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Studies on the Chemistry of Lichens

III *. Long-Chain Tetrahydroxy Fatty Acids from Some Norwegian Lichens

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Norwegian lichen species have been studied with regard to content of higher tetrahydroxy fatty acids. The lichens are found on the bark of trees, on rocks, and on the ground. The hydroxy acids isolated are of the same type as those previously recorded in *Haematomma ventosum*. Infra-red spectra of the acids in solid state have been obtained in the region from 4 000 to 700 cm^{-1} and confirm the similarity.

Semi-solid and solid fatty acids were obtained by reduction with hydrogen iodide and zinc. Paper chromatographic analysis of the products obtained by periodic acid oxidation of ventosic acid from *Haematomma ventosum* showed the presence of tetrahydroxy *heneicosanoic* acid ($C_{21}H_{42}O_6$) and tetrahydroxy *docosanoic* acid ($C_{22}H_{44}O_6$) in the lichen.

Work on *Parmelia centrifuga*, has pointed out the possibility of the presence of tetrahydroxy *tricosanoic* acid ($C_{23}H_{46}O_6$) in the lichen.

Systematic investigations of Norwegian lichen species were made during the period 1955 to 1959. The presence of higher tetrahydroxy fatty acids in some lichens was of special interest. In 1957 ventosic acid was obtained from *Haematomma ventosum* and reported [1] as a tetrahydroxy *docosanoic* acid with the composition $C_{22}H_{44}O_6$. Later uniform substances have been isolated from the lichen species *Parmelia centrifuga, Parmelia encausta, Parmelia furfuraceae, Parmelia physodes, Cetraria nivalis, Cetraria delisei, Cladonia alpestris, Alectoria ochroleuca*, and *Siphula ceratites*.

The plant material was collected in different parts of the country and before the extraction, the material was dried and ground into coarse powder. The extraction process was carried out first with ether for about 24 h, and subsequently with acetone for 48 h in a Soxhlet extractor designed to take 400—500 g of the material. As a rule the main portion of the acids were separated already

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under the acetone extraction and could therefore easily be isolated in a pure condition.

After the solvents had been removed from these extracts, the highly coloured solids were examined separately in the usual manner [2,3]. Analytical data will be given only for those compounds for which values have not been reported in the literature. From *Parmelia centrifuga* a relatively larger portion of sugar alcohol and from *Siphula ceratites* a new lichen substance with the probable formula $C_{19}H_{21}O_6$ have been isolated. The investigations are being continued and complete data will be given in a forthcoming paper.

The hydroxy fatty acids which have been isolated so far, at once distinguished themselves by an especially low solubility in the common organic solvents. The acids are less soluble in alcohols, more soluble in warm dioxane, pyridine and glacial acetic acid. Glacial acetic acid has in all cases proved to be the most suitable recrystallization solvent, from which an easily filterable product has been obtained.

The hydroxy fatty acids are colourless, amorphous substances with melting points in the region 180—193° (*cf.* Table 1). Occasionally two different melting points for the same hydroxy acid have been observed. This is probably due to the presence of *cistrans* isomerism, and is otherwise known from tetrahydroxy stearic acid.

The lichen species *Haematomma ventosum* and *Parmelia centrifuga* are distinguished by a particularly high amount of these acids, a fact which gave rise to a further investigation. In both cases the hydroxy acids were reduced to the corresponding fatty acids. However, these seemed not to be identical, but differed with regard to solubility, melting points and by paper chromatographic analysis (Tables 2 and 3).

In *Parmelia physodes* hydroxy fatty acids have been established by means of solubility, X-ray, and infra-red spectra. However, the sample was contaminated with a tenacious impurity which rendered purification extremely difficult and wasteful. The yield of the crude product was too low and further analysis was not possible.

RESULTS AND DISCUSSION

In Table 1 a series of experimental results of the isolated hydroxy fatty acids, or acid-mixtures, from nine different lichen species are listed. The average composition is: C 66.33 %; H 11.05 %.

It is interesting to compare these results with those reported by Zellner [4,5], who isolated the lichen substances *Hypogymnole* I and II from *Parmelia physodes*.

Compound	M.p.	Found by Zellner	
Hypogymnole I	190°	C 64.39 %	H 11.12 %
Hypogymnole II	218°	C 63.64 %	H 11.16 %

Table 1. Analysis of the isolated tetrahydroxy fatty acids.

