Procedure. To 1.0 ml of an aqueous solution of the sample containing from 15 to 30 μg of the LPS were added 0.4 ml of distilled apyrogenic water and 0.6 ml of a solution of the reagent (a solution of 2.5 mg of the dye in 25 ml of ethanol is stable on storage in the dark at +4-6°C for 5-6 days). The reaction mixture was carefully stirred and the optical density was measured at 22°C 15 min after the addition of the reagent on a Gilford 240 spectrophotometer (ℓ = 1.0 cm) at 467 nm relative to a comparison solution consisting of 1.4 ml of apyrogenic distilled water and 0.6 ml of the solution of the reagent.

The amount of LPS in the sample was determined from the specific absorption index calculated previously.

A calibration curve (Fig. 2) was plotted on the basis of the proposed method. Each point on the curve represents the mean of five determinations.

SUMMARY

1. A procedure has been developed for the quantitative spectrometric determination of the bacterial lipopolysaccharide of Salmonella $typhi_{4446}$ which eliminates the use of an antioxidant.

2. The proposed procedure is characterized by high sensitivity, good reproducibility, and simplicity of performance.

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NEW QUINONE FROM A MARINE SPONGE OF THE ORDER DICTYOCERATIDA

N. M. Rebachuk, V. A. Denisenko, and S. A. Fedoreev

UDC 547.567+593.4

Two pigments of quinoid nature, one of which has been identified as ilimaquinone, have been isolated from a hexane extract of a marine sponge Hyatella sp., family Spongiidae, order Dictyoceratida. The second was a new benzoquinone of the drimane series with the composition $C_{22}H_{32}O_5$, which has been called hyatoquinone. Its structure has been established on the basis of spectral characteristics and chemical transformations.

From a hexane extract of a freeze-dried preparation of the marine sponge Hyatella sp., family Spongiidae, order Dictyoceratida, we have isolated a fraction containing several substances of quinoid nature. The physicochemical properties of the main component of this fraction coincided completely with the properties given in the literature for ilimaquinone (I) [1].

The other quinone (I), which we have called hyatoquinone (II) proved not to have been described previously. Its spectral characteristics were largely similar to those of ilimaquinone, which indicated a closeness of their structures. The elementary analysis of hyato-

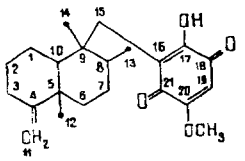
Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, USSR Academy of Sciences, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 793-796, November-December, 1987. Original article submitted May 14, 1987.

 0009-3130/87/2306-0656$12.50

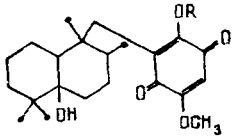
TABLE 1. Comparison of the ^{13}C NMR Spectra of Compounds (I)*, (II), and (III)

C	I	II	III	C	I	II	III
1	33.0[a]	31.4[a]	31.3[a]	13	17.9[c]	17.7[b]	17.8[b]
2	28.6[a]	27.7[a]	27.6[a]	14	17.3[c]	17.2[b]	17.0[b]
3	36.7	22.8[a]	23.1[a]	15	32.4	32.9	34.0
4	153.4	42.9	43.5	16	117.4	117.8	134.2
5	43.3[b]	75.4	75.1	17	160.5	153.3	151.0
6	28.0[a]	35.7	35.8	18	182.4	182.3	181.7
7	23.2[a]	22.4[a]	22.5[a]	19	102.1	102.0	105.2
8	38.2	38.0	38.0	20	161.8	161.6	159.7
9	40.5[b]	41.9	42.1	21	182.0	181.9	179.1
10	50.2	42.1	42.7	22	56.8	56.7	56.6
11	102.6	23.9[b]	24.2[b]	23			167.5
12	20.6[c]	17.8[b]	18.0[b]	24			20.4

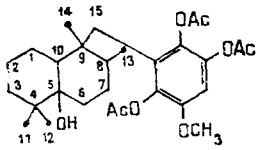
*Taken from the literature [1] (on a correct assignment of the chemical shifts, the figures for C-4 and C-17 must be interchanged); a, b, c – assignment ambiguous.



I



II. R = H
III. R = Ac



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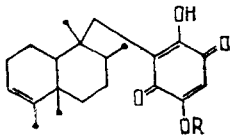
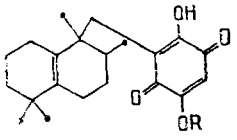
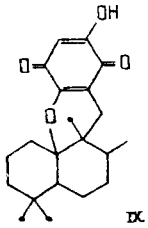


V. R = CH_3
VI. R = H



VII. R = H
VIII. R = CH_3



IX

quinone corresponded to the composition $C_{22}H_{32}O_5$, which agrees with the results of mass spectrometric analysis – 378 (M^+). Its fragmentation under electronic impact largely repeated that of the (I) molecule.

