EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 7
335-370
Year: 2024

ISOLATION AND STRUCTURAL CHARACTERIZATION OF TRIDEPSIDES FROM POLAR FRACTION OF LICHEN (*PERMETREMA PARLATUM*)

Ajoy K. Bauri[1]*, Ines Castro Dionicio[2], Eric Salinas Arellano[2], Jonathan G. Jeyaraj[2], Sabine Foro[3] and Esperanza J. Carcache de Blanco[2]*

[1]Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India.
[2]College of Pharmacy, The Ohio State University, Ohio, Columbus, OH 43210, USA.
[3]Institute of Materials Science, Darmstadt University of Technology, Alarich-Weiss-Strasse 2, D-64287, Darmstadt, Germany.

***Corresponding Author: Ajoy K. Bauri**
Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India.

Article Received on 26/05/2024 **Article Revised on 16/06/2024** **Article Accepted on 06/07/2024**

ABSTRACT
Phytochemical investigation of more polar fraction of lichen (*P. parlatum*) led to the isolation of four known tridepsides (**1**-**4**) along with a new depside (**5**). They were identified as β-hydroxy methyl ester of gyrophoric acid, methyl ester of lasallic acid, methyl ester of crustinic acid, methyl ester of hiascic acid and 4-carboxy lecanoric acid. The structures of known tridepsides (**1**-**4**) were identified in comparison with their ^{1}H NMR, ^{13}C NMR and HRESI-MS spectral data available in literature. The structural determination of new depside (**5**) was carried out both 1D, 2D NMR spectroscopy and HRESI-MS spectrometry. In *vitro*, anti-proliferative activity assay of these compounds was conducted against various human cancer cell lines in comparison with therapeutically prescribed authentic drug paclitaxel as control using SRB assay.

KEYWORDS: Lichen; isolation; tridepsides; structural characterization by spectral study; anti-proliferative activity; SRB assay.

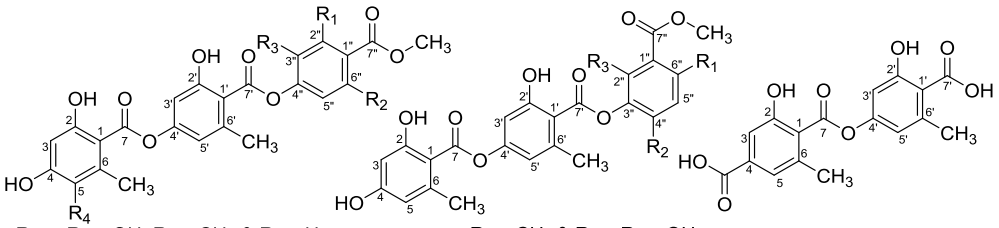
INTRODUCTION
Lichens are beautiful composite organism. In India, lichen represented by 2532 species under 324 genera and 78 families including 541 endemic species.[1] It belongs to the family *Parmeliaceae*, genus *Permotrema*, and species *parlatum* in the plant kingdom. Lichens are very pollution sensitive bio-organism and survive in pollution free zone or at least polluted area.[2,5] It is familiar by different names in Indian vernacular such as 'Chhareela' (meaning 'spreader'), 'Shilapuspa' (where 'Shila' means 'rock' and 'puspa' stands for 'flower'), and 'Pathorphool' (where 'Pathor' stands for 'rock' and 'phool' denotes 'flower'). It is commonly referred to as 'Dagadphool' (where 'Dagad' means 'rock' and 'phool' stands for 'flower') in Marathi language. Based on the habitat, lichens are classified into two categories such as rock lichen and wood lichen. Rock lichens have grown on basalt rock or stone in mountains and wood lichens have grown on the stem or branches of the trees. Amongst, some species of them are very useful in different aspects such as food supplements, an active ingredient for preparation of exotic spices, herbal medicines[6,7], source of natural dyes for coloring of textiles and fabrics especially wool and silk.[8,10] We have procured a lichen specimen identified as *Permotrema parlatum* from Munner in Kerala State of South India for its chemical profiling. This species of lichen survives pollution free area at high altitude of mountains on black basalt rocks under the drastic environmental condition.[11,13] Lichens act as bio-indicator to measure the level of environmental pollution and capable to detect multi elements (C, N, Pb, Hg *etc.*) isotopic signature in atmospheric contamination.[14,16] It has wide applications as an active ingredient for preparation of exotic spices and botanical supplement for preparation of polyherbal medicine prescribed by tribal people of India for treatment of various kinds of ailments including cancer.[6,7] For phytochemical investigation of secondary metabolites, sequential solvent extraction of lichen was conducted using diethyl ether, and methanol. The solvents were removed using rota-vapor at reduced pressure to yield crude extracts. These crude extracts were fractionated by column chromatography over silica gel with gradient solvent elution using a binary mixture of solvent methanol in chloroform to yield several fractions. The fractions with similar TLC profiles were combined and further purified by column chromatography over silica gel, by gel permeation chromatography (GPC) over sephadexLH20, high performance chromatography (HPLC) and preparative

thin layer chromatography (PTLC) to afford compounds (**1**-**5**). The structural characterization of these isolated metabolites has been determined by chemical, spectral, and spectrometric study. In *vitro*, anti-proliferative assay of isolated metabolites of lichen from more polar fractions/extracts was evaluated against five different types of human cancer cell lines such as cervical cell line (Hela), breast cancer cell line (MDA-MB-231), pancreas cancer cell line (HPAC), prostate cancer cell line (DU-145), colon cell line (HT-29) to check the efficacy of cell cytotoxicity by SRB assay in comparison with paclitaxel as control. This manuscript presents the isolation and structural characterization of isolates (**1**-**5**) from more polar fraction/extract of lichen (*P. parlatum*) and evaluation of anti-proliferative activity against different human cancer cell lines.

RESULTS AND DISCUSSION

The extensive study of more polar fractions has carried out to investigate its chemical constituents. It was found that the major chemical constituents present into more polar fraction of lichen is tripeptides. Tripeptides area class of compounds composed of three units of hydroxy benzoic acid or their congeners linked to an ester bond. Mostly, lichen is major source of these classes of compounds. But this class of compounds has also been reported from higher plants belongs to different families such as *Ericaceae*, *Lamiaceae*, *Papaveraceae*, *Myristacae*, *and Fabaceae*[17,22], etc. In general, the chemical constituents present in more polar fraction of *P. parlatum* are credited various biological activities in different aspects such as anti-proliferative[24,28], anti-cancer[27,33], anti-tumor[29], anti-miotic[33], anti-microbial[28,33], anti-oxidant[34,36], anti-bacterial[37], anti-fungal[37], anti-inflammatory[37,39], neuro-protective activity[40,45], UV protectant[46], anti-HIV[47], anti-diabetic[43,44,48], hyper-glycaemia[48], anti-pyretic[37,39], anti-mycobacterial[36,39], free radical scavenging[34], cytotoxic[24,37,42], flavoring agents in exotic spices in Indian cuisine.[49]

More polar fraction/extract of *P. parlatum* was fractionated by column chromatography over silica gel with gradient solvent elution by using a binary mixture of methanol in chloroform to yield eight fractions. Fractions with similar TLC profiles were combined and further purified by column chromatography over silica gel, gel permeation chromatography (GPC) over sephadexLH20, high performance liquid chromatography (HPLC) followed by preparative thin layer chromatography (PTLC) to afford known tridepsides (**1**-**4**)[39,50,54] along with new a depsides (**5**).[39,50,54] The major chemical constituents (**1**-**5**) isolated from polar fraction/extract were depicted in figure 1. The structural characterization of these compounds was carried out by spectrometric and spectroscopic methods.



R_1 = R_3 = OH, R_2 = CH_3 & R_4 = H;
β-Hydroxy-Methyl Ester of Gyrhophpric Acid (**1**)

R_1 = R_4 = OH, R_2 = CH_3 & R_3 = H;
Methyl Ester of Hiascic Acid (**4**)

R_1 = CH_3 & R_2 = R_3 = OH;
Methyl Ester of Lasallic Acid (**2**)

R_1 = R_2 = OH, R_3 = CH_3;
Methyl Ester of Crustinic Acid (**3**)

Compound (**5**)
4-carboxy lecanoric acid

Figure 1: Chemical structure of tridepsides (1-4) along with a new depside (5).

When sprayed with aqueous 10% H_2SO_4 on micro TLC plate followed by heating at 120 ºC for 5 min exhibited peculiar red colored spots. It indicated that it may be depside class of compound or its analogue. The UV spectra of these compounds were very similar, with absorptions at 211, 268 and 300 nm, indicating similar chromophores. Spraying with neutral alcoholic $FeCl_3$ solution on TLC plates gave very dark green spots, suggesting that these compounds were phenolic with vicinal carbonyl groups.[55] These compounds also showed batho-chromic shifts of about 35-40 nm upon the addition of anhydrous $AlCl_3$ at an acidic pH.[56] ^{1}H NMR spectra coupled with mass spectral fragments arising from cleavage of ester bond provided a clear indication of the nature and substitution pattern of the benzene rings of the depsides.

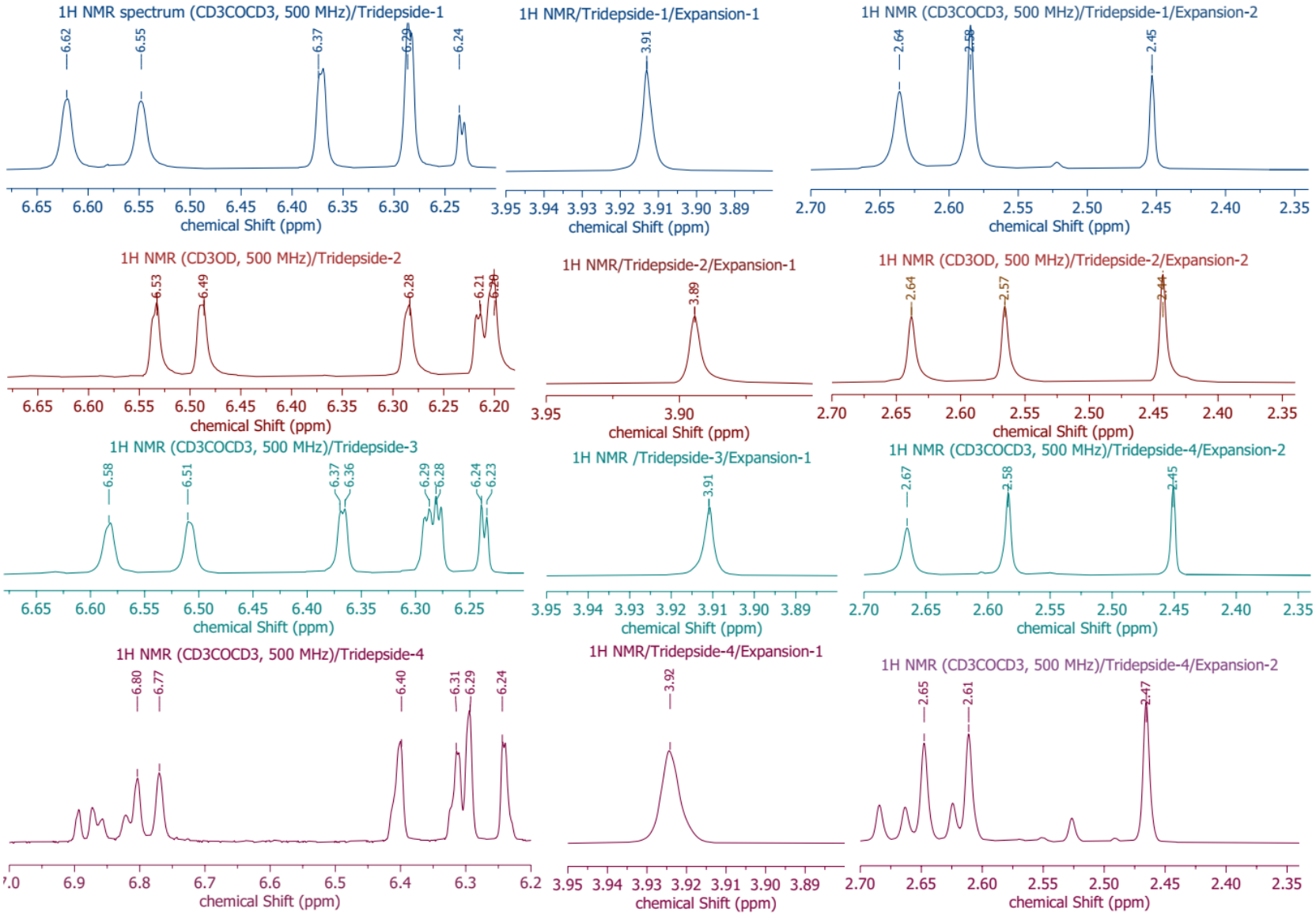


Figure 2: Expansion of ^{1}H NMR spectra ($CDCl_3$, 500 MHz) of known tridepsides (1-4) isolated from lichen (*P. parlatum*).

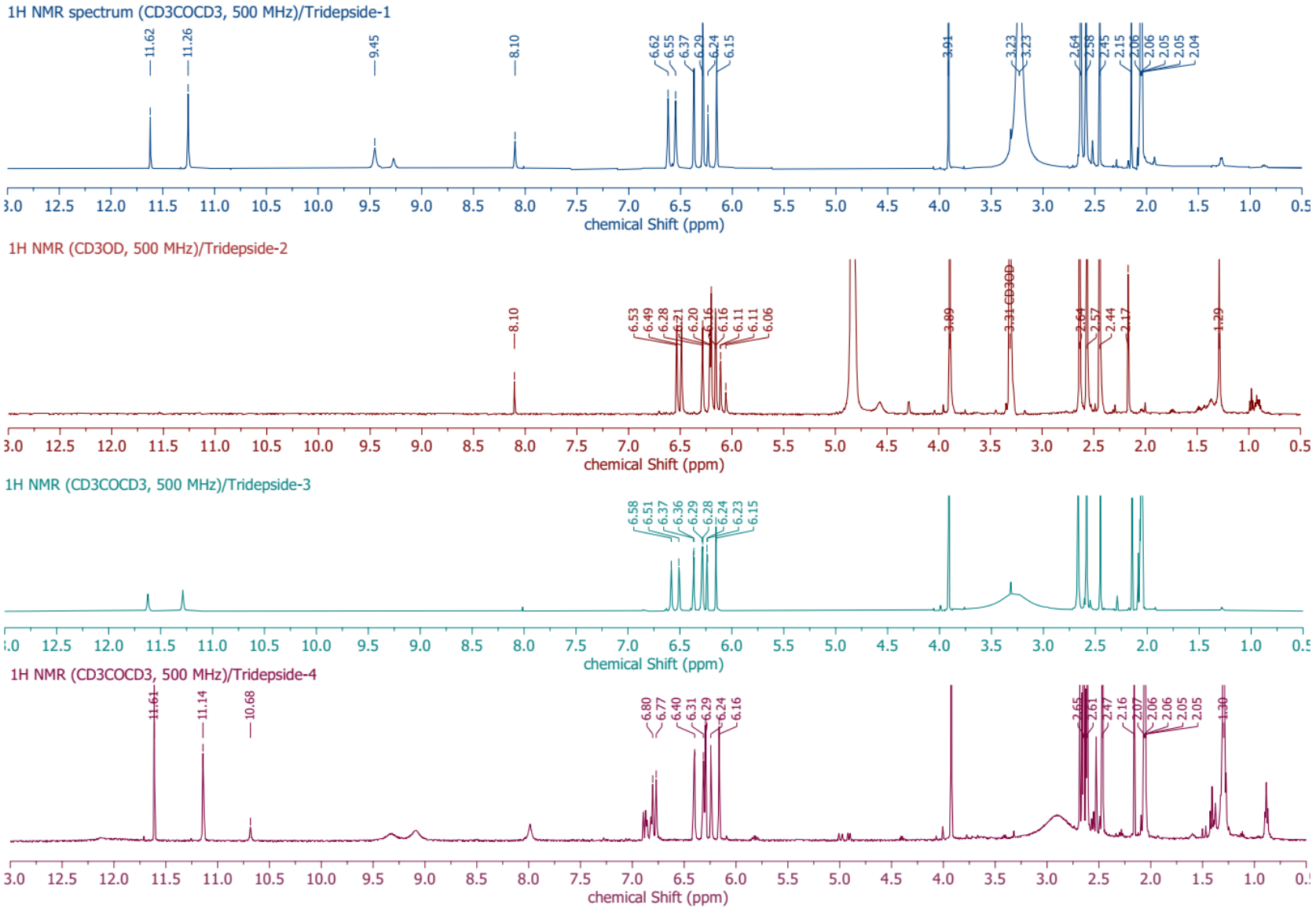


Figure 3: ^{1}H NMR spectra ($CDCl_3$, 500 MHz) of known tridepsides (1-4) isolated from more polar fraction of lichen (*P. parlatum*).

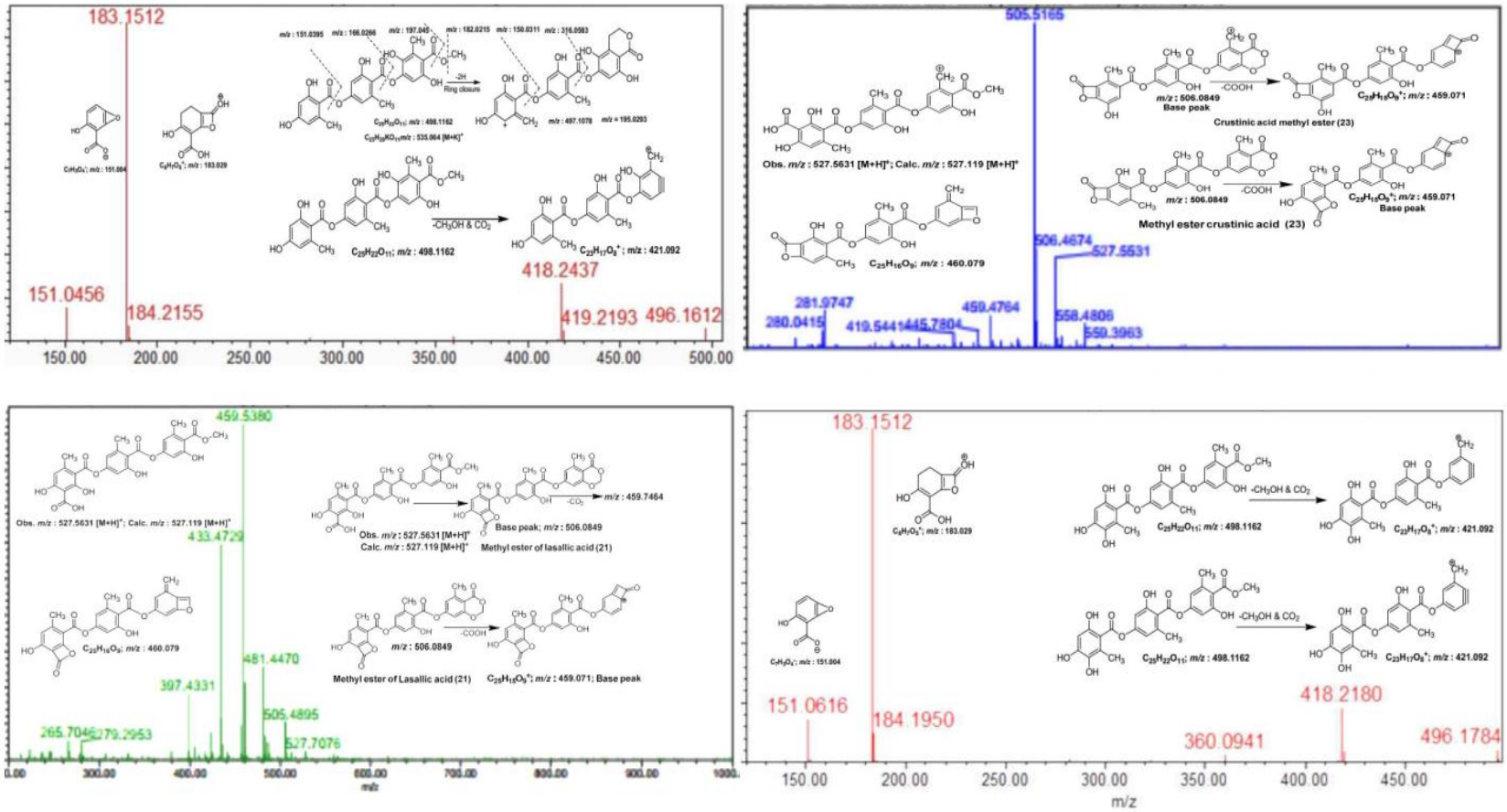


Figure 4: HRESI-MS spectra of known tridepsides (1-4) isolated from lichen (*P. parlatum*).

