Steroids from the Antarctic Octocoral Anthomastus bathyproctus

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · AUGUST 2005

Impact Factor: 3.8 · DOI: 10.1021/np050080q · Source: PubMed

Steroids from the Antarctic Octocoral *Anthomastus bathyproctus*

Gonzalo G. Mellado,[†] Eva Zubía,*[,†] María J. Ortega,[†] and Pablo J. López-González[‡]

Departamento de Química Orgánica, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Apartado 40, 11510-Puerto Real (Cádiz), Spain, and Departamento de Fisiología y Zoología, Facultad de Biología, Universidad de Sevilla, Reina Mercedes 6, 41012-Sevilla, Spain

Received March 7, 2005

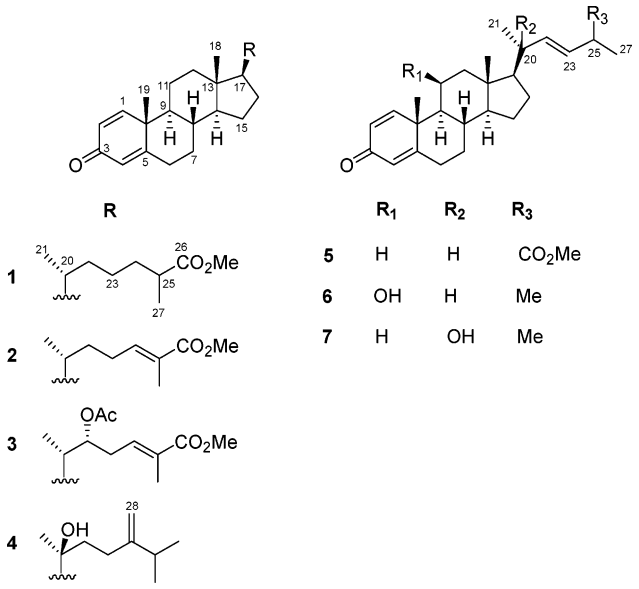
Seven new steroids, compounds **1**−**7**, were isolated from the Antarctic octocoral *Anthomastus bathyproctus*. The structures of the new metabolites have been established by analysis of their spectroscopic data. The in vitro cytotoxicity has been tested against three human tumor cell lines.

It is widely recognized that marine invertebrates of the class Octocorallia (Alcyonaria) from tropical and temperate waters have been a prolific source of new metabolites, most of them terpenoid and steroidal derivatives.[1] Although the chemical accounts on Antarctic octocorals are scarce, the reported results suggest that the secondary metabolism of these organisms is also characterized by compounds derived through the mevalonate pathway. Thus, in recent years, several sesquiterpenes[2] and polyoxygenated steroids[3] have been isolated from different Antarctic and sub-Antarctic octocorals.

As a part of our search for bioactive metabolites from marine invertebrates, we have examined specimens of the alcyonacean octocoral *Anthomastus bathyproctus* Bayer 1993 collected in the South Shetland Islands (Antarctica). This study has resulted in the isolation of new steroids, compounds **1**−**7**, all of them displaying a cross-conjugated ketone system in the A ring of the tetracarbocyclic nucleus, while their side chains belong to the cholestane, ergostane, and 24-norcholestane types.

Frozen specimens of *A. bathyproctus* were extracted with acetone/MeOH (1:1). After evaporation under reduced pressure, the aqueous residue was extracted with Et_2O and the organic extract subjected to column chromatography eluting with hexane/Et_2O mixtures of increasing polarity, then $CHCl_3$/MeOH, and finally MeOH. Fractions eluted with hexane/Et_2O mixtures (1:1 to 3:7), which showed mild cytotoxic activity, were subjected to HPLC separation to yield the new compounds **1**−**7**.

