QUINOID PIGMENTS OF ECHINODERMATA

# VII. ANTHRAQUINONES OF THE STARFISH *Henricia leviuscula*

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We first detected anthraquinone pigments similar to the pigments of crinoids in the starfish *Echinaster echinophorus* from the Caribbean Sea [1, 2]. This interesting fact induced us to make a search for anthraquinone derivatives in the starfish of the Sea of Japan, and also some representatives of starfishes of the family Echinasteridae from the collection of the Zoological Museum of the Academy of Sciences of the USSR.

The presence of anthraquinones in alcoholic extracts of the animals was judged from the appearance of a red coloration of the spots of chromatograms when they were sprayed with solutions of alkali and magnesium acetate. Below we give information on the distribution of anthroquinones in starfish:

| Order, family | Species | Presence of anthraquinones |
|---|---|---|
| Phanerozonia | | |
| Luidiidae | *Luidia guinaria bispinosa* | — |
| Spinulosa | | |
| Asterinidae | *Patiria pectenifera* | — |
| Echinasteridae | *Echinaster echinophorus* [1, 2] | + |
| | *Henricia knipowitschi* * | + |
| | *H. leviuscula* * | + |
| | *N. ochotensis* * | + |
| | *H. sanguinolenta* * | + |
| Forcipulata | | |
| Asteriidae | *Aphelasterias japonica* | — |
| | *Asterias amurensis* | — |
| | *Distolasterias nipon* | — |
| | *Evasterias echinosoma* | — |
| | *Lisostrosoma anthosticta* | — |

Thus, anthraquinone pigments have been detected so far only in starfishes of the family Echinasteridae.

In the present paper we report the structures of the pigment of the starfish *Henricia leviuscula* (Stimpson). This is a small five-rayed starfish of claret color which is found in temperate and cold water in the seas of the Pacific Ocean. When the animals were extracted with acetone or ethanol, a yellow pigment passed into solution. TLC of the extract in system 1 showed the presence of two pigments in it.

The more mobile (I) had a $R_f$ value identical with that of the 1,6,8-trihydroxy-3-n-propyl-9,10-anthraquinone isolated from the starfish *Echinaster echinophorus*. A comparison of the spectra characteristics of pigment (I) with an authentic sample of 1,6,8-trihydroxy-3-n-propyl-9,10-anthraquinone, and also a mixed melting point, showed that the compounds were identical.

The second substance remained at the start in system 1 and in system 2 it had an $R_f = 0.21$. Its elementary composition corresponded with the formula $C_{17}H_{13}O_9SNa$; it was readily soluble in water and was not reextracted into ethyl acetate from the aqueous solution at pH 7, which permitted the assumption that the pigment included a sulfate group [3]. Heating with water

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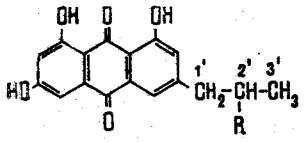
led to the partial loss of sodium, and in the presence of traces of an acid pigment (II) it readily formed sulfate ion and isorhodoptilometrin (III), which is known from the sea lily *Ptilometra australis* [4] and the starfish *Echinaster echinophorus* [1].



I. R = H
II. R = $OSO_3Na$
III. R = OH

To establish the position of the sulfate group we compared the $^{13}C$ NMR spectrum of pigment (II) and that of isorhodoptilometrin (III), the assignment of the signals of compound (III) being made on the basis of the information given by Berger and Jadot [5] (δ, ppm; 0 — TMS; $C_5D_5N$):

