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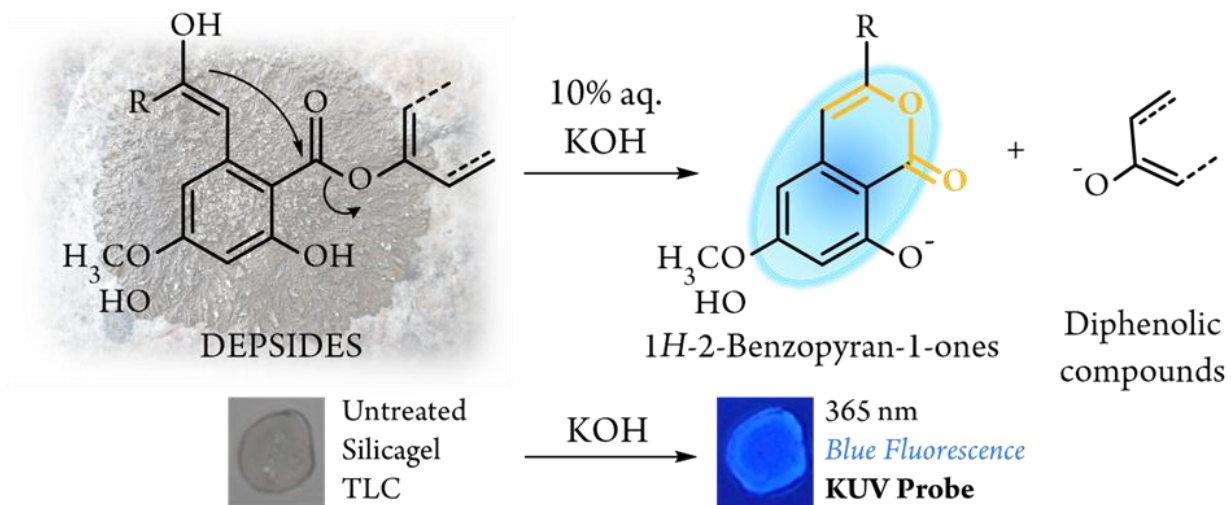
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# 1 Graphical Abstract



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4 **Intramolecular transesterification of depsides yields fluorescent 1H-**  
5 **isochromen-1-ones: application as a chemical probe for lichen**  
6 **determination.**

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13 **Abstract:** The reactivity of eight purified depsides obtained from six european  
14 lichens and that display as 2-oxoalkyl chain in *ortho*-position of the ester bond was  
15 explored. These depsides were found to lead to 1H-Isochromen-1-ones, which  
16 exhibit a distinctive blue fluorescence at 365 nm, in the presence of a 10% aqueous  
17 solution of KOH. A mechanistic explanation, involving the formation of an enolate  
18 intermediate and intramolecular transesterification, was proposed and validated by  
19 DFT. By exploiting this fluorescent phenomenon, we conceived a chemical probe (the  
20 **KUV probe**) that is useful for lichen determination, as exemplified on a selection of  
21 European *Porpidia* species.

22 **Keywords:** Depsides,  $\delta$ -keto esters, enolate, transesterification, fluorescent  
23 1H-Isochromen-1-ones, chemical lichen determination, KUV probe.

24 **Abbreviations:** Reagent **K** or **K**: 10% aq KOH solution; **KUV probe**: Addition  
25 of **K** on the acetonic lichen extract on a support (white paper or TLC plate) or on the  
26 lichen medulla followed by observation at 365 nm. If a blue fluorescence appears:  
27 **KUV +**.

28

## 29 **1. Introduction**

30 Lichens arise from a symbiotic relationship between a fungus and a  
31 photosynthetic partner (algae or cyanobacteria) and are considered a biological entity  
32 rather than a systematic one as their classification is based on the nature of the fungi.  
33 Then, the analysis of the lichen specialized metabolites along with morphological and  
34 genetical data of fungi (Asahina and Shibata, 1954; Calcott et al., 2018; Culberson,  
35 1969; Elix et al., 1984; Huneck and Yoshimura, 1996) are widely used for the taxa  
36 and species determination, leading to the chemosyndrome concept. In this context,  
37 the study of the aromatic depsides, generated by the polyketide synthase (PKS)  
38 enzymatic complexes (Armaleo et al., 2011; Herbst et al., 2018; Walsh and Tang,  
39 2017), has been shown to resolve numerous taxonomic problems among lichens.

40 In a previous publication (Hellou et al., 2019), we have shown that two  
41 depsides extracted from *Xanthoparmelia delisei* or *X. loxodes*, the glomelliferic acid **1**  
42 and the glomellic acid **2**, exhibited a strong fluorescence at 365 nm when a solution  
43 of 10% aqueous KOH (the **K** spot reagent in lichenology) was added (Figure 1). This  
44 addition of **K** reagent is thereafter referenced as the **KUV probe**. An unusual  
45 structural feature of these two lichen specialized metabolites when compared to other  
46 depsides is that they are substituted on the C-6 of the A ring by a 2-oxoalkyl  
47 chain. This observation prompted us to explore their reactivity to rationalize the  
48 fluorescence observed and to broaden the use of the KUV probe onto other similar  
49 depsides from various lichens.

50 In this paper, the chemical behaviour of eight depsides treated with the KUV  
51 probe was explored and the fluorescent compounds isolated and fully characterized,  
52 leading to their identification as isochromen-1-ones (1*H*-2-benzopyran-1-ones). To  
53 explain their formation under basic conditions, a mechanistic explanation,  
54 supported by DFT calculations, is presented. Finally, we highlight the interest of the  
55 KUV probe in the field of lichenology for lichen determination and exemplify its use,  
56 both out in the field by direct application on the medulla or in the laboratory on TLC,  
57 on a selection of European *Porpidia* species.

## 58 **2. Results and discussion**

### 59 **2.1. Selection and purification of $\delta$ -keto ester-containing depsides**

60 Using the literature data (Huneck and Yoshimura, 1996), the depsides **1-7** and  
61 **4'** reported in Figure 2 were selected and extracted from the corresponding lichens  
62 using acetone, followed by purification by column and/or thin layer chromatography  
63 using silica gel with the solvent system C (toluol/acetic acid, 85/15) as the mobile  
64 phase.

## 65 **2.2. Reactivity of $\delta$ -keto containing lichen metabolites in basic conditions**

66 The cleavage of the ester bond between **A** and **B** rings of the depsides  
67 (Figure 1) has been previously performed for structure elucidation purposes. Along  
68 with saponification (5% KOH or NaOH at 100°C) and alcoholysis (MeOH or *t*-BuOH)  
69 at high temperature, acidic hydrolysis (96% H<sub>2</sub>SO<sub>4</sub>, then H<sub>2</sub>O at 0°C) has been  
70 widely used (Culberson et al., 1987).

71 In this study, the selected depsides were allowed to react overnight with the  
72 reagent **K** at room temperature in order to mimic the conditions used for **1** and **2**  
73 (Figure 1). After neutralization with 10M aq. HCl, used to minimize the total water  
74 amount, two series of aromatic products (Figure 3) were obtained:

75 - tri-substituted 1*H*-Isochromen-1-ones: glomelline (**8**) (Huneck and Follmann,  
76 1973), 4-*O*-methylolivetonide (**9**) (Asai et al., 2017; Saeed, 2011), insignine (**10**) (Elix  
77 et al., 1996) and olivetonide (**11**) (Huneck et al., 1970; Kaji et al., 1986; Sanchez,  
78 1994), fluorescent at 365nm (**F**) **in basic conditions**. These heterocycles were  
79 obtained by rearrangement of the  $\delta$ -keto-ester moiety of the ring **A**.

80 - and various diphenolic acids (**12-16**) corresponding to the **B** ring after ester  
81 hydrolysis.

82 As demonstrated with the glomelliferic acid **1**, the same complete reaction was  
83 observed if the neutralization was carefully performed using a saturated NH<sub>4</sub>Cl  
84 solution (pH: 4-5).

85 In the case of **4'**, bearing a second  $\delta$ -keto-ester moiety on the **B** ring, three  
86 compounds were isolated by chromatography: along with **9** and **15** (previously  
87 obtained from **4**), the fluorescent 2-*O*-methylolivetonide **17** (Asahina and Fujikawa,  
88 1935) was also obtained. This isomer of **9** is resulting of the rearrangement of the  $\delta$ -  
89 keto-ester moiety of the **B** ring (Figure 4).

90 At this stage, we wanted to investigate if identically substituted depsidones,  
91 linked by a supplementary ether bridge between C-2 and C-5', could also exhibit this  
92 specific reactivity. For this purpose, the same experimental conditions have then  
93 been applied to the  $\alpha$ -alectoronic acid **18** (*Ochrolechia parella*) (Millot et al., 2007)  
94 and to the  $\alpha$ -collatolic acid **19** (*Tephromela atra*) (Millot et al., 2008) (Figure 5A).  
95 Their  $\delta$ -keto-ester moiety was not affected and neither the  $\beta$ -alectoronic acid nor the  
96  $\beta$ -collatolic acid were obtained. In one last experiment, the importance of the keto  
97 function was demonstrated. Thus, the perlatolic acid **20** (2'-deoxoglomelliferic acid)  
98 (Elix, 1974) remained unchanged when treated with **K** in the same conditions.

### 99 **2.3. Optical properties of the 1H-Isochromen-1-ones**

100 The UV and fluorescence spectra (Figure 6) of the isochromen-1-ones **9** and  
101 **11** were recorded in methanol (blue curves). The addition of NaOH (red curves),  
102 induced a phenate formation in *ortho* of the C=O that results in a bathochromic (near  
103 350 nm) and hypsochromic effect (near 240 nm) for compound **9**. The effects were  
104 inverted for **11**, due to the ionization of the second phenol in *para* of the C=O. The  
105 blue fluorescence emission is represented by the green curve.

### 106 **2.4. Chemistry: Mechanism**

107 Under basic condition, the observed isochromen-1-ones formation led us to  
108 propose, in accordance with the literature (Duan and Herndon, 2008; Sudarshan and  
109 Aidhen, 2017), the formation of an enolate anion (**I**) that reacts in an intramolecular  
110 transesterification to yield the isochromen-1-one derivatives (Figure 7A). The keto-  
111 enolate equilibrium was displaced by the cyclisation and the transformation was  
112 complete. Alternatively, the more usual ester saponification (**II**) followed by  
113 dehydration could be proposed, but this mechanism needs more drastic conditions  
114 and would lead to hydroxy-lactone intermediates which were never isolated during  
115 our numerous experiments. Interestingly, the same ester cleavage, but probably  
116 involving an *ortho* effect leading to ketene formation (**III**), was observed in the mass  
117 spectrometer (Figure 7B).

