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ALIPHATIC COMPOUNDS IN SOME LICHENS

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Key Word Index—*Cetraria cucullata*; *C. nivalis*; *Cladonia gonecha*; lichens; *n*-alkanols; *n*-alkanoic acids; hexa(α-hydroxyisovalerate); ribitol.

Abstract—The presence of free fatty acids in *Cetraria nivalis* and *Cladonia gonecha* has been demonstrated. Saturated wax esters occur in *Cetraria cucullata* and *C. nivalis*, whilst free alkanols were found in *C. cucullata*. Triglycerides in *C. nivalis* were detected by their MS fragmentation patterns. Hexa(α-hydroxyisovalerate) is present in *C. nivalis* and *Cl. gonecha*, and the presence of ribitol in *C. nivalis* was demonstrated by identification of its acetonide in an acetone extract.

INTRODUCTION

Work on lichen compounds has indicated that compounds which are extracted with ether and acetone constitute a complex mixture of difficultly separable substances, and that these substances form a major part of the extracts. It has not been possible to disentangle this problem, but the present work indicates that aliphatic compounds account for a major part of the extracts.

Culberson [1] states that long-chain aliphatic compounds have not been adequately characterised from lichens. The only works she quotes, are those, of Klima [2] dealing with *Alectoria ochroleuca* Ehrh. (= *Alectoria ochroleuca* (Hoffm.) Mass.), and of Wagner and Friedrich [3], dealing with the lichens *Parmelia physodes*, *Usnea barbata* and *Cetraria islandica*. Klima isolated di- and tetrahydroxystearic acids after alkaline hydrolysis and oxidation and concluded that oleic and linoleic acids had been present in his unsaponified material. Wagner and Friedrich (1965) used a TLC method and their results suggest that C_{16} acids with 1 and 3 double bonds and C_{18} with 1, 2 and 4 double bonds were the major unsaturated acids with traces of unsaturated C_{20} and C_{22} acids. Neither work reports saturated fatty acids.

RESULTS AND DISCUSSION

Cetraria cucullata. The MS of the material melting at 67–70° (see Experimental) indicated a mixture of esters. Table 1 gives the observed M^+ with relative intensities and measured compositions together with the observed, related fragment acids (observed as $C_nH_{2n+1}O_2^+$ but tabulated as $C_nH_{2n}O$).

GLC and MS of the saponified mixture confirmed the presence of the acids as found by MS of the mixture of esters and indicated that the even-numbered *n*-alkanols C_{16}–C_{28} were present. From the data in Table 1 it was concluded that the acids and alkanols are randomly combined to give the saturated wax esters. This idea obtained some support from metastable scanning, when it was found that all fragment acids in the wax ester mixture originated from all observed M^+.

Table 1. Observed composition of wax esters and related fragment acids (as $C_nH_{2n}O_2$) with their relative intensities in the MS of material (mp 67–70°) from *Cetraria cucullata*

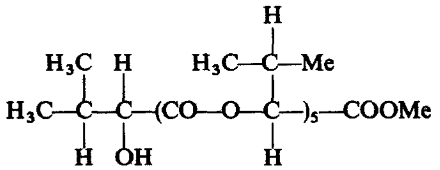
Esters Rel. intensity		Acids Rel. intensity	
0.2	$C_{50}H_{100}O_2$	2	$C_{26}H_{52}O_2$
0.5	$C_{48}H_{96}O_2$	5	$C_{24}H_{48}O_2$
1	$C_{46}H_{92}O_2$	9	$C_{22}H_{44}O_2$
2	$C_{44}H_{88}O_2$	9	$C_{20}H_{40}O_2$
2	$C_{42}H_{84}O_2$	4	$C_{18}H_{36}O_2$
0.5	$C_{40}H_{80}O_2$	*	$C_{16}H_{32}O_2$

* Complex peak with the protonated acid as the most volatile constituent; true intensity therefore cannot be given.

MS and GLC of the material melting at 75–78° (see Experimental) indicated the presence of the even-numbered alkanols C_{22}–C_{28}, again with a *ca* random distribution.

Cladonia gonecha. GLC of the distillates from the alkaline extracts (see Experimental) indicated the presence of the Me esters of palmitic, oleic and stearic acids as the major constituents.

The combined information from the PMR, MS and IR of the gum isolated from the Na_2CO_3 extract (see Experimental) indicated that the isolated gum was Me hexa(α-hydroxyisovalerate) (1).



$$H_3C-\underset{H}{\overset{H_3C}{C}}-\underset{OH}{\overset{H}{C}}-(CO-O-\underset{H}{\overset{H_3C-\overset{H}{C}-Me}{C}})_5-COOMe$$

1

Cetraria nivalis. The C_6H_6 eluates of the extracts obtained with alkali (see Experimental) by GLC and MS were shown to contain palmitic, oleic, stearic, nonadecanoic, and eicosanoic acids in the approximate proportions (11:4:4:4:1). This lichen contained trace amounts of hexa(α-hydroxyisovalerate).