Lichen species	Yield of purified hydroxy acid, %	Melting point	Analysis, values obtained % C	% H	Characteristic strong peaks in the X-Ray diffraction pattern (Å)			
Haematomma ventosum	0.5	184°	65.29	10.88	32.5	—	4.55	4.05
Parmelia centrifuga	0.5	191°	64.77–64.37	10.84–10.68	31.6	20.5	4.54	4.04
Parmelia furfuraceae	0.2	191–193°	65.68–66.45	10.82–10.92	33.0	22.1	4.56	4.05
Parmelia encausta	—	180°	65.58	11.00	—	22.1	4.57	4.06
Cetraria nivalis	0.2	181°	66.16–66.78	11.02–11.02	—	—	—	—
Cetraria delisei	0.03	184–186°	66.98	11.23	—	—	—	—
Cladonia alpestris	0.2	191°	66.31–67.64	11.18–11.32	32.4	21.5	4.54	4.05
Siphula ceratites	0.1	182°	68.39	11.61	—	23.6	4.51	4.04
Alectoria ochroleuca	0.05	179°	67.06	11.21	33.2	23.2	4.54	4.04

In the present work the attempts made to resolve the acid-mixture from *Parmelia physodes* failed. Zellner indicated that these two compounds might be *higher aliphatic alcohols*. Today it is fairly obvious that Zellner's *Hypogymnoles* are identical with the isolated hydroxy fatty acids.

The X-ray diffraction patterns show two characteristic peaks at 4.54 and 4.05 Å. With the normal fatty acids the two corresponding peaks are found at 4.1 and 3.7 Å. The technique for the X-ray analysis has already been described in Part II of this series, and two diffraction patterns have been recorded in the same paper.

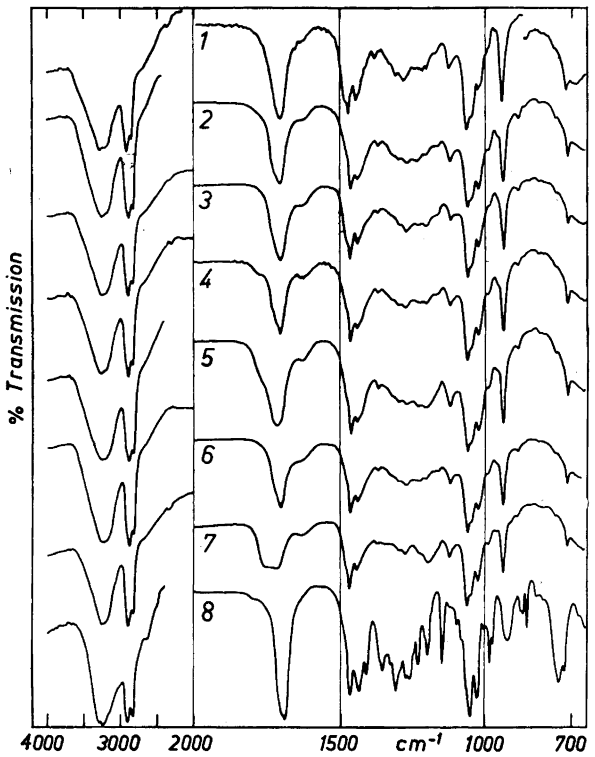
The infra-red absorption spectra of the hydroxy acids in Fig. 1 show a surprisingly close similarity. Bayzer [6] reported broad absorption bands at 3 200—3 300 cm^{-1} for C_{18}-hydroxy acids due to the valence bands of the OH-groups. The hydroxy acids in Fig. 1 show a broad unresolved OH-band around 3 270 cm^{-1}. Further, it is worth noting that this band shows a constant intensity, compared with the well known asymmetric carbon-hydrogen stretching band around 2 900 cm^{-1}. According to Bayzer, all isolated hydroxy acids should contain four OH-groups in the molecule. In all of the IR analyses carried out, the ratio of hydroxy acid to KBr has been nearly constant, 1.5 mg/200 mg. *Previously ventosic acid from Haematomma ventosum has been shown to contain four OH-groups.*

Susi [7] pointed out that the constant position of the 3 270 cm^{-1} band would be expected to depend on a similar position of the hydroxyl groups in the molecule. The associated carboxyl group exhibits a weak band at 2 700 cm^{-1}. The carboxyl band appears to be constant at 1 700 cm^{-1}.