118 DFT calculations were undertaken to assess differences in chemical reactivity  
119 between depsides and depsidones. Thus, under neutral conditions, an intramolecular  
120 hydrogen bond between the ester carbonyl and the OH-2 results in an energetically  
121 favoured planar conformation (Figure 8A), which is in accordance with the crystal

122 structure of atranorine (Brassy et al., 1982), a close structural analogue. Upon  
123 addition of **K**, deprotonation of the OH-2 disrupt the planar conformation and position  
124 the ester carbonyl group in a perpendicular conformation relative to the phenyl ring **A**  
125 that is suitable for a nucleophilic attack by the enolate (Figure 8B). Interestingly, the  
126 highest energy observed in the relaxed coordinate scans displays a geometry close  
127 to the transition state of the proposed mechanism.

128 On the contrary, in the case of the depsidones **18** and **19**, the supplementary  
129 ether function between the **A** and **B** rings locks the compounds in a wide V-shaped  
130 conformation (118° angle, Figure 7C). The fixed geometry of the intra-cyclic ester of of  
131 the depsidone core then prevents a nucleophilic attack by the enolate as the angle  
132 between the two isn't suitable (Figure 7D) and results in an electronical clash,  
133 explaining the absence of reactivity.

## 134 2.5. Interest in Lichenology

135 As previously reported (Hellou et al., 2019), the KUV probe allows the easy  
136 distinction between *Xanthoparmelia delisei* (Duby) O. Blanco, A. Crespo, Elix, D.  
137 Hawksw. & Lumbsch (**KUV+**) and *X. pulla* (Ach.) O. Blanco, A. Crespo, Elix, D.  
138 Hawksw. & Lumbsch (**KUV-**) as well as between *X. loxodes* (Nyl.) O. Blanco, A.  
139 Crespo, Elix, D. Hawksw. & Lumbsch (**KUV +**) and *X. verruculifera* (Nyl.) O. Blanco,  
140 A. Crespo, Elix, D. Hawksw. & Lumbsch (**KUV-**). Therefore this concept will be  
141 extended to other lichens using a selection of European *Porpidia* species (Friday,  
142 2005).

143 - A positive KUV probe was obtained with *Porpidia cinereoatra* (Ach.) Hertel  
144 and Knoph, *P. tuberculosa* (Sm.) Hertel and Knoph, *P. speirea* (Ach.) Kremp var  
145 *speirea*, *P. melinodes* (Körb.) Gowan & Ahti, *P. contraponenda* (Arnold) Knoph and  
146 Hertel, *P. irrigua* Orange and *P. rugosa* (Taylor) Coppins and Fryday whereas a  
147 negative KUV probe was obtained with the other *Porpidia* species containing stictic  
148 acid, norstictic acid (i.e. *P. macrocarpa* (DC.) Hertel and Schwab and *P.*  
149 *platycarpoides* (Bagl.) Hertel or without metabolites (i.e. *P. hydrophila* (Fr.) Hertel &  
150 A.J. Schwab) (Fryday, 2005).

151 - Most interestingly, lichenologists out in the field can use the KUV probe  
152 directly on the medulla of the lichen or using absorbant paper (without blanching  
153 agent), allowing quick determination of species with morphological similarities (Figure

154 10A). Of note, in the case of lichens containing atranorine (e.g. *Furfuracea* var.  
155 *ceratea*), which emits a strongly yellow fluorescence upon addition of reagent K, the  
156 TLC methods presented below should be used.

157 - This probe can also be used in the laboratory as a revealing agent for TLCs  
158 (Figure 10B). Deposition of one drop of the K reagent on the various spots of the TLC  
159 after elution of the acetonic extract of **Co**, **I**, **C**, **S**, **T**, **M** and **R** revealed a blue  
160 fluorescence at 365 nm with the exception of **16** and of the 2'-O-methylperlatolic acid.  
161 Interestingly the methyl ester of **15** has the same behaviour as **4'** and the KUV probe  
162 was positive. The **spot 8** observed by Orange [Orange, 2014] was corresponding to  
163 the oxo derivative of **5** (Figure 11). To the best of our knowledge such a keto function  
164 in position  $\omega$ -1 of the alkyl chain was only observed in aliphatic acids such as  
165 dihydropertusaric, muronic and isomuronic acids. The presence of **9**, **16** and of the  
166 methyl ester of **15** was probably due to the extraction and/or the TLC process.

### 167 **3. Conclusion**

168 This study illustrates the interest of both natural products and chemistry. The  
169 diversity resulting from the numerous pathways of the specialized metabolism offers  
170 interesting structural, mechanistic or synthetic challenges to chemists. Thus, the  
171 intramolecular transesterification occurring in some depsides bearing a 2-oxoalkyl  
172 chain in C-6 (ring A) has been elucidated. Reciprocally, the results of the chemical  
173 studies allow analysis and comprehension of biosynthetic processes and also provide  
174 useful tools to biologists. In our case the structure elucidation of some metabolites of  
175 European *Porpidia* species has been performed and a new easy to use chemical  
176 probe has been validated and proposed to lichenologists.

177



## 178 **4. Experimental**

### 179 **4.1. Reagents**

180 All reagents were of high quality and were purchased from commercial suppliers.  
181 They were either used as received without further purification or were purified/dried  
182 according standard procedures.

### 183 **4.2. Lichen material and identification**

184 The lichen specimens used in this study were collected from different locations of  
185 France and UK (Table 1).

186 Morphological characters were examined with a Müller stereomicroscope (Gx10) and  
187 anatomical details of the samples were studied with an Olympus CX41 compound  
188 light microscope.

### 189 **4.3. Extracts and sample preparation**

190 Lichen thalli (often with mineral) were ground and extracted three times with warm  
191 acetone. After drying the solvent was evaporated under reduced pressure. (Process  
192 not optimized)

### 193 **4.4. TLC analysis**

194 Specialized metabolites were identified by thin-layer chromatography (TLC) using  
195 standards. The extracts and standards were loaded onto 10 × 20 cm aluminium  
196 sheets coated by Silica gel 60F254 (Merck). Thereafter the plates were developed  
197 with solvent C (toluene/acetic acid 85/15) and solvent G (toluene/ethylacetate/formic  
198 acid 139/83/8) for the *Porpidia* species study. All TLC plates were observed under  
199 UV at 254 and 365 nm (Fischer bioblock Scientific 12W, 6W-365nm, 6W-254nm).  
200 Indicative R<sub>f</sub> values in solvents C are given for each product. The R<sub>f</sub> values of the  
201 classical references are: atranorine: 0.61; norstictic acid: 0.28; stictic acid: 0.12.

### 202 **4.5. Purifications using Liquid-Chromatography**

203 Silicagel 60 Merck and solvent system C (toluol/acetic acid, 85/15) were used for the  
204 purification of the different extracts. The combined fractions with the selected  
205 metabolite were washed once with water in order to eliminate the acids, dried over  
206 MgSO<sub>4</sub> and evaporated under reduced pressure. Preparative TLC were also  
207 performed using the same solvents. (Process not optimized)

208

209 **4.6. High Resolution Mass Spectrometry (HRMS) and Tandem Mass**  
210 **Spectrometry (MS-MS)**

211 Mass spectra of standards were recorded at the CRMPO-Scanmat (Rennes, France)  
212 on an Orbitrap Thermo Fisher Scientific Q-Exactive instrument with an ESI  
213 (ElectroSpray Ionization) source in negative mode by direct introduction at 5-10  
214  $\mu\text{g.mL}^{-1}$ . Samples were prepared in methanol at  $10 \mu\text{g.mL}^{-1}$ .

215 **4.7.  $^1\text{H}$  and  $^{13}\text{C}$  NMR**

216 Spectra were recorded at 300/75 MHz or 500/100 MHz (cryoprobe) respectively,  
217 using TMS as an internal standard. Shifts ( $\delta$  values) are given in parts per million  
218 (ppm), coupling constants ( $J$  values) are given in Hertz (Hz), and multiplicity of  
219 signals are reported as follows: s (singlet), d (doublet), t (triplet), q (quadruplet), quint  
220 (quintet), sext (sextet), m (multiplet), br. s (broad singlet), dt (doublet of triplet), td  
221 (triplet of doublet).

222 **4.8. DFT studies**

223 All calculations were carried with Gaussian 16 program (Frisch et al., 2016) and the  
224 results visualized in Maestro (Schrödinger, LLC, New York, NY, 2021). Simplified  
225 depsides and depsidones representative of their respective core scaffold were used  
226 to avoid unnecessary costly optimizations of the long aliphatic side chains. Structure  
227 optimizations of molecular structures in ground states and relaxed coordinate scans  
228 were carried out at the B3LYP-D3 level of theory in combination with the 6-31G(d,p)  
229 basis and using PCM solvent models. The vibrational frequencies were computed at  
230 the same level to check whether each optimized structure is an energy minimum (no  
231 imaginary frequency) or a transition state (single imaginary frequency).

232 **4.9. UV and Fluorescence studies**

233 2 mg of the isochromen-1-ones **9** and **11** were dissolved in MeOH to obtain  $10^{-5}$   
234  $\text{mol.L}^{-1}$  solutions. Native UV spectra were recorded using a Jenway 7415 UV  
235 spectrometer (Cole-Parmer Instrument Company). Second spectra were recorded  
236 after addition of one drop of  $1 \text{ mol.L}^{-1}$  NaOH.

237 The emission fluorescence spectrum of these solutions was then measured between  
238 360 and 600 nm using a Horiba Jobin-Yvon Fluorolog 3 spectrometer with an  
239 excitation at 240 nm.

240 **4.10. Reactions with KOH:** n x 5 mg of the metabolite were allowed to react  
241 under stirring with n x 100  $\mu$ L of aqueous 10% KOH at room temperature overnight.  
242 After acidification with aqueous 10M HCl under cooling an extraction was performed  
243 using diethylether (2 x 5 mL). The organic layer was dried over MgSO<sub>4</sub> and the  
244 solvent was evaporated under vacuum. The conversion was checked by TLC and the  
245 purification was achieved either by preparative column chromatography or TLC.  
246 Number of attempts > 3. Quantitative reactions for **1-7** and no reaction for **18-19**.

247 **4.11. Conversion study:** In order to confirm that the transesterification was  
248 complete the reaction was performed with a mixture (40 mg) of glomelliferic acid  
249 (89%) + perlatolic acid (11%) and **K** (0.8 mL). To avoid any eventual acidic  
250 deshydration the reaction mixture was treated with a saturated aq NH<sub>4</sub>Cl solution (6  
251 mL/pH 5-6) and extracted with ether (2 X 10 mL). The organic layer was dried over  
252 MgSO<sub>4</sub> and evaporated under reduced pressure giving 35mg of a colored oil (87%).

