UNIVERSITE DE NEUCHATEL
FACULTE DES SCIENCES

Etude Phytochimique de: *Usnea articulata* (L.) Hoffm., et *Usnea hesperina* Mot.

Contribution à la synthese de phtalides, depsides et depsidones

Thèse présentée à la Faculté des Sciences par

Mohamed Hareed Ali

Chimiste diplômé (M.Sc.) de
l'Université de East Anglia, England
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docteur ès sciences

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Phytochemical Investigation on: *Usnea articulata* (L.) Hoffm., and *Usnea hesperina* Mot.

Contribution to the Syntheses of Phthalides, Depsides and Depsidones

A Thesis Submitted for the Doctor of Philosophy
to the Faculty of Sciences of the
University of Neuchâtel

By

Mohamed Hareed Ali M.Sc (University of East Anglia England)

Institute of Chemistry
University of Neuchâtel

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IMPRIMATUR POUR LA THÈSE

Etude phytochimique de *Usnea articulata* (L.) Hoffm. et *Usnea hesperina* Mot. - Contribution à la synthèse de phtalides, depsides et depsidones

de Monsieur Ali Mohamed Hareed

UNIVERSITÉ DE NEUCHÂTEL

FACULTÉ DES SCIENCES

La Faculté des sciences de l'Université de Neuchâtel sur le rapport des membres du jury,

Messieurs R. Tabacchi, R. Deschenaux,

Ph. Clerc (Genève) et S. Hüneck (Halle).

autorise l'impression de la présente thèse.

Neuchâtel, le 7 octobre 1993

Le doyen :

A. Robert

DEDICATION

TO MY WIFE AND PARENTS

ASHA HARED (JAMA) ALI

HARED ALI HASAN

MARYAN SALAH YUSUF

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CONTENTS

Etude phytochimique de : *Usnea articulata* (L.) Hoffm. et *Usnea hesperina* Mot.

Contribution à la synthèse de phtalides, depsides et depsidones

RESUME

Le premiere partie de ce travail concerne l'étude de la composition chimique de deux espèces de lichen du genre Usnea (Ascomycotina):

(a) *Usnea articulata* (L.) Hoffm. (Fig. 4) et
(b) *Usnea hesperina* Mot. (fig. 54)

La seconde partie traite de la synthèse de phtalides, depsides, éthers diaryliques, et depsidones lactoniques.

PARTIE ANALYTIQUE

L' étude de la composition chimique des lichens susmentionnés a été entreprise.
Le but primaire revenait à isoler les metabolites secondaires contenus dans les deux espèces de lichen.
Usnea articulata (L.) Hoffm.(1 kg.), (provenant des Iles Canaries) a été extrait successivement à l' éther, à l' hexane puis à l' acétone.
La partition de l' extrait éthéré en acides forts, acides faibles et partie neutre, suivie de purifications par chromatographie a permis d' isoler 20 composés (voir liste):
dérivés monophénoliques, dérivés dibenzofuraniques, depsides, depsidones, triterpènes et stérols.

Composés isolés du lichen: *Usnea articulata* (L.) Hoffm.

I- Dérivé dibenzofuranique
- 1- (+) Acide usnique

II- Depsidones
- 1- Acide fumarprotocétrarique
- 2- Acide protocétrarique

III- Depsides
- 1- Acide barbatique
- 2- Acide diffractaique
- 3- Atranorin
- 4- Chloroatranorin
- 5- Acide 4-O-deméthyldiffractaique

IV- Dérivés monophénoliques
- 1- Acide rhizonique
- 2- Acide 4-O-méthylhaematommique
- 3- Everninate de méthyle
- 4- Orcinol monométhyl éther

V- Triterpènes
- 1- Lupéol
- 2 - Acetate de tylolupenyle

VI- Sterols
- 1- Peroxyde d' ergostérol
- 2- Ergostérol
- 3- Lanostérol
- 4- Cholestérol
- 5- Stigmastérol
- 6- β-Sitostérol

La structure de ces produits a été établie à l'aide de méthodes chimiques, et spectroscopiques. Dans certains cas, les structures ont été confirmées par comparaison avec un'échantillon authentique.

Une démarche analogue a permis l'étude, pour la première fois, d'*Usnea hesperina* Mot. (750 g), (provenant des Iles Canaries). Les constituants principaux ont été isolés et identifiés, a savoir:

Depsidones: acide protocétrarique, acide fumarprotocétrarique et acide virensique

Dérivés dibenzofuraniques: (+) acide usnique

Dérivés monophénoliques: acide 4-O-methylorsellinique et methylhaematommate monomethyl éther

Triterpènes: nor-30-hopan-3,22-dione, acide ursolique et peroxide d' ergostérol.

PARTIE SYNTHETIQUE

La deuxieme partie de ce travail a consisté en la synthèse de divers depsides, éther diaryliques et depsidones contenant un cycle hydroxylactonique.

Outre la méthode classique, trois voies synthétiques furent étudieés, chacune d' entre elles pouvant reproduire, du moins partiellement un possible processus biomimétique. La première voie comprend le couplage oxydatif d' une benzophénone, la seconde un réarrangement de depsides selon Smiles, et la troisième une réaction d' Ullmann.

La principale difficulté dans la synthèse des tels depsides et depsidones lactoniques reside dans l' élaboration du noyau phtalique. Bien que bon nombre de phtalides soient des substances naturelles et des intermédiaires importants dans la synthèse de benzophénones, depsides, et depsidones lactoniques, peu de méthodes existent pour leur synthèse et les rendements sont souvent très faibles. Aussi, notre objectif premier était d' elaborer une méthode adéquate pour la synthèse des phthalides en question.

La préparation de ces noyaux phtaliques a rendu possible la synthèse de trois nouveaux depsides lactoniques.

La réaction d' Ullmann appliquée aux phénols et bromophtalides appropriés a rendu possible la synthèse de 2 nouveaux éthers diaryliques. Au contraire, la synthèse de depsidones lactoniques n' a pas pu être réalisée, bien que les trois voies synthétiques aient été utilisée en alternance et les conditions de réaction constamment modifiées.

Phytochemical investigation on *Usnea articulata* (L.) Hoffm. and *Usnea hesperina* Mot.

Contribution to the Syntheses of Phthalides, Depsides and Depsidones

ABSTRACT

The chemical composition of the two above mentioned rare lichens have been investigated.

I- The plant material of the lichen *Usnea articulata* (L.) Hoffm. (1kg), collected from the Canary islands, was extracted with ether, hexane, and acetone subsequently. Partition of the ether extract into acids and neutral fractions, followed by chromatographic separation provided 20 typical lichen compounds consisting of monophenolic derivatives, depsides, depsidones, dibenzofuran derivatives, triterpenes, and sterols.

The structures of these have been established by chemical, chromatographic, and spectroscopic methods. The presence of some compounds have been confirmed by comparison with authentic samples.

II- The lichen *Usnea hesperina* Mot.(750 g), collected from the Canary islands, have been investigated for the first time following the same method as above. The result of this is presented below. The following nine lichen substances have been isolated and identified as the main constituents of this lichen. Some of these compounds are very rare in the genera *Usnea*.

Depsidones: protocetraric acid , fumarprotocetraric acid , virensic acid .

Dibenzofuran derivative: usnic acid.

Monophenolic derivatives: 4-O-methylorsellinic acid, methylhaematommate mononethyl ether.

Triterpenes, and sterols: nor-30-hopan-3,22-dione, ursolic acid and ergosterol peroxide.

III- In addition, the syntheses of phthalides, lactonic depsides and depsidones have been carried out as part of this study. Three lactonic depsides **49**,**50**, and **51**, haved been prepared. Attempts to synthesize lactonic depsidones failed even though three main methods (oxidative coupling of dibenzophenone, Smiles rearrangement, and Ullmann reaction) were employed exhaustively. The Ullmann reaction provided two lactonic diphenyl ethers **62** and **67**.

1.1 INTRODUCTION

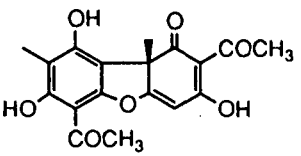
Lichen substances (Fig. 1, 2, 3) are metabolic products of lichens, which are the result of a symbiotic association between algae and fungi [1]. Most of these compounds occur only in lichens, whereas others are also found in non-lichen forming fungi and higher plants. We know very little about the origin and the precursors of the individual substances.

Under certain conditions some lichen fungi are able to synthesize the compounds present in the corresponding lichens by themselves [2]. Parietin (**1**), rhodocladonic acid (**2**), pulvinic acid (**3**), usnic acid (**4**) and didymic acid (**5**) could be established in lichen cultures[2, 3], (Fig 2).

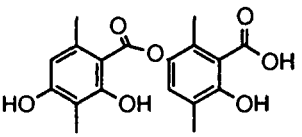
The mycelia of fungi generated only simple phenols, including orsellinic acid (**6**) and haematommic acid (**7**), which could not be established in the corresponding lichens [1]. Obviously by symbiosis, the simple phenols synthesized by the fungus are interlinked into depsides, and depsidones; the simple phenols are chemically changed before or after they have been linked. This means that the phycobiont not only generates the energy required by the fungus for forming the phenols, but later also takes part in their metabolism [4].

The physiology of the isolated fungal and algal symbionts are broadly dealt with by Ahmadjian and Culberson [5, 6]. As lichens are organisms which grow very slowly, lichen substances which possess antibiotic activity may have the function of protecting the organism from attack by other fungi [7, 8].

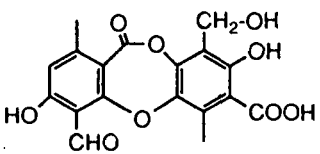
CHARACTERISTIC LICHEN PRODUCTS



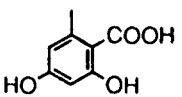
(+) - USNIC ACID



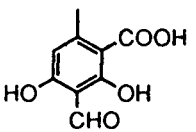
DEPSIDE



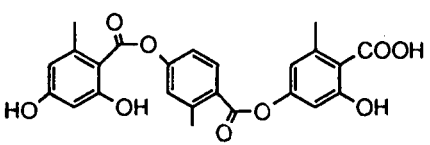
DEPSIDONE



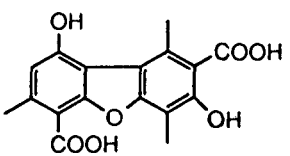
ORSELLINIC ACID



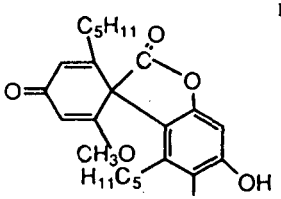
HAEMATOMMIC ACID



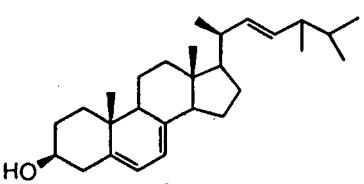
TRIDEPSIDE



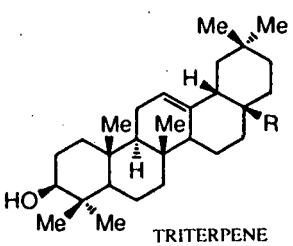
DIBENZOFURAN



DEPSONE



STEROL



TRITERPENE

Figure 1

The List of the Compounds Presented in the Figures 2, 3

1- Parietin
2- Rhodocladonic acid
3- Pulvinic acid
4- (+)-Usnic acid
5- Didymic acid
6- Orsellinic acid
7- Haematommic acid
8- Picrolichenic acid
9- Gyrophoric acid
10- Lobaric acid
11- Diploicin
12- Variolaric acid
13- Grayanic acid
14- Olivetoric acid
15- Physodic acid
16- Sphaerophoric acid
17- 4-O-methylbarbatic acid
18- Hypoprotocetraric acid
19- Lecideoidin
20- Colensoic acid
21- Vittatolic acid
22- Virensic acid
23- Protocetraric acid
24- Salazinic acid
25- Nornotatic acid
26- Imbricaric acid
27- Strepsilin
28- Pophyrilic acid
29- Squalene
30- α- Amyrins
31- β- Amyrins
32- Ursolic acid
33- Oleanolic acid
34- Limonin
35- β-Sitosterol
36- Stigmasterol
37- Campesterol
38- Ergosterol

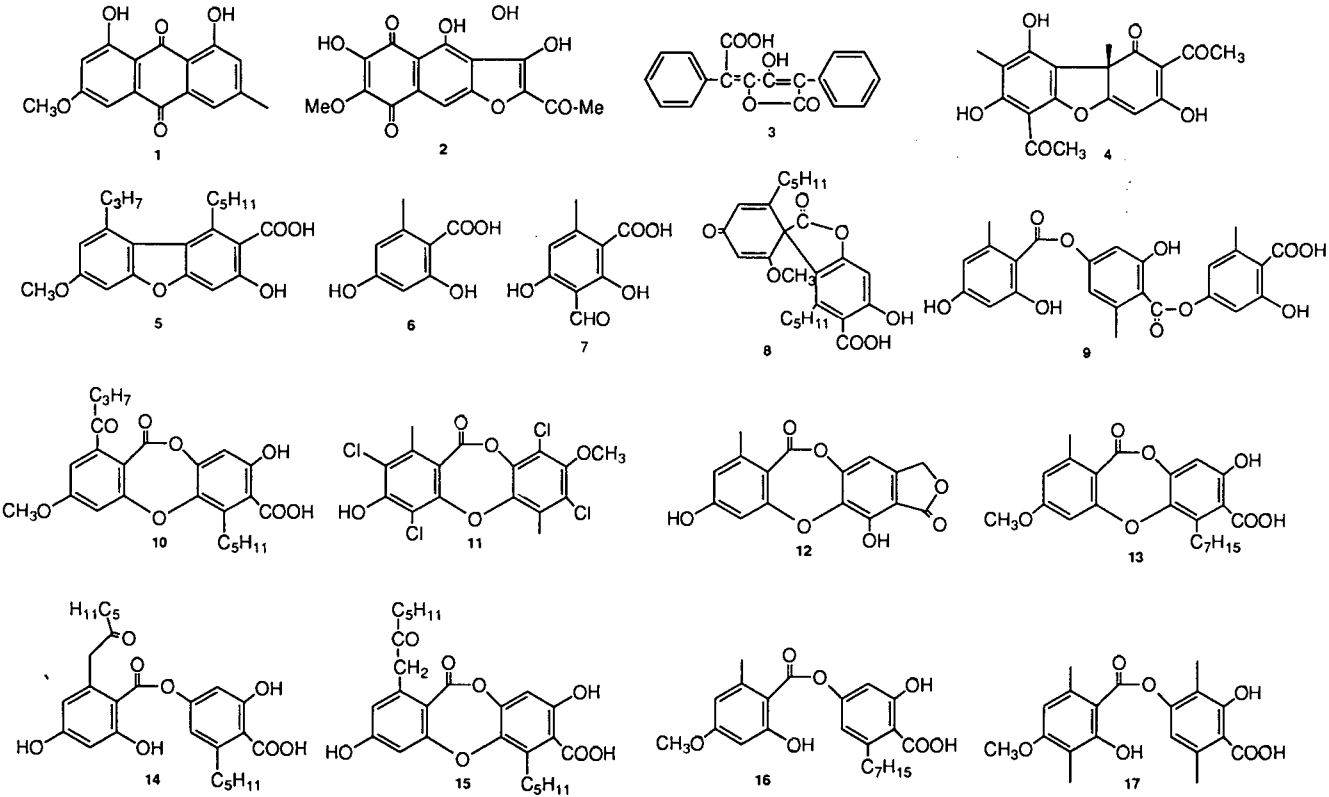
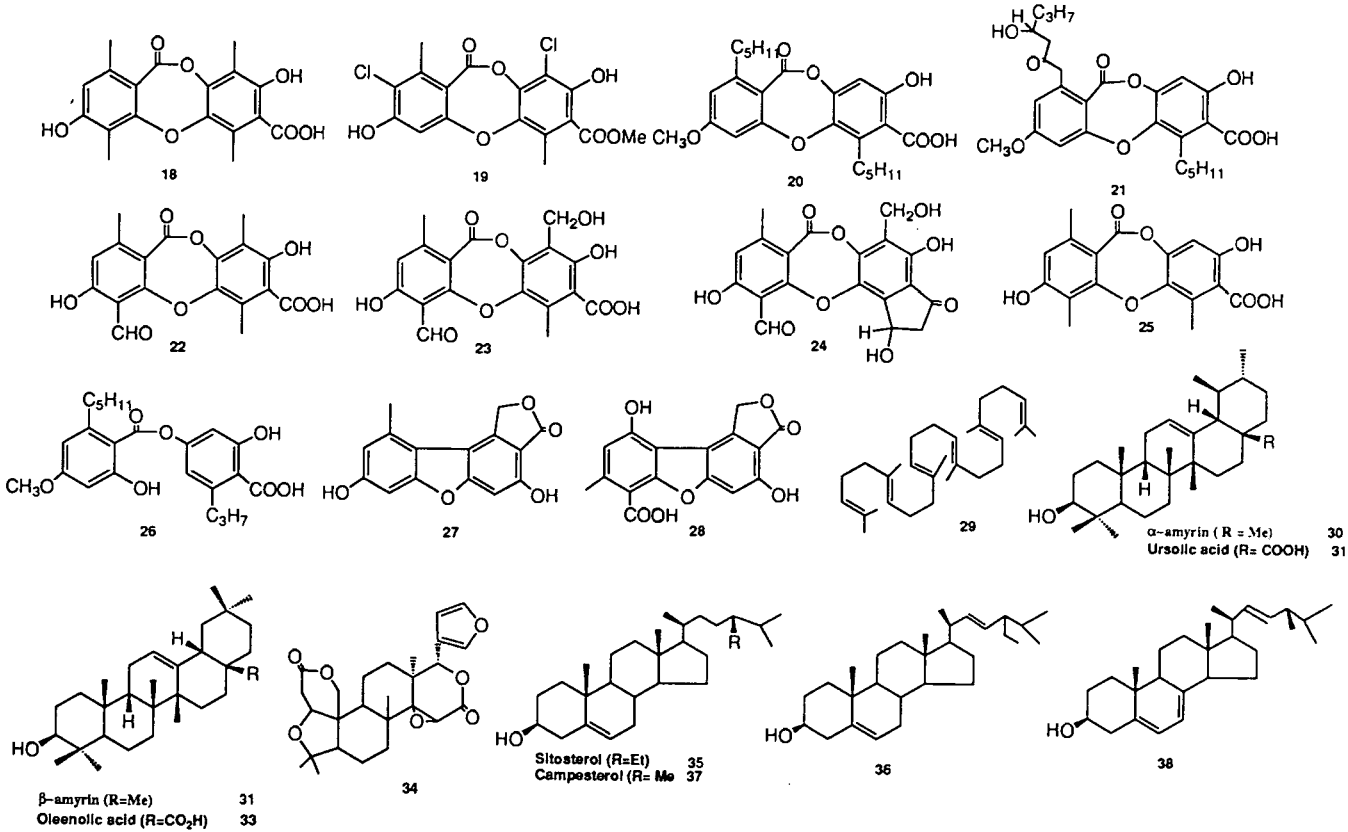


Figure 2


18
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27
28
29
α-amyrin (R = Me) 30
Ursolic acid (R= COOH) 31
β-amyrin (R=Me) 31
Oleenolic acid (R=CO2H) 33
34
Sitosterol (R=Et) 35
Campesterol (R= Me 37
36
38

Figure 3

When isolating lichen substances, it is important to identify without any doubt the lichen in question and to use homogenous material. As the taxonomy of lichens is very difficult , the test material should first be identified by an expert and subsequently a voucher specimen should be preserved.

Work in lichen chemistry is further complicated by the fact that numerous species exist with chemical strains confined to geographical districts of wider or narrower boundaries [9]. These chemically different, but morphologically identical plants, can be distinguished only by chemical tests or by inspecting a specimen under UV. light. For rapid chemical examination, either thalline reactions are carried out or thin layer chromatogram tests are made. The most important reagents for thalline reactions are: potassium hydroxide (KOH), sodium hypochlorite (NaOCl) and p-phenylenediamine (PD).

For isolating lichen substances, the dried and pulverized lichens are extracted with organic solvents, or in the case of polysacharides with water. Separation of the constituents can often be achieved by using different solvent systems consecutively (e.g. benzene, toluene, hexane, dimethylether, ethylacetate, acetone and etc). Further processing of the extracts depends on the substances involved.

In many cases lichen acids become separated during the extraction process and can then be purified by recrystallization. Otherwise, an etheral extract is shaken consecutively with sodiumbicarbonate ($NaHCO_3$), sodiumcarbonate (Na_2CO_3) and sodiumhydroxide (NaOH) solutions which separate acids, phenols and neutral portions respectively. Na_2CO_3 solution extracts compounds possessing relatively strong acids and phenolic hydroxyl groups. Column- type chromatographic separation of the individual lichen substances has often been applied in certain special cases [10, 11].

Lichens produce many unusual secondary products not found in other plants [12], (scheme 1). At the start chemical interest in lichen substances was generated by the uniqueness of many of the aromatic products. In more recent years, this interest has been increased by the discovery that some lichen products are biologically active [13], and that a great many of them can contribute to the solution of the enormous task of identifying and classifying the known species of lichens (about 15000 species).

Today the interest in lichen is heightened even further by a large series of biologically intriguing studies on the biosyntheses of the products, the distribution of the chemical races in nature and the physiology of both intact lichens and the isolated fungal and algal components in culture [14].

Although serious scientific study of the lichen products started about the middle of the 19th century, man has known of the lichens as useful sources of chemical products for centuries. Perhaps in classical times, and certainly in the middle ages, lichens were used as a source of dye stuffs for textiles, an application that persists to the present day in the rural hand weaving industries of many countries [15].

The chemists litmus paper was obtained by fermenting species of *Ochrolechia* and *Roccella* with Na_2CO_3 or K_2CO_3 in urine, saturated with ammonium carbonate $(NH_4)_2CO_3$; although synthetic litmus was prepared from orcinol as early as 1864. Certain lichen products are still an important commodity in the perfume and soap industries. As far as we are aware lichen compounds have not been widely exploited as medicinals, but the sodium salt of usnic acid (4) has apparently found relatively wide applications as an antibiotic in many countries [16].

In early scientific studies on lichen substances, large quantities of materials were extracted to obtain the pure compounds contained in lichens. As a result of these studies, hundreds of compounds were isolated and identified. The highest point of this period came in 1907 with the publication of Zopf's 450 page book [17]. Accelerated progress towards the elucidation of the structural formulae of the compounds came in a long series of papers by Yasuhiko Asahina and his students in Japan [18].

The chemistry of lichen products, with special emphasis on the problems of determining the chemical structures was summarized in Asahina and Shibata's book "Chemistry of Lichen Substances", of which an English translation appeared in 1954 [19]. In addition, the Culberson's published three important books: : "Chemical and Botanical Guide to Lichen Products", 1969, and in "theFirst and Second Supplements" [12, 20] .

1.1 CHARACTERISTIC LICHEN PRODUCTS.

Lichen forming fungi produce compounds many of which are identical or closely related to those produced by non-lichen forming fungi [5]. The most distinguishing feature of the compounds produced only by lichen, is the high proportion of acetate-polymalonate derived aromatic phenols composed of two or three phenolic (orcinol and β-orcinol types) units joined by esterification or by oxidative coupling or by both [1, 12].

The depsides, depsidones, dibenzofurans, usnic acid (4) and the depsone picrolichenic acid (8), all appear to be produced by such mechanisms and all are characteristic to lichens [21] (Fig. 1).

Other aromatic compounds of acetate-polymalonate origin such as chromones, xanthones, and anthraquinones, which are probably formed by internal cyclization of a folded polyketide chain, are often identical to only minor derivatives of products of non-lichen forming fungi or higher plants [5].

Information on the syntheses of lichen products by lichen forming fungi cultured in the absence of the algal partner is limited [4]. The substances detected from culture are few and include pulvinic acid derivatives [22], (E. A. Thomas, 1939), [23], phenolic acid units, chromones, and anthraquinones, [24]. It is noted that none of these compound types are particular to lichen forming fungi [4].

Although the characteristic lichen substances have not been found in cultures of the isolated lichen fungus, these substances are formed in the intact lichen under even bizarre experimental conditions [1]. For example, studies on the biosynthesis of the lichen substances have repeatedly demonstrated that intact lichens can synthesize depsides, tridepsides, depsidones and usnic acids under conditions such as total submersion in a liquid, [25, 26]; an environmental situation completly alien to the normal ecology of the plants. Furthermore, Mosbach and Jacobsson [27], prepared a cell free system from *Evernia prunastri* that synthesized evernic acid from acetyl coenzyme-A and malonyl coenzyme-A.

Depsides, depsidones and dibenzofuran derivatives, in particular the usnic acids (4), are the most characteristic lichen products, as they are found almost exclusively in these organisms. The biogenesis of depsides involves intramolecular esterification of two molecules of the polyketide derivative hydroxy-benzoic acid [1, 28].

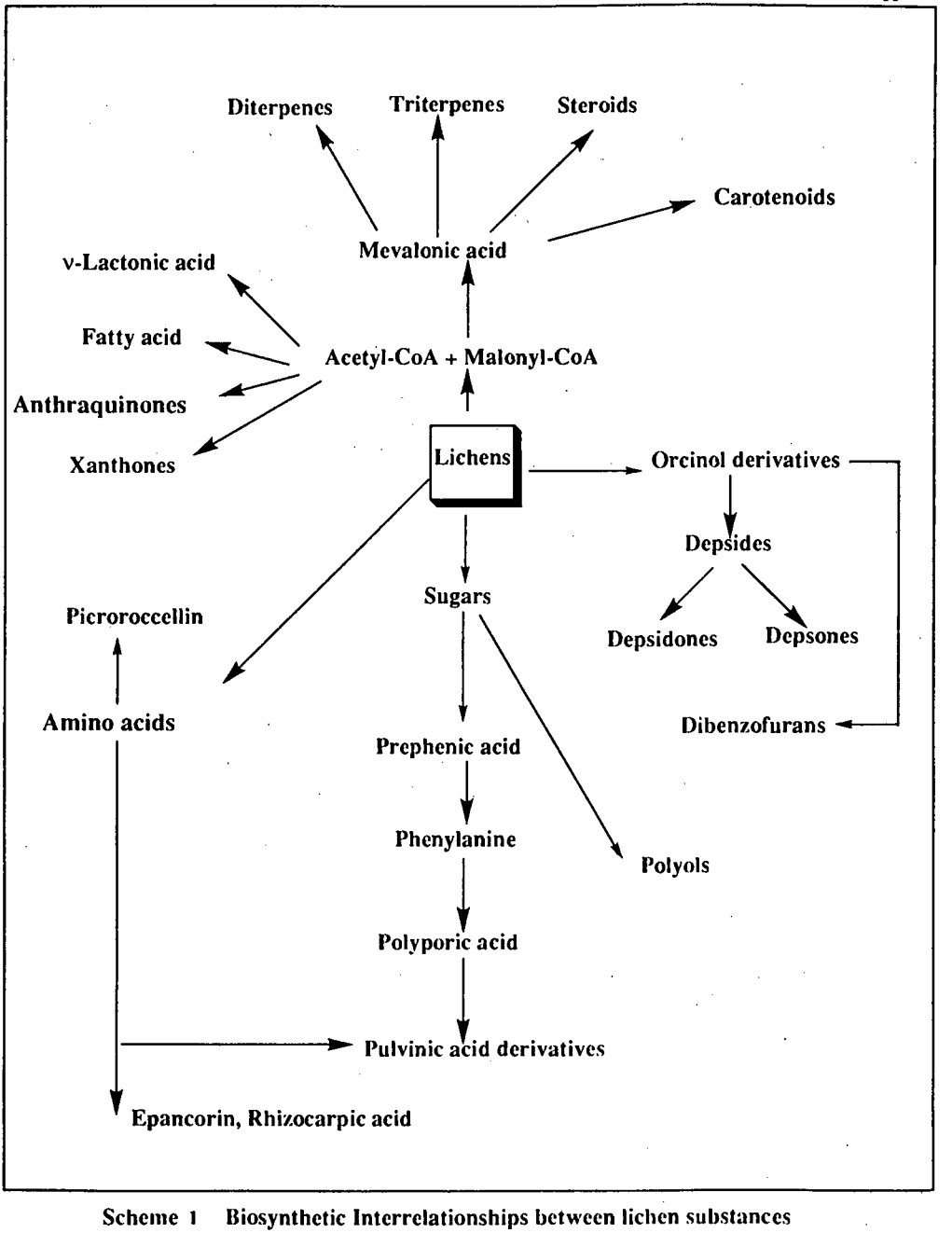
1.1.1 DIARYL AND TRIARYL ESTER ORCINOL SERIES

The most common acid units derived by the acetate polymalonate pathway and combined to form the characteristic lichen substances are of two types: (a) the orcinol type units, represented by orsellinic acid (6) and a number of related units ; (b) the β-orcinol type units which have an extra C-1 substituent at the 3-position [11].

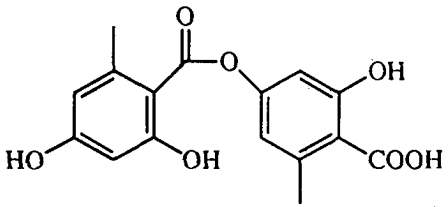
While compounds from these two types of units are similar in many ways, differences in their structures and especially in their distribution among the lichens, suggests that the usual tendency to consider the orcinol and β-orcinol compounds separately, probably has a biosynthetic justification [29]

The most common fate of acetate-polymalonate derived phenolic acids in lichen is intramolecular esterification of two or three similar or identical units [30]. In the most cases, the carboxylic acid of one unit is joined to the hydroxyl para to the carboxylic acid of the second unit [31].

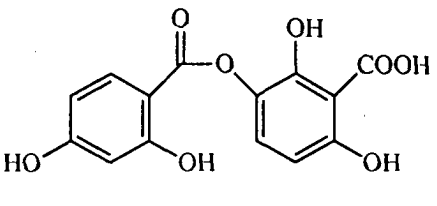
Such esterifications lead to the formation of para-depsides. A second esterification reaction leads to the formation of tridepsides, such as gyrophoric acid (**9**). If the ester linkage joins the first two units to a position meta to the carboxylic acid of the second ring, a meta-depside results [1].



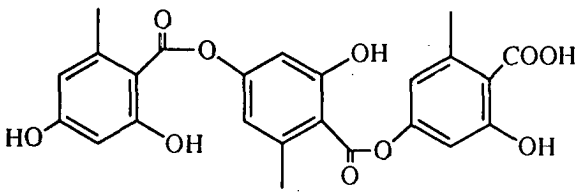
Scheme 1 Biosynthetic Interrelationships between lichen substances



PARA-DEPSIDE a



META-DEPSIDE b



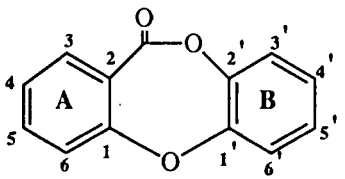
TRIDEPSIDE 9

Total synthesis of depsides has provided a definitive method for the structure elucidation of all groups of depsides and is also a useful alternative to the classical hydrolytic degradation procedure. Furthermore, in cases where complex mixtures of homologous depsides or simply lack of enough lichen material make isolation of depsides impractical, TLC comparison of synthetic material with the natural mixture has enabled the identification of a number of new depsides [32].

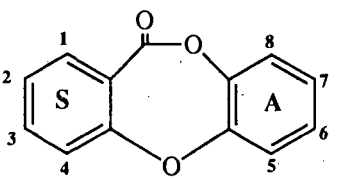
The use of the new condensing reagents, such as trifluoroacetic anhydride or dicyclohexylcarbodiimide, has streamlined the synthesis of depside linkage from the appropriate substituted benzoic acid and a phenol. Where necessary, the phenolic and carboxyl groups of the precursors are protected by O-benzylation or other appropriate protecting group units, after depside ester bond formation has been achieved. Catalytic hydrogenolysis of the so formed O-benzyl depside esters subsequently gives the natural depside.[1].

1.1.2 DEPSIDONES.

The depsidones are based on the 11H dibenzo[b, e] [1, 4] dioxepin-11one ring system and are numbered systematically as in the formulae below [19, 33, 34]. For convenience the benzoid rings are designated as A and B or as S and A according to the structures **a** and **b** (S portion = Säureglied and A portion= Alkoholglied). The depsidone system was numbered by Neelakantan and others [35] according to structure **a** and by Dean [33] according to structure **b**.

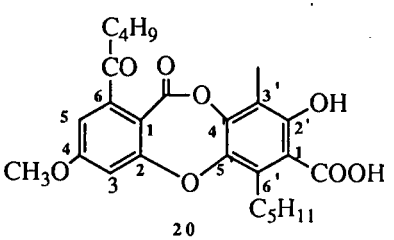


a



b

Unfortunately, the numbers of equivalent positions in desides and depsidones do not coincide. To overcome this difficulty, Culberson [36] considered the depsidones as 2-O-5- dihydroxy derivatives of depsides. Another possibility would be to combine depsidone and above numbering. According to this procedure lobaric acid (**10**) would be 2'-hydroxy-4- methoxy- n-pentyl- 6-n-valeryl-1'-carboxydepsidone.



20

Depsidones are the second largest group of the lichen metabolites [21] and are believed to arise by oxidative cyclization of depsides.The biogeneses of depsidones was first explained by the attractive theory that they arose from intramolecular phenolic oxidative coupling of para-depsides

[37, 38]. Oxidative coupling of phenols is often encountered among fungal products of many classes [39].

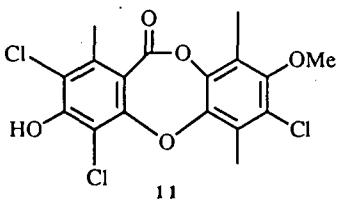
The fact that depsidones are characteristic for lichens is probably the result of the uniqueness of the lichen depsides from which they are formed [40]. Although depsidones are a chemically distinct class of compounds, biosynthetically they are simply depside derivatives [34]. The most common orcinol type of depsidones have an α-or β-keto group in the side chain of the first ring [41]. It is well known that this functional group has a strong effect upon the ester linkage between the two rings, since enol lactones form readily [34, 38].

A similar interaction may aid depsidone formation by stabilizing a conformation favorable to cyclization. In diploicin (**11**), variolaric acid (**12**) and grayanic acid (**13**), the first ring is an orsellinic acid unit lacking any oxygen function that could conceivably affect the cyclization. It is apparently because of the great difficulty of creating polysubstituted diphenyl ethers by classical methods that depsidone synthesis faced considerable obstacles [42].

Oxidative cyclization of depsides to depsidones usually join the two hydroxyls of ring A and the 5'-position of ring B in spite of the fact that most orcinol depsides also have free the 3'- position [37]. The mechanism of the cyclization reaction may involve intramolecular coupling of a phenoxyl radical and an aryl radical, leading to the formation of the ether linkage of the depsidone structure [35]. Olivetoric acid (**14**) and physodic (**15**); Sphaerophoric acid (**16**) and grayanic acid (**13**); and 4-O-methylbarbatic acid (**17**) and hypoprotocetraric acid (**18**) are depside depsidone pairs with exactly corresponding structures [43].

Of the orcinol type compounds, derivatives of orsellinic acid frequently show variations of structures that are also observed among the β-orcinol type units with longer side chains [44]. The biosynthsis of these substances is believed to proceed from acetate to orsellinic acid derivatives and then to esters (depsides) coupling to two of these derivatives [45]. Intramolecular oxidative coupling [3, 39] then creates the characteristic diphenyl ether ring of the depsidones.

The depsidones may be classified into: (a) compounds in which both benzenoid rings are derived from the polyketide orsellinic acid [44], chlorination and O-methylation as in lecideoidin (**19**) or decarboxylation as in diploicin (**11**) being common secondary modifications. Homologues of orsellinic acid (**6**) as in colensoic acid (**20**) or analogues with oxygenated side chains as in vittatolic acid (**21**), are also encountered.



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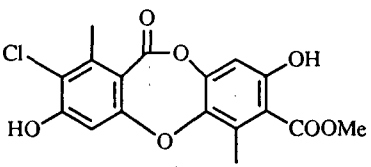

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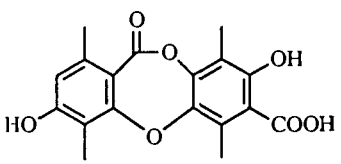
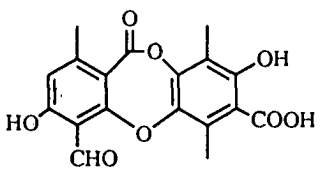
(b) The second class of depsidones contains those compounds in which both rings are derived from C-methylated polyketide β-orcinol carboxylic acid; the simplest member being hypoprotocetraric acid (**18**).



18

Side chain oxidation is very common in this group as illustrated by the series of: virensic acid (**22**), protocetraric acid (**23**) and salazinic acid (**24**), in which the degree of side chain oxidation increases [43].



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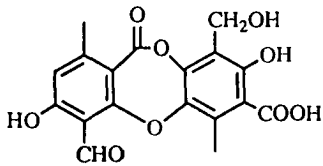

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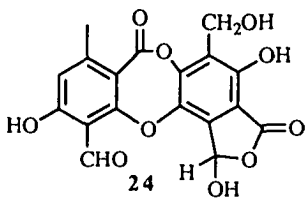

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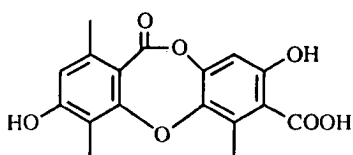
(c) The third class of depsidones includes those compounds in which one ring is derived from orsellinic acid (6) and the other from β–orcinol carboxylic acid, as in the simplest member, nornotatic acid (25).



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I.I.3 DIBENZOFURAN DERIVATIVES

The best known lichen products are the usnic acids (4) [12, 46]; yellow pigments produced in the upper cortex of many species from phytogenetically widely distinct families. These pigments are known only in lichens. A laboratory culture of the fungal components of *Cladonia cristatella* was claimed to synthesize usnic acids (4) [47]. However repeated attempts to confirm this report have failed (Ahmadjian, 1964) [5].

The usnic acids appear therefore, to be among the compounds which are the most characteristic of lichens and which are apparently not synthesized in cultures of the isolated fungal components and are formed by reactions uniting two or three phenolic units.

Cyclization of the aromatic ring of the methylethylphloroacetophenone precursor of the usnic acid [48], is basically different from the orsellinic acid (6) cyclization leading to the precursor of dibenzofuran derivatives.
However the oxidative coupling and cyclodehydration reactions of pairs of units are similar in the two cases [49]. With usnic acid (4) a C-methyl substituent prevents rearomatization of one ring [12, 37].
Unlike usnic acids (4), the phenolic units involved in the production of the true lichen dibenzofurans are derived by the orsellinic acid type by cyclization [12]. These units are potential starting materials for either depsides or dibenzofurans, and in fact, are found in both groups of compounds.

Didymic acid (5) and a number of meta-depsides are formed from the same basic units as the para-depside imbricaric acid (26). The dibenzofurans strepsilin (27) and porphyrilic acids (28), have the same phenolic units as the depsidone variolaric acid (12).
The dibenzofurans appear to be formed by carbon-carbon coupling and cyclodehydration of two of such acetate-polymalonate derived phenolic acid units:[48].

1.1.4 THE TERPENOIDS AND STEROIDS

An enormous range of plant substances are covered by the word 'terpenoids', a term which is used to indicate that all such substances have a common biosynthetic origin. Thus the terpenoids are based on the isoprene molecule and their carbon skeletons are built up from the union of two or more of these C-5 units [50]. They are then classified according to whether they contain two (C-10), three (C-15), four (C-20), six (C-30) or eight (C-40) such units (Fig. 2, 3).

The terpenoids range from the essential oil components, the volatile mono and sesquiterpenes (C-10), through the less volatile diterpens (C20), to the involatile triterpenes and sterols and carotenoid pigments. Each of these various classes of terpenoids are significant in either plant growth, metabolism or ecology [51].

Most natural terpenoids have cyclic structures with one or more functional groups (hydroxyl, carboxyl, carbonyl and etc.), so that the final steps in their syntheses involves cyclization and oxidation or other structural modifications [52]. Terpenoids are generally lipid soluble and are located in the cytoplasm of the plant cells. Terpenoids are normally extracted from the plant tissues with light petroleum, ether, chloroform or acetone, and can be separated by chromatography on silica gel or alumina using as eluents the same solvents as above. There is, however, often difficulty in detection on a microscale, since all (except carotenoids) are colourless and there are no sensitive universal chromatogenic reagents for them. Reliance often has to be placed on tentatively non-specific detection on TLC. plates with vanilline reagent and conc. H_2SO_4 and then heating [53].
Isomerism is common among the terpenoids and pairs of isomers may be isolated from the plants. In addition, terpenoids are mostly alicyclic compounds and because the cyclohexane ring is usually twisted, in the so called 'chair form', different geometric conformations are possible on the substitution around the ring.

A considerable number of quite different functions have been ascribed to plant terpenoids [54, 55]. Their growth regulating properties are very well documented. Two of the major classes of growth regulators are the sesquiterpenoid abscicins and the ditrpenoids based on gibberellin [56, 57]. The important contribution of carotenoids to the colour of plants is well known. The importance of monosesquiterpenes in providing the plant with many of their distinictive smells and odours is also well known.

Triterpenoids are compounds with a carbon skeleton based on six isoprene units and which are derived biosynthetically from the acyclic C-3O hydrocarbon squalene (**29**). They have relatively complex cyclic structures, most being either alcohols, aldehydes or carboxylic acids [50]. They are

colourless, crystalline, often higher melting optically active substances, which are generally difficult to characterize because of their lack of chemical reactivity.

Widely used tests are the Liebermann Burchard reaction (acetic anhydride and conc. H_2SO_4) and Godin reagent (vanilline solution and conc. H_2SO_4), which produce a blue green color with most triterpenes and sterols [58].
Triterpenoids can be divided into at least four groups of compounds [53]: (a) true triterpenes, (b) steroids, (c) saponins and (d) cardiac glycosides. The latter two groups are essentially triterpenes or steroids but which occur mainly as glycosides.

Many triterpenes are known in plants, and some are known to be widely distributed [59] such as the pentacyclic triterpenes α– and β–amyrin (**30, 31**) and the derived acids, ursolic acid (**32**) and oleanolic acid (**33**).
These and related compounds occur mainly in the waxy coatings of leaves and on fruits such as apple and pear, and may serve a protective function in repelling insects and microbial attack. Triterpenes are also found in resin and barks of trees and in latex. Certain triterpenes are notable for their tastes, particularly for their bitterness. Limonin (**34**), the lipid soluble bitter principle of citrus fruits, is a case in point.

Sterols are triterpenoids which are based on the cyclopentane perhydrophenanthrene ring system. At one time sterols were considered to be animal substances (such as sex hormones, bile acids etc.), but in recent years, an increasing number of such compounds have been detected in plant tissue.
The substances were therefore given the generic name sterol (Gr. sterol= solid + ol) [53].
Indeed, three so called phytosterols are probably ubiquitos in occurrence in higher plants: sitosterol (**35**), formerly known as β–sitosterol, stigmasterol (**36**), and campesterol (**37**).

These common sterols occur both as free or as simple glycosides. Certain sterols are confined to lower plants; one example is ergosterol (**38**) found in lichens, yeasts and many fungi [60, 61].

2. PHYTOCHEMICAL INVESTIGATION ON TWO LICHEN SPECIES OF THE GENERA USNEA(ASCOMYCOTINA)

2.1 *USNEA ARTICULATA* (L.) HOFFM.

2.1.1 INTRODUCTION

This study was undertaken as a continuation of a research work on the chemistry of lichen species carried out in our laboratory. The present research work involved an investigation of the chemical components contained in the lichen species: *Usnea articulata* (L.) Hoffm. Very little research work has been done on this lichen, and very few compounds isolated from this lichen have been reported [1, 2, 3, 4, 5].

This study provides detailed information of a number of supplementary components isolated and identified from the lichen under investigation. To isolate and purify the individual components, different chromatographic techniques were employed. The methods of isolation and identification of the components contained in this lichen are described in the following pages. The types of components isolated were a complex mixture of depsides, depsidones, usnic acid, monophenolic derivatives, triterpenes, and sterols.

Considerable quantities of common fatty acids and triglycerides were detected but were not investigated. The concentration of sterol components was extremely small. Some of the detected sterols resulted as traces in a complex mixture, hence isolation of a sufficient quantity for spectroscopic analyses failed.

A considerable amount of potassium oxalate was detected by microchemical analyses but was not fully investigated. In this research work 20 compounds were isolated or detected from the lichen under investigation (Fig. 53). Seventeen of these compounds were fully identified. One triterpene **14** and two sterols **19, 20** were detected by TLC and identified by MS, but could not be identified by NMR spectroscopy, due to the low concentration of these substances in the lichen extracts.

All the chemical substances which were known previously, as components of the lichen under investigation are listed below, and shown in the references presented below [1-5].

1- Barbatic acid, salazinic acid and potassium oxalate: Schulte (1905) [1].
2- Salazinic acid (as usnaric acid) and (+)- usnic acid: Zopf (1907) [2] and Thies (1932) [3].
3- Fumarprotocetraric acid and usnic acid: Hawksworth (1972) [4].
4- GLC methods for quantitative analyses of soluble carbohydrates: Holligan and Drew (1971) [5].

The primary aim of this research work was to isolate and identify the chemical substances contained in this lichen, particularly: depsides, depsidones, steriods and triterpenes. Subsequent large scale extraction and isolation of the lichen substances, yielded a number of common and rare lichen components presented in the following pages.

Hence it is expected that the findings of the present research work will be an important contribution to the knowledge of the lichen speces *Usnea articulata* (L.) Hoffm.

2.1.2 MATERIALS AND METHODS

The main study was carried out with 1kg of the lichen material. This lichen species *Usnea articulata* (L). Hoffm. (Figure 4) was collected on the from Canary islands by Dr. Ph. Clerc [46].

Usnea articulata (L.) Hoffm.

Figure 4

Extraction

Several solvent systems of differing polarities were employed for the extraction of the chemical substances from the lichen, such as ether, hexane, acetone, and subsequently methanol (scheme 2, 3). The dried and finely powdered lichen material was extracted with ether twice and hexane (24 h) by soxhlet extractor. TLC analyses showed that the two ether extracts and that of hexane were very similar, hence were combined together. On concentration of these extracts, a considerable amount of yellow crystalline substances precipitated and was then filtered off. Subsequent extraction of the lichen with acetone and finally with methanol afforded a mixture consisting mainly of two major components which precipitated after the concentration of the acetone and methanol extracts to one third of the original volume. The acetone extract afforded more grey-white substances which readily precipitated upon concentration of the extract. On standing in the refrigerator for two days, both the acetone and methanol extracts afforded more grey-white substance which was filtered off. The grey-white precipitate was subsequently identified as fumarprotocetraric acid (2).

Separation

Several common techniques were employed to isolate the chemical components contained in the lichen under investigation. Well known techniques for the isolation of depsides, depsidones and the other mixtures of the lichen substances were used including: (a) Fractional crystallization (b) Preparative layer chromatography and (c) column chromatography. The following three well known standard solvent systems were used to analyse the components in the lichen:

(I) Toluene-Dioxane-Acetic acid (180: 45: 5)
(II) Hexane-Ether-Formic acid (6: 4: 1)
(III) Toluene-Acetic acid (20: 4)

Preliminary TLC Analyses

TLC analyses of the crude hexane and ether extracts indicated the presence of four major components and several minor ones. Many of the minor components were overlapping. The TLC analyses indicated the presence of triterpenoid compounds, though in trace amounts.

