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Chemical Investigation of the Lichen Species *Alectoria ochroleuca, Stereocaulon vesuvianum* var. *pulvinatum* and *Icmadophila ericetorum*

Yngve Solberg

Chemical Research Laboratory, Agricultural University of Norway, Ås-NLH

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Lichens, Lipids, Phenols

The three lichen species *Alectoria ochroleuca, Stereocaulon vesuvianum* var. *pulvinatum* and *Icmadophila ericetorum* have been chemically investigated with regard to their content of aliphatic and aromatic compounds. Tetrahydroxy fatty acids were isolated in all three species. A monoacetylated pentol, higher fatty acids and an unsaturated triglyceride were detected as new constituents of *A. ochroleuca* and *I. ericetorum*. *S. vesuvianum* and *I. ericetorum* both contained phenolic components which not previously have been discussed in literature. Chemical and spectroscopic evidences are presented in these studies.

Introduction

During our research on various constituents of lichens, we studied large samples of the species *Alectoria ochroleuca, Stereocaulon vesuvianum* var. *pulvinatum* and *Icmadophila ericetorum*. *S. vesuvianum* has been investigated several times previously [1–3], the common constituents reportedly being atranorin, lobaric and stictic acid. Asahina [4] reported the existence in some kinds of lichens of constictic acid with or without stictic acid, and he pointed out non-identity of this acid with protocetraric acid. Stictic acid was the only substance detected by him in the species *S. vesuvianum*. Nakamura [5] isolated constictic acid and worked out its structure. Two chemical strains (**I** and **II**) of *S. vesuvianum* reportedly containing stictic acid and atranorin, and porphyrilic acid and atranorin respectively, are described by Dahl and Krog [6]. The occurrence of porphyrilic acid in an arctic species of *Stereocaulon* belonging to the *S. vesuvianum* assemblage is reported by Fox *et al.* [7]. The purpose of the present study was to re-examine the species, and it was found to contain porphyrilin and a new C21 depsidone, besides atranorin, stictic and constictic acid. The name *vesuvianic* acid was adopted for the new depsidone. Samples of the species were collected in 1969 and 1975 and the same substances were detected in both cases.

It was previously supposed that *I. ericetorum* contained thamnolic and icmadophiliaic acid only [1, 2]. In our research we isolated higher fatty acids, oleo-dipalmitin and two new phenols as new constituents of this lichen, in addition to thamnolic acid.

The characteristic and attractive mountain lichen *A. ochroleuca* has previously been examined by several workers [1] and found to contain diffractaic and usnic acid, friedelin, arabitol, choline, enzymes, and an unidentified aliphatic carboxylic acid. An earlier report from our laboratory [8] briefly mentions the presence of tetrahydroxy fatty acid. Upon re-examination of this lichen a monoacetylated pentol with the composition $C_{24}H_{48}O_6$ was isolated. This is a new class of lipids in lichens.

A mixture of higher homologues of tetrahydroxy fatty acids was isolated from all three species. By means of high resolution mass spectrometry and comparison with standards, new details of these compounds have been found and are discussed in this paper.

The occurrence of depsides and depsidones already known in the species investigated has been disregarded.

This paper is dedicated to my wife Dagny, in recognition of her many valuable contributions of lichen samples.

Requests for reprints should be sent to Yngve Solberg, Chemical Research Laboratory, Agricultural University of Norway, P.O. Box 31, *N-1432 Ås-NLH*, Norway.

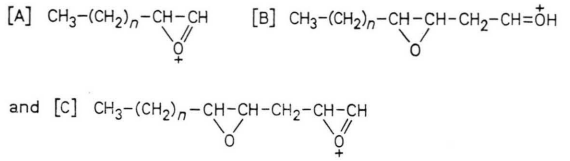
Results and Discussion

Isolation and structural determination of the tetrahydroxy fatty acids

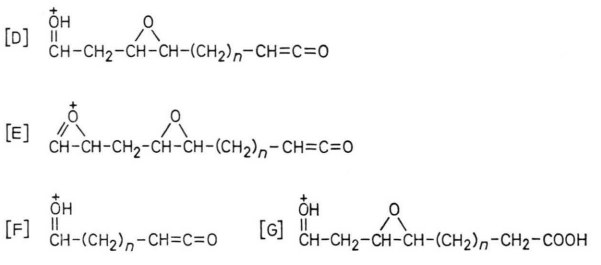
The process of extraction from the lichen samples was carried out as described in earlier communica-

tions from our laboratory [8–12]. As a rule the greater part of the hydroxy acids was separated during acetone extraction and could therefore easily be isolated in a relatively pure condition. The hydroxy acid fractions isolated from the three species concerned were purified by recrystallization twice from boiling acetic acid. The melting points and infrared spectra harmonized well with previous accounts [8–11]. However, the elementary analysis of the hydroxy acid fractions isolated from *I. ericetorum* and *S. vesuvianum* showed a higher carbon content (67.9 and 67.8%) and a proportionally lower oxygen content (20.3 and 20.9% respectively). Analyses of the same acid fractions from ten samples of other lichens gave the average values C 65.9% and 22.9%.

