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CHARACTERISTICS OF THE FAR-EASTERN LICHEN *Cetraria islandica*

L. S. Stepanenko, I. F. Skirina, P. S. Dmitrenok, and S. V. Khotimchenko

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It has been established that the form Cetraria islandica *var.* polaris *Rassad. is characteristic for the northern regions of the Russian Far East while the related variety* Cetraria laevigata *Rassad. grows in the Maritime Territory.*

Iceland moss *Cetraria islandica* L. Ach. is a well-known widespread lichen. A difficulty of its species determination consists in its extreme polymorphism and the similarity of its morphological characteristics to those of the related species *Cetraria laevigata* Rassad. We have attempted to determine the distributions of these species in the territory of the Far East and the Gorno-Altai Autonomous Region. (Herbarium specimens of lichens are stored in the Pacific Ocean Institute of Geography, Far-Eastern Division of the Russian Academy of Sciences.)

We have investigated for the first time, specimens of Iceland moss gathered in various regions of Magadan province. Forms correponding to *Cetraria islandica* var. *polaris* Rassad, with canaliculkate lobes having a larger or smaller number of pseudocyphellae, sometimes almost without them, predominated. In Magadan province, *C. laevigata* is fairly large, with a characteristic white band at the edge of the lobes and sometimes with maculae. The specimens of Iceland moss gathered in the Kabarovsk Territory and in the mountains of the Gorno-Altai Autonomous Oblast are represented mainly by the narrow-lobed form *C. islandica* var. *polaris*. Collections from the Amur region in the environs of the village of Dipkun revealed the presence of the species *C. laevigata*. In the Maritime Territory we encounted only the typical form *C. larvigata* Rassad.

The lichenologist K. A. Rassadina, who discovered and characterized the species *C. laevigata*, speaks in his publications of the evolution of *C. islandica* into *C. laevigata*, the center of distribution of the latter being Siberia [1]. Analysis of Far-Eastern specimens of lichens has shown that the typical form *Cetraria islandica* L. Ach. and the transitional form *C. islandica* var. *polaris* are found in Magadan province. On moving to the east (Kamchatka, Paramushir Island) and the south (Khabarovsk Territory) the narrow-lobed form *C. islandica* var. *polaris* becomes predominating, while only *Cetraria laevigata* grows in the southern regions of the Far East, in Amur province and Maritime Territory.

We have studied for the firt time the chemical compositions of Far-Eastern specimens of *C. islandica* var. *polaris* and *C. laevigata*. We had established previously that the main component of acetone and ethyl acetate extracts of Iceland moss (samples 1 and 2) are fumarprotocetraric (FPC) and protocetraric (PC) acids. In addition to these, substances less polar than FPC were present on the TLC plate, and it was our task to elucidate the nature of these substances.

An investigation of a $CHCl_3$ extract of the samples established the presence of ursolic acid and ergosterol peroxide in Iceland moss for the first time; ergosterol [2] and α-amyrin [3] had been found previously. Ergosterol is characteristic for lichens of the *Cetraria* genus and the Parmeliaceae family. Cetraric acid or cetrarin was also detected. The presence of cetrarin in Iceland moss was first reported by O. Hesse, who considerd that cetrarin is formed from FPC under the action of ethanol during the isolation of the lichen substances [4]. The use of $CHCl_3$ for extracting lichen substances excluded the possibility of the formation of cetraric acid from FPC or PC, and we regard it as a native substance.

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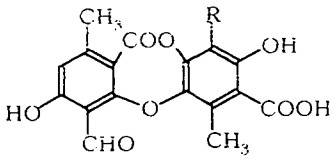
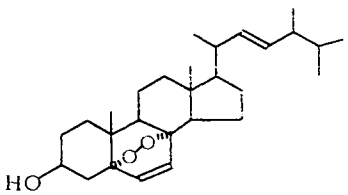
TABLE 1. TLC of Lichen Substances

Name of the substance	R_f in systems		Coloration with reagents	
	G	B	1% $FeCl_3$	10% H_2SO_4
Ursolic acid	0.68	0.63	Blue	Crimson
Cetraric acid	0.67	0.56	Brown	Brown
Fumarprotocetraric acid	0.51	0.26	Brown	Gray
Protocetraric acid	0.43	0.14	Brown	Gray
Physodalic acid	0.71	0.47	Brown	Gray
Ergosterol peroxide	0.48	0.40	Dark gray	Purple



Fumarprotocetraric acid
R=CH_2OOCCH=$CHCOOH$
Protocetraric acid R=CH_2OH
Cetraric acid R=$CH_2OC_2H_5$



Ergosterol peroxide

Ursolic acid and ergosterol peroxide have not been reported previously for Iceland moss, possibly because of difficulties in their identification. On Silufol plates in solvent systems B and G used in the chemistry of lichen substances [5] they had close R_f values. R_f values were determined relative to the position of atranorin on a TLC plate (Table 1).

