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FOUR NOVEL C_{28} STEROLS FROM LOBOPHYTUM DEPRESSUM

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Abstract

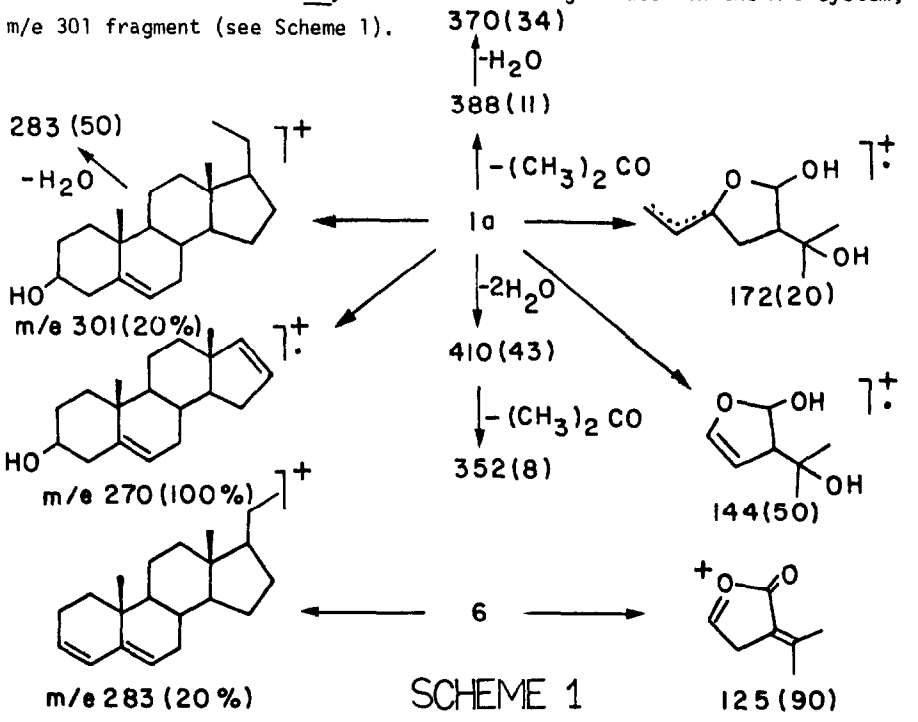
Four novel polyoxygenated C_{28}-sterols have been identified from the soft coral L.depressum (the Gulf of Eilat, the Red Sea): the 22,28-lactol of 24-methylcholest-5-en-3β,22(R),25-triol--28-al (1), 24-methylcholest-5-en-3β,22(R),25,28-tetraol(2) and the corresponding 5,6β-epoxides (3 and 4).

Recently we have reported the isolation and characterization of 11-acetyl-$PGF_{2\alpha}$ and the then unknown prostaglandin 11-acetyl-18-acetoxy-$PGF_{2\alpha}$ from the soft coral Lobophytum depressum[1]. From the CH_2Cl_2 extract of this soft coral, collected in the Gulf of Eilat, the Red Sea, we succeeded in isolating a series of new polyoxygenated C_{28} sterols, the structure of four of which follows[2].

A host of novel marine sterols, most of which have unique alkylation patterns in the side chain, have been isolated and characterized during the recent years[3,4]. In Alcyonaceans[4], by far the most abundant sterols are the 24-methylcholesterols and 24-methylenecholesterols to which the present compounds are closely related. Dichloromethane extracts of freeze-dried specimens of L.depressum, afforded after repetitive silica gel and Sephadex LH-20 column chromatography, several polar steroid fractions[5]. Acetylation and further chromatographic purifications of these fractions provided four different polyacetates, in order of polarity, compounds 1b, 2b, 3b, and 4b. All compounds were found to be homogeneous according to ordinary, and silver nitrate impregnated, silica gel TLC.

