3-*epi*-Aplykurodinone B, a New Degraded Sterol from *Aplysia fasciata*

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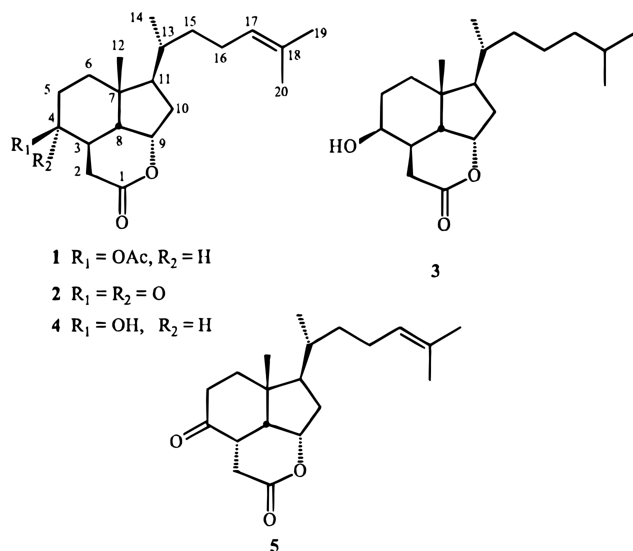
The anaspidean mollusk *Aplysia fasciata* from Río San Pedro (Cádiz, Spain) contains the known degraded sterols aplykurodinone B (**2**) and aplykurodin B (**4**) together with the new 3-*epi*-aplykurodinone B (**5**). The structure of **5** was elucidated by interpretation of spectral data, and its relative stereochemistry was established using NOEDS experiments. The new compound **5** exhibited cytotoxicity against four tumor cell lines.

In 1992, Cimino and co-workers reported the isolation and characterization of the ichthyotoxic degraded sterols 4-acetylaplykurodin B (**1**) and aplykurodinone B (**2**) from the anaspidean mollusk *Aplysia fasciata* Poiret (Aplysiidae) collected in the Bay of Naples, Italy.[1] Two related isoprenoids, aplykurodin A (**3**) and aplykurodin B (**4**), had been previously isolated from *Aplysia kurodai*.[2] Because the cytotoxicity of aplykurodins against tumor cell lines had not been tested, we investigated specimens of *A. fasciata* in the course of studying pharmacologically active metabolites from marine organisms of the southern coast of Spain. Our collection afforded the known compounds aplykurodinone B (**2**) and aplykurodin B (**4**), together with the new compound 3-*epi*-aplykurodinone B (**5**).



Three specimens of *A. fasciata* were collected by hand in the intertidal zone of Río San Pedro, Cádiz, Spain. The specimens were carefully dissected into parapodial lobes, mantles (external parts), and internal viscera. The combined Et_2O-soluble fractions of Me_2CO extracts of the external parts were chromatographed on Si gel followed by final purification using HPLC to afford (in order of increasing polarity): aplykurodinone B (**2**, 0.058% dry wt), aplykurodin B (**4**, 0.029% dry wt), and 3-*epi*-aplykurodinone B (**5**, 0.044% dry wt). Comparison of spectral data with the literature values allowed identification of the known compounds aplykurodinone B (**2**)[1] and aplykurodin B (**4**).[2]

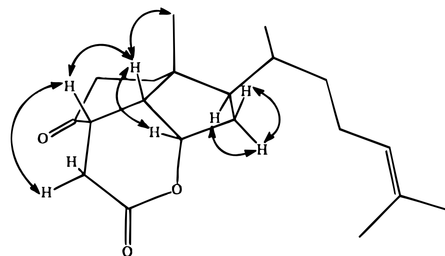


Figure 1. NOE correlations for 3-*epi*-aplykurodinone B (**5**).

3-*epi*-Aplykurodinone B (**5**) was isolated as an amorphous powder. The molecular formula, $C_{20}H_{30}O_3$, was obtained from the high resolution mass measurement and indicated that compound **5** was an isomer of aplykurodinone B (**2**). The ^{13}C-NMR signals (131.6 (s), 124.3 (d), 35.3 (t), 32.2 (d), 25.6 (q) 25.0 (t), 18.2 (q), and 17.7 (q)) were consistent with the presence of a 1,5-dimethylhex-4-enyl side chain. The IR absorptions at 1725 and 1730 cm^{-1}, together with the ^{13}C-NMR singlets at δ 210.5 and 172.2, were assigned to a ketone and a δ-lactone carbonyl group, respectively. These data, together with the general similarities in both ^{1}H- and ^{13}C-NMR spectra indicated that the structure of compound **5** was closely related to that of aplykurodinone B (**2**). However, the slight differences observed in H-3 and H-9 proton signals at δ 3.01 (1H, ddd, $J =$ 10.7, 4.2, 3.1 Hz) and 4.62 (1H, dd, $J = 7.3$, 5.6 Hz), respectively, and the significant downfield shift of H-8 resonance at δ 2.70 (1H, dd, $J = 10.6$, 7.5 Hz) suggested a different stereochemistry of the δ-lactone ring.