Fractionation of the Extract

The crude hexane and ether extracts were treated with ice cold 5N $NaHCO_3$, 5N Na_2CO_3 and 5N NaOH respectively, in order to separate the weak acids, the strong acid and the neutral fractions. Large quantities of yellow and grey-white substances precipitated at the beginning of the partition

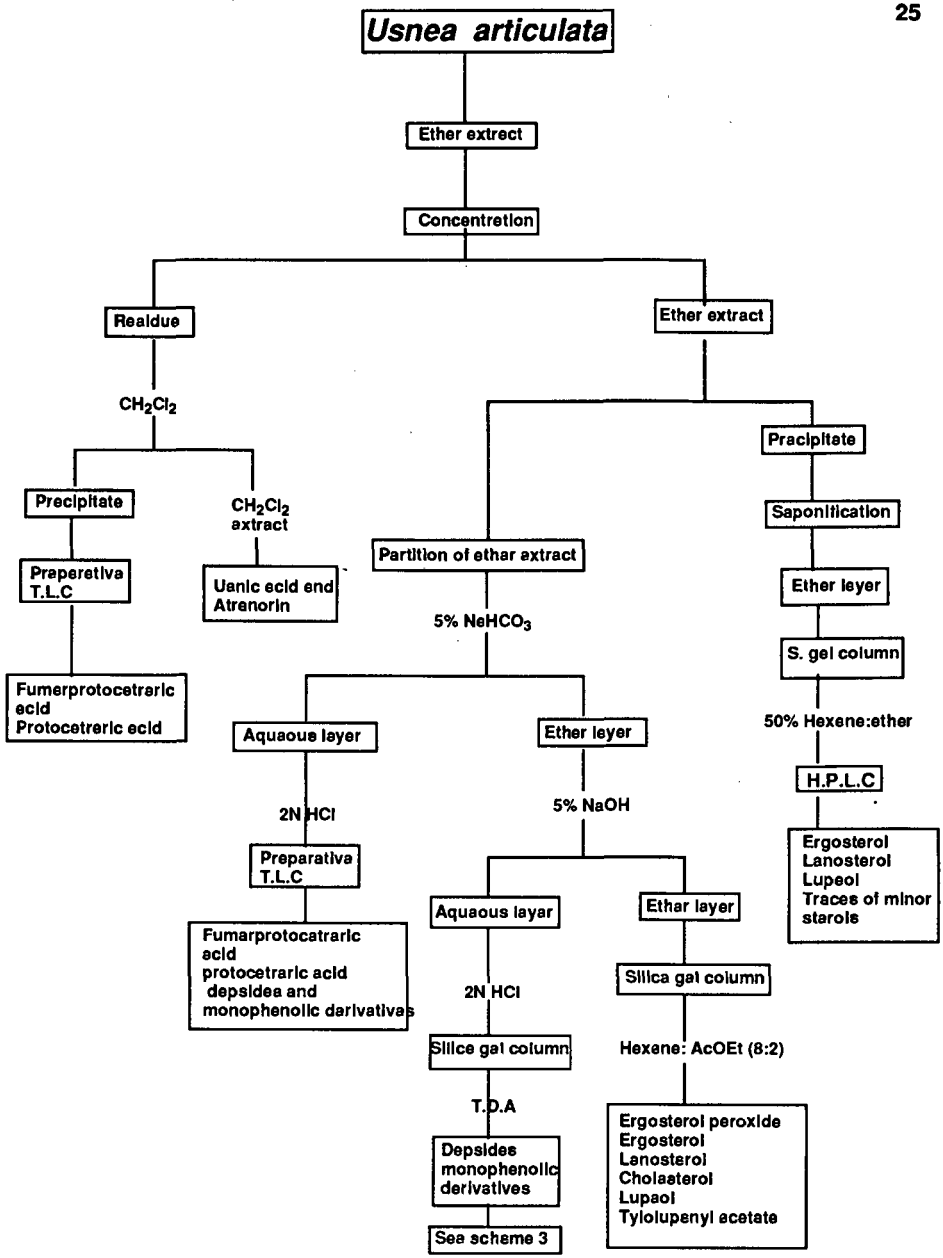
of the crude extracts. These were later identified as (+)- usnic acid (**1**) and fumarprotocetraric acid (**2**). These two major components comprised approximately 5.5% of the dry weight.

Barbatic acid (**4**) occured in this lichen in a relatively small amount (0.212%) of the crude extracts. The acid fractions were separately subjected on silica gel column eluted with different solvent mixtures and with different proportions of the individual solvents. The isolated individual compounds were purified on a Sephadex LH-20 column. Components with very close Rf values were separated by silica gel preparative plates, eluted with one of the three common standard solvent systems. TLC analyses of the neutral fraction revealed components of different concentrations and with very close Rf values. Considerable difficulties were encountered in separating some overlapping components. An attempt to separate such components by TLC or column chromatography failed to produce pure compounds. Hence reverse phase HPLC was employed. This allowed the isolation of the following compounds in a fairly pure state:
(I) diffractaic acid (**5**), which was admixed with barbatic acid (**4**)
(II) lanosterol (**17**) which was admixed with ergosterol peroxide (**15**), ergosterol (**16**) and cholesterol (**18**).

Ergosterol peroxide (**15**) is the most abundant neutral component in this lichen (40 mg or 0.004%). The other detected sterols and triterpenes such as cholesterol and tylolupenyl acetate, occurred in a very complex mixture and isolation of pure individual components was extremely difficult or impossible. An attempt to isolate a sufficient amount of each of the detected components for full spectroscopic analyses failed due to the very small concentration of such components in the extracts.

Identification of the Isolated Compounds

Identification of the isolated individual compounds was based on their physical properties, microanalytical, chromatographic and spectroscopic analyses. The spectroscopic data presented here confirmed the presence of the proposed structures. In particular the ^{1}H-NMR and ^{13}C-NMR spectra were very informative and useful. Characteristic substitution patterns were observed in the ^{1}H-NMR spectra. The appearance in the ^{1}H-NMR spectra of resonances typical for certain substituent groups, such as aromatic protons, were very informative The fragmentation pattern observed in the mass spectra also provided useful information.


Usnea articulata
Ether extract
Concentretion
Realdue
Ether extract
CH_2Cl_2
Precipitate
CH_2Cl_2 extract
Preperetiva T.L.C
Uanic ecid end Atranorin
Fumerprotocetrarlc ecid
Protocetrerlc ecid
Partition of ethar extract
5% $NaHCO_3$
Aquaous layer
Ether leyer
2N HCl
Preparativa T.L.C
Fumarprotocatrarlc acid
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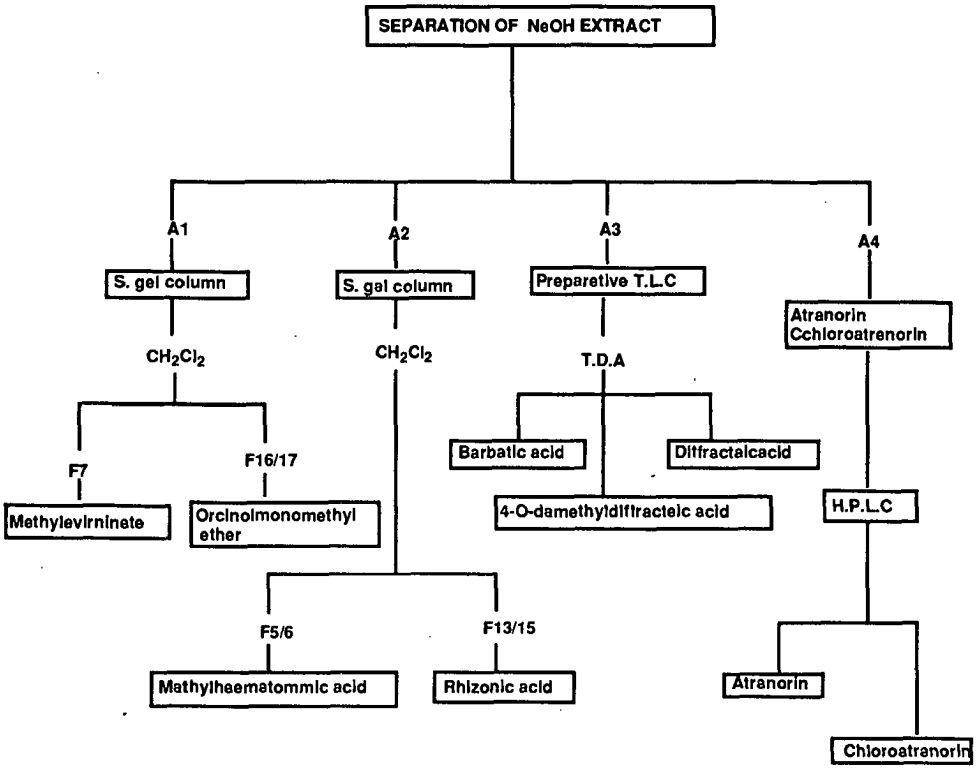
depsidea and monophenolic derivatives
5% NaOH
Aquaous layar
Ethar layer
2N HCl
Silice gal column
T.D.A
Depsides monophenolic derivatives
See scheme 3
Silica gal column
Hexene: AcOEt (8:2)
Ergosterol peroxide
Ergosterol
Lanosterol
Cholasterol
Lupaol
Tylolupenyl acetate
Praclpitate
Saponification
Ether leyer
S. gel column
50% Hexene:ether
H.P.L.C
Ergosterol
Lanosterol
Lupeol
Traces of minor sterols

Scheme 2


SEPARATION OF NeOH EXTRACT
A1
A2
A3
A4
S. gel column
S. gel column
Preparetive T.L.C
Atranorin
Cchloroatrenorin
CH2Cl2
CH2Cl2
T.D.A
F7
F16/17
Barbatic acid
Diffractaicacid
Methylevirninate
Orcinolmonomethyl ether
4-O-damethyldiffractelc acid
H.P.L.C
F5/6
F13/15
Methylheematommic acid
Rhizonic acid
Atranorin
Chloroatranorin

Schame 3

2.1.3 RESULTS AND DISCUSSION

2.1.3.1 DIBENZOFURAN DERIVATIVES AND DEPSIDONES

The isolation and identification of three common lichen substances is discussed in this chapter and the results are presented below. The structures of these compounds are presented at the end of this chapter; namely usnic acid (**1**), fumarprotocetraric acid (**2**) and protocetraric acid (**3**).
The spectra for the isolated compounds are presented at the end of this chapter.

(+)- USNIC ACID (1)

Compound **1** was the major component in the lichen under investigation. The yellow precipitates obtained after the concentration of the hexane and ether extracts were combined together and purified on silica gel column (4.52 %),(m.p. 201-203), (lit. 203-204°C) [10, 13]. A small amount was then recrystallized from acetone for spectroscopic analyses. It was identified as (+) Usnic acid (**1**) on the basis of spectroscopic and polarimetric data.
Usnic acid (**1**) has a characteristic yellow colour and can easily be detected by chromatographic analyses; however it was identified mainly on the basis of spectroscopic analyses. The fragmentation pattern observed in the mass spectra both EI and CI (Fig. 5, 6) of **1** indicated the presence of a dibenzofuran derivative. Probably the compound most easily recognized in lichen mass spectral analyses is usnic acid [6]. Its mass spectrum exhibited three prominent peaks at m/z 233, and a peak at m/z 260 with the parent peak appearing at m/z 344.

The ^{1}H-NMR spectrum of **1** was particularly informative and is in agreement with the proposed structure. Two signals corresponding to two CH_3-CO- groups were observed together with one aromatic methyl group resonance. One proton resonance assigned to H-C-4, together with signals attributed to 3 hydroxyl groups were also observed, (fig. 7).

Useful and supporting evidence was also obtained from the ^{13}C-NMR spectrum (Fig. 8) which revealed signals due to the presence of carbonyl carbons (179.86 and 164.4 ppm), the alcoholic carbons (158.05 and 155.77 ppm), (table 1). It appears that carbons located with the same environment show the same chemical shifts.

Analyses
^{1}H-NMR($CDCl_3$ - 200 MHz) : 1.77 ppm (s, 3H, CH_3, C-11); 2.12 (s, 3H, CH_3-Ar, C-8); 2.85 (s, 6H, $2CH_3CO$-, C-2 and C-6), 6.05 (s, 1H, H-C-4); 11.02 (s,1H, HO-C-3): 13.30 (s, 1H, HO-C-7); 15.85 (s, 1H, HO-C-9)

EI Mass spectrum (m/z, I%) 345 (M^{+}, 6), 344 (M, 32), 260 (32), 234 (17), 233 (100), 232 (23), 215 (14), 231 (24), 204 (4), 161 (6), 149 (5), 115 (6), 97 (6), 84 (9), 83 (17), 77 (11), 71 (12), 69 (26), 57 (23), 55 (19), 43 (47).

Table 1 ^{13}C-NMR Chemical Shift Data of Usnic Acid

C(1)	164.40	CH_3(11)	28.27
C(2)	163.98	C(11)	59.61
C(3)	155.77	C(12)	104.53
C(4)	102.07	C(13)	98.95
C(6)	109.51	$\underline{C}OCH_3$(2)	179.86
C(7)	158.05	CO$\underline{CH_3}$(2)	31.99
C(8)	105.79	$\underline{C}OCH_3$(6)	179.32
C(9)	156.93	CO$\underline{CH_3}$(6)	31.69
C(10)	109.76	CH3(8)	8.01

FUMARPROTOCETRARIC ACID (2)

Compound **2** was isolated mainly from the acetone extract as a very polar and sparingly soluble substance in almost all organic solvents and soluble only in hydrated acetone (80-90%). The precipitate of the acetone extract was recrystallized from acetone/methanol. The resulting ash-white substance (1.48.%) was isolated and identified as Fumarprotocetraric acid (**2**) on the basis of its spectroscopic data (m.p. 254-257), (lit. 250-260°C) [10, 13]. The mass spectrum failed to provide the expected information concerning the structure **2**. A parent peak was not observed in the spectrum and the fragmentation peaks observed could not be assigned. The mass spectrum revealed signals which corresponded to units of ions formed by recombination of various fragments. However, the spectrum did reveal the presence of certain characteristic fragment peaks typical of depsidones, such as m/z 179, 178 and 177. The ^{13}CNMR spectrum (Fig. 9) in DMSO of **2** was very useful and showed the presencc of the expected signals such as an aldehyde group signal at 191.68 ppm, carboxylic group at 170.20 ppm, and signals for methyl, methylene and methine groups.

The ^{1}H-NMR spectrum was particularly useful in determining the sequence of the substituent groups of compound **2**. The following prominent substituent groups were observed: two C-methyl groups, one aromatic proton resonance, one resonance assigned to the aldehyde group (OHC-Ar), together with two resonances attributed to the side chain vinylic protons (Fig. 10).

Thc IR spectrum (Fig. 11) is in agreement with the proposed structure showing the following absorptions: 3420 cm^{-1} (broad hydrated OH), 3100 cm^{-1}(aromatic C=C), 2910 cm^{-1}(aliphatic

side chain), 1745 cm^{-1}(depsidone CO), 1705 cm^{-1}(acid CO), 1660 cm^{-1} (aldehyde CO), 1500-1420 cm^{-1} (C=C side chain aliphatic vinyl group), 1300-1210 cm-1(broad ether group Ar-O-Ar absorption)

The results presented here were compared with those of the existing literature [7]. In the light of the results presented here, **2** was identified as the depsidone fumarprotocetraric acid (**2**)

Analyses

^{1}H-NMR (DMSO- 200 MHz) : 2.44 ppm (s, 3H,CH_3 -Ar), 2.48 (s,3H, CH_3-Ar), 2.51 (broad, DMSO), 5.31 (s, 2H, Ar-CH_2-O-), 6.65 (s, 2H, vinylic protons,-CH=CH-COOH), 6.84 (s,1H, H-Ar), 10.57 (s,1H, OHC-Ar), 11.97 (br, s, HOOC-Ar).

PROTOCETRARIC ACID (**3**)

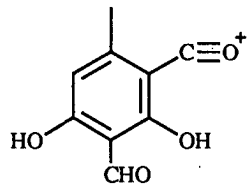
Compound **3** was obtained admixed with fumarprotocetracic acid (**2**) which precipitated upon saturation of the acetone extract. Similar to fumarprotocetraric acid (**2**), **3** is sparingly soluble in all organic solvents. It was isolated by silica gel column chromatography and preparative TLC as a white crystalline substance (0.0135%), (m.p. 244-246), (lit. 245-246°C) [8, 13], and was identified as protocetraric acid (**3**) on the basis of its spectroscopic and chromatographic data.

The ^{1}H-NMR spectrum (Fig. 12) indicated that **3** was probably a derivative of fumarprotocetraric acid (**2**), given the appearance of the following substituents: two distinct C-methyl group signals, one aromatic proton resonance, two singlet signals corresponding to the Ar-CH_2-OH protons, and one proton signal attributed to an aldehyde group.

The ^{1}H-NMR spectral evidence substantiated the similarity in the structures of compounds **2** and **3** and also indicated the required location of the aldehyde group, since it did not reveal the presence of a methoxy group.

The ^{13}C-NMR spctrum of **3** (Fig. 13) provided characteristic signals indicating the presence of an aldehyde group, carboxylic group and the expected number of methyl, methylene and methine groups.The ^{13}C-NMR data of compound **3** were compared with with that of the literature [17] as presented in table 2.

Further information regarding the structure of **3** was obtained form the fragmentation pattern observed in the mass spectrum (Fig. 14) which showed certain characteristic fragments and the molecular mass m/z 374. Of particular importance is the presence of the fragment at m/z 179, arising from the S-ring. This is an indication that the aldehyde group is most probably located at the 3-position. The fragment peak at m/z 179 (3b) is due to the fragmentation of the molecular ion



(3 b) m/z 179

The data presented here confirm the presence of the proposed structure, [8].

Analyses

^{1}H-NMR (CD_3OD- 200 MHz) : 2.47 ppm (s, 3H, CH_3-Ar), 2.675 (s, 3H, CH_3-Ar), 3.325 (br, s, CD_3OD), 4.81 (s, 2H, Ar-CH_2-OH), 5.11 (br, s, DOH), 6.75 (s, 1H, H-Ar), 10.72 (s, 1H, Ar-CHO)

Mass spectrum (m/z, I%) 374 (M, 5), 357 (14), 356 (23), 340 (12), 330 (9), 313 (38), 312 (52.6), 299 (33), 298 (14), 285 (28.6), 284 (32), 271 (26), 258 (84), 257 (27), 244 (12), 243 (17), 230 (65), 179 (51), 178 (32), 165 (23), 164 (13), 151 (100), 150 (29), 138 (19), 137 (16), 122 (16), 121 (12), 107 (15), 106 (28), 85 (22), 84 (36), 77 (34), 65 (26), 51 (64), 50 (80), 45 (100).

The main fragments of protocetraric acid

374 - (H_2O) 18 = 356

374 - (CO_2) 44 = 330

356- (CH_3) 15 = 341

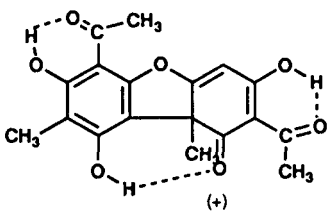
356 - (CO_2) 44 = 312

374 - (CO_2) 44 = 330

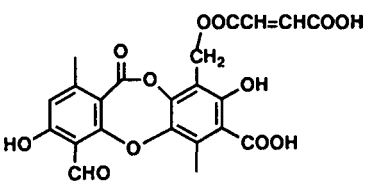
330 - (CH_3) 15 = 312

Table 2 ^{13}C-NMR Chemical shift data of protocetraric acid compared with that of the literature[17].

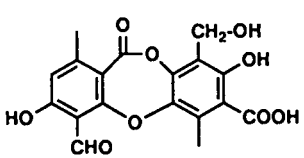
Protocetraric acid				literature			
C(1)	112.9	C(1)'	116.29	C(1)	112.1	C(1)'	115.5
C(2)	165.52	C(2)'	156.58	C(2)	165.6	C(2)'	156.1
C(3)	112.00	C(3)'	112.9	C(3)	111.6	C(3)'	113.2
C(4)	163.90	C(4)'	145.61	C(4)	164.6	C(4)'	145.8
C(5)	117.22	C(5)'	141.73	C(5)	117.3	C(5)'	142.1
C(6)	152.45	C(6)'	131,59	C(6)	152.3	C(6)'	132.5
C(7)	161.79	C(7)'	170.94	C(7)	160.7	C(7)'	170.7
C(8)	21.72	C(8)'	14.78	C(8)	21.4	C(8)'	14.9
C(9)	191.92	C(9)'	57.67	C(9)	191.7	C(9)'	56.8



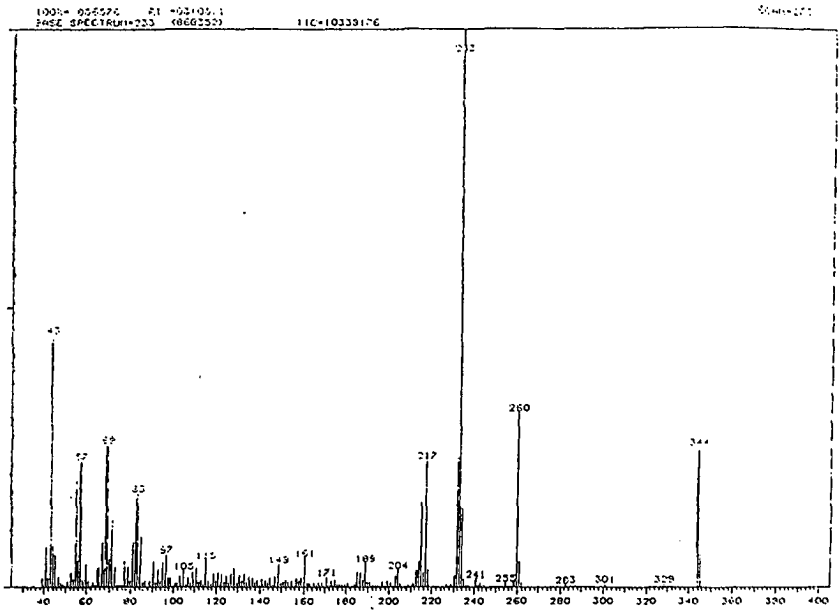
1



2



3



Figrure 5

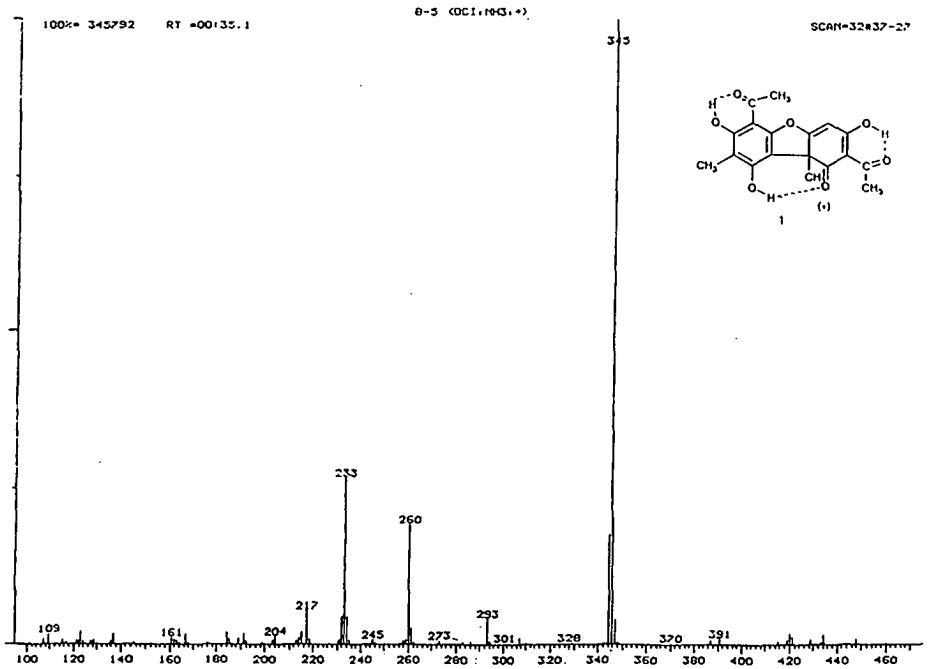


Figure 6

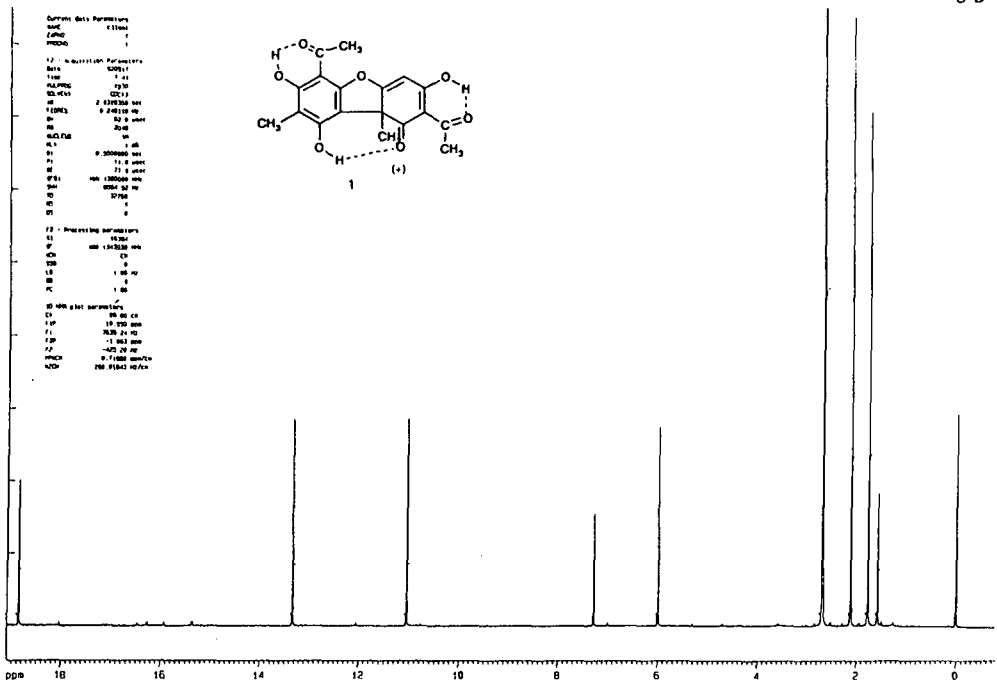


Figure 7

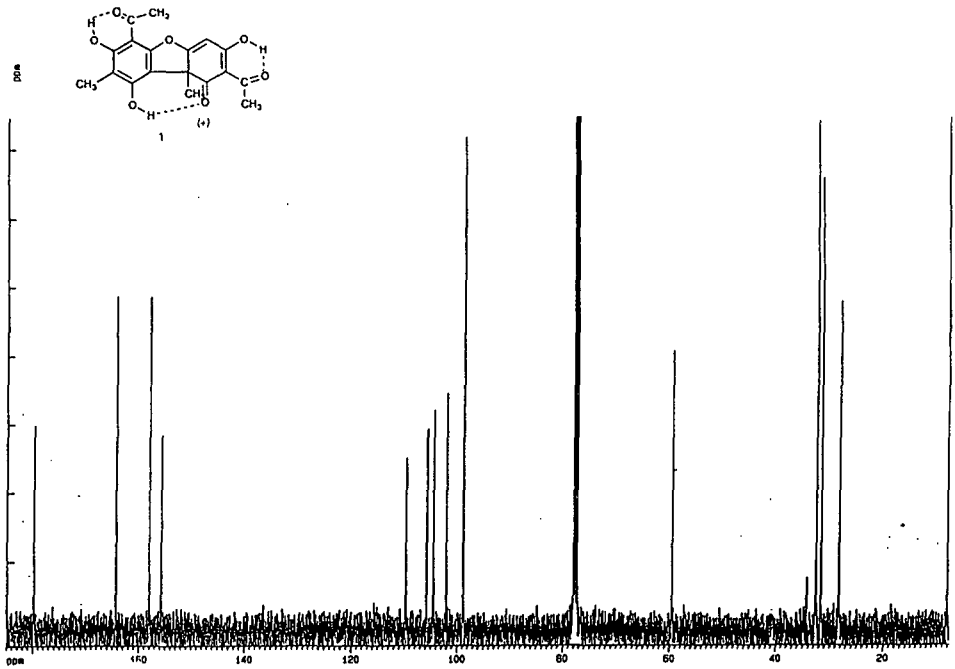
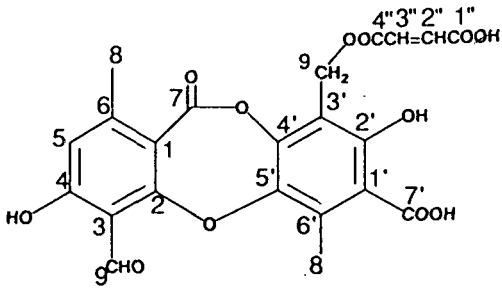


Figure 8



13CNMR Chemical Shift Data of Fumarprotocetraric Acid

C(1)	112.15	C(1)'	116
C(2)	165.57	C(2)'	155.6
C(3)	111.9	C(3)'	113.3
C(4)	164.6	C(4)'	145.6
C(5)	117.2	C(5)'	142.1
C(6)	152.11	C(6)'	132.20
C(7)	160.86	C(7)'	170.20
C(8)	21.48	C(8)'	14.7
C(9)	191.68	C(9)'	56.8

C(1)" 163.99, C(2)" 132.5, C(3)" 135.02, C(4)" 164.41

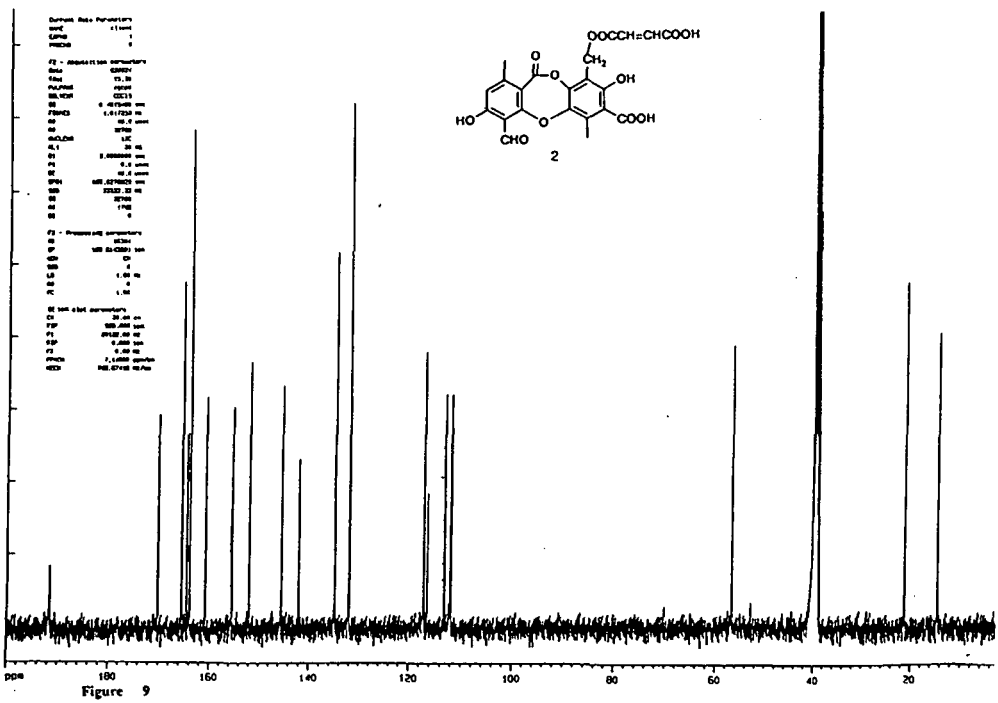


Figure 9

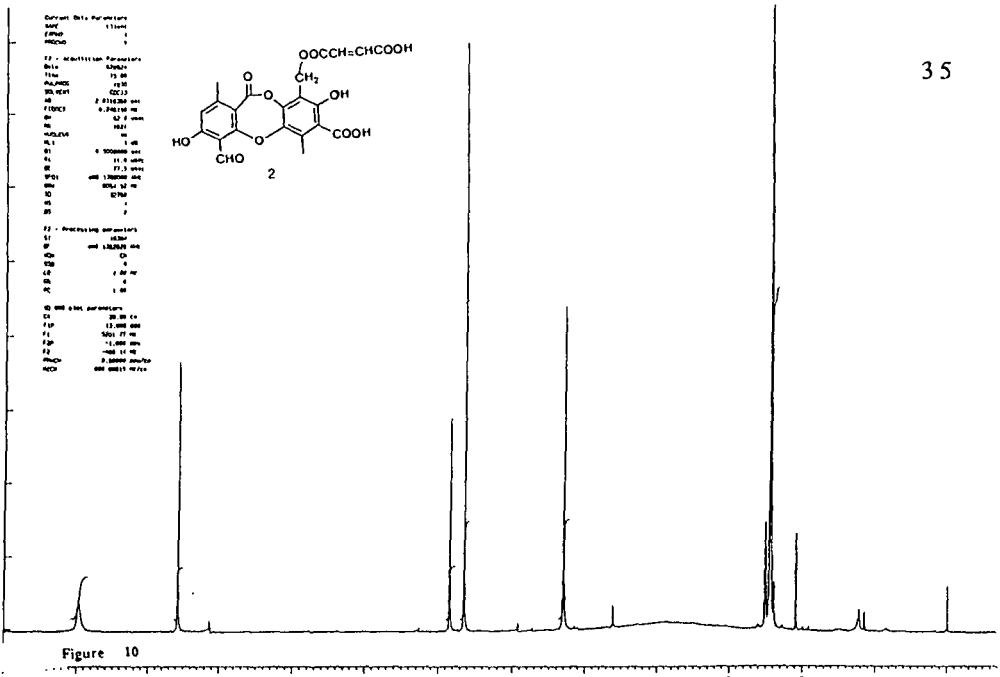


Figure 10

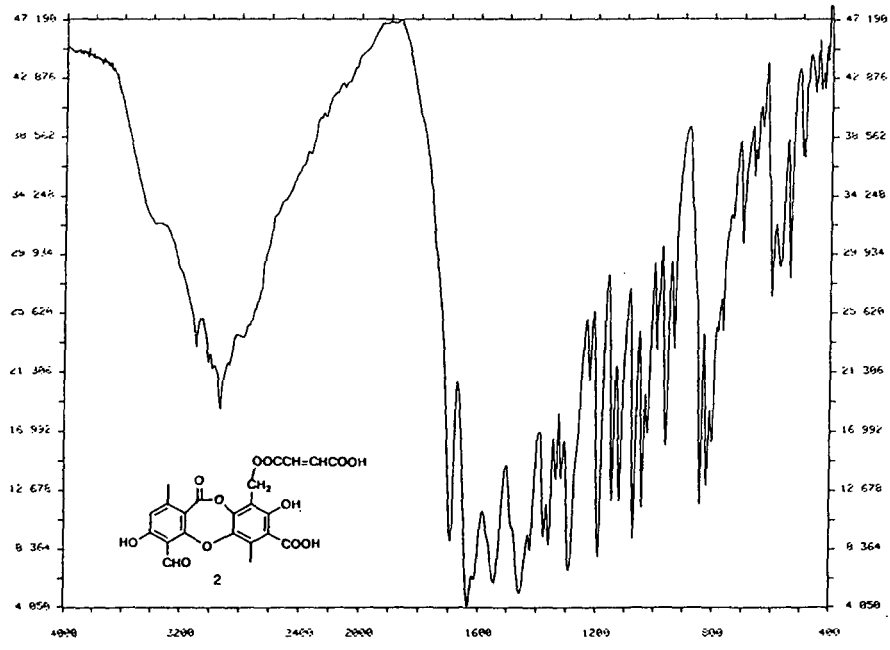


Figure 11

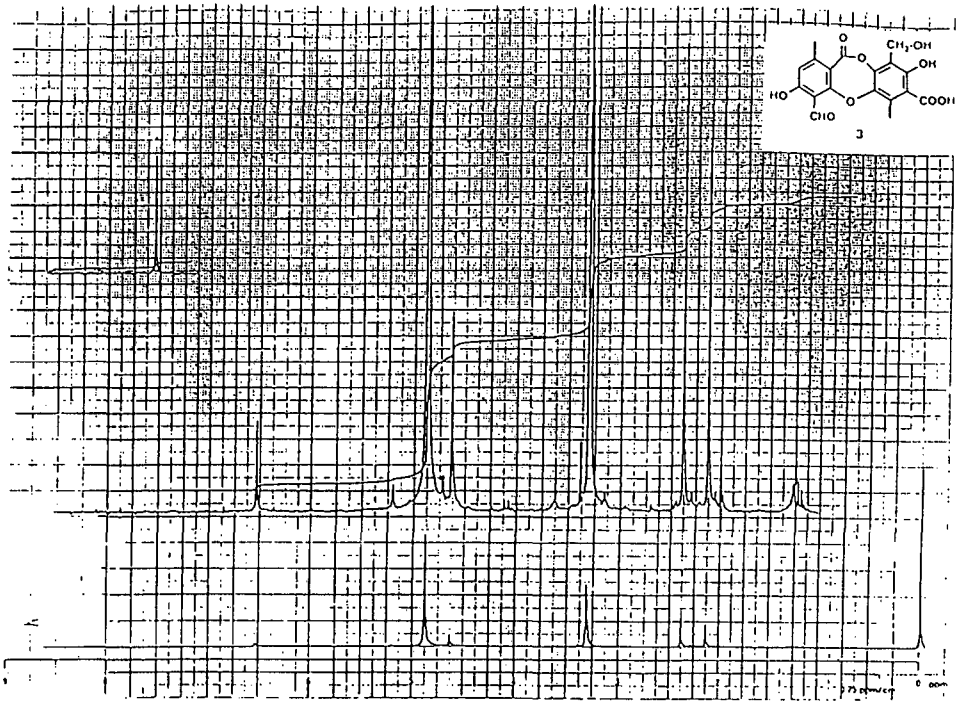


Figure 12

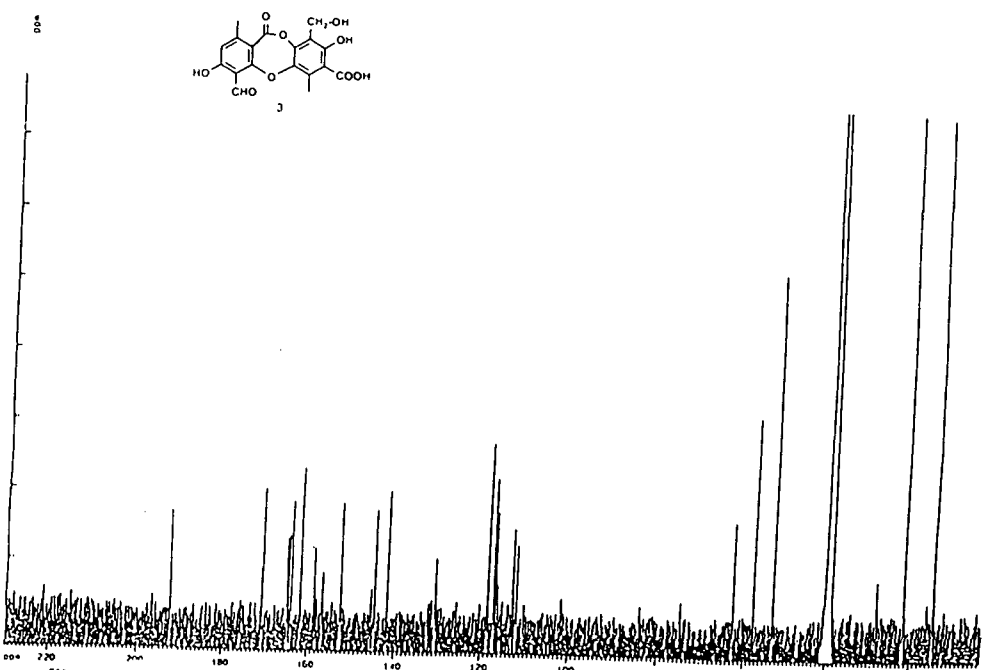
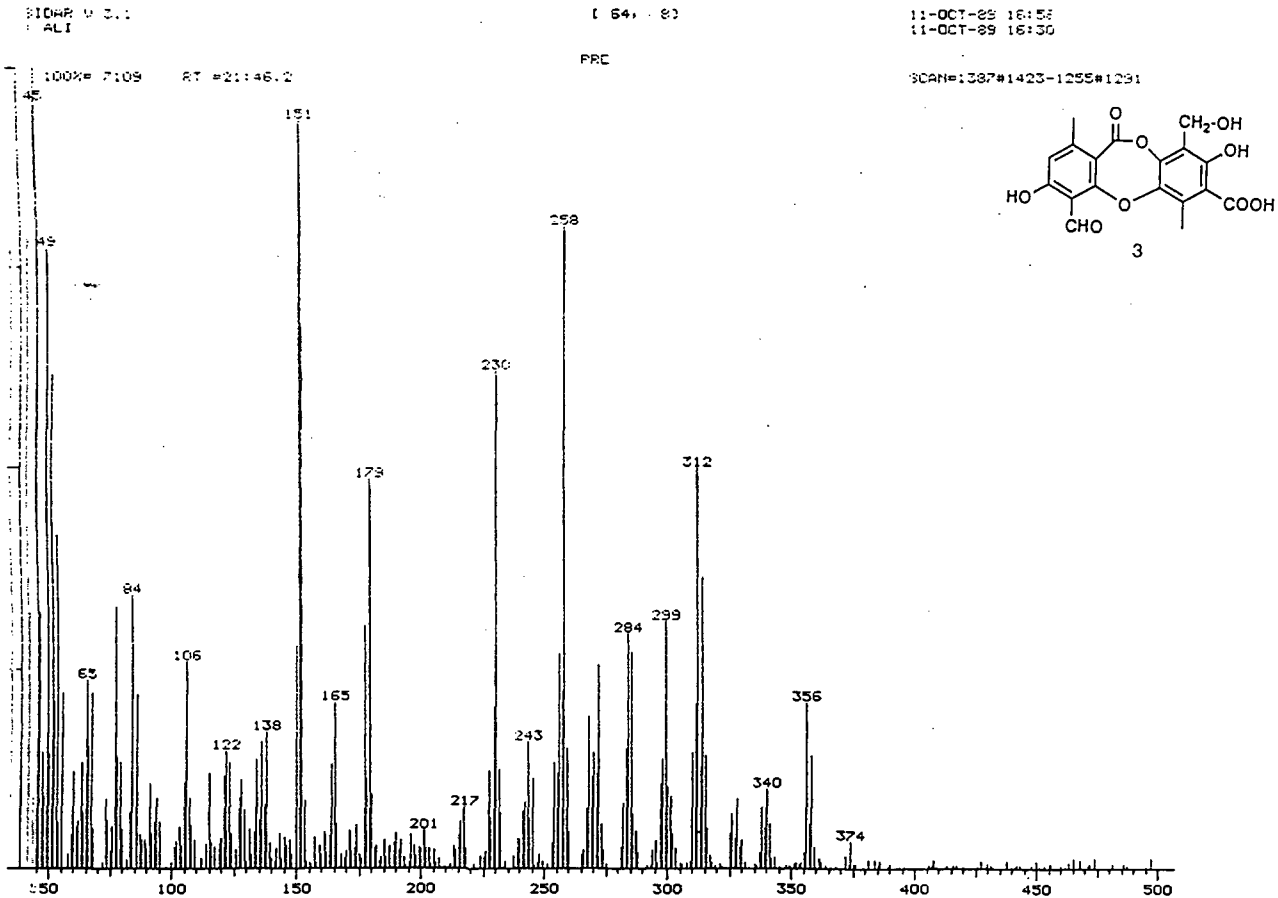


Figure 13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PRC
SCAN=1387#1423-1255#1291
11-OCT-89 16:58
11-OCT-89 16:30
45
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84
65
106
122
138
151
165
173
201
217
230
243
258
284
299
312
340
356
374
50
100
150
200
250
300
350
400
450
500
O
CH2-OH
OH
HO
CHO
COOH
3

Figure 14

2.1.3.2 D E P S I D ES

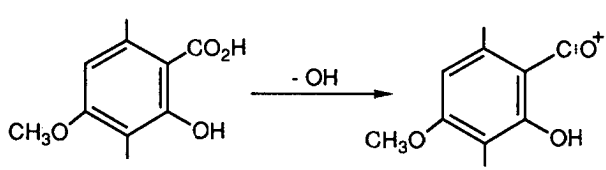
The results on the following five depsides, isolated from the acid part of the lichen extract, are discussed in this chapter; namely barbatic acid (**4**), diffractaic acid (**5**), atranorin (**6**), chloroatranorin (**7**) and comp.(**8**).
The spectra of the isolated depsides are presented at the end of this chapter

BARBATIC ACID (4)

Compound **4** was isolated from the acid extracts and was identified as the depside barbatic acid (**4**) on the basis of spectroscopic and chromatographic evidence. Although found in a small quantity, barbatic acid is present in many of lichen species belonging to the genus *Usnea* as well as *Ochrolechia* [9].

Barbatic acid was isolated in a considerable yield of about 0.021% of the total extract of the lichen material. Compound **4** was isolated and purified by silica gel column chromatography and preparative TLC using standard solvents as eluents (m.p 186-188), (lit. 187-189°C) [10].

Spectroscopic analyses of **4**, suggested the presence of a depside. The mass spectrum indicated the presence of a depside compound having molecular mass of m/z 360. The ^{1}H-NMR was particularly informative and showed the signals of the following substituents, characteristic of a depside compound: four distinict C-methyl group signals, one methoxy group signal and resonances of two aromatic protons. Since the two proton signals are well separated singlets, they must be located in different rings (fig. 15). Further information regarding the structure of compound **4** was obtained from the fragmentation pattern in the mass spectrum (Fig.16). This indicated the location of the methoxy group revealed by the ^{1}H-NMR spectrum. Of particular importance are the fragment peaks at m/z 196 and 179 arising from the S-ring. This is an indication that the methoxy group must be located at the 4-position of compound **4.**



m/z 196 m/z 179

Many compounds, particularly depsides exhibit M^+ ions that are of low intensity or are too weak to be observed [10]. Thus the mass spectrum of **4** was amplified ten times . The data presented here is identical in all aspects with that of the literature [11].

Analyses

^{1}H-NMR ($CDCl_3$- 200 MHz) : 2.08 ppm (s, 3H, CH_3-Ar), 2.25 (s, 3H, CH_3-Ar), 2.62 (s,3H, CH_3-Ar), 2.75 (s, 3H, CH_3-Ar), 3,96 (s, 3H, CH_3-O-Ar), 6.42 (s, 1H, H-Ar), 6.50 (s, 1H, H-Ar).

Mass spectrum (m/z, I%) 360 (M, 21), 196 (44), 180 (11), 179 (100), 178 (3), 164 (5), 150 (5), 136 (7), 123 (5), 108 (4), 107 (6), 91 (6), 77 (7), 67 (3), 55 (3), 53 (4), 39 (3).

DIFFRACTAIC ACID (5)

The second depside contained in this lichen exhibited quite similar physical and chemical properties to barbatic acid (**4**). It was identified as diffractaic acid (**5**) by chromatographic and spectroscopic studies. Diffractaic acid (**5**) is a β–orcinol type compound and occured in this lichen extract admixed with barbatic acid (**4**), usnic acid (**1**), and some monophenolic derivatives. Historically diffractaic acid was first assumed to be barbatic acid (**4**), but it was subsequently identified by Asahina et al [12] as diffractaic acid (**5**).

The physical properties of **5**, including its melting point, are similar to those of barbatic acid. It can only be distinguished by spectroscopic analyses. Diffractaic acid was isolated subsequently by preparative TLC and was purified on a column of Sephadex LH-20, (m.p. 188-191), (lit. 189-190°C) [10, 13]. It was identified on the basis of spectroscopic analyses as follows.

The mass spectral fragmentation pattern of **5** showed the presence of a depside with a molar mass m/z 374. The ^{1}H-NMR spectrum was particularly useful in determining the nature of the substituents on both rings. This revealed considerable similarities between barbatic acid (**4**) and compound **5**. The most significant difference between compounds **4** and **5** is that the former possesses only one methoxy group, while two methoxy groups were observed in the latter. Both possess four methyl groups. Two lower field and two higher field C-methyl signals were observed in the ^{1}H-NMR spectrum of **5** (fig. 17). The low field methyl signals are due to the presence of two methyl groups adjacent to the carbonyl functional groups. One of the aromatic proton resonances, appearing in the ^{1}H-NMR spectrum of **5**, can be ascribed to the S-ring (6.57 ppm). The remaining aromatic proton resonance (6.42 ppm) must be due to a proton attached to the A-ring.

This was further confirmed by the fragmentation pattern (fig. 18) observed in the mass spectrum, which exhibited the expected depside characteristic peaks at m/z 210, 193, 177, 166 and 164 which resulted after the break down of the molecular ion. The final confirmation of the presence of diffractaic acid was obtained by comparison of the data presented here with that of the literature [10].

Analyse

^{1}H-NMR ($CDCl_3$ - 200 MHz) : 2.10 ppm (s, 6H, $2CH_3$-Ar), 2.62 (s, 3H, CH_3-Ar), 2.72 (s,3H, CH_3-Ar), 3.86 (s, 3H, CH_3-O-Ar), 3.96 (s, 3H,CH_3-O-Ar), 6.42 (s, 1H, H-Ar), 6.57 (s, 1H, H-Ar), 12.34 (s, 1H, ArCOOH).

Mass Spectrum (m/z, I%) 374 (21), 346 (26), 331 (33), 316 (17), 311 (11), 300 (31), 210 (30), 193 (2), 177 (3), 166 (44), 164 (100), 152 (3), 139 (100), 136 (52), 123 (25), 108 (9), 91 (23), 77 (18).

ATRANORIN (6)

The presence of compound **6** in the sample was inferred from TLC analyses. This was obtained admixed with usnic acid (**1**), and traces of chloroatranorin (**7**). TLC analyses of a fraction A4 of acid extract indicated the presence of three components of different concentrations, and the spots of the two major components were overlapping. Usnic acid, chloroatranorin and compound **6** were separated by silica gel column chromatography with dichloromethane as eluent. The resulting white crystalline substance was further purified by TLC (m.p. 194-196), (lit. 196°C) [10]. This was identified as atranorin (**6**) on the basis of the spectroscopic data presented below.

Atranorin (**6**) occurs in many lichens, usually as one of the major components, but in this lichen of the genera *Usnea*, it is present in a relatively very small amount (0.0011%).
The spectroscopic analyses of **6** revealed the presence of a depside compound.

The ^{1}H-NMR spectrum (Fig. 19) provided useful information regarding the structure of compound **6**. This revealed the signals of the existing functional groups as follows: three distinct C-methyl group resonances, one methoxy group resonance, two well separated aromatic proton resonances, and one proton signal attributed to an aldehyde group resonance. In addition, signals due to three hydroxyl group resonances were observed.
The mass spectrum showed the molecular mass ion at m/z 374, (Fig. 20). In addition certain characteristic fragment peaks, typical of depsides, were noted such as m/z 196, 179, 178, 165, and 164.
Comparison of the results presented here with that of the literature confirmed the presence of the depside atranorin [13].

