NMR spectra were taken on a Varian XL-100 spectrometer (CCl_4, internal standard TMS, τ scale).

SUMMARY

The neutral lipids of the second vegetation phase of *Helleborus abchasicus* are enriched with hydrocarbons and free fatty acids. Fatty acid methyl esters have been detected in the seed oil of this plant.

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AROMATIC METABOLITES OF LICHENS OF THE FAMILY PARMELIACEAE.

I. DEPSIDONES

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UDC 547.982+581.192.2+582.29

(+)-Usnic acid, the depside atranorin, and the depsidones fumarprotocetraric, α-alectoronic, and α-collatolic acids, and also the phenoxyisocoumarin derivatives β-alectoronic and β-collatolic acids have been isolated by extraction with hexane and chloroform from lichens *Asahinea chrysantha, A. scholanderi,* and *Parmelia birulae*. This is the first time that β-alectoronic acid has been detected in lichens. The structures of the compounds have been established by spectral and chemical methods.

Continuing a chemical study of lichens of the family Parmeliaceae, from two of its representatives *Asahinea chrysantha* (Tuck.), W. Culb. et C. Culb. and *Parmelia birulae* Elenk., together with alectoronic acid (I) [1], we have isolated a previously undescribed compound isomeric with it. For this we propose the name β-alectoronic acid (III), to distinguish it from the acid known previously, which we propose to rename α-alectoronic acid.

We have also studied the lichen *Asahinea scolanderi* (Llano) W. Culb. et C. Culb, which, in addition to the α-collatolic acid (II) known for this species [2], also contained β-collatolic acid (VI). The latter was first obtained by Asahina by the alkaline hydrolysis of α-

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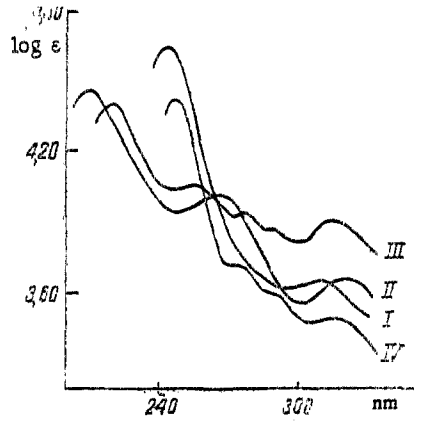


Fig. 1. UV spectra of the alectoronic and collatolic acids in ethanol: I) α-alectoronic acid; II) α-collatolic acid; III) β-alectoronic acid; IV) β-collatolic acid.

collatolic acid, and it proved to be identical with a minor component of the lichen *Cetraria collata* (Nyl) [2]. This isolated report on the presence of β-collatolic acid in a natural material was not included in the well-known reviews on the chemical components of lichens [3-5].

Since we isolated these isomeric acids from lichens that had been stored in the dry state for 2-3 years, the fact that they are native compounds was checked by careful TLC investigations of extracts from fresh samples, and this confirmed their presence in the natural material.

The use of neutral solvents and mild conditions of extraction apparently excludes the possibility of the appearance of artefacts in the process of isolation of these substances.

The α-alectoronic and α-collatolic acids and the other components of the lichens investigated were identified from the melting points, absorption characteristics, and PMR, ^{13}C NMR, and mass spectra of the substances isolated and of some of their derivatives. Their amounts in the lichens are given below (% on the dry raw material).

Compound	*Asahinea chrysantha*	*Asahinea scholanderi*	*Parmelia birulae*
α-Alectoronic acid	0,25	0,01	5,10
β-Alectoronic acid	0,31	0 01	0,57
α-Collatolic acid	—	0,04	Tr.
β-Collatolic acid	—	0,02	2,43
Usnic	1,30	0,01	—
Atranorin	0 09	0,02	0,90
Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	0,01	—	0,19
Fumarprotocetraric acid	—	—	0,18

The β-alectoronic and β-collatolic acids isolated gave the ions $M^+ - H_2O$ (494 and 508, respectively), just like α-alectoronic and α-collatolic acids, but they differed considerably in their chromatographic mobilities, melting points, and spectral characteristics.