Compound **1** was isolated as a solid whose molecular formula $C_{28}H_{42}O_3$ was established by HRCIMS. The ^{1}H NMR spectrum displayed one *O*-methyl signal at δ 3.64 (3H, s) as well as signals at δ 1.22 (3H, s), 1.14 (3H, d, J = 6.8 Hz), 0.89 (3H, d, J = 6.4 Hz), and 0.73 (3H, s) attributable to four methyl groups of a steroidal metabolite. Furthermore, the ^{13}C NMR spectrum displayed, in addition to the *O*-methyl signal at δ 51.5 (q), 27 signals that suggested the presence of a cholestane framework. The ^{13}C NMR signal at δ 186.4 (s) was assigned to a conjugated carbonyl carbon, while those of three methines at δ 156.0, 127.4, and 123.8 and one quaternary carbon at δ 169.4 represented two double bonds. These chemical shifts matched with the presence of a cross-conjugated ketone in ring A of the tetracyclic nucleus.[4] The signal at δ 177.4 (s) was assigned to an ester carbonyl. This signal was correlated in the HMBC spectrum with the *O*-methyl at δ 3.64



and with the methyl at δ 1.14 (3H, d, J = 6.8 Hz), indicating the presence of a methoxycarbonyl group at the end of the cholestane side chain. The cross-peaks observed in the NOESY spectrum [Me-19 (δ 1.22) with H-6ax (δ 2.46) and H-8 (1.60); Me-18 (δ 0.73) with H-12eq (δ 2.02), H-8, and H-20 (δ 1.38); Me-21 (δ 0.89) with H-12eq] were in agreement with the usual configuration of the natural cholestane steroids (8β,9α,10β,13β,14α,17β,20*R*),[5] while the configuration at C-25 remains undetermined. All these data established the structure methyl 3-oxocholesta-1,4-dien-26-oate for compound **1**. Marine steroids displaying a fully oxidized C-26 have been described only from the gorgonian *Acalycigorgia inermis*[6] and the starfish *Myxoderma platyacanthum*.[7]

Compound **2** was isolated as a solid of molecular formula $C_{28}H_{40}O_3$, as deduced by HRCIMS. The NMR spectra of **2** suggested it was a dehydro derivative of compound **1**. In particular, the signals at δ_H 6.74 (tq, J = 7.7 and 1.3 Hz), δ_C 143.0 (s), and δ_C 127.2 (d) indicated the presence in **2** of an additional double bond. This unsaturation was located at C-24,C-25 upon observation of the multiplicity and downfield shift of the Me-27 signal at δ 1.83 (bs), as well as the upfield shift of the ester carbonyl at δ 168.8. In addition, the NOE effect association between Me-27 (δ 1.83) and H-23 (δ 2.20) was in agreement with an *E* geometry for the C-24,C-25 double bond. These data and the remaining spectroscopic evidence led to the structure methyl (24*E*)-3-oxocholesta-1,4,24-trien-26-oate for compound **2**.

* To whom correspondence should be addressed. Tel: +34 956016021. Fax: +34 956016193. E-mail: eva.zubia@uca.es.
[†] Universidad de Cádiz.
[‡] Universidad de Sevilla.

10.1021/np050080q CCC: $30.25
Published on Web 06/22/2005

The NMR spectra of compound **3** were similar to those of compound **2** except for the presence of the signals due to an acetoxyl group [δ_H 2.02 (3H, s), δ_C 170.6 ($-OCOCH_3$) and 21.3 ($-OCOCH_3$)] linked to a methine [δ_H 4.97 (dt, J = 10.5 and 3.1 Hz) and δ_C 75.4 (d)]. The cross-peaks in the COSY spectrum between the methine at δ 4.97 and the allylic protons H-23 at δ 2.37 (1H, m) and 2.25 (bdd, J = 14.0 and 7.0 Hz) defined the location of the secondary *O*-acetyl group at C-22. The 22*R* absolute configuration was deduced taking into account that a substituent at C-22 deshields C-20 and C-23 (β effects) with respect to the unsubstituted compound, and the magnitude of these effects is significantly different for the 22*R* and 22*S* epimers.[8] Thus, a comparison of the ^{13}C resonances of the side chains of the 22*R* and 22*S* epimers of cholest-5-ene-3β,22-diol diacetate[9] with those of the nonfunctionalized side chain of cholesterol benzoate[8] shows that signals due to C-20 and C-23 in the 22*R* isomer are deshielded by 3.5 and 1.1 ppm, respectively, while in the 22*S* isomer these carbons are deshielded by 3.1 and 6.1 ppm, respectively. Following this, a comparison of the ^{13}C NMR data of **3**, carrying an acetoxyl group at C-22, with those of **2** reveals that the C-20 and C-23 resonances in **3** are deshielded by 3.4 and 1.6 ppm, respectively, in agreement with a 22*R* configuration. All these data led to the structure methyl (22*R*,24*E*)-22-acetoxy-3-oxocholesta-1,4,24-trien-26-oate for compound **3**.