Analyses

^{1}H-NMR ($CDCl_3$ 200 MHz) : 2.09 ppm (s, 3H, CH_3-Ar), 2.54 (s, 3H.CH_3-Ar), 2.63 (s, 3H, CH_3-Ar), 3.96 (s, 3H, CH_3-O-Ar), 6.40 (s, 1H, H-Ar), 6.52 (s, 1H, H-Ar), 10.36 (s,1H, Ar-CHO), 11.96, 12.34, 12.56 (s, 1H each, HO-Ar and Ar-COOH).

Mass spectrum (m/z, I%) 374 (M, 20), 212 (9), 197 (9), 196 (79), 179 (91), 178 (35), 164 (100), 136 (43), 121 (7), 108 (5), 107 (15), 97 (7), 79 (29), 77 (35), 67 (24), 53 (34), 43 (11), 39 (30)

CHLOROATRANORIN (7)

The presence of compound **7** in the lichen under investigation was inferred by TLC analyses. A very small amount of **7** was obtained admixed with usnic acid (**1**) and atranorin.(**6**) An attempt to isolate a sufficient amount for ^{1}H-NMR analyses failed. However, it was possible to isolate enough for mass spectrometry analysis. Hence compound **7** was identified from mass spectral evidence.

The mass spectral analyses indicated that compound **7** was a depside having molecular mass m/z 408 (fig. 21). This also showed certain typical depside fragment ion peaks such as m/z 197, 196, 179, 165 and 164. In addition, the presence of fragment peaks due to chlorinated fragmentions were noted at m/z 213, and 211. The fragmentation pattern shown by the mass spectrum of **7** indicated the close relationship between compounds **6** and **7**.

The comparison between the data presented here and that of the literature confirmed the presence of chloroatranorin (**7**), [7,10].

Analyses

Mass spectrum (m/z, I%) 410 (2, isotopic peak), 409 (2), 408 (M, 15), 374 (15), 343 (3), 215 (10), 213 (26), 212 (9), 211 (9), 197 (13), 196(96), 179(100), 178 (7), 177 (38), 165 (28), 164 (91), 150 (7), 137 (5), 136 (44), 135 (28), 121 (6), 108 (7), 107 (19), 79 (34), 77 (43), 69 (29), 53 (57), 43 (26), 39 (44).

4-O-DEMETHYLDIFFRACTAIC ACID (8)

In the course of our investigation on the lichen *Usnea articulata* (L.) Hoffm., we isolated an interesting rare depside identified as 4-O-demethyldiffractaic acid (**8**). Particular emphasis was give to the analyses of the depside 8, because it is the second time that the occurrence of this compound in a lichen species was reported [14]. It is also the first time that depside **8** was isolated from genus *Usnea* The structural elucidation and analyses of the depside are described below.

Compound **8** occurred in the acid extract as a white microcrystalline substance. The ether soluble residue of the $NaHCO_3$ extract was dissolved in ethyl acetate and separated by silica gel column chromatography and preparative TLC, eluting with the standard solvent system: toluene - acetic acid (20:4). It was further purified on a column of Sephadex LH-20. The depside was crystallized from acetone as white substance, (m.p. 206-208°C), (lit. 207-209°) [14]

Compound **8** was coloured red with $FeCl_3$ in ethanol, but it gave a negative result with p-phenylenediamine indicating the absence of an aldehyde group.
The IR spectrum, (fig. 22) of **8** showed absorption bands at 3413 cm^{-1} (-OH group), 1658 cm^{-1} (pentacyclic C=O group), 1617 cm^{-1} (depside C=O group), 1597-1577 cm^{-1} (aryl C=C group), 1499- 1367 cm^{-1} (esters and ethers Ar-O-Ar and Ar-O-R groups).

The CI mass spectrum, (Fig. 23) of **8** showed a prominent peak at m/z 378 ($M+ NH_4$) and a quasi molecular ion peak at m/z 361 (M + 1) and a base peak at m/z 317 (M^+ / CO_2), while its EI mass spectrum showed a weak molecular ion peak at m/z 360 and a base peak at m/z 316 (M, CO_2). Furthermore, the CI spectrum revealed certain characteristic depside fragments such as m/z 197, 196, 179, 177, 164. Of particular importance is the presence of the fragment ion at m/z 196, arising from the S-ring of the depside. Although depsides rarely exhibit a molecular ion in the positive ion spectra, daughter ions originating from the rings S and A, rings were always observed and naturally, are indicative of the substitution pattern in the depside rings [10].

The ^{1}H-NMR spectrum (Fig. 24) indicated the presence of two aromatic protons, one methoxy group, one hydrogen bonded hydroxyl group, and four aromatic methyl group. In fact both the MS and ^{1}H-NMR spectra of depside **8** appeared to be similar to those of common depsides, barbatic acid (**4**), and diffractaic acid (**5**). However , its R*f* value and melting point results (table 4) were quiet different from those of the common desides **4**, **5**. Furthermore, unlike barbatic acid (**4**) and diffractaic acid (**5**), 4-O-demethyldiffractaic acid (**8**) is sparingly soluble in most organic solvents.

The ^{13}C-NMR spectrum (fig. 25), provided useful information which was in agreement with the spectroscopic results presented above, and confirmed the presence of a depside structurally related

to the depsides **4** and **5**. This showed signals of 19 carbon types in addition to the TMS at 0.0 ppm and a multiplets due to DMSO centred at 39.31-40.14 ppm. The spectrum indicated from higher to lower field, the molecular fragments, such as four methyl group signals at 7.93, 9.25, 22.88, and 23.29 ppm, a methoxy group signal at 55.68 ppm, a prominent signal appearing at 169.20 ppm corresponding to the depside ester group and a signal appearing at the lowest shift at 173.12 ppm assigned to the carboxyl group.

This evidence indicated that compound **8** was an uniquely substituted depside, particularly the location of the methoxy group, seemed to be the determining factor of the structure of the depside **8**. Comparison of the data presented here and that of the literature substantiated the presence of depside **8** [8, 10, 13, 14]

Analyses

^{1}H-NMR (DMSO-400 MHz) : 2.11 ppm (s, 3H, CH_3-Ar), 2.13 (s, 3H, CH_3-Ar), 2.38 (s, 3H, CH_3-Ar), 2.56 (s, 3H, CH_3-Ar), 3.84 (s, 3H, CH_3-O-Ar), 6.45 (s, 1H, H-Ar), 6.61 (s, 1H, H-Ar), 11.017 (s, 1H, HO-Ar).

Mass Spectrum (m/z, 1%), (Cl, NH_4^+), 378 (M+ NH_3), 361 (60), 360 (2), 317 (32), 214 (4), 197 (9) 196 (3), 182 (9), 181(39), 179 (78), 178 (3) 167 (3) 165 (3),154 (4), 153 (12), 139 (100), 138 (11), 123 (4) 108 (3).

The ^{13}C-NMR data of 4-O-demethyldiffractaic acid (**8**) was compared with those of the related depsides such as barbatic acid (4) and diffractaic acid (**5**) [15] as shown in table 3.

Table 3 ^{13}C-NMR chemical shifts of the depsides 4, 5 and 8

4-O-Demethyldiffractaic acid (in DMSO)		Barbatic acid (in $CDCl_3$)		Diffractaic acid (in $CDCl_3$)	
C(1)	116.32	C(1)	119.2	C(1)	119.4
C(2)	163.9	C(2)	160.2	C(2)	159.5
C(3)	108.7	C(3)	115.8.	C(3)	116.1
C(4)	161.05	C(4)	157	C(4)	156.4
C(5)	112.47	C(5)	108.6	C(5)	108.4
C(6)	139.96	C(6)	135.3	C(6)	134.8
C(7)	169.2	C(7)	165.7	C(7)	165.5
C(8)	23	C(8)	19.8	C(8)	19.5
C(9)	7.93	C(9)	7.93	C(9)	8.8
CH_3O	55.68	CH_3O	55.5	CH_3O	61.7
C(1')	115.18	C(1')	116.5	CH_3O	55.7
C(2')	159.91	C(2')	161.7	C(1')	116.5
C(3')	106.67	C(3')	111	C(2')	161.7
C(4')	149	C(4')	152	C(3')	111
C(5')	114.01	C(5')	115.8	C(4')	152.4
C(6')	139.4	C(6')	139.3	C(5')	115.8
C(7')	173.1	C(7')	173.4	C(6')	139.6
C(8')	22.8	C(8')	23	C(7')	173.4
C(9')	9.24	C(9')	9.0	C(8')	23.0
				C(9')	9.0

Thin layer chromatography has been used in distinguishing depside **8** form the other related depsides as shown in the table below [8].

The melting point and TLC data of 4-O-demethyldiffractaic acid (**8**) were also compared with those of the related depsides **4**, and **5** as presented in the table 4 [8, 10, 13].

Table 4 Comparison of TLC and melting point data

Standard R*f* values in three independent TLC solvent systems

A, Benzene- dioxane- acetic acid (180: 45. 5).

B, n-Hexane - diethyl ether- formic acid (130: 80: 20)

C, Toluene- acetic acid (200: 30)

		TLC Data		Melting Point Data
Compound	R*f*(A)	R*f*(B)	R*f*(C)	(m.p)
4-O-demethyldiffractaic acid	34	45	30	206-208
Literature (13)	34	34	31	207-209
Barbatic acid	44	69	52	187°C
Diffractaic acid	43	64	51	189-191°C

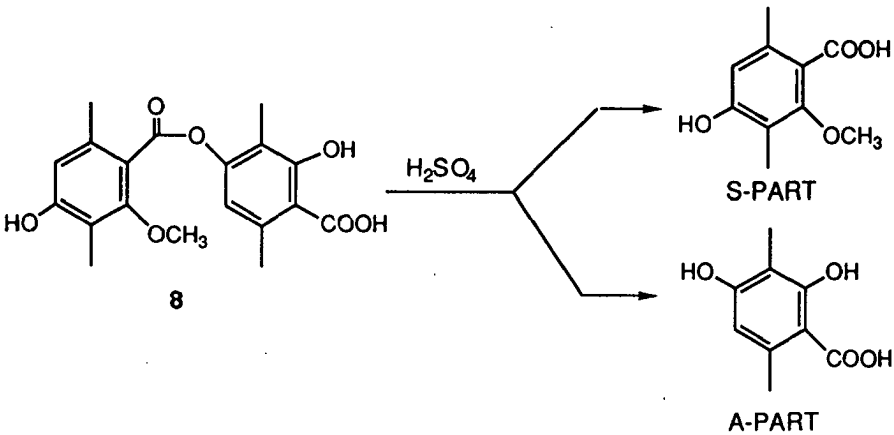
DEGRADATION OF 4-O-DEMETHYLDIFFRACTAIC ACID (8)

In order to investigate further the overall structure and the distribution of the substituents in the S- and A-parts of the depside, 5 mg of **8** were hydrolysed with ice cold H_2SO_4. The TLC analyses of this showed two main spots indicating the formation of two products (p1 and p2), resulting most probably from the fragmentation of compound **8**. It was assumed that the two products formed were the S-part and the A-part of the depside **8**. The spectroscopic analyses of product-1 and product -2 are presented below.

The ^{1}H-NMR spectrum (Fig. 26) of the S-part showed the following substituent groups: two aromatic methyl group signals at 2.12 and 2.55 ppm, one methoxy group signal at 3.86 ppm, and one aromatic proton signal at 6.39 ppm.

The mass spectrum of the S-part (Fig. 27) gave a molecular ion peak at m/z 196 and certain characteristic fragment peaks such as m/z 179, 178 and163. The information gathered from the ^{1}H-NMR and mass spectra (Fig. 26, 27) led us to propose that the structure of product-1 is the one shown below, corresponding to the S-part of the depside in question.

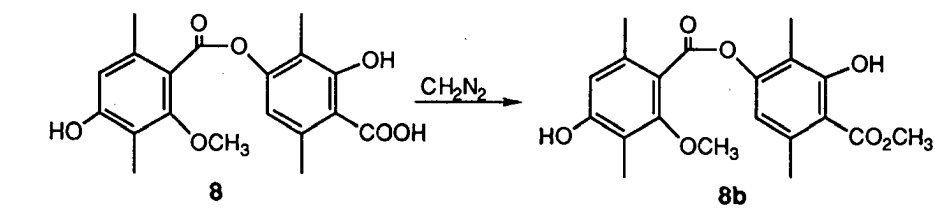
The mass and ^{1}H-NMR spectral analyses (Fig. 28, 29) of the hydrolysis products of the depside **8** also showed the presence of product -2, corresponding to the A-part of the depside in question. The ^{1}H-NMR spectrum of part-2, showed signals corresponding to two aromatic methyl group and one aromatic proton, (Fig. 28) and its mass spectrum showed molecular ion peak at m/z 182 (Fig. 29)



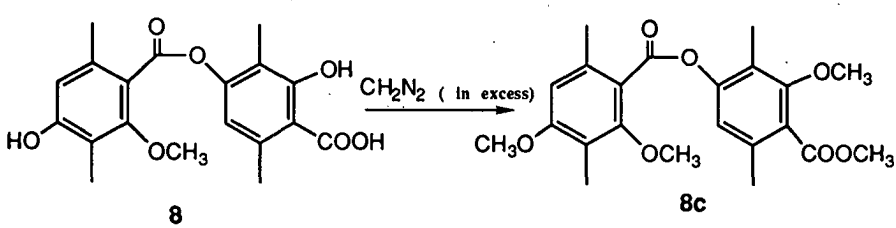
8

METHYLATION OF DEPSIDE 8

The depside **8** was converted into the corresponding methyl ester by brief treatment with ethereal diazomethane in order to facilitate purification. The ^{1}H-NMR spectrum (Fig. 30) of the methyl ester so obtained revealed four aromatic mehyl groups, two aromatic protons and two O-methyl groups. However, methylation of depside **8** with excess of diazomethane gave rise to the corresponding tetramethoxy ester **8c**. The ^{1}H-NMR spectrum of totally methylated depside **8c** (Fig. 31) showed signals of four methoxy groups. This led us propose that compound **8** was a depside containing a free carboxylic and two phenolic groups.

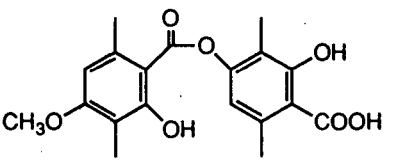


8 **8b**



8 **8c**

THE STRUCTURES OF THE DEPSIDES 4, 5, 6, 7, 8



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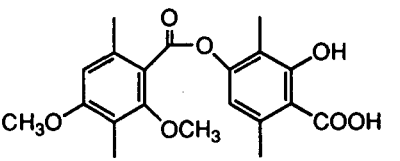

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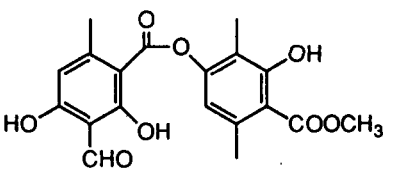

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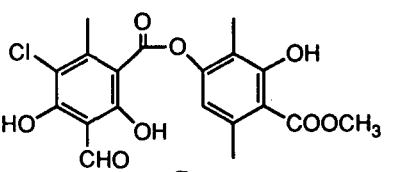

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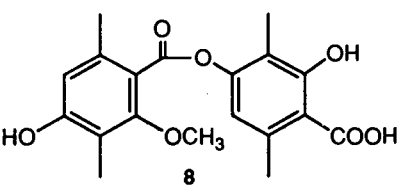

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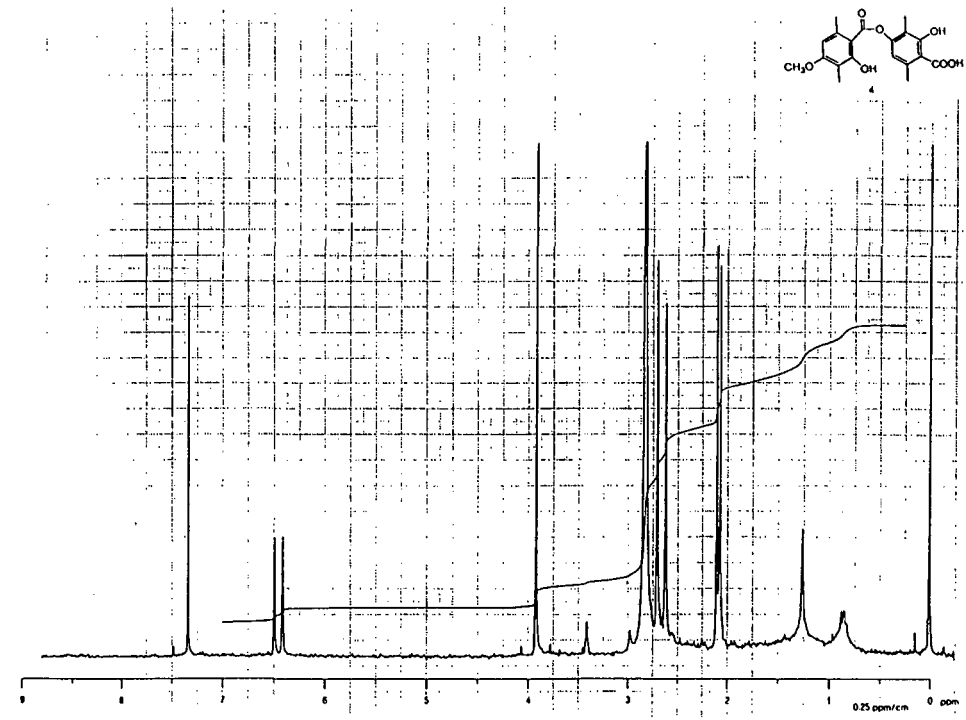


Figure 15

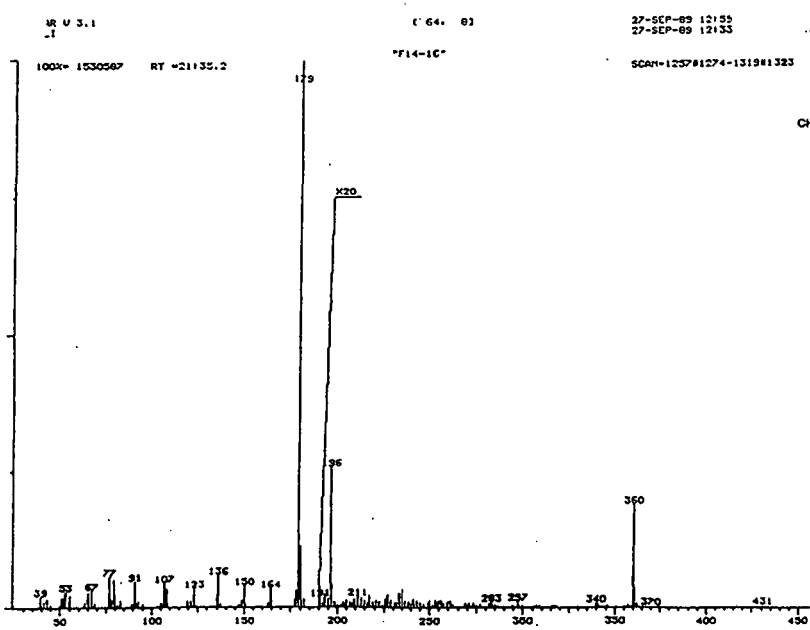


Figure 16

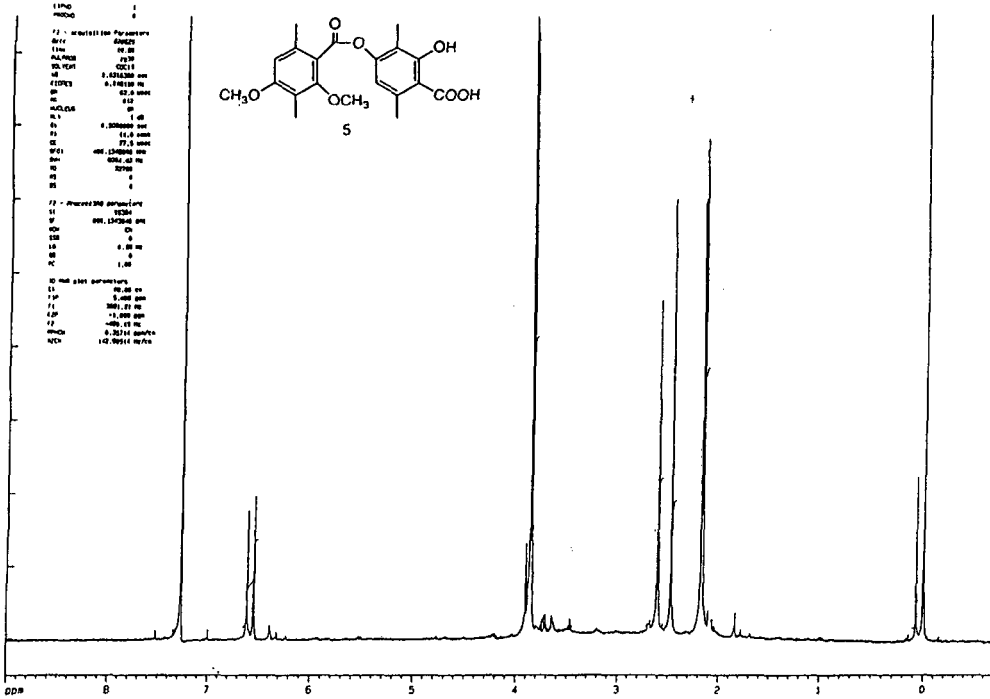
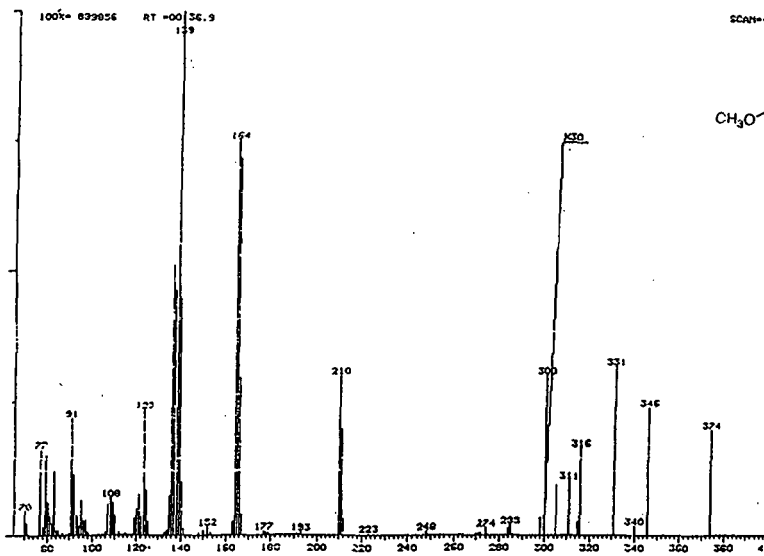


Figure 17



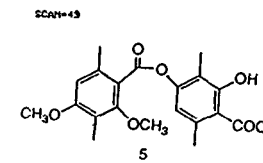


Figure 18

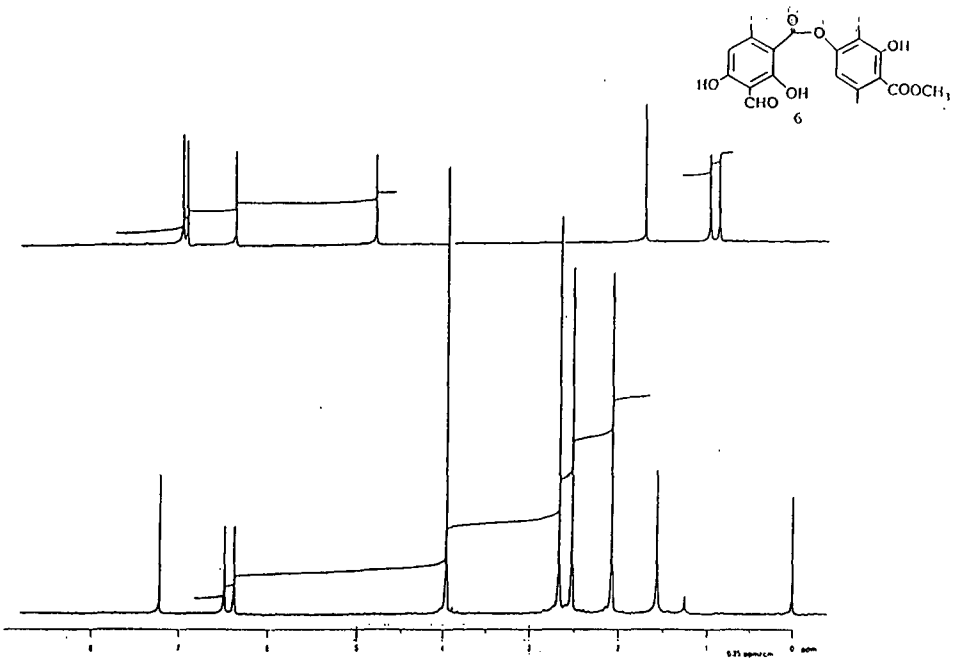


Figure 19

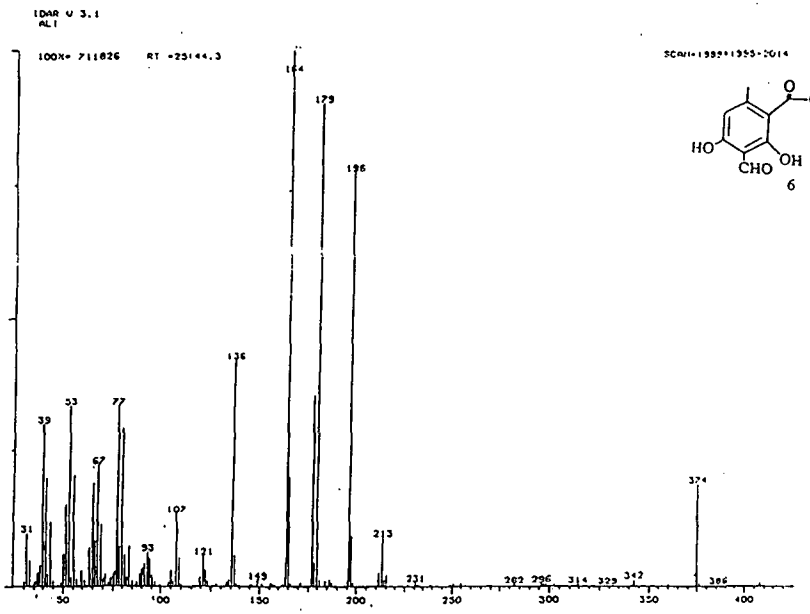
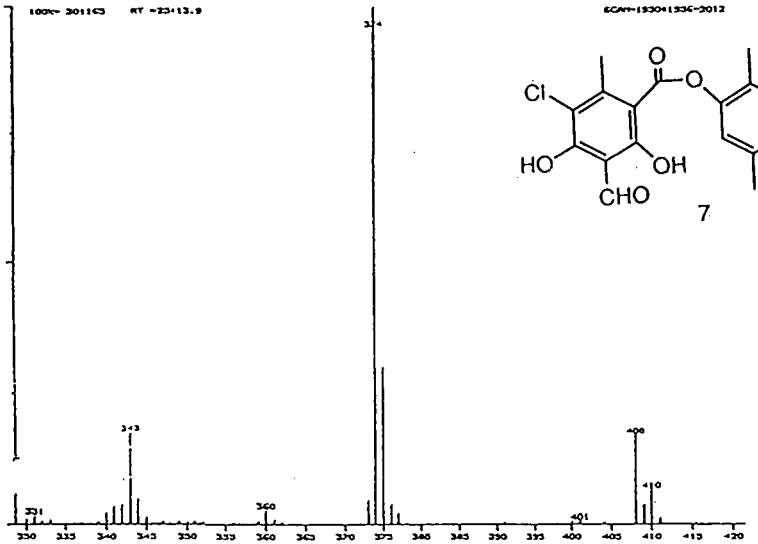


Figure 20



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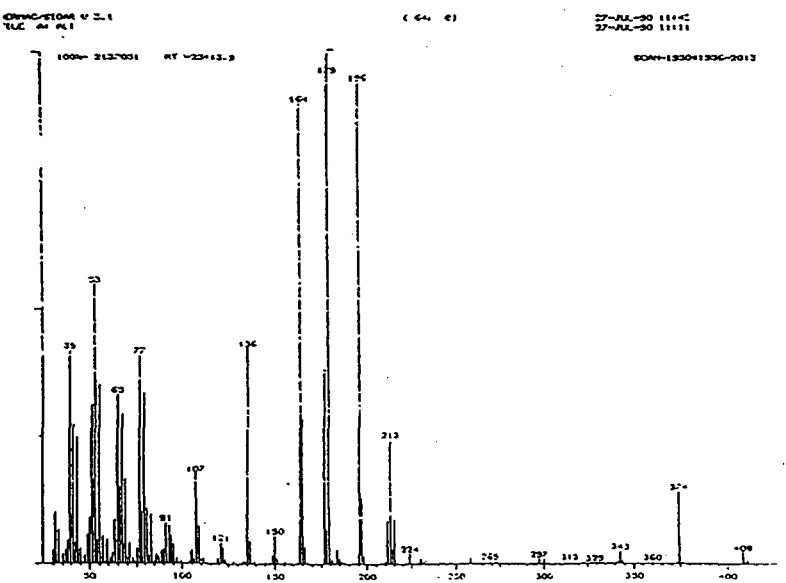
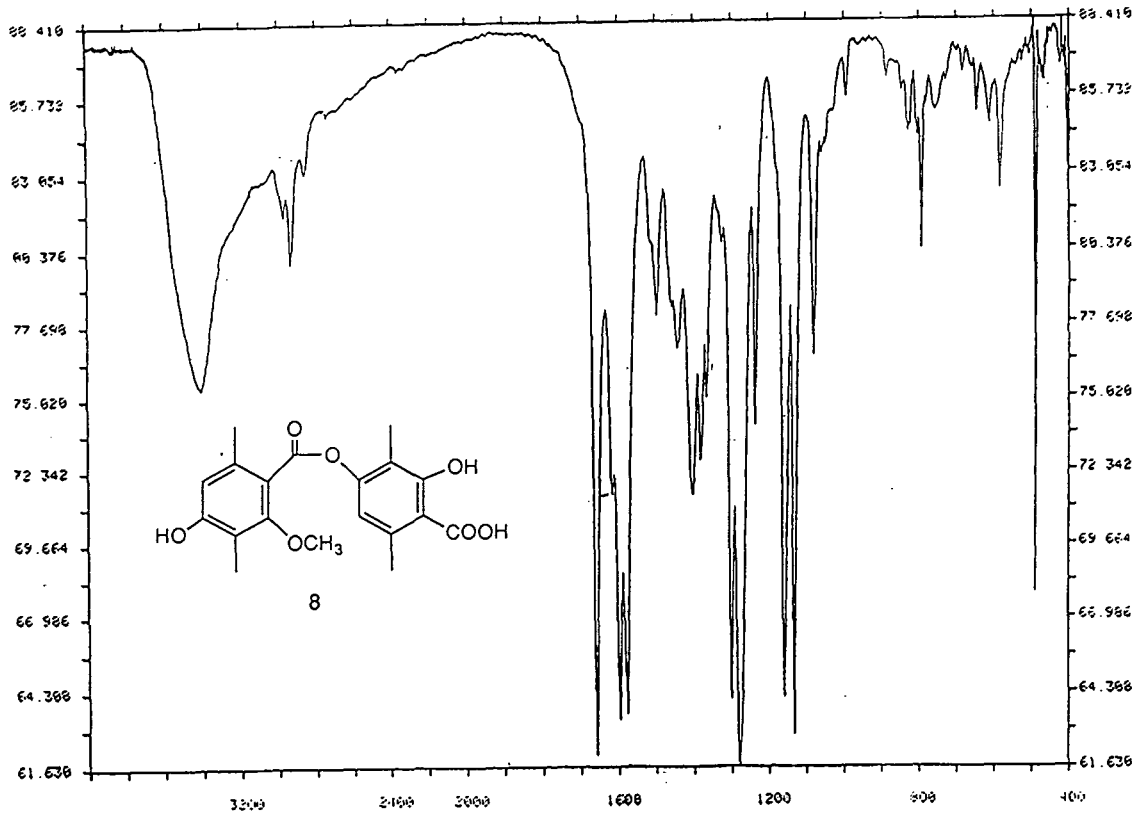


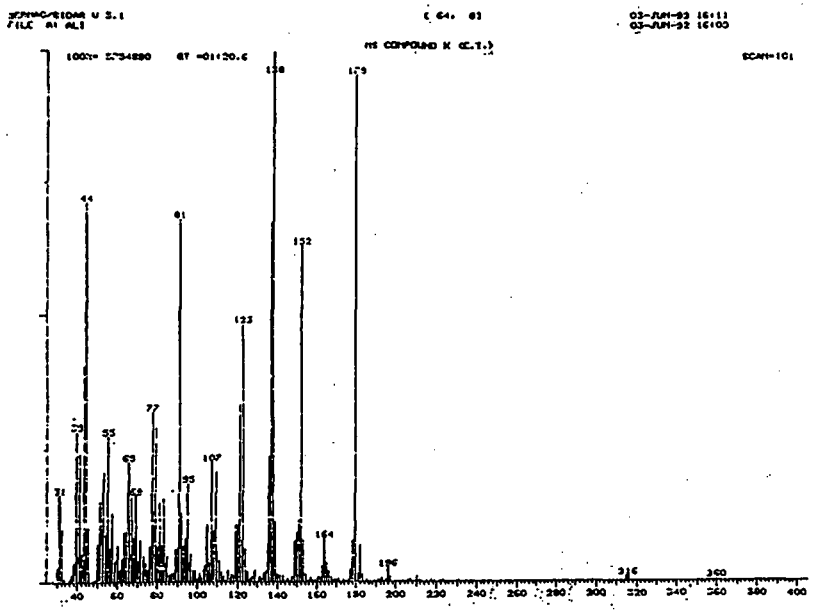
Fgure 21

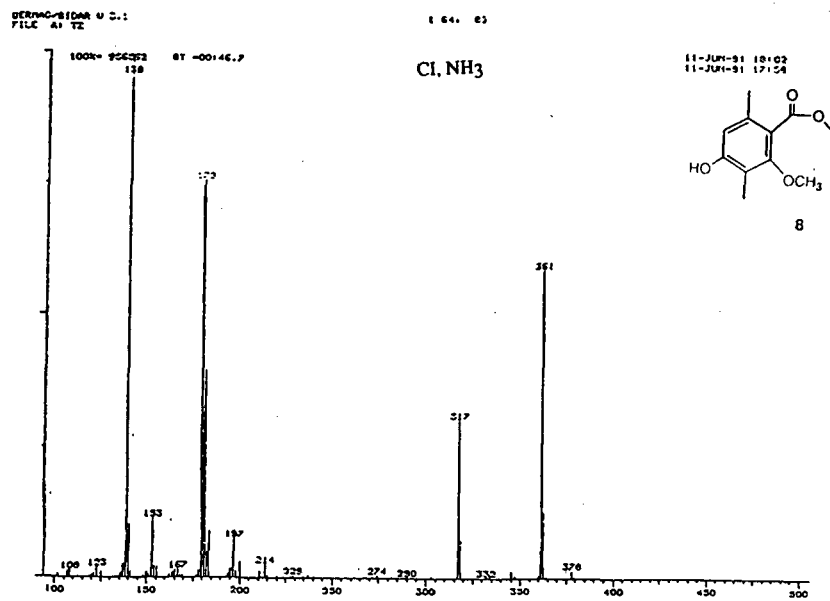


PEAK THRESHOLD = 0.50

CM-1	%T
3416.00	75.385
2974.00	81.620
2934.00	79.910
2853.00	83.160
1458.00	82.860
1617.00	71.465
1597.00	83.280
1577.00	83.515
1499.00	78.620
1445.00	76.785
1404.00	71.450
1383.00	73.675
1367.00	74.990
1351.00	64.860
1288.00	81.60
1238.00	73.95
1159.00	84.185
1132.00	82.730
1081.00	76.540
994.00	85.645
947.00	86.335
831.00	84.510
805.00	84.315
793.00	88.295
758.00	85.210
684.00	86.495
645.00	85.130
611.00	84.760
584.00	82.430
567.00	87.695
489.00	87.685
471.00	86.195
456.00	87.455
427.00	86.735
412.00	86.615

Figure 22





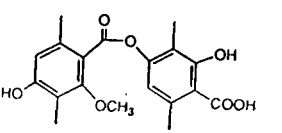


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Figure 23

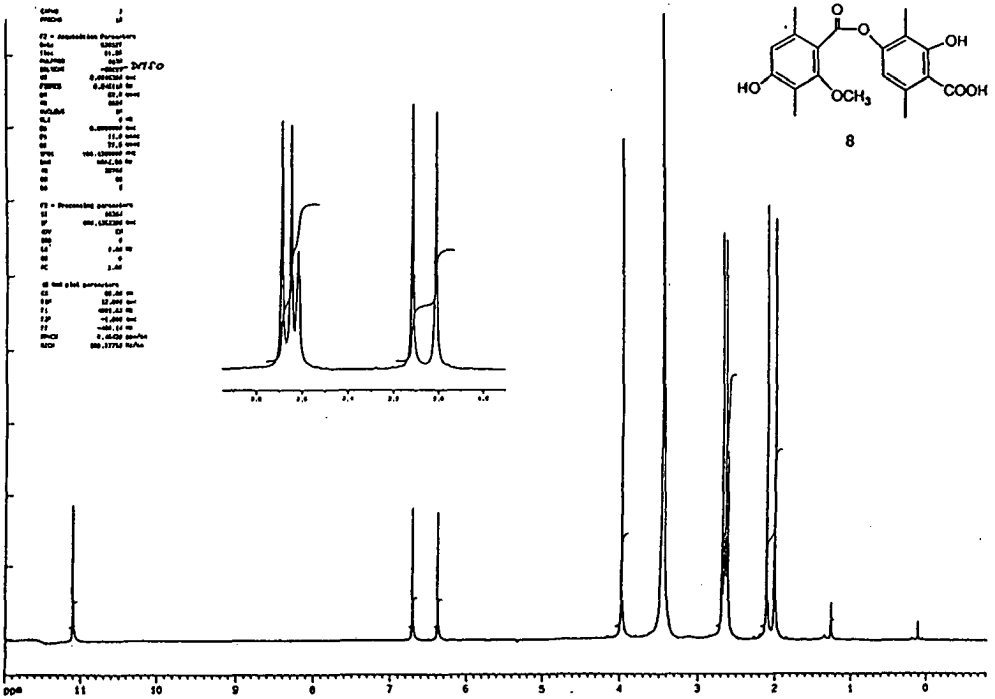


Figure 24

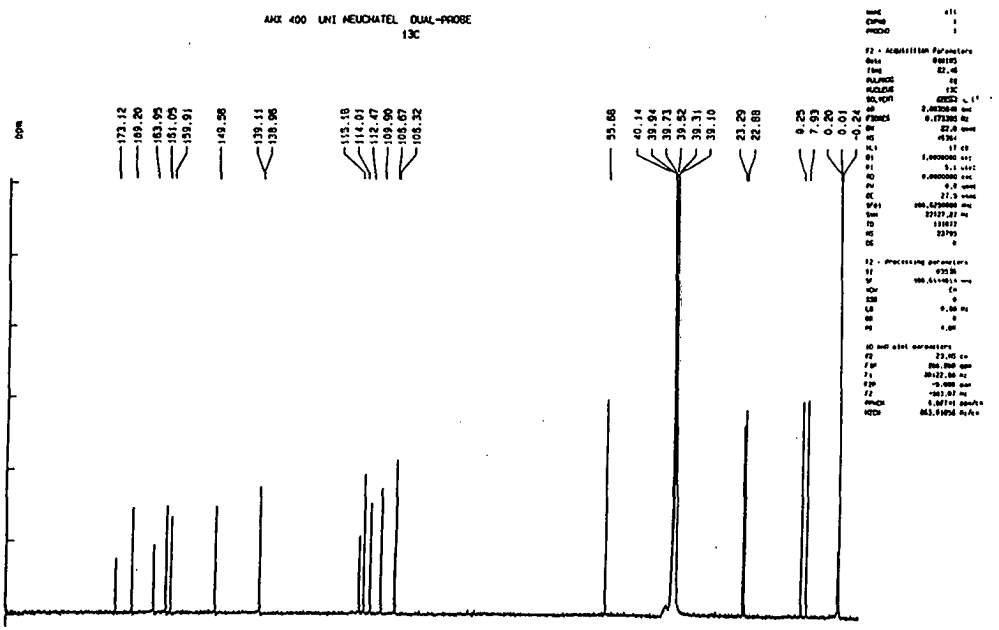


Figure 25

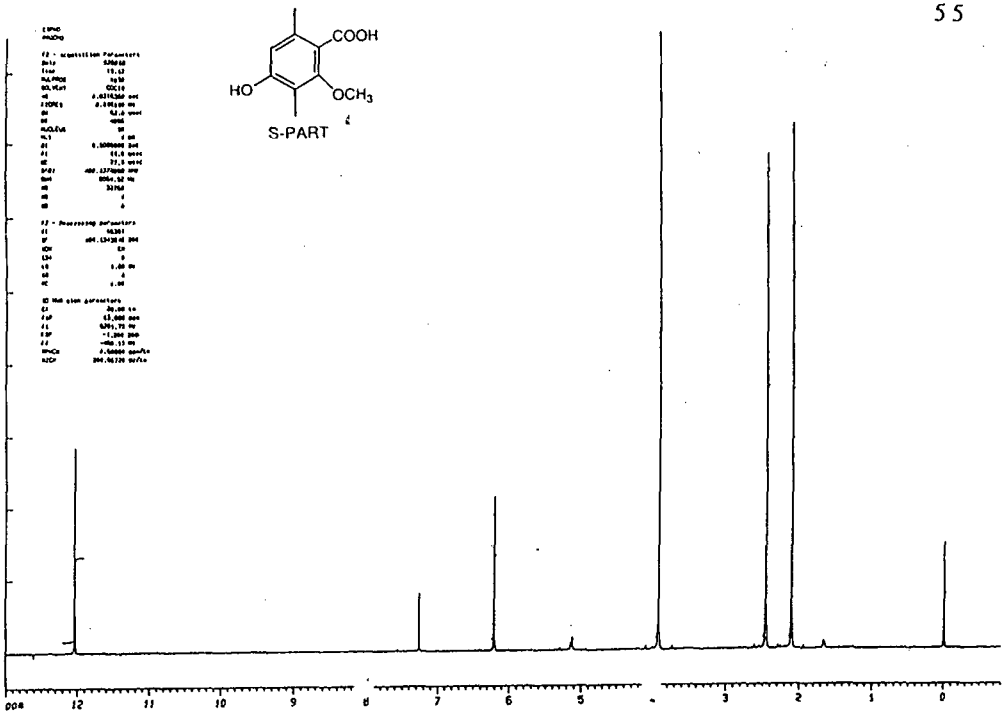


Figure 27

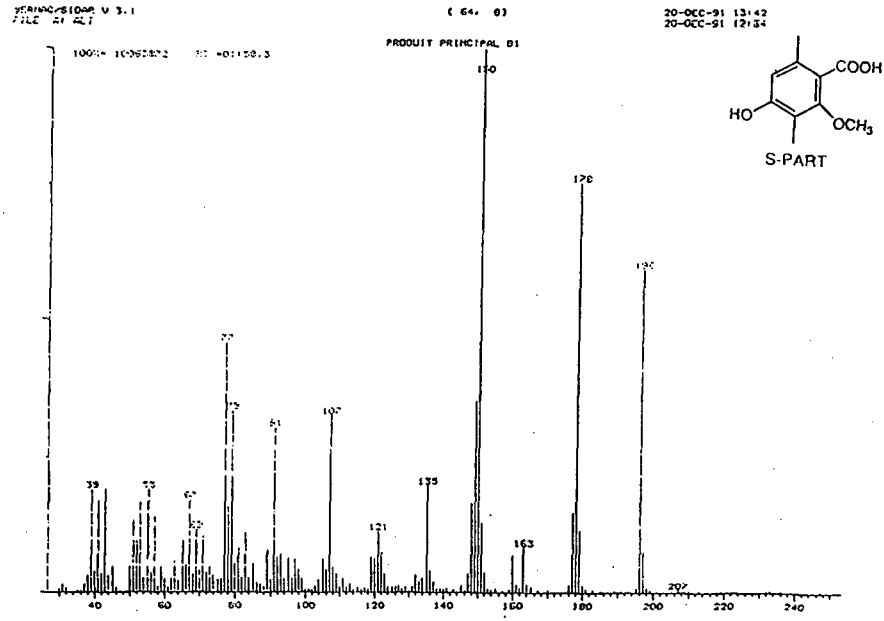


Figure 28

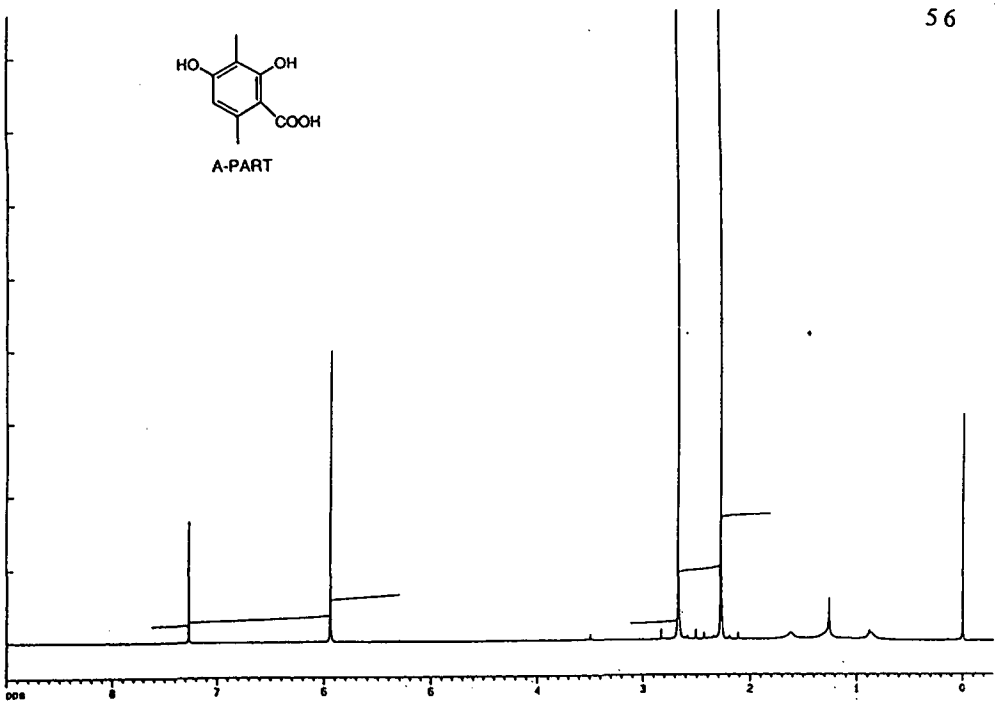


Figure 28

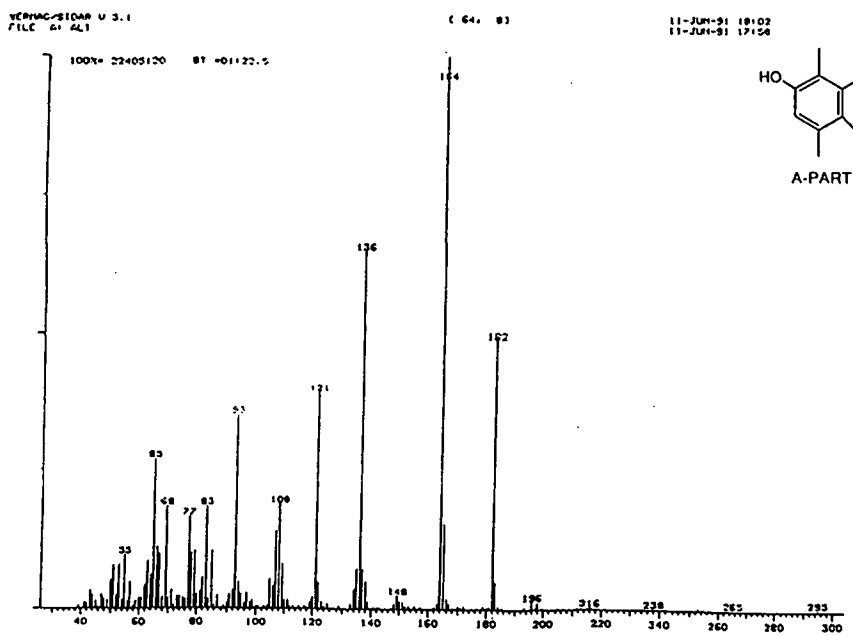
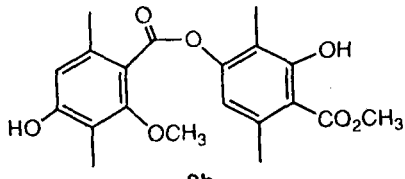
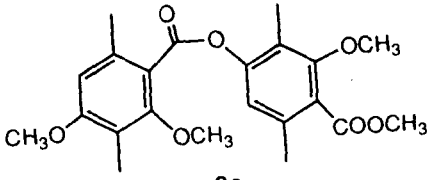


Figure 29



8b

Figure 30



8c

Figure 31

2.1.3.3 MONOPHENOLIC DERIVATIVES

The results on the identification of the following four monophenolic lichen components are discussed in this chapter and the structures of the compounds are presented at the end of this chapter.