A comparison of the PMR spectra of the α- and β-alectoronic acids showed that the general pattern of the spectrum differed for the β isomer by the absence of the signals of two protons of a benzyl group (C-7, 4.04 ppm) and by the appearance of a one-proton singlet at δ 6.31 ppm. Likewise, for β-collatolic acid a singlet appeared at δ 6.39 ppm and the signal at δ 4.08 ppm in the spectrum of α-collatolic acid had disappeared.

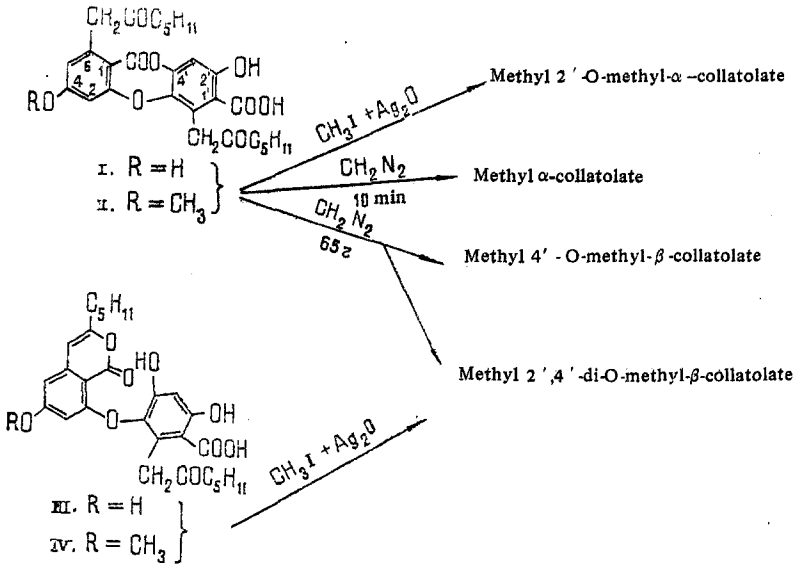
Such differences in the signals of the protons show different types of substitution at the C-7 carbon atom in the α and β forms of the acids, which was confirmed by their ^{13}C NMR spectra. While the number of signals of carbon atoms in the spectra of the two forms were preserved, for the β acids there was a decrease in the number of carbon atoms in saturated aliphatic chains.

Thus, in place of the signal of a benzyl carbon at δ 48.1 ppm and of three signals of aromatic tertiary carbons (δ 106.4, 107.7, 117.9 ppm) in α-alectoronic acid, in the spectrum of its β isomer the signals of four tertiary carbons appeared (δ 102.1, 102.9, 103.7, 104.8 ppm).

Additional information on the structural features of the isomeric acids isolated was obtained from the results of absorption spectra and of studies of methylation products.

The UV spectra of β-alectoronic and β-collatolic acids differed sharply from the spectra of lichen depsides and depsidones (see Fig. 1). The IR spectra of these acids likewise did not have the characteristic absorption bands in the carbonyl region (1710-1750 cm^{-1}), which shows the absence of depside bonds from their molecules.

The products obtained by the methylation of the alectoronic and collatolic acids that had been isolated were characterized in detail by chemical and spectral methods and exhibited properties corresponding completely to those found previously for these compounds by Asahina and Elix [5, 6]. The results of these experiments are shown in the scheme.



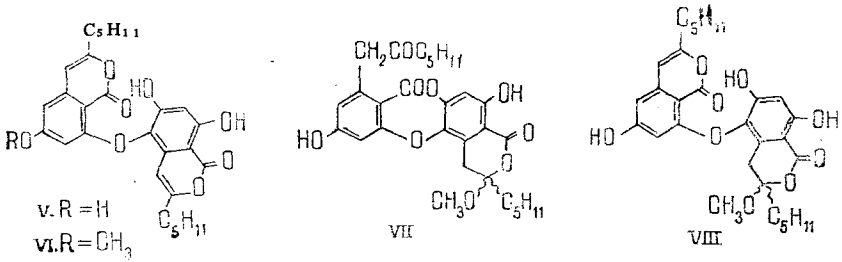
Products of the methylation of alectoronic and collatolic acids.