Compound **4** was isolated as a solid whose molecular formula $C_{28}H_{42}O_2$ was established by HRCIMS. Although the NMR spectra of **4** were reminiscent of those of the previously described compounds, several diagnostic differences were readily observed. Thus, the spectra of **4** lacked the signals due to the *O*-methyl group, suggesting that this steroid had a C_{28} framework. The signal due to the carboxyl carbon was also absent, while the signals due to a *gem*-disubstituted double bond [δ_H 4.74 (1H, bs) and 4.67 (1H, d, J = 1.3 Hz); δ_C 156.2 (s) and 106.4 (t)] and to a fully substituted carbon atom linked to an oxygenated function [δ_C 75.0 (s)] were evident. In the COSY spectrum the olefinic methylene signals at δ 4.74 and 4.67 showed cross-peaks with a methine at δ 2.23 (1H, hept, J = 6.9 Hz), which in turn was correlated with a *gem*-dimethyl group at δ 1.03 (6H, d, J = 6.9 Hz). These correlations indicated the presence of an isopropyl group at the side chain terminus and the location of the double bond at C-24,C-28 of an ergostane skeleton. On the other hand, the sp^3 carbon bearing the oxygenated function (δ_C 75.0) was identified as C-20, which had to be linked to a hydroxyl group that gave rise to the IR absorption at 3420 cm^{-1}. This proposal was supported by the NMR signals generated by Me-21 at δ_H 1.30 (3H, s) and δ_C 26.2 (q). The remaining signals were in full agreement with the presence in **4** of a tetracyclic nucleus identical to that of compounds **1**−**3**. With respect to the configuration at C-20, we propose an *S* absolute configuration based on the chemical shift of Me-21 at δ_H 1.30 and the NOESY correlation between Me-21 and H-12eq [δ 2.13 (1H, ddd, J = 12.8, 3.6 and 3.3 Hz)].[10] All these data led to the structure (20*S*)-20-hydroxyergosta-1,4,24(28)-trien-3-one for compound **4**.

The NMR spectra of compound **5** contained, in addition to the signals of a steroidal nucleus identical to that of the compounds described above, those attributable to Me-21 [δ_H 1.00 (3H, d, J = 6.6 Hz)], Me-27 [δ_H 1.22 (3H, d, J = 7.0 Hz)], H-20 [δ_H 2.06 (1H, m)], H-25 [δ_H 3.06 (δ_H 1H, quint, J = 7.1 Hz)], and one methoxycarbonyl group [δ_H 3.67 (3H, s, $COOCH_3$); δ_C 51.7 ($COOCH_3$) and 175.6 ($COOCH_3$)]. The remaining signals were due to a *trans*-disubstituted double bond [δ_H 5.43 (1H, dd, J = 15.3 and 7.4 Hz) and 5.34 (1H, dd J = 15.3 and 8.2 Hz); δ_C 138.0 (d) and 126.5 (d)]. Furthermore, in the COSY spectrum the olefinic protons at δ 5.34 and 5.43 were correlated with H-20 and H-25, respectively. These data indicated that compound **5** was a 24-norcholestane containing an *E*-double bond at C-22,C-23 and a methoxycarbonyl group at C-26. Analysis of the NOESY spectrum confirmed that compound **5** displayed around the tetracyclic nucleus and C-20 the usual configuration of a cholestane derivative, while the configuration at C-25 remains undetermined. The structure methyl (22*E*)-3-oxo-24-norcholesta-1,4,22-trien-26-oate was therefore proposed for compound **5**.

The molecular formula of compound **6**, $C_{26}H_{38}O_2$, together with the NMR spectra indicated that compound **6** was also a 24-norcholestane metabolite related to compound **5**. However, the absence of the signals due to the methyl ester, together with the ^1H NMR signal at δ 0.93 (6H, d, J = 6.7 Hz), was consistent with the presence of an isopropyl group at the end of the side chain. This assignment was confirmed by the COSY correlations between the *gem*-dimethyl group at δ 0.93 and a methine proton at δ 2.18 (1H, heptd, J = 6.7 and 5.8 Hz, H-25), which in turn was correlated with the olefinic proton at δ 5.27 (1H, dd, J = 15.2 and 6.4 Hz, H-23). A second distinctive feature of the NMR spectra of compound **6** was the presence of the signals of a methine linked to a hydroxyl group [δ_H 4.34 (1H, bs) and δ_C 70.6 (d)]. An HMBC correlation between H-12eq (δ 2.08) and the carbon resonance at δ 70.6 located this hydroxyl group at C-11. Furthermore, the multiplicity of the H-11 signal and the NOESY correlations between this signal and those of H-12eq and H-12ax were in agreement with an equatorial orientation of H-11 and therefore an axial hydroxyl group. The spectroscopic evidence was consistent with the structure (22*E*)-11β-hydroxy-24-norcholesta-1,4,22-trien-3-one for compound **6**.

