Chemical Constituents of the Lichen *Cladina macaronesica*

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Column chromatography of the acetone extract of the lichen *Cladina macaronesica* (Sephadex LH-20, silica gel and silver nitrate-impregnated silica gel) afforded eight triterpenes identified by chemical and spectral means. α-Amyrenone, lupenone, taraxerol, taraxerone and *iso*-arborinol acetate were isolated for the first time from lichens and (−)-usnic acid and five mononuclear phenolic compounds were also obtained, four for the first time as natural products. The possible transformation of perlatolic acid into these phenolic compounds is briefly outlined.

Introduction

Cladina macaronesica (Ahti) Follm. & Hern.-Padr. (Cladoniaceae Zenker, Lecanorales Nannf.) [1] is an interesting lichen species, arboriform, robust, greenish- to greyish-yellow in colour, which grows in more or less dense pads 6–8 cm high. It has a very ramified stalk with predominantly dichotomous hollow ramifications. The lichen is commonly found in the Canary Islands in the undergrowth of green mountain-tops (evergreen woods) and on the banks of the roads and tracks surrounding these areas and often characterizes the bryolichenous terricole communities of these soils seen best in clearings and other exposed hillside sites. *Cladina macaronesica* is native to the Macaronesia and is found in the Azores, Madeira and the Canary Islands of Tenerife, Gran Canaria, Gomera, Hierro and Palma.

As part of an intensive study of the huge variety of Canarian lichens, a chemical analysis of this interesting plant was made and yielded two types of substances: one consisting of a series of triterpenes and a sterol obtained from the hexane and benzene extracts, called hereafter H_1, H_2, H_3, H_6 and B_1; and the other of the lichenous substances H_4, H_5, B_2, B_3, Cl_1 and Cl_2 isolated from the hexane, benzene and chloroform extracts, respectively (see Experimental section).

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Results and Discussion

Isolation and structure determination of the triterpenes

Most of the triterpenes isolated from lichens are pentacyclic hopane derivatives [2] such as zeorin known to be the commonest lichen triterpene. Exceptionally, dammarane-type triterpenes are also found, such as diacetylpyxinol, a stictane-type triterpene, and the following pentacyclic triterpenes with oleanane, ursane and lupane skeletons: friedelin, isolated from the lichens *Alectoria ochroleuca, Cetraria curcullata, Stereocaulum paschale* and *Cetraria nivalis* [3, 4], taraxerene, isolated from *Cladonia deformis* [5], ursolic acid isolated from the lichens *Cladonia arbusculata, Cladonia impexa* and *Cetraria nivalis,* and α-amyrin, lupeol and cerin obtained from the lichen *Cetraria nivalis* [6, 7].

Compound H_1

This was isolated from the Sephadex LH-20 chromatography of the hexane extract as a mixture of three triterpenes which were separated by TLC impregnated with silver nitrate and denominated H_{1a}, H_{1b} and H_{1c} for the purposes of this study. The IR spectrum of all three substances

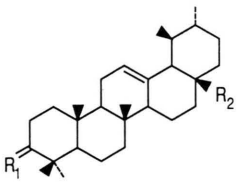
showed an absorption band at ν_{max} 1700 cm^{-1} characteristic of a carbonyl group. MS showed the molecular ion at *m/z* 424, in accordance with the molecular formula $C_{30}H_{48}O$.

Triterpene H_{1a}

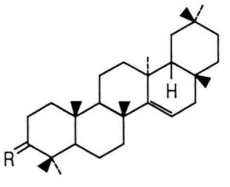
Only a small quantity was obtained. Study of the MS indicated a Δ^{12}-unsaturated pentacyclic triterpene with a base peak at *m/z* 218 typical of retro-Diels Alder fragmentation [8]. The ^1H NMR spectrum is very similar to that of α-amyrin (**2**) differing in that here the hydroxyl on C-3 is replaced by a carbonyl group as can be seen clearly in the IR spectrum. Substance H_{1a} is assumed to be α-amyrenone (**1**) [9] isolated from the first time from a lichen.