The brief action of solutions of alkali on the preparations of α-alectoronic and α-collatolic acids obtained (according to Asahina [2, 7]) led to the formation of the β isomers which were identical with the substances obtained from the same lichens.

The hydrolysis with concentrated sulfuric acid of the α and β isomers was accompanied by the formation of alectorone (V) and of collatolone (VI), respectively, according to PMR and mass spectroscopy.

The cleavage of the depsidone studied to phenolcarboxylic acids under the action of sodium and liquid ammonia took place extremely smoothly. The Shorygin reaction [8] has apparently not previously been used in the study of lichen substances.

When the lichen *A. chrysantha* was subjected to prolonged extraction with boiling methanol, in place of the expected α- and β-alectoronic acids their pseudoesters (VII) and (VIII), respectively, were isolated. The first of them was first obtained by Elix et al. [6] by the methylation of alectoronic acid with methanol in the presence of sulfuric acid. This fact shows the impermissibility of the use of hot extraction with alcohols to obtain the native components of lichens.



In conclusion, it must be pointed out that the simultaneous presence of normal depsidones and their lactonic (phenoxyisocoumarin) forms that we have detected in three species of lichens of the family Parmeliaceae is not a distinguishing feature of these species alone. Recently, Foo and Galloway [9] have also isolated from the lichen *Xanthoparmelia scabrosa* the normal depsidones loxodin and norlobaridone, and also the lactones loxodinol and lorlobariol isomeric with them. It is likely that other lichens containing depsidones with oxoalkyl substituents may also be found to contain the lactone forms isomeric with them.

EXPERIMENTAL

The lichens were collected in the Ten'ki region of the Magadan province in 1976-1977 and again in 1981.* Herbarium samples were stored in the Department of Plant Systematics and Geobotany of Tartu University. The species were determined by Ch. Kh. Trass and L. A. Knyazheva.

Melting points were determined on a Boëtius stage (and are uncorrected). Mass spectra were taken by the direct introduction of sample at 70 V on a LKB-9000 S instrument. PMR and ^{13}C NMR spectra were recorded on a Bruker HX-90 E instrument with a working frequency of 90 MHz for ^{1}H and 22.63 MHz for ^{13}C (δ, ppm; 0 – TMS). Absorption spectra were recorded on a Specord UV-Vis instrument in ethanol, and IR spectra on a Specord IR-75 instrument. TLC was performed on Silufol in the following systems: 1) benzene–dioxane–acetic acid (18 : 4.5 : 0.5); 2) hexane–diethyl ether–formic acid (13 : 8 : 2); and 3) toluene–acetic acid (20 : 3) [10]. Column chromatography was carried out on silica gel of types L and KSK.

Extraction and Isolation of the Substances. The comminuted air-dry samples of lichens were extracted in a Soxhlet apparatus first with hexane and then with chloroform. On cooling, the concentrated hexane extracts deposited yellow crystals of (+)-asahinic acid, which were identified from the coincidence of the properties with those of an authentic sample (melting point, TLC, etc). The low value of the specific rotation of the preparation of usnic acid from the lichen *P. birulae* – $[\alpha]_{Hg}^{20}$ 412° (c 0.05; chloroform) may indicate contamination with (–)-usnic acid.