Table 2 gives the results of an investigation by the TLC method of Far-Eastern (plus one sample from the Altai) and some European samples of Iceland moss. The main component of all the samples was FPC. Ergosterol peroxide was characteristic for three forms of *Cetraria*: *C. islandica* L. Ach., Far-Eastern form; *C. islandica* var. *polaris*; and *C. laevigata*.

Ursolic acid was present in the Asian samples 1-12 but not in all the samples of typical European *C. islandica*. Protocetraric acid was found in both forms of Iceland moss but was absent from *C. laevigata*. Cetraric acid was detected in individual samples from the Far East and Europe and is not characteristic for any particular species. The substances that we have isolated for the first time were characteristic for the Far-Eastern samples that we studied, and their detection in Iceland moss is of interest for understanding the properties of this widely distributed lichen, which is used for medicinal purposes.

In addition to the compounds mentioned, in some forms of *C. islandica* a substance similar in its R_f values and colorations with reagents to physodalic acid was detected that was absent from *C. laevigata*; a substance of steroid nature was present in European lichens.

A comparison of the chemical compositions of the samples from Asia (1-12), including the species *C. islandica var. polaris* and *C. laevigata*, and some European specimens of *C. islandica* L. Ach. (13-16) showed that the main difference of the former was the absence of the lichesterinic acids that are characteristic for European lichens. In the *C. laevigata* samples, not only lichesterinic acids but also PC and physodalic acid were absent. It is interesting that in a number of samples of Iceland moss gathered in Alaska only FPC was detected, and there were no diterpene acids [6]. This fact agrees with our results and is probably explained by the similar growth conditions and geographical situation of *Cetraria islandica* var. *polaris* in the Far East and in Alaska.

In reports of the 1940s and 1950s information was given on the presence of usnic acid in Iceland moss, but numerous literature sources later denied the presence of usnic acid in this lichen [2]. We also failed to find it in the samples from Europe and Asia that we investigated. Unfortunately, in publications relating to the medicinal properties of Iceland moss, usnic acid is named as one of the main components responsible for the medicinal properties of the lichen [7].

Aliphatic acids were determined in the Magadan samples 1 and 2 (Table 3). In sample 2, the bulk (66.8%) was made up of the saturated acids from palmitic, C:16, to lignoceric, C:24, the main ones being palmitic (C:16), stearic (C:18), and behenic (C:22). Unsaturated acids made up 26.3% of the total fatty acids, the main one quantitatively being oleic, C:18:1. In sample 1, saturated acids amounted to 48.1% of all the fatty acids, the main ones among them again being the C:16, C:18, and C:22 species; among the 32.9% of unsaturated acids the main ones were oleic and linoleic, C:18:2. Thus, in the samples studied the amounts of saturated and unsaturated acids were close or the saturated ones predominated. According to Solberg [3], in European specimens unsaturated acids predominated (43.5%), with oleic and linoleic together making up 42.2%, while

TABLE 2. Chemical Compositions of Different Forms of *C. islandica* and *C. laevigata* from Various Growth Sites

Sample No. and collection site	Proto-cetraric acid	Fumarproto-cetraric acid	Lichest-erinic acid	Ergosterol peroxide	Cetraric acid	Ursolic acid
1. Kulu, Magadan prov.	0.6%	5.9%	–	+	–	+
2. Palatka, Magadan prov.	0.3%	4.5%	–	0.05%	0.022%	0.1%
3. "Snezhnaya dolina" ["Snowy valley"], Magadan prov.	–	+++	–	+	–	+
4. R. Yudoma, Kharabovsk Terr.	+	+++ Σ 5.0%*	–	+	+	+
5. Ayan, Khabarovsk Terr.	+	+++ Σ 6.4%*	–	+	–	+
6. Ayan, airport, Khabarovsk Terr.	–	4.7%	–	+	–	+
7. Kronotskii reservation, Kamchatka	–	+++	–	Tr.	Tr.	+
8. Paramushir Island, Sakhalin prov.	–	+++	–	+	+	+
9. Yailo, Altai AO [Autonomous Oblast]	–	+++	–	+	+	+
10. Dipkun, Amur prov.	–	+++	–	+	–	+
11. Mt. Kolumbe, Maritime Terr.	–	5.4%	–	+	Tr.	+
12. R. Rudnaya, Maritime Terr.	–	5.1%	–	+	+	Tr.
13. St. Petersburg	–	4.9%	+	+	Tr.	–
14. Ust'-Narva, Leningrad prov.	+	+++ Σ 4.6%*	+	+	+	–
15. Vaigach Island, Arkhangel prov.	+	+++ Σ 4.7%*	+	+	+	–
16. Tallinn, Estonia	+	+++ Σ 5.3%*	+	+	–	+

*Sum of the FPC and PC acids.