Compound **7** was obtained as a solid whose molecular formula $C_{26}H_{38}O_2$, obtained by HRCIMS, indicated that it was an isomer of compound **6**. Furthermore, the NMR spectra of both steroids were closely similar except for the absence in the spectra of compound **7** of the signals due to the hydroxylated methine at C-11 and the presence of a signal attributable to a fully substituted carbon atom linked to a hydroxyl group [δ_C 75.0 (s)]. These data, together with the ^1H NMR signal of Me-21 [δ 1.30 (3H, s)], indicated the location of the hydroxyl group at C-20. The *S* configuration of this chiral center was proposed upon observation of the NOESY correlation between Me-21 and H-12eq [2.13 (1H, ddd, J = 12.9, 3.7, and 3.3 Hz)].[10] Therefore, the structure (20*S*,22*E*)-20-hydroxy-24-norcholesta-1,4,22-trien-3-one was proposed for compound **7**.

The new compounds from *A. bathyproctus* were tested in assays directed to detect in vitro cytotoxicity against the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A-549 (lung carcinoma), and HT-29 (colon adenocarcinoma). Compounds **2**−**5** displayed weak activity as inhibitors of cell growth. The individual cell line identifiers and the corresponding GI_{50} values (μM) obtained for each active compound were as follows. Compound **2**: MDA-MB-231 (21.2), A-549 (16.5), HT-29 (15.3); compound **3**: MDA-MB-231 (18.4); compound **4**: A-549 (23.4); compound **5**: MDA-MB-231 (22.4), A-549 (21.4), HT-29 (20.2).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra were obtained on a Philips PU 8710 spectrophotometer, and

Table 1. ^{1}H NMR Data for Compounds **1**−**7**[a]

