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METABOLIC PROFILING OF LICHENS (*PERMOTREMA PERLATUM*) COLLECTED FROM DIFFERENT HILL STATIONS

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ABSTRACT

Lichens are beautiful bio-organisms in nature. They are symbiotic association of algae and fungus or cyano-bacteria and belong to *Parmeliaceae* family. Amongst, some of lichen species are edible and used as spices as well as folklore medicine to cure different kind of ailments. Specimens of lichen (*Permotrema parlatum*) have been collected from different hill stations of South India to combat their bioactive chemical constituencies. Chemical investigation revealed that different classes of compounds **1-12** present in the diethyl ether and methanol extracts of lichens collected from different localities. Amongst them, atranorin, was present as major chemical constituent in all lichen samples in variable amounts (2.0-0.02 % of its dry weight). The structural determinations of these compounds were carried out by means of ^{1}H & ^{13}C NMR and HRESI-MS in comparison with their data reported in literature. The structural confirmation was secured by X-ray diffraction study of crystallized compounds as applicable. The chemical composition of all lichens is very resembled, but with variable in relative abundance except lichen sample procured from Munnar hill station located in Kerala State of South India. Interestingly, lichen collected from Munnar in Kerala contained chloroatranorin and a very small amount of a fluoroatranorin along with other aforesaid metabolites in the lichen samples collected from other hill stations.

KEYWORDS: Lichens; a comparative study of their metabolites; phenols, depsides and tridepsides; their isolation and structural characterization by NMR & HRESI-MS.

INTRODUCTION

Lichen is a beautiful composite of micro-organisms. They are a symbiotic association of algae and fungus or cyano-bacteria and survive on mutual condition under drastic environmental conditions. In India, the lichens are represented by 2532 species under 324 genera and 78 families including 541 endemic species.[1,2] Based on their habitat, they are classified into two types such as rock lichen and wood lichen. They belong to poisonous and non-poisonous classes. Amongst, some of them are non-toxic and edible, used as food supplements, spices and herbal medicines,[3-4] active ingredient for preparation natural dyes for coloring of textiles and fabrics especially wool and silk.[5-9] They are major bio-organism which act as bio-indicator to measure environmental pollution,[10] and acts as bio-indicator to trace heavy metals in atmosphere.[11] Lichens are unable to survive at high levels of environmental pollution. They survive in least polluted area or pollution free.[12]

This manuscript focuses on the metabolic profiling of lichen (*P. parlatum*) collected from five hill stations of Eastern and Western Ghats Mountains of South India. The lichen, *P. parlatum* belongs to the family *Parmeliaceae*, genus *Permotrema*, and species *parlatum* in the plant kingdom. In various Indian vernaculars, it is familiar in different names such as 'Chhareela' or Chhabeli in Hindi, 'Shilapuspa' in Sanskrit, and 'Pathorphool' in Oria, Bengali. It is commonly referred to as 'Dagadphool' in Marathi and in Mumbai region. These lichens are survived on black basalt rocks in high temperature and high altitude in the mountains and referred as rock lichen.[13-15] We have selected an edible rock lichen very commonly used for exotic spices and an active ingredient for preparation herbal medicine to cure different kinds of ailments.[3-4] These specimens were collected from five different hill stations of South India identified as *Permotrema parlatum* for their metabolic profiling. It is widely used as an active ingredient for preparation of exotic spices and botanical supplement for

preparation of polyherbal medicines by tribal people in some part of India for treatment of various kinds of ailments including cancer. That is why generically, it is called as herbal spice. We have collected five specimens of same variety of lichens from five different hill stations of South India *viz.* Mahabaleswar (Maharashtra), Madikari (Karanataka), Amboli Ghat (Western Maharashtra, nearby Goa), Munnar (Kerala), Dodda Betta Peak in Ootty (Tamil Nadu) at high altitude of Western and Eastern Ghats Mountains to investigate their chemical compositions. In this regard, sequential solvent extractions of defatted lichens were carried out using diethyl ether and methanol. Isolation of secondary metabolites has been carried out by column chromatography over silica gel with gradient solvent elution by using a binary solvent system with changing its polarity described details in literature.[16] The final purification of individual constituent was done by preparative TLC followed by crystallization as applicable. Structural determination was secured by chemical, spectral, and spectrometric study.

RESULTS AND DISCUSSION

The details phyto-chemical investigation of different extracts of lichens collected from five hill stations of South India has carried out. It was found that the major and common secondary metabolites present in all specimens of lichens were phenols, depsides and tridepsides. Amongst them, atranorin was major constituent in all the samples in variation with the relative abundances along with phenols atraric acid, methyl ester orsellinic acid and β-orsellinic acid. These phenolic acids were present in substantial quantities in all the extracts of lichens whereas β-resorcyclic acid was available in Munnar sample only. Trace amount of tridepsides has also been isolated from methanol extract. Depsides and tridepsides are a type of compounds composed by phenolic precursors or their congeners linked by an ester bond. Mainly, lichen is major source of these class of compounds. Besides lichens, higher plants belonging to family *Ericaceae*,[17] *Lamiaceae*,[18-19] *Papaveraceae*,[20] *Myristacae*,[21] and *Fabaceae*,[22] *etc.* are also biosynthesized these classes of compounds.

The chemical constituents isolated from *P. parlatum* credited various biological activities like anti-proliferative,[23-32] anti-cancer,[23-32] anti-tumor,[29, 33] anti-oxidant,[34-36] anti-miotic,[23-32, 37, 38] neuroprotective,[39-43] UV-protectant,[44] anti-viral,[45] anti-mycobacterial,[46] anti-microbial,[23, 24] anti-fungal,[23,24,31] anti-diabetic,[42,47] anti-inflammatory,[34-36] anti-pyretic,[48] free radical scavenging property,[34-36] cytotoxic,[39-43] anti-HIV,[45] hyperglycemic,[47] flavoring agents in exotic spices in Indian cuisines,[49] natural dying agents especially for wool and silk.[5-9] Some of lichen has been shown to biosynthesize chloroatranorin along with atranorin, depsides and tridepsides.[50-54] It has also been credited as anti-bacterial,[23,24] and anti-biotic activities.[50-54] Some of secondary metabolites present in lichens act as very good preventive agent for neuron degeneration like Alzheimer's disease, Parkinson disease by performing cholinesterase activity.[39-43] Lichens are very much sensitive against the pollution, act as bio-indicator to detect toxic heavy metals in atmosphere. They are unable to survive in polluted area,[10-13] and survive in least polluted area or pollution free zone such as at high altitude of the mountains and dense forest area.[56-58]

Extraction, Isolation and Structural Elucidation

The lichen samples were (100 gm each sample) collected from Mahabaleswar (Maharashtra), Madikari (Karanataka), Amboli Ghat (Western Maharashtra, nearby Goa), Munnar (Kerala), Dodda Betta Peak in Ootty (Tamil Nadu) at high altitude of Western and Eastern Ghats Mountains to investigate their chemical constituents from their different organic extracts. These shade dried lichens were subsequently pulverized and extracted. These pulverized lichens were extracted with freshly distilled hexane, diethyl ether and methanol, sequentially. The phyto-chemical analysis of diethyl ether extract revealed mainly depsides (atranorin), phenols (atraric acid, methyl ester of orsellinic acid mainly) as major chemical constituents along with trace amount tridepsides. But sample procured from Munnar from Kerala State of South India contained atranorin, atraric acid, methyl ester of orsellinic acid as major chemical constituents along with chloroatranorin, trace amount of fluoroatranorin.[16] The chemical constituents present in the methanol extracts were mainly tridepsides as minor constituents along with very trace amount usnic acid in all specimens of lichens. On preliminary investigation, a comparative study was also carried out on Co-TLC by using different kinds of solvent systems. The TLC was visualized under exposure of both long as well as short range wavelength of UV radiation, expose in iodine vapor in an iodine chamber, by using 10 % aqueous H_2SO_4 sprayed on TLC plate followed by heating at 120 ºC for five min. These spots were displaying a peculiar red color spots on microplate while spraying with 10% aqueous H_2SO_4 on TLC plate followed by heating at 120 ºC for five min.[59] These colored reactions on TLC micro plates reflected that they belong to depsides, tridepsides class of compounds is present in all lichen extracts. Three absorption peaks were appeared at approximately at 215, 263 and 301 nm in UV spectra (methanol) predicted the presence of benzoyl ester in the compound, therefore it was a depside or its derivatives.[60] Spraying with neutral alcoholic $FeCl_3$ solution on TLC plates visualized a very dark green spot suggesting that these compounds contained phenolic hydroxyl group adjacent to carbonyl group.[59] These compounds also showed bathochromic UV shifts of about 35-40 nm upon the addition of anhydrous $AlCl_3$ at an acidic pH. Based on the above evidences, it was inferred that it is a depside class or its derivatives.[60] In high resolution mass spectrometry, it was also evidenced the presence of base peak observed in mass fragmentations at *m/z* 177.05/183.15/196.15.[61]

Finally, the isolation of major constituents present in all crude extracts of diethyl ether and methanol was carried out by column chromatography over silica gel, gel permeation (GPC) over Sephadex LH20 with gradient solvent elution by using a binary mixture of solvent system. Final purification was carried out preparative thin layer chromatograph (PTLC) followed by crystallization.[16] The structural characterization of the individual isolates was carried out using spectroscopic and spectrometric methods. The structures of known compounds were identified in comparison with their ^{1}H & ^{13}C NMR and mass data reported in literature and confirmed by single crystal X-ray diffraction study as applicable. The chemical structure of all the major isolates segregated from different extracts of lichens collected from five hill stations are depicted below in figure 1.

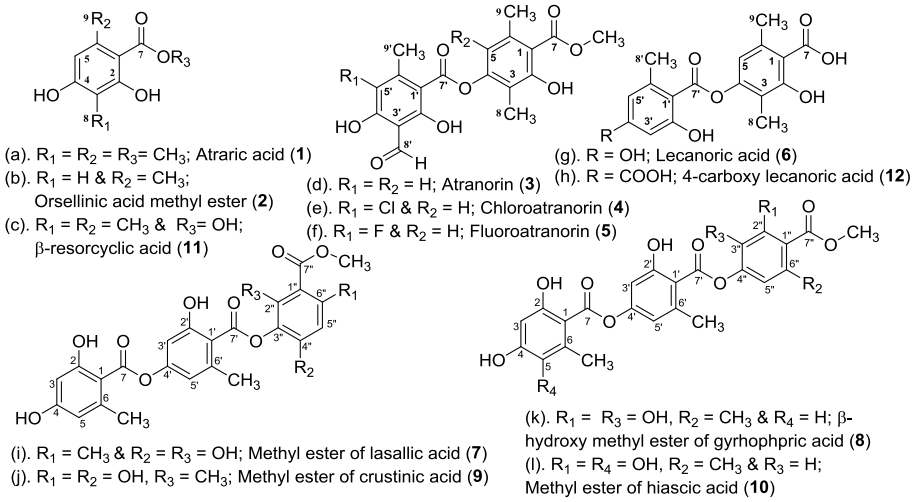

Figure 1: Chemical structure of the major metabolites 1-12 isolated from five lichens.

Among them, some of the metabolites were crystals in nature and their crystal structures were confirmed by single X-ray diffraction study.[16] The crystal structures of chemical constituents **1-3** and **5** were depicted in figure 2.

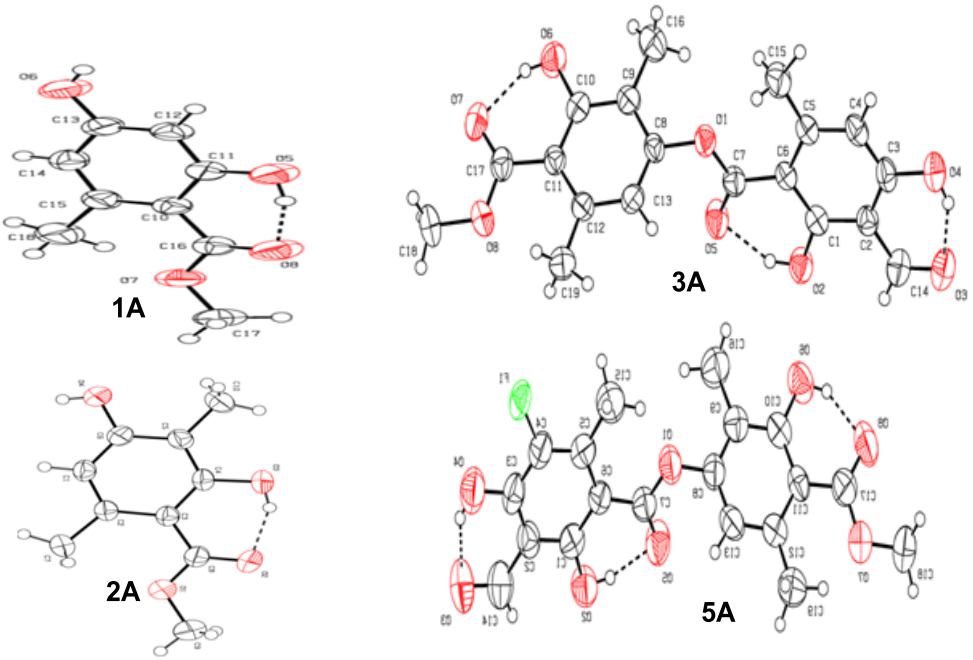

Figure 2: ORTEP of very common phenols 1A, 2A & depsides 3A and rare depsides 5A.

SUMMARY OF THE WORK AND CONCLUSION

Lichen (*P. parlatum*) is an iconic condiment in India for preparation of various exotic spices, locally known as 'Byrani masale' widely used in various Indian cuisine. Vegetative parts of lichen were collected from five different hill stations from South India to study metabolic profiling them in the lieu of biodiversity and climatic variations. In this connection, at least twelve

compounds belong to phenol, depsides and tridepsides classes that were isolated from diethyl ether and methanol extract of these five defatted lichen extracts. The structural determination of these isolates was conducted by chemical, spectral and spectrometric analysis in comparison with spectral data reported in literature.[16] Atranorin was present as major chemical constituent in all the lichens with variable relative abundances along with trace amount phenols atraric acid, methyl ester of orsellinic acid and β-resorcyclic acid. The yield of crude extract in organic solvents was maximum in the sample collected from Medikari a hill station located in Corgi district of Karnataka and was minimum in the specimen collected from Aamboli Ghat, Kolhapur district in Maharashtra. Interestingly, fluoroatranorin and chloroatranorin was found in association with atranorin in diethyl ether extract of lichen collected from Munnar in Kerala State and their assignment was carried out by HRESI-MS and MS/MS analyses reported in literature.[16, 62]

Table 1: Qualitative analysis of chemical constituents in lichens collected from five different hill stations in South India.

Hill stations / Compounds	Chemical constituents isolated from ether extracts of lichen collected from different hill stations of South India										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**9**	**10**	**11**	**12**
Medikari (KS)	√	√	√	×	×	√	√	√	√	√	√
Munnar (KA)	√	√	√	√	√	√	√	√	√	√	√
M. Baleshwar (MH)	√	√	√	×	×	×	√	×	√	√	√
Doda Beta Peak (TN)	√	√	√	×	×	×	√	×	√	√	√
Amboli (GA)	√	√	√	×	×	×	√	×	√	√	√

KS = Karnataka State; KA = Kerala State; MH = Maharashtra State; TN = Tamil Nadu State; GA = Goa State

Table 2: Relative abundance (%) of extractable amount in lichen collected from different states in South India.

