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STEROIDS FROM THE SEA RASPBERRY GERSEMIA RUBIFORMIS
24-NORCHOLESTA-1,4,22-TRIEN-12β-OL-3-ONE AND ITS ACETATE

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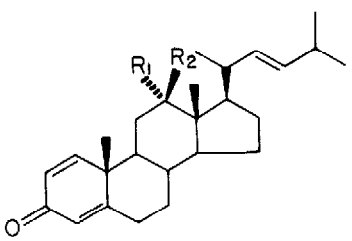
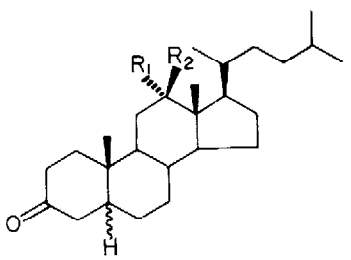
Abstract: Two new 26 carbon steroids with 12β oxygen functions were isolated from the soft coral Gersemia rubiformis and their structures determined from their spectral data and chemical transformations.

In previous publications[1] we reported the isolation of two novel C_{21} steroids from the Alcyonacean coral Gersemia rubiformis (Pallas). We now wish to report the isolation from the same animal of two unusual C_{26} steroids, 24-norcholesta-1,4,22-trien-12β-ol-3-one (1) and its 12β-acetoxy derivative 2. Standard 3β-hydroxy sterols with the 24-norcholesterol side chain are widely distributed in marine invertebrates[2] but C_{26} steroids with other functionalities in the ring system are much less common. Sheikh and Djerassi identified 24-norcholesta-4,22-dien-3-one in the sponge Stelleta clarella.[3] Steroids with an oxygen function at C-12 are rarely encountered in marine species although 24-methylcholestan-3β,5α,6β,12β,25-pentol 25-monoacetate has been isolated from the Alcyonacean coral Sarcophyton elegans.[4] Pregnanes incorporating a $\Delta^{1,4}$-dien-3-one functionality in ring A have recently been isolated from Alcyonacean[1,5] and related[6] corals but this is the first report of such a system in a marine steroid of more than 21 carbons.





1 R_1 = H, R_2 = OH

2 R_1 = H, R_2 = OAc

3 R_1, R_2 = O

4 R_1 = H, R_2 = OH

5 R_1, R_2 = O

Samples of Gersemia rubiformis were collected at Admiral's Cove and Bay Bulls, Newfoundland. The acetone extract of the freeze-dried animals was fractionated by liquid chromatography on silica gel using a stepped gradient of hexanes:chloroform:isopropanol. The acetate 2 and alcohol 1 were contained in separate fractions which eluted with chloroform:isopropanol = 97:3. Fractions rich in the alcohol were subjected to further column chromatography on silica gel (toluene:acetone = 9:1) followed by preparative TLC on alumina (toluene:acetone = 3:1). Final purification by preparative TLC on silica gel (hexanes:isopropanol = 9:1) afforded the alcohol as a colorless oil in 0.009% yield based on the dry weight of the animal. Fractions rich in the acetate were purified in a similar way [column chromatography on silica gel (toluene:acetone = 9:1), preparative TLC on alumina (toluene:acetone = 9:1) and silica gel (hexanes:isopropanol = 19:1)] to afford the acetate as a colorless oil (0.008% of the dry weight of the animal. Both the alcohol and acetate were accompanied by a C_{27} homolog present as about 5% of the mixture which could be separated from the main C_{26} compound by preparative GLC.

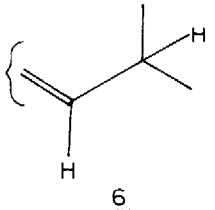
High resolution mass spectrometry established the molecular formula of the alcohol $C_{26}H_{38}O_2$ (m/e 382.2867; calc 382.2872). The presence of the cross conjugated ketone in ring A was indicated by absorptions characteristic[1] of this function in the IR (λ_{max} 1660, 1620, 1595 cm^{-1}) and UV (λ_{max} 244 nm, ε = 12,000) spectra.[7] In the ^1H NMR spectrum[7] signals assigned to the protons at C-1 (δ 7.03, 1H, d, J = 10 Hz), C-2 (δ 6.22, 1H, dd, J = 10, 2 Hz), and C-4 (δ 6.07, 1H, bs, $w_{\frac{1}{2}}$ = 4 Hz) appeared in a pattern typical[1,8] of $\Delta^{1,4}$-dien-3-ones.

