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QUINONES OF THE LICHEN *CETRARIA CUCULLATA*

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Key Word Index—*Cetraria cucullata*; Parmeliaceae; lichen; new naphthoquinones; anthraquinones; aliphatic acids.

Abstract—Two new quinones, 7,7′-bis (1,4,5,8-tetrahydroxy-3-ethylnaphthalene-2,6-dione) and its monomeric analogue, five anthraquinones, parietin, fallacinal, emodin, endocrocin and emodic acid, as well as (−)-usnic acid, (+)-lichesterinic and protolichesterinic acids were isolated from the lichen *Cetraria cucullata.*

INTRODUCTION

Cetraria cucullata (Bell.) Ach. is an abundant lichen species found in the north east of the USSR, which is grazed by reindeer. Its chemical composition has been little investigated. Recent publications [1–4] have reported that it contains ascorbic, usnic and protolichesterinic acids, the terpenoid friedelin, structurally unknown pigments and some microelements.

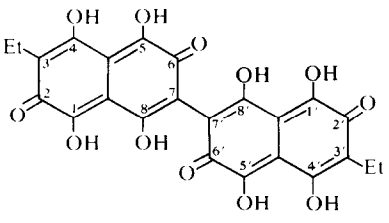
The present paper gives the results of an investigation of the secondary metabolites, mainly the pigments, located in the lower tips of the thallus of this lichen.

RESULTS

Extraction of the thallus with ethyl ether, benzene and acetone did not remove all the pigments. The residual pigments were bound in stable metal–organic complexes and were extracted by methanol containing hydrochloric acid (99:1).

TLC of the total extract gave usnic acid, and red, yellow and yellow-orange pigments. A red polar pigment (**1**) was the predominant coloured compound (0.20% by wt of coloured thallus tips).

Electron spectra data of pigment **1** were very similar to those of the natural hydroxynaphthoquinones mompain, 2,5,7,8-tetrahydroxy-1,4-naphthoquinone, and echinochrome A, 2,3,5,7,8-pentahydroxy-6-ethyl-1,4-naphthoquinone [5]. When treated with alkali, magnesium acetate, zirconyl nitrate and ammonia, the pigment changed colour to blue-violet, blue-violet, violet and lavender, respectively. It was decolourized by sodium hydrosulphite and the colour was restored in air. These data indicated that the pigment was a hydroxyquinone, apparently a hydroxynaphthoquinone. The ^{13}C NMR spectrum of **1** showed signals for two aliphatic (*q* and *t*) and 10 aromatic (*s*) carbons, two of which were carbonyl C atoms. None of the aromatic carbon atoms had a proton substituent. This observation was confirmed by the absence of signals in the aromatic region of the ^{1}H NMR. The triplet at δ 1.23 and the quartet at δ 2.66 (which interacted with each other) were assigned to the protons in the ethyl side-chain and the signal at δ 11.33 was attributed to the presence of chelated OH groups. The IR spectrum of pigment **1** contained a complex band at 1598 cm^{-1} with shoulders at 1572, 1580 and 1620 cm^{-1}. A band at 1620 cm^{-1} was thought to represent the stretching vibration of quinonoid carbonyl groups bounded by intramolecular hydrogen bonds. The MS of pigment **1** exhibited major fragments at *m/z* 500 $[M+2]^+$ (7%), 499 $[M^+ + 1]^+$ (28%), 498 $[M^+]^+$ (100%), 497 (M − 1], 480 $[M - H_2O]^+$, m* 462.6 (498 → 480), 470 $[M^+ - CO]$, m* 443.6 [498 → 470] and 452 $[M - CO - H_2O]^+$, m* 434.7 (470 → 452). The elemental composition ($C_{24}H_{18}O_{12}$) of M^+ was established by high resolution MS (Found 498.076, calcd. 498.080.) The elemental composition and the presence of only 12 carbon signals in the ^{13}C NMR spectrum indicated that pigment **1** must contain two



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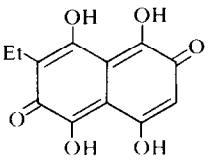

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identical fragments. This was confirmed by the presence of a fragment peak at m/z 249 $[M]^+$ in the MS.

