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ANTHRAQUINONES OF THE LICHEN *Asahinea chrysantha*

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From a hexane extract of the dry lichen we have isolated six anthraquinones: chrysophanol (I), islandicin (II), cynodontin (III), emodin (IV), a tetrahydroxymethylanthraquinone (V), and a pentahydroxymethylanthraquinone (VI). The structures of (I) and (IV) were confirmed by direct comparison with authentic samples. The structures of (II) and (III) were established by the aid of UV, IR, PMR, and mass spectra. Pigments (V) and (VI) were isolated from a carbonate extract. Pigment (V): mp > 320°C; UV spectrum (nm) 258, 283, 310, 447, 500, 533; mass spectrum: 286 (M^+ 100%), 270, 258, 257, 241, 229, 216, 213, 212, 211, 201, 161, 155, 137, 115, 105, 97. Pigment (VI): mp 315°C; UV spectrum (nm): 247, 261, 302, 500, 540, 565, 578; IR spectrum (cm^{-1}): 1587, 3492; mass spectrum: 302 (M^+, 100%), 286, 274, 245, 228, and the metastable ions 248.6, 219.1, and 192.5. The positions of the β-hydroxyls in the molecules of (V) and (VI) have not been definitively established.

In the territory of the Soviet Union, the genus *Asahinea* is represented by two mass species: *A. chrysantha* (Tuck.) Culb. et Culb. and *A. scholanderi* (Llono) Culb. et Culb., occupying enormous areas of the northeastern part of the country. Both species are char-characteristic for mountain tundras [1]. The high-mountain lichens are arousing special interest because of their capacity for protecting the cells of their organisms from ultraviolet radiation. It is assumed that the colored lichen substances act as filters protecting the phycobionts from the effects of radiation [2].

It is known that lichens of the genus *Asahinea* contain usnic, alectoronic and α-collatolic acids and pink pigments the nature of which has not so far been established [3]. In the present paper we consider the isolation of the pigments of the lichen *Asahinea chrysantha* and the determination of their structures.

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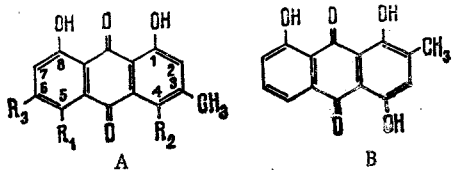
By chromatography on silica gel and Sephadex LH-20 we isolated six pigments from a hexane extract of the lichen thallus. The change in color under the action of alcoholic solutions of KOH and of magnesium acetate showed that the pigments were hydroxylated anthraquinones.

The bathochromic shifts in comparison with the spectrum of anthraquinone observed in the absorption spectra of the pigments showed the presence in them of α-hydroxy groups forming chelate bonds with the quinoid carbonyls.

The mass spectra of all the pigments contained the peaks of the molecular ions with the maximum intensity and the peaks of the fragments $[M - CO]^+$ and $[M - CO - HCO]^+$, which is characteristic for anthraquinones. The structures of the pigments isolated are represented by the generalized formula A (see formula).



The structures and colors of the pigments are as follows:

No. of the pigment	Structure A			Name of the pigment	Colors of the spots on a chromatogram		
	R_1	R_2	R_3		pigment	+KOH + $Mg(OAc)_2$	
I	H	H	H	Chrysophanol	Yellow	Pink	Pink
II	H	OH	H	Islandicin	Orange	Violet	Crimson
III	OH	OH	H	Cynodontin	Crimson	Pale blue	Pale blue
IV	H	H	OH	Emodin	Yellow	Pink	Pink
V	OH	H	OH	A tetrahydroxymethylanthraquinone	Orange	Violet	Crimson
VI	OH	OH	OH	A pentahydroxymethylanthraquinone	Crimson	Deep blue	Violet

The identification of pigment (I) as chrysophanol was confirmed by a direct comparison of the substance isolated with an authentic sample (TLC, melting points, spectra).

