Guggulsterol-like steroids from the Mediterranean gorgonian *Leptogorgia sarmentosa*

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Summary. Several polyoxygenated steroids (**1**, **2**, **3**) have been isolated from the marine gorgonian *Leptogorgia sarmentosa*. One of these (**1**) is the known guggulsterol III, previously found in the pharmacologically active resin from the tree *Commiphora mukul*; the others, **2** and **3**, have not been found before in nature, and are closely related to **1**. During the structural work apparent anomalies in the CMR-spectra of **1** were observed.

The resinous exudate from the tree *Commiphora mukul* possesses a variety of pharmacological activities; anti-inflammatory, anti-rheumatic and hypocholesterolemic, some of which are due to the presence, among the secondary metabolites, of a series of steroids, named guggulsterols[3]. One of these, guggulsterol III (**1**), has also been found, along with 2 closely related steroids (**2** and **3**), in extracts of the Mediterranean gorgonian *Leptogorgia sarmentosa*, which previously[4] yielded as a major component of the very abundant mixture of steroids the highly unstable compound **4** which easily looses its side chain.

In this paper we wish to report the structural elucidation, based on spectroscopic and chemical evidence, of the 2 steroids newly found in nature, namely **3**, 16-epi-guggulsterol III (cholest-4en-16α,20ξ-diol-3-one) and **2**, cholest-4en-20ξ-ol-3,16-dione, and some additional data for the known compound **1**, with remarks about the unexpected strongly upshifted resonance of C-17 in its CMR-spectrum.

The ether soluble fraction from the acetone extracts of the coelenterata *L. sarmentosa*, yielded, after chromatography on silica gel, the previously reported **4** and a series of related steroids currently under investigation, and also **3** (0.003% dry weight, TLC R_f 0.3, light petroleum-diethyl ether, 3:7) and a less polar mixture (R_f 0.5) resolved by preparative HPLC (μ Bondapak C-18, $CH_3OH:H_2O$, 8:2) into **1** and **2** (0.004% and 0.003% dry wt, respectively). All the spectral data for **1**, including the CMR-spectrum (table), fitted well with the structure of guggulsterol III[3].

2 showed the following spectral data: $C_{27}H_{42}O_3$ (high resolution mass spectrometry of the $M^+ - H_2O$ taken in conjunction with the NMR-data). UV (MeOH) 242 (ε 15.800) nm; IR ($CHCl_3$) 3450, 1730, 1660, 1610 cm^{-1}; PMR ($CDCl_3$) 5.74 (H-4, 1H, s), 2.21 (H-17, 1H, s), 1.26 (H-21, 3H, s), 1.22 (H-19, 3H, s), 0.97 (H-18, 3H, s), 0.87 (H-26 and H-27, 6H, d, J = 6.2 Hz) δ; MS: 396 ($M^+ - H_2O$, 50%), 381 (75%), 329 (86%, 20-22 cleavage from M^+), 286 (66%, 17-20 cleavage + 1H), 271 (100%) m/z; CMR (table).

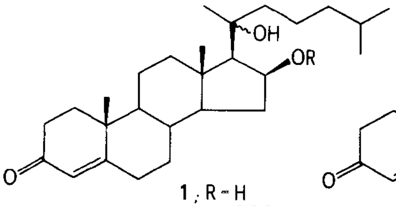
The structure **2**, suggested on the basis of the above spectral evidence, was definitively confirmed by chemical correlation with **1**, which by treatment with Corey's reagent[5] yielded a ketone identical to **2**.

The following evidence suggested for **3** the structure of 16-epi-guggulsterol III. $C_{27}H_{44}O_3$ (high resolution mass

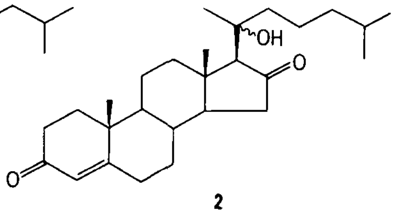
CMR chemical shifts[a] (in ppm from internal TMS) of **1**, **5**, **2**, **3**, **6**

C	**1**	**5**	**2**	**3**	**6**
C-12	40.2	40.2	39.2	39.8	40.2
C-13	42.9	43.4	42.6	48.6	44.8
C-14	54.0	54.0	50.4	53.7	53.2
C-15	37.2	35.0	39.2	35.0	34.2
C-16	73.8	77.6	220.0	73.0	75.9
C-17	60.1	60.4	71.3	68.1	62.8
C-18	14.9	14.6	14.6	15.3	14.6
C-19	17.4	17.4	17.3	17.4	17.3
C-20	76.8	75.7	74.0	76.0	75.7
C-21	26.8	26.5	25.4	25.7	26.5
C-22	44.4	44.4	42.5	44.0	44.8

Spectra were determined in $CDCl_3$ on a Varian XL-100 FT spectrometer operating at 25.20 MHz. [a] The remaining chemical shifts are fully consistent with those reported for 3-keto-Δ-4-steroids[6].



1; R = H
5; R = $COCH_3$



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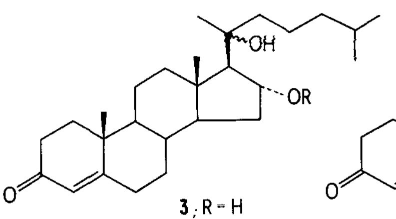
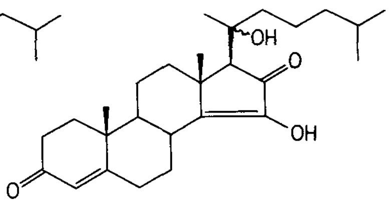


3; R = H
6; R = $COCH_3$



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spectrometry and NMR). UV (MeOH) 242 (ε 14.900) nm; IR ($CHCl_3$) 3450, 1660, 1610 cm^{-1}; PMR ($CDCl_3$) 5.70 (H-4, 1H, s), 4.55 (H-16, 1H, m, w/2 15 Hz), 1.26 (H-19, 3H, s), 1.13 (H-21, 3H, s), 0.84 (H-26 and H-27, 6H, d, J = 6.2 Hz), 0.77 (H-18, 3H, s) δ. MS: 398 (3%, $M^+ - H_2O$), 383 (5%), 331 (10%), 313 (50%), 295 (23%), 271 (100%) m/z; CMR (table).

