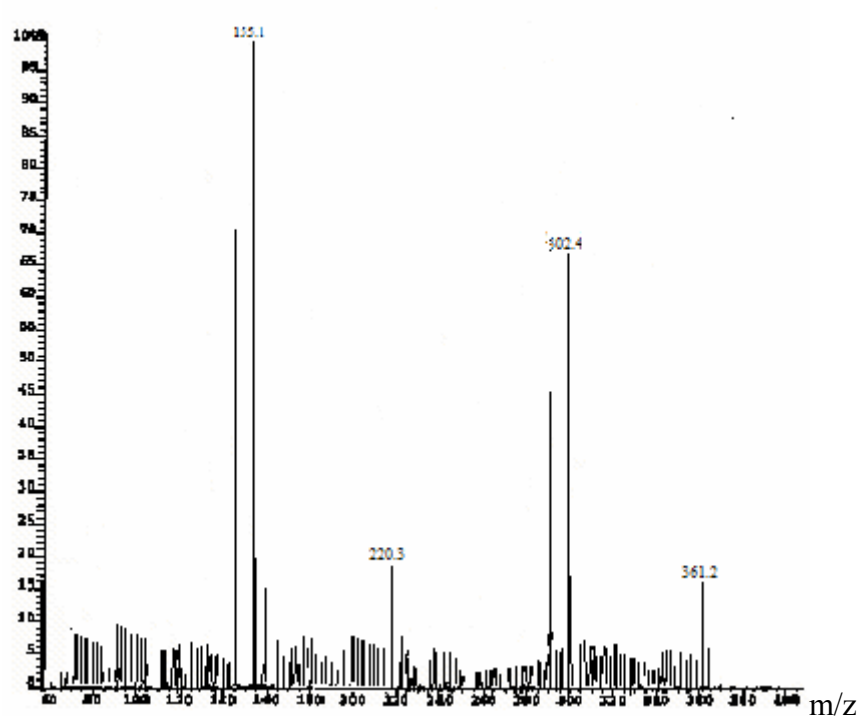


Figure 38 MS of compound 26

The MS of compound 26 displayed a molecular ion peak at m/z 360.2, which is consistent with the molecular formula $C_{22}H_{32}O_4$. The m/z 302.1 resulted from the cleavage of C-O bond at C-13 of compound 26. Further fragmentation gave the base peak at m/z 154.1 as shown in figure 40.