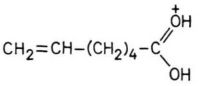
As reported in part XI and XIV of this series [11, 12] characteristic fragment ions are observed in mass spectra of tetrahydroxy fatty acids. In the present spectra the most significant peaks correspond to the fragment ions



where the values of n are 6, 7, 8, and 9. In the fraction from *I. ericetorum* the value of 10 was also observed in the formulas B and C. The fragment ion m/e 199 ($C_{12}H_{23}O_2$, $n = 7$ in B) dominates in all the spectra so far investigated. Accurate mass determinations of 41 ions in spectra of hydroxy fatty acid fractions from ten different species and of five standards explain other prominent fragments in the spectra of *A. ochroleuca*, *S. vesuvianum* and *I. ericetorum*. These fragments all containing the carbonyl portion of the parent molecules, may be represented by the structures D to G, with $n = 6$, 7, 8, and 9.



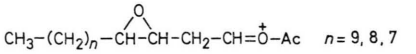
In the lower part of the mass spectra the fragment m/e 129 ($C_7H_{13}O_2$) is especially noteworthy. This corresponds to the structure



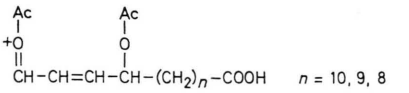
which upon the elimination of water produces the ion m/e 111 [$CH_2=CH-(CH_2)_3-CH=C=\overset{+}{O}H$].

A sufficient amount of the hydroxy fatty acid fraction was available from *S. vesuvianum* to allow an examination of the product obtained by oxidation with HJO_4 [8]. The greater part of the distillable monoaldehydes obtained by this procedure proved to be *n*-octanal, *n*-nonal, and *n*-decanal (TLC, LrMS).

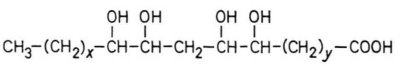
Acetylation of the same acid fraction gave a tetraacetyl mixture, m.p. about 43 °C. Outstanding peaks in the HrMS of the acetate mixture occurred at m/e 269(50), 255(16), and 241(100) corresponding to the fragments



Other important fragments were seen at m/e 355, 341, and 327 being due to



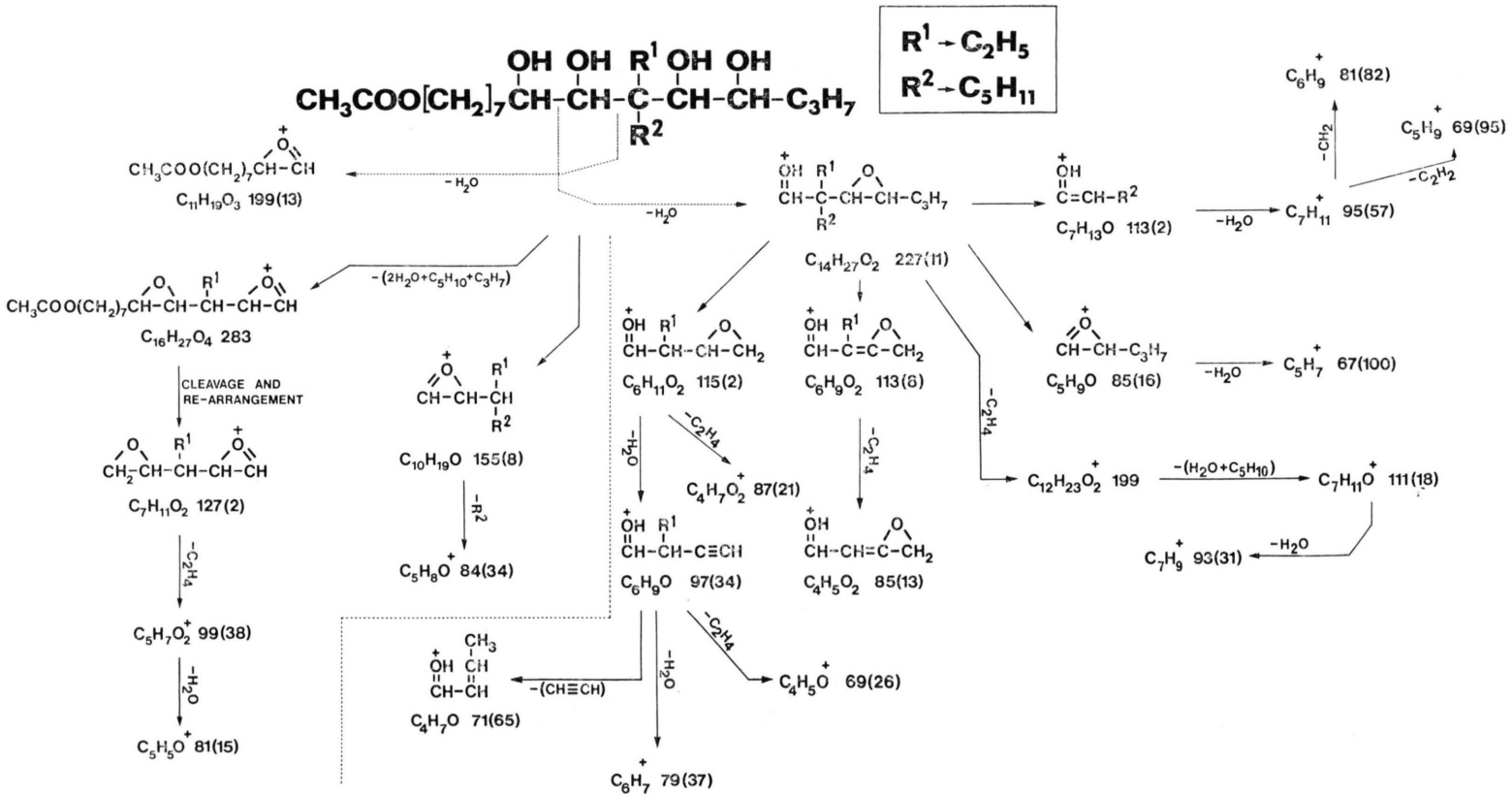
As a result of the data reported above (including elementary analyses) it must be concluded that the tetrahydroxy fatty acids in the isolated mixtures may be expressed more suitable by the following structural formula than by that reported in earlier communications.



