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J. Nat. Prod., **1991**, 54 (1), 273-275• DOI: 10.1021/np50073a033 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 1, pp. 273-275, Jan-Feb 1991

STEROIDS FROM *NEOSIPHONIA SUPERTES*, A MARINE FOSSIL SPONGE

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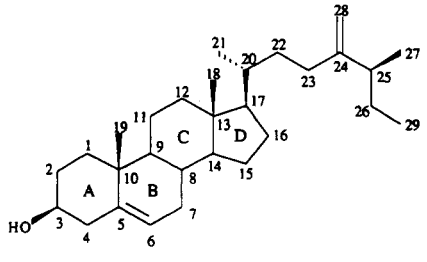
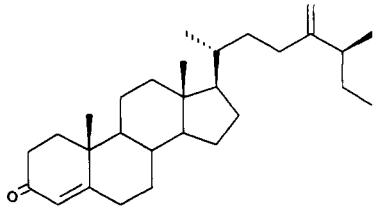
ABSTRACT.—The sponge *Neosiphonia supertes* contains 24(28)-dehydroaplysterol [**1**] and the new steroid (25*S*)-26-methyl-24-methylenecholest-4-en-3-one [**2**].

In the wake of the marine program S.M.I.B. (Marine Products of Biological Interest) directed by CNRS and ORSTOM in New Caledonia, we now have investigated the steroidal content of a neocaledonian sponge, *Neosiphonia supertes* Sollas (Theonellidae) (1,2). Two known sterols were obtained, β-sitosterol and 24(28)-dehydroaplysterol [**1**] (3,4), together with a new compound, namely (25*S*)-26-methyl-24-methylenecholest-4-en-3-one [**2**].

The dried hexane extract was chromatographed on a Si gel column and eluted with $CHCl_3$–*n*-C_6H_{14} (7:3) to yield a crude sterol fraction that was further purified by means of semipreparative hlpc on μ-C_{18} column and preparative tlc on Si gel glass plates. Three compounds were obtained: β-sitosterol, 24(28)-dehydroaplysterol [**1**], and (25*S*)-26-methyl-24-methylenecholest-4-en-3-one [**2**]. β-Sitosterol and 24(28)-dehydroaplysterol [**1**] were identified by direct comparison of their spectral properties (ir, uv, eims, ^{1}H nmr) with those available (4,5). The assignments of the ^{13}C-nmr values for **1** summarized in Figure 1 have been supported by an heteronuclear 2D correlation experiment. These results are in agreement with those already published (6).



1



2

The high resolution mass spectrum of compound **2** contains the molecular ion species *m/z* 410.3585, with *m/z* 312, 269, 229, 124 ions. The *m/z* 229 ion is generated by the loss of ring D bearing the side chain and an extra hydrogen. This fragmentation is diagnostic of a steroid bearing a side chain at C-17 (7). The *m/z* 124 and 149 peaks are characteristic of Δ^4-3-keto steroid, resulting from fission of 6-7 and 9-10 bonds in ring B with the charge remaining on either the hydrocarbon or the oxygen-containing fragment (8). The *m/z* 269 peak corresponds to the loss of the side chain together with two hydrogens from the steroid nucleus. The *m/z* 312 $[M - C_7H_{14}]^+$ intense peak is diagnostic of a C-24(28) double bond; this fragmentation is rationalized by a McLafferty type of rearrangement (9,10).

