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STEROIDAL KETONES FROM THE SPONGE *GEODIA CYDONIUM*

ANNA MIGLIUOLO, VINCENZO PICCIALLI, and DONATO SICA*

Dipartimento di Chimica Organica e Biologica, Università di Napoli, via Mezzocannone 16, 80134 Napoli, Italy

ABSTRACT.—Four 4-ene-3,6-diketosteroids **1–4** have been isolated from the sponge *Geodia cydonium* along with 4-ene-3-ketosteroids **5–8**. The structure of these compounds was deduced by analysis of spectral data. Partial synthesis of compound **1** confirmed the structure assignment of 4-ene-3,6-diketones.

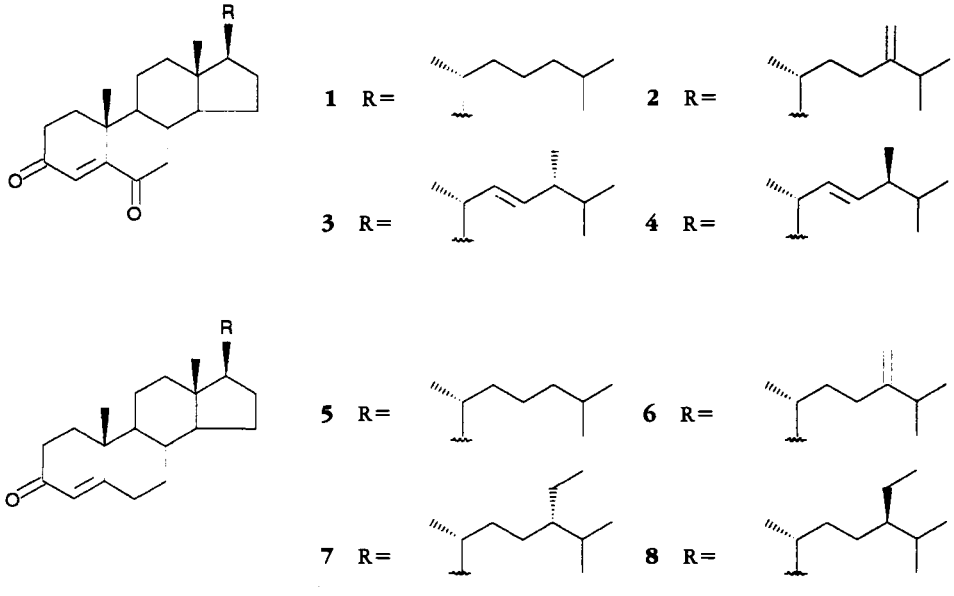
As part of our continuing research on polyoxygenated steroids from sponges (1–6) we now report the isolation and the characterization of steroidal 4-ene-3,6-diketones **1–4** obtained from the marine sponge *Geodia cydonium* (Jameson) (order Choristida,

rather common metabolites of biological importance in marine organisms (7–13).

RESULTS AND DISCUSSION

The Et_2O-soluble material from the Me_2CO and $CHCl_3$-MeOH (1:1) extracts of the sponge was chromatographed on a Si gel column. The fractions eluted with $CHCl_3$ slightly before sterols were further separated by normal phase hplc on Si gel into the 4-ene-3,6-diketo- and 4-ene-3-keto-steroid fractions. Final separation of the above frac-

4-ENE-3,6-DIKETOSTEROIDS.—Steroid **1** had the molecular formula $C_{27}H_{42}O_2$ deduced by hrms. The mass spectrum contained the molecular ion at *m/z* 398 with significant fragment ions at *m/z* 285 $[M - C_8H_{17}]^+$ and 243 $[M - 42 - C_8H_{17}]^+$ indicating the presence of a C_8H_{17} saturated side chain. The ^{1}H-nmr spectrum contained a signal at δ 6.17 for a trisubstituted double bond (bs, H-4) and five methyl resonances of a cholestane structure: singlets at δ 0.72 and 1.17 (H_3-18 and H_3-19, respectively) and doublets at δ 0.93 (H_3-21) and 0.87 (H_3-26 and H_3-27). The ^{13}C-nmr spectrum confirmed the presence of a carbon-carbon double bond (δ 160.8, 125.2) and indicated the presence of two conjugated carbonyl functions (δ 199.1, 201.9). In the ir spectrum