Hitherto the values of x have been found to be 6, 7, 8, 9, and 10, and of y 7, 8, 9, and 10. The possibility of branching in the $(CH_2)_y$ part of the molecule has been appraised, but excluded because of the low $C-CH_3$ value (mean of six samples = 3.4%).

Isolation of the monoacetylpentol from Alectoria ochroleuca

The lichen constituents of *A. ochroleuca* were separated by successively diminishing the volume of acetone extract. A more soluble fraction obtained by working up the residue from the mother liquor was identified as a monoacetylpentol, $C_{24}H_{48}O_6$, in the text named as *Ochr.-X*. This conclusion was supported by elemental, IR, NMR and MS analyses.

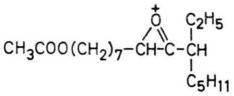


Scheme 1. The major dissociation paths of the monoacetyl pentol, Ochr.-X, isolated from *Alectoria ochroleuca*.

The IR spectrum of Ochr.-X displayed characteristic bands of an unbranched aliphatic ester. A strong stretching vibration band at 1240 cm^{-1} clearly indicated the presence of an *acetate* group. This absorption is confirmed by a strong singlet in the NMR spectrum at δ 2.00, corresponding to a methyl group in an *acetoxy* group. In LrMS a dominant ion was found at *m/e* 43 which may correspond to the *acetate* fragment CH_3CO or to alkyl-ion C_3H_7. Integration of the NMR spectrum and the intensity of the strong and sharp methyl band at 1375 cm^{-1} in IR suggested the presence of more than one methyl group. This was confirmed by elementary $C-CH_3$ analysis.

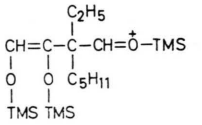
The HrMS failed to show the parent ion and the base peak of the compound was observed at *m/e* 67 corresponding to the alkyl radical $C_5H_7^+$. The highest mass was found at *m/e* 283(3.4) $C_{16}H_{27}O_4$, this being due to $[M-2\,H_2O-C_3H_7-C_5H_{10}]$. Furthermore, elimination of one and two molecules of water succesively from ion 283 produced the fragments *m/e* 265(8.8) and *m/e* 247(7.7) respectively. Ion *m/e* 283 is represented by the molecular structure given in Scheme 1.

The ion at *m/e* 311(0.7) observed in a LrMS might be assumed to have a structure of

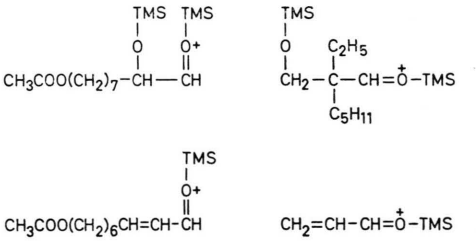


This ion eliminated a C_5H_{10} moiety to lead to the *m/e* 241(5.1) $C_{14}H_{25}O_3$ ion, which is still capable of eliminating one C_2H_4 group, very easily giving the abundant *m/e* 213(34) $C_{12}H_{21}O_3$ ion. Other major ions were observed at *m/e* 195(23) $C_{12}H_{19}O_2$ and *m/e* 135(13) $C_{10}H_{15}$ arising through successive loss of water and acetic acid respectively from the *m/e* 213 ion. The significant ion at *m/e* 227(11) $C_{14}H_{27}O_2$ (Scheme 1) is derived by cleavage of the parent molecule and loss of water. Other typical details of the fragmentation pattern are given in Scheme 1.