Triterpene H_{1b}

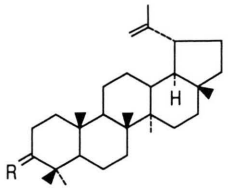
Isolated as a solid, m.p. 240–242 °C. This substance was identified from its spectral data (IR, ^1H NMR and MS) as taraxerone (**3**) [10]. This same substance was isolated by Bohlmann *et al.* [11] but could not be demonstrated to be a genuine component of the lichen in view of the difficulty of separating it from the bark of *Alnus glutinosa* and *A. incana,* which contain taraxerone and taraxerol, respectively. As our lichen material was gathered at ground level (see Experimental) H_{1b} is unequivocally a lichen product, isolated as such for the first time.



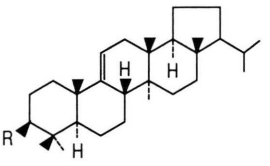
H_{1a} **1** R_1 = O ; R_2 = Me
H_{3a} **2** R_1 = β-OH, H; R_2 = Me
H_6 **8** R_1 = β-OH, H; R_2 = COOH
8a R_1 = β-OH, H; R_2 = COOMe



H_{1b} **3** R = O
H_{3b} **4** R = β-OH, H
4a R = β-OAc, H



H_{1c} **5** R = O
H_{3c} **6** R = β-OH, H



H_2 **7** R = OAc
7a R = OH

Triterpene H_{1c}

Obtained as a crystalline solid, m.p. 160 °C. The MS showed a molecular ion peak at *m/z* 424 and a prominent peak at *m/z* 205 characteristic of lupane derivative triterpenes. The ^1H NMR is similar to that of lupeol (**6**) differing only in having a carbonyl group at C-3 instead of a hydroxyl. Comparison of the spectral data obtained from compound H_{1c} with data given for lupenone (**5**) [12] indicated that the two were identical. Lupenone (**5**) is here isolated for the first time from a lichen.

Substance H_2

Isolated directly as an acetate [IR (1720 and 1250 cm^{-1}) and ^1H NMR spectra (δ 2.05, 3H, s)] from the Sephadex LH-20 chromatography of the hexane extract. In low resolution MS, the molecular ion was observed at *m/z* 468 which agrees with the molecular formula $C_{32}H_{52}O_2$. Alkaline hydrolysis gave an alcohol, **7a**, with a MS (M^+ *m/z* 426, $C_{20}H_{50}O$) for a triterpene with a hydroxy group and a double bond. The ^1H NMR spectral data of **7a** clearly showed a broad doublet at δ 5.23 corresponding to a vinyl proton and a double doublet at δ 3.21 characteristic of a geminal proton to a β-equatorial hydroxyl. In the methyl region there are two notable doublets at δ 0.89 and 0.83 assigned to the methyls of an isopropyl radical. The MS has fragments typical of Δ^7, Δ^8 or $\Delta^{9(11)}$-unsaturated pentacyclic triterpenes, with methyls at C-13 and C-14. A comparison of the percentage of the fragmentations in the mass spectrum of this product with those given by Nishimoto *et al.* [13] shows that it is an exact match with an arborane-skeleton triterpene with a double bond at $\Delta^{9(11)}$. Both the physical and spectral data coincide with those for iso-arborinol (**7a**) [14] which is here reported for the first time from a lichen. It is nonetheless surprising to find triterpenes formed by two different biogenetic routes in the same lichen.

Substance H_3

Also obtained from the Sephadex LH-20 chromatography. TLC with silver nitrate-impregnated

silica gel gave a mixture of three products H_{3a}, H_{3b} and H_{3c}. H_{3a} had m.p. 183 °C and a M^+ at *m/z* 426 ($C_{30}H_{50}O$); its ^1H NMR and MS (see Experimental) agreed with those of α-amyrin (**2**) [7]. When treated with acetic anhydride-pyridine, the triterpene mixture yielded a compound in needles, m.p. 300–302 °C, MS *m/z* 486 $[M]^+$ ($C_{32}H_{52}O_2$). On the basis of the ^1H and MS spectral data (see Experimental) this substance was identified as taraxerol acetate (**4a**). Alkaline hydrolysis of **4a** gave taraxerol H_{3b} (**4**) [10], isolated here for the first time from a lichen. H_{3c} was isolated in the form of needles, m.p. 202–207 °C and identified as lupeol (**6**) [7] from its ^1H NMR and MS spectral data (see Experimental).