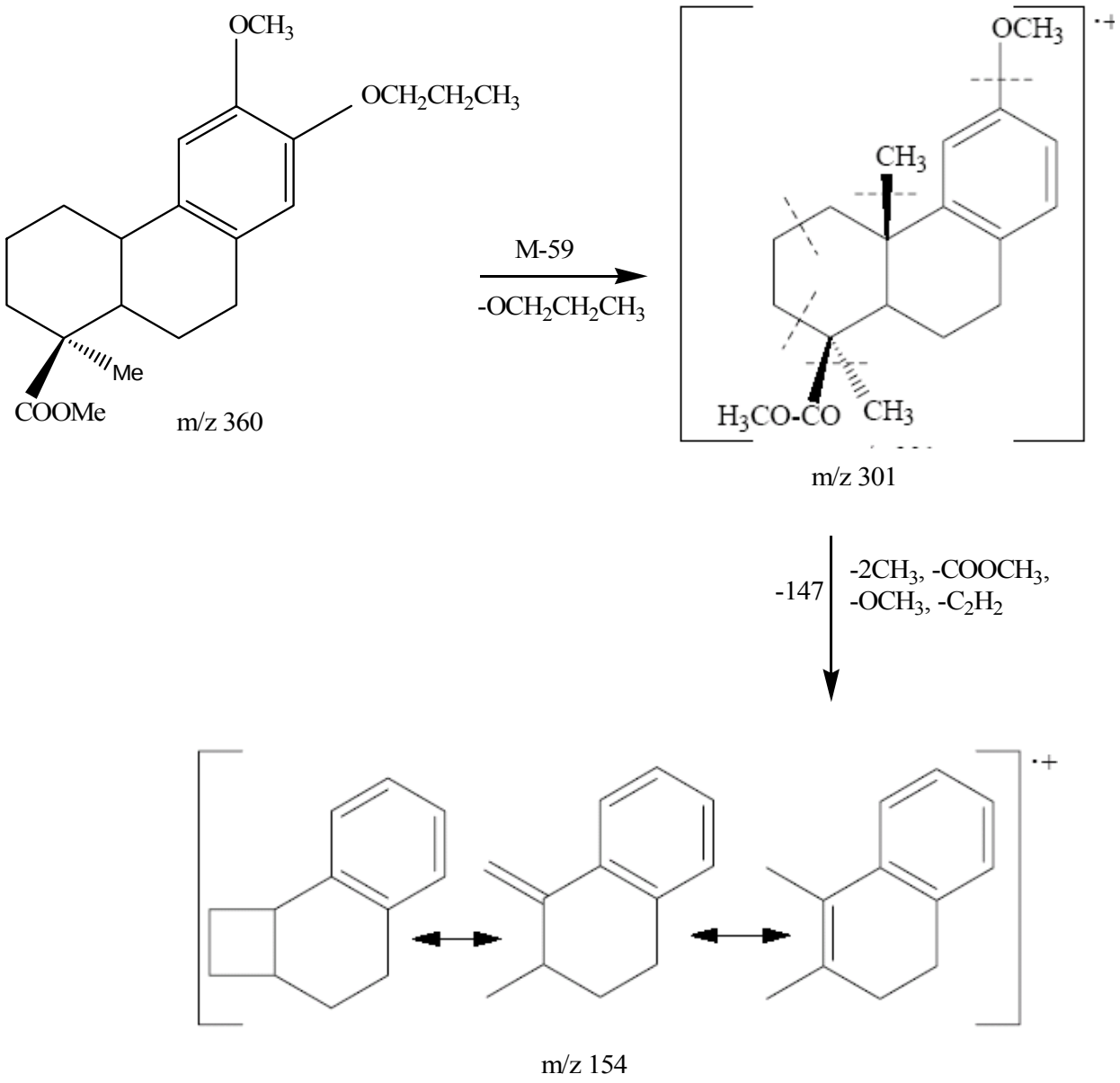


Figure 39 Basic fragmentation pattern of compound 26

Therefore the compound 26 can be confirmed as 13-propoxy methyl-O-methyl podocarpate. Structure of compound 26 is shown in figure 40.

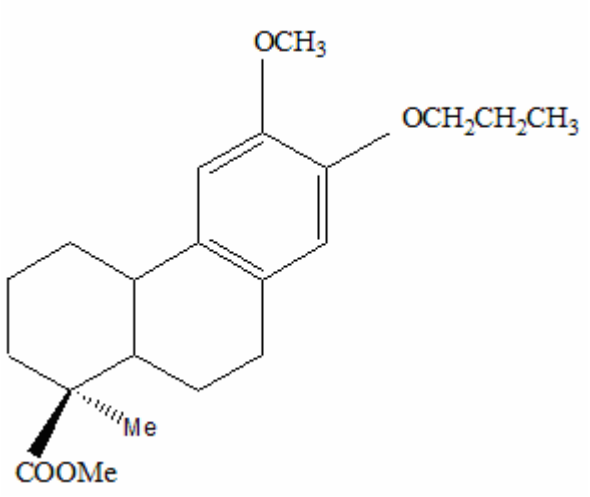


Figure 40 Structure of compound 26

In the summary the reaction of 13-iodo methyl-O-methyl podocarpate (23) with aliphatic alcohols gave ether derivatives of podocarpic acid in high yield (between 84 and 88 %). Three new ether derivatives of podocarpic acid were synthesized and their activity against cancer will be tested by National Cancer Institute against 60 cancer cell lines and activity against tuberculosis will be performed by National Institute for Allergies, Infections and Diseases. Thus this work may result in a new drug lead useful for the treatment of human ailments/diseases.