only one band at 1686 cm^{-1} was present for the carbonyl groups. Strong peaks at *m/z* 136, 137 (fission of bonds 6–7 and 9–10) and 259 (fission of bonds 7–8 and 9–10) in the mass spectrum of **1** suggested that it was a 4-ene-3,6-diketone (14,15). This was supported by the uv spectrum, which showed absorption at λ max 250 nm (16), and by ^{1}H-nmr experiments, which facilitated the identification of the structural fragment (from C-1 to C-7) containing the enedione moiety. Two deshielded resonances at δ 2.54 (ddd) and 2.45 (bddd) were assigned to protons attached to a methylene carbon (C-2) α to the unsaturated carbonyl-containing function. Support for this connectivity came from the observation of a long range coupling between the proton at δ 2.45 and the olefinic proton at δ 6.17. The multiplicities of the resonances at δ 2.54 and 2.45 required that these protons have two vicinal neighbors (H_2-1). Indeed double resonance experiments demonstrated that they each were coupled with two protons resonating at δ 2.15 and 1.91, which showed to be coupled with each other and with no other protons. Resonances at δ 2.68 and 2.04 (both double doublets) were assigned to the protons of a methylene group (CH_2-7) attached to the other side of the enedione portion. The multiplicities of these signals required that they have only one vicinal neighbor (H-8). The ^{13}C-nmr spectrum of **1** showed that the side chain of this steroid was of the cholestane type (17). All the above observations were consistent with the cholest-4-ene-3,6-dione structure **1** for this compound.

Compound **1** has not been found as a naturally occurring steroid but has been previously synthesized (18). An authentic specimen prepared from cholesterol according to a procedure described in the literature (18) was identical in all respects with the naturally occurring steroid **1**.

The presence of the *m/z* 285 (loss of the side chain), 243 $[M-42-\text{side chain}]^+$, 136, and 137 peaks in the mass spectra of all four enediones **1–4** indicated that they possessed a common 4-ene-3,6-dione structure in their steroidal skeleton and differed only in the side chain. This was confirmed by their ^{1}H-nmr spectra that showed identical chemical shift values for the H_2-2, H-4, H_2-7, H_3-18, and H_3-19 resonances.

Steroid **2** had the molecular formula $C_{28}H_{42}O_2$ deduced by hrms. The ions at *m/z* 285 $[M-C_9H_{17}]^+$ and 243 $[M-42-C_9H_{17}]^+$ established the presence of a C_9H_{17} monounsaturated side chain. The ^{1}H-nmr spectrum displayed methyl doublets at δ 1.03 and 1.02 (H_3-26 and H_3-27) and 0.97 (H_3-21) and two broad singlets for a terminal methylene group at δ 4.72 and 4.66 (H_2-28) also evident in the ^{13}C-nmr spectrum (δ 106.1 and 156.6), suggesting that this sterol was the previously described 24-methylenecholest-4-ene-3,6-dione (19).

Compound **3** had the molecular formula $C_{28}H_{42}O_2$ (from hrms). Ions at *m/z* 285 $[M-C_9H_{17}]^+$ and 243 $[M-42-C_9H_{17}]^+$ indicated for the new compound a side chain having one degree of unsaturation and a C_9H_{17} composition. The ^{1}H-nmr chemical shifts for the side chain protons of this ketosteroid are consistent with those of an authentic sample of brassicasterol. Evidence supporting the above observation was obtained from spin decoupling experiments. Irradiation of the allylic proton at δ 2.01 (m, H-20) collapsed the double doublet at δ 5.13 (H-22) to a doublet (J = 15.4 Hz) and the H_3-21 methyl doublet at δ 1.029 to a singlet. The H-22 proton at δ 5.13 was also coupled to the olefinic double doublet at δ 5.20 (H-23). Irradiation of the allylic proton at δ 1.45 (H-25) collapsed the two methyl doublets centered at δ 0.83 and 0.81 (H_3-26 and H_3-27) to singlets, showing the presence of one isopropyl group. These data, in conjunction with the presence of a further methyl resonance at δ 0.91 (H_3-28), established a (22*E*,24*R*)-24-methylcholesta-4,22-diene-3,6-dione structure **3** for this steroid. The configuration of the Δ^{22} double bond was established to be *E* on the basis of the value (15.4 Hz) of the H-22–H-23 coupling constant.