253 The TLC and <sup>1</sup>H NMR (see SI) of this crude reaction mixture showed that the  
254 glomelliferic acid **1** gave only the glomelline **8** and the phenolic compound **12**  
255 whereas the perlatolic acid remained unchanged (confirmed with pure perlatolic acid  
256 by TLC).

257 *Remark:* Along with the desired and purified compounds some minor specialized  
258 products as well as trace of starting material can be observed in very small amounts.  
259 Then, the conversions can be always considered as > 90%. Considering the little  
260 amounts of starting material yields are not given.

#### 261 **4.10. Spectroscopic data**

262 Glomelliferic acid 1 [552-49-8] C<sub>25</sub>H<sub>30</sub>O<sub>8</sub> MW: 458.5 g/mol

263 2-Hydroxy-4-[[2-hydroxy-4-methoxy-6-(2-oxopentyl)benzoyl]oxy]-6-pentylbenzoic acid

264 **<sup>1</sup>H NMR** (300MHz, CDCl<sub>3</sub>): 11.30 (s, 1H), 11.26 (s, 1H), 6.64 (d, *J* = 2.4 Hz, 1H), 6.52  
265 (d, *J* = 2.4 Hz, 1H), 6.47 (d, *J* = 2.4 Hz, 1H), 6.30 (d, *J* = 2.4 Hz, 1H), 4.05 (s, 2H),  
266 3.84 (s, 3H), 2.97 (t, *J* = 7.8 Hz, 2H), 2.41 (t, *J* = 7.2 Hz, 2H), 1.58 (m, 4H), 1.36 (m,  
267 4H), 0.90 (t, *J* = 7.3 Hz, 3H), 0.85 (t, *J* = 7.4 Hz, 3H). **<sup>13</sup>C NMR** (80MHz, CDCl<sub>3</sub>):  
268 207.3, 174.4, 168.7, 166.6, 165.3, 165.0, 154.5, 150.0, 138.9, 116.2, 113.5, 109.7,  
269 108.7, 104.2, 100.1, 55.6, 51.3, 44.6, 36.5, 31.9, 31.4, 22.5, 17.1, 14.0, 13.7. **HRMS**  
270 [M- H]<sup>-</sup> C<sub>25</sub>H<sub>29</sub>O<sub>8</sub> calc. 457.18679 found. 457.1868; [B]<sup>-</sup> C<sub>12</sub>H<sub>15</sub>O<sub>4</sub> calc. 223.09758

271 found. 223.0977; [B- H<sub>2</sub>O]<sup>-</sup> C<sub>12</sub>H<sub>13</sub>O<sub>3</sub> calc. 205.08702 found. 205.0872; [A- CO<sub>2</sub>]<sup>-</sup>  
272 C<sub>11</sub>H<sub>15</sub>O<sub>2</sub> calc. 179.10775 found. 179.1080. **Rf C:** 0.42.

273 Glomellic acid 2 [52589-14-7] C<sub>25</sub>H<sub>28</sub>O<sub>9</sub> MW: 472.47 g/mol

274 2-Hydroxy-4-[[2-hydroxy-4-methoxy-6-(2-oxopentyl)benzoyl]oxy]-6-(2-oxopentyl)benzoic acid

275 <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): 11.18 (s, 1H), 6.65 (d, *J* = 2 Hz, 1H), 6.50 (d, 1H), 6.47  
276 (d, *J* = 2.5 Hz, 1H), 6.30 (d, *J* = 2.5 Hz, 1H), 4.04 (s, 2H), 3.84 (s, 3H), 3.22 \*(s, 2H),  
277 2.39 (t, *J* = 7.5 Hz, 2H), 1.98\* (m, 2H), 1.57 (m, *J* = 7.5 Hz 2H), 1.00 (t, *J* = 7.3 Hz,  
278 3H), 0.84 (t, *J* = 7.3 Hz, 3H). <sup>1</sup>H NMR (300MHz, CD<sub>3</sub>COCD<sub>3</sub>): 11.04 (s, 1H), 6.70 (d,  
279 *J* = 2 Hz, 1H), 6.66 (d, 1H), 6.49 (d, *J* = 2.5 Hz, 1H), 6.47 (d, *J* = 2.5 Hz, 1H), 4.19 (s,  
280 2H), 3.88 (s, 3H), 3.45 \*(s, 2H), 2.47 (t, *J* = 7.5 Hz, 2H), 1.58\* (m, 2H), 1.55 (m, *J* =  
281 7.5 Hz 2H), 0.97 (t, *J* = 7.3 Hz, 3H), 0.81 (t, *J* = 7.3 Hz, 3H). <sup>13</sup>C NMR (80MHz,  
282 CD<sub>3</sub>COCD<sub>3</sub>): 207.1, 169.7, 166.7, 165.9, 164.0, 156.5, 141.6, 141.1, 114.0, 113.7,  
283 109.7, 106.5, 109.7, 100.6, 56.1, 51.2, 44.6, 43.5, 37.2, 17.6, 17.5, 14.3, 14.0. **HRMS**  
284 [M- H]<sup>-</sup> C<sub>25</sub>H<sub>27</sub>O<sub>9</sub> calc. 471.16606 found. 471.1657; [A]<sup>-</sup> C<sub>12</sub>H<sub>13</sub>O<sub>5</sub> calc. 237.07685  
285 found. 237.07690. **Rf C:** 0.33.

286 Confluentic acid 3 [6009-12-7] C<sub>28</sub>H<sub>36</sub>O<sub>8</sub> MW: 500.23 g/mol

287 2-Methoxy-4-[[2-hydroxy-4-methoxy-6-(2-oxoheptyl)benzoyl]oxy]-6-pentylbenzoic acid

288 <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): 11.31 (s, 1H), 6.61 (d, *J* = 2 Hz, 1H), 6.55 (d, *J* = 2 Hz,  
289 1H), 6.47 (d, *J* = 2 Hz, 1H), 6.30 (d, *J* = 2 Hz, 1H), 4.07 (s, 2H), 3.88 (s, 3H), 3.85 (s,  
290 3H), 2.74 (t, *J* = 8.1 Hz, 2H), 2.41 (t, *J* = 7.6 Hz, 2H), 1.62 (m, 2H), 1.53 (m, *J* = 7.5  
291 Hz, 2H), 1.34 (m, 4H), 1.21 (m, 4H), 0.89 (t, *J* = 7 Hz, 3H), 0.82 (t, *J* = 7 Hz, 3H). <sup>13</sup>C  
292 NMR (80MHz, CDCl<sub>3</sub>): 207.3, 169.2, 168.6, 166.6, 164.9, 157.9, 151.5, 145.3, 138.9,  
293 115.4, 113.4, 104.3, 103.1, 100.1, 56.4, 55.5, 51.3, 42.5, 33.9, 31.7, 31.3, 30.8, 23.3,  
294 22.4, 14.0, 13.8. **HRMS** [M- H]<sup>-</sup> C<sub>28</sub>H<sub>35</sub>O<sub>8</sub> calc. 499.23374 found. 499.2336 (0 ppm)  
295 [A]<sup>-</sup> C<sub>13</sub>H<sub>17</sub>O<sub>4</sub> calc. 237.11323 found. 237.1131 (1 ppm). **Rf C:** 0.40.

296 2'-O-Methylmicrophyllinic acid 4 [79579-62-7] C<sub>30</sub>H<sub>38</sub>O<sub>9</sub> MW: 542.62 g/mol

297 2-Methoxy-4-[[2-hydroxy-4-methoxy-6-(2-oxoheptyl)benzoyl]oxy]-6-(2-oxoheptyl)benzoic acid

298 <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): 11.17 (s, 1H), 6.74 (d, *J* = 2.1 Hz, 1H), 6.63 (d, *J* = 2.1  
299 Hz, 1H), 6.47 (d, *J* = 2.5 Hz, 1H), 6.30 (d, *J* = 2.5 Hz, 1H), 4.07 (s, 2H), 4.03 (s, 2H),  
300 4.00 (s, 3H), 3.85 (s, 3H), 2.59 (t, *J* = 7.5 Hz, 2H), 2.42 (t, *J* = 7.5 Hz, 2H), 1.61 quint,  
301 *J* = 7Hz 2H), 1.54 (quint, *J* = 7Hz 2H), 1.30 (m, 4H), 1.22 (m, 4H), 0.89 (t, *J* = 6.7 Hz,

302 3H), 0.83 (t,  $J = 7$  Hz, 3H), .  $^{13}\text{C NMR}$  (80MHz,  $\text{CDCl}_3$ ): 207.4, 168.9, 166.6, 165.0,  
303 140.8, 138.8, 113.5, 105.1, 104.1, 100.1, 57.0, 55.6, 51.2, 42.5, 31.4, 31.3, 29.7,  
304 23.4, 23.2, 22.5, 22.5, 13.9, 13.8. **HRMS**  $[\text{M}^- \text{H}]^-$   $\text{C}_{30}\text{H}_{38}\text{O}_9$  calc. 541.24431 found.  
305 541.2442  $[\text{A}]^-$   $\text{C}_{15}\text{H}_{19}\text{O}_5$  calc. 279.1238 found. 279.1235. Ortho effect: loss of  $\text{CH}_3\text{OH}$ .  
306 **Rf C**: 0.33.

307 Methyl, 2'-O-methylmicrophyllinate 4', Gustastatin [720698-33-9]  $\text{C}_{31}\text{H}_{40}\text{O}_9$  MW:  
308 556.64

309 Methyl, 2-Methoxy-4-[[2-hydroxy-4-methoxy-6-(2-oxoheptyl)benzoyl]oxy]-6-(2-  
310 oxoheptyl)benzoate

311  $^1\text{H NMR}$  (500MHz,  $\text{CDCl}_3$ ): 11.29 (s, 1H), 6.58 (d,  $J = 1.9$  Hz, 1H), 6.56 (d,  $J = 1.9$   
312 Hz, 1H), 6.46 (d,  $J = 2.3$  Hz, 1H), 6.29 (d,  $J = 2.9$  Hz, 1H), 4.06 (s, 2H), 3.87 (s, 3H),  
313 (s, 3.84, 3H), 3.82 (s, 3H) 3.70 (s, 2H), 2.43 (t,  $J = 7.2$  Hz, 4H), 1.56 (m, 2H), 1.53 (m,  
314 2H), 1.28 (m, H), 1.24 (m, 2H), 1.22 (m, 2H), 1.19 (m, 2H), 0.87 (t,  $J = 7$  Hz, 3H),  
315 0.83 (t,  $J = 7$  Hz, 3H).  $^{13}\text{C NMR}$  (100MHz,  $\text{CDCl}_3$ ): 207.6, 206.7, 169.3, 167.6, 166.7,  
316 165.0, 158.5, 151.4, 139.0, 135.5, 121.8, 116.3, 113.5, 104.6, 104.4, 100.2, 56.4,  
317 55.7, 52.5, 51.4, 47.7, 42.6, 42.3, 31.5, 31.4, 23.5, 23.4, 22.6, 14.1, 14.0. Attributions  
318 using 2D NMR experiments. **HRMS**  $[\text{M}^+ \text{Na}]^+$   $\text{C}_{31}\text{H}_{40}\text{O}_9\text{Na}$  calc. 579.25645 found.  
319 579.2563 (0 ppm)  $[\text{AH}^+ \text{Na}]^+$   $\text{C}_{16}\text{H}_{22}\text{O}_5\text{Na}$  calc. 317.13594 found. 317. **Rf C**: 0.50.

