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1 Graphical Abstract



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

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13 Abstract: The reactivity of eight purified depsides obtained from six european lichens and that display as 2-oxoalkyl chain in ortho-position of the ester bond was 14 15 explored. These depsides were found to lead to 1H-Isochromen-1-ones, which exhibit a distinctive blue fluorescence at 365 nm, in the presence of a 10% aqueous 16 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.

Keywords: Depsides, δ-keto esters, enolate, transesterification, fluorescent
 1*H*-Isochromen-1-ones, chemical lichen determination, KUV probe.

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

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 complexe (Armaleo et al., 2011; Herbst et al., 2018; Walsh and Tang, 2017), has been shown to resolve numerous taxonomic problems among lichens. 39

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 addition of K reagent is thereafter referenced as the KUV probe. An unusual 44 45 structural feature of these two lichen specialized metabolites when compared to other depsides is that they are substituted on the C-6 of the A ring by a 2-oxoalkyl 46 47 chain. This observation prompted us to explore their reactivity to rationalize the fluorescence observed and to broaden the use of the KUV probe onto other similar 48 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 (1H-2-benzopyran-1-ones). To explain their formation under basic conditions, a mechaninistic explanation, 53 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, both out in the field by direct application on the medulla or in the laboratory on TLC, 56 on a selection of European Porpidia species. 57

58 2. Results and discussion

59 **2.1. Selection and purification of** δ **-keto ester-containing depsides**

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

65 **2.2. Reactivity of** δ-keto containing lichen metabolites in basic conditions

The cleavage of the ester bond between **A** and **B** rings of the depsides (Figure 1) has been previously performed for structure elucidation purposes. Along with saponification (5% KOH or NaOH at 100°C) and alcoholysis (MeOH or *t*-BuOH) at high temperature, acidic hydrolysis (96% H_2SO_4 , then H_2O at 0°C) has been widely used (Culberson et al., 1987).

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

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

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

As demonstrated with the glomelliferic acid **1**, the same complete reaction was observed if the neutralization was carefully performed using a saturated NH_4CI solution (pH: 4-5).

In the case of **4**', bearing a second δ -keto-ester moiety on the **B** ring, three compounds were isolated by chromatography: along with **9** and **15** (previously obtained from **4**), the fluorescent 2-*O*-methylolivetonide **17** (Asahina and Fujikawa, 1935) was also obtained. This isomer of **9** is resulting of the rearrangement of the δ 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 been applied to the α -alectoronic acid **18** (Ochrolechia parella) (Millot et al., 2007) 93 94 and to the α -collatolic acid **19** (*Tephromela atra*) (Millot et al., 2008) (Figure 5A). Their δ -keto-ester moiety was not affected and neither the β -alectoronic acid nor the 95 96 β-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 1*H*-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 inversed 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).

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

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

On the contrary, in the case of the depsidones **18** and **19**, the supplementary ether function between the **A** and **B** rings locks the compounds in a wide V-shaped conformation (118° angle, Figure 7C). The fixed geometry of the intra-cyclic ester of the depsidone core then prevents a nucleophilic attack by the enolate as the angle between the two isn't suitable (Figure 7D) and results in an electronical clash, 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. platycarpoides (Bagl.) Hertel or without metabolites (i.e. P. hydrophila (Fr.) Hertel & 149 A.J. Schwab) (Fryday, 2005). 150

Most interestingly, lichenologists out in the field can use the KUV probe
directly on the medulla of the lichen or using absorbant paper (without blanching
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 revelating agent for TLCs (Figure 10B). Deposition of one drop of the K reagent on the various spots of the TLC 158 159 after elution of the acetonic extract of Co, I, C, S, T, M and R revealed a blue fluorescence at 365 nm with the exception of **16** and of the 2'-O-methylperlatolic acid. 160 161 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 ω -1 of the alkyl chain was only observed in aliphatic acids such as dihydropertusaric, muronic and isomuronic acids. The presence of 9, 16 and of the 165 166 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.

178 **4. Experimental**

179 **4.1. Reagents**

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

183 **4.2. Lichen material and identification**

The lichen specimens used in this study were collected from different locations ofFrance 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

Lichen thalli (often with mineral) were ground and extracted three times with warm
acetone. After drying the solvent was evaporated under reduced pressure. (Process
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 x 20 cm aluminium sheets coated by Silica gel 60F254 (Merck). Thereafter the plates were developed 196 197 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 Rf values in solvents C are given for each product. The Rf values of the 201 classical references are: atranorine: 0.61; norstictic acid: 0.28; stictic acid: 0.12.