3. Experimental

3.1 Preparation of methyl-O-methyl podocarpate (22)

25 g of crude podocarpic acid was weighed in a 300 mL beaker and 25 g of ice was added into it. Then 25 mL of methanol was added to the beaker. This mixture was stirred then added 12 g pellets of sodium hydroxide. The solution was continually stirred to dissolve completely the podocarpic acid and sodium hydroxide, then it was cooled to 15^{0}C in an ice bath. 21.5 mL of dimethyl sulfate was added into this solution in a period of 1 hour[52]. At the end this period, this solution solidified. This mixture was stirred for an addition of 30 minutes then added 50 mL water then it was filtered. The solid was dissolved in 50 mL water then filtered and dried to obtain 22 g a white solid. This solid was recrystallized to obtain 20 g of methyl-O-methylpodocarpate with m.p. of 125C. Yield was 82%. IR (Perkin Elmer Spectrometer, $CHCl_3$):3000, 2950, 2900, 2860, 1720, 1600, 1540, 1490, 1460, 1400, 1360, 1300, 1240, 1200, 1190, 1150, 1060, 1020950, 760, 740 cm^{-1}. ^{1}H-NMR (Mercury 300 MHz): 6.95 (d), 6.8 (s), 6.65 (d), 3.85 (s), 3.65 (s), 2.8 (m), 2.25 (m), 1.95 (m), 1.6 (m), 1.5 (m), 1.4 (m), 1.22 (m), 1.2 (s), 1.15 (m), 1.05 (m), 1.00 (s) ppm. ^{13}C-NMR (Mercury 300 MHz): 178, 158, 150, 130, 128, 114, 112, 56, 52, 51, 44, 39.5, 38, 31, 28, 25, 22.5, 20.5, 20 ppm. MS: 302(44), 287(6), 228(16), 227(100), 173(6), 170(23), 147(10), 121(6), 91(4).

3.2 Preparation of 13-Iodomethyl-O-Methylpodocarpate (23)

3.025 g of methyl-O-methylpodocarpate was weighed and transferred into a 500 mL volumetric flask, and then it dissolved in 60 mL of acetic acid. In a separated beaker, 2 g of mercury (II) acetate was weighed and dissolved in 60 mL of acetic acid, and then this content was added into the flask above. This solution was heated to 70C and stirred for 15 minutes. A solution of iodine was prepared by dissolving of 7.614 g of iodine in 240 mL of warm acetic acid. This iodine solution was then added drop wise in a period of 45 minutes into the flask while the temperature maintained at 70C during this period. At the end of this period, the solution was stirred for an addition 1 hour then cooled the flask to 15C in an ice bath, the solution was filtrated and the filtrate was added into 500 mL of cold water in a 1L beaker. The resulting precipitate was filtered to yield 3 g of product. The product was recrystallized from acetone to obtain 2 g of pure product with m.p.149C, yield95%. IR (Perkin Elmer Spectrometer, $CHCl_3$): 3000, 2940, 2850, 2400, 1720, 1600, 1495, 1470, 1440, 1390, 1350, 1300, 1250, 1200, 1150, 1050, 950, 900, 800, 750, 650 cm^{-1}.^{1}H-NMR (Mercury 300 MHz):7.45(s), 6.65 (s), 3.95 (s), 3.85 (s), 2.70 (m), 2.20 (m), 1.95(m), 1.6 (m), 1.5 (m), 1.25 (s), 1.15 (m), 1.02 (s), 0.95 (m) ppm. ^{13}C-NMR (Mercury 300 MHz): 178, 156, 150, 140, 131, 108, 83, 56.5, 52.5, 52, 44, 39, 38.5, 32, 31.5, 29.5, 23, 21, 20 ppm. HRMS (Finnegan Spectrometer): 428(100), 413(8), 381(3), 368(3), 353(77), 313(4), 287(6), 272(6), 227(15), 211(4), 172(6), 140(5),129(8),115(6),101(3),91(2).

3.3 Preparation of 13-methoxy methyl-O-methyl podocarpate (24)

2.14 g 13-iodo methyl-O-methyl podocarpate was weighed in a 100 mL round bottom flask and dissolved in 0.32 g of N, N-dimethyl formamide. 0.405 g of sodium methoxide was added to 0.34g of methanol in a separate beaker. The content inside the beaker was added to the flask and magnetic stirrer was added. Then the flask was heated by using heating mantle with continuous stirring till the temperature of solution reached 110C. At 110C, 0.1 g Copper (I) iodide was added. Then condenser was connected and reflux at 110C for four hours while monitoring the reaction with TLC. After the completion of the reaction, the solution was allowed to cool. The precipitate formed and filtered. The precipitate was washed with 100 mL ethyl acetate. The filtrate was evaporated to obtain 2 g of solid. Normal phase open column chromatography was performed to obtain 0.58 g 13-methoxy methyl-O-methyl podocarpate with m.p 143C. Yield was 84%. IR (Perkin Elmer Spectrometer, $CHCl_3$): 3000, 2940, 2850, 2400, 1720, 1600, 1495, 1470, 1440, 1390, 1350, 1300, 1250, 1200, 1150, 1050 cm^{-1}. ^{1}H NMR (Mercury 300 MHz): 7.52(s), 7.32(s), 6.73(s), 3.95(s), 3.85(s), 3.62(s), 2.80(m), 2.28(m), 2.02(m), 1.64(m), 1.30(s), 1.22(s), 0.81(s) ppm. ^{13}C NMR (Mercury 300 MHz): 178, 158, 151, 148, 146.1, 132, 110.5, 57.2, 52, 50.2, 44.3, 38, 36, 30, 28, 22, 20, 18 ppm. MS (Finnegan spectrometer): 333.4(30), 332(14), 302.4(82), 220.3(18), 155.1(100).

