A STUDY OF AROMATIC METABOLITES OF LICHENS OF THE FAMILY PARMELIACEAE. II. PIGMENTS

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The following anthraquinones have been isolated from the lichen Asahinea scholanderi (Llano) W. Culb et C. Culb: chrysophanol, emodin, islandicin, cynodontin, and 1,4,5,6(or 7),8-pentahydroxy-3-methylanthraquinone. From the lichen Parmelia birulae Elenk. have been isolated the anthraquinone islandicin and the new violet pigment biruloquinone – 1,4,8-trihydroxy-2-methoxy-7-methyl-9,10-phenanthraquinone-5-carboxylic acid 5→4-lactone – the first ortho-phenanthraquinone detected in lichens.

Lichens of the family Parmeliaceae are widespread in the North East of the USSR and occupy wide areas of the forest-tundra and barren peaks. The massive distribution of high-mountain lichens demonstrates an example of their unique adaptability to conditions of low temperatures, briefness of the vegetation period, and prolonged and intense ultraviolet irradiation.

A study of the life-protecting systems of these astonishing plant symbionts should lead to an understanding of the mechanism that they use for protection from damaging radiation. Important functions in such a mechanism can be ascribed to the quinoid pigments.

An investigation of the chemical components of lichens of the genus Cetraria [1] revealed a wide set of anthraquinone pigments and their simultaneous presence with naphthoquinones with unusual structures. Lichens of the genus Asahinea also contain a wide series of anthraquinone pigments. We have previously isolated from Asahinea chrysantha six polyhydroxyanthraquinones [2]. Anthraquinones identical with or related to them have now been detected in a close species – A. scholanderi (Llano) W. Culb et C. Culb (Table 1).

Pigments (I-IV and VI), were found to be identical with compounds isolated previously by a comparison of their spectral characteristics and by the absence of depressions of the melting points of mixed samples.

In the preceding communication, we did not determine the position of the β-hydroxyl in pigment (VI) [2]. The PMR spectrum of the pentaacetate obtained from it has enabled us to show that the β-hydroxyl in this anthraquinone is located in position 6 or 7; position 6 is more likely from biosynthetic considerations. A doublet of the proton of the methyl group at 2.19 ppm (J = 1.1 Hz) and a quartet of aromatic protons with the same SSCC at 7.33 ppm show their interaction and ortho arrangement.

From the lichen A. scholanderi we isolated yet another pigment (VII), apparently a tetrahydroxymethylanthraquinone, m/z 286 (M^+, 100%). On methylation with diazomethane, it gave a monomethyl ether with m/z 300 (M^+, 100%); its structure has not yet been definitively established. A minor pigment (VIII) was not identified.

The mass species Parmelia birulae, which is endemic for the North East of the USSR, has not previously been investigated chemically. It contains a set of phenolic metabolites similar to those of lichens of the genus Asahinea [3]. The only anthraquinone detected in it (II) amounted to not more than 0.01% of the weight of the dry lichen. However, the main pigment of P. birulae Elenk. (0.01%) was a new quinone: 1,4,8-trihydroxy-2-methoxy-7-methyl-9,10-phenanthraquinone-5-carboxylic acid 5 → 4-lactone, the first phenanthraquinone detected in lichens, for which we propose the name biruloquinone (see scheme on following page).

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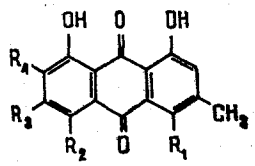
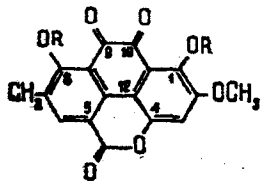
TABLE 1. Amounts and Structures of the Pigments of Lichens of the Family Parmeliaceae

Compound	R_1	R_2	R_3	R_4	Colors of solutions of the pigments	mp, °C	Amount, %	
							A. chrysantha	A. scholanderi
I. Chrysophanol	H	H	H	H	Yellow	194–195	$7\cdot10^{-4}$	$7\cdot10^{-5}$
II. Islandicin	OH	H	H	H	Orange	217–219	$1\cdot10^{-2}$	$2\cdot10^{-2}$
III. Cynodontin	OH	OH	H	H	Crimson	264	$3\cdot10^{-3}$	$2\cdot10^{-3}$
IV. Emodin	H	H	OH	H	Yellow	254–255	$7\cdot10^{-4}$	$7\cdot10^{-5}$
V. 5-Hydroxyemodin	H	OH	OH	H	Orange	>320	$1\cdot10^{-5}$	Her
VI.	OH	OH	H*	OH*	Crimson	>320	$3\cdot10^{-3}$	$3\cdot10^{-4}$
VII.	—	—		—	Orange	298	None	$2\cdot10^{-4}$
VIII.	—	—	—	—	Crimson	—	None	Tr.

