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QUINONES OF *CETRARIA ISLANDICA*

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Abstract—Two known quinones, 3-ethyl-2,7-dihydroxynaphthazarin and 6,6′-bis(3-ethyl-2,7-dihydroxy-naphthazarin) and one new quinone, 2-*O*-(3′-ethyl-1′,2′,3′,4′-tetrahydro-5′,7′,8′-trihydroxy-1′,2′,4′-triketo-3′-naphthyl)-3-ethyl-7-hydroxynaphthazarin, were isolated from the red thallus tips of the lichen *Cetraria islandica* var. *polaris*. © 1997 Elsevier Science Ltd

INTRODUCTION

Many scientists have investigated the chemical composition of the lichen *Cetraria islandica* (L.) Ach. beginning from the XIX century till to day. However, so far the nature of the red pigments of the lichen thallus tips has not been elucidated. Recently, we have reported on some peculiarities of the chemical composition of *C. islandica* var. *polaris* Rassad., which is a variety of *C. islandica* (L.) Ach. and a typical lichen of the North regions of the Russian Far East [1]. The completely investigated samples from the Magadan district have bright orange-red thallus tips. We report here on the isolation and structure elucidation of the pigments.

RESULTS AND DISCUSSION

The bright-coloured tips form 10–18% of the dry thallus weight. The pigments are difficult to extract and are dissolved only after maceration with 1–5% HCl in ethyl acetate. Earlier, acidic maceration was used for the isolation of the bisnaphthoquinone (cuculuquinone) from red thallus tips of the lichen *Cetraria cucullata* (Bell.) Ach. [2, 3]. It is interesting that similar pigments (echinochromes, spinochromes) are also found in echinoderms. These pigments are bonded with calcium in the skeletons of the animals and the preliminary acid processing is necessary for their extraction with solvents [4]. Apparently, the lichen pigments are also bound with metals very firmly as in echinoderms. Determination of the ash in the different parts of the thallus showed that the coloured tips had ash contents 1.5 times higher than those of the upper and middle parts.

TLC of the benzene extract of coloured tips exhibited three principal pigments: P1 red (R_f 0.56), P2 crimson (R_f 0.32), and P3 orange (R_f 0.26) (see Experimental System 3). Treatment with reagents for hydroxyquinone structures ($Mg(OAc)_2$ and NaOH) changed P1 and P2 to blue. They were decolourized with $Na_2S_2O_4$ and the colour was restored in air, suggesting the hydroxyquinonoid structure of the pigments. Colour alterations of P3 were not specific unlike P1 and P2. Impregnation of the Silufol sheets with oxalic acid solution was necessary for successful TLC separation of the pigments. This is further indication of some likeness of these pigments to echinochrome and cuculoquinone [5].

The UV, ^{1}H NMR and mass spectra of P1 and its derivatives were found to be the same as those for 3-ethyl-2,7-dihydroxynaphthazarin [6], so that P1 was identified as **1**.

The UV, IR and NMR spectra of P2 were the same as those of P1 excluding the aromatic protons signal, which was absent in the NMR spectrum of P2. The positive and negative FAB mass spectra [7, 8] showed intense peaks at *m/z* 498 (42%), which was equal to double the M_r of P1 minus two protons. Characteristic fragment ions of P2 at *m/z* 248–250 were observed in the NI mode only. These results suggested that P2 was a symmetric dehydrodimer of P1 formed by C—C linkage. The spectral characteristics of P2 were like those of 6,6′-bi(3-ethyl,2,7-dihydroxynaphthazarin), which was first isolated and identified from deep-sea holothuria; therefore P2 has the same structure **2** [9].

In contrast to P1 and P2, P3 was very sensitive to alkali. Na_2Co_3 (1–5%) induced its decomposition, producing P1 (M_r 250) (confirmed by TLC, NMR and

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MS). $NaBH_4$ induced the same effect. These results suggest that P1 is a moiety of P3. We failed to obtain methyl ether, acetyl ester and *o*-phenylenediamine derivatives of P3; it was destroyed to give a multicomponent mixture that suggested a complex structure.

Spectral methods were used to determine the structure of P3. The EI mass spectrum of P3 showed the most intensive peak at *m/z* 250 like P1. The negative and positive ion FAB mass spectra exhibited four ion peaks of low intensities at *m/z* 497–500 (4–22%), and fragment ion peaks of high intensities at *m/z* 247–251 (42–100%). The results of NI-PI-FABMS and EIMS showed that the M_r of P3 could not be determined by these techniques. However, Liquid Secondary Ion Mass Spectrometry (LSIMS) revealed an intensive peak at *m/z* 497 (100%), and another peak at *m/z* 247 (6%) for $[M-H]^-$, $[M/2-2H]^-$ ions, respectively. These results suggest that the M_r of P3 was 498.