Substance H_6

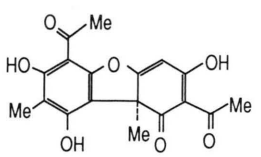
Was obtained in copious yield from the hexane and chloroform extracts (see Experimental) and purified as methyl ester, m.p. 106–107 °C. Both the physical and spectroscopic constants were in agreement with those of ursolic acid methyl ester. (**8a**) [7].

Substance B_1 was identified as β-sitosterol [15].

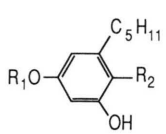
Isolation and structural determination of the lichenous substances

Substance H_4

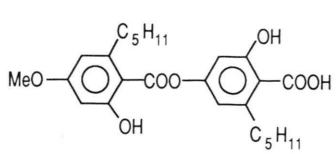
Like H_6 this compound was also obtained from the hexane and chloroform extracts (see Experimental) proving to be an oily substance which, when treated with ferric chloride, turned greenish-yellow. The IR spectrum exhibited absorption bands due to a chelated hydroxy group (3350 cm^{-1}), a carbonyl group (1650 cm^{-1}) and an aromatic ring (1610, 1550 cm^{-1}). In the MS spectrum the molecular ion at *m/z* 266 $[M]^+$ agreed with a molecular formula of $C_{15}H_{22}O_4$. The IR, ^1H NMR and MS spectra were in perfect accordance with those given for ethyl-2-hydroxy-4-methoxy-6-pentylbenzoate (**10**) isolated earlier by Solberg *et al.* from *Icmadophila ericetorum* [16]. Ethanol was used in the earlier extraction process although the possibility of **10** being an extraction artifact was not taken into account. The product now isolated, however, never came into contact with ethanol.



H_5 **9**



H_4 **10** R_1 = Me, R_2 = COOEt
B_2 **11** R_1 = Me, R_2 = H
B_3 **12** R_1 = Me, R_2 = COOH
12a R_1 = Me, R_2 = COOMe
Cl_1 **13** R_1 = H , R_2 = H
Cl_2 **14** R_1 = H , R_2 = COOH



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Substance H_5

This compound was obtained in abundance from the hexane and benzene extracts as a crystalline yellow substance, m.p. 195–197 °C. Its physical and spectroscopic properties coincided with those of (−)-usnic acid (**9**) [17].

Substance B_2

This new substance was isolated from the benzene extract (see Experimental) as a brown oil which turned orange-brown when drops of sulphuric acid were added. The IR spectrum showed absorption bands due to a hydroxy group (3580 cm^{-1}) and an aromatic ring (1610, 1590 cm^{-1}). In the MS, the molecular ion was at *m/z* 194 $[M^+]$ ($C_{12}H_{16}O_2$) and characteristic fragments at *m/z* 152 $[M-C_3H_6]^+$ and 138 $[M-C_4H_8]^+$ indicated the presence of a pentyl radical. Its ^1H NMR spectrum had signals for three aromatic hydrogens at δ 6.34, 6.29 and 6.26 which appeared as doublets with coupling constants typical of meta hydrogens (J = 1.3 Hz), a signal at δ 3.76 assigned to a methoxyl on an aromatic ring, and the signals for a pentyl radical. From the foregoing B_2 could be identified as 3-methoxy-5-pentylphenol (**11**). This structure was ratified by a study of the ^{13}C NMR spectrum (DEPT) (see Experimental).

Substance B_3

This substance was obtained in the same way as the above from the benzene extract, and crystal-

lized as needles, m.p. 123 °C, turning deep yellow with sulphuric acid and dark green with ferric chloride. Its IR spectrum had absorption bands at 3480 cm^{-1} for a hydroxy group, a broad band between 3300 and 2400 cm^{-1} characteristic of an acid group and typical aromatic bands at 1610 and 1570 cm^{-1}. The MS of B_3 showed the molecular ion at *m/z* 238 for a formula of $C_{13}H_{18}O_4$. Significant fragments at *m/z* 182 $[M-C_4H_8]^+$ and 164 $[M-C_4H_8-H_2O]^+$ indicated the presence of a pentyl radical, confirmed in the ^1H NMR spectrum (see Table I). Treatment with CH_2H_2 gave the methyl ester **12a** in the ^1H NMR spectrum of which a singlet at δ 11.74 was observed corresponding to a chelated hydroxyl, a signal which was not seen in the spectrum of the acid, plus two *meta* aromatic protons at δ 6.33 and 6.29 which appeared as a singlet at δ 6.35 in the spectrum of B_3. In view of these findings and biogenetic considerations [18], the structure of 2-hydroxy-4-methoxy-6-pentylbenzoic acid (**12**) was allotted to this new substance. This structure is in line with the ^{13}C NMR spectral (DEPT) data (see Experimental).

