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A NEW CYTOTOXIC DIHYDROXY STEROL FROM THE SOFT CORAL *ALCYONIUM PATAGONICUM*

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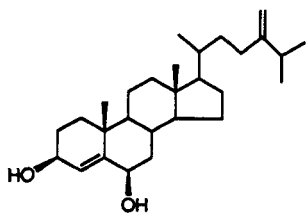
ABSTRACT.—A new sterol, 24-methylenecholest-4-ene-3β,6β-diol [**1**] was isolated from the soft coral *Alcyonium patagonicum* collected from the South China Sea. Its structure was determined by spectral analysis. Compound **1** was cytotoxic against the P-388 cell line with an IC_{50} value of 1 μg/ml.

Diterpenoids (1–8), sesquiterpenoids (9–12), and steroidal glycosides (13,14) have been reported from soft corals of various species of the genus *Alcyonium*, in the family Alcyoniidae. Until recently the species *A. patagonicum* (May) had not been investigated chemically and our initial studies resulted in the isolation of the diterpene patagonicol as a major component of the non-polar lipid extracts (15). In this paper we report that further study on the more polar fractions of the extracts of this soft coral has led to the isolation of a new cytotoxic sterol, 24-methylenecholest-4-ene-3β,6β-diol [**1**].

Compound **1**, mp 236–237°, $[\alpha]_D$ +20.5° (c=0.15, MeOH), which was obtained as fine needles from the EtOAc-soluble portion of an EtOH extract of *A. patagonicum*, displayed a molecular ion at m/z 414.3489 by hreims, corresponding to $C_{28}H_{46}O_2$. In the lreims of **1**, peaks were observed at m/z 396 [M^+-H_2O] and 378 [M^+-2H_2O], corresponding to successive losses of 18 mass units, which indicated the presence of two hydroxyl groups. The ir spectrum showed an absorption at 3300 cm^{-1}, supporting the presence of hydroxyl groups, and bands at 1640 and 880 cm^{-1} revealing the existence of a terminal methylene group.



1

The nmr spectra of **1** supported the existence of a terminal methylene group [2H, δ 4.65 br s and 4.71 br s; ^{13}C δ 105.71 (t) ($C=CH_2$), 157.05 (s)] and showed the presence of an additional trisubstituted double bond (1H, br s, δ 5.54; ^{13}C δ 128.33 (=CH-), 147.36 (s)], as well as an isopropyl group (3H, d, δ 1.01 ppm and 3H, d, δ 1.02). Also present were singlets at δ 0.71 and 1.26 corresponding to Me-18 and Me-19, respectively. Two one-proton signals at δ 4.18 (ddd, J=10, 5, and 2 Hz) and 4.23 (br t, J=2.5 Hz) denoted the presence of two oxygenated methine protons; in agreement with this the ^{13}C-nmr spectrum showed two signals at δ 73.76 and 67.66. The presence of a conventional 24-methylene steroid side-chain was indicated by the isopropyl methyl doublet at δ 1.01, 1.02 that was coupled to an allylic methine multiplet at δ 2.23, along with the additional secondary methyl signal at δ 0.93 (d, J=6.4 Hz), and by comparison of ^{13}C-nmr data with those of other 24-methylene sterols (13, 17, 18). Further confirmation of a 24-methylene-substituted side-chain was derived from a prominent hreims peak at m/z 312.2484 corresponding to a fragment ion which would be expected from McLafferty-type cleavage

at C-22, C-23 and loss of H_2O from the nucleus. Thus, both hydroxyl groups were confined to the nucleus.

The ^1H-^1H COSY nmr spectrum of **1** showed a correlation between the resonance at δ 5.54 (H-4) and the signal at δ 4.18 (ddd, J=10, 5, and 2 Hz, H-3α) which in turn was coupled to a pair of geminally coupled protons (δ 2.00 and 1.53). Irradiation of the broad singlet at δ 5.54 simplified the ddd at δ 4.18 into a dd, thus establishing the partial structure -CH_2-CH(OH)-CH=C. In a conventional steroid nucleus this can only be accommodated as the C-2 through C-5 unit. The alcohol at C-3 was assigned a 3β configuration based on the observed coupling constants for H-3 (J=10, 5, and 2 Hz).