The weight of the molecular ion of pigment (II), M^+ 270, is 16 units higher than for chrysophanol and, consequently, its molecule must contain three hydroxyls. The fact that pigment (II) does not interact with sodium carbonate and does not give a methyl ether with an ethereal solution of diazomethane shows the absence of β-hydroxyls. The position in the IR spectrum of the carbonyl absorption band at 1602 cm^{-1} [5] and of signals at δ 11.52, 11.48, and 10.25 ppm in the PMR spectrum confirms the presence of three chelated hydroxyls in the molecule.

The chemical shifts of three aromatic protons, δ 7.85, 7.65, and 7.24 ppm having ortho and meta spin–spin coupling constants, and of the singlet of an aromatic proton at δ 7.10 ppm shows that the methyl group is present in the most hydroxylated nucleus.

All the spectral characteristics given above can be satisfied by two structures: those of islandicin and of digitopurpone (B). The melting points of the pigment isolated and of its triacetate coincided with literature figures for islandicin [6a], while digitopurpone has a lower melting point of 209-211°C [6d]. Furthermore, hitherto the latter has been isolated only from higher plants, and islandicin is distributed in various fungi and molds and was first isolated from a culture of the fungus *Penicillium islandicum* [7]. Thus, pigment (II) is islandicin.

According to the results of mass spectrometry (M^+ 286), the molecule of pigment (III) should contain four hydroxy groups and a methyl group. The substance isolated gave no reaction for β-hydroxyls and, consequently, all four hydroxyls are present in the β-positions with respect to the quinoid carbonyls. This was confirmed by the presence of a single carbonyl absorption band in the IR spectrum at 1576 cm^{-1}, which is characteristic for tetraperi-hydroxyanthraquinones [5].

The PMR spectrum of pigment (III) contains the signals of a methyl group present in an aromatic ring at δ 2.37 ppm and of two pairs of chelated hydroxyls at δ 12.42 and 12.39 ppm. To increase its solubility, the pigment was subjected to silylation; in the PMR spectrum of the trimethylsilyl ether obtained the H-6 and H-7 protons gave a common singlet at δ 6.84 ppm and the H-2 proton a singlet at δ 6.76 ppm. All the spectral characteristics given correspond to the structure of cynodontin.

Considerable difficulties were encountered in the chromatographic separation of chysophanol, islandicin, and cynodontin, which differ in the numbers of hydroxy groups. These difficulties arose because of the presence of strong hydrogen bonds of the hydroxyls with the quinoid carbonyls leading to similarity of the chromatographic parameters of these compounds.

In a sodium carbonate extract from a hexane extract of the lichen, in addition to the main pigment (VI), two minor pigments (IV) and (V) were detected. In contrast to the anthraquinones already considered, pigment (V) gave a monomethyl ether (M^+ 316) with a dilute ethereal solution of diazomethane, which indicates the presence of a β-hydroxyl in it. The fact that the four other hydroxy groups are present in the α-positions to the quinoid carbonyls was confirmed by the carbonyl absorption at 1584 cm^{-1} in the IR spectrum of the pigment. The high-resolution mass spectrum unambiguously gave the empirical formula $C_{15}H_{10}O_7$, and the nature of the fragmentation under electron impact did not contradict the proposed formula and structure.

The appearance of a hydroxyl in the β-position greatly changes the physicochemical properties of a pentahydroxyanthraquinone as compared with α-hydroxylated anthraquinones: the melting point rises and the already low solubility in organic solvents falls. An inadequate amount of the substance isolated and its poor solubility did not enable us to obtain derivatives other than the methyl ether or additional spectral characteristics for this pigment and definitively to establish the mutual positions of the β-hydroxyl and methyl group in its molecule.

By a comparison of its properties with those of an authentic sample, pigment (IV) was identified as emodin.

The orange pigment (V) had the molecular weight of a tetrahydroxymethylanthraquinone, 286 (mass spectrum), and a melting point above 320°C. Its capacity for interacting with sodium carbonate showed the presence of β-hydroxyls. A comparison of the melting points with literature figures for emodin derivatives [8] permitted the assumption that the pigment isolated had the structure of 5-hydroxyemodin (mp > 310°C). However, a direct comparison of pigment (V) and the product of the oxidation of emodin [9] by TLC in various solvent systems showed that pigment (V) differed chromatographically from the 5-hydroxyemodin obtained synthetically. Having less than 1 mg of pigment (V), we were unable to obtain other characteristics for determining its structure.

