NEW SESQUITERPENE QUINONES FROM MARINE SPONGES OF THE ORDER DICTYOCERATIDA

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Isospongiaquinone and a new sesquiterpene quinone - dictyoceratidaquinone - have been isolated from a dictyoceratidan marine sponge. Ilimaquinone and the new aminoquinone, a sesquiterpenylamylaminohydroxyquinone, have been isolated from a sponge Spongia *sp. Synthetic analogs of natural aminoquinones have been obtained by replacing the methoxy group in ilimaquinone and isospongiaquinone by an amine group.*

Among marine terpenoids of mixed biogenesis, a considerable proportion is composed of sesquiterpene quinones and hydroxyquinones. The majority of them have been isolated from marine sponges of the order Dictyoceratida [1]. We have investigated the sponges from the collections of the scientific-research ships "Professor Bogorov" and "Academik Oparin". (See scheme on following page.)

From an unidentified sponge of the order Dictyoceratida we have isolated isospongiaquinone (I) and a new compound (II), which has been called dictyoceratidaquinone. Compound (II) had the molecular formula $C_{22}H_{30}O_4$. The absorption spectrum and the typical behavior of the quinone in an alkaline solution of sodium dithionite followed by reoxidation in the air showed the presence of a hydroxyquinone structure. This was confirmed by the formation of a monoacetate with M^+ 400, $[\alpha]_D^{25}$ +6.6° (c 0.51; $CHCl_3$). The structure of the quinoid fragment was easily established from an analysis of the PMR spectrum, which showed a singlet signal of a quinoid proton at δ 5.86 ppm (H-19), methoxyl resonance at δ 3.87 ppm, an exchange one-proton singlet of a hydroxy group at δ 7.35 ppm, and two one-proton doublets at δ 2.67 and 2.40 ppm (CH_2-15). These facts confirmed the presence in compound (II) of the partial structure (III), which was further confirmed by a strong ion in the mass spectrum of (II) at m/z 168.

The ^{13}C NMR spectrum confirmed the presence of a quinoid fragment: 182.5 (s, C-17), 181.8 (s, C-20), 161.1 (s, C-18), 152.1 (s, C-21), 118.8 (s, C-16), 102.2 (d, C-19), 56.6 (q, OMe) and furnished considerable additional information on the structure of the sesquiterpenoid part of the molecule. The absence of signals from sp^2-hybridized carbon atoms (apart from the signals of the quinoid nucleus) excluded the presence of a C=C bond in the sesquiterpenoid fragment, which showed the

TABLE 1. ^{13}C NMR Spectra of Compounds (V), (VI), and (VII)

Atom	V	VI	VII	Atom	V	VI	VII
C-18	182,2 s	182,7 s	183,0 s	C-6	33,0 t	33,1 t	33,1 t
C-21	181,8 s	178,0 s	179,8 s	C-15	32,2 t	32,8 t	32,8 t
C-4	160,3 s	160,2 s	160,3 s	C-7	27,7 t	28,8 t	28,8 t
C-17	153,2 s	157,0 s	155,0 s	C-2	28,0 t	28,1 t	28,2 t
C-20	161,6 s	150,4 s	150,6 s	C-1	23,3 t	23,3 t	23,4 t
C-16	117,4 s	113,6 s	114,5 s	C-12	20,6 q	20,6 q	20,6 q
C-11	102,4 t	102,4 t	102,5 t	C-13	17,9 q	17,9 q	17,9 q
C-19	102,0 d	91,6 d	95,8 d	C-14	17,4 q	17,2 q	17,2 q
C-10	50,3 d	50,3 d	50,4 d	C-22	56,8 q	41,2 t	
C-9	43,4 s	43,0 s	43,2 s	C-23		37,0 t	
C-5	40,5 s	40,5 s	40,6 s	C-24		26,1 t	
C-8	38,2 d	38,2 d	38,3 d	C-25		23,3 t	
C-3	36,7 t	36,8 t	36,9 t	C-26		17,4 q	