Substance Cl_1

This new substance was isolated from the chloroform extract as a reddish-brown oil which turned yellow when treated with sulphuric acid. Its IR spectrum had three characteristic absorption bands at ν_{max} 3580, 3340 and 1600 cm^{-1} for two hydroxy groups and an aromatic ring, respectively. MS showed the molecular ion at *m/z* 180 $[M]^+$ agreeing with $C_{11}H_{16}O_2$, and also a fragment characteristic of the pentyl radical at *m/z* 124 $[M-C_4H_8]^+$. The aromatic protons appeared as two broad singlets at δ 6.26 (2H) and 6.19 (1H) in the ^1H NMR spectrum, showing that the unoccupied positions on the ring were meta to each other. The above data match the structure of 3-hydroxy-5-pentylphenol (**13**).

Substance Cl_2

This was also isolated from the chloroform extract, as a brown oil, $C_{11}H_{18}O_2$, turning yellow when treated with sulphuric acid. Its IR spectrum revealed absorption bands at ν_{max} 3300 cm^{-1} (hydroxyl), 3200–2500 (carboxyl), 1630 (carbonyl)

Table I. ^1H NMR data of the mononuclear phenolic compounds isolated from *Cladina macaronesica*[a].

mpounds	H_4[b]	B_2[b]	B_3[b]	B_3[b] methyl ester	Cl_1[b]	Cl_2[c]
$-(CH_2)_4-\underline{Me}$	0.89 (t, *J* = 7.0)	0.89 (t, *J* = 6.3)	0.91 (t, *J* = 6.9)	0.90 (t, *J* = 6.6)	0.86 (t, *J* = 6.5)	0.91 (t, *J* = 6.4)
$-(CH_2)_2-(\underline{CH_2})_2-Me$	1.30 (m)	1.32 (m)	1.36 (m)	1.32 (m)	1.26 (m)	1.35 (m)
$-CH_2-\underline{CH_2}-C_3H_7$	1.52 (m)	1.58 (m)	1.60 (m)	1.53 (m)	1.51 (m)	1.57 (m)
$-\underline{CH_2}-C_4H_9$	2.85 (t, *J* = 7.5)	2.51 (t, *J* = 7.5)	2.93 (t, *J* = 7.4)	2.84 (t, *J* = 7.0)	2.38 (t, *J* = 7.6)	2.88 (t, *J* = 7.5)
$-\underline{H}$	6.28 (1H, d, *J* = 2.0)	6.26 (1H, d, *J* = 1.3)	6.35 (2H, s)	6.29 (1H, d, *J* = 2.6)	6.19 (1H, s)	6.14 (1H, d, *J* = 2.0)
	6.33 (1H, d, *J* = 2.0)	6.29 (1H, d, *J* = 1.3)	–	6.33 (1H, d, *J* = 2.6)	6.26 (2H, s)	6.19 (1H, d, *J* = 2.0)
	–	6.34 (1H, d, *J* = 1.2)	–		–	–
ers	3.80 (3H, s, Ar–O<u>Me</u>)	3.76 (3H, s, Ar–O<u>Me</u>)	3.82 (3H, s, Ar–O<u>Me</u>)	3.80 (3H, s, Ar–O<u>Me</u>)	–	–
	1.41 (3H, t, *J* = 7.26, Ar–$COOCH_2-\underline{Me}$)	–	–	3.92 (3H, s, Ar–COO<u>Me</u>)	–	–
	4.40 (2H, q, *J* = 7.26, Ar–$COOCH_2$–Me)	–	–	–	–	–
	11.86 (1H, s, Ar–O<u>H</u>)	–	–	11.74 (1H, s, Ar–O<u>H</u>)	–	–

[a] Abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.
[b] Taken in $CDCl_3$.
[c] Taken in CD_3OD.

and 1600, 1550 cm^{-1} (aromatic). A study of the MS spectrum (see Experimental) showed the pentyl radical observed in the compounds described above and this was also visible in the ^1H NMR spectrum. These data agree perfectly with the structure 2,4-dihydroxy-6-pentylbenzoic acid (**14**) for this new substance.