| Carbon atom | Isorhodoptilometrin (III) | Pigment (II) | Δδ |
|---|---|---|---|
| C-1 | 162,5 | 162,0 | —0,5 |
| C-2 | 114,5 | 114.5 | 0,0 |
| C-3 | 150.5 | 148,5 | —2,0 |
| C-4 | 108,7 | 108.8 | +0,1 |
| C-5 | 110,0 | 110.3 | +0,3 |
| C-6 | 167,3 | 167,0 | —0,3 |
| C-7 | 109,5 | 109.6 | +0,1 |
| C-8 | 165,8 | 165,4 | —0,4 |
| C-9 | 181,8 | 180,3 | —1,5 |
| C-10 | 190,5 | 189,0 | —1,5 |
| C-11 | 136,0 | 135,5 | —0,5 |
| C-12 | 121,6 | 121,5 | —0,1 |
| C-13 | 125,2 | 125,6 | +0,4 |
| C-14 | 133,5 | 133,0 | —0,5 |
| C-1′ | 46,5 | 43,4 | —3,1 |
| C-2′ | 67,5 | 74.1 | +6,6 |
| C-3′ | 24,0 | 20,6 | —3,4 |

As is well known, the introduction of a sulfate ester group into, for example, a monosaccharide molecule causes a downfield shift of the signals of the corresponding carbon atom by 7-9 ppm and an upfield shift of the resonance signals of the β-carbon atoms by 1.2-2.1 ppm. The influence of a sulfate group on the signals of more remote carbon atoms is very slight and does not have a regular nature [6, 7]. The position of the sulfate group at position 2' in pigment (II) is confirmed by its similar influence on the signals of the carbon atoms of the side chain.

The sulfation of isorhodoptilometrin (III) with the pyridine—sulfur trioxide complex in pyridine gave a compound having the same R*f* as the natural pigment (II). A mixture of the two compounds showed no depression of the melting point. Below, we give the PMR spectra of isorhodoptilometrin, of the product of its sulfation, and of pigment (II) (δ, ppm; 0 — TMS; $C_5D_5N$):

| Compound | *H-2* | *H-4* | *H-5* | *H-7* | $CH_2$– | *CH*— | $CH_3$ |
|---|---|---|---|---|---|---|---|
| Isorhodoptilometrin (III) | 7,70 | 7,98 | 7,43 | 6,99 | 2,92 d | 4,29 m | 1,38 d |
| Product of its sulfation | 7,60 | 7,95 | 7,48 | 6,94 | 3,26 m | 5,39 m | 1,49 d |
| Pigment (II) | 7,60 | 7,91 | 7,43 | 6,95 | 3,22 m | 5,30 m | 1,42 d |

The facts given confirm that (II) is the sodium salt of isorhodoptilometrin 2-sulfate. The detection of this type of compound in starfish is not unexpected. Thus, Erdman and Thomson [8] have postulated the possibility of the presence of water-soluble anthraquinone O-sulfates in sea lilies.

## EXPERIMENTAL

The apparatus used and the other experimental details have been described previously [1].

Extraction and Chromatography. The animals were collected in July 1978, in Vostok Bay, Sea of Japan. Shortly after they had been caught, the animals were placed in ethanol or acetone. Forty specimens (134 g) were exhaustively extracted with ethanol. After evaporation of the extract in vacuum, 12.5 g of a dark brown viscous substance readily soluble in water was obtained. An aqueous solution was extracted with ether, and the resulting extract was chromatographed on a column of Sephadex LH-20 in chloroform. This yielded 13 mg of pigment (I).

Then the aqueous solutions were repeatedly extracted with n-butanol and, after elimination of the solvent in vacuum, the residue was chromatographed in a thin layer of Woelm silica gel in system 2 [ethyl acetate—methanol—water (100:16.5:13.5)]. The zone with $R_f$ 0.21 was repurified on a column of Sephadex LH-20 in methanol. This gave 33.5 mg of pigment (II).

