Highly Hydroxylated Marine Steroids from the Starfish *Archaster typicus*

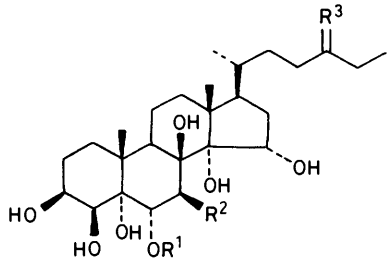
Raffaele Riccio,* Olinda Squillace Greco, and Luigi Minale*
Dipartimento di Chimica delle Sostanze Naturali, Università, via L. Rodinò 22, 80138 Napoli, Italy
Dominique Laurent and Daniel Duhet
Centre Orstom, B.P. A5, Nouméa, New Caledonia

Seven new marine polyhydroxysteroids, (**1**)—(**7**), of which four have a 27-norcholestane skeleton, have been isolated by droplet counter-current chromatography and reversed-phase h.p.l.c. from the starfish *Archaster typicus*. Characterization was accomplished by FAB m.s., and ^{1}H and ^{13}C n.m.r. spectroscopy including two-dimensional proton–carbon chemical-shift correlation (direct and long range) n.m.r. spectroscopy.

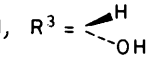
The past few years have seen the structural elucidation of a number of echinoderm saponins.[1–3] Among echinoderms, asteroids (starfishes) have been shown to contain the largest variety of saponins, which can be conveniently divided into three classes: sulphated steroidal glycosides 'asterosaponins,' steroidal cyclic glycosides, and glycosidated polyhydroxysteroids.[4] Often these saponins are accompanied by small amounts of unusual polyhydroxylated steroids.[3,4] The extracts from one such starfish, *Archaster typicus*, collected off Nouméa, New Caledonia, were found to contain large amounts of novel polyhydroxylated steroids as compared with the steroidal glycosidic fraction. In this report we describe the isolation and structure elucidation of seven new polyhydroxysteroids.

Separation of the aqueous extracts from *A. typicus* was achieved by using the following successive chromatographic steps: (i) recovery of the polar material from the extracts on a column of Amberlite XAD-2 (methanol as eluting solvent), (ii) chromatography of the methanol eluate on a column of Sephadex LH-60 to separate the sulphated asterosaponins from the polyhydroxysteroids, (iii) droplet counter-current chromatography (DCCC), and (iv) high performance liquid chromatography (h.p.l.c.) on a C_{18} bonded-phase to obtain 27-nor-5α-cholestane-3β,4β,5,6α,7β,8,14,15α,24α-nonaol (**1**) (0.004% yield), its 6α-sulphate derivative (**2**) (0.0003% yield), 27-nor-5α-cholestane-3β,4β,5,6α,8,14,15α,24α-octaol (**3**) (0.003% yield), 3β,4β,5,6α,8,14,15α-heptahydroxy-27-nor-5α-cholestan-24-one (**4**) (0.0001% yield), (24*E*)-5α-cholest-24-ene-3β,6α,8,14,15α,26-hexaol 15-sulphate (**5a**) (0.007% yield), (24*E*)-5α-cholest-24-ene-3β,4β,6α,8,14,15α,26-heptaol 15-sulphate (**6a**) (0.006% yield), and (24*E*)-5α-cholest-24-ene-3β,4β,5,6α,7β,8,14,-15α, 26-nonaol 6-sulphate (**7**) (0.0001% yield). The yields are based on the fresh weight of the animals.

Compound (**1**) had $[\alpha]_D$ +37.6° (*c* 0.8 in MeOH), m.p. 288—290 °C. Electron-impact mass spectrometry showed no molecular ion. The highest peak was observed at *m/z* 466. In the fast-atom bombardment (FAB) mass spectrum molecular-ion species were observed at *m/z* 503 (*M* + H) and 525 (*M* + Na); further major peaks at *m/z* 467, 449, and 431, corresponding to the loss of two, three, and four molecules of water from *M* + H, were also observed. A DEPT (distortionless enhancement by polarization transfer)[5] ^{13}C n.m.r. spectrum indicated that compound (**1**) contained four methyl groups, eight methylene, three methine, and two quaternary carbons, together with six CHO and three C–O groups (Table 1). A total of 26 carbon resonances was observed. Taken together these data suggested a nonahydroxynorcholestane structure with one of the five methyl groups typical of a sterol missing. Thus the highest peak observed in the e.i. mass spectrum (*m/z* 466) corresponded to loss of two molecules of water from the molecular formula $C_{26}H_{46}O_9$. The ^{1}H N.m.r. spectrum (CD_3OD; Table 2)