The IR spectrum of hyatoquinone, like that of ilimaquinone, had absorption bands at 3360 cm^{-1} (hydrogen bonded hydroxyl) and at 1666 and 1646 cm^{-1} (quinoid carbonyls) and differed from the latter only by the presence of an absorption band at 3608 cm^{-1}, which is characteristic for an alcoholic hydroxyl group.

A comparison of the PMR spectra (7.58 ppm – exchangeable one-proton single; 5.87 ppm, s – aromatic proton; 3.8 ppm, s – methoxy group) and also of the ^{13}C NMR spectra (Table 1) of compounds (I) and (II) unambiguously showed that the quinoid moieties of the ilimaquinone and hyatoquinone molecules were identical. The preparation of acetyl derivatives – the monoacetate (III) and the triacetate (IV) – confirmed this conclusion.

In the high-field region (C-1–15) of the ^{13}C NMR spectrum of hyatoquinone there were the signals of four primary carbon atoms, six secondary, two tertiary, and three quaternary, one of the last (75.4 ppm) being linked to the oxygen of a hydroxy group (compare the IR

spectrum). As can be seen from the IR spectra of compounds (III) and (IV), the acetylation of this hydroxy group did not take place under the usual conditions.

In the PMR spectrum of (II), with overall similarity to the spectrum of (I), there was no multiplet at 4.44 ppm assigned to the hydrogen atoms of an exomethylene group, but an additional singlet of a methyl group appeared at 0.96 ppm. Thus, the substance under investigation contained four methyl groups, three of them (singlets in the PMR spectrum) being attached to the two remaining quaternary carbon atoms and one (doublet, 0.95 ppm) to a tertiary carbon atom. In light of all that has been said above, it is possible to suggest two variants of the structure of this part of the hyatoquinone molecule with the location of a hydroxy group at either the fifth or the sixth carbon atom.

The dehydration of (II) with the aid of p-toluenesulfonic acid led to the formation, as the main products, of isospongiaquinone (V) and of Δ^5-dehydroxyhyatoquinone (VIII), a compound the structure of which is known [2, 3], and also of a number of minor products (VI, VII, IX), some of which must be assigned to the products of the transformation of isospongiaquinone (compounds (VI) and (IX); see [3]). It may be concluded from this that the position of the hydroxy group on the fifth carbon atom is the most likely.

Experiments on the detection of a nuclear Overhauser effect (NOE) in $CDCl_3$ showed that the methyl group at C-8 (doublet at 0.95 ppm) was spatially close to the methyl group giving a signal at 0.82 ppm, i.e., the latter was located at C-9. Consequently, the signals at 1.03 and 0.96 ppm corresponded to the two remaining methyl groups at C_4. The observation of a NOE in DMSO-d_6 showed that the alcoholic hydroxy group (3.61 ppm) was spatially close to the methyl group giving a signal at 0.90 ppm (1.03 ppm in $CDCl_3$). This enabled us to state unambiguously that the alcoholic hydroxy group was present on the fifth carbon atom.

On the basis of the facts presented, structure (II) has been adopted for hyatoquinone.

EXPERIMENTAL

Melting points were determined on a Boëtius stage. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Electron impact mass spectra were obtained on a LKB-9000S spectrometer with direct introduction of the sample in the ion source at ionizing voltages of 12 and 70 eV, and absorption spectra were obtained on a Cary-219 spectrophotometer. IR spectra were obtained on a Specord IR-75 spectrophotometer, and NMR spectra on HX 90E and WM 250 spectrometers with working frequencies of 90 and 250 MHz for ^1H and 22.6 and 62.9 MHz for ^{13}C.

Isolation of Ilimaquinone (I) and Hyatoquinone (II). A freeze-dried preparation of the sponge was ground and was extracted with hexane. The extract was freed from the bulk of the lipid impurities by chromatography on a column of Florisil with hexane as eluent. The quinone fraction was eluted from the column with hexane–acetone–acetic acid (10:3:0.1) and was separated on a column of silica gel by gradient elution with chloroform containing increasing concentrations of ethanol. The ilimaquinone (I) isolated (2.5% of the dry weight of the sponge) crystallized from hexane in the form of light brown needles, mp 112-114°C.

Hyatoquinone (II) (0.2% of the dry weight of the sponge) crystallized from aqueous ethanol in the form of flat elongated light orange needles. It was soluble in hexane, chloroform, acetone, and ethanol, and practically insoluble in water. mp 68.5-69.5°C. $C_{22}H_{32}O_5$. $[\alpha]_D^{18}$ +34° (c 1.0; ethanol). UV spectrum (nm): $\lambda_{max}^{C_2H_5OH}$ 288, 434 (log ε 4.17, 2.73). IR spectrum (cm^{-1}): $\nu_{max}^{CHCl_3}$ 3608, 3360, 1666, 1644, 1612; mass spectrum (m/z): 376 (M^+), 358, 191, 168, 135, 121, 109, 95; PMR spectrum ($CDCl_3$, ppm): 7.58 (s, 1 H), 5.87, (s, 1 H), 3.88 (s, 3 H), AB-system (δ_A = 2.62, 1 H, δ_B = 2.47, 1 H, J_{AB} = 13.6 Hz), 1.03 (s, 3 H), 0.96 (s, 3 H), 0.95 (d, 3 H, J = 6 Hz), 0.83 (s, 3 H).

