Studies on the Chemistry of Lichens. XI. Chemical Investigations of Five Norwegian *Alectoria* Species

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Five Norwegian *Alectoria* species have been studied with regard to their chemical content. A mixture of higher tetrahydroxy fatty acids and 5,7-dihydroxy-6-methyl phthalide have been isolated from *A. nigricans*. Fumarprotocetraric acid was identified as the main component of *A. bicolor*. From *A. fremontii* was isolated a crystalline sterol, of the composition $C_{29}H_{48}O_2$, fremontol, for which a probable structure is proposed. A mixture of higher homologues of tetrahydroxy fatty acid alkyl esters was isolated from the same lichen. Atranorin and the aminosulfonic acid taurine, were detected in all the species investigated.

A number of species of the *Alectoria* have been chemically studied by different research workers. Recently a survey was given by Huneck and Follmann¹ concerning the secondary metabolites of 21 species of the genus *Alectoria*. Hawksworth²⁻⁴ reported extensive investigation of the same genus. Alectorialic acid, thamnolic acid, and atranorin were reported to be the frequently occurring chemical constituents of these lichens. The excellent publications of Culberson⁵⁻⁶ have been of great value to the present work.

In a previous communication from this laboratory⁷ investigations on the occurrence of alectorialic and decarboxyalectorialic acid in *A. nigricans* and *Parmelia alpicola* have been reported. The structure of alectorialic acid has recently been established by Persson and Santesson.⁸

The present report describes a chemical examination of the species *Alectoria nigricans*, *A. capillaris*, *A. nadvornikiana*, *A. bicolor*, and *A. fremontii*. No attention has been given to the occurrence of sugar alcohols in the species studied.

Alectoria nigricans (Ach.) Nyl. *Investigation of the tetrahydroxy fatty acids.* The hydroxy acids were obtained as a white powder, m.p. 181–182 °C from the acetone extract of the lichen (fraction 1; found C 65.85; H 11.05; O 22.77). Analyses of hydroxy acids from five other lichen species (*Haematomma ventosum*, *Ochrolechia tartarea*, *Lecanora myrinii*, *Ramalina siliquosa*, and *Parmelia alpicola*) in our laboratory gave the average values C 65.97; H 11.01; O 22.82. On account of the close similarity that always has been found by our laboratory⁹ with regard to the elemental composition and the infrared spectra of these hydroxy compounds, and on the basis of the publication of Susi,¹⁰ it is expected that the four hydroxyl groups have the same positions (9,10,12,13) in the acid molecules in all the investigated cases.

The NMR spectra of the isolated tetrahydroxy fatty acids from *A. nigricans* in DMSO- d_6 at 73 °C, exhibited signals corresponding to methyl-protons (δ 0.87) and straight chain methylene-protons (δ 1.28).

It was found expedient to carry out investigations on the acid mixture and this sufficed to unravel the structures of the components. Four homologous species are discernible in the mass spectrum of the mixture. The interpretation of the low resolution mass spectrum has been made by comparing it with the spectra of authentic di- and tetrahydroxystearic acid. The spectrum showed series of strong peaks corresponding to ions of the empirical formula $C_nH_{2n+1}^+$, $C_nH_{2n}^+$, and $C_nH_{2n-1}^+$. Base peaks were found at masses 57(C_4H_9), 71(C_5H_{11}), and 97(C_7H_{13}), but no peak for a molecular ion.

Table 1. Masses (m/e) and relative abundances (%) of significant fragment-ions in the low resolution mass spectrum of tetrahydroxy fatty acid mixture isolated from *Alectoria nigricans*, $\text{CH}_3-(\text{CH}_2)_n-\text{CH}(\text{OH})-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}(\text{OH})-(\text{CH}_2)_7-\text{COOH}$, and of 9,10,12,13-tetrahydroxy-stearic acid ($n=4$).