Sample collected from hill stations Western and Eastern Ghats Mountains	Amount of lichen Used for extraction	Extractable amt, % of extracts				Duration, temp. for percolation		Duration, temp. for soxhlation	
		Diethyl ether		Methanol		Percolation	Temp (ºC)	Soxhlation	Temp (ºC)
Medikari (KS)	10 gm	500 mg	5.0	Less	150	24-h x 3 days	25	12-hx3 days	45/70
Munnar (KA)	10 gm	350 mg	3.5	Less	100	24-h x 3 days	25	12-hx3 days	45/70
Mahabaleshwar (MH)	10 gm	250 mg	2.5	Less	90	24-h x 3 days	25	12-hx3 days	45/70
Doda Beta Peak (TN)	10 gm	350 mg	3.5	Less	110	24-h x 3 days	25	12-hx3 days	45/70
Amboli (GA)	10 gm	150 mg	1.5	Less	75	24-h x 3 days	25	12-h x 3 days	45/70

EXPERIMENTAL SECTION

Plant material

The specimens of *P. parlatum* was collected from different hill stations (Mahabaleswar hill station in Maharashtra, Medikari hill station in Karnataka, Amboli Ghat hill station nearby Goa, Dodda Betta Peak located in Ootty hill station in Tamil Nadu, Munnar hill station in Kerala) from Western and Eastern 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 each sample was deposited in the Herbarium of the Landscape & Cosmetic Maintenance Division, 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 acetone-d_6 or DMSO-d_6 on a Bruker Avance 200 (Switzerland), Varian 500AR (USA), Varian 600 AR (USA) at TIFR, Colaba, Mumbai, NOVA-470 (VT, Blacksburg, USA) spectrometer using residual $CHCl_3$ / HDO as an internal standard. Chemical shifts are given in ppm (δ_C, δ_H & δ_F), relative to residue $CHCl_3$ / HDO (7.25 & 77.00 / 4.78 or 3.30 & 49.00 ppm) and for ^{19}F NMR, trifluoro acetic acid (-76.55 ppm) used as internal standard and recorded by using NOVA-470 from Virginia State University, Blacksburg, USA. 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. Eluents were water and acetonitrile both containing 0.1% formic acid at a flow rate of 0.35 mL/min. A gradient from 2 v% to 98 v% organic phases was used. The gradient time was set to 7 min. The column temperature was 30 ºC, the auto sampler temperature was set to 4 ºC and the injection volume was 2 µL. Peaks were detected with a single quadrupole mass spectrum (QDa, Waters). Capillary voltage was set to 0.8 kV. The probe temperature was held at constantly 450 ºC. For the gas flow the default instrument setting of a consumption rate of nitrogen of 1200 L/h was used. Positive and negative scan mode in a range of 120-1000 *m/z* with a cone voltage of 15 V was used as screening method. The sampling rate was 10 points per second in scan mode. Crystal data were

collected at 293 ºK on an Oxford Diffraction Xcalibur™ Single Crystal X-ray Diffractometer with Sapphire CCD Detector, Enhance (Mo) or Cu-K\αX-ray source, and graphite monochromator using ω scans (TU, Darmstadt, Germany). The structures were solved by direct methods and refined using SHELXL97. Silica gel 60 (230-400 mesh, Merck) was used for analytical TLC. Silica gel 60 (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 110 °C for 5 min, DNP in EtOH for detection of aldehyde functionalities, 10% aqueous H_2SO_4 followed by heating at 110 °C for 5 min and neutral $FeCl_3$ in MeOH for visualization of phenols and chelated phenols.[59]

Extraction and Isolation

Freshly dried 100 g lichen (*P. parlatam*) each specimen was crushed to powder and extracted with hexane, diethyl ether and methanol (350 ml each) three times at room temperature for 12h. Removal of solvents afforded a grey colored solid substance. The isolation of chemical constituents was done by column chromatography over silica gel with gradual solvent elution using a binary mixture of solvent ethyl acetate in hexane followed by methanol in chloroform as described earlier.[16] The analysis of diethyl ether extract revealed the presence of mainly depsides and phenols such as atranorin, atraric acid and methyl ester of orsellinic acid as major chemical constituents of samples collected from different hill stations (Mahabaleswar hill station in Maharashtra, Medikari hill station in Karnataka, Amboli Ghat hill station nearby Goa, Doda Betta Peak located in Ootty hill station in Tamil Nadu) from Western and Eastern Ghats Mountains. Additional metabolites chloroatranorin and fluoroatranorin were also isolated from ether extract of the specimen collected at Munnar hill station in Kerala. The spent of lichen was extracted with methanol (350 ml each) 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 sixteen 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 case the volume of aliquot collected is more than 250 mL. Each and every fraction has been monitored by TLC to visualize their chemical constituencies. The fractions having similar chemical profiles were combined and further purified by using repetitive column chromatography on open column over silica gel, gel permeation chromatography (GPC) over Sephadex LH20 with gradual solvent elution, preparative thin layer chromatography (PTLC) yielded tridepsides named as β-hydroxy methyl gyrophorate, methyl ester of lasallic acid, hiascic acid, and crustinic acid along with very trace amount of usnic acid and lecanoric acid will be published somewhere else.[62]

Compound **1** (Atraric acid is a common phenol to all the lichens procured from five hill stations): light yellow prism shaped crystal (EtOAc-hexane); mp 175 ºC; UV (MeOH) λ_{max} (log ε): 216 (2.68), 263 (2.04) and 301 (0.78) nm; IR (neat) ν_{max} (cm^{-1}): 3305.4, 2956.34, 1614.18, 1581.34, 1313.29, 1258.53, 1158.04, 1107.9, 1060.66, 95.09, 951.69, 852.38, 829.24, 799.35, 699.07; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 1; EIMS (*m/z*) 196.07au; ORTEP diagram in figure 3. CCDC deposition number of crystal of compound **10**: 2272303

Compound **2** (Orsellinic acid methyl ester is a common phenol to all the lichens procured from five hill stations): prism shaped orange crystal (EtOAc-hexane); crystallized as twin molecule; mp 143 ºC; UV (MeOH) λ_{max} (log ε): 215 (2.07), 263 (1.30) and 301 (0.48) nm; IR (neat) ν_{max} (cm^{-1}): 3286.11, 2916.81, 2849.31, 1738.51, 1647.88, 1443.46, 1158.8, 1171.54, 1065.48, 985.0, 943.0, 724.13; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) and data, see Table 1; EIMS (*m/z*) 182.05 au; ORTEP diagram in figure 4. CCDC deposition number of crystal of compound **11**: 2271330.

Compound **3** (β-resorcyclic acid is a phenol available in the lichen procured from Medikari hill station located in Corgi district of Karnataka State): colorless soils substance; mp 179 ºC; UV (MeOH) λ_{max} (log ε): 215 (2.07), 263 (1.30) and 301 (0.48) nm; IR (neat) ν_{max} (cm^{-1}): 3286.11, 2916.81, 2849.31, 1738.51, 1647.88, 1443.46, 1171.54, 1065.48, 950.0, 943.0, 724.13 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 1; EIMS (*m/z*) 168.04 au.

Compound **4** (Atranorin is common depside to all the lichens procured from five hill stations): colorless needle (EtOAc-hexane): mp 195 ºC; UV (MeOH) λ_{max} (log ε): 211(0.14), 253 (0.08) and 277 (0.08) nm; IR (neat) ν_{max} (cm^{-1}): 3454.4, 2925.5, 1626.6, 1594.8, 1518.6, 1454.0, 1186.9, 1238,0, 1110.8, 1040.4, 792.6, 722.2; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 1; HRESI-MS (negative mode), *m/z*: 373.0924 Da; ORTEP diagram in figure 5. CCDC deposition number of crystals of compound **4**: 2291117 or 2271314.

Compound **5** (Fluoroatranorin was present only in the lichen procured from Munnar, Kerala): colorless needle (EtOAc-hexane); mp199.5 ºC; UV (MeOH) λ_{max} (log ε): 211 (0.19), 253 (0.11) and 276 (0.11) nm; IR (neat) ν_{max} (cm^{-1}): 3450.8, 2928.4, 1724.0, 1599.8, 1445.4, 1417.4, 1269.9 (very strong and sharp peak due at C-F absorption), 1112.7, 1073.2, 750.8; ^{1}H NMR ($CDCl_3$, 500 MHz), ^{13}C NMR ($CDCl_3$, 125 MHz) and ^{19}F NMR ($CDCl_3$, 470 MHz) data, see Table 1; EIMS (*m/z*) 437.0873 au ($HCOO^-$ as adduct); ORTEP diagram in figure 1. CCDC reference number of crystals of compound **5**: 2271302.

Compound **6** (Chloroatranorin was present only in the lichen procured from Munnar, Kerala State): colorless needle (EtOAc-hexane); mp 205 ºC; UV (MeOH) λ_{max} (log ε): 211 (0.19), 253 (0.11) and 276 (0.11) nm; IR (neat) ν_{max} (cm^{-1}): 3450.8, 2928.4, 1724.0, 1599.8, 1445.4, 1417.4, 1112.7, 1073.2, 750.8; ^{1}H NMR ($CDCl_3$, 500 MHz), ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; HRESI-MS (*m/z*) 407.0543 au.

Compound **7** (Lecanoric acid was present in all specimen of the lichens): colorless solid substance; mp 180 ºC; UV (MeOH) λ_{max} (log ε): 211 (0.27), 269 (0.11) and 304 (0.06) nm; IR (neat) ν_{max} (cm^{-1}): 3364, 1738.9, 1612, 1580, 1502, 1443, 1311, 1261, 1199, 1159, 1112, 1060, 994, 952, 835, 799, 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; LRESI-MS: *m/z*: 318.074 au.

Compound **8** (β-Hydroxy methyl ester of gyrophoric acid, HMEGA was present in all specimens of the lichens, its relative abundance is higher in respect to other tridepsides): 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, 2956, 1607, 1457, 1317.3, 1252, 1208, 1148, 177, 993, 879, 830, 800, 732, 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.: $C_{26}H_{24}O_{10}$; HRESI-MS (positive mode): obs. *m/z* 496.1784 au $[M-2H]^+$ and calc. *m/z* 498.116 au; base peak 183.151 au (100 % intense peak).

Compound **9** (Methyl ester lisallic acid, MELA was present in all specimens of the lichens): 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 (100 % intense peak in mass spectrum).

Compound **10** (3-carboxy lecanoric acid was present in all specimens of lichens): brown solid substance; mp 195 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, 1612, 1461, 1317, 1252, 1210, 1178, 1150, 1078, 993, 879, 830, 800, 731, 682, 627, 527; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) and data, see Table 1; M. F.: $C_{17}H_{14}O_9$; LR-ESI-MS: obs. *m/z*: 318.074 $[M-CO_2]^+$ au; calc. *m/z*: 362.290 au.

Compound **11** (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.: $C_{25}H_{22}O_{11}$; HRESI-MS (positive mode): obs. *m/z*: 527.563 $[M+H]^+$; calc. *m/z*: 527.111 au; base peak at *m/z* 459.538 au (100 % intense peak).

Compound **12** (Methyl ester of hiascic acid, MEHA was present in all specimens of the lichens, relative abundance is comparatively less): 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 (100 % intense peak in mass spectrum).

Table 3: ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) of compounds 1-3.

Position	1 Atraric acid		2 Methy ester of orsellinic acid		11 β-resorcyclic acid	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		107.93		105.79	-	105.93
2	-	158.15	-	163.78	-	163.92
3	-	101.84	6.28, d (2.2), 1H	101.70	6.32, d (2.2), 1H	101.84
4	-	163.30	-	166.12	6.43, dd (8.0, 2.2), 1H	166.26
5	6.21, s, 1H	110.66	6.23, d (2.2), 1H	112.46	-	112.60
6	-	140.29	-	144.49	7.78, d (8.0), 1H	144.63
7	-	172.75	-	173.38	-	173.52
$C_{7'}$-CH_3(8)	3.92, s, 3H	51.97	3.92, s, 3H	52.06	3.92, s, 3H	52.20
C_3-CH_3(10)	2.46, s, 3H	7.79	-	-	-	-
$C_{6'}$-CH_3(9)	2.10, s, 3H	24.26	2.49, s, 3H	24.17	-	-
C_2-OH	12.04, s, 1H	-	11.71, s, 1H	-	12.74, s, 1H	-
C_4-OH	-	-	-	-	9.74, s, 1H	-

Table 4: ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) of compounds 3-6 & 12.

Positions	3 Atranorin		4 Fluoroatranorin		5 Chloroatranorin		6 Lecanoric acid		12 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_C	δ_H (*J* in Hz)	δ_C
1	-	116.01	-	115.72	-	115.54	-	108.26	-	109.45
2	-	162.87	-	164.57	-	169.63	-	167.75	-	164.16
3	-	108.56	-	109.04	-	109.04	6.12, d (2.2), 1H	110.34	6.31, d (2.2), 1H	101.84
4	-	167.49	-	164.79	-	164.29	-	161.15	-	166.59
5	6.40, s, 1H	110.27	6.43, s, 1H	107.92	-	107.92	615, d (2.2), 1H	100.34	6.40, d (2.2), 1H	104.83
6	-	152.42	-	149.03	-	144.37	-	142.70	-	144.80
7	-	169.68	-	169.64	-	169.09	-	165.26	-	166.82
C6-CH_3	2.69, s, 3H	25.54	2.70, s, 3H	21.09	2.46, s, 3H	21.19	2.24, s 3H	21.29	2.64, s, 3H	24.34
C3-COR*	10.36, s, 1H	193.83	10.29, s, 1H	194.34	10.36, s, 1H	193.91	-	173.24	9.33, brs, 1H	173.67
C2-OH	12.55, s, 1H	-	12.04, s, 1H	-	12.03, s, 1H	-	10.74, s, 1H	-	11.98. s, 1H	-
C4-OH/R_1	11.94, s, 1H	-	11.70, s, 1H	-	11.70, s, 1H	-	10.49, s, 1H	-	11.14, s, 1H	-
1'	-	112.85	-	115.54	-	115.72	-	115.89	-	111.31
2'	-	169.09	-	169.79	10.36, s, 1H	157.29	-	160.69	-	165.44
3′	-	116.01	-	116.31	-	116.31	6.22, d (2.2), 1H	113.44	6.80, d (2.2), 1H	112.90
4'	-	151.99	-	151.41	-	151.57	-	151.80	-	155.13
5′	6.51, s, 1H	116.78	-	115.72	6.21, s, 1H	109.59	6.29, d (2.2), 1H	107.50	6.77, d (2.2), 1H	117.34
6′	-	139.86	-	136.58	-	136.55	-	140.60	-	144.63
7′	-	172.18	-	169.74	-	169.74	-	167.75	-	170.33
C3'-CH_3	2.09, s, 3H	9.35	2.10, s, 3H	21.02	2.10, s, 3H	21.98	2.16, s, 1H	21.77	2.61, s, 3H	24.34
C6′-CH_3	2.55, s, 3H	23.98	2.59, s, 3H	9.32	2.10, s, 3H	9.32	-	-	-	-
C7'-OCH_3	3.99, s, 3H	52.31	3.99, s, 3H	52.35	3.92, s, 3H	51.98	-	-	-	-
C2′-OH	10.50, s, 1H	-	12.04, s, 1H	-	12.03, s, 1H	-	-	-	11.98, s,1H	-

*R is H or OH in C3-COR. When R is H in compound **3-5** indicating aldehyde; R_1 is OH/COOH at C4 depsides indicating lecanoric acid/4-carboxy lecanoric acid in compound **6**/**12**

Table 5: ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) of compounds 7-10.