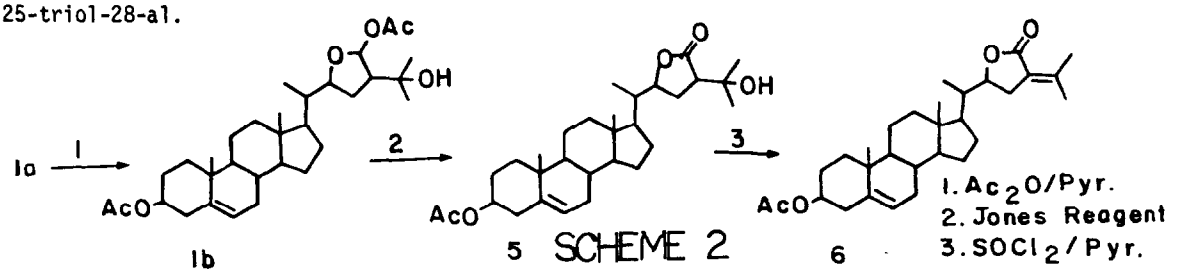
Compound 1a, $C_{28}H_{46}O_4$ m/e 446 (1%) was obtained as an amorphous compound following mild basic hydrolysis (1% KOH, MeOH) of diacetate 1b. The 3β-hydroxy-Δ^5-steroidal structure of this compound (1a) could be unequivocally established from the mass spectrum fragmentation pattern of 1a (as well as of 1b[6]) (see Scheme 1), and from the ^{13}C-NMR spectrum. This spectrum possesses the characteristic resonance lines of the carbon atoms of rings A-D in a 3β-acetoxy-Δ^5-sterol[7,8]. The latter observation locates the remaining three oxygen atoms in the steroid side chain. The ^{1}H-NMR spectrum of compound 1b[9] reveals, beside the well-known signals of the 3β-OAc-Δ^5 site (δ 4.58ddt (H-3), δ 2.31bd (H-4,H-4') and δ 5.37bd (H-6)), two additional low-field protons at δ 6.23d (J=3.0 Hz) and 4.23dt (J=11.4 and 4.8 Hz). Two methyl carbinols observable at δ 1.21s and 1.22s (3H each) suggest a tert. 25-hydroxyl in 1a (and 1b). The various oxygenated carbon signals give rise, in the ^{13}C-NMR spectrum, to four lines: δ74.0d, 82.3d, 71.0s and 100.0d. Among these lines the δ 71.0 singlet agreeswell with the above suggested 25-hydroxylated terminus, while the latter line indicates a carbon atom bearing two oxygens (of the ketal or acetal type). Obtaining a diacetate (1b) from 1a in spite of the three $\geq$CH-O- signals, observable in the ^{13}C-NMR spectrum, can be best explained by the existence of a lactol. This moiety also explains: a) the δ 100.0 doublet in the ^{13}C-NMR spectrum (C-28), b) the low-field doublet at δ 6.23 in the ^{1}H-NMR spectrum

(H-28), c) the six unsaturations of 1a, five of which being located in the A-D system, and d) the inter alia, m/e 301 fragment (see Scheme 1).



SCHEME 1

Proof of the proposed lactol was obtained by the unexpected possibility of diacetate 1b undergoing a Jones oxidation to give the corresponding lactone (5)[10] (see Scheme 2). Obtaining a γ-lactone[11], together with the above mentioned spectral data, can only be explained by a lactol between C-22 and C-28. Final unequivocal proof of the lactol and distinction between the two possible C-22/28 lactols (C-22 or C-24 alkylation), was obtained from the αβ-unsaturated lactone 6[12] - the elimination product of lactone 5 (Scheme 2). Most important in the structure elucidation of lactone 6 were the m/e 125 and m/e 283 peaks in the mass spectrum (see Scheme 1). Thus, the structure of compound 1a was confirmed to be the 22,28-lactol of 24-methylcholest-5-en-3β,22,25-triol-28-al.

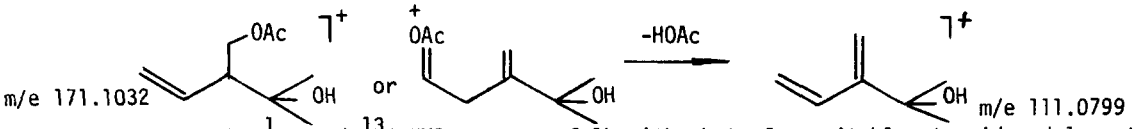


SCHEME 2

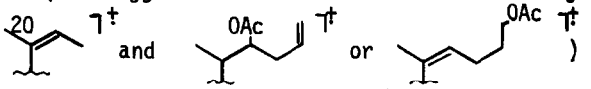
The second, noncrystalline, compound which was eluted from the chromatographic column (2b) was a monohydroxy triacetate (δ 2.03, 2.04 and 2.06, 3H each, m/e 514.3673 ($C_{32}H_{50}O_5$, M-60), 454.3493 (M-2x60), 436.3397 (M-2x60-18) and 376.3151 (M-3x60-18)). As in the case of compound 1b, the 3β-OAc-Δ^5-cholestane skeleton of 2b was determined from the ^{1}H-, ^{13}C-NMR and mass spectral data[13]. Obtaining as parent peak, the M-HOAc fragment (m/e 514,6.4%) is expected from a 3β-OAc-Δ^5 compound[6b].

