established that the hydroperoxyoctadecadienoyl radical is localized mainly in the α position of the oxy-TGs. On the basis of the fatty-acid composition and the results of enzymatic hydrolysis, the proportions of the 15 possible types of oxytriglycerides have been calculated.

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QUINOID PIGMENTS OF ECHINODERMATA

V. PIGMENTS OF THE SEA URCHIN *Strongylocentrotus drŏebachiensis*

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UDC 547.567:539.9

The sea urchin *Strongylocentrotus drŏebachiensis* (O. F. Müll) is a species that is widely distributed in the boreal regions of the world ocean. The presence of spinochromes A, B. C, D, and E [1] and also of a substance which has been identified as anhydroethylidene-3,3'-bis-(2,6,7-trihydroxynaphthazarin) [2] has been established among the quinoid pigments of its shell and needles.

In an investigation of the pigments of animals of the same species caught in the Bering Sea, we also isolated spinochromes A, C, D, and E, and two other substances — X and Y. Substance X predominated in the total pigment extract. The chromatographic mobility and the absorption, mass, and PMR spectra of the pigment and its hexamethyl ether were similar to those of the binaphthoquinone isolated previously from the sea urchin *Strongylocentrotus intermedius* [3], for which the structure of ethylidene-6,6'-bis(2,3,7-trihydroxynaphthazarin) has been proposed.

These indices also do not differ greatly from those found by Mathieson and Thomson for a binaphthoquinone (and its hexamethyl ether, respectively) isolated for the first time from the sea urchin *Spatangus purpureus* [2]. However, these authors considered that a more probable structure of this substance was that of ethylidene-3,3'-bis(2,6,7-naphthazarin).

A comparison of the chromatographic mobilities (in two chromatographic systems) and absorption spectra of substance X and the binaphthoquinones from *Strongylocentrotus* and *Spatangus purpureus*,* and also their hexamethyl ethers, showed no differences whatever between them. Practically the only difference between the binaphthoquinones mentioned is found in their melting points. However, it is possible that this difference could be caused by the presence of impurities and by the tendency of these compounds to form extremely stable solvated crystals. Thus, we previously gave for the hexamethyl ether of pigment X mp 71-73°C [4], while on extremely prolonged drying in a pistol over P_2O_5 it was found to increase and finally reached 134-136°C, i.e., it coincided with the figure given previously [3].

Mathieson and Thomson gave as one of the indications of the proposed structure of the binaphthoquinone the coincidence of its properties with the properties of the binaphthoquinone obtained by the action of acetaldehyde on spinochrome D [2]. We have repeated this synthesis, using spinochrome D obtained from the shell of the sea urchin under investigation. The reac-

*A sample of the binaphthoquinone was kindly given to us by Prof. Thomson.

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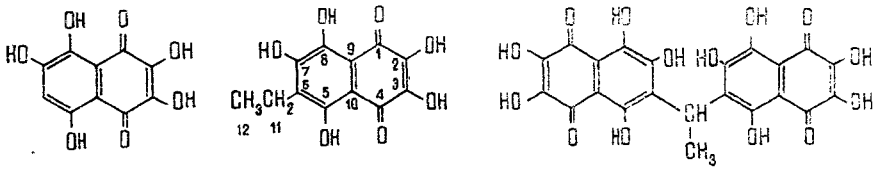
tion product was identical with pigment X in relation to its R_f values, melting point (>310°C and mass and absorption spectra.

Thus, there are sufficient grounds for assuming that substance X and the binaphthoquinones isolated previously from sea urchins have identical structures. Since the available information did not permit the reliable determination of the position at which the ethylidene bridge in the molecules of these pigments binds the symmetrical naphthazarin fragments, we recorded the ^{13}C NMR spectra of spinochrome D, echinochrome A, and pigment X (a plus sign shows that the assignment is not unambiguous):



Spinochrome D Echinochrome A Binaphthoquinone

C atoms	^{13}C chemical shifts, ppm (TMS)		
$1^{(-4)}$	179.0	174.6	170.2
$2^{+(3)}$	141.1	139.9	139.5
$3^{+(2)}$	142.2	142.1	142.6
$4^{+(1)}$	181.4	176.1	170.7
5	161.2	164.6	168.8
6	108.5	124.8	124.8
7	157.1	155.3	161.5
8	151.2	153.5	155.6
9	109.3	106.6	105.9
10	102.0	102.0	102.0
11	—	15.8	27.6
12	—	12.7	17.4

In the ^{13}C NMR spectrum of spinochrome D two signals in the weak field close to 180 ppm are assigned to C-1 and C-4. Three signals in the strong field — 102.0, 108.5, and 109.3 ppm — are assigned to C-6, C-9, and C-10, each of which has an OH group in the ortho position. The remaining five signals belong to carbon atoms to which hydroxy groups are attached.