The presence of an hydroxyl group in the molecule was demonstrated by a large peak in the mass spectrum at m/e 364 corresponding to loss of water from the parent ion and an hydroxyl absorption (λ_{max} 3400-3600 cm^{-1}) in the IR spectrum. A signal in the ^1H NMR spectrum (δ 3.40, 1H, dd, J = 5,10 Hz) assigned to the proton on the hydroxyl carbon established that the alcohol was secondary and this was confirmed by the doublet in the off-resonance ^{13}C NMR spectrum at δ 78.6 assigned to this carbon. In addition, the ^1H NMR spectrum displayed a signal at δ 5.35 (2H, m) indicative of a disubstituted double bond, and singlets at δ 0.74 (3H) and δ 1.20 (3H) assigned to the C-18 and C-19 methyl groups respectively. In the 400 MHz spectrum the C-21 methyl group appeared as a doublet at δ 1.02 (3H, J = 6Hz) and the C-25 and C-26 methyl groups as a doublet at δ 0.95 (6H, J = 6 Hz). A multiplet (1H) at δ 2.25 was assigned to the proton at C-24 and irradiation of this signal caused the doublet at δ 0.95 to collapse to a singlet and caused partial collapse of the vinyl proton multiplet at δ 5.35. This demonstrated the presence of the part structure 6 and established the position of the double bond at C-22. Since no secondary positions were available in the side chain the hydroxyl group had to be located in the steroid nucleus.

Hydrogenation of 1 over 5% Pd/C afforded a mixture of 5α and 5β-3-ones 4 ($C_{26}H_{44}O_2$, m/e 388.3366, calc 388.3341) in a ratio of 1:3 (by GLC). The mass spectrum of this product confirmed that the hydroxyl function was in the ring system showing a base peak at m/e 271 corresponding to loss of water plus a saturated side chain of C_7H_{15}.

The hydroxyl group could be assigned to one of six secondary positions in the steroid nucleus, C's 6,7,11,12,15, or 16. Oxidation of the hydroxy compound 1 with Jones' reagent

afforded the diketone 3 ($C_{26}H_{36}O_2$, m/e 380.2726, calc 380.2715) whose IR spectrum showed a new carbonyl absorption at λ_{max} 1705 cm^{-1} typical of a saturated six ring ketone.[9a] The UV spectrum (λ_{max} 244 nm, no change in base) indicated that the chromophore of the starting material had not been extended. These data allowed elimination of positions 6,7,15, and 16 from further consideration. The presence of a carbonyl function at C-11 causes a considerable downfield shift of the C-1 proton doublet in the ^1H NMR spectrum of an 11-keto-$\Delta^{1,4}$-dien-3-one such as 7, in which this doublet occurs at δ 7.68.[10] This downfield shift was not observed in the spectrum of the oxidation product of 1, the C-1 proton doublet appearing at δ 6.85. Thus the hydroxyl group could not be located at C-11 and was tentatively assigned to C-12.



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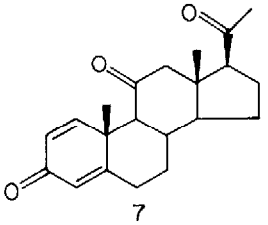

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Steroids with C-12 ketones and 17-alkyl side chains are known[11] to undergo a characteristic mass spectral fragmentation involving loss of ring D and transfer of a hydrogen from C-20 to the oxygen at C-12, resulting in a fragment corresponding to loss of the side chain plus 40 mass units. In the oxidation product 3 this fragmentation was masked by the more facile ring B cleavage[12] promoted by the $\Delta^{1,4}$-dien-3-one system in ring A. However, Jones' oxidation of the hydrogenated alcohol 4 afforded the 3,12-dione 5 ($C_{26}H_{42}O_2$, m/e 386.3174, calc 386.3185) whose mass spectrum displayed a base peak ($C_{16}H_{23}O_2$, m/e 247,1686, calc 247.1698) arising by loss of the side chain plus 40 mass units and establishing the location of the oxygen function at C-12.

The stereochemistry of the hydroxyl group at C-12 was ascertained from the ^1H NMR spectrum of 1. The double doublet at δ 3.40 assigned to the C-12 proton displayed coupling constants (for coupling with the two protons at C-11) which were typical of one axial-axial (J = 10 Hz) and one axial-equatorial (J = 5 Hz) coupling in a system with an electronegative substituent in an equatorial position.[9b] Thus the C-12 proton was axial and the hydroxyl group equatorial (β) and the structural assignment of 1 is complete.

The acetate 2 was characterized by an IR absorption at 1735 cm^{-1} and a sharp three proton singlet at δ 2.09 in the ^1H NMR spectrum. The C-12 proton appeared as a double doublet (1H, J = 5,10 Hz) at δ 4.60. The mass spectrum showed a tiny molecular ion at m/e 424 and a large fragment at m/e 364 arising by loss of acetic acid. In other respects the spectral data were similar to those of the hydroxy compound 1. Treatment of 1 with acetic anhydride in pyridine afforded the acetate 2 which was identical in all respects to the naturally occuring compound.

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