Silylation of Ochr.-X. Trimethylsilyl derivative of the isolated compound Ochr.-X, was found to be useful in the structure determination. The TMS ether did not exhibit the molecular ion but quite prominent ions were seen at *m/e* 517 and 427(0.4), corresponding to $[M-C_5H_{10}-C_3H_7-(CH_3)_3SiOH]$ and $[M-C_5H_{10}-C_3H_7-2(CH_3)_3SiOH]$ respectively from a compound of molecular weight 720 (432 + 4 TMS). A more intensive fragment ion at *m/e* 417(2.1) $C_{11}H_{18}O_3[Si(CH_3)_3]_3$ may be due to the structure



Four other prominent ions found at masses 361(0.3), 317(0.1), 271(4.6) and 129(35) are represented by the structures



Periodate oxidation of Ochr.-X. By oxidation of Ochr.-X with HJO_4 it was confirmed that the four free hydroxyl groups have neighboring positions in pairs. The aldehydes obtained by oxidation were converted into 2,4-dinitrophenylhydrazones and two fractions of the adduct were isolated by PLC. Interpretation of the HrMS of the two fractions revealed that *7-formyl-heptanol-1-acetate*, $CH_3COO(CH_2)_7CHO$, and 2,2-ethyl-pentyl-propanedial were obtained by the oxidation. Data from HrMS are given in Table I.

Table I. Elemental composition and relative intensities of the 2,4-dinitrophenylhydrazones of the aldehyde components obtained by oxidation of the isolated acetylpentol from *Alectoria ochroleuca*, Ochr.-X.

Assignments	Composition *	R.I. ** [%]
The dihydrazone of 2,2-ethyl-pentylpropanedial, M^+	$C_{22}H_{26}N_8O_8$	2.7
$[M-C_2H_4]^+$	$C_{20}H_{22}N_8O_8$	3.2
$[M-2\,H_2O-C_2H_4-C_5H_{10}]^+$	$C_{15}H_8N_8O_6$	2.4
$[M-2,4\,(NO_2)_2PhNH]^+$	$C_{16}H_{22}N_5O_4$	6.6
$[M-C_2H_4-2,4\,(NO_2)_2PhNH]^+$	$C_{14}H_{18}N_5O_4$	7.5
$[M-C_2H_4-C_5H_{11}-2,4\,(NO_2)_2PhNH]^+$	$C_9H_7N_5O_4$	7.8
The 2,4-dinitrophenylhydrazone of 7-formyl-heptanol-1-acetate, M^+	$C_{16}H_{22}N_4O_6$	—

* The elemental compositions have been obtained from accurate mass measurements. The results quoted are accurate to within ±0.1 to 3.3 millimass units (^{12}C = 12.000000).

** Relative intensities as % of intensity of base peak, *m/e* 183, corresponding to 2,4-dinitroaniline.

Thus from these chemical and spectral studies, the isolated monoacetyl pentol isolated from *Alectoria ochroleuca*, designated as Ochr.-X, is finally assigned the structure shown in Scheme 1 and established to be *8,9,11,12-tetrahydroxy-10,10-ethylpentyl docosanol acetate*, $C_{24}H_{48}O_6$. Homologous species were not discernible in the mass spectrum.

Isolation and identification of phenols from Stereocaulon vesuvianum var. pulvinatum

Three phenol derivative (**I, II, III**) were isolated from this species by PLC of the acetone-soluble part of the extract. A molecular ion of *compound* ***I*** in MS was observed at *m/e* 402(3). The compound reacted with PD and H_2SO_4 on the TL chromatograms and was identified as *constictic acid*.