In the spectrum of echinochrome A, in the strong field of the aromatic region there are only two signals, at 102.0 and 106.6 ppm, and there is a signal at 124.8 ppm which is absent from the spectrum of spinochrome D. This permits the signals at 108.5 ppm in the spectrum of spinochrome D and at 124.8 ppm in the spectrum of echinochrome A to be assigned to C-6, which is in harmony with the α effect of an ethyl substituent for benzene [5].

The signal of one of the angular C-atoms in the spectrum of echinochrome A (106.6 ppm) is shifted upfield by 2.7 ppm as compared with the analogous signal in the spectrum of spinochrome D. This shift of the signal corresponds to the influence of an ethyl group in the para position in benzene [5]. Consequently, the signals at 109.3 ppm in the spectrum of spinochrome D and at 106.6 ppm in the spectrum of echinochrome A relate to C-9. The greater screening of C-10 than of C-9 is a consequence of the para influence of the hydroxy group at C-7.

Two signals close to 140 ppm do not undergo the influence of the ethyl substituent, which permits them to be assigned to C-2 and C-3 and confirms the assumption that in echinochrome A the ethyl substituent is present in the benzenoid, and not the quinoid, ring. This is also indicated by the small difference in the chemical shifts of the C-1 and C-4 signals in the spectrum of this compound (1.5 ppm), while for 3-hydroxy-2-methylnaphthazarin this difference amounts to 6 ppm [6].

In the spectrum of echinochrome A obtained without suppression of spin–spin coupling with the protons, an appreciable broadening of the lines and a decrease in the amplitude of the signals at 124.8, 155.3, and 164.6 ppm caused by H–C-11–C-6, H–C-11–C-6–C-5, and H–C-11–C-6–C-7 long-range spin–spin coupling are observed, This permits the unambiguous assignment of the signal at 153.5 ppm to C-8.

A study of the rate of deuterium exchange in echinochrome A showed that the minimum value of the rate is observed for the hydroxyl at the C atom the signal of which is located

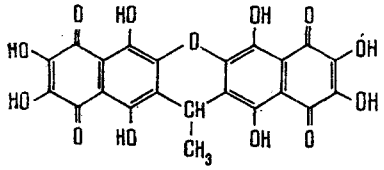
at 155.3 ppm, somewhat higher rates for the hydroxyls at C-2 and C-3, and the maximum rate for C-8 and the C atom with the signal at 164.5 ppm. The hydroxyls and C-5 and C-8 participate in the formation of a chelate H bond, as a consequence of which they possess the maximum and similar capacities for deuterium exchange. Consequently, the signal at 164.6 ppm belongs to C-5 and the signal at 155.3 ppm to C-7.

The assignment of the C-5, C-7, and C-8 signals in the spectra of spinochrome D and the binaphthoquinone was made by comparison with the spectrum of echinochrome A.

In the aromatic region, the spectrum of the binaphthoquinone contains signals of double intensity in comparison with the signals of the sp^3-hybridized atoms in the strong field, which shows the symmetry of the molecule. The coincidence of the chemical shifts of the C-2, C-3, C-6, C-9, and C-10 signals in the spectrum of the binaphthoquinone with the corresponding signals in the spectrum of echinochrome A, together with the fact that the compound is symmetrical, shows a bridge structure of this compound (see the PMR spectrum) and also the predominance on its composition of the tautomer with the C-6,6' linkage of the naphthazarin fragments.

It must be mentioned that on passing from spinochrome D to echinochrome A and further to the binaphthoquinone, in the ^{13}C NMR spectrum there is an upfield shift of the C-1 and C-4 signals and a downfield shift of the C-5 and C-8 signals, i.e., their gradual levelling. It is possible that the replacement of a proton by an ethyl radical or a more voluminous substituent via an ethylidene bridge affects the magnitudes of the chemical shifts of these carbon atoms.

Pigment Y is present in minor amounts in the total pigment extract of the given species of sea urchin. In the mass spectrum of pigment Y, the peak of the molecular ion with m/e 484 is observed. The PMR spectrum (DMSO-d_6) contains the signals from the protons of an ethylidene bridge at 1.36 and 4.35 ppm. From its chromatographic behavior, melting point, and adsorption and mass spectra, pigment Y is identical with the anhydro derivative formed on heating pigment X with concentrated sulfuric acid. These facts permit pigment Y to be assigned the structure of anhydroethylidene-6,6'-bis(2,3,7-trihydroxynaphthazarin):



EXPERIMENTAL

For general remarks, see [4].

The NMR spectra were taken on a Brüker HX-90 Fourier spectrometer with working frequencies of 90 MHz for ^1H and 22.63 MHz for ^{13}C at 30°C with TMS as internal standard. Melting points were determined on a Boëtius stage. The animals, caught in Provideniya Bay, Bering Sea, were kindly given to us by V. L. Khetchikov.

