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AVAROL AND ISOAVAROL FROM A PACIFIC OCEAN SPONGE *Dysidea* SP.

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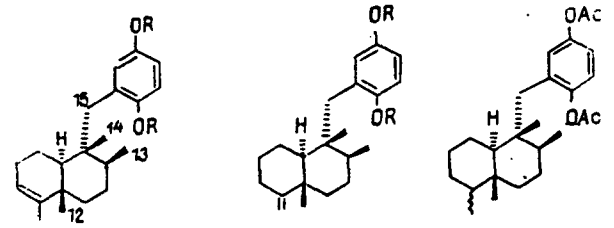
UDC 547.91:639.29

Two hydroquinone-group-containing terpenoids have been isolated from a Pacific Ocean sponge *Dysidia*, sp. One of them has been identified by physicochemical methods as the previously known avarol. The second, isoavarol, obtained for the first time, differs from avarol by the 4(11)-position of the double bond.

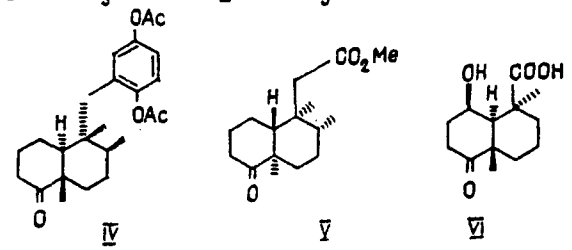
Continuing investigations of sponge metabolites [1], from an alcoholic extract of *Dysidia* sp. we have isolated by column chromatography on silica gel a difficultly separable mixture of two hydroquinone-group-containing terpenoids (I) and (II). We obtained the individual compounds by acetylating the mixture and using HPLC for separating the acetates (Ia) and (IIa). The acetate (Ia) was converted into the dimethyl ether (Ib). A comparison of the ^1H and ^{13}C NMR spectra and the physical constants of this compound with literature information for the dimethyl ether of avarol [2] enabled (I) to be identified as avarol.



I. R = H
Ia. R = Ac
Ib. R = CH_3

II. R = H
IIa. R = Ac
IIb. R = CH_3

III a,b



IV V VI

The mass spectrum of (IIa) was close to the spectrum of (Ia), but their ^1H and ^{13}C NMR differed substantially. The ^1H NMR spectrum of (IIa) lacked the signal of the olefinic protons at C3 present in the spectrum of (Ia) but, at the same time, there were two signals in the form of triplets at 4.40 and 4.46 ppm, which are characteristic for the protons of an exomethylene group. Correspondingly, in the ^{13}C NMR spectrum of (IIa) the signals of an exocyclic double bond appeared in the form of a singlet at 159.3 and a triplet at 103.0 ppm.

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Branch, USSR Academy of Sciences, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 358-361, May-June, 1990. Original article submitted August 8, 1989.

 0009-3130/90/2603-0296$12.50

We assumed that (II) was a compound isomeric with avarol, differing by the position of the double bond in the sesquiterpenoid fragment. The 4(11) position of the double bond in (IIa) was established with the aid of NOE experiments. Irradiation of the methylene group at δ 2.51 ppm (CH_3-15) gave responses of the signals with δ 0.98 (CH_3-13) and δ 0.85 ppm (CH_3-14), but gave no responses of the protons of the exomethylene group. When the latter were irradiated (doublet of triplets, δ 4.43 ppm), only responses of the δ 2.13 and δ 2.09 ppm multiplets (protons at C-6) and of the δ 1.45 ppm multiplet (equatorial proton at C-3) were observed. This excluded the alternative 8(13) position of the double bond.

The mutual location of the methyl groups in (IIa) was also established by NOE experiments. Action on the δ 0.85 ppm signal (CH_3-14) gave responses at δ 1.06 ppm (CH_3-12) of the δ 0.98 ppm doublet (CH_3-13) and of the δ 2.51 ppm quartet (CH_3-15), while irradiation of the δ 1.06 ppm signal (CH_3-12) gave a response at δ 0.85 ppm (CH_3-14). These results showed that all three methyl groups had either the α- or the β-orientation.

