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A NEW BIOACTIVE DERIVATIVE OF AVAROL FROM THE MARINE SPONGE *DYSIDEA AVARA*[1]

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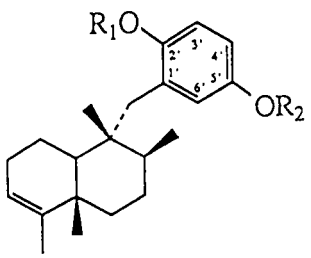
ABSTRACT.—A new derivative of avarol, monoacetyl avarol [**2**], has been isolated from the sponge *Dysidea avara*. The structural elucidation and biological activities are reported.

Avarol [**1**], a sesquiterpene hydroquinone, and its quinone, avarone [**4**], the main secondary metabolites from the marine sponge *Dysidea avara* Schmidt (Keratosa) (1,2), have been reported to have a wide variety of biological activities, including in vivo antileukemic activity (3) and inhibition of replication of the HIV virus in vitro (4).

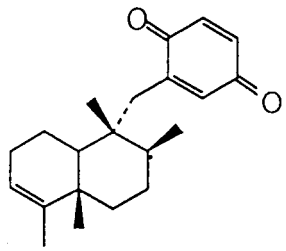
In the course of preparing derivatives of avarol and/or avarone, we have extracted a large amount of *D. avara*, collected in different places. From this sponge collected at Capo Miseno, Naples, we have isolated a new compound **2**, a monoacetyl derivative of avarol.

We report here the isolation, structural elucidation, and biological activities of the new compound **2**, in comparison with avarol [**1**] and avarone [**4**].

Avarol monoacetate [**2**] was obtained as a solid (mp 151–153°). The ^{1}H nmr and ^{13}C nmr of the terpenoid moiety were coincident with those previously reported for avarol (2). The presence in



1 $R_1=R_2=H$
2 $R_1=H, R_2=Ac$
3 $R_1=R_2=Ac$



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the ^{1}H nmr spectrum of a methyl singlet at δ 2.26 and in the ^{13}C-nmr spectrum of two signals at δ 169.5 (CO) and 21.0 (Me) suggested the presence of an acetyl group in the molecule, and this was confirmed by the ir spectrum with bands at 1760 and 1205 cm^{-1}.

While Ac_2O/pyridine acetylation of avarol affords its diacetyl derivative **3**, treatment of avarol with NaOAc and Ac_2O in Et_2O gives exclusively a monoacetyl derivative, identical in all respects to the natural compound **2**. Finally, acetylation of **2** with Ac_2O/pyridine gave the same diacetyl derivative **3** obtained from avarol.

The 5′ position of the acetyl group was established by considering the acetylation shifts on signals in the ^{13}C-nmr spectra of **2** and **3**, as reported in Table 1.

Brine shrimp (5) and potato disc (6) assays were used to determine the cytotoxic and antitumor activities, respectively, and the results are reported in Table 2.

The new compound **2** was the most cytotoxic of the compounds tested, with an activity twice that of avarol.

The brine shrimp assay shows excellent agreement with traditional cell cul-

[1]Dedicated to the memory of Prof. E. Lederer, 1908–1988.

TABLE 1. ^{13}C-nmr Chemical Shifts of Aromatic Signals for Compounds **2** and **3**.

Carbon	Compound	
	2	**3**
C-1′	126.7	132.2
C-2′	152.3	147.4
C-3′	116.0	122.9
C-4′	120.0	119.9
C-5′	144.3	147.4
C-6′	125.6	125.3

ture assays; in fact, the ratio of activity of avarol to that of avarone in this assay was 1.28, while in L-5178y and L-1210 cell assays it was 1.50 and 1.16, respectively (3,7).

order of elution): avarone [**4**] (450 mg), avarol monoacetate [**2**] (21 mg), and avarol [**1**] (3 g).

