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ISOLATION AND STRUCTURE ELUCIDATION OF LOBOPHYTOSTEROL, DEPRESOSTEROL AND THREE OTHER CLOSELY RELATED STEROLS

FIVE NEW C_{28} POLYOXYGENATED STEROLS FROM THE RED SEA SOFT CORAL *LOBOPHYTUM DEPRESSUM*

S. CARMELY and Y. KASHMAN*
Department of Chemistry, Tel-Aviv University, Ramat Aviv 69978, Israel

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Abstract—A series of polyoxygenated sterols were isolated from the soft coral *Lobophytum depressum*. The structure of five of these sterols was elucidated and determined to be; 5β,6β - epoxy - 24ξ - methylchloestan - 3β,22(R),25 - triol (**2a**), 22(R),28 - oxido - 24ξ - methylcholest - 5 - en - 3β,25,28 - triol (**1c**, lobophytosterol), 24ξ - methyl - cholest - 5 - en - 3β,22(R),25,28 - tetraol (**1b**, depresosterol), and 5β,6β - epoxy derivatives of lobophytosterol and depresosterol, compounds **2c** and **2b** respectively. The structures were determined mainly on the basis of the fully interpreted ^1H and ^{13}C NMR spectra as well as the mass spectrum and several chemical transformations which resulted in unambiguous structures. All identified compounds belong to the C_{28} category and are the first examples of marine sterols possessing the C_{28}-atom in various oxidative states.

24-Methylchloesterols and 24-methylenechloesterols are by far the most abundant sterols in Alcyonaceans.[1] Many of these sterols possess the 5α,6α-dihydroxy grouping while others contain in addition the 25-hydroxyl (e.g. lobosterol).[2]

The above C_{28} sterols are believed to be obtained, in the animal tissue, by methylation, as in other known alkylations, of the cholesterol side-chain. Some animals and probably some coelenterates, too, have also the ability to dealkylate C_{28} (and C_{29}) sterols to produce cholesterol.[1]

In connection with the demethylation ability, it is of particular interest to discover new sterols which possess oxygenated C-28 groupings which may be intermediates in the demethylation pathway.

The present work describes 5 new sterols all belonging to the polyoxygenated C_{28} category in which the C-28 atom appears as a CH_3, CH_2OH or CHO group.[3]

All five compounds were isolated from the CH_2Cl_2 extract of freeze-dried specimens of *Lobophytum depressum* collected in the Gulf of Eilat (The Red Sea). Repetitive silica gel and Sephadex LH-20 column chromatography afforded several fractions containing *inter alia* polar steroids as well as our previously reported $PGF_{2\alpha}$ derivatives.[4] Acetylation of the above fractions and further chromatographic purifications yielded five steroidal polyacetates, compounds **4d, 3f, 3e, 4f** and **4e**, in their order of polarity. All compounds were found to be homogeneous according to ordinary and silver nitrate impregnated silica gel tlc.

The first compound to be eluted from the chromatographic column, compound **4d**, $C_{32}H_{52}O_6$, was determined, on the basis of its NMR (Tables 1 and 2) and mass spectrum (Table 3), to be 5β,6β - epoxy - 24ξ - methylchloestan - 3β,22(R),25 - triol diacetate (Fig. 1). The steroidal 3β-OAc-5β,6β-epoxy AD ring system of this compound was established by comparison of its ^{13}C δ-values with those of 3β-OAc-5β,6β-epoxy cholesterol.[a] Carbons C-1 to C-15 of **4d** were found to be in excellent agreement with those of the model compound (±0.2 ppm) (Table 2). A small chemical shift difference in C-16 (1.1 ppm) and to some extent a larger difference in C-17 (3.1 ppm) point furthermore to structural changes in the nearby surrounding of the side chain. Next, a 22-acetoxy grouping was suggested by the *m/e* 172 $(C_9H_{16}O_3]^+$, 11%) and *m/e* 112 (172-HOAc, 100%) fragments, in the mass spectrum of compound **4d**, obtained due to a cleavage of the C_{20}–C_{22} bond (in a fragmentation process which involves a hydrogen migration). The 22 alcohol could have been confirmed by comparison of the ^{13}C δ-values ($CDCl_3$) of C_{17}, C_{20} and C_{21} of compound **2a** (the natural triol from which diacetate **4d** was obtained) with those of the corresponding resonance lines of an epimeric pair of 22-hydroxy cholesterols.[b,6] Furthermore this comparison established not only the location of the hydroxyl but also its absolute configuration at C-22 to be the R configuration.[c]

The location of the third hydroxyl in compound **4d**, the tertiary one (which does not undergo acetylation under the usual Ac_2O/Pyr. r.t. conditions which acetylates the C-3 and C-22 OH groups), was deduced from: (a) the *m/e* 59 fragment ($(\overset{+}{C}H_3)_2C{=}H$, 10%); (b) from the ^1H δ-values of Me-26 and Me-27 (Table 1); and (c) from the ^{13}C δ-values of the side chain terminus (Table 2) which were compared with a suitable aliphatic model compound.[d]