4.5. Purifications using Liquid-Chromatography

Silicagel 60 Merck and solvent system C (toluol/acetic acid, 85/15) were used for the purification of the different extracts. The combined fractions with the selected metabolite were washed once with water in order to elimine the acids, dried over MgSO₄ and evaporated under reduced pressue. Preparative TLC were also performed using the same solvents. (Process not optimized)

2094.6. High Resolution Mass Spectrometry (HRMS) and Tandem Mass210Spectrometry (MS-MS)

- Mass spectra of standards were recorded at the CRMPO-Scanmat (Rennes, France)
 on an Orbitrap Thermo Fisher Scientific Q-Exactive instrument with an ESI
 (ElectroSpray Ionization) source in negative mode by direct introduction at 5-10
 µg.mL⁻¹. Samples were prepared in methanol at 10 µg.mL⁻¹.
- 215 **4.7.** ¹H and ¹³C NMR

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

2 mg of the isochromen-1-ones 9 and 11 were dissolved in MeOH to obtain 10⁻⁵
mol.L⁻¹ solutions. Native UV spectra were recorded using a Jennway 7415 UV
spectrometer (Cole-Parmer Instrument Company). Second spectra were recorded
after addition of one drop of 1 mol.L⁻¹ NaOH.

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

4.10. Reactions with KOH: n x 5 mg of the metabolite were allowed to react under stirring with n x 100 μ L of aqueous 10% KOH at room temperature overnight. After acidification with aqueous 10M HCl under cooling an extraction was performed using diethylether (2 x 5 mL). The organic layer was dried over MgSO₄ and the solvent was evaporated under vacuum. The conversion was checked by TLC and the purification was achieved either by preparative column chromatography or TLC. Number of attempts > 3. Quantitative reactions for **1-7** and no reaction for **18-19**.

4.11. Conversion study: In order to confirm that the transesterification was complete the reaction was performed with a mixture (40 mg) of glomelliferic acid (89%) + perlatolic acid (11%) and **K** (0.8 mL). To avoid any eventual acidic deshydratation the reaction mixture was treated with a saturated aq NH₄Cl solution (6 mL/pH 5-6) and extracted with ether (2 X 10 mL). The organic layer was dried over MgSO₄ and evaporated under reduced pressure giving 35mg of a colored oil (87%).

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

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

- 261 **4.10. Spectroscopic data**
- 262 <u>Glomelliferic acid</u> **1** [552-49-8] C₂₅H₃₀O₈ MW: 458.5 g/mol
- 263 2-Hydroxy-4-[[2-hydroxy-4-methoxy-6-(2-oxopentyl)benzoyl]oxy]-6-pentylbenzoic acid

¹H NMR (300MHz, CDCl₃): 11.30 (s, 1H), 11.26 (s, 1H), 6.64 (d, J = 2.4 Hz, 1H), 6.52 (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), 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, 4H), 0.90 (t, J = 7.3 Hz, 3H), 0.85 (t, J = 7.4 Hz, 3H). ¹³C NMR (80MHz, CDCl₃): 207.3, 174.4, 168.7, 166.6, 165.3, 165.0, 154.5, 150.0, 138.9, 116.2, 113.5, 109.7, 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 [M- H]⁻ C₂₅H₂₉O₈ calc. 457.18679 found. 457.1868; [B]⁻ C₁₂H₁₅O₄ calc. 223.09758