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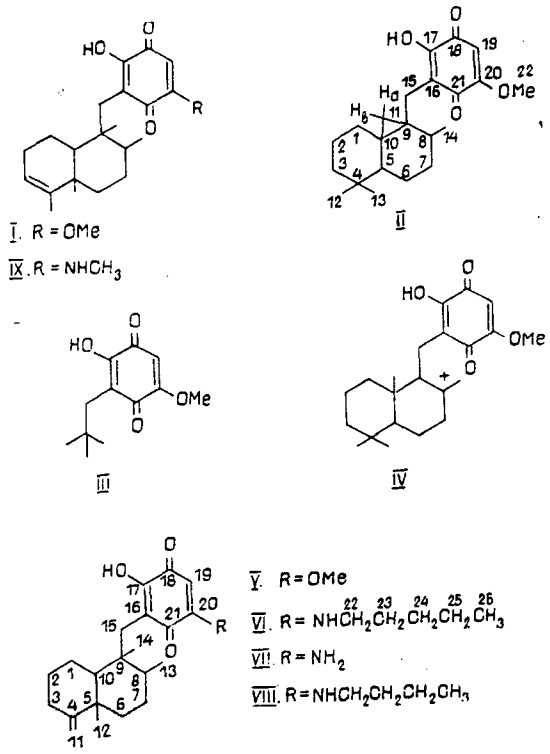

tricyclic structure of the sesquiterpene moiety. On the other hand, the signal of a methylene group at the δ 16.8 ppm showed the presence of a cyclopropane fragment [2], and this was confirmed by the PMR spectrum, which included two one-proton doublet signals in the strong field at (ppm) 0.31 (J = 5.2 Hz) and 0.32 (J = 5.25 Hz) which is characteristic for a strained cyclopropane nucleus [3]. In addition to these signals, the PMR spectrum contained the signals of three methyl groups, one of which (δ 0.65 ppm) interacted with the H8 one-proton quintet at δ 1.98 ppm, and a series of poorly resolved multiplets. The totality of the spectral results did not permit the position of the cyclopropane ring to be identified unambiguously, but if it is assumed that sesquiterpene quinones in sponges are formed from the common intermediate (IV) [1], this leads to a presumption of the alternative structure (II) for dictyoceratidaquinone.

A sponge, *Spongia* sp., contained two sesquiterpene quinones. The main, yellow, compound was identified as ilimaquinone (V). The second substance (VI), with the molecular formula $C_{26}H_{39}NO_3$ had a molecular peak M^+ 413. The UV absorption spectrum (323, 498 nm) and the IR spectrum (ν 1641, 1588 cm^{-1}) showed the presence of a quinoid ring in the molecule. In addition, the IR spectrum showed the presence of hydroxy and amino groups (ν 3271 and 3460 cm^{-1}, respectively).

The upfield shift of the signal of the H-19 proton in the PMR spectrum of (VI) (δ 5.38 ppm) as compared with the signal of the corresponding proton in (V) (δ 5.85 ppm) showed the presence of an amino group in the ortho position to it [4]. A comparison of the PMR and ^{13}C NMR spectra of (VI) and (V) showed that the sesquiterpene moiety in compound (VI) was similar to that in (V) and had the drimane structure, which was confirmed by the presence of a fragmentary peak at m/z 191 in the mass spectrum of (VI).

The aminoquinone smenospongine has been isolated previously from a marine sponge *Smenospongia* sp. [4]. A comparison of literature information for smenospongine and compound (VI) showed their considerable similarity. A difference in the values of the molecular masses by 70 units, and also the presence in the ^{13}C NMR spectrum of (VI) of additional signals of the carbon atoms from four methylene and one methyl groups showed the presence of an n-amyl chain in the amino group. This was confirmed by the presence of a two-proton multiplet in the PMR spectrum at δ 3.16 ppm (NH—CH_2—) and by a signal at δ 41.2 ppm (C-22) in the ^{13}C NMR spectrum.

