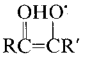
Consistent with this is the observation that bovine catalase (250 units/ml) inhibited the formation of both components whereas copper-zinc superoxide dismutase (330 units/ml) enhanced the formation of both components and indicates that hydrogen peroxide is a precursor of the hydroxyl radical. Autoxidising GCHO therefore produces 1-hydroxylalkyl (R ·) and hydroxyl (· OH) radicals at pH 8.6.

The free radical production from GCHO, as judged by spin trapping, showed a peculiar pH profile (fig. 2, b): below pH 8.2, only DMPO-R was observed, figure 2, c. This is consistent with a pKa of 8.2 for the ene-diol of GCHO. Above this pH the ene-diolate anion predominates and rapidly reduces dioxygen by a redox mechanism (Equations 2+3); the ene-diol is short-lived. Below pH 8.2, the unionized ene-diol predominates, autoxidation is slower and hydroxyl radicals produced are scavenged by a free radical addition mechanism (Equation 7). Hydroxyl radicals are also scavenged by the free aldehyde throughout the pH range (Equation 6). Both ene-diol oxy radicals  $\overset{OH}{\overset{\|}{RC}}=\overset{O^{\cdot}}{\overset{|}{C}}R'$ and superoxide radicals $O_2\cdot$ are included in the mechanism of α-hydroxyketone autoxidation (scheme) yet these radicals were not observed by spin-trapping. This can be expected from the relatively low sensitivities of detection by spin-trapping for these radicals[12,13]. Evidence of ene-diol oxy radical intermediates comes from the ESR spectrum of α-hydroxyketones at high pH, under anaerobiosis[14]. Superoxide involvement was indicated indirectly; superoxide dismutase decreased the rate of cytochrome c reduction (38%) and oxygen consumption (40%) and stimulated DMPO-R and DMPO-OH production from GCHO. A generalized, overall mechanism for α-hydroxyketone autoxidation is given in the scheme. The ene-diol oxy and R radicals may disproportionate (Equations 5 and 8) to a keto/enol reactant and an α-dicarbonyl product molecule. Products from the overall reaction, α-dicarbonyl compounds and hydrogen peroxide are both potentially toxic to biological systems. Low levels of hydroxypyruvaldehyde-3-phosphate have been detected in erythrocytes[15]. The addition of metal ion chelators to autoxidizing GCHO reduced the rate of oxygen consumption by about 25% (fig. 1, a), suggesting that trace metal ions may catalyze α-hydroxyketone autoxidation, although their involvement does not appear to be obligatory. Metal ions may co-ordinate to cis-ene diol and ene-diol-oxy radicals to facilitate electron transfer to dioxygen.

Our observations suggest that α-hydroxyketones become susceptible to autoxidation at physiological temperatures, albeit dependent on the availability of the ene-diol tautomer. In many systems, this has not been hitherto appreciated. We suggest that phenomena observed in some experimental systems using α-hydroxyketones may not be ascribed to the substrate but to the intermediates and products of their autoxidation processes.

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C-18 Hydroxy steroids from the Mediterranean gorgonian *Leptogorgia sarmentosa*[1]

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Summary. Five new polyoxygenated steroids, 24-methylcholesta-1,4,22E-trien-16β,18,20ξ-triol-3-one (**1**), cholest-1,4,22E-trien-16β,18,20ξ-triol-3-one (**2**), 24-methylcholesta-4,22E-dien-16β,18,20ξ-triol-3-one (**3**), cholesta-4,22E-dien-16β,18,20ξ-triol-3-one (**4**) and 27-nor-24-methylcholesta-4,22E-dien-16β,18,20ξ-triol-3-one (**5**), have been found in a marine organism, the gorgonian *Leptogorgia sarmentosa.*

The polyoxygenated steroids are largely present in the secondary metabolism of marine organisms[2] but they rarely show a hydroxy group at C-18. A pregnane derivative with this structural feature has been reported[3], together with its C-18 acetate, from the telestacean octocoral *Telesto riisei,* while another C-18 acetate derivative has been found[4] in the hydroid *Eudendrium.*

Continuing our researches on the steroids of Mediterranean gorgonians[5–7] we have now found in *Leptogorgia sarmentosa* a series of steroids (**1–5**) characterized by oxidation patterns which include a C-18 hydroxy group.