The infra-red spectra are nearly identical above 1 500 cm^{-1}. Curiously enough only a few, effaced bands are found in the region 1 180—1 350 cm^{-1} compared with the spectra of sativic acid (No. 8, Fig. 1).

The bands at 1 465 cm^{-1} and at 715 cm^{-1} in the spectra are attributed to bending and rocking methylene vibrations [8,9].

Spectra of mono-, di-, tetra- and hexahydroxy stearic acid are reported by Bayzer. These spectra show a close similarity to those obtained in the present paper.



Figs. 1—8. Infrared spectra of tetrahydroxy acids from *Haematomma ventosum*(1), *Alectoria ochroleuca*(2), *Parmelia centrifuga*(3), *Cladonia alpestris*(4), *Cetraria nivalis*(5) *Cetraria delisei*(6), *Parmelia furfuraceae*(7) and *Sativic* acid(8). All substances are pressed in KBr-disks.

Several paper-chromatographic analyses of the hydroxy fatty acids have been tried without success.

Reduction to fatty acids. Upon treatment of ventosic acid from *Haematomma ventosum* and the hydroxy acid component from *Parmelia centrifuga* with hydrogen iodide and zinc, as described in the earlier publication, the com-

Table 2. Fatty acids obtained by the reduction of the hydroxy acids isolated from *Haematomma ventosum* (H.v.) and *Parmelia centrifuga* (P.c.).

Fatty acid and the related methyl ester	Melting point	Found by analysis C	H	CH_3O	Calculated C	H	CH_3O
Docosanoic acid (H.v.)	74—75°	77.54	12.82		77.55	13.02	
» » methyl ester	52—53°	78.10	13.01	9.89	77.90	13.08	8.75
Tricosanoic acid (P. c.)	69—70°	77.92	13.03		77.90	13.08	
» » methyl ester	53.5°	78.06	13.09	7.97	78.19	13.12	8.42

Table 3. Ascending paper chromatography of the fatty acids obtained by the reduction of hydroxy fatty acids from *Haematomma ventosum* (H.v.) and *Parmelia centrifuga* (P.c.). 50 μg of the fatty acid was chromatographed. The paper was impregnated with A) *n*-undecan (0.23), B) liquid paraffin (0.18), and C) liquid paraffin (0.16). Paper S & S 2043 b. Temperature 47°, 4 h.

Fatty acids	A	B	C
Autentic n-*docosanoic* acid	—	0.35	—
Docosanoic acid (H.v.)	0.18	0.35	0.45
Tricosanoic acid (P.c.)	0.13	0.27	0.35

pounds are converted into *n*-fatty acids. The fatty acids were white, crystalline substances and the elementary results are given in Table 2.

Paper chromatography of the fatty acids. During the period 1954 to 1958, Kaufman and Wagner [10–12] described some paper chromatographic methods for higher *n*-fatty acids. These methods have been useful in the present chromatographic investigations. The results, which are given in Table 3, confirm the fact that *n*-docosanoic acid (C_{22}) is the main component of the reduction products from ventosic acid. The reduction product from *Parmelia centrifuga* is the higher homologous tricosanoic acid (C_{23}). These observations are in agreement with the elementary analysis (Table 2).

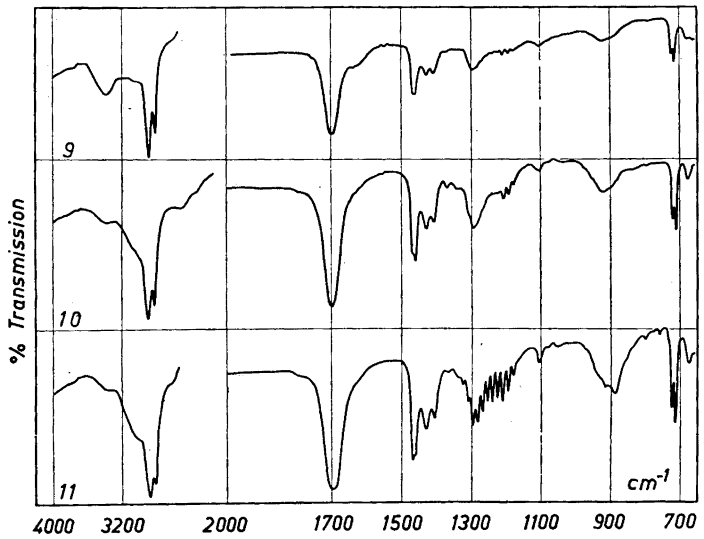
Infra-red analysis of the fatty acids. The infra-red absorption spectra of the fatty acids are shown in Fig. 2. Examination of the spectra of the free acids by the potassium bromide disk technique has definite advantages, and the spectra recorded in this paper show distinctly that by the classical reduction of the hydroxy fatty acids with hydrogen iodide and zinc, long-chain normal fatty acids have been obtained.