Indian workers [10] have proposed one of the schemes of evolution of lichen and mold polyhydroxyanthraquinones having a 1,4-dihydroxy system: helminthosporin, islandicin, and cynodontin, which may be related by the common simpler precursor chrysophanol and be formed by additional stages of its oxidation in the para position.

A biogenetic affinity of emodin and islandicin has been shown by Gattenbeck [11] and of emodin and chrysophanol by Harris et al. [12], and therefore the detection of a pentahydroxyanthraquinone, which is in the further stage of oxidation, should not be unexpected.

Islandicin and cynodontin have not previously been detected in lichens, these substances being known as products of the metabolism of molds [13, 14], but this only confirms the generality of biosynthesis of anthraquinones for lichens and free-living fungi.

EXPERIMENTAL

The lichen was collected in the Magadan province in August, 1976.* Melting points were determined on a Boetius stage (they are uncorrected). Mass spectra were taken on a LKB-9000S instrument by the direct introduction of the sample at an ionizing voltage of 70 eV, and the high-resolution mass spectrum on a MS-902 instrument: PMR spectra were taken on a Bruker HX-90E spectrometer with a working frequency of 90 MHz (δ, ppm, 0 — TMS; abbreviations adopted; s, singlet; t, triplet; m, multiplet), absorption spectra on a Specord UV-VIS, and IR spectra on a Specord IR-75.

Extraction and Isolation of the Substances. The air-dry lichen thallus was comminuted and extracted in a Soxhlet apparatus successively with hexane and methanol. The methanol extract was reextracted with hexane. The hexane extracts were combined.

When the hexane extract was concentrated, the solution deposited a yellow precipitate of usnic acid having, after recrystallization, mp 201-203°C, $[\alpha]_D^{20}$ +498° ($CHCl_3$), the identity of the substance being confirmed by a mixed melting point with an authentic sample of (+)-usnic acid.

The mother liquor was evaporated, the residue was dissolved in ether, and the ethereal solution was treated with a 5% aqueous solution of sodium carbonate. The carbonate solution was separated off, acidified, and extracted with ether, after evaporation of the extract, a fraction of acid pigments with a crimson color was obtained. An orange-colored neutral fraction was obtained from the ethereal solution containing the substances not reacting with sodium carbonate.

Pigments of the Neutral Fraction. TLC on Silufol in the hexane—chloroform (1:1) system showed that this fraction contained three pigments: a yellow one (I), an orange one (II), and a crimson one (III) with close R_f values — 0.76, 0.82, and 0.80, respectively. After repeated separation on a column containing silica gel in the hexane—methylene chloride (30:1) system followed by crystallization, 1 mg of chrysophanol, 22 mg of islandicin, and 45 mg of cynodontin were obtained in the pure form from 150 g of dry lichen.

Chrysophanol (I), yellow crystals with mp 194-195°C (literature: 196°C [6c]). It was identified by direct comparison with an authentic sample of chrysophanol (TLC, mass spectrum, absence of a depression of the melting point of the mixture).

Islandicin (II), bronze-red plates, mp 217-219°C (literature figure: 218°C [6a]). Absorption spectrum: λ_{max}(EtOH), nm: 234, 253, 294, 479, 510, 532, 550. IR spectrum, ν_{max}($CHCl_3$), cm^{-1}: 1602. PMR spectrum (CCl_4), δ, ppm: 2.21 (3 H, s, CH_3), 7.10 (1 H, s, H-2), 7.65 (1 H, t, H-6, $J_{6,5}$ = 8.2 Hz. $J_{6,7}$ = 7.6 Hz), 7.85 (1 H, m, H-5, $J_{5,7}$ = 1.5 Hz, $J_{5,6}$ = 7.5 Hz), 7.24 (1 H, m, H-7, $J_{7,5}$ = 1.4 Hz, $J_{7,6}$ = 8 Hz), 11.52 (1 H, s, OH), 11.48 (1 H, s, OH), 10.25 (1 H, s, OH). Mass spectrum, m/e: 270 (M^+, 100%), 253, 242, and 213, metastable ions 237.0, 216.9, 186.5.