Fragment-ions	$n=4$ m/e	%	$n=6$ m/e	%	$n=7$ m/e	%	$n=8$ m/e	%	$n=9$ m/e	%
I	101	14	129	65	143	13	157	9	171	13
I-H ₂ O	83	58	111	69	125	31	139	21	153	13
II	113	70	141	27	155	36	169	17	183	21
III	157	85	185	14	199	93	213	16	227	40
III-H ₂ O	139	43	167	12	181	21	195	9	209	13
III-2H ₂ O	121	40	149	38	163	39	177	7	191	12
IV	169	18	197	14	211	12	225	9	239	8

The most significant peaks in the spectrum of the acid mixture correspond to the fragment-ions

and

where n has the values of 6, 7, 8, and 9. The masses of all the different fragments and their relative abundances are listed in Table 1. The elemental composition of the fragments has been confirmed by high resolution mass spectrometry. The fragments given by 9,10,12,13-tetrahydroxystearic acid are also included in the same table.

It should be emphasized that the epoxi-structures depicted above are only formal representations.

Three important metastable ions are found in the spectrum corresponding to the following fragmentation processes:

The metastable ion at m/e 133.5 indicates that the ion m/e 163 is formed, in part, by a one-step loss of two molecules of water.

High resolution mass spectrometry has shown that the ion at m/e 99 (96 %) corresponds to the fragment

As a result of the data reported above it must be concluded that the hydroxy fatty acids isolated from *A. nigricans* contained significant proportions of 9,10,12,13-tetrahydroxy fatty acids with chain length of C_{20} , C_{21} , C_{22} , and C_{23} . The presence of such hydroxy acids with chain length of C_{21} , C_{22} , and C_{23} in the lichens *Parmelia centrifuga* and *Haematomma ventosum* has been pointed out in an earlier publication.⁹

Alectoria capillaris (Ach.) Croub. and *A. nadvornikiana* (Gyeln.) separately were extracted with acetone. TLC and IR of the different fractions which were obtained by working up the residues, demonstrated that the species both contained the components *atranorin*, *barbatolic acid*, *alectorialic acid*, and decomposition products of these compounds. Mass spectrometry confirms the conclusion obtained by TLC. Dominant and characteristic peaks are

found at m/e 194 (36 %), corresponding to 5,7-dihydroxy-6-formyl phthalide (A), 176 (36 %, $A-H_2O$), 166 (79 %, $A-CO$), and 137 (34 %, $A-CO-CHO$).

In addition to the compounds mentioned above, *A. capillaris* is reported to contain norstictic, psoromic, and salazinic acid. These compounds have not been detected in the sample collected here.

Alectoria bicolor (Ehrh.) Nyl. A sparsely soluble acid obtained by extraction of this lichen was identified as *fumarprotocetraric acid* by comparison with an authentic sample using TLC. This conclusion was supported by IR and MS analyses of the acid. A dominant peak in the mass spectrum at m/e 116 corresponds to ionized fumaric acid. A metastable peak was observed at m/e 66.6 in the pattern of the isolated fumarprotocetraric acid. The same metastable peak by authentic acid was found at 66.6, and by authentic fumaric acid at m/e 66.7. The attached process is

Three products, A, B, and C, positive to *p*-phenylenediamine were isolated by PLC from the acetone-soluble part of the extract, Fraction 2. On the basis of colour reactions, TLC, UV, and MS analyses the products were found to consist of *fumarprotocetraric* and *decarboxythamnolic acid* (A), *decarboxyalectorialic acid* (B), and *atranorin* (C).

On further investigation of Fraction 2, an unsaturated sterol compound was isolated. TLC on silica gel with solvent C revealed a spot with R_F less than 0.20. Spraying with sulfuric acid and heating gave a gray-bluish coloured spot. Extraction of the sterol-zone from preparative plates, afforded a residue sufficient for TLC and MS analyses. The lower part of the mass spectrum revealed the characteristic sterol fragmentation known from the literature.¹¹ The molecular ion which corresponds to the formula $C_{28}H_{44}O_2$, was observed at m/e 412 (6 %). The most important observations from the mass spectrum are summarized in Table 2. Owing to the lack of sufficient material, a complete structural determination of this sterol could not be accomplished.