1,6,8-Trihydroxy-3-n-propyl-9,10-anthraquinone (Pigment I). Yellow plates from $CHCl_3$, mp 217-219°C (according to the literature: 215-217°C [1], 219-221.5°C [9]); $R_f$ 0.43 [system 1: hexane—ether (2:1)]. $\lambda_{max}^{MeOH}$, nm: 223, 252, 268, 291, 308 (sh.), 441, 460 (sh.) (log ε 4.50, 4.23, 4.23, 4.24, 4.02, 4.27, 4.23); $\nu_{max}^{CHCl_3}$, $cm^{-1}$: 3400 (β-OH), 1676 ($CO_{free}$), 1628 ($CO_{chel.}$), 1610 (C=C). Mass spectrum, m/e: 298(100), 283(20), 271(12), 270(75), 269(15), 253(5), 242(12), 241(40), 228(5), 213(15), 185(5), 157(8), 139(15), 128(12), 115(12). PMR spectrum (δ, $CD_3OD$), ppm: 0.89 (t, J = 7 Hz, $CH_3$), 1.65 (m, J = 7.9 Hz, $CH_2$-2'), 2.68 (t, J = 7.9 Hz, $CH_2$-1'), 6.54 (d, J = 2.3 Hz, $H_{ar}$), 7.06 (d, J = 1.1 Hz, $H_{ar}$), 7.16 (d, J = 2.3 Hz, $H_{ar}$), 7.54 (d, J = 1.1 Hz, $H_{ar}$).

Na Salt of Isorhodoptilometrin 2'-Sulfate (Pigment II). Yellow amorphous powder, mp 187°C (after prolonged drying over $P_2O_5$), $R_f$ 0.21 (in system 2). Found: Na 4.25, S 7.56%. Calculated for $C_{17}H_{13}O_9SNa$: Na 5.52, S 7.69%. $\lambda_{max}^{H_2O}$: 222, 254, 267, 291, 449 nm (log ε 4.38; 4.16, 4.14; 4.11; 3.86). IR spectrum: $\nu_{max}^{KBr}$: 3430, 1676, 1628, 1610, 1562, 1460, 1390, 1271, 1230, 1212, 1169, 1072, 1031, 936, 769 $cm^{-1}$.

Pigment (II) (7.6 mg) was heated in the water bath with 2 ml of 10% HCl for 15 min, the mixture was extracted with ether, and the extract was evaporated in vacuum. This gave 5.4 mg of isorhodoptilometrin (III). On crystallization from methanol, the isorhodoptilometrin formed solvated crystals with mp 244-246°C, and only after prolonged drying at 60°C over $P_2O_5$ did the melting point rise to 272-274°C (according to the literature [4] 275-277°C).

From the aqueous solution after hydrolysis 4.0 mg of $BaSO_4$ (calculated: 4.25 mg) was obtained by the action of $BaCl_2$.

Sulfation of Isorhodoptilometrin. The pyridine—sulfur trioxide complex was obtained by a standard method [10]. A solution of 25 mg of isorhodoptilometrin (III) in 0.4 ml of absolute pyridine was treated with 25 mg of the complex. After 15 h, the reaction mixture was decomposed with ice water, neutralized with $NaHCO_3$, and evaporated to dryness. The residue was transferrred to a TLC plate (Woelm silica gel) and was separated in system 2. The zone with $R_f$ 0.21 yielded 30 mg of a substance with mp 187°C, identical with the natural pigment (II): $\lambda_{max}^{H_2O}$: 222, 254, 267, 290, 448 nm (log ε 4.37; 4.16; 4.15; 4.11; 3.87). $\nu_{max}^{KBr}$: 3430, 1676, 1631, 1610, 1561, 1470, 1390, 1270, 1231, 1210, 1177, 1029, 932, 750 $cm^{-1}$.

## SUMMARY

A preliminary study has been made of starfish of the family Echinasteridae for their content of anthraquinone pigments.

A sulfated anthraquinone — the Na salt of isorhodoptilometrin 2'-sulfate has been isolated from the starfish *Henricia leviuscula*.