3.4 Preparation of 13-ethoxy methyl-O-methyl podocarpate (25)

2.14 g 13-iodo methyl-O-methyl podocarpate was weighed in a 100 mL round bottom flask and dissolved in 5 mL of N, N-dimethyl formamide. 0.51 g of sodium ethoxide was added to 0.54g of ethanol in a separate beaker. The content inside the beaker was added to the flask and magnetic stirrer was added. Then the flask was heated by using heating mantle with continuous stirring till the temperature of solution reached 110C. At 110C, 0.1 g Copper (I) iodide was added. Then condenser was connected and reflux at 110C for four hours while monitoring the reaction with TLC. After the completion of the reaction, the solution was allowed to cool. The precipitate formed and filtered. The precipitate was washed with 100 mL ethyl acetate. The filtrate was evaporated to obtain 2 g of solid. Normal phase open column chromatography was performed to obtain 0.64 g 13-ethoxy methyl-O-methyl podocarpate with m.p 146C. Yield was 88%. IR (Perkin Elmer Spectrometer, $CHCl_3$): 3000, 2940, 2850, 2400, 1720, 1600, 1495, 1470, 1440, 1390, 1350, 1300, 1250, 1200, 1150, 1050 cm^{-1}. ^{1}H NMR (Mercury 300 MHz): 7.45(s), 7.25(s), 4.04(m), 3.95(s), 3.85(s), 2.80(m), 2.28(m), 2.02(m), 1.64(m), 1.30(s), 1.22(s), 0.81(s) ppm. ^{13}C NMR (Mercury 300 MHz): 178, 150, 142, 140,132, 108, 106, 65.2, 56, 52.2, 44.3, 40, 36, 30, 28, 22, 20, 18, 14 ppm. MS (Finnegan spectrometer): 347.4(25), 346 (10), 302.4(58), 220.3(18), 155.1(100).

3.5 Preparation of 13-propoxy methyl-O-methyl podocarpate (26)

2.14 g 13-iodo methyl-O-methyl podocarpate was weighed in a 100 mL round bottom flask and dissolved in 5 mL of N, N-dimethyl formamide. 0.55 g of sodium propoxide was added to 0.54g of propanol in a separate beaker. The content inside the beaker was added to the flask and magnetic stirrer was added. Then the flask was heated by using heating mantle with continuous stirring till the temperature of solution reached 110C. At 110C, 0.1 g Copper (I) iodide was added. Then condenser was connected and reflux at 110C for four hours while monitoring the reaction with TLC. After the completion of the reaction, the solution was allowed to cool. The precipitate formed and filtered. The precipitate was washed with 100 mL ethyl acetate. The filtrate was evaporated to obtain 2.2 g of solid. Normal phase open column chromatography was performed to obtain 0.67 g 13-propoxy methyl-O-methyl podocarpate with m.p 148C. Yield was 84%. IR (Perkin Elmer Spectrometer, $CHCl_3$): 3000, 2940, 2850, 2400, 1720, 1600, 1495, 1470, 1440, 1390, 1350, 1300, 1250, 1200, 1150, 1050 cm^{-1}. ^{1}H NMR (Mercury 300 MHz): 6.82(s), 6.73(s), 4.01(m), 3.95(s), 3.85(s), 3.12(m), 1.80(m), 1.60(m), 1.40(s), 1.02(s), 0.81(s) ppm. ^{13}C NMR (Mercury 300 MHz): 178, 146, 144, 142, 132, 110.5, 108.2, 70.4, 58, 53.8, 52, 44.3, 38, 36, 32, 30, 28, 22, 20, 19, 18, 14 ppm. MS (Finnegan spectrometer): 361.2(16), 360(5), 302(68), 220.3(18), 155.1(100).

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