Journal of Natural Products
Vol. 53, No. 3, pp. 699–702, May-Jun 1990

BIOLOGICAL ACTIVITIES OF AVAROL DERIVATIVES, 1. AMINO DERIVATIVES

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Abstract.—Nine amino derivatives, compounds **3**–**11**, of avarone were synthesized. Their antibacterial and cytotoxic activities are evaluated, and the results of a prescreen for antitumor effects are reported.

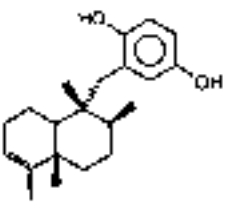
Recent studies have revealed that avarol (**1**) and avarone (**2**), previously isolated from the marine sponge *Dysidea avara* Schmidt (Dictyoceratida) (1,2), show a wide variety of biological activities. Both compounds are potent antileukemic agents, in vitro and in vivo (3,4). They were determined to be neither direct mutagens nor premutagens, and they displayed antimutagenic activity (5). Both avarol and avarone inhibit replication of the etiological agent of acquired immune deficiency syndrome (AIDS) (6). These interesting properties and the previous finding that 3′-methylamino (**5**) and 4′-methylamino (**6**) derivatives of avarone, from *D. avara* (7), also show interesting biological properties, prompted us to prepare other amino derivatives of avarone and evaluate their antibacterial and antitumor activity.

We wish to describe herein the synthesis of nine amino derivatives from avarone by using Me_3SiN_3, methylamine, ethylamine, alanine, 6-aminopurine (adenine), and glucosamine to obtain a wide series of amino derivatives of avarone, with different polarities. Brine shrimp lethality (8) was used as an indicator of cytotoxicity. This assay was demonstrated to be in excellent agreement with L5178y (mouse lymphoma cells) and L1210 (leukemia cells) assays, using avarol (**1**) and avarone (**2**) (9). A prescreen for antitumor activity utilized the potato disc assay, by which the inhibition of crown gall tumors on potato discs inoculated with *Agrobacterium tumefaciens* was determined (10).

Amino derivatives were generally obtained by slowly adding the amine hydrochloride, dissolved in basic solution, to a dilute solution of avarone (**2**), obtained by Ag_2O oxidation of avarol (**1**), in EtOH or EtOH-H_2O (1:1). Using either trimethylsilyl azide or ethylamine, two isomers were obtained with substitution at 3′ (**3**,**7**) and 4′ (**4**,**8**) of the benzoquinone ring, as previously described (7) for methylamine derivatives (**5**,**6**). Using alanine and glucosamine, only one derivative was obtained, with substitution at 4′ (**9** and **10**, respectively).

The position of the substituent was determined by the analysis of ^{1}H-nmr spectra. Signals of protons in the benzoquinone ring are doublets in 3′-substituted compounds and singlets in 4′-substituted compounds. Finally, when 6-aminopurine was used, the addition of two adenine nitrogens takes place so that a pyrazine ring is formed. The ^{1}H-nmr spectra, showing in the sp^2 region two protons of purine (δ 8.05 and 7.84), one vinylic proton (δ 5.08) due to sesquiterpenoid moiety, and one proton singlet (δ 6.48) attributable to the original benzoquinone ring, suggest 3′,4′ substitution. We suggest that the process takes place by addition of the adenine amino group at 4′, followed by the reaction of the pyrrolic nitrogen at 3′, yielding the cyclized product **11**.

All derivatives were tested in an-



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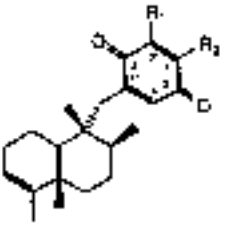
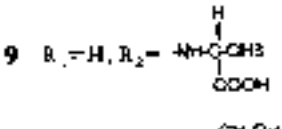
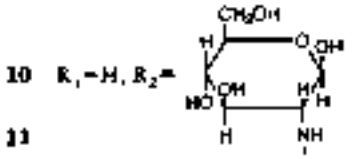


2 $R_1=R_2=H$
3 $R_1=NH_2$, $R_2=H$
4 $R_1=H$, $R_2=NH_2$
5 $R_1=NHMe$, $R_2=H$
6 $R_1=H$, $R_2=NHMe$
7 $R_1=NHEt$, $R_2=H$
8 $R_1=H$, $R_2=NHEt$

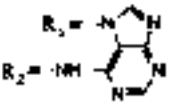


9 $R_1=H$, $R_2=$



10 $R_1=H$, $R_2=$

11



tibacterial, brine shrimp, and potato disc assays; the results are reported in Table 1.