- 271 found. 223.0977; $[B H_2O]^- C_{12}H_{13}O_3$ calc. 205.08702 found. 205.0872; $[A CO_2]^-$
- 272 $C_{11}H_{15}O_2$ calc. 179.10775 found. 179.1080. **Rf** C: 0.42.
- 273 <u>Glomellic acid</u> **2** [52589-14-7] C₂₅H₂₈O₉ MW: 472.47 g/mol
- 274 2-Hydroxy-4-[[2-hydroxy-4-methoxy-6-(2-oxopentyl)benzoyl]oxy]-6-(2-oxopentyl)benzoic acid
- 275 ¹**H NMR** (300MHz, CDCl₃): 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). ¹H NMR (300MHz, CD₃COCD₃): 11.04 (s, 1H), 6.70 (d, 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). ¹³**C** NMR (80MHz,
- 282 CD₃COCD₃): 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 $[M-H]^{-}C_{25}H_{27}O_{9}$ calc. 471.16606 found. 471.1657; $[A]^{-}C_{12}H_{13}O_{5}$ calc. 237.07685
- 285 found. 237.07690. **Rf** C: 0.33.
- 286 <u>Confluentic acid</u> **3** [6009-12-7] C₂₈H₃₆O₈ MW: 500.23 g/mol
- 287 2-Methoxy-4-[[2-hydroxy-4-methoxy-6-(2-oxoheptyl)benzoyl]oxy]-6-pentylbenzoic acid
- ¹**H NMR** (300MHz, CDCl₃): 11.31 (s, 1H), 6.61 (d, J = 2 Hz, 1H), 6.55 (d, J = 2 Hz, 288 289 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 Hz, 2H), 1.34 (m, 4H), 1.21 (m, 4H), 0.89 (t, J = 7 Hz, 3H), 0.82 (t, J = 7 Hz, 3H). ¹³C 291 292 **NMR** (80MHz, CDCl₃): 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]⁻ $C_{28}H_{35}O_8$ calc. 499.23374 found. 499.2336 (0 ppm) 295 [A]⁻ C₁₃H₁₇O₄ calc. 237.11323 found. 237.1131 (1 ppm). **Rf** C: 0.40.
- 296 <u>2'-O-Methylmicrophyllinic acid</u> **4** [79579-62-7] C₃₀H₃₈O₉ MW: 542.62 g/mol
- 297 2-Methoxy-4-[[2-hydroxy-4-methoxy-6-(2-oxoheptyl)benzoyl]oxy]-6-(2-oxoheptyl)benzoic acid
- 298 ¹**H NMR** (300MHz, CDCl₃): 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**C NMR** (80MHz, CDCl₃): 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** [M- H]⁻ C₃₀H₃₈O₉ calc. 541.24431 found. 305 541.2442 [A]⁻ C₁₅H₁₉O₅ calc. 279.1238 found. 279.1235. Ortho effect: loss of CH₃OH. 306 **Rf** C: 0.33.

307 <u>Methyl, 2'-O-methylmicrophyllinate 4', Gustastatin</u> [720698-33-9] C₃₁H₄₀O₉ 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**H NMR** (500MHz, CDCl₃): 11.29 (s, 1H), 6.58 (d, J = 1.9 Hz, 1H), 6.56 (d, J = 1.9312 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**C** NMR (100MHz, CDCl₃): 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 using 2D NMR experiments. HRMS [M+ Na]⁺ C₃₁H₄₀O₉Na calc. 579.25645 found. 318

- 319 579.2563 (0 ppm) $[AH + Na]^+ C_{16}H_{22}O_5Na$ calc. 317.13594 found. 317. **Rf** C: 0.50.
- 320 <u>2'-O-methylsuperphyllinic acid</u> **5** [108529-20-0] C₃₄H₄₆O₉ 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**H NMR** (300MHz, CDCl₃): 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**C** NMR 326 (100MHz, CDCl₃): 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 [M-H]⁻ C₃₄H₄₅O₉
- 329 calc. 597.30691 found. 597.3069. [A]⁻ C₁₇H₂₃O₅ calc. 307.15455 found. 307.1546. **Rf**
- 330 C: 0.44.
- 331 <u>Olivetoric acid</u> **6** [491-47-4] C₂₆H₃₂O₈ MW: 472.52 g/mol
- 332 2-Hydroxy-4-[[2-hydroxy-4-hydroxy-6-(2-oxoheptyl)benzoyl]oxy]-6-pentylbenzoic acid