Thus, on the basis of the results obtained, the sesquiterpenylamylaminohydroxyquinone was ascribed structure (VI).

For aminoquinones isolated previously from sponges [4, 5], the question of their possible artefactual origin has been discussed in the literature [5]. We have confirmed the possibility of the formation of such aminoquinones from compounds (I) and (V). For this purpose we used the known reaction of the replacement of methoxy groups in quinones by amino groups [6].

When (V) was treated with an aqueous alcoholic solution of ammonia, the aminoquinone (VII), identical in spectral characteristics with smenospongine [4] was obtained. The reaction of (V) with n-butylamine gave a homolog of compound (VI) — the sesquiterpenylbutylaminohydroxyquinone (VIII) — while compound (I) with methylamine gave 17-hydroxy-20-methylaminoavarone (IX) — a hydroxylated analog of 3′-methylaminoavarone [5].

The sesquiterpene moiety of the aminoquinone synthesized did not differ according to the PMR spectra from that of the initial quinones. Changes were observed in the chemical shifts of the signals of the quinoid nucleus. The PMR spectra of all the aminoquinones obtained lacked the signal of a methoxy group and exhibited in the δ 3-ppm region the signal of a methylene or methyl group attached to nitrogen. In addition, the H-19 signal was shifted upfield, which showed the presence of an amino group in the ortho position to it.

Thus, the combined presence of compounds (V) and (VI) in the sponge *Spongia* sp. and of (V) and (VII) in *Smenospongia* sp. [4], together with the ease of formation of aminoquinones under the conditions of the experiment, does not exclude the possibility of their artefactual origin.

EXPERIMENTAL

IR spectra were taken on a Specord-75 IR instrument, UV spectra on a Cary 219, NMR spectra on a Bruker WM-250 instrument in $CDCl_3$ (δ scale, 0 — TMS), and mass spectra on an LKB-9000 S with direct introduction of the sample into the ion source at an ionizing energy of 70 eV. Angles of rotation were determined on a Perkin—Elmer 141 polarimeter, and melting points on a Boëtius stage. The analyses of all the compounds corresponded to the calculated figures.

Isolation of Quinones (I) and (II). A sponge of the order Dictyoceratida was collected during the 19th voyage of the scientific-research ship "Professor Bogorov" in 1984 on the Saya de Malha bank in the Indian Ocean. The freeze-dried sponge (39 g) was extracted with CH_2Cl_2, the solvent was driven off in vacuum, and the resulting yellow oil was chromatographed on a column of Florisil in $CHCl_3$. Two fractions having the same R_f value on TLC in all the solvent systems used were obtained. Each fraction was additionally chromatographed on a column of Sephadex LH-20 in the CH_2Cl_2—hexane (10:1) solvent system.

The first fraction yielded compound (I) (0.23% on the dry weight of the sponge) having characteristics identical with those given in the literature [1]. The second fraction yielded the quinone (II) (0.16% on the dry weight of the sponge).