320 2'-O-methylsuperphyllinic acid 5 [108529-20-0]  $\text{C}_{34}\text{H}_{46}\text{O}_9$  MW: 598.72 g/mole

321 2-Methoxy-4-[[2-hydroxy-4-methoxy-6-(2-oxononyl)benzoyl]oxy]-6-(2-oxononyl)benzoic acid

322  $^1\text{H NMR}$  (300MHz,  $\text{CDCl}_3$ ): 11.17 (s, 1H), 6.73 (d,  $J = 1.7$ , 1H), 6.63 (d,  $J = 1.7$  Hz,  
323 1H), 6.46 (d,  $J = 2.3$  Hz, 1H), 6.29 (d,  $J = 2.3$  Hz, 1H), 4.06 (s, 2H), 4.02 (s, 2H), 3.99  
324 (s, 3H), 3.84 (s, 3H), 2.59 (t,  $J = 7.5$  Hz, 2H), 2.41 (m, 2H), 1.60 (m, 2H), 1.51 (m,  
325 2H), 1.28-1.20 (m, 16H), 0.87 (t,  $J = 7$  Hz, 3H), 0.85 (t,  $J = 7$  Hz, 3H), .  $^{13}\text{C NMR}$   
326 (100MHz,  $\text{CDCl}_3$ ): 207.7, 207.3, 169.0, 166.7, 165.7, 165.2, 159.4, 152.5, 141.2,  
327 139.0, 119.4, 119.4, 113.6, 105.2 104.2, 100.2, 57.2, 55.7, 51.4, 49.2, 43.0, 42.7,  
328 31.8, 29.3, 29.3, 29.3, 29.2, 23.9, 23.7, 22.8 22.7, 14.2, 14.2 **HRMS**  $[\text{M}^- \text{H}]^-$   $\text{C}_{34}\text{H}_{45}\text{O}_9$   
329 calc. 597.30691 found. 597.3069.  $[\text{A}]^-$   $\text{C}_{17}\text{H}_{23}\text{O}_5$  calc. 307.15455 found. 307.1546. **Rf**  
330 **C**: 0.44.

331 Olivetoric acid 6 [491-47-4]  $\text{C}_{26}\text{H}_{32}\text{O}_8$  MW: 472.52 g/mol

332 2-Hydroxy-4-[[2-hydroxy-4-hydroxy-6-(2-oxoheptyl)benzoyl]oxy]-6-pentylbenzoic acid

333 **<sup>1</sup>H NMR** (300MHz, CD<sub>3</sub>COCD<sub>3</sub>): 6.61 (s\*, 1H), 6.54 (s\*, 1H), 6.38 (s, 1H), 4.16 (s,  
334 2H), 3.06 (t\*, *J* = 8.6 Hz, 3H), 2.48 (t, *J* = 7.8 Hz, 2H), 1.64 (m, 2H), 1.50 (quint, *J* =  
335 7.3 Hz, 2H), 1.33 (m, 6H), 1.25 (m, 6H), 0.86 (t, *J* = 7.3 Hz, 3H), 0.81 (t, *J* = 7.3 Hz,  
336 3H). **<sup>13</sup>C NMR** (80MHz, CD<sub>3</sub>COCD<sub>3</sub>): 207.2, 170.0, 166.6, 164.0, 149.5, 141.7, 116.1,  
337 114.3, 109.1, 105.1, 102.7, 51.4, 42.7, 32.8, 32.5, 31.2, 24.0, 23.3, 23.2, 14.5, 14.3.  
338 **HRMS** [M- H]<sup>-</sup> C<sub>26</sub>H<sub>31</sub>O<sub>8</sub> calc. 471.20244 found. 471.2026; [A]<sup>-</sup> C<sub>12</sub>H<sub>15</sub>O<sub>4</sub> calc.  
339 223.09758 found. 223.0977. **Rf C**: 0.22.

340 Arthoniaic acid 7 [25556-24-5] C<sub>29</sub>H<sub>36</sub>O<sub>9</sub> MW: 528.59 g/mole

341 2-Methoxy-4-[[2-hydroxy-4-hydroxy-6-(2-oxoheptyl)benzoyl]oxy]-6-(2-oxoheptyl)benzoic acid  
342 **<sup>1</sup>H NMR** (300MHz, CDCl<sub>3</sub>): 11.16 (s, 1H), 6.89 (d, *J* = 2 Hz, 1H), 6.68 (d, *J* = 2 Hz,  
343 1H), 6.38 (s, 1H), 4.15 (s, 2H), 3.88 (s, 3H), 3.86 (s, 2H), 2.50 (q, *J* = 7.7 Hz, 4H),  
344 1.52 (m, 4H), 1.4-1.1 (m, 8H), 1.25 (t, *J* = 7 Hz, 3H), 1.22 (t, *J* = 7 Hz, 3H). **<sup>13</sup>C NMR**  
345 (80MHz, CDCl<sub>3</sub>): 207.4, 170.2, 168.1, 166.7, 164.3, 158.8, 152.1, 141.5, 136.6,  
346 123.3, 117.4, 114.4, 105.5, 104.9, 102.7, 56.7, 51.4, 47.7, 42.7, 42.5, 32.1, 32.0,  
347 24.0, 23.1, 14.2, 14.2. **HRMS**: [M- H]<sup>-</sup> C<sub>29</sub>H<sub>35</sub>O<sub>9</sub> calc. 527.22866 found. 527.2286; [A]<sup>-</sup>  
348 C<sub>14</sub>H<sub>17</sub>O<sub>5</sub> calc. 265.10815 found. 265.1079. **Rf C**: 0.14, **G**: 0.37.

349 Glomelline 8 [52589-15-8] C<sub>13</sub>H<sub>14</sub>O<sub>4</sub> MW: 234.1 g/mol

350 8-Hydroxy-6-methoxy-3-propyl-1*H*-2-benzopyran-1-one

351 **<sup>1</sup>H NMR** (300MHz, CDCl<sub>3</sub>): 11.14 (s, 1H), 6.47 (d, *J* = 2.3 Hz, 1H), 6.33 (d, *J* = 2.3  
352 Hz, 1H), 6.19 (s, 1H), 3.88 (s, 3H), 2.49 (t, *J* = 7.3 Hz, 2H), 1.74 (sext, *J* = 2.3 Hz,  
353 2H), 1.01 (t, *J* = 7.3 Hz, 3H). **<sup>13</sup>C NMR** (80MHz, CDCl<sub>3</sub>): 166.8, 166.5, 163.6, 157.8,  
354 139.4, 104.1, 101.0, 100.2, 100.00, 55.6, 35.2, 20.2, 13.5. **HRMS** [M+ H]<sup>+</sup> C<sub>13</sub>H<sub>15</sub>O<sub>4</sub>  
355 calc. 235.09648 found. 235.0964. **Rf C**: 0.57.

356 4-O-methylolivetonide 9 [3734-55-2] C<sub>15</sub>H<sub>18</sub>O<sub>4</sub> MW: 262.23 g/mol

357 8-Hydroxy-6-methoxy-3-pentyl-1*H*-2-benzopyran-1-one

358 **<sup>1</sup>H NMR** (300MHz, CDCl<sub>3</sub>): 11.15 (s, 1H), 6.48 (d, *J* = 2.3 Hz, 1H), 6.32 (d, *J* = 2.3  
359 Hz, 1H), 6.19 (s, 1H), 3.88 (s, 3H), 2.50 (t, *J* = 7.5 Hz, 2H), 1.71 (m, *J* = 7.5 Hz, 2H),  
360 1.39-1.27 (m, 4H), 0.93 (t, *J* = 7 Hz, 3H). **<sup>13</sup>C NMR** (80MHz, CDCl<sub>3</sub>): 166.8, 165.5,  
361 163.6, 158.1, 139.5, 103.9, 101.0, 100.2, 55.7, 33.3, 31.1, 26.5, 22.4, 14.0. **HRMS**  
362 [M+H]<sup>+</sup> C<sub>15</sub>H<sub>19</sub>O<sub>4</sub> calc. 263.12778 found. 263.1278. **Rf C**: 0.61.

363 Insignin 10 [108598-79-4] C<sub>17</sub>H<sub>22</sub>O<sub>4</sub> MW: 290.35 g/mol

364 8-Hydroxy-6-methoxy-3-heptyl-1*H*-2-benzopyran-1-one  
365 <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): 11.13 (s, 1H), 6.46 (d, *J* = 2.3 Hz, 1H), 6.31 (d, *J* = 2.3  
366 Hz, 1H), 6.17 (s, 1H), 3.86 (s, 3H), 2.49 (t, *J* = 7.5 Hz, 2H), 1.59 and 1.29 (m, 10H),  
367 0.89 (t, 3H). <sup>13</sup>C NMR (80MHz, CDCl<sub>3</sub>): 163.7, 103.9, 101.0, 100.1, 55.7, 33.3, 31.7,  
368 29.0, 26.8, 22.6, 19.2, 14.0. HRMS [M+H]<sup>+</sup> C<sub>17</sub>H<sub>24</sub>O<sub>4</sub> calc. 291.15908 found.  
369 291.1591 [M + H – H<sub>2</sub>O]<sup>+</sup> C<sub>17</sub>H<sub>21</sub>O<sub>4</sub> calc. 273.14852 found. 273.1486. Rf C: 0.60.

370 Olivetone **11** [3734-54-1] C<sub>14</sub>H<sub>16</sub>O<sub>4</sub> MW: 248.27 g/mol  
371 6,8-Dihydroxy-3-pentyl-1*H*-2-benzopyran-1-one  
372 <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): 11.15 (s, 1H), 6.38 (d, *J* = 2 Hz, 1H), 6.26 (d, *J* = 2 Hz,  
373 1H), 6.15 (s, 1H), 2.49 (t, *J* = 7 Hz, 2H), 1.66 (m, 2H), 1.36 (m, 4H), 0.91 (t, *J* = 7 Hz,  
374 3H). <sup>13</sup>C NMR (80MHz, CDCl<sub>3</sub>): 166.7, 164.1, 163.3, 158.6, 140.2, 103.9, 102.2,  
375 101.9, 33.4, 31.3, 26.9, 22.5, 14.1. HRMS [M+H]<sup>+</sup> C<sub>14</sub>H<sub>17</sub>O<sub>4</sub> calc. 249.11213 found.  
376 249.1122. Rf C: 0.36.