Pigment **1** gave an octaacetate ($M^+ = 824$), suggesting the presence of eight OH groups in the molecule, but only a tetramethoxy derivative ($M^+ = 554$) was obtained by methylation with diazomethane. Hence, there were only four non-hydrogen-bonded hydroxyl groups. The positions of the ethyl groups, belonging to the two symmetrical parts of the molecule, were determined from the ^{13}C NMR data. The existence of long-range C—C—C—H coupling constants for the signals at δ 127.3 (C—Et), 153.3 (C—OH) and 172.3 (C=O) indicated that the ethyl substituents were located between the carbonyl and hydroxyl functions of the quinonoid rings. An attempt to obtain an anhydro derivative of the pigment by treatment with conc. H_2SO_4 at 110° [6] was unsuccessful and the pigment was recovered unchanged. This excluded 1,2- and 1,4-naphthoquinonoid structures for the fragment. Hence, as a possible naphthoquinonoid system, a 2,6-naphthoquinone (*amphi*-naphthoquinone) system seemed the most likely. The location of the carbonyls in positions 1 and 5 was improbable due to the ease of transformation in 1,4-quinones [7]. For a 2,6-naphthoquinonoid fragment with four hydroxyls and an ethyl group there are four isomeric structures (**2a**–**2d**). The 1,1′ dimer of **2a** should give a binaphthoquinone with six hydroxyl groups capable of reaction with diazomethane, and unlike the natural pigment its IR spectrum should contain an absorption band for non-chelated carbonyl groups. The dimer of **2b** would form an anhydro derivative very easily. Thus our experimental data did not correspond to structures **2a** and **2b**. Of the isomers **2c** and **2d**, the preferred one was **2d**, the coupling of which in its free position 7 leads to structure **1** and does not contradict the experimental data. The corresponding product of isomer **2c** could not be definitely discounted. Confirmation of structure **1** as 7,7′-bis (1,4,5,8-tetrahydroxy-3-ethylnaphthalene-2,6-dione) requires an X-ray study which is in progress.

The UV spectrum of pigment **2** was similar to that of pigment **1**. The colour reactions were identical and the MS contained analogous fragments. Methylation with diazomethane formed a dimethyl ether. The identification of pigment **2** as 1,4,5,8-tetrahydroxy-3-ethylnaphthalene-2,6-dione, the monomeric unit of pigment **1**, was confirmed by dimerization of the dimethyl ether of pigment **2** to give a product identical to the tetramethyl ether of pigment **1**.

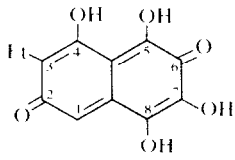
A naphthoquinonoid pigment (**3**) was isolated in very small quantities and its structure was not established.

The presence of a series of anthraquinones along with the new naphthoquinones in *C. cucullata* was unexpected. They were seen as a yellow zone on a Sephadex column and were isolated together (35 mg). The main component of this mixture was emodin, which was separated by repeated chromatography. Crystallization of the rest of the yellow pigments produced a polar anthraquinone, mp >310°, m/z 314 $[M]^+$, which was identified as endocrocin. Among the minor components the anthraquinone mixture parietin, fallacinal and emodic acid were identified by comparison (MS and TLC) with known samples.

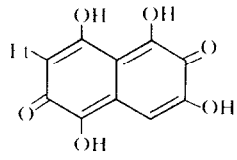
The other six pigments, one red, one yellow and four yellow-orange, were present in small amounts and not identified. In the course of the separation and purification of these pigments, a mixture of other colourless compounds, mainly fatty acids, was obtained. GLC of the methyl esters of these acids showed that (+)-lichesterinic and protolichesterinic acids were the principal components.

DISCUSSION

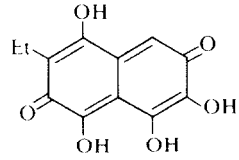
In Thomson's monograph [5] there is no mention of natural *amphi*-naphthoquinones, and data on these compounds have not appeared in the following years (Sci. Cit. Index). Although *amphi*-naphthoquinone is an unstable substance [9], its 1,5-dihydroxy derivative is



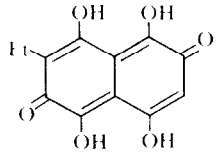
2a



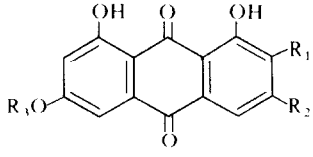
2b



2c



2d



	R_1	R_2	R_3	
4	H	Me	H	Emodin
5	COOH	Me	H	Endocrocin
6	H	Me	Me	Parietin
7	H	CHO	Me	Fallacinal
8	H	COOH	H	Emodic acid

stable due to mesomerism [10]. The polyhydroxynaphthoquinone **1** isolated in the present work is also stable at normal temperatures in the solid state and in solution.

Naphthoquinones in lichens are evidently not rare [11–14], but information on binaphthoquinones has so far not been published, though the same compounds are known as fungal metabolites [15,16].