Pigment X — ethylidene-6,6'-bis(2,3,7-trihydroxynaphthazarin). R_f 0.23. mp > 310°C (needles from aqueous methanol). Absorption spectrum: $\lambda_{max}^{CH_3OH}$ 266, 342, 482, 528 nm (log ε 4.44, 4.14, 4.06, 3.91).

Mass spectrum: m/e 264, 238, 210, 192, 181, 168, 153, 140, 136, 128, 125, 108. ^1H NMR spectrum (acetone-d_6, δ, ppm): 1.77 (doublet, J = 7.5 Hz, 3 H, CH_3), 4.84 (q, J = 7.5 Hz, H, CH). The ^{13}C NMR spectrum is given in the table.

Hexamethyl Ether of Ethylidene-6,6'-bis(2,3,7-trihydroxynaphthazarin). R_f 0.23; mp 134-156°C (needles from aqueous methanol). Absorption spectrum: $\lambda_{max}^{CHCl_3}$ 247, 326, 483, 509, 545 nm (log ε 4.59, 4.21, 4.15, 4.25, 4.12). ^1H NMR spectrum ($CDCl_3$, δ, ppm): 3.99, 4.07, 4.10 (18 H, ar. CH_3O); 1.72 (doublet, J = 7.5 Hz, 3 H, CH_3); 4.84 (q, J = 7.5 Hz, 1 H, CH); 12.95 and 13.36 (2 H, peri-OH).

Ethylidene-bis-6'-bis(2,3,7-trihydroxynapthazarin) from Spinochrome D. To 10 mg of spinochrome D in 5 ml of acetic acid was added 1 ml of acetaldehyde. The mixture was heated

in a sealed tube in the water bath for 2 h. After cooling and dilution with water, the reaction product was extracted with ether and was purified by PTLC. In its R_f value, melting point, and absorption and mass spectra, the substance coincided completely with the natural sample given above.

Pigment Y — Anydroethylidene-6,6'-bis(2,3,7-trihydroxynaphthazarin). R_f 0.30; sparingly soluble in all the solvents tested, mp > 310°C. Absorption spectrum: $\lambda_{max}^{CH_3OH}$ 212, 269, 320, 469, 490, 525 nm. Mass spectrum: m/e 484 (M^+). ^1H NMR spectrum (DMSO-d_6, δ, ppm): 1.6 (doublet, J = 6.8 Hz, 3 H, CH_3); 4.35 (q, J = 6.8 Hz, 1 H, CH).

Pigment X (10 mg) was heated in 5 ml of concentrated sulfuric acid for 10 min in a boiling-water bath. The mixture was poured onto ice, and the reaction product was extracted with ether and purified by PTLC. Yield 5 mg. The natural and synthetic samples were identical in their R_f value, melting point, and absorption and mass spectra.

SUMMARY

From a mixture of the pigments of the sea urchin *Strongylocentrotus dröebachiensis*, in additionto the known spinochromes A, C, D, and E, we have isolated a binaphthoquinone and its anhydro derivative. On the basis of ^{13}C NMR spectra the position of the ethylidene bridge symmetrically binding the two naphthazarin fragments of the binaphthoquinone has been definnitely established, and the structure of this compound has been determined as ethylidene-6,6'-bis(2,3,7-trihydroxynaphthazarin).

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STRUCTURE OF THE SESQUITERPENE LACTONE ELEGIN

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We have previously reported the isolation from *Saussurea elegans* Ledeb. of a new sesquiterpene lactone elegin (I) with the composition $C_{19}H_{23}O_6Cl$, mp 158-159°C, $[\alpha]_D^{24}$ + 82.5° (c 2.125; ethanol), M^+ 382 [1]. In the present paper we give proof of its structure.

The PMR spectrum of elegin contains the following characteristic signals: singlet at 1.90 ppm — protons of a methyl group on a double bond; doublets at 5.47 ppm (J = 2.5 Hz) and 6.08 ppm (J = 3.5 Hz) — protons of an exocyclic methylene of a γ-lactone ring; singlet at 4.88 ppm — protons of an exocyclic methylene at C-10; singlets at 5.51 and 6.12 ppm — protons of a vinylmethylene at C-17; doublets with their centers at 4.26 and 4.68 ppm ($J_1 = J_2$ = 11 Hz; $Cl—\underline{CH_2}—\underset{|}{C}—OH$); doublet at 7.25 ppm (J = 5 Hz; OH at C-3); and singlets at 6.70 ppm (OH at C-4). Analysis of the PMR spectrum and the formation of chamazulene when elegin was dehydrogenated over selenium (200-220°C, 10-12 min) showed that it belonged to the sesquiterpene lactones of the guaiane series.

The acetylation of (I) with acetic anhydride in pyridine yielded a monoacetate with the composition $C_{21}H_{25}O_7Cl$ (II), mp 168-170°C, M^+ 424. The acetylation of (I) with acetyl chlo-

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