The gorgonian was extracted with acetone at room temperature. The extract, after concentration in vacuo, was partitioned between diethyl ether and water. The ethersoluble fraction was chromatographed on silica gel to yield, along with the previously[6,7] reported steroids, several related compounds.

Two fractions, homogeneous in TLC (diethyl ether: light petroleum, 8:2, A, R_f 0.2, B, R_f 0.3), were submitted to preparative HPLC purification (μ Bondapak C-18, CH_3OH: H_2O, 7:3, flow 2 ml/min) which split each of them into 2 fractions (from A: **1**, 0.003%, r.t. 14.5 min, and **2**, 0.003%, r.t. 10.6 min; from B: **3**, 0.003%, r.t. 23.3 min, and the mixture of **4** and **5**, 0.004%, r.t. 16.4 min).

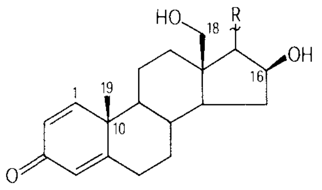
The compound **1** had the molecular formula $C_{28}H_{42}O_4$ (HRMS of the $M^+ - H_2O$ taken in conjunction with NMR data). Assuming a normal steroidal skeleton, the UV [λ_{max} (MeOH) 244 nm (ε = 12.000)] and IR (ν_{max} 1654, 1622 and 1604 cm^{-1}) data suggested the presence of a ring A dienone, confirmed by the PMR spectrum, δ7.08 (1H, d, J = 10 Hz), 6.22 (1H, dd, J = 10 and 2 Hz), 6.06 (1H, bs) and CMR data (C-1, 155.8; C-2, 127.6; C-3, 186.3; C-4, 123.9; C-5, 168.9; C-10, 43.6). In the PMR spectrum only 1 singlet attributable to angular methyls was present. It resonated at δ1.26 (CMR δ18.7) and was assigned to the C-19 protons by comparison with model compounds[8]. This assignment was confirmed by the presence in the MS spectrum of the base peak at m/z 121 due to a ring A fragment obtained by cleavage across ring B. On the other hand the evidence for a hydroxy group at C-18 derived from the absence from the PMR spectra of the singlet due to C-18 H's, substituted by an AB quartet at δ3.9 (2H, J_{AB} = 12 Hz). The corresponding triplet at δ60.1 in the off-resonance CMR spectrum of **1** and at δ62.3 in that of its acetyl derivative, confirmed the suggested functionalization. The PMR spectrum contained also a singlet (3H) at δ1.42, due to C-21 H's, strongly downshifted mainly for the influence of a hydroxy group at C-20. This arrangement found further support in the CMR spectrum showing signals at δ28.6 (q) and 76.0 (s) for C-21, and C-20, respectively. The PMR spectrum also showed evidence for a disubstituted double bond (δ5.62, 2H) and for three methyl doublets (J = 7 Hz) at δ0.96, 0.87 and 0.85. These groups were arranged in the side chain as depicted in **1** on the basis of the MS spectrum[9], showing significant fragments at m/z 353 and 327 due to cleavages between C-23 and C-24 and between C-20 and C-22, respectively, from the ion at m/z 424 ($M^+ - H_2O$). The comparison of the CMR resonances (table) of the carbons in the side chain of **1** with those of model compounds[10] allowed us to assign the E stereochemistry to the double bond.

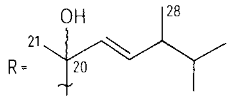
The comparison of the NMR spectra of **1** with those previously reported[7,11] for guggulsterol III (**6**) allowed us to locate the last hydroxy group at C-16 (PMR: δ4.52, H-16, w/2 = 18 Hz; CMR: δ74.0, C-16) with a β-orientation. The resonance of C-17 was particularly diagnostic; as with guggulsterol III[7], the value was strongly upshifted (δ62.7) by the influence of hydrogen bonds among the surrounding hydroxy groups. The unusual very small upshift observed after acetylation (only 0.9 ppm) confirms this hypothesis. The comparison of the NMR spectra of **1** with those of guggulsterol III (**6**) added further support to the location of the hydroxy group at C-18 in **1**, since the C-18 signals exhibited in the spectra of **6** (PMR δ1.15; CMR δ14.9) are lacking in those of **1**.