Compound **4** had molecular formula $C_{28}H_{42}O_2$. The close similarity of the mass

and ^{1}H-nmr spectra of compounds **4** and **3** suggested that the two steroids must be C-24 epimers. The H_3-21 doublet in the ^{1}H-nmr spectrum of **4** was shifted upfield (δ 1.019) (20) when compared to the corresponding H_3-21 signal (δ 1.029) for the steroid **3**; hence **4** must be formulated as (22*E*,24*S*)-24-methylcholesta-4,22-diene-3,6-dione.

4-ENE-3-KETOSTEROIDS.—The mass spectra of steroids **5–8** exhibited intense peaks at *m/z* 229 $[M-42-\text{side chain}]^+$ and 124 (fission of the 6–7 and 9–10 allylic bonds) characteristic of Δ^4-3-ketosteroids (15,21). The presence of *m/z* 271 $[M-\text{side chain}]^+$ and 229 $[M-42-\text{side chain}]^+$ peaks in the mass spectra of all four steroids indicated that they possessed identical nuclei, with the structural difference between these compounds being confined to the nature of the side chains. This was confirmed by their ^{1}H-nmr spectra that showed identical chemical shift values for the H-4, H_3-18, and H_3-19 protons.

Steroids **5** and **6** were shown to be identical with the earlier reported 4-ene-3-ketosteroids (9).

Steroids **7** and **8** could not be separated by reversed-phase hplc. They each had the molecular formula $C_{29}H_{48}O$ and a $C_{10}H_{21}$-saturated side chain. Their ^{1}H-nmr spectrum was composed of signals from the two epimers at C-24. The side chain methyl signals for both isomers were assigned by comparison with those of authentic samples of sitosterol and clionasterol.

Mixtures of C-24 epimeric 24-ethylcholest-4-en-3-ones have previously been isolated from marine organisms (11).

Compound **6** is the major component of the 4-ene-3-ketosteroid mixture.

From a biosynthetic point of view it seems probable that 4-ene-3,6-diketosteroids **1–4** could derive from the corresponding 4-ene-3-ketosteroids which, in turn, are likely biogenetically related to the Δ^5-3β-monohydroxysterols present in the sponge (22).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on a WM-400 spectrometer in $CDCl_3$ solutions. ^{1}H chemical shifts were refereenced to the residual $CHCl_3$ signal (7.26 ppm). ^{13}C chemical shifts were referenced to $CDCl_3$ (77.0 ppm). Low resolution mass spectra were determined at 70 eV with an AEI MS 30 mass spectrometer. High resoluton mass spectra were recorded on a Kratos MS 50 spectrometer. Ft-ir spectra were obtained with a Perkin-Elmer 1760-X FT-ir. Uv spectra were recorded using a Perkin-Elmer Model 550S spectrophotometer and MeOH solvent. Hplc was carried out on a Varian 2510 pump and a Waters Associates R403 differential refractometer. Melting points were determined on a Kofler apparatus and are uncorrected.

EXTRACTION AND ISOLATION.—The sponge *G. cydonium*, identified by Dr. G. Corriero, University of Genova, was collected by hand at depths of 10 to 15 m near Bacoli in the Bay of Naples in September 1989. A voucher specimen is on file at our laboratories. The freshly collected sponge (202 g dry wt after extraction) was extracted one time with Me_2CO and two times with $CHCl_3$-MeOH (1:1). Removal of the solvents under reduced pressure left an aqueous suspension which was extracted with Et_2O. Evaporation of the Et_2O layer afforded 2.04 g of crude extract which was fractionated on an open Si gel column (200 g, 3 cm diameter) using $CHCl_3$ as eluent; 100-ml fractions were collected. Fractions 9–11 (one spot on tlc), eluted slightly before monohydroxylated sterols, contained 4-en-3-one and 4-ene-3,6-dione steroids that were separated by normal phase hplc on a Si gel column (Hibar LiChrosorb Si-60, 250 × 4 mm) using hexane-EtOAc (9:1) as the mobile phase. The more polar fraction obtained from this separation, containing 4-ene-3,6-dione steroids (4.5 mg), was fractionated by reversed-phase hplc on a Hibar Superspher RP-18 (250 × 4 mm) column eluted with MeCN to give pure **1** (2.2 mg), **2** (1.0 mg), **3** (0.4 mg), and **4** (0.7 mg). The fraction containing 4-en-3-one steroids (7 mg) was subjected to reversed-phase hplc on the above column using MeOH as eluent to give steroids **5** (2.0 mg), **6** (3.5 mg), and **7** and **8** (together 1.0 mg), which were identified from their ^{1}H-nmr and mass spectral data.