LSIMS technique was also used to check the alkaline hydrolysis products of P3. Different conditions were used to produce various fragment ions of this pigment, but only the peaks at *m/z* 249 (100%) $[M/2]^+$ and $[M/2]^-$ were obtained. Thus, P3 was a dehydrodimer of P1 in which two moieties (each M_r 249) were not connected by a C-C linkage, since it was so easily destroyed with hydrolytic reagents.

The IR spectra of P3 contained very strong absorption bands which were absent from the spectra of P1 and P2: a band at 1652 cm^{-1} (>C=O) showed a free carbonyl group; one at 1432 cm^{-1} (—CH_2—C—O) corresponded to an active -CH_2-group. Very strong bands at 1284 cm^{-1}, 1208 cm^{-1} and a medium one at 1032 cm^{-1} (≥C—O—C) indicated the existence of an ether linkage. Therefore, P3 was a dimer with an ether linkage between the monomeric units.

The ^1H NMR and ^{13}C NMR spectra of P3 contained double the number of signals observed in P1 and established the asymmetry of P3. These spectra exhibited signals like P1, which belong to P3-N moiety having a naphthazarin structure, the remaining signals belong to the other moiety P3-T (Table 1).

On the basis of literature data, it was suggested that the structure and properties of P3 were similar to 'lapachol peroxide' (**5**), suggesting that P3 was formed through C-O coupling of the radicals according to the scheme of Thomson for lapachol oxidation [4]. Thus, lapachol (**4**) was oxidized with PbO_2 in HOAc to give the dimeric 'lapachol peroxide' [10]. The last was sensitive to alkali like P3 and was hydrolysed with 2% $NaHCO_3$ into lapachol. 'Lapachol peroxide' resulted from a coupling reaction involving the radicals **4b** and **4c** (Scheme **1**). It should be noted that neither monomeric unit has a lapachol structure.

In contrast to 'lapachol peroxide', P3 was probably formed by coupling of the radicals analogous to **4a** and **4b**. In this case, only P3-N kept the naphthazarin structure of P1, while the other moiety was transformed into a triketotralin (P3-T). The structure 2-*O*-(3′-ethyl-1′,2′,3′,4′-tetrahydro-5′,7′,8′-trihydroxy-1′,2′,4′-triketo-3′-naphthyl)-3-ethyl-7-hydroxynaphthazarin is suggested for P3, which is called islandoquinone. An accurate assignment of the relevant carbons was realized for P1 only, while the chemical shifts in P3 were determined according to the suggested structure **3**.

Some lichenologists consider the red colour of the *Cetraria* thallus tips (*C. cucullata*, *C. islandica*) to be

Table 1. ^1H and ^{13}C NMR spectral data for pigments

	P1		P2	P3-N*			P3-T†	
Position	δ_H	δ_C	δ_H	δ_H	δ_C	Position	δ_H	δ_C
1		174.5			183.6	1′		190.6
2		153.9			111.0	2′		193.1
3		126.7			128.0	3′		83.0
4		179.3			177.2	4′		189.3
5		165.2			146.0	5′		145.0
6	6.66*s*	109.0‡		6.89*s*	110.3§	6′	6.20*s*	107.5
7		155.9			156.2	7′		157.8
8		157.0			156.7	8′		158.0
9		109.0‡			110.3§	9′		107.5
10		102.9			103.8	10′		93.0
2-OH	6.92*bs*		6.85*bs*			2′-OH		
3-$CH_2\underline{CH_3}$¶	1.18*t*(7.3)	12.4	1.18*t*(8.0)	1.37*t*(7.2)	11.5	3′-$CH_2\underline{CH_3}$	1.05*t*(7.3)	5.8
3-$\underline{CH_2}CH_3$¶	2.68*q*(7.3)	15.4	2.72*q*(8.0)	2.36*q*(7.2)	16.0	3′-$\underline{CH_2}CH_3$	1.79*q*(7.3)	27.0
5-OH	13.13*s*		13.42*s*	13.42*s*				
8-OH	11.72*s*		11.78*bs*	11.52*s*			10.97*bs*	