*The opposite arrangement is possible





IX. R = H
X. R = COCH$_3$

A pigment (IX) isolated from a chloroform extract of the lichen was soluble in dioxane and pyridine and sparingly soluble in methanol, ethanol, and dimethyl sulfoxide. The blue-violet color of a solution of the pigment changed to red-violet under the action of alkali and to deep blue under the action of magnesium acetate; dithionite caused the decoloration of the pigment, but the color was restored on standing in the air.

Under the action of diazomethane, (IX) did not form methyl ethers; but an acetylating mixture gave a diacetate (X). With o-phenylenediamine in $CHCl_3$ an orange-colored adduct (XI) was obtained, the mass-spectrum of which corresponded to that of a quinoxaline derivative. The electronic spectrum of (IX) was very close to that of piloquinone, a dihydroxy-9,10-phenanthraquinone derivative isolated from the fungus Streptomyces pilosus [4, 5].

In the carbonyl region of the IR spectrum of the pigment there were two bands at 1728 and 1628 cm^{-1}, corresponding to the carbonyl of a lactone group and the absorption of chelated quinoid carbonyls.

The mass spectrum of (IX) was characterized by the presence of the ions M^+ 326 (100%); (M^+ + 1), 327; and (M^+ + 2), 328 (6%) and by a low intensity of the majority of fragmentary ions, which is typical for orthoquinones with condensed carbon skeletons. The largest fragments with m/z 297 and 280, corresponded to the elimination of HCO and CO groups and were accompanied by metastable ions. In the high-resolution mass spectrum for the m/z 326 ion a value of 326.0375 was measured, the calculated figure for $C_{17}H_{10}O_7$ being 326.0426.

The PMR spectrum of (IX) taken in deuterochloroform, contained the signal of the protons of a methyl group attached to an aromatic ring in the form of a doublet at 2.87 ppm (J = 0.9 Hz), a methoxy group (4.03 ppm), two aromatic protons at 6.96 (q, J = 0.9 Hz), and 7.02 ppm (s), and two singlet signals of the protons of hydroxy groups at 12.50 and 12.66 ppm. Because of the poor solubility of the pigment it was impossible to obtain a satisfactory ^{13}C NMR spectrum; the spectrum of the diacetate also permitted only limited information to be obtained.

In the spectrum, 17 signals (out of 21) were reliably observed; the remaining ones either overlapped with those observed or were absent because of long relaxation times and the small amount of substance. Signals at 20.6 and 21.0 ppm belonged to the carbon of CH_3 groups of acetyl substituents, a signal at 24.6 ppm to a CH_2

group attached to an aromatic ring, and one at 56.9 ppm to a methoxy group.

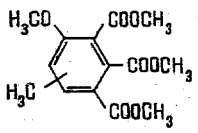
The signals of the α-dicarbonyl (quinoid) grouping coincided and were present at 175.6 ppm, and those of the C atoms of the carbonyls of the acetyl substituents did so at 168.4 ppm. The C-3 and C-6 carbons resonated at 106.9 and 128.3 ppm, respectively. The signal of the C-7 atom, and also the signals of the quaternary carbons bound to oxygen atoms, C-1, C-2, C-4, and C-8, were present in the 149.7-157.7 ppm region. The C-12 atom is present in ortho and para positions to carbon atoms bearing oxygen substituents; a signal at 109.1 ppm must be ascribed to this carbon atom.

The signal of the carboxy group of the δ-lactone, apparently absent from the spectrum, is probably located at 168.4 ppm, coinciding with the signals of the carbonyls of the acetyl substituents, as indicated by its anomalously high intensity.

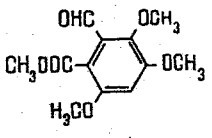
Thus, pigment (IX) is undoubtedly an o-quinone with two symmetrically arranged chelated hydroxyls, and its skeleton consists of 16 carbon atoms. The proposed structure of the 9,10-phenanthraquinone with a δ-lactone ring is not contradicted by the facts given above. However, the positions of the quinoid carbonyls and of the methyl and methoxyl groups and of the carboxyl of the lactone group required additional proofs. They were obtained from the results of an investigation of the products of the oxidation of (X) by alkaline hydrogen peroxide.

After methylation with diazomethane, these products were separated by micropreparative gas chromatography, and the fractions obtained were characterized by their mass spectra and by a second gas-chromatographic comparison with a series of standard esters of aromatic methoxy acids on phases of different polarities.