Compound ***II*** was not soluble in $NaHCO_3$, it reacted negatively with PD, but produced a brown colour reaction with H_2SO_4 on the TL chromatograms. It turned yellow when dissolved in a solution of KOH, and green when aqueous NaClO was added to an alcoholic solution of the compound. Base peak at *m/e* 270 in LrMS corresponded to the molecular ion of composition $C_{15}H_{10}O_5$. Other significant peaks were found at *m/e* 255 ($M-CH_3$), 252 ($M-H_2O$), 242 ($M-CO$), 226 ($M-CO_2$), and 225 ($M-COOH$). Metastable peaks for the following three significant fragmentation processes were found:

		Found m*	Calcd
m/e 270 $\xrightarrow[-CHO]{m^*}$	*m/e* 241 (86)	215.2	215.1
m/e 241 $\xrightarrow[-CO]{m^*}$	*m/e* 213 (16)	188.5	188.3
m/e 213 $\xrightarrow[-CO]{m^*}$	*m/e* 185 (10)	160.5	160.7

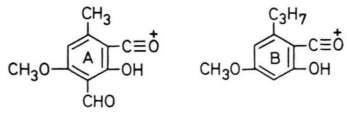
Colour reactions, TLC, UV and IR (Exp. part), and MS permit the assumption that the compound may be the dibenzofuran derivative *porphyrilin* (decarboxy porphyrilic acid), $C_{15}H_{10}O_5$. It is very likely that the isolated compound is produced from porphyrilic acid in the course of extraction and working up.

Compound ***III***. Colour reactions and R_f-values revealed that this compound was a new PD-positive substance, which we designated as *vesuvianic* acid. Ultraviolet analysis harmonized well with the bimodal spectra usually found in β-orcinol-depsidones. The absorbancy was much higher at short than at long wavelengths. Infrared analysis of vesuvianic acid indicated the presence of several characteristic frequencies such as aromatic ester, γ-lactone and aldehyde. The mass spectrum exhibited the molecular ion as base peak at *m/e* 414, and this confirms that vesuvianic acid highly probable is a depsidone. The relative abundance of M^+, $M+1$, and $M+2$ (100, 22.4, 4.7 respectively) are in accord with the formula $C_{21}H_{18}O_9$. Also in the mass spectrum of the depsidone stictic acid, isolated from this species, the M^+ was found as the base peak. Peaks corresponding to $M-H_2O$, $M-CH_3$ and $M-OH$ were detected as characteristic signals in the spectrum. Ions at *m/e* 41 and 43 are responsible for the fragments $C_3H_5^+$ and $C_3H_7^+$. The fragmentation due to $M-46$ confirmed by the appropriate metastable peak at *m/e* 327.2 may be regarded as a two-step process, H_2O+CO, or an elimination of formic acid from the parent molecule. Similar fragmentation in the MS of salazinic and stictic acid has previously been detected at our laboratory. The most important observations from the mass spectrum are summarized in Table II. Owing to the lack of significant material, a complete structural determination of this compound could not be accomplished. However, the analytical and spectral results above have established that vesuvianic acid is probably a *depsidone-aldehyde* with the molecular formula $C_{21}H_{18}O_9$. Further, it is evident that substituents such as C_3H_7, OH and γ-lactone groups are present in the molecule. A prominent peak at *m/e* 193(27) may be respon-

Table II. Mass fragmentation of vesuvianic acid isolated from *Stereocaulon vesuvianum* var. *pulvinatum*. The results are based on LrMS.

m/e	R.I. [%]	Structure	Fragment−ion
385	9	$C_{20}H_{17}O_8$	$M-CHO$
371	8	$C_{18}H_{11}O_9$	$M-C_3H_7$
370	27	$C_{20}H_{18}O_7$	$M-CO_2$
369	43	$C_{20}H_{17}O_7$	$M-COOH$
368	79	$C_{20}H_{16}O_7$	$M-H_2O-CO$ (A)
341	27	$C_{19}H_{17}O_6$	$M-CO_2-CHO$
340	27	$C_{19}H_{16}O_6$	$A-CO$
339	17	$C_{19}H_{15}O_6$	$A-CHO$
325	22	$C_{17}H_9O_7$	$A-C_3H_7$
324	25	$C_{19}H_{16}O_5$	*m/e* 341 − OH
		$C_{18}H_{12}O_6$	*m/e* 339 − CH_3
313	19	$C_{18}H_{17}O_5$	$M-CO_2-CHO-CO$
312	24	$C_{18}H_{16}O_5$	$A-2\,CO$
311	13	$C_{18}H_{15}O_5$	$A-CHO-CO$
297	14	$C_{16}H_9O_6$	$A-CHO-C_3H_6$

sible for the fragment A or B, formed by the splitting of the parent molecule.



Isolation and identification of fatty acids from Icmadophila ericetorum

The slightly soluble tetrahydroxy fatty acids obtained by extraction of this species have been discussed in the first part of this paper. Four compounds **A**, **B**, **C**, and **D**, were isolated by working up the acetone-soluble part of the extract.

The IR spectrum of compound A (soluble in chloroform) displayed bands characteristic of saturated, long-chain fatty acids. The spectrum was nearly identical with that of authentic *n*-C30 acid observed at our laboratory. A series of homologue molecular ions appeared in a HrMS. Furthermore, ions corresponding to the characteristic fragments represented by the formula $(CH_2)_nCOOH$ could all be assigned.