Dictyoceratidaquinone (II) was crystallized from hexane to form yellow needles, mp 159-161°C, $[\alpha]_D^{25}$ −12.8° (c 0.21; $CHCl_3$). Mass spectrum, m/z (%): 358 (M^+, 100), 189 (50). 168 (100), 105 (40). UV spectrum (λ_{max}^{MeOH}, nm) 215, 286, 425 (log ε 3.95, 4.20, 2.53); ($\lambda_{max}^{MeOH\text{-}NaOH}$, nm): 218, 240 sh, 288, 522 (log ε 4.32, 3.96, 4.11, 3.13). PMR: 7.35 (1H, br.s, OH), 5.86 (1H, s, H-19), 3.87 (3H, s, OCH_3), 2.67 and 2.40 (2H, dd, J = 14 Hz, CH_2-15), 1.98 (1H, m, J = 7 Hz, H-8), 1.7-0.69 (11H, m), 0.83 (3H, s, CH_3-12), 0.85 (2H, s, CH_3-13), 0.65 (3H, d, J = 7 Hz, CH_3-14), 0.31 (1H, d, J = 5.2 Hz, H_a-11), 0.53 (1H, d, J = 5.2 Hz, H_b-11). ^{13}C NMR: 182.5 (s, C-17), 181.8 (s, C-20), 161.1 (s, C-18), 152.1 (s, C-21), 118.8 (s, C-16), 102.0 (d, C-19), 56.6 (q, OMe), 35.0 (s), 28.0 (s), 27.1 (s), 30.9 (q), 20.5 (q), 19.8 (q), 48.4 (d), 29.1 (d), 16.8 (t), 18.1 (t), 21.9 (t), 29.0 (t), 30.9 (t), 34.0 (t), 42.6 (t).

Isolation of the Quinones (V) and (VI). The sponge *Spongia* sp. was collected during the fifth voyage of the scientific-research ship "Akademik Oparin" in 1987 on the shores of Vietnam. The freeze-dried sponge (400 g) was extracted with $CHCl_3$, the solvent was driven off in vacuum, and the resulting yellow oil was separated on a column of Florisil in $CHCl_3$ and then on a column of Sephadex LH-20 in $CHCl_3$. This gave ilimaquinone (V) (0.3% on the dry weight of the sponge) and quinone (VI) (0.03% on the dry weight of the sponge).

The sesquiterpenylamylaminohydroxyquinone (VI) was crystallized from aqueous ethanol (in the form of red needles), mp 131-134°C. Mass spectrum, m/z (%): 413 (M^+, 10), 223 (100), 209 (3), 191 (10), 180 (8), 166 (15), 152 (20). UV spectrum (λ_{max}^{MeOH}, nm): 210, 323, 498 (log ε 4.38, 4.13, 2.86). IR spectrum (ν_{max}^{KBr}, cm^{-1}): 1588, 1641, 3271, 3460. PMR: 8.45 (1H, br.s, OH), 6.45 (1H, s, NH), 5.39 (1H, s, H-19), 4.45 (2H, m, CH_2-11), 3.17 (2H, m, CH_2-22), 2.44 (1H, dd, J = 14 Hz, CH_2-15), 2.4-0.85 (17H, m), 1.05 (3H, s, CH_3-12), 0.95 (3H, d, J = 6 Hz, CH_3-13), 0.83 (3H, s, CH_3-14), 0.98 (3H, t, J = 6 Hz, CH_3-26), 0.78 (1H, dd, J = 11.5 Hz, J = 2 Hz, H-10). The ^{13}C NMR spectrum is given in Table 1.

Synthesis of Smenospongine (VII). A solution of 112 mg of (V) in 200 ml of 50% aqueous ethanol was treated with 0.1 ml of a 14% aqueous solution of ammonia and 0.1 ml of pyridine. The reaction mixture was left at room temperature for 20 h. Then the alcohol was evaporated off in vacuum, the aqueous residue was extracted with $CHCl_3$, the solvent was evaporated off, and the dry residue was chromatographed on LH-20 in $CHCl_3$. The eluate containing the main component furnished (VII) (yield 85%).

After crystallization from $CHCl_3$, red crystals were obtained with mp 203-205°C (according to the literature: 153-155°C [4]). Mass spectrum, m/z (%): 343 (M^+, 4), 191 (20), 153 (100), 124 (50), 107 (30). UV spectrum (λ_{max}^{MeOH}, nm): 210,

316. PMR: 8.12 (1H, br.s, OH), 5.62 (1H, s, H-19), 4.4 (2H, m, CH_2-11), 2.46 (2H, dd, J = 14 Hz, CH_2-15), 1.05 (3H, s, CH_3-12), 0.98 (3H, d, J = 6 Hz, CH_3-13), 0.84 (3H, s, CH_3-14), 0.79 (1H, dd, J = 11.5 Hz, J = 2 Hz, H-10), 2.35-0.85 (11H, m). The ^{13}C NMR spectrum is given in Table 1.