Alectoria fremontii (Tuck.) From a smaller Acta Chem. Scand. B 29 (1975) No. 2

Table 2. Mass fragmentation of the sterol isolated from *Alectoria bicolor*. The side-chain is designated R.

m/e	%	Fragment-ion
397	3	$M-CH_3$
394	6	$M-H_2O$
383	5	$M-29$
379	7	$M-CH_3-H_2O$
376	6	$M-2H_2O$
369	3	$M-C_3H_7$
287	4	$M-R$ ($R=C_9H_{17}$)
285	5	$M-(R+2H)^a$
269	8	$M-R-H_2O$
267	7	$M-(R+2H)-H_2O$
251	18	$M-R-2H_2O$
249	7	$M-(R+2H)-2H_2O$
228	100	$M-R-C_3H_5-H_2O^b$
227	41	$M-R-C_3H_5-H_2O^b$

^a C_9 -unsaturated side-chain. ^b Cleavage of ring D.

sample of the lichen *vulpinic acid* was isolated by PLC on Woelm SiO_2 plates and solvent C. TLC of the isolated and authentic vulpinic acid on activated SiO_2 plates proved their identity. The solvents used were hexane-chloroform-acetone (2:3:1) and solvent C.

From the greater part of the lichen material three fractions were obtained according to solubility. TLC of the light petroleum extracted part of the residue (I) showed a complex mixture of components. Four of these components were isolated and investigated by TLC, IR, and MS.

Fremontii 1 gave a yellow colour with *p*-phenylenediamine and a faint yellow colour with sulfuric acid on the chromatograms. With solvent A the component has an R_F -value quite different from that of alectorialic, norstictic, or thamnolic acid.

Its mass spectrum showed a probable molecular peak at m/e 372. Peaks for $M-H_2O$ (m/e 354), $M-CO_2$ (m/e 328), $M-CO_2-CO$ (m/e 300), and $M-CO_2-CHO$ (m/e 299) were present. The substance must be regarded as unidentified.

Fremontii 2 gave a strong yellow colour with *p*-phenylenediamine, gray-black or brown colour with sulfuric acid and heat on TLC plates. Yellow colour with KOH, and a yellow-brown colour with ferric chloride in ethanolic solution. A long series of known lichen substances have

been cochromatographed with Fremontii 2, but it is still unidentified.

Fremontii 3. By means of TLC with three different solvents and spraying with *p*-phenylenediamine, sulfuric acid and bisdiaz. benzidine, the component was identified as *atranorin*.

Fremontii 4 gave a dark blue colour with phosphomolybdic acid, and gray-green or green colour with sulfuric acid on TLC plates. Ergosterol and sitosterol, in comparison, gave brown and violet colour reactions with the same reagent, respectively. The compound was recrystallized from ethanol-water (4:1). Faint-yellow-coloured crystals, yield 3.8 mg, showed only one spot on the chromatograms and gave an unambiguous and easily interpretable mass spectrum.

The compound, for which the name *fremontol* is proposed, was soluble in conc. sulfuric acid with a strong red colour, and the solution fluoresced yellow-green or green in UV-light. An acetic anhydride solution gave a blue-bluegreen-green reaction with one drop of sulfuric acid; Liebermann-Burchard reaction. A chloroform solution gave a pale yellow colour reaction with tetranitromethane, indicating that fremontol was an unsaturated sterol.

The IR spectrum, Fig. 1, of the compound displays characteristic bands of a sterol, and exhibits marked similarity with the spectrum of ergosterol. The spectrum contains bands at 3390 and 3520 cm^{-1} corresponding to more than one hydroxyl group. The small band at 1735 cm^{-1} is a reflection of the strong hydrogen bonded hydroxyl group at 3390 cm^{-1} . The spectrum

Table 3. Mass fragmentation of *fremontol* isolated from *Alectoria fremontii*. The fragments A and B are explained in Fig. 2.