The stereochemistry of 3-*epi*-aplykurodinone B (**5**) was determined by using NOEDS experiments (Figure 1). Irradiation of the H-3 signal caused enhancement on the H-8 signal, and irradiation of H-8 signal enhanced the H-3 and Me-12 signals, defining the geometry of the 3,8-ring junction. Furthermore, the mutual enhancements observed upon irradiation of H-8 and H-9 signals indicated the *cis* orientation of these protons and defined a 8,9-ring junction stereochemistry identical to that of **1**–**4**. The stereochemistry at C-13 was proposed identical to that of the known cometabolites of *A. fasciata*[1,2] on biogenetic grounds. It was therefore assumed that compound **5** was the C-3 epimer of aplykurodinone B (**2**).

It had been suggested that the aplykurodins (**1**–**2**) might be biogenetically derived from a parent sterol by

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a degradative loss of ring-A carbon atoms through 5,6 and 9,10 oxidative cleavages.[1] The stereochemistry at C-8 of aplykurodins (**1**–**5**) would require a 14β proton orientation in these biosynthetic precursors. This stereochemistry, though unusual, has been described in a sterol from a marine sponge.[3]

The new compound 3-*epi*-aplykurodinone B (**5**) was tested against P-388 mouse lymphoma, A-549 human lung carcinoma, HT-29 human colon carcinoma, and MEL-28 human melanoma tumor cell lines, exhibiting mild in vitro cytotoxicity with ED_{50} values of 2.5 μg/mL in all cases.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer 881 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian 400 at 400 MHz and 100 MHz, respectively, using $CDCl_3$ as solvent. The resonances of residual $CHCl_3$ at δ_H 7.26 and δ_C 77.0 were used as internal reference for ^{1}H and ^{13}C-NMR spectra, respectively. Mass spectra were measured on a VG 12250 or on a Kratos MS 80RFA spectrometer. In HPLC separations, LiChrosorb Si-60 was used in normal-phase mode using a differential refractometer. All solvents were distilled from glass prior to use.

Collection, Extraction, and Isolation Procedures. Three specimens of *A. fasciata* (6.85 g dry wt) were collected by hand in the intertidal zone of Río San Pedro, Cádiz, Spain. A voucher specimen is deposited at Departamento de Biología Animal, Biología Vegetal y Ecología, Universidad de Cádiz (no. 9603). The material was carefully dissected into parapodial lobes, mantles, and internal viscera. All these sections were separately soaked with Me_2CO at room temperature, the solvent evaporated, and the resulting residue extracted with Et_2O. Preliminary TLC analysis of the Et_2O extracts showed the presence of compounds **2**, **4**, and **5** only in the parapodial lobes and mantles. These two extracts were therefore combined (0.7 g) and subjected to SiO_2 column separation eluting with mixtures of increasing polarity from hexane to Et_2O and subsequently $CHCl_3$–MeOH (8:2). Medium polar fractions contained compound **2** (4 mg, 0.058% dry wt). Fractions eluted with hexane–Et_2O (3:7) contained a mixture of compounds that was further separated by HPLC in normal-phase mode (LiChrosorb 5 μ, 5 mm $\times$ 25 cm; hexane–EtOAc, 1:1) affording compound **4** (2 mg, 0.029% dry wt) and compound **5** (3 mg, 0.044% dry wt).

3-*epi*-Aplykurodinone B (5): amorphous powder; $[\alpha]^{25}_D$ −98.0° (*c* 0.2, $CHCl_3$); IR (dry film) ν_{max} 1730 (C=O), 1725 (C=O), 1220 (CO) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.06 (1H, tq, J = 7.2, 1.3 Hz, H-17), 4.62 (1H, dd, J = 7.3, 5.6 Hz, H-9), 3.01 (1H, ddd, J = 10.7, 4.2, 3.1, H-3), 2.96 (1H, dd, J = 15.7, 2.9 Hz, H-2α), 2.70 (1H, dd, J =10.6, 7.5 Hz, H-8), 2.42 (1H, m, H-5), 2.37 (1H, ddd, J = 19.0, 5.8, 4.1 Hz, H-5), 2.15 (1H, dd, J = 15.7, 4.2 Hz, H-2β), 2.13 (1H, d, J = 5.6 Hz, H-10α), 2.04 (1H, m, H-16), 2.00 (2H, m, H-6), 1.88 (1H, m, H-16), 1.77 (1H, m, H-10β), 1.74 (1H, m, H-11), 1.59 (3H, br s, H-20), 1.56 (3H, br s, H-19), 1.50 (1H, m, H-13), 1.41 (1H, dddd, J = 16.5, 6.5, 6.5, 3.2 Hz, H-15), 1.11 (3H, s, H-12), 1.10 (1H, m, H-15), 0.97 (3H, d, J = 7.2 Hz, H-14); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 210.5 (s, C-4), 172.2 (s, C-1), 131.6 (s, C-18), 124.3 (d, C-17), 79.4 (d, C-9), 52.1 (d, C-8), 45.8 (d, C-11), 43.2 (s, C-7), 42.3 (d, C-3), 35.5 (t, C-10), 35.3 (t, C-15), 34.7 (t, C-5), 32.2 (d, C-13), 31.8 (t, C-6), 30.4 (t, C-2), 25.6 (q, C-19), 25.0 (t, C-16), 24.9 (q, C-12), 18.2 (q, C-14), 17.7 (q, C-20); EIMS (70 eV) *m/z* (rel int) 318 (13), 300 (12), 259 (16), 205 (72), 83 (89), 69(100); HREIMS *m/z* 318.2195 calcd for $C_{20}H_{30}O_3$, 318.2203.

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