The IR-spectra of long chain fatty acids as a class have certain characteristic features which have been discussed by other authors [9,13–16].

The carbonyl stretching band of the carboxyl acid group appeared at very nearly the same wave number, 1 700 cm^{-1}. The two strong bands at 2 900 and 2 830 cm^{-1} are attributable to the asymmetrical and symmetrical methylene stretching and vibrations, respectively.

Strong complex absorption occurs between 880 and 930 cm^{-1}. The bands at 1 460—1 470 cm^{-1} and at 720 cm^{-1} in the spectra of the fatty acids are attributed to bending and rocking methylene vibrations, respectively. The splitting of the 720 cm^{-1} rocking band into two components, in the solid phase spectra, is in accordance with the observations of Stein and Sutherland [17] for *n*-paraffins below the transition point. Some or all of the absorption bands occurring between 1 460 and 1 400 cm^{-1} can probably be attributed also to methylene bending vibrations.

Of particular interest for the study of the spectra is the absence of progression of bands between 1 180—1 350 cm^{-1} spaced at approximately equal intervals of 15—20 cm^{-1}, (compare with spectra of pure *n*-docosanoic acid in the same figure). The progression bands are almost certainly associated with



Figs. 9—11. Infrared spectra of *docosanoic* acid and reduced hydroxy acids from *Haematomma ventosum* and *Parmelia centrifuga*. All substances are pressed in KBr-disks.

methylene wagging and/or twisting vibrations. The introduction of substituents along the molecule-chain alters the appearance of these bands. If the degree of substitution is excessive, the characteristic appearance of the progression is lost. In this work, branched acids have not been established. Infrared spectra of monocarboxylic acids are fairly insensitive in relation to homologous. Therefore, the lack of bands in the region 1 180—1 350 cm^{-1} can scarcely be due to the presence of fatty acid mixtures.

Oxidation of ventosic acid from Haematomma ventosum. A sufficient amount of ventosic acid was available from *Haematomma ventosum* and enabling an

Table 4. R_x values of the 2,4-dinitrophenylhydrazones obtained with the aldehydes from the oxidation of the hydroxy compounds with HIO_4. Paper: S & S 2043b, impregnated with 2-phenoxyethanol. Solvent *n*-heptan and water.

2,4-Dinitrophenylhydrazone of	R_x		
Autentic *n*-decanal			1.00
n-Nonal from 9,10-dihydroxystearic acid		0.89	
n-Hexanal from 9,10,12,13-tetrahydroxy stearic acid	0.58		
Azelaic half-aldehyde ($OHC \cdot (CH_2)_7 \cdot COOH$) from 9,10-dihydroxy stearic acid	0.18		
Azelaic half-aldehyde from 9,10,12,13-tetrahydroxy stearic acid	0.19		
Oxidation products from ventosic acid (*Haematomma ventosum*)	0.17	0.86	1.02

examination of the products obtained by oxidation with HIO_4. In this way the position of the four OH-groups in the molecule were determined.

A sulphuric acid solution of KIO_4 was added to a suspension of ventosic acid in 96 % ethanol. Within a short time and by continuous shaking an almost clear solution with a characteristic smell of higher aldehydes was obtained. After concentration, the aqueous solution was steam distilled and the volatile aldehyde compounds converted into 2,4-dinitrophenylhydrazones. The main amount of the distillate proved to be *n*-decanal. However, small amounts of hydrazones were obtained by careful examination of the filtrate. By chromatographic analysis of the last fraction mentioned, the presence of *n*-nonal and azelaic-half-aldehyde was demonstrated.