<i>m/e</i>	% Abundance	Fragment-ion
413	4	M-CH ₃
411	5	M-OH
410	9	M-H ₂ O
397	11	M-CH ₃ O
396	31	M-CH ₃ OH
395	6	M-CH ₃ -H ₂ O
394	10	M-2OH
392	4	M-2H ₂ O
385	5	M-CH(CH ₃) ₂
378	5	M-CH ₃ OH-H ₂ O
377	7	M-CH ₃ -2H ₂ O
367	5	M-CH(CH ₃) ₂ -H ₂ O
363	21	M-CH ₃ OH-CH ₃ -H ₂ O
287	4	M-B
271	8	M-C ₁₀ H ₁₉ -H ₂ O
269	8	M-B-H ₂ O
255	8	M-B-CH ₃ OH
253	19	M-C ₁₀ H ₁₉ -2H ₂ O
251	28	M-B-2H ₂ O ^a
239	11	M-B-CH ₃ O-OH
229	7	M-A-H ₂ O
215	9	M-A-CH ₃ OH
211	13	M-A-2H ₂ O
197	13	M-A-CH ₃ OH-H ₂ O

^a Ionised nucleus.

shows also olefinic unsaturation (1650, 965, and 935 cm^{-1}) and characteristic bands at 857 and 820 cm^{-1} associated with a trisubstituted double bond. It exhibits a split absorption band at 1380 cm^{-1} corresponding to δ_s CH(CH₃)₂.

Fig. 1. Infrared spectra of fremontol (A) and ergosterol (B) isolated from *Alectoria fremontii*.

The bands at 1070 and 1040 cm^{-1} and the characteristic bands between 850 and 790 cm^{-1} may be attributed to a 3β -hydroxysteroid.

The mass spectrum (Table 3) was typical for that of sterol, and the lower part of it was quite similar to that of cholesterol, ergosterol, and stigmasterol.¹¹ The lack of peak at $M-72$ indicated that the nucleus in the sterol molecule must have an unsaturated character; Ryhage and Stenhagen.¹² The spectrum exhibited a molecular peak at m/e 428 (7 %), and the sterol was assigned the formula $\text{C}_{29}\text{H}_{48}\text{O}_2$. Of value are the ions $(M-\text{H}_2\text{O})$, $(M-2\text{H}_2\text{O})$, $(M-\text{H}_2\text{O}-\text{CH}_3)$, and $(M-2\text{H}_2\text{O}-\text{CH}_3)$, which unambiguously indicate that fremontol must be a diol. Base peak in the spectrum was observed at m/e 69 corresponding to the frag-

ment-ion $\text{CH}_2-\text{CH}=\text{C}(\text{CH}_3)_2$. The dominant peaks at m/e 396 and 363 due to the ions $(M-\text{CH}_3\text{OH})$ and $(M-\text{CH}_3\text{OH}-\text{CH}_3-\text{H}_2\text{O})$ should be noticed.

Probably a hydroxyl group is to be found in position C-18. Fragmentation due to the loss of CH_3OH might indicate the presence of a methyl ether. However, additional IR and MS data did not support this assumption. Among other things, neither a peak in the mass spectrum at m/e 381 ($M-47$) nor loss of methanol from the $(M-\text{CH}_3)$ fragment, was observed.

The usual peaks due to the loss of the side-chain are observed, and a peak at $M-\text{CH}(\text{CH}_3)_2$, supported interpretation of the IR band at 1380 cm^{-1} . The isopropyl group corresponds to the end of the side-chain. The peak at m/e 251, due to the ionized nucleus formed with loss of the side-chain and two molecules of water from the molecule-ion, indicates the presence of two hydroxyl groups. Other important processes caused by cleavage of the molecule as

shown in Fig. 2 are found as well defined peaks in the spectrum.

Owing to the remarkable similarity of IR and the lower part of MS of fremontol, and those of cholesterol, ergosterol, and stigmasterol, it can be assumed that fremontol has a molecular structure as drawn in Fig. 2, and belongs to the group of C_{29} -sterols. The band at 965 cm^{-1} in the IR spectrum showed the side-chain to have a double bond (*trans*) in 22–23 position; Spiteller-Friedmann and Spiteller.¹¹

It is essential to underline that the fixation of the second hydroxyl group in C-18, and the nuclear double bond at 5,6-position are to some extent arbitrary, and is therefore marked with a questionmark in the figure. However, MS and IR support the postulated structure. The small yield of fremontol prevented a complete determination of the molecular structure.

