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FURTHER BIOACTIVE DERIVATIVE OF AVAROL FROM *DYSIDEA AVARA*

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Abstract.- A new bioactive derivative of avarol, 6'-hydroxy,5'-acetyl-avarol (**3**), has been isolated from the marine sponge *Dysidea avara* and characterized mainly by spectral analysis. Its biological activities are also reported. The occurrence of diacetyl-avarol (**1b**) as natural metabolite is reported.

In the course of preparing derivatives of avarol (**1a**) and/or avarone (**2**), the main secondary metabolites from the sponge *Dysidea avara* Schmidt (Dyctioceratida)[1,2] that showed a wide variety of biological activities including *in vivo* antileukemic activity,[3] we made an extensive collection of *D. avara*, from different locations in Naples' bay. From this sponge collected at Torre Annunziata (Naples, Italy) we have isolated together with avarol and avarone, two new metabolites, 6'-hydroxy,5'-acetyl-avarol (**3**) and diacetyl-avarol (**1b**), for the first time as natural metabolite.

In this paper we wish to report the structural elucidation, based mainly on spectroscopic evidence including 2D-NMR and biological activities of these new metabolites.

The diethyl ether soluble fraction from the acetone extract was chromatographed on Si gel, using increasing concentrations of diethyl ether in light petroleum as eluent. Four sesquiterpenoids were isolated. In order of polarity, these were avarone (**2**), diacetyl-avarol (**1b**), avarol (**1a**) and 6'-hydroxy,5'-acetyl-avarol (**3**). The spectral data of **1a** and **2** were in excellent agreement with published values.[1,2]

Diacetyl-avarol (**1b**): m.p. 92-94°, (n-hexane); $[\alpha]_D$ = +12.5 (c = 1.0, $CHCl_3$); molecular formula $C_{25}H_{34}O_4$ (based on HRMS of the parent ion). It was recognized as a diacetyl derivative of avarol by IR (ν_{max} 1760, 1205 cm^{-1}) and NMR data. ^1H-NMR spectrum showed two acetyl groups at δ 2.30 and 2.26, confirmed by the presence in

the ^{13}C-NMR spectrum of two singlets at δ 168.8 and 168.7 and two quartets at δ 21.0 and 20.8. Acetylation of avarol with acetic anhydride and pyridine gave a diacetyl derivative identical in all respects to the natural compound **1b**.

TABLE 1 - ^{1}H and ^{13}C-NMR data of 6'-hydroxy,5'-acetyl-avarol (**3**)[a]

C	δ^{13}C	m[b]	δ^{1}H at C[c] m (J in Hz)	δ^{1}H correlated to C[d]
1	20.3	t	1.95 m	
2	27.3	t	2.02 m	
3	120.6	d	5.16 m	
4	144.5	s	---	1.55 (H-15's)
5	38.5	s	---	1.55 (H-15's), 1.03(H-14's)
6	35.9	t	1.63 m, 1.12 m	
7	28.5	t	1.35 m	
8	39.1	d	1.60 m	0.83 (H-13's)
9	42.7	s	---	0.90 (H-12's), 0.83 (H-13's)
10	49.7	d	1.52 m	
11	35.9	t	2.74, 2.66 ABq (14.2)	0.90 (H-12's)
12	18.2	q	0.90 s	
13	16.2	q	0.83 d (6.5)	
14	20.0	q	1.03 s	
15	18.1	q	1.55 br s	
1'	116.2	s	---	
2'	153.6	s	---	2.74, 2.66 (H-11's)
3'	106.9	d	6.28 d (8.7)	
4'	119.7	d	6.81 d (8.7)	
5'	132.4	s	---	
6'	147.1	s	---	2.74; 2.66 (H-11's)
$CO\underline{C}H_3$	20.9	q	2.32 s	
$\underline{C}OCH_3$	169.8	s	---	2.32 ($COC\underline{H}_3$)

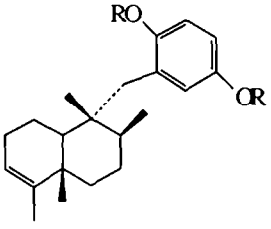
[a] Measured in $CDCl_3$ solution with TMS as internal standard.

[b] By DEPT sequence.

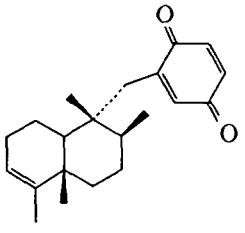
[c] The assignments were aided by homonuclear and heteronuclear 2D experiments.

[d] By long-range HETCOR experiment (J = 8 Hz).