377 2,4-dihydroxy-6-pentylbenzoic acid **12** [491-72-5] C<sub>12</sub>H<sub>16</sub>O<sub>4</sub> MW: 224.1 g/mol  
378 <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): 11.52 (s, 1H), 6.30 (d, *J* = 2.6 Hz, 1H), 6.28 (d, *J* = 2.6  
379 Hz, 1H), 5.16 (s, 1H), 2.92 (m, 2H), 1.59 (m, 2H), 1.35 (m, 4H), 0.90 (t, *J* = 7.1Hz,  
380 3H). <sup>13</sup>C NMR (80MHz, CD<sub>3</sub>COCD<sub>3</sub>): 173.1, 166.3, 162.5, 149.0, 110.8, 103.4, 100.8,  
381 36.3, 31.9, 31.6, 22.3, 13.4. HRMS [M- H]<sup>-</sup> C<sub>12</sub>H<sub>15</sub>O<sub>4</sub> calc. 223.0976 found. 223.0974;  
382 [M-CO<sub>2</sub>- H]<sup>-</sup> C<sub>11</sub>H<sub>15</sub>O<sub>2</sub> calc. 179.1078 found. 179.1077. Rf C: 0.24.

383 2,4-dihydroxy-6-(2-oxopentyl)benzoic acid **13** [196926-65-5] C<sub>12</sub>H<sub>14</sub>O<sub>5</sub> MW: 238.24  
384 g/mol  
385 <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): 6.32 (d, *J* = 1.3 Hz, 1H), 6.24 (d, *J* = 1.3 Hz, 1H), 3.14\* (s,  
386 2H), 1.96 (m, *J* = 7.5 Hz, 2H), 1.5 (m, *J* = 7.5 Hz, 2H), 1.00 (t, *J* = 7.5 Hz, 3H).  
387 Presence of residual solvents.

388

389 4-hydroxy-2-methoxy-6-pentylbenzoic acid **14** [53530-22-6] C<sub>13</sub>H<sub>18</sub>O<sub>4</sub> MW: 238.28  
390 g/mol  
391 <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): 6.27 (s, 2H), 4.00 (s, 2H), 3.92 (s, 3H), 2.72\* (t, 2H), 1.65  
392 (m, 2H), 1.33 (m, 4H), 0.90 (t, *J* = 6.8 Hz, 3H). <sup>13</sup>C NMR (80MHz, CDCl<sub>3</sub>): 211.1,

393 165.6, 160.8, 160.5, 142.3, 115.5, 108.3, 98.7, 56.8, 50.1, 43.0, 31.4, 23.2, 22.5,  
394 14.0. **HRMS** [M- H]<sup>-</sup> C<sub>15</sub>H<sub>18</sub>O<sub>4</sub> calc. 237.11323 found. 237.1134. **Rf C**: 0.24.

395 4-hydroxy-2-methoxy- 6-(2-oxoheptyl) benzoic acid 15 [193617-80-0] C<sub>15</sub>H<sub>20</sub>O<sub>5</sub> MW:  
396 280.20 g/mol

397 <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): 11.52 (s, 1H), 6.30 (d, *J* = 2.6 Hz, 1H), 6.28 (d, *J* = 2.6  
398 Hz, 1H), 5.16 (s, 1H), 2.92 (m, 2H), 1.59 (m, 2H), 1.35 (m, 4H), 0.90 (t, *J* = 7.1Hz,  
399 3H). <sup>13</sup>C NMR (80MHz, CD<sub>3</sub>COCD<sub>3</sub>): 173.1, 166.3, 162.5, 149.0, 110.8, 103.4, 100.8,  
400 36.3, 31.9, 31.6, 22.3, 13.4. **HRMS** [M- H]<sup>-</sup> C<sub>15</sub>H<sub>19</sub>O<sub>5</sub> calc. 279.1238 found. 279.1235;  
401 [M-CO<sub>2</sub>- H]<sup>-</sup> C<sub>14</sub>H<sub>19</sub>O<sub>3</sub> calc. 235.13397 found. 235.1340. **Rf C**: 0.08.

402 Methyl, 4-hydroxy- 2-methoxy-6-(2-oxononyl) benzoate 16 [133362-68-2] C<sub>17</sub>H<sub>24</sub>O<sub>5</sub>  
403 MW: 308.37 g/mole

404 **HRMS** [M-H]<sup>-</sup> C<sub>17</sub>H<sub>23</sub>O<sub>5</sub> calc. 307.1551 found. 307.1550. See Figure 6

405 2-O-methylolivetonide 17 [1195517-47-5] C<sub>15</sub> H<sub>18</sub> O<sub>4</sub> MW: 262.13 g/mol

406 6-hydroxy-8-methoxy-3-pentyl-1*H*-2-benzopyran-1-one

407 <sup>1</sup>H NMR (300MHz, CDCl<sub>3</sub>): 6.43 (d, *J* = 2 Hz, 1H), 6.34 (d, *J* = 2 Hz, 1H), 6.06 (s,  
408 1H), 3.90 (s, 3H), 2.44 (t, *J* = 7.5 Hz, 2H), 1.66 (m, *J* = 7.5 Hz, 2H), 1.33 (m, 4H),  
409 0.89 (t, *J* = 7 Hz, 3H). <sup>13</sup>C NMR (80MHz, CDCl<sub>3</sub>): 163.9, 163.3, 160.9, 159.1, 142.6,  
410 103.2, 102.9, 102.2, 98.7 56.2, 33.4, 31.3, 26.6, 22.5, 14.1. **HRMS** [M-H]<sup>-</sup> C<sub>15</sub>H<sub>17</sub>O<sub>4</sub>  
411 calc. 261.11323 found. 261.1131.

412 α-Alectoronic acid 18 [54226-87-8] C<sub>28</sub>H<sub>32</sub>O<sub>9</sub> MW: 512 .54 g/mol **Rf C**: 0.09. (Millot  
413 et al, 2007).

414 α-Collatolic acid 19 [522-52-1] C<sub>29</sub>H<sub>34</sub>O<sub>9</sub> MW: 526 .56 g/mol **Rf C**: 0.21. (Millot et al,  
415 2008).

416 Perlatolic acid 20 [529-47-5] C<sub>25</sub>H<sub>32</sub>O<sub>7</sub> MW: 444.51 g/mol **Rf C**: 0.48. (Elix, 1974)

417 **UNKNOWN COMPOUNDS OF ORANGE** (See Figure 6 and Table 2)

418 **Spot 2** Methyl, Microphyllinate CAS: [858027-82-4] C<sub>30</sub>H<sub>39</sub>O<sub>9</sub> MW: 542.60 g/mol,  
419 Methyl, 4-[[2-hydroxy-4-methoxy-6-(2-oxoheptyl)benzoyl]oxy]-6-(2-oxoheptyl)benzoate

420 **HRMS**: [M-H]<sup>-</sup> C<sub>30</sub>H<sub>38</sub>O<sub>9</sub> found. 541.2444 C<sub>15</sub>H<sub>20</sub>O<sub>5</sub> 279.1238



421 **<sup>1</sup>H NMR** (300MHz, CDCl<sub>3</sub>) :11.35 (s, 1H), 11.22, (s, 1H), 6.71 (d, *J* = 2.4 Hz, 1H),  
422 6.56 (d, *J* = 2.4 Hz, 1H), 6.46 (d, *J* = 2.4 Hz, 1H), 6.29 (d, *J* = 2.4 Hz, 1H). Mixture  
423 with **4'** (See SI).

424 **Spot 7** Methyl, 4-hydroxy-2-methoxy-6-(2-oxoheptyl)benzoate

425 [846606-93-7] C<sub>16</sub>H<sub>22</sub>O<sub>5</sub> MW: 294.34 g/mole (García-Fortanet et al., 2005)

426 **HRMS** [M-H]<sup>-</sup> C<sub>16</sub>H<sub>21</sub>O<sub>5</sub> calc. 293.13945 found. 293.1394 [M-H-CH<sub>3</sub>]<sup>-</sup> C<sub>15</sub>H<sub>17</sub>O<sub>4</sub> calc.  
427 261.11323 found. 261.1130.

428 **Spot 8** 2'-O-methyl-8'''-oxosuperphyllinic acid C<sub>34</sub>H<sub>44</sub>O<sub>10</sub> MW: 612.30 g/mol

429 2-Methoxy-4-[[2-hydroxy-4-methoxy-6-(2,8-dioxononyl)benzoyl]oxy]-6-(2-oxononyl)benzoic  
430 acid

431 **<sup>1</sup>H NMR** (300MHz, CDCl<sub>3</sub>): 11.16 (s, 1H), 6.73 (d, *J* = 1.6 Hz, 1H), 6.62 (d, *J* = 1.6  
432 Hz, 1H), 6.46 (d, *J* = 2.5 Hz, 1H), 6.30 (d, *J* = 2.5 Hz, 1H), 4.06 (s, 2H), 4.02 (s, 2H),  
433 3.99 (s, 3H), 3.85 (s, 3H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.43 (m, 2H), 2.41 (t, *J* = 7.2 Hz,  
434 2H), 2.14 (s, 3H), 1.63 (m, 2H), 1.58 (m, 2H), 1.52 (m, 2H), 1.31 (m, 2H), 1.26 (m,  
435 2H), 1.2 (m, 2H), 1.2 (m, 2H), 1.2 (m, 2H), 0.85 (t, *J* = 7 Hz, 3H).

436 **<sup>13</sup>C NMR** (100MHz, CDCl<sub>3</sub>): 209.4, 207.6, 206.8, 169.0, 166.7, 165.5, 165.2, 159.4,  
437 152.5, 141.0, 139.0, 119.4, 177.3, 113.6, 105.3, 104.3, 103.0, 57.2, 55.7, 51.4, 49.2,  
438 43.7, 42.7, 42.4, 31.8, 30.1, 29.3, 29.2, 28.7, 23.9, 23.5, 23.2, 22.7, 14.2. Attributions  
439 using 2D experiments. [M-H]<sup>-</sup> C<sub>34</sub>H<sub>43</sub>O<sub>10</sub> calc. 611.28617 found. 611.2862 [A]<sup>-</sup>  
440 C<sub>17</sub>H<sub>21</sub>O<sub>6</sub> calc. 321.1344 found. 321.1347.