From the IR and HrMS results, compound **A** was identified as a mixture of *straight chain, saturated fatty acids homologues* of the general formula $C_nH_{2n}O_2$, where n represents all the values from 34 to 26. The acids C30, C32 and C34 dominate in the mixture. Hitherto such lipids have not been detected in lichen material.

Triglyceride from Icmadophila ericetorum

Small amounts of a lipid component were isolated by PLC-technique. The mass spectrum was highly characteristic, and all the major peaks were interpreted in terms of triglyceride structure by high resolution measurements.

Mixed triglycerides have mass spectra in which each acyloxy group is manifested by an ion $(M-RCOO)^+$. In the spectrum of the isolated compound B there were two peaks at *m/e* 577(63) $C_{37}H_{69}O_4$ and *m/e* 551(28) $C_{35}H_{67}O_4$ corresponding to the loss of two different acyloxy groups from the molecular ion. The two RCO^+ peaks at *m/e* 239(88) $C_{15}H_{31}CO$ and *m/e* 265(94) $C_{17}H_{33}CO$ confirmed this identification. Monounsaturation in the acyl moiety caused the abundant formation of $(C_{17}H_{33}CO-1)^+$ at *m/e* 264(81). Peak at *m/e* 576(19) $C_{37}H_{68}O_4$ indicated the loss of the palmitic acid fragment.

Other outstanding peaks occurring at masses 467 (8) $C_{28}H_{51}O_5$, 423(10) $C_{26}H_{47}O_4$, 281(57), and 256 (24) are due to the ions $[M-C_{15}H_{31}CO-C_9H_{18}]^+$, $[M-C_{15}H_{31}COO-C_{11}H_{22}]^+$, $C_{17}H_{33}COO^+$, and $C_{15}H_{31}COOH^+$ respectively.

The MS study revealed an additional type of fragments at *m/e* 393(32) $[C_{17}H_{33}CO+128]$, 367 (29) $[C_{15}H_{31}CO+128]$, 339(82) $[C_{17}H_{33}CO+74]$, and 313(72) $[C_{15}H_{31}CO+74]$. These type of ions, all containing the glycerol portion of the TG, are discussed by Lauer [13] and Litchfield [14] and confirmed the conclusions above. $(M-RCOOCH_2)^+$ fragments are not seen in the spectrum and therefore the positions of the fatty acids are not established. Double-bond mobility during fragmentation prevents the direct determination of double-bond location by this means.

On the basis of the results obtained from HrMS it is likely, that the isolated lipid (compound B) must be an *oleo-dipalmitin*, $C_{53}H_{100}O_6$, MW 832.

Isolation of phenol compounds from Icmadophila ericetorum

Evaporation of the preceding filtrates by working up the acetone extract left a dark-coloured, noncrystalline, solid residue, which was treated with ethanol. Two products **C** and **D**, positive to $FeCl_3$, FBB, diaz. sulph. and H_2SO_4 corresponding to phenol derivatives, were isolated by PLC. These compounds did not contain aldehyde groups.

The IR spectrum of compound **C** displayed bands due to aromatic hydroxyl and ester. High resolution mass spectrometry indicated the empirical formula $C_{26}H_{34}O_7$, MW 458. It is assumed that compound **C** is a depside or depsidone, but the structure must be regarded as unidentified.

*Compound **D*** homogeneous in TLC turned light yellow with diaz. sulph., gray-brown with sulfuric acid on TL plates, but no reaction with PD. Its UV spectrum displayed characteristic absorptions of a monocyclic phenol. The main IR frequencies in the higher part of the spectrum were detected at 1707 cm^{-1} (ester-carbonyl). This value is somewhat below the range of aromatic ester, probably due to intermolecular hydrogen bonding. Other bands are found at 1645, 1610, 1575 (aromatic) and 1253 cm^{-1} (ether/ester). The absorption in the region $2000-1667\ cm^{-1}$ compared with figures reported by Silverstein [15], and out-of-plane bending bands due to isolated hydrogen observed at 847, 830 and

Table III. Mass fragmentation of Icma D isolated from *Icmadophila ericetorum*. The results are based on accurate mass measurements.

m/e	R.I. [%]	Composition	Fragment−ion
221	16	$C_{13}H_{17}O_3$	$M-OC_2H_5$
220	57	$C_{13}H_{16}O_3$	$M-HOC_2H_5$
210	36	$C_{11}H_{14}O_4$	$M-C_4H_8$
196	5	$C_{10}H_{12}O_4$	$M-C_5H_{10}$
192	13	$C_{11}H_{12}O_3$	$M-C_4H_8-H_2O$
177	14	$C_{10}H_9O_3$	m/e 192 $-CH_3$
165	12	$C_9H_9O_3$	$M-OC_2H_5-C_4H_8$
164	100	$C_9H_8O_3$	$M-HOC_2H_5-C_4H_8$
137	15	$C_8H_9O_2$	m/e 165 $-CO$
135	21	$C_8H_7O_2$	m/e 164 $-CHO$