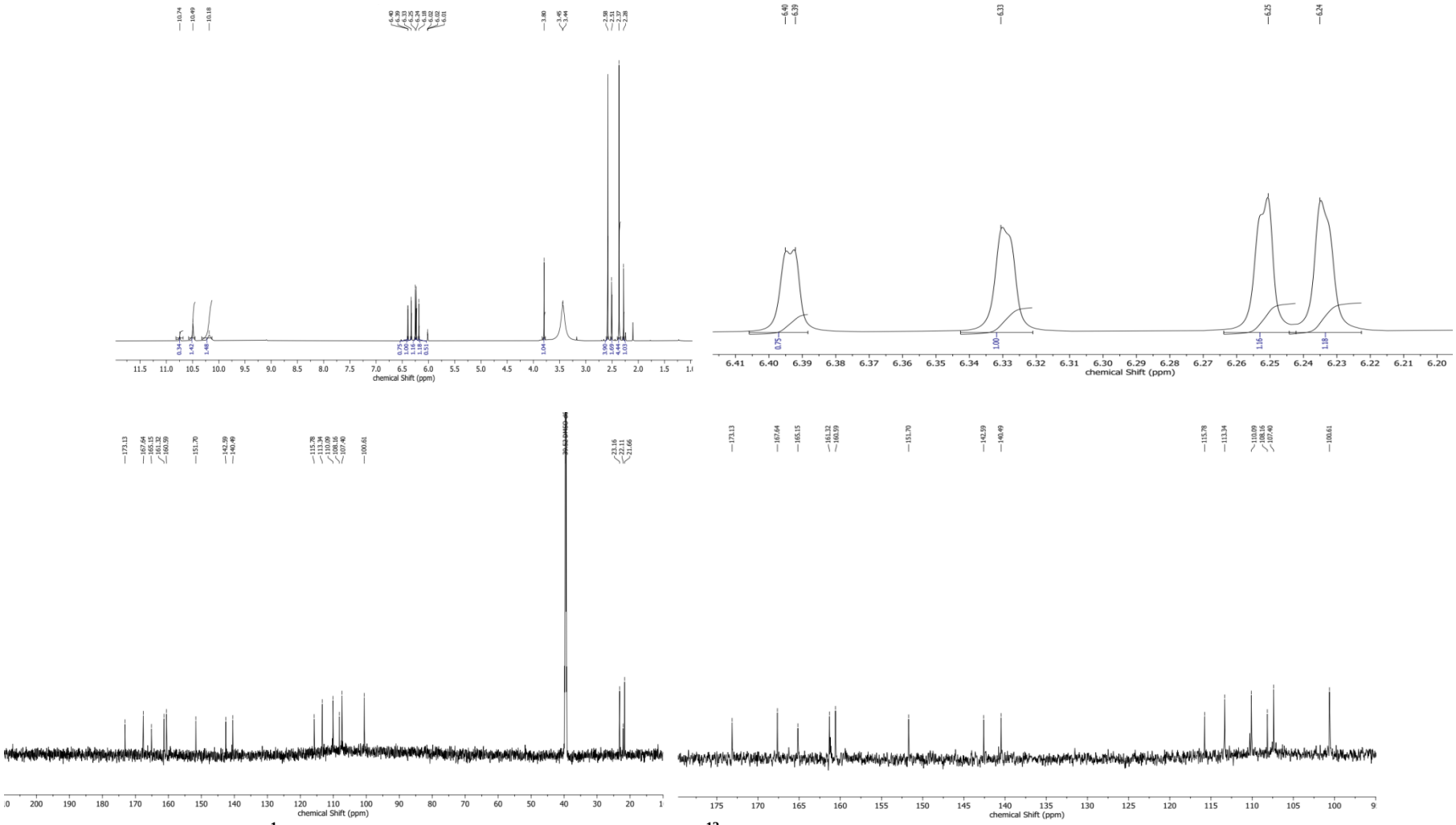
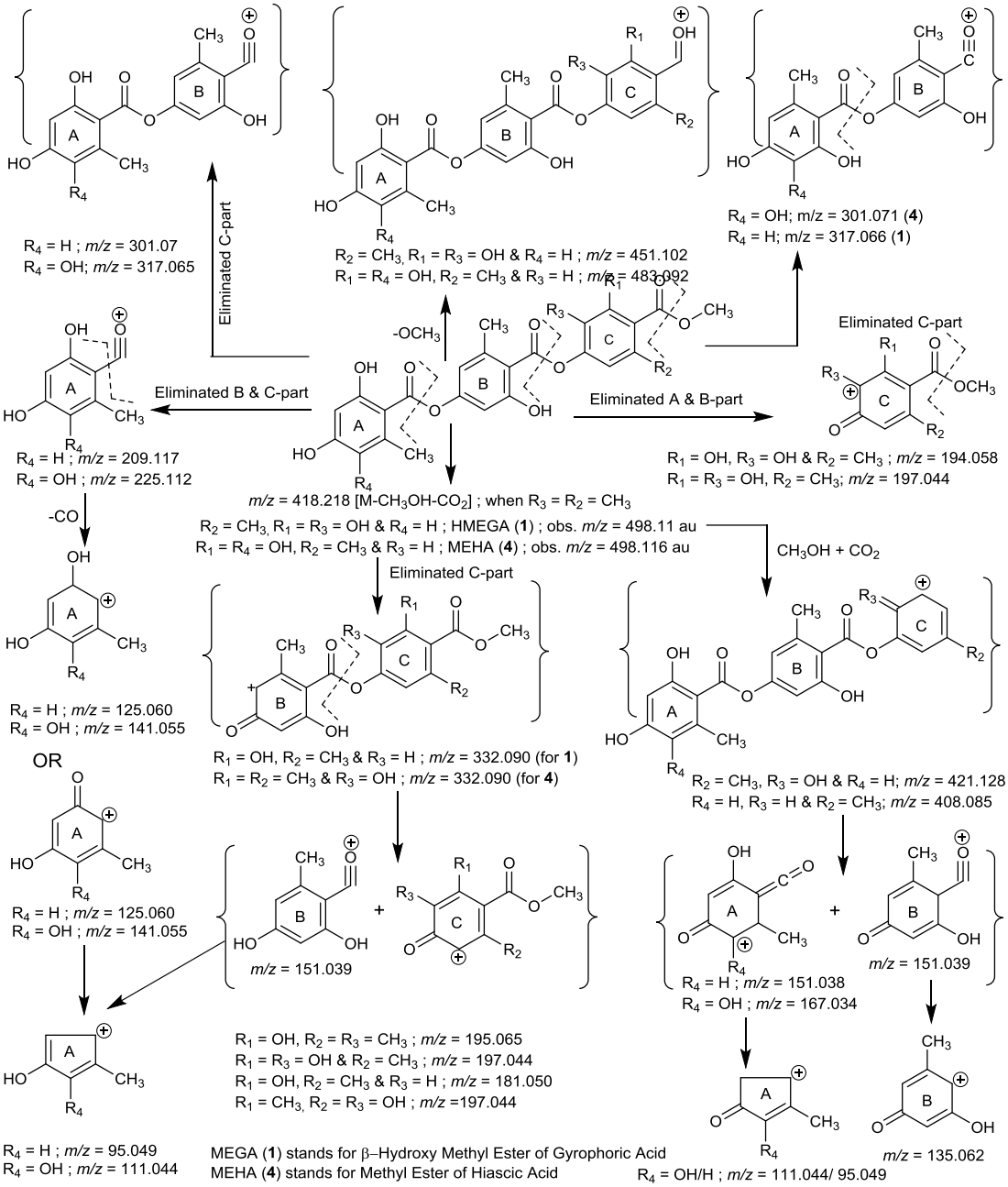
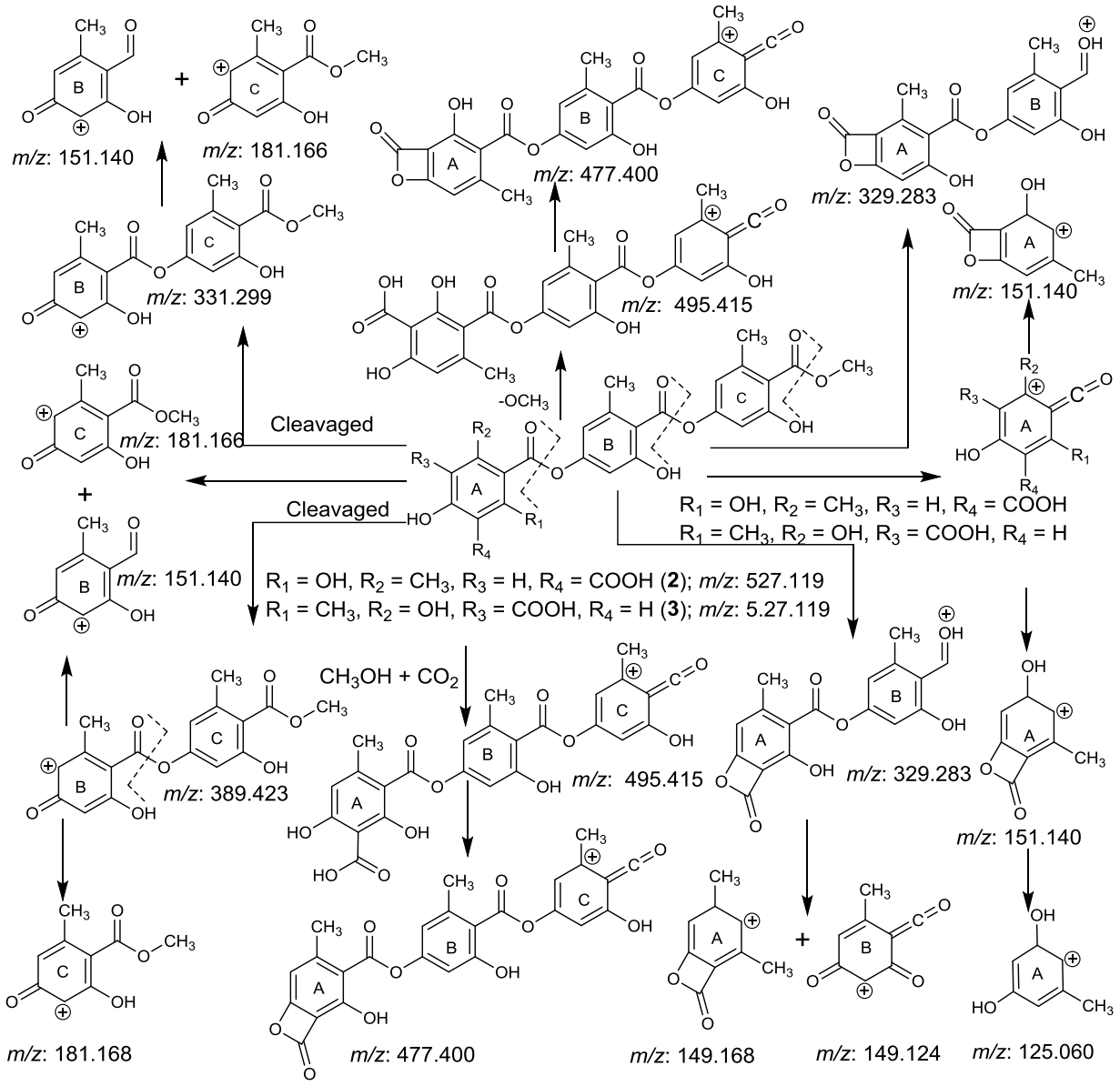


Figure 5: ^{1}H NMR spectrum (CD_3SOCD_3, 800 MHz) and ^{13}C NMR (CD_3SOCD_3, 201 MHz) of new depside 4-carboxy lecanoric acid.

The structure of tridepsides from lichen (*P. parlatum*) were deduced from their spectral data. The IR spectrum showed two carbonyl absorption due to ester linkage at around 1706 and 1651 cm^{-1}. In the ^{1}H NMR spectrum, signal was exhibited corresponding to an isolated one proton singlet at 12.54 assigned to a phenolic function at C-2 associated with an ester group as same as depsides and proton singlet at 9.94 assigned to phenolic groups at position C-4 and C-5. Aromatic protons at 3', 3", 5' and 5" appeared respectively as series of four doublets in the range of δ_H 6.0-7.0 ppm all with coupling constant, *J* values around 2.0-2.5 Hz and one proton singlet appeared in the range of δ_H 6.00-6.70 ppm corresponding signals as singlet due to proton located at position C-3 in tridepsides. A methoxyl function was detected as singlet at δ_H 3.90 ± 0.1 ppm and three methyl groups appeared as singlets at δ_H 2.65-2.27 ppm. ^{13}C NMR (please see figure & table) confirmed the presence of three methyl groups at positions 6, 6' and 6" at 24.3-23.8 ppm; one methoxyl group at δ_H 51.0-52.00 ppm, and five non-substituted aromatic carbons. The mass spectra of these depsides exhibited a molecular ion, in agreement with similar results on other tridepsides derived from orsellinic acid and orcinol as precursors. The principal ion fragments at *m/z* corresponded to rings formed by rupture of triphenyl ester linkages. The fragment due to ring A/B (*m/z*: 151 and 125/123) are the only ones which explain the presence of a methyl group. Moreover, the reciprocal meta coupling in ^{1}H NMR spectrum refer to aromatic protons on C-3, C-5 positions in ring A and on C-3', C-5' positions in ring B in compound **1**; on C-3', C-5' positions in ring B and on C-3', C-5' positions in ring C in compound **4** respectively. The nature of substituent and substitution pattern in ring C of the tridepside **1** and A ring of tridepside **4** is relatively different that is assigned by mass fragmentation (*m/z*: 151/167, *m/z*:181/182/184/184 and *m/z*:194/195/197). Same criteria are also valid for tridepside **2** and **3**. This assignment was confirmed by comparison with ^{13}C NMR spectra of other tridepsides with same B and C ring structure. Finally, it is well known that all the para depsides derived bio-genetically from β-orcinol are always methylated at positions C-6 and C-6'.[52,54] The interpretation of mass fragmentation of tridepsides isolated from lichen was illustrated in scheme 2 and 3 depicted below.[57]



Scheme 1: Interpretation of HRESI-MS of tridepsides 1 and 4.



MELA stands for Methyl Ester of Lasallic Acid (**2**) MECA stands for Methyl Ester of Crustinic Acid (**3**)

Scheme 2: Interpretation of HRESI-MS of tridepsides 2 and 3.

Compound **1** isolated from more polar fraction of diethyl ether / methanol extract of lichen eluted with 20% methanol in chloroform from column chromatography over silica gel followed by gel permeation chromatography (GPC) over sephadexLH20 with a mixture of binary solvents system methanol in chloroform. On removal of solvent, a brown solid substance was obtained and seemed to be orcinol type of tridepside by NMR spectral analysis. The molecular formula of this tridepside was determined as $C_{25}H_{22}O_{10}$ by HRESI-MS spectroscopy and was identified β-hydroxy methyl ester of gyrophoric acid (mol. wt: 498.11 au) and its spectral was compared with its published spectral value of the related compounds available in literature.[24,35,51,54,57] The structure of the compound **1** was depicted in figure1.

Compound **2** was separated as crude product from more polar fraction of the diethyl ether extract or methanol extract of lichen eluting with 20-25% methanol in chloroform by column chromatography over silica gel followed by gel permeation chromatograph (GPC) over sephadexLH20 using a binary solvent system by changing its polarity to yield a brown solid substance. This brown solid substance was further passed over florisil through open column chromatography to avail a light brown color solid substance designated as compound **2**. The molecular formula of this compound has been determined as $C_{25}H_{22}O_{11}$ by HRESI-MS spectroscopy. Based on the spectral evidences, the structure of the compound **2** was assigned as methyl ester of lasallic acid[19,2] and its structure was depicted in figure 1.

Compound **3** segregated from more polar fraction of diethyl ether or methanol extract of lichen eluted with 30% methanol in chloroform by column chromatography over silica gel to afford a solid substance. This product was purified by gel permeation chromatography (GPC) over sephadexLH20 to yield light pink colored solid substance. This light pink solid substance was further purified on open column chromatography over florisil to remove a trace amount polymeric material associated into it. It was further purified by column chromatography over silica gel eluted with a binary mixture of solvent methanol in chloroform to afford light pink color solid substance. The molecular formula of this compound was established as $C_{25}H_{22}O_{11}$ by NMR and HRESI-MS spectrometric study. The structure of the compound **3** was assigned as methyl ester of crustinic acid and its structure was depicted in figure 1.

Compound **4** was obtained from diethyl ether extract or methanol extract of lichen by column chromatography over silica gel eluting with 30-35% methanol in chloroform as solvent system. It was further purified by gel permeation chromatography over sephadexLH20 to afford a brown solid containing compound **4** as major constituent. This brown solid was further purified by open chromatography over silica gel to afford compound **4** as pure product. The NMR spectral analysis revealed that it may be orcinol type of depside. The molecular formula of this compound was determined as $C_{25}H_{22}O_{11}$ (mol. wt.: 498.44 au) by study of NMR and HRESI mass spectrometry. Based on the above spectral evidences, the structure of the compound **4** has been secured as methyl ester of hiascic acid in comparison with reported spectral data published in literature.[19,32] The structure of the compound **4** was depicted in figure 1.

Compound **5** was obtained from diethyl ether extract or methanol extract of lichen by column chromatography over silica gel eluting with 30-35% methanol in chloroform as solvent system. It was further purified by gel permeation chromatography over sephadexLH20 to appear as a brown solid containing compound **5** as minor constituent. This brown solid was further purified by preparative thin layer chromatography to yield compound **5** as almost pure product. The NMR spectral analysis revealed that it may be orcinol type of depside. The molecular formula of this compound was deduced as $C_{17}H_{14}O_9$ (mol. wt: 362.063 au) by study of 1D, 2D NMR and HRESI mass spectrometry. Based on the above spectral evidences, the structure of the compound **5** was assigned as 4-carboxy lecanoric acid in comparison with published spectral data reported in literature.[24, 35, 51-54, 57] The structure of the compound **5** was depicted in figure 1.

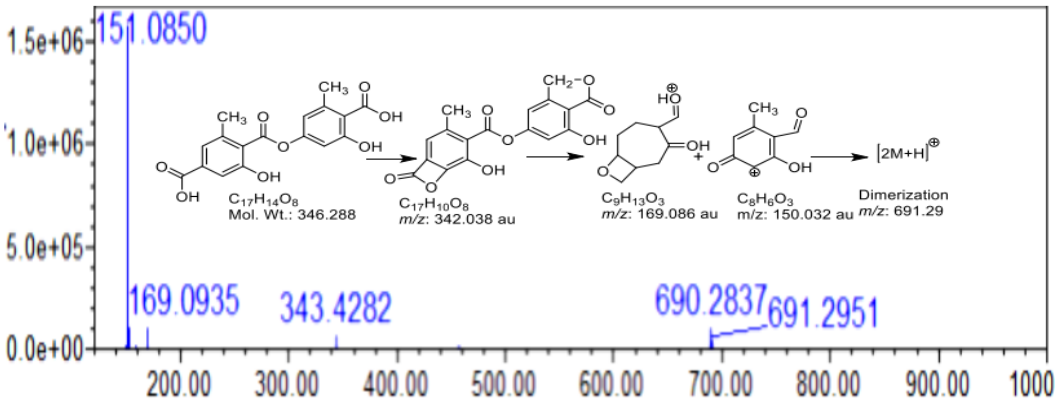


Figure 5: HRESI-MS spectrum of compound 5 (4-carboxy lecanoric acid).

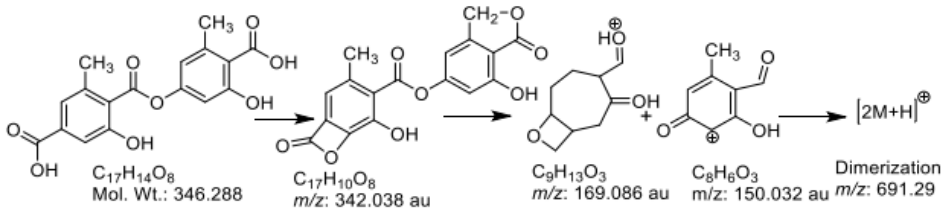


Figure 6: Interpretation of HRESI-MS spectrum of compound 5 (4-carboxy lecanoric acid).

BIOASSAY OF ANTI-PROLIFERATIVE ACTIVITY

The evaluation of anti-proliferative activity of the aforesaid tridepsides and depside (**1-5**) was performed against different human cancer cell lines using sulfo-rhodamine B (SRB) at College of Pharmacy, The Ohio State University, Ohio, Columbus, USA.[58,59] The sulfo-rhodamine B (SRB) cell cytotoxicity assay is one of the most widely used methods to detect cell viability. This assay is independent of cell metabolic activity. The incorporated dye released from stained cells after washing is directly proportional to the cell biomass and can be measured at 460 nm. Five different types of human cancer cell lines such as cervical cell line (Hela), breast cancer cell line (MDA-MB-231), pancreas cancer cell line (HPAC), prostate cancer cell line (DU-145),

colon cell line (HT-29) were used to carry out bioassay for cell cytotoxicity study (Table1 not shown).

Sample preparation. Test samples and control (paclitaxel) were dissolved in 100% DMSO to prepare stock solutions of 10 mg/ml. Dilutions were prepared using 10% DMSO in water and 100% water.

Cell culture. Cervical cancer cell line (Hela), pancreatic (HPAC), prostate (DU-145), colon (HT-29) and breast cancer cell line (MDA-MB-231) were obtained from American Type Culture Collection, Manassas, VA, USA. Monolayer cells were cultured using T75 tissue culture flasks in Roswell Park Memorial Institute medium (RPMI) or Dulbecco's Modified Eagle Medium (DMEM), containing 10% fetal bovine serum and 1% antibiotic-antimycotic from Gibco. Cells were kept at 37 °C and in an atmosphere with 5% of CO_2.

Anti-proliferative assays. Anti-proliferative activity of test samples was evaluated in triplicate on cancer cells, using a sulfo-rhodamine-B (SRB) assay as reported previously in three independent experiments.

EXPERIMENTAL SECTION

Plant Material

The specimen of *P. parlatum* was collected from Munnar in Kerala of Western Ghats Mountains in May, 2018. The material was authenticated by Dr. Hussain Barbhuiya, Landscape and Cosmetic Maintenance Section, A & SED Division, Bhabha Atomic Research Centre, Trombay, Mumbai. A voucher specimen of sample was deposited in the Herbarium of the Landscape and Cosmetic Maintenance Section, BARC, Mumbai-400085.

General Experimental Procedures

Melting points were determined using a Buchi melting point apparatus (Model Number-M560). Specific rotations were obtained using a JASCO DIP 1000 digital polarimeter. UV spectra were measured on Shimadzu UV-2100 UV-Vis spectrophotometer. NMR spectra were recorded in $CDCl_3$ or CD_3OD or CD_3COCD_3 or CD_3SOCD_3 on a Bruker Avance 200 (Switzerland), Varian 500 AR (USA), Varian 800 AR (USA) at National NMR Facility, TIFR, Colaba, Mumbai spectrometer using residual $CHCl_3$/HDO as an internal standard. Chemical shifts are given in ppm (δ_C, and δ_H), relative to residue $CHCl_3$/HDO (7.25 & 77.00/4.78 or 3.30 & 49.00 ppm). The low-resolution and high-resolution mass analyses were performed with a UPLC system (ACQUITY UPLC, Waters) at College of Pharmacy, The Ohio State University, Ohio, Columbus, USA. Silica gel (230-400 mesh, Merck) was used for analytical TLC. Silica gel (70-230 mesh, Merck) was used for column chromatography. All compounds were visualized in TLC by using vanillin-perchloric acid-EtOH followed by heating at 120 °C for 5 min, DNP in EtOH, 10% aqueous H_2SO_4 followed by heating at 120 °C for 5 min and neutral $FeCl_3$ in MeOH.