CMR chemical shifts[a] (in δ from internal TMS) of **1–5**

C	**1**	**2**	**3**	**4 and 5**	
C-13	47.8	47.8	47.6	47.4	
C-14	53.0	53.0	54.1	53.9	
C-15	36.4	36.4	36.4	36.2	
C-16	74.0	74.0	74.2	74.1	
C-17	62.7	62.6	62.8	62.6	
C-18	60.1	60.1	60.1	60.1	
C-19	18.7	18.7	17.4	17.4	
C-20	76.0	75.6	75.4	75.5	
C-21	28.6	28.4	28.7	28.5	
C-22	136.8	138.8	136.9	138.7	136.1
C-23	131.3	125.9	131.2	125.5	132.7
C-24	42.7	41.6	42.9	41.6	38.1
C-25	36.4	34.1	36.4	34.3	29.8
C-26	18.8	22.2	19.8	22.3	11.8
C-27	20.1	22.2	20.1	22.3	
C-28	17.7		17.7		20.3

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 in literature[8,10].





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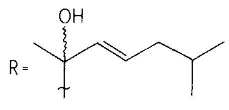

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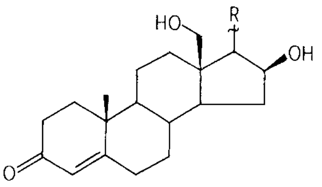

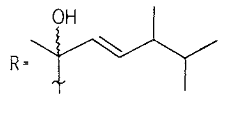

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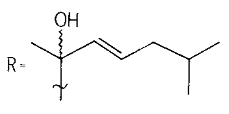

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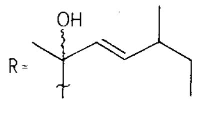

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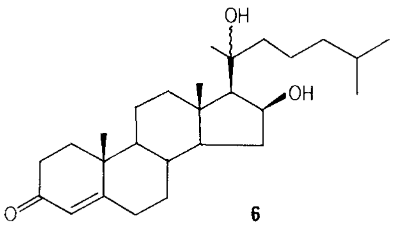

6

The compound **2** ($C_{27}H_{40}O_4$) showed the following spectral data: UV λ_{max} (CH_3OH) 244 nm ($\varepsilon = 11.800$); IR ν_{max} 3450, 1654, 1622 and 1604 cm^{-1}; MS m/z 410 (5%, $M^+ - H_2O$), 395 (11%), 353 (20%), 327 (12%), 253 (53%), 121 (100%); PMR: δ ($CDCl_3$), 7.07 (H-1, d, J = 10 Hz), 6.21 (H-2, dd, J = 10 and 2 Hz), 6.05 (H-4, bs), 5.68 (2H), 4.56 (H-16, w/2 = 18 Hz), 3.92 (2H, ABq J = 12 Hz), 1.43 (3H, 21-H's, s), 1.24 (3H, 19-H's, s), 0.88 (6H, d, J = 7 Hz, isopropyl Me's); CMR (table).

The above data were very similar to those of **1**, significant differences being observed in the MS (m/z 410, $M^+ - H_2O$), PMR (absence of the 3H doublet at δ0.96) and CMR (absence of the off-resonance quartet due to C-28 at δ17.7) spectra; these differences were easily explained by the absence of the methyl group at C-24.

The structure **3** has been suggested on the basis of the following evidence: UV λ_{max} (CH_3OH) 241 nm ($\varepsilon = 15.000$), IR ν_{max} 3450, 1660 and 1610 cm^{-1}; MS m/z 426 (4%, $M^+ - H_2O$), 411 (51%), 383 (12%), 355 (82%), 329 (32%), 255 (100%), 124 (14%), 123 (18%); PMR δ ($CDCl_3$) 5.74 (H-4, s), 5.65 (2H), 4.57 (H-16, w/2 = 18 Hz), 3.92 (2H, ABq, J = 12 Hz), 1.43 (3H, 21-H's, s), 1.22 (3H, 19-H's), 0.96 (3H, d, J = 7 Hz, 28-H's), 0.87 (3H, d, J = 7 Hz), 0.85 (3H, d, J = 7 Hz); CMR (table).