805 cm^{-1}, convinced us of the presence of substitution in the position 1, 2, 3, and 5 in the aromatic ring. Easy elimination of ethanol from the molecular ion in the HrMS unambiguously indicated that the ester group is adjacent to a hydroxyl group. Furthermore, the mass spectrum of compound **C** showed a parent ion at *m/e* 266.1511 (22). [$C_{15}H_{22}O_4$ requires 266.1517.) Mass number and relative intensities of other prominent fragment ions are summarized in Table III, where the compound is designated as *Icma D*.

Owing to the results of UV, IR and HrMS, the reasonable structure for compound **C** is considered to be *2-pentyl-4-methoxy salicylic acid ethyl ester*. Previously only one monocyclic phenol derivative, ethyl orsellinate, has been isolated from lichen material [2].

Experimental

Methods. Unless otherwise stated the determinations of melting points, the recording of UV, IR, MS and ^1H NMR spectra, and the performance of microanalyses and extraction technique were accomplished as described in previous papers [11, 12, 16]. Thin layer chromatographic testing (TLC) of the phenol compounds was carried out on precoated plates (Woelm, Merck, Germany); Silica gel GF/254 was used as adsorbant. The solvents A, B, C used in the identification attempts were the same as those reported by Culberson [17]. The spots were examined in UV light and visualized with 10% H_2SO_4 (heat by ca. 110 °C), *p*-phenylenediamine (PD), fast blue B (FBB), $FeCl_3$ in ethanol, and diazotized sulphanilic acid. Solvent C and benzene-heptane (75 + 25) were used in TLC of the 2,4-dinitrophenylhydrazones.

Silylation of Ochr.-X isolated from *Alectoria ochroleuca* and LrMS of the silylether was performed by Shrader Analytical, Detroit, USA.

The relative intensities of the fragment ions in mass spectrometric analyses are given in parenthesis in the text and figures. Trimethylsilyl is abbrevated to TMS.

Isolation of the lichen substances

The tetrahydroxy fatty acid mixtures were obtained by acetone extraction of all the three species investigated in this work. These were separated out of the extract prior to a diminishing of the volume. The acids were collected and purified by recrystallisation twice from boiling acetic acid. MS analyses of the hydroxy acids isolated and of authentic samples of 12-hydroxymethylstearate, 9,10-dihydroxymethylstearate and 9,10,16-trihydroxy palmitic acid are based on accurate measurements. Furthermore, MS comparisons were made with tetrahydroxy fatty acids isolated from the lichen species *Ramalina siliquosa, Ochrolechia tartarea, Parmelia centrifuga, Haematomma ventosum, Parmelia alpicola, Cetraria delisei,* and *Lecanora myrini.*

Alectoria ochroleuca (Hoffm.) Mass. This lichen (14.7 kg) was collected in Southern Norway. After filtration of the tetrahydroxy acids (m.p. 179 °C) the acetone solution was concentrated stepwise to obtain the known constituents of this species (diffractaic acid, usnic acid, sugar alcohols). The dark residue was partly purified by washing with cold ethyl acetate, ethanol and chloroform. The insoluble remainder was then extracted with hot ethanol, the solution treated with activated charcoal and filtered. A colourless and crystalline substance which separated from the ethanolic solution was recrystallized from dilute acetic acid (140 + 170), Ochr.-X. Yield 663 mg of a saturated lipid, m.p. 164 – 165 °C. By elementary analyses of the purified and dried sample the composition of the compound was found to be $C_{24}H_{48}O_6 \cdot H_2O$. [Found: C 64.1, H 10.4, O 25.6. Calcd: C 63.9, H 11.2, O 25.3.] IR (KBr): 3300 (broad, unresolved OH), 2915 and 2850 (CH stretch), 1735 (ester C = O), 1465 and 720 (CH_2 bend. & rock vibr.), 1440 (CH_3 asym. bend. vibr.), 1375 (CH_3 sym. bend. vibr.), 1240 (CH_3CO stretch. vibr.), and 1060 cm^{-1} (C – O stretch).

^1H NMR (60 MHz, DMSO – d_6): δ 4.12 (broad multiplet corresponding to methine protons of sec. alcohol groups and of $COOCH_2$), 2.00 (CH_3CO), 1.30 (CH_2), 0.87 (CH_3, distorted triplets).

Oxidation of Ochr.-X. A sulfuric acid solution of KJO_4 was added to a suspension of Ochr.-X

(286 mg) in ethanol. By continuous agitation at room temperature a completely clear solution with a characteristic smell of aldehydes was obtained within 22 hours. The details of the procedure have been described previously [8]. The aldehydes were trapped as their 2,4-dinitrophenylhydrazones and separated by PLC. HrMS data of the isolated hydrazones are summarized in Table I.