To determine the absolute stereochemistry in the sequiterpene moiety, (IIa) was subjected to ozonolysis, as a result of which the ketone (IV) was obtained. In the CD spectrum of (IV), a negative maximum, $\theta_{298} = -491$, was observed in the form of a shoulder at θ_{283} [sic]. For comparison we made use of literature information on the CD spectra of the ketones (V) [3] with $\theta_{298} = +455$ and (VI) [4] with $\theta_{293} = -2520$. The comparison showed that the absolute stereochemistry in (II) coincided with that in compound (VI) and, consequently, with that in avarol.

The position of the double bond and the stereochemistry in the (II) molecule were confirmed by the production from (Ia) and (IIa), after catalytic hydrogenation, of one and the same mixture of stereoisomeric dihydro derivatives (IIIa and b). At the same time, (Ia) gave (IIIa) as the predominating stereoisomer after hydrogenation while (IIa) gave mainly (IIIb).

So as far as we are aware, compound (II) has not been described previously. We propose to call it isoavarol.

EXPERIMENTAL

The sponge was gathered off the north-eastern coast of Australia at a depth of 30 m during the seventh voyage of the Scientific Research Vessel Akademik Oparin in August, 1988.

Melting points were determined on a Boetius stage. ^1H and ^{13}C NMR spectra were obtained on a Bruker WM-250 spectrometer in $CDCl_3$ using TMS as internal standard, and mass spectra were obtained on a LKB-9000S instrument at an ionizing voltage of 70 eV and a source temperature of 250°C. For high-performance liquid chromatography we used a Du Pont 8800 chromatograph, a Zorbax ODS column (4.6 × 250 mm), and the eluent acetonitrile–water (4:1). (UV detector λ = 290 nm.)

Isolation of Compounds (I) and (II). The comminuted sponge (dry weight 100 g) was extracted twice with ethanol. The extract was concentrated in vacuum. The residue was chromatographed on a column of silica gel (hexane–chloroform), and then the fraction containing a mixture of (I) and (II) was crystallized several times from hexane–chloroform. This gave 300 mg (0.33%) of a crystalline substance. Mass spectrum, m/z (%): 314 (M^+, 8), 191(50), 175(36), 135(38), 124(90), 121(62), 107(85), 95(100).

Separation of the Mixture of (I) and (II). The mixture was acetylated with the aid of HPLC. This gave the acetate (Ia), mp 122-123°C (ethanol), $[\alpha]_{578}^{20}$ +15° (c 0.2; chloroform). Mass spectrum, m/z (%): 398 (M^+, 2), 383(1), 328(1), 191(90), 177(52), 149(24), 135(40), 121(45), 109(88), 107(70), 95(100).

^1H NMR spectrum (δ, ppm): 0.83 s (3H), 0.96 d (3H, J=6 Hz), 1.02 s (3H), 1.52 d (3H, J=2 Hz), 2.30 s (3H), 2.33 s (3H), 2.56 AB qu (2H, J=14 Hz), 5.15 m (1H), 6.9 d (1H, J=2 Hz), 6.99 d (1H, J=2 Hz), 7.0 s (1H).

^{13}C NMR spectrum (δ, ppm): 17.6 (C-13, C-14), 18.1 (C-12), 19.9 (C-1), 20.2 (C-11), 21.1 (2 × CH_3COO), 26.5; 27.8 (C-2 and C-7), 35.9 (C-6), 36.3 (C-8), 38.1 (C-15), 38.5 (C-9), 42.0 (C-5), 46.3 (C-10), 120.2 (C-3), 125.3; 122.9; 125.3 (C-3', C-4', C-6'), 132.5 (C-1'), 144.1 (C-4), 147.3; 147.5 (C-2' and C-5'), 168.8; 169.0 ($COCH_3$).

The Acetate (IIa), mp 133-134°C (ethanol), $[\alpha]_{578}^{20}$ −30° (c 0.2; chloroform).