Five of these lichenous substances are mono-aryl compounds and four are reported here for the first time. Literature search showed that few such compounds have been isolated from lichens in spite of their apparent intermediary role in the biosynthesis of depsides [19]. Occasionally they have been regarded as artifacts of the depsides found in the same lichens, partially hydrolyzed during the extraction process; and elsewhere, as natural products. In this instance, perlatolic acid (**15**) could well be the origin of the phenolic compounds isolated and it remains to be seen if they are evolved within the lichen as the result of action by the hydrolase and decarboxylase enzymes there present, or during extraction. To clarify this point, the acetone extract was chromatographed directly on Sephadex LH-20 and perlatolic acid (**15**) was obtained – this substance was not found in the previous study – plus the mono-aryl compounds B_3 and B_4. Perlatolic acid was refluxed with silica gel in benzene for 4 h and gave the mono-aryl compounds B_2, B_3, Cl_1 and Cl_2. This bore out the thesis that the products obtained in the first study could be the result of chemical degradation of perlatolic acid due to its great lability [20, 21].

Experimental

Melting points were obtained on a Kofler apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer model 257 spectrophotometer. ^1H NMR spectra were taken at 200 MHz and MS were obtained using a direct inlet system at 70 eV. The plant material was collected from the ground in La Gomera in July 1988 in the region of Tajaque (hilltop heath) at 1200 m. Voucher specimens are on file in the TFC Lich. Herbarium (No. 1939) of the Departamento de Biología Vegetal, Botánica of the Universidad de La Laguna.

Isolation of Compounds

The lichen material once dried was ground to give 684 g of a fine powder which was macerated with acetone. The acetone extract was concentrated at reduced pressure to give 184 g of a syrupy liquid. This was impregnated with silica gel and extracted in a Soxhlet with hexane, benzene and chloroform giving a hexane extract (900 mg), a benzene extract (4.3 g) and a chloroform extract (2.5 g). All the extracts were chromatographed on a Sephadex LH-20 column using *n*-hexane–$CHCl_3$–MeOH (2:1:1) as eluant and yielded H_1 (85 mg); H_2 (64 mg); H_3 (33 mg); H_6 (125 mg); B_1 (70 mg); and the lichenic substances H_4 (17 mg); H_5 (123 mg); B_2 (120 mg); B_3 (200 mg); Cl_1 (50 mg) and Cl_2 (63 mg).

Substance H_1

Isolated as a mixture from Fractions 3–4 of the Sephadex LH-20 chromatography of the C_6H_{14} extract, extracted by re-chromatography on silica gel impregnated with $AgNO_3$ using C_6H_{14}–C_6H_6 (1:1) as eluant to afford the triterpenes H_{1a} (5 mg), H_{1b} (25 mg) and H_{1c} (10 mg).

α-Amyrenone (H_{1a}): amorphous powder; ^1H NMR ($CDCl_3$) δ: 0.50–1.10 (24H, 8 × Me), 5.15 (1H, t, J = 3.5 Hz, H-12); MS m/z (rel. int.): 424 $[M]^+$ (21), 409 $[M-Me]^+$ (12), 218 (100), 205 (14), 203 (28), 189 (18); 133 (16).

Taraxerone (H_{1b}): m.p. 240–242 °C (EtOH); $[\alpha]_D^{25}$ + 12.6° (*c* 1.03, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 0.83 (3H, s, Me), 0.91 (6H, s, 2 × Me), 0.95 (3H, s, Me), 1.06 (3H, s, Me), 1.08 (6H, s, 2 × Me), 1.13 (3H, s, Me), 5.55 (1H, dd, J = 4.0, 7.0 Hz, H-15); MS m/z (rel. int.): 424 $[M]^+$ (100), 409 $[M-Me]^+$ (51), 300 (97), 285 (62), 204 (90), 189 (40).

