ANTHRAQUINONE PIGMENTS OF THE STARFISH *Echinaster echinophorus*. IV

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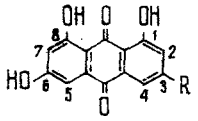
Echinoderms are producing agents of a peculiar group of pigments of quinoid nature. Of the five classes of echinoderms (sea urchins, starfish brittle stars, sea cucumbers, and crinoids), the chemical nature of the pigments of sea urchin has been most studied. In their needles and tests a large number of naphthoquinoid derivatives including binaphthoquinones have been found [1, 2]. Naphthoquinoid pigments have also been detected in sea cucumbers [3], brittle stars [4], and starfish [4]. Only in the crinoids has a different type of quinoid pigment been found – anthraquinone derivatives [5-13].

In the present paper we report the isolation of anthraquinone pigments from the starfish Echinaster echinophorus (Lamarck) from the Caribbean Sea.

The column chromotography on Sephadex LH-20 of an ethanolic extract from the starfish Echinaster echinophorus yielded three compounds consisting of derivatives of 1,6,8-trihydroxy-9,10-anthraquinone:



A. R = $-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_3$ (1′ 2′ 3′)

B. R = $-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-CH_2-CH_2-CH_3$ (1′ 2′ 3′ 4′ 5′)

C. R = $-CH_2-CH_2-CH_3$ (1′ 2′ 3′)

On thin-layer chromatography (TLC) on Silufol plates, the pigments formed yellow-orange spots which changed color to red when they were sprayed with alkali and an ethanolic solution of magnesium acetate. These color reactions, and also electronic spectra, gave grounds for assuming that the pigments were hydroxylated derivatives of anthraquinone.

The solubility of the substances isolated in aqueous Na_2CO_3 showed the presence of a β-hydroxy group in their structure. This was confirmed by the ease of formation of a monoethyl ether by the action on the pigments of an ethereal solution of diazomethane.

The IR spectra of the pigments showed two absorption bands of quinoid carbonyls: 1630 cm^{-1} for a chelated carbonyl and 1670 cm^{-1} for a free carbonyl, i.e., the pigments isolated are derivatives of 1,8-dihydroxy-9,10-anthraquinone.

The PMR spectra of the pigments contained doublets from two pairs of aromatic protons located in the meta position with respect to one another. Of the remaining at C-3 and C-6 of the anthraquinone nucleus one must be occupied by the hydroxy group methylatable with diazomethane.

In the ^{13}C NMR spectrum of substance A there are signals from 17 carbon atoms, three signals (δ 24.0, 46.5, 67.5) being located in the strong field showing that the side chain contains three carbon atoms to one of which (δ 67.5) an oxygen atom is attached.

The strong peak of the molecular ion M^+ 314 in the mass spectrum confirms the presence of a side chain with the composition C_3H_7O. The loss of 44 units, leading to the formation of the main peak with m/e 270, shows the presence of a hydroxy group in position C-2′ of the side chain. Its position at C-1′ would have led, through α-cleavage, to the formation of a main peak with m/e 285, as has been observed for rhodoptilometrin. [13]. The structure of the 2′-hydroxypropyl side chain was confirmed by the presence in the PMR

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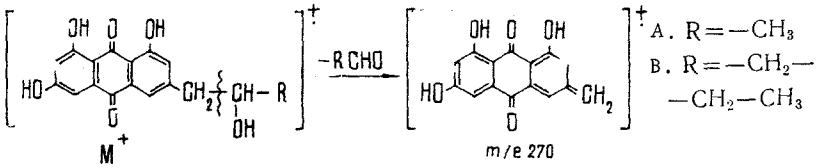
spectrum of a doublet from two benzyl protons (δ 2.92), a doublet from three methyl protons (δ 1.38), and a one proton methine multiplet (δ 4.30). For all of them, the spin–spin coupling constants J = 6.2 Hz.

Thus, substance A has the same structure as the isorhodoptilometrin isolated from the quinoid Ptilometra australis [8] but differs considerably from the latter in its melting point. The methylation of substance A with diazomethane leads to a monomethyl ether which has a PMR spectrum and melting point close to those of the monomethyl ether of isorhodoptilometrin – nalgiovensin [8, 14].

In the ^{13}C NMR spectrum of substance B there are signals from 19 carbon atoms, of which 5 relate to the aliphatic carbons of a side chain: δ 14.4, 19.4, 40.4, 45.1, 71.2. The signal at δ 71.2 is due to a carbon atom to which oxygen is attached.