4'-Methylamino and 4'-ethylamino derivatives were the most cytotoxic of compounds tested, with an activity comparable to that of avarol, previously reported (9).

The compounds **3–11** tend to remain in the quinone form; in fact, hydroquinones obtained by $NaBH_4$ reduction of amino derivatives **3–11** of avarone are immediately oxidized by air to the original quinones.

We believe that the stability of the quinone form of amino derivatives is at least in part responsible for diminution of some aspects of biological activities, because apparently they can be reduced in biological medium only with difficulty. As stated by Müller *et al.* (11), one aspect of the biological activity of the avarone/avarol couple is associated with formation of superoxide anion radicals via semiquinones.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage microscope and are uncorrected. ^{1}H-nmr spectra were measured on a WM 500 Bruker spectrometer (TMS as internal standard). Only chemical shifts of quinone ring protons are re-

TABLE 1. Biological Activity.

Compound	Bioassay		
	Brine shrimp (LC_{50} ppm)[a]	Potato disc (% Inhibition)[b]	Antibacterial activity vs *Staphylococcus aureus* (MIC, μg/ml)
Avarone [2]	0.14 (0.07/0.32)	63 (64/62)	1.56
3'-Amino-avarone [3]	0.24 (0.12/0.43)	53 (55/51)	50
4'-Amino-avarone [4]	0.25 (0.11/0.41)	55 (57/55)	50
3'-Methylamino-avarone [5]	2.4 (0.8/14.4)	48 (50/46)	no activity[c]
4'-Methylamino-avarone [6]	0.34 (0.19/0.58)	57 (58/56)	no activity
3'-Ethylamino-avarone [7]	0.81 (0.25/2.3)	54 (56/52)	no activity
4'-Ethylamino-avarone [8]	0.27 (0.11/0.52)	38 (40/36)	no activity
4'-Alanino-avarone [9]	2.0 (1.3/3.2)	38 (40/36)	6.25
4'-Glucosamino-avarone [10]	11.1 (6.4/21.3)	38 (40/36)	6.25
3'-Adenine adduct of avarone [11]	20.2 (16/207)	27 (29/25)	12.50
Penicillin G Na	—	—	0.04

[a]95% confidence levels in parentheses.
[b]Values of two determinations in parentheses.
[c]More than 100 μg/ml.

powed because all other signals belonging to the sesquiterpenoid portion and other structural moieties were earlier reported (1,2) or are generally known. Uv spectra were obtained on a Varian DMS 90 spectrophotometer. Gc was carried out on Merck Si gel 60 and Sephadex LH-20.

MATERIALS.—Avarol (**1**) was isolated from *D. avara* (1) which was collected in the Bay of Naples, Italy; a voucher specimen is maintained in the collection of the Italian institute. Avarone (**2**) was prepared from avarol by oxidation with Ag_2O as previously described (1). Methylamine hydrochloride (BDH), trimethylsilyl azide (Merck), ethylamine, D,L-alanine, and glucosamine (Fluka AG), and 6-aminopurine (Sigma) were used for the synthesis.

SYNTHESIS OF **3** AND **4**.—Avarone (115 mg) was treated with Me_3SiN_3 in absolute EtOH as previously described (12), and 50 mg of **3** and 60 mg of **4** were obtained.

3'-Azido-avarone (**3**).—Mp 78–80° (pentane); uv λ max (MeOH) 287 (5600), 478 (1600); ^{1}H nmr ($CDCl_3$) δ 6.52 (H-6', d, J = 2.2 Hz), 5.69 (H-4', d, J = 2.2 Hz).

4'-Azido-avarone (**4**).—Mp 84–85° (pentane); uv λ max (MeOH) 286 (5600), 475 (900); ^{1}H nmr ($CDCl_3$) δ 6.56 (H-6', sl), 5.69 (H-4', sl).

SYNTHESIS OF **5** AND **6**.—Avarone (100 mg) was treated with $MeNH_2$·HCl as previously described (7), and 63 mg of **5** and 110 mg of **6** were recovered.