SPECTRAL DATA.—*Cholest-4-ene-3,6-dione* [**1**].—Mp 123–125° (MeOH) [lit. (18) 124–125°]; ir ($CHCl_3$) ν max 1686 cm^{-1}; uv λ max (MeOH) 250 nm (ε = 12300); ^{1}H nmr ($CDCl_3$, 400 MHz) δ 6.17

(1H, bs, 4-H), 2.68 (1H, dd, J = 14.6 and 3.7 Hz, Ha-7), 2.54 (1H, ddd, J = 17.5, 17.5 and 5.1 Hz, Ha-2), 2.45 (1H, bddd, J = 17.5, 5.1, and 3.2 Hz, Hb-2), 2.15 (1H, m, partially overlapped to other signals, Ha-1), 2.04 (1H, dd, J = 14.6 and 13.0 Hz, Hb-7), 1.91 (m, overlapped to other signals, Hb-1), 1.17 (3H, s, H_3-19), 0.93 (3H, d, J = 6.6 Hz, H_3-21), 0.87 (6H, d, J = 6.7 Hz, H_3-26 and H_3-27), 0.72 (3H, s, H_3-18); ^{13}C nmr ($CDCl_3$, 100.1 MHz) δ 35.5 (C-1), 33.8 (C-2), 199.1 (C-3 or C-6), 125.2 (C-4), 160.8 (C-5), 201.9 (C-6 or C-3), 46.6 (C-7), 34.0 (C-8), 50.8 (C-9), 39.0 (C-10), 20.7 (C-11), 39.3 (C-12), 42.3 (C-13), 55.8 (C-14), 23.6 (C-15 or C-23), 27.8 (C-16), 56.4 (C-17), 11.7 (C-18), 17.3 (C-19), 35.3 (C-20), 18.5 (C-21), 35.9 (C-22), 23.8 (C-23 or C-15), 39.6 (C-24), 27.8 (C-25), 22.4 (C-26 or C-27), 22.7 (C-27 or C-26) [carbon multiplicities were determined through DEPT experiments; nuclear carbon assignments were facilitated using cholest-4-en-3-one (17) as a model compound]; lreims *m/z* (rel. int.) $[M]^+$ 398 (25), $[M-Me]^+$ 383 (9), $[M-CH_2CO]^+$ 356 (9), [M − side chain]$^+$ 285 (16), [M − side chain − 28]$^+$ 257 (12), [rings C and D, and side chain]$^+$ 247 (21), [M − 42 − side chain]$^+$ 243 (42), $[C_8H_9O_2]^+$ 137 (100), $[C_8H_8O_2]^+$ 136 (55); hreims *m/z* 398.3170 ($C_{27}H_{42}O_2$ requires 398.3185).

24-Methylenecholest-4-ene-3,6-dione [**2**].—Mp 130–132° (MeOH); ir ν max 1686 cm^{-1}; uv λ max (MeOH) 250 nm (ε = 12300); ^{1}H nmr ($CDCl_3$, 400 MHz) δ 6.17 (1H, bs, H-4), 4.72 (1H, bs, Ha-28), 4.66 (1H, bs, Hb-28), 2.68 (1H, dd, J = 14.6 and 3.7 Hz, Ha-7), 2.54 (1H, ddd, J = 17.7, 17.7, and 5.1 Hz, Ha-2), 2.45 (1H, bddd, J = 17.7, 5.1, and 3.2 Hz, Hb-2), 2.23 (1H, septet, J = 6.7 Hz, H-25), 2.15 (1H, m, partially overlapped to other signals, Ha-1), 2.04 (1H, dd, J = 14.5 and 12.8 Hz, Hb-7), 1.91 (m, overlapped to other signals, Hb-1), 1.16 (3H, s, H_3-19), 1.03 and 1.02 (3H each, d's, both J = 6.7 Hz, H_3-26 and H_3-27), 0.97 (3H, d, J = 6.7 Hz, H_3-21), 0.72 (3H, s, H_3-18); ^{13}C nmr ($CDCl_3$, 100.1 MHz) δ 35.5 (C-1), 33.8 (C-2), 199.5 (C-3 or C-6), 125.5 (C-4), 161.0 (C-5), 202.4 (C-6 or C-3), 46.8 (C-7), 34.0 (C-8), 50.9 (C-9), 39.1 (C-10), 20.8 (C-11), 39.8 (C-12), 42.5 (C-13), 55.8 (C-14), 23.9 (C-15), 28.0 (C-16), 56.5 (C-17), 11.9 (C-18), 17.5 (C-19), 35.6 (C-20), 18.6 (C-21), 34.5 (C-22), 30.9 (C-23), 156.6 (C-24), 34.2 (C-25), 21.8 (C-26 or C-27), 22.0 (C-27 or C-26), 106.1 (C-28); lreims *m/z* (rel. int.) $[M]^+$ 410 (6), $[M-Me]^+$ 395 (6), $[M-CH_2CO]^+$ 368 (3), $[M-C_6H_{12}]^+$ 326 (48), $[M-C_6H_{12}-CH_3]^+$ 311 (36), 285 (9), [M − side chain − 2H]$^+$ 283 (31), [rings C and D, and side chain]$^+$ 259 (31), 257 (62), 243 (31), 137 (100), 136 (22); hreims *m/z* 410.3170 ($C_{28}H_{42}O_2$ requires 410.3185).