The acetone-soluble product (II) gave a positive Liebermann-Burchard reaction identical with that of ergosterol. In the mass spectrum the molecular-ion was observed at m/e 396. The IR spectrum and the elemental analysis confirmed that the isolated compound was identical with ergosterol.

Water- and acetone-insoluble fraction (III). After purification of this fraction by recrystallization, the component sublimed in the temperature interval 182–185 °C. It did not give any of the usual colour reactions. The main IR absorption bands in the higher part of the spectrum were detected at 1735 (ester-carbonyl), 1468 (CH_3 and CH_2 hydrogen bending), 1175 (C–O stretch), 1062 (C–O stretch), 940 and 720 cm^{-1} . The last band was weak and unsplit. The lower part of the spectrum (Fig. 3) shows the intensity of the hydroxyl band as compared with the asymmetric carbon-hydrogen stretching band around 2900 cm^{-1} . The figure demonstrates that the chainlength of the esters in the mixture obviously must be appreciable larger than that of stearic acid.

The high sublimation interval, lack of IR signal between 1600 and 1650 cm^{-1} , and no hydrogenation in the system dioxane/ PtO_2 , proved that the esters were saturated.

The mass spectrum of the ester mixture has no molecular ion, which is in accordance with the observation of Ryhage and Stenhagen.¹² The fragmentation in the lower part of the

Fig. 2. Preliminary structure of fremontol isolated from *Alectoria fremontii*.

Fig. 3. Infrared spectrum of the tetrahydroxy fatty acid alkyl esters (a) isolated from *Alectoria fremontii*, compared with spectra of dihydroxystearic acid (b) and tetrahydroxystearic acid (c).

spectrum is nearly identical with the spectrum reported for the hydroxy acids of *A. nigricans*. Outstanding peaks in the spectrum of the ester mixture occur at m/e 213 (25 %), 199 (100 %), 185 (25 %), and 183 (50 %). Other important fragments were

The following processes were confirmed by the appropriate metastable peaks

The whole series of the ions $(\text{CH}_2)_x-\text{COO}-(\text{CH}_2)_y\text{CH}_3$ ($x+y$ up to and including 12) with intensities 11 % and higher, were recognized. The peak corresponding to the case of $x+y=12$, was observed at m/e 227 (45 %).

Considering the IR-spectrum given in Fig. 3 and spectra reported earlier,⁹ it seems probable that the esters are of relatively high molecular weights. The ratio between the contents of carbon and oxygen in the molecule itself is found to be about 4.0. This value agreed well with the molecular weight of 558, calculated for $\text{C}_{33}\text{H}_{66}\text{O}_6$. From the present results, the recrystallized fraction III most probably contains a mixture of *tetrahydroxy fatty acid alkyl ester* homologues of the general formula $\text{C}_n\text{H}_{2n}\text{O}_6$, where $n=32, 33$, and 34 are given as tentative values. The four hydroxyl groups may be located in positions as demonstrated for the molecule

where z has the values of 6, 7, and 8, and $(x+y=18)$

The five *Alectoria* species were finally extracted with water and the extracts worked up as described by Solberg.¹³ It is worth noting that 2-aminoethanesulfonic acid (taurine) was present in all of them and in amounts as follows: *Alectoria nigricans* 50 ppm; *A. capillaris* 196 ppm; *A. nadvornikiana* 212 ppm; *A. bicolor* 128 ppm; *A. fremontii* 171 ppm.

EXPERIMENTAL

Materials and methods. Elemental analyses were performed by Alfred Bernhardt, Mikro-analytisches Laboratorium, Elbach-BRD. The melting points have not been corrected. IR spectra were all taken in KBr pellets; NMR spectra were run in deuteriochloroform, deuterium oxide, and dimethyl sulfoxide- d_6 . Chemical shifts were recorded in δ [ppm downfield from internal standard tetramethylsilane (TMS)].

The mass spectra recorded in this communication were obtained by direct sample insertion into the ion source of the mass spectrometer. Ionising energies of 70 eV were used, and the temperature of the solid heater and in the chamber was lying between 250 and 280 °C. The relative abundances are given in parenthesis.