Extraction and Isolation

Freshly dried 100 g lichen (*P. parlatam*) was crushed to powder and extracted with diethyl ether (350 ml each) three times at room temperature for 12h. Removal of solvents afforded a grey colored solid substance viscous residue. The analysis of diethyl ether extract has been carried out previously. The spent of vegetative part of lichen was extracted with methanol (350 ml) three times at room temperature for 12h. Removal of methanol afforded a brown and viscous residue. The residue obtained from methanol extract was fractionated over silica gel (250 g, 230-400 mesh, Aldrich, USA) and eluted with a step gradient of chloroform and mixtures of methanol in chloroform to furnish six fractions. The volume of each aliquot collected approximately 50-150 mL. Fractions were monitored by TLC, and fractions having similar chemical profiles were combined. In some specific cases, the volume of aliquot collected is more than 250 mL. Each and every fraction has been monitored by TLC to visualize its profile. The fractions having similar chemical profiles were combined and further purified by using repetitive column chromatography (CC) on open column over silica gel, gel permeation chromatography (GPC) over sephadexLH20 with gradual solvent elution, preparative thin layer chromatography (PTLC) as applicable.

Fractions 1 eluted with hexane and ethyl acetate (10-30%) in hexane. The solvents were evaporated to furnish a negligible mass.

Fractions 2 eluted with ethyl acetate (35%) in hexane and followed by 10% methanol in chloroform. The solvents were evaporated to furnish a blackish mug residue (A). This blackish mug was a mixture of few compounds monitored by TLC using different solvent system. When sprayed with aqueous 10% H_2SO_4 on micro TLC plate and heated at 120 ºC for 5 min exhibited peculiar color. This indicated that it belongs to depside/tridepside class of compounds. This mixture of compounds was further purified by column chromatography sequentially over silica gel and then over sephadexLH-20 column eluted with 10% methanol in chloroform to yield sub-fractions $2A_1$-$2A_{15}$.

Sub-fraction $2A_5$ was monitored by TLC using different solvent systems. It was sprayed with 10% aqueous H_2SO_4 on TLC subsequently heated at 120 °C for 5 min. It was observed that sub-fractions $2A_5$ contained a major constituent. It has been further purified on a small silica gel open column followed by passed over short florisil column to afford colorless compound. Preliminary investigation revealed that it was tridepside and designated as compound **1**. The structure of the compound **1** has been secured by means of by chemical, spectral and spectrometric methods and identified as a β-hydroxy methyl ester of gyrophoric acid.

Fractions 3 eluted with 20% methanol in chloroform and evaporated to furnish a blackish mug residue (3A) and it

was a mixture of few compounds along with polymeric substance, checked by TLC. When sprayed with aqueous 10% H_2SO_4 on micro TLC plate and subsequently heated at 120 ºC for 5 min gave peculiar color indicating that it belongs to depside/tridepside class of compounds. This impure product was further purified by column chromatograph over silica gel and then over sephadexLH-20 column eluted with 10% methanol in chloroform to yield sub-fractions $3A_1$-$3A_{10}$.

Sub-fractions $3A_3$-$3A_4$ was combined and passed over florisil eluted with 10-15% methanol in chloroform as solvent system. The solvent was evaporated to yield compound **2**. Structural characterization of the compound **2** has been determined by chemical, spectral and spectrometric methods and designated as a methyl ester of lasallic acid.

Fractions 4 eluted with 20-25% methanol in chloroform and evaporated to furnish a mug residue (4A) and it looked like a mixture of few compounds along with polymeric substance, checked by TLC. When sprayed with aqueous 10% H_2SO_4 on micro TLC plate and heated at 120 ºC for 5 min, it displayed a peculiar color indicating that it may be depside/tridepside class compounds. This was further purified by chromatography over silica gel followed by sephadexLH-20 column by using 10-20% methanol in chloroform as eluting solvent system to yield sub-fractions $4A_1$-$4A_7$.

Sub-fractions $4A_2$-$4A_3$ was monitored by TLC followed by performed with various spray reagents. Preliminary investigation revealed that since chemical profiling of sub-fractions $4A_2$-$4A_3$ was almost similar and hence they combined. It was further passed through a small bed florisil column and eluted with 15% methanol in chloroform. The solvent was removed under reduced pressure to obtain a very light pink color substance designated as compound **3**. Its structural characterization has been carried out by spectrometric and spectroscopic methods and identified as a tridepside known as methyl ester of hiascic acid **3**.

Fractions 5 was eluted with 25% methanol in chloroform and evaporated to furnish a mug residue (5A). It seemed to be a mixture of few compounds along with polymeric substance as monitored by TLC using different kinds of solvent systems. Amongst them, one compound was major constituent. When sprayed with aqueous 10% H_2SO_4 on micro TLC plate and heated at 120 ºC for 5 min, it displayed a peculiar color securing that it belongs to depside class compounds. This was further chromatographed sequentially over silica gel followed by gel permeation over sephadexLH-20 with the help of 10-20% methanol in chloroform as eluting solvent to yield sub-fractions $5A_1$-$5A_8$.

Sub-fractions $5A_2$-$5A_3$ was monitored by TLC by using different kind solvent system followed by with different spray reagents (*viz.* 10% aqueous H_2SO_4, neutral $FeCl_3$, DNP reagent, Anis-aldehyde spray *etc*.). Since, chemical constituents of these sub-fractions were similar with variation of relative abundance, hence they were combined. The combined product was passed over a short florisil column by using with 15% methanol in chloroform as eluting solvent system to afford a compound **4**. The structural characterization of this compound **4** has been carried out by spectrometric, spectroscopic methods and identified a known compound known as methyl ester of crustinic acid.

Fractions 6 was eluted with 25% methanol in chloroform and evaporated to furnish a brown residue coded as (6A). It seemed to be a single compound associated with polymeric substance as monitored by TLC. When sprayed with aqueous 10% H_2SO_4 on micro TLC plate and heated at 120 ºC for 5 min, it displayed a peculiar color securing that it belongs to depside class of compound. This was further chromatographed sequentially over silica gel followed by gel permeation over sephadexLH-20 with the help of 10-20% methanol in chloroform as eluting solvent to yield sub-fractions $6A_1$-$6A_9$. Sub-fractions $6A_3$-$6A_4$ was same monitored by TLC by using different kind of solvent system and spray reagents (*viz*. 10% aqueous H_2SO_4, neutral $FeCl_3$, DNP reagent, Anisaldehyde spray *etc*.). Since, chemical constituent of these sub-fractions were similar, hence they were combined. The combined product was passed over a short florisil column by using with 15% methanol in chloroform as eluting solvent system to afford compound **5**. The structural characterization of this compound **5** was carried out by 1D NMR, 2D NMR (HMBC and HSQC) and HRESI-MS methods and assigned as 4-carboxy lecanoric acid.[39,50,54]

CONCLUSION

The lichen (*P. parlatum*) specimen was collected from Munnar hill station in Kerala State from South India for its chemical profiling. The major chemical constituent present in more polar fraction are tridepsides (**1**-**4**) along with a new depside (**5**) which were assigned as β-hydroxy methyl gyrophorate, methyl ester of lasallic acid, methyl ester of hiascic acid, methyl ester of crustinic acid and 4-carboxy lecanoric acid respectively. The structural determination of new depside (**5**) was carried out by means of 1D and 2D NMR spectroscopy. The evaluation of anti-proliferative activity against the various human cancer cell lines was conducted in comparison with therapeutically prescribed drug paclitaxel as control using sulfo-rhodamine B (SRB) assay. In this connection, five different types of human cancer cell line such as cervical cancer cell line (Hela), pancreatic cell line (HPAC), prostate cancer cell line (DU-145), colon cell line (HT-29) and breast cancer cell line MDA-MB231 were used to carry out bio-assay for cell cytotoxicity study. This manuscript deals with the isolation of known tridepsides (**1**-**4**), their structural characterization by spectroscopic, spectrometric methods. The anti-proliferative activity of compounds

(**1**-**5**) against these aforesaid human cancer cell lines was tested. But inhibition of cell proliferative activity of these compounds (**1**-**5**) are not good enough (Table not shown). It was also speculated that halogenated tridepsides may be present in this specimen procured from Munnar hill station.[53] But unfortunately, we are unable to trace halogenated tridepsides in more polar extract of above specimen though the halogenated depsides was present its in medium polar diethyl ether extract.

Compound **1** (β-Hydroxy Methyl Ester of Gyrophoric Acid, HMEGA): light pink color solid substance; mp. 210 ºC; UV (MeOH) λ_{max} (log ε): 211 (0.67), 267 (0.29) and 304 (0.16) nm; IR (neat) ν_{max} (cm^{-1}): 3610.0, 2956.3, 1607, 1457, 1317.3, 1252.0, 1208.0, 1148.0, 177.5, 993.7, 879.0, 830.0, 800.0, 732.5, 681, 562, 526; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) and data, see Table 1; M. F.: $C_{26}H_{24}O_{10}$; HR-ESI-MS (positive mode): obs. *m/z*: 496.1784 au $[M-2H]^+$& calc. *m/z*: 498.116 au; base peak 183.151 au.

Compound **2** (Methyl Ester Lisallic Acid, MELA): light pink color solid substance; mp 235 ºC; UV (MeOH) λ_{max} (log ε): 211 (1.21), 268 (0.51) and 303 (0.30) nm; IR (neat) ν_{max} (cm^{-1}): 3605, 1607, 1457, 1317, 1252, 1208, 1148, 1177, 993, 897, 830, 800, 732, 681, 562, 626; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) and data, see Table 1; $C_{25}H_{22}O_{11}$; HRESI-MS (positive mode): obs. *m/z*: 527.707 $[M+H]^+$; calc. *m/z*: 527.111 au ; base peak *m/z* 505.516 au.

Compound **3** (Methyl Ester of Crustinic Acid, MECA):brown solid substance; mp 215 ºC; UV (MeOH) λ_{max} (log ε): 210 (0.81), 267 (0.33) and 303 (0.18) nm; IR (neat) ν_{max} (cm^{-1}): 3364, 1612, 1580, 1502, 1443, 1379, 1311, 1261, 1199, 1159, 1112, 1060, 994, 952, 835, 799, 754, 700, 754, 700, 622, 574, 523; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) and data, see Table 1; M.F.: $C_{25}H_{22}O_{11}$; HR-ESI-MS (positive mode): obs. *m/z*: 527.563 $[M+H]^+$; calc. *m/z*: 527.111 au; base peak at *m/z*: 459.538 au.

Compound **4** (Methyl Ester of Hiascic Acid, MEHA): brown solid substance; mp 200 ºC; UV (MeOH) λ_{max} (log ε): 208 (1.61), 263 (0.64) and 30 (0.22) nm; IR (neat) ν_{max} (cm^{-1}): 3516, 3393, 2918, 2849, 1706, 1651, 1581, 1450, 1409, 1379, 1266, 1197, 1163, 1108, 1077, 1026, 967, 934, 863, 820, 802, 728, 645, 610, 585, 540; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) and data, see Table 1; M. F.: $C_{24}H_{22}O_{11}$; HRESI-MS (positive mode): obs. *m/z*: 496.1784 au & cal. *m/z*: 498.116 $[M-2H]^+$au; value of base peak *m/z*: 183.029 au.

Compound **5** brown solid substance; mp195 ºC; UV (MeOH) λ_{max} (log ε): 210 (0.81), 267 (0.33) and 303 (0.18) nm; IR (neat) ν_{max} (cm^{-1}): 3536, 1708, 1612, 1461, 1317, 1252, 1210, 1178, 1150, 1078, 993, 879, 830, 800, 731, 682, 627, 527; ^{1}H NMR (CD_3SOCD_3, 800 MHz) and ^{13}C NMR (CD_3SOCD_3, 201 MHz) and data, see Table 1. HRESI-MS (positive mode): obs. *m/z*: 346.4282 au $[M+H]^+$; cal. *m/z*: 347.077 $[M+H]^+$ au; value of base peak *m/z*: 151.085 (100%) and peak was appeared at 691.2957 au $[M+H]^+$ due to dimmer formation while on mass fragmentation.

Table 1: ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) of compound (1-5) isolated from polar fraction of lichen.

Positions	1 (HMEGA)		2 (MELA)		3 (MECA)		4 (MEHA)		5 (4-carboxy lecanoric acid)	
	δ_H(*J* in Hz)	δ_C	δ_H(*J* in Hz)	δ_C	δ_H(*J* in Hz)	δ_C	δ_H(*J* in Hz)	δ $cccc_C$	δ_H(*J* in Hz)	δccc_C
1	-	108.17	-	108.17	-	108.25	-	109.32	-	144.80
2	-	160.08	-	159.40	-	163.18	-	159.19	-	164.16
3	6.24, d (2.2), 1H	100.53	6.28, d (1.8), 1H	100.52	6.37, d (1.9), 1H	101.52	6.31, s, 1H	100.57	-	109.45
4	-	166.60	-	166.59	-	163.78	-	166.72	6.80, d (2.2), 1H	166.59
5	6.29, d (2.2), 1H	101.48	6.20, d (1.8), 1H	108.17	-	108.05	-	101.54	-	101.84
6	-	140.40	-	144.81	-	144.73	-	140.54	6.77 d (2.2), 1H	117.34
7	-	167.07	-	170.40	-	166.55	-	170.25	6.40, d (2.2), 1H	173.67
8 (CH_3)	2.45, s, 3H	21.30	2.44, s, 3H	24.20	2.45, s, 3H	24.09	2.47, s, 3H	24.26	-	
9 (CH_3)	-	-	-	-	-	-	-	-	-	-
C2-OH	11.62, s, 1H	–	10.30, s, 1H	-	11.62, s, 1H	–	11.60, s, 1H	–	-	–
C4-OH	9.45, s, 1H	–	-	-	9.33, s, 1H	–	11.13, s, 1H	–	-	–
C5-OH	-	–	-	-	-	–	9.32, s, 1H	–	-	–
1'	-	116.13	-	116.13	-	115.40	-	117.44	-	144.63
2'	-	156.43	-	159.40	-	159.09	-	154.94	-	170.33
3′	6.62, d (2.2), 1H	104.73	6.49 d (2.2), 1H	101.47	6.58, d (2.2), 1H	100.52	6.40, d (2.2), 1H	101.47	6.31, d (2.2), 1H	104.83
4'	-	159.14	-	166.09	-	152.65	-	166.18		166.82
5′	6.65, d (2.2), 1H	108.64	6.53, d (2.2), 1H	112.67	6.51, d (2.2), 1H	108.25	6.77, d (2.2), 1H	112.23		111.31
6′	–	144.13	-	144.56	-	144.53	-	144.69	-	
7′	-	170.43	–	172.40	-	166.31	-	172.85	-	
8'	2.64, s, 3H	21.30	2.64, s, 3H	24.00	2.58, s, 3H	24.21	2.61, s, 3H	23.84	-	
C2′-OH	9.27, s, 1H	–	-	–	11.28, s, 1H	-	12.42, s, 1H	-	-	-
1"	-	112.67	-	-	-	112.11	-	117.16	-	
2"	-	159.14	-		-	159.09	-	164.08	-	
3"	6.62, d (2.2), 1H	108,17	6.53, d (2.2), 1H		6.24, d (1.9), 1H	112.55	6.29, d (2.2), 1H	101.48	-	
4"	-	153.45	-	163.19	-	163.18	-	159.19	-	
5"	6.37, d (2.2), 1H	116.13	6.49, d (2.2), 1H	155.02	6.28, d (1.9), 1H	104.70	6.31, d (2.2), 1H	155.02	-	
6"	-	139.56	-		-	144.10	-	144.22	-	
7"	-	172.78	–	174.80	-	170.58	-	173.73		
8"	2.58, s, 3H	24.00	2.57, s, 3H	23.73	2.66, s, 3H	24.29	2.65, s, 3H	24.11	2.64	24.34
9"	-	-	-	-	-	-	2.18, s, 3H	22.99	2.60	23.2
C7"-OCH_3	3.91, s, 3H	51.96	3.89, s, 3H	51.96	3.91, s, 3H	52.01	3.92, s, 3H	52.04		-

ASSOCIATED CONTENT

Supporting Information Contents

Annexure 1: Supporting Information (Spectroscopic data consisting IR, UV, ^{1}H NMR,^{13}C NMR, ^{1}H, ^{13}C-HMBC, ^{1}H, ^{13}C-HSQC, HRESI-MS of compounds (**1-5**), were enclosed in attached file.

AUTHOR INFORMATION

Corresponding Author
Tel: +91-22-2559-0232. Fax: +91-22-5560-750. Email: abauri@rediffmail.com.

ORCID
Ajoy Bauri: 0000-0002-0941-5638
ORCID
Esperanza J. Carcache de Blanco: 0000-0002-9519-3292
ORCID
Ines Castro Dionicio: 0000-0003-4349-3477
ORCID
Eric Salinas Arellano: 0000-0002-7393-0630
ORCID
Sabine Foro: 0000-0001-8341-0205
ORCID
Jonathan G. Jeyaraj: 0000-0002-7059-6140

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

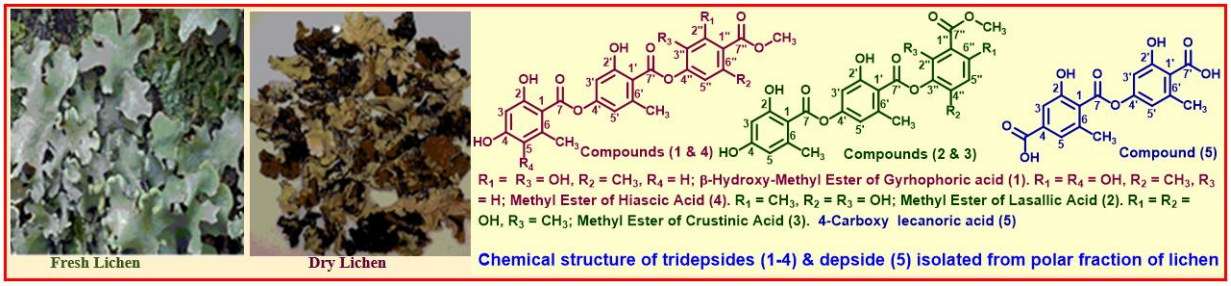
ACKNOWLEDGEMENTS

We express our sincere gratitude to Prof. David G. I. Kingston, Department of Chemistry, M/C 0212, Virginia Tech, Blacksburg, Virginia 24061, USA for fostering to collaborate with Prof. Esperanza J. Carcache de Blanco, College of Pharmacy, The Ohio State University, Ohio, Columbus, USA. We are thankful to Dr. Dmitriy Uchenik and Dr. Deepa Krisnan, OSU College of Pharmacy instrumental facility, for allowing acquisition of HRESI-MS data and Mrs. Mamata V. Joshi, National NMR Facility, TIFR, Colaba, Mumbai-400-005 for her excellent effort to acquire 2D-NMR data. Authors are very much grateful to Head of Division, Bio-Organic Division and Group Director of Bio-science Group for their continuous encouragement and ethical support.

AUTHOR CONTRIBUTION STATEMENT

AKB performed isolation, structural characterization and drafted the manuscript. SF contributed analysis tools and analyzed the XRD data of the crystals, structure analysis and computation. IC JGJ, and ESA contributed to the samples/reagents/materials/analysis tools and analyzed anti-proliferative assay the data. EJCB supervised biological experimental, analyzed the data of anti-proliferative assay, reviewed the manuscript, and managed to acquire HR-ESI-MS and LR-ESI-MS data with the help of IC and ESA.