441

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## 453 **Declaration of competing interest**

454 The authors declare that they have no known competing financial interests or  
455 personal relationships that could have appeared to influence the work reported in this  
456 paper.

## 457 **Associated content**

458 Supporting Information: NMR spectra, UV fluorescence spectra of 9 and 11,  
459 examples of TLC, HRMS of spot 8.

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561

562 **Figure 1.** Molecular structures of glomelliferic acid **1** and glomellic acid **2** and their  
563 fluorescent behaviour upon addition of **K**. Key 2-oxoalkyl motif highlighted in blue.

564 **Figure 2.**  $\delta$ -keto ester-containing depsides considered in this study.

565 **Figure 3.** Reactivity of depsides with **K**.

566 **Figure 4.** Reactivity of the methyl, 2'-O-methylmicrophyllinate **4'** with **K**.

567 **Figure 5. A** Reactivity of the  $\alpha$ -alectoronic acid **18** and the  $\alpha$ -collatolic acid **19** with **K**.

568 **B** Structure of the perlatolic acid **20**.

569 **Figure 6.** UV and fluorescence spectra of the isochromen-1-ones **9** and **11**.

570 **Figure 7. A.** Depsides rearrangement mechanisms with **K**; **B.** MS fragmentation.

571 **Figure 8.** Relaxed coordinate scans of simplified depsides under **A.** neutral and **B.**  
572 basic conditions; **C.** Energy minimized structure of simplified depsidone under basic  
573 conditions; **D.** Manually adjusted dihedral angle to position the enolate close to the  
574 ester carbonyl group.

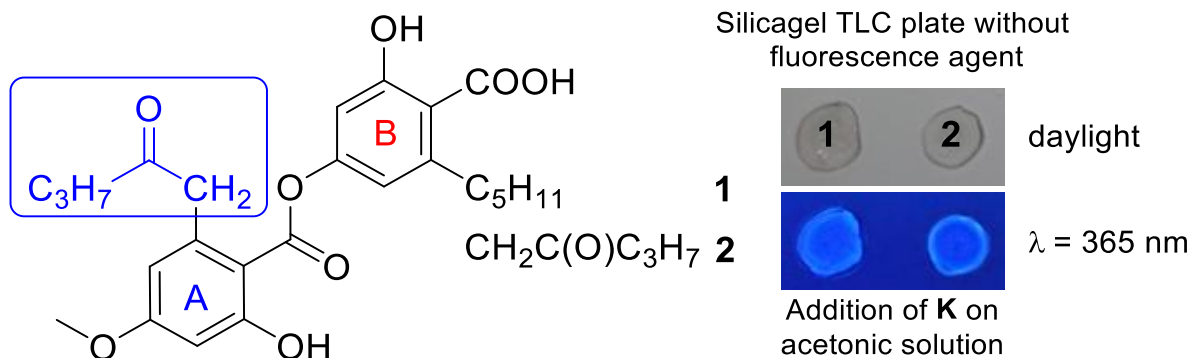
575 **Figure 9.** KUV probe on extracts of *P. cinereoatra* **C**, *P. speirea* var *speirea* **S**, *P.*  
576 *irrigual* **I**, *P. macrocarpa* **Ma** and *P. platycarpoides* **P**. Extracts were spotted and **K**  
577 was added (right) or not (left).

578 **Figure 10. A.** Direct application of the KUV probe on the medulla of *P. cinereoatra*;  
579 **B.** KUV probe on the eluted TLC (solvent G) of *Porpidia cinereoatra* **C**, *P.*  
580 *tuberculosa* **T**, *P. speirea* var *speirea* **S**, *P. melinodes* **M**, *P. contraponenda* **Co**, *P.*  
581 *irrigua* **I** and *P. rugosa* **R**

582 **Figure 11.** Structural data of compound **s8** (NMR in acetone-  $d_6$ )

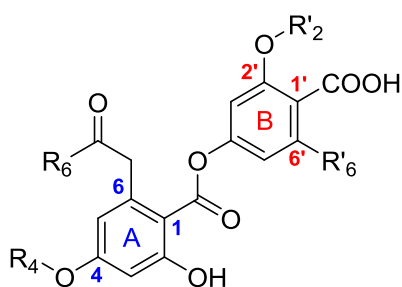
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584 **Figure 1**



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586 **Figure 2**

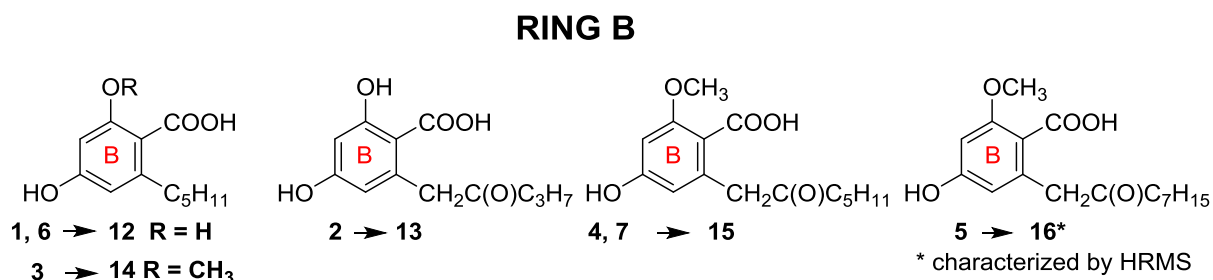
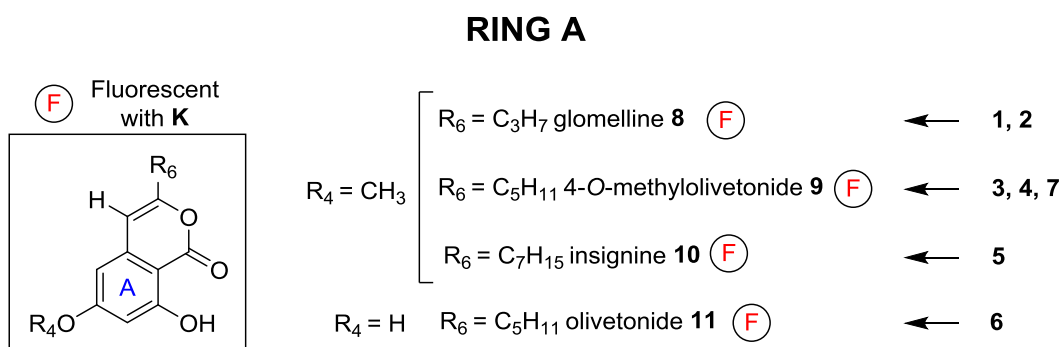


| Depsides                       | ID | R <sub>4</sub>  | R' <sub>2</sub> | R <sub>6</sub>                 | R' <sub>6</sub>                                    | Lichens                                       | Ref |
|--------------------------------|----|-----------------|-----------------|--------------------------------|--|---|-----|
| Glomelliferic acid             | 1  | CH <sub>3</sub> | H               | C <sub>3</sub> H <sub>7</sub>  | C <sub>5</sub> H <sub>11</sub>                     | <i>Xanthoparmelia loxodes</i>                 | 1   |
| Glomellic acid                 | 2  | CH <sub>3</sub> | H               | C <sub>3</sub> H <sub>7</sub>  | CH <sub>2</sub> C(O)C <sub>3</sub> H <sub>7</sub>  | <i>Xanthoparmelia loxodes</i>                 | 1   |
| Confluent acid                 | 3  | CH <sub>3</sub> | CH <sub>3</sub> | C <sub>5</sub> H <sub>11</sub> | C <sub>5</sub> H <sub>11</sub>                     | <i>Porpidia cinereoatra</i>                   | 2   |
| 2'-O-Methylmicrophyllinic acid | 4  | CH <sub>3</sub> | CH <sub>3</sub> | C <sub>5</sub> H <sub>11</sub> | CH <sub>2</sub> C(O)C <sub>5</sub> H <sub>11</sub> | <i>Porpidia cinereoatra</i>                   | 2   |
| Methyl ester of 4              | 4' | CH <sub>3</sub> | CH <sub>3</sub> | C <sub>5</sub> H <sub>11</sub> | CH <sub>2</sub> C(O)C <sub>5</sub> H <sub>11</sub> | <i>Porpidia irrigua</i>                       | 2   |
| 2'-O-Methylsuperphyllinic acid | 5  | CH <sub>3</sub> | CH <sub>3</sub> | C <sub>7</sub> H <sub>15</sub> | CH <sub>2</sub> C(O)C <sub>7</sub> H <sub>15</sub> | <i>Porpidia rugosa</i>                        | 2   |
| Olivetoric acid                | 6  | H               | H               | C <sub>5</sub> H <sub>11</sub> | C <sub>5</sub> H <sub>11</sub>                     | <i>Pseudevernia furfuracea (var. ceratea)</i> | 3   |
| Arthoniaic acid                | 7  | H               | CH <sub>3</sub> | C <sub>5</sub> H <sub>11</sub> | CH <sub>2</sub> C(O)C <sub>5</sub> H <sub>11</sub> | <i>Pachnolepia pruinata</i>                   | 4   |

1 (Elix and Whitton, 1989); 2 (Orange, 2014; Elix *et al.*, 1996, Elix and Wardlaw, 1997); 3 (Culberson *et al.*, 1977; Pérez *et al.*, 2016); 4: (Huneck *et al.*, 1970)