Positions	7 β-hydroxy methyl gyrophorate		9 Methyl ester of lasallic acid		8 Methyl ester of crustinic acid		10 Methyl ester of hiascic acid	
	δ_H (*J* in Hz)	δ_C (GA)	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	-	108.17	-	108.17	-	108.25	-	109.32
2	-	160.08	-	159.4	-	163.18	-	159.19
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
4	-	166.60	-	166.59	-	163.78	-	166.72
5	6.29, d (2.2), 1H	101.48	6.20, d (1.8), 1H	108.17	-	108.05	-	101.54
6	-	140.40	-	144.81	-	144.73	-	140.54
7	-	167.07	-	170.4	-	166.55	-	170.25
8 (CH_3)	2.45, s, 3H	21.3	2.44, s, 3H	24.2	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	11.62	11.60, s, 1H	–

C4-OH	9.45, s, 1H	-	-	-	9.33, s, 1H	6.15 (OH)	11.13, s, 1H	-
C5-OH	-	-	-	-	-	-	9.32, s, 1H	-
1'	-	116.13	-	116.13	-	115.40	-	117.44
2'	-	156.43	-	159.4	-	159.09	-	154.94
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
4'	-	159.14	-	166.09	-	152.65	-	166.18
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
6′	-	144.13	-	144.56	-	144.53	-	144.69
7′	-	170.43	-	172.4	-	166.31	-	172.85
8'	2.64, s, 3H	21.3	2.64, s, 3H	24.0	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	-	112.67	-	112.11	-	117.16
2"	-	159.14	-	139.56	-	159.09	-	164.08
3"	6.62, d (2.2), 1H	108,17	6.53, d (2.2), 1H	156.43	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	116.13	6.28, d (1.9), 1H	104.70	6.31, d (2.2), 1H	155.02
6"	-	139.56	-	144.56	-	144.10	-	144.22
7"	-	172.78	-	174.80	-	170.58	-	173.73
8"	2.58, s, 3H	24.0	2.57, s, 3H	23.73	2.66, s, 3H	24.29	2.65, s, 3H	24.11
9"	-	-	-	-	-	-	2.18, s, 3H	22.99
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

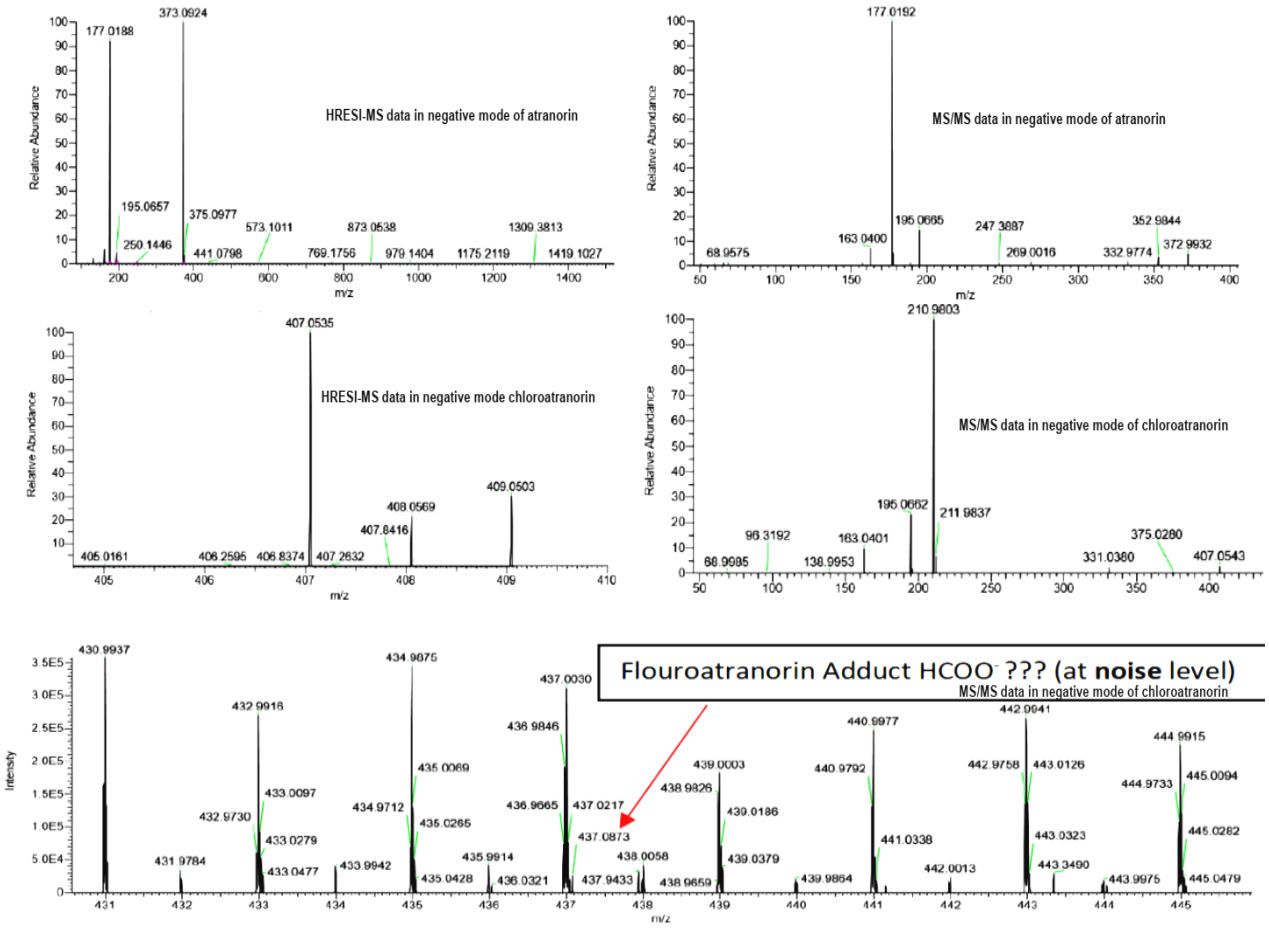

Figure 3: HRESI-MS (in negative mode) and MS/MS spectra of atranorin, chloroatranorin and a rare depside fluoroatranorin respectively.

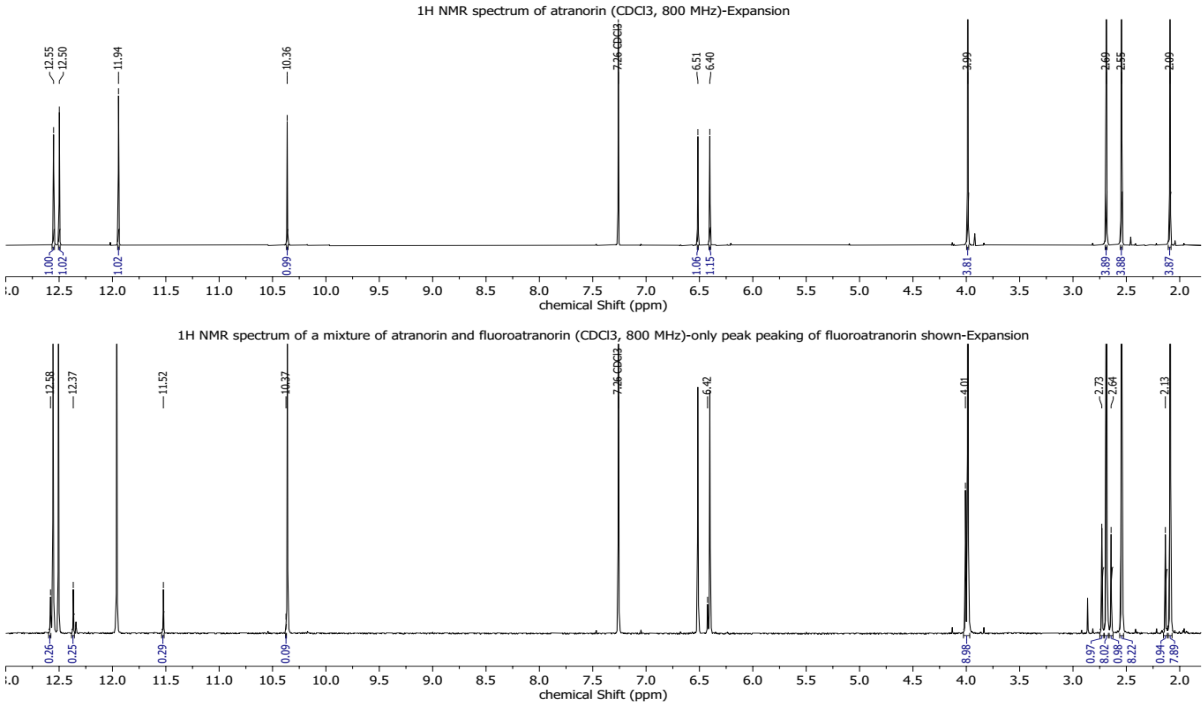

Figure 4: ^{1}H NMR spectra ($CDCl_3$, 800 MHz) of atranorin and a rare depside fluoroatranorin respectively.

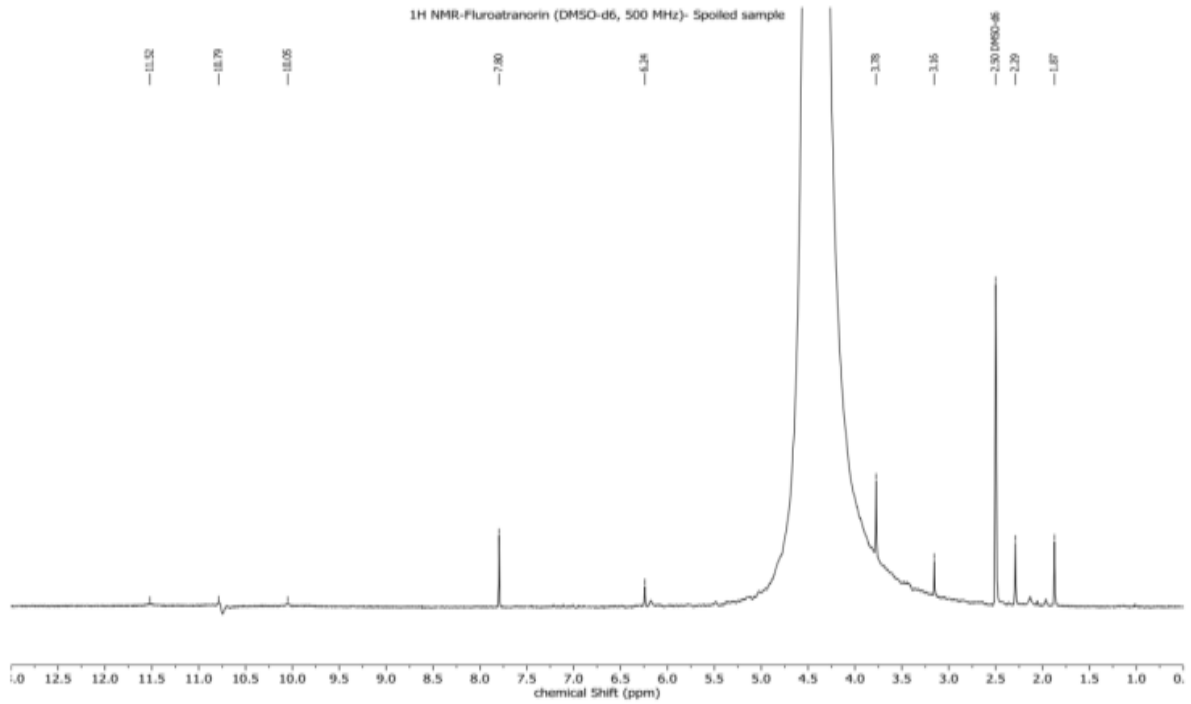

Figure 5: ^{1}H NMR spectrum (CD_3SOCD_3, 500 MHz) of fluoroatranorin, a rare depside isolated from P. parlatum.

ASSOCIATED CONTENT

Supporting information

Annexure-1: Supporting Information (Spectroscopic data consisting IR, UV, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, DEFT, HMBC, EIMS, HRESI-MS of compounds **1-12**, X-ray diffraction of compounds **1-3** & **5** were enclosed in attached file. Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (CCDC). Copies of the data can be obtained, free of charge, on application to Director, CCDC, 12 Union Road, Cambridge CB2, 1EZ, UK (fax: +44-(0)1223-336033 or email: deposit@ccdc.cam.ac.uk

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

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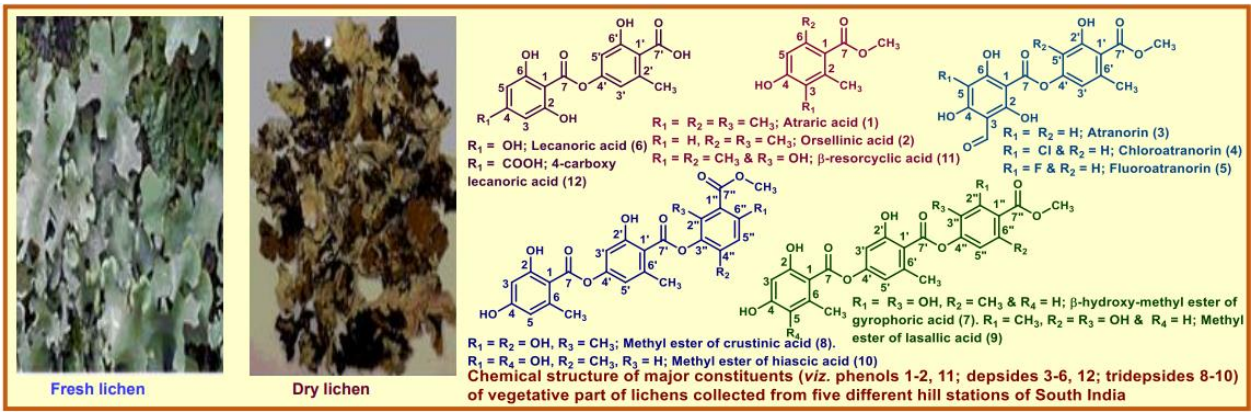
AUTHOR CONTRIBUTION STATEMENT
AKB performed isolation, structural characterization, chemical transformations/reactions and drafted the manuscript. SF contributed analysis tools and analyzed the XRD data of the crystals, structure analysis and computation. IC, ESA and JGJ contributed HR-ESI-MS and LR-ESI-MS data. EJCB reviewed the manuscript and managed for acquiring HR-ESI-MS and LR-ESI-MS data with the help of her team, IC, ESA and JGJ.

GRAPHICALABSTRACT



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Appendix A: Supplementary Information

Entry number	Legendary title
Figure S1	^{1}H NMR ($CDCl_3$, 500 MHz) spectrum of compound **1** (atraric acid)
Figure S2	^{13}C NMR ($CDCl_3$, 125 MHz) spectrum of compound **1**
Figure S3	^{1}H NMR ($CDCl_3$, 500 MHz) spectrum of compound **2** (methyl orsellinic acid)
Figure S4	^{13}C NMR ($CDCl_3$, 125 MHz) spectrum of compound **2**
Figure S5	^{1}H NMR ($CDCl_3$, 500 MHz) spectrum of compound **3** (atranorin)
Figure S6	^{13}C NMR (CD_3COCD_3, 125 MHz) spectrum of compound **3** (atranorin)
Figure S7	HRESI-MS spectrum of compound **3** (atranorin) in negative mode
Figure S8	^{1}H NMR (CD_3OD, 500 MHz) spectrum of compounds **3**, **4** & **5**
Figure S9	Stacking plot of ^{13}C NMR spectrum of chloroatranorin, **4** (CD_3SOCD_3, 125 MHz) and atranorin, **3** ($CDCl_3$, 125 MHz)
Figure S10	^{19}F NMR (CD_3SOCD_3, NOVA 470 MHz) spectrum of compound **5** (F-atranorin)
Figure S11	HRESI-MS and MS/MS spectra of compounds **3** (atranorin) and **4** (chloroatranorin)
Figure S12	Stacking plot of 1H NMR spectrum ($CDCl_3$, 500 MHz) atranorin, **3**, a mixture of atranorin **4** and fluoroatranorin), **5**
Figure S13	HRESI-MS and MS/MS spectrum of compound **5** (fluoroatranorin)
Figure S14	^{1}H NMR (CD_3COCD_3, 500 MHz) spectrum of compound **6** (lecanoric acid)
Figure S15	^{13}C NMR (CD_3COCD_3, 125 MHz) spectrum of compound **6**
Figure S16	LRESI-MS spectrum of compound **6** (lecanoric acid)
Figure S17	^{1}H NMR spectrum (CD_3OD, 500 MHz of compound **7 (**methyl ester LA)
Figure S18	^{13}C NMR spectrum (CD_3OD, 125 MHz of compound **7** (methyl ester LA)
Figure S19	LRESI-MS spectrum of compound **7** (methyl ester LA)
Figure S20	^{1}H NMR spectrum (CD_3OD, 500 MHz of compound **8** (methyl ester GA)
Figure S21	^{13}C NMR spectrum (CD_3OD, 125 MHz of compound **8** (methyl ester GA)
Figure S22	LRESI-MS spectrum of compound **8** (methyl ester GA)
Figure S23	^{1}H NMR spectrum (CD_3COCD_3, 500 MHz of compound **9** (methyl ester CA)
Figure S24	^{13}C NMR spectrum (CD_3COCD_3, 125 MHz of compound **9** (methyl ester **CA)**
Figure S25	LRESI-MS spectrum of compound **9** (methyl ester CA)
Figure S26	^{1}H NMR spectrum (CD_3COCD_3, 500 MHz of compound **10** (methyl ester HA)
Figure S27	^{13}C NMR spectrum (CD_3COCD_3, 125 MHz of compound **10** (methyl ester HA)
Figure S28	LRESI-MS spectrum of compound **10** (methyl ester HA)
Figure S29	^{1}H NMR (CD_3COCD_3, 500 MHz) of compound **11** (β-Resorcyclic acid)
Figure S30	Expansion of ^{1}H NMR (CD_3COCD_3, 500 MHz) of compound **11** (β-Resorcyclic acid)
Figure S31	Exoansion of ^{1}H NMR (CD_3COCD_3, 500 MHz) of compound **11** (β-Resorcyclic acid)
Figure S32	^{1}H NMR spectrum (CD_3COCD_3, 500 MHz of compound **12** (4-carboxy LA)
Figure S33	^{13}C NMR spectrum (CD_3COCD_3, 125 MHz of compound **12** (4-carboxy LA)
Figure S34	LRESI-MS spectrum of compound **12** (4-carboxy lecanoric acid)
Figure S35	Interpretation of LRESI-MS spectrum of compound **12**
Figure S36	Joint interpretation of HRESI-MS of methyl ester of lasallic acid, **7** and methyl ester of crustinic acid, **9**