Synthesis of Sesquiterpenylbutylaminohydroxyquinone (VIII). A solution of 63 mg of (V) in 100 ml of 50% aqueous ethanol was treated with 0.1 ml of n-butylamine. After 24 h the alcohol was evaporated off in vacuum, the aqueous residue was extracted with $CHCl_3$, and the solvent was evaporated off. Yield 90%. After crystallization from $CHCl_3$, red crystals were obtained with mp 141-143°C. Mass spectrum, m/z (%): 399 (M^+, 5), 209 (100), 191 (5), 180 (25), 166 (12), 152 (20). UV spectrum (λ_{max}^{MeOH}, nm): 210, 322, 500. PMR: 8.45 (1H, br.s, OH), 6.43 (1H, m, NH), 5.39 (1H, s, H-19), 4.45 (2H, m, CH_2-11), 3.16 (2H, m, CH_2-22), 2.45 (2H, dd, J = 14 Hz, CH_2-15), 1.05 (3H, s, CH_3-12), 0.96 (3H, d, J = 6 Hz, CH_3-13), 0.83 (3H, s, CH_3-14), 0.79 (1H, dd, J = 11.5 Hz, J = 2 Hz, H-10), 0.85 (3H, t, CH_3-25), 2.4-0.9 (11H, m).

Synthesis of 17-Hydroxy-20-methylaminoavarone (IX). A solution of 20 mg of (I) in 40 ml of 50% aqueous ethanol was treated with 100 mg of $CH_3NH_2 \cdot HCl$ and 0.5 ml of pyridine. After 20 h, the alcohol was driven off in vacuum and the aqueous residue was extracted with $CHCl_3$. The solvent was eliminated and the dry residue was chromatographed on LH-20 in $CHCl_3$. This gave 15 mg of (IX). Crystallization from aqueous methanol gave red crystals with mp 215-217°C. Mass spectrum, m/z (%): 357 (M^+, 5), 191 (20), 167 (100). UV spectrum (λ_{max}^{MeOH}, nm): 221, 294, 322, 500. PMR: 8.4 (1H, br.s, OH), 6.45 (1H, m, NH), 5.38 (1H, s, H-19), 5.14 (1H, br.s, H-3), 2.92 (3H, d, J = 5 Hz, N—CH_3), 2.56 (2H, dd, J = 14 Hz, CH_2-15), 1.54 (3H, d, J = 2 Hz, CH_3-11), 1.00 (3H, s, CH_3-14), 0.97 (3H, d, J = 6.1 Hz, CH_3-13), 0.83 (3H, s, CH_3-12), 2.1-0.95 (9H, m).

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ECDYSTEROIDS OF A CULTURE OF TISSUES AND CELLS OF *Ajuga turkestanica*

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The possibility has been studied of obtaining ecdysteroids — ecdysterone and turkesterone — with the aid of a culture of the tissues and cells of the plant Ajuga turkestanica. *Conditions have been selected under which the yield of ecdysteroids in the culture of tissues and cells is comparable with amounts of the same substances in the organs of the whole plant.*

It is known that ecdysterone has been isolated from the leaves from *Ajuga turkestanica* (Rgl.) Brig (family Labiatae) while in the roots, in addition to the ecdysteroid mentioned, turkesterone (11α-hydroxyecdysterone) has been detected [1].

We have considered the possibility of producing ecdysteroids with the aid of a culture of the tissues and cells of this plant. To induces callusogenesis and the growth of the cell culture we investigated several modifications of nutrient media. The variants that proved to be the best are given in the Experimental section.

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