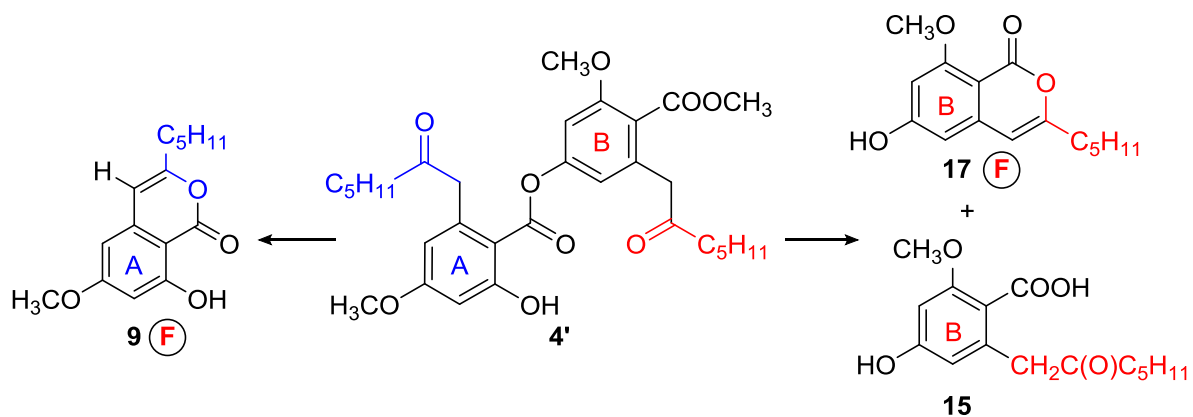
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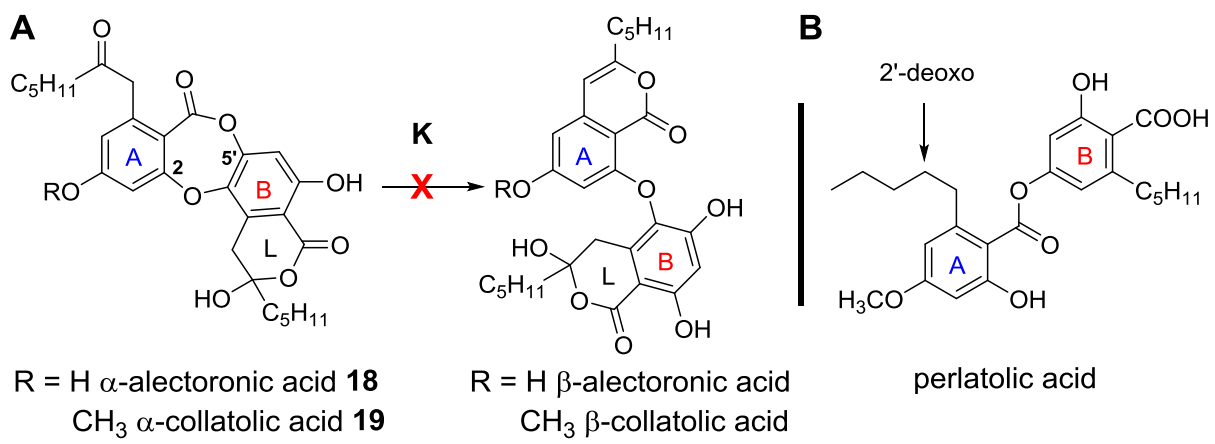
593 **Figure 4.**



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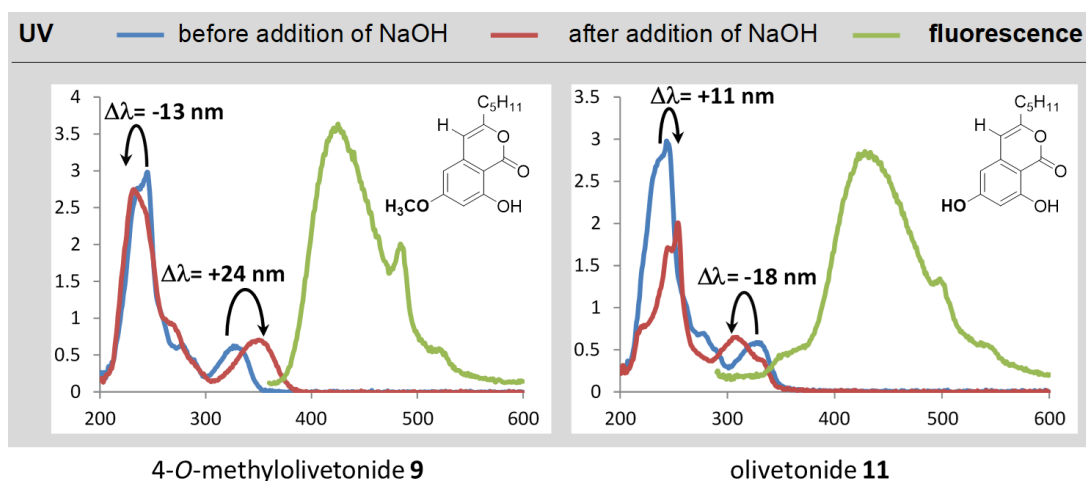
596 **Figure 5.**





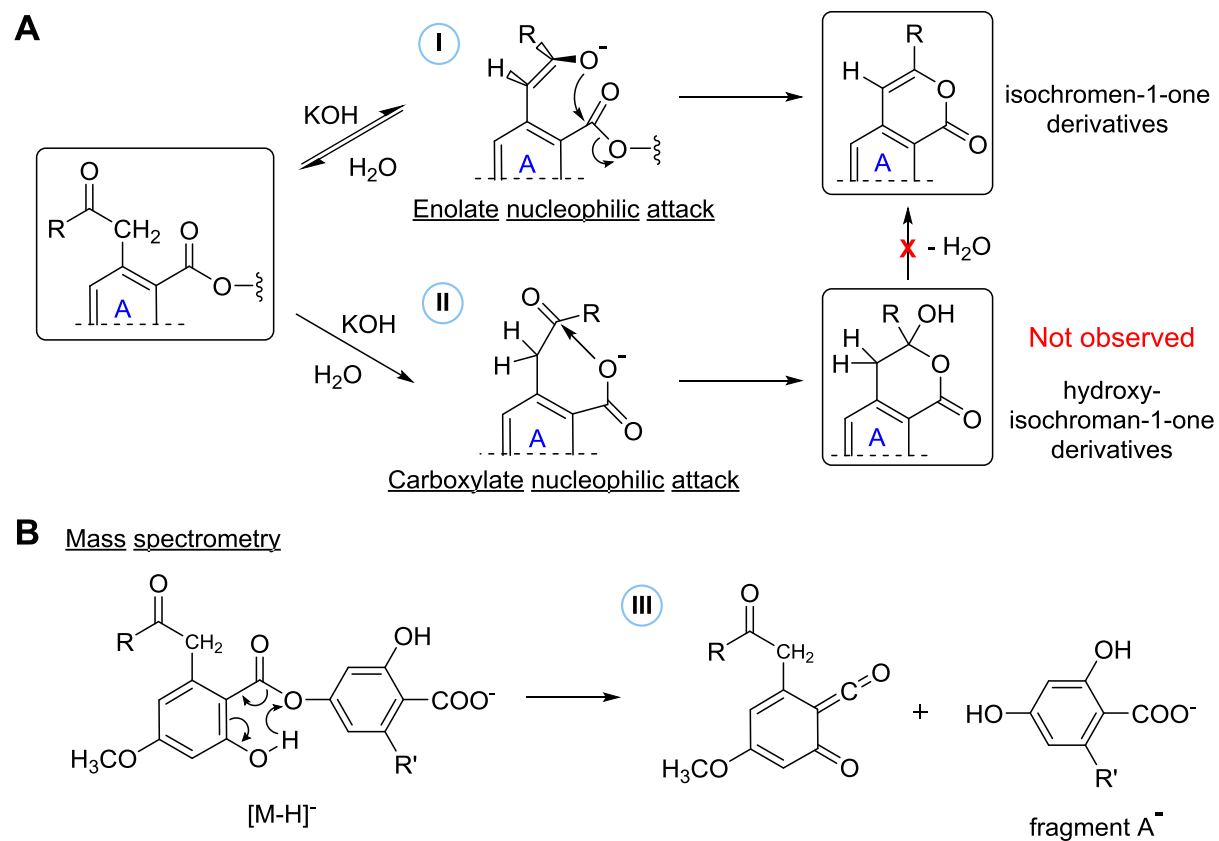
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599 **Figure 6.**



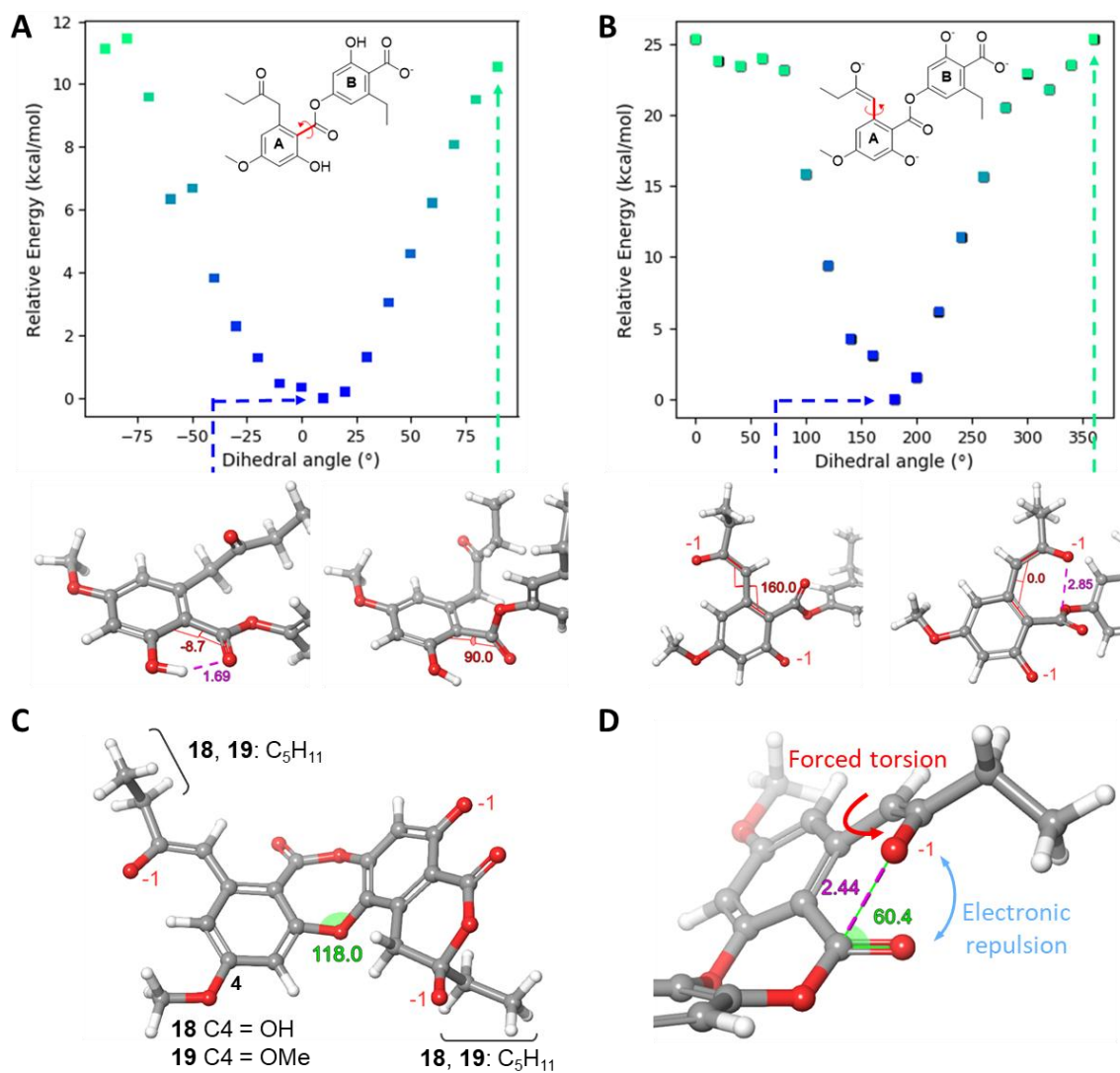
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601 **Figure 7.**