The remaining hydroxyl group in **1** could be placed at C-1, C-6, or C-12, based on the limited multiplicity (triplet, J=2.5 Hz) of the associated methine proton signal at δ 4.23. However, the OH group at any of these positions must be axially oriented, so that the corresponding oxymethine proton would have only a small vicinal coupling. The possible structure with a hydroxyl group at C-1 was excluded since no correlations were observed between the signal at δ 4.23 and the H-2 signals in the ^1H-^1H COSY spectrum. A choice between C-6 and C-12 could be made from solvent-induced shifts in pyridine-d_5 vs. $CDCl_3$. In pyridine-d_5 the methyl singlet signals appeared at δ 0.71 and 1.53 vs. δ 0.71 and 1.26 in $CDCl_3$. This large downfield shift (Δ=0.27) for the low-field methyl signal is consistent with a 6β OH/Me-19 arrangement, but not a 12α-OH (no expected Me shifts). Thus, the structure of **1** was assigned as 24-methylenecholest-4-ene-3β,6β-diol. The absence of a 4,6α-H coupling is consistent with a nearly 90° angle between the H-6 σ bond and the π orbitals (19). The chemical shifts assigned ($CDCl_3$) to Me-18 (δ 0.71) and Me-19 (δ 1.26) are in good agreement with the respective calculated values (0.75 and 1.26 ppm) using Arnold's substituent increment parameters for 4-cholesten-3β,6β-diol (20).

Sterol **1** was cytotoxic to murine leukemia cells (P-388), with an IC_{50} value of 1 μg/ml. This new sterol adds to the extensive list of hydroxylated sterols, some of which are also cytotoxic, isolated from marine sources (21,22).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—General experimental procedures were as described previously (15).

ANIMAL MATERIAL.—Specimens of *A. patagonicum* were collected off the Xisha Islands in the South China Sea in 1988. A voucher specimen (No. 88-28) is held at Zhongshan University.

EXTRACTION AND ISOLATION.—Chopped, sun-dried specimens (3.5 kg) were extracted with EtOH at room temperature. The EtOH extract was concentrated *in vacuo* and partitioned between EtOAc and H_2O. The organic phase was separated and concentrated *in vacuo* to give a residue which was subjected to flash chromatography on Si gel, eluting with EtOAc/petroleum ether with gradually increasing amounts of EtOAc. Compound **1** was obtained from the fractions eluted with 55% EtOAc in petroleum ether, after further flash chromatography on Si gel with Me_2CO in petroleum ether as eluent, and finally recrystallization of purified fractions from Me_2CO.

*24-Methylenecholest-4-en-3*β,6β-diol [**1**].—Obtained as fine needles (Me_2CO) (2.3 mg); mp 236–237°; $[\alpha]_D$ +20.5° (c=0.15, MeOH); ir (neat) ν max 3300, 1640, 1460, 1370, 1030, 880 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 0.71 (3H, s, Me-18), 0.93 (3H, d, J=6.4 Hz, Me-21), 1.01, 1.02 (6H, d, J=6.8 Hz, Me-26, -27), 1.12 (m, H-7), 1.26 (3H, s, Me-19), 1.53 (m, H-2), 1.90 (m, H-7), 2.00 (m, H-2), 2.23 (m, J=6.8 Hz, H-25), 4.18 (1H, ddd, J=10, 5, and 2 Hz, H-3α), 4.23 (1H, br t, J=2.5 Hz, H-6α), 4.65 (br s, H-28), 4.71 (br s, H-28), 5.54 (br s, H-4); ^1H nmr (pyridine-d_5, 300 MHz) δ 0.70 (3H, s, Me-18), 0.98 (3H, d, J=6.5 Hz, Me-21), 1.05 (d, J=6.9 Hz, Me-26, -27), 1.53 (s, Me-19), 4.56 (2H, m, H-3 and H-6), 4.84 (br s, H-28), 4.85 (br s, H-28), 6.00 (1H, br s, H-4); ^{13}C nmr (125 MHz, $CDCl_3$/CD_3OD) δ 157.05 (C-24), 147.36 (C-5), 128.33 (C-4), 105.71 (C-28), 73.76 (C-3), 67.66 (C-6), 56.17 (2C), 54.37, 42.43, 39.72, 38.94, 36.77, 36.54, 35.61 (C-20), 34.53 (C-22), 33.67 (C-25), 30.88 (C-23), 30.19, 28.56, 28.02, 23.99, 21.75 (C-26), 21.62 (C-27), 21.12, 20.81 (C-19), 18.49 (C-21), 11.82 (C-18); hreims m/z 414.3489 (30) (calcd for $C_{28}H_{46}O_2$,

414.3498) $[M]^+$, 312.2484 (100) (calcd for $C_{22}H_{32}O$, 312.2453), $[M-C_6H_{12}-H_2O]^+$; eims *m/z* 414 (8) $[M^+]$, 399 (13) $[M^+-Me]$, 396 (24) $[M^+-H_2O]$, 381 (19) $[M^+-H_2O-Me]$, 378 (12) $[M^+-2H_2O]$, 367 (8), 343 (8), 330 (19) $[M^+-C_6H_{12}]$, 312 (65) $[M^+-H_2O-C_6H_{12}]$, 297 (28), 287 (17), 269 (39), 243 (19), 229 (24), 189 (13), 175 (23), 161 (28), 135 (29), 121 (38), 109 (46), 107 (49), 95 (71), 93 (49), 81 (69), 69 (76), 55 (100), 43 (60).

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