RHIZONIC ACID (4-O- methyl- β– orcinol carboxylic acid) (9)

Compound **9** was isolated from the acid fraction of the lichen extract by silica gel column chromatography with dichloromethane as eluent. The yellow crystalline substance separating as fraction (F13/15) was collected (m.p. 208-210), (lit. 210°C) [10, 13]. It was identified as rhizonic acid on the basis of spectroscopic and chromatographic analyses.

Preliminary TLC analyses and the spectroscopic spectral evidence indicated the presence of a monophenolic derivative. In particular, the ^{1}H-NMR spectrum of compound **9** showed signals for the following substituent groups (Fig. 32) : two C-methyl group signals, one methoxy group resonance, one aromatic proton resonance and a lower shifted single proton resonance assigned to a hydroxyl group. The mass spectrum of **9** provided useful information concerning the structure of the proposed compound and showed certain characteristic fragment ion peaks and the molecular mass peak at m/z 196 (Fig. 33).

Analyses

^{1}H-NMR ($CDCl_3$ - 200 MHz) : 2.103 ppm (s, 3H, CH_3-Ar), 2.45 (s, 3H, CH_3-Ar), 3.93 (s, 3H, CH_3-O-Ar), 5.17 (s,br, 1H, H-O-Ar non H-bonded), 6.21 (s, 1H, H-Ar), 12.04 (s, 1H, HOOC-Ar).

Mass spectrum (m/z, I%) 197 (M^+, 20), 196 (M, 98), 165 (67), 164 (100), 163 (22), 137 (6), 136 (45), 122 (3), 121 (14), 108 (7), 93 (14), 77 (9), 65 (9), 51(6), 39 (8).

4-O-METHYLHAEMATOMMIC ACID (10)

Compound **10** was isolated from the NaOH extract by silica gel column chromatography with dichloromethane as eluent. The white crystalline substance which migrated as fraction F5/6 (0.0025%) was collected and purified on a column of Sephadex LH-20, (m.p. 112-114) [12]. Compound **10** was identified as 4-O-methylhaematommic acid (**10**) on the basis of the spectroscopic results presented below.

Both the ^{1}H-NMR and MS spectra indicated the presence of a monophenolic derivative [12]. The ^{1}H-NMR spectrum showed the signals of the following substituent groups: one C-methyl group resonance, one methoxyl group resonance, one aromatic proton resonance and one proton resonance, attributed to an aldehyde group, together with two hydroxyl group signals (Fig. 34). The mass spectrum was consistent with the proposed structure and showed the molecular mass ion peak at m/z 210 together with certain characteristic fragment peaks such as m/z 182, 179, and 177 (fig. 35).

Analyses

^{1}H-NMR ($CDCl_3$ - 200 MHz) : 2.53 ppm (s,3H, CH_3-Ar), 3.96 (s, 3H, CH_3-O-Ar), 6.29 (s,1H,H-Ar), 10.34 (s,1H, Ar-CHO), 12.41 and 12.88 (s, 1H each, H-O-Ar and Ar-COOH).

Mass spectrum (m/z, I%) 210 (M, 66.5), 182 (20), 179 (16), 178 (34, 177 (14), 165 (4), 151 (13), 150 (100), 122 (31), 121 (9), 108 (4), 94 (24), 81(8), 77 (12), 69 (15), 65 (20), 53 (16), 43 (10), 39 (12).

METHYL EVERNINATE (Methyl 2- hydroxy-4-methoxy-6-methylbenzoate) (**11**)

Compound **11** was present in both the neutral and the acid extracts. It was isolated by silica gel column chromatography with dichloromethane as eluent. The crystalline substance migrating as fraction F7, (m.p. 66-68), (lit. 67-68°C) [13], was collected and identified as methyleverninate. The spectroscopic analyses of **11** indicated the presence of a monophenolic derivative. In particular, the ^{1}H-NMR spectrum showed prominent signals for the following substituent groups: one C-methyl group, two methoxyl groups, two aromatic protons and a hydroxyl group (Fig 36).

The mass spectrum was likewise indicative of the presence of a monophenolic derivative, revealing characteristic phenolic compound fragment ion peaks and a parent peak at m/z 196 (Fig. 37). The data presented here are consistent with the presence of the proposed compound. Methyl everninate is a monophenolic compound that occurs freely in many lichens [13,15].

Analyses

^{1}H-NMR ($CDCl_3$ - 200 MHz) : 2.5 ppm (s, 3H, CH_3-Ar), 3.83 (s, 3H,CH_3-O-Ar), 3.96 (s, 3H, CH_3-OOC- Ar), 6.32 (s,1H, H-Ar), 6.38 (s, 1H, H-Ar), 11.78 (s, 1H, H-O-Ar).

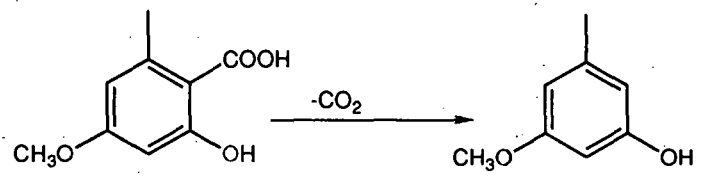
Mass spectrum (m/z, 1%) 197 (M^+; 69), 196 (M, 42), 165 (19), 164 (63), 163 (6), 137 (9), 136 (100), 135 (12), 121 (5), 108 (23), 107 (26), 91(6), 79 (23), 77 (20), 67 (6), 53 (13), 43 (6).

ORCINOLMONOMETHYL ETHER (12)

Compound **12** was isolated from the NaOH extract by silica gel column chromatography eluted by dichloromethane. The yellow substance, separating as fraction F16/17, (m.p 127-128°C) [17], was collected and identified as orcinol monomethyl ether on the basis of its spectroscopic analyses. Both the chromatographic and spectroscopic analyses provided useful information concerning the structure of **12.**

In particular, the ^{1}H-NMR spectrum was indicative and showed signals for the following substituent groups: one C-methyl group, one methoxy group, three aromatic protons and one hydroxyl group signals, (Fig. 38). The mass spectrum of **12** (Fig. 39) indicated the presence of a monophenolic structure. A parent peak was observed at m/z 138 corresponding to the molar mass of the proposed structure. Both the MS and ^{1}H-NMR spectral evidence were consistent with the presence of orcinol monomethyl ether.

Compound **12** exists in the majority of lichen materials as a free component [15], but it could have resulted after the decarboxylation of a monophenolic derivative such as β–orcinol carboxylic acid.



β–ORCINOL CARBOXYLIC ACID

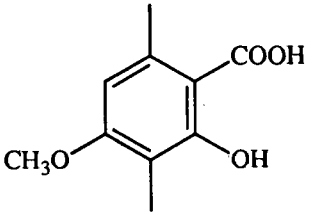
ORCINOL MONOMETHYL ETHER

Analyses

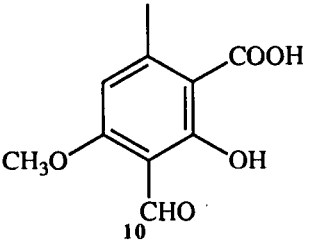
^{1}H-NMR ($CDCl_3$ - 200 MHz) : 2.25 ppm (s,3H, CH_3-Ar), 3.82 (s,3H, CH_3-O-Ar), 5.21 (s; br, 1H non H-bonded H-O-Ar), 6.27, 6.30, 6.35 (s,1H each, 3H-Ar).

Mass spectrum (m/z, 1%) 139 (M^+, 13), 138 (M, 100), 137 (9), 123 (6), 110(28), 109 (28), 107 (27), 95 (9), 79 (10), 77 (12), 69 (8), 67 (17), 55 (10), 43 (5), 39 (9).

Below are presented the structures of the four monophenolic derivatives identified.



9



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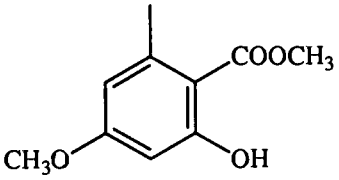
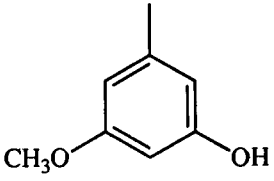


11



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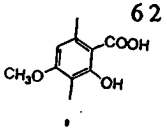


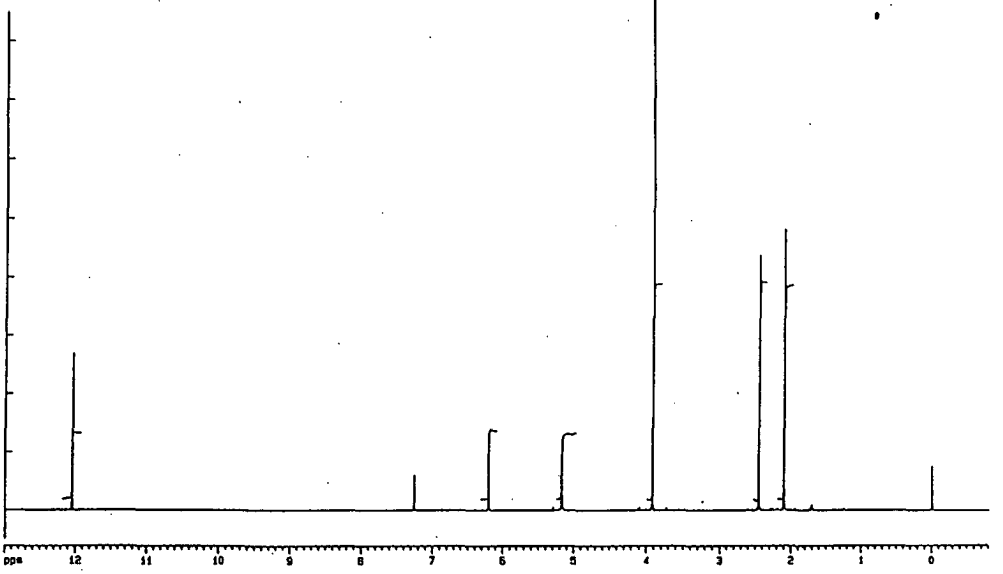


Figure 32

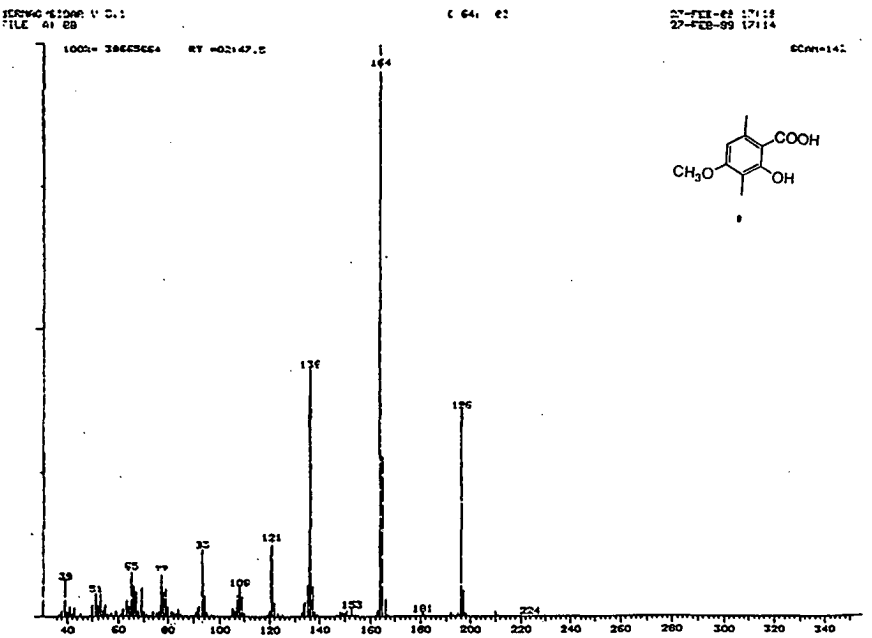


Figure 33

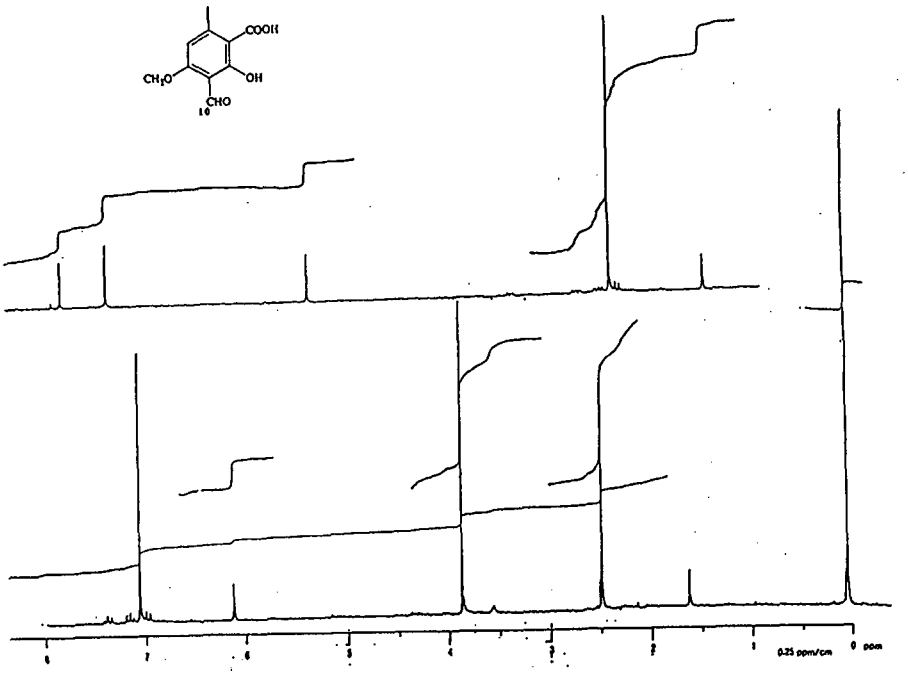


Figure 34

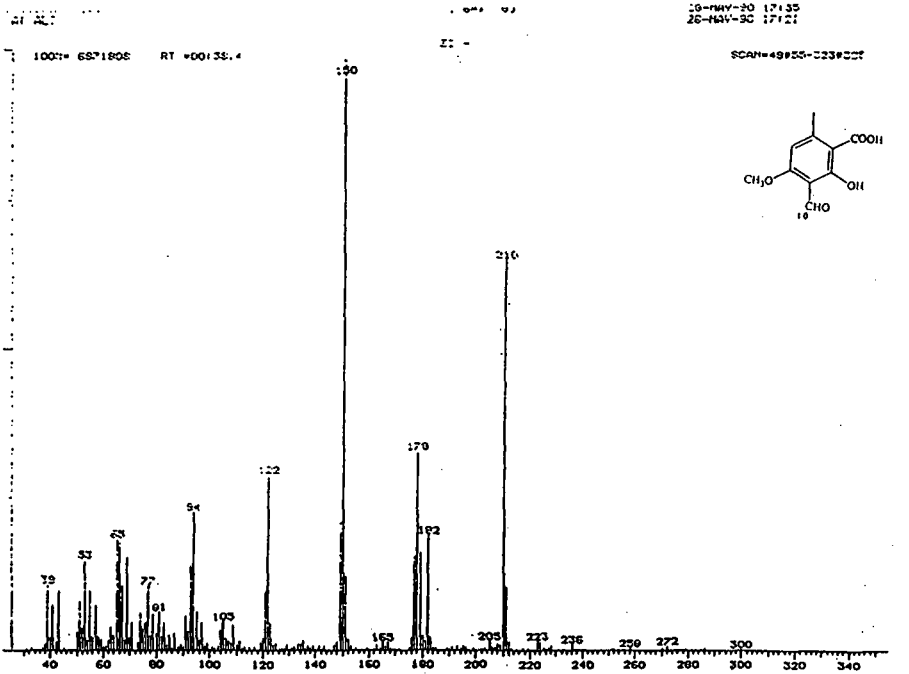


Figure 35

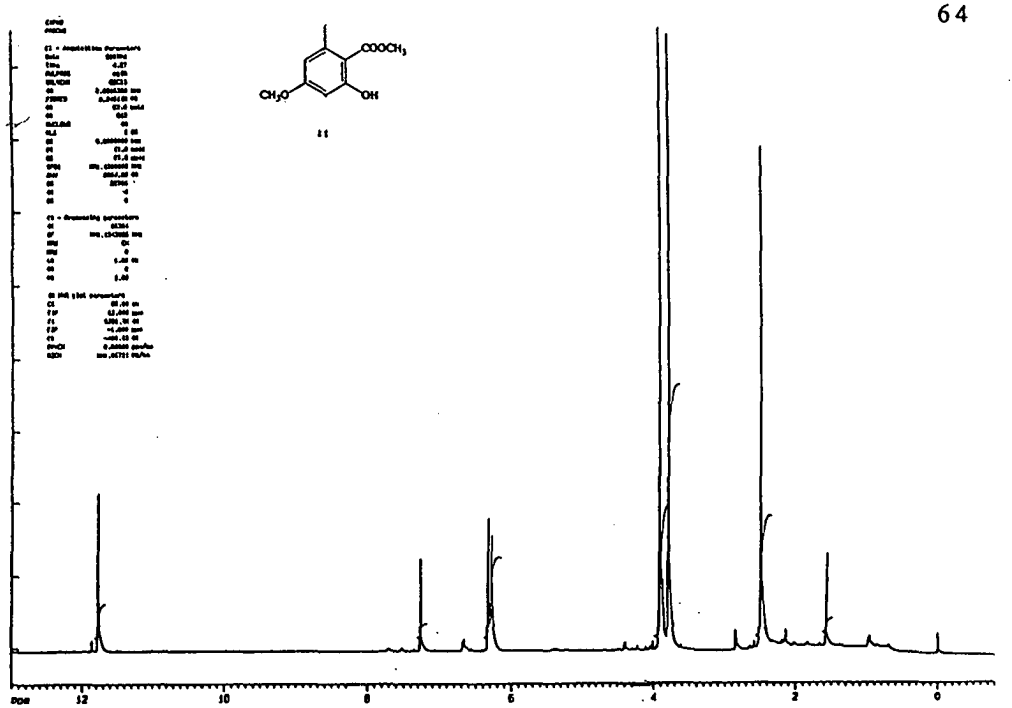


Figure 36

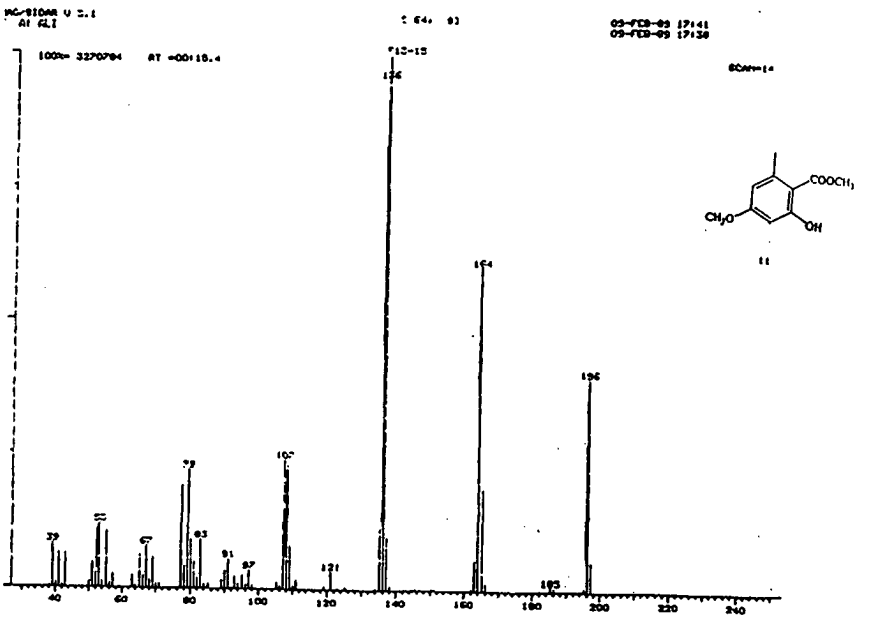


Figure 37

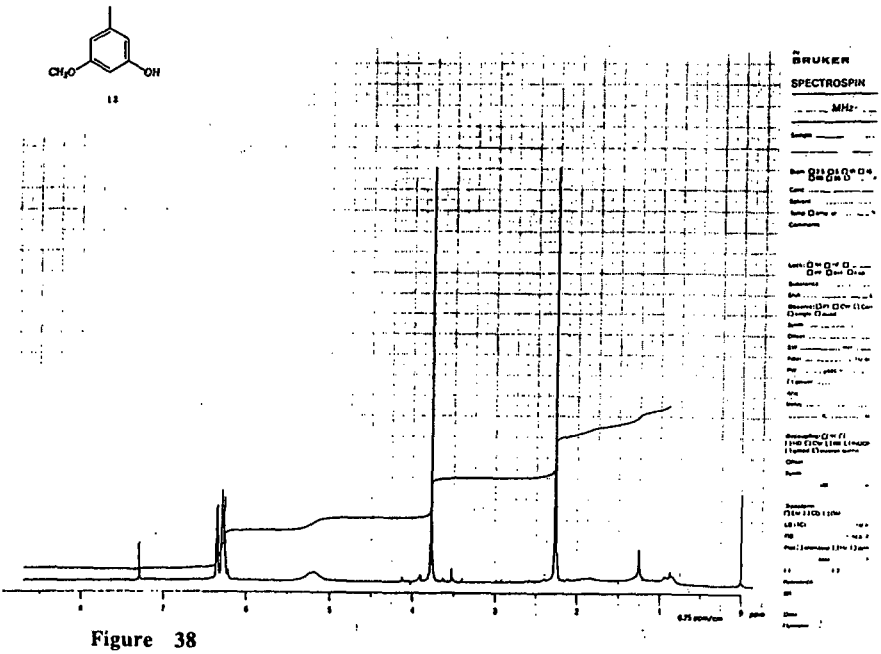


Figure 38

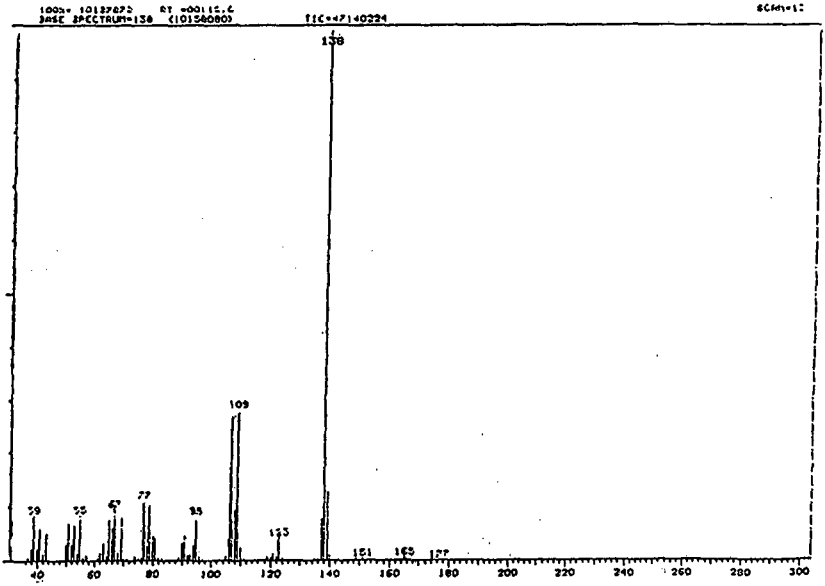


Figure 39

2.1.3.4 COMPONENTS OF THE NEUTRAL PART

The following triterpenes and sterols were isolated and identified from the neutral extract of the lichen under investigation (Fig. 53).

I- Triterpenes: lupeol (**13**), and tylolupenyl acetate (**14**).
II- Sterols: ergosterol peroxide (**15**), ergosterol (**16**), lanosterol (**17**), and cholesterol (**18**).

III- Two more sterol compounds were detected by TLC and MS but were not fully identified. These are compound **19** and compound **20.**
The spectra for the above listed compounds are presented at the end of this chapter.

LUPEOL (13)

Compound **13** was isolated from the neutral extract of the lichen under investigation. It was separated by silica gel column chromatography and preparative TLC, then purified on a column of Sephadex LH-20. A very small amount of **13** was isolated from the lichen (35mg). The isolated white crystalline substance **13** (m.p. 273-274), lit. (m.p. 274), [19],was identified as Lupeol (**13**) on the basis of its chromatographic and spectroscopic studies [1].

The ^{1}H-NMR spectrum (Fig. 40) revealed sharp signals as singlets and doublets between 0.73 ppm and 1.62 ppm, corresponding to secondary and tertiary methyl group resonances. The singlet signal which appeared at 1.62 ppm was assigned to the methyl group attached to the isopropylene side chain. The other methyl group signals appeared at relatively higher field and fell within a small chemical shift. The two broad doublets which appeared at 4.56 - 4.68 ppm, of relative intensity of one proton each, indicated the presence of two olefinic protons, corresponding to the side chain vinyl proton resonances. The two vinylic protons appeared at relatively higher fields as two broad doublets instead of one singlet as expected. This is probably due to a long range coupling of the olefinic protons to the proton at C-19. This information indicated the location of the double bond at C-29. The multiplet centered at 3.25 ppm, was assigned to the α-proton adjacent to the 3β- hydroxyl group. The ^{1}H-NMR spectrum also showed a multiplet at 2.25 ppm, with relative intensity of one proton. This was assigned to the C-18 proton.
The mass spectrum of **13**, provided useful information and showed a molecular ion peak at m/z 426, and several typical triterpenoid characteristic peaks were also observed (Fig. 41). The fragments at m/z 234 and 207 derived from the characteristic triterpenoid cleavage at ring C of the parent molecule, and the ions at m/z 191 and 189 [234- isopropylene group]$^+$, strongly

confirmed the presence of **13.** The peak at m/z 203 [234 -CH3 -OH]$^+$ also pinpointed the location of the hydroxyl group.

The final confirmation of the presence of **13** was obtained from the comparison of the results presented here with those of the literature [72,73].

Analyses

^{1}H-NMR spectrum ($CDCl_3$- 200 MHz) : 0.73, 0.85, 0.90, 0.925, 0.98, 1.05, 1.08 ppm (s, 3H each, 6 x CH_3), 1.62 (S, 3H, vinylic CH_3, C-30), 1.25- 1.375 (m, 23H, methylene and methine protons), 2.25 (m,1H, H-C-18), 3.25 (m, 1H, H-C-3), 4.56- 4.68 ppm (s,1H each,-C=CH_2, C-29).

Mass spectrum (m/z,I%) 427 (M^+, 21), 426 (M, 74), 411 (28), 393(7), 383 (6), 315 (17), 313 (6), 301 (3), 300 (6), 297 (9), 273 (4), 272 (6), 271 (4), 257 (17), 247 (11), 235 (5), 234 (21), 229 (18), 220 (15), 218 (86), 207 (77), 205 (31), 204 (50), 203 (53), 191 (42), 190 (45), 189 (100), 175 59), 163 (36), 161(55), 151(18).

TYLOLUPENYL ACETATE (**14**)

Compound **14** occurred admixed with lupeol (**13**). This was detected by TLC which showed the presence of two overlapping spots of different concentrations. Compound **14** was separated from the mixture by reverse phase HPLC (1.2mg). However it was not possible to isolate a sufficient amount for ^{1}H-NMR analysis.

This was identified as tylolupenyl acetate (**14**) by mass spectral analysis. There is a great similarity between the mass spectrum of Lupeol (**13**) and that of tylolupenyl acetate and other structurally related triterpenoids. The mass spectrum of **14** showed the molecular mass ion peak at m/z 468, and the base peak at m/z 419/418 (M - 60- acetate) and other typical triterpenoid fragment ion peaks such as m/z 410/409, 393, 313, 257, 207, 205, 191, 189, (fig. 42).

In general the acetoxy derivative of triterpenoids show stronger substituent elimination than the hydroxyl group [18]. The driving force for the acetic acid elimination is due to rotation of the CH_3-COO group, with the carbonyl oxygen being the hydrogen acceptor [72]. The prominent peak at m/z 393 resulted from the loss of acetate and methyl groups 393 [M^+ - acetate and methyl (60 + 15)], and the peak at m/z 367 resulted from the loss of acetate and propyl groups 367 [M^+ - acetate and C_3H_7 group]. The peaks appearing at m/z 207 and 205 resulted from ring A and ring B fragments. These were derived from the characteristic triterpenoid fragmentation of the parent molecule. Subsequent loss of water from the peak at m/z 207 led to the formation of fragment ions at m/z 189/191. The prominent peak at m/z 213 indicated the presence of an isopropyl group 213

(257 - isopropyl group). This provided important information concerning the presence of a side chain and the overall structure of the compound in question. The results presented here are in agreement with the literature[74, 75]. and confirmed the presence of tylolupenyl acetate (**14**)

Analyses

Mass spectrum (m/z, I%) 469 (M^+, 12), 468 (M, 36), 453 (31), 441 (20), 423 (28), 410 (47), 409 (100), 393 (22), 367 (8), 313 (5), 301 (14), 287 (24), 273 (21), 271 (11), 257 (14), 241 (14), 229 (9), 219 (11), 207 (15), 205 (15), 204 (12), 191 (20), 189 18), 175 (9), 149 (11), 135 (17), 123 (17),121 (21), 109 (28),107 (22).

ERGOSTEROL PEROXIDE (5α,8α,- epidioxy- 5α-ergosta- 6, 22-dien-3-β-ol) (**15**)

The presence of compound **15** in the neutral part of the lichen extract was detected by preliminary TLC anlyses. This compound was separated from the neutral fraction by silica gel column chromatography with a hexane and ethyl acetate (8:2) mixture as eluent. The white crystalline substance migrating as F27/28 (85mg), was collected, (m.p. 180-182), (lit. 182), [1, 12]. It was identified as egosterol peroxide on the basis of chromatographic and spectroscopic data.

The spectroscopic analyses indicated the presence of a sterol. In the mass spectra of all the epidioxy sterols, an intense peak was observed due to the loss of O_2 from the molecular ion, persumably by a retro Diels-Alder type of fragmentation [41]. In the MS of **15,** the resulting diene from the epidioxy sterol showed fragmentations characteristic of Δ5,7 sterol. Hence, the intense fragment peak appearing at m/z 396 corresponded to the loss of an oxygen molecule (O_2). It was assumed that the loss of the oxygen molecule could have been derived from a sterol peroxide, since the ^{1}H-NMR spectrum (Fig. 43) of **15** did not reveal the presence of a methoxyl group [41]. The M S exhibited further typical sterol fragmentation patterns such as the fragment peaks at m/z 363, 271, 253 and 213 (Fig. 44).

Supporting evidence for the proposed structure of **15** came from the ^{1}H-NMR studies. This was particularly informative and compatible with the structure of ergosterol peroxide. The AB system centered at 6.25 and 6.52 ppm, corresponded to the signals of the double bond protons at Δ6,7. The methyl group signals appeared between 0.81 and 1.01 ppm. The proton attached at the α–position to the 3β–hydroxyl group gave a characteristic signal at 3.98 ppm. The ^{1}H-NMR spectrum was also useful in establishing the nature of the side chain present in this epidioxy sterol. The C(22) and C(23) olefinic proton signals appeared at 5.21 ppm, as multiplets, and the signals of C(21), C(26) and C(27) methyl protons appeared as doublets in a relatively lower region. The signal due to the C(20) proton appeared at 2.26 ppm as a broad triplet clearly separated from the methylene and methine envelope.

Final confirmation of the presence of ergosterol peroxide (**15**) was obtained from the comparison of the data presented here and that of the literature [73, 76].

Analyses

^{1}H-NMR ($CDCl_3$- 200 MHz) : 0.81 ppm (s, 3H, CH_3, C-18), 0.83 (S, 3H, CH_3, C(19)), 0.88 (d, 3H, CH_3, C(21)), 0.91 (d, 3H, CH_3, C(28)), 0.99 -1.01(d, 6H, $2CH_3$, C(26) and C(27)), 103- 210 (m, 20H, 7xCH_2 + 6CH, methylene and methine groups), 2.26 (br, t, 1H, H-C(20)), 3.98 (m, 1H, H-(C3)), 5.21(m, 2H, C-22 and C-23 protons), 6.24 (d, 1H, H-C-6), 6.52 (d, 1H, H-C-7).

Mass spectrum (m/z, I%) 428 (M, 4), 410 (4), 397 (5), 396 (52), 378 (4), 363 (20), 339 (3), 337 (11), 301 (3), 271 (11) 253 (21), 251 (17), 239 (7), 231 (8), 198 (7), 188 (5), 171 (7), 155 (14), 149 (3), 128 (14), 121 (16), 109 (36) 108 (32), 97 (37), 95 (53), 81 (69), 77 (41), 69 (100), 56 (50), 55 (100), 43 (79).

ERGOSTEROL (24- METHYL- CHOLEST-5, 7,-22-TRIEN- 3β–OL) (**16**)

The presence of **16** was detected by TLC analyses and was isolated admixed with ergosterol peroxide (**15**). It was separated from the mixture by silica gel column chromatography and by reverse phase HPLC (36mg), (m.p. 145-147°C), (lit. 147°C) [67, 1].

This was identified as ergosterol (**16**) on the basis of its spectroscopic analyses presented below. The spectroscopic data obtained are consistent with the presence of a sterol substance. The mass spectrum revealed a typical sterol fragmentation. Of particular interest is the presence of the characteristic peaks at m/z 367, 253, 251, 235, 213 and the molecular mass ion peak observed at m/z 396 (Fig. 46).

The ^{1}H-NMR spectrum (Fig. 45) of **16** was particularly informative and compatible with the proposed structure. The sharp signals which appeared at 0.82 - 1.06 ppm were assigned to the angular and side chain methyl groups. The doublet signal which appeared at 1.05-108 ppm corresponded to the two methyl groups attached to the terminal side chain. The broad envelope at 1.22 -2.07 ppm was attributed to the methylene and methine groups. The mutiplet centred at 3.63ppm was assigned to the α-proton adjacent to the 3β-hydroxyl group. The AB system centered at 5.39 and 5.57 ppm corresponded to the C(6) and C(7) protons of the dien group. The ^{1}H-NMR spectrum was also useful in establishing the nature of the side chain present in the sterol **16.** The C(22) and C(23) olefinic proton signals appeared at 5.17-5.21 ppm as multiplets and C(20) proton resonated at 2.26 ppm as a multiplet.

The spectroscopic results of **16** presented here are very similar to those of ergosterol peroxide (**15**) and of other related compounds of the sterol family [77]. This confirmed the presence of the proposed structure.

Analyses

^{1}H-NMR ($CDCl_3$) : 0.82 ppm (s, 3H, CH_3-C-18), 0.84 (s, 3H, CH_3-C-19), 0.86 (d, 3H, CH_3-C-21), 0.91-0.94 (d, 6H, CH_3-C-26 and CH_3-C-27), 1.04 (s, 3H, CH_3-C-28), 1.22- 2.07 (m, 21H, 7 x CH_2 + 6H, methylene and methine envelope), 2.26 (m, 1H, H-C-20)), 3.63 (m, 1H, H-C-3), 5.17-5.21 (m, 2H, C-22 and C-23 protons), 5.39 (dd, 1H, H-C-6) 5.57 (dd, 1H, H-C-7).

Mass Spectrum (m/z, 1%) 397 (M^+, 2), 396 (M, 52), 363 (14), 337 (8), 313 (2), 311(4), 300 (2), 299 (7), 271 (3), 269 (6), 253 (7), 251 (28), 247 (6), 243 (5), 235 (9), 227 (11), 222 (11), 221 (7), 217 (10), 213 (18),197 (13), 189 (15), 188 (15), 169 (11), 155 (6), 149 (3), 147 (11), 135 (9), 121 (13), 109 (17), 97 (24), 91)32), 81 (57), 71 (23), 69 (42), 67 (48), 53 (100), 43 (37).

LANOSTEROL (17)

Compound **17** was obtained from the neutral part of the lichen extract. It was detected by TLC analyses and isolated by silica gel column chromatography with hexane and ethyl acetate (8:2) as eluent. The substance migrating as fraction F13/15, (8mg) was collected and identified as lanosterol (**17**) on the basis of the spectroscopic data presented below.

The ^{1}H-NMR spectrum (Fig. 47) of **17** was very informative and particularly useful in establishing the presence of the unsaturated side chain. The signals due to the methyl groups were observed at 0.70-1.20 ppm. The angular methyl groups appeared at higher field region. The two methyl groups adjacent to the terminal side chain double bond, appeared at a lower field (2.10 -2.17 ppm). The broad envelope which appeared at 1.12 - 1.65 ppm, was assigned to the methylene and methine group resonances. The sharp signal appearing at 3.95 ppm,was assigned to the α-proton adjacent to the 3β- hydroxyl group. The singlet resonance appearing at 6.25 ppm was assigned to the vinylic proton at the side chain.

The mass spectrum (Fig. 48) was likewise useful and consistent with the structure of **17.** This showed a typical sterol fragmentation pattern such as m/z 411, 393, 271, 205, 191, and the molecular mass peak at m/z 426 . Finally the comparison of the results presented here with those of the literature [67, 87] strongly confirmed the presence of Lanosterol (**17**).

Analyses

The ^{1}H-NMR ($CDCl_3$- 200 MHz) : 0.70 ppm (s, 3H, CH_3, C-13), 0.82 (s, 3H, CH_3, C-20), 0.92 (d, 3H, CH_3, C-14), 1.10 (s, 3H, CH_3, C-18), 1.15 (s, 3H, CH_3, C-19), 1.17 (s, 3H, CH_3, C-10), 2.10- 2.17 ppm (d, 6H, $2CH_3$, C-26, 27). 1.225- 1.65 ppm (m, 23H, methylene and methine groups), 3.95 (s, 1H, H-C-3), 6.25 (s, 1H, H-C-24).

Mass spectrum (m/z, I%) 427 (M^+, 6), 426 (M, 28), 393 (7), 383 (7), 355 (2), 313 (6), 301 (3), 299 (2), 287 (8), 271 (6), 269 (16), 257 (11), 256 (11), 255 (16), 229 (14), 218 (33), 207 (20), 205 (34), 203 (38), 191 (37), 189 (41), 175 (25), 161 (30), 149 (28), 136 (67), 120 (61), 109 (68), 107 (81), 96 (100), 81 (83), 79 (49) 69 (68), 55 (74), 43 (85).

CHOLESTEROL (18)

The presence of cholesterol (**18**) in the neutral part of the extract was detected by TLC analyses. It occurred admixed with several sterol components. It was difficult to isolate enough pure amounts of the individual components in the extract for spectroscopic analyses, due to very low concentration.

The mass spectral evidence for the sample showed a mixture of **18** and some sterol components, such as stigmasterol (**19**) and β–sitosterol (**20**) . However, the MS of the sample mixture indicated that **18** was the major component.

About 2 mg of compuond **18** were separated after many attempts to isolate enough material for spectroscopic analyses. It was identified by ^{1}H-NMR and mass spectral evidence (Fig. 49, 50) as cholesterol (**18**).
The ^{1}H-NMR spectrum (Fig. 49) revealed typical sterol signals although not well resolved. The signal appearing at 3.52 ppm was assigned to the C(3) α-proton. The multiplet at 5.33 ppm was attributed to the olefinic proton at C(6). The singlet signals which appeared at higher field, around 0.79-0.95 ppm, indicated the presence of tertiary and secondary methyl groups. The multiplet which appeared at 2.25 ppm was assigned to the C(20) proton resonance. The broad envelope centred at 1.01- 1.99 ppm showed the methylene and methine signals.

The mass spectrum of **18** (Fig. 50) revealed certain sterol characteristic fragmentation peaks, such as m/z 368, 355, 273, and 213. In the cholesterol mass spectrum, the molecular mass peak at m/z 386 is not always observed, or appears as a very weak peak, due to the easy loss of water from the parent molecule [79].
Hence the loss of water from the parent molecule is the result of the formation of the peak at m/z 368. Loss of water and a methyl radical led to the formation of the peak at m/z 355, and loss of the methyl radical from the parent molecule led to the formation of the fragment peak at m/z 371. The peaks appearing at m/z 284 / 285 indicated the possible cleavage of the bond at C(20)- C(22).

Analyses

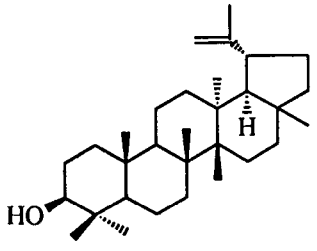
^{1}H-NMR ($CDCl_3$ - 400 MHz) : 0.79, 0.825, 0.85, 0.90 and 0.95 ppm (m, 5 methyl group signals), 1.01-1.99 ppm (br., m, methylene and methine group signals), 2.27 (m, 1H, H-C-20), 3.52 (br., d, 1H, H-C-3), 5.33 (m, 1H, vinylic proton at C-6).

Mass spectrum (m /z, 1%) m/z 386 (M, 4), 371 (6), 356 (5, 355 (4), 344 (17), 315 (7), 301 (5), 300 (3), 285 (10), 284 (23), 275 (10), 273 (14), 259 (27), 256 (31), 255 (7), 250 (58), 247 (13), 233 (44), 219 (22), 218 (22), 217 (38), 213 (20), 207 (15), 205 (17), 203 (22), 191 (31), 189 (20), 177 (30), 167 (36), 163 (40), 153 (100), 151 (98).

STIGMASTEROL (19), AND β–SITOSTEROL (20)

The presence of the sterols **19** and **20** in the mixture was detected by TLC and identified by mass spectral evidence. They occurred admixed with cholesterol and other sterols. The mass spectral evidence of the sample mixture indicated the presence of some sterol components.

The appearence of certain sterol characteristic fragment peaks in the MS (Fig. 51, 52), such as the peak at m/z 368, showed that cholesterol is the main component in the mixture. Those at m/z 412 and 414, showed the existence of stigmasterol (**19**) and β–sitosterol (**20**), respectively . Perhaps the most common characteristic peak of the steroids (Δ5) is m/z 247, which is present in most sterols. Other contributing fragments are m/z 313, 257, 217 and 213.



