*Journal of Natural Products*
*Vol. 52, No. 6, pp. 1331-1333, Nov-Dec 1989*



# A NOVEL CONJUGATED KETOSTEROID FROM THE MARINE SPONGE *DICTYONELLA INCISA*

PATRIZIA CIMINIELLO, ERNESTO FATTORUSSO,* SILVANA MAGNO, ALFONSO MANGONI,

*Dipartimento di Chimica delle Sostanze Naturali, Via Montesano 49, 80131 Napoli, Italy*

and MAURIZIO PANSINI

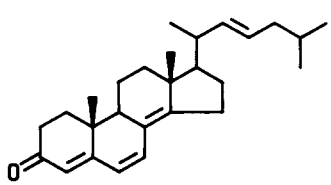
*Istituto di Zoologia, Via Balbi 5, 16126 Genova, Italy*

ABSTRACT.—The structure of (22*E*)-cholesta-4,6,8(14),22-tetraen-3-one [**1**], isolated from the Demospongia *Dictyonella incisa*, has been elucidated by spectroscopic analyses ($^1$H-nmr, uv, ir, and ms), and confirmed via synthesis.

Many new sterols have been isolated from marine organisms in the last two decades. Commonly encountered features in these compounds include additional oxygenation of both the nucleus and the side chain, and also side chains extensively modified by alkylation and degradation (1). Our continuing investigation on metabolites from marine invertebrates has resulted in the isolation of a new ketosteroid **1**, characterized by the highly unsaturated 4,6,8(14),22-tetraen-3-one functionality.

Compound **1** has been isolated from the Mediterranean sponge *Dictyonella incisa* Schmidt (2). The sponge is cushion-shaped to massive, up to about 10 cm in diameter, fleshy, rather soft, with an uneven, grooved surface. This species, of the family Hymeniacidonidae (Porifera, Demospongiae), has been found till now only in the Mediterranean Sea between 2 and 50 m depth.

The difficulties associated with the collection of sufficient quantities of this material, together with the low concentration of **1** in the sponge, allowed us to isolate only minute amounts of this compound; therefore its structure elucidation has been based upon spectroscopic data (uv, ir, ms, and $^1$H nmr). Synthesis of **1** by oxidation of cholesta-5,7,22-trien-3β-ol confirmed the proposed structure.



**1**

The sponge was extracted with MeOH; the $Et_2O$-soluble material from the MeOH extract after repeated chromatographies on $SiO_2$ and RP-18 gave 2 mg of the pure compound **1**. The hrms of **1** displayed a molecular ion at *m/z* 378.2927, corresponding to the molecular formula $C_{27}H_{38}O$, which requires 9 unsaturations. The 250 MHz $^1$H-nmr spectrum showed a series of methyl resonances at δ 1.05 (3H, d, $J = 6.5$ Hz), 0.99 (3H, s), 0.96 (3H, s), and 0.87 (6H, d, $J = 6.5$ Hz), clearly indicative of a steroidal structure. Lack of the 3β-OH group was shown by the absence of signals in the region 3–5 δ. The ir spectrum contained bands at ν max 1650, 1640, and 1585 $cm^{-1}$, in accordance with an unsatured carbonyl functionality, thus establishing the nature of the sole oxygen atom implied by the molecular formula. Additional evidence for the enone chromophore was provided by the uv absorption at λ max 349 nm, which indicated also that the carbonyl group must be conjugated with at least three double bonds.

The location of this functionality on a steroidal nucleus was deduced from an analysis of the low field region of the $^1$H-nmr of **1**, which displayed three 1H signals, related to one another from coupling information obtained by decou-

pling experiments. Irradiation at δ 6.03 collapsed the broad doublet at δ 6.60 ($J$=9.5 Hz) into a broad singlet and sharpened the broad singlet at δ 5.74. Conversely, irradiation at δ 5.74 sharpened the broad doublet at δ 6.03 ($J$=9.5). From the above data it was evident that the signals at δ 6.60 and 6.03 were due to two cis-oriented adjacent vinyl protons. These data indicated that they must be connected to the third olefinic proton through an unprotonated $sp^2$ carbon atom in a conjugated double bond system which, according to the uv and nmr evidence, must be extended to a further fully substituted double bond.

Combination of the foregoing data led to the location of the carbonyl group at C-3 and the three carbon-carbon double bonds at positions 4, 6, and 8(14). The alternative location of the third double bond between C-8 and C-9 was ruled out since the uv absorption calculated for such a system on the basis of the rules of enone absorption (3) would be much higher (λ max 385) than the obtained value [349 nm; calculated for a 4,6,8(14)-trien-3-one, 356 nm].