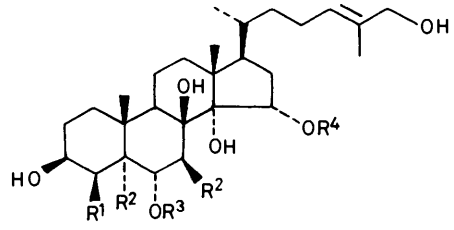


(**1**) R^1 = H, R^2 = OH, R^3 = 

(**2**) R^1 = $SO_3^-Na^+$, R^2 = OH, R^3 = 

(**3**) R^1 = H, R^2 = H, R^3 = 

(**4**) R^1 = H, R^2 = H, R^3 = O



(**5a**) R^1 = H, R^2 = H, R^3 = H, R^4 = $SO_3^-Na^+$
(**5b**) R^1 = H, R^2 = H, R^3 = H, R^4 = H
(**6a**) R^1 = OH, R^2 = H, R^3 = H, R^4 = $SO_3^-Na^+$
(**6b**) R^1 = OH, R^2 = H, R^3 = H, R^4 = H
(**7**) R^1 = OH, R^2 = OH, R^3 = $SO_3^-Na^+$, R^4 = H

confirmed the presence of four methyl groups at δ_H 1.18 (s, 18-H_3), 1.34 (s, 19-H_3), 0.96 (t, *J* 7 Hz, 26-H_3), and 0.89 (d, *J* 7 Hz, 21-H_3). The triplet (3-H) at δ_H 0.96 indicated the presence of one ethyl group. Irradiation of the multiplet around δ_H 1.48 caused collapse of the triplet at δ_H 0.96 to a singlet, and also simplified the hydroxymethine multiplet at δ_H 3.40 (24-H), thus suggesting a 24-hydroxy-27-nor side-chain as the most probable structure. In addition ^{13}C n.m.r. signals for (**1**) (assignments based on 2D

Table 1. 2D (^{13}C–^{1}H chemical-shift correlation) n.m.r. data for compound (**1**) in [$^{2}H_5$]pyridine

Position	δ_C	DEPT	δ_H	J_{HH} (Hz)	$^{2}J^{a}$	$^{3}J^{a}$
1	32.9	CH_2	1.61; 2.10			1.91 s (19-H_3)
2	26.7	CH_2	2.00			
3	68.2	CH	4.73 m			
4	72.6	CH	4.84 d	4		
5	78.3	C				1.91 s (19-H_3)
6	70.8	CH	4.87 brs			
7	73.5	CH	4.87 brs			
8	79.7	C				
9	40.7	CH	3.06 brd	11		1.91 s (19-H_3)
10	39.7	C			1.91 s (19-H_3)	
11	18.0	CH_2	1.56; 2.08			
12	37.6	CH_2	2.00; 2.12			
13	48.5	C			1.41 s (18-H_3)	1.41 s (18-H_3)
14	83.8	C				
15	69.2	CH	4.76 dd	5, 9.5		
16	35.0	CH_2	1.76; 2.21			
17	51.0	CH	2.35 ddd	8.5, 8.5, 10.0		1.41 s; 0.96 d (18-H_3; 21-H_3)
18	16.9	CH_3	1.41 s			
19	17.3	CH_3	1.91 s			
20	35.5	CH	1.45		0.96 d (21-H_3)	
21	18.9	CH_3	0.96 d	7		
22	32.6	CH_2	1.12; 1.81			0.96 d (21-H_3)
23	34.4	CH_2	1.52; 1.71			
24	73.0	CH	3.63 m	$w_{\frac{1}{2}}$ 17.5	1.58 (25-H_2)	1.04 t (26-H_3)
25	30.6	CH_2	1.58		1.04 t (26-H_3)	
26	10.3	CH_3	1.04 t	7.5	1.58 (25-H_2)	

[a] The ^{2}J and ^{3}J columns give ^{13}C–^{1}H cross correlations through two and three bonds respectively.