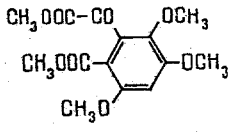
As a result, the esters of 6-methoxy-4(5)-methylbenzene-1,2,3-tricarboxy acid (XIII), of 1-formyl-3,4,6-trimethoxybenzoic acid (XIII), and of 3,4,6-trimethoxyphthalonic acid (XIV) were identified.



XII



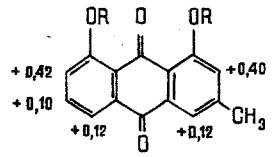
XIII



XIV

The presence of compound (XII) among the oxidation products unambiguously shows the (5 → 4) position of the lactone group in the molecule of (IX) and the position of the methyl substituent at C-6 or C-7. The position of the methyl (and that of the methoxy) group was established definitively by a comparison of the chemical shifts of the aromatic protons in compounds (IX) and (X). When the hydroxy groups of (IX) were acetylated, rises in the chemical shifts of the signals of the aromatic protons by 0.11 and 0.14 ppm were observed. Using as model compounds chrysophanol (I) and its diacetate (XV) we observed a similar rise in the chemical shifts of the protons in the meta and para positions but not of those in the ortho position to a hydroxyl, where the magnitude of this rise was considerably greater (about 0.4 ppm).

The methyl and methoxy constituents in (IX) are located in positions 7 and 2, respectively.



I. R=H
XV. R=COCH3

Phenanthraquinones are extremely rare compounds among natural quinones. The fungal metabolites piloquinone and hydroxypiloquinone [4, 5] are probably the only authentically known representatives of them. The discovery of biruloquinone once more confirms the analogy of the biogenetic pathways in free-living fungi and lichens.

EXPERIMENTAL

The sites where the samples of lichens were collected and the basic instrumental technique used in their investigation were described in the first communication [3]. GLC was performed on a GC-5AP instrument with a flame-ionization detector in the analytical experiments and with a thermal conductivity detector in the preparative experiment. The columns were charged with the phases OV-1, OV-17, and QF-1 (3% on Gas-Chrom Q). The temperature was programmed from 170 to 295°C, 6 deg/min, and the carrier gas was argon.

Isolation of the Pigments. The comminuted air-dry lichen Asahinea scholanderi was extracted successively with hexane, acetone, and methanol. After the evaporation of the solvents, the combined residue was dissolved in acetone and chromatographed on a short column filled with basic magnesium carbonate. The pigments formed colored lakes and the bulk of the impurities were removed with the solvent.

The pigments were extracted with acidified methanol, and they were precipitated from methanolic solution with an excess of copper acetate. The precipitate, after being washed with hexane, chloroform, and water, was treated with dilute HCl and extracted with ether. The combined pigments were separated by column chromatography on silica gel and Sephadex LH-20 [2]. From a hexane extract of the lichen P. birulae (712 g), 7 mg of the antraquinone (II) was obtained by the method described above.

The chloroform extract of this lichen was also chromatographed on magnesium carbonate, giving 70 mg of a crude violet pigment. It was purified in the form of the copper complex, and also by repeated crystallization from dioxane and a mixture of dioxane with $CHCl_3$.

Anthraquinones from A. scholanderi. Substances (I)-(IV) proved to be identical with the chrysophanol, emodin, islandicin, and cynodontin that had been isolated from A. chrysantha [2], having the same chromatographic behavior in standard systems (A, B, and C – see [6]) and the same changes in coloration under the action of solutions of caustic soda and magnesium acetate, and they showed no depressions of mixed melting points.

Compound (VI) was identical with the pentahydroxymethylanthraquinone likewise isolated from A. chrysantha. Acetylation with a mixture of acetic anhydride and pyridine gave the pentaacetate: mp 233-235°C (decomp.) PMR spectrum, ppm: 2.19 (3 H, d, J = 1.1 Hz, CH_3Ar); 2.33, 2.39, 2.41, 2.44, 2.48 (each 3 H, s, CH_3CO); 7.16 (1 H, s, HAr); 7.33 (1 H, q, J = 1.1 Hz, HAr).

Islandicin from P. birulae. Orange-red crystals from hexane, mp 217-219°C. Mass spectrum, m/z: 270, 253, 242, 224, 213; metastable ions at 237.1, 216.9, and 187.5. The chromatographic behavior in standard systems [3, 6], the change in coloration under the action of caustic soda and magnesium acetate, decoloration with sodium dithionite, and the absence of a depression in a mixture with an authentic sample completely proved the structure of the pigment isolated.