The comparison of the above spectral data with those of **1** revealed a strong structural analogy between the 2 steroids, with changes mainly due to the conformational difference that results from a different localization, in **3**, of the β-oriented hydroxy group at C-16 of **1**. This group in **3** can be located only on the D ring and has to show an α orientation. In fact the comparison of the MS- and CMR-data of **3** with those of model compounds[3,6] excludes the localization of the hydroxy group either on the side chain or on the rings A, B and C. In addition the PMR-spectrum of **3**, compared with that of **1**, showed a strongly upshifted resonance of the C-18 methyl, 0.77 δ, justificable[7] only with an α orientation of the hydroxy group at C-15 or at C-16. The definitive assignment at C-16 arised from the oxidation, as seen for **1**, of **3** to **2**.

During this structural work we have observed that, in **1** and **3**, the ^{13}C chemical shift of C-17 is strongly influenced by the stereochemistry of the hydroxy group at C-16. In fact the CMR-spectrum of **3** shows for C-17 a shielding (68.1 ppm) in agreement with the predicted values, while in **1** the value for C-17 is strongly upshifted (60.1 ppm). The observed anomaly may be due to the possibility that in **1** a hydrogen linkage may be formed between the hydroxy groups at C-20 and C-16.

The acetylation at room temperature of **1** and **3** confirmed this hypothesis. In fact the CMR-spectra of both the acetyl derivatives, **5** and **6**, besides allowing the assigning of the chemical shifts to all the D ring carbons, showed significant resonances for C-17 (60.4 and 62.8 ppm, respectively). The 1st value was particularly informative; in disagreement with the expected upshift due to the acetylation it was weekly downshifted. This unusual shift can be well justified by the above suggested intramolecular interaction.

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A new furanoterpene from a *Spongia* sp.

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Summary. A new C21 furanoterpene, oxygenated at C(8), has been isolated from a *Spongia* sp.; its structure has been established by chemical and spectroscopic studies. The stereochemistry of tetradehydrofurospongin-1 is discussed.

A number of C21 furanoterpenes have been isolated from sponges of the family Spongiidae (order Dictyoceratida)[1]. Most have been obtained from 3 related Mediterranean sponges, *Spongia officinalis, S. nitens* and *Hippospongia communis* and can be considered to be based on anhydrofurospongin-1 (**1**) with oxygenation at the center of the prenyl chain and varying degrees of unsaturation e.g. furospongin-1 (**2**) and nitenin (**3**)[2]. Some Australian *Spongia* species have been shown to contain C21 furanoterpenes, in particular tetradehydrofurospongin-1 (**4**)[3] and the unsymmetrically oxygenated furospongenol (**5**)[4].

We now wish to report the isolation and structural elucidation of a new C21 furanoterpene isolated from a *Spongia* sp. found off the Western Australian coast.

A specimen of the sponge (found at a depth of 15 m east of Gun Island, Southern Abrolhos Group, Western Australia)[5] was collected and frozen for transport. On thawing the diced sponge was extracted twice with CH_2Cl_2 : MeOH (1:1) and the extract was partitioned by the addition of H_2O. Fractionation of the CH_2Cl_2 soluble extract on silica gel afforded, in the CH_2Cl_2 : AcOEt (3:1) fraction, **6** (1.3% yield based on wet weight of sponge) as an unstable oil, $[\alpha]_D^{20} - 10°$ (c = 0.4, $CHCl_3$), $C_{21}H_{26}O_3$ (elemental analysis).

The ^1H-NMR-spectrum (270 MHz, $CDCl_3$) suggested the presence of 2 β-substituted furan rings [4 multiplets at δ 7.30 (3H), 7.20 (1H), 6.49 (1H), 6.27 (1H)]. This was supported by the ^{13}C-NMR-spectrum (20.1 MHz, $CDCl_3$) (table) which showed signals for 4 furan α-carbons (doublets at δ 139.0, 140.0, 142.7, 143.5) and 4 furan β-carbons (doublets at δ 107.7, 112.2; singlets at δ 124.5, 125.0). The ^1H-NMR-spectrum included a broad signal at δ 2.70 for a doubly allylic mehtylene which was shown to be coupled (J 7 Hz) to 2 vinylic protons resonating at δ 5.58 and with long-range coupling to the furan protons at δ 7.30. 7.20 and 6.27. The presence of a tertiary carbinol system was deduced from IR (ν_{max}^{film} 3400 cm^{-1}), ^1H-HMR [δ 1.86 (D_2O exchangeable), 1.29 (3H, s)] and ^{13}C-NMR evidence (δ 72.6, C-OH). Significant downfield shifts were observed for the vinyl multiplet (δ 5.58 to 6.0) and the methyl singlet (δ 1.29 to 1.8) when the ^1H-NMR-spectrum was recorded in the presence of Tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato]europium (III). The CI_{CH_4} mass spectrum of **6** showed, besides the $M^+ + 1$ ion, significant peaks at m/z 309 ($M^+ + 1 - H_2O$) and 175 ($M^+ - C_9H_{11}O_2$), the latter arising from fragmentation α to the tertiary hydroxyl group. The EI mass-spectrum did not show an M^+, the highest