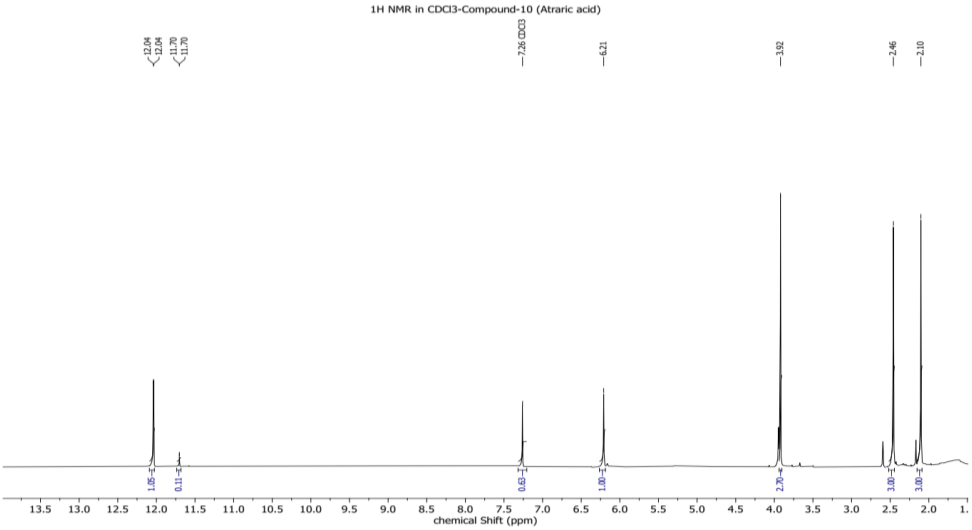

Figure S1: ^{1}H NMR ($CDCl_3$, 500 MHz) of compound 1 (atraric acid).

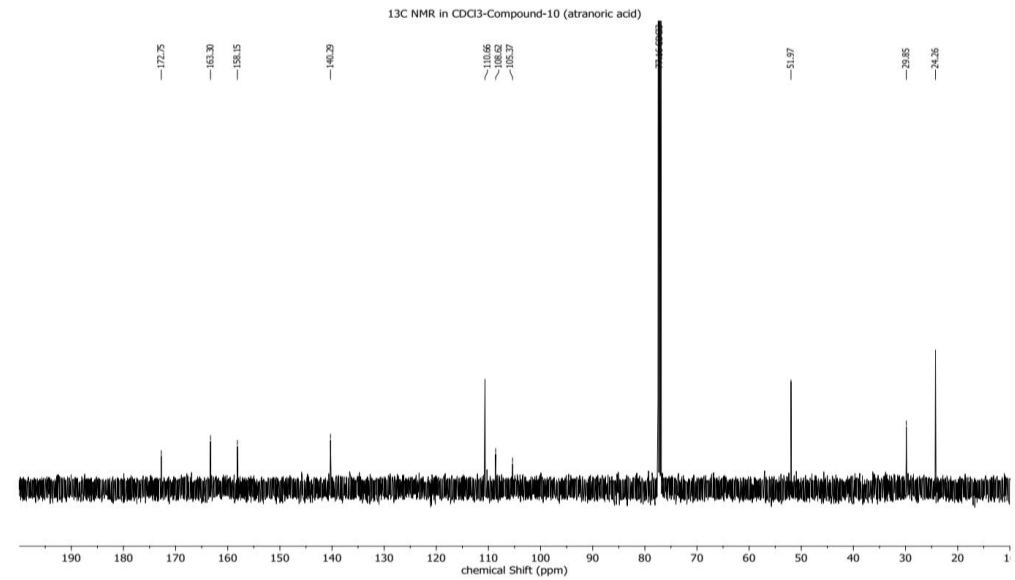

Figure S2: ^{13}C NMR (CD_3OD, 125 MHz) of compound 1 (atraric acid).

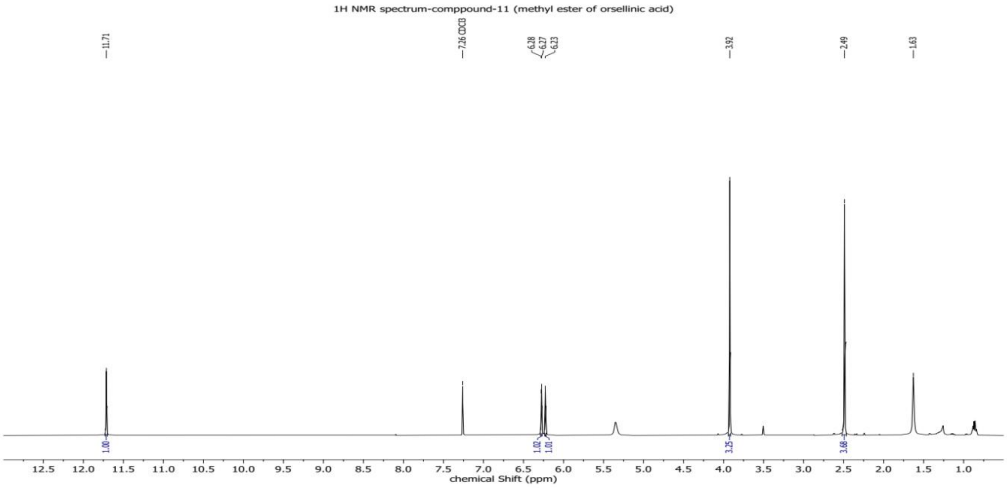

Figure S3: ^{1}H NMR ($CDCl_3$, 500 MHz) of compound 2 (methyl ester of orsellinic acid).

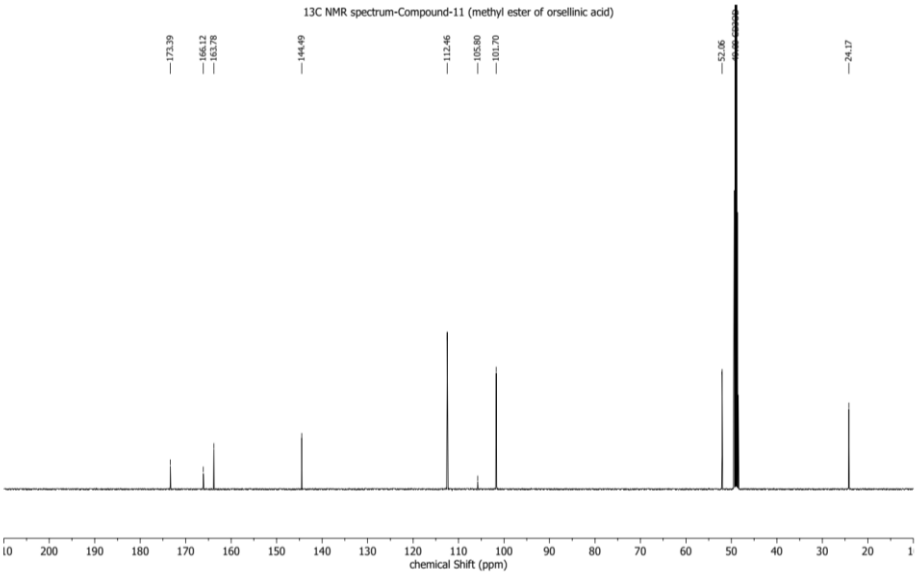

Figure S4: ^{13}C NMR ($CDCl_3$, 125 MHz,) of compound 2 (methyl ester of orsellinic acid).

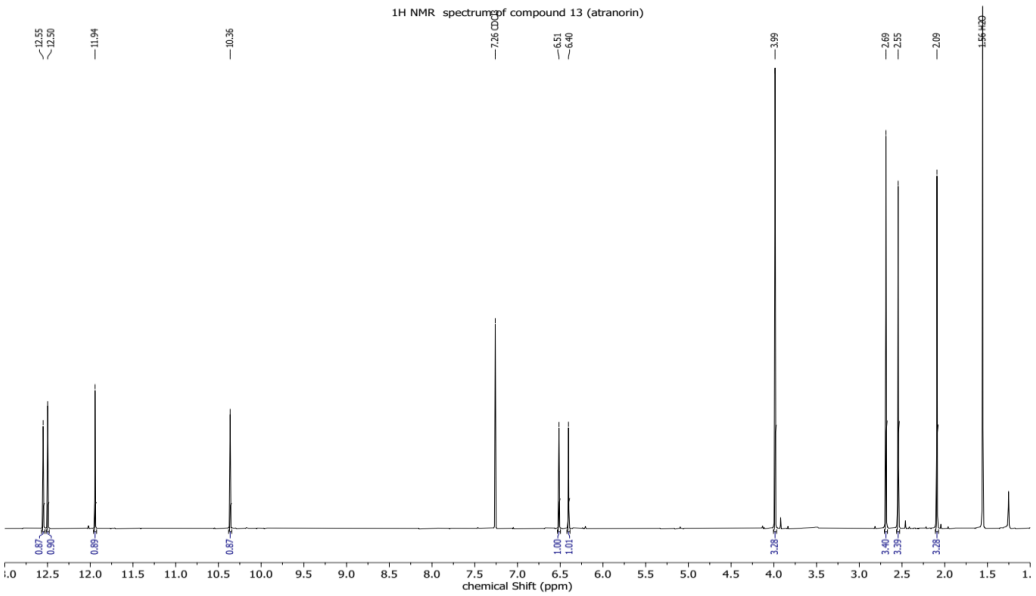

Figure S5: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 3 (atranorin).

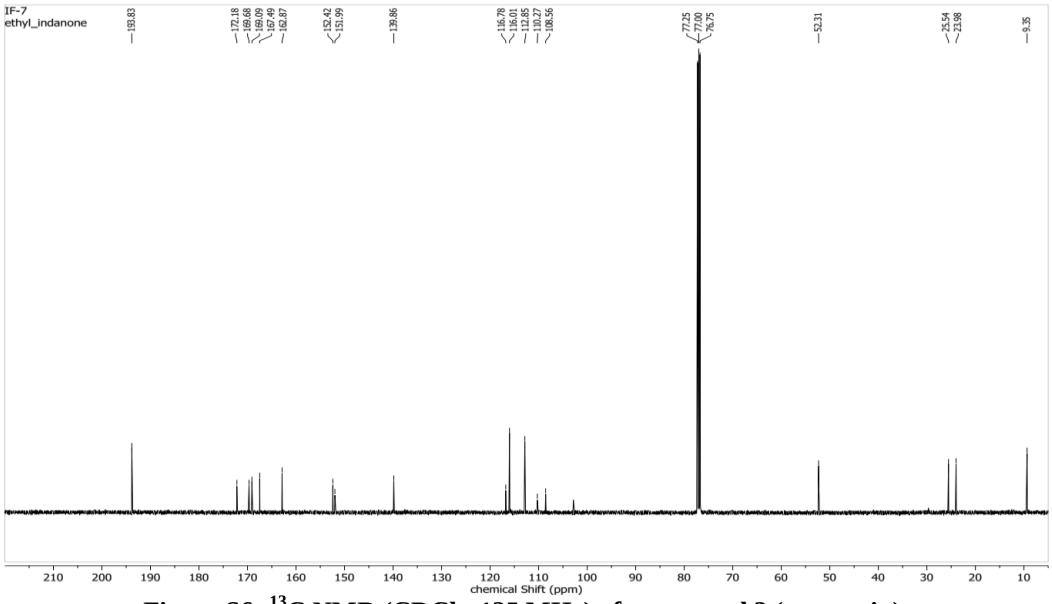

Figure S6: ^{13}C NMR ($CDCl_3$, 125 MHz) of compound 3 (atranorin).

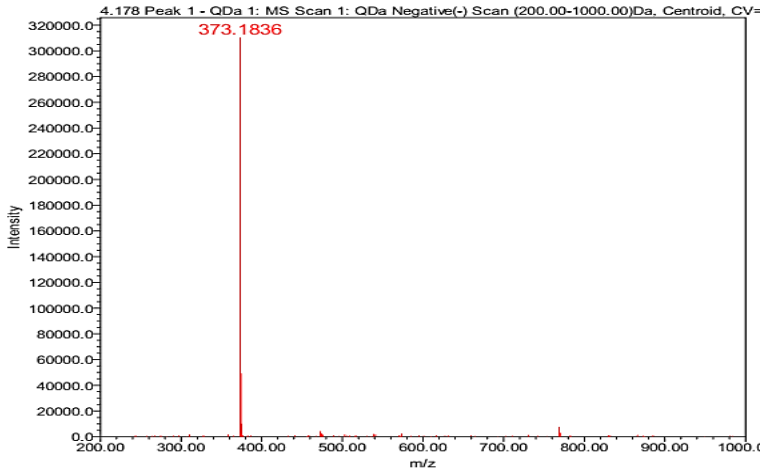

Figure S7: LRESI-MS spectrum of compound 3 (atranorin) in negative mode.

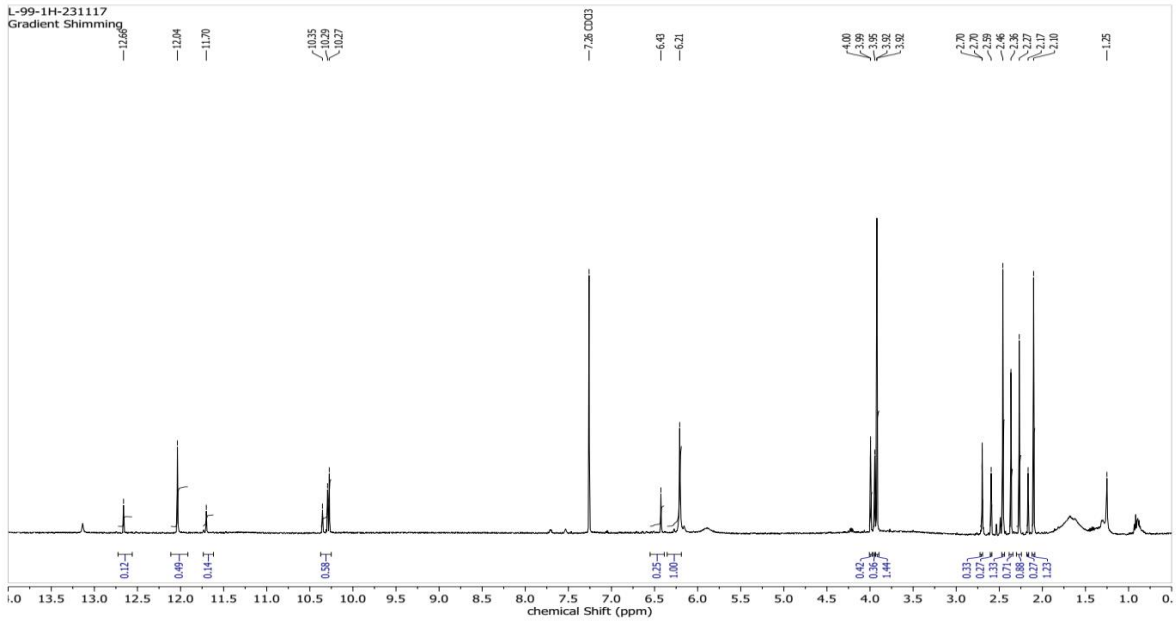

Figure S8: ^{1}H NMR spectrum (CD_3OD, 500 MHz) of a mixture of compound 5 (fluoroatranorin) along with compound 4 (chloroatranorin) and compound 3 (atranorin).