Hyatoquinone Acetate (III). This was obtained by the acetylation of hyatoquinone with a mixture of pyridine and acetic anhydride (1:1) at room temperature. It crystallized from aqueous ethanol in the form of lemon-yellow needles. $C_{24}H_{34}O_6$. mp 135-137°C. UV spectrum (nm): $\lambda_{max}^{C_2H_5OH}$ 276, 372 (log ε 4.04, 2.79). IR spectrum (cm^{-1}): $\nu_{max}^{CHCl_3}$ 3608, 1776, 1680, 1660, 1608. Mass spectrum (m/z): 420 (M^+ + 2), 418 (M^+), 402, 400, 376, 191. PMR spectrum ($CDCl_3$, ppm): 5.90 (s, 1 H), 3.85 (s, 3 H), 2.62 (d, 1 H), 2.41 (d, 1 H), 2.32 (s, 3 H), 1.03 (s, 3 H), 0.95 (s, 3 H), 0.89 (d, 3 H, J = 6 Hz), 0.82 (s, 3 H).

Hyatoquinone Triacetate (IV). This was obtained by the acetylation of hyatoquinone with a mixture of pyridine and acetic anhydride (1:1), with the addition of zinc dust, at room temperature. It crystallized from aqueous ethanol in the form of small colorless needles. mp 73-75°C. UV spectra (nm): $\lambda_{max}^{C_2H_5OH}$ 283 (log ε 3.36). IR spectrum (cm^{-1}): $\nu_{max}^{CHCl_3}$ 3592, 1768, 1600. Mass spectrum (m/z): 504 (M^+), 486, 462, 444, 420, 296, 254, 209, 191, 169. PMR spectrum ($CDCl_3$, ppm): 6.70 (s, 1 H), 3.79 (s, 3 H), 2.43 (d, 2 H), 2.32 (s, 3 H), 2.28 (s, 3 H), 2.26 (s, 3 H), 1.03 (s, 3 H), 0.96 (s, 3 H), 0.85 (s, 3 H), 0.82 (d, 3 H, J = 6 Hz).

Dehydration of Hyatoquinone (II). A mixture of 100 mg of hyatoquinone and 30 ml of benzene with three small crystals of p-toluene sulfonic acid was boiled for 40 min. After cooling, the solution was washed with water, dried, and evaporated. The mixture of dehydration products was separated on a column of silica gel and they were identified on the basis of spectroscopic characteristics.

This gave 23 mg of a product with characteristics identical with those of isospongiaquinone (V) [2], 10 mg of a substance (IX) identical with quinone (18a) from [2], and also 4 mg of the product of the demethyloxylation of isospongiaquinone (VI) with the following characteristics. Mass spectrum (m/z): 344 (M^+), 191, 107, 95. IR spectrum (cm^{-1}): $\nu_{max}^{CHCl_3}$ 3340, 1635, 1360. PMR spectrum (ppm): 7.85 (br.s, 2 H), 6.03 (s, 1 H), 5.13 (br.s, 1 H), 2.62 (d, 1 H), 2.46 (d, 1 H), 1.57 (s, 3 H), 1.01 (s, 3 H), 0.97 (d, 3 H), 0.84 (s, 3 H). In addition, 34 mg of Δ^5-dehydroxyhyatoquinone (VIII), identical with compound (8) from [3], was isolated, together with 5 mg of the product of its demethoxylation (VII), having the following characteristics. Mass spectrum (m/z): 346 ($M^+ + 2$), 344 (M^+), 328, 326, 272, 191. IR spectrum (cm^{-1}): $\nu_{max}^{CHCl_3}$ 3360, 1638, 1460, 1360. PMR spectrum ($CDCl_3$, ppm): 7.76 (br.s, 2 H), 6.03 (s, 1 H), 2.70 (d, 1 H, J = 13 Hz), 2.56 (d, 1 H, J = 13 Hz), 1.01 (s, 3 H), 0.96 (s, 3 H), 0.85 (s, 3 H), 0.80 (d, 3 H, J = 6.6 Hz).

SUMMARY

Two quinoid pigments have been isolated from a hexane extract of the marine sponge Hyatela sp., one of which was identified as ilimaquinone while the other was a new benzoquinone of the drimane series which we have called hyatoquinone. Its structure has been established on the basis of spectrum characteristics and chemical transformations.

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