GRAPHICAL ABSTRACT



REFERENCES

1. ENVIS Resource Partner on Biodiversity; Botanical Survey of India, Kolkatta, West Bengal; Ministry of Environment, Forest and Climate Change, Govt. of India.
2. E. S. Hansen. Vertical distribution of lichens on mountain Aucellabjerg, northeastern, Green land. Arctic *and Alpine Research*, 1996; 28: 111-117.
3. A. Abas, A. Awang. Air pollution assessments using lichen bio-diversity index (LBI) in Kuala Lumpur, Malaysia. *Pollut. Res*., 2017; 36: 241-248.
4. Z. Bozkurt. Determination of airborne trace elements in an urban area using lichen as bio-indicator. *Environ. Monit. Assess*., 2017; 189: article number 573.
5. M. Conti, G. Cecchetti. Biological monitoring: Lichen as bio-indicators of air pollution assessment-a review. *Eviron. Pollut*., 2001; 114: 471-492.
6. W. A. Elkhateeb, G. M. Daba. Lichens, an alternative drug for modern diseases. *Inter. J. Res. Pharma and Biosciences*, 2019; 6: 5-9.
7. P. C. Schalock. Lichen Extracts. *Dermatitis*, 2009; 20: 53-54.
8. Shukla, D. K. Upreti, S. Nayak, P. Tiwari. Natural dyes from Himalayan lichen. *Indian J. Tradit. Knowledge*, 2014; 13: 195-201.
9. A. R. Horwood. Lichen Dying To-day: The Revival of an Ancient Industry. *Science Progress in the Twentieth Century*, 1928; 23: 279-283.
10. R. RoyChowdhury, S. Maiti, R. V. Adivarekar, R. S. Singhal. Sustainable dying of silk using an acetylshkonin-based natural colourant from lichen Parmotrema perlatum *Green Chemistry*, 2024; 26: 904-917.
11. S. Nayaka, D. K. Upreti. *Sahydri E-News Western Ghats Biodiversity Information System*. Issue *XVI*, 2005.

http://wgbis.ces.iisc.ernet.in/biodiversity/Sahyadri_enews/newsletter/issue16/main_index.htm
12. S. Nayaka, S. Josheph, S. K. Rajaram, S. Natesan. Lichens of the *Sirimalai hillls* Eastern Ghats with one genus and six species new to India. *Studies in fungi*, 2021; 6: 204-212.
13. S. Nayaka, A. M. Reddy, P. Ponumurugan, A. Devi, G. Ayyapapadasan, D. K. Upreti. Eastern Ghat's biodiversity reserves with unexplored lichen wealth. *Current Science*, 2013; 104: 821-825.
14. H. Root, L. Geiser, S. Jovan, P. Neitlich. Epiphytic micro-lichen indication of air quality and climate in interior mountain of pacific Northwest USA. *Ecol. Indic*., 2015; 53: 95-105.
15. R. Daimari, P. Bhuyan, S. Hussain, S. Nayaka, M. A. J. Mazumdar, R. R. Hoque. Lichen as bio-indicators of air pollution assessment-a review. *Environ. Monit. Asses*., 2020; 192: article no, 37.
16. J. P. G. Barre, G. Deletraz, C. Sola-Larranaga, J. M. Santamaria, S. Berail, O. Donard, D. Amouroux. Multi elements isotopic signature (C, N, Pb, Hg etc) in epiphytic lichens to discriminate atmospheric contamination as a function of land-use characteristics (Pyrenees Atlantiques SW, France). *Environ. Pollut*., 2018; 243: 961-971.
17. M. Ono, C. Masuoka, M. Koto, M. Tateishi, H. Komatsu, H. Kobayashi, K. Igoshi, Y. Ito, M. Okawa, T. Nohara. Anti-oxidant ortho benzoyloxy phenyl acetic acid, vaccihein A from fruit of Rabbiteye blueberry (Vaccinium ashei). *Chem. Pharm. Bull*., 2002; 50: 1416-1417.
18. G. Zgorka, K. J. Glowniak. Variation of free phenolic acids in medicinal plants belonging to the Lamiaceae family. *Pharm. Biomed. Anal*., 2001; 2: 79-87.
19. E. Skrzypczak-Pietraszek, J. Pietraszek. Chemical profile and seasonal variation of phenolic acid content in bastard balm (*Melittis melissophyllum* L., Lamiaceae). *J. Pharm. Biomed. Anal.*, 2012; 66: 154-161.
20. M. Hillenbrand, J. Zapp, H. Becker. Depsides from the petals of *Papaver rhoeas. Planta Med*., 2004; 70: 380-382.
21. K. A. Reynertson, A. M. Wallace, S. Adachi, R. R. Gill, H. Yang, M. J. Basile, J. D'Armiento, I. B. Weinatein, E. J. Kennelly. Bioactive depsides and anthocyanin from Jaboticaba (Myrciaria caulifolra). *J. Nat. Prod*., 2006; 69: 1228-1230.
22. L. John, T. P. Clausen, D. Grapov, P. D. Coley, T. A. Kursar. Galloyl Depsides of Tyrosine from young leaves of Inga laurina. *J. Nat. Prod*., 2007; 70: 134-136.
23. K. C. S. Kumar, K. Muller, 'Depsides an nonredox inhibitors of leukotriene B4 biosynthesis and HaCaT cell growth 2, Novel analogues of obtusatic acid. *Euro. J. Med. Chem*., 2000; 35: 405-411.
24. K. C. S. Kumar, K. Muller. Anti-proliferative and cytotoxicity activity of gyrophoric acid, usnic acid and diffractaic acid on human kertinocyte growth. *J. Nat. Prod.*, 1999; 62: 821-823.
25. U-V. Isabel, G-B. Elena, P. K. Divakar, G-D. Maria Pilar. Lichen depsides and tridepsides: progress in pharmacological approaches. *J. Fungi*, 2023; 9: 116.
26. J. M. Gerrard, D. A. Peterson. Structure of the active site of prostaglandin syntheses from studies of depsides: an alternate view. *Prostaglandins Leukot Med.*, 1984; 13: 139-142.
27. T. T. Ngugen, S. Yoon, Y. Yang, H. B. Lee, S. Oh, H.-H. Jeong, J.-J. Kim, S. T. Yee, F. Crisen, C. Moon, K. Y. Lee, K. K. Kim, J.-S. Hur, H. Kim. Lichen secondary metabolites in Flavocetraria cucullata exhibit anti-cancer effect on human cancer cell line through the induction of apoptosis and suppression of tumorigenic potential. *PloS One*, 2014; 9: e111575.
28. B. Sepulveda, M. C. Chamy, M. Piovano, C. Areche. Anti-cancer potential of lichen secondary metabolites. *J. Chil. Chem. Soc.*, 2013; 52: 1750-1752.
29. C. A. F. Alexandrino, N. K. Honda, M. de F. C. Matos, L. C. Portugal, P. R. B. de Souza, R. T. Perdomo, R. de C. A. Guimaraes, M. C. T. Kadri, M. C. B. Lima Silva, D. Bogo. Anti-tumor effect of depsidones from lichens on cell lines and experimental murine melanoma. *Revista Brasileira de Farmacognosia*, 2019; 29: 449-456.
30. G. Shrestha, L. L. St. Clair. lichen: a promising source of anti-biotic and anti-cancer drugs. *Phyto chem Rev*., 2013; 12: 229-244.
31. M. A. O'Neill, M. Mayer, K. E. Murray, H. M. L. Rolim-Santos, N. S. Santos-Magalhaes, A. M. Thompson,V. C. L. Appleyard. Does usnic acid affect microtubules in human cancer cells? *Braz. J. Biol.*, 2010; 70: 659-664.
32. M. Kello, M. Goga, K. Kotorova, D. Sebova, R. Frenak, L. Tkacikova, J. Mojzis. Screening evaluation of anti-proliferative, anti-microbial and antioxidant activity of lichen extract and secondary metabolites. *Plants* (basel), 2023; 12: 611.
33. A. Basile, D. Raigano, S. Loppi, A. Di Santi, A. Nebbioso, S. Sorbo, B. Conte, L. Paoli, F. DeRuberto, A. M. Molinari, L. Altucci, P. Bontempo. Anti-proliferative, anti-bacterial and anti-fungal activity of lichen *Xanthoriaparietina* and its secondary metabolites Parietin. *Int. J. Mol. Sci*., 2015; 16: 7861-7875.
34. P. A. S. White, R. C. Oliveira, A. P. Oliveira, M. R. Serafini, A. A. Araujo, D. P. Gelain, J. C. Moreira, J. R. Almedia, J. S. Quintans, L. J. Quintans-Junior, M. R. Santos. Anti-oxidant activity and mechanisms of action of natural compounds isolated from lichen. *Molecules*, 2014; 12: 14496-14527.
35. C. Fernadez-Moriano, M. P. Gomez-Serranillos, A. Crespo. Anti-oxidant potential of lichen species and their secondary metabolites-a systematic review. *Pharm. Biol*., 2016; 54: 1-17.
36. J. Elecko, M. Vilkova, R. Frenak, D. Routray, D. Rucova, M. Backor, M. Goga. A comparative study of isolated secondary metabolites from lichens and

their anti-oxidative properties. *Plants*, 2022; 11: 1077.
37. K. Mullar. Pharmaceutical relevant metabolites from lichen. *App. Microbiol. Biotech*, 2001; 56: 9-16.
38. M. J. Cacott, D. F. Ackerley, A. Knight, R. A. Keyzers, J. G. Owen. Secondary metabolites in the lichen symbiosis. *Chem. Soc. Rev*., 2018; 47: 1730-1760.
39. V. P. Zambare, L. P. Christopher. Biopharmaceutical potential of lichens. *Pharm. Biol*., 2012; 50: 778-798.
40. C. Fernadez-Moriano, P. K. Divakar, A, Crespo, M. P., Gomez-Serranillos. Neuro-protective activity and cytotoxic potential of two Parmeliaceae lichen: Identification of active compounds. *Phytomedicine*, 2015; 15: 847-855.
41. V. Sieteiglesias, E. E. Gonzalez-Burgos, P. Bermejo-Bescos, C. Fernadez-Moriano, A. Crespo, P. K. Divakar, M. P. Gomez-Serranillos. Lichen of parmelioid clade as promising multi-target neuro-protective agents. *Chem. Res. Toxicol*., 2019; 32: 1165-1177.
42. C. Fernadez-Moriano, P. K. Divakar, A. Crespo, M. P. Gomez-Serranillos. Protective effects of lichen metabolites evernic and usnic acids against redox impairment mediated cytotoxicity in central nervous system like cells. *Food Chem. Toxicol*., 2017; 105: 262-277.
43. G-B. Elena, C. Fernadez-Moriano, M. P. Gomez-Serranillos. Current knowledge of Parmelia: Ecological interest, phyto-chemistry, biological activities and therapeutic potential. *Phytochemistry*, 2019; 112051.
44. M. Kosanik, B. Rankovic. Anti-neurodegenerative and anti-diabetic activities of lichens. *Lichen Secondary Metabolites*, 2019; 215-236.
45. S-S. Elzbieta, M-C. Aleksandra, P. Zalewski, D. Swajgier, B-W. Ewa, B. Kapron, T. Plech, M. Zarowski, C-P. Judyta. Lichen derived compounds and extracts as biologically active substances with anti-cancer and neuro-protective properties. *Pharmaceuticals*, 2021; 14: 1293-1317.
46. K.-H. Nguyen, M. C. Krugler, N. Gouanlt, S. Tomasi. UV-protectant metabolites from lichens and their symbiotic partners. *Nat. Prod. Rep.*, 2013; 30: 1490-1508.
47. N. Neamati, H. Hong, A. Mazumdar, S. Wang, S. Sunder, M. C. Nieklam, G. W. Milne, B. Proksa, Y. Pommier. Depsides and desidones as inhibitors of HIV-1 integrase: discovery of novel inhibitors through 3D data base searching. *J. Med. Chem*., 1997; 40: 942-951.
48. A. P. Devi, T-H. Duong, S. Ferron, M. A. Beniddir, M-H. Dinh, V-K. Nguyan, N-K-T. Pham, D-H. Mac, J. Boustie, W. Chavasiri, P-L. Pogam. Salanzic acid derived depsidones and diphenyl ester with α-glucose inhibitory activities from the lichen Parmotrema dilatatum. *Planta Med*., 2020; 86: 1216-1224.
49. J. Daniel, T. Raphael. Lichen extracts as raw materials in perfumery. *Flavour Frag., J*., 2009; 24: 49-61.
50. I. Urena-Vacas, E. Gonzalez-Burgos, P. K. Divakar, M. P. Gomez-Serranillos. Lichen depsides and tridepsides: Progress in pharmacological approaches. *Journal of Fungi*, 2023; 9: 116-148.
51. V. B. Tatimula, K. N. Killari, H. Polimati, A. Ketha. Chemical and biological investigation on Mangrove associated lichens (Manglicolous lichena) from India. *Indian J. Pharm. Sci*., 2021; 83; 186-194.
52. G. Singh, A. Calchera, D. Merges, H, Valim, J. Otte, I. Schmitt, F. D. Grande. A candidate Gene Cluster for the Bioactive Natural ptoduct Gyrophoric acid in Lichen-Forming Fungi. *Microbiology Spectrum*, 2022; 10: e00109-22.
53. A. K. Bauri, I. C. Dionicio, E. S. Arellano, J. G. Jaraj, S. Foro, E. J. Carcache de Blanco. A New Rare Halogenated Depside from Lichen and study of its Anti-Proliferative Activity. *Chem. Bio-diversity*, 2024; e202301874.
54. G. Singh, D. Armaleo, F. D. Grande, I. Schmitt. Depsides and Depsidone Synthesis in Laconized Fungi Comes into Focus through a Genomes-Wide Comparison of Olivetoricand Physodic Acid Chemotypes of Pseudevernia furfuracea *Biomolecules*, 2021; 11: 1445-1450.
55. F. M. Dean. *Naturally Occurring Oxygen Ring Compounds*. Butterworth: London, UK, 1963; 288-289.
56. T. J. Mabry, K. R. Markham, M. B. Thomas. *The Systematic Identification of Flavonoids*. Springer-Verlag: New York, 1970; 36-38.
57. S. Huneck, C. Djerssi, D. Becher, D. Barber, M. V. Ardenne, K. Steinfelder, R. Tummler. Interpretation of mass spectra of depsides and tridepsides. *Tetrahedron*, 1968; 24: 2707-2755.

Appendix A: Supplementary Information Contents.

Entry No.	Legendary title
Figure S1	^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound **1** (β-hydroxy methyl ester of gyrophoric acid)
Figure S2	Expansion of ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound **1** (β-hydroxy methyl ester of gyrophoric acid)
Figure S3	^{13}C NMR spectrum (CD_3COCD_3, 500 MHz) of compound **1** (β-hydroxy methyl ester of gyrophoric acid)
Figure S4	Expansion of ^{13}C NMR ($CDCl_3$, 500 MHz) spectrum of compound **1** (β-hydroxy methyl ester of gyrophoric acid)
Figure S5	UV absorption spectrum (MeOH) of compound **1** (β-hydroxy methyl ester of gyrophoric acid)
Figure S6	IR absorption spectrum (neat) of compound **1** (β-hydroxy methyl ester of gyrophoric acid)
Figure S7	HRESI-MS spectrum of compound **1** (β-hydroxy methyl ester of gyrophoric acid)
Figure S8	^{1}H NMR spectrum (CD_3OD, 125 MHz) of compound **2** (methyl ester of lasallic acid)
Figure S9	Expansion of ^{1}H NMR spectrum (CD_3OD, 125 MHz) of compound **2** (methyl ester of lasallic acid)
Figure S10	Expansion of ^{1}H NMR (CD_3OD, 125 MHz) spectrum of compound **2** (methyl ester of lasallic acid)
Figure S11	^{13}C NMR ($CDCl_3$, 125 MHz) spectrum of compound **2** and its expansion (methyl ester of lasallic acid)
Figure S12	UV absorption spectrum (MeOH) of compound **2** (methyl ester of lasallic acid)
Figure S13	IR absorption spectrum (neat) of compound **2** (methyl ester of lasallic acid)
Figure S14	HRESI-MS spectrum of compound **2** (methyl ester of lasallic acid)
Figure S15	^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound **3** (methyl ester of crustinic acid)
Figure S16	Expansion of ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound **3**
Figure S17	^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound **3** (methyl ester of crustinic acid)
Figure S18	Expansion of ^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound **3** (methyl ester of crustinic acid)
Figure S19	UV absorption (MeOH) spectrum of compound **3** (methyl ester of crustinic acid)
Figure S20	IR absorption spectrum (neat) of compound **3** (methyl ester of crustinic acid)
Figure S21	HRESI-MS spectrum of compound **3** (methyl ester of crustinic acid)
Figure S22	^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound **4** (methyl ester of hiascic acid)
Figure S23	Expansion of ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound **4** (methyl ester of hiascic acid).
Figure S24	^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound **4** (methyl ester of hiascic acid)
Figure S25	Expansion of ^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound **4** (methyl of hiascic acid)
Figure S26	UV absorption (MeOH) spectrum of compound **4** (methyl ester of hiascic acid)
Figure S27	IR absorption spectrum of compound **4** (methyl ester of hiascic acid)
Figure S28	HRESI-MS spectrum of compound **4** (methyl ester of hiascic acid)
Figure S29	General interpretation of cleavage of ester bond of tridepside in mass spectrometry
Figure S30	Joint interpretation of mass fragmentations of tridepsides **1** and **4** in HRESI mass spectrometry
Figure S31	Interpretation of mass fragmentation of compound **4** (methyl ester of hiascic acid)
Figure S32	Interpretation of cleavage ester bond of tridepsides, compound **2** (methyl ester of lasallic acid) and compound **3** (methyl ester of crustinic acid)
Figure S33	^{1}H NMR spectrum (CD_3SOCD_3, 800 MHz) of compound **5** (4-carboxy lecanoric acid)
Figure S34	Expansion of ^{1}H NMR spectrum (CD_3SOCD_3, 800 MHz) of compound **5** (4-carboxy lecanoric acid)
Figure S35	^{13}C NMR spectrum (CD_3SOCD_3, 201 MHz) of compound **5** (4-carboxy lecanoric acid)
Figure S36	Expansion of ^{13}C NMR spectrum (CD_3SOCD_3, 201 MHz) of compound **5** (4-carboxy lecanoric acid)
Figure S37	UV absorption spectrum (MeOH) of compound **5** (4-carboxy lecanoric acid)
Figure S38	IR spectrum (neat) of compound **5** (4-carboxy lecanoric acid)
Figure S39	Interpretation of LRESI-MS spectrum of compound **5** (4-carboxy lecanoric acid)
Figure S40	General interpretation of mass fragmentation of compound **5** (4-carboxy lecanoric acid)
Figure S41	^{1}H, ^{13}C-HSQC spectrum (CD_3SOCD_3, 800 & 201 MHz) of compound **5** (4-carboxy lecanoric acid)
Figure S42	Expansion of ^{1}H, ^{13}C-HMBC spectrum (CD_3SOCD_3, 800 MHz & 201 MHz) of compound **5** (4-carboxy lecanoric acid)
Figure S43	Expansion of ^{1}H, ^{13}C-HMBC spectrum (CD_3SOCD_3, 800 & 201 MHz) of compound **5** (4-carboxy lecanoric acid)
Figure S44	Expansion of ^{1}H, ^{13}C-HMBC spectrum (CD_3SOCD_3, 800 MHz & 201 MHz) of compound **5** (3-carboxy lecanoric acid)
Figure S45	Graphical abstract

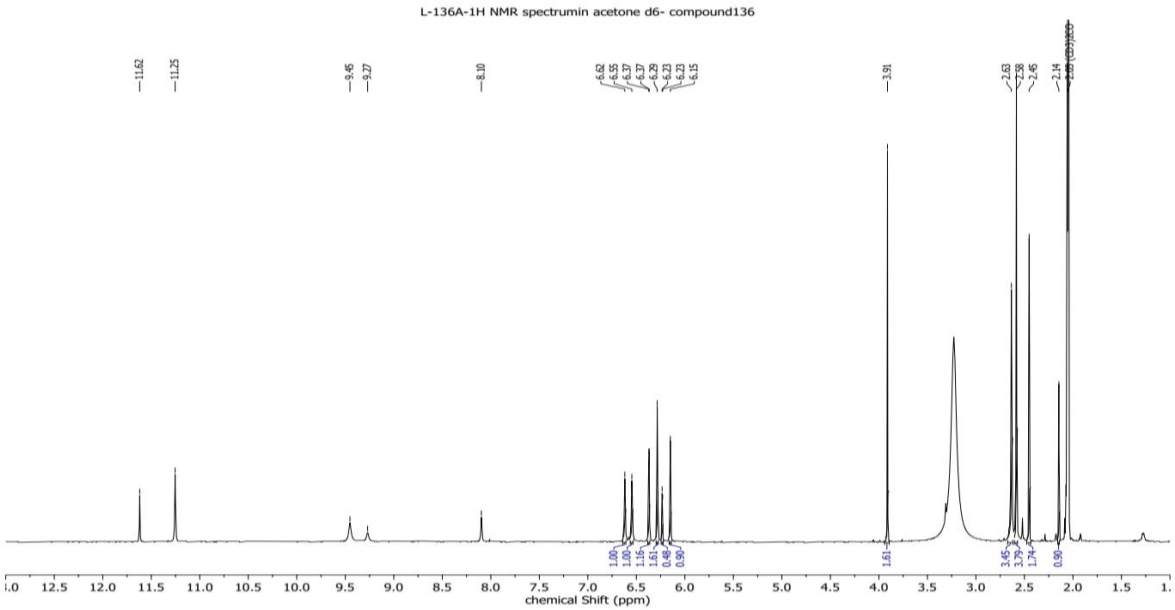


Figure S1: ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound 1 (β-hydroxy methyl ester of gyrophoric acid).

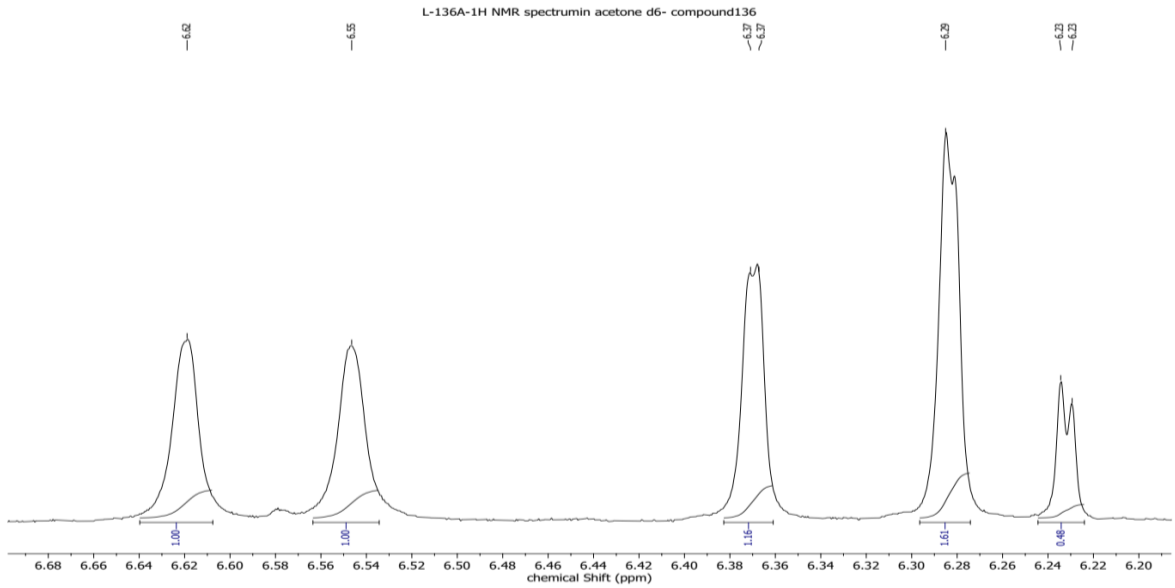


Figure S2: Expansion of ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound 1 (β-hydroxy methyl ester of gyrophoric acid).

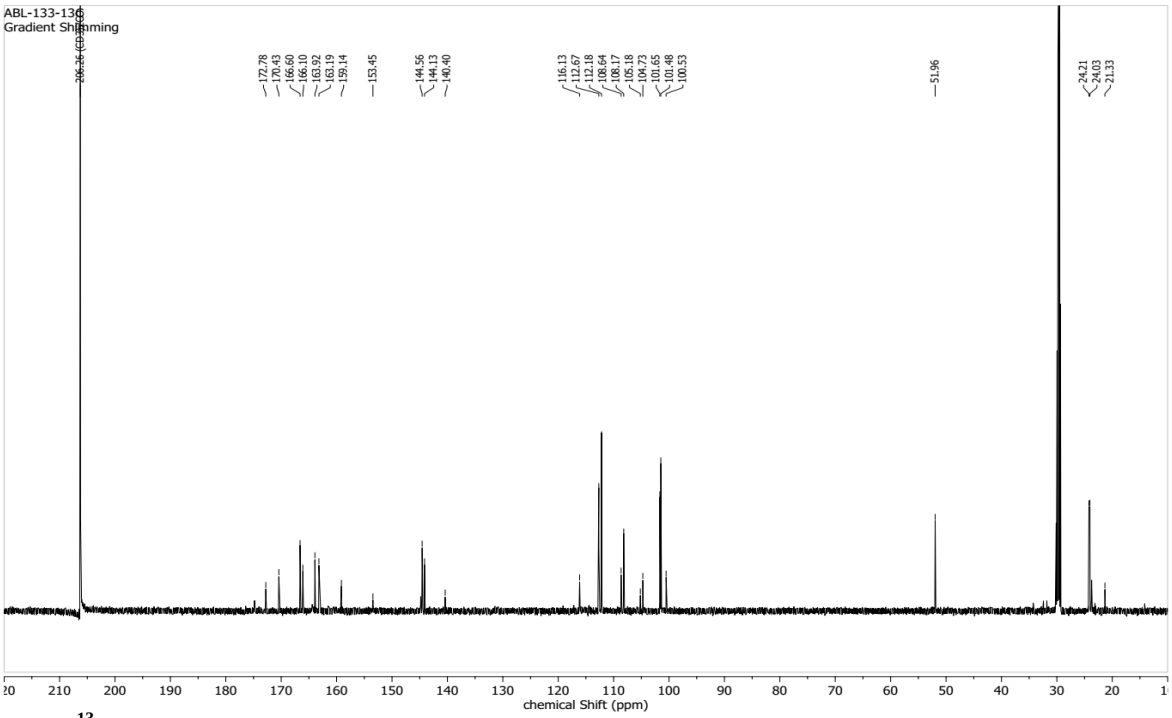


Figure S3: ^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound 1 (β-hydroxy methyl ester of gyrophoric acid).