Mass spectrum, m/z (%): 398 (M^+, 5), 383(2), 328(3), 191(98), 177(40), 149(21), 135(30), 121(36), 109(85), 107(74), 95(100).

^1H spectrum (δ, ppm): 0.85 s (3H), 0.98 d (3H, J=6 Hz), 1.06 s (3H), 2.28 s (3H), 2.30 s (3H), 2.51 AB qu (2H, J=14 Hz), 4.43 at (2H, J_1=13 Hz, J_2=2 Hz, J_3=2 Hz,), 6.87 d(1H, J=2 Hz), 6.97 d (1H, J=2 Hz), 6.98 s (1H).

^{13}C NMR spectrum (δ, ppm): 17.47 (C-14), 17.71 (C-13), 20.6 (C-12), 21.06 (2 × CH_3COO), 23.3 (C-7), 27.7 (C-6), 28.2 (C-2), 33.0 (C-1), 36.5 (C-15), 36.6 (C-3), 38.0 (C-8), 40.3 (C-9), 42.3 (C-5), 48.5 (C-10), 103.0 (C-11), 119.9; 122.9; 125.9 (C-3', C-4', C-6'), 132.0 (C-1'), 147.0; 147.5 (C-2', C-5'), 159.3 (C-4), 167.3 ($\underline{C}OCH_3$), 168.9 ($\underline{C}OCH_3$).

The dimethyl ether of avarol was obtained by the method described previously [2]; mp 80-81°C (methanol), $[\alpha]_{578}^{20}$ +6° (c 0.12; chloroform).

Mass spectrum, m/z (%): 342 (M^+, 2), 191(10), 152(68), 121(30), 95(100).

^1H NMR spectrum (δ, ppm): 0.86 s (3H), 0.99 d (3H, J=6 Hz), 1.02 s (3H), 1.50 d (3H, J=2 Hz), 2.68 AB qu (2H, J=14 Hz), 3.72 s (3H), 3.75 s (3H), 5.13 m (1H), 6.71 m (3H).

Hydrogenation of the Acetate (Ia). The substance (20 mg) was hydrogenated in methanol over Adams catalyst for 5 h. The catalyst was filtered off, the solution was evaporated, and the mixture of two substances obtained was separated with the aid of HPLC. Two individual compounds were obtained, which were crystallized from ethanol:

(IIIa) (80%), mp. 125-127°C (ethanol), $[\alpha]_{578}^{20}$ −18° (c 0.1; chloroform). ^1H NMR spectrum (δ, ppm): 0.67 d (3H, J=7 Hz), 0.79 s (3H), 0.81 s (3H), 0.95 d (3H, J=6 Hz), 2.30 s (3H), 2.32 s (3H), 2.50 AB qu (2H, J=1.4 Hz), 6.88–6.98 m (3H);

(IIIb) (20%), mp 142-143°C (ethanol), $[\alpha]_{578}^{20}$ −30° (c 0.2; chloroform). ^1H NMR spectrum (δ, ppm): 0.75 d (3H, J=7 Hz), 0.79 s (3H), 0.94 d (3H, J=6 Hz), 1.03 s (3H), 2.29 s (3H), 2.31 s (3H), 2.50 AB qu (2H, J=14 Hz, 6.90–7.00 m (3H).

Hydrogenation of the Acetate (IIa). The substance (12 mg) was hydrogenated in a similar manner to that described above. A mixture of (IIIa) and (IIIb) in a ratio of 1:4 was obtained.

Ozonolysis of the Acetate (Ia). A current of ozone was passed through a solution of 15 mg of (Ia) in 2 ml of methanol and 0.5 ml of chloroform cooled to −70°C for 25 min. The solution was evaporated, and the substance obtained (IV) was recrystallized from ethanol, mp 129-130°C.

Mass spectrum, m/z (%): 400 (M^+, 2), 314(2), 298(1), 208(18), 193(100), 175(48), 166(35), 149(40), 135(33), 123(41).

CD spectrum (c 0.2; alcohol); $\theta_{298} = -491$.

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