Table 2. ^{1}H N.m.r. data (250 MHz; CD_3OD) for compounds (**1**)—(**7**) (J in Hz).

	(1)	(2)	(3)	(4)	(5a)	(5b)	(6a)	(6b)	(7)
3-H	4.03 m	4.03 m	4.06 m	4.06	3.52 m	3.52 m	3.48 ddd (4, 4, 11)	3.48 ddd (4, 4, 11)	4.03 m
4-H	3.98 d (4)	4.08 d (4)	3.96 d (4)	3.96 d (4)			4.28 t ($w_{\frac{1}{2}}$ 7.5)	4.28 t ($w_{\frac{1}{2}}$ 7.5)	4.08 d (4)
6-H	4.05 d (9)	4.84 d (10)	4.37 dd (4.8, 12)	4.37 dd (5, 12)	3.67 ddd (4, 11, 11)	3.66 ddd (4, 11, 11)	4.13 ddd (4, 11, 11)	4.12 ddd (4, 11, 11)	4.84 d (10)
7-H_α	4.10 d (9)	4.40 d (10)	2.24 t (12.5)	2.24 t (12.5)					4.40 d (10)
7-H_β			1.85 dd (5, 12.5)	1.85 dd (5, 12.5)	2.31 dd (4, 12.5)	2.13 dd (4, 12.5)	2.38 dd (4, 12.5)	2.20 dd (4, 12.5)	
9-H	2.32 brd (11)	2.35 brd (11)	2.26 brd (11)	2.26 brd (11)					2.35 brd (11)
15-H	4.39 dd (5, 9.5)	4.43 dd (5, 9.5)	4.46 dd (4.2, 9.5)	4.46 dd (5, 9.5)	4.97[a]	4.45 dd (5, 9.5)	4.97[a]	4.45 dd (5, 9.5)	4.43 dd (5, 9.5)
18-H_3	1.18 s	1.18 s	1.14 s	1.13 s	1.19 s	1.15 s	1.18 s	1.14 s	1.18 s
19-H_3	1.34 s	1.41 s	1.33 s	1.33 s	1.06 s	1.06 s	1.22 s	1.22 s	1.41 s
21-H_3	0.89 d (7)	0.89 d (7)	0.88 d (7)	0.85 d (7)	0.91 d (7)	0.90 d (7)	0.90 d (7)	0.90 d (7)	0.91 d (7)
24-H	3.40 m	3.40 m	3.40 m		5.40 brt (6.5)	5.40 brt (6.5)	5.40 brt (6.5)	5.40 brt (6.5)	5.41 brt (6.5)
25-H_2				2.50 q (7)					
26-H_3	0.96 t (7)	0.96 t (7)	0.96 t (7)	1.04 t (7)	3.94 s	3.94 s	3.93 s	3.94 s	3.94 s
27-H_2					1.67 brs	1.67 brs	1.67 brs	1.67 brs	1.67 brs

[a] Partly under solvent signal.

^{13}C–^{1}H chemical-shift cross-correlation spectroscopy; Table 1) were in close accord (for carbons 26—24) with model linear secondary alcohols.[6a] Comparison of the ^{13}C data with those for 24-substituted sterols[7] allowed the side-chain structure of compound (**1**) to be established.

Continuing now with the analysis of the ^{1}H n.m.r. data for compound (**1**) in CD_3OD (Table 2), the downfield AB quartet (J_{AB} 9 Hz) at δ_H 4.05 and 4.10 indicated a structural element with *trans*-(diequatorial), vicinal, secondary hydroxy groups, both adjacent to carbons without protons. The spectrum also contained a doublet at δ_H 3.98 (1 H, J 4 Hz) and a multiplet at δ_H 4.03, each coupled to the other, thereby indicating the presence of two *cis*-vicinal secondary groups — one adjacent to a carbon bearing two protons and the other to one without a proton. The large coupling constants ($w_{\frac{1}{2}}$ 20 Hz) associated with the signal at δ_H 4.03 are consistent for an axial proton, while the small