A comparison of the mass spectra of pigments A and B and also of 1,6,8-trihydroxy-3-(2'-hydroxypentyl)-9,10-anthraquinone, which has been isolated from the crinoid Comanthus bennetti [13], shows that the hydroxy group in the 5-carbon side chain of pigment B is located at C-2'. This follows from the formation under electron impact of one and the same fragment with m/e 270, which rises by α-cleavage of the side chain in the following way:



The normal structure of the hydroxypentyl group is confirmed by the PMR spectrum of B. A triplet from the three protons of a methyl group with δ 0.94 (C-5'), the two-proton doublet of a benzylmethylene group with δ 2.92 (C-1'), the multiplet of a methine proton with δ 4.14 (C-2'), and a multiplet from four methylene protons δ 1.64 (C-3' and C-4') confirm that pigment B is 1,6,8-trihydroxy-3-(2'-hydroxypentyl)-9,10-anthraquinone, which is analogous to the pigment from the crinoid Comanthus bennetti [13].

The third pigment, C, chromatographically the most mobile (R_f 0.66) has an electronic spectrum repeating those of substances A and B and, consequently, this substance must also be a derivative of 1,6,8-trihydroxy-9,10-anthraquinone.

The molecular ion M^+ 298 differs by 16 units from that of pigment A (M^+ 314) and the considerably lower intensity of the fragmentary ion with m/e 270 (45%) indicates the absence of a hydroxy group from the side chain.

The PMR spectrum of substance C shows a three-proton triplet at δ 0.85 of a methyl group, a triplet of the two protons of a benzylmethylene group with δ 2.52, and a methylene multiplet at δ 1.54, which are characteristic for a n-propyl radical. A comparison of the melting points, PMR, IR, and mass spectra of substance C with those given for 1,6,8-trihydroxy-3-propyl-9,10-anthraquinone [8, 13, 14] shows the identity of these compounds.

EXPERIMENTAL

The absorption spectra were taken on a Shimadzu MPS-5000 instrument in MeOH, the IR spectra on UR-20 and Specord IR-75 instruments, the NMR spectra in C_5D_5N solution on a Brüker HX-90E spectrometer with a working frequency of 22.63 MHz for ^{13}C nuclei and 90 MHz for protons (δ, ppm; 0 – TMS; abreviations adopted: s – singlet: d – doublet; t – triplet; m – multiplet), and the mass spectra on an LKB-9000 instrument with the direct introduction of the sample into the ion source at an ionizing voltage of 70 eV. The melting points were determined on a Boëtius stage. The R_f values were determined by TLC on Silufol plates in the chloroform–hexane–methanol (10:3:2) system.

Extraction and Chromatography. The animals (1 kg) were extracted with ethanol for 7 days. The extract was concentrated in vacuum and it gave 28 g of a dark brown substance readily soluble in water. Exhaustive extraction with diethyl ether of an aqueous solution and subsequent concentration of the ethereal extract in vacuum gave 0.75 g of a yellow oil. The ethereal extract was separated on a column of Sephadex LH-20 in the chloroform–methanol (3:2) system. Of the five fractions obtained, two (I, 70 mg, and II, 2 mg) gave a positive color reaction for quinones (red coloration with a solution of alkali and an ethanolic solution of magnesium acetate).

The rechromatography of fraction I on a column of Sephadex LH-20 in chloroform gave three pigments: A – 30 mg; B – 21 mg; C – 12 mg.

Fraction (II) contained a fourth pigment (R_f 0.10) in amount insufficient to determine its structure.

1,6,8-Trihydroxy-3-(2'-hydroxypropyl)-9,10-anthraquinone (A). Orange needles from $CHCl_3$–MeOH, mp 244-246°C (literature information for isorhodoptilometrin 275-277°C [8], 272-272.5°C [14]), R_f 0.54.