The residue from the evaporation of the mother liquors was dissolved in benzene. The colorless crystals that deposited on cooling were identified, after purification, by comparison with an authentic sample of atranorin: mp 197°C ($CHCl_3$–ether); according to the literature [3a]: 196°C (acetone). A mixed sample showed no depression of the melting point. In systems 1, 2, and 3, the R_f value of the preparation and of the standard coincided. In the case of the lichens *P. birulae* and *A. chrysantha* the unpurified atranorin precipitate contained as an impurity a substance, apparently of pure nature, which on a thin-layer chromatogram was colored by sulfuric acid bright crimson and gave a blood-red coloration with the Salkowsky reagent [11].

The chloroform extracts were separated by crystallization from various solvents and by gel chromatography on Sephadex LH-20 in chloroform. The extracts from the lichens *A. chrysantha* and *P. birulae* formed on chromatography a pale yellow zone from an eluate of which, after recrystallization, methyl 2,4-dihydroxy-3,6-dimethylbenzoate was obtained with mp 143-144°C; according to the literature [3b]: 143-144°C. PMR spectrum ($CDCl_3$), ppm: 2.44 (6 H, s, 2 CH_3); 3.92 (3 H, s, $COOCH_3$); 6.19 (1 H, s, ArH). Mass spectrum, m/z: 196 (M^+), 165, 164, 137, 136.

Fumarprotocetraric Acid. This was isolated from a chloroform extract of the lichen *P. birulae* and was separated from the alectoronic acids by fractional crystallization from benzene. It decomposed at about 250°C. Mass spectrum, m/z: 358, 340, 314, 299, 258, 257, 243, 116. The chromatographic behavior and the coloration and the action of 10% H_2SO_4 and of solutions of p-phenylenediamine and of $FeCl_3$ coincided completely with those of an authentic sample.

α-Alectoronic Acid. This was isolated from a chloroform extract of *P. birulae*. mp 192-193°C (benzene); according to the literature [3c]: 193°C (benzene). ν_{max}^{KBr}: 3390, 3207, 1728, 1709, 1680 cm^{-1}. PMR spectrum (CD_3COCD_3), PPM: 0.89, 0.92 (3 H each, t, CH_3); 1.20-1.64 (12 H, m, 6 CH_2); 2.26 (2 H, m, $CH_2C_4H_9$); 2.53 (2 H, t, $CH_2C_4H_9$); 3.62 (2 H, broad s, CH_2);

*The duplicate samples of lichens were collected by Yu. B. Korolev (Institute of Biological Problems of the North of the Far Eastern Scientific Center of the Academy of Sciences of the USSR).

4.04 (2 H, s, Ar – CH_2CO); 6.68, 6.78 (1 H each, d, j = 2.4 Hz, ArH); 6.71 (1 H, s, ArH) Mass spectrum, m/z (%): 494 (12), 468 (10), 450 (5), 371 (6), 370 (23), 369 (4), 352 (1), 245 (3), 249 (2), 248 (2); and the metastable ions 432.7 and 334.9.

β-Alectoronic Acid. This was isolated from the lichen *P. birulae*. It was separated from the α isomer by the addition of chloroform to an ethereal solution of the mixture, and also by preparative layer chromatography. White powder, $C_{28}H_{22}O_9$, mp 138-139°C (chloroform). On irradiation with UV light, it gave a weak violet fluorescence. Color reactions: $FeCl_3$ – gray-violet; $Ca(OCl)_2$ – on Silufol, bright yellow; in solution, red. $\lambda_{max}^{CH_3OH}$ nm: 212, 238 sh., 245, 278 sh., 290, 314 (log ε 4.44, 4.64, 3.83, 3.89). ν_{max}^{KBr}, cm^{-1}: 3387 (broad), 1688, 1683, 1662. PMR spectrum, ppm (CD_3COCD_3): 0.84, 0.91 (3 H each, t, J = 6.2 Hz, CH_3); 1.20-1.70 (12 H, m, 6 CH_2); 1.89 (2 H, m, $CH_2C_4H_9$); 2.49 (2 H, t, J = 7.2 Hz, $CH_2C_4H_9$); 3.15 (2 H, broad s, CH_2); 6.18, 6.52 (1 H each, d, J = 2.0 Hz, ArH); 6.31 (1 H, s, =CH); 6.48 (1 H, s, ArH). Mass spectrum, m/z (%): 495 (9), 594 (34), 468 (10), 450 (5), 371 (10), 370(29), 369 (6), 352 (5); 254 (3), 249 (4), 248 (4).