- ¹H NMR (300MHz, CD₃COCD₃): 6.61 (s*, 1H), 6.54 (s*, 1H), 6.38 (s, 1H), 4.16 (s, 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 =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, 3H). ¹³C NMR (80MHz, CD₃COCD₃): 207.2, 170.0, 166.6, 164.0, 149.5, 141.7, 116.1, 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. HRMS [M- H]⁻ C₂₆H₃₁O₈ calc. 471.20244 found. 471.2026; [A]⁻ C₁₂H₁₅O₄ calc. 223.09758 found. 223.0977. **Rf** C: 0.22.
- 340 <u>Arthoniaic acid</u> 7 [25556-24-5] C₂₉H₃₆O₉ MW: 528.59 g/mole
- 341 2-Methoxy-4-[[2-hydroxy-4-hydroxy-6-(2-oxoheptyl)benzoyl]oxy]-6-(2-oxoheptyl)benzoic acid
- ¹H NMR (300MHz, CDCl₃): 11.16 (s, 1H), 6.89 (d, J = 2 Hz, 1H), 6.68 (d, J = 2 Hz, 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), 1.52 (m, 4H), 1.4-1.1 (m, 8H), 1.25 (t, J = 7 Hz, 3H), 1.22 (t, J = 7 Hz, 3H). ¹³C NMR (80MHz, CDCl₃): 207.4, 170.2, 168.1, 166.7, 164.3, 158.8, 152.1, 141.5, 136.6, 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, 24.0, 23.1, 14.2, 14.2. HRMS: [M- H]⁻ C₂₉H₃₅O₉ calc. 527.22866 found. 527.2286; [A]⁻ C₁₄H₁₇O₅ calc. 265.10815 found. 265.1079. **Rf** C: 0.14, G: 0.37.
- 349 <u>Glomelline</u> **8** [52589-15-8] C₁₃H₁₄O₄ MW: 234.1 g/mol
- 350 8-Hydroxy-6-methoxy-3-propyl-1*H*-2-benzopyran-1-one

351 ¹**H NMR** (300MHz, CDCl₃): 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). ¹³**C** NMR (80MHz, CDCl₃): 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]⁺ C₁₃H₁₅O₄
- 355 calc. 235.09648 found. 235.0964. Rf C: 0.57.
- 356 <u>4-O-methylolivetonide</u> **9** [3734-55-2] C₁₅H₁₈O₄ MW: 262.23 g/mol
- 357 8-Hydroxy-6-methoxy-3-pentyl-1*H*-2-benzopyran-1-one
- ¹H NMR (300MHz, CDCl₃): 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). ¹³**C** NMR (80MHz, CDCl₃): 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]⁺ C₁₅H₁₉O₄ calc. 263.12778 found. 263.1278. **Rf** C: 0.61.
- 363 Insignin 10 [108598-79-4] C₁₇H₂₂O₄ MW: 290.35 g/mol

364 8-Hydroxy-6-methoxy-3-heptyl-1*H*-2-benzopyran-1-one

¹**H** NMR (300MHz, CDCl₃): 11.13 (s, 1H), 6.46 (d, J = 2.3 Hz, 1H), 6.31 (d, J = 2.3Hz, 1H), 6.17 (s, 1H), 3.86 (s, 3H), 2.49 (t, J = 7.5 Hz, 2H), 1.59 and 1.29 (m, 10H), 0.89 (t, 3H). ¹³**C** NMR (80MHz, CDCl₃): 163.7, 103.9, 101.0, 100.1, 55.7, 33.3, 31.7, 29.0, 26.8, 22.6, 19.2, 14.0. **HRMS** [M+H]⁺ C₁₇H₂₄O₄ calc. 291.15908 found. 291.1591 [M + H – H₂O]⁺ C₁₇H₂₁O₄ calc. 273.14852 found. 273.1486. **Rf** C: 0.60.

- 370 <u>Olivetonide</u> **11** [3734-54-1] C₁₄H₁₆O₄ MW: 248.27 g/mol
- 371 6,8-Dihydroxy-3-pentyl-1*H*-2-benzopyran-1-one
- 372 ¹**H NMR** (300MHz, CDCl₃): 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). ¹³C NMR (80MHz, CDCl₃): 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]⁺ C₁₄H₁₇O₄ calc. 249.11213 found.
 376 249.1122. Rf C: 0.36.
- 377 <u>2,4-dihydroxy-6-pentylbenzoïc acid</u> **12** [491-72-5] C₁₂H₁₆O₄ MW: 224.1 g/mol

¹**H** NMR (300MHz, CDCl₃): 11.52 (s, 1H), 6.30 (d, J = 2.6 Hz, 1H), 6.28 (d, J = 2.6Hz, 1H), 5.16 (s, 1H), 2.92 (m, 2H), 1.59 (m, 2H), 1.35 (m, 4H), 0.90 (t, J = 7.1Hz, 3H). ¹³**C** NMR (80MHz, CD₃COCD₃): 173.1, 166.3, 162.5, 149.0, 110.8, 103.4, 100.8, 36.3, 31.9, 31.6, 22.3, 13.4. **HRMS** [M- H]⁻ C₁₂H₁₅O₄ calc. 223.0976 found. 223.0974; [M-CO₂- H]⁻ C₁₁H₁₅O₂ calc. 179.1078 found. 179.1077. **Rf** C: 0.24.