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[a] 3β-Acetoxy-5β,6β-epoxy-cholestane was synthesized according to Levine and Wall[5a] and was measured by us in $CDCl_3$ solution. The 5,6-epoxy configuration could have been determined also on basis of the chemical shift and coupling constants of the 6α-proton.[5b]

[b]	C-17	C-20	C-21
2a	53.4	42.6	12.3
22R	53.2	42.6	12.5
22S	52.6	40.3	11.6

[c] One can arrive at the same conclusion by comparison of the C-22 and C-23 ^{13}C δ-values in the 22-alcohol to that of the 22-acetate.[7]

[d] 3,7-Dimethylocta-1,7-diol is one of the suitable model compounds.[8]

Table 1. ^{1}H NMR chemical shifts and multiplicity data (270 MHz, $CDCl_3$)

H at C	4d			3e			4e			3f			4f		
	δ	mult	J,Hz	δ	mult	J,Hz	δ	mult	J,Hz	δ	mult	J,Hz	δ	mult	J,Hz
3	4.76	m		4.60	ddt	12.0, 4.0, 8.0	4.77	m		4.58	dt	12.0, 4.0, 8.0	4.77	m	
4				2.31	brd	8.0				2.31	brd	8.0			
6	3.07	d	1.9	5.37	brd	3.8	3.08	d	1.9	5.37	brd	4.3	3.08	d	1.9
18	0.64	s		0.68	s		0.64	s		0.70	s		0.66	s	
19	1.00	s		1.02	s		1.00	s		1.02	s		1.00	s	
21	0.91	d	6.7	0.94	d	6.8	0.91	d	6.8	0.94	d	6.8	0.92	d	6.7
22	4.99	dt	10.2, 2.5	5.04	dt	11.2, 2.0	5.02	dt	11.2, 2.0	4.23	dt	11.4, 4.8	4.23	dt	11.4, 4.6
24										2.42	dt	9.3, 3.0	2.42	dt	9.3 3.0
26,27	1.12, 1.19	s, s		1.22, 1.25	s, s		1.21, 1.24	s, s		1.21, 1.22	s, s		1.21, 1.22	s, s	
28	0.91	d	6.7	4.10, 4.15	ABXsys.	15.4, 4.4, 5.0	4.10, 4.15	ABXsys	15.4, 5.0, 4.5	6.23	d	3.0	6.23	d	3.0
OAc	2.02	s		2.03	s		2.02	s		2.03	s		2.03	s	
OAc	2.04	s		2.04	s		2.03	s		2.06	s		2.06	s	
OAc				2.06	s		2.06	s							

Table 2. ^{13}C NMR chemical shifts of the sterols (22.63 MHz, $CDCl_3$).

C	Mult.	2a	3e	3f	3j	4d	4e	4f	4k
1	t	32.2*	38.2	38.2	38.1	32.4	32.5	32.5	32 5
2	t	32.7*	27.8	27.8	27.8	29.3	29.4	29.4	29.1
3	d	69.5	74.0	74.0	74.0	71.4	71.4	71.4	71.3
4	t	41.3	37.1	37.0	37.0	36.7	36.8	36.8	36.6
5	s	63.0	139.7	139.7	139.7	62.5	62.5	62.6	62.4
6	d	63.8	122.6	122.5	122.6	63.6	63.6	63.6	63 4
7	t	31.1	31.9	31.9	31.8	29.8	29.8	29.8	29 7
8	d	37.3	31.8	31.9	31.8	38.0	38.1	38.1	37 9
9	d	51.5	50.1	50.0	50.0	51.0	51.1	51.1	51 0
10	s	35.0	36.7	36.6	36.7	35.0	35.1	35.1	34.9
11	t	22.0	21.1	21.0	21.1	21.9	22.0	22.0	22 0
12	t	39.9	39.7	39.6	39.6	39.8	39.8	39.8	39.8
13	s	42.3	42.8	42.9	42.6	42.6	42.8	42.9	42.2
14	d	56.0	56.3	56.3	56.4	55.8	55.9	55.8	56 1
15	t	24.4	24.4	24.4	24.3	24.2	24.4	24.3	24.2
16	t	27.6	27.2	27.5	27.0	27.2	27.2	27.4	28.1
17	d	53.4	53.1	53.8	52.9	53.2	53.1	53.8	56.1
18	q	11.8	11.9	11.8	11.8	11.8	11.8	11.7	11.7
19	q	17.1	19.4	19.3	19.4	17.0	17.1	17.0	17.0
20	d	42.6	39.7	37.2	39.1	39.8	39.8	37.2	35 7
21	q	12.3	13.0	12.3	13.0	12.9	12.9	12.4	18.7
22	d/t	71.0	77.9	82.3	75.6	78.3	77.7	82.3	36 1
23	t	29.8	25.2	26.5	29.6	25.0	25.1	26.5	23.8
24	d/t	42.6	46.4	57.0	130.1	43.1	46.4	57.0	39.5
25	s/d	73.2	72.8	71.0	128.4	73.6	72.7	71.0	27.2
26	q	29.6	28.4	28.6	19.4	28.4	28.4	28.6	22.7
27	q	26.5	27.2	26.7	21.1	27.2	27.2	26.8	22.7
28	q/t/d	16.0	66.0	100.0	66.6	16.9	66.0	100.0	
CH_3-Ac	q		21.0	21.5	21.1	21.6	21.1	21.5	21.2
CH_3-Ac	q		21.5	21.5	21.5	21.3	21.5	21.5	
CH_3-Ac	q		21.5		21.5		21.5		
CO-Ac	s		170.6	170.6	170.5	170.6	170.6	171.0	170.4
CO-Ac	s		171.0	171.0	170.5	171.0	171.0	171.0	
CO-Ac	s		171.0		171.1		171.0		

*These signals may be interchanged.