All evaporation of extracts and solutions was performed with a rotating evaporator under reduced pressure.

Thin layer chromatography. Thin layer chromatography (TLC) and preparative layer chromatography (PLC) of the depside, depsidone, and sterol components were carried out on precoated plates (Woelm, Germany); Silica gel F 254/366 was used as adsorbant. The solvents (A, B, C) used were the same as reported by Culberson and Kristinsson.¹⁴ The sterols were chromatographed with the solvent system chloroform-methanol (95+5).

Isolation of the lichen substances

Alectoria nigricans. The lichen (500 g) was collected on Reinøy, Troms, and extracted in a Soxhlet extractor with acetone for 12 h. A colourless solid which slowly separated from the acetone solution was collected, washed with ethanol, recrystallized twice from boiling glacial acetic acid and dried for several hours at about 80 °C; yield 0.36 g (Fraction 1). The fraction was identified as a mixture of tetrahydroxy fatty acids.

The acetone solution was concentrated to a smaller volume, and an almost colourless solid was deposited; yield 11.2 g (Fraction 2). After repeated crystallizations from diluted acetic acid and ethanol, colourless needles was obtained, m.p. 178.5 °C. The fraction was identified as alectorialic acid by TLC, IR, MS, and NMR.

The mother liquor from the acetone extract was evaporated and the residue subjected to a preliminary investigation by TLC, solvents A and C. In addition to several known components an unknown PD-negative compound, AN-X, with relatively low R_F -value was seen on the chromatograms. Preparative chromatography of the residue and repeated crystallizations from hot water and diluted acetic acid yielded 129 mg colourless needles, m.p.

224–225 °C (d) (Fraction 3). (Found: C 60.00; H 4.42; O 35.56. Calc. for $C_9H_8O_4$: C 60.00; H 4.47; O 35.52), M^+ 180. AN-X was fairly soluble in organic solvents, and one active hydrogen atom was detected by analysis.

Main IR bands: 3460, 3300, 1740, 1690, 1630, 1610 cm^{-1} . UV in ethanol gave: λ_{max} at 260 nm, λ_{min} at 237 nm.

The NMR (acetonitrile- d_3) of AN-X at 100 MHz showed a singlet at δ 2.04, due to an aryl-methyl group, a two-proton singlet at δ 5.16 arising from a C-CH₂-O group, and one aromatic ring proton singlet at δ 6.50. AN-X was identified as *5,7-dihydroxy-6-methyl phthalide*.

The 5,7-dimethoxy-6-methyl phthalide crystallized from methanol as colourless needles, m.p. 169.4 °C. The 4-bromo-5,7-dihydroxy-6-methyl phthalide was purified by recrystallization from dilute ethanol, giving colourless needles melting between 155 and 160 °C.

Alectoria capillaris. This lichen (37 g) was collected from spruce at Golå, Oppland and Brydal, Hedemark, 1970–1971, and extracted with acetone. The solvent was distilled off and the green coloured residue macerated on a filter with light petroleum and acetone. A sparsely soluble product was obtained (0.73 g). Colour reactions, IR, and MS were in agreement with those of barbatolic acid.

The acetone soluble part of the residue was investigated by TLC with solvents A, B, and C. The chromatograms were sprayed with *p*-phenylenediamine (PD), sulfuric acid (10 %) and bisdiazotized benzidine. 5,7-Dihydroxy-6-methyl and 5,7-dihydroxy-6-formyl phthalide both gave a dark brown colour with the benzidine reagent.

Alectoria nadvornikiana. The material used in this investigation was collected at Hauge in Dalane, Rogaland, 1969, and extracted with acetone. By concentration the solution a residue (147 mg) was obtained. The fraction was investigated by TLC with the same solvents and spraying reagents as mentioned for *A. capillaris*. Mass spectrometry confirmed the conclusions obtained by TLC.