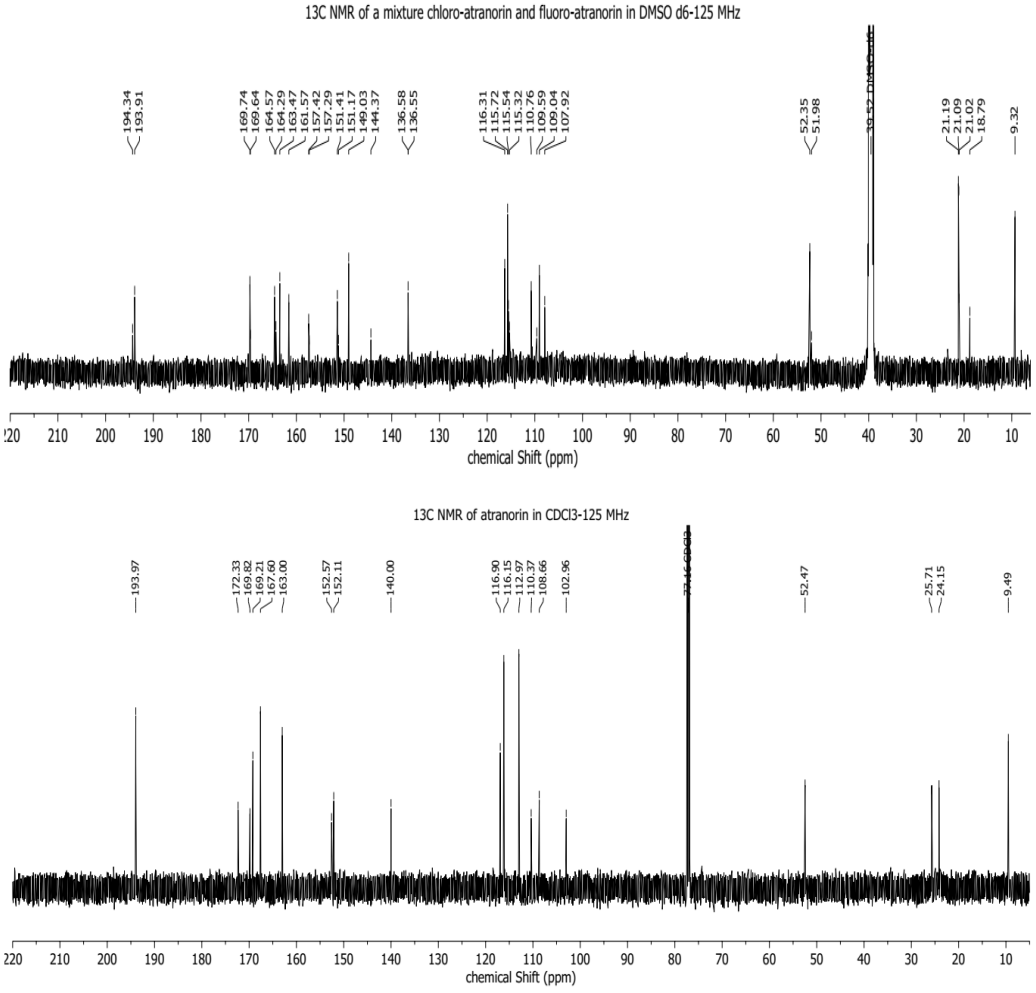

Figure S9: Stacking plot of ^{13}C NMR spectrum of chloroatranorin (CD_3SOCD_3, 125 MHz) and atranorin ($CDCl_3$, 125 MHz).

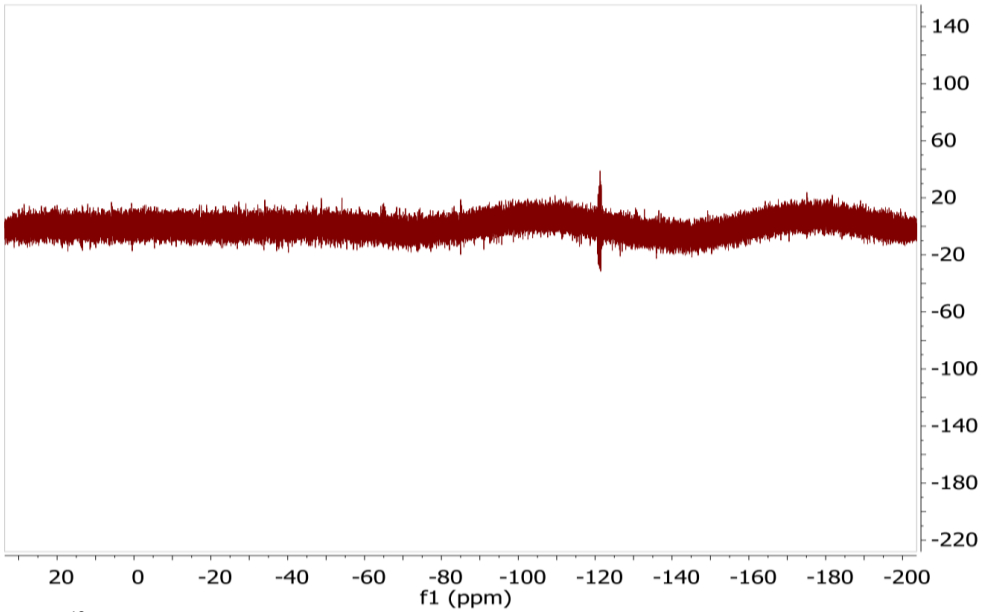

Figure S10: ^{19}F NMR spectrum (CD_3SOCD_3-d_6, Nova 470 MHz) of compound 5 (fluoroatranorin).

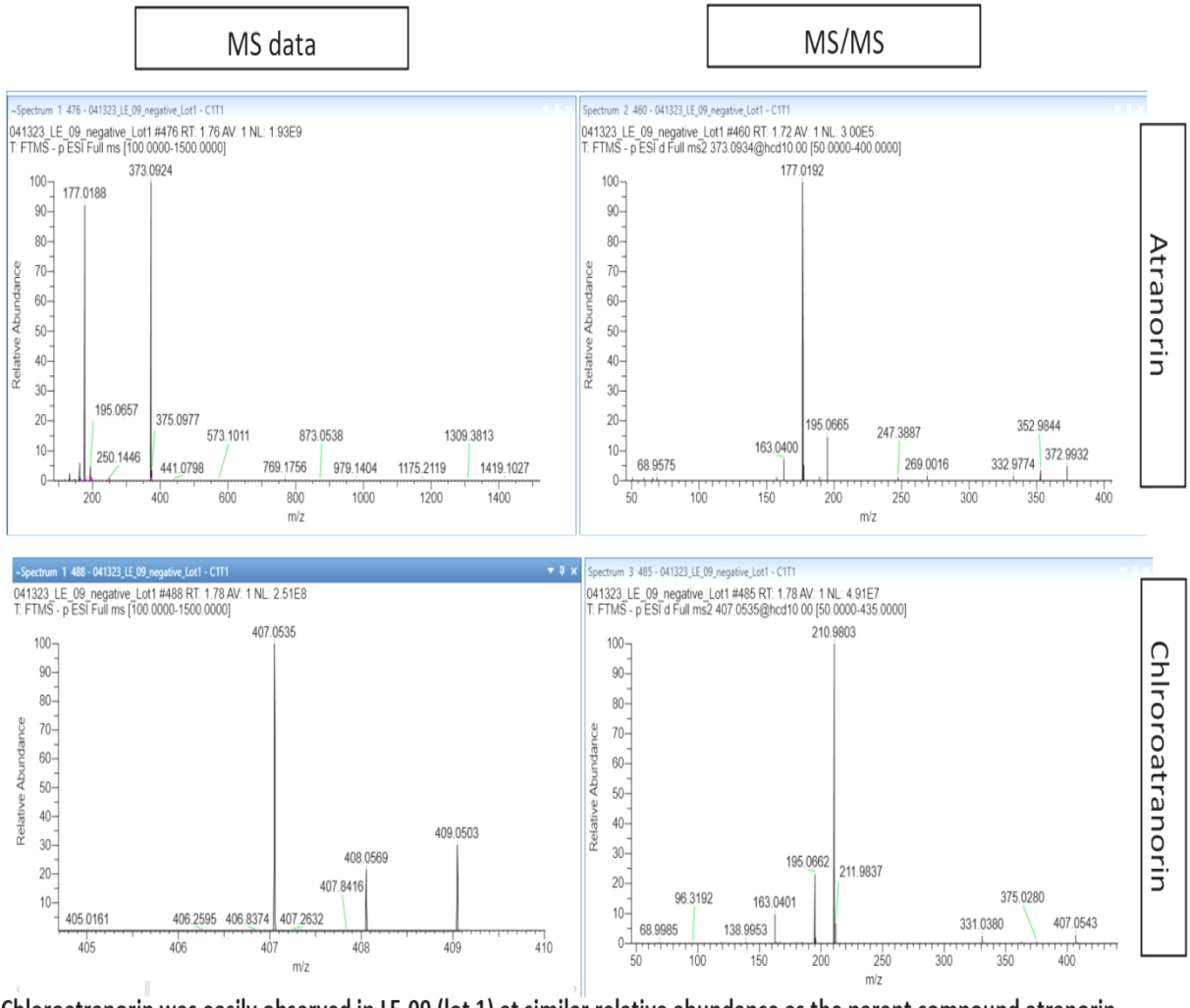

Figure S11: HRESI-MS spectrum and MS/MS data of compound 3 (atranorin) and compound 4 (chloroatranorin).

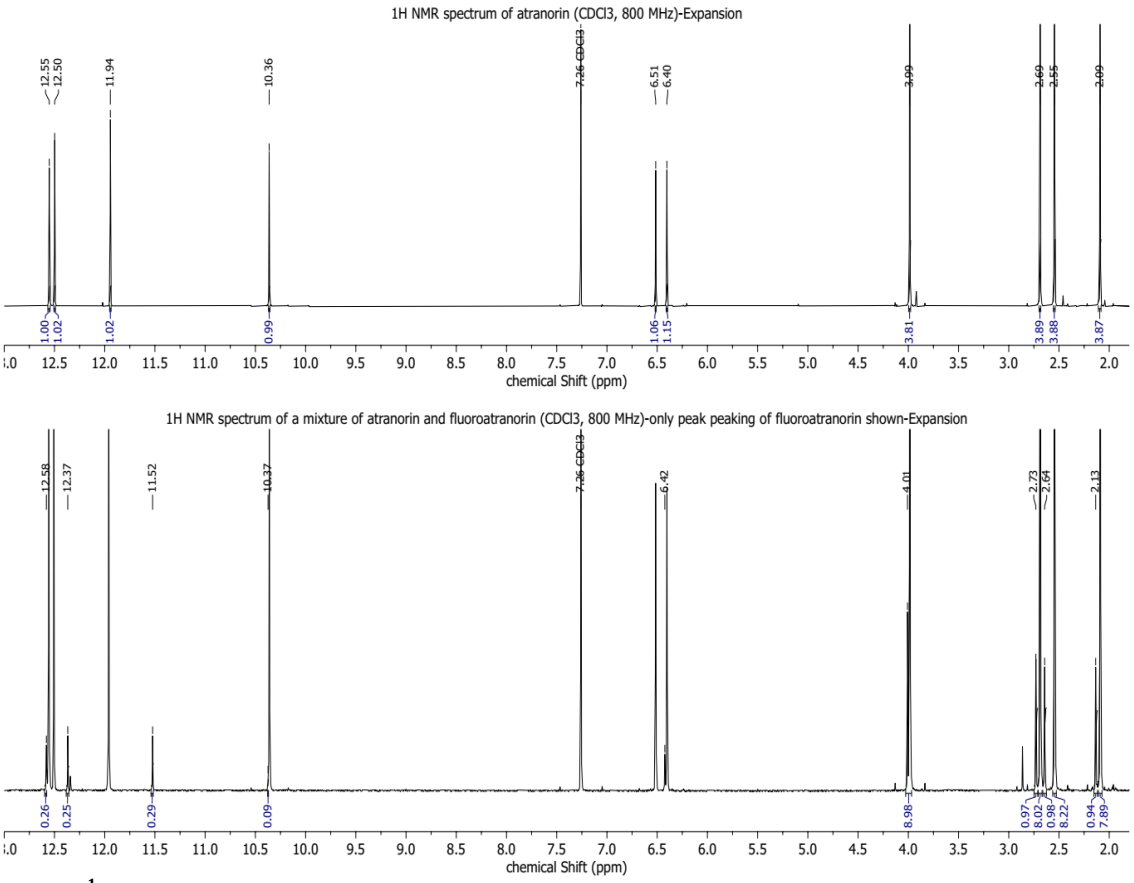

Figure S12: ^{1}H NMR spectrum ($CDCl_3$, 800 MHz) of atranorin 4 and a mixture of atranorin 4 and fluoroatranorin 5 (appearance small peaks in bottom spectrum indicates peaks for 5).

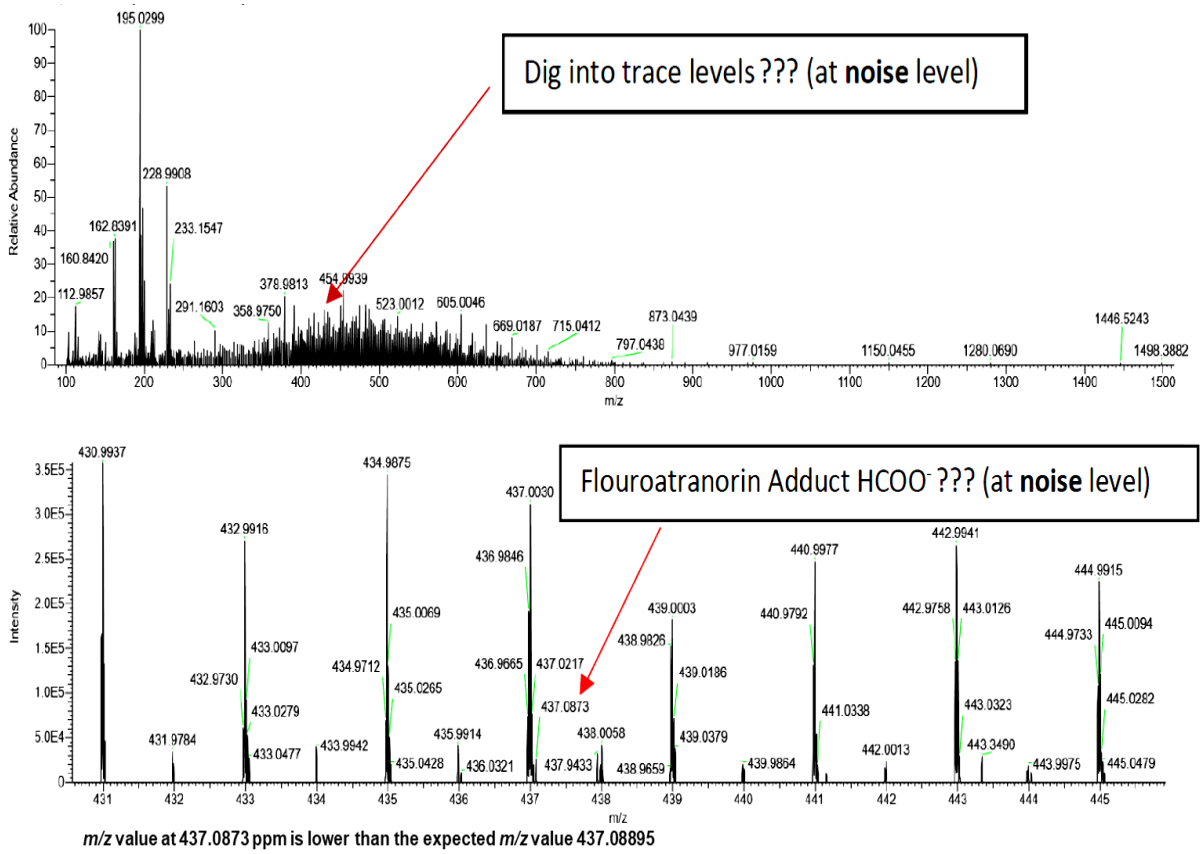

Figure S13: HRESI-MS and MS/MS spectrum of compound 5 (fluoroatranorin).