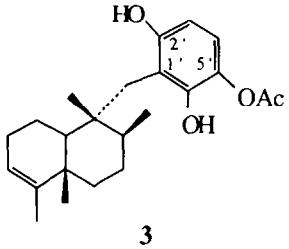
Compound **3**, was obtained as solid: m.p. 113-115° (n-hexane/diethyl ether); $[\alpha]_D$ = +16 (c = 0.8, $CHCl_3$); molecular formula $C_{23}H_{32}O_4$ (based on HRMS of the parent ion). The analysis of NMR data (Table 1) of **3** strongly suggested that the new compound showed the same sesquiterpenoid carbon skeleton of avarol (**1a**), linked to a disubstituted hydroquinone ring. The presence in the ^{1}H-NMR spectrum of two hydroquinone protons (δ 6.81 and 6.28) with a coupling constant of 8.7 Hz localized the fourth substituent in 6' position of the aromatic ring. The presence in the ^{1}H-NMR spectrum of a methyl singlet at δ 2.32 and in the ^{13}C-NMR spectrum of two signals at δ 169.8 (CO) and 20.9 (Me) suggested the presence of an acetyl group in the molecule, and this was confirmed by the IR spectrum with bands at 1746 and 1230 cm^{-1}.



1a R=H
1b R=Ac



2



3

Acetylation of **3** with Ac_2O/pyridine gave a triacetate. The 5' position of the acetyl group, in the compound **3**, was established by considering the acetylation shifts on signals in the ^{13}C-NMR spectra of **3** and its triacetyl derivative. Particularly the large down-shift of C-1' from δ 116.2 to δ 128.3 is in accord with two β acetylation shift effects.

The ^{13}C-NMR shifts of the aromatic ring of **3** are in excellent agreement with those obtained from 5'-monoacetyl-avarol[4] by adding the increment of an OH group in 6' position.

Brine shrimp[5] and potato disc[6] assays are used as in-house assays substituting for 9KB and 9PS cytotoxicities and for P388 and 3PS *in vivo* antileukemic activities respectively, and the results are reported in Table 2.

The diacetyl-avarol (**1b**) showed an activity comparable to avarol in both assays, while the compound **3** was less cytotoxic and more active in the potato disc assay.

TABLE 2. Biological activities of compounds **1b** and **3** in comparison with avarol (**1a**) as determined by brine shrimp and potato disc assays.

Compound	Brine shrimp assay LD_{50} (ppm)	Potato disc assay % inhibition
Diacetyl-avarol (**1b**)	0.15 (0.25/0.10)[a]	55 (56/54)[b]
6'-hydroxy,5'-acetyl-avarol (**3**)	1.30 (2.46/0.69)	76 (77/75)
Avarol (**1a**)	0.18 (0.32/0.10)	64 (66/62)

[a] Confidence levels (95%) in parentheses.

[b] Values of two determinations in parentheses.

EXPERIMENTAL

Melting points were measured on a Kofler apparatus and are uncorrected. UV spectra were obtained on a Varian DMS 90 spectrophotometer. IR spectra were recorded on a Bio-Rad FTS-7 FT-IR spectrometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter, using a 10-cm microcell. Low-resolution and high-resolution mass spectra were recorded on an AEI MS-50 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 500 and 125 MHz, respectively, with TMS as internal standard on a Bruker WM 500 instrument, under Aspect 2000 control. The 2D-NMR spectra were obtained using Bruker's microprograms. Si gel chromatography was performed using pre-coated Merck F_{254} plates and Merck Kieselgel 60 powder.

Extraction of *D. avara* and isolation of compounds.

The sponge *D. avara* (Schmidt) (80 g, dry weight after extraction), collected in March 1989 at Torre Annunziata, Naples (Italy) at depth of 15 meters, was extracted with cold acetone, the solvent was removed *in vacuo*, and the aqueous residue was extracted with diethyl ether. The ether extract was evaporated *in vacuo* to obtain an oil (10 g), which was fractionated on Si gel column by step gradient from light petroleum 40-70° to diethyl ether. Fractions with the same TLC profile were combined. Four fractions were recovered. The less polar fraction was further chromatographed on a Si gel column, eluted with n-hexane/diethyl ether (9:1), to give

2 (720 mg). From the second fraction, after crystallization from n-hexane, was recovered **1b** (56 mg). The third fraction was crystallized from $CHCl_3$, yielding **1a** (6.4 g). The last fraction, after crystallization from n-hexane/diethyl ether, gave **3** (64 mg).

Avarone (2).

UV λ_{max} (CH_3OH) 246, 315 and 440 nm; other spectroscopic data were in excellent agreement with published values.[1]

Diacetyl-avarol (1b).