The olefinic region of the $^1$H-nmr spectrum of **1** comprises also 2H multiplets at δ 5.29, indicative of an isolated double bond which was situated in the side chain at C-22 on the basis of the following decoupling experiment: irradiation at δ 2.11 (tentatively the frequency of H-20) simplified the further coupled AB system centered at δ 5.29 (H-22 and H-23) and collapsed the doublet at δ 1.05 ($H_3$-21) to a singlet. What remained to be established was the stereochemistry of the C-22 double bond, which was assigned as *E*, based on the observed vicinal coupling (H-22/H-23) of 15.5 Hz.

The highly unsaturated 4,6,8(14),22-tetraen-3-one functionality, to our knowledge, has never been so far encountered in naturally occurring sterols. Some sterols related to **1**, however, have been synthesized by oxidation of the corresponding 5,7-dien-3β-ol compounds (4,5). Following this experimental procedure we have prepared **1** in larger quantities by treatment of cholesta-5,7,22-trien-3β-ol with *p*-benzoquinone and aluminium tert-butoxide in dry toluene under reflux for 1 h. An extensive nmr analysis on synthetic **1** was carried out. All the resonances in the $^1$H-nmr spec-

TABLE 1. $^{13}$C- and $^1$H-nmr Spectral Data of Compound **1** in $CDCl_3$ solution.[a]

| Position | $\delta_C$ | $\delta_H$ |
|---|---|---|
| 1a | 34.2[b] | 2.02[d] |
| 1b | | 1.80[d] |
| 2a | 34.1[b] | 2.53 (ddd, $J$ = 18, 18, and 5 Hz) |
| 2b | | 2.39[d] |
| 3 | 199.2 | |
| 4 | 123.0 | 5.74 (bs) |
| 5 | 164.2 | |
| 6 | 124.5 | 6.03 (bd, $J$ = 9.5 Hz) |
| 7 | 133.9 | 6.60 (bd, $J$ = 9.5 Hz) |
| 8 | 124.5 | |
| 9 | 44.4 | 2.12[d] |
| 10 | 36.7 | |
| 11a | 19.0 | 1.24[d] |
| 11b | | 1.67[d] |
| 12a | 35.6 | 1.31[d] |
| 12b | | 2.12[d] |
| 13 | 44.0 | |
| 14 | 155.9 | |
| 15a | 25.4 | 2.40[d] |
| 15b | | 2.40[d] |
| 16a | 27.8 | 1.50[d] |
| 16b | | 1.82[d] |
| 17 | 55.7 | 1.28[d] |
| 18 | 18.9 | 0.96 (s) |
| 19 | 16.6 | 0.99 (s) |
| 20 | 39.2 | 2.11[d] |
| 21 | 22.3 | 1.05 (d, $J$ = 6.5 Hz) |
| 22 | 137.3 | 5.25[e] |
| 23 | 127.1 | 5.33[e] |
| 24 | 41.9 | 1.84[d] |
| 25 | 28.5 | 1.50[d] |
| 26 | 21.2[c] | 0.87 (d, $J$ = 6.5 Hz) |
| 27 | 21.1[c] | 0.87 (d, $J$ = 6.5 Hz) |

[a]Assignments were based on COSY, DEPT, XHCORR, and COLOC experiments.

[b,c]These resonances may be reversed.

[d]Submerged by other signals.

[e]Further coupled AB system, $J_{AB}$ = 15.5 Hz.

trum were assigned through information gleaned from COSY plot and difference double resonance (DDR) experiments conducted in $CDCl_3$ solution, while two-dimensional $^{13}C$-$^1H$ shift correlation (XHCORR and COLOC) and DEPT experiments allowed the assignment of all carbon signals (see Table 1).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Eims of **1** was obtained at 70 eV on a Kratos MS80 mass spectrometer. Ft-ir spectra were recorded on a Bruker IFS-48 spectrophotometer with $CHCl_3$. Uv spectra were performed on a Beckman DU70 spectrometer with EtOH. Optical rotations were measured on a Perkin-Elmer 192 polarimeter, with a 10-cm microcell in $CHCl_3$. $^1H$- and $^{13}C$-nmr spectra were determined on a Bruker WM-250 spectrometer in $CDCl_3$ solution using a 5-mm $^1H/^{13}C$ dual-tuned probe. $^1H$ chemical shifts are referenced to the residual $CHCl_3$ signal ($\delta$ 7.26). $^{13}C$ chemical shifts are referenced to the solvent ($\delta$ 77). The multiplicities of $^{13}C$ resonances were determined by DEPT experiments that were performed using polarization transfer pulses of 90° and 135°, obtaining in the first case only signals for -CH groups and in the other case positive signals for -CH and -Me and negative ones for $-CH_2$ groups. Polarization transfer delays were adjusted to an average C-H coupling of 125 Hz. $^{13}C$-$^1H$ shift-correlated 2D nmr spectra via $^1J$ (XHCORR) and via $^{2,3}J$ (COLOC) were carried out with Bruker microprograms, by adjusting delays to give maximum polarization transfer for $J_{CH} = 125$ Hz and $J_{CH} = 8$ Hz, respectively. Medium pressure liquid chromatography (mplc) was performed on a Büchi 861 apparatus using an $SiO_2$ (230–400 mesh) column. Hplc was performed on a Varian 5020 apparatus equipped with an uv detector, using Hibar LiChrosorb Si60 (7 × 250 mm) and Superspher C-18 (4 × 250 mm) columns.