Table 2. Observed composition of wax esters and fragment acids (as $C_nH_{2n}O_2$) with relative intensities in the MS of crystalline material (mp 75–85°) from *Cetraria nivalis*

Esters Rel. intensity		Esters Rel. intensity		Acids Rel. intensity	
2	$C_{52}H_{104}O_2$	21	$C_{44}H_{88}O_2$	10	$C_{24}H_{48}O_2$
3	$C_{50}H_{100}O_2$	16	$C_{42}H_{84}O_2$	19	$C_{22}H_{44}O_2$
6	$C_{48}H_{96}O_2$	8	$C_{40}H_{80}O_2$	23	$C_{20}H_{40}O_2$
12	$C_{46}H_{92}O_2$	1	$C_{38}H_{76}O_2$	15	$C_{18}H_{36}O_2$
2	$C_{45}H_{90}O_2$			42	$C_{16}H_{32}O_2$

MS of the material melting at 75–85° (see Experimental) indicated a mixture of esters. Table 2 lists the measured M^+ and the fragment acids with their abundances. The MS contained peaks also at higher *m/e* ([illegible] 744) but of intensities less than 1%. Again a random distribution and combination of alkanols and alkanoic acids are indicated. GLC of the saponified material confirmed the presence of the acids and indicated the presence of the even-numbered alkanols C_{22}–C_{28}.

MS of the first yellow zone obtained by column chromatography of the neutral part of the Me_2CO extract (see Experimental) contained M^+ and important fragments as given in Table 3. Saponification of the material furnished a small neutral fraction, a large acid fraction and some glycerol. By GLC the neutral fraction was shown to contain C_{22}, C_{23}, C_{28}, C_{30} *n*-alkanols. The acid fraction was shown to contain palmitic, oleic, linolic and stearic acids in the ratio of *ca* (2.5:64:16:1).

The composition of the peaks at *m/e* 882 and 880 indicated the presence of triglycerides containing stearic, oleic and linolic acids in appropriate combinations, whilst the composition of the peaks at *m/e* 856 and 854 likewise indicated triglycerides composed of palmitic, oleic and linoleic acids. The peaks *m/e* 603, 577, 399 and 262 are the strongest ones in the clusters of peaks with differences of one a.m.u. between the peaks of each cluster. In support of this contention MS were determined of the triglycerides trimyristin, triolein, trilinolein and trilinolenin, which all showed the same fragmentation pattern as above with clusters of peaks with one a.m.u. difference between the peaks in the regions of major fragments.

The fragmentation patterns are in agreement with those reported by Lauer *et al.* [4] for saturated triglycerides, except that the fragments [M^+–H_2C–O–OC–R] and [R–CO + 115] appear to be of lower abundance (1% or less) than reported by these authors. These authors also noted that the fragment [R–CO + 74] gave a prominent peak in the MS. In the present work it was found that the abundance of this peak decreased as the unsaturation increased: Trimyristin, 42%—triolein, 24%—trilinolein, 8%—trilinolenin, 2%.

Table 3. M^+ and important fragments with relative intensities in the MS of the first yellow zone of the neutral part of the acetone extract of *Cetraria nivalis*

m/e	Rel. intensity	Composition	Found	Calculated
884	4	not measured		
882	7	$C_{57}H_{102}O_6$	882.7659	882.7676
880	6	$C_{57}H_{100}O_6$	880.7494	880.7520
856	1	$C_{55}H_{100}O_6$	856.7504	856.7520
854	1	$C_{55}H_{98}O_6$	854.7370	854.7363
603	45	$C_{39}H_{71}O_4$	603.5340	603.5352
577	16	$C_{37}H_{69}O_4$	577.5183	577.5196
339	20	$C_{21}H_{39}O_3$	339.2901	339.2899
262	59	$C_{18}H_{30}O$	262.2298	262.2297
255	1	not measured		

The distillate from the fraction eluted with C_6H_6–Et_2O (1:1) (see Experimental) on 5% Carbowax 20M at 185° showed one peak with traces of another, whilst the distillate from the fraction eluted with Et_2O showed two peaks with R_t's of *ca* 4 and 4.5 min. On co-injection the single peak was indistinguishable from the faster of the two peaks. IR showed no C=O group, and PMR signals only in the C–CH_3 and O–Me regions, but a 2,4-dinitrophenylhydrazone was obtained from the material. These facts together with MS data suggested an acetonide of a pentitol of which ribitol and D-arabitol are common in lichens [1, p. 73–74]. Acetonides of the two pentitols were prepared as described by Nakagawa *et al.* [5]. By GLC they could not be distinguished on 5% Carbowax 20M or on a mixture of 1.5% XE 60 and 1.5% ethylene glycol succinate, but after acetylation they were readily separated on the latter column. In both cases two or more peaks were visible. The main component of the distillate from the material eluted with C_6H_6–Et_2O (1:1) after acetylation was indistinguishable from the faster, main peak of the acetylated acetonide of ribitol. It seems likely that this compound has been formed during extraction of the lichen by catalysis of free acids. Nevertheless, it does demonstrate the occurrence of ribitol in *C. nivalis*, which has not been previously reported.