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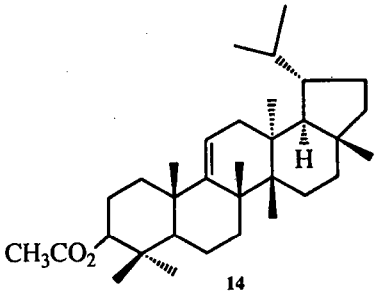

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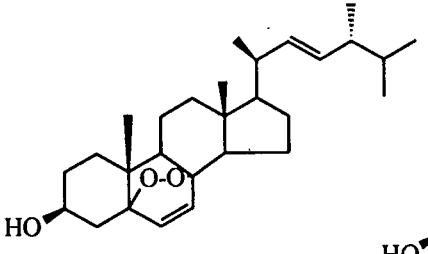

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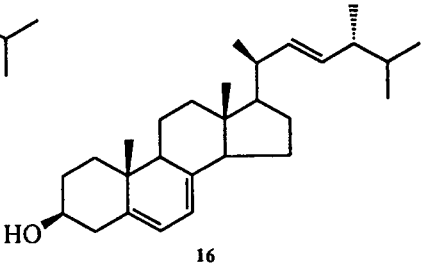

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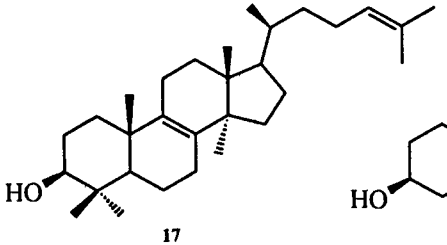

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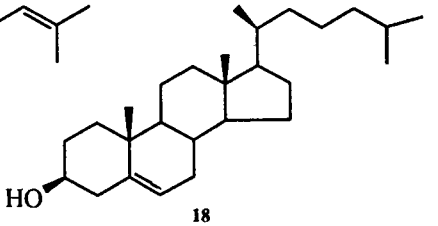

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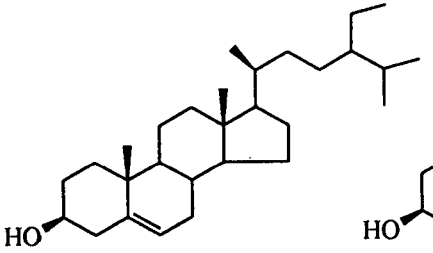

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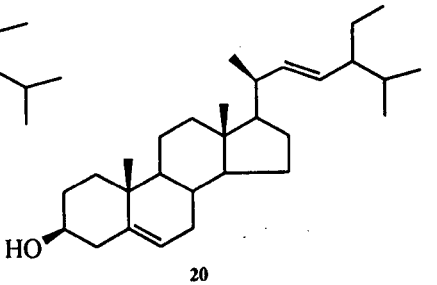

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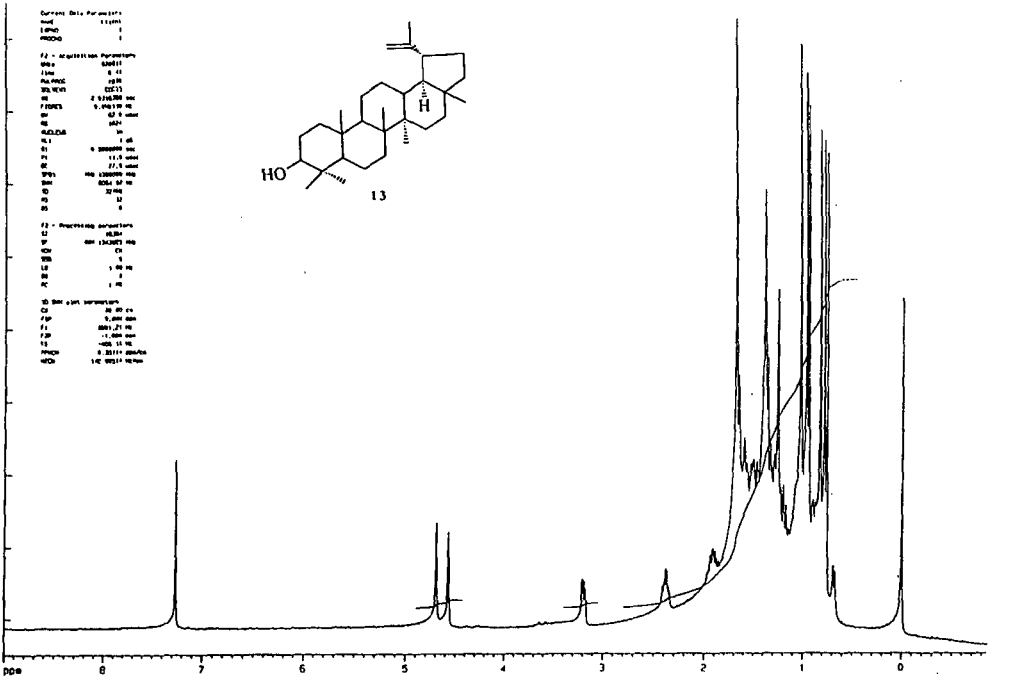

Figure 40

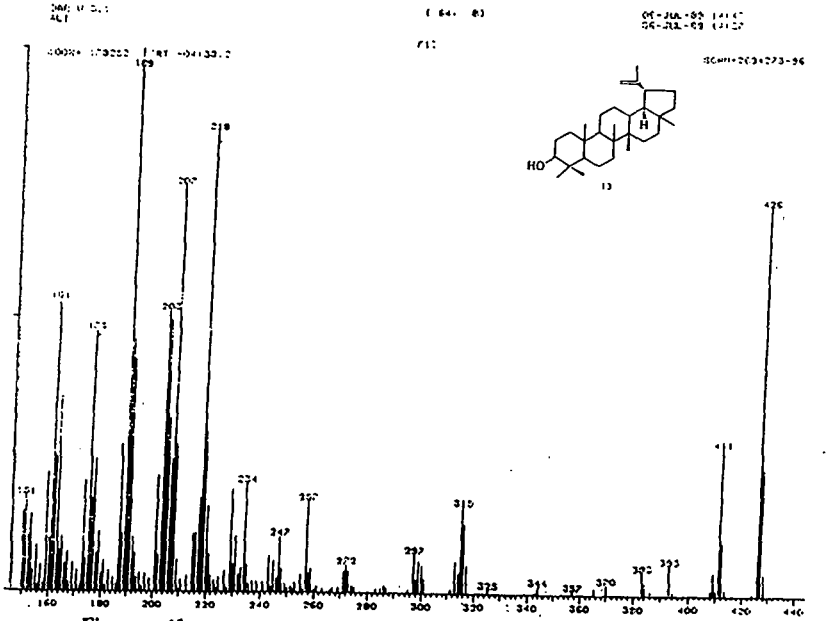

Figure 41

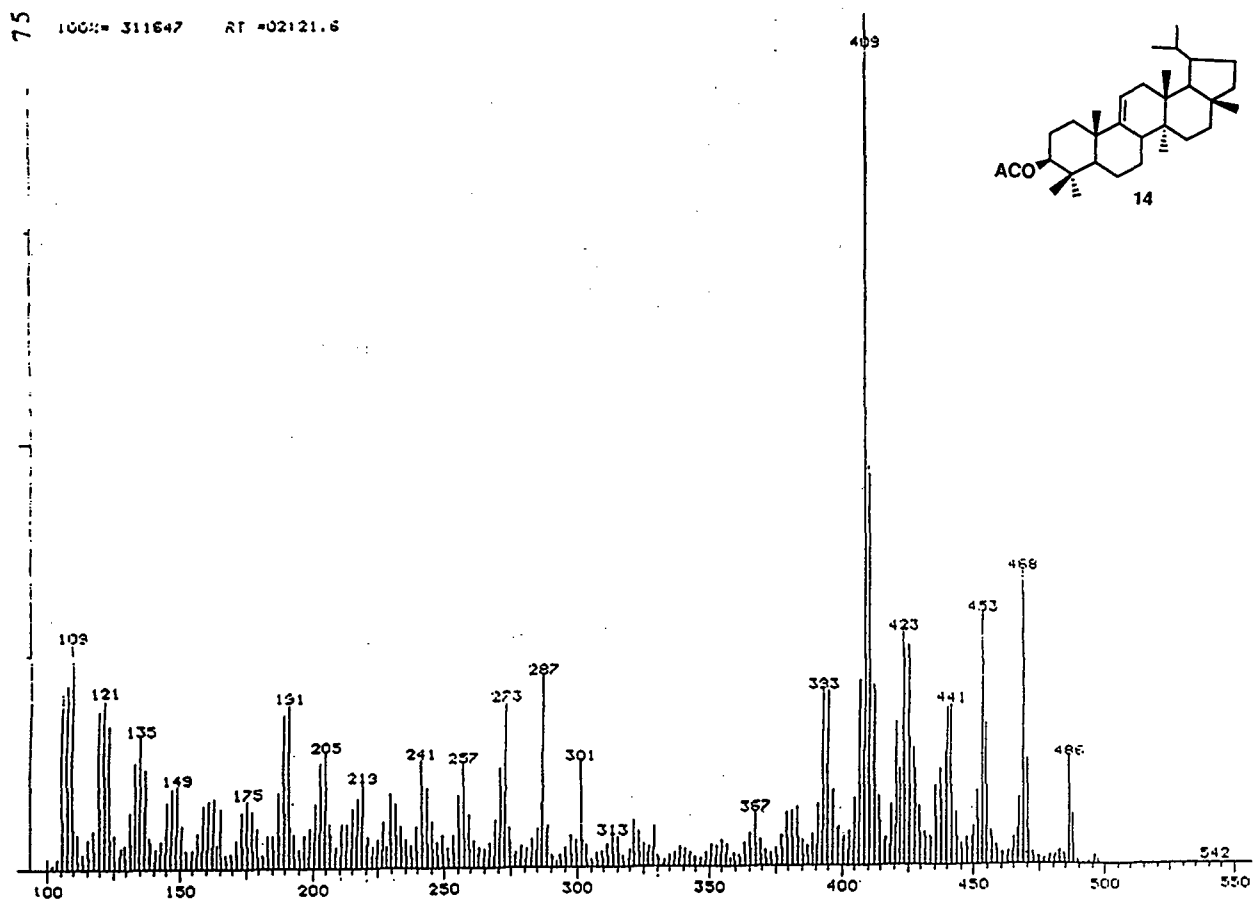
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Figure 42

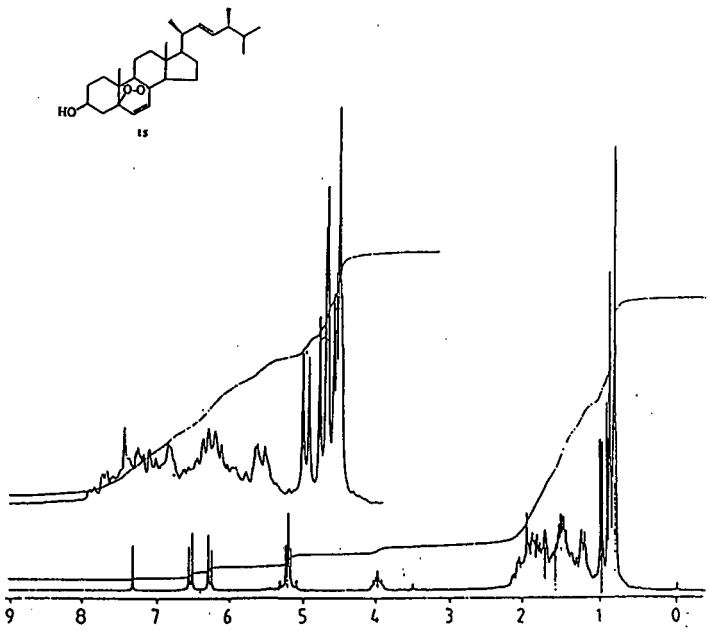


Figure 43

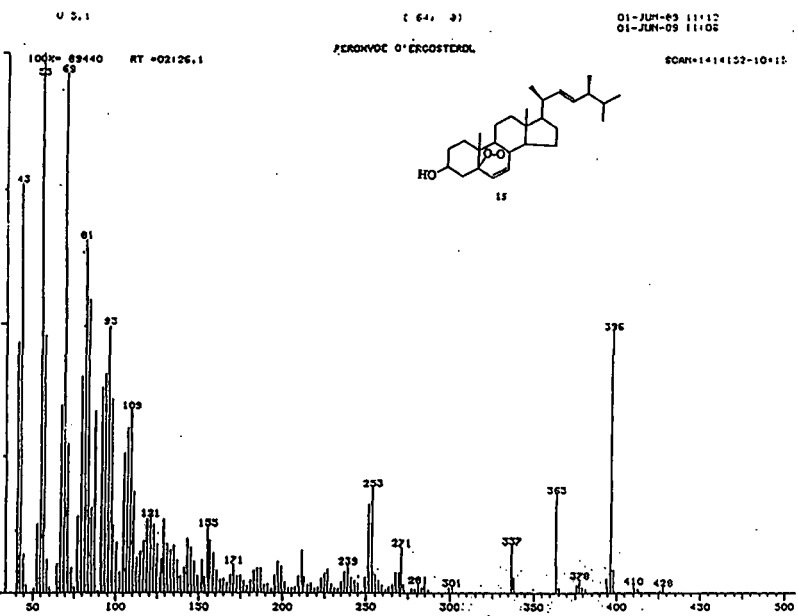
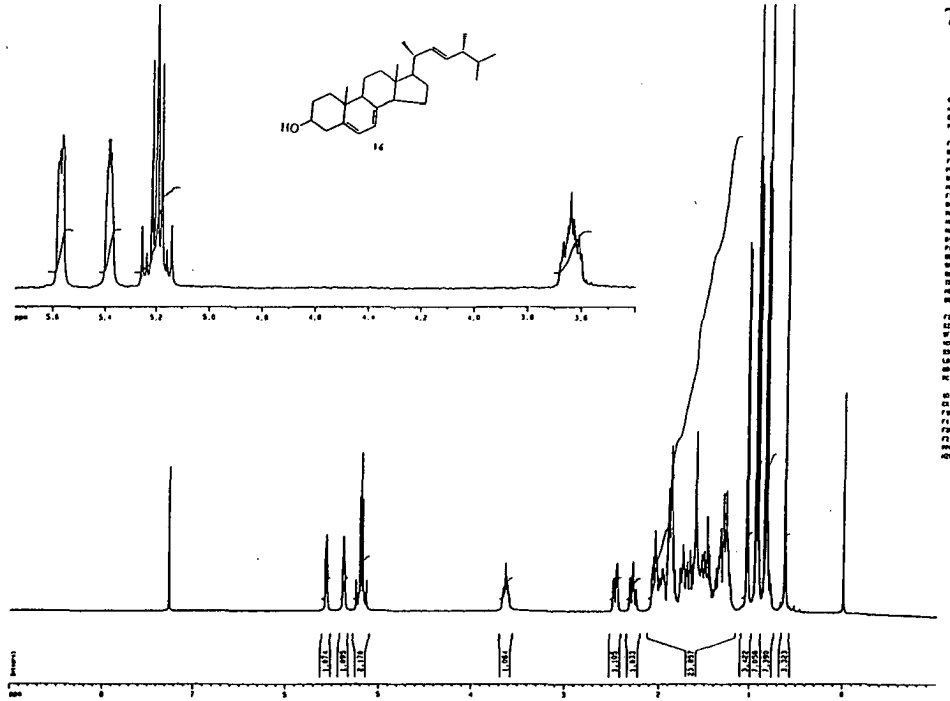


Figure 44



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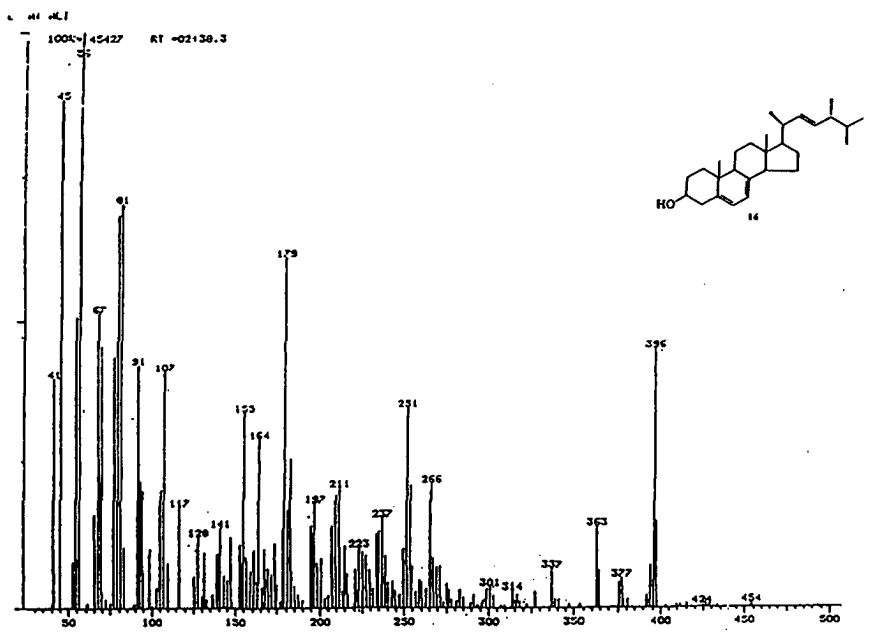


Figure 46

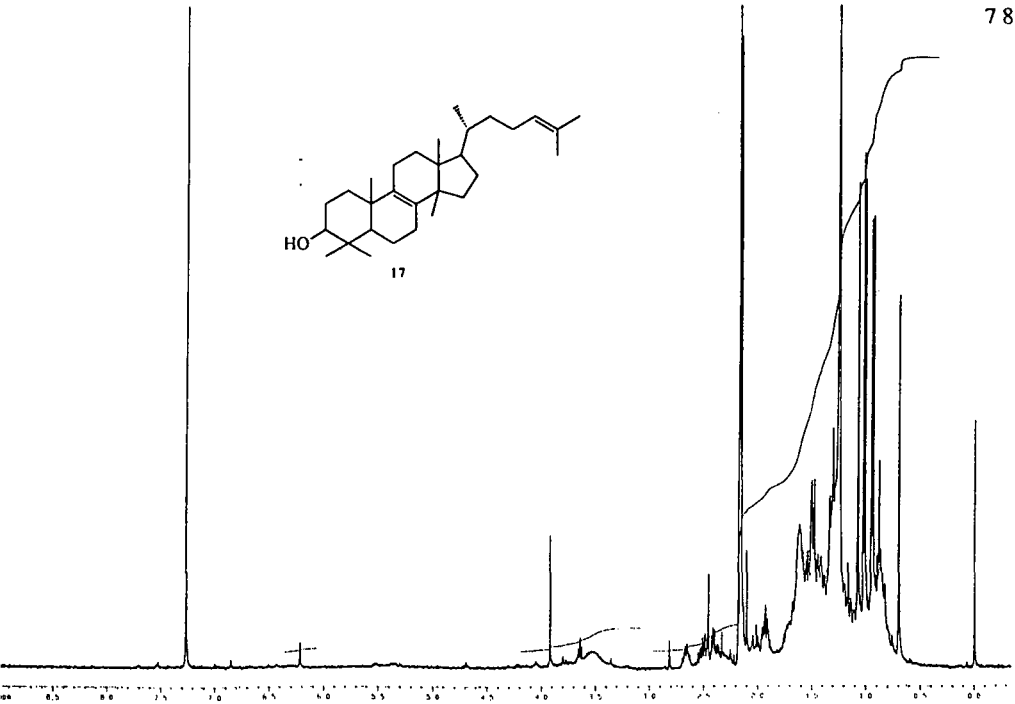


Figure 47

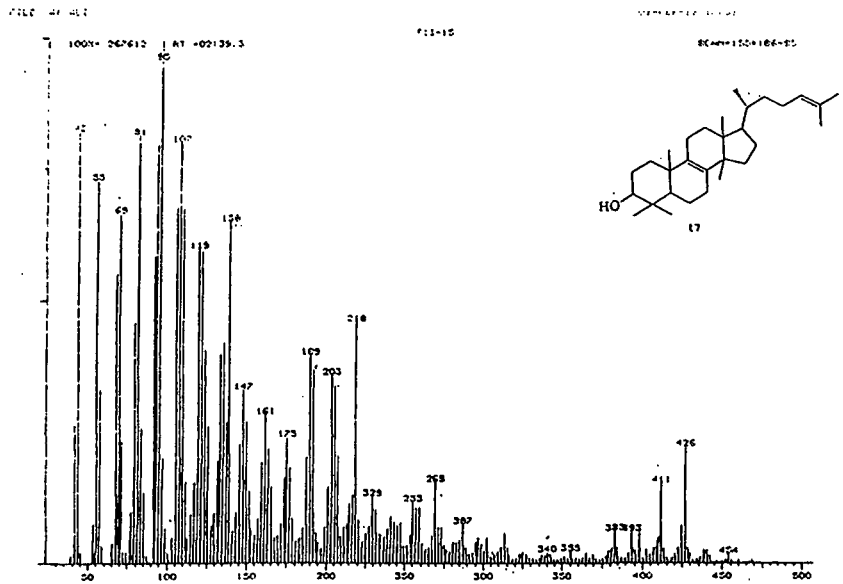


Figure 48

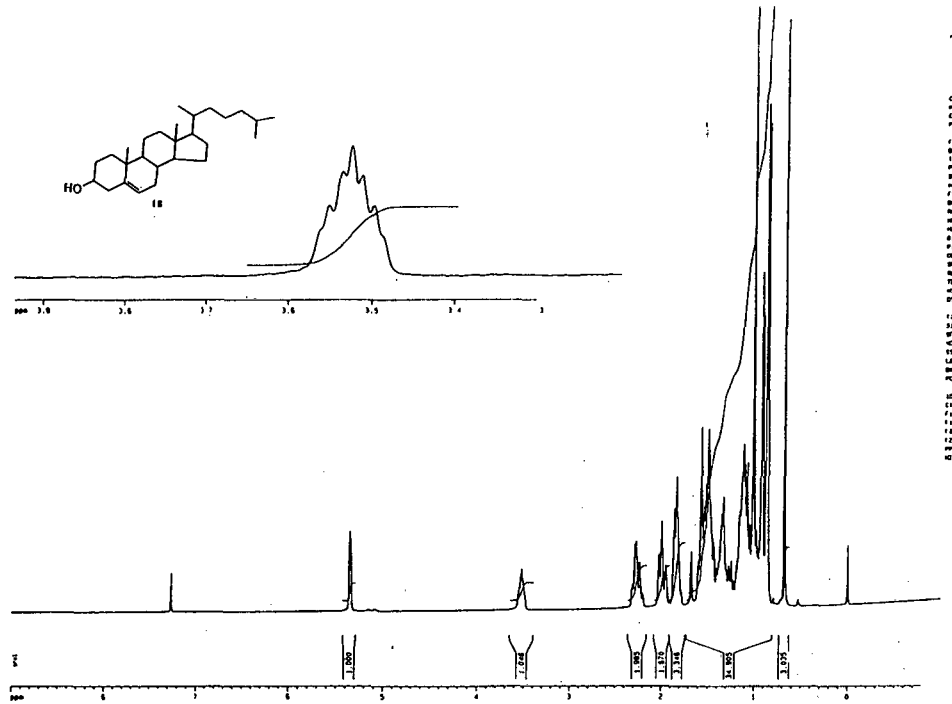


Figure 49

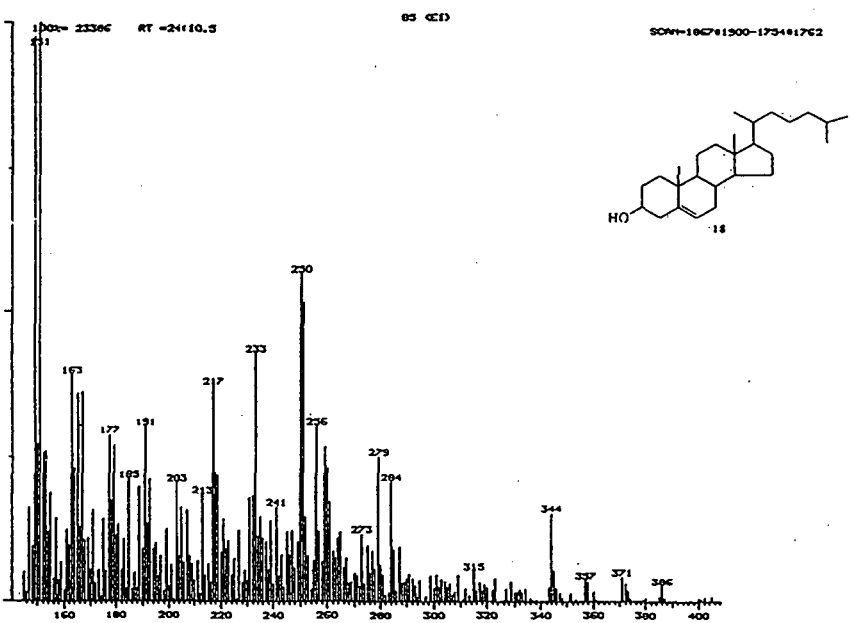


Figure 50

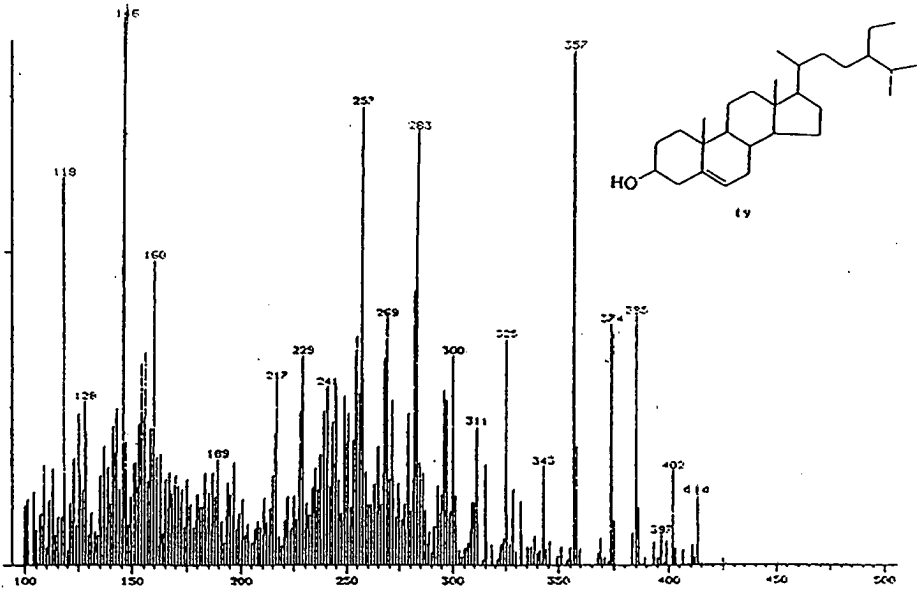


Figure 51

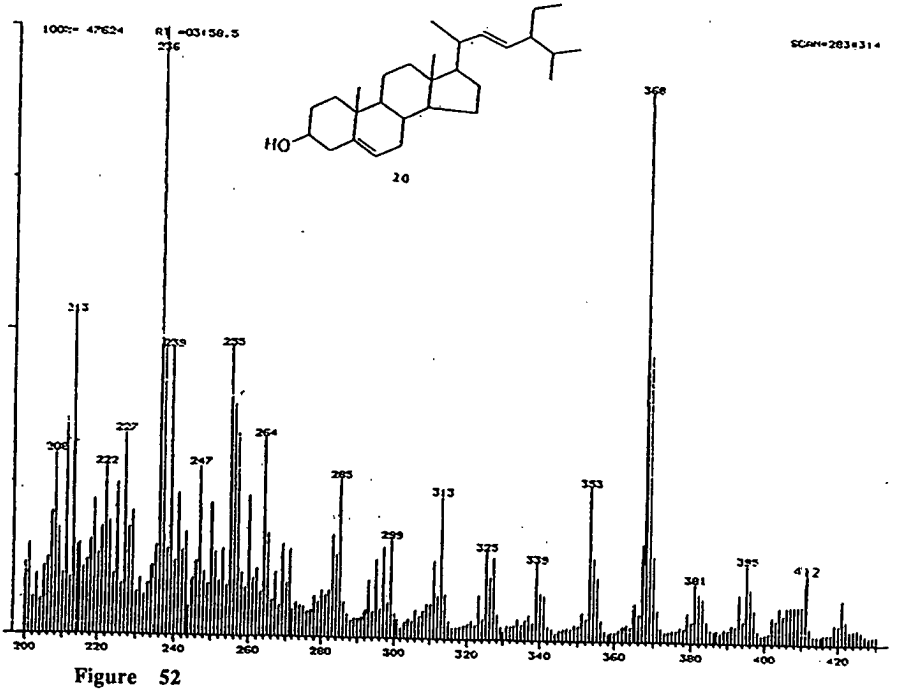


Figure 52

Compounds Isolated from *Usnea articulata* (L.) Hoffm.

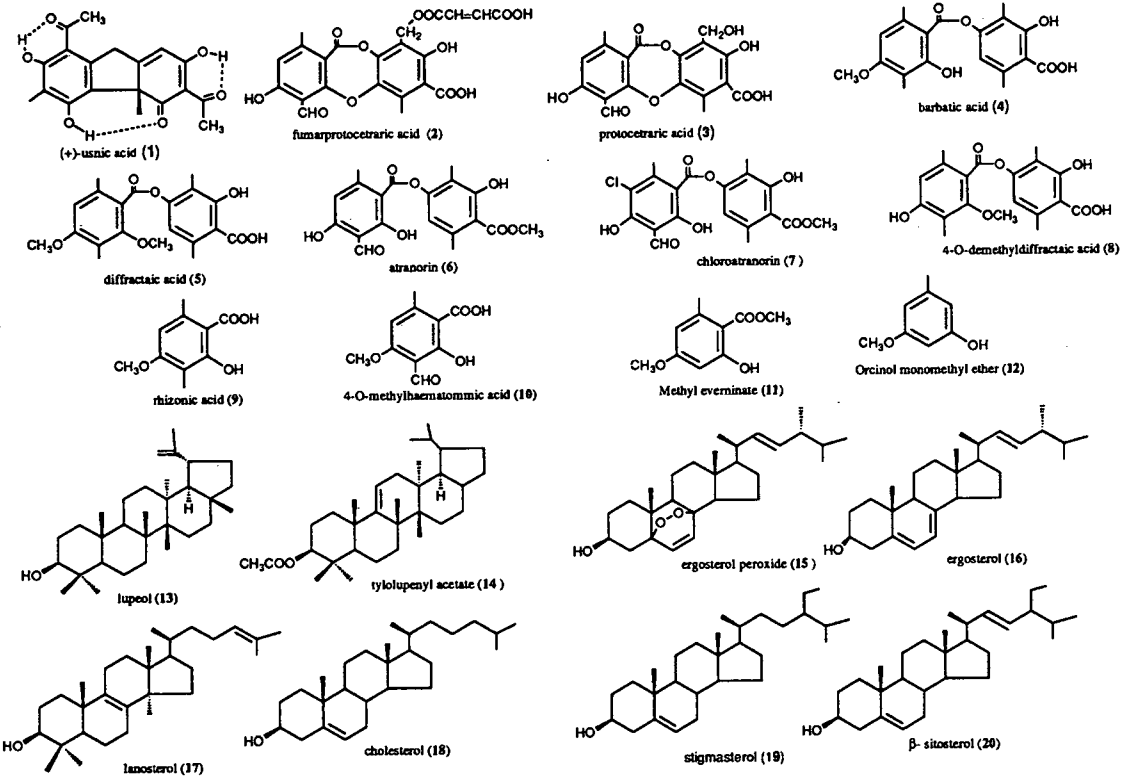


Figure 53

THE FIRST PHYTOCHEMICAL ANALYSIS OF *USNEA HESPERINA* MOT.

2.2 *USNEA HESPERINA* MOT.

2.2.1 INTRODUCTION

The primary aim of this research work was to isolate and identify the chemical substances contained in this lichen which was not investigated previously. Several solvent systems of different polarities were employed for the extraction of the chemical components from the lichen.
Subsequent large scale extraction of the lichen components led to the isolation of a number of common lichen substances such as: depsidones, a dibenzofuran derivative, monophenolic derivatives, one common sterol and two very rare triterpenes.

Several techniques were employed to isolate the chemical components from the lichen under investigation, for example fractional crystallization, preparative TLC, column chromatography, and HPLC (reverse phase). The following three standard [8] solvent mixtures were mainly employed to isolate and purify the lichen substances:

A- Toluene-Dioxane-AcOH (180: 45: 5)
B- Touene-Ether-Formic acid (6: 4: 1)
C- Toluene-AcOH (20: 4)

Usnea hesperina Mot.

Figure 54

2.2.2 MATERIAL AND METHODS

For the purpose of isolation of the chemical constituents from the lichen *Usnea hesperina* Mot. (Fig. 54), collected by Dr. Ph. Clerc [46] from Canary Islands, the plant material (750 g) was dried and powdered. This was extracted subsequently with ether, hexane and acetone using a soxhlet extractor (24 h each), (scheme 4).

The TLC analyses showed that the ether and hexane extracts contained similar components, and were combined together and evaporated to dryness. The resulting brown residue was treated with diethylether. The ether insoluble yellow residue consisted mainly of usnic acid (**4**) (4.5 g- 0.60%).

The acetone extract afforded mainly a grey white substance which precipitated upon concentration of the extract. This grey white precipitate consisted mainly of the major component in this lichen, protocetararic acid (**1**) (5.21g- 0.70%), admixed with a small amount of fumarprotocetraric acid (**2**). It also contained a large amount of potassium oxalate.

The crude ether extract was treated with 5N $NaHCO_3$, 5N Na_2CO_3 and 5N NaOH, in order to separate the strong acids, the weak acids, and the neutral part.

The acid extracts were subjected to silica gel column chromatography eluted by different standard solvent mixtures and with different proportions of the individual sovents. The individually isolated compounds were further purified by TLC and later passed through a Sephadex LH- 20 column. In addition HPLC (reverse phase C18) was employed to separate components with very close Rf values.

The neutral fraction was subjected to silica gel column chromatography and subsequently preparative TLC. Successive elution with a ligroine / ethylacetate mixture (9:1- 5:5) gave two rare triterpenes and one common sterol compound (Scheme 4).

2.2.3 RESULTS AND DISCUSSION

In this first study on the lichen *Usnea hesperina* Mot., the following nine lichen substances were isolated and identified (Fig. 66).

I- Three depsidones: protocetraric acid (**1**), fumarprotocetraric acid (**2**) and virensic acid (**3**).

II- Usnic acid (**4**).

III- Monophenolic derivatives: 4-O-methylorsellinic acid (**5**) and methylhaematommate monomethyl ether (**6**).

IV- Triterpenes: nor-30-hopan-3,22- dione (**7**), ursolic acid (**8**).
V- Sterol: ergosterol peroxide (**9**).

Identification of the isolated individual compounds was based on their physical properties (table 5, 6), [1, 12] microchemical analyses, chromatographic and spectroscopic analyses. Protocetraric acid (**1**) occurred as the major component in the lichen under investigation and usnic acid (**4**) occurred as the second one. Relatively small amounts of fumarprotocetraric acid (**2**) occurred admixed with protocetraric acid (**1**).

After subsequent separation of the grey-white mixture by column and preparative TLC, 47 mg of compound **2** was isolated and identified. The third depsidone **3** occurred in the acid extracts and the fraction (F3/6), migrating as a light orange substance, was collected (67 mg). This very rare depsidone in the lichen genus of *Usnea* was identified as virensic acid (**3**).

Two rare triterpenes **7**, **8** and one common sterol were isolated from the neutral part of the extract. These were later identified as :

I- nor- 30-hopan- 3-,22-dione (**7**).
II- ursolic acid (**8**).
II- ergosterol peroxide (**9**).

The lichen *Usnea hesperina* Mot. was investigated for the first time and the findings of this investigation are presented below. All the compounds presented in this part of the research work have never been reported before as components of the lichen under investigation. Hence

it is expected that the findings of the present research work will be an important contribution to the knowledge of the phytochemistry of the lichen *Usnea hesperina* Mot.

The two triterpene compounds **7** and **8** are very rare in the lichen of the genus *Usnea,* but relatively common in some lichen species and occur in few higher plants [79].

The discussion and results on protocetraric acid (**1**), fumarprotocetraric acid (**2**) (50 mg), and usnic acid (**4**) (4.5 g) were presented in the first part of this chapter 2.1.3.1, (Figs. 6-15) and those for ergosterol peroxide (**9**) (5 mg) were presented in part 2.1.3.4 (Figs. 43 & 44)

The results on the following three compounds, isolated from the acid part of the lichen extract, are discussed in this chapter: namely the depsidone virensic acid (**3**) and two monophenolic derivatives; 4-0-methyl orsellinic acid (**5**) and methylhaematommate monomethyl ether (**6**). The structures of these compounds are presented at the end of this chapter.

VIRENSIC ACID (3)

Compound **3** is a rare depsidone which occurs in only a few lichen species [80]. It was present in the acid extracts and was isolated by repeated silica gel column chromatography and preparative TLC. The white substance, migrating as fraction (F3/6), was collected (174 mg-0.0232%) and purified on a column of Sephadex LH-20, (m.p.247-249°C), (lit. 248-250°C), [1, 12]. It was identified as virensic acid (**3**) on the basis of the spectroscopic data presented below.

The fragmentation pattern in the mass spectrum (Fig. 56) showed certain characteristic fragment ion peaks typical of a depsidone compound, that is m/z 314, 258, 179 and 177 in addition to a molecular ion peak at m/z 358 (scheme 5). The ^{1}H-NMR spectrum (Fig. 55) revealed prominent signals for the following substituent groups: three distinct C-methyl group resonances, one aromatic proton resonance and one proton signal assigned to an aldehyde group. The comparison of these results with those of the literature [81] confirmed the presence of virensic acid. Further comparison of this data with that of an authentic sample of virensic acid substantiated the presence of the proposed structure The authentic sample was provided by the courtesy of Dr.S. Huneck (Institut für Biochemie der Pflanzen, Halle / Saale).

Analyses

^{1}H-NMR (CD_3OD - 200 MHz) : 2.18 ppm (s, 3H, CH_3-Ar, C-6), 2.47 (s, 3H, CH_3-Ar, C-3'), 2.62 (s, 3H, CH_3-Ar, C-6'), 6.75 (s, 1H, H-Ar, H-C-5), 10.75 (s,1H, Ar-CHO, C-3).

Mass spectrum (m/z, 1%) 358 (M, 86), 340 (11), 314 (100), 313 (8), 312 (28), 299 (28), 286 (13), 285 (23), 284 (14), 272 (33), 271 (23), 258 (85), 257 (45), 245 (20), 230 (68), 229 (16), 201 (6), 190 (5), 179 (14), 178 (11), 177 (20), 136 (6), 115 (8), 106 (16), 91 (12), 87 (8), 77 (50), 69 (10), 65 (14), 53 (26), 51 (20), 44 (95).

EVERNINIC ACID OR 4-O - METHYL ORSELLINIC ACID (2- hydroxy-4-methoxy-6-methylbenzoic acid) (5)

Compound 5 was isolated from the acid fractions by silica gel column chromatography eluted by dichloromethane. The white crystalline substance separating as fraction (F14/17-B1) was collected (18 mg), (m.p. 168-169°C), (lit. 170°C) [12]. This was identified as 4-O-methyl orsellinic acid on the basis of its spectroscopic data. The spectroscopic analyses of 5 indicated the presence of a monophenolic derivative. In particular, the ^{1}H-NMR spectrum (Fig. 57) showed prominent signals for the following substituent groups: one methoxyl group, one methyl group, two aromatic proton resonances, and a signal attributed to a hydroxyl group.

The mass spectrum (Fig. 58) was likewise indicative of a monophenolic derivative, revealing certain characteristic fragment ion peaks and a parent peak at m/z 182. The prominent peaks appearing at m/z 164 and 136 indicated the presence of a typical orsellinic acid unit. The loss of water from the parent molecule at m/z 164 (M - H_2O) confirmed the presence of a carboxylic acid group.

Analyses

^{1}H-NMR ($CDCl_3$ - 200 MHz) : 2.57 ppm (s, 3H, CH_3-Ar, C-6), 3.85 (s, 3H, CH_3-O-Ar, C-4), 6.35 (s, 2H, H-Ar, H-C-3,5), 11.52 (s, 1H, Ar-OH, C-2).

Mass spectrum (m/z, 1%) 182 (M, 51), 165 (16), 164 (100), 137 (7), 136 (68), 135 (7), 121 (42), 108 (21), 107 (14), 93 (37), 83 (20), 77 (19), 69 (20), 65 (28), 55 (12).

METHYLHAEMATOMMATE MONOMETHYL ETHER (Methyl-3-formyl-2-hydroxy 4-methoxy-6-methylbenzoate) (6)

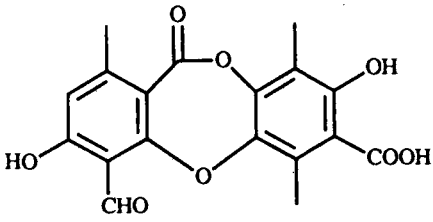
Compound **6** occurred both in the weak acidic and neutral fractions, and was isolated by silica gel column chromatography eluted by dichloromethane. The white crystalline substance migrating as fraction (F5/6) was collected (27mg- 0.0036%), (m.p. 147-149°C), (lit. 149°C) [84] . This was identified as methylhaematommate monomethyl ether on the basis of the spectroscopic data presented below.

The spectroscopic results of **6** indicated the presence of a monophenolic derivative. The ^{1}H-NMR spectrum (Fig. 59) showed the signals of the following substituent groups: one C-methyl group, two well separated methoxy groups, one aromatic proton resonance, attributed to an aldehyde group, together with a signal assigned to a hydroxyl group. The mass spectrum (Fig. 60) was consistent with the proposed structure, and showed a molecular mass peak at m/z 224 and certain characteristic fragment peaks such as: m/z 210, 196, 179, and 178.

Analyses

1H-NMR ($CDCl_3$- 200 MHz) : 2.52 (s, 3H, CH3-Ar), 3.86 (s, 3H, CH3O-Ar), 3.97 (s, 3H, CH_3OOC-Ar), 6.47 (s, 1H, H-Ar), 10.35 (s, 1H, OHC-Ar), 12.42 (s, 1H, HO-Ar).

Mass spectrum (m/z,1%) 224 (7), 210 (53), 196 (3), 182 (17), 179 (13), 178 (31), 163 (2), 151 (12), 150 (100), 137 (2), 122 (13), 109 (3), 94 (11), 77 (8), 69 (24), 55 (21), 43 (14).



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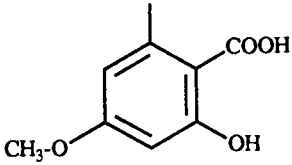

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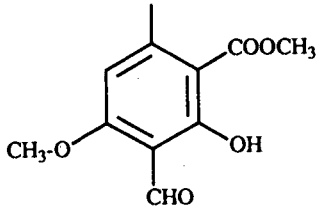

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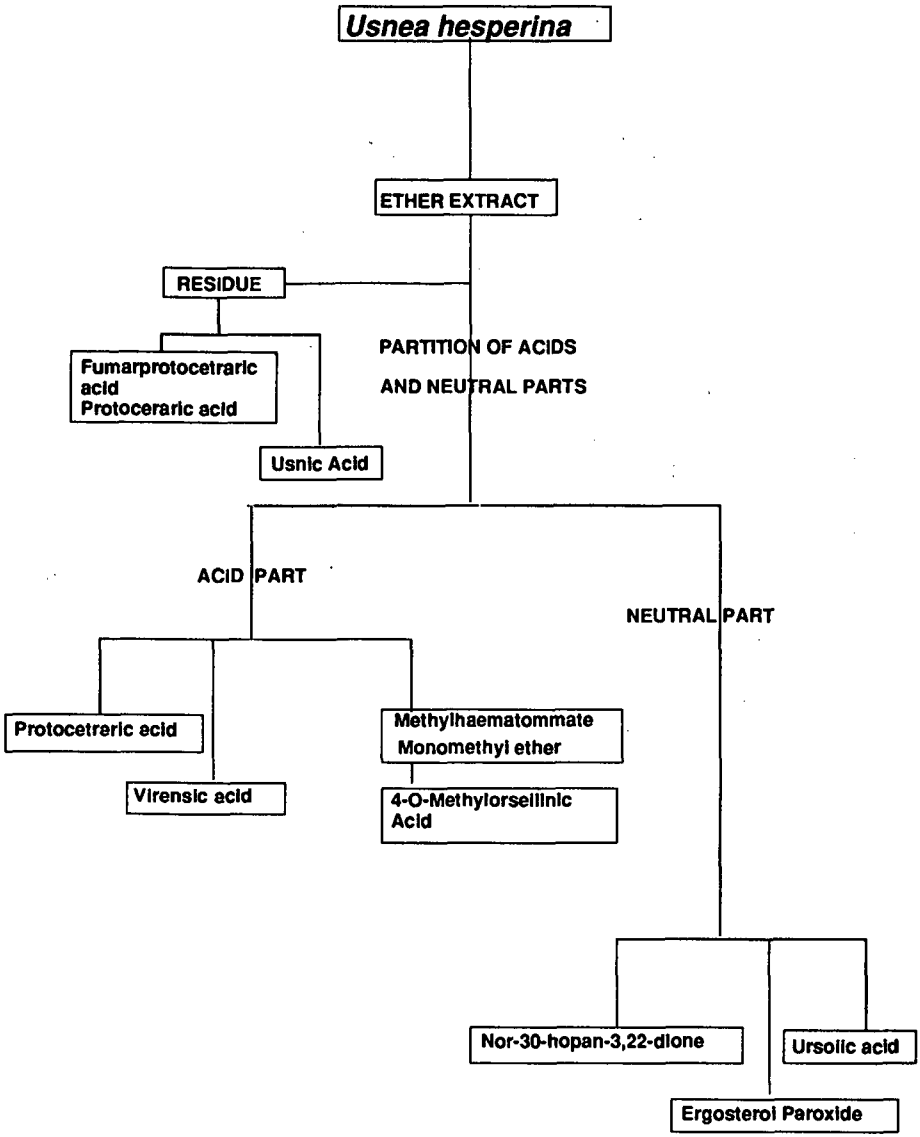

Usnea hesperina
ETHER EXTRACT
RESIDUE
Fumarprotocetraric acid
Protoceraric acid
Usnic Acid
PARTITION OF ACIDS
AND NEUTRAL PARTS
ACID PART
NEUTRAL PART
Protocetreric acid
Virensic acid
Methylhaematommate
Monomethyl ether
4-O-Methylorsellinic Acid
Nor-30-hopan-3,22-dione
Ursolic acid
Ergosterol Peroxide

Scheme 4

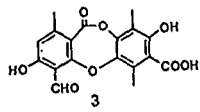


Figure 55

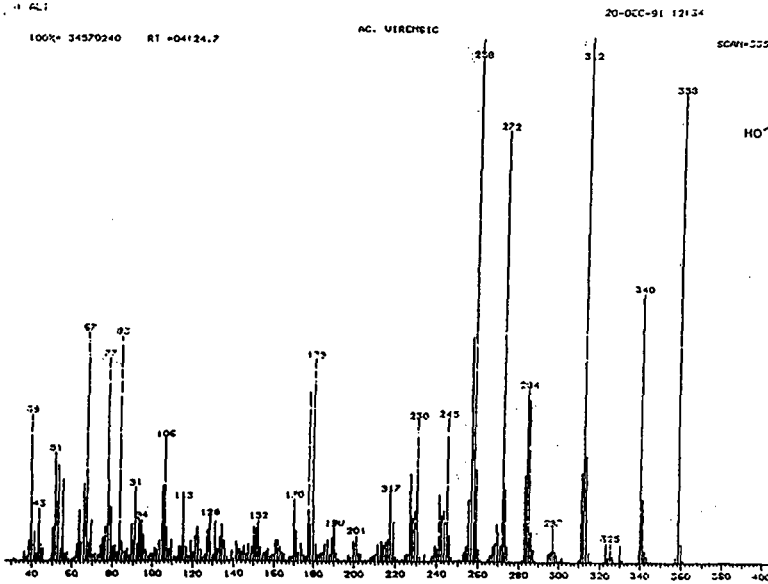
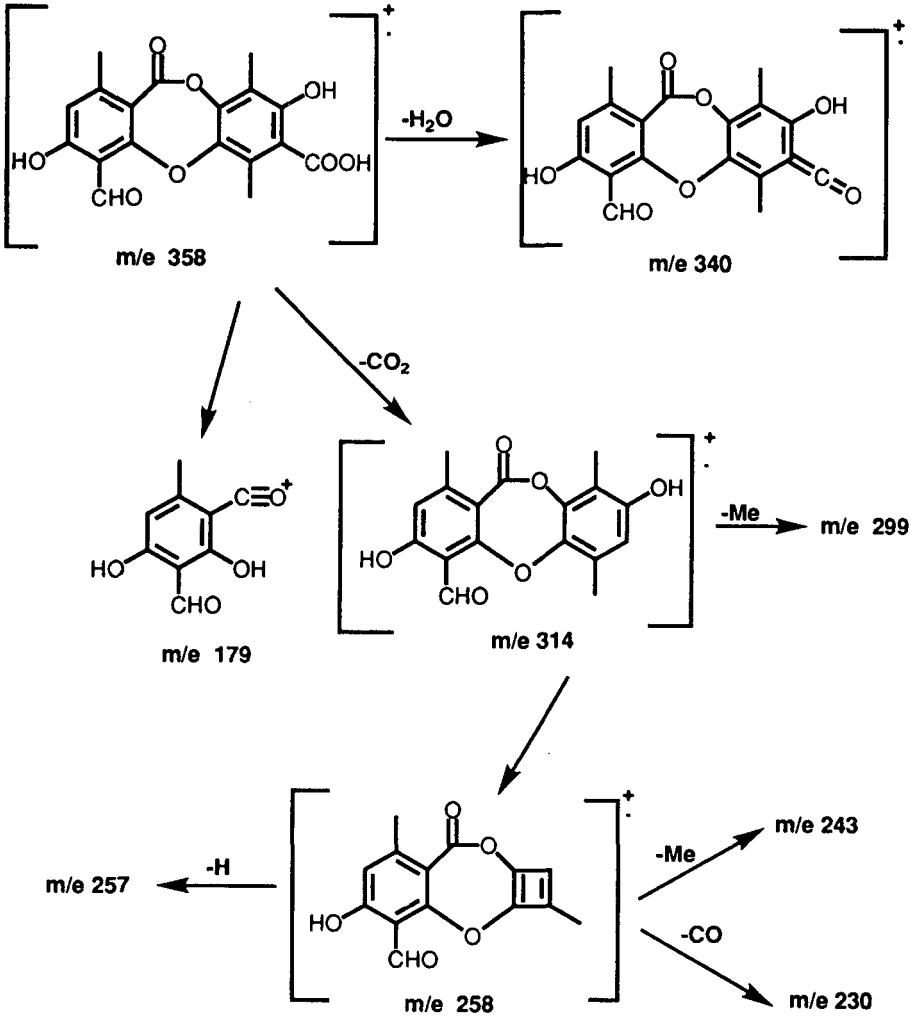


Figure 56


m/e 358
-H2O
m/e 340
-CO2
m/e 179
m/e 314
-Me
m/e 299
-H
m/e 257
m/e 258
-Me
m/e 243
-CO
m/e 230

Scheme 5

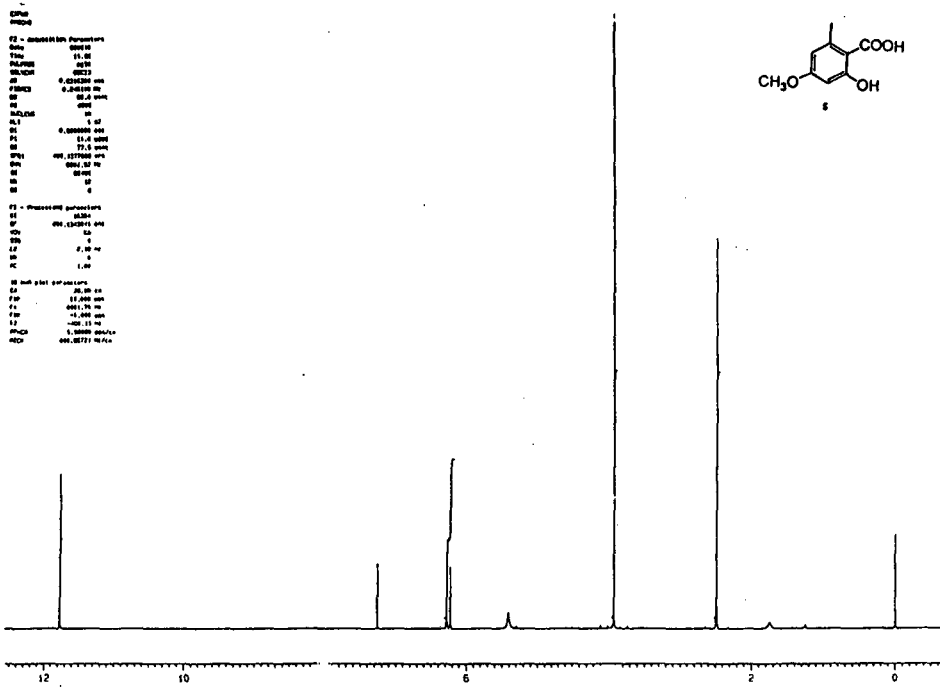


Figure 57

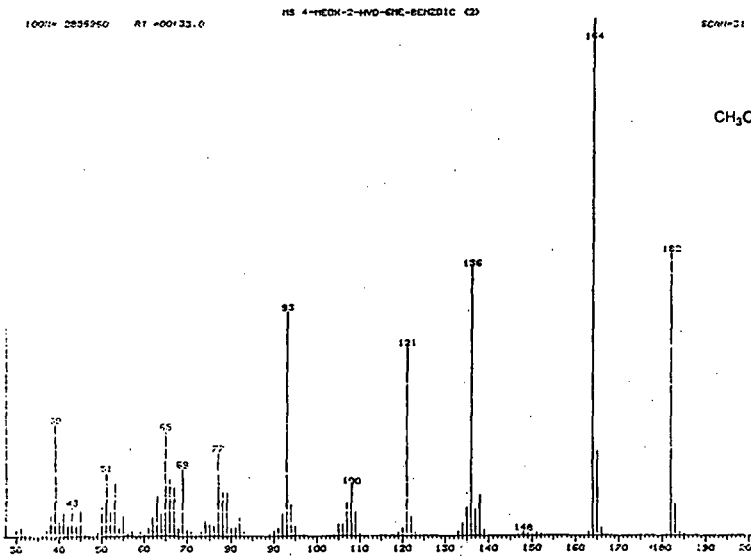


Figure 58

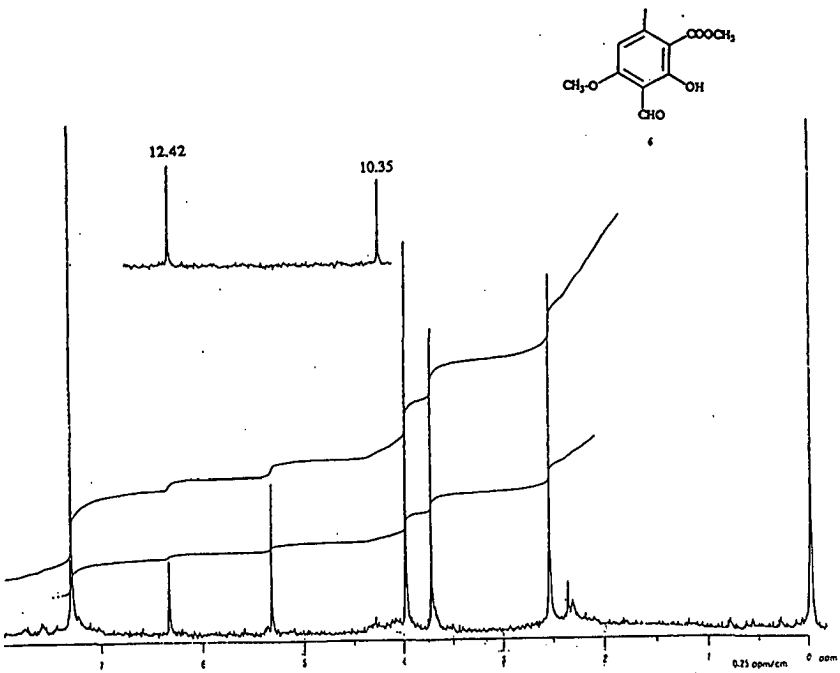


Figure 59

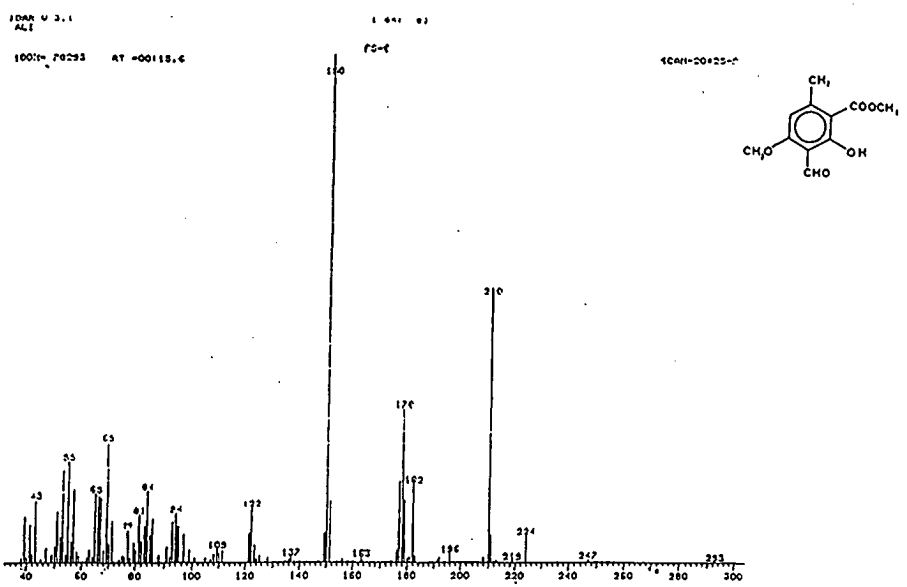


Figure 60

2.2.4. COMPONENTS FROM THE NEUTRAL PART

The results on the following two triterpenes **7** and **8** and a sterol **9** isolated from the neutral part of the lichen extract, are discussed in this chapter; namely nor-30-hopan-3,22-dione (**7**), ursolic acid (**8**) and ergosterol peroxide (**9**). The results and discussion of compound **9** are presented in part 2.1.3.4 of this chapter (Figs. 43 and 44).
The structures of these compounds are presented at the end of this chapter. The spectra for the isolated compounds are also presented at the end of this chapter.