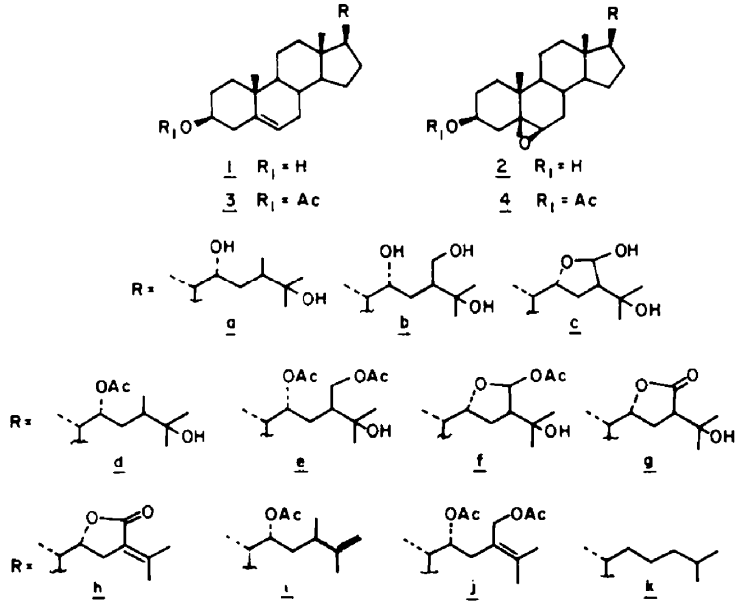


Fig. 1. Structure of the sterols.

Table 3. Partial mass spectrum fragments of the sterol acetates (see note Scheme 2)

Fragment	3e[a]	3f[b]	4d[b]	4e[c]	4f[b]
M^+		530(<1)	532(2)	590(5)	546(3)
M^+-AcOH	514.3673(6.4)	470(7)	472(10)		486(72)
M^+-AcOH-H_2O		452(10)	454(12)	512(31)	468(90)
M^+-2AcOH	454.3493(1.1)	410(5)	412(3)	470(17)	426(17)
M^+-2AcOH-H_2O	436.3397(2.9)	392(8)	394(9)	452(24)	408(34)
M^+-(C_{20} to C_{28})			330(12)	330(45)	
			329(17)	329(100)	329(5)
M^+-(C_{20} to C_{28})-AcOH	254.2006(3.5)		270(12)	270(29)	270(11)
	253.1947(5.3)	253(5)	269(18)	269(5)	
C_{22} to C_{28}			172(11)		
(C_{22} to C_{28})-AcOH	171.1032(6.3)	126(10)	112(100)	170(35)	126(20)
C_{25} to C_{27}	59.0504(1.1)'	59(17)	59(10)	59(58)	59(21)

a. H.R., EI, 70eV ; b. L.R., EI, 12eV ; c. L.R., EI, 14eV.

In paranthesis are given the percentages of the relative fragment intensities.

For more non-general fragments - see Experimental.

Further comparison of the ^{13}C-chemical shifts of **2a** with those of the side chain of ecdysone (22R,25 diol) and makisterone-A(20,22R,25 triol, 24-Me)[9] (all measured in d_5-Pyridine)[e] indicated clearly that the additional side chain methyl (whose presence was determined by the mass spectrum and ^{1}H NMR spectrum) must be at C-24.

Comparison of **2a** with ecdysone exhibits the following effects: (a) a γ-effect on C-22,26 and 27 (−0.9, −2.9 and −0.4 ppm respectively); (b) a β-effect on C-23 and 25 (+4.7 and +3.3 ppm respectively); and (c) an α-effect on C-24 (+1 ppm). At the same time comparison of compound **2a** with makisterone-A reveals similar chemical shifts of the C-atoms of the side chain terminus, in both compounds. It is interesting to note that in both latter compounds one of the C-26, 27 methyl groups is by *ca.* 2 ppm (in comparison to ecdysone) more upfield shifted, by the 24-methyl group, than the other methyl, pointing to a conformation of the side chain end in which the more affected methyl is skew to the C-24–Me while the other one is anti. The absolute configuration of C-24 should have been possible to be determined by comparison of the NMR data of compound **2a** or **4d** with that of a suitable model whose structure was unequivocally established by an X-ray analysis. However, as the δ-values of the epimers depend on the conformer populations, each group which will influence this population (and of course also the magnetic anisotropy) will change also the δ-values. Thus, the presence of two OH-groups in the side chain of **2a** is expected to strongly influence the chemical shifts, and only compounds with exactly the same substitution pattern (with no additional hydroxyl as in makisterone-A) should be reliable comparison models.[10] Lack of such epimers prevented us from determining the C-24 configuration in **2a**.[f]