The distillates of the Me esters from the acidic fractions from the Me_2CO extract contained palmitic, oleic, linoleic and stearic acids in the ratio of *ca* (2.5:7:33:1) whilst the larger residue contained the saturated acids C_{20} and C_{22}–C_{26}, inclusive, in the ratios of *ca* (1:3:2.5:5:5:2:2).

EXPERIMENTAL

GLC analyses (FID, N_2, 20 ml per min) of alkanols and Me alkanoates were performed with the stationary phases 5% SE 30 and 5% Silar 5 rcp at 190° and 250°, or at 170° and 220°, respectively. Extractions were done in a glass Soxhlet apparatus for 24 hr with Et_2O and 96 hr with Me_2CO. Identification of alkanols and acids (as Me esters) were obtained by co-injection with authentic substances and analysis of the MS of the mixtures, including accurate mass determinations. Coating of Si gel with oxalic acid was done by mixing 650 g of Si gel with 1 l. of 0.5 N oxalic acid and drying the mixture at 100°. Petrol refers to a fraction of bp 45–65°.

Cetraria cucullata (*Bell.*) *Ach.* The lichen (2.5 kg, air dried) was collected at Falksnydden, Driva, Oppdal, some 140 km south of Trondheim. The neutral material (12.6 g) was chromatographed on Al_2O_3. An intermediate fraction (100 ml) between the petrol and C_6H_6 eluates contained 570 mg, which afforded 30 mg of crystalline material mp 67–70° ($CHCl_3$–MeOH). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1733, PMR suggested saturated, aliphatic material. C_6H_6–Et_2O (1:1) eluted 700 mg, which also furnished a crystalline material from $CHCl_3$–MeOH, mp 75–78°; PMR suggested aliphatic material, IR 3300 cm^{-1}. The material which melted at 67–70° (10.5 mg) was saponified and separated into acid and neutral fractions.

Cladonia gonecha (*Ach.*) *Asah.* The lichen (1 kg, air dried) was collected in a peat bog along the railway line between Agle and Lurudal, 200 km north of Trondheim. The Et_2O extract was treated successively with a 5% soln of $NaHCO_3$, a M soln of Na_2CO_3 and a 0.5 M soln of NaOH until no further colouration of the extracts could be seen, to give 3.35, 2.14 and 0.36 g, respectively, with 6.2 g remaining as neutral matter. The sample was then extracted with Me_2CO. The Me_2CO soln was concentrated, poured into H_2O and extracted with Et_2O. The Et_2O extract on extraction with 0.5 N NaOH gave 5.3 g acids and 7.4 g neutral material. The acids from the Me_2CO extract contained much unstable material, which may have been phenolic since solns of the extract on standing deposited large amounts of a brown, amorphous ppt. The soluble portion of this extract was chromatographed on Si gel. Materials eluted with C_6H_6 and with $CHCl_3$ were combined (1.27 g) and chromatographed on Si gel–oxalic acid. After 220 ml of C_6H_6 had passed through the column, 839 mg were eluted with 2230 ml of C_6H_6. Conversion to Me esters and rechromatography on Si gel furnished 493 mg eluted with 75 ml C_6H_6 and 97 mg eluted with the next 60 ml. At 110–135°/0.2 T they furnished 422 and 91 mg of distillate, respectively.

$NaHCO_3$ extract. The acids were chromatographed on Si gel/oxalic acid. $CHCl_3$–MeOH (1:1) eluted 1.3 g (after oxalic acid had been removed). It was treated with CH_2N_2 and separated into acidic and neutral fractions. The neutral fraction afforded a distillate (110 mg) at a temp. up to 170°/0.05 T.