Pigments of the lichen *C. cucullata* include both naphtho- and anthraquinones, which are rarely found together in plants and which are interesting from a biogenetic point of view. The very complicated set of quinonoid pigments present in the lichen *C. cucullata* further alerts our attention to the biological function of this class of compounds.

EXPERIMENTAL

Mp (uncorr.); ^{1}H NMR 90 MHz; ^{13}C NMR 22,63 MHz; MS: 70 eV, direct inlet; GLC: dual columns 100 × 0.3 cm, packed with 3% OV-17, 200–300°, 6°/min, FID; TLC: Si gel developed with (1) hexane–Et_2O–HCOOH (13:8:2), (2) C_6H_6–Me_2CO (7:3), (3) C_6H_6–Et formate–HCOOH (75:24:1); Silufol sheets impregnated with 2% soln of oxalic acid. Compounds located with 10% H_2SO_4 (100°), 1% ethanolic KOH, 1% $Mg(OAc)_2$ and UV (254 and 355 nm).

Lichen samples. Voucher specimens deposited at Tartu State University: *C. cucullata*, Magadan district, July 1976; *C. ornata* and *C. nigricans*, Sikhote Alin, July 1977; *Xanthoria aureola*, coast of Peter the Great Bay, May 1980.

Extraction and fractionation. Air-dried coloured tips of the thallus (900 g, 30% dry wt) were extrd first with Me_2CO and then with 1% HCl–MeOH. Usnic acid (300 mg), mp 201–202° (C_6H_6), $[\alpha]_D^{20}$ −489° ($CHCl_3$) was sepd out by concn of the extracts. Recrystallization of the main portion of the extracts from various solvents (C_6H_6, MeOH, Et_2O, hexane) gave the following fractions: A, dark red residue (3.0 g) containing polar pigment **1**; B, concentrate of red and yellow pigments (60 mg); and C, combined residues of colourless substances (35.5 g).

Purification of pigment **1**. Crude pigment was precipitated in hot MeOH containing $Cu(OAc)_2$ (3 g). The ppt. was washed with H_2O and C_6H_6 and decomposed with 10% HCl. Extraction with Et_2O gave 1.8 g of fine red crystals, mp 265° (dec.). UV λ_{max}^{MeOH} nm (log ε): 216 (3.95), 242 (4.12), 270 (3.80), 333 (3.62), 457 *sh* (3.42), 494 (3.52), 525 (3.57), 562 (3.41); IR: ν_{max}^{KBr} cm^{-1}: 3527, 3400, 2968–2872, 1598, 1460, 1413, 1298; ^{1}H NMR ($CDCl_3$): δ 1.23 (*t*, Me), 2.66 (*q*, CH_2-Ar), 11.33 (*s*, OH); ^{13}C NMR (DMSO-d_6): δ 12.5(*q*), 16.1 (*t*), 102.9 (*s*), 109.9 (*s*), 115.5 (*s*), 127.3 [*s* (*br*)], 153.3 [*s* (*br*)], 154.9 (*s*), 165.3 (*s*), 166.0 (*s*), 171.2 (*s*), 172.3 [*s* (*br*)]; MS *m/z* (rel. int.): 499 (26), 498 [M]$^+$ (100), 497 (31), 483 (4), 482 (10), 481 (12), 480 (18), 479 (8), 470 (12), 455 (13), 452 (22), 451 (15), 424 (6), 409 (7), 249 (9), 207 (7), 181 (17), 97 (7), m* at 462.6, 443.6, 434.7. (Found: C, 57.99; H, 3.71. $C_{24}H_{18}O_{12}$ requires: C, 57.83; H, 3.62%.)

Tetramethyl ether of pigment **1**. 5 mg **1** was treated with CH_2N_2 in Et_2O. The methylated product was separated by TLC (system 1), to give 2 mg tetramethyl ether, mp 263–266° (Et_2O). MS *m/z* (rel. int.): 554 [M]$^+$ (100), 553 (88), 539 (78), 538 (61), 524 (16), 523 (40), 522 (26), 507 (11), 493 (6), 479 (5), 277 (13).

Octaacetate of pigment **1**. 52 mg **1** treated with 30 ml Ac_2O containing 3 drops of H_2SO_4 for 48 hr at room temp. The mixture was then poured onto ice and extrd with $CHCl_3$. Purification by CC on cellulose (CH_2Cl_2) gave 42 mg octaacetate, mp 115–118° (Et_2O) *m/z* (rel. int.) MS: 834 [M]$^+$ (**3**); UV λ_{max}^{MeOH} nm (log ε): 207 (4.43), 254 (4.22), 277 (4.07); IR ν_{max}^{KBr} cm^{-1}: 1777, 1675, 1167; ^{1}H NMR ($CDCl_3$): δ 2.38 (*s*, MeCO).