atom no.	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[c]
1	7.05 (d, 10.0)	7.05 (d, 10.0)	7.04 (d, 10.3)	7.05 (d, 10.0)	7.05 (d, 10.2)	7.26 (d, 10.2)	7.05 (d, 10.1)
2	6.22 (dd, 10.0, 1.9)	6.22 (dd, 10.0, 2.0)	6.23 (dd, 10.3 1.8)	6.23 (dd, 10.0, 1.8)	6.22 (dd, 10.2, 1.9)	6.26 (dd, 10.2, 1.9)	6.23 (dd, 10.1, 1.8)
3							
4	6.06 (bt, 1.5)	6.07 (bt, 1.5)	6.07 (bt, 1.4)	6.07 (bt, 1.3)	6.07 (bt, 1.6)	6.00 (bt, 1.6)	6.06 (bt, 1.5)
5							
6	2.36 (ddd, 13.2, 4.2, 2.6)α, 2.46 (m)*β*	2.35 (ddd, 13.0, 4.4, 2.6)α, 2.46 (dddd, 13.3, 13.3, 5.1, 1.5)*β*	2.37 (m)α, 2.47 (dddd, 13.3, 13.3, 5.1, 1.4)*β*	2.36 (ddd, 13.3, 4.3, 2.6)α, 2.47 (dddd, 13.3, 13.3, 5.1, 1.3)*β*	2.35 (ddd, 13.3, 4.2, 2.6)α, 2.46 (dddd, 13.5, 13.3, 5.0, 1.4)*β*	2.31 (ddd, 13.3, 4.9, 1.8)α, 2.55 (dddd, 13.7, 13.5, 5.4, 1.5)*β*	2.35 (ddd, 13.2, 4.3, 2.8)α, 2.46 (dddd, 13.5, 13.5, 5.2, 1.5)*β*
7	1.04 (m)α, 1.84 (m)*β*	1.02 (m)α, 1.94 (m)*β*	1.04 (m)α, 1.94 (m)*β*	1.04 (m)α, 1.96 (m)*β*	1.05 (m)α, 1.94 (m)*β*	1.04 (m)α, 2.04 (m)*β*	1.02 (m)α, 1.94 (m)*β*
8	1.60 (m)	1.60 (m)	1.62 (m)	1.62 (m)	1.60 (m)	2.02 (m)	1.62 (m)
9	1.04 (m)	1.06 (m)	1.04 (m)	1.04 (m)	1.08 (m)	1.04(m)	1.04 (m)
10							
11	1.66 (m)	1.68 (m)	1.68 (m)	1.68 (m)	1.68 (m)	4.34 (bs)	1.68 (m)
12	1.14 (m)α, 2.02 (m)*β*	1.15 (m)α, 2.04 (ddd, 12.8, 3.6, 3.3)*β*	1.18 (m)α, 2.02 (m)*β*	1.20 (m)α, 2.13 (ddd, 12.8, 3.6, 3.3)*β*	1.18 (m)α, 2.00 (ddd, 12.9, 3.6, 3.2)*β*	1.44 (m)α, 2.08 (m)*β*	1.20 (m)α, 2.13 (ddd, 12.9, 3.7, 3.3)*β*
13							
14	0.98 (m)	1.00 (m)	1.00 (m)	1.00 (m)	1.00 (m)	0.96 (m)	0.98 (m)
15	1.16 (m), 1.58 (m)	1.15 (m), 1.56 (m)	1.18 (m), 1.62 (m)	1.20 (m), 1.64 (m)	1.10 (m), 1.56 (m)	1.15 (m), 1.58 (m)	1.14 (m), 1.64 (m)
16	1.24 (m), 1.82 (m)	1.26 (m), 1.84 (m)	1.62 (m), 1.80 (m)	1.77 (m), 1.68 (m)	1.25 (m), 1.63 (m)	1.28 (m), 1.64 (m)	1.64 (m)
17	1.08 (m)	1.10 (m)	1.14 (m)	1.47 (m)	1.15 (m)	1.08 (m)	1.50 (dd, 9.5, 9.5)
18	0.73 (s)	0.72 (s)	0.75 (s)	0.93 (s)	0.75 (s)	0.97 (s)	0.87 (s)
19	1.22 (s)	1.23 (s)	1.23 (s)	1.23 (s)	1.23 (s)	1.45 (s)	1.22 (s)
20	1.38 (m)	1.42 (m)	1.88 (m)		2.06 (m)	1.98 (m)	
21	0.89 (d, 6.4)	0.95 (d, 6.4)	0.97 (d, 6.8)	1.30 (s)	1.00 (d, 6.6)	0.99 (d, 6.7)	1.30 (s)
22	0.98 (m), 1.32 (m)	1.15 (m), 1.52 (m)	4.97 (dt, 10.5, 3.1)	1.54 (m)	5.34 (dd, 15.3, 8.2)	5.13 (ddd, 15.2, 8.3, 1.3)	5.51 (d, 15.7)
23	1.16 (m), 1.32 (m)	2.08 (m), 2.20 (m)	2.25 (bdd, 14.0, 7.0), 2.37 (m)	2.01 (m)	5.43 (dd, 15.3, 7.4)	5.27 (dd, 15.2, 6.4)	5.48 (dd, 15.7, 5.5)
24	1.38 (m), 1.58 (m)	6.74 (tq, 7.7, 1.3)	6.68 (bt, 7.5)				
25	2.42 (m)			2.23 (hept, 6.9)	3.06 (quint, 7.1)	2.18 (heptd, 6.7, 5.8)	2.27 (heptd, 6.8, 5.5)
26				1.03 (d, 6.9)		0.93 (d, 6.7)	0.98 (d, 6.8)
27	1.14 (d, 6.8)	1.83 (bs)	1.85 (bs)	1.03 (d, 6.9)	1.22 (d, 7.0)	0.93 (d, 6.7)	0.98 (d, 6.8)
28				4.74 (bs), 4.67 (d,1.3)			
OMe	3.64 (s)	3.73 (s)	3.73 (s)		3.67 (s)		
OAc			2.02 (s)				

[a] Spectra recorded in $CDCl_3$, *J* in Hz. Assignments aided by COSY, HSQC, HMBC, and NOESY experiments. [b] Spectra recorded at 400 MHz. [c] Spectra recorded at 600 MHz.