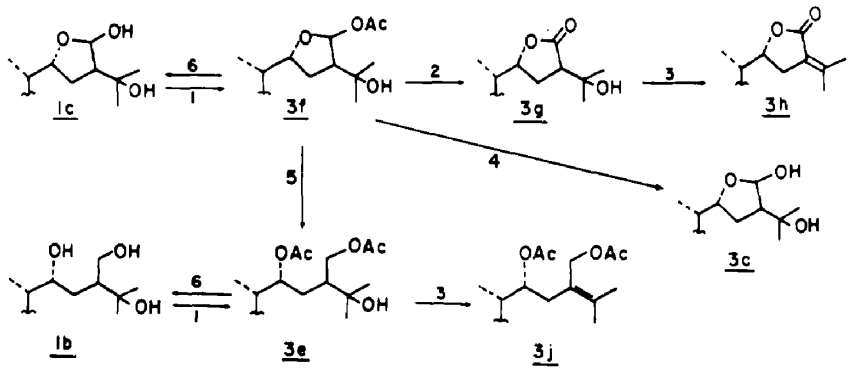
Lobophytosterol (**1c**) (Fig. 1) the second steroid which was purified, $C_{28}H_{46}O_4$, *m/e* 446 (1%), was obtained as an amorphous substance following mild basic hydrolysis (1% KOH, MeOH) of diacetate **3f**. The 3β-acetoxy-Δ^5-steroidal moiety of compound **3f** was unequivocally established by the ^{13}C NMR spectrum (possessing the characteristic resonance lines of the corresponding carbon atoms of rings A–D), as well as from the mass spectrum (see Tables 2 and 3 and Experimental).

The ^{1}H and ^{13}C NMR spectra of compound **3f** (Tables 1 and 2) suggested also the 25-hydroxy and 22,28-lactol functions in the side chain of lobophytosterol (**1c** and in its diacetate **3f**). Most characteristic for the lactol were the methinoxy group which did not undergo acetylation, and the hemiacetal carbon which gives rise to the δ 100.0 doublet in the ^{13}C NMR and the 6.23d signal in the proton NMR spectrum.

Proof of proposed lactol and its location in the side chain was obtained by oxidation of lobophytosterol acetate (**3f**) to lactone **3g** (ν_{max} 1745 cm^{-1}) and the concurrent water elimination to give the α,β-unsaturated lactone **3h** (ν_{max} 1750 cm^{-1}) (Scheme 1). Compound **3f** could have been oxidised, although the esterified lactol group, a fact which can be explained due to the acidic oxidation conditions which were strong enough to hydrolyse at first place the labile lactol acetate. Water

	C-20	21	22	23	24	25	26	27	28
2a	43.1	12.6	71.8	29.6	43.1	71.8	26.4	29.2	16.6
ecdysone	41.5	13.2	72.7	24.9	42.1	68.5	29.3	29.6	—
makisterone-A	—	—	74.8	34.5	41.8	72.2	26.2	28.3	15.5

[f]The C-24 configuration of 24-alkylated sterols may prove to be a valuable clue to their origin (the soft coral vs the unicellular symbiotic alga).[1]



Scheme 1. Chemical transformations of lobophytosterol and depresosterol.

elimination from compound **3g** with $SOCl_2$/Pyr resulted in the unsaturated lactone **3h**. The relatively low carbonyl absorption of the γ-lactone in the IR spectrum of compound **3g** agrees well with the neighbouring 25-hydroxyl (a hydrogen bond) and, indeed after the water expulsion to give the α,β-unsaturated lactone, the CO stretching is shifted to higher wave length (rather than to lower one, as is usually observed during such eliminations). Obtaining the exocyclic α,β-unsaturated lactone, possessing the isopropylidene group (δ 1.87 (Me) and δ 2.25 (Me) ppm) established unequivocally the structure of the side chain of lobophytosterol.[8]

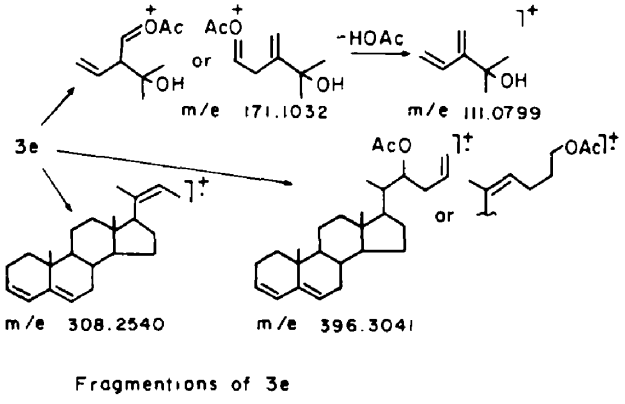
Most important in the structure elucidation of lactone **3g** were the *m/e* 125 and *m/e* 283 peaks in the mass spectrum resulting from the C-20/22 bond cleavage.[3]