Leucoacetate of pigment **1**. 10 mg **1** in 3 ml EtOAc was reduced by PtO_2 (1 mg)/H_2 until colourless (16 min). 0.5 ml Ac_2O containing 3 drops of C_5H_5N was added and the mixture warmed for 30 min at 50°. After purification on a cellulose column 7 mg leucoacetate **1** was obtained, mp 170–172° (HOAc), IR ν_{max}^{MeOH} cm^{-1}: 1774, 1165.

Isolation of other pigments. TLC of fraction B on Si gel showed the presence of three red, six yellow and four yellow-orange pigments which were all decolourized by $NaHSO_3$ and showed typical colour reaction of hydroxyquinones. Fraction B (60 mg) was sepd on a column of Sephadex LH-20 ($CHCl_3$–EtOH, from 1:1 to 0:1). Two red zones gave pigments **2** and **3** (3 and 1 mg). Repeated chromatography of the yellow zone (25 mg) gave emodin. The rest was separated by prep. TLC, but only endocrocin was isolated (1 mg).

Minor anthraquinones were identified by comparison (MS and TLC) with authentic samples.

Pigment **2**. Crystallization (EtOH) gave 2 mg red crystals, mp 183–185°. UV λ_{max}^{MeOH} nm: 217, 241 (sh), 266, 485 (sh), 517, 555; MS *m/z* (rel. int.): 250 [M]$^+$ (100), 235 (37), 232 (10), 217 (12), 209 (2), 207 (3), 203 (5), 193 (5), 156 (7), 142 (5), 137 (5), 124 (5). Methylation of pigment **2** with CH_2N_2 gave a dimethyl ether, mp 163–164°, MS *m/z*: 278 [M]$^+$, 263, 262, 250, 249.

Dimethyl ether of **2** in CH_2Cl_2 at 20° was treated (16 hr) with $FeCl_3$–SiO_2, prepared according to [17]. After addition of 1 ml H_2O and 0.2 ml of HCl, the reaction product was isolated by extraction with CH_2Cl_2. The extract contained a substance identical (TLC and MS) with the tetramethyl ether of pigment **1**.

Pigment **3**. Collected from Sephadex column in very small quantity. UV λ_{max}^{MeOH} nm: 220, 278, 378, 487, 514.

Emodin. Crystallization (EtOH) gave 15 mg, mp 254–255°. UV λ_{max}^{MeOH} nm: 254, 269, 290, 436; MS *m/z*: 270 [M]$^+$, 242, 213. According to these data and R_f value, the pigment was identical with an authentic sample of emodin isolated from *Rumex acetosa*.

Endocrocin. A polar yellow pigment, mp >310°. MS *m/z*: 314 [M]$^+$, 270, 269, 253, 242, 213, 44 (base peak). Colour reaction and chromatographic behaviour were identical to those of a sample of endocrocin isolated from *C. ornata*.

Parietin, fallacinal, emodic acid. These pigments were present in small amounts. They were identified by comparison (MS and TLC) with samples extrd from *Xanthoria aureola*. Parietin, *m/z* 284 [M]$^+$, 256, 241; fallacinal, *m/z* 300 [M]$^+$, 256, 255, 242.

(+)-Lichesterinic and protolichesterinic acids. GLC of the Me esters (CH_2N_2) of fraction C showed the presence of eight components. The RR_fs of the two main components were the same as those of the Me esters of lichesterinic and protolichesterinic acids. Crystallization (C_6H_6) of fraction C gave 20 g, colourless prisms, mp 122–123°, $[\alpha]_D^{22}$ +34°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1662, 1699, 1756; MS *m/z* (rel. int.): 324 [M]$^+$ (16), 281 (15), 279 (68), 261 (10), 251 (10), 155 (22), 112 (22). These data correspond to those of (+)-lichesterinic acid [18].

Authentic samples. Emodin (mp 254–255°, C_6H_6) was obtained from the $NaHCO_3$ fraction of an Et_2O extract of the roots of *R. acetosa*. Endocrocin (mp >310°) was obtained from the Me_2CO extract of the lichen *C. ornata*. IR ν_{max} cm^{-1}: 3392, 1717, 1660, 1615. Parietin, fallacinal and emodic acid were extrd from the lichen *X. aureola* [19]. (+)-Protolichesterinic acid was obtained from the Me_2CO extract of the lichen *C. nigricans*, as colourless needles (C_6H_6), mp 106–107°, $[\alpha]_D^{22}$ +12.7°. (+)-Lichesterinic acid was obtained by isomerization of (+)-protolichesterinic acid according to ref. [20], mp 123–124°, $[\alpha]_D^{22}$ +30°.

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