TABLE 3. Compositions of the Monobasic Aliphatic Acids in Samples 1 and 2 (% of the total fatty acids)

Saturated acids	1	2	Unsaturated acids	1	2
14:0	2.8	4.0	14:1	1.4	–
15:0	1.3	1.6	–	–	–
16:0	22.6	38.1	16:1	3.0	2.8
17:0	1.2	1.0	–	–	–
18:0	9.1	10.5	18:1	13.6	20.8
–	–	–	18:2	9.8	2.7
20:0	1.7	2.8	20:1	2.6	–
21:0	1.3	–	–	–	–
22:0	6.1	6.1	–	–	–
24:0	2.0	1.6	24:1	1.2	–

there was only 9.2% of saturated acids, with 6.2% of palmitic; i.e., the amount of monobasic unsaturated acids several times exceeded the amount of saturated acids.

EXPERIMENTAL

^{1}H NMR spectra were taken on a Bruker WM-250 spectrometer, δ scale, standard TMS, in $CDCl_3$. NI FAB mass spectra were obtained on a LKB 2091 with an Ion Tech. Saddl field. Cs ionizer [sic] and by direct injection into a LKB 9000 S with ionizing energies of 15 and 70 eV. Angles of rotation were determined on a Perkin-Elmer 141 polarimeter, and melting points on a Boëtius stage.

TLC of the Lichen Samples. Previously dried samples of lichens (1.0 g) were ground and extracted exhaustively first with $CHCl_3-CH_3COCH_3$ (2:1) and then with CH_3COCH_3. Solutions of the same concentration — 0.46% — were prepared from the extracts and these were used for TLC. Identical amounts of the sample solutions were deposited on Silufol plates. Amounts of FPC and of FPC + PC were determined from the weights of the dried acetone extracts plus the residue insoluble in $CHCl_3-CH_3COCH_3$. To detect lichesterolic acids we used system E: hexane–ethyl acetate (75:25), and the other lichen substances were analyzed in systems B and G [8]: B — hexane–diethyl ether–formic acid (130:80:20); G — toluene–ethyl acetate– formic acid (139:83:8).

As the reagents for detecting the substances on the chromatograms we used: a 1% alcoholic solution of $FeCl_3$, a 10% solution of H_2SO_4, a 1% alcoholic solution of *p*-phenylenediamine (reagent for the aldehyde group), and a 0.04% solution of Bromocresol Green in a 0.01 M alcoholic solution of NaOH (reagent for aliphatic acids). The best reagent was $FeCl_3$: the brown coloration of cetraric acid appeared immediately after spraying; ergosterol was colored dark gray on heating; and the blue color of ursolic acid appeared some minutes after heating.

The positions on the plates of the substances being analyzed were determined with the aid of standard samples of fumarprotocetraric, protocetraric, physodalic, lichesterinic, and protolichesterinic acids and atranorin.

Samples of the lichen Iceland moss, *Cetraria islandica*, gathered in Magadan province and Khabarovsk Territory, on Kamchatka, in the Gorno-Atai AO, and on the Kurile islands we assigned to the variety *Cetraria islandica* var. *polaris* Rassad. Magadan province: 1) environs of Kulu (Verkhne-Kolimskoe upland); 2) environs of the village of Palatka; 3) "Snezhnaya dolina" ("Snowy valley") 25 km from Magadan, Khabarovsk Territory; 4) upper reaches of the R. Yudoma; 5) environs of the village of Ayan, airport region; 7) village of Yailo, Gorno-Altai AO; 8) Kronotskii reservation, Kamchatka; 9) Paramushir Island, Sakhalin province.

The samples from Maritime territory and Amur province belonged to the species *Cetraria laevigata* Rassad.: 10) environs of the village of Dipkul, Amur province, mountains of Sitokhe-Alinya, Maritime territory; 11) Kolumbe; 12) valley of the R. Rudnaya; and the other samples from the towns of Oblachnaya, Él'dorado, Rossypnaya, Lysaya, Snezhnaya, and Perlitova, the Gorbusha pass, and the town of Ptich'ya. Samples 13-16 from the European part of Russia and the former USSR belonged to the typical *Cetraria islandica* L. Ach. The lichen samples were gathered within a radius of 5 km from the points shown in Table 2.