The preparations of α- and β-alectoronic acids isolated from the lichens *A. chrysantha* and *A. scholanderei* showed analogous spectral characteristics, and their mixtures with the corresponding acids from *P. birulae* gave no depression of the melting point.

α-Collatolic Acid. This was isolated from a chloroform extract of the lichen *A. scholanderei*. mp 89-90°C (aqueous acetic acid); 124-125°C (chloroform–hexane); according to the literature [3d]: 124-125°C. $\lambda_{max}^{C_2H_5OH}$, nm: 210, 316 (log ε 4.45, 3.99, 3.46). $\nu_{max}^{CHCl_3}$, cm^{-1}: 1741, 1723, 1686. PMR spectrum, (CD_3COCD_3) ppm: 0.89 (6 H, m, 2 CH_3); 1.20-1.56 (12 H, m, 6 CH_2); 2.15 (2 H, m, $CH_2C_4H_9$); 2.54 (2 H, t, $CH_2C_4H_9$); 3.71 (2 H, broad s, CH_2); 3.87 (3 H, s, OCH_3); 4.08 (2 H, s, $ArCH_2CO$); 6.73 (1 H, s, ArH); 6.79 (1 H, d, J = 2.6 Hz, ArH); 6.92 (1 H, d, J. 2.6 Hz, ArH). Mass spectrum, m/z (%): 510 (5), 509 (20), 508 (61), 484 (2), 482 (32), 464 (5), 411 (7), 410 (24), 385 (18), 384 (78), 383 (13); 368 (10), 366 (5), 286 (4), 262 (5), metastable ions: 448.0 and 348.8.

β-Collatolic Acid. This was isolated from a chloroform extract of the lichen *A. scholanderei*. mp 117°C (ethanol), 117-118°C (benzene). $\nu_{max}^{C_2H_5OH}$ nm: 248, 276, 290, 320 (log ε 4.42, 3.70, 3.57, 3.62). $\nu_{max}^{CHCl_3}$, cm^{-1}: 1694, 1676, 1662. PMR spectrum (CD_3COCD_3), ppm: 0.87 (6 H, m, 2 CH_3); 1.25-2.00 (14 H, m, 7 CH_2); 2.52 (2 H, t, j = 7.9 Hz, $CH_2C_4H_9$); 3.20 (2 H, broad s, CH_2); 3.79 (3 H, s, OCH_3); 6.26 (1 H, d, J = 2.3 Hz, ArH); 6.39 (1 H, s, =CH); 6.48 (1 H, s, ArH); 6.66 (1 H, d, J = 2.3 Hz, ArH). Mass spectrum m/z (%): 509 (4), 508 (11), 483 (13), 482 (41), 464 (7), 386 (4), 385 (24), 384 (100), 383 (15), 366 (3), 286 (3), 283 (5), 262 (6); metastable ions: 448.0, 348.8, 330.0. R_f values on chromatography in three standard systems on Silufol:

Acid	System		
	1	2	3
α-Alectoronic	0.78	0.11	0.16
β-Alectoronic	0.70	0.06	0.07
α-Collatolic	0.87	0.20	0.40
β-Collatolic	0.82	0.10	0.26

Methyl 2'-O-Methyl-α-collatolate. This was obtained by the Purdie methylation (CH_3I + Ag_2O) in chloroform of (I) and (II): mp 140-141°C (ether). Mass spectrum, m/z: 554 M^+, 523, 522, 456, 424.

Methyl α-Collatolate. This was obtained by the brief action of an ethereal solution of diazomethane on (I) and (II); mp 119-120°C (ether). Mass spectrum, m/z: 540 M^+, 509, 508, 442, 410.