EXTRACTION AND ISOLATION OF **1**.—Specimens of *D. incisa* were collected from the rocky areas of the Portofino Promontory (Eastern Ligurian Coast) at ca. 10 m depth during November 1988. They were frozen when still alive at −18° and then dispatched to the laboratory. Reference specimens are deposited at the Istituto di Zoologia dell'Università di Genova. Freshly collected animals (dry wt after extraction 40 g) were homogenized and extracted with MeOH (500 ml × 5) at room temperature. The MeOH extracts were evaporated in vacuo and partitioned between $H_2O$ and $Et_2O$. Evaporation of the combined $Et_2O$ extracts afforded 1.5 g of an oily residue, which was chromatographed by mplc on $SiO_2$ column using a solvent gradient system from petroleum ether to $Et_2O$. The combined fractions eluted with petroleum ether-$Et_2O$ (7:3) showed the presence of a highly fluorescent product. These fractions were rechromatographed by hplc on a Si gel column (LiChrosorb Si60, 7 × 250 mm), using $CHCl_3$ as eluent. The fraction containing the bulk of the enone **1** (7 mg) was purified by reversed-phase hplc on an RP-18 column (Superspher C-18, 4 × 250 mm; eluent MeOH), thus obtaining 2 mg of **1** as an amorphous solid: $[\alpha]^{25}D$ 705°; hrms *m/z* 378.2927 (calcd for $C_{27}H_{38}O$, 378.2913); low resolution ms *m/z* (rel. int.) $[M]^+$ 378 (80), $[M - \text{side chain}]^+$ 267 (100), 214 (60), 173 (58); uv λ max (EtOH) 349 nm, $\epsilon = 26000$; ir ν max ($CHCl_3$) 1650, 1640, 1585 $cm^{-1}$; $^{13}C$ and $^1H$ nmr see Table 1.

SYNTHESIS OF **1**.—Cholesta-5,7,22-trien-3β-ol (100 mg) and *p*-benzoquinone (200 mg) were dissolved in 20 ml of toluene (sodium-dried). Aluminium tert-butoxide (100 mg) was added, and the mixture was boiled under reflux for 1 h. After being cooled, the solution was filtered and the dark solid washed with warm $C_6H_6$. The combined filtrate and washings were extracted with dilute aqueous NaOH and the layers separated by centrifugation. After washing with $H_2O$ the organic phase was dried ($CaCl_2$) and distilled under high vacuum to obtain a residue that was first separated on tlc [$SiO_2$, $CHCl_3$-$Et_2O$ (9:1)]. The fluorescent band (uv light) at $R_f$ 0.6 was scraped, eluted with $Et_2O$, and evaporated to dryness. It yielded 25 mg of a product that was further purified on reversed-phase hplc with MeOH. The synthetic product (12 mg) was identical to the naturally occurring **1** on the basis of their chromatographic and spectral properties.

## ACKNOWLEDGMENTS

This work is a result of a research supported by M.P.I. (Italy). Mass spectra data were provided by "Servizio Spettrometria di Massa del CNR e dell'Università di Napoli." The assistance of the staff is gratefully appreciated.

## LITERATURE CITED

1. D.J. Faulkner, *Nat. Prod. Rep.*, **5**, 613 (1988), and literature cited herein.
2. O. Schimdt, *Arch. Mikrosk. Anat. Entwicklungsmech.*, **18**, 280 (1880).
3. A.I. Scott, "Interpretation of Ultraviolet Spectra of Natural Products," Pergamon Press, Oxford, 1984, p. 58.
4. J. Elks, *J. Chem. Soc.*, 468 (1954).
5. D.H.R. Barton and T. Bruun, *J. Chem. Soc.*, 2748 (1951).

*Received 24 April 1989*