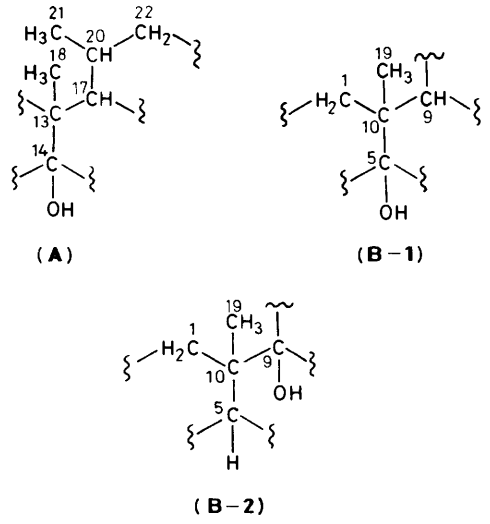
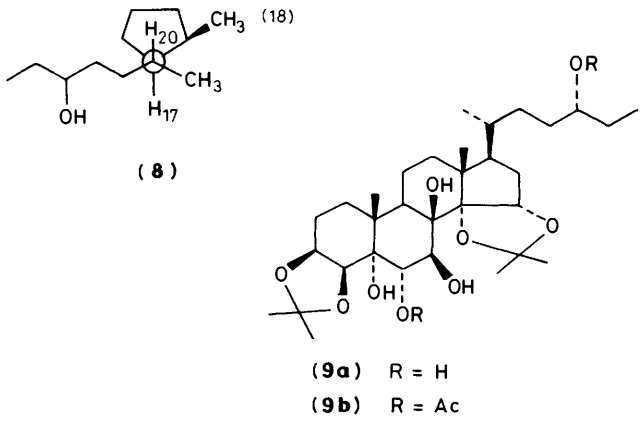


Figure. Partial structures derived from ^{13}C–^{1}H (long-range) chemical-shift correlation spectrum of compound (**1**)

coupling constant (J 4 Hz) of the doublet at δ_H 3.98 is consistent for an equatorial proton. There are several ways such fragments and the remaining three tertiary and one secondary hydroxy groups can be put into a steroid skeleton. The use of two-dimensional (2D) proton–carbon chemical-shift correlation spectroscopy,[8] especially long range, proved to be fruitful and allowed the structure (**1**) to be assigned confidently. First, the direct ^{13}C–^{1}H shift correlation was established (see Table 1). Intense long-range heterocorrelation peaks were then observed with the proton signals due to the methyl resonances. Beginning with the singlet at δ_H 1.41, we observed points of intensity in the contour plot which correlated with the carbon resonances at δ_C 48.5 (C; C-13), 83.8 (C; C-14), and 51.0 (CH; C-17) p.p.m. Furthermore the methyl doublet at δ_H 0.96 (21-H_3) was observed to correlate with the carbon resonances at δ_C 35.5 (CH; C-20), 51.0 (CH; C-17), and 32.6 (CH_2; C-22) p.p.m. This established that the two four-carbon fragments are connected through C-17 (CH; δ_C 51.0 p.p.m.), and provided an unequivocal assignment of the methyl singlet at δ_H 1.41 (18-H_3). Accordingly, one tertiary hydroxy group is placed at C-14 [Figure; part structure (**A**)]. The C-26 methyl triplet at δ_H 1.04 gave points of intensity in the contour plot which correlate with the carbon resonances at δ_C 30.6 (CH_2; C-25) and 73.0 (CH; C-24) p.p.m., thus providing final confirmation of the side-chain structure. The remaining methyl signal, the C-19 methyl singlet at δ_H 1.91, was observed to correlate with the carbon resonances at δ_C 32.9 (CH_2; C-1), 39.7 (C; C-10), 40.7 (CH; C-9 or C-5), and 78.3 (C; C-5 or C-9) p.p.m., thus indicating the alternative substructures B-1 or B-2. Of the three high-field methine carbon resonances (δ_C 35.5, 40.7, and 51.0 p.p.m.), two have been assigned to C-17 (51.0 p.p.m.) and C-20 (35.5 p.p.m.), while the remaining one must be due to C-5 or C-9. Thus one tertiary hydroxy group must be located at C-8. Further, the fact that C-1 is unsubstituted indicated that the *cis*-diol system, revealed by the ^{1}H n.m.r. spectrum (δ_H 3.98, d, J 4 Hz; δ_H 4.03 m; in CD_3OD), must be positioned at C-3β and C-4β. The multiplicity of the absorption at δ_H 3.98, which was determined to be coupled only to the C-3 methine, indicated C-5 to be quaternary. Thus the third tertiary hydroxy group must be placed at C-5, ruling out the alternative partial structure B-2. As a direct consequence the *trans*-diol system giving rise to the AB pattern in the ^{1}H n.m.r. spectrum (CD_3OD) must be positioned at C-6 and C-7. In ^{1}H nuclear Overhauser enhancement experiments, including the spectral subtraction technique