Comparison of these data with those for **1** allowed the conclusion that in **3** there was a different arrangement of the ring A, characterized by the presence of a Δ^4-3-ketone.

The same chromophore was found in the mixture of **4** and **5**, which were not separable by the usual chromatographic techniques. The MS data[12] were in accordance with a C-27 steroid (m/z 412, $M^+ - H_2O$) characterized by the same oxidation pattern as **3**, but the presence in the PMR spectrum[12] of an AB quartet at δ3.91 and of 5 signals attributable to methyls, suggested the possibility of a 1:1 mixture of 2 compounds. The CMR spectrum (table) confirmed this suggestion; it showed all the signals easily assignable, by comparison with ^{13}C resonances of **2** and **3**, to the carbon skeleton of **4** and, in addition, signals at δ136.1 (d), 132.7 (d), 38.1 (d), 29.8 (t), 20.3 (q) and 11.8 (q) due to the presence of a further component with the same carbon nucleus of **4** but with the side chain arranged as depicted in **5**.

The reported spectral data exclude for all the new compounds a possible C-19 hydroxylation. In fact in the CMR spectra (table) the resonances of the C-18 functionalized carbons are constant at δ60.1, while those of C-19 are strongly influenced by the arrangement of the ring A.

All these unusual C-18 hydroxy steroids are biogenetically related to guggulsterol III (**6**), which occurs at the same time in the same organism[7], and which was previously found in the biologically active extracts of the three *Commiphora mukul*[11].

1 This work has been done with the financial support of 'Progetto Finalizzato der la Chimica Fine e Secondaria', C.N.R., Roma. The authors are grateful to Zoological Station of Naples for collecting the gorgonian. Thanks are also due to Mr A. Crispino and Mr G. Scognamiglio for their technical assistance.

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12 Spectral data for **4** and **5**: Ms m/z 412 (3%, $M^+ - H_2O$), 397 (29%), 355 (23%), 329 (5%), 255 (100%), 124 (13%), 123 (16%): PMR: δ ($CDCl_3$) 5.72 (H-4, s), 5.62 (2H, unresolved m), 4.56 (H-16), w/2 = 18 Hz), 3.91 (2H, ABq, J = 12 Hz), 1.42 (H-21, 3H, s), 1.21 (H-19, 3H, s), 0.98 (28 H's of **5**, 1.5H, d, J = 7 Hz), 0.88 (isopropyl methyl protons of **4**, 3H, d, J = 7 Hz), 0.85 (26H's of **5**, 1.5H, t, J = 7 Hz).

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Reversible impairment of hepatobiliary function induced by streptozotocin in the rat[1]

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Summary. The effect of streptozotocin (SZ) on hepatobiliary function was studied in rats on the 1st, 7th and 15th days of treatment. Serum glucose increased significantly on the 1st day, and then remained high. Bile flow, bile acids output and BSP biliary excretion were significantly decreased on the 1st day of treatment, whereas serum sorbitol dehydrogenase was increased. All the parameters tested apart from serum glucose tended to normalize with time. The results suggested a transient toxic effect of SZ on the hepatocyte.

Streptozotocin (SZ) is a highly effective cytotoxic agent for pancreatic β-cells[3] probably due to a high capacity of these cells for accumulating the compound[4]. SZ administration in animals results in an irreversible diabetic state which develops by 24 h post-injection[5]. Besides being an effective diabetogenic substance, SZ possesses carcinogenic, antibacterial and antitumoral properties[4]. Despite the enormous amount of information concerning metabolic disorders in the liver related to SZ-induced diabetes very little is known regarding the hepatobiliary function in SZ-treated animals. It was reported that SZ-induced diabetes in rats showed a decrease in total pool of bile acids[6] as well as in bile flow and biliary excretion of drugs[7].

Furthermore, some modifications of the hepatocyte membrane[8] and a decrease in UDP-glucuronyltransferase activity related to an alteration in the membrane environment of