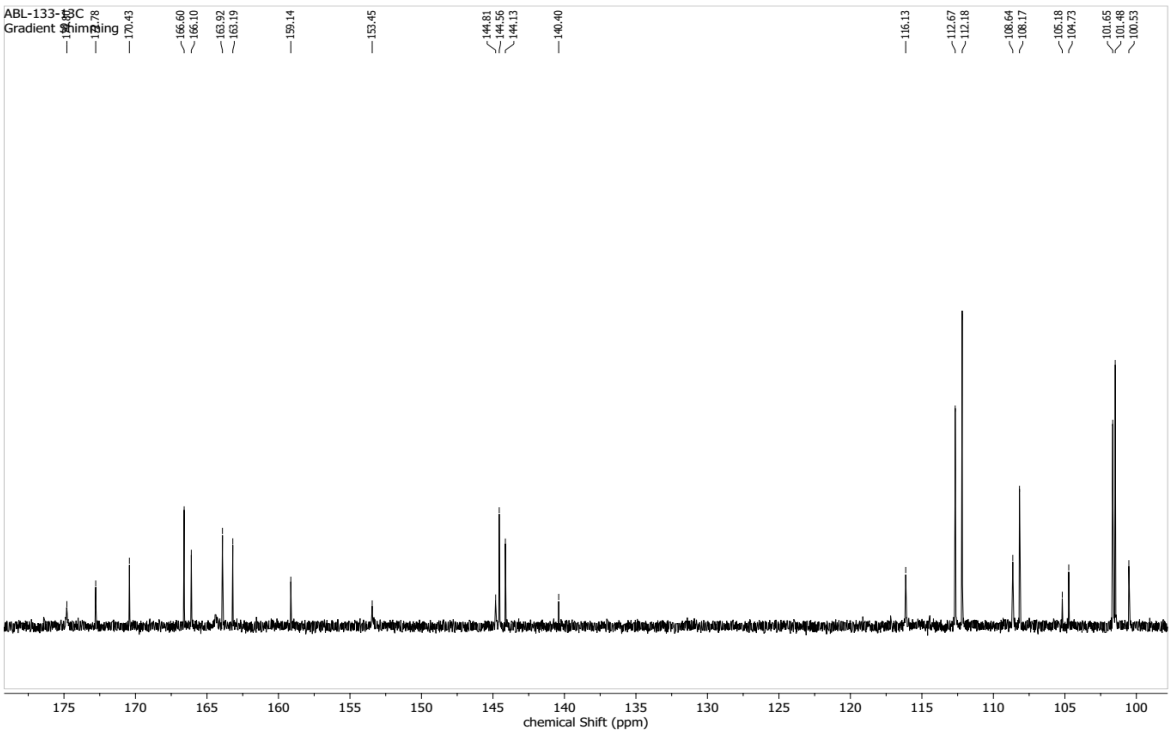


Figure S4: Expansion of ^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound 1 (β-hydroxy methyl ester of gyrophoric acid).

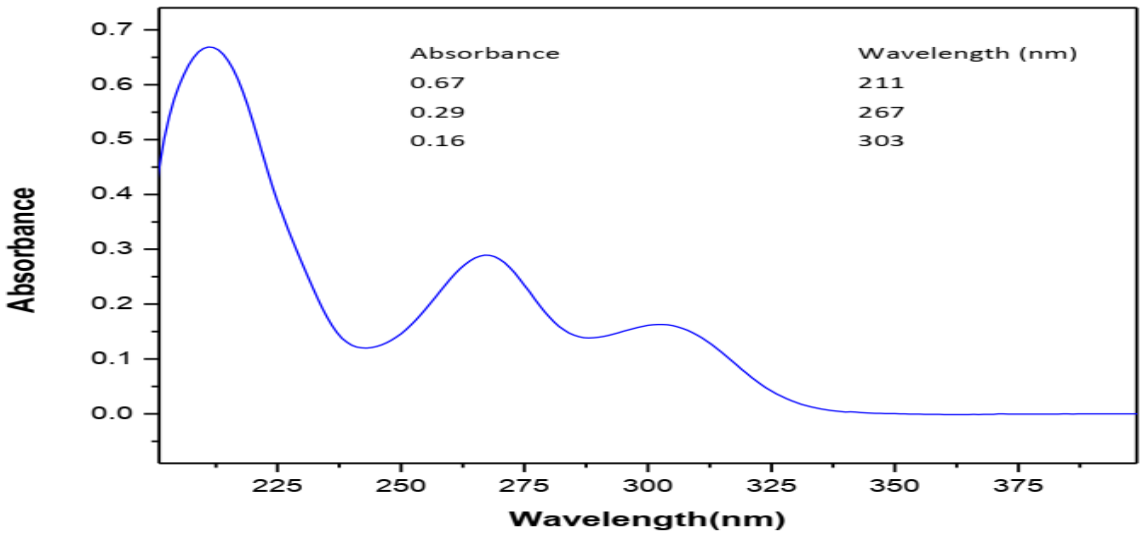


Figure S5: UV absorption spectrum (MeOH) of compound 1 (β-hydroxy methyl ester of gyrophoric acid).

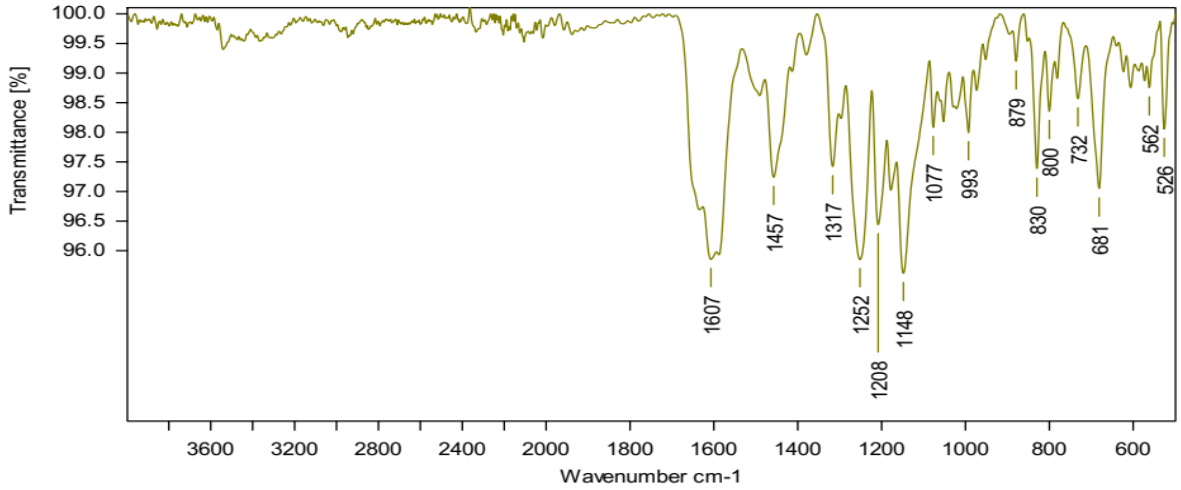


Figure S6: IR absorption spectrum (neat) of compound 1 (β-hydroxy methyl ester of gyrophoric acid).

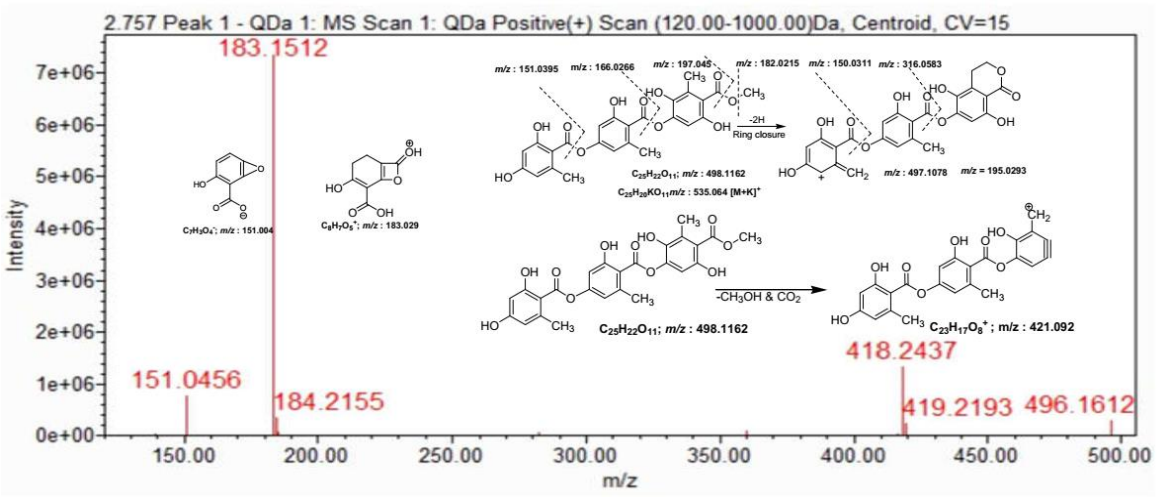


Figure S7: HRESI-MS spectrum of compound 1 (β-hydroxy methyl ester of gyrophoric acid).

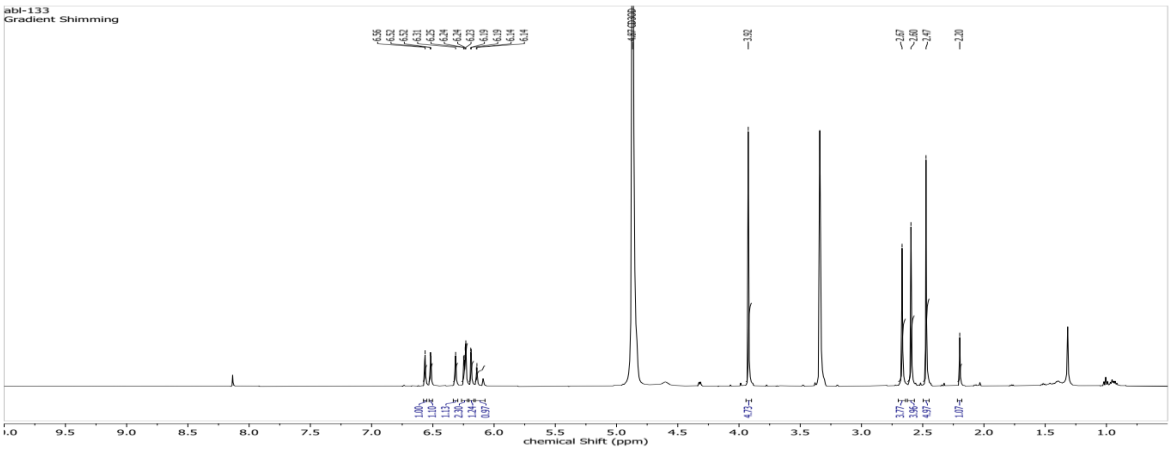


Figure S8: ^{1}H NMR spectrum (CD_3OD, 125 MHz) compound 2 (methyl ester of lasallic acid).

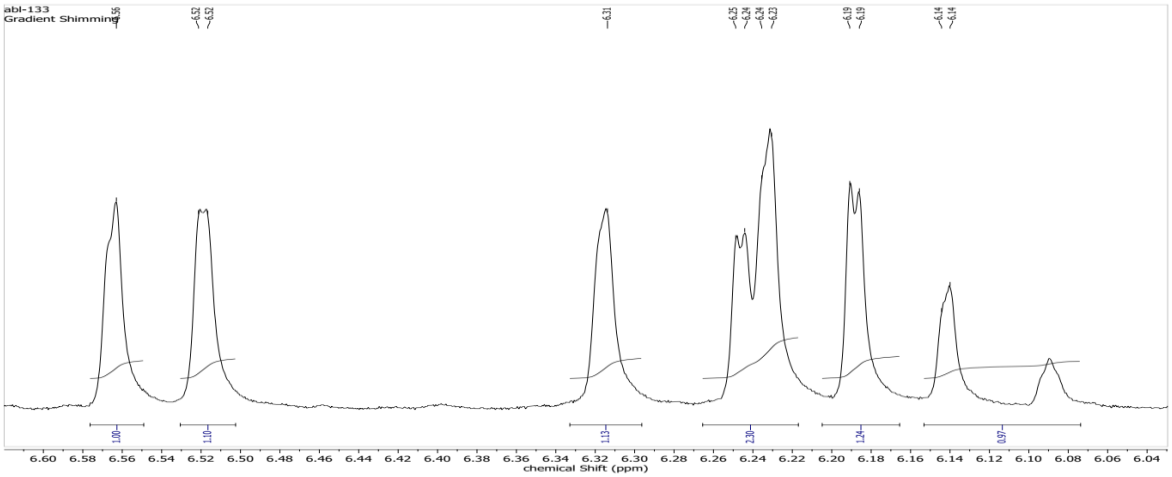


Figure S9: Expansion of ^{1}H NMR spectrum (CD_3OD, 125 MHz) compound 2 (methyl ester of lasallic acid).

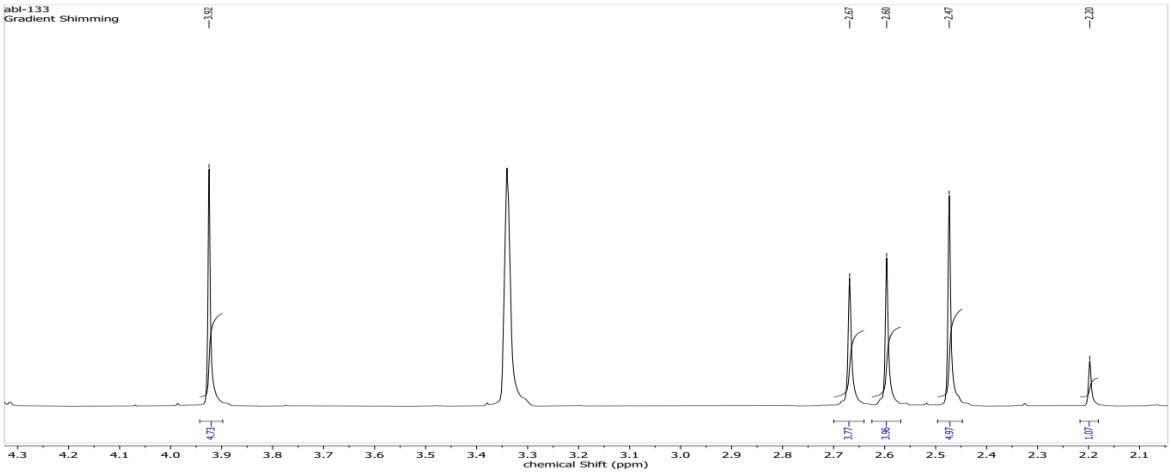


Figure S10: Expansion of ^{1}H NMR spectrum (CD_3OD, 125 MHz) compound 2 (methyl ester of lasallic acid).

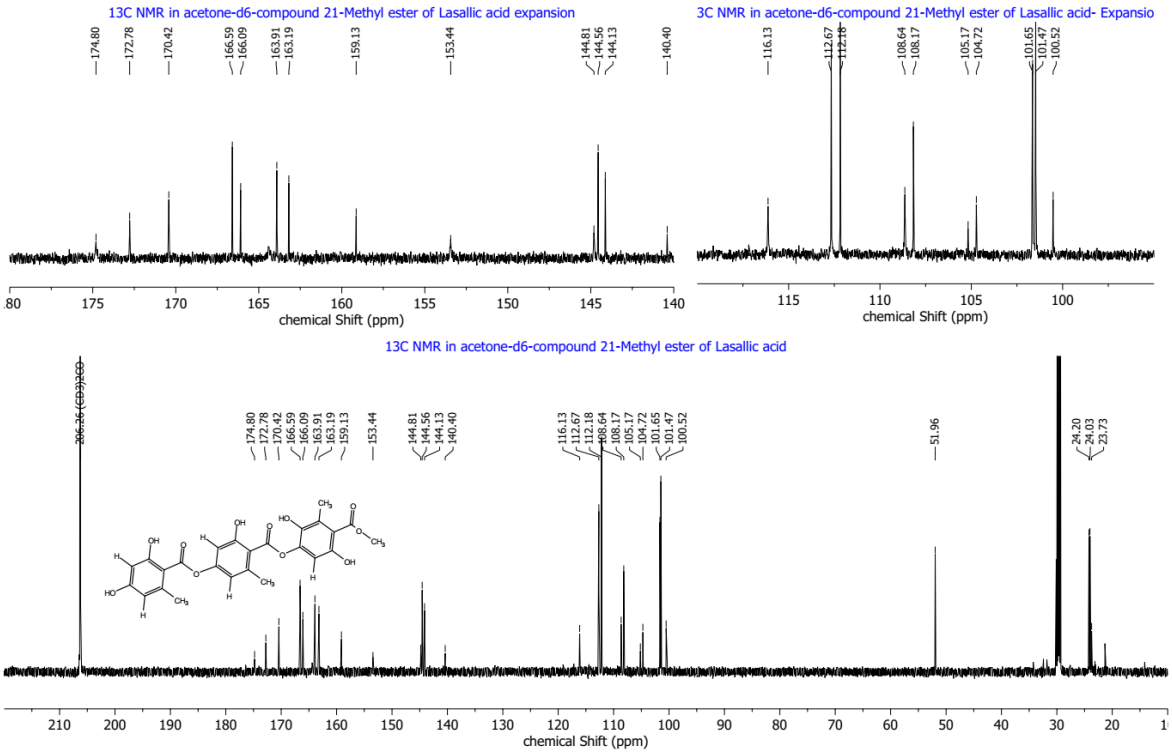


Figure S11: ^{13}C NMR spectrum ($CD_3COCD_3$125 MHz) of compound 2 (methyl ester of lasallic acid).

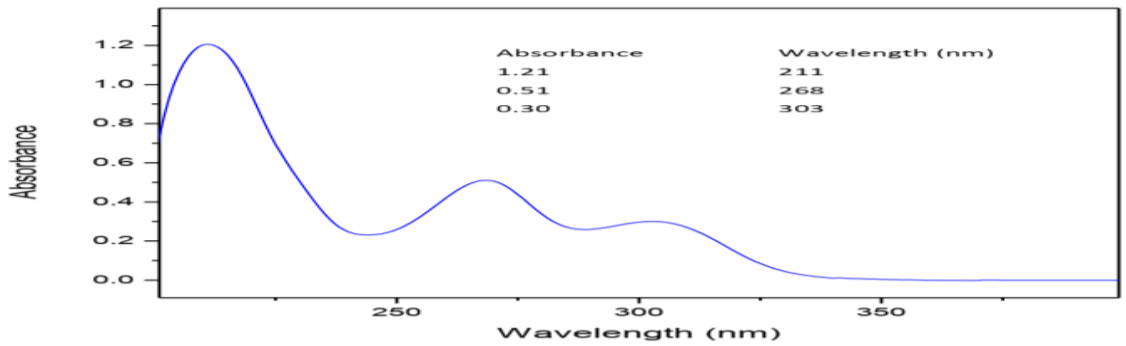


Figure S12: UV absorption spectrum (MeOH) of compound 2.

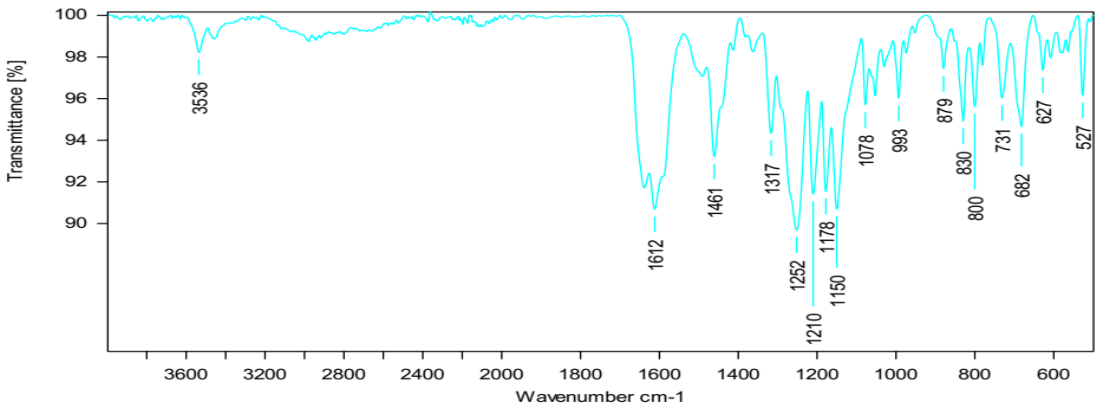


Figure S13: IR absorption spectrum (neat) of compound 2 (methyl ester of lasallic acid).