M.p. 92-94° (n-hexane); $[\alpha]_D$ = +12.5 (c = 1.1, $CHCl_3$); I.R. ν_{max} ($CHCl_3$) 3090, 1760, 1205 cm^{-1}; EIMS m/z (%): 398.2460 (18) [M^+ calc. for $C_{25}H_{34}O_4$, 398.2458], 356 (10), 314 (5), 299 (8), 191 (100), 135 (25), 123 (15); ^{1}H-NMR δ ($CDCl_3$): 6.90 (2H), 6.80 (1H), 5.14 (1H), 2.56 (2H, ABq, J = 14.0 Hz), 2.28 (3H, s), 2.24 (3H, s), 1.51 (3H, br s), 1.00 (3H, s), 0.95 (3H, d, J = 6.0 Hz), 0.83 (3H, s); ^{13}C-NMR δ ($CDCl_3$): 168.8 (s), 168.7 (s), 147.6 (s), 147.4 (s), 144.0 (s), 132.2 (s), 125.3 (d), 122.9 (d), 120.3 (d), 119.9 (d), 46.4 (d), 41.9 (s), 38.4 (s), 38.2 (t), 36.3 (d), 35.9 (t), 27.8 (t), 26.4 (t), 21.0 (q), 20.9 (q), 20.0 (q), 19.8 (t), 17.8 (q), 17.6 (q), 17.4 (q).

Avarol (1a).

M.p. 148-149° ($CHCl_3$); $[\alpha]_D$ = +6.2 (c = 4.1, $CHCl_3$); other spectroscopic data were in excellent agreement with published values.[1,2]

6'-hydroxy,5'-acetyl-avarol (3).

M.p. 113-115° (n-hexane/diethyl ether); $[\alpha]_D$ = +16 (c = 0.8, $CHCl_3$); I.R. ν_{max} ($CHCl_3$) 3500, 1746, 1468, 1230, 1205 cm^{-1}; EIMS m/z (%) 372.2314 (4) [M^+ calc. for $C_{23}H_{32}O_4$, 372.2302], 330 (3), 299 (4), 191 (100), 182 (35); ^{1}H and ^{13}C-NMR (Table 1).

Acetylation of avarol.

A solution of avarol (50 mg) in pyridine (3 ml) and acetic anhydride (0.3 ml) was kept at reflux for 30 min. The excess reagents were removed *in vacuo*, and the residue was crystallized from n-hexane, yielding diacetyl-avarol (45 mg), identical in all respects to the natural compound **1b**.

Acetylation of 3.

A solution of **3** (15 mg) in pyridine (2 ml) and acetic anhydride (0.2 ml) was kept at reflux for 30 min. The excess reagents were removed *in vacuo*, and the residue was chromatographed on Si gel column, eluted with n-hexane/diethyl ether (8:2) to give a triacetyl derivative of **3** (13 mg).- I.R. ν_{max} ($CHCl_3$) 1760, 1746, 1468, 1230, 1205 cm^{-1}; EIMS m/z (%): 456 (2), 414 (2), 331 (2), 266 (5), 224 (25), 191 (100), 182 (35), 135 (20), 123 (10); ^{1}H-NMR δ ($CDCl_3$): 7.08 (1H, d, J = 8.9 Hz), 7.01

(1H, d, J = 8.9 Hz), 5.18 (1H, m), 2.66 (1H, d, J = 14.0 Hz), 2.34 (1H, d, J = 14.0 Hz), 2.31 (3H, s), 2.30 (3H, s), 2.26 (3H, s), 1.57 (3H, br s), 1.02 (3H, s), 0.86 (3H, s), 0.65 (3H, d, J = 6.5 Hz); ^{13}C-NMR δ ($CDCl_3$): 168.7 (s), 168.1 (s), 167.6 (s), 147.8 (s), 144.4 (s), 142.5 (s), 140.2 (s), 128.3 (s), 120.9 (d), 120.5 (d), 120.1 (d), 52.1 (d), 42.9 (s), 40.3 (d), 38.7 (t), 38.6 (s), 36.2 (t), 28.5 (t), 27.2 (t), 21.2 (q), 20.6 (q), 20.5 (q), 20.3 (t), 19.8 (q), 18.1 (q), 18.0 (q), 15.5 (q).

Biological activities.

Brine shirmp (*Artemia salina*) assay was performed in DMSO (1% final volume) using 10x3 animals/dose suspended in artificial sea water (5 ml) as previously reported.[5] After 24 h the live animals were noted and the data were analyzed by the Finney program[7] obtaining LD_{50} values with 95% confidence intervals.

The potato disc antitumor assay was performed using 2 mg of each compounds, dissolved in 0.5 ml of DMSO, 1.5 ml of sterile distilled water and 2 ml of *Agrobacterium tumefaciens* suspension are added. Sterile potato discs (n = 25, ø = 1.8 cm, h = 0.5 cm) were used for each compound and 25 µl of the suspension added to each disc. Tumors were counted after 16-18 days and the data were compared with control discs.[6]

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