IR spectra were recorded on a Perkin-Elmer FT-IR System Spectrum BX spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 400 or on a Varian INOVA 600 spectrometer using $CDCl_3$ as solvent. Proton chemical shifts were referenced to the residual $CHCl_3$ signal at δ 7.26. ^{13}C NMR spectra were referenced to the central peak of $CDCl_3$ at δ 77.0. COSY, HSQC, HMBC, and NOESY experiments were performed using standard Varian pulse sequences. Low-resolution mass spectra were recorded on a Finnigan Voyager GC8000^{top} spectrometer. High-resolution mass spectra (HRMS) were obtained on a Autospec-Q spectrometer. Column chromatography was carried out on Merck silica gel 60 (70−230 mesh). HPLC separations were performed on a LaChrom-Hitachi apparatus equipped with LiChrospher Si-60 (Merck) columns in normal-phase mode and LiChrosorb RP-18 (Merck) columns in reversed-phase mode using a differential refractometer RI-71. All solvents were spectral grade or were distilled prior to use.

Collection and Identification. Specimens of *Anthomastus bathyproctus* Bayer 1993 were collected on the *R/V Polarstern* cruises ANT XVII/3 (EASIZ-III, Ecology of Antarctic Sea Ice Zone, March 17 to May 11, 2000) sponsored by the Alfred Wegener Institut für Polar und Meeresforschung (Bremerhaven) under the auspices of the Scientific Committee for Antarctic Research (SCAR). All the material of *A. bathyproctus* was collected using a bottom trawl at Deception Island (South Shetland Islands, Antarctica) in May 2000. The colonies were sorted on board and washed in running sea water to avoid external contamination from other organisms collected in the same haul, and immediatly frozen at −27 °C. Taxonomy: phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonacea, family Alcyoniidae. A voucher specimen is stored at the collection of the BEIM group (Biodiversity and Ecology of Marine Invertebrates), University of Seville (Spain), under the code BEIM-ANT-426.

Extraction and Isolation. Frozen specimens of *A. bathyproctus* (107.5 g) were chopped and extracted with 2 L of acetone/MeOH (1:1) at room temperature. The solution was filtered and evaporated under reduced pressure to obtain an aqueous residue that was partitioned between H_2O and Et_2O. The organic layer was evaporated to dryness to give an orange oil (3.1 g), which was chromatographed on a SiO_2 column using solvents of increasing polarities from hexane to Et_2O, then $CHCl_3$/MeOH (8:2), and finally MeOH. The fraction eluted with hexane/Et_2O (1:1) was subjected to normal-phase HPLC using hexane/EtOAc (85:15) to yield compound **1** (3.2 mg, 0.003% dry wt) and a mixture that was further separated by reversed-phase HPLC using MeOH/H_2O (92:8) to yield compounds **5** (4.3 mg, 0.004% dry wt) and **2** (2.3 mg, 0.002% dry wt). The fraction of the general chromatography eluted with hexane/Et_2O (4:6) was subjected to HPLC with hexane/EtOAc (75:25), yielding compound **6** (2.6 mg, 0.002% dry wt). Finally, the fraction of the general chromatography eluted with hexane/Et_2O (3:7) was subjected to HPLC using hexane/EtOAc (7:3) to give compounds **4** (2.4 mg, 0.002% dry wt), **7** (1.2 mg, 0.001% dry wt), and **3** (2.3 mg, 0.002% dry wt). Final purification of compounds **4**, **7**, and **3** was performed by reversed-phase HPLC eluting with MeOH/H_2O (8:2).

Methyl 3-oxocholesta-1,4-dien-26-oate (1): white amorphous powder; $[\alpha]^{25}_D$ −8.8 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 244 (3.96) nm; IR (film) ν_{max} 2933, 2854, 1738, 1663, 1463, 1377 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$,100 MHz), see Table 2; EIMS *m/z* 426 (8) $[M]^+$, 122 (36), 88 (100); HRCIMS *m/z* 427.3192 $[M + H]^+$ (calcd for $C_{28}H_{43}O_3$, 427.3212).

Methyl (24*E*)-3-oxocholesta-1,4,24-trien-26-oate (2): white amorphous powder; $[\alpha]^{25}_D$ +8.4 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 223 (4.11), 238 (4.02) nm; IR (film) ν_{max} 2936, 2854, 1715, 1663, 1437, 1268 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m/z* 424 (18) $[M]^+$, 392 (9), 269 (18), 122 (100); HRCIMS *m/z* 425.3060 $[M + H]^+$ (calcd for $C_{28}H_{41}O_3$,425.3056).