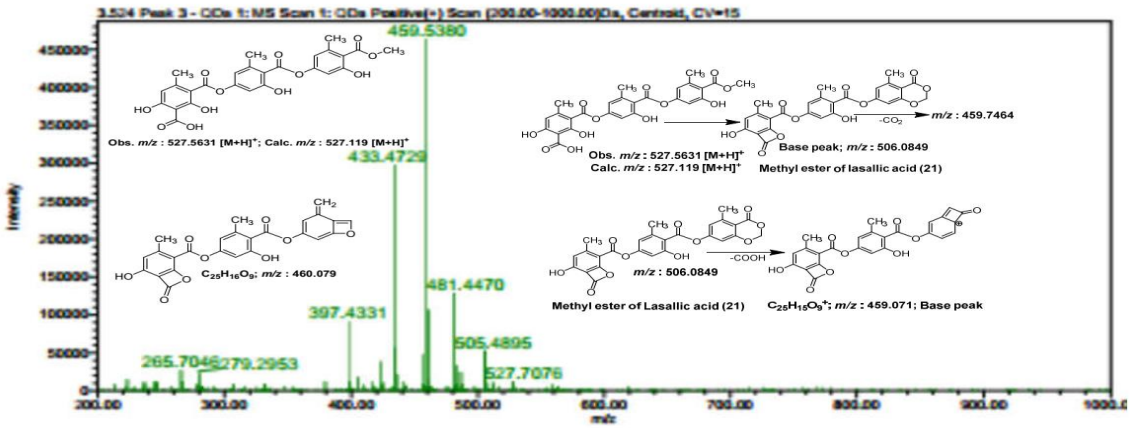


Figure S14: HRESI-MS spectrum of compound 2 (methyl ester of lasallic acid).

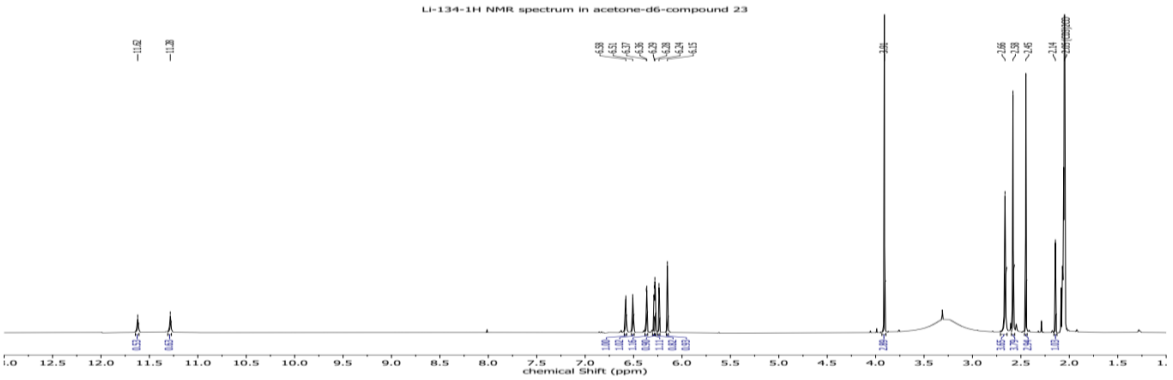


Figure S15: ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound 3 (methyl ester of crustinic acid).

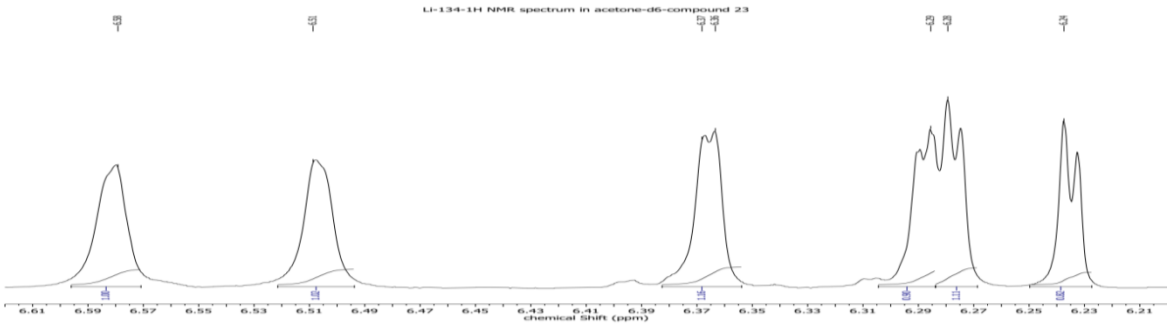


Figure S16: Expansion of ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound 3.

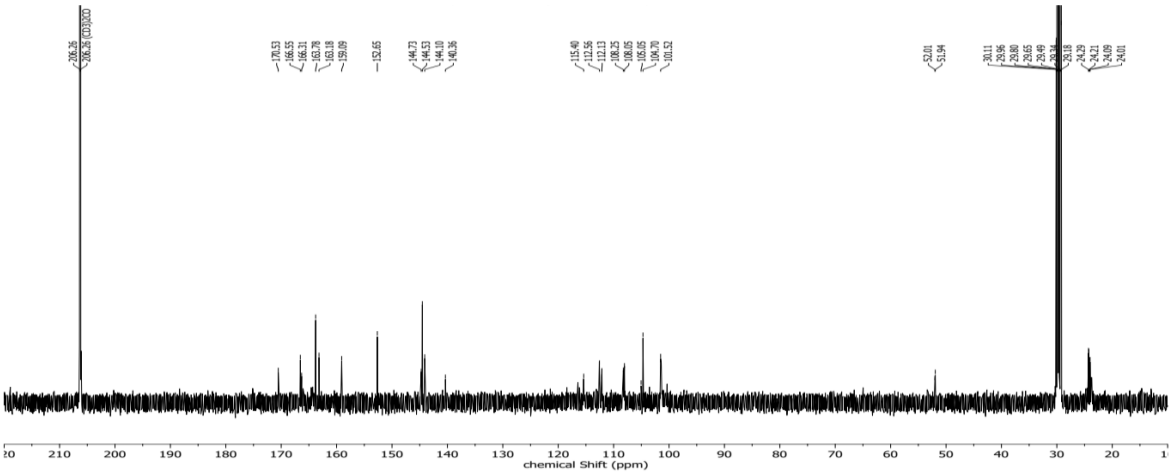


Figure S17: ^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound 3 (methyl ester of crustinic acid).

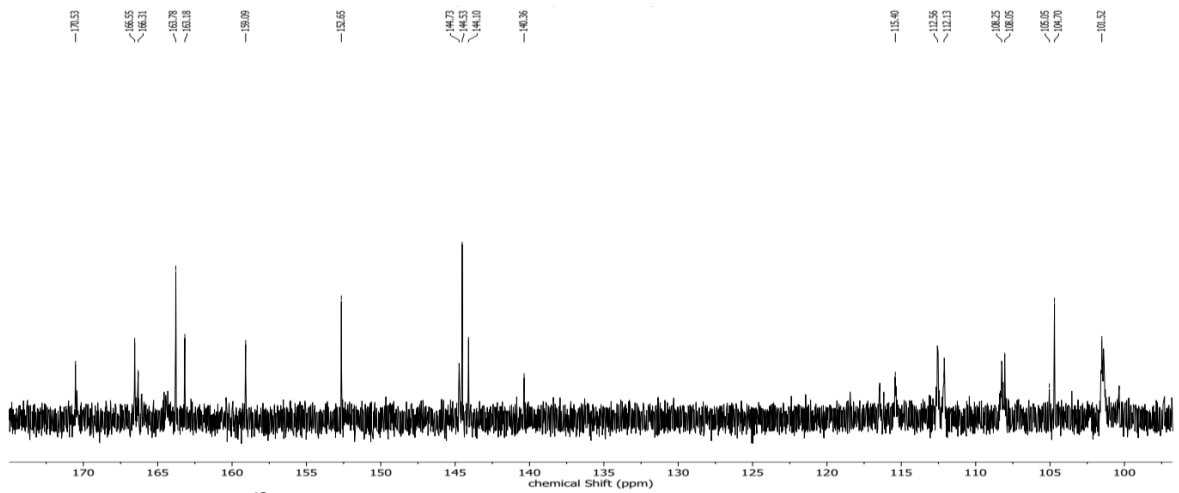


Figure S18: Expansion of ^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound 3 (methyl ester of crustinic acid).

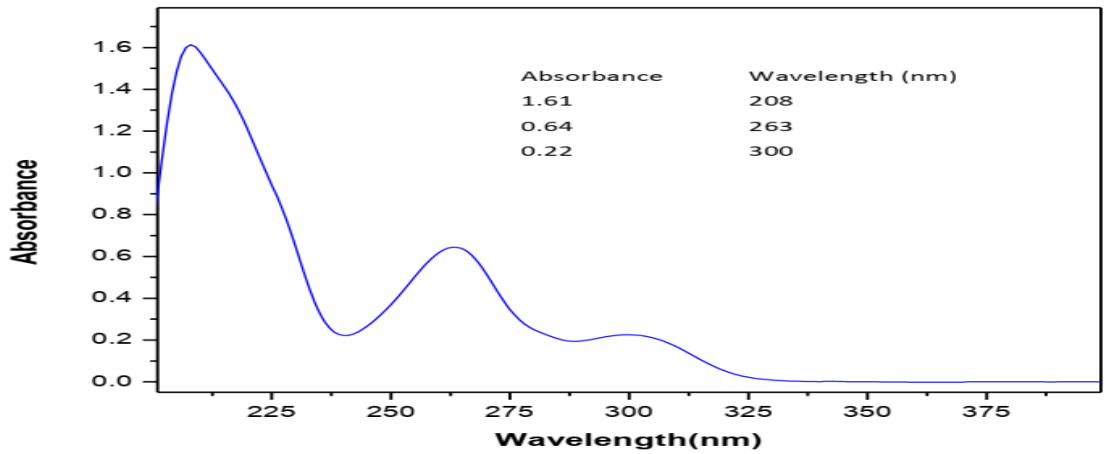


Figure S19: UV absorption spectrum of compound 3 (methyl ester of crustinic acid).

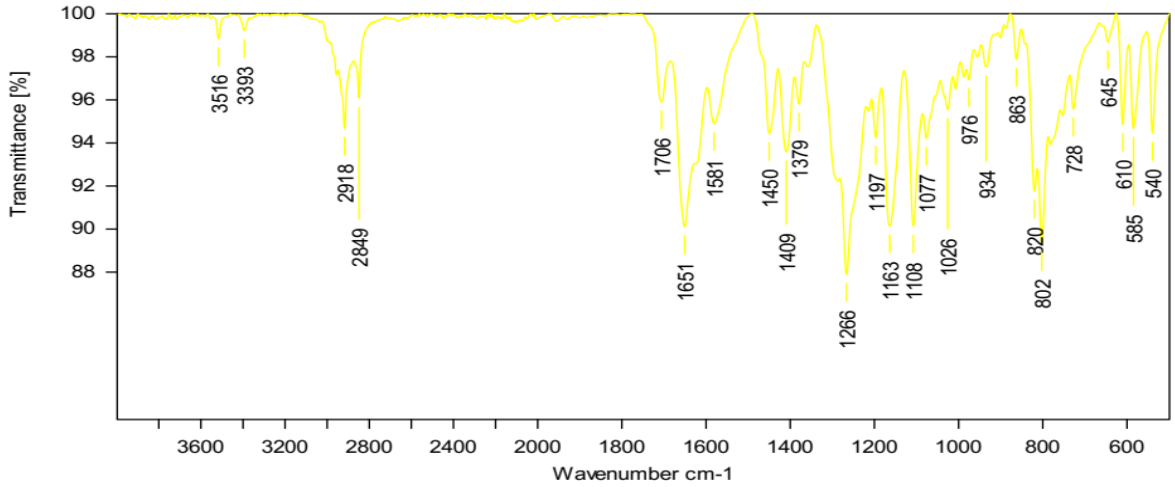


Figure S20: IR spectrum (neat) of compound 3 (methyl ester of crustinic acid).

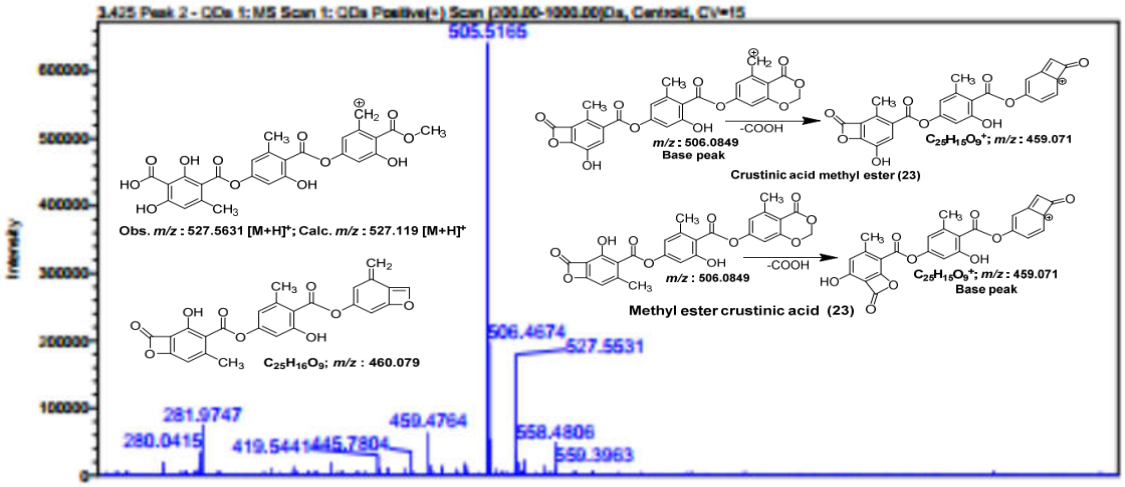


Figure S21: HRESI-MS spectrum of compound 3 (methyl ester of crustinic acid).

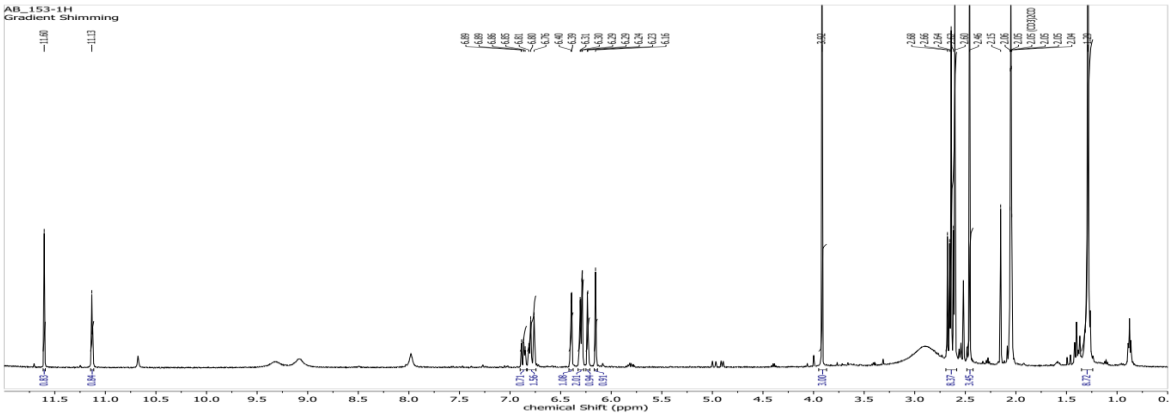


Figure S22: ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound 4 (methyl ester of hiascic acid).

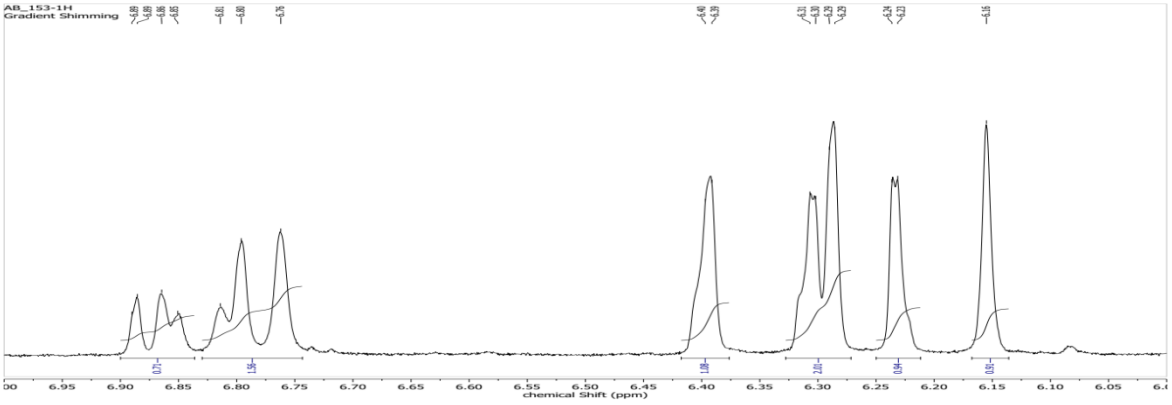


Figure S23: Expansion of ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound 4 (methyl ester of hiascic acid). [Note: trace amount impurity has been contaminated with this desired product]

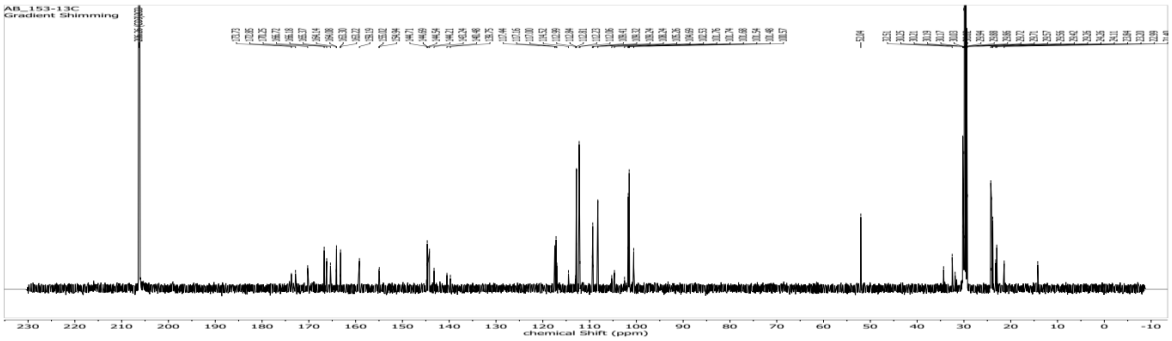


Figure S24: ^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound 4 (methyl ester of hiascic acid).

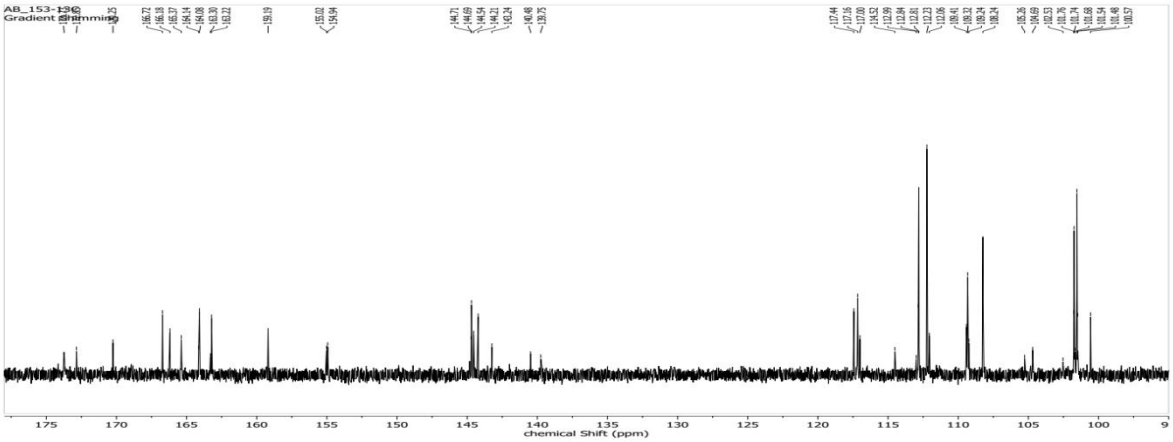


Figure S25: Expansion of ^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound 4 (methyl of hiascic acid).

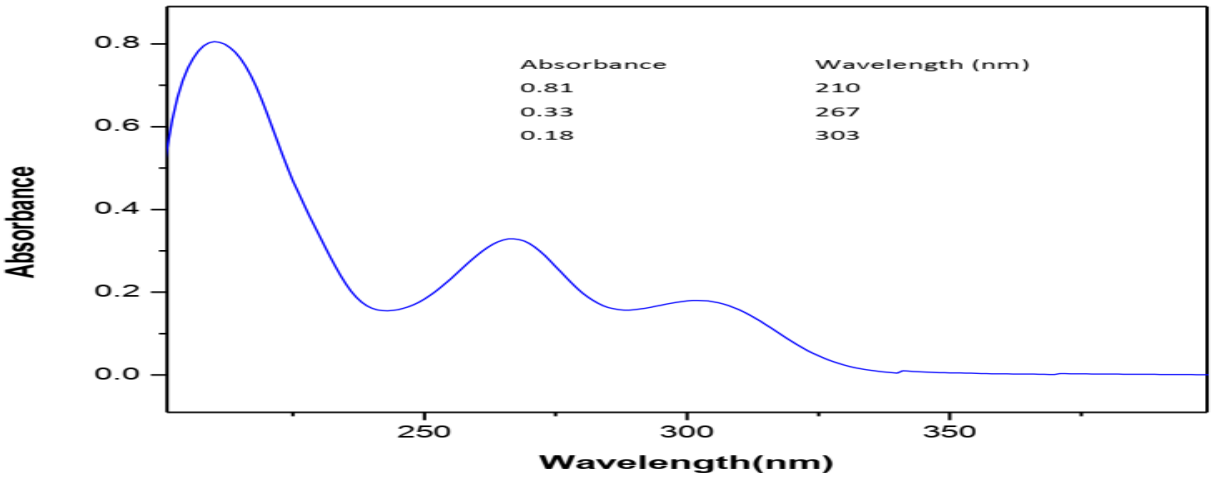


Figure S26: UV absorption spectrum (MeOH) of compound 4 (methyl ester of hiascic acid).