NOR-30-HOPAN- 3, 22-DIONE (7)

Compound **7** was obtained from the neutral part of the lichen extracts. It was detected by TLC analyses and was isolated by silica gel column chromatography with ligroine and ethylacetate (8:2) as eluent. The crystalline substance migrating as fraction (F13/15) was collected (39 mg-0.0104%), (m.p. 258-260°C). This was identified as nor-30-hopan-3, 22-dione (**7**) on the basis of the spectroscopic data presented below.

The appearance of six tertiary and one secondary methyl group resonances (0.79, 0.86, 0.935, 0.951, 1.011, 1.07 and 2.15 ppm) in the ^{1}H-NMR spectrum (Fig.61) suggested the triterpenoid nature of **7**. The broad envelope appearing at 1.25-1.503 ppm corresponds to the methylene and methine group resonances. The doublet signal appearing at 1.44 - 1.503 ppm was attributed to the H-C-21 proton resonance.

The mass spectrum (Fig. 62), showed the molecular ion peak at m/z 426 and exhibited significant characteristic fragment peaks at m/z 232, 205, 191 and 189. These fragments show the presence of a typical hopane skeleton. The absence of an H-C-3 proton resonance and an olefinic proton absorption in the ^{1}H-NMR led to the assignment of the structure of compound **7** as Nor -30-hopan-3,22-dione. Furthermore the presence of the significant fragment peaks at m/z 205, 191 and 189 in the MS, showed the C ring fragmentation and substantiated the presence of the proposed compound. The comparison of the data presented here with that of the literature [67, 82] confirmed the presence of the proposed structure.

Analyses

^{1}H-NMR ($CDCl_3$- 200 MHz) : 0.79 ppm (s, 3H, C-28), 0.86 ppm (s, 3H, C-25), 0.94 (s, 3H, C-27), 0.951 (d, 3H, C-24), 1.01 (d, 3H, C-26), 1.07 (s, 3H, C-23), 2.155 (s, 3H, C-30), 1.25-1.53 (br, m, 25H, methylene and methine groups), 1.44- 1.53 (d, 1H, H-C-21).

Mass spectrum (m/z, I%) 427 ($M^{+\cdot}$ 7), 426 (M, 16%), 411 (11), 393 (7), 384 (5), 244 (2), 232 (4), 220 (8), 206 (27), 205 (40), 203 (7), 191(100), 189 (7), 187 (7), 175 (11), 174 (8), 173 (15), 163 (26), 149 (18), 135 (17), 121 (17), 108 (13), 107 (21), 95 (25), 81 (23), 67 (27), 55 (11), 43 (40).

URSOLIC ACID (8)

Compound **8** occurred both in the acidic and neutral lichen extracts. This was separated by silica gel column chromatography and preparative TLC with ligroine and ethyl acetate (8 : 2) as eluent, and purified further on a column of Sephadex LH-20. The white crystalline substance, migrating as fraction (F 17/21), was collected (245 mg- 0.033%), (m.p. 285-287°C), (lit. 285-291°C), [1,12]. Compound **8** was identified as ursolic acid on the basis of its chromatographic and spectroscopic data.

The ^{1}H-NMR spectrum (Fig.63) showed seven tertiary methyl group signals; five as singlets (0.862, 0.885, 0.95, 1.02, 1.08 ppm) and two as doublets (0.83, 1.01 ppm), indicating the presence of a pentacyclic triterpenoid skeleton, most probably of the ursene series. The ^{1}H-NMR spectrum also showed a broad triplet signal at 3.22 ppm, corresponding to the H-C-3 proton resonance and a broad triplet signal at 5.17 ppm, corresponding to the vinylic proton resonance (H-C-12). The broad envelope appearing at 1.25 -1.75 ppm, corresponds to the methylene and methine group resonances. The broad doublet signal appearing at 1.81 ppm corresponds to the H-C-18 proton resonance.

Useful information concerning the ring system and the substituent mode in compound **8** was obtained from the mass spectrum. The MS (Fig. 64) showed a molecular ion peak at m/z 456 and several characteristic triterpenoid fragment peaks such as m/z 248, 220, 207, 204, 190, and 189, denoting the retro Diels-Alder cleavage of fragments commonly found in the spectra of urs- 12- ene derivatives possessing a hydroxyl group in rings A/B and hydroxyl or carboxyl groups in rings D/E (Scheme 8). Furthermore, the MS exhibited diagnostically important peaks at m/z 412/411 (M - CO_2^+) and m/z 207, 203 and 190, which are common fragment peaks to many naturally occurring triterpenes. These peaks indicated further losses of water and carboxylic groups.
Further supporting evidence concerning the structure of ursolic acid (**8**) was obtained from its ^{13}C-NMR sectrum (Fig. 65). This revealed thirty carbon type signals including characteristic signals due to one carboxylic acid group (178.30 ppm), a trisubstituted double bond carbons (138.20 and 124.50 ppm, and one alcoholic carbon (78.96 ppm), indicating the presence of β-hydroxyl group at the 3-position. The spectrum also showed the expected signals due to methyl, methylene and methine groups.

The comparison of the data presented here with that of the literature [83, 84] confirmed the presence of the proposed compound. Further comparison of these data with those for an authentic sample substantiated the presence of ursolic acid (**8**). The authentic sample was provided by the courtesy of Dr. S. Huneck. (Institut für Biochemie der Pflanzen, Halle / Saale).

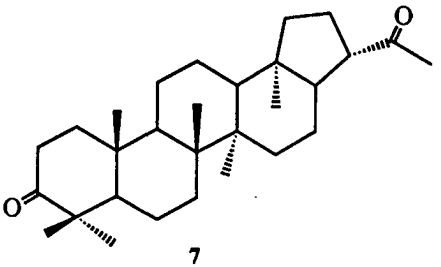
Analyses

^{1}H-NMR ($CDCl_3$ - 200 MHz) : 0.83 ppm (d,3H, -CH_3), 0.862 (s, 3H, -CH_3), 0.875 (s, 3H, -CH_3) 0.95 (s, 3H, -CH_3), 1.01 (s, 3H, -CH_3), 1.03 (d, 3H, -CH_3), 1.081 (s, 3H, -CH_3), 1.25 - 1.75 (br, m, 21H, methylene and methine group), 1.81 (br., d, 1H, H-C-18), 3.22 (br., t, 1H, H-C-3), 5.17 (br, t, 1H, H-C-12).

Mass spectrum (CI, NH_4^+) (m/z, I%) 474 (M^+ + NH3, 42), 457 (M^+, 61), 456 (13), 440 (11), 439 (100, M- H_2O), 438 (46), 412 (M - COOH, 21), 411 (33), 395 (3), 345 (31), 248 (22), 220 (4), 207 (10), 204 (18), 203 86), 191(3), 190 (8), 189 (3), 185(2), 147 (4), 129 (3), 119 (7), 106 (6), 105 (10)

Table 6 ^{13}C-NMR Spectral Data for Ursolic acid (8)

C(1)	38.87	C(16)	26.98
C(2)	27.54	C(17)	47.02
C(3)	78.96	C(18)	39.28
C(4)	41.65	C(19)	38.39
C(5)	45.45	C(20)	38.4
C(6)	18.03	C(21)	26.98
C(7)	33.0	C(22)	38.51
C(8)	41.3	C(23)	15.22
C(9)	46.83	C(24)	13.95
C(10)	38.24	C(25)	17.02
C(11)	23.81	C(26)	16.84
C(12)	124.58	C(27)	25.6
C(13)	138.2	C(28)	178.30
C(14)	41.32	C(29)	18.00
C(15)	29.01	C(30)	16.02



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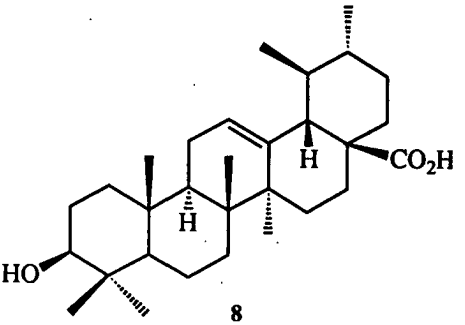

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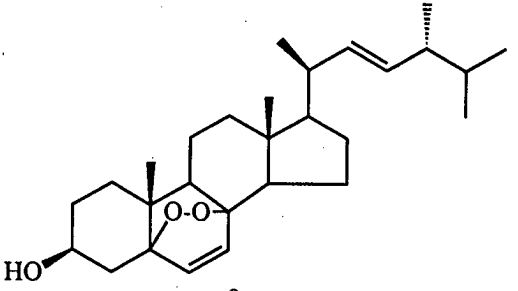

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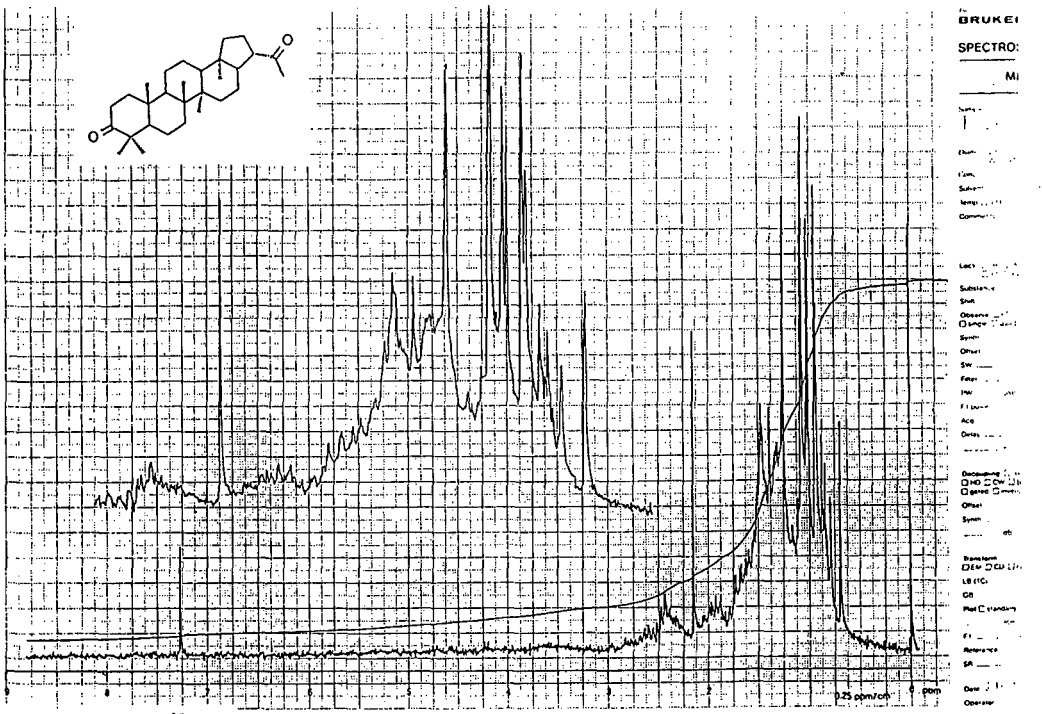

Figure 61

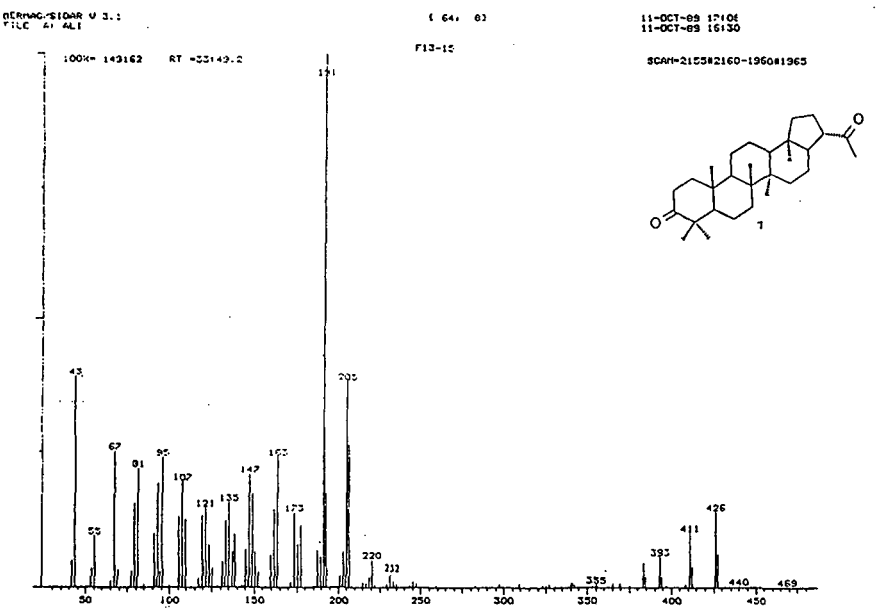

Figure 62

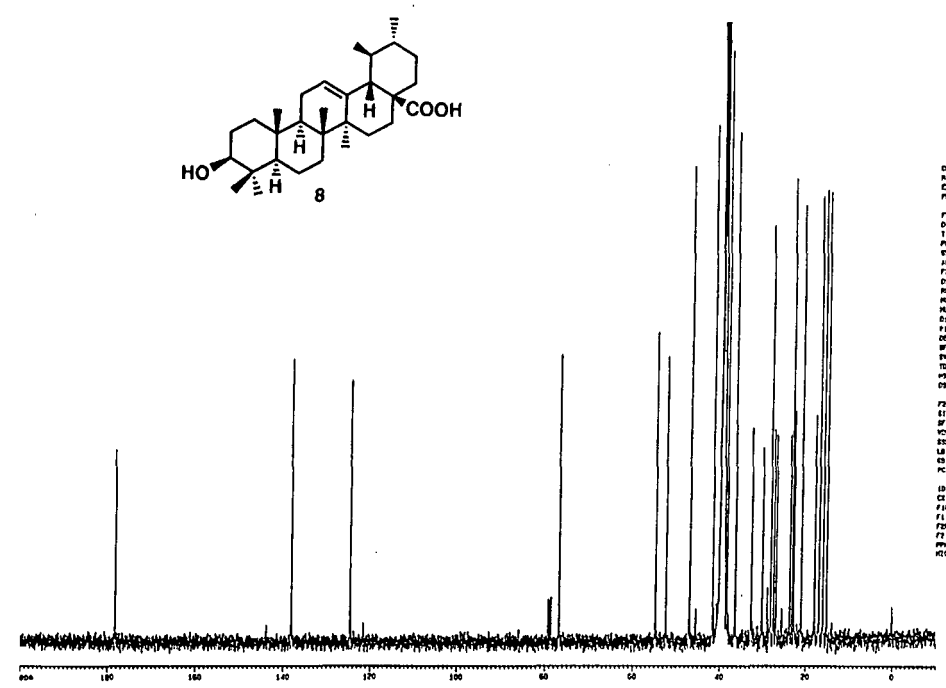

Figure 65

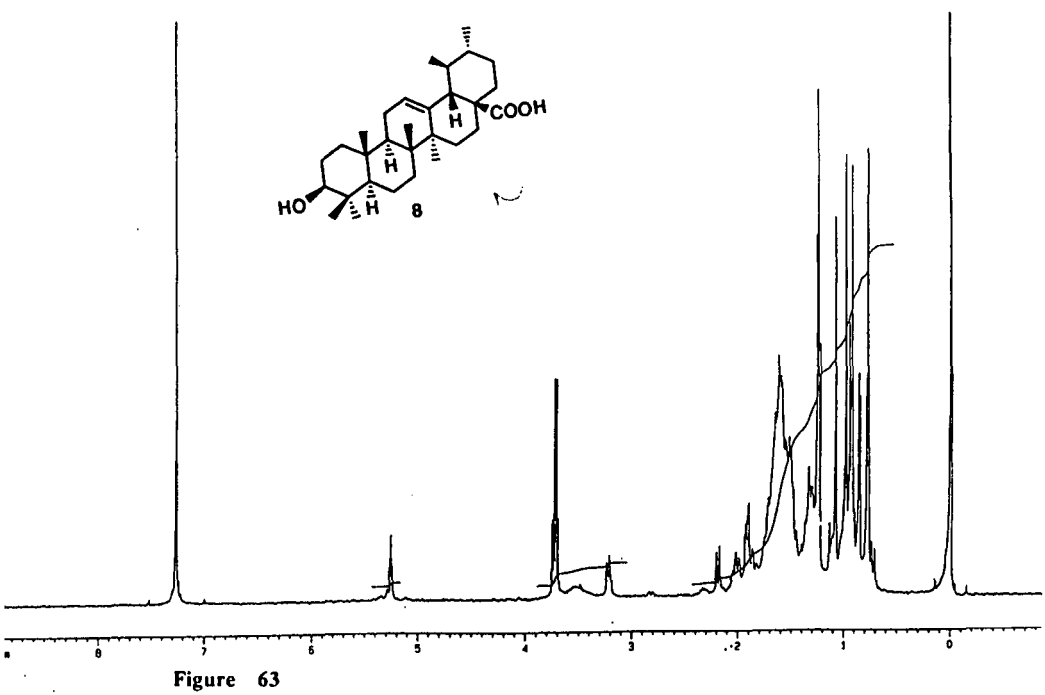

Figure 63

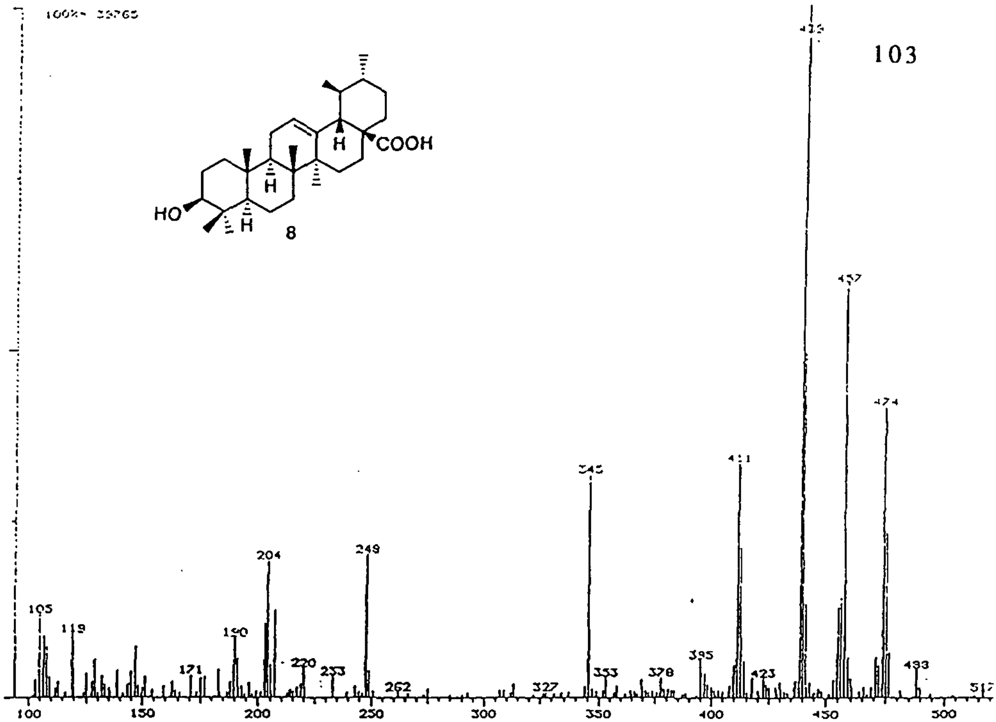
100%= 33765
HO
COOH
8
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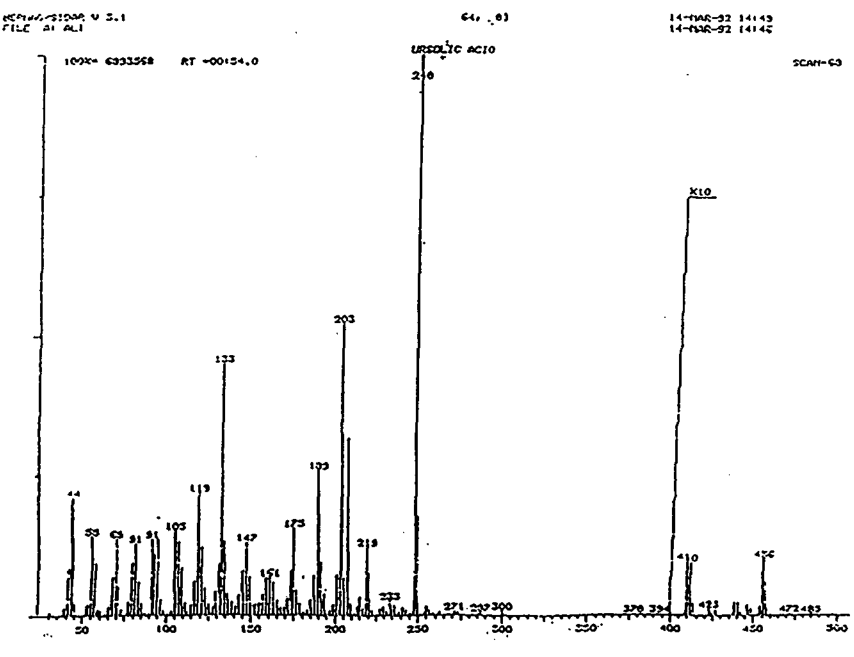
Figure 64


URSOLIC ACID
14-MAR-92
SCAN=63
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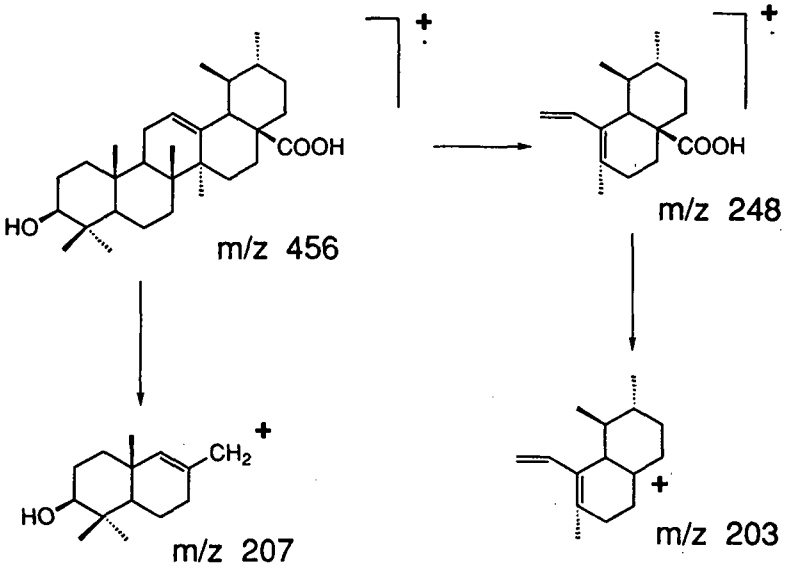


Scheme 6 Mass Spectral Fragmentation pattern of Ursolic Acid

TABLE 5

The melting points of the compounds isolated from the lichen-Usnea hesperina were compared with those of the literature [10, 13] as presented below

Compounds isolated from U. hesperina.	m.p of U.h	m.p, Lit.
Protocetraric acid	244-246°C	245-250°C
Fumarprotocetraric acid	249-252°C	250-260°C
Virensic acid	246-248°C	248-250°C
Usnic acid	201-203°C	203-204°C
4-O-methyl orsellinic acid	168-169°C	170°C
Methylhaema. monomethyl ether	147°C	149°C
Nor-30-hopan-3,22-dione	258-260°C	260-262°C
Ursolic acid	284-286	285-291°C
Ergosterol peroxide	179-181°C	180-181°C

U.H- Usnea hesperina

Lit.- Literature

Methylhaema = Methylhaematommate

TABLE 6

Comparison of the Rf values of the compounds isolated from the lichen Usnea hesperina Mot. with that of the literature [8, 10].

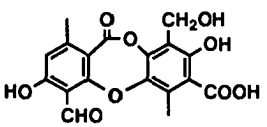
TLC data for the 9 isolated substances Rf x 100 Values

Compounds	solvent	A in U.H.	A in Lit.	B in U.H	B in Lit.	C in U.H	C in Lit.
Protocetraric acid		3	3	17	19	6	4
Fumarproto. acid		1.5	1.5	23	24	6	7
Virensic acid		24	26	47	57	36	38
Usnic acid		68	70	68	70	70	71
4-O-methylorsellinic acid		42				53	
Methylhaematommate		56				64	
Nor-30-hopan-3,22-dione		61				67	
Ursolic acid		56	59	54	50	54	52
Ergosterol peroxide		50		57		61	

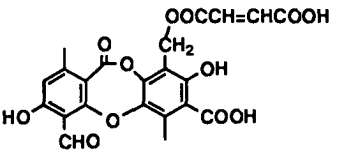
Composition of the standard solvents: (A) toluene- dioxane- acetic acid (180: 45: 5); (B) hexane-diethyl ether-formic acid (6:4:1): and (C) toluene-acetic acid (20:4).

U.H - Usnea hesperina Mot.; Lit.-Literature.

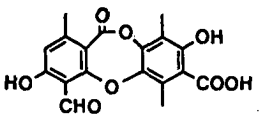
Structures of the Compounds Isolated from *Unsea hesperina* Mot.



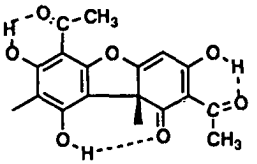
Protocetraric acid (1)



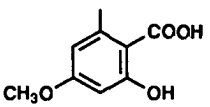
Fumarprotocetraric acid (2)



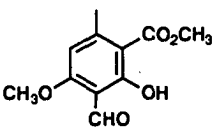
Virensic acid (3)



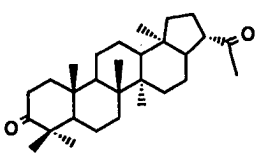
(+) Usnic acid (4)



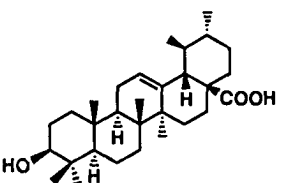
4-O-Methyl orsellinic acid (5)



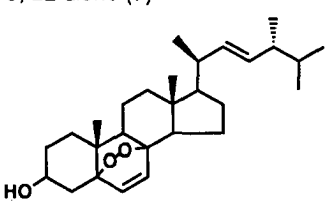
Methylhaematommate monomethyl ether (6)



Nor-30-hopan-3, 22 dione (7)



Ursolic acid (8)



Ergosterol peroxide (9)

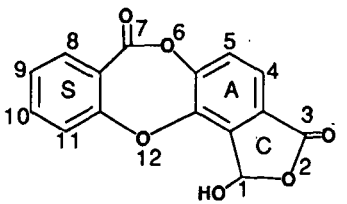
Figure 66

3 CONTRIBUTION TO THE SYNTHESES OF PHTHALIDES, DEPSIDES, DIARYLE ETHERS AND DEPSIDONES CONTAINING A HYDROXYLACTONIC RING

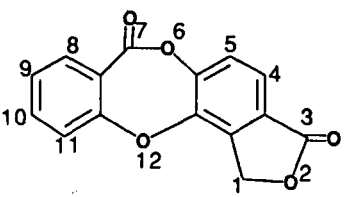
3.1 INTRODUCTION

In this research work we were attempting to synthesize a group of depsides and depsidones that have never been prepared to date. Besides the classical methods, three different synthetic routes were attempted, all of which may be biomimetic. The first route employs oxidative coupling of a benzophenone, while the second route makes use of the Smiles rearrangement of depsides and the third route makes use of the Ullmann reaction.

Approximately 15 depsidones exist which contain a hydroxylactonic cycle C attached to cycle A (structure I). The A and C cycles, when considered as a single unit, are known as hydroxyphthalide. The basic structure of depsidones containing this cycle is known as isobenzofurobenzodioxepine (structure II).



I

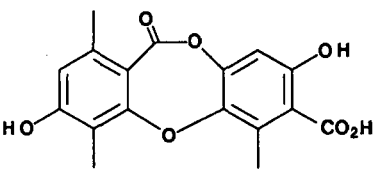


II

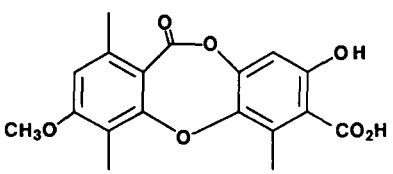
With two exceptions, all the depsidones containing a hydroxyphthalide cycle also possess two structural groups (an S cycle and the A + C cycles) derived from β–orcinol (Table 8), No 1-13). Methylstictic acid (**13**) is a particular case, since it contains a methylated hydroxyphthalide cycle.

Menegazziaic acid (**14**) contains an S cycle which is derived from an orcinol group that contains an additional phenolic group in the 11-position.

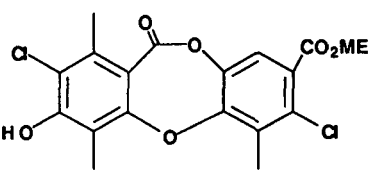
The substictic acid is formed from an S cycle derived from a β–orcinol group and an [A + C] cycle derived from the orcinol group. Only three other depsidones possess these two structural groups: nornotatic acid, notatic acid, and eriodermine. The substictic acid is thus the fourth known structure in this class of compounds, but it is the only example which contains a hydroxyphthalide cycle [85].



16 Nornotatic acid



Notatic acid



Eriodermine

The depsidones that contain hydroxyphthalides [85, 86, 87] are listed below (Table 8) The α–acetylsalazinic acid (**11**) is also known as galbinic acid [44].

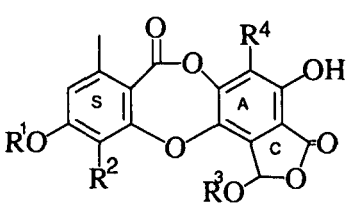


Table 8

No	R^1	R^2	R^3	R^4	Trivial Names
1	H	CH_3	H	CH_3	Hyposalazinic acid
2	CH_3	CH_3	H	CH_3	Hypostictic acid
3	CH_3	CH_3	H	CH_2OH	Hypoconstictic acid
4	H	CH_2OH	H	CH_3	Connorstictic acid
5	CH_3	CH_2OH	H	CH_3	Cryptostictic acid
6	H	CH_2OH	H	CH_2OH	Consalazinic acid
7	H	CHO	H	CH_3	Norstictic acid
8	CH_3	CHO	H	CH_3	Stictic acid
9	H	CHO	H	CH_2OH	Salazinic acid
10	CH_3	CHO	H	CH_2OH	Constictic acid
11	H	CHO	H	CH_2OAc	α- Acetylsalazinic acid
12	CH_3	CHO	H	CH_2OAc	α–Acetylconstictic acid
13	CH_3	CHO	CH_3	CH_3	Methylstictic acid
14	CH_3	OH	H	CH_3	Menegazziaic acid
15	CH_3	CHO	H	H	Substictic acid

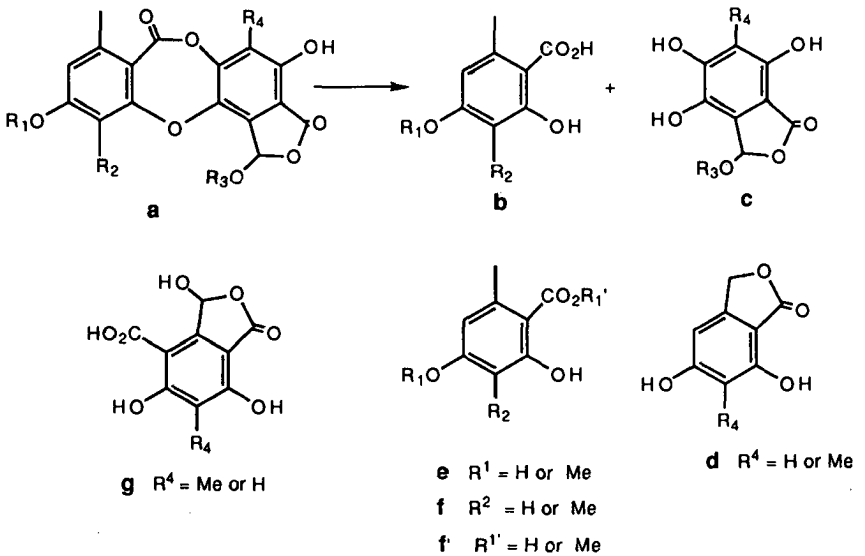
The majority of the compounds in this family can be derived from the depsidones **7**, **8**, and **10**. Stictic acid (**8**), for example, leads to the formation of compounds **5**, **2**, **9**, **3**, **6**, **11** and **14**, whereas norstictic acid (**7**) leads to the formation of compounds **1**, **2**, **3** and **4**. However substictic acid (**15**), requires a unique synthetic route.

Syntheses of some of the above mentioned natural depsides, diphenyl ether and depsidones, was the major aim of this part of our research work. Numerous previous reports on similar research work were consulted, particularly that of C. Pulgarin [96] who conducted considerable work on phthalides, and lactonic depsides and depsidones. It appears that all the work conducted in this area had some setbacks. However, we adopted different approaches than that of the previous researchers in the syntheses of phthalides, depsides and depsidones, and modified slightly the

three general routes for the syntheses of lactonic depsides and depsidones. This modification provided better yield.

3.2 THE STRATEGY ATTEMPTED FOR THE TRANSFORMATION OF BENZOPHENONE TO GRISADIENDIONE AND THEN TO DEPSIDONE

The retro synthetic analyses of depsidones **a** results in its decomposition into the synthons **b** to **c**

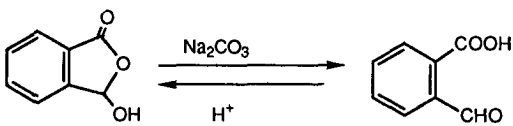


Scheme 7

Synthons **e** and **f** are the starting materials for the syntheses of depsides and depsidones. The substituent R^1 (H or CH_3) is determined from the rest of the substituents R^2 and R^4 in the final depsidones (see the list , table.8). If R^1 = H, the starting material is methyl orsellinate (e), and if R^2 = Me, then the starting material is methyl- β-orcinol carboxylate (**f**).

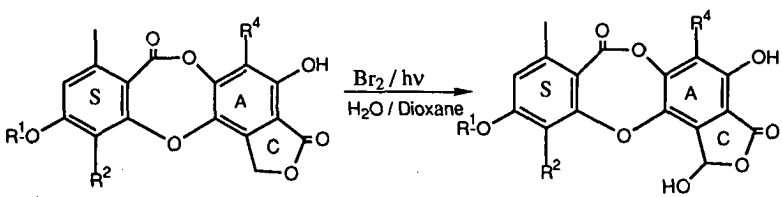
Condensation of the two units **b** and **c**, by analogy with the Friedel-Crafts reaction, results in a benzophenone. Similarly condensation of **e** and **c** with trifluoroacetic anhydride results in a depside. The two phenols within unit **b** and the orthophenol in the acid function of **c** must be protected in the form of benzylic ether units. The second phenol within the acid **c** is similarly protected (iso-Pro, AcO, MeO, and etc) in order to favourably orient the subsequent oxidative coupling.

This protective group must remain labile, since it will be subsequently removed. The hydroxyphthalide **g** is a precursor much closer to **a** than synthon **c**, but it is certainly less stable. It is known that hydroxyphthalides dimerize in solution under the action of light [88]. They are very labile and open easily in slightly basic solution to give phthaladehydic acids [89].



It is therefore preferable to form the hydroxyphthalide from a depsidone already containing a phthalide group in the last step.

By interconversion of the functional groups, the residues R^1 to R^4 of the hydroxyphthalide are finally obtained in order to conduct total syntheses of all the depsidones required.

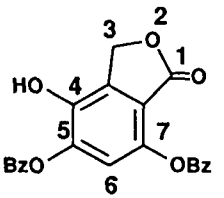


3.3 SYNTHESES OF PHTHALIDES GROUPS

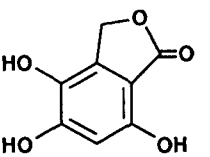
Two main synthetic routes for the syntheses of the groups of phthalides are discussed here and presented as Group I and Group II

3.3.1 SYNTHETIC ROUTES FOR GROUP I.

Our primary objective in this research work was to design an appropriate method for the synthesis of the following phthalides **16, 17, 18,** which are the necessary percursors for the synthesis of the required depsides and depsidones.The structures of the monophenolic and phthalide compounds are presented in the following scheme 8



16



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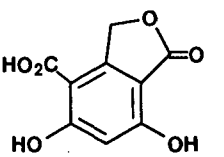


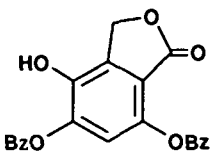
18

The difficulty in the syntheses of depsides and depsidones of this kind was envisaged to be construction of the phthalide moiety. Although many phthalides [isobenzofuran-1-(3H)-ones] are naturally occurring substances and are important intermediates in the syntheses of benzophenones, naphthalenes, anthraquinones, and certain depsides and depsidones, the methods available for their syntheses are few and the yields are often low [90, 91].

For the syntheses of the phthalides **16, 17** and **18,** we required methylorsellinate (**e**) and methyl β-orcinolate (**f**), (Scheme 7) as precursors.

Synthesis of 5,7-dibenzyloxy- 4-hydroxyphthalide (5,7-Dibenzyloxy- 4-hydroxy-isobenzofuran-1 (3H)-one

As the majority of the depsides and depsidones containing hydroxyphthalide cycles carry methyl, hydroxymethylene, acetoxymethylene groups etc , at the 6-position (R^4 in table 7), it was necessary to design appropriate methods for the syntheses of phthalides having a methyl group at the 6-position (R^4, depsidone structures). To establish an appropriate method for the syntheses of the particular phthalides, which were considered precursors for the syntheses of depsides and depsidones, we attempted the following synthetic routes. The key steps to the main route for the synthesis of the phthalide are presented in scheme 8. The spectroscopic properties of the these phthalides **25**, **28** and **16**, are presented in the following (fig. 67 - 71).



16

Synthetic Procedure

A convenient method for the syntheses of phthalides which are precursors for the syntheses of depsides and depsidones, containing a hydroxylactonic ring, is described below (scheme 8).

Certain aspects of the syntheses of methylorsellinate (**29**) deserve some comment. The best method for the conversion of the dihydro compound **21** into methylorsellinate appeared to be bromination of **21** and subsequent debromination.

Treatment of the dehydroester **21** with 3 mol equiv. of bromine for an extended period gave the dibromo derivative **22**. This underwent smooth debromination and afforded the expected methylorsellinate (**12**). Photobromination of the dibromo ester (**22**) gave the tribromo compound **23** which was cyclized to the dibromo lactone **24**. Debromination of **24** gave 5,7-dihydroxyphthalide (**25**) which was benzylated to give 5,7 dibenzyloxyphthalide (**27**). Formylation of compound **27** gave the aldehyde **28**. Baeyer-Villiger oxidation [45, 92] of the resultant formate gave the desired phenol **16**.(scheme 8).

Acetylation of methyl 2,4-dihydroxy-6-methylbenzoate (**29**) gave the diacetoxy compound **30**. Photobromination of the diacetate gave methyl 2,4-diacetoxy-6-bromomethylbenzoate (**31**) which

was cyclized to form two major lactone products; 5,7-dihydroxyphthalide (**25**) and 5-hydroxy-7-acetoxyphthalide (**32**).

EXPERIMENTAL

Methyl Dihydro Orsellinate (21)

Sodium (46 g, 2 mol), was added with stirring to dry methanol (750 ml). After the reaction had subsided, methylacetoacetate (**19**) (232 g, 2 mol) and methyl crotonate (**20**) (200 g, 2 mol) were added in turn and the mixture was stirred and heated under reflux for 48 h. The bulk of the methanol was removed, and ether (800 ml) was added to the stirred and ice cold residue. The precipitated salt was collected by filtration and washed with ether (400 ml). It was dissolved in water (1l) cooled to 0°C, and acidified with ice cold conc. hydrochloric acid. The crystalline precipitate (78%) was separated by filtration, washed with ice cold water and dried in vacuo. A small sample was recrystallized from methanol, forming prisms (m.p. 122- 124°C).

Methyl -3,5-Dibromo-2,4- Dihydroxy-6-Methylbenzoate (22)

Bromine (157 g, 3 mol) in acetic acid (60 ml) was added to a warm stirred solution of the Dihydro Orsellinate (**21**) (60 g, 0.33 mol) in acetic acid (190 ml) at such a rate that the temperature was 40-50°C. The mixture was then stirred for 2 h and set aside for 20 h. Water was added and the dibromo compound **22** was separated by filtration, then washed with water and dried in vacuo. This gave higher yield 105g, 94%, m.p. 102-104°C.

^{1}H-NMR ($CDCl_3$ - 200 MHz) : 2.68 ppm (s, 3H, Ar-CH_3), 3.97 (s, 3H, CH_3OOC-Ar), 7.05 (br, 1H, H-O-Ar), 12.82 (s, 1H, H-O-Ar).

MS (m/z, I%) 342 (M^+, 9), 340 (18), 338 (17), 310 (31), 308 (100), 306 (83), 280 (21), 252 (24), 229 (24), 199 (24), 187 (5), 171 (24), 155 (6), 143 (20), 117 (11), 91 (41), 77 (12), 67 (19), 63 (37), 53 (23).

Synthesis of Methyl-3,5-Dibromo-2,4-Dihydroxy-6-Bromomethylbenzoate (23)

The dibromo compound **22** (14 g, 41 mmol) was heated under reflux in carbon tetrachloride (CCl_4, 250 ml) over a 300W incandescent lamp or UV lamp during the dropwise addition of bromine (7 g, 82 mmol) in carbon tetrachloride (75 ml) for 1 h. The mixture was stirred under

reflux for a further 6 h. .After cooling the solution, the solvent was removed under reduced pressure. The semi-solid orange residue gave 12.75g, 75% yield,it was further purified by silica gel column chromatography.

^{1}H-NMR $CDCl_3$ - 200 MHz) : 3.97 ppm (s, 3H, CH_3OOCAr), 5.12 (s, 2H, Br-CH_2-Ar), 12.31 (s, 1H, HO-Ar)
MS (m /z, I%) 419 (M^+, 4), 418.(22), 388 (15), 386 (43), 384 (15), 359 (10), 341 (53), 339 (100), 337 (56), 324 (7), 309 (47), 307 (70), 304 (31), 295 (36), 281 (42), 279 (85), 277 (42), 251 (11), 225 (8), 199 (16), 170 (7), 147 (9), 119 (5), 91 (23), 77 (11), 63 (33), 51 (13)

4,6-Dibromo-5,7-Dihydroxyphthalide (24)

Methyl 3,5-dibromo-6-bromomethyl-2,4-dihydroxybenzoate (23) (7.5 g, 18 mmol) was heated at reflux in dioxane (300 ml) and water (300 ml) for 16 h. The dioxane was evaporated under reduced pressure, and the residual aqueous solution was allowed to stand over night. The precipitate which formed was separated and washed with hexane and dried thoroughly. This was recrystallized from chloroform / methanol (3:1) and gave 4,6-dibromo- 5,7- dihydroxyphthalide (24) as prisms with high yield 4.9 g, 85%, (m.p. 228-231°C).

The aqueous filtrate was extracted with ethyl acetate and the extract was washed in turn with saturated brine and water. Removal of the solvent gave more compound 24.

^{1}H-NMR (CD_3OD - 200 MHz) : 5.12 ppm (S, 2H, -CH_2-lactone), 8.56 (br. 1H, HO-Ar), 9.35 (br, 1H, HO-Ar).

MS. (m/z, 1%) 325 (M^+, 24), 324 (M, 47), 321 (23), 297 (49), 295 (100), 293 (52), 266 (3), 239 (6), 215 (5), 199 (5), 187 (10), 171 (3),.131 (8), 117 (5), 107 (6), 90 (11), 79 (13), 77 (20), 69 (8).

5,7- Dihydroxyphthalide (25)

A stirred solution of the dibromo phthalide (24) (5.5 g, 17 mmol) in dioxane (112 ml) and aqueous 2N NaOH (112 ml) was cooled to 0°C and treated in portions with Nickel-Aluminium alloy (8.3 g). Stirring was continued at O°C for a further 1.50 h. The suspension was filtered off and the acidified filtrate was extracted with ethylacetate. The extract was washed in turn with saturated brine and water and dried over $MgSO_4$. Removal of the solvent gave 5.7-dihydroxyphthalide (25), with relatively high yield 2.60 g, 93% , m.p. 148-150°C.

^{1}H-NMR (CH_3OD - 200 MHz) : 5.21ppm (s, 2H, -$\underline{CH_2}$- lactone group), 6.35 (s, 2H, 2 x H-Ar)

MS (m/z, I%) 167 (M^+, 11), 166 (M, 54), 165 (17), 149 (6), 138 (10) 137 (100), 121 (7) 108 (12),.81(14), 79 (5), 77 (3), 69 (30), 63 (9), 53 (11).

5,7-Dibenzyloxyphthalide (27)

Dihydroxyphthalide (7) (3 g, 18 mmol), dry potassium carbonate (4.56 g, 36 mmol) and benzylchloride (4.56 g, 36 mmol) were stirred at 60 °C (bath) in dry dimethylformamide (DMF) (100 ml) for 18 h. The mixture was poured into cold dilute hydrochloric acid and extracted with ethyl acetate (3 times). The extract was washed in turn with water and saturated brine then dried over $MgSO_4$. The compound was recrystallized from light petroleum as prisms, yield 5.25 g, 84%, (m.p. 209-211°C).

^{1}H-NMR ($CDCl_3$) : 5.12 ppm (2 x s, 4H, 2 x -$\underline{CH_2}$-Ar), 5.18 (s, 2H, -CH_2- lactone), 6.52 (s, 2H, 2 x H-Ar), 7.43 (m, 10H, 2 x Ar)

MS (m/z, I%) 347 (M^+, 11), 346 (M, 35), 328 (13), 327 (7), 255 (7), 240 (15), 238 (8), 225 (3), 211 (7), 209 (4), 181 (37), 167 (4), 151 (7), 91 (100), 65 (16).