3'-Methylamino-avarone (**5**).— Mp 152–155° (hexane); uv λ max (MeOH) 292 (5000), 486 (1700); ^{1}H nmr ($CDCl_3$) δ 6.37 (H-6', d, J = 2.3 Hz), 5.40 (H-4', d, J = 2.3 Hz).

4'-Methylamino-avarone (**6**).—Mp 161–164° (hexane); uv λ max (MeOH) 290 (4900), 488 (1000); ^{1}H-nmr ($CDCl_3$) δ 6.35 (H-6', s), 5.41 (H-3', s).

SYNTHESIS OF **7** AND **8**.—A solution of avarone (340 mg) in EtOH (100 ml) was saturated with anhydrous $EtNH_2$ and kept at room temperature for 1 h. After elimination of the solvent, the mixture was separated by chromatography over Si gel and eluted with petroleum ether-Et_2O (7:3). The less polar component was 4'-ethylamino-avarone (**7**) (165 mg): mp 122–124° (hexane); uv λ max (MeOH) 290 (6700), 484 (1500); ^{1}H-nmr ($CDCl_3$) δ 6.34 (H-6', s), 5.41 (H-3', s). The more polar component was 3'-ethylamino-avarone (**8**) (135 mg): mp 110–112° (hexane); uv λ max (MeOH) 257 (34000), 277 (9000), 348 (4000), 490 (2400); ^{1}H nmr ($CDCl_3$) δ 6.35 (H-6', s, J = 2.2 Hz), 5.39 (H-4', d, J = 2.2 Hz).

SYNTHESIS OF **9**.—D,L-Alanine (1 g) was dissolved in a saturated solution of $NaHCO_3$ (100 ml), added to a solution of avarone (250 mg) in EtOH (100 ml), and stirred for 24 h at room temperature. After elimination of EtOH, the remaining aqueous solution was extracted with $CHCl_3$; the extract was chromatographed on a Si gel column to give, by elution with $CHCl_3$-MeOH (85:15), **9** (200 mg): mp 153–156° ($CHCl_3$/MeOH); uv λ max (MeOH) 260 (59000), 278 (25000), 486 (7100); ^{1}H nmr ($CDCl_3$ + CD_3OD) δ 6.50 (H-6', sl, 5.55 (H-4', sl).

SYNTHESIS OF **10**.—Glucosamine hydrochloride (1 g), dissolved in a saturated solution of $NaHCO_3$ (100 ml), was added dropwise with stirring to a solution of avarone (240 mg) in EtOH (100 ml), and the reaction solution was stirred at room temperature for 24 h. After the usual workup the residue was chromatographed on a Si gel column to give, by elution with $CHCl_3$-MeOH (9:1), **10** (100 mg): mp 203–205° ($CHCl_3$/MeOH); uv λ max (MeOH) 260 (16000), 276 (11000), 492 (4000); ^{1}H nmr ($CDCl_3$ + CD_3OH) δ 6.63 (H-6', s), 5.92 (H-3', s).

SYNTHESIS OF **11**.—6-Aminopurine (adenine) (1 g) dissolved in a saturated solution of $NaHCO_3$ (100 ml) was added to a solution of avarone (280 mg) in EtOH (100 ml). After the usual workup, the $CHCl_3$ extract was chromatographed on a Sephadex LH-20 column (2 × 100 cm) to give, by elution with MeOH, **11** (130 mg): mp > 300° with decomposition ($CHCl_3$-MeOH); uv λ max (MeOH) 266 (29000), 496 (450); ^{1}H nmr (CD_3OD + $CDCl_3$) δ 6.48 (H-6', sl).

BIOASSAYS.—The brine shrimp lethality assay and the potato disc assay were performed as described (8,10). The antimicrobial activity toward the Gram-positive bacterium *Staphylococcus aureus* ATCC 25293 was determined by the serial dilution method (15). The lowest concentration of the compound inhibiting macroscopically detectable bacterial growth was taken as the minimum inhibitory concentration (MIC). The results are reported in Table 1.

ACKNOWLEDGMENTS

This work was partially supported by Progetto Finalizzato Chimica Fine II, CNR, Rome. We thank Mr. C. Iodice for skillful technical assistance and Mr. A. Crispino and Mr. A. Traburco for collection of the sponge.

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Received 8 September 1989