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# STRUCTURE AND CONFIGURATION OF CAUFERININ

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Continuing a study of the coumarins of the roots of *Ferula conocaula* Korov. [1, 2], from the total extractive substances we have extracted a new terpenoid coumarin with the composition $C_{22}H_{32}O_6$, $M^+$ 416, mp 204-206°C, $[\alpha]_D^{20}$ +37.5° (c 0.5; ethanol), which we have called cauferinin. It is readily soluble in acetone, ethanol, and pyridine, sparingly soluble in chloroform and water, and insoluble in ether.

The UV spectrum has maxima at $\lambda_{max}$ 218, 244, 255, and 328 nm (log ε 4.22, 3.80, 3.85, and 4.08) which are characteristic for a 7-hydroxy-substituted coumarin chromophore, and the IR spectrum has absorption bands of an OH group (3400 $cm^{-1}$), of the carbonyl of an α-pyrone (1713 $cm^{-1}$), and of an aromatic nucleus (1618, 1515 $cm^{-1}$).

Treatment of cauferinin with acetic anhydride in pyridine yielded cauferinin diacetate with the composition $C_{28}H_{36}O_8$, $M^+$ 500, the IR spectrum of which retained the absorption band of an OH group. These facts show that the terpenoid part of cauferinin contains three hydroxy groups, one of which is tertiary while the other two are secondary. The presence of hydroxy groups was also confirmed by the mass spectrum of cauferinin [m/e 416 ($M^+$), 398 ($M - H_2O$)$^+$, 380 ($M - 2H_2O$)$^+$, 255 ($M - RO$)$^+$, 237 ($M - RO - H_2O$)$^+$, 219 ($M - RO - 2H_2O$)$^+$, 201 ($M - RO - 3H_2O$)$^+$, 161 ($RO$)$^+$, 162 ($ROH$)$^+$], in which there are fragments corresponding to the successive elimination of three molecules of water.

The PMR spectrum ($C_5D_5N$) of cauferinin has the signals of methyl groups on quaternary carbon atoms — singlets at 1.04 (3H), 1.37 (6 H), and 1.85 ppm (3 H) — and a multiplet at 3.57 ppm (2 H) due to hemihydroxylic protons. A multiplet in the 4.25 ppm region (2 H) represents the methylene protons on a —$CH_2$—O—R grouping. In addition, in the weak-field region there are one-proton doublets at 6.20 and 7.57 ppm (J = 10 Hz), 7.16 ppm (J = 2.5 Hz), and 7.32 ppm (J = 8.5 Hz) due to the $H_3$, $H_4$, $H_8$, and $H_5$ protons, respectively; a quartet at 6.92 ppm (1 H, $J_{ortho}$ = 8.5 Hz, $J_{meta}$ = 2.5 Hz) is due to the $H_6$ proton of the coumarin ring.

It must be mentioned that the PMR spectrum of cauferinin diacetate has signals with similar chemical shifts and multiplicities to those of cauferin diacetate [2] and differs only by the fact that in place of the signals of the protons of an exocyclic methylene at 4.56 and 4.89 ppm (in cauferin diacetate) there is a singlet at 1.28 ppm caused by the presence of a hemihydroxylic methyl group. From the facts given above, it may be concluded that cauferinin is cauferin hydrated at $C_2'$ [2]. This is in harmony with the mass spectrum of cauferinin which includes a peak with m/e 398 corresponding to the fragment after the ejection of one molecule of water.

The dehydration of cauferinin with sulfuric acid in ethanol yielded an anhydro derivative with the composition $C_{24}H_{30}O_5$, $M^+$ 398. A comparison of the physicochemical constants and spectral characteristics (IR, PMR) of the latter and of cauferin showed that they were completely identical. Consequently, in the cauferinin molecule there are, as in cauferin, hydroxy groups at $C_4'$ and $C_6'$ and they are oriented equatorially. The substituent —$CH_2$—OR at $C_1'$ also has the equatorial orientation.

The formation of cauferin in the dehydration of cauferinin also showed the equatorial orientation of the hydroxy group and the axial orientation of the methyl group at $C_2'$, since it is just in this case that the formation of an exocyclic double bond is observed [3, 4].

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