Acetyl Derivative of Islandicin. A mixture of 3 mg of islandicin and 1 ml of acetic anhydride (with one drop of pyridine) was left at room temperature for 48 h. Then it was poured onto ice and extracted with ether. After crystallization from methanol, pale yellow crystals of islandicin triacetate were obtained with mp 206-207°C (according to the literature: 208°C [7]). Mass spectrum, m/e: 396, 354, 336, 312, 294, 270 (100%), 242, 241, 213, 185.

Cynodontin (III), dark red crystals, mp 264°C (according to the literature: 263.5°C [6b]). IR spectrum, ν_{max}(KBr), cm^{-1}: 1576. PMR spectrum ($CDCl_3$), δ, ppm: 2.37 (3 H, s, CH_3), 12.39 (2 H, s, OH), 12.42 (2 H, s, OH). Mass spectrum, m/e: 286 (M^+, 100%), 270, 257, 229. Trimethylsilyl ether of cynodontin: PMR spectrum (CCl_4), δ, ppm: 6.76 (1 H, s, H-2), 6.84 (2 H, s, H-6, H-7).

Pigments of the Acid Fraction. The carbonate fraction was separated on Sephadex LH-20 in chloroform, and the crimson, yellow, and orange zones were collected.

Emodin (IV). Crystallization from methanol of the substance from the yellow zone gave about 1 mg of orange needles with mp 254-255°C (according to the literature: 255°C [6e]).

*A sample of *Asahinea chrysantha* (Tuck). Culb. et Culb. is kept in the herbarium of the Tartu State University of the Estonian SSR. The species of lichen was determined by Professor Kh. Kh. Trass.

Absorption spectrum, λ_{max}, nm: 254, 268, 290, 459. The identity of the pigment isolated as emodin was confirmed by a direct comparison with an authentic sample of emodin (TLC, no depression of the melting point with the sample of emodin).

Pigment (V). The substance from the orange zone was dissolved in 0.5 ml of chloroform, and the addition of 2 ml of hexane led to the precipitation of red crystals, which wer filtered off to give about 1 mg of a pigment with mp > 320°C. Absorption spectrum, λ_{max} (EtOH), nm: 258, 283, 310, 447, 500, 533. Mass spectrum, m/e: 286 (M^+, 100%), 270, 258, 257, 241, 229, 216, 213, 212, 211, 201, 161, 155, 137, 115, 105, 97.

Pentahydroxymethylanthraquinone (VI). Three crystallizations from ethanol of the substance from the crimson zone yielded 5 mg of small brownish crystals with mp > 315°C slightl soluble in ethanol and insoluble in other organic solvents. Absorption spectrum, λ_{max}(EtOH) nm: 247, 261, 302, 500, 540, 565, 578. IR spectrum, ν(KBr), cm^{-1}: 1584, 3492. Mass spectrum, m/e: 302 (M^+, 100%), 286, 274, 245, 228, and metastable ions at 248.6, 219.1, 192.5.

Methylation of the Pentahydroxymethylanthraquinone. A methanolic solution of 1 mg of the pigment was treated with a dilute ethereal solution of diazomethane at 8°C, dark brown crystals with mp > 320°C deposited. Mass spectrum, m/e: 316 (M^+, 100%), 301, 273, 245, 217, 189.

SUMMARY

1. Six anthraquinones have been isolated from the lichen *Asahinea chrysantha*: Chrysophanol, islandicin, cynodontin, emodin, a tetrahydroxymethylanthraquinone, and a pentahydroxymethylanthraquinone.

2. The structures of the substances isolated have been established by spectral methods The mutual positions of the methyl and β-hydroxy groups in the molecules of the tetra- and pentahydroxymethylanthraquinones have not yet been definitively established.

3. The detection in the lichen investigated of a series of polyhydroxymethylanthraquinones present in various stages of oxidation confirms the generality of the biosynthesis of anthraquinones in lichens and fungi.

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