Biruloquinone (IX) from P. birulae. Dark violet crystals (from dioxane); mp 307-308°C. Absorption spectrum, nm: λ_{max}^{CHCl} 290, 307, 320, 440, 568 nm (log ε 4.03, 4.17, 41.2, 3.33, 3.42); $\lambda_{max}^{CH_3OH}$ 234, 268, 323, 403, 565 nm. $\nu_{max}^{CHCl_3}$: 3080, 2960, 2927, 2840, 1728, 1628, 1600, 1555, 1473, 1366, 1195, 784 cm^{-1}. Mass spectrum, m/z (%): 328(6), 327(19), 326(100), 298(11), 297(23), 280(20), 269(14), 252(9), 241(15); metastable ions: 270.6, 264.0, 226.8, 215.9. R_f in standard solvent systems: A – 0.80; B – 0.13; C – 0.30 (on Silufol).

Biruloquinone Diacetate (X). Compound (IX) (31 mg) was treated with 1 ml of acetic anhydride and 1 ml of pyridine. The mixture was kept at room temperature for 1 h, whereupon the color of the solution became orange. The crude acetylation product was crystallized from $CHCl_3$ with the addition of hexane. Yellow crystals, mp 132-134°C. PMR spectrum ($CDCl_3$), ppm: 2.47 and 2.48 (each 3 H, s, CH_3CO); 2.94 (3 H, D, J = 0.9 Hz, CH_3Ar); 3.98 (3 H, s, OCH_3); 7.07 (1 H, q, J = 0.9 Hz, HAr); 7.16 (1 H, s, HAr).

Quinoxaline Derivative of Biruloquinone (XI). A solution of 2 mg of (IX) in 5 ml of $CHCl_3$ was treated with 1.5 mg of o-phenylenediamine and three drops of acetic acid. After 3 days, the precipitate of the adduct that had deposited was washed with ether and was recrystalized from chloroform. mp > 340°C. Mass spectrum, m/z (%) 400(3), 399(22), 398 M^+ (73), 383(13), 381(4), 380(19), 369(10), 368(4), 365(10), 355(40), 299(10), 149(100); metastable ion 364.7.

Oxidation of Biruloquinone Diacetate. To 9 mg of (X) in 1 ml of methanol were added, in 0.1-ml portions, 1 ml of 0.3 M KOH in methanol and 0.4 ml of 29% H_2O_2. The mixture was stirred at room temperature for 1.5 h, and then at 50°C for 1 h. The residual hydrogen peroxide was decomposed by boiling. The methanol was evaporated off in vacuum, 2 ml of water added, and the mixture was extracted with ether. The aqueous solution was acidified with H_2SO_4 and was saturated with Na_2SO_4, and the acidic oxidation products were reextracted with methyl ethyl ketone. After the solvent had been eliminated, the residue was treated with an ethereal solution of diazomethane. The mixture of methyl ethers obtained was investigated with the aid of analytical and micropreparative gas chromatography and chromato-mass spectrometry. The gas chromatogram had four main peaks. Mass spectrum of the first substance, m/z: 254 (M^+), 239 ($M^+ - CH_3$), 226 ($M^+ - CHO + H$), 223 ($M^+ - CH_3O$), 195 ($M^+ - CH_3OCO$), 194 (m/z 223 – CH_3O); the substance was identified as the permethylate of a formyltrihydroxybenzoic acid.

When the second main component was subjected to comparative chromatography on columns with phases with different polarities, it proved to have retention times close to those of the trimethyl ether of 4-methoxyhemimellitic acid but differed from the latter by 14 mass units. Its mass spectrum, m/z: 296 (M^+), 266 ($M^+ - CH_3 + H$), 265 ($M^+ - CH_3O$) 250 (m/z 265 − CH_3), 237 ($M^+ - CH_3OCO$), 236 ($M^+ - CH_3OCO - H$), 221 (m/z 236 − CH_3), 207 (m/z 237 − CH_3 + H), 149; the substance was interpreted as the permethylate of 4-hydroxymethylbenzene-1,2,3-tricarboxylic acid.

Mass spectrum of the third substance, m/z: 312 (M^+), 281 ($M^+ - CH_3O$), 253 ($M^+ - CH_3OCO$), 225 (m/z 253 − CO). The substance was interpreted as the permethylate of a trihydroxyphthalonic acid. The fourth substance (m/z 298) was probably a product of incomplete methylation.

SUMMARY

1. A chloroform extract of the lichen Parmelia birulae has yielded the anthraquinone islandicin and the new violet pigment biruloquinone, 1,4,8-trihydroxy-2-methoxy-7-methyl-9,10-phenanthraquinone-5-carboxylic acid (5→4)-lactone, the first ortho-phenanthraquinone from a lichen.

2. A hexane extract of the lichen Asahinea scholanderi has yielded seven anthraquinone pigments, including: chrysophanol, islandicin, cynodontin, emodin, and 1,4,5,6(or 7),8-pentahydroxy-3- methylanthraquinone.

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