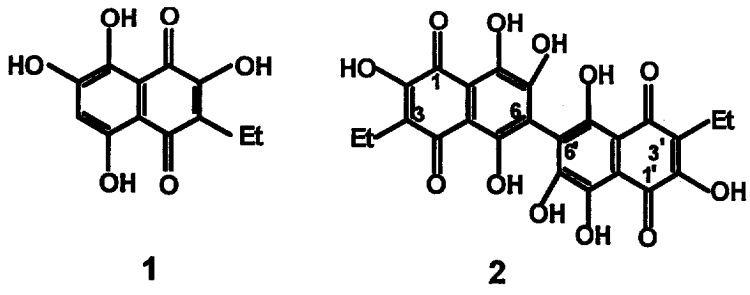
* Naphthazarin moiety of P3.

† Triketotetralin moiety of P3.

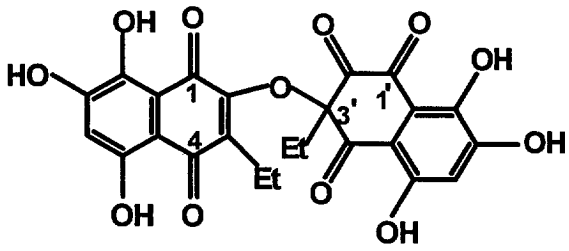
‡ One signal of double intensity due to C-6 and C-9 of P1.

§ One signal of double intensity due to C-6 and C-9 of P3-N moiety.

¶ Coupling constants (*J* in Hz).



1 2



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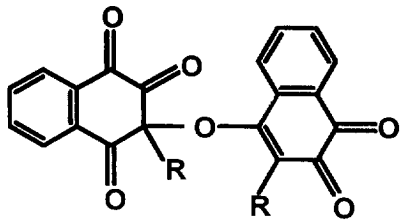

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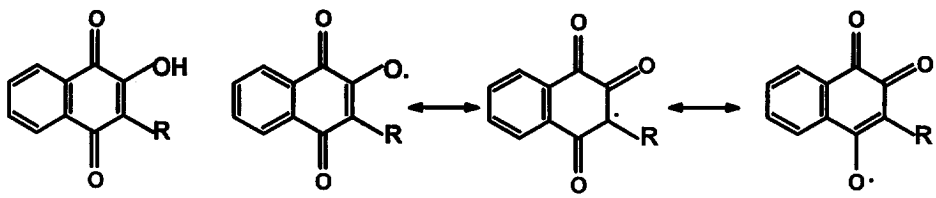

4 4a 4b 4c

Scheme 1.

an evidence of their drying off [11]. This opinion seem to be erroneous. On the contrary, we observed the longest and brightest coloured tips of both lichens when its thallus reached the largest dimension and often had an apothecium.

EXPERIMENTAL

NMR: 250 MHz for ^1H and 63 MHz for ^{13}C, in $CDCl_3$, DMSO-d_6 solns, using TMS as int. standard; IR: KBr; UV-VIS: MeOH; mp: uncorr.; MS *m/z* rel. int. in %; EIMS: direct inlet at 15 and 70 eV (LBK 9000 S); FABMS and LSIMS: LKB 2091 with Ion Tech. FAB gun and Cs mini-ionizer. The sensitivity was demonstrated to be higher in the NI mode than in the PI one and exceeded 10 ng. LSIMS: 1 μl 10^{-2}–10^{-3} M/l solns of compounds, modified glycerine as matrix. TLC: Silufol sheets impregnated with 2% soln of oxalic acid and developed with (1) hexane–Et_2O–HCOOH (13:8:2), (2) toluene–EtOAc–HCOOH

(139:83:8), (3) C_6H_6–HCOOEt–HCOOH (75:24:1). Compounds located with 1% ethanolic KOH or 1% $Mg(OAc)_2$.

Lichen samples of Cetraria islandica *var.* polaris *Rassad.* Magadan district, Russia (July 1991). Voucher specimens deposited in the Herbarium of the Pacific Institute of Geography Far East Division of Russian Academy of Sciences.

Extraction and fractionation. Air-dried coloured tips of the tallus (1,5 kg) were extracted with EtOAc contained 2% HCl and after evapn of solvent the residue was reextracted with C_6H_6 and MeOH. The crude extract (7 g) of pigments was sepd by DCCC with upper and lower phases of system $CHCl_3$–MeOH–H_2O (5:6:3.5), under 8 atm., in 7 days. The pigments were isolated from differently coloured frs of the eluate and recrystallized. Yields: P1 0.01%, P2 0.001%, P3 0.0004%.