Absorption spectrum, λ_{max}, nm: 224.5, 254, 268, 290.5, 304 sh., 441, 461 sh. (log ε 4.49, 4.22, 4.23, 4.23, 4.03, 4.27, 4.23). IR spectrum (KBr), λ_{max}, cm^{-1}: 3480, 3400, 1670, 1630, 1620. Mass spectrum: m/e 314 (100%), 299 (2), 286 (22), 272 (28), 271 (100) 270 (100), 269 (30), 253 (16), 242 (36), 241 (50), 228 (30), 213 (28), 185 (10), 157 (10), 139 (34), 128 (16). PMR spectrum, δ, ppm: 1.38 (d, J. = 6.2 Hz, CH_3), 4.30 (m, J = 6.2 Hz, CH), 2.92 (d, J = 6.2 Hz, CH_2), 6.91 (d, J = 1.7 Hz, H_{ar}), 7.38 (poorly resolved doublet, H_{ar}), 7.61 (poorly resolved doublet, H_{ar}, 7.93 (poorly resolved doublet, H_{ar}). ^{13}C-NMR, δ: 24.0; 46.5; 67.5; 108.7; 109.5; 110; 114.4; 121.6; 125.2; 133.5; 136; 150.5; 162.5; 165.8; 167.3; 181.8; 190.5.

1,8-Dihydroxy-3-(2'-hydroxypropyl)-6-methoxy-9,10-anthraquinone. A. The monoethyl ether of substance A was obtained by the action of an ethereal solution of diazomethane on it; orange needles from $CHCl_3$–hexane, mp 192-192°C (literature information: 196-197°C [8], 194.5°C [14]).

Absorption spectrum, λ_{max}, nm: 225.5, 254.5, 267, 288, 304 sh., 435, 455 sh. (log ε 4.56, 4.27, 4.28, 4.30, 4.04, 4.23, 4.18. IR spectrum ($CHCl_3$), ν_{max}, cm^{-1}: 3690, 3610, 1670, 1625, 1610. Mass spectrum: m/e 328 (33%), 314 (2), 300 (4), 285 (16), 284 (100), 270 (2), 269 (3), 256 (4), 255 (6), 241 (4), 227 (3), 213 (2), 196 (2), 168 (2), 152 (2), 140 (3), 129 (2), 128 (2). PMR spectrum, δ, ppm: 1.39 (d, J = 5.8 Hz, CH_3), 2.93 (d, J = 6.2 Hz, CH_2), 3.76 (s, OCH_3), 4.32 (m, J = 6.2 Hz, CH), 6.88 (d, J = 2.6 Hz, H_{ar}), 7.43 (d, J = 1.4 Hz, H_{ar}), 7.50 (d, J = 2.6 Hz, H_{ar}) 7.97 (d, J = 1.4 Hz, H_{ar}).

1,6,8-Trihydroxy-3-(2'-hydroxypentyl)-9,10-anthraquinone (B). Orange needles from MeOH, mp 193-195°C, R_f 0.58.

Absorption spectrum, λ_{max}, nm: 224.5, 254, 268, 290.5, 304 sh., 441, 461 sh. (log ε 4.53, 4.25, 4.27, 4.06, 4.31, 4.27). IR spectrum (KBr), ν_{max}, cm^{-1}: 3470, 3380, 1670, 1630, 1620. Mass spectrum: m/e 342 (100%), 314 (4), 299 (12), 286 (12), 272 (19), 271 (100), 270 (100), 269 (16), 253 (8), 242 (17), 241 (30), 228 (14), 213 (11), 185 (4), 157 (4), 139 (5), 128 (8). PMR spectrum, δ, ppm: 0.94 (t, J = 6.4 Hz, CH_3), 1.64 (m 2CH–3' and –4'), 2.92 (d, J = 6.1 Hz, CH_2–1'), 4.14 (m, J = 5.2 Hz, CH–2'), 7.61 (d, J = 2.3 Hz, H_{ar}), 7.95 (d, J = 1.1 Hz, H_{ar}), 6.91 (d, J = 2.3 Hz, H_{ar}), 7.42 (d, J=1.1 Hz, H_{ar}). ^{13}C NMR spectrum, δ, ppm: 14.4; 19.4; 40.4; 45.1; 71.2; 108.7; 109.6; 110.1; 114.4; 121.7; 125.4; 133.5; 136; 150.9; 162.5; 165.8; 167.4; 182; 190.6.