- 383 <u>2,4-dihydroxy-6-(2-oxopentyl)benzoïc acid</u> **13** [196926-65-5] C₁₂H₁₄O₅ MW: 238.24
 384 g/mol
- 385 ¹**H NMR** (300MHz, CDCl₃): 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 <u>4-hydroxy-2-methoxy-6-pentylbenzoïc acid</u> 14 [53530-22-6] C₁₃H₁₈O₄ MW: 238.28
 390 g/mol
- 391 ¹**H NMR** (300MHz, CDCl₃): 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). ¹³**C NMR** (80MHz, CDCl₃): 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]⁻ C₁₅H₁₈O₄ calc. 237.11323 found. 237.1134. Rf C: 0.24.
- 395 <u>4-hydroxy-2-methoxy- 6-(2-oxoheptyl) benzoic acid</u> 15 [193617-80-0] C₁₅H₂₀O₅ MW:
 396 280.20 g/mol
- ¹H NMR (300MHz, CDCl₃): 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). ¹³**C NMR** (80MHz, CD₃COCD₃): 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** $[M-H]^{-}C_{15}H_{19}O_{5}$ calc. 279.1238 found. 279.1235;
- 401 $[M-CO_2 H]^- C_{14}H_{19}O_3$ calc. 235.13397 found. 235.1340. **Rf** C: 0.08.
- 402 <u>Methyl, 4-hydroxy- 2-methoxy-6-(2-oxononyl) benzoate</u> **16** [133362-68-2] C₁₇H₂₄O₅
- 403 MW: 308.37 g/mole
- 404 **HRMS** [M-H]⁻ C₁₇H₂₃O₅ calc. 307.1551 found. 307.1550. See Figure 6
- 405 <u>2-O-methylolivetonide</u> **17** [1195517-47-5] C₁₅ H₁₈ O₄ MW: 262.13 g/mol
- 406 6-hydroxy-8-methoxy-3-pentyl-1*H*-2-benzopyran-1-one
- 407 ¹**H NMR** (300MHz, CDCl₃): 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). ¹³**C NMR** (80MHz, CDCl₃): 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]⁻ C₁₅H₁₇O₄
- 411 calc. 261.11323 found. 261.1131.
- 412 <u> α -Alectoronic acid</u> **18** [54226-87-8] C₂₈H₃₂O₉] MW: 512 .54 g/mol **Rf** C: 0.09. (Millot 413 et al, 2007).
- 414 <u>α-Collatolic acid</u> 19 [522-52-1] C₂₉H₃₄O₉ MW: 526 .56 g/mol Rf C: 0.21. (Millot et al,
 415 2008).
- 416 <u>Perlatolic acid</u> **20** [529-47-5] C₂₅H₃₂O₇ 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** <u>Methyl, Microphyllinate</u> CAS: [858027-82-4] C₃₀H₃₉O₉ 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^{]-} C₃₀H₃₈O₉ found. 541.2444 C₁₅H₂₀O₅ 279.1238

- 421 ¹**H NMR** (300MHz, CDCl₃) :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** <u>Methyl, 4-hydroxy-2-methoxy-6-(2-oxoheptyl)benzoate</u>
- 425 [846606-93-7] C₁₆H₂₂O₅ MW: 294.34 g/mole (García-Fortanet et al., 2005)
- 426 **HRMS** [M-H]⁻ C₁₆H₂₁O₅ calc. 293.13945 found. 293.1394 [M-H-CH₃]⁻ C₁₁₅H₁₇O₄ calc.
- 427 261.11323 found. 261.1130.
- 428 **Spot 8**<u>2'-O-methyl-8'''-oxosuperphyllinic acid</u> C₃₄H₄₄O₁₀ MW: 612.30 g/mol
- 429 2-Methoxy-4-[[2-hydroxy-4-methoxy-6-(2,8-dioxononyl)benzoyl]oxy]-6-(2-oxononyl)benzoic430 acid
- 431 ¹**H NMR** (300MHz, CDCl₃): 11.16 (s, 1H), 6.73 (d, J = 1.6 Hz, 1H), 6.62 (d, J = 1.6432 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), 0.85 (t, J = 7 Hz, 3H).
- 436 ¹³**C NMR** (100MHz, CDCl₃): 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. Attibutions 439 using 2D experiments. $[M-H]^{-}$ C₃₄H₄₃O₁₀ calc. 611.28617 found. 611.2862 $[A]^{-}$ 440 C₁₇H₂₁O₆ calc. 321.1344 found. 321.1347.
- 441