Stereocaulon vesuvianum var. *pulvinatum*. (Schaer.) Duncan for study was collected at Prestholtskarvet, Hallingskarvet, Buskerud. Complete extraction of the lichen sample (780 g) with acetone for 50 hours, yielded 2.3 g of tetrahydroxy fatty acid mixture, m.p. 178 °C. Acetylation of the mixture gave a tetraacetyl product, m.p. about 43 °C. Found 28.0% CH_3CO and an average MW of 604 (osmometric in pyridine). Calcd on $C_{33}H_{58}O_{10}$: CH_3CO 28.0%, MW 615.

After evaporation of the acetone solution to about 300 ml, 2.3 g of a mixture of atranorin and stictic acid precipitated. The filtrate was evaporated to a small volume and examinated by TLC. More stictic aicd, atranorin, atranol, and several other lichen constituents were found to be present in the concentrated solution. Three phenolic compounds **I**, **II**, **III** were separated by PLC, solvent C and subjected to further investigations.

Compound ***I*** was identified as constictic acid.

Compound ***II*** was identified as porphyrilin (decarboxyporphyrilic acid). IR(KBr): 1735 ($\alpha\beta$-unsaturated γ-lactone), 1610, 1590, 1510, 1465, 1443, 1335, 1140 cm^{-1}. UV(EtOH): λ_{max} 240, 258, 307 nm, shoulder 273, 296 nm, λ_{min} 249, 284 nm.

Compound ***III*** had the following chromatographic behaviours in solvent C:

Stictic acid	$R_f = 0.30$
Psoromic acid	$R_f = 0.52$
Compound **III**	$R_f = 0.65$.

The compound did not emit CO_2 from a solution of $NaHCO_3$. On the TL plates it turned orange with PD and the same colour with sulfuric acid. This compound was found to be a new depsidone which was named vesuvianic acid. UV(EtOH): λ_{max} 237, 311 nm, λ_{min} 292 nm, shoulder at 270 nm. IR(KBr): 3430 (intr. mol. bonded OH), 1730 (aromatic ester C=O and lactone), 1700 (C=O of aldehyde and other possible carbonyl groups), 1440 and 1380 cm^{-1} (CH_2, CH_3).

Icmadophila ericetorum (L.) Zahlbr. (611 g) was collected in Alvdal, Hedemark, during the summers of 1974–1975 and extracted with acetone for 14 hours. Thamnolic acid, tetrahydroxy fatty acids, and straight chain fatty acids were isolated as the insoluble part by tetrament of the residue with cold EtOH.

Thamnolic acid was recrystallized from dioxane, yield 0.4%.

Tetrahydroxy fatty acids, yield 0.5%, m.p. 194 °C.

Higher fatty acids (compound **A**), yield 0.0005%. IR(KBr): 2910 and 2845 (alkyl groups), 1710 (strong, C=O), 1410 (CH_2-group in α-position to the carbonyl), and 728–718 cm^{-1} (CH_2 rock. twist., orthorhombic packing of the molecule chain). HrMS (*m/e*, % R.I.): Homologous molecular ions at 508(26), 494(11), 480(100), 466(21), 452 (59), 438(9), 424(4), 410(4), and 396(1).

By subjecting the ethanol-soluble part of the extract to PLC, solvent B, oleo-dipalmitin (compound **B**) and two aromatic compounds, **C** and **D**, were isolated.

Compound ***C***. IR(KBr): 3350, 1660 cm^{-1}. HrMS (formula, *m/e*, % R.I):

$M - H_2O$ [$C_{26}H_{32}O_6$, 440(23)],
$M - CH_3OH$ [$C_{25}H_{30}O_6$, 426(3)],
$M - H_2O - CO$ [$C_{25}H_{32}O_5$, 412(9)],
$M - H_2O - OCH_3$ [$C_{25}H_{29}O_5$, 409(15)],
$M - CH_3OH - CO_2$ [$C_{24}H_{30}O_4$, 382(33)],
$M - H_2O - C_3H_7 - CO_2$ [$C_{22}H_{25}O_4$, 353(19)],
$M - H_2O - C_4H_9 - CO_2$ [$C_{21}H_{23}O_4$, 339(100)] and
$M - H_2O - C_5H_{10} - CO_2$ [$C_{20}H_{22}O_4$, 326(50)].

Compound ***D***. UV(EtOH): λ_{max} 260 nm ($\log \varepsilon$ 4.6), 300 nm ($\log \varepsilon$ 2.6). IR(KBr): 1707 (C=O ester), 1645, 1610, 1575 (aromatic). In Table III the compound is designated as *Icma D*.

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