Na_2CO_3 extract. As above C_6H_6–$CHCl_3$ (1:1) eluted 498 mg, and $CHCl_3$–MeOH (1:1) eluted material, which after treatment with CH_2N_2 was separated into acid (260 mg) and neutral (39 mg) fractions. The neutral fraction gave a distillate (30 mg) at 125–150°/0.05 T. The material eluted with C_6H_6–$CHCl_3$ was treated with CH_2N_2 and repeatedly chromatographed on Si gel and finally by TLC $PF_{254+366}$/oxalic acid. The plates were developed twice with C_6H_6–$CHCl_3$ (1:1). From the "empty" space between start and the first zone visible under the UV 47 mg of Me hexa(α-hydroxyisovalerate) were isolated as a slightly coloured gum, $[\alpha]_D + 44°$ (*c*, 1.39, $CHCl_3$); PMR (60 MHz, $CDCl_3$) 1.08 (36H, *d*, *J* 6 Hz), 2.34 (6H, very broad *m*) (*iso*-Pr groups), 3.73 (3H, *s* (–COOMe), 4.13 (1H, *d*, *J* 4 Hz) ($\underline{H}$–Ç–OH), 5.01 (5H, overlapping *d*) (H–Ç–OOCR); MS showed M^+ at *m/e* 632 (1) ($C_{31}H_{52}O_{13}$), M^+-42 (7) ($C_{28}H_{46}O_{13}$), M^+-116 (12) ($C_{26}H_{44}O_{10}$), M^+-31 (3) ($C_{30}H_{49}O_{12}$) and subsequent losses of 100 ($C_5H_8O_2$) terminating at *m/e* 201 (100). Metastable peaks were present corresponding to all losses of 100. In the MS *m/e* 73 (C_4H_9O) was also base peak.

Cetraria nivalis (*L.*) *Ach.* Lichen (1 kg, air dried) was collected at Ålmenberget, Oppdal, and extracted with Et_2O and Me_2CO as described for *Cl. gonecha.* The Et_2O extract gave 1.04, 0.68 and 0.53 g after extraction with $NaHCO_3$, Na_2CO_3 and NaOH, respectively, with 2.71 g remaining as neutral material. The acid fractions were treated with CH_2N_2 and separated into acid (discarded) and neutral fractions, 332, 402 and 558 mg, respectively. The neutral esters were separated on Si gel to give 8, 31 and 71 mg, respectively, eluted with C_6H_6. Subsequent elution with Et_2O furnished 335 mg from the Na_2CO_3 extract. MS of this fraction indicated the presence of Me hexa(α-hydroxyisovalerate). Purification on Si gel and oxalic acid and development with C_6H_6–$CHCl_3$ (1:1) finally afforded 59 mg of slightly coloured gum, $[\alpha]_D + 41°$ (c, 2.20); PMR, IR($CHCl_3$) and Ms appeared identical with those of the compound isolated from *Cl. gonecha* (above).

Me_2CO extract. After sparingly soluble materials had been removed by filtration, the conc Me_2CO soln was poured into H_2O, extracted with Et_2O, and the Et_2O extract treated with 0.5 N NaOH soln to give 4.5 g of acids and 5.2 g of neutral material. Neutral material was chromatographed on Al_2O_3. The C_6H_6 eluate was separated into a first (1.7 g) and a second (138 mg) yellow zone. Only the MS of the first yellow zone contained peaks with an even number of hydrogen atoms, indicating that they might be M^+. C_6H_6–Et_2O (1:1) and Et_2O eluted 1.1 and 0.9 g, respectively, which furnished distillates at 90°/0.1 T. The identity of this material as the acetonide of ribitol is discussed above. A sample (109 mg) of the first yellow zone was hydrolysed with KOH in C_6H_6-MeOH (1:10). The reaction mixture was evaporated to dryness and the residue was treated with H_2O and Et_2O. The aq layer was acidified with H_2SO_4 and again treated with Et_2O. The separated acid and neutral fractions contained 89 and 6 mg, respectively. The aq soln was neutralised with KOH and evaporated to dryness. Residue was treated with EtOH, filtered and the filtrate evaporated to *ca* 1 ml, and weighed amounts of hexadecanol and octadecanol were added. After conversion to the acetates, cf. ref. [6], the reaction mixture was evaporated to dryness and subjected to GLC on 5% DEGS at 170° or 160°. The presence of triacetin was demonstrated by co-injection with the authentic substance, and the amount of glycerol in the original sample was found as 8.3 and 8.5 mg, based on hexadecanol and octadecanol, respectively. The acidic substances (4.5 g, above) were treated with MeOH. Some (1.9 g) did not dissolve and was removed by filtration. The remainder was treated with CH_2N_2 and chromatographed on Si gel. The first 400 ml of C_6H_6 eluted 127 mg, which afforded a distillate (98 mg) at 105–135°/0.2 T as a colourless oil, and a residue (18 mg) as a faintly yellow paste. The next 450 ml of C_6H_6 eluted 20 mg which furnished a distillate at 90–142°/0.2 T. The neutral material from the Et_2O extract (2.71 g) was chromatographed on Si gel. Petrol–C_6H_6 (1:1) eluted 144 mg, which from $CHCl_3$–MeOH gave 50 mg of crystalline material mp 75–85°. Saponification of this material (10 mg) afforded an acid (4.4 mg) and a neutral (6.9 mg) fraction.

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