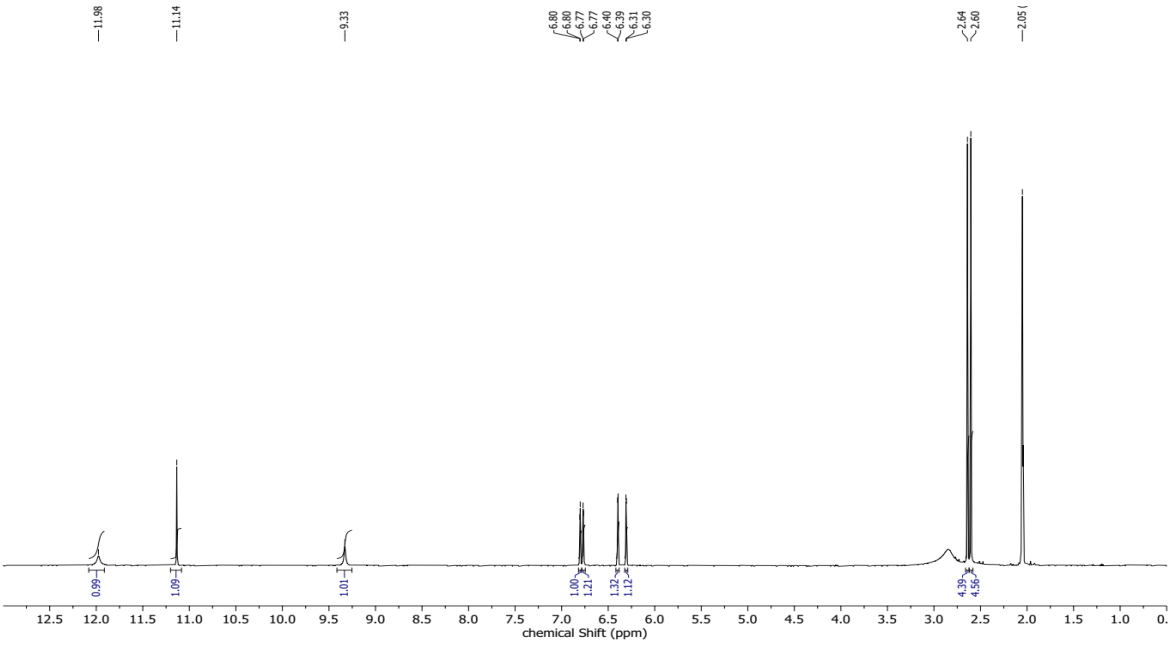

Figure S14: ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound 6 (lecanoric acid).

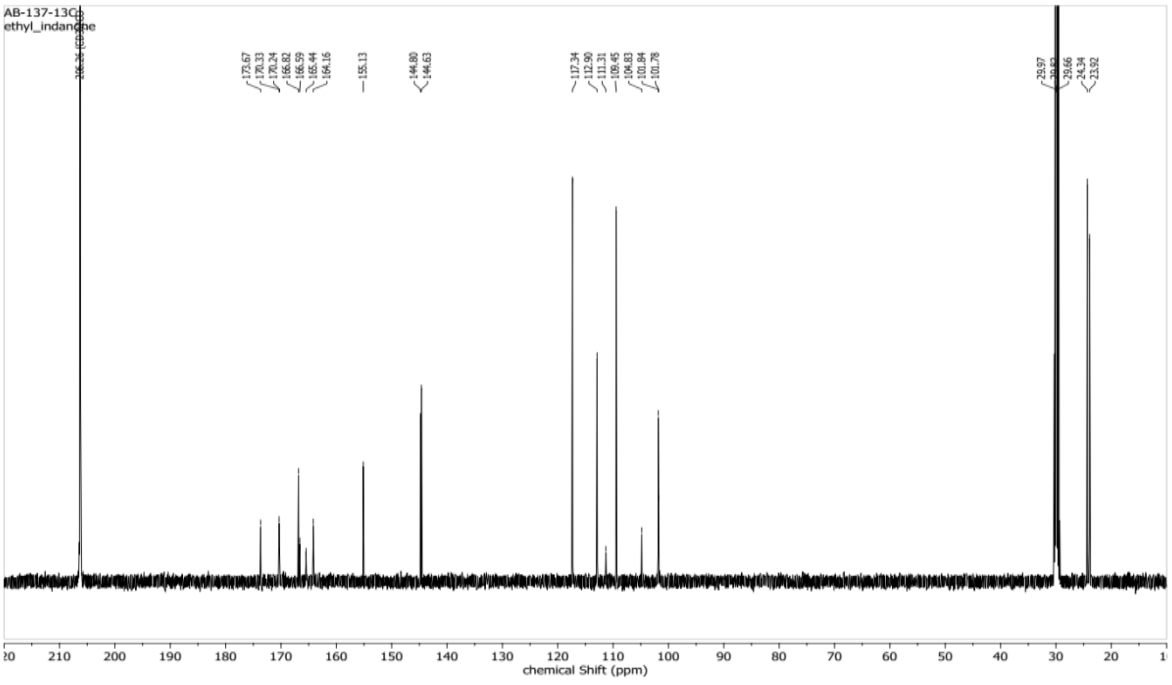

Figure S15: ^{13}C NMR spectrum (CD_3OD, 125 MHz) of compound 6 (Lecanoric acid).

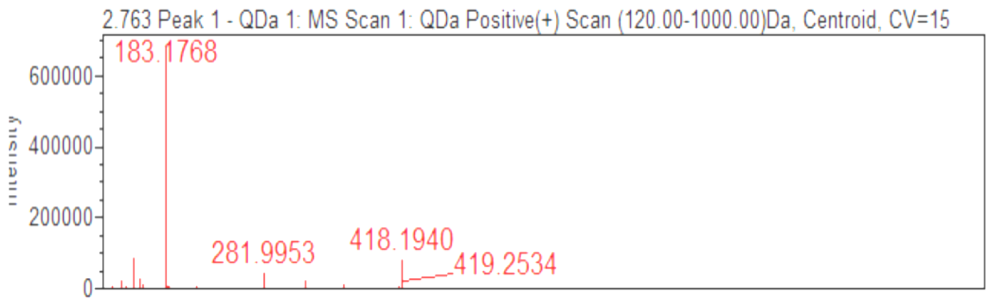

Figure S16: LRESI-MS spectrum of compound 6 (lecanoric acid); obs. mass: $[M+Ru]^-$: 418.1940 ($C_{16}H_{14}O_7Ru$); calc. mass: 418.9704 ($C_{16}H_{13}O_7Ru$).

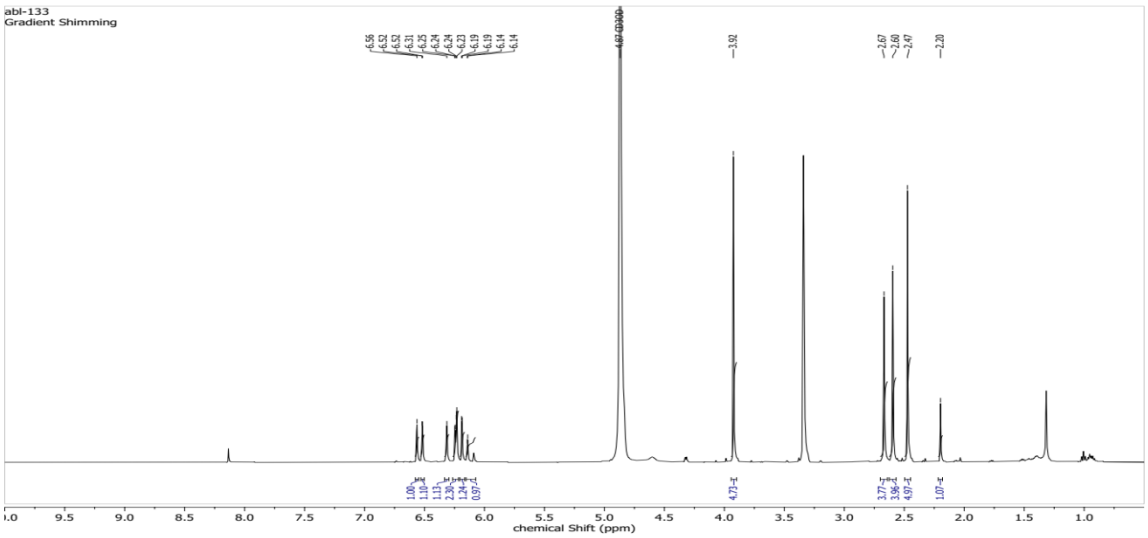

Figure S17: ^{1}H NMR spectrum (CD_3OD, 500 MHz) compound 7 (methyl ester of lasallic acid).

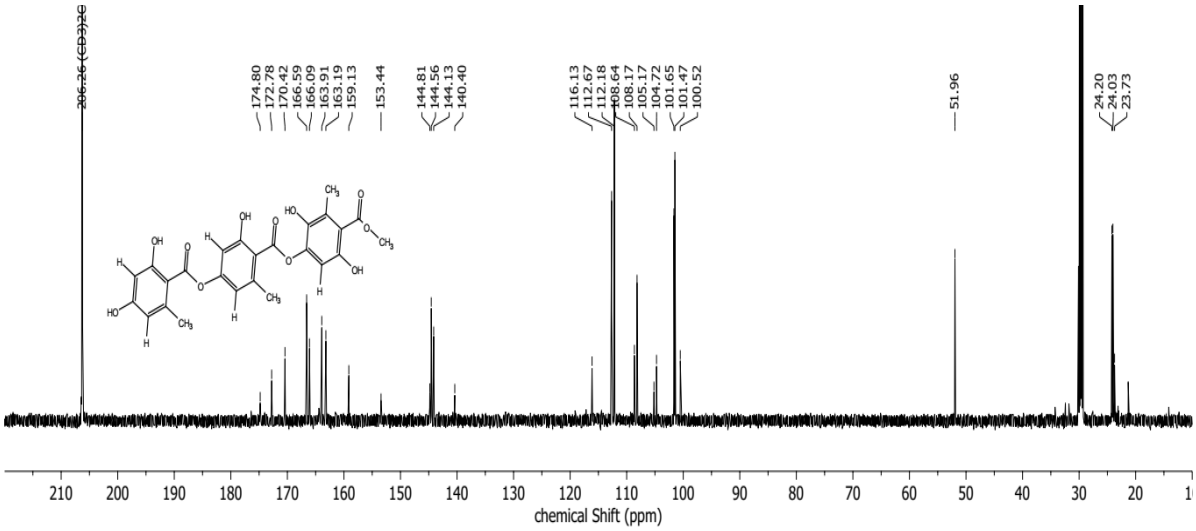

Figure S18: ^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound 7 (methyl ester of lasallic acid).

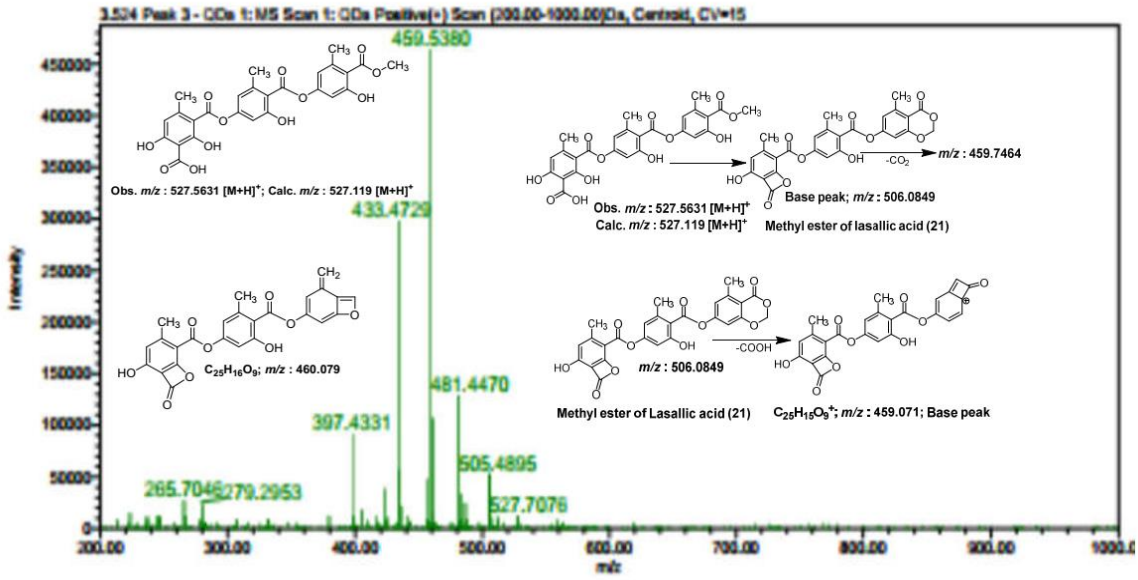

Figure S19: HRESI-MS spectrum of compound 7 (methyl ester of lasallic acid).

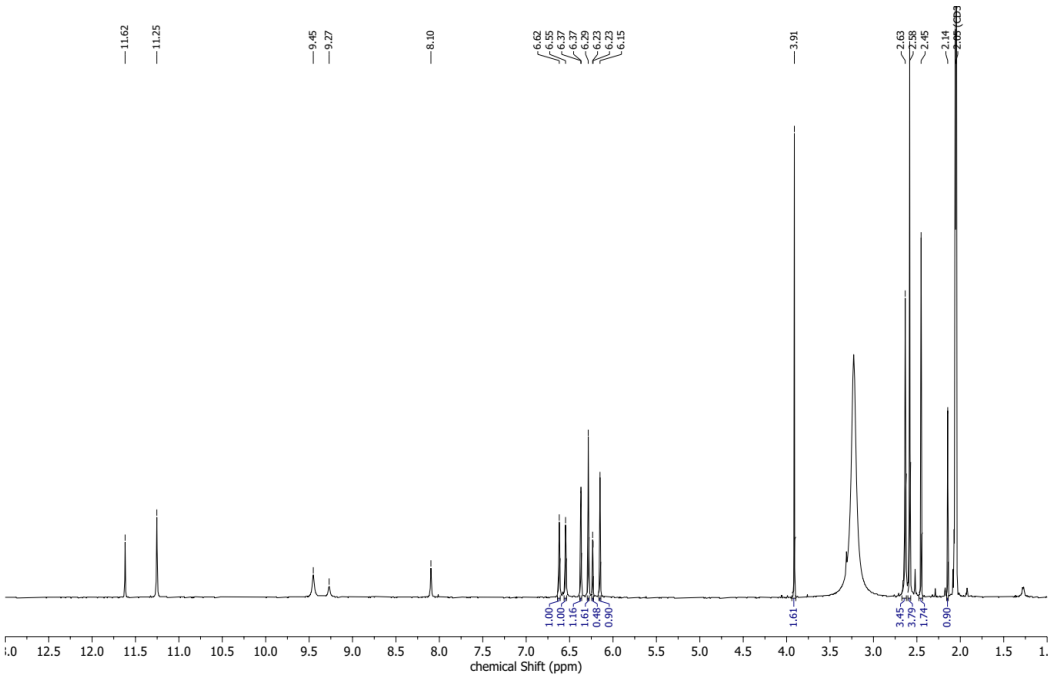

Figure 20: ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound 8 (β-hydroxy methyl ester of gyrophoric acid).