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453 **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this 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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- 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.** δ -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 α -alectoronic acid **18** and the α -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.
- Figure 8. Relaxed coordinate scans of simplified despides under A. neutral and B.
 basic conditions; C. Energy minimized structure of simplified depsidone under basic
 conditions; D. Manually adjusted dihedral angle to position the enolate close to the
 ester carbonyl group.
- Figure 9. KUV probe on extracts of *P. cinereoatra C, P. speirea* var speirea S, *P. irrigual I, P. macrocarpa Ma* and *P. platycarpoides P.* Extracts were spotted and K
 was added (right) or not (left).
- Figure 10. A. Direct application of the KUV probe on the medulla of *P. cinereoatra*;
 B. KUV probe on the eluted TLC (solvent G) of *Porpidia cinereoatra* C, *P. tuberculosa* T, *P. speirea* var speirea S, *P. melinodes* M, *P. contraponenda* Co, *P. irrigua* I and *P. rugosa* R
- 582 Figure 11. Structural data of compound s8 (NMR in acetone- d6)
- 583





586 Figure 2



Depsides	ID	R₄	R'₂	R ₆	R' 6	Lichens	Ref
Glomelliferic acid	1	CH₃	н	C_3H_7	C_5H_{11}	Xanthoparmelia loxodes	1
Glomellic acid	2	CH₃	Н	C_3H_7	CH ₂ C(O)C ₃ H ₇	Xanthoparmelia loxodes	1
Confluentic acid	3	CH₃	CH₃	C_5H_{11}	C ₅ H ₁₁	Porpidia cinereoatra	2
2'-O-Methylmicrophyllinic acid	4	CH_3	CH₃	C_5H_{11}	$CH_2C(O)C_5H_{11}$	Porpidia cinereoatra	2
Methyl ester of 4	4'	CH_3	CH₃	C_5H_{11}	$CH_2C(O)C_5H_{11}$	Porpidia irrigua	2
2'-O-Methylsuperphyllinic acid	5	CH₃	CH₃	C ₇ H ₁₅	$CH_2C(O)C_7H_{15}$	Porpidia rugosa	2
Olivetoric acid	6	Н	Н	C_5H_{11}	C_5H_{11}	Pseudevernia furfuracea (var. ceratea)	3
Arthoniaic acid	7	Н	CH_3	C_5H_{11}	$CH_2C(O)C_5H_{11}$	Pachnolepia pruinata	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)

589 Figure 3

RING A







- 596 Figure 5.

















610 Figure 10.



611 612

613 Figure 11.



no.	δ C	δΗ	no.	δ C	δΗ
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		
HRI	MS { <mark>\$8</mark> [M-⊢	l] = 611.2862 (C ₃₄	H ₄₃ O ₁₀) →	[A] ⁻ = 321.1	347 (C ₁₇ H ₂₁ O ₆)
	 5 [M-H]	¯ = 597.3069 (C ₃₄ ⊦	$H_{45}O_9) \longrightarrow$	[A] = 307.1	1546 (C ₁₇ H ₂₃ O ₅)

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

Species	Lichen (g)	Extract (mg)	Compound (mg)	Location	Date	Collector / herb. ref.
Xanthoparmelia loxodes (Nyl.) O. Blanco et al. (Parmeliaceae)	8	220	1: 152 2: 25 20: 8	France, Port Lépine, Trélévern (22660), acidic coastal rocks near the tower, Alt 15m	December 2019	P. Uriac
Porpidia cinereoatra (Ach.) Hertel & Knoph (Lecideaceae)	2	170	3 : 95 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
Porpidia rugosa (Taylor) Coppins & Fryday (Lecideaceae)	0.47	46	5 : 26 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 Chaudefour, Chambon sur Lac (63790), coniferous trees, Alt. 1450m July 1961 Herb. J. C. Massé 15017		Herb. J. C. Massé 15009
Pachnolepia pruinata (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
Ochrolechia parella (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
Porpidia contraponenda (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
Porpidia melinodes (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
Porpidia tuberculosa (Sm.) Hertel & Knoph (Lecideaceae)				France, near the Chapelle Ste Trépine, acidic rocks, St Aignan (56480), Alt. 179m	March 2019	P. Uriac
Porpidia macrocarpa (DC.) Hertel & A.J. Schwab (Lecideaceae)				France, La touche Guérin, Paimpont (35380), red schist, Alt : 189m	January 2020	P. Uriac
<i>Porpidia platycarpoid</i> es (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