Avarol monoacetate [**2**].—Mp 151–153° (*n*-hexane/Et_2O); $[\alpha]^{25}D$ +11 (c = 2.3, $CHCl_3$); ir ($CHCl_3$) 3090, 1760, and 1205 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 6.80 (2H), 6.70 (1H), 5.12 (m, 1H), 2.68 (ABq, J = 14.0 Hz, 2H), 2.26 (s, 3H), 1.52 (br s, 3H), 1.02 (s, 3H), 0.99 (d, J = 6.0 Hz, 3H), 0.86 (s, 3H); ^{13}C nmr ($CDCl_3$) δ 169.5 (s), 152.3 (s), 144.3 (s), 144.1 (s), 126.7 (s), 125.6 (d), 120.5 (d), 120.0 (d), 116.0 (d), 46.6 (d), 42.1 (s), 38.6 (s), 38.2 (t), 36.7 (d), 36.1 (t), 28.0 (t), 26.6 (t), 21.0 (q), 20.2 (q), 20.0 (t), 17.9 (q), 17.5 (q), 17.4 (q); ms *m/z* $[M]^+$ 356 (30%), 191 (100%), 175 (27%), 122 (28%), 107 (52%), 95 (97%).

Avarol [**1**].—Mp 149–150° ($CHCl_3$); $[\alpha]^{25}D$ +6.2 (c = 4.0, $CHCl_3$); other spectroscopic data were in excellent agreement with published values (1,8).

TABLE 2. Biological Activity of Compounds **1**, **2**, and **4** as Determined by Brine Shrimp and Potato Disc Assays.

Compound	Brine shrimp assay LC_{50} (ppm)	Potato disc assay % Inhibition
Avarol [**1**]	0.18 (0.10/0.32)[a]	64 (66/62)[b]
Avarol monoacetate [**2**]	0.09 (0.05/0.18)	59 (61/57)
Avarone [**4**]	0.14 (0.07/0.26)	63 (64/62)

[a]Confidence levels (95%) in parentheses.
[b]Values of two determinations in parentheses.

EXPERIMENTAL

GENERAL PROCEDURES.—Melting points were determined using a Kofler hot-stage microscope and are uncorrected. ^{1}H- and ^{13}C-nmr spectra were measured on a WM 500 Bruker spectrometer (δ ppm/TMS). Ms spectra were taken on an AEI MS-50 instrument. Ir spectra were obtained on a Perkin-Elmer 257 spectrometer. Optical rotation was measured on a Perkin-Elmer Model 141 polarimeter, using a 10-cm microcell. Cc was carried out on Merck Si-gel 60.

MATERIAL.—The sponge *D. avara* was collected at Capo Miseno (Naples, Italy) and identified by Dr. G. Bavestrello of the Istituto di Zoologia dell'Universita di Genova, Italy; a voucher specimen is maintained in the collection of our institute.

EXTRACTION AND ISOLATION.—Fresh material (50 g dry wt after extraction) was extracted with cold Me_2CO, the solvent was removed, and the aqueous residue was extracted 3 times with Et_2O. After evaporation of the solvent in vacuo, the residue (6 g) was applied to a column of Si gel. The column was eluted with a solvent gradient system from petroleum ether to Et_2O to yield (in

PERACETYLATION.—Peracetylation of **1** and **2** was carried out as previously described for **1** (2).

PARTIAL ACETYLATION OF **1**.—To 100 mg of avarol in Et_2O, three drops of Ac_2O and 50 mg of NaOAc were added, stirred at reflux for 4 h, and stirred at room temperature overnight. After filtration and elimination of solvent, the residue was chromatographed on a Si gel column using petroleum ether-Et_2O (4:1) as eluent, giving **2** (45 mg); mp 151–152° (*n*-hexane/Et_2O); $[\alpha]^{25}D$ +11 (c = 3.1, $CHCl_3$).

BIOLOGICAL EVALUATION.—The brine shrimp lethality assay and potato disc assay were performed in our laboratory by the literature methods (5,6).

ACKNOWLEDGMENTS

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