The third compound which was eluted from the chromatographic column, was the triacetate (**3e**) of depresosterol (**1b**) (Fig. 1) (δ 2.03, 2.04 and 2.06, 3H-each, and *m/e* 514.3673 ($C_{32}H_{50}O_5$, M-60), as well as peaks standing for M-2×60, M-2×60-18 and M-3×60-18, see Tables 1 and 3). As above in the case of compound **3f**, the 3β-OAc-Δ⁵-cholestane skeleton as well as the C(OH)$(CH_3)_2$ side chain terminus of depresosterol, were determined from the NMR and mass spectral data (Tables 1–3). Beside the 25-carbinol which was evident from the *m/e* 59.0504 ($Me_2C{=}\overset{+}{O}H$) and 296.3041 (M-2HOAc-58) fragments, the peaks at *m/e* 171 and 111 strongly suggest two additional OH groups i.e. a 22,25,28-triol (see Scheme 2).

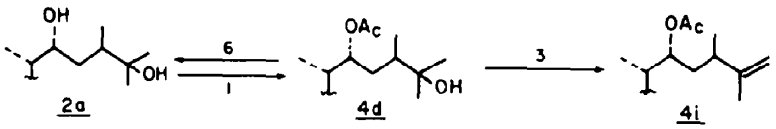
Comparing the ^{13}C NMR spectrum of compound **3e** with that of compound **4d** containing the 22(R) acetoxy grouping reveals an excellent agreement of C-13 to C-18 and C-20 to C-22 (Table 2) thus confirming the 22(R) acetoxy moiety (see above for the 22R/22S determination).

The location of the primary alcoholic group was the last structural feature which had to be established. The chemical shift of C-26 and C-27, together with several fragmentations in the mass spectrum *vide supra* (see Scheme 2) could be best rationalized by a 28-methylenoxy group. Unequivocal proof for the location of the latter group was obtained from (a) H_2O elimination from compound **3e** (see Scheme 1, and Experimental) to give compound **3j**, possessing the tetrasubstituted Δ^{24} double bond (^1H NMR, δ: 1.74s (Me_{26}), 1.67s (Me_{27}) and 4.00

[8]As in case of compound **2a**, and for the same reasons, the absolute configuration of C-24 could not be determined.



Scheme 2. Mass spectrum fragmentations of **3e**. (Four further fragmentations as well as the fragmentations of the other sterols see Experimental. Many of the fragmentation patterns follow known ones in the steroid field[12,13]).



Scheme 3. Chemical transformations of compound **4d**.

and 4.03s (CH_2OAc))[a] and (b) by reduction of lobophytosterol (**3f**) with $NaBH_4$ to obtain after concurrent acetylation the triacetate (**3e**) of depresosterol (Scheme 1).

The last two sterols which were obtained in a pure state compounds **4e** and **4f**, were determined to be 5β,6β-epoxy depresosterol and 5β,6β-epoxy lobophytosterol respectively (Fig. 1). Their structures were unequivocally established by comparison of their side chain data with the parent Δ^5-sterols (**3e** and **3f** respectively) while the steroidal ring system was in full agreement with that of 3β-acetoxy-5β,6β-epoxy cholestane as well as compound **4d** (see Tables 1–3).

To the best of our knowledge the above sterols are the first examples of marine 22,28-oxygenated sterols and are of special interest from the biogenetical point of view *vide supra*.

EXPERIMENTAL

IR spectra were recorded on a Perkin-Elmer Model 177 spectrophotometer. Low Resolution Mass Spectra were recorded on a Dupont 21-491B spectrometer and high Resolution Mass Spectra were taken on a Varian MAT 731 instrument. Optical rotations were measured on Bellingham and Stanley Polarimeter, using a 10 cm microcell. Melting points were determined on a Thomas Hoover capillary m.p. aparatus and are reported uncorrected. ^1H NMR spectra were recorded on Brucker WH-270 and Brucker WH-90 NMR spectrometers, and ^{13}C NMR spectra were recorded on a Brucker WH-90 (22.63 MHz) NMR spectrometer; all chemicals shifts are reported with respects to TMS (δ_0).

Collection and extraction of the soft coral

The soft coral *Lobophytum depressum* (Tixier-Durivault, 1966) was collected in the Gulf of Eliat (The Red Sea) in April 1978. The soft coral was deep frozen immediately after collection, and then freeze-dried to give the dry material (1 kg). Dry soft coral (1/2 kg) was first extracted with petrol ether in a soxhlet for 24 hr and then with methylenechloride. Evaporation of the methylenechloride extract gave a red gum (5.4 gr. 1.1% dry weight).