5,7-Dibenzyloxy- 4-Formylphthalide (28)

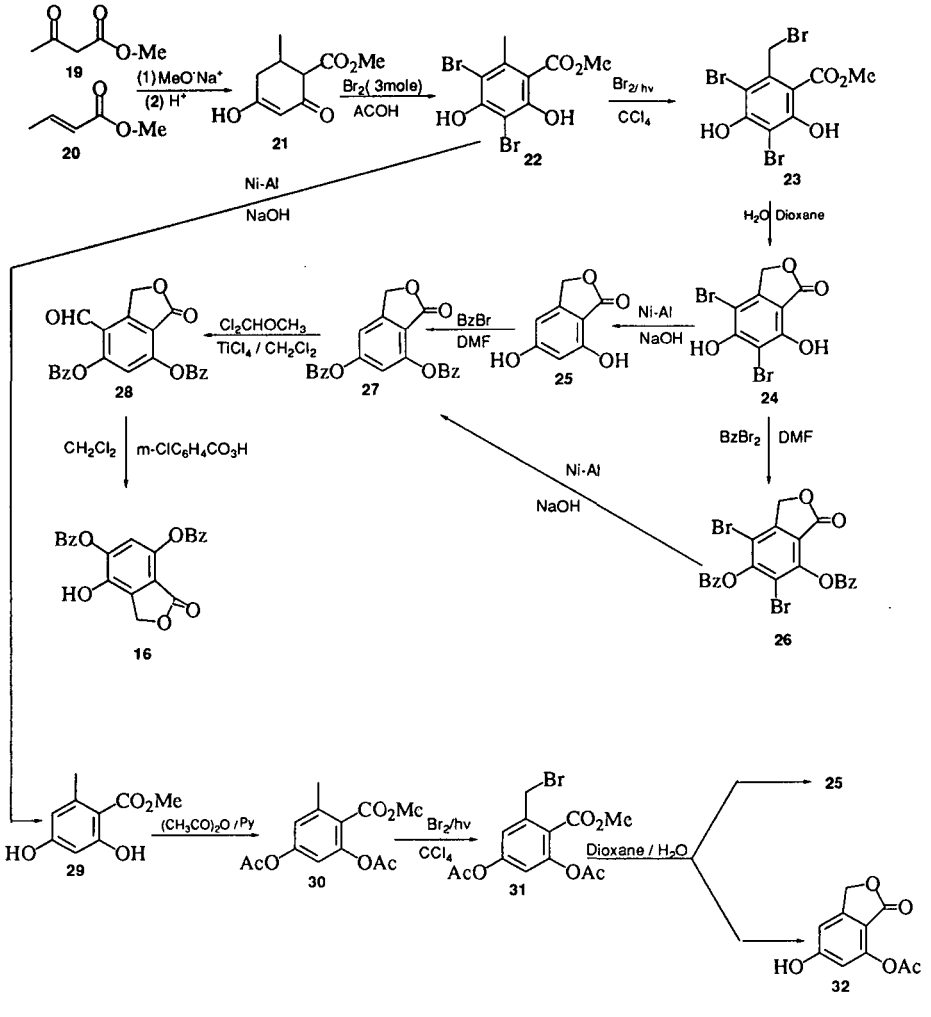
A stirred solution of the dibenzyloxyphthalide (27), (1.4 g, 4 mmol) and dichloromethyl methyl ether (5 g, 43 mmol) in dichloromethane (50 ml) was treated dropwise, over 1h at 0°C, with a solution of titanium(IV) chloride (6 g) in dichloromethane (25 ml). The solution was then stirred at 0°C for 1 h and at room temperature for 5 h. An excess of cold hydrochloric acid was added to the stirred solution and the precipitate was separated by filtration. The organic phase was separated from the filtrate and washed with saturated brine. Removal of the solvent gave more product. The combined crude mixture was separated by silica gel column chromatography. The resulting aldehyde compound was recrystallized from methanol and formed needles, yield 0.93 g, 62%, m.p. 172-174°C.

^{1}H-NMR ($CDCl_3$- 200 MHz) : 5.11 and 5.21 ppm (2s, 2H each, 2 x -$\underline{CH_2}$-Ar), 5.25 (s, 2H, -CH_2- lactone group), 6.56 (s, 1H, H-Ar), 7.43 (m, 10H, 2 x Ar).

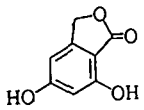
4-Hydroxy - 5,7- dibenzyloxyphthalide (16)

A solution of the aldehyde **28** (0.70g, 1.87 mmol) and m-chloroperbenzoic acid (85%, 1.2 g) in acetic acid (30 ml), was stirred at 90 - 100°C (bath) for 2 h. The residue left upon removal of the solvent was dissolved in dichloromethane and washed in turn exhaustively with saturated aqueous sodium hydrogen carbonate and saturated brine.

The solvent was removed and the crude product was dissolved in warm methanol (50 ml), and stirred at room temperature under nitrogen for 1 h with aqueous 10% sodium hydroxide (20 ml). The solution was acidified with dilute hydrochloric acid, and most of the methanol was removed under reduced pressure. The residue was extracted with dichloromethane and the extract was washed with saturated brine. The crude product was recrystallized from methanol and dichloromethane, and gave the phenol **11** as needles, yield 0.41g 61%, m.p. 228-231°C.


19
20
(1) MeO⁻Na⁺
(2) H⁺
21
Br2(3mole)
ACOH
22
Br2/ hv
CCl4
23
Ni-Al
NaOH
H2O Dioxane
24
Ni-Al
NaOH
25
BzBr
DMF
27
Cl2CHOCH3
TiCl4 / CH2Cl2
28
CH2Cl2
m-ClC6H4CO3H
16
BzBr2
DMF
Ni-Al
NaOH
26
29
(CH3CO)2O / Py
30
Br2/hv
CCl4
31
Dioxane / H2O
25
32

Scheme 8



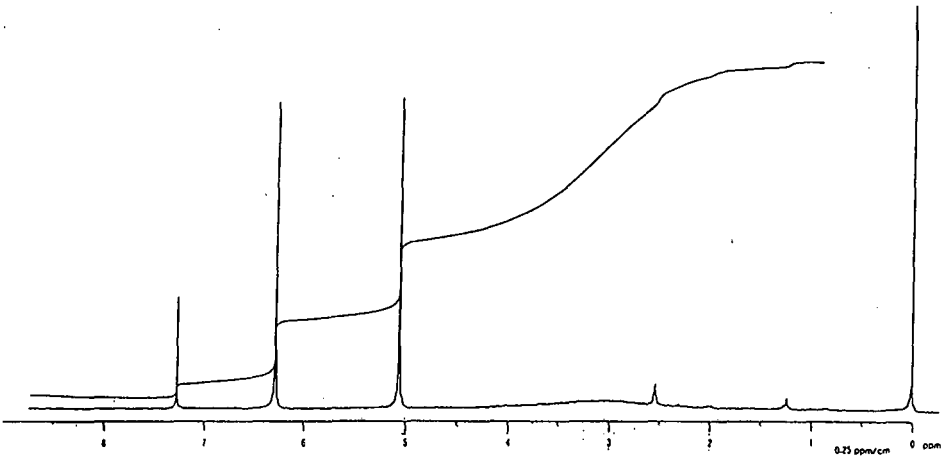
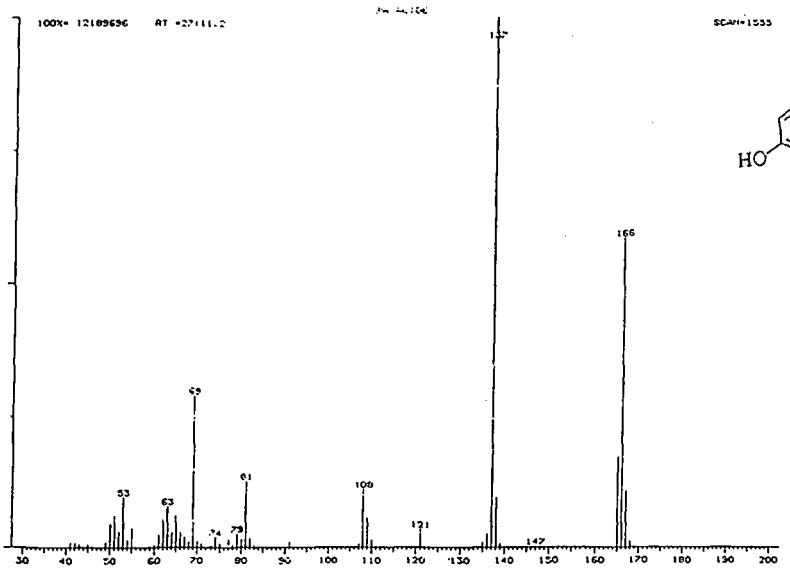


Figure 67



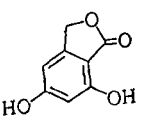


Figure 68

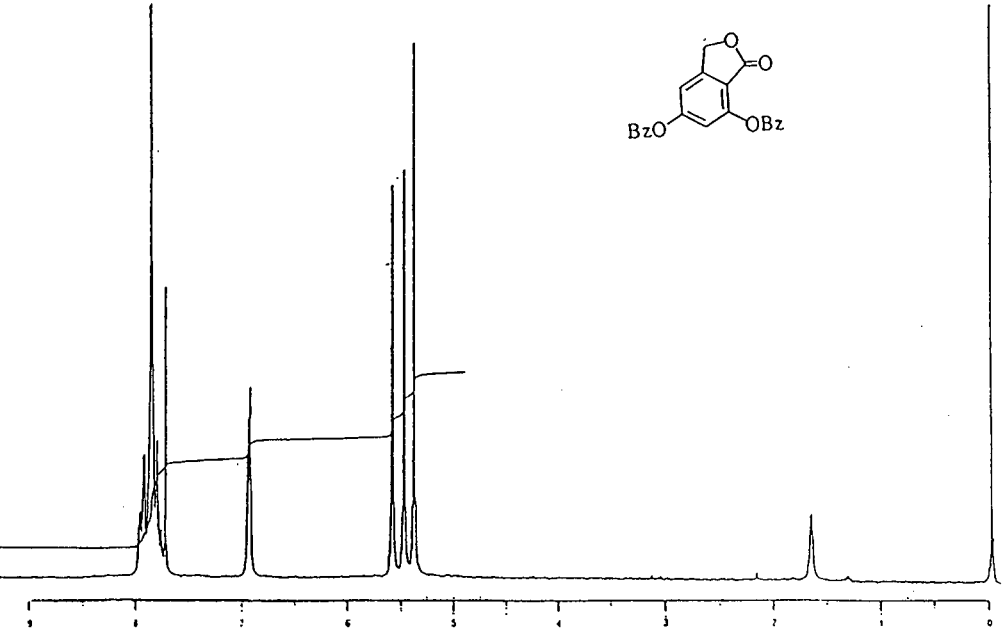


Figure 69

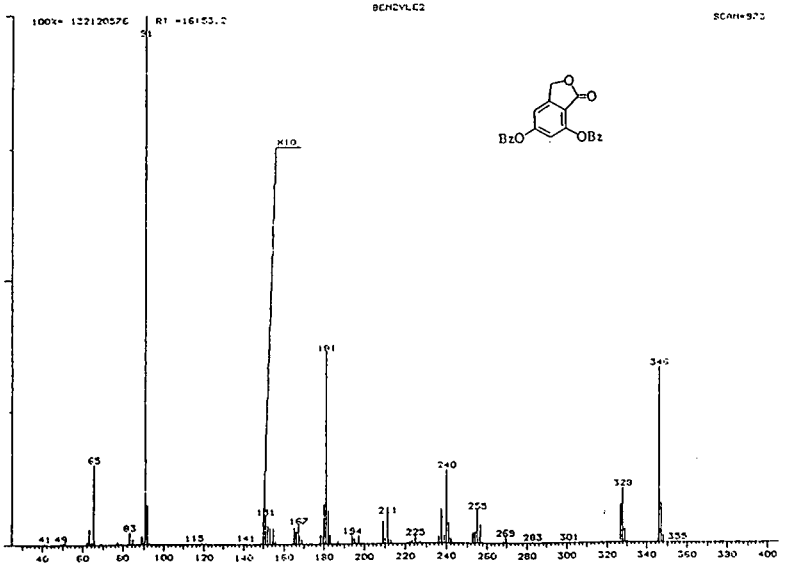
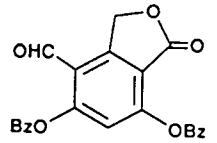


Figure 70



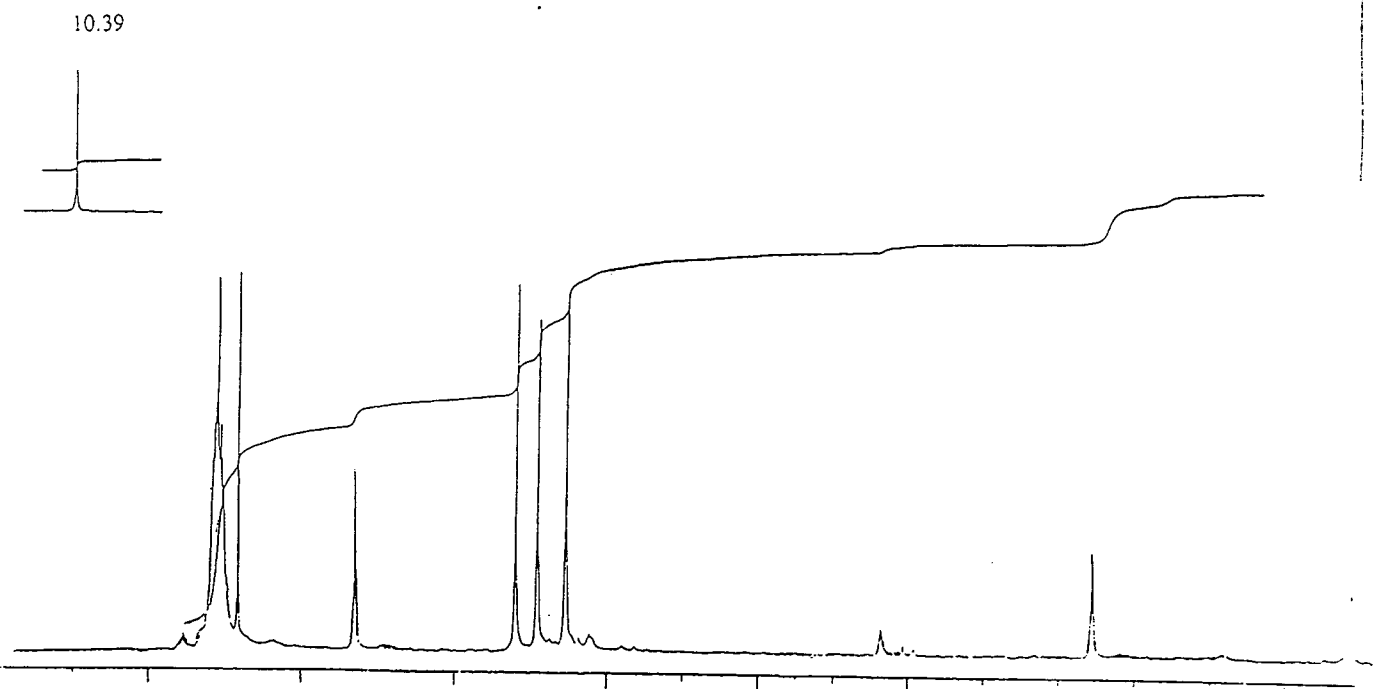
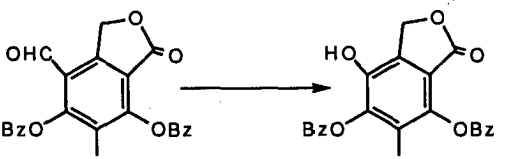


Figure 71

3.3.1 SYNTHETIC ROUTE FOR GROUP II PHTHALIDES

The second alternative method for the syntheses of phthalides with an appropriate substituent and protecting groups is discussed below.

5,7-DIBENZYLOXY-4-HYDROXY-6-METHYLISOBENZOFURAN-1-(3H)-ONE (34)



33 34

In this part of the syntheses of the phthalide group **II**, we designed an appropriate synthetic route for the synthesis of the required compound 5,7-dibenzyloxy-4-hydroxy-6-methylphthalide (**34**). For this purpose we utilized methyl-2,4-dihydroxy-3,6-dimethylbenzoate (**35**) as a convenient precursor for the syntheses of the phthalides in question. We undertook the sequence of reactions outlined in the scheme 9

Acetylation of methyl-β–orcinolate (**35**) with two equivalents of acetic anhydride in pyridine gave the diacetyl compound **36.** Photobromination of the acetyl compound with one equivalent of bromine yielded a mixture of products consisting mainly of the monobrominated compound **37** at the 6-position (41%) and the dibrominated compound **38** at the 3 and 6-positions. Cyclization of the bromobenzyl **3** by treating with dioxane and water (1:1) gave the lactone compounds **39** and **40**. Benzylation of compound **40** gave the dibenzyloxy phthalide **41**. The resultant phthalide on formylation gave the aldehyde **33**. Debenzylation of the formate **33** gave the aldehyde **43.** Baeyer-Villiger oxidation of the resultant formate gave the desired phenol **34.**

Benzylation of compound **35** gave dibenzyloxy ester **44.** Photobromination of this gave three brominated isomers **45, 46, 47**. Cyclization of methyl 6-bromomethane-2,4-dibenzyloxy-3-methylbenzoate (**45**), by treating with dioxane and water (1:1), gave the dibenzyloxyphthalide **41**. The spectroscopic analyses of some of the second group compounds **36, 41** and **43**, are presented in the following (fig. 72 - 77).

2,4- Diacetoxy-3,6-Methylbenzoate (36)

To a stirred solution of methyl-2,4-dihydroxy-3,6-dimethylbenzoate (35), (52 g, 266 mmol) in dry pyridine (60 ml) was added acetic anhydride (49 g, 533 mmol). The resulting mixture was refluxed at 90°C for 4 h. The reaction process was monitored by TLC until traces of the starting product had almost disappeared. The cooled solution was acidified with dilute hydrochloric acid and extracted with ether (3 x 500 ml.). The ether solution was washed with water and saturated brine and dried over $MgSO_4$. Removal of the solvent gave plates, yield 69.56 g, 94%, m.p. 85-87°C.

^{1}H-NMR ($CDCl_3$- 200 MHz) : 1.98 ppm (s, 3H, CH_3-Ar, C-3), 2.30 (3, 3H, AcO-Ar), 2.35 (s, 3H, AcO-Ar), 2.40 (s, 3H, CH_3-Ar, C-6), 3.94 (s, 3H, Ar-$CO_2\underline{CH_3}$), 6.53 (s, 1H, H-Ar).

MS (m/z, 1%) 280 (6), 249 (22), 239 (13), 238 (66), 206 (13), 196 (44), 179 (3), 167 (24), 164 (100), 136 (16), 107 (6), 91 (3), 77 (8), 67 (5), 53 (6), 43 (100).

2,4 - Diacetoxy-6-Bromomethyl-3-Methylbenzoate (37)

The diacetate compound 36, (14 g, 50 mmol) was heated under reflux in carbon tetrachloride, 250 ml, over a 300W incandescent lamp during the dropwise addition of bromine (7.9 g,100 mmol) in carbon tetrachloride (75 ml) for 1 h. The stirred mixture was heated under reflux for a further 6 h. After cooling the solution, the solvent was removed under reduced pressure. TLC analyses of the crude substance indicated a mixture of a number of compounds with two major compounds 37 and 38. The mixture was separated by silica gel column chromatography eluting with ethyl acetate / hexane (3:7). Compound 37 separated as a semi-solid orange substance with yield 5.81 g, 39%

^{1}H-NMR ($CDCl_3$- 200 MHz) : 1.98 ppm (s, 3H, CH_3-Ar), 2,30 (s, 3H, CH_3CO2Ar), 2.35 (s, 3H, CH_3CO_2Ar), 3.97 (s, 3H, CH_3OOC-Ar), 5.12 (s, 2H, Br-$\underline{CH_2}$-Ar), 6.54 (s, 1H, H-Ar).

5,7- Dihydroxy-6-Methylphthalide (39)

The diacetoxy bromo compound 37 (4 g, 11 mmol) was refluxed in dioxane (150 ml) and water (150 ml) for 16 h. The dioxane was evaporated under reduced pressure, and the residual aqueous solution was allowed to stand for a short time. The precipitate was separated and washed with hexane and dried thoroughly. The aqueous filtrate was extracted with ethyl acetate and gave more phthalides. TLC analysis of the crude product indicated the presence of two major products; 5,7-dihydroxy-3-methylphthalide (39), (1.25 g, 62%) and 7-acetoxy-5-hydroxy-3-methylphthalide

(**40**), 24%. The mixture was separated by silica gel column chromatography eluting with ethyl acetate / ligroine (3:7). The major compound **39** was collected and recrystallized from chloroform / methanol (3:1) and gave the dihydroxyphthalide as prisms, m.p. 218-220°C.

^{1}H-NMR (CD_3OD - 200 MHz) : 2.15 ppm (s, 3H, CH_3-Ar) 5.12 (S, 2H, -CH_2-lactone), 6.54 (s, 1H, H-Ar).
MS (m/z, 1%) 181(M^+, 16), 180 (86), 163 (8), 162 (100), 151(43), 134 (9), 122 (5), 107 (3), 106 (9), 95 (16), 93 (8), 83 (19), 77 (19), 69 (15), 67 (27).

5,7-Dibenzyloxy -6-Methylphthalide (**41**)

Dihydroxyphthalide (**39**) (3 g, 16.6 mmol), dry potassium carbonate (4.26 g, 33 mmol) and benzyl chloride (4.2g, 33 mmol) were stirred at 60°C (bath) in dry dimethylformamide (DMF) (100 ml) for 18 h. The mixture was poured into cold dilute hydrochloric acid and extracted with ethyl acetate (3 times). The extract was washed in turn with water and saturated brine and dried over $MgSO_4$. The product recrystallized from light petroleum as prisms, yielded 5.7 g, 95%, (m.p. 209-211°C).

^{1}H-NMR ($CDCl_3$ - 200 MHz) : 2.16 ppm (s, 3H, CH_3-Ar), 5.15 ,5.19 (2s, 2 x 2H, 2 x-CH_2-Ar), 5.28 (s, 2H, -CH_2- lactone group).

MS (m/z, I%) 361(M^+, 8), 360 (21), 269 (7), 181 (9), 91 (100), 77 (28), 65 (30), 53 (23).

5,7-Dibenzyloxy-4-Formyl-6-Methylphthalide (**33**)

A stirred solution of the dibenzyloxyphthalide (**41**) (2.25 g, 6.25 mmol) and dichloromethyl methyl ether (8 g, 62.5 mmol) in dichloromethane (70 ml) was treated dropwise over 1h at 0°C with a solution of titanium(IV) chloride (11 g) in dichloromethane (40 ml). The solution was then stirred at 0°C for 1 h and at room temperature for 5 h. An excess of cold hydrochloric acid was added to the stirred solution and the precipitate formed was separated by filtration. The organic phase was washed with saturated brine and water. Removal of the solvent gave more product. The combined crude mixture was separated by silica gel column chromatography. The resulting aldehyde was recrystallized from methanol giving needles, yield 52%, m.p. 146-150°C.

Analyses
^{1}H-NMR ($CDCl_3$) : 2.08 ppm (s, 3H, CH_3-Ar), 5.09 (2s, 4H, 2 x CH_2-Ar), 5.17 (s, 2H, -CH_2- lactone), 7.79 (m, 10H, 2 x Ar), 10.11 (s, 1H, Ar-CHO).

5,7- Dibenzyloxy-4-Hydroxy- 6-Methylphthalide (34)

A solution of the aldehyde **33** (1.3 g, 3.35 mmol) and m-chloroperbenzoic acid (85%, 2.2 g, 10.86 mmol) in acetic acid (50 ml) was stirred at 90 - 100°C (bath) for 2 h. The residue left upon removal of the solvent was dissolved in dichloromethane and washed exhaustively with saturated aqueous sodium hydrogen carbonate and saturated brine. The solvent was removed and the crude product was dissolved in warm methanol (60 ml), and stirred at room temperature under nitrogen for 1 h with aqueous 10% sodium hydroxide (25 ml). The solution was acidified with dilute hydrochloric acid, and most of the methanol was removed under reduced pressure. The residue was extracted with dichloromethane and the extract was washed with saturated brine. The crude product was crystallized from methanol and gave the phenol **34** as prisms, yield 1.26 g, 48%, m.p. 217- 220°C).

Analyses

^{1}H-NMR ($CDCl_3$ 400 MHz) : 2.32 ppm (s, 3H, CH_3-Ar), 5.11, and 5.17 (2s, 2H each, 2 x CH_2-Ar), 5.21 (s, 2H, lactone methylene group), 11.37 (s, 1H, HO-Ar).

MS. (m/z, 1%) 377 (8), 376 (28), 361 (22), 345 (32), 342 (21), 329 (3), 317 (15), 285 (18), 254 (35), 252 (16), 244 (5), 225 (15), 197 (7), 181 (100),180 (38), 167 (10), 164 (16).

5,7-Dihydroxy-4-Formyl-6-Methylphthalide (42)

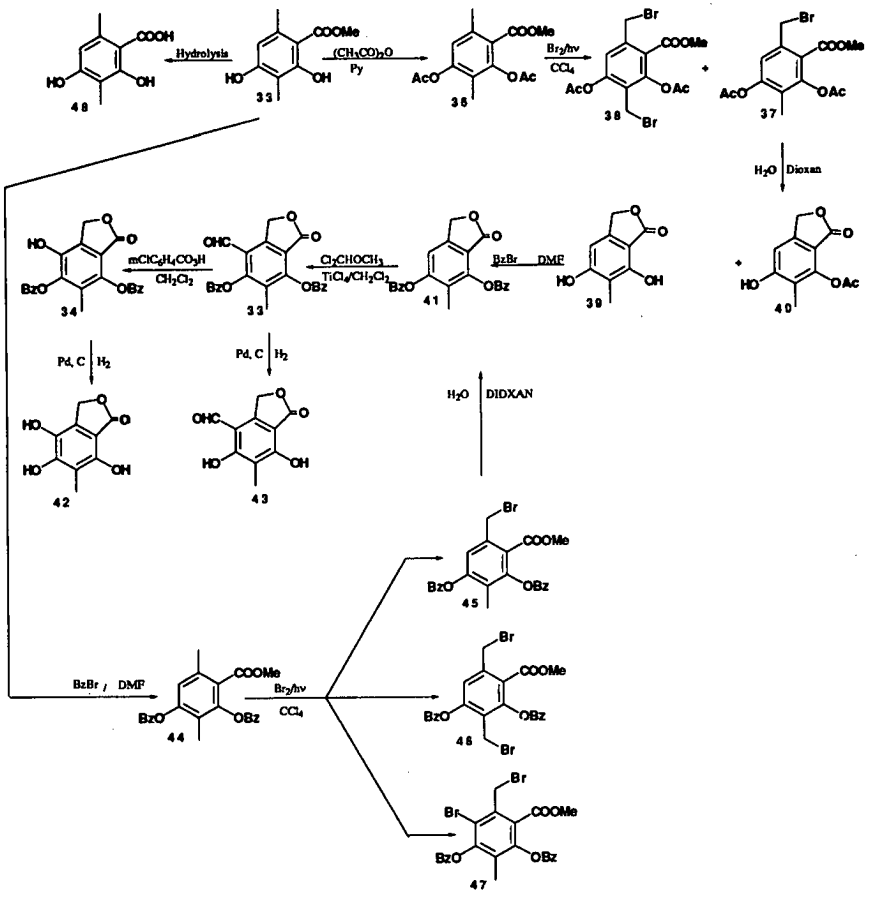
Hydrogenolysis of **33** (1g, 4.8 mmol) over palladium on carbon provided the aldehyde **42**, after the usual work up, yield 0.47 g, 81%, m.p. 157°C

Analyses

1HNMR ($CDCl_3$) 2.08 ppm (s, 3H, CH_3-Ar), 5.18 (s, 2H, -CH_2- lactone),10, 11 (s, 1H, CHO-Ar). MS (m/z, I%), 209 (8), 208 (65), 190 (10), 180 (5), 179 (19), 164 (26), 151 (16) ,134 (8), 122 (4), 105 (6), 89 (5), 77 (10), 65 (12), 52 (10), 39 (25), 36 (100).

5,7-DIBENZYLOXY-6-BROMMETHYL-3-METHYLBENZOATE (44)

Photobromination of compound **44** provided number of isomers (**45, 46, 47**) including the interested compound **45**. Cyclization of this with dioxane and water (1:1) gave the expected phthalide **41**.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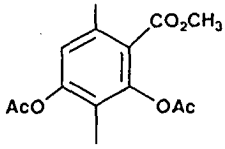
HO
OH
48
Hydrolysis
COOMe
HO
OH
33
(CH3CO)2O
Py
COOMe
AcO
OAc
35
Br2/hv
CCl4
Br
COOMe
AcO
OAc
38
Br
Br
COOMe
AcO
OAc
37
H2O
Dioxan
mClC6H4CO3H
CH2Cl2
Cl2CHOCH3
TiCl4/CH2Cl2
BzBr
DMF
Pd, C
H2
H2O
DIDXAN
BzO
OBz
OAc
34
33
41
39
40
42
43
BzBr / DMF
44
45
46
47

Scheme 9



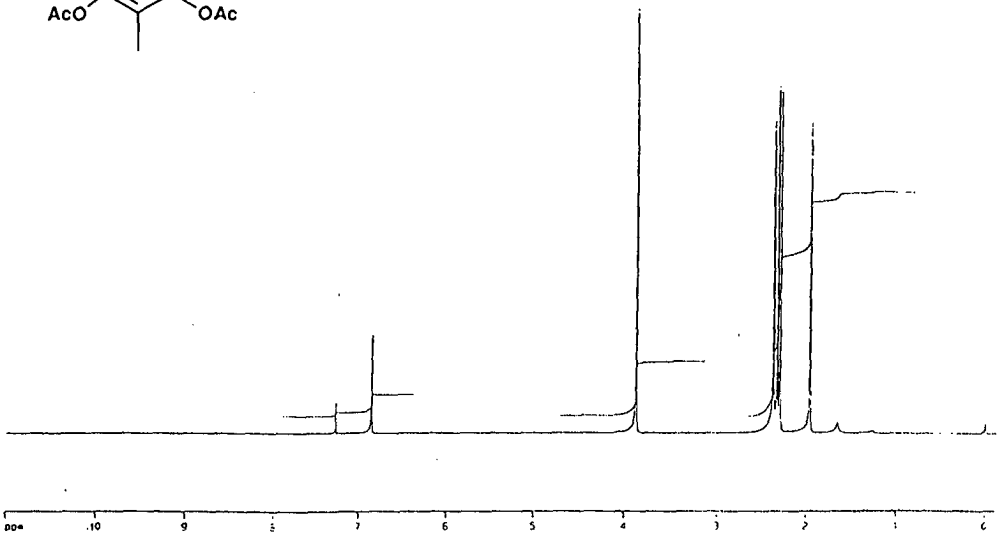


Figure 73

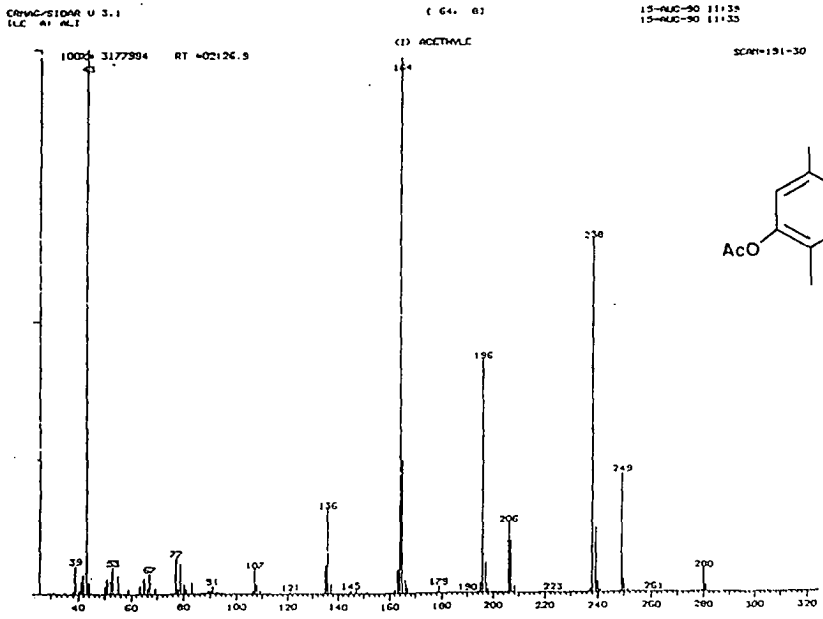


Figure 74

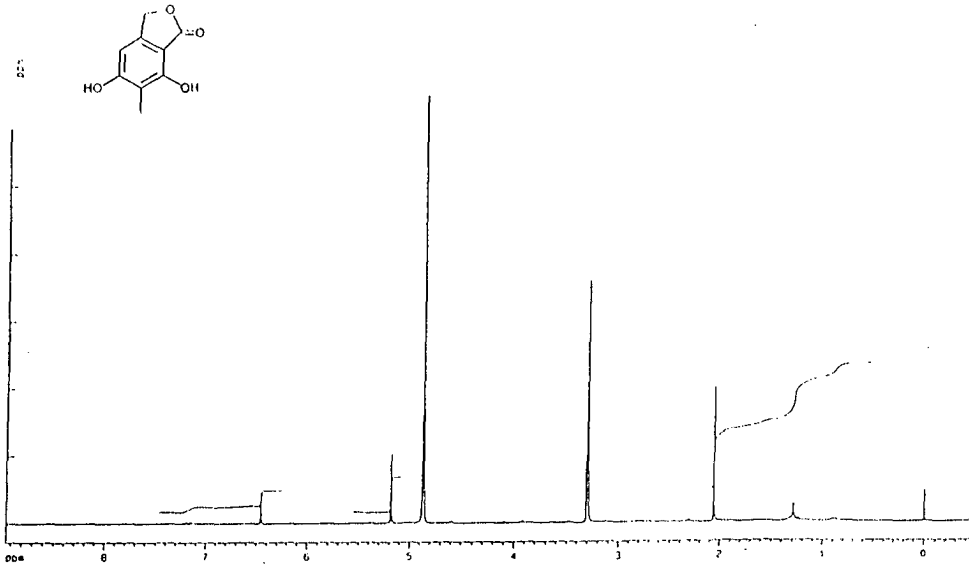


Figure 69

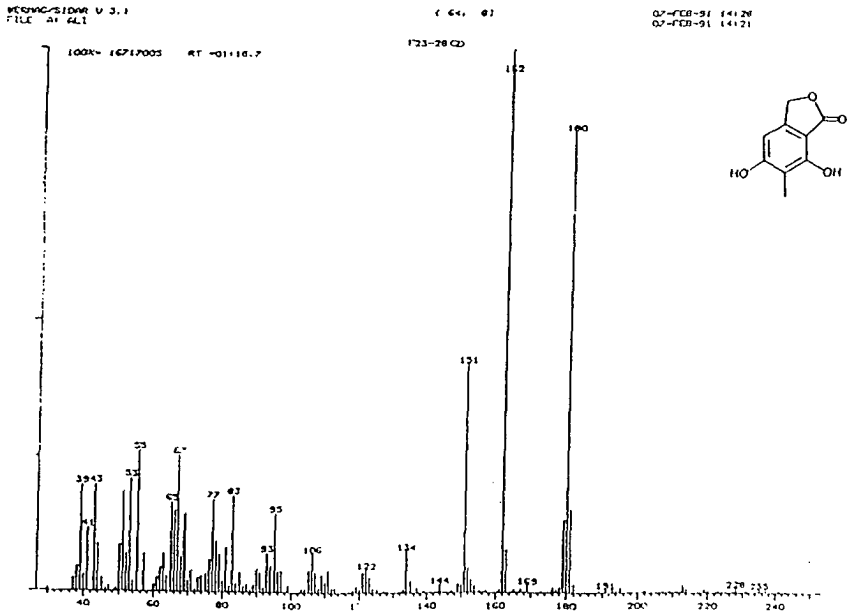


Figure 70

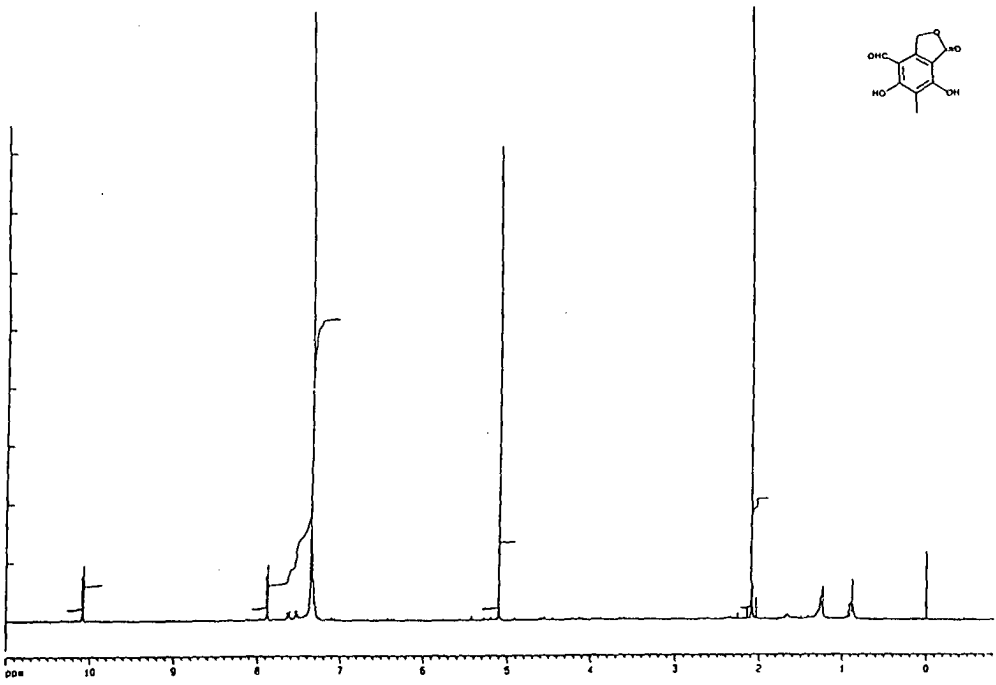


Figure 71

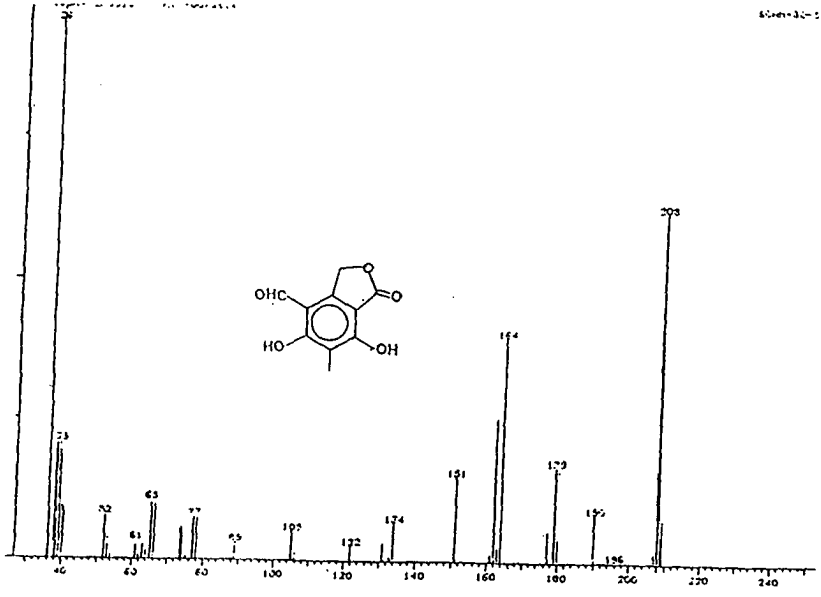


Figure 72

3.4 SYNTHESES OF THREE LACTONIC DEPSIDES

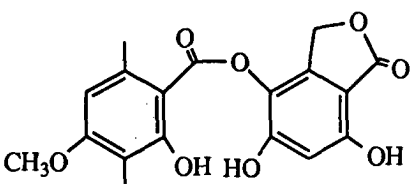
In this part of the chapter we also present results which are expected to throw light on the methods of syntheses of the following three lactonic depsides **49, 50, 51,** which are intended to be used as precursors for the syntheses of lactonic depsidones.

The syntheses of this group of lichen metabolites was undertaken using appropriate protecting groups. The preparation of the necessary precursors, with the appropriate substituents and protective groups, have been considered the most important part for the syntheses of lactonic depsides, intended to be used as precursors for the syntheses of lactonic depsidones in the final step of this work. Hence, the mononuclear precursors for the syntheses of the required depsides were almost all prepared and are presented in the schemes 8 and 9.

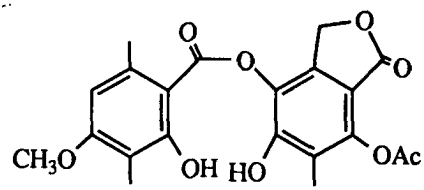
The final step for the syntheses of the lactonic depsides is the esterification between benzoic acids and the phthalide groups. Condensation between the substituted benzoic acid **43** and the phenol derivatives **16** by treatment with trifluoroacetic anhydride followed by catalytic hydrogenolysis gave the corresponding depside **49** in good yield. Similarly, condensation between benzoic acid **53** and dibenzyloxyphthalide **55** followed by debenzylation gave the corresponding depsides **50.**

In addition, condensation between benzoic acid **57** and the phthalide **58** in the same method as above, gave the depside **59.** Subsequent hydrogenolysis of this ester over palladium on carbon afforded the corresponding depside **51** in low yield. The above procedure represents an appropriate method for the syntheses of the lactonic lichen depsides of this kind.

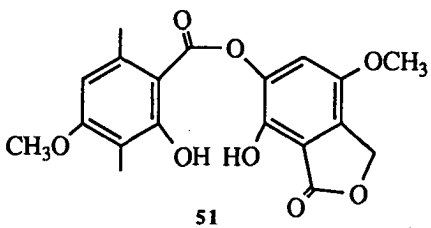
The spectroscopic properties, both ^{1}H-NMR and Mass spectra of these depsides **49, 50, 51** are presented in the following (fig. 78-83)



49



50

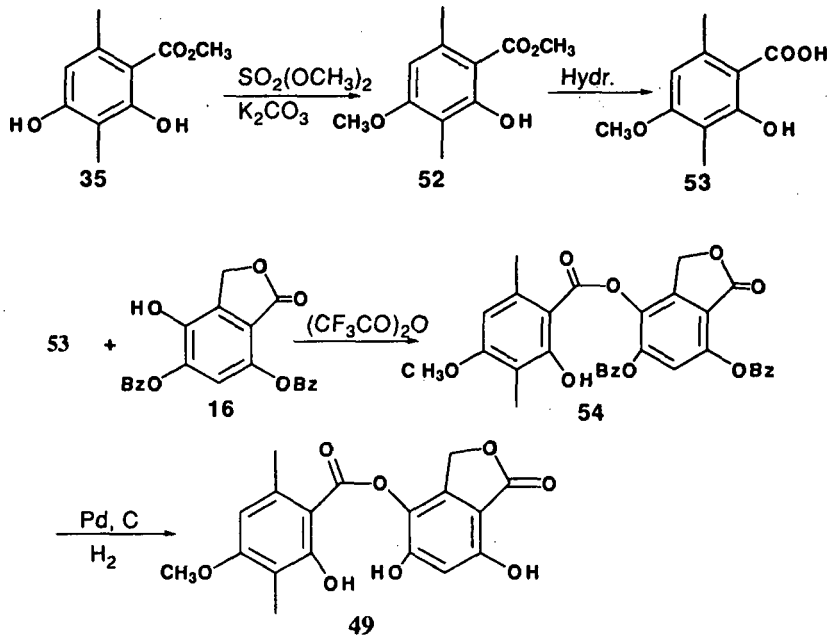


51

3.4.1 SYNTHETIC ROUTES FOR THE THREE DEPSIDES 49, 50, 51

SYNTHESIS OF DEPSIDE 49

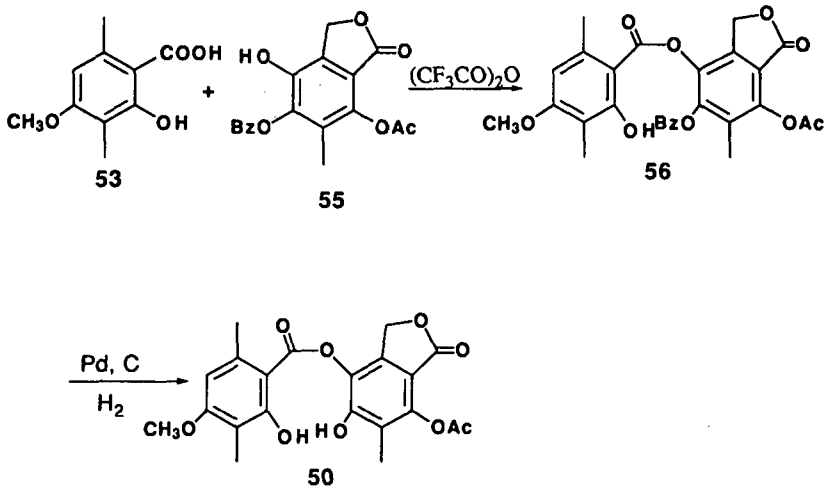
Esterification of 2-hydroxy-4-methoxy-3,6-dimethylbenzoic acid (**53**) with 5,7-dibenzyloxy-4-hydroxyphthalide (**16**) and subsequent debenzylation gave the depside **49**, (scheme 10).



Scheme 10

SYNTHESIS OF DEPSIDE 50

Condensation of 3,6-dimethyl-2-hydroxy-4-methoxylbenzoic acid (**53**) with 7-acetoxy-5 benzyloxy-4-hydroxy-6-methylphthalide (**55**) in the presence of trifluoroacetic anhydride yielded the depside **56**. Hydrogenolysis of this gave the depside **51**, (scheme 11).

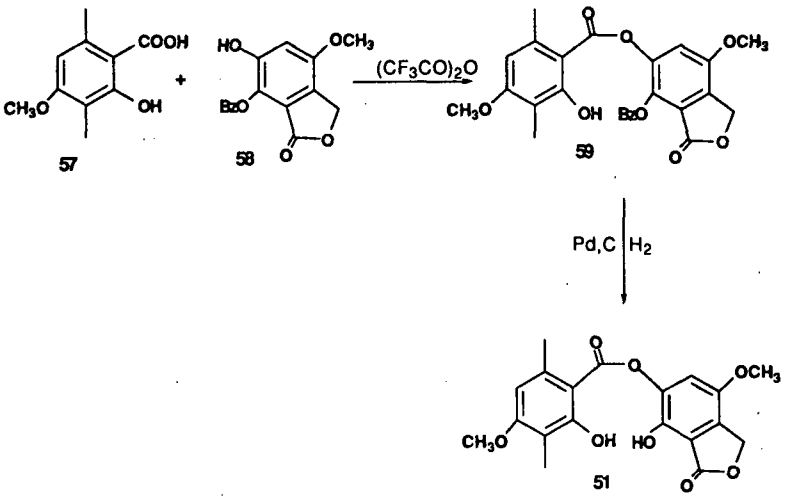


Scheme 11

SYNTHESIS OF DEPSIDE 51

Condensation of 3,6-dimethy-2-hydroxy-4-methoxybenzoic acid (**57**) with 7-benzyloxy-6-hydroxy-4-methoxyphthalide (**58**) gave the depside **59.** Debenzylation of this yielded the depside **51**, (scheme 12). Phthalide **58** was synthesized in a similar proceture as those shown in the two schemes 8 and 9 . This has been prepared specially for the synthesis of depside **51**

Synthesis of Depside 51



Scheme 12

3.4.2 Experimental

SYNTHESIS OF DEPSIDE 49

A solution of 3,6-dimethyl-2-hydroxy-4-methoxybenzoic acid (**53**), (1.5 g, 7.6 mmol) and 5,7-dibenzyloxy-4-hydroxyphthalide (**16**), (2.87 g, 7.6 mmol) in anhydrous toluene (40 ml) and trifluoroacetic anhydride (10 ml) was allowed to stand at room temperature for 2.5 h. The solvent was then removed and the residue was diluted with water and extracted with ethyl acetate. The extract was washed with saturated brine and water and dried over $MgSO_4$. The solvent was removed and the crude mixture was separated and purified over silica gel plates eluted with ethyl acetate / light petroleum (3 : 7). The slow moving major band yielded the depside ester **54**. Subsequent hydrogenolysis of **54** with palladium on carbon provided the corresponding depside **49**, which was recrystallized from dichloromethane / light petroleum giving prisms, yield 1.87 g, 68%, m.p. 106-108°C.

^{1}HNMR ($CDCl_3$ 200 MHz) : 2.12 (*s*, 3H, CH_3-Ar), 2.45 (s, 3H, CH_3-Ar), 3.94 (*s*, 3H, CH_3O-Ar), 5.14 (s, 2H, -CH_2- lactone methylene group), 6.21(s, 2H, 2 aromatic protons).

Mass spectrum (m/z, I%) 361 (M^+, 5), 360 (M, 67), 342 (5), 283 (3), 254 (15), 251 (11), 225 (5), 196 (3), 195 (4), 181(65), 180 (8), 165 (3), 163 (7), 105 (5), 91 (100), 77 (3), 65 (5).

SYNTHESIS OF DEPSIDE 50

A solution of 2-hydroxy-3,6-dimethyl-4-methoxybenzoic acid (**53**), (1.2 g, 6.12 mmol), 7-acetoxy-5-benzyloxy-4-hydroxy-6-methylphthalide (**55**) (2.01 g, 6.12 mmol) in anhydrous toluene (45 ml) and trifluoroacetic anhydride (11 ml) were allowed to stand at room temperature for 2.5 h. The sovent was removed, and the residue was diluted with water and extracted with ethyl acetate. The extract was washed with saturated brine and water and fairly dried. The solvent was evaporated, and the crude mixture was separated and purified over silica gel column chromatography eluted with ethyl acetate / light petroleum (2:8). The fractions migrating as F 6/12 were collected and gave the ester **56**. Catalytic hydrogenolysis of this ester provided the expected depside **50**. This was recrystallized from dichloromethane and gave prisms, yield 1.70 g, 67%, m.p.127- 129°C.