(NOEDS),[9] on compound (**1**), intense enhancement of the proton at δ_H 4.05 (d, J 9 Hz) was observed when the C-19 methyl protons singlet was irradiated, while strong enhancement of the proton at δ_H 4.10 (d, J 9 Hz) was observed when the C-9 proton (δ_H 2.32, br d, J 11 Hz) was then irradiated. These experiments provided unequivocal assignments of the signals at δ_H 4.05 (6-H) and 4.10 (7-H), and fixed the hydroxy group at C-6 on the 'α' face and that at C-7 on the 'β' face.

Finally, the isolated secondary hydroxy group was located at C-15 from the chemical shift and multiplicity (dd, J 9.5, 3 Hz) of the signal at δ_H 4.39 in the ^{1}H n.m.r. spectrum, and from ^{13}C n.m.r. data. The highfield methylene signal at δ_C 18.0 p.p.m. in the ^{13}C n.m.r. spectrum was assigned to C-11, shifted upfield by an effect due to the hydroxy group at C-8, and eliminated the possibility of an hydroxy group located there or at C-12. The shift values for C-17 (δ_C 51.0 p.p.m.) and C-20 (δ_C 35.5 p.p.m.) eliminated the possibility of an hydroxy group located at C-16.[10] The hydroxymethine proton at C-15 showed nuclear Overhauser enhancement on irradiation of the C-18 methyl protons in a NOEDS experiment,[9] thereby fixing the 18-H_3 and C-15 protons on the same 'β' face of the steroid. Further evidence for the structural assignment with 14α,15α-diol groups was provided by the formation of a bis-acetonide from (**1**). An analysis of the ^{1}H n.m.r. spectrum of the acetonide (**9a**) and comparison with that of the natural sterol (**1**) indicated that acetonide formation had occurred at the 3,4- and 14,15-positions. The resonances associated with 3-H and 4-H showed downfield shifts to δ_H 4.30 [*vs.* 4.03 in (**1**)] and δ_H 4.40 [*vs.* 3.98 in (**1**)], respectively, while the signal for 15-H was observed as a triplet at δ_H 4.45 (J 7.5 Hz) [*vs.* 4.39, dd in (**1**)]. Treatment of compound (**9a**) with excess of acetic anhydride in pyridine gave a diacetate (**9b**), whose ^{1}H n.m.r. spectrum contained an isolated doublet at δ_H 5.50 (J 10 Hz) assigned to 6-H_β, thus confirming that the hydroxy group at C-6 was not involved in the acetonide formation. Acetylation had also occurred at the C-24 hydroxy group (δ_H 4.74, m).

The 5α,14α-cholestane skeleton assigned to compound (**1**) follows from the ^{13}C n.m.r. data, especially the chemical shift of the angular methyl carbons at high field (δ_C 16.9 and 17.3 p.p.m.) only compatible with a *trans* stereochemistry of the ring junctions,[11] the multiplicities observed in the ^{1}H n.m.r. spectrum, and the results of the NOEDS experiments.