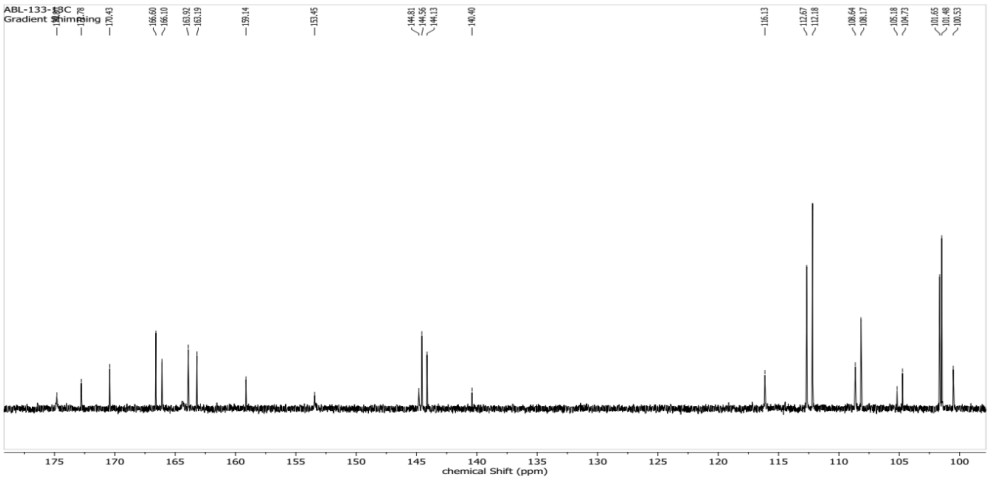

Figure S21: ^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound 8 (β-hydroxy methyl ester of gyrophoric acid).

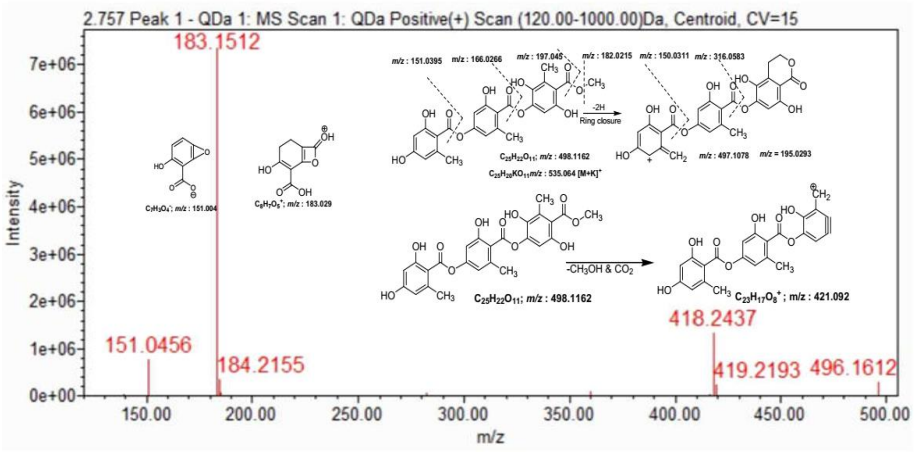

Figure S22: HRESI-MS spectrum of compound 8 (β-hydroxy methyl ester of gyrophoric acid).

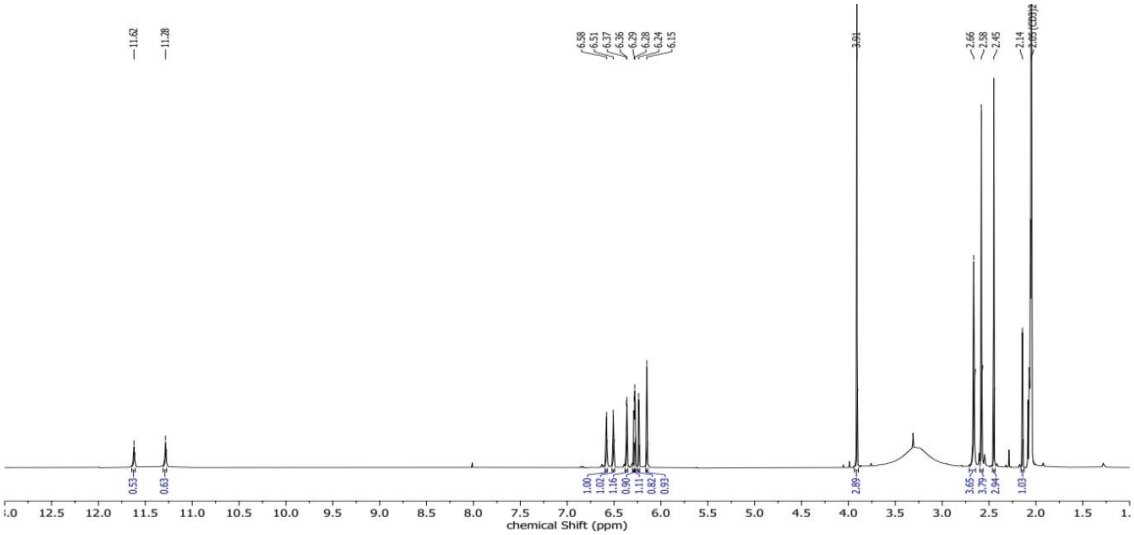

Figure S23: Expansion of ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound 9 (methyl ester of crustinic acid).

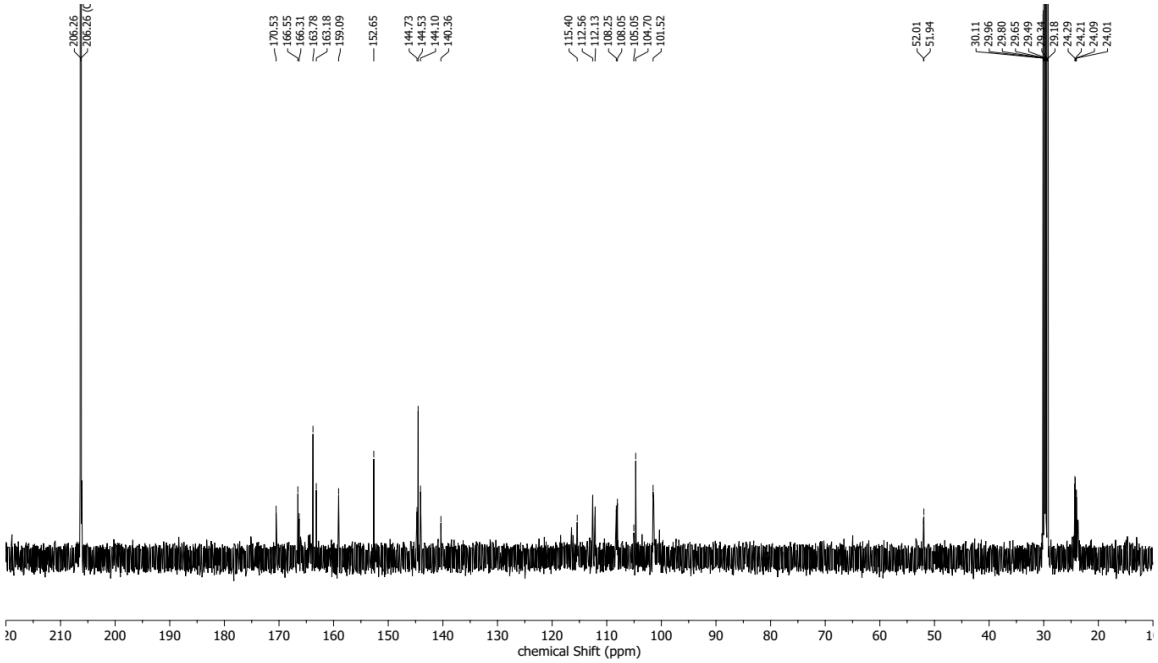

Figure S24: ^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound 9 (methyl ester of crustinic acid).

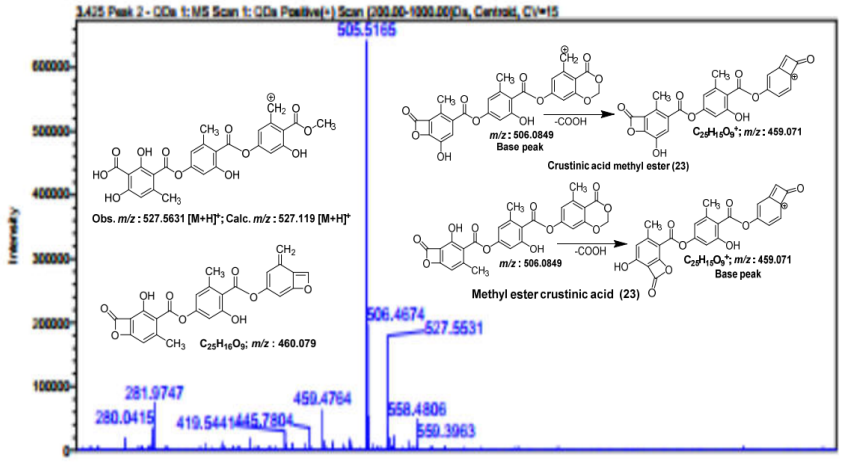

Figure S25: HRESI-MS spectrum of compound 9 (methyl ester of crustinic acid).

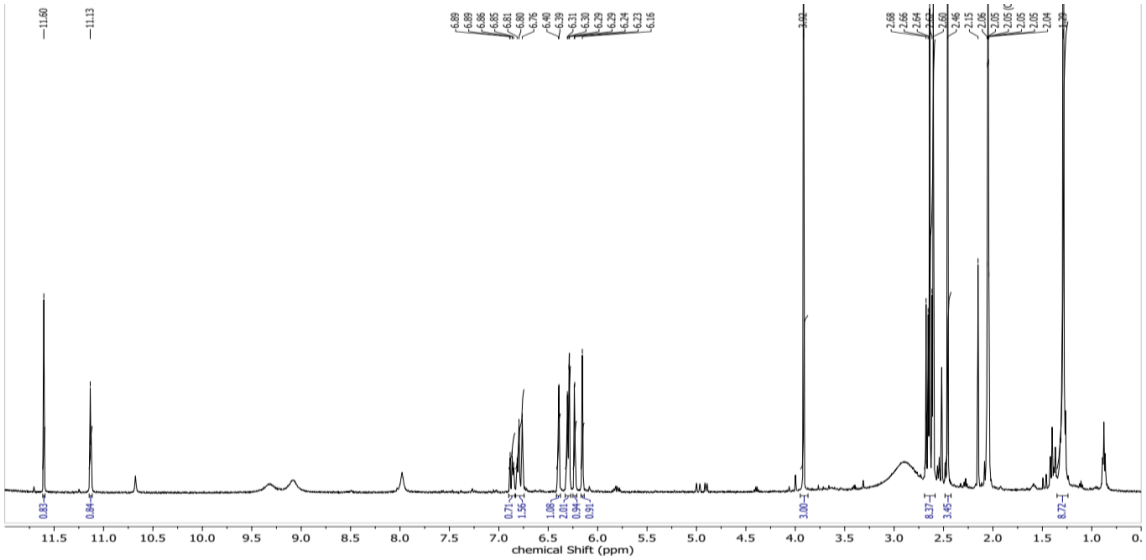

Figure S26: ^{1}H NMR spectrum (CD_3COCD_3, 500 MHz) of compound 10 (methyl ester of hiascic acid).

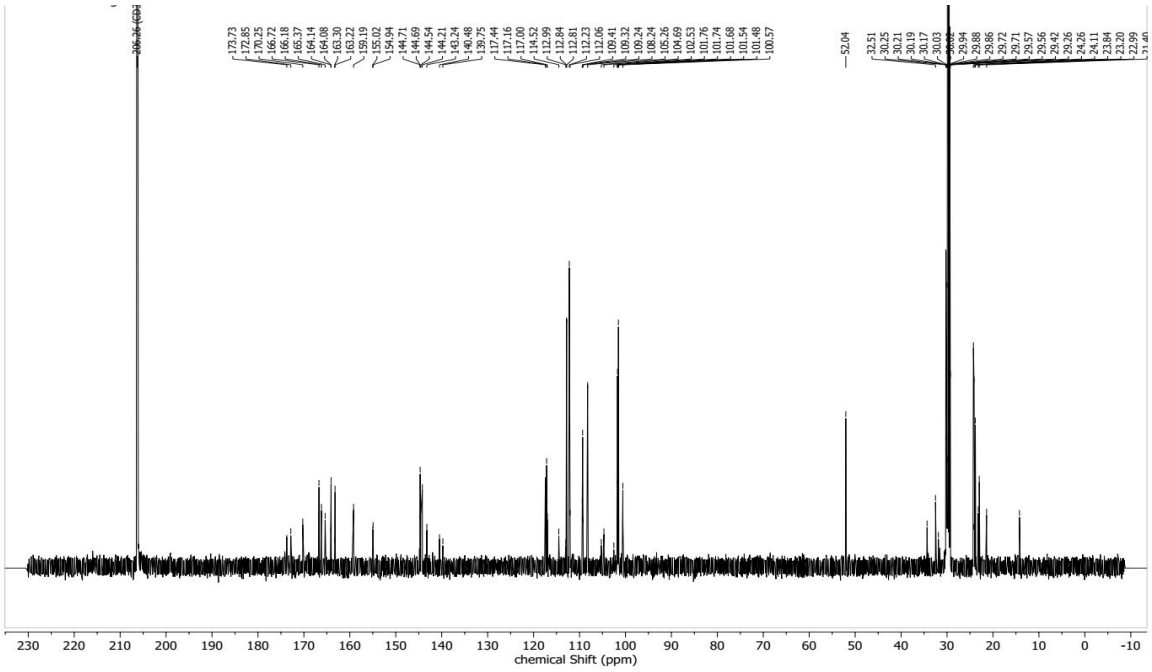

Figure S27: ^{13}C NMR spectrum (CD_3COCD_3, 125 MHz) of compound 10 (methyl ester of hiascic acid).

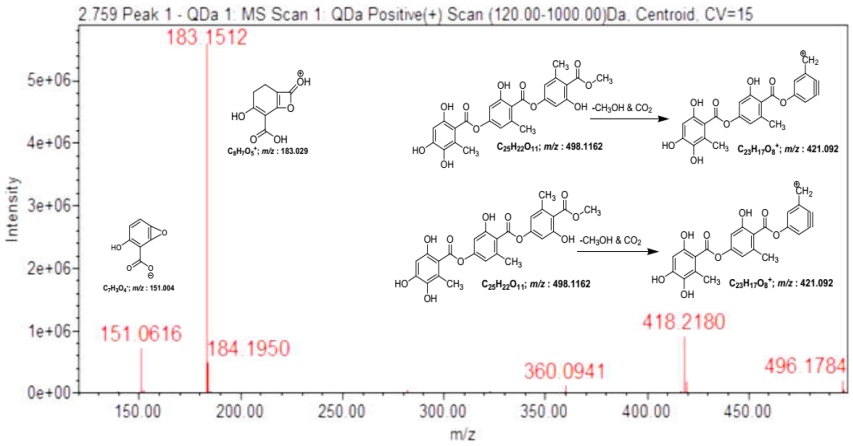

Figure S28: HRESI-MS spectrum of compound 10 (methyl ester of hiascic acid).