The ^{1}H-NMR spectrum of compound 2b suggests the same $C(OH)(CH_3)_2$ side chain terminus for 2 as in 1 and in addition one primary acetate (δ 4.12 ABX system, J_{AX}=4.4, J_{BX}=5.0 and J_{AB}=15.4 Hz, 2H) and one secondary acetate (δ 5.04dt J=11.0 & 2.0 Hz,1H). The 25-carbinol is also supported

by the m/e 59.0504 ($(CH_3)_2C=\overset{+}{O}H$) and 396.3041(M-2HOAc-58) fragments. Furthermore, the m/e 171 (6.3%) and m/e 111 (10%) fragments in the mass spectrum, strongly suggest the 22,25,28-triol:



Comparison of the ^{1}H- and ^{13}C-NMR spectra of 1b with that of a suitable steroid model containing the 3β,22-diacetoxy-Δ^5 grouping[14], reveals an excellent agreement of the C-18,19 and 21 proton signals as well as the C-1 to C-22 carbon lines[15], confirming unequivocally the sec. hydroxyl location at C-22. Furthermore, the latter agreement determines also the R configuration of C-22-OH. It has been shown, that the ^{13}C-NMR δ-values of C-16 to C-22 constitute a very delicate probe for distinguishing between the R and S configurations of C-22[16].

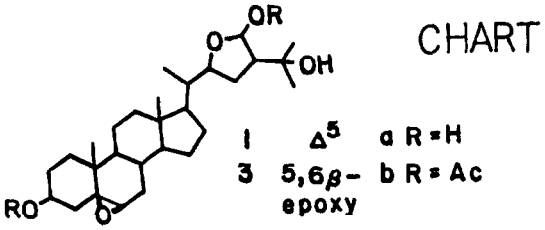
The proposed attachment of the $-CH_2OAc$ group to C-24 (rather than to the second possible location at C-23) is based on the following arguments: a) a shift of 0.5-2.2 ppm of C-26 and C-27, in comparison to a model carbinol[17], which is best explained in the case of 2b by the neighbouring C-28 substituent, b) appearance in the mass spectrum of peaks at m/e 308.2540 ($C_{23}H_{32}^{+}$, 2.3%),m/e 396.3041 ($C_{27}H_{40}O_2$,2.4%) as well as others (the suggested structure of the former being the $\Delta^{3,5}$ systems with the following side chains: 

) and c) the possibility of obtaining compound 1a from 2a by reduction of the lactolic aldehyde to the primary alcohol.

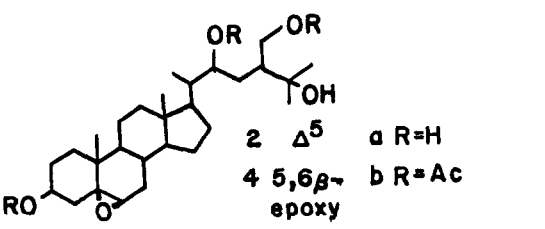
Of special interest is the 22(R) configuration in the above compounds which is thus in accordance with the reported natural 22-hydroxy steroids[18].

The two additional steroids which were obtained in a pure state, compounds 3b and 4b were found to be closely related to compounds 1b and 2b respectively. The ^{13}C- and ^{1}H-NMR spectra confirmed unequivocally the same side chains in 3b and 4b as in 1b and 2b respectively[19]. The NMR spectra indicate also the difference between the two pairs of compounds, that is, replacement of the Δ^5-double bond of 1b and 2b by a 5,6β-epoxide[20] (see chart).

CHART



1 Δ^5 a R=H
3 5,6β-epoxy b R=Ac



2 Δ^5 a R=H
4 5,6β-epoxy b R=Ac

To the best of our knowledge, the above steroids are the first examples of marine 22,28-oxygenated C_{28} steroids. As mentioned above, the until recently reported steroids from soft corals, all belong to the C_{28} category. All possess the conventional C_{28} skeleton, many possess the 5α,6β-hydroxy grouping while others contain in addition the 25-hydroxyl. Thus, compounds 1-4 fit into the same C_{28} category and are the first examples of a new oxidation pattern.