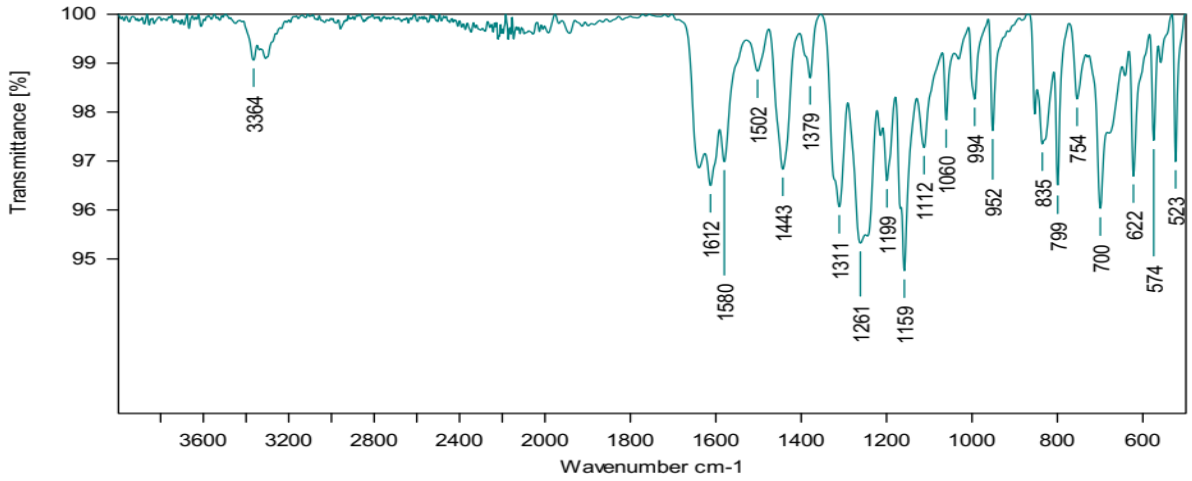


Figure S27: IR spectrum of compound 4 (methyl ester of hiascic acid).

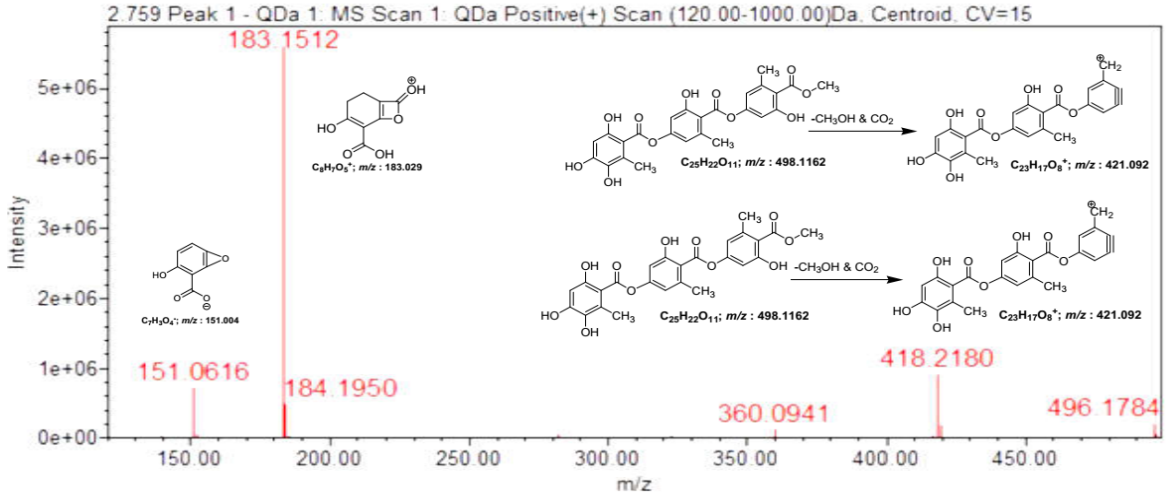


Figure S28: HRESI-MS spectrum of compound 4 (methyl ester of hiascic acid).

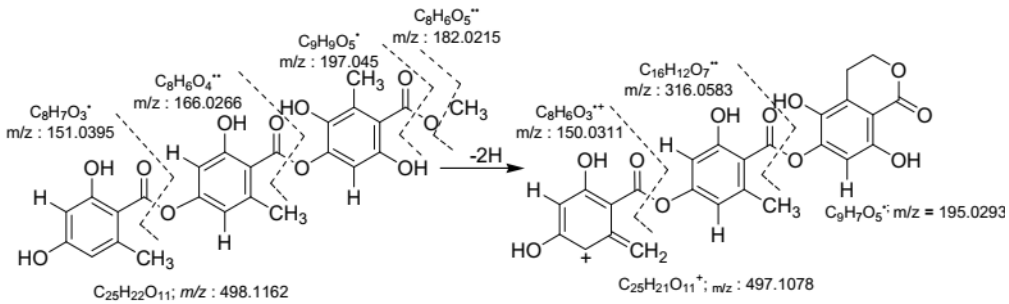


Figure S29: General interpretation of cleavage of ester bond of tridepside in mass spectrum.

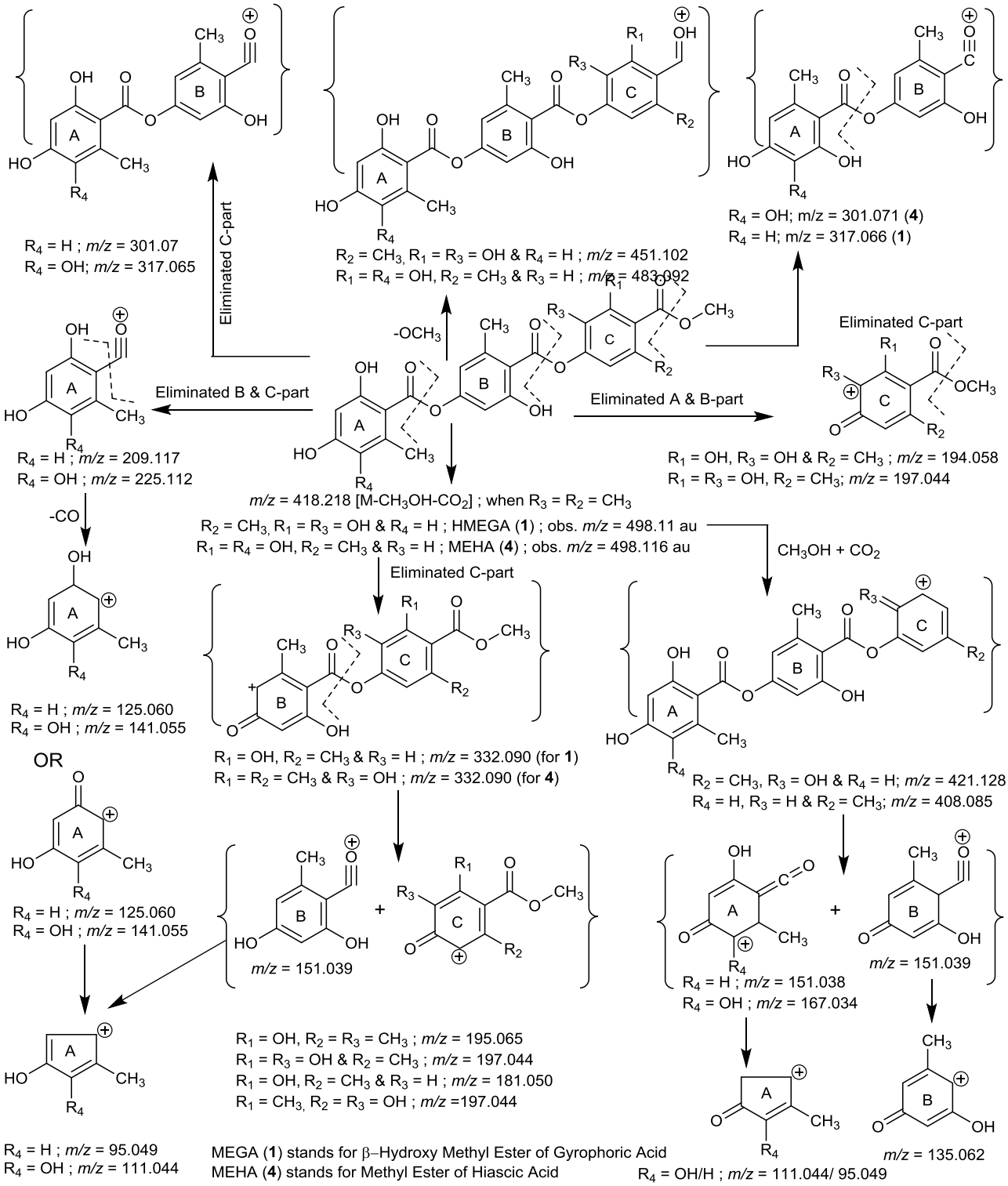


Figure S30: Joint interpretation of mass fragmentations of tridepsides 1 and 4 in HRESI mass spectrometry.

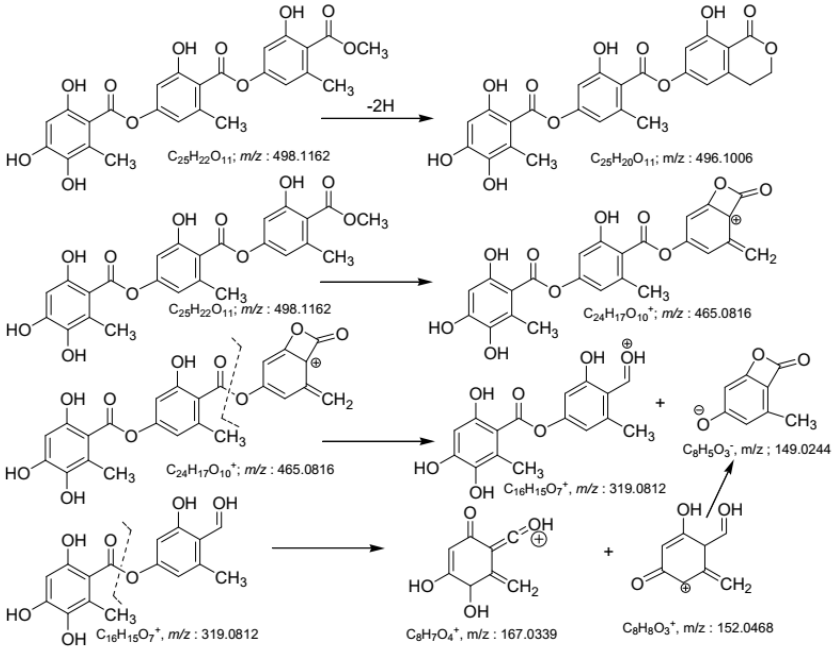
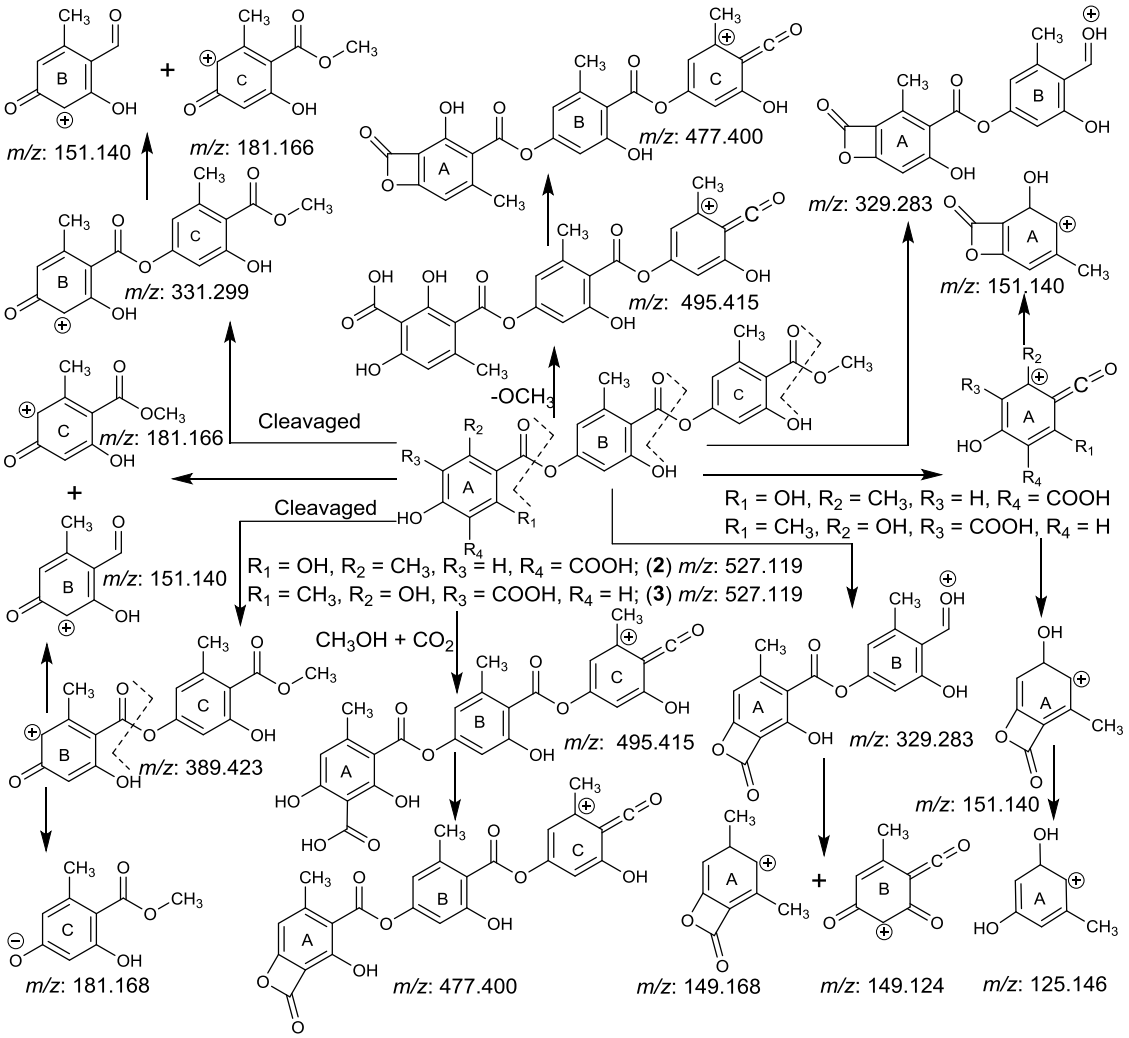


Figure S31: Interpretation of mass fragmentation of compound 4 (methyl ester of hiascic acid).



MELA denotes methyl ester of lasallic acid (**2**) and MECA stands for methyl ester of crustinic acid (**3**)

Figure S32: Interpretation of cleavage ester bond of tridepsides, compound 2 (methyl ester of lasallic acid) and compound 3 (methyl ester of crustinic acid).

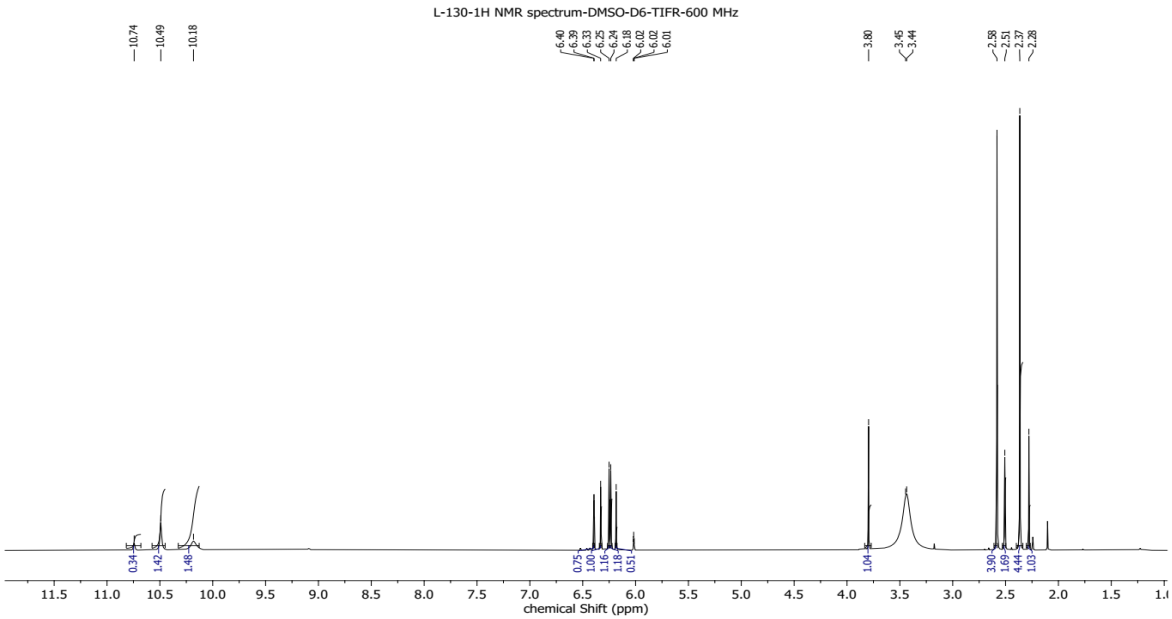


Figure S33: ^{1}H NMR spectrum (CD_3SOCD_3, 800 MHz) of compound 5 (4-carboxy lecanoric acid).

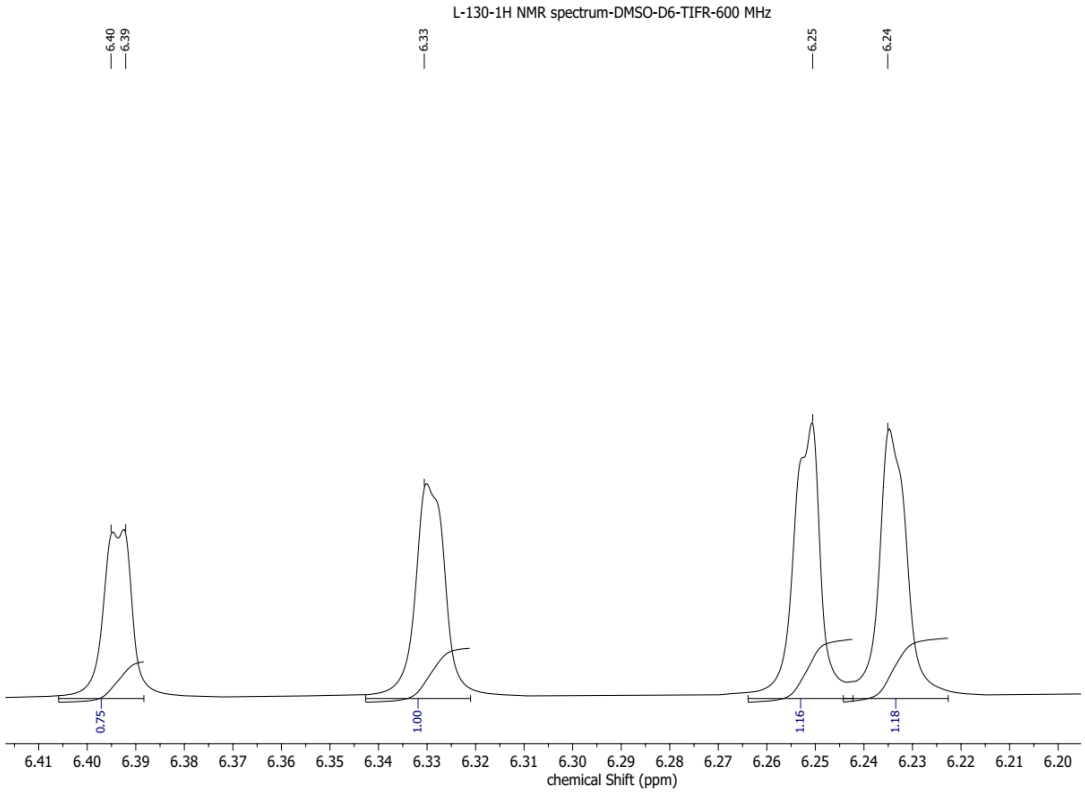


Figure S34: Expansion of ^{1}H NMR spectrum (CD_3SOCD_3, 800 MHz) of compound 5 (4-carboxy lecanoric acid).

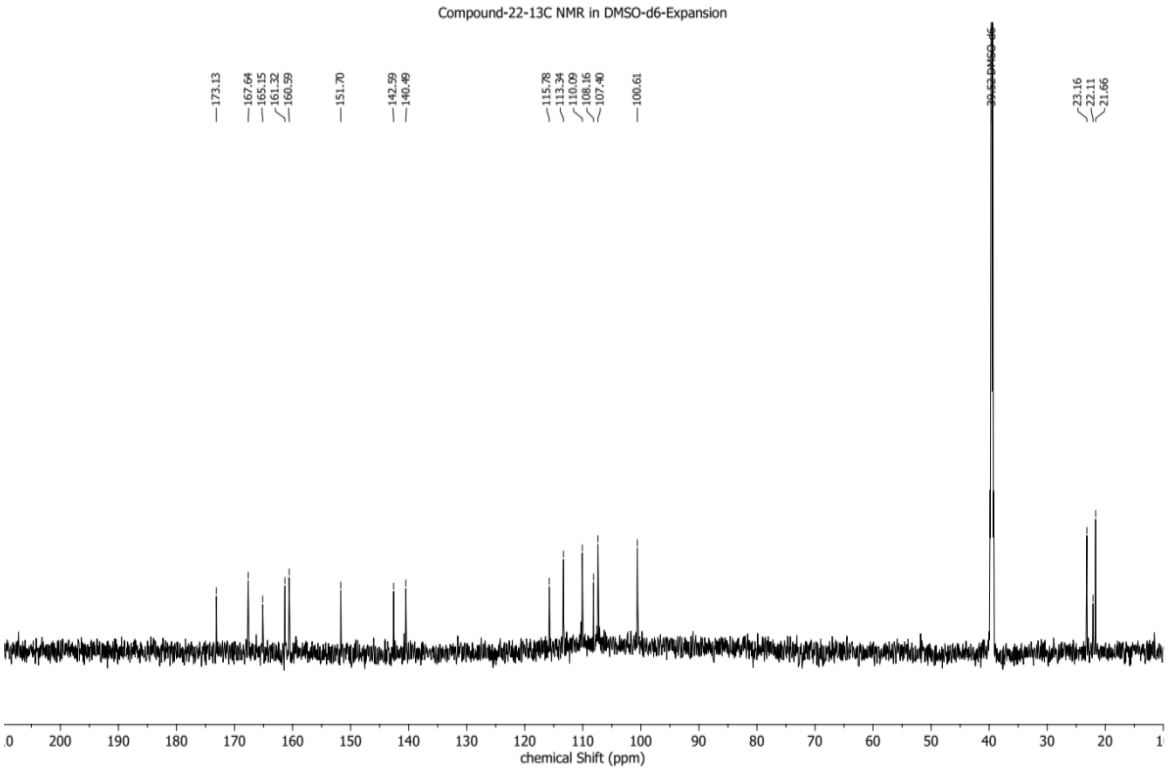


Figure S35: ^{13}C NMR spectrum (CD_3SOCD_3, 201 MHz) of compound 5 (4-carboxy lecanoric acid).