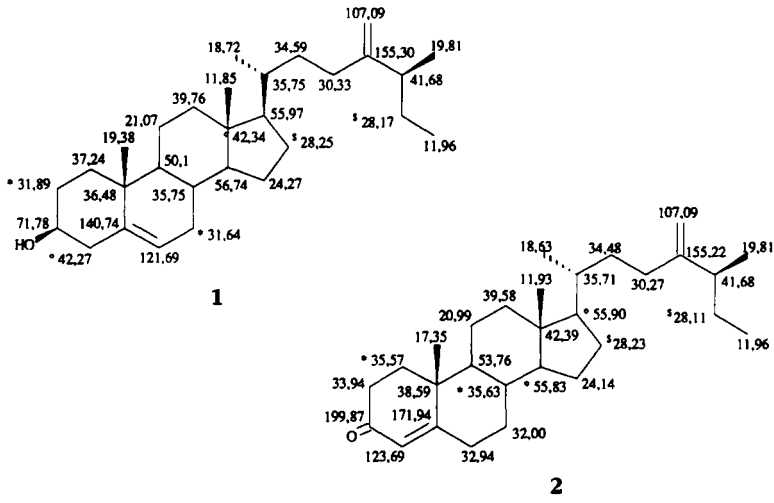


FIGURE 1. ^{13}C-nmr values for compounds **1** and **2**. Values with the same superscript are interchangeable.

The ^1H-nmr spectrum of **2** is close to that of **1** with specific protons at δ 4.69 (CH_2-28), 1.00 (Me-27), 0.94 (Me-21), and 0.83 ppm (Me-29). However, a singlet at 5.72 ppm (H-4) indicates a Δ^4 unsaturation (11). The presence of a keto function is also deduced from the ir spectrum (ν CO 1677 cm^{-1}), and its location at C-3 is suggested by the ^{13}C- and ^1H-nmr spectra (12). The completed ^{13}C assignments are shown in Figure 1. The structure of **2** was unambiguously confirmed through a chemical correlation. Oppenauer oxidation of **1** led to a keto compound identical (ir, eims, ^1H-nmr, and [α]D) with **2**. Therefore, **2** was identified as (25*S*)-26-methyl-24-methylenecholest-4-en-3-one.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Büchi 510 apparatus and are uncorrected. Uv spectra were obtained on a Hitachi spectrophotometer. Ir spectra were obtained on a Perkin-Elmer 580 spectrometer. Optical rotations were measured on a Schmidt-Haensch Polartronic I polarimeter, using a 10-cm microcell. High-resolution mass spectra were recorded on a Varian Mat. 11 spectrometer. ^1H-nmr and ^{13}C-nmr spectra were recorded at 300 and 125 MHz, respectively, with TMS as internal standard on Bruker AMWB 300 and Bruker AMWB 500 instruments. The ^1H-^{13}C correlation nmr spectra were obtained using Bruker's microprograms. Tlc chromatographies were performed using Merck Kieselgel 60 H Si gel and precoated Merck F_{254} glass plates. Preparative hplc purifications were carried out on a Waters apparatus equipped with μ-C_{18} column (7.8 mm i.d. × 30 cm) and refractive index and uv detectors. The purity of sterols was checked with a Carlo Erba Vega GC 6000 gas chromatograph equipped with a SE30/OV1 (0.22 mm i.d. × 25 m) capillary column and a flame-ionization detector (carrier gas N_2, oven temperature 250°).

EXTRACTION AND ISOLATION OF COMPOUNDS.—The *N. superba* specimens were collected (by dredging at about 500 m deep off New Caledonia shores by the R/V VAUBAN) and lyophilized. A voucher specimen (reference R1408) has been deposited at the Centre ORSTOM of New Caledonia. The dried sponge material (500 g) was extracted with *n*-hexane at room temperature. The hexane extract (2.2 g) was applied on a Si gel column and eluted with $CHCl_3$–*n*-C_6H_{14} (7:3). Fractions with the same tlc profile were combined. Two steroidal fractions were recovered. The first one (22 mg) was subjected to preparative tlc [$CHCl_3$–*n*-C_6H_{14} (8.5:1.5)] yielding **2** (15 mg). The second one (610 mg) was subjected to preparative reversed-phase hplc (MeOH, flow rate 4 ml per min) yielding **1** (25 mg) and β-sitosterol (1.5 mg). The purity of **1** and β-sitosterol was checked through gc.