Lupenone (H_{1c}): m.p. 160 °C (EtOH); $[\alpha]_D^{25}$ + 60.0° (*c* 0.54, $CHCl_3$); ^1H NMR ($CDCl_3$) δ: 0.70 (3H, s, Me), 0.84 (3H, s, Me), 0.86 (3H, s, Me), 0.93 (3H, s, Me), 0.98 (3H, s, Me), 1.65 (3H, s, Me–C=), 4.48 (1H, d, J = 2.0 Hz, H-29), 4.60 (1H, d, J = 2.0 Hz, H-29); MS m/z (rel. int.): 424 $[M]^+$ (39), 409 $[M-Me]^+$ (15), 381 $[M-C_3H_7]^+$ (4), 218 (17), 205 (51), 189 (22).

Iso-arborinol acetate (H_2)

Isolated from Fractions 3–4 of the Sephadex LH-20 chromatography and purified by re-chromatography on silica gel using C_6H_{14}–C_6H_6 (1:1) as eluant. M.p. 285 °C (MeOH–EtOAc); $[\alpha]_D^{25}$ + 48.0° (*c* 0.26, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1}: 1730, 1250, 1170, 1145, 1030, 970: ^1H NMR ($CDCl_3$) δ:

0.50–1.10 (24H, 8 × Me), 2.05 (3H, s, OAc); 4.49 (1H, dd, J = 4.0, 10.0 Hz, H-3), 5.23 (1H, deformed d, H-11).

Alkaline hydrolysis of *iso*-arborinol acetate

50 mg of H_2 was refluxed for 24 h with a solution of 5% NaOH. After the usual work-up, *iso*-arborinol (**7a**) (40 mg) was obtained: m.p. 300 °C (MeOH–$CHCl_3$); $[\alpha]_D^{25}$ + 37.0° (*c* 0.27, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 0.75 (3H, s, Me), 0.76 (3H, s, Me), 0.80 (3H, s, Me), 0.81 (3H, s, Me), 0.83 (3H, d, J = 6.0 Hz, $\underline{Me}_2CH$–), 0.89 (3H, d, J = 6.0 Hz, $\underline{Me}_2CH$–), 0.98 (3H, s, Me), 1.03 (3H, s, Me), 3.21 (1H, dd, J = 4.0, 10.0 Hz, H-3), 5.23 (1H, deformed d, H-11).

Substance H_3

Isolated as a mixture from Fractions 5–8 of the Sephadex LH-20 chromatography of the C_6H_{14} extract. Re-chromatography on silica gel impregnated with $AgNO_3$ with C_6H_6–EtOAc (9:1) as eluant afforded H_{3a} (15 mg), H_{3b} (26 mg) and H_{3c} (10 mg).

α-Amyrin (H_{3a}): m.p. 183 °C (EtOH); $[\alpha]_D^{25}$ + 83.0° (*c* 0.62, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 0.50–1.10 (24H, 8 × Me), 3.22 (1H, dd, J = 5.0, 11.0 Hz, H-3), 5.12 (1H, t, J = 4.0 Hz, H-12); MS *m/z* (rel. int.): 426 $[M]^+$ (11), 411 $[M-Me]^+$ (3), 218 (100), 207 (12), 203 (23), 189 (19), 133 (16).

Taraxerol (H_{3b}) was purified with Ac_2O–Py giving a solid: m.p. 300 °C (MeOH); $[\alpha]_D^{25}$ + 12.0° (*c* 1.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 0.81 (3H, s, Me), 0.85 (3H, s, Me), 0.87 (3H, s, Me), 0.90 (6H, s, 2 × Me), 0.95 (6H, s, 2 × Me), 1.08 (3H, s, Me), 2.06 (3H, s, OAc), 4.45 (1H, dd, J = 5.0, 10.0 Hz, H-3), 5.53 (1H, dd, J = 4.0, 7.0 Hz, H-15); MS *m/z* (rel. int.): 468 $[M]^+$ (45), 453 $[M-Me]^+$ (14), 393 $[M-Me-HOAc]^+$ (8), 344 (18), 329 (9), 298 (37), 284 (5), 269 (9), 218 (100), 204 (42), 189 (49).