The remaining feature needed to establish the structure fully is the stereochemistry at C-20 and C-24. The common 20*R* (20-H_α) configuration tentatively proposed for compound (**1**) and the related sterols (**2**)—(**7**) is based on the chemical shifts of the C-21 methyl protons. The proton shifts for the C-21 methyl group are sensitive to the stereochemistry at C-20 and our values ranging from δ_H 0.88 [(**1**)—(**3**)] to 0.91 [(**5a**)—(**7**)] in CD_3OD were in better agreement with the value δ_H 0.91 reported for (20*R*)-cholesterol as compared with δ_H 0.81 for the

Table 3. ^{13}C N.m.r. data (62.9 MHz; [$^2H_5$]pyridine) for compounds (1)—(7).[a]

Carbon	(1)	(2)	(3)	(4)	(5a)	(5b)	(6a)	(6b)	(7)
1	32.9	32.7	32.8	33.2	38.7	39.3	38.9	40.0	32.7
2	26.7	27.0	26.8	27.1	31.1	31.8	25.5	26.9	27.1
3	68.2	67.5	68.5	68.7	70.6	71.2	72.2	73.1	67.7
4	72.6	72.7	72.6	72.9	32.3	32.9	68.2	69.2	72.6
5	78.3	78.5	78.1	78.3	52.3	53.3	55.6	57.1	78.5
6	70.8	79.8	65.8	66.0	66.6	66.7	64.0	64.2	79.8
7	73.5	72.8	40.0	40.2	43.8	45.8	44.3	46.7	72.9
8	79.7	79.7	78.8	79.1	78.4	79.1	78.4	79.4	79.9
9	40.7	40.5	41.1	41.4	47.6	48.3	48.6	49.6	40.5
10	39.7	40.4	39.8	40.0	36.6	36.3	36.7	36.6	40.4
11	18.0	18.0	18.0	18.3	18.2	18.7	17.6	18.8	18.0
12	37.6	37.0	39.8	39.5	35.7	40.0	35.6	40.4	36.5
13	48.5	48.7	48.0	48.3	46.9	47.9	46.9	48.3	48.7
14	83.8	83.3	84.4	84.6	84.4	84.2	84.4	84.5	83.3
15	69.2	68.9	69.1	69.3	76.2	68.9	76.5	69.1	68.9
16	35.0	35.1	35.0	35.2	33.9	34.8	33.9	35.1	35.3
17	51.0	50.9	51.1	51.2	50.7	51.0	50.6	51.3	50.8
18	16.9	16.7	17.2	17.4	16.8	17.0	16.8	17.3	16.7
19	17.3	17.4	17.4	17.6	13.9	14.4	16.8	17.6	17.4
20	35.5	35.6	35.4	35.0	34.8	35.0	34.7	35.3	35.2
21	18.9	18.9	18.8	18.6	18.0	18.5	18.0	18.5	18.7
22	32.6	32.7	32.5	30.6	36.6	37.0	36.7	37.5	37.0
23	34.4	34.6	34.4	40.3	24.4	24.7	24.6	25.0	24.9
24	73.0	73.1	73.0	210.9	124.9	125.3	125.1	125.5	125.5
25	30.6	30.7	30.5	35.8	134.9	135.8	135.1	135.8	135.8
26	10.3	10.3	10.2	8.1	67.6	68.0	67.7	68.3	68.2
27					13.4	13.7	13.4	14.0	13.9

[a] The assignments were aided by DEPT and in compounds (1), (3), (5a), and (6a) were also confirmed by 2D ^{13}C–^1H cross-correlation spectroscopy.

corresponding (20*S*)-isomer.[12] We note that the signal for the C-21 methyl group of the 24-oxosteroid (4) is shifted to δ_H 0.85 and it seems reasonable to assume that such an effect is due to the 24-carbonyl rather than to a change of stereochemistry at C-20. We also note that hydroxy groups at the 14α- and 15α-position induce a downfield shift of the resonance of the proton at C-17, which resonates as an isolated signal at δ_H 2.35 (in [$^2H_5$]pyridine) and is observed as a ddd with *J* 8.5, 8.5, and 10 Hz. The large coupling constant H-17/H-20 is consistent with a 20*R* configuration, assuming an *anti* conformation with the side-chain projecting to the right (*anti* relative to C-13) as depicted in structure (**8**). To determine the configuration at C-24 we have applied Horeau's method[13] to compound (**9a**). Following esterification with excess of racemic α-phenylbutyric anhydride, a preponderance of (+)-α-phenylbutyric acid was recovered, thus indicating the 24*R* stereochemistry. Analysis of the ester fraction revealed that α-phenylbutyric anhydride indeed reacted regioselectively at C-24.