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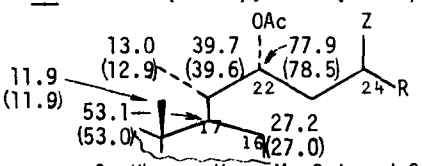
References and Notes

1. S. Carmely and Y. Kashman, Tetrahedron Letters, 21, 875 (1980).
2. Noteworthy is the fact that no cembranoids whatsoever were revealed in L. depressum, in contrast to other Lobophyton species.
3. L. Minale and G. Sodano in "Marine Natural Products Chemistry", D.J. Faulkner and W.H. Fenical ed., Plenum Press, New York 1977, p. 87.
4. F.J. Schmitz, in "Marine Natural Products" Vol. I, P.J. Scheuer ed., Academic Press, New York 1978, p. 241.
5. The crude extract was eluted from a silica gel column using solvents of gradually increasing polarity from petrol ether to ethylacetate. The LH-20 column was prepared and eluted with $CHCl_3$:MeOH, 1:1.

6a. The parent peak in the mass spectrum of 1b is M^+-60 as expected for a 3β-OAc-Δ^5 steroid (ref. 6b)

6b. Z.V. Zaretskii, "Mass Spectrometry of Steroids", Wiley & Sons, 1976.

7. All ^{13}C-NMR spectra were performed on the acetates (because of the low solubility of the parent alcohols) in $CDCl_3$ solutions, with a Bruker WH-90(22.63 MHz) instrument.
8. J.W. Blunt and J.B. Stothers, 9, 439 (1977).
9. ^{1}H-NMR spectra were recorded on a Bruker 270 MHz instrument in $CDCl_3$ solutions.
10. The acidity of the Jones reagent is strong enough for hydrolysing the lactol acetate which can then undergo oxidation to the lactone.
11. ν_{max} 1745 cm^{-1} (the relatively low value is best explained by hydrogen bonding with C-25-OH); ^{1}H-NMR: 0.73s,0.92d,1.03s,1.25s,1.29s,2.03s (3H each), 4.45 ddd(J=10.4, 6.0 and 5.2 Hz,H-22), 4.58m (H-3) and 5.35bd(J=4 Hz,H-5).
12. Compound 6 was obtained by $SOCl_2$/Pyr elimination of 5 at r.t; M.S.(EI,12eV):m/e 408(M^+-60, 59%) and 122(100%); ν_{max} 2990,1750,1735 cm^{-1}, ^{1}H-NMR: 1.87s and 2.25s (Me's 26 and 27), 2.62 (ABX system, H-23,23') and 4.54dt(J=8.0 and 3Hz,H-22).
13. The series of fragments obtained in the mass spectrum conform with the known steroid fragmentation pattern [6b].
14. The Δ^5-3β,22(R),24-triol m which was prepared according to the method of E.J. Corey and H.E. Ensley, J. Org.Chem., 38, 3187 (1973) served as a proper ^{13}C-NMR model compound.
15. ^{1}H-NMR of 2b: δ 0.68s(Me-18), 1.02s(Me-19) and 0.99d(Me-21); ^{13}C-NMR:



2b R=C(OH)Me$_2$ Z=CH$_2$OAc
(m)[14] R=CH$_3$ Z=OAc

16. Y. Letourneux, O. Khuong-Huu, M. Gut and G. Lukacs, J. Org. Chem., 40, 1674 (1975).
17. 3,7-Dimethylocta-1,7-diol is one of the suitable model compounds;F. Bohlman and R. Zeisberg, Org. Mag . Res., 7, 426 (1975).
18. K. Nakanishi et al (Eds) "Natural Product Chemistry", Academic Press, N.Y. 1974, Vol I, Chapter 6.
19. Compound 3b, M.S.(EI,14eV) m/e 590(M^+,5%); ^{1}H-NMR 3.08d(J=1.9Hz,H-6) and 4.76(H-3) full identity with the side chain signals of compound 1b. Compound 4b, M.S.(EI,12eV) m/e 546 (M^+,3%); ^{1}H-NMR 3.08d (J=1.9Hz,H-6) and 4.76m(H-3), full identity of the side chain signals with those of compound 2b.

20a. 3β-Acetoxy-5,6β-epoxy-cholestane serving as a ^{13}C-NMR model, was prepared according to S.G. Levine and M.G. Wall, J. Am. Chem. Soc., 81, 2826 (1959). The measured carbons 1 to 15 are in full agreement (±0.2ppm) with those of compounds 3b and 4b (32.5,29.1,71.3,36.6,62.4, 63.4,29.7,37.9,51.0,34.9,22.0,39.8,42.2,56.1 and 24.2).

20b. The 6α-proton of 3b and 4b can be also used for the determination of the epoxide stereochemistry; A.D. Cross, J. Am. Chem. Soc., 84, 3206 (1962).

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