Alectoria bicolor. The material (19.6 g) was collected at Hauge in Dalane, Rogaland, and refluxed with acetone for 2 h. The residue treated with cold acetone gave 54 mg of a sparsely soluble product which was relatively easily soluble in hot 80 % acetone. It gave a reddish brown colour with FeCl_3 , a yellow colour with KOH, and an orange-red with PD. TLC with solvents A and C, and with butanol-acetone-water (5:1:2), followed by spraying with PD and sulfuric acid unambiguously demonstrated the presence of fumarprotocetraric acid.

The acetone-soluble part of the extract was at first investigated by TLC. Three dominant spots (A, B, C) were noticed when the chromatograms were sprayed with PD. Small amounts of these products were isolated subsequently by PLC.

Product A. The bright yellow colour with PD on TLC plates was persistent. This is an important sign as several PD-positive substances at first may produce a yellow colour which darkens more or less after a short period of time. Colour reactions with sulfuric acid was gray, dark gray or gray-black. The spot gave a bluish-white fluorescence in UV-light. Hawksworth¹⁵ has reported decarboxythamnolic acid as having the same property.

The mass spectrum is rather complicated and in all probability the product A contained fumarprotocetraric acid and decarboxythamnolic acid.

Product B. On the basis of chromatographic behaviour, colour reactions and UV absorption in ethanol ($\lambda_{\max}$ 375 nm, $\lambda_{\min}$ 326 nm) this product was identified as decarboxyalectorialic acid. R_F -values with solvents A and C are given below:

Compound	$R_F \times 100$	
	Solv. A	Solv. C
Alectorialic acid	59	33
Decarboxyalectorialic acid	62	42
Substance isolated	62	42
Psoromic acid (standard)	67	43

Product C. Chromatographic properties, colour reactions with PD and sulfuric acid on TLC plates, and the UV absorption (compared with an authentic sample) established that product C is identical with atranorine.

Alectoria fremontii. This species was collected from *Picea exelsa* at Kvernmoen, Alvdal, Hedemark, 1971–1972. The material was divided into two portions, a smaller one (70 g) which was particularly rich in yellow soredia, and a greater part (1.8 kg) poor in soredia. The two portions were separately dealt with according to the usual procedure. The second and greater part of the material was refluxed with acetone for 22 h. Evaporation of the filtered extract left a dark green, amorphous, solid residue, which was divided into three fractions (I, II, III) according to solubility.

TLC of the light petroleum extracted part of the residue (I) showed a complex mixture of components. Some of these components were only present as traces and not studied. However, four components were isolated by PLC from the extract and named Fremontii 1, 2, 3, and 4. These components were purified further by a second PLC technique. Fremontii 4 was identified as a new sterol and named fremontol. The substance was chromatographed on thin layer plates with different solvents and compared with ergosterol. The R_F -values ($\times 100$) are reported below:

Solvent	$R_F \times 100$	
	Fremontol	Ergosterol
A	61	73
C	39	45
CHCl_3 —MeOH(95:5)	45	60
Benzene— CHCl_3 (1:1)	69	69

By recrystallizing the acetone-soluble product (II) of the original residue three times from diluted acetic acid, colourless and silky crystals were separated, yield 10.5 mg. (Found: C 81.97; H 11.23; O 6.26. Calc. for ergosterol, $\text{C}_{28}\text{H}_{44}\text{O} \cdot \frac{1}{2}\text{H}_2\text{O}$: C 82.29; H 11.18; O 6.53). In day-light the product decomposes relatively fast in an acetone solution and the decomposition products were just the same as those obtained with authentic ergosterol. In the mass spectrum of the isolated ergosterol there are peaks for $(\text{M}-\text{H}_2\text{O})$ m/e 378 and $(\text{M}-\text{CH}_3-\text{H}_2\text{O})$ m/e 363 in addition to the molecular ion. IR of the isolated ergosterol is given on the same chart as that of fremontol (Fig. 1).

The part of the residue insoluble in cold acetone (III), was crystallized from boiling acetone, giving a faintly yellow product, yield 154 mg. (Found: C 70.91; H 11.24; O 17.72; $\text{C}-\text{CH}_3$ 2.13. Calc. for $\text{C}_{33}\text{H}_{56}\text{O}_6$: C 70.92; H 11.90; O 17.18). The fraction was identified as a mixture of tetrahydroxy fatty acid alkyl esters.

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