(22E,24R)-24-Methylcholesta-4,22-diene-3,6-dione [**3**].—Ir ($CHCl_3$) ν max 1686 cm^{-1}; uv λ max 250 nm (ε = 12300); ^{1}H nmr ($CDCl_3$, 400 MHz) δ 6.17 (1H, bs, H-4), 5.20 (1H, dd, J = 15.4 and 6.8 Hz, H-23), 5.13 (1H, dd, J = 15.4 and 7.5 Hz, H-22), 2.67 (1H, dd, J = 14.5 and 3.6 Hz, Ha-7), 2.54 (1H, ddd, J = 17.5, 17.5, and 5.1 Hz, Ha-2), 2.45 (1H, bddd, J = 17.5, 5.1, and 3.2 Hz, Hb-2), 2.15 (1H, m, overlapped to other signals, Ha-1), 2.04 (1H, dd, J = 14.5 and 12.8 Hz, Hb-7), 1.91 (m, overlapped to other signals, Hb-1), 1.17 (3H, s, H_3-19), 1.029 (3H, d, J = 6.7 Hz, H_3-21), 0.91 (3H, d, J = 6.7 Hz, H_3-28), 0.83 and 0.81 (3H each, d's, both J = 6.7 Hz, H_3-26 and H_3-27), 0.72 (3H, s, H_3-18); lreims *m/z* (rel. int.) $[M]^+$ 410 (20), 395 (4), 368 (6), $[M-C_3H_7]^+$ 367 (16), 285 (75), 283 (40), 259 (15), 257 (31), 243 (32), 137 (100), 136 (20); hreims *m/z* 410.3199 ($C_{28}H_{42}O_2$ requires 410.3185).

(22E,24S)-24-Methylcholesta-4,22-diene-3,6-dione [**4**].—Ir ($CHCl_3$) ν max 1686 cm^{-1}; uv λ max 250 nm (ε = 12300); ^{1}H nmr ($CDCl_3$, 400 MHz) δ 6.17 (1H, bs, H-4), 5.20 (1H, dd, J = 15.4 and 6.8 Hz, H-23), 5.13 (1H, dd, J = 15.4 and 7.5 Hz, H-22), 2.67 (1H, dd, J = 14.5 and 3.6 Hz, Ha-7), 2.54 (1H, ddd, J = 17.5, 17.5, and 5.1 Hz, Ha-2), 2.45 (1H, bddd, J = 17.5, 5.1, and 3.2 Hz, Hb-2), 2.15 (1H, m, overlapped to other signals, Ha-1), 2.04 (1H, dd, J = 14.5 and 12.8 Hz, Hb-7), 1.91 (m, overlapped to other signals, Hb-1), 1.17 (3H, s, H_3-19), 1.019 (3H, d, J = 6.7 Hz, H_3-21), 0.91 (3H, d, J = 6.7 Hz, H_3-28), 0.83 and 0.81 (3H each, d's, both J = 6.7 Hz, H_3-26 and H_3-27), 0.72 (3H, s, H_3-18); lreims *m/z* $[M]^+$ 410 (11), 395 (2), 368 (10), 367 (10), 285 (25), 283 (21), 259 (7), 257 (16), 243 (15), 137 (100), 136 (22); hreims *m/z* 410.3176 ($C_{28}H_{42}O_2$ requires 410.3185).

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