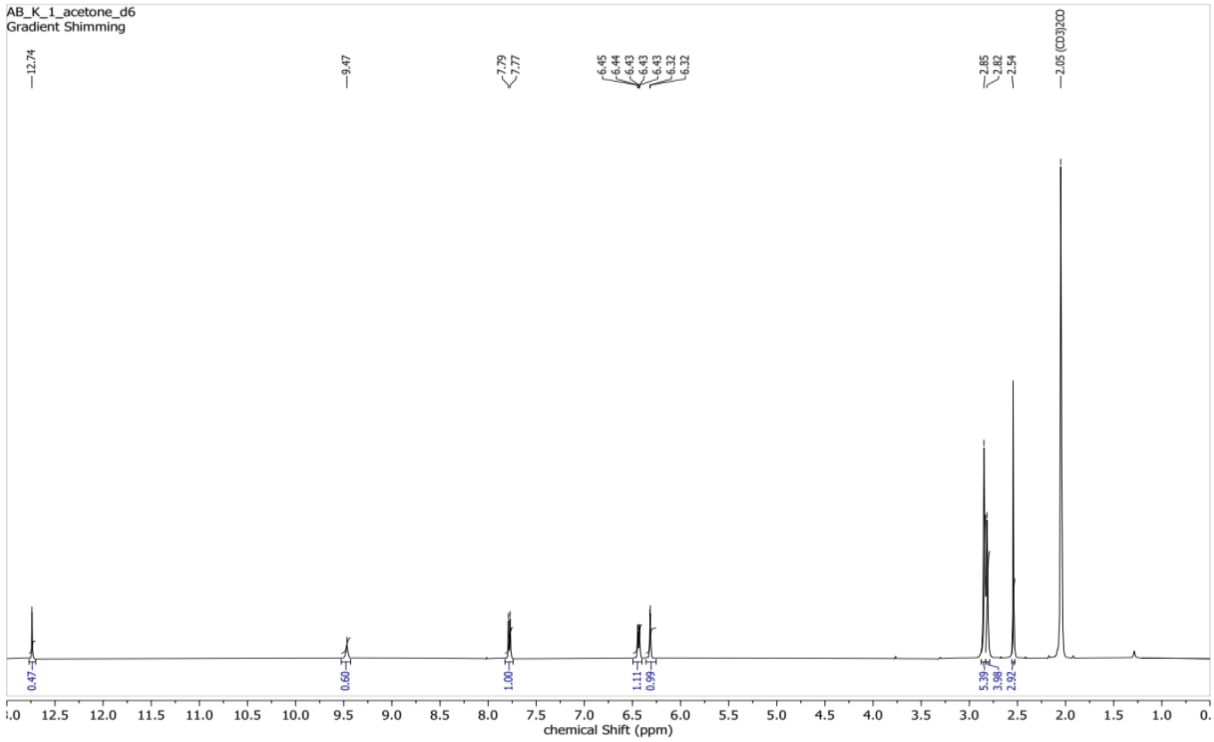

Figure S29: ^{1}H NMR (CD_3COCD_3, 500 MHz) of compound 11 (β-Resorcyclic acid).

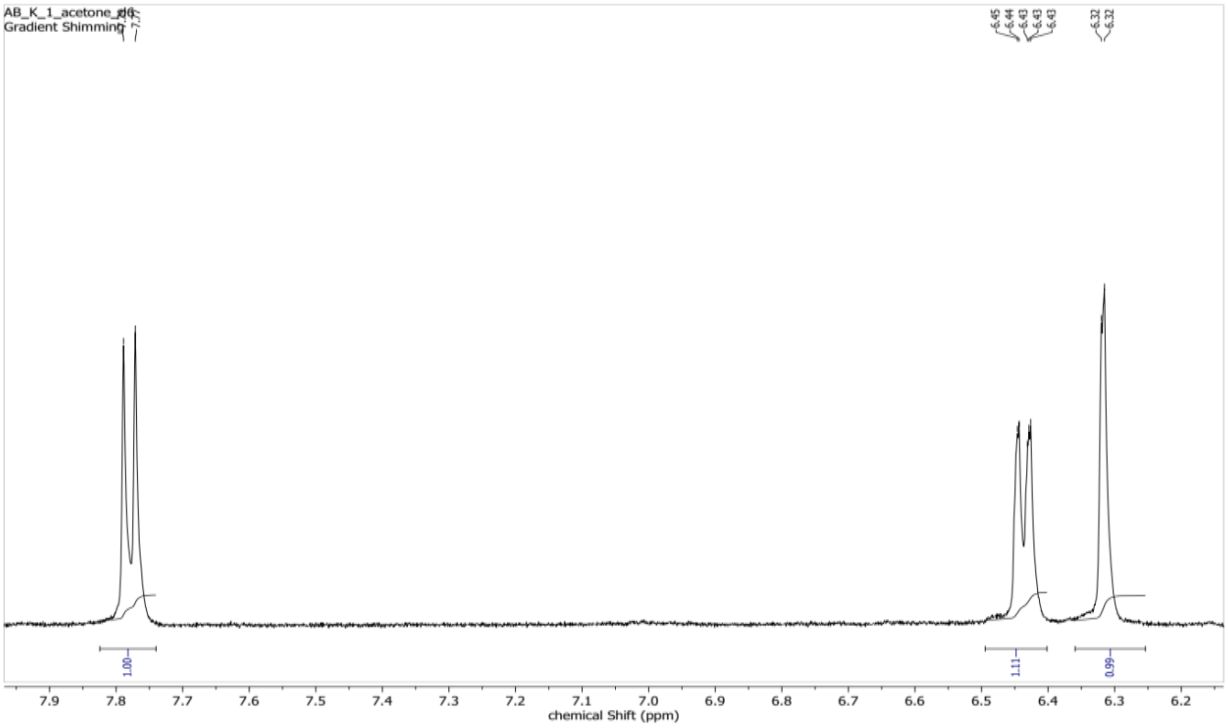

Figure S30: Expansion of ^{1}H NMR (CD_3COCD_3, 500 MHz) of compound 11 (β-Resorcyclic acid).

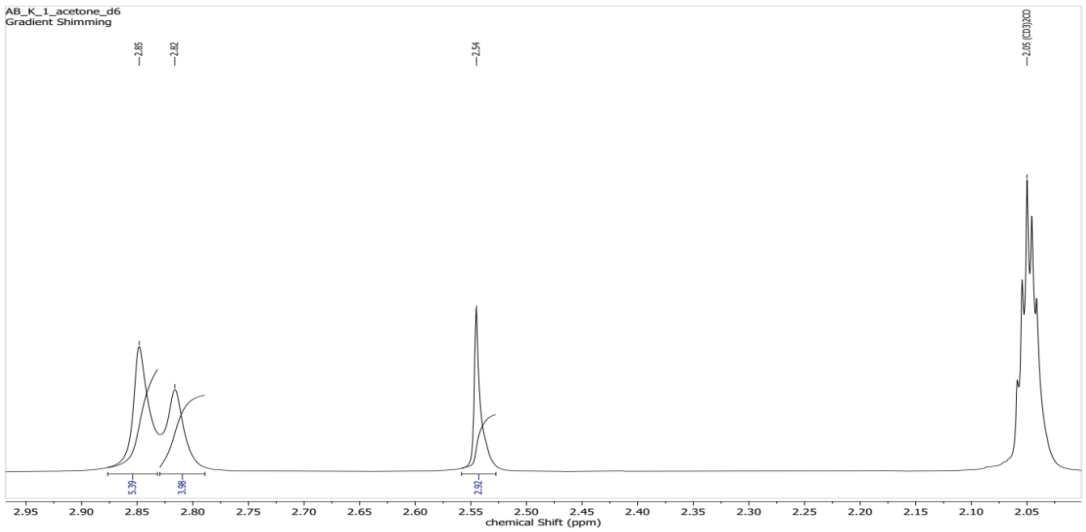

Figure S31: Expansion of ^{1}H NMR (CD_3COCD_3, 500 MHz,) of compound 11 (β-Resorcyclic acid).

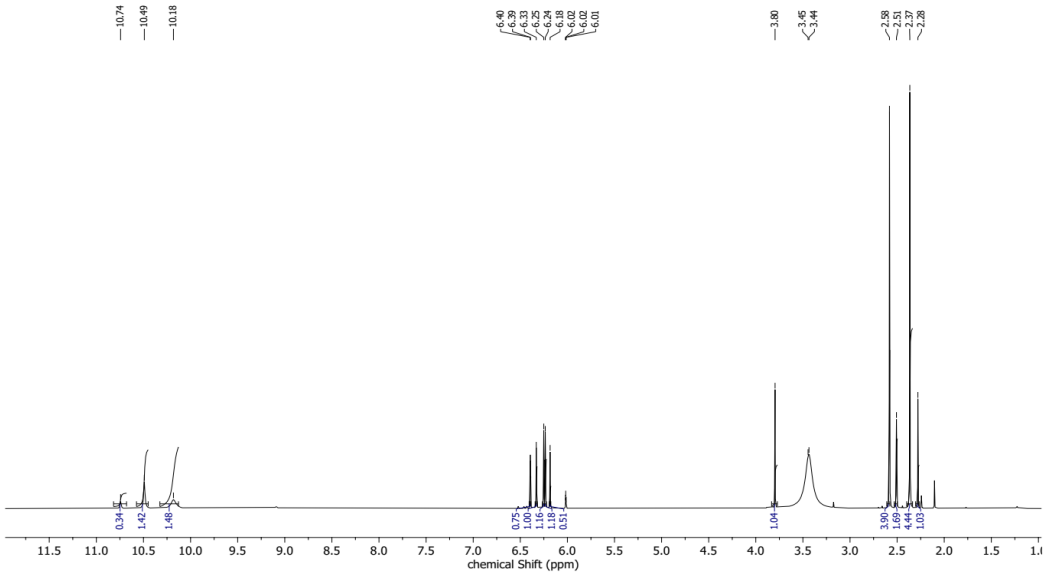

Figure S32: ^{1}H NMR spectrum (CD_3SOCD_3, 800 MHz) of compound 12 (4-carboxy lecanoric acid).

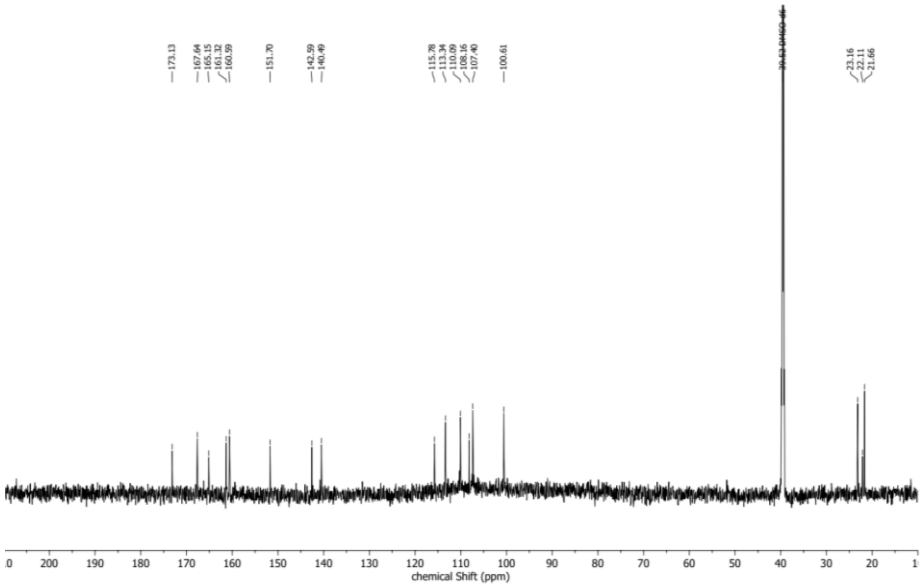

Figure S33: ^{13}C NMR spectrum (CD_3SOCD_3, 201 MHz) of compound 12 (4-carboxy lecanoric acid).

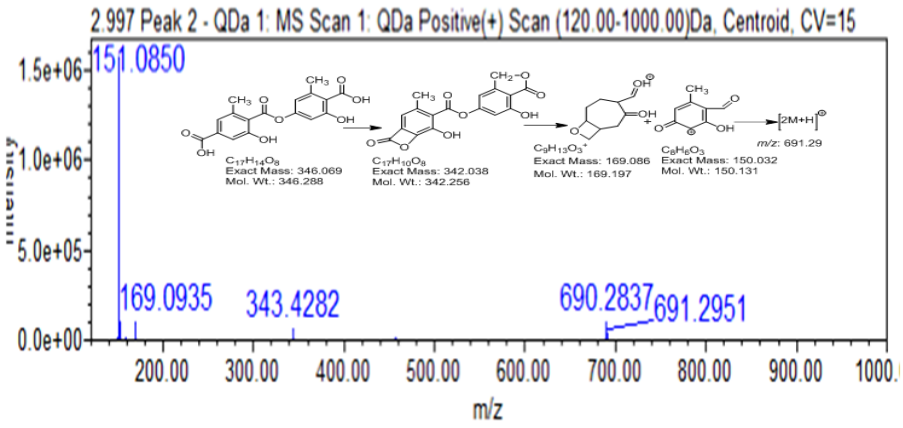

Figure S34: LRESI-MS spectrum of compound 12 (4-carboxy lecanoric acid).

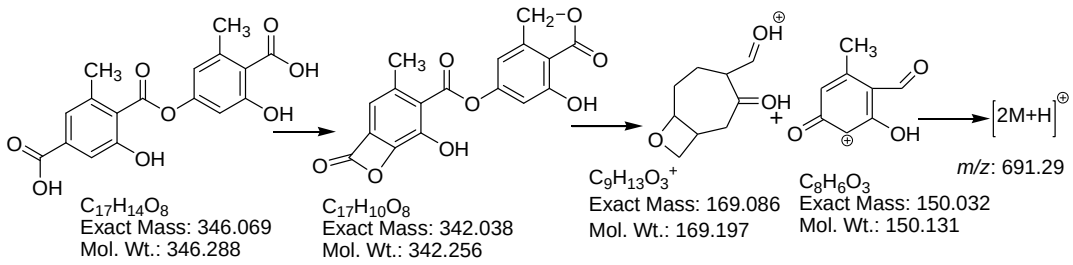

Figure 35: Interpretation of HRESI-MS spectrum of compound 12 (4-carboxy lecanoric acid).

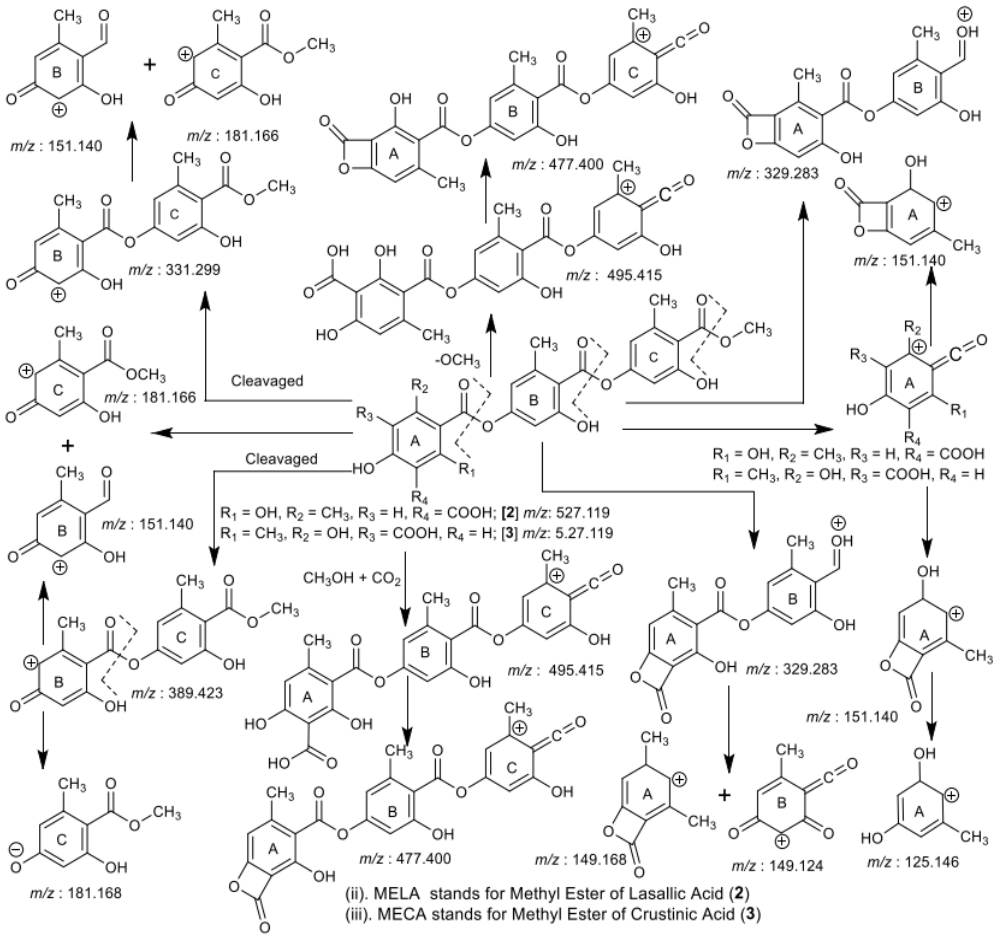

Figure S36: Joint interpretation of HRESI-MS of methyl ester of lasallic acid 7 and methyl ester of crustinic acid 9.