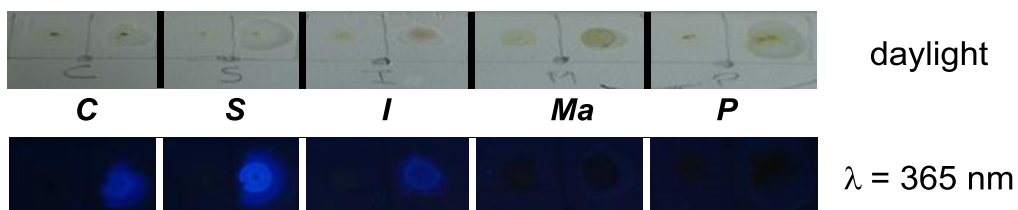
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605 **Figure 8.**



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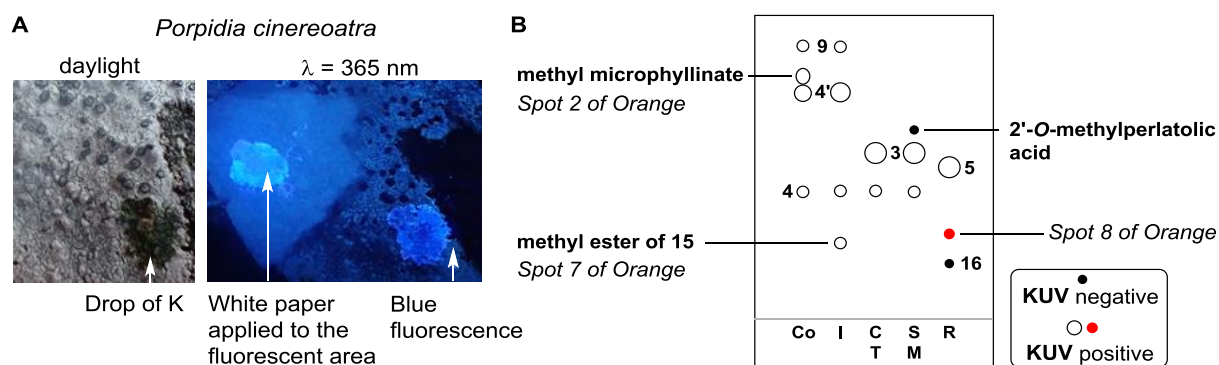
607 **Figure 9.**



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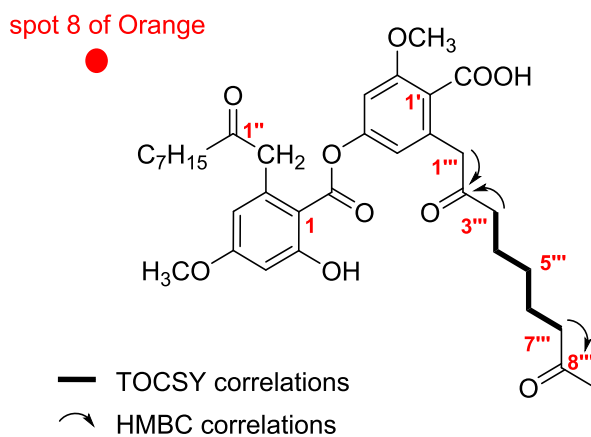
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610 **Figure 10.**



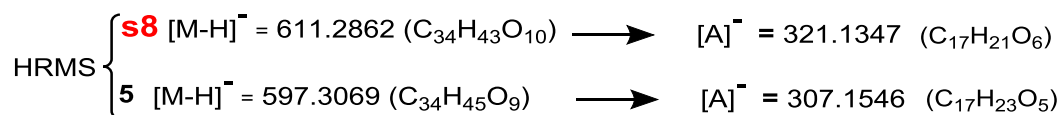
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613 **Figure 11.**



| no. | $\delta \text{ C}$ | $\delta \text{ H}$ | no.    | $\delta \text{ C}$ | $\delta \text{ H}$ |
|-----|--------------------|--------------------|--------|--------------------|--------------------|
| 1   | 104.3              |                    | 5''*   | 29.2               | 1.20, m            |
| 2   | 166.7              |                    | 6''*   | 29.3               | 1.20, m            |
| 3   | 100.3              | 6.46, d (2.5 Hz)   | 7''    | 31.8               | 1.20, m            |
| 4   | 165.2              |                    | 8''    | 22.7               | 1.26, m            |
| 5   | 113.6              | 6.30, d (2.5 Hz)   | 9''    | 14.2               | 0.85, t (7 Hz)     |
| 6   | 139.0              |                    | 1'''   | 49.2               | 4.02, m            |
| 7   | 165.5              |                    | 2'''   | 206.8              |                    |
| 1'  | 117.3              |                    | 3'''   | 42.4               | 2.60, t (7 Hz)     |
| 2'  | 159.4              |                    | 4'''   | 23.2               | 1.63, m            |
| 3'  | 105.3              | 6.73, d (1.6 Hz)   | 5'''   | 28.7               | 1.31, m            |
| 4'  | 152.5              |                    | 6'''   | 23.5               | 1.58, m            |
| 5'  | 119.4              | 6.62, d (1.6 Hz)   | 7'''   | 43.7               | 2.43, m            |
| 6'  | 141.0              |                    | 8'''   | 209.4              |                    |
| 7'  | 169.0              |                    | 9'''   | 30.1               | 2.14, s            |
| 1'' | 51.4               | 4.06, m            | 4-OMe  | 55.7               | 3.85, s            |
| 2'' | 207.6              |                    | 2'-OMe | 57.2               | 3.99, s            |
| 3'' | 42.7               | 2.41, m            | 2-OH   |                    | 11.16, s           |
| 4'' | 23.9               | 1.52, m            |        |                    |                    |

\*overlapped





615 **Table 1.** List of taxa with sampling and collecting details.

| Species   | Lichen (g) | Extract (mg) | Compound (mg)            | Location  | Date          | Collector / herb. ref.                             |
|---|------------|--------------|--------------------------|---|---------------|--|
| <i>Xanthoparmelia loxodes</i> (Nyl.) O. Blanco et al. (Parmeliaceae)                  | 8          | 220          | 1: 152<br>2: 25<br>20: 8 | France, Port Lépine, Trélévern (22660), acidic coastal rocks near the tower, Alt 15m  | December 2019 | P. Uriac   |
| <i>Porpidia cinereoatra</i> (Ach.) Hertel & Knoph (Lecideaceae)                       | 2          | 170          | 3: 95<br>4: 19           | France, Néant sur Yvel (56430), cliff of acidic rocks, Alt. 53m   | March 2021    | J. Esnault, AFL                                    |
| <i>Porpidia irrigua</i> Orange (Lecideaceae)  | 0.77       | 62           | 4': 36                   | France, La touche Guérin, Paimpont (35380), red schist, Alt : 189m  | January 2020  | J. Esnault, AFL                                    |
| <i>Porpidia rugosa</i> (Taylor) Coppins & Fryday (Lecideaceae)                        | 0.47       | 46           | 5: 26<br>Spot 8: 4       | Ireland, West Galway (V.C. H16), Maam Cross, lake west of bridge, On steep rocks by lake, occasionally inunded, Alt. 40m, N.Grid: 02(L)/9760.4605             | February 2013 | Herb. A. Orange 23767                              |
| <i>Pseudevernia furfuracea</i> (var <i>ceratea</i> ) (Ach.) D. Hawksw. (Parmeliaceae) | 2          | 50           | 6: 23                    | Vallée de Chaudéfour, Chambon sur Lac (63790), coniferous trees, Alt. 1450m July 1961 Herb. J. C. Massé 15017   |               | Herb. J. C. Massé 15009                            |
| <i>Pachnolepia pruinata</i> (Torss.) Frisch & G. Thor (Arthoniaceae)                  | 0.67       | 120          | 7: 10                    | France, Bill point, Séné (56860), Pinus maritimus near the sea  | October 2020  | J. Y. Monnat, AFL                                  |
| <i>Ochrolechia parella</i> (L.) A. Massal. (Ochrolechiaceae)                          | 8          | 100          | 18: 20                   | France, walls of the bridge of the Gacet, Betton (35830), vertical acid rocks, Alt. 35m   | November 2019 | P. Uriac   |
| <i>Tephromela atra</i> (Huds.) Hafellner (Tephromelataceae)                           | 12         | 120          | 19: 50                   | France, Lancieux F22770, coastal acidic rocks on the beach near the Point du rocher, Alt 5m   | July 2020     | P. Uriac   |
| <i>Porpidia contraponenda</i> (Arnold) Knoph & Hertel (Lecideaceae)                   | 0.27       | 16           |                          | Wales, Caernarvonshire (V.C 49), NW of Capel Curig, Afon Llugwy above Glan Llugwy. On rocks in stream, upper riparian zone, Alt. 425m, N. Grid: 268717.361411 | July 2017     | Herb. A. Orange 23767                              |
| <i>Porpidia melinodes</i> (Körb.) Gowan & Ahti (Lecideaceae)                          |            |              |                          | Wales, Caernarvonshire, (V.C 49), Bethesda, Penrhyn Quarry, on old drystone wall in heathland, Alt.415m, N.Grid: 261130.363519                                | February 2019 | Herb. A. Orange 24381                              |
| <i>Porpidia tuberculosa</i> (Sm.) Hertel & Knoph (Lecideaceae)                        |            |              |                          | France, near the Chapelle Ste Trépine, acidic rocks, St Aignan (56480), Alt. 179m   | March 2019    | P. Uriac   |
| <i>Porpidia macrocarpa</i> (DC.) Hertel & A.J. Schwab (Lecideaceae)                   |            |              |                          | France, La touche Guérin, Paimpont (35380), red schist, Alt : 189m  | January 2020  | P. Uriac   |
| <i>Porpidia platycarpoides</i> (Bagl.) Hertel (Lecideaceae)                           |            |              |                          | France, plage de la Fresnaye, St Cast-Le Guildo (22380), cliff near the sea, Alt 5m.  | August 2020   | P. Uriac   |
| <i>Porpidia speirea</i> var <i>speirea</i> (Ach.) Kremp. 1861 (Lecideaceae)           |            |              |                          | France, col d'Iléhou, Estaing (65400), calcareous rocks, Alt: 2200m   | July 2018     | J. L. Farroux, AFL / Herb. J. L. Farroux SP2018-80 |