Isolation of the Lichen Substances. A $CHCl_3$ extract of 43.7 g of the lichen of sample 2 deposited a precipitate (0.1 g) consisting of FPC and PC; the weight of evaporated extract was 0.4 g. On treatment of this extract with benzene, 0.028 g of

ursolic acid was obtained. The extract (0.3 g) was then chromatographed on a column of Sephadex LH-20 in $CHCl_3$: aliphatic components issued first, then ergosterol peroxide and ursolic and cetraric acids.

The lichen substances were purified by recrystallization and the aliphatic acids were converted into methyl esters by their treatment with a 1% solution of Na in MeOH and then with a 5% solution of HCl in MeOH, and these were analyzed by GLC on a Shimadzu-9A chromatograph. GLC conditions: capillary column (0.25 mm × 30 m) coated with Supelcowax 10M, column temperature 220°C, detector temperature 240°C, carrier gas helium, rate of flow 40 ml/min.

Ergosterol peroxide — yield 0.05% of the weight of the lichen, mp 178-181°C (methanol). $[\alpha]^{20}_{578}$ — 25° (c 0.084; $CHCl_3$). PMR spectrum (250 MHz, $CDCl_3$, ppm): 0.82 (3H, d, J = 6.5 Hz), 0.83 (3H, s), 0.84 (3H, d, J = 4.5 Hz), 0.89 (3H, s), 0.92 (3H, d, J = 4.5 Hz), 1.01 (3H, d, J = 4.5 Hz), 1.4-1.7 (14H, m, $7CH_2$), 1.95 (1H, m, J = 6.0 Hz, CH), 4.0 (1H, m, H-3), 5.20 (2H, q, J = 7.5 Hz), 6.25 (H, d, J = 8.5 Hz), 6.53 (H, d, J = 8.5 Hz) [9]. NI FAB mass spectrum, *m/z* (%): 429 $(M + H)^-$, (100), 428 $(M^-$, 80), 427 $(M-H)^-$, (56). EI mass spectrum: 428 M, 410 $(M-H_2O)$, 396 $(M-O_2$, ergosterol).

Ursolic acid — yield 0.1% of the weight of the lichen; melting behavior: at 227°C it changed from shapeless crystals into rectangular plates, which melted at 245-248°C. $[\alpha]^{20}_{578}+60°$ (c 0.084; chloroform) [10]. NI FAB mass spectrum, *m/z* (%): 455 $(M-H)^-$, (100), 456 $(M^-$, 58). PMR spectrum (250 MHz, $CDCl_3$, ppm): 0.78 (3H, s), 0.81 (3H, s), 0.86 (3H, d, J = 6.4 Hz), 0.99 (3H, s), 1.09 (3H, s), 1.42-1.78 (9 CH_2-, m), 3.22 (1H, d, J = 10 Hz, H-3), 5.28 (H, t, J = 3.6 Hz, H-12).

Cetraric acid — yield 0.022% on the weight of the lichen; heating behavior: darkened above 190°C (benzene–hexane) [4]. EI FAB mass spectrum: *m/z* (%): 401 $(M-H)^-$, (100), 314 — the PC ion (24). PMR spectrum: 1.09 (3H, t, J = 4.5 Hz), 2.51 (3H, s), 2.67 (3H, s), 3.66 (2H, q, J = 7.1 Hz), 4.76 (2H, s), 6.72 (H, s), 10.71 (OH, s), 12.2 (H, s, OH).

REFERENCES

1. K. A. Rassadina, *Cetraria* of the USSR. Cryptogamous Plants, No. 5 [in Russian]. Trudy Bot. In-ta im. V. L. Komarova, Izd. AN SSSR, Leningrad (1950), p. 171.
2. C. F. Culberson, Chemical and Botanical Guide to Lichen Products, University of North Carolina Press, Chapel Hill (1969), p. 276.
3. Y. Solberg, J. Hattori Bot Lab., **60**, 391 (1986).
4. W. Karrer, Konstitution und Vorkommen der organischen Pflanzenstoffe, Birkhäuser Verlag, Basel and Stuttgart (1976), p. 425.
5. C. F. Culberson, J. Chromatogr., **72**, 113 (1972).
6. H. Krog, The Macrolichens of Alaska, Norsk Polarinst. Skr., Oslo, **144**, 1 (1968).
7. V. G. Minaeva, Medicinal Plants of Siberia [in Russian], SO AN SSSR, Tsentr. Sib. Botsad, izd. 5, Novosibirsk, Nauka (1991), p. 195.
8. J. Elix, J. Johnston, and J. Parker, J. Mycotaxon, **31**, No. 1, 89 (1988).
9. L. I. Strigina and V. N. Sviridov, Khim. Prir. Soedin., 551 (1976).
10. G. Nicollier, R. Tabacchi, J. Gavin, J. L. Breton, and A. G. Gonzalez, Helv. Chim. Acta, **62**, 807 (1979)