*P*1 (3-*ethyl*-2,7-*dihydroxynaphthazarin*). Fine red crystals, mp 182–185° (hexane). UV λ_{max}^{MeOH} nm (log ε): 232(4.16), 268(3.86), 318(3.69), 442(3.31)sh, 479(3.42)sh, 511(3.49), 551(3.33)sh; IR: ν_{max}^{KBr} cm^{-1}: 3528, 3428, 2978-2860, 1604, 1480, 1444, 1336, 1304, 1268. ^1H and ^{13}C NMR: Table; MS *m/z* (rel. int.): EIMS, 250 $[M]^+$(100); NI FABMS, 251 $[M+H]^-$(20), 250 $[M]^-$(100), 249 $[M-H]^-$ (80); PI FABMS, 252 $[M+2H]^+$ (65), 251 $[M+H]^+$(100), 250 $[M]^+$(25); LSIMS, 249 $[M-H]^-$(100); ^1H and ^{13}C NMR: Table.

2,7-*Dimethyl ether of P*1. Prepared with CH_2N_2, needles of cherry colour, mp 145–147° (hexane). ^1H NMR δ: 3,95 (3H, *s*, OCH_3), 4.078 (3H, *s*, OCH_3), 1.10 (3H, *t*, J = 7.5 Hz), 2.75 (2H, *q*, J = 7.5 Hz), 6.28 (H, *s*), 12.82 (OH, *s*), 13.32 (OH, *s*).

2,5,7,8-*Tetraacetate of P*1. ^1H NMR δ: 2.34–2.45 (12H, 4*s*, 4Me), 1.08 (3H, *t*, J = 7.5 Hz), 2.49 (2H, *q*, J = 7.5 Hz), 7.33 (H, *s*).

*P*2 6,6′-*bis*(3-*ethyl*-2,7-*dihydroxynaphthazarin*). Fine raspberry colour crystals, mp 155–157° (benzene). UV λ_{max}^{MeOH} nm (log ε): 216 (4.42), 238 (4.45), 270 (4.20)sh, 318 (3.97), 452 (3.72)sh, 514 (3.85), 552 (3.65)sh; IR ν_{max}^{KBr} $cm^{)1}$: 3520, 3418, 2978–2853, 1602, 1460, 1388, 1334, 1294; PI FABMS *m/z* (rel. int.): 500 $[M+2H]^+$ (95), 499 $[M+H]^+$ (100), 498 $[M]^+$ (42); NI FABMS *m/z* (rel. int.): 499 $[M+H]^-$ (11), 498 $[M]^-$ (20), 497 $[M-H]^-$(13), 250 $[M/2+H]^-$(35), 249 $[M/2]^-$(100), 248 $[M/2-H]^-$(55); ^1H NMR: Table.

2,2′,7,7′-*Tetramethyl ether of P*2. Prepared with CH_2N_2. EIMS *m/z* (re. int.): 554 $[M]^+$ (100).

*P*3, *Islandoquinone*, (2-O-(3′-*ethyl*-1′,2′,3′,4′-*tetrahydro*-5′,7′,8′-*trihydroxy*-1′,2′,4′-*triketo*-3′-*naphthyl*)-3-*ethyl*-7-*hydroxynaphthazarin*). Orange crystals, mp 243–244° (benzene). UV λ_{max}^{MeOH} nm (log ε): 251 (4.57), 314 (4.33), 403 (4.17), 414 (4.16)sh, 474 (3.87)sh, 506 (3.69)sh; IR ν_{max}^{KBr} cm^{-1}: 3340, 2971-2856, 1652, 1610, 1432, 1364, 1284, 1208, 1176, 1032; MS *m/z* (rel. int.): EIMS, 250 (100); PI FABMS, 500 $[M+2H]^+$(22), 499 $[M+H]^+$(10), 251 $[M/2+2H]^+$(40), 250 $[M/2+H]^+$(52), 249 $[M/2]^+$(4); NI FABMS, 499 $[M+H]^-$(5), 498 $[M]^-$(40), 497 $[M-H]^-$(4), 249 $[M/2]^-$(100), 248 $[M/2-H]^-$(42), 247 $[M/2-2H]^-$ (15); NI LSIMS, 497 $[M-H]^-$ (100), 247 $[M/2-2H]^-$ (6); ^1H and ^{13}C NMR: Table.

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