1,6,8-Trihydroxy-3-propyl-9,10-anthraquinone (C). Orange needles from $CHCl_3$–hexane, mp 215-217°C (literature information: 219.5-221.5°C [13], 216-216.5°C [14]), R_f 0.66. Absorption spectrum, λ_{max}, nm: 224, 254, 267.5, 291, 304 sh., 441, 460 sh. Log ε 4.51, 4.23, 4.24, 4.25, 4.04, 4.29, 4.25). IR spectrum ($CHCl_3$), ν_{max}, cm^{-1}: 3690, 3610, 3570, 1670, 1630, 1610. Mass spectrum: m/e 298 (100%), 283 (12), 281 (16), 279 (6), 271 (8), 270 (45), 269 (10), 253 (4), 242 (6), 241 (17), 228 (3), 213 (6), 185 (3), 157 (3), 139 (6), 128 (5). PMR spectrum. δ, ppm: 0.85 (t, J = 7.1 Hz, CH_3), 1.54 (m, J=7.9 Hz, CH_2–2'), 2.52 (t, J = 7.9 Hz, CH_2–1'), 7.00 (d, J = 2.2 Hz, H_{ar}), 7.57 (poorly resolved doublet, H_{ar}), 7.71 (d, J = 2.6 Hz, H_{ar}), and 7.77 (poorly resolved doublet, H_{ar}).

SUMMARY

1. Anthraquinone pigments forming derivatives of 1,6,8-trihydroxy-9,10-anthraquinone have been found in the starfish Echinaster echinophorus (Lamarck).

2. The structures of the pigments isolated are identical with those of pigments of sea lilies.

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COUMARIN COMPOSITION OF *Seseli grandivittatum*

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The genus Seseli, numbering 60 species, 16 of which grow in the Caucasus [1] has been studied inadequately from the chemical point of view, although many species of this genus are characterized by containing coumarins of various structures with high biological activity [2, 3].

We have investigated a sample of the roots of Seseli grandivittatum,* collected in the Nakhichevan Autonomous Soviet Socialist Republic in the flowering period and at the beginning of the ripening of the fruit. The coumarin compositions of these samples did not differ from one another and they contained mainly the following substances: (I), $C_{29}H_{50}O$, mp 128-129.5°C, M^+ 414; (II) $C_{14}H_{14}O_4$, mp 180-181.5°C, $[\alpha]_D^{22}$ −8.98° (c 2.56; chloroform), M^+ 246; (III), $C_{19}H_{20}O_5$, $[\alpha]_D^{22}$ −83.2° (c 6.05; chloroform), $n_D^{21.5}$ = 1.5742, M^+ 328; (IV) $C_{19}H_{20}O_5$, M^+ 328; and (V), $C_{19}H_{22}O_6$, mp 143-145°C M^+ 346, substances (II) and (III) predominating in both cases.

Substance (I) gave a positive reaction for sterols and corresponded in chemical composition and constants to β-sitosterol, as was confirmed by the preparation of an acetyl derivative (VI), $C_{31}H_{52}O_2$ with mp 131.5°C, and by the results of IR, PMR, and mass spectroscopy.

According to its IR and PMR spectra, substance (II) is a linear 3',4'-dihydropyanocoumarin with a hydroxy group in position 3', the properties of which are identical with those of (−)-3'R-decursinol [4, 5]. Compound (II) has not previously been found in nature. The presence of one hydroxy group in its molecule was shown by the preparation of a monoacetyl derivative (VII), $C_{16}H_{16}O_5$, with mp 141-142°C, and M^+ 288, and by the results of the IR and PMR spectroscopy of (VII).

Substance (III) consists of a viscous oil readily soluble in chloroform and is new, not having been described previously in the literature. We have called it grandivittin. The IR spectrum of (III) has, besides others, a broad absorption band at 1710-1750 cm^{-1} showing the presence in the molecule of (III) of, in addition to the CO of an α-pyrone ring, an ester grouping, which is confirmed by the results of its treatment with solutions of alkalis and acids.

The structure of (III) is unambiguously determined by the characteristics of its PMR spectrum in which, beside the signals of the protons of a 6,7-disubstituted coumarin nucleus (6.08 and 7.46 ppm, doublets, H-3 and H-4, J = 10 Hz; 7.06 and 6.64 ppm, singlets, H-5 and H-8), there are the signals of four methyl groups, two of which are attached to a carbon atom bearing an oxygen function (1.33 ppm, singlet, $-C(O)(CH_3)_2$) and two of which are on a double bond (1.84 and 2.06, singlets, $-C=C(CH_3)_2$), and the signals of a methylene

*During the preparation of this paper for the press, a report [6] appeared on the isolation of osthole and two angular pyranocoumarins (edultin and anomalin) from Seseli grandivittatum, but we found no such compounds in samples that we have investigated.

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