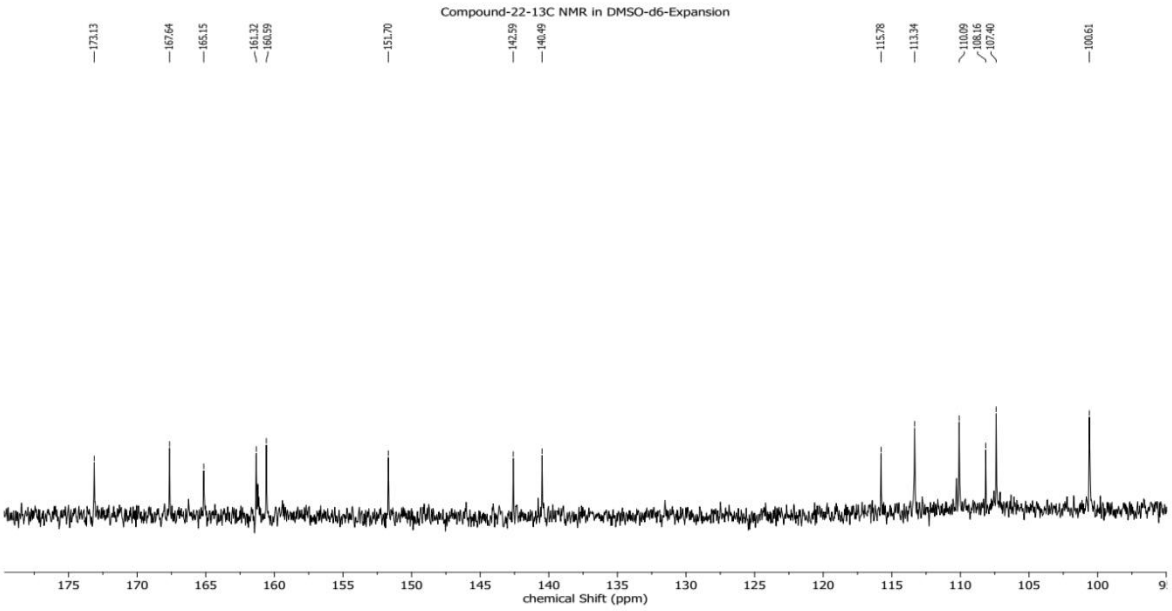


Figure S36: Expansion of ^{13}C NMR spectrum (CD_3SOCD_3, 201 MHz) of compound 5 (4-carboxy lecanoric acid).

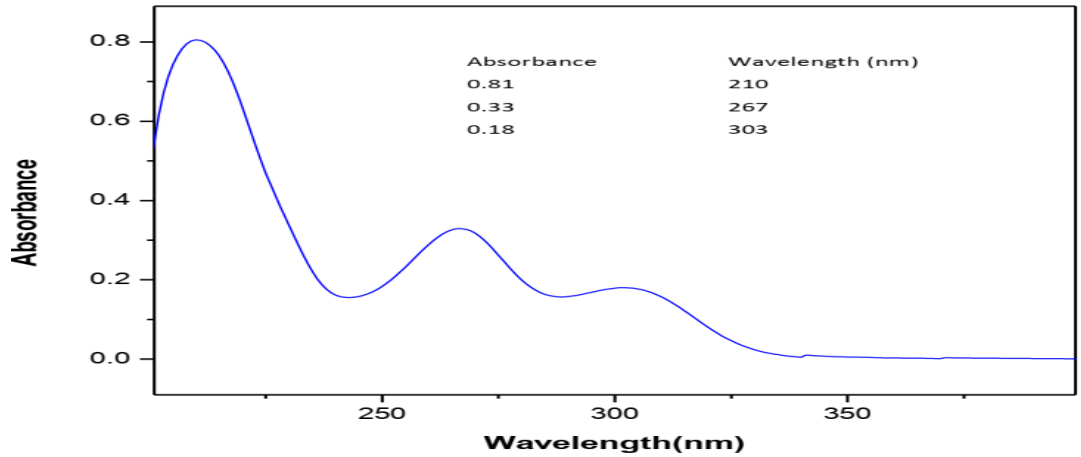


Figure S37: UV absorption spectrum (MeOH) of compound 5 (4-carboxy lecanoric acid).

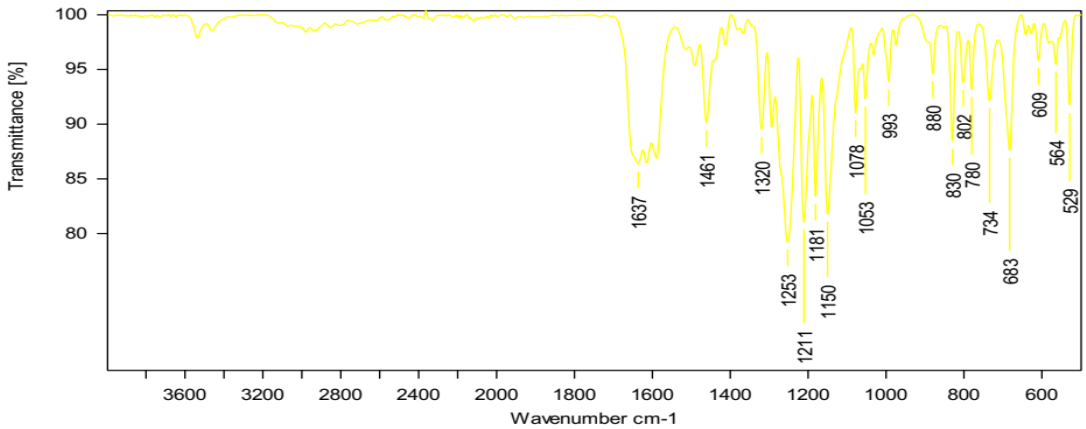


Figure S38: IR spectrum (neat) of compound 5 (4-carboxy lecanoric acid).

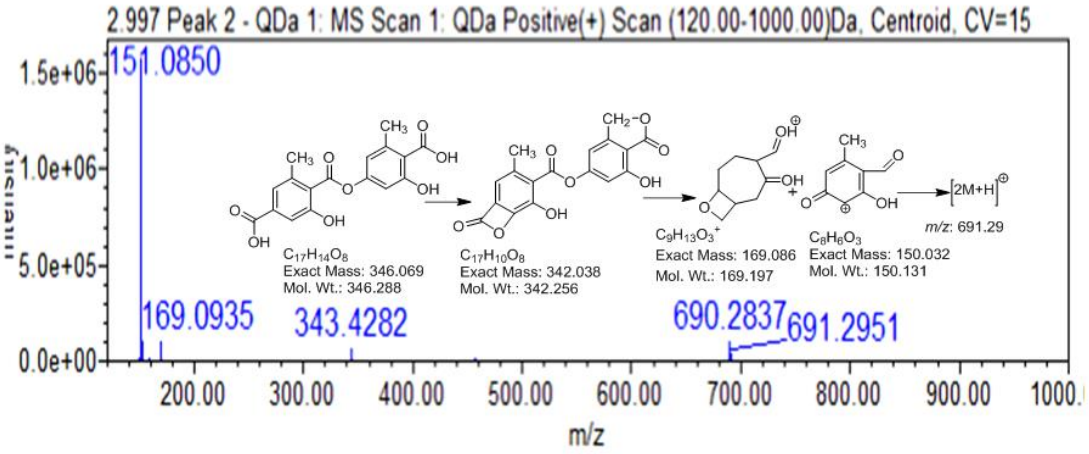


Figure S39: Interpretation of LRESI-MS spectrum of compound 5 (4-carboxy lecanoric acid).

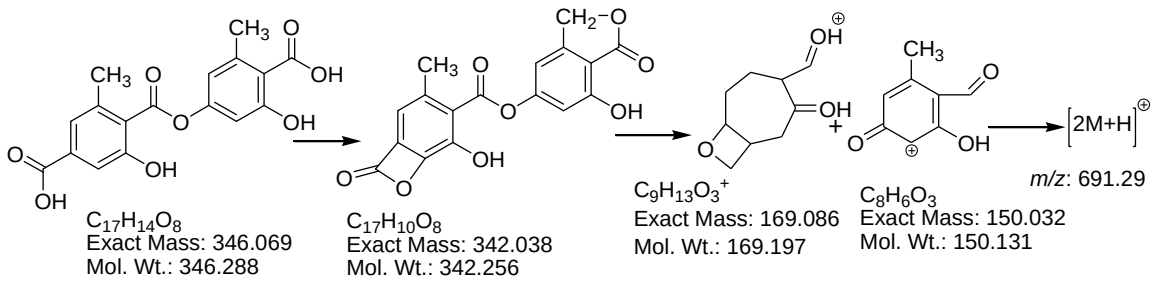


Figure 40: General interpretation of mass fragmentation of compound 5 (4-carboxy lecanoric acid).

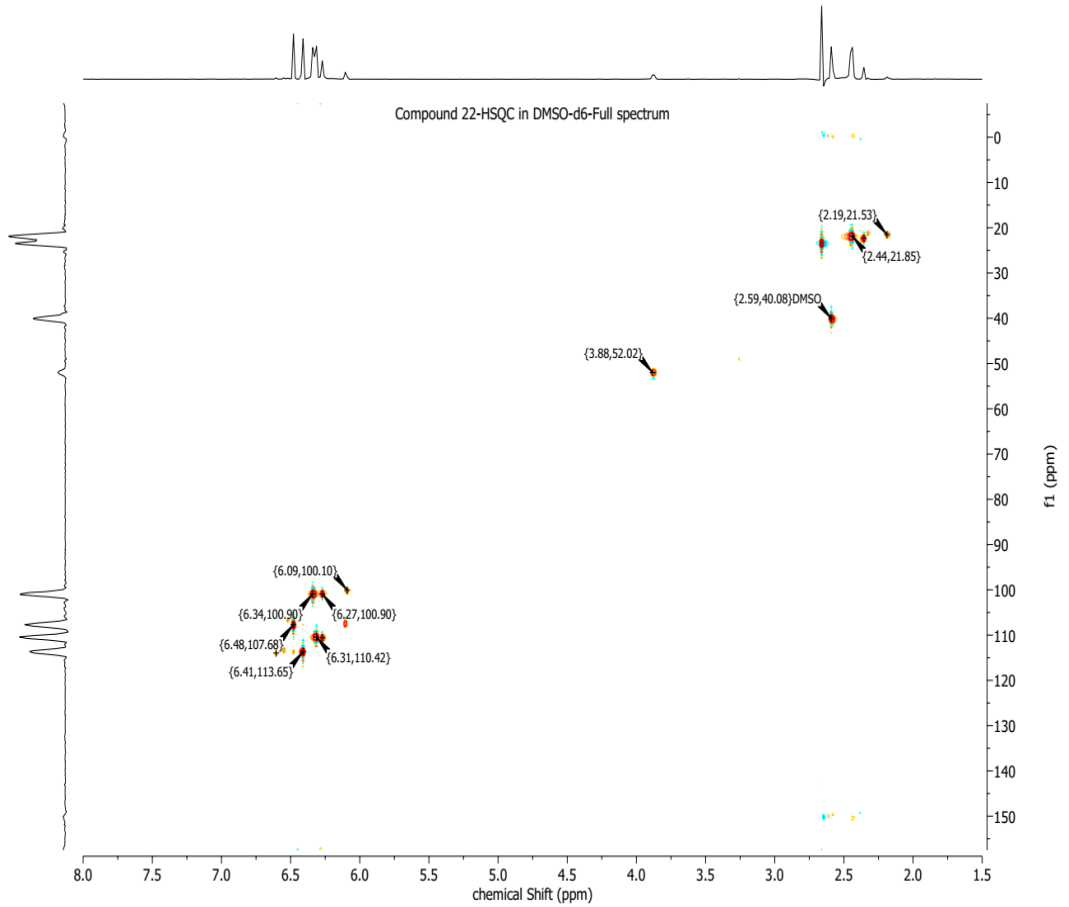


Figure S41: ^{1}H, ^{13}C-HSQC spectrum (CD_3SOCD_3, 800 & 201 MHz) of compound 5 (4-carboxy lecanoric acid).

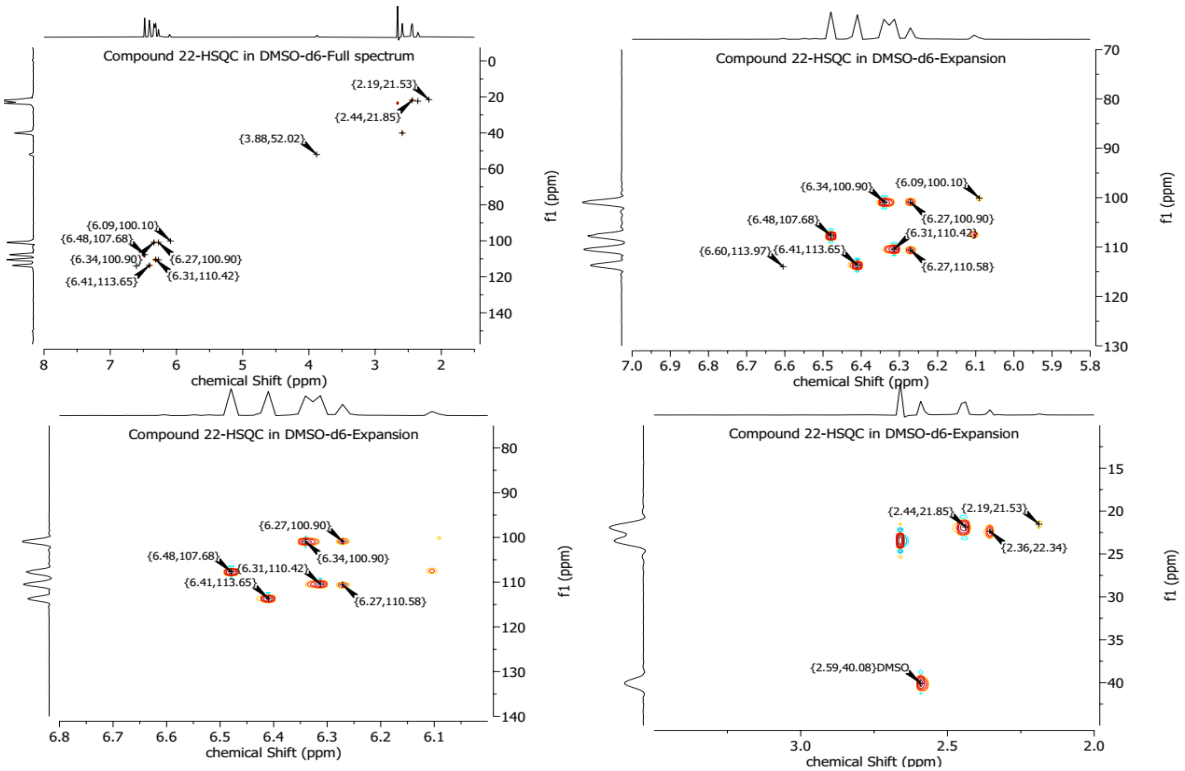


Figure S42: Expansion of ^{1}H, ^{13}C-HMBC spectrum (CD_3SOCD_3, 800 MHz & 201 MHz) of compound 5 (4-carboxy lecanoric acid).

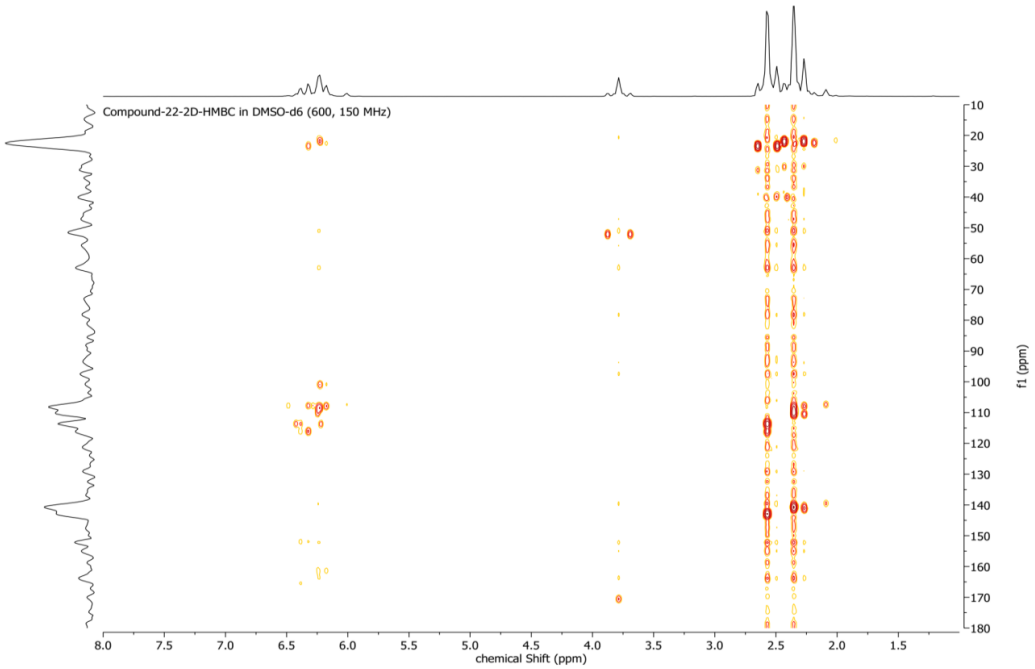


Figure S43: Expansion of ^{1}H, ^{13}C-HMBC spectrum (CD_3SOCD_3, 800 & 201 MHz) of compound 5 (4-carboxy lecanoric acid).

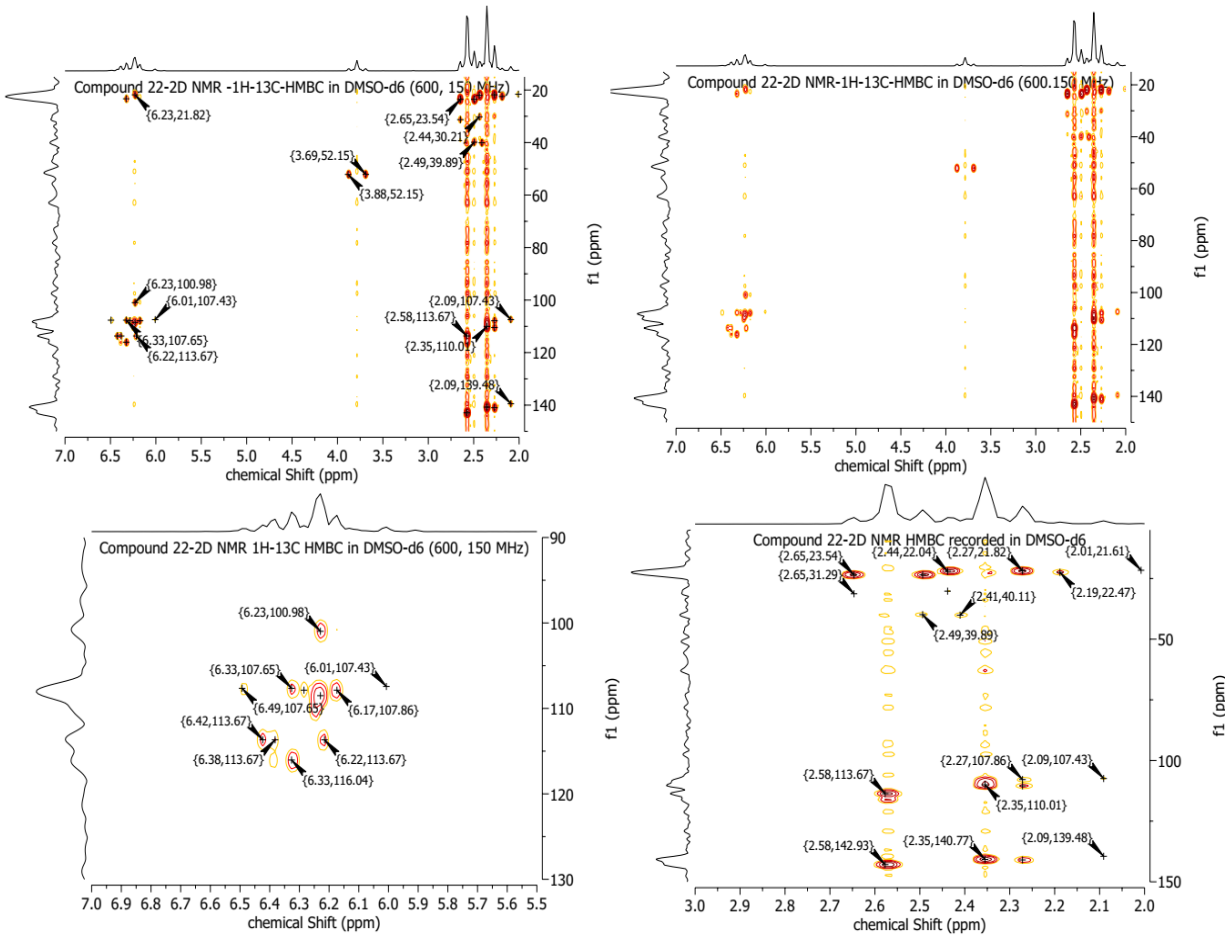


Figure S44: Expansion of ^{1}H, ^{13}C-HMBC spectrum (CD_3SOCD_3, 800 MHz & 201 MHz) of compound 5 (3-carboxy lecanoric acid).

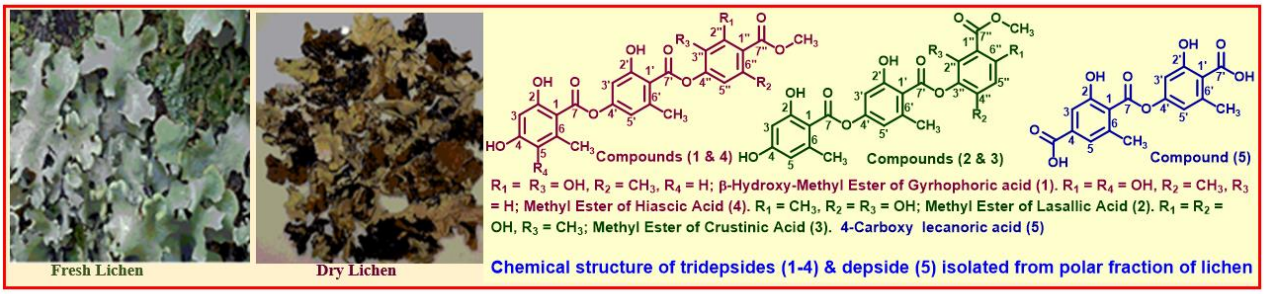


Figure S45: Graphical abstract.