Methyl 4'-O-Methyl-β-collatolate. This was the main product obtained from the prolonged action of an ethereal solution of diazomethane on (I) and (II); mp 68-70°C (ether); mass spectrum, m/z: 554 M^+, 523, 522, 457, 456.

Methyl 2', 4'-Di-O-methyl-β-collatolate. This was the main product formed in the Purdie methylation of (III) and (IV). A small amount of it was found in the products of the prolonged methylation of (I) and (II) with diazomethane; mp 128-130°C (ether); mass spectrum, m/z: 568 M^+, 470, 438.

Alectorone (V). A solution of 20 mg of (I) in eight drops of concentrated H_2SO_4 was left for 15 h, and then ice was added and it was extracted with ether. After separation by TLC in the benzene—dioxane—acetic acid (260 : 9 : 1) system, the product was isolated from the upper zone. Colorless crystals, mp 180-182°C. λ_{max}, nm: 248, 262, 282, 298, 334 (log ε 4.64, 3.57, 3.28, 2.38, 3.19). PMR spectrum ($CDCl_3$), ppm: 0.86 (6 H, m, 2 CH_3); 1.20-1.80 (12 H, m, 6 CH_2); 2.26 (2 H, t, $CH_2C_4H_9$); 2.50 (2 H, t, $CH_2C_4H_9$); 6.20 (1 H, s, =CH); 6.37 (1 H, s, = CH); 6.49 (1 H, d, J = 2.0 Hz, ArH); 6.51 (1 H, s, ArH); 6.55 (1 H, d, J = 2.0 Hz, ArH). Mass spectrum, m/z: M^+ 494 (100%).

Collatolone (VI). This was obtained from (II) by the method described above. Colorless crystals, mp 140-142°C. λ_{max}, nm: 240, 248, 262, 282, 298, 334 nm (log ε 4.26, 4.68, 3.57, 3.27, 2.34, 3.17). PMR spectrum (C_5D_5N), ppm 0.78 (6 H, m, 2 CH_3); 1.08-1.65 (12 H, m, 6 CH_2); 2.28 (2 H, t, $CH_2C_4H_9$); 2.36 (2 H, t, $CH_2C_4H_9$); 3.79 (3 H, s, OCH_3); 6.17 (1 H, s, ArH); 6.70 (1 H, s, ArH); 6.72 (H, s, = CH); 6.75 (1 H, s, = CH); 6.88 (1 H, s, ArH). Mass spectrum, m/z: M^+ 508 (100%).

Pseudoester of α-Alectoronic Acid (VII). This was isolated from the solution obtained by the prolonged extraction of the dry lichen *A. chrysantha* with hot methanol in a Zaitsev extractor. Colorless crystals, mp 155-156°C (chloroform—hexane); according to the literature [6]: 153-155°C. The spectral characteristics corresponded to those given in the literature.

Pseudo-Ester of β-Alectoronic Acid (VIII). This was isolated simultaneously with (VII). Colorless crystals, mp 174-176°C. PMR spectrum (C_5D_5N), ppm: 0.87 (6 H, m, 2 CH_3); 1.18-1.70 (12 H, m, 6 CH_2); 1.74 (2 H, m, $CH_2C_4H_9$); 2.36 (2 H, t, $CH_2C_4H_9$); 3.04 (1 H, d, J = 17 Hz, gem. H); 3.20 (3 H, s, aliph. OCH_3); 3.41 (1 H, d, J = 17 Hz, gem. H); 6.07 (1 H, s, = CH); 6.55 (2 H, s, 2ArH); 6.61 (1 H, s, ArH). Mass spectrum, m/z (%): 527 (5), 526 (15, M^+), 525 (14), 496 (9), 495 (37), 494 (100).

SUMMARY

Together with the known lichen depsidones α-alectoronic and α-collatolic acids the presence of the β-alectoronic and β-collatolic acids, phenoxyisocoumarin derivatives isomeric with them, has been detected in a natural material.

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