Lupeol (H_{3c}): m.p. 207 °C (MeOH–EtOAc); $[\alpha]_D^{25}$ + 22.2° (*c* 0.54, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 0.76 (3H, s, Me), 0.79 (3H, s, Me), 0.83 (3H, s, Me), 0.94 (3H, s, Me), 0.97 (3H, s, Me), 1.03 (3H, s, Me), 1.68 (3H, s, $\underline{Me}$–C=), 3.18 (1H, dd, J = 5.0, 10.0 Hz, H-3), 4.56 (1H, d, J = 2.0 Hz, H-29), 4.69 (1H, d, J = 2.0 Hz, H-29); MS *m/z* (rel. int.): 426 $[M]^+$ (18), 411 $[M-Me]^+$ (6), 220 (6), 218 (25), 207 (29), 191 (16), 189 (35).

Ursolic acid (H_6)

Isolated from Fractions 13–18 of the Sephadex LH-20 chromatography of the C_6H_{14} extract and purified by treatment with an ether solution of CH_2N_2 produced from N-nitroso-N-methylurea and NaOH: m.p. 106–107 °C solidifying and re-melting at 170–171 °C (C_6H_{14}–$CHCl_3$); $[\alpha]_D^{25}$ + 67.0° (*c* 1.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 0.72 (3H, s, Me), 0.75 (3H, s, Me), 0.83 (3H, d, J = 6.4 Hz, $\underline{Me}$–CH); 0.89 (3H, s, Me), 0.91 (3H, d, J = 6.4 Hz, $\underline{Me}$–CH), 0.96 (3H, s, Me), 1.05 (3H, s, Me), 3.18 (1H, dd, J = 5.0, 10.0 Hz, H-3), 3.58 (3H, s, COOMe), 5.22 (1H, t, J = 3.6 Hz, H-12); MS *m/z* (rel. int.): 470 $[M]^+$ (5), 441 $[M-COOMe]^+$ (4), 262 (100), 207 (22), 203 (68), 189 (17), 133 (35).

Ethyl-2-hydroxy-4-methoxy-6-pentylbenzoate (H_4)

Isolated from Fractions 9–12 of the Sephadex LH-20 chromatography of the C_6H_{14} extract; yellow oil; IR ν_{max} ($CHCl_3$) cm^{-1}: 3350, 1650, 1610, 1570; ^{1}H NMR ($CDCl_3$) see Table I; MS *m/z* (rel. int.): 266 $[M]^+$ (34), 221 $[M-C_2H_5O]^+$ (13), 220 $[M-C_2H_6O]^+$ (50), 210 $[M-C_4H_8]^+$ (53), 196 $[M-C_5H_{10}]^+$ (4), 192 $[M-C_4H_8-H_2O]^+$ (30), 177 $[M-C_4H_8-H_2O-Me]^+$ (21), 165 $[M-C_2H_5O-C_4H_8]^+$ (13), 164 $[M-C_2H_6O-C_4H_8]^+$ (100), 135 $[M-C_2H_6O-C_4H_8-CHO]^+$ (26).

(–)-Usnic acid (H_5)

Isolated from Fractions 13–18 of the Sephadex LH-20 chromatography of the C_6H_{14} extract: m.p. 195–197 °C (MeOH); $[\alpha]_D^{25}$ – 495° (*c* 1.85, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 1.59 (3H, s, Me), 2.11 (3H, s, Me–Ar), 2.67 (3H, s, Me–CO–), 2.68 (3H, s, Me–CO–), 5.98 (1H, s, H–Ar), 11.05 (1H, s, HO–Ar), 13.33 (1H, s, HO–Ar), 18.86 (1H, s, HO–Ar); MS *m/z* (rel. int.): 344 $[M]^+$ (85), 260 (72), 233 (100), 217 (18).

3-Methoxy-5-pentylphenol (B_2)

Isolated from Fractions 16–25 of the Sephadex LH-20 chromatography of the C_6H_6 extract: brown oil; IR ν_{max} ($CHCl_3$) cm^{-1}: 3580, 1610, 1590; ^{1}H NMR ($CDCl_3$) see Table I; ^{13}C NMR (50.32 MHz, $CDCl_3$) δ: 160.8 (C-3), 108.2 (C-6), 156.7 (C-1), 98.9 (C-2), 145.9 (C-5), 106.9 (C-4), 65.3 (OMe), 36.1 (C-1′), 31.6 (C-2′), 30.9 (C-3′),

22.6 (C-4′), 14.1 (C-5′); MS *m/z* (rel. int.): 194 $[M]^+$ (21), 152 $[M-C_3H_6]^+$ (13), 138 $[M-C_4H_8]^+$ (100).