Isolation of Steroles **3e**, **3f**, **4d**, **4e** *and* **4f**. The crude extract (5.4 gr) was applied to a column of Sephadex LH-20 using chloroform/methanol 1:1 as eluant to obtain in fractions 9–12 (200 ml each fraction), among other compounds, a mixture of polyoxygenated sterols (~500 mg). The residue obtained after evaporation of the combined fractions (9–12) was acetylated with Ac_2O/Pyr in the usual manner. The pyridine was then evaporated *in vacuo*, and the residue (~600 mg) chromatographed on a silica gel H column, using solvents of gradually increasing polarity. Fraction 3, eluted with petrol ether/methylene chloride 1:1 gave compound **4d** (70 mg), fraction 4, eluted with petrol ether/methylene chloride 1:2 gave compound **3f**, (55 mg), fraction 6, eluted with petrol ether/methylene chloride 1:4 gave **3e** (110 mg), fraction 7, eluted with methylene chloride gave **4f** (13 mg) and fraction 8, eluted with methylene chloride/ethyl acetate 9:1 gave **4e** (48 mg).

(22*R*) - 5β,6β - *Epoxy* - 24 - *methyl* - 5α - *cholestan* - 3β,22,25 - *triol* - 3,22 - *diacetate* (**4d**). An oil, $[\alpha]_D^{24} - 2°$ (c, 3.4, $CHCl_3$); IR (Neat) 3550, 2965, 1735, 1475, 1360, 1260, 1240, 1120 cm^{-1}; M.S. (EI, 12 eV) *m/e* 532 (M^+, $C_{32}H_{52}O_6$, 2%), 514 (4), 472 (10), 454 (12), 412 (3), 394 (9), 371 (5), 354 (9), 330 (12), 329 (17), 311 (8), 310 (10), 299 (12), 270 (12), 269 (18), 172 (11), 149 (27), 123 (49), 112 (100%), 95 (54), 83 (18), 81 (13), 59 (10).

(22*R*, 24ξ, 28ξ) - 22,28 - *Oxido* - 24 - *Methylcholest* - 5 - *en* - 3β,25,28 - *triol* - 3,28 - *diacetate* (**3f**). An oil, $[\alpha]_D^{24} + 11°$ (c, 1.0, $CHCl_3$); IR ($CHCl_3$) 3500, 2960, 1740, 1470, 1380, 1260, 1180, 1030, 1000 cm^{-1}; M.S. (EI, 12 eV) *m/e* 530 (M^+, $C_{32}H_{50}O_6$, > 1%), 470 (7), 455 (13), 452 (10), 410 (5), 392 (8), 382 (10), 358 (6), 312 (4), 298 (4), 282 (7), 253 (5), 172 (45), 126 (10), 114 (100), 109 (37), 98 (85), 84 (72), 82 (85), 59 (17).

(22*R*, 24ξ) - 24 - *Methylcholest* - 5 - *en* - 3β,22,25,28 - *tetraol* - 3,22,28 - *triacetate* (**3e**). An oil, $[\alpha]_D^{24} - 6°$ (c, 1.1, $CHCl_3$); IR (Neat) 3500, 2970, 1730, 1380, 1180, 970 cm^{-1}; M.S. (H.R., EI, 70 eV) *m/e* 514.3673 (M^+–AcOH, $C_{32}H_{50}O_5$, 6.4%), 4.4.3493 (1.1), 436.3397 (2.9), 396.3041 (2.4), 379.2977 (1.2), 376.3151 (1.9), 322.2642 (3.5), 254.2006 (3.5), 253.1947 (5.3), 228.1911 (1.7), 227.1792 (1.8), 213.1623 (3.8), 171.1032 (6.3), 159.1168 (4.4), 145.1016 (4.0), 122.0733 (8.1), 111.0799 (10.3), 108.0570 (20.5), 94.0408 (100%), 59.0504 (1.1).

(22*R*, 24ξ, 28ξ) - 5β,6β - *Epoxy* - 22,28 - *Oxido* - 24 - *methyl* - 5α - *cholestan* - 3β,25,28 - *triol* - 13,28 - *diacetate* (**4f**). An oil, $[\alpha]_D^{24} + 57°$ (C, 1.3, $CHCl_3$); IR (Neat) 3500, 2965, 1740, 1470, 1380, 1270, 1190 cm^{-1}; M.S. (EI, 12 eV) *m/e* 546 (M^+, $C_{32}H_{50}O_7$, 3%), 486 (72), 471 (100%), 468 (90), 426 (17), 408 (34), 329 (5), 317 (5), 270 (11), 244 (10), 190 (20), 162 (9), 142 (31), 126 (20), 108 (46), 82 (77), 59 (21).

(22*R*, 24ξ) - 5β,6β - *Epoxy* - 24 - *methyl* - 5α - *cholestan* - 3β,22,25,28 - *tetraol* - 3,22,28 - *triacetate* (**4e**). An oil, $[\alpha]_D^{24} + 22°$ (c, 0.4, $CHCl_3$); IR (Neat) 3500, 2975, 1735, 1375, 1260, 1140 cm^{-1}; M.S. (EI, 14 eV) *m/e* 590 (M^+, $C_{34}H_{54}O_8$, 5%), 572 (12), 512 (31), 470 (17), 452 (24), 432 (29), 410 (29), 397 (31), 329 (100%), 303 (24), 281 (22), 270 (29), 269 (5), 258 (22), 243 (22), 170 (35), 148 (39), 138 (41), 59 (58).