For comparison by the chromatographic analysis, di- and tetrahydroxy stearic acid also were oxidized in the same manner, and the following substances were obtained:

Compound	Oxidation products
9,10-dihydroxy stearic acid	*n*-nonal azelaic half-aldehyde
9,10,12,13-tetrahydroxy-stearic acid	*n*-hexanal azelaic half-aldehyde

Chiefly the method of King [18] was used in the oxidation with HIO_4. The hydrazones were separated on paper S & S 2043b, impregnated with 2-phenoxyethanol, (Lynn [19]). The results are summarized in Table 4.

Polymerization products constituted the main part of the non-volatile matter produced under the steam distillation. They were not further examined.

All things considered the facts given in Tables 3 and 4 lend support to the conclusion that a mixture of 9,10,12,13-tetrahydroxy docosanoic acid ($C_{22}H_{44}O_6$) and 9,10,12,13-tetrahydroxy heneicosanoic acid ($C_{21}H_{42}O_6$) must be present in the lichen. By the reduction of the mixture of the two tetrahydroxy acids, and the repeated recrystallization of the reduction products, the heneicosanoic acid was lost. As already mentioned, only docosanoic acid has been recognized on the paper chromatogram. It is, therefore, likely that only a small amount of tetrahydroxy heneicosanoic acid is present in the lichen.

It is known that mixtures of the normal fatty acids C_{21} and C_{22} give melting points in the region 74.9—75.2° [20]. This is in agreement with the melting point found in the present work.

Nor has the reduction product of the tetrahydroxy acid from *Parmelia centrifuga* any distinct melting point. There are some indications that a mixture of two acids is present, with tricosanoic acid (C_{23}) as the main component.

Reduction of tetrahydroxy acids from the lichen species *Parmelia encausta*, *Parmelia furfuraceae*, *Cetraria nivalis* and *Cladonia alpestris* have also been made. Solubilities, melting points and paper chromatographic analysis undoubtedly indicated the presence of mixtures. Work is in progress with a view to establishing the different tetrahydroxy acids in these lichens.

At times it has been supposed that these tetrahydroxy fatty acids might be compounds caused by the extraction of the lichens. However, an intense research in this field, has yielded no facts supporting this hypothesis. It is therefore reasonable to assume that the acids may be a result of biochemical oxidations in the lichens. In 1932 oleic- and linoleic acid were isolated from *Alectoria ochroleuca* by Klima [21].

EXPERIMENTAL

Extraction and isolation of the lichen substances from the different lichen species were carried out according to the procedure described in Part II. The analytical results are summarized in Table 1.

In the same paper reduction with HI and Zn, and methylation of the fatty acids obtained are also described in detail. The analytical results of the fatty acids are given in Table 2.

Oxidation of ventosic acid. The double amount of KIO_4 in N H_2SO_4 (40°) was added to a suspension of ventosic acid in 96 % ethanol (20°), and the mixture shaken for 30 min. The reaction mixture was then filtered, diluted with the same volume of water, and extracted with ether. The ether extract was filtered, concentrated, and distilled with steam. Only polymerisation products could be detected in the distillation remainder.

The aldehydes in the distillate were converted into 2,4-dinitrophenylhydrazones. The crude adduct obtained was filtered off and recrystallized several times from hot 96 % ethanol. The pure hydrazone crystallized as yellow prisms; m.p. 104—105° undepressed when mixed with the authentic hydrazone of *n*-decanal. (Found: C, 7.01; H 7.31; N 16.70. Calc. for $C_{16}H_{24}O_4N_4$: C 57.13; H 57.19; N 16.66.)

The crude hydrazone product obtained by concentration of the filtrate above was crystallized, and melted in the 76—93° range. This mixture of hydrazones was subjected to chromatographic investigations. The results are summarized in Table 4.

The oxidation of di- and tetrahydroxy stearic acids was carried out according to the method described by King [18]. The oxidation products were converted into 2,4-dinitrophenylhydrazones, purified by extraction with petroleum ether (60—70°) and recrystallized from ethanol.

In the X-ray diffraction analysis, a Philips X-ray Geiger Counter Spectrometer with $CuK\alpha_1$ radiation was used.

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