2-Hydroxy-4-methoxy-6-pentylbenzoic acid (B_3)

Isolated from Fractions 16–25 of the Sephadex LH-20 chromatography of the C_6H_6 extract: m.p. 123 °C ($CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1}: 3480, 3300–2400, 1630, 1610, 1570; ^1H NMR ($CDCl_3$) see Table I; ^{13}C NMR (50.32 MHz, $CDCl_3$) δ: 176.2 (COOH), 103.5 (C-5), 166.8 (C-4), 111.4 (C-1), 165.2 (C-2), 99.0 (C-3), 149.9 (C-6), 55.5 (OMe), 36.8 (C-1′), 32.1 (C-2′), 31.5 (C-3′), 22.6 (C-4′), 14.2 (C-5′); MS *m/z* (rel. int.): 238 $[M]^+$ (26), 220 $[M-H_2O]^+$ (33), 192 $[M-C_2H_4-H_2O]^+$ (25), 182 $[M-C_4H_8]^+$ (39), 177 $[M-C_3H_7-H_2O]^+$ (5), 164 $[M-C_4H_8-H_2O]^+$ (100), 137 $[M-C_4H_8-H_2O-CHO]^+$ (28).

Methylation of 2-hydroxy-4-methoxy-6-pentylbenzoic acid

20 mg of B_3 was treated with CH_2N_2 in the same way as H_6 yielding methyl ester **12a** (19 mg): yellow oil; ^1H NMR ($CDCl_3$) see Table I; MS *m/z* (rel. int.): 252 $[M]^+$ (41), 220 $[M-MeOH]^+$ (47), 196 $[M-C_4H_8]^+$ (72), 192 $[M-C_2H_4-MeOH]^+$ (27), 177 $[M-C_3H_7-MeOH]^+$ (20), 164 $[M-C_4H_8-MeOH]^+$ (100), 135 $[M-C_4H_8-MeOH-CHO]^+$ (22).

3-Hydroxy-5-pentylphenol (Cl_1)

Isolated from Fractions 23–30 of the Sephadex LH-20 chromatography of the $CHCl_3$ extract: reddish-brown oil; IR ν_{max} ($CHCl_3$) cm^{-1}: 3580, 3340, 1600; ^1H NMR ($CDCl_3$) see Table I; MS *m/z* (rel. int.): 180 $[M]^+$ (29), 124 $[M-C_4H_8]^+$ (100).

2,4-Dihydroxy-6-pentylbenzoic acid (Cl_2)

Isolated from Fractions 23–30 of the Sephadex LH-20 chromatography of the $CHCl_3$ extract: brown oil; IR ν_{max} (film) cm^{-1}: 3350, 3200–2500, 1630, 1600, 1590; ^1H NMR (CD_3OD) see Table I: MS *m/z* (rel. int.): 224 $[M]^+$ (50), 206 $[M-H_2O]^+$ (60), 178 $[M-C_2H_4-H_2O]^+$ (42), 168 $[M-C_4H_8]^+$ (48), 160 $[M-C_4H_8-H_2O]^+$ (100), 124 $[M-C_4H_8-CO_2]^+$ (48).

Hydrolysis and decarboxylation of perlatolic acid (**15**)

About 24 g of dried, ground lichen was macerated with acetone (250 ml) for one month and, after the acetone had been eliminated under reduced pressure, was chromatographed on Sephadex LH-20 using $C_6H_{14}-CHCl_3-MeOH$ (2:1:1) as eluant to afford perlatolic acid (**15**) (30 mg). This acid was mixed with silica gel and refluxed with C_6H_6 for 4 h, filtered and concentrated. A TLC assay with C_6H_6-acetone (7:3) as eluant showed that perlatolic acid had been almost completely transformed to the mono-aryl compounds B_2, B_3, Cl_1 and Cl_2.

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