Dehydration of **4d**. A cold solution of thionylchloride in pyridine (1 ml) was added to a cold solution (0°) of **4d** (27 mg) in pyridine (1 ml), the reaction mixture was kept at 0° for 30 min. The pyridine was then evaporated in vacuo to obtain **4i** (20 mg, 80% theoretical) as an oil, IR ($CHCl_3$) 2945, 2875, 1740, 1730, 1465, 1440, 1380, 1365, 1255, 1020, 960 cm^{-1}, ^1H NMR (270 MHz $CDCl_3$) δ 0.63 (3H, s), 0.88 (3H, d, J = 6.8 Hz), 1.00 (3H, s), 1.02 (3H, d, J = 7.0 Hz), 1.64 (3H, s), 2.01 (3H, s), 2.03 (3H, s), 3.08 (1H, d, J = 1.9 Hz), 4.63 (1H, s), 4.73 (1H, s), 4.76 (1H, m), 4.79 (1H, dt, J = 10.3, 3.2 Hz). M.S. (EI, 12 eV) *m/e* 514 (M^+, $C_{32}H_{50}O_5$, 1%), 499 (10), 454 (30), 439 (100), 394 (12), 339 (10), 269 (14), 124 (22), 96 (74), 85 (30), 83 (37), 79 (20).

Dehydration of **3e**. Dehydration of **3e** (11 mg) in the same manner as described above, gave **3j** (8 mg, 85% theoretical) as an oil; IR ($CHCl_3$) 2950, 1730, 1460, 1440, 1350, 1250, 1030 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.67 (3H, s), 0.91 (3H, d, J = 6.8 Hz), 1.01 (3H, s), 1.67 (3H, s), 1.74 (3H, s), 2.03 (6H, s), 2.05 (3H, s), 2.31 (2H, d, J = 8.0 Hz), 4.00, 4.03 (2H, ABsys.), 4.60 (1H, m), 4.86 (1H, dt, J = 11.1, 3.0 Hz), 5.37 (1H, brd, J = 3.8 Hz). M.S. (EI, 12 eV) *m/e* 496 (M^+–AcOH, $C_{32}H_{48}O_4$, 100%), 454 (10), 436 (16), 376 (13), 329 (11), 254 (13), 253 (19), 122 (21), 111 (13), 108 (18), 94 (58), 85 (13), 83 (18).

Treatment of **3f** *with sodium borohydride at R.T.* $NaBH_4$ (10 mg) was added to a solution of **3f** (9 mg) in methanol (5 ml) and the solution was stirred at room temperature for 2 hr. The solvent was then evaporated and the residue partitioned between water (5 ml) and chloroform (3 × 10 ml). The combined chloroform phase was dried over $MgSO_4$ and the solvent evaporated to give **3e** (7 mg, 80% theoretical); IR ($CHCl_3$) 3500, 2960, 1735, 1470, 1360, 1260, 1030, 995 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 0.71 (3H, s), 0.93 (3H, d, J = 6.5 Hz), 1.02 (3H, s), 1.20 (3H, s), 1.25 (3H, s), 2.03 (3H, s), 2.31 (2H, brd, J = 8.0 Hz), 4.15 (1H, dt,

[a]Compound **4d** yields upon the same elimination conditions, the Δ^{25} compound (**4i**) possessing a side chain end previously identified in a marine sterol[11] (see Scheme 3).

J = 11.7, 4.5 Hz), 4.62 (1H, m), 5.01 (1H, d, J = 3.0 Hz), 5.37 (1H, brd, J = 3.9 Hz).

Jones Oxidation of **3f**. Jones reagent (2 drops) was added to a solution of **3f** (10 mg) in acetone (2 ml), and the reaction mixture was stirred at 0° for 10 min. Excess reagent was destroyed by addition of methanol. The reaction mixture was then worked up as usual to obtain **3g** (9 mg, 95% theoretical) as an oil. IR ($CHCl_3$), 3500, 2960, 1745, 1725, 1470, 1380, 1260, 1180, 1030, 965 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.73 (3H, s), 0.92 (3H, d, J = 6.8 Hz), 1.03 (3H, s), 1.25 (3H, s), 1.29 (3H, s), 2.03 (3H, s); 2.31 (2H, d, J = 8.0 Hz), 2.76 (1H, dd, J = 12.0, 9.2 Hz), 4.45 (1H, ddd, J = 10.4, 6.0, 5.2 Hz), 4.58 (1H, m), 5.35 (1H, brd, J = 4.0 Hz).