(25*S*)-26-METHYL-24-METHYLENECHOLEST-5-EN-3β-OL [**1**].—$[\alpha]^{20}D$ −40° (*c* = 1, $CHCl_3$); mp 128°; uv λ max (MeOH) 212 nm; ir ν max (liquid film) 3440 cm^{-1} (OH); eims *m/z* (%) $[M]^+$ 412.3708 ($C_{29}H_{48}O$ required 412.3705) (10), 315 (23), 314 (91), 299 (29), 281 (27), 271 (34), 231 (15), 229 (28), 213 (26), 161 (24), 159

(29), 147 (22), 145 (36), 135 (21), 133 (31), 131 (21), 121 (28), 120 (20), 119 (30), 109 (30), 107 (50), 105 (41), 97 (21), 95 (50), 93 (41), 91 (32), 83 (34), 81 (58), 79 (33), 69 (60), 67 (35), 57 (25), 55 (100), 43 (23), 41 (43); ^{1}H nmr (δ, ppm, $CDCl_3$), 5.35 (1H, m, H-6), 4.69 (2H, s, CH_2-28), 3.52 (1H, m, H-3), 1.01 (3H, s, Me-19), 1.00 (3H, d, J = 6.8 Hz, Me-27), 0.95 (3H, d, J = 6.5 Hz, Me-21), 0.83 (3H, t, J = 7.4 Hz, Me-29), 0.68 (3H, s, Me-18); ^{13}C nmr ($CDCl_3$, 125 MHz) see Figure 1.

(25*S*)-26-METHYL-24-METHYLENECHOLEST-4-EN-3-ONE [**2**].—$[\alpha]^{20}$D +42° (c = 1, $CHCl_3$); uv λ max (MeOH) 242 nm (log ε 4.31); ir ν max (liquid film) 1677 cm^{-1} (C=O); eims *m/z* (%) $[M]^+$ 410.3538 ($C_{29}H_{46}O$ required 410.3585) (17), 313 (46), 312 (100), 297 (39), 269 (44), 231 (20), 229 (24), 227 (25), 149 (23), 148 (20), 147 (27), 124 (44), 123 (30), 121 (31), 119 (30), 109 (36), 107 (28), 105 (46), 95 (49), 93 (35), 91 (24), 83 (34), 82 (24), 81 (42), 79 (25), 69 (66), 67 (31), 57 (35), 55 (80), 43 (50), 41 (47), 28 (29), 18 (22); ^{1}H nmr (δ, ppm, $CDCl_3$) 5.72 (1H, s, H-4), 4.69 (2H, s, CH_2-28), 1.18 (3H, s, Me-19), 1.00 (3H, d, J = 6.9 Hz, Me-27), 0.94 (3H, d, J = 6.5 Hz, Me-21), 0.83 (3H, t, J = 7.4 Hz, Me-29), 0.71 (3H, s, Me-18); ^{13}C nmr ($CDCl_3$, 125 MHz) see Figure 1.

PREPARATION OF (25*S*)-26-METHYL-24-METHYLENECHOLEST-4-EN-3-ONE [**2**].—Compound **2** was prepared from (25*S*)-26-methyl-24-methylenecholest-5-en-3β-ol [**1**] by Oppenauer oxidation (13). A mixture of **1** (100 mg), cyclohexanone (1 ml), dry toluene (25 ml), and aluminum isopropoxide (100 mg) was refluxed with vigorous stirring for 30 min. Removal of solvent followed by preparative tlc furnished the corresponding Δ^4-3-one **2**.

The synthetic (25*S*)-26-methyl-24-methylenecholest-4-en-3-one [**2**] had $[\alpha]^{20}$D, ir, eims, and ^{1}H-nmr data identical to those of the natural **2**.

ACKNOWLEDGMENTS

We thank Pr. Lévi for his kind identification of the sponge and Dr. Kelecom for providing the reprint of reference 6.

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Received 7 June 1990