Methyl (22*R*,24*E*)-22-acetoxy-3-oxocholesta-1,4,24-trien-26-oate (3): white amorphous powder; $[\alpha]^{25}_D$ −4.9 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 221 (4.25), 238 (4.19) nm; IR (film) ν_{max} 2938, 1715, 1662, 1240 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m/z* 482 (14) $[M]^+$, 422 (8), 390 (12), 269 (26), 121 (100); HRCIMS *m/z* 483.3084 $[M + H]^+$ (calcd for $C_{30}H_{43}O_5$, 483.3110).

Table 2. ^{13}C NMR Data for Compounds **1**−**7**[a]

atom no.	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[c]
1	156.0	155.9	155.8	155.9	156.0	156.3	155.9
2	127.4	127.5	127.5	127.5	127.4	127.8	127.5
3	186.4	186.4	186.4	186.4	186.4	186.6	186.4
4	123.8	123.8	123.9	123.8	123.8	122.3	123.8
5	169.4	169.4	169.2	169.3	169.4	170.3	169.3
6	32.9	32.9	32.9	32.9	32.9	32.1	32.9
7	33.7	33.7	33.6	33.6	33.6	34.0	33.5
8	35.5[e]	35.5[d]	35.5	34.9	35.5	31.1[d]	34.9
9	52.4	52.3	52.3	52.3	52.4	55.8	52.4
10	43.6	43.6	43.6	43.6	43.6	44.1	43.6
11	22.9	22.8	22.8	22.7	22.8	70.6	22.7
12	39.4[d]	39.5	39.5	39.9	39.3	49.9	39.9
13	42.7	42.7	43.1	43.0	42.7[d]	41.7	43.1
14	55.4	55.4	55.1	55.6	55.5[e]	56.8[e]	55.5
15	24.4	24.4	24.4	23.9	24.3	24.3	23.9
16	28.1	28.1	27.0	22.3	28.2	27.9	23.1
17	56.0	55.9	53.1	57.9	55.6[e]	56.5[e]	59.9
18	12.0	12.1	12.1	13.7	12.2	14.8	14.0
19	18.7	18.7	18.7	18.7	18.7	21.1	18.7
20	35.6[e]	35.6[d]	39.0	75.0	39.8	39.8	75.0
21	18.5	18.4	13.0	26.2	20.2	20.7	29.2
22	35.6[e]	34.6	75.4	42.4	138.0	133.0	134.7
23	23.7	25.4	27.0	28.9	126.5	135.3	133.4
24	34.1	143.0	138.3	156.2			
25	39.5[d]	127.2	129.5	33.9	42.6[d]	30.9[d]	30.7
26	177.4	168.8	168.3	21.9	175.6	22.7	22.5[d]
27	17.0	12.3	12.6	21.9	17.7	22.7	22.4[d]
28				106.4			
O*CH*$_3$	51.5	51.8	51.8		51.7		
OCO*CH*$_3$			21.3				
O*CO*CH$_3$			170.6				

[a] Spectra recorded in $CDCl_3$. Assignments aided by HSQC and HMBC experiments. [b] Spectra recorded at 100 MHz. [c] Spectra recorded at 150 MHz. [d,e] Values with the same superscript in the same column are interchangeable.

(20*S*)-20-Hydroxyergosta-1,4,24(28)-trien-3-one (4): white amorphous powder; $[\alpha]^{25}_D$ +5.6 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 246 (4.03) nm; IR (film) ν_{max} 3420, 2932, 2853, 1661, 1620, 888 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m/z* 411 (1) $[M + H]^+$, 392 (2), 83 (100); HRCIMS *m/z* 411.3273 $[M + H]^+$ (calcd for $C_{28}H_{43}O_2$, 411.3263).

Methyl (22*E*)-3-oxo-24-norcholesta-1,4,22-trien-26-oate (5): white amorphous powder; $[\alpha]^{25}_D$ +8.5 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 245 (4.01) nm; IR (film) ν_{max} 2937, 2869, 1738, 1665 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m/z* 410 (9) $[M]^+$, 351 (4), 269 (17), 122 (100); HRCIMS *m/z* 411.2891 $[M + H]^+$ (calcd for $C_{27}H_{39}O_3$, 411.2899).