Dehydration of **3g**. Dehydration of **3g** (7 mg) in the same manner as described above gave **3h** (6 mg, 92% theoretical) as an oil. IR ($CHCl_3$) 2990, 1750, 1735, 1380, 1265, 1120, 1050, 875 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.73 (3H, s), 0.82 (3H, d, J = 6.4 Hz), 1.02 (3H, s), 1.87 (3H, s), 2.04 (3H, s), 2.25 (3H, s), 2.31 (2H, brd, J = 8.0 Hz), 2.62 (2H, ABX system), 4.54 (1H, dt, J = 8.0, 3.0 Hz), 4.60 (1H, m), 5.38 (1H, brd, J = 3.8 Hz). M.S. (EI, 12 eV) *m/e* 408 (M^+–AcOH, $C_{28}H_{40}O_2$, 59%), 355 (37), 283 (20), 259 (40), 208 (70), 191 (61), 179 (93), 165 (77), 149 (48), 138 (60), 125 (90), 122 (100), 110 (91), 86 (95).

Conversion of **3f** *to* **3e**. Sodium Borohydride (15 mg) was added to a solution of **3f** (15 mg) in methanol (5 ml) and the solution was boiled to reflux for 2 hr. The solution was acidified with acetic acid and the solvent was then evaporated. Acetylation of the residue in the usual manner (Ac_2O/Pyridine) and filtration through silica gel gave an oily substance (12 mg, 85% theoretical) identical in all respects with **3e**.

Basic Hydrolysis of Compounds **3e**, **3f**, **4d**, **4e** *and* **4f**. Each one of these compounds was dissolved in 0.5N methanolic KOH (5 ml) and was allowed to stand at room temperature for 1 hr. The solution was then neutralized (10% aq. HCl), and the solvent removed under vacuo. The residue was dissolved in water (1 ml) and extracted with 9:1 chloroform/ethanol solution (3 × 3 ml). Evaporation of the combined fractions gave a non-crystallizing solid. Hydrolysis of **3e** (15 mg) gave **1b** (7 mg) as an amorphic solid. m.p. 198°–201°. Hydrolysis of **3f** (15 mg) gave **1c** (10 mg) as an amorphic solid. m.p. 193–194.5°; M.S. (EI, 70 eV) *m/e* 446 (M^+, $C_{28}H_{46}O_4$, 1%), 410 (43), 388 (11), 370 (34), 355 (8), 352 (8), 337 (8), 301 (20), 283 (50), 271 (54), 270 (100%), 254 (20), 228 (23), 214 (20), 198 (25), 172 (19), 114 (50), 132 (40), 120 (60), 108 (85), 86 (90), 80 (95).

Hydrolysis of **4d** (25 mg) gave **2a** (21 mg) as an amorphic solid m.p. 210–212°; ^1H NMR (90 MHz, $CDCl_3$) δ 0.66 (3H, s), 0.92 (6H, d, J = 6.4 Hz), 1.00 (3H, s), 1.12 (3H, s), 1.18 (3H, s), 3.05 (1H, d, J = 1.9 Hz), 3.59 (1H, dt, J = 10.1, 2.5 Hz), 3.82 (1H, m). Hydrolysis of **4e** (10 mg) gave **2b** (4 mg) as an amorphic solid m.p. 228–229°; ^1H NMR (90 MHz, $CDCl_3/CD_3OD$) δ 0.70 (3H, s), 0.94 (3H, d, J = 7.5 Hz), 0.99 (3H, s), 1.16 (3H, s), 1.19 (3H, s), 3.11 (1H, d, J = 1.9 Hz), 3.31 (2H, ABX system), 3.55 (1H, dt, 10.3, 2.5 Hz), 3.78 (1H, m).

Hydrolysis of **4f** (13 mg) gave **2c** (5 mg) as an amorphic solid, m.p. 223–224.5°.

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REFERENCES

[1]L. J. Goad, *Marine Natural Products* (Edited by P. J. Scheuer), Vol. II, p. 76. Academic Press, New York (1978).
[2]B. Tursch, C. Hootele, M. Kaisin, D. Losman and R. Karlsson, *Steroids* **27**, 137 (1976).
[3]Y. Kashman and S. Carmely, *Tetrahedron Letters* in press.
[4]S. Carmely and Y. Kashman, *Ibid.* **21**, 875 (1980).
[5a]S. G. Levine and M. G. Wall, *J. Am. Chem. Soc.* **81**, 2826 (1959); [b]A. D. Cross, *Ibid.* **84**, 3206 (1962).
[6]Y. Letourneux, O. Khuong-Huu, M. Gut and G. Lukacs, *J. Org. Chem.* **40**, 1674 (1975).
[7]G. Cimino, S. De Rosa, S. De Stefano and G. Sodano, *Tetrahedron Letters* **21**, 3303 (1980).
[8]F. Bohlmann and R. Zeisberg, *Org. Mag. Res.* **7**, 426 (1975).
[9]J. Krepinsky, J. A. Findlay, B. Danieli, G. Palmisano, P. Beynon and S. Murakami, *Ibid.* **10**, 255 (1977).
[10]N. Koizumi, Y. Fujimoto, T. Takeshita and N. Ikekawa, *Chem. Pharm. Bull.* **27**, 38 (1979).
[11]I. Rubinstein and L. J. Goad, *Phytochemistry* **13**, 481, 485 (1974).
[12]Z. V. Zaretskii, *Mass Spectrometry of Steroids*. Wiley, New York (1976).
[13]C. Djerassi, *Pure and Appl. Chem.* **50**, 171 (1979).