^{1}H-NMR ($CDCl_3$ 200 MHz) : 2.08 (s, 3H, CH_3-Ar), 2.24 (s, 3H, CH_3-Ar), 2.27 (s, 3H, CH_3-Ar), 2,42 (s, 3H, CH_3CO_2-Ar), 3.94 (s, 3H, CH_3O-Ar), 4.94 (s, 2H, -CH_2- lactone methylene group), 6,76 (s, 1H, H-Ar).

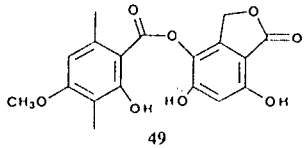
Mass spectrum (m/z, I%), 416 (7), 414 (8), 317 (5), 298 (6), 276 (7), 274 (11), 249 (100), 239 (24), 238 (51), 214 (4), 207 (13), 196 (41), 181 (3), 165 (11), 164 (40), 136 (19), 135 (8), 119 (5), 107 (8), 91 (4), 77 (11).

SYNTHESIS OF DEPSIDE 51

The depside **51** was synthesized in the same procedure as above.
Condensation of 3,6-dimethyl-2-hydroxy-4-methoxybenzoic acid (**57**), (1.21 g, 6.15 mmol) with 7-benzyloxy-6-hydroxy-4-methoxyphthalide (**58**), (1.76 g, 6.15 mmol) gave the depside **59**. Catalytic hydrogenolysis gave depside **51** with relatively low yield 1.31 g, 57%.

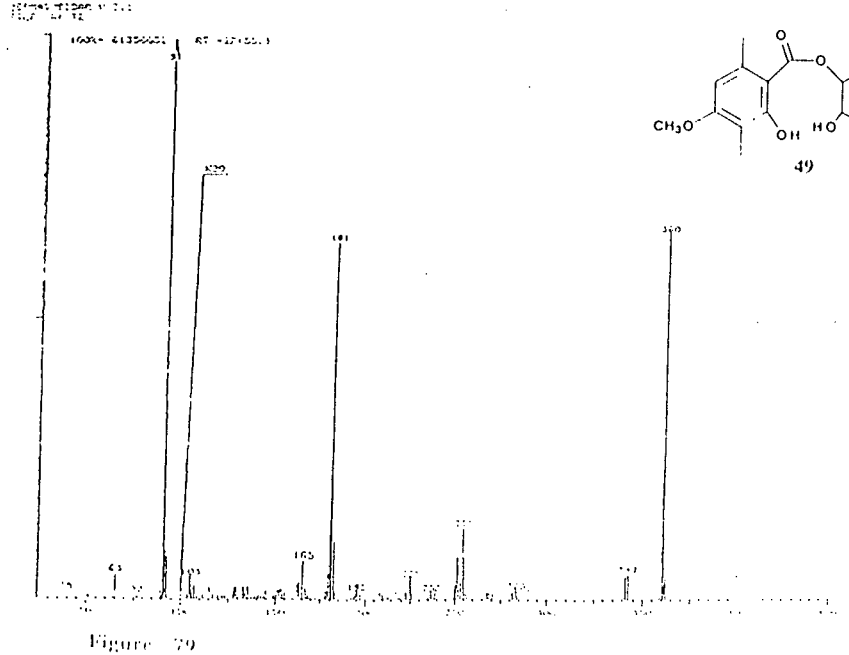
^{1}H-NMR ($CDCl_3$ 400 MHz) : 2.18 (s, 3H, CH_3-Ar), 2.57 (s, 3H, CH_3-Ar), 3.84 (s, 3H, CH_3O-Ar), 3.86 (s, 3H, CH_3O-Ar), 5.17 (s, 2H, -CH_2- lactone group), 6.49 (s, 2H, 2 x H-Ar).

Mass Spectrum (m/z, I%) 374 (M, 2), 358 (4), 318 (3), 264 (3), 21 (3), 196 (18), 182 (21), 181 (7), 180 (5), 166 (17), 164 (100), 156 (16), 153 (10), 151 (15), 150 (21), 141 (3), 110 (3), 101 (14), 95 (3), 83 (31), 77 (2), 69 (3), 59 (46), 43 (100).



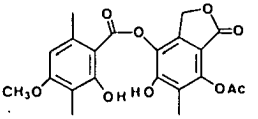
49

Figure 78



49

Figure 79



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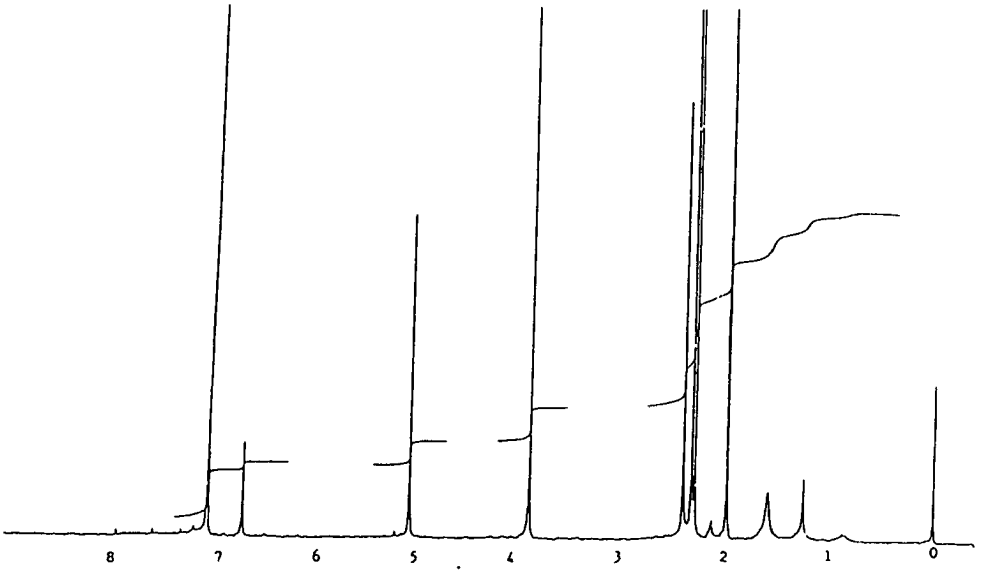
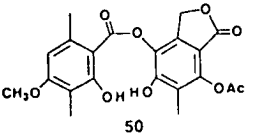


Figure 80



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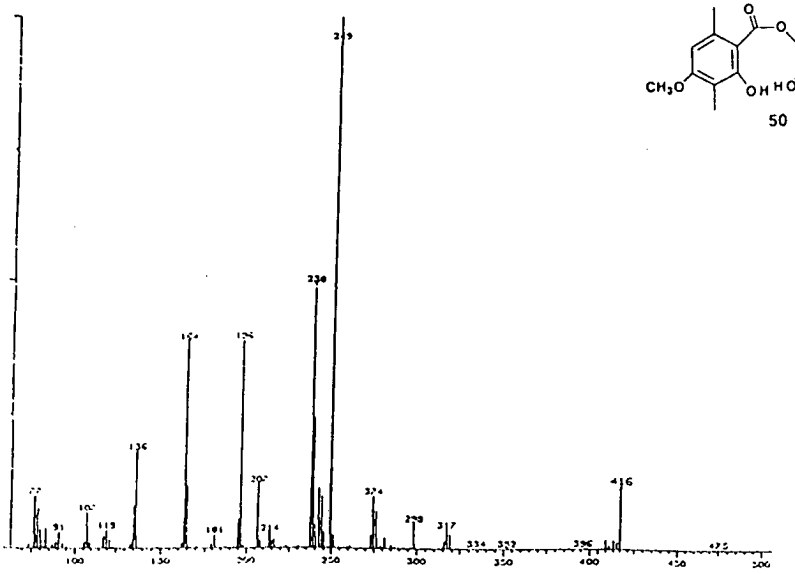
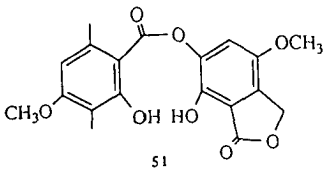


Figure 81



51

Figure 82

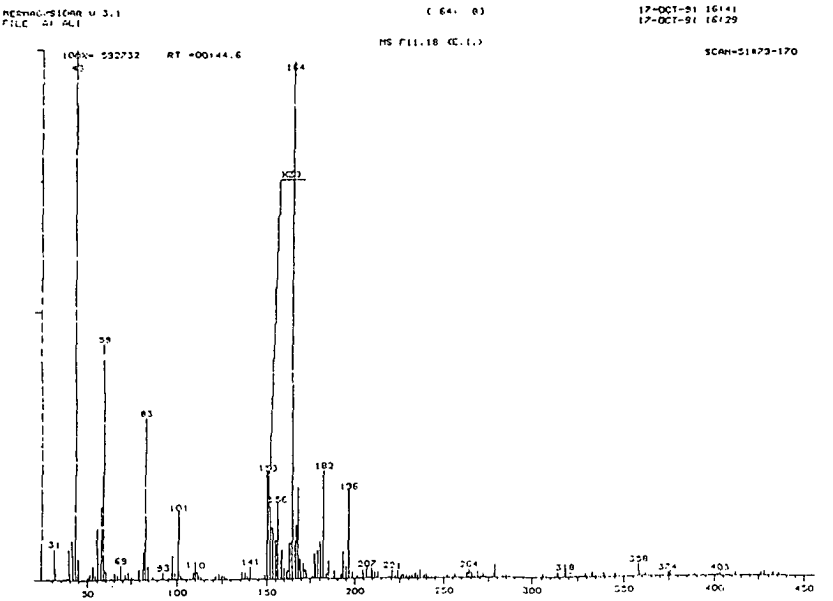


Figure 83

3.5 ATTEMPTS TO SYNTHESIZE NATURAL LACTONIC DEPSIDONES AND DIPHENYL ETHERS

Several attempts were made to synthesize natural depsidones containing a hydroxyphthalide cycle utilising the following three synthetic routes:

1- Oxidative Coupling of Benzophenones.
2- Smile's Rearrangement.
3- Ullmann Reaction.

Our primary objective in this part was to design an appropriate method for the syntheses of natural depsidones containing a lactonic cycle.

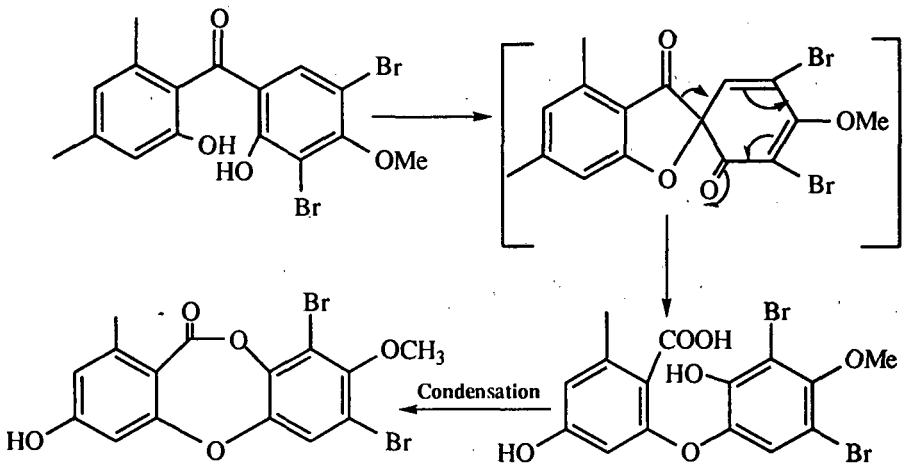
Before proceeding to the three main synthetic routes, the classical method was attempted. Upon treatment of a depside with copper bronze in pyridine, no trace of the required cyclized product could be detected. A variety of depsides failed to cyclize to the required depsidones under these reaction conditions. Hence, the use of the classical synthetic route was abandonned.

The First Synthetic Route: Oxydative Coupling of Benzophenones

This synthetic route is based on benzophenone-grisadienedione-depsidone interconversion. The most important part of this reaction route is the preparation of an appropriate benzophenone which is a precursor for the syntheses of the required depsidones.

Although benzophenones are stable and important reactives, the short-lived intermediate grisadienediones are comparatively unstable [45]. Condensation of an appropriately substituted benzoic acid with a phenol is expected to provide a normal benzophenone. Oxidative coupling of the benzophenone was claimed to yield grisadienedione. The resulting grisadienedione could then be converted into the corresponding depsidone [92, 93].

The Mechanism of the Benzophenone-Grisadienedione-Depsidone Interconversion

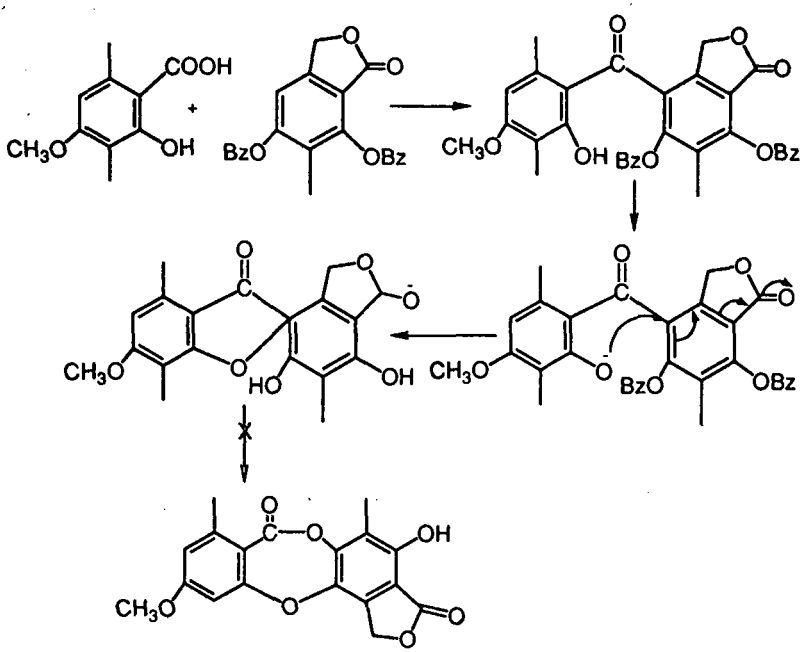


Depsidone

Scheme 13

In this research work, an attempt was made to synthesize depsidones containing a lactone group, following the above mentioned theoretical basis. The Friedel-Craft reaction between the benzoic acid and dibenzyloxyphthalide and subsequent hydrogenolytic debenzylation gave a mixtue of three products. Immediate treatment of the crude mixture with potassium hexacyanoferrate (III) in aqueous potassium carbonate solution (two min), provided a mixture consisting mainly of four products. Separation of the mixture into the individual components, failed to provide the expected result and no traces of the required depsidone could be detected in this reaction mixture.

It is believed that the expected grisadienedione formation did not take place, or did not undergo rearrangement to the corresponding depsidone. Probably, the presence of the lactonic cycle inhibited the formation of the grisadienedione or conversion of this to the depsidone. The opening of the lactonic cycle, which could have possibly taken place during the reaction process, would be another possible cause which may have inhibited the formation of grisadienedione. However, this reaction route is open to speculation in the future studies.



Scheme 14

The Second Synthetic Route: Smiles Rearrangement

As mentioned previously, diphenyl ethers constitute a relatively rare group of lichen metabolites. Few of the known derivatives appear to be derived catabolically from co-occurring depsidones [94, 95].

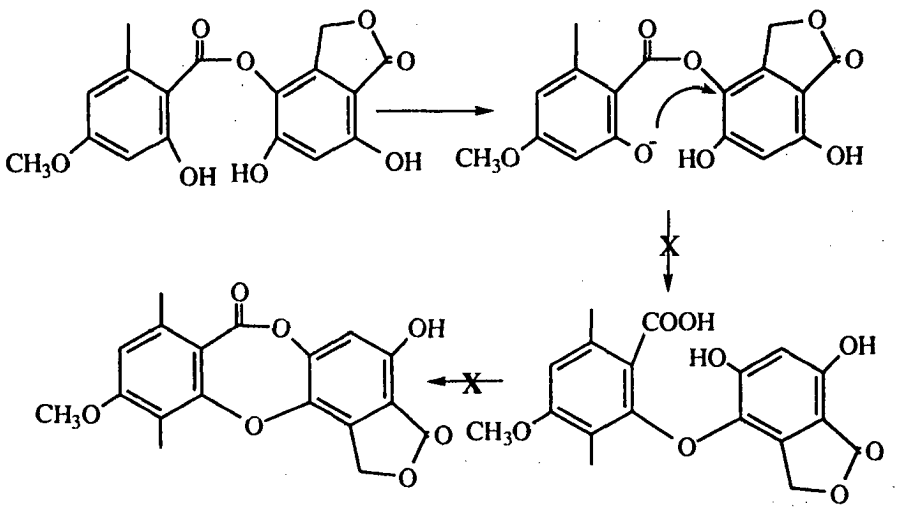
Previous research work on the syntheses of depsidones containing a lactonic-C cycle did not give credence to the proposal that Smiles rearrangement of appropriate depsides would provide a viable biosynthetic pathway to such lactonic depsidones [37, 96].

In this work several attempts were made to synthesize this type of depsidone by employing Smiles rearrangement and using appropriate depsides (scheme 14).

Smiles rearrangement of the depside was carried out by treating it with anhydrous lithium

carbonate in dry dimethylsulfoxide solution at room temperature. Despite repeated attempts to modify the reaction conditions, the results of this reaction route did not provide the expected compound. In actual fact, the TLC analyses of the crude mixture showed the presence of several substances consisting mainly of the starting material and fragments of the depside Chromatographic separation of the mixture did not show traces of the expected result, hence it was assumed that the Smile's rearrangement was not occurring.

Hydrolysis of the crude reaction mixture and subsequent esterification with trifluoroacetic anhydride did not lead to the expected cyclization.



scheme 15

The reason why a similar Smile's rearrangement of an appropriate depside, which did not contain a lactonic cycle (see scheme 13), provided a viable biosynthetic pathway to the corresponding diphenyl ether formation, but not in the case of a depside which contains a lactone group, requires further speculation. It is assumed that in the case of this kind of depsides, it is due to the presence of the lactonic cycle which is inhibiting the rearrangement or the formation of the expected diphenyl ether.

3.6 SYNTHESES OF TWO DIARYL ETHERS

The Third Route: Ullmann Reaction

Following the failure of the first two synthetic routes so far attempted, we reverted to the third alternative. For further attempts of the syntheses of the required depsidones, we utilized an Ullmann diphenyl ether syntheses. The ether so obtained was modified by selective introduction and conversion of the substituent groups [97].

For the syntheses of the required diaryl ethers, we employed the following bromophthalides and the phenols as appropriate precursors. The Ullmann reaction of the phenol **35** and the bromophthalide **60**, (Scheme 16) by treatment with dry potassium carbonate and dry pyridine led to the formation of the diphenyl ether **61**. Catalytic hydrogenolysis of **3** with palladium on carbon gave diphenyl ether, 5,7-dihydroxy-6-methyl-4-(-5-hydroxy-2-methoxycarbonyl-3,6-dimethylphenoxy) phthalide (**62**) in very low yield. Hydrolysis of this ether and subsequent condensation with trifluoroacetic anhydride failed to lead to cyclization to the corresponding depsidone **63** under this reaction condition, despite repeated attempts to modify the reaction procedure and condition.

Similarly the Ullmann reaction of the phenol **64** with the bromophthalide **65** (scheme) by treatment with dry potassium carbonate and dry pyridine led to the formation of the diphenyl ether **66**. Hydrogenolysis of **66** over palladium on carbon, provided the diaryl ether, 5-hydroxy-7-isopropyl-6-methyl-4-(-5-acetoxy-2-methoxycarbonyl-3,6-dimethylphenoxy) phthalide (**67**). Hydrolysis of this ether and subsequent condensation with trifuoroacetic anhydride, failed to cyclize to the corresponding depsidone **68**. The spectroscopic analyses of the two diaryl ethers **62**, **67** are presented in the following (fig. 84-87).

3.6.1 Experimental

SYNTHESIS OF DIARYL ETHER 62

The phenol **35** (1.3 g, 5.75 mmol), the bromo compound **60** (2.14 g, 5.75 mmol), and dry finely divided potassium carbonate (2.4 g) in dry pyridine (8 ml) were stirred and heated gradually to 130 °C (bath) under nitrogen. Copper (II) oxide was added and the mixture was stirred and heated

at 150 °C (bath) for 20 h. The cooled mixture was diluted with hot dichloromethane and filtered. The filtrate was washed with dilute hydrochloric acid and with saturated brine. The crude product was chromatographed over a silica gel column with 20% ethylacetate / hexane as eluent. Hydrogenolysis of compound **61** provided the diaryl ether **62** with very low yield (0.27 g, 11%).

^{1}H-NMR ($CDCl_3$) 2.08 (s, 3H, CH_3-Ar), 2.31 (s, 3H, CH3-Ar), 2.42 (s,3H, CH_3-Ar), 3.97 (s, 3H, $\underline{CH_3}COOAr$), 5.18 (s, 2H, -$\underline{CH_2}$-, lactonic group), 7.12 (s, 1H, H-Ar), 7.86 (s, 1H, HO-Ar).
Mass Spectrum (m/z, I%) 374 (M, 15), 360 (14), 337(11), 323 (10), 273 (34), 255 (75), 211 (32), 210 (18), 196 (77), 181 (3), 180 (12), 179 (72), 178 (26), 165 (94), 164 (100), 150 (5), 149 (9), 136 (34), 108 (18), 107 (12), 77 (28), 69 (21), 57 (30), 43 (39).

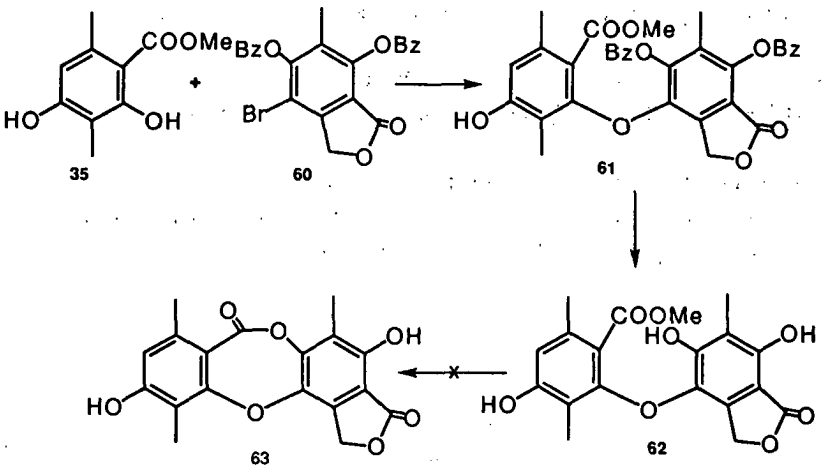
SYNTHESIS OF DIARYL ETHER 67

The second diaryl ether **67** was synthesized in a similar method as above.
The acetyl compound **64** (0.5 g, 2.1 mmol), the bromophthalide **65** (0.82 g, 0.21 mmol) and dry finely divided potassium carbonate (0.92 g) in dry pyridine (3 ml) were heated gradually to 130°C (bath) under nitrogen. Copper (II) oxide was added and the mixture was stirred and heated at 150°C (bath) for 20 h. Hydrogenolysis of compound **66** provided the diaryl ether **67** with very low yield (0.11 g, 11%).

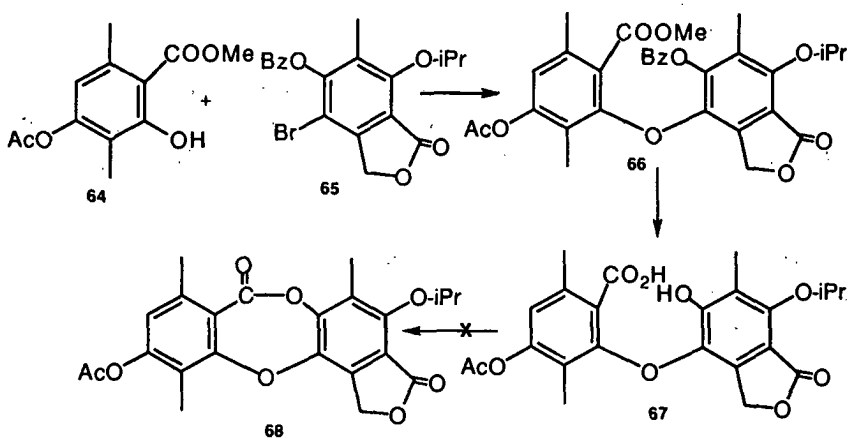
^{1}H-NMR ($CDCl_3$ 400 MHz) : 1.36 (d, 6H, $(CH_3)_2\underline{CH}$-, isopropyl group), 2.08 (s, 3H, CH_3-Ar), 2,11 (s, 3H, CH_3-Ar), 2.26 (s, 3H, CH_3-Ar), 2.38 (s, 3H, $\underline{CH_3}COO$-Ar), 3.96 (s, 3H, $\underline{CH_3}OOC$-Ar), 4.75 (m, 1H, -$\underline{CH}$-, isopropyl methine group), 5.38 (s, 2H, -$\underline{CH_2}$-, lactone group), 6.18 (s, 1H, H-Ar).

Mass spectrum (m/z, I%), 459 (M^+,19), 458 (37), 440 (11), 414 (6), 391 (5), 379 (13), 349 (10), 323 (19), 300 (9), 275 (100), 244 (75), 243 (75), 216 (42), 187 (9), 186 (11), 107 (6), 91 (2), 77 (13), 67 (7), 51 (12), 39 (14).

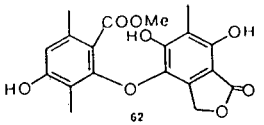
Synthesis of the diaryl ether 5,7-dihydroxy-6-methyl-4-(-5-hydroxy-2-methoxycarbonyl-3,6-dimethylphenoxy)phthalide (62), (scheme 16).



Synthesis of the diaryl ether 5,7-dihydroxy-6-methyl-4-(-5-hydroxy-2-methoxycarbonyl-3,6-dimethylphenoxy)phthalide (67), (scheme 17).



Scheme 17



62

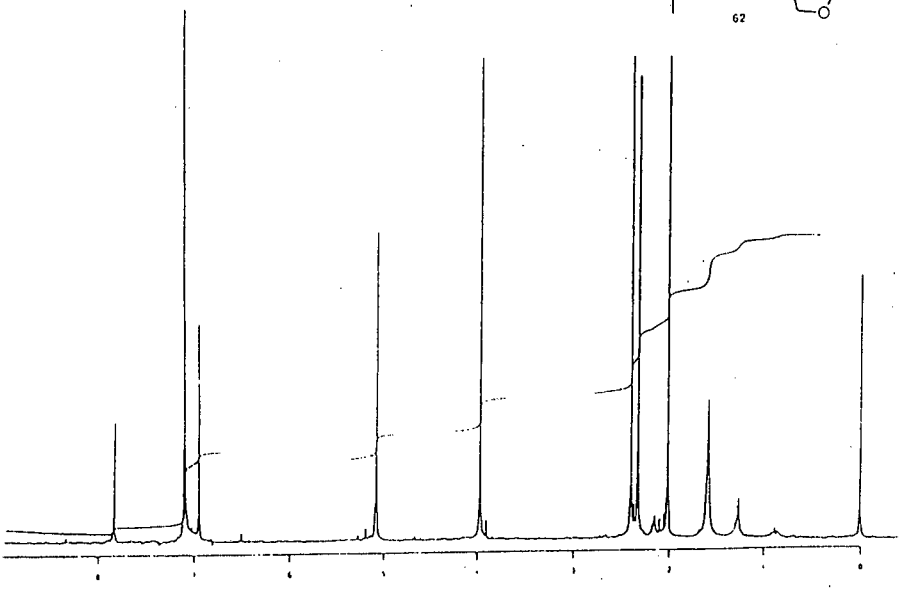
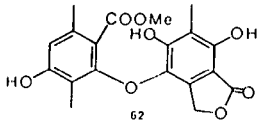


Figure 84



62

Figure 85

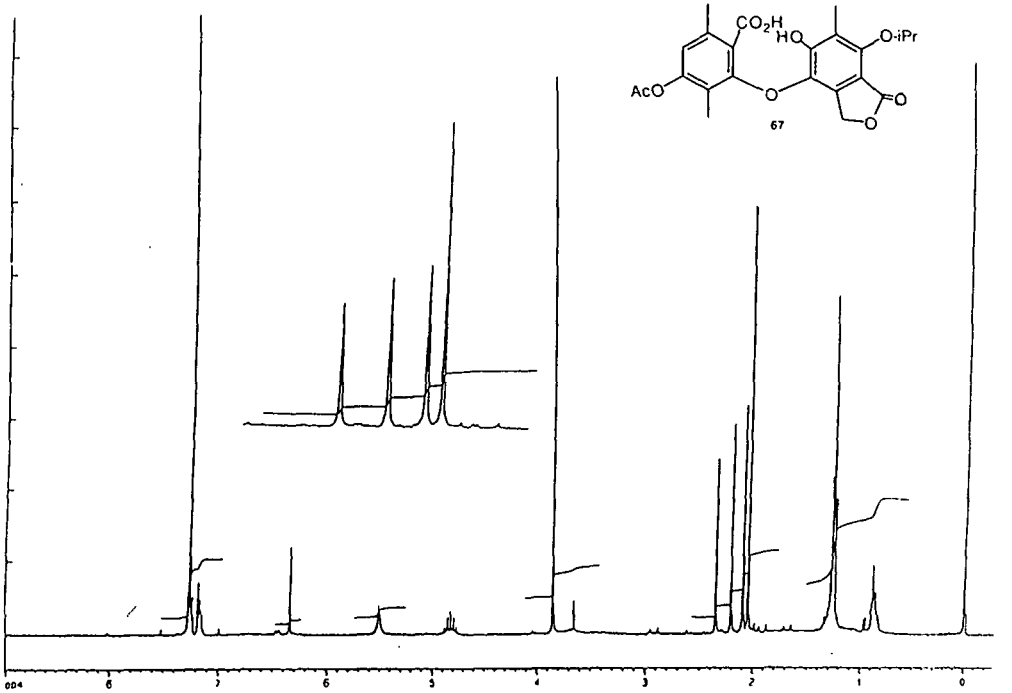


Figure 86

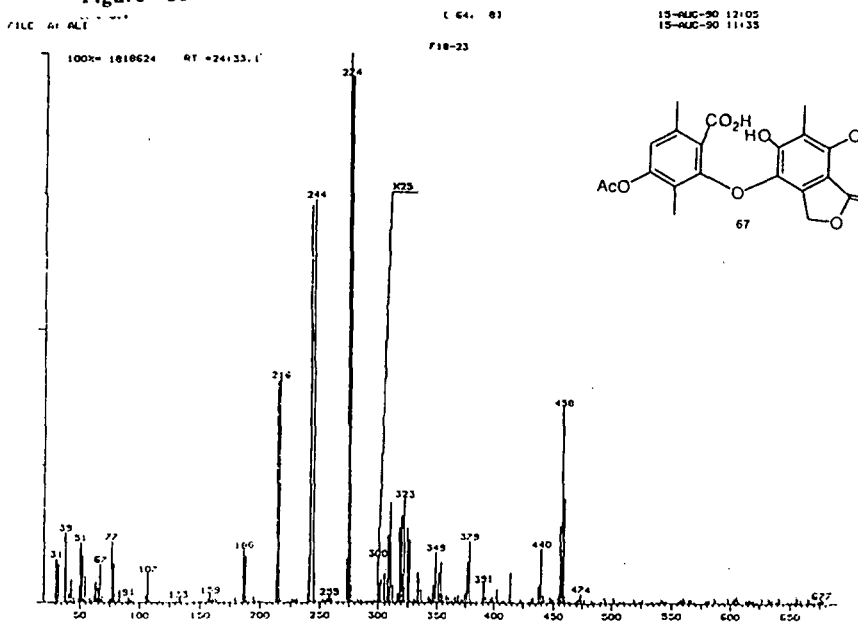


Figure 87

4 EXPERIMENTAL PART

4.1 Extraction

The dried and finely ground lichen materials were extracted in a soxhlet extractor with dry ether, hexane, and acetone subsequently.

4.2 Chromatographic Techniques

Different chromatographic methods: TLC, LC, and HPLC were utilized for the separation of the crude mixtures. The three common standard solvent systems were mainly employed as eluents. Preparative-layer chromatograms were carried out on thick layer plates (20 x 20 by 0.2 mm or 0.1 mm) using often silica gel, 60 F_{254} (Merck) as absorbent. Bands were detected by exposure to short wave length ultraviolet and visible light.
Glass columns of different sizes were employed for the separation of the main crude products. Different absorbents, particularly silica gel 0.015-0.040mm (Merck), and Sephadex LH-20 were utilized as stationary phase. HPLC reverse phase was utilized to separate compounds of very close *Rf* values.

4.3 Spectroscopy

The IR spectra were recorded as KBr pellets in a Perkin-Elmer Spectrometer model 521or 1701. The ^{1}H-NMR spectra were recorded at 200 MHz and 400 MHz on a Brucker WP-200 and AMX-400. The chemical shifts were measured on the δ scale relative to TMS as an internal standard. Mass spectra were determined on Nermag R30-10 instrument operating at 70 eV in EI or CI mode.

5. CONCLUSION

5.1 THE ANALYTICAL PART

This research work involved an investigation of the chemical components in two rare lichen species: (a) *Usnea articulata* (L.) Hoffm. (Fig. 5) and (b) *Usnea hesperina* Mot. (Fig. 54).

The primary aim of this research work was to isolate the secondary metabolites contained in this lichens.

Very little research work was done on the lichen *Usnea articulata* (L.) Hoffm., and very few components had been isolated from this, and reported perviously, such as usnic acid [1,2, 3, 4]. This study provides a detailed information of a number of supplementary lichen components isolated from the lichen under investigation. The types of components isolated from this lichen were in a complex mixture of monophenolic derivatives, depsides, depsidones, dibenzofuran derivative, triterpenes and sterols. Considerable quantities of fatty acids, triglycerides, and potassium oxalate contained in this lichen but were not further investigated.

In this research work 20 components were isolated form *Usnea articulata* (L.) Hoffm. Seventeen of this compounds were fully identified. The presence of one triterpene, tylolupenyl acetate and two sterols, stigmasterol and β-sitosterol have been detected by TLC. and identified by mass spectrometry.

Usnea hesperina Mot. has been analysed for the first time. Large scale extraction of the lichen led the isolation of nine compounds including some very rare components. These consisted of depsidones, dibenzofuran derivative, monophenolic derivatives, one common sterol and two triterpenes.

Identification of the isolated individual compounds from both the two lichen was based on their physical properties, microchemical analyses, chromatographic and spectroscopic analyses.

Compounds Isolated From *Usnea articulata* (L.) Hoffm.

I- Dibenzofuran derivative
- 1- (+) Usnic Acid

II- Depsidones
- 1- Fumarprotocetraric Acid
- 2- Protocetraric acid

III- Depsides
- 1- Barbatic Acid
- 2- Diffractaic Acid
- 3- Atranorin
- 4- Chloroatranorin
- 5- 4-O-Demethyldiffractaic acid

IV- Monophenolic Derivatives
- 1- Rhizonic acid
- 2- 4-O-Methylhaematommic Acid
- 3- Methyl Everninate
- 4- Orcinol Monomethyl Ether

V- Triterpenes
- 1- Lupeol
- 2- Tylolupenyl Acetate

VI- Steroids
- 1- Ergosterol Peroxide
- 2- Ergosterol
- 3- Lanosterol
- 4- Cholesterol
- 5- Stigmasterol
- 6- β-Sitosterol

Compounds Isolated From *Usnea hesperina* Mot.

I- Depsidones
- 1- Protocetraric Acid
- 2- Fumarprotocetraric Acid
- 3- Virensic Acid

II- Dibenzofuran Derivative
- 1- (+) Usnic Acid

III- Monophenolic Derivatives
- 1- Methylhaematommate Monomethyl Ether
- 2- 4-O-Methyl Orsellinic Acid

IV- Triterpenes
- 1-Nor-30-hopan-3,22-dione
- 2- Ursolic Acid

V- Steriods
- 1- Ergosterol Peroxide

5.2 SYNTHETIC PART

The third part of this study involved attempts to synthesize group of depsides, diaryl ethers and depsidones containing hydroxylactonic cycle that up to date had not been prepared.
Besides the classical method, three main synthetic routes were attempted, all of which may be biomimetic. The first route employs oxidative coupling of a benzophenone. The second route makes use of the Smiles rearrangement of depsides, and the third route makes use of the Ullmann reaction.
The difficulties in the syntheses of lactonic depsides and depsidones of these kinds, was envisaged to be the construction of the phthalide moiety. Although many phthalides are naturally occurring substances, and are important intermediates in the syntheses of benzophenones, and certain lactonic depsides and depsidones, the methods available for their syntheses are few and the yields are often very low.
Hence our primary objectve in this part of the work was to design an appropriate method for the syntheses of the required phthalides. To establish an appropriate method for the syntheses of the particular phthalides, we utilized the synthetic routes presented in the schemes (10, 11). The preparation of these phthalides made possible the syntheses of three new, synthetically interesting lactonic depsides **49**, **50**, and **51**.

The Ullmann reaction between appropriate phenols and bromophthalides yielded two new diaryl ethers. However, several attempts to synthesize lactonic depsidones failed, even though all the three main synthetic routes were utilized repeatedly and the reaction condition modified.

6. REFERENCES

[1] S. Huneck, "Lichen substances". In Progress in Phytochemistry. Ed. L. Reinhold and Y. Liwschitz **1968**, 1, 223. J. Wiley and Sons, London.
[2] E. A.Thomas. Beitr. Kryptogamenflora Schweiz, **1939**, 9, 1.
[3] H. Castle and F. Kubsch. Arch. Biochemistry, **1949**, 23, 158.
[4] S. Shibata, T. Natori, and S. Udagawa, List of fungal products, **1964**, C. Thomas, Springfield.
[5] V. Ahmadjian, Bryologist, **1964**, 67, 87-98.
[6] C. F. Culberson and D. Armaleo, Experimental Mycology, **1992**, 16, 52-63.
[7] A. M. Cruickshank, and Dawn, R. Perrin. "Pathological function of phenolic compounds in plants", in Biochemistry of phenolic compounds, **1964** , 511. Ed. J. B. Harborne. Academic Press, New York.
[8] D. Hess, Z. Botan., **1960**, 48, 36.
[9] M. E. Hale, Jr., Bryologist, **1955**, 58, 242; **1956**, 59, 114 ; **1958**, 81, 231.
[10] B. Akermark, H. Erditman, and C. A . Wachtmeister, Acta Chem. Scand., **1959**, 13, 1855.
[11] T. R. Seshadri and G. B. V. Subramanian., J. Indian Soc.,**1963**, 40, 7.
[12] C. F. Culberson, The First Supplement to Chem. Bot. Guide Lich. Prod. The American Bryological and Lichenological Society, USA **1970**.
C. F. Culberson, Culberson, W. L. and Johnson, A., The second supplement to Chem. Bot. Guide Lich. prod. **1977**, 9, 89. The American Bryological and Lichenological Society Missouri Bot. Garden, St. Louis, USA.
[13] D. H. Lewis, and D. C. Smith. New Phytol. **1967**, 66, 143
[14] D. H. Lewis, and D. C. Smith. New Phytol, **1967**, 66. 185, 204.
[15] G. A. Perez-Liano, Botan. Rev. **1944**, 10 , 1.
[16] N. Y. Lazarev, and V. P. Savicz, New Antibiotic Binan or Sodium Salt of Usnic Acid (Botanical and medical investigation), **1957**. Akademiia Nauk SSSR, Botanicheskii Institut Im.V. L. Komarova, Moscow.
[17] W. Zopf, Die Flechtenstoffe in chemischer, botanischer, pharmakologischer und technischer, Beziehung. Gustav Fischer. Jena, **1907**.
[18] Y. Asahina, Series papers by Asahina and his students.**1936-1940.**
[19] Y. Asahina, and S. Shibata, Japan Society for the promotion of science, **1954**, Tokyo.
[20] C. F. Culberson, Chemical and Botanical Guide to Lichen Products. University of North Carolina Press, Chapel Hill, **1969.**
[21 R. Thomas. Biochem. Jour. **1961**, 78, 748.
[22] K. Mosbach, Acta chem. Scand , **1967**, 21, 2331.
[23] E.A.Thomas, . Berit. Kryptogamenflora. Schweiz, **1939**, 1, 1.
[24] E.A. Thomas, Ber. Schweiz. Botan. Ges. **1936**, 45, 191.
[25] K. Mosbach,. Biochem. Biophysic. Res. Commun., **1964**, 17, 363.
[26.] C. Leuckert, Mycologia, **1990**, 82 (3), 370
[27] K. Mosbach, and G. Jacobsson, in Abstract of papers presented at the 5th International Symposium on the Chemistry of Natural Products, **1968**, 109.
[28] K. Mosbach, Angew. Chem. Int. Edn., **1969**, 8, 240.
[29] C. F. Culberson, W. L. Culberson, and T. L. Esslinger, Bryologist, **1977**, 80, 125.
[30] S. Gatenbeck, On the biosynthesis of sulochrin and geodin. In Abstracts of papers presented at the 5th international symposium on the chemistry of natural products, London, **1968**, 114.
[31] D. O. Chester, J. A. Elix, Aust. J. Chem., **1978**, 31, 2745.
[32] D. O. Chester, and J. A. Elix, Aust. J. Chem., **1979**, 32, 1399.
[33] F. M. Dean, Natural occurring oxygen ring compounds, London, Butterworths, **1963.**
[34] C. F. Culberson, Bryologist, **1970**, 73, 177.
[35] S. Neelakantan, T. R. Seshadri, and S. S. Subramanian, Tetrahedron, **1962**, 13, 597.

[36] C. F. Culberson, Phytochemistry, **1966**, 5, 815.
[37] D. H. D. Barton, and T. Cohen. Some biogenetic aspects of phenolic oxidation. Festschrift, A. Stoll, **1957**, 117. Birkhäuser, Basel.
[38] H. Erdtman, and C. A. Wachtmeister. Phenoldehydrogenation as a biosynthetic reaction, in Festschrift, A. Stoll. **1957**, 144. Rirkhäuser, Basel.
[39] A. I. Scott, Quart. Rev. London, **1965**, 1
[40] W. L. Culberson, C. F. Culberson, and A. Johnson, Bryologist, **1981**, 84, 16.
[41] E. G. Sundholm, and S. Huneck, Chemica Scripta, **1981**, 18, 233.
[42] I. J. Culberson, and M. V. Sargent, Aust. J. Chem., **1981**, 34, 2701.
[43] T. Sala, and M. V. Sargent, J. Chem. Soc., Perkin Trans. I, **1981**, 877.
[44] J. A. Elix, and A. Engkanian, Aust. J. Chem., **1976**, 29, 203.
[45] J. B. Hendrickson, M. V. Ramsay, and T. Ress Kelly, J. Amer. Chem. Soc., **1972**, 6834
[46] K. H. Chanee, and D. L. Tibbett, Bryologist, **1978**, 76, 208.
[47] H. Castle, and F. Kubsch, Arch. Biochem., **1949**, 23, 158.
[48] J. P. Kutney, and I. H. Sanchez, Cand. J. Chem., **1977**, 55, 1088.
[49] J. A. Elix, and D. Tronson, Aust. J. Chem., **1973**, 26, 1093.
[50] J. B. Harborne, Phytochemical Methods, ed. **1973**, 109.
[51] R. B. Herbert, Biosynthesis of secondary metabolites, Chapman and Hall, London **1981**, 251.
[52] A. A. Newman, Chemistry of terpenes and terpenoids. Academic Press, London, **1972**.
[53] R. Neher, TLC. of steroids and related compounds. Ed. E. Stahl, London, **1969**, 311.
[54] J. Verghese, Perfume flavours, **1981**, 6, 23.
[55] H. Inouye, Planta Medica, **1978**, 33, 193.
[56] J. MacMillan, Diterpenes. The Gibberellins in "Aspects of terpenoid chemistry and biochemistry", **1971**,153. Ed.T.W. Goodwin.
[57] W. Sandermann, Terpenoids: structure and distribution in Comparative Biochemistry, **1962**, Vol. III, 503. Ed. Florkin, M., and Masan, H. S., Academic Press, London.
[58] D. L. Dreyer, Phytochemistry, **1966**, 5, 367.
[59] M. J. Kulshreshtha, Phytochemistry, **1972**, 11, 2369.
[60] R. D. Bennettel, J. of Chromtogr., **1966**, 21, 488; Phytochemistry, **1966**, 5, 231.
[61] J. D. Weete, Phytochemistry, **1973**, 12, 1843.
[62] F. Schulte, Beih. Botan. Zentralblatt, **1905**, 18, 1.
[[63] W. Thies, Systematische Verbreitung und Vorkommen der Flechtenstoffe (Flechtensäuren). **1932**, 429, in G. Klein, ed., Handbuch der Pflanzenanalyse 3. Band spezielle Analyse 2. Teil, Organische Stoffe II.Julius Springer, Wien.
[64] D. L. Hawksworth, The natural history of Slapton Ley Nature Reserve. IV. Lichens. Field study. London, **1972**, 3, 535.
[65] P. W. Holligan, and Drew, E. A. New Phytol. **1971**, 70, 271.
[66] H. Budzikiewicz, J. M. Wilson et C. Djerassi, J. Amer. Chem. Soc. **1963**, 85, 3688.
[67] P. Allemand, "Contribution à l' Etude de la Composition Chimiques de Lichens". Thèse Université de Neuchâtel, **1988**.
[68] C. F. Culberson, J. of Chromtogr., **1972**, 72, 113-125.
[69] W. R. Allison and G. T. Newbold. J. Chem. Soc, part VI, **1959**, 3335.
[[70] Y. Nishitoba, H. Nishimura, T. Nisshiyama and J. Mizutani, Phytochemistry, **1987**, 26, (12), 3181.
[71] J. A. Elix, D. O. Chester, K. L. Gaul, J. L Parker and J. H. Wardlaw, Aust. J. Chem. **1989**, 42, 1191
[[72] M. Shamma, R. E. Glick, and R. O. Mumma, J. of org. chem., **1962**, 27, 4512.

[73 J. Gunziger, "Etude de la Composition Chimique de Pseudeverina furfuracea" (L.) Ach. Synthèse d' un depside et d'un depsidone. Thèse, Université de Neuchâtel , **1985.**
[74] J. Schmidt and S. Huneck, Organic Mass Spectrometry, **1979,** 14, 12
[75] H. Ageta, K. Shiojima, K. Masuda and T. Lin, Tetrahedron Lett., **1981,** 22, 2289.
[76] Z. A. Wojciechowski, L. J. Goad and T. Goodwin. Phytochemistry, **1973,** 12, 1433.
[77] C. F. Culberson, and H. Kristinsson, J. of Chromatogr., **1970,** 46, 85.
[78] L. B. Keir, Journal of Pharm. Soc., **1963,** 50, 471 ; **1963,** 52, 465.
[79] S. Safe, L. M. Safe and W. S. G. Maass, Phytochemistry, **1975,** 14, 1821.
[80] B. C. Sekula, and Nes, R. W., Phytochemistry , **1980,** 19, 1509.
[81] P. S. Rao, and T. R. Seshadri, Proc. Indian Acad. Sci.,**1967,** 66A, 1.
[82] G. Tsoupras , R. Tabacchi, Sur la presence de triterpenes dans quelques lichens, Université de Neuchâtel , **1988.**
[83] Munehiro Nakatani, Yuji Miyazaki, Takashi Iwashita, Hideo Naoki, and Tsunao Hase, Phytochemistry, **1989,** 28, (5), 1479.
[84] G. Nicollier, Isolement, Identification et quelques synthèses, de composés de la mousse de chêne Evernia prunastri (L.) Ach., Thèse Université de Neuchâtel, **1979.**
[85] J. A. Elix, K. L. Gaul, P. W. James, and O. W. Purvis, Aust. J. Chem., **1987,** 40, 417.
[86] S. Shimada, T. Saitoh, U. Sankawa, & S. Shibata, Phytochemistry, **1990,** 29, 328.
[87] T. Zesiger, Travail de diplome, Université de Neuchâtel , **1986.**
[88] K. F. Cohen, J. T. Pinhey , and R. J. Smith, Tetrahedron Lett., **1968)** 4729.
[89] J. Kagan, J. Org. Chem., **1967,** 32, 4060.
[90] B. M. Trost, G. T. Rivers, and J. M. Gold. J. Org. Chem., **1980,** 45, 1835.
[91] S. Mahalingam, P. C. Kuzma, J. Y. C. Lee, and T. M. Harris, J. Amer. Chem. Soc., **1985,** 107, 7760.
[92] M. Mahan Mohandru and Alireza Tajbakhsh, J.Chem..Soc. Perkin Trans. I, **1963,** 2249.
[93] A. I. Scott, Proc. Chem. Soc., **1958,** 195; N. L. Wendler, J. Org. Chem., **1963,** 28, 2752.
[94] L. Y. Foo, and Gwyn, S. A., Experientia, **1978,** 34, 970.
[95] L. Y. Foo, and Galloway, D. J., Phytochemistry, **1979,** 18, 1977.
[96] C. Pulgarin, Synthèses de depsides et depsidones. Approche biomimétique. Thèse, Université de Neuchâtel, **1989.**
[97] N. M. Rama, M. V. Sargent, and J. A. Elix, J.C.S. Perkin Trans. I, **1975,** 1922.