(22*E*)-11*β*-Hydroxy-24-norcholesta-1,4,22-trien-3-one (6): white amorphous powder; $[\alpha]^{25}_D$ +4.0 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 241 (4.05) nm; IR (film) ν_{max} 3390, 2934, 2867, 1658, 1615 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; EIMS *m/z* 382 (7) $[M]^+$, 267 (17), 261 (44), 147 (52), 122 (100); HRCIMS *m/z* 383.2940 $[M + H]^+$ (calcd for $C_{26}H_{39}O_2$, 383.2950).

(20*S*,22*E*)-20-Hydroxy-24-norcholesta-1,4,22-trien-3-one (7): white amorphous powder; $[\alpha]^{25}_D$ +11.1 (*c* 0.09, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 245 (4.19) nm; IR (film) ν_{max} 3443, 2935, 2865, 1661, 1621, 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2; EIMS *m/z* 383 (5) $[M + H]^+$, 364 (5), 270 (64), 122 (100); HRCIMS *m/z* 383.2943 $[M + H]^+$ (calcd for $C_{26}H_{39}O_2$, 383.2950).

Cytotoxicity Assays. Compounds **1**−**7** were tested against the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A-549 (lung carcinoma), and HT-29 (colon adenocarcinoma). Cytotoxicity assays were performed by PharmaMar.

A colorimetric type of assay using sulforhodamine B (SRB) reaction has been adapted for a quantitative measurement of cell growth and viability following the method described in the literature.[11]

Acknowledgment. This research was supported by grants from MCYT (research project PPQ2003-02928) and from Junta de Andalucía (FQM-285). Cytotoxicity assays were performed through a cooperation agreement with PharmaMar. Field work was supported by a grant from CICYT (research project ANT99-1608-E). P.J.L.-G. acknowledges the valuable assistance of the officers and crew of the *R*/*V Polarstern*, the cruise leader, and steering committee on board during the EASIZ-III cruise. G.G.M. acknowledges a fellowship from Junta de Andalucía (ATT-04-2003).

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**−**7**. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2005**, *22*, 15−61, and previous reviews of this series. (b) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19,* 1−48, and previous reviews of this series.

(2) (a) Gavagnin, M.; Mollo, E.; Castelluccio, F.; Crispino, A.; Cimino, G. *J. Nat. Prod.* **2003**, *66,* 1517−1519. (b) Ike, K. B.; Baker, B. *J. Nat. Prod.* **2003**, *66*, 888−890. (c) Rodríguez Brasco M. F.; Seldes, A. M.; Palermo, J. A. *Org. Lett.* **2001**, *3*, 1415−1417. (d) Palermo, J. A., Rodríguez Brasco, M. F.; Spagnuolo, C.; Seldes, A. M. *J. Org. Chem.* **2000**, *65,* 4482−4486.

(3) Mellado, G. G.; Zubía, E.; Ortega, M. J.; López-González, P. J. *Steroids* **2004**, *69*, 291−299.

(4) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*, 3rd ed.; VCH: Weinheim, 1990; p 346.

(5) (a) Goad, L. J.; Akihisa, T. *Analysis of Sterols*; Blackie Academic & Professional: London, 1997; pp 1−42. (b) Hall, L. D.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1980**, *102*, 5703−5711. (c) Nes, W. R.; Varkey, T. E.; Krevitz, K. *J. Am. Chem. Soc.* **1977**, *99*, 260−262.

(6) Rho, J.-R.; Lee, H.-S.; Seo, Y.; Cho, K. W.; Shin, J. *Bull. Korean Chem. Soc.* **2000**, *21*, 518−520.

(7) Finamore, E.; Minale, L.; Riccio, R.; Rinaldo, G.; Zollo, F. *J. Org. Chem.* **1991**, *56*, 1146−1153.

(8) Letourneux, Y.; Khuong-Huu, Q.; Gut, M.; Lukacs, G. *J. Org. Chem.* **1975**, *40*, 1674−1675.

(9) Kluge, A. F.; Maddox, M. L.; Partridge, L. G. *J. Org. Chem.* **1985**, *50*, 2359−2365.

(10) (a) Garrido, L.; Zubía, E.; Ortega, M. J.; Salvá, J. *Steroids* **2000**, *65*, 85−88. (b) Aiello, A.; Fattorusso, E.; Menna, M. *Steroids* **1991**, *56*, 513−517.

(11) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

NP050080Q