Compound (**2**) had $[\alpha]_D$ +56.2 (*c* 1 in MeOH). The FAB mass spectrum gave molecular-ion species at *m/z* 643 (*M* + K) and *m/z* 627 (*M* + Na), and fragments at *m/z* 525 and 507, interpreted as due to the loss of $NaHSO_4$ from species with *m/z* 643 and 627, respectively. *M* is the molecular weight of the sodium salt. Solvolysis using dioxane–pyridine[14] afforded the nonaol (**1**). This established that compound (**2**) is a sodium sulphated derivative of (**1**). An examination of ^1H and ^{13}C n.m.r. spectra (Tables 2 and 3) immediately located the sulphate group at C-6 or C-7. In the ^1H n.m.r. spectrum the AB quartet for 6-H and 7-H, observed at δ_H 4.05 and 4.10 in (**1**), was found to be shifted downfield to δ_H 4.84 (d, *J* 10 Hz) and 4.40 (d, *J* 10 Hz) in sulphate (**2**). In a NOEDS experiment, irradiation of the C-19 methyl singlet caused an intense enhancement of the sulphoxymethine proton at δ_H 4.84, while enhancement of the doublet at δ_H 4.40 was observed when the 9-H resonance at δ_H 2.35 (br d, *J* 11 Hz) was irradiated. These experiments placed the sulphate group at C-6 in compound (**2**).

Compound (**3**) had $[\alpha]_D$ +43.4° (*c* 0.8 in MeOH), m.p. 294 °C. This compound is closely related to (**1**) although it lacks the 7β-hydroxy group. In the e.i. mass spectrum the highest-mass ion observed (*m/z* 468) corresponded to loss of water from the molecular formula $C_{26}H_{46}O_8$. In the FAB mass spectrum a molecular-ion species was observed at *m/z* 509 (*M* + Na); intense peaks at *m/z* 469, 451, 433, 415, 397, and 379 for stepwise water loss from *M* + H were also observed. The ^1H n.m.r. spectrum of compound (**3**) showed signals virtually identical with those observed in the spectrum of (**1**), except for the AB quartet for 6-H and 7-H which was found to be replaced by a one-proton double doublet (*J* 12, 4.8 Hz) at δ_H 4.37. Enhancement of this latter methine signal was observed when the C-19 methyl resonance was irradiated in a NOEDS experiment, thus providing the assignment of this signal to 6-H_β. We note that the removal of the 7β-hydroxy group also resulted in a small shift of the signals for 15-H [δ_H 4.46 in (**3**) *vs.* 4.39 in (**1**)]. The structure (**3**) also accounted for the shielded carbons observed in the ^{13}C n.m.r. spectrum at δ_C 65.8 (CH) and 40.0 (CH_2) p.p.m., assigned to C-6 and to the adjacent methylene carbon (C-7), respectively.

Compound (**4**) had $[\alpha]_D$ +40.0° (*c* 0.5 in MeOH). This compound is the 24-keto derivative of octaol (**3**). In the e.i. mass spectrum a series of peaks at *m/z* 466, 448, 430, 412, and 394 corresponding to sequential loss of water from the molecular formula $C_{26}H_{44}O_8$ was observed. A ^{13}C n.m.r. peak at 210.9 (s) p.p.m., coupled with an i.r. absorption at 1 700 cm^{-1}, indicated that compound (**4**) possessed a non-conjugated ketone. The isolated ethyl group that gave rise to a methylene protons signal at δ_H 2.50 (2 H, q, *J* 7 Hz) coupled to a methyl signal at δ_H 1.04 (3 H, t, *J* 7 Hz) must be attached at the carbonyl carbon. ^{13}C N.m.r. signals assigned to the side-chain carbons (Table 3) are in agreement with a 24-oxo-27-nor side-chain structure.[6b] The remaining signals in the spectrum of ketone (**4**) are identical with those assigned to (**3**), and the resonances associated with the hydrogen atoms of the steroid nucleus have chemical shifts essentially identical in both spectra.

Compound (**5a**) had $[\alpha]_D$ +54.5° (*c* 1.0 in MeOH). The presence of a sulphate function in compound (**5a**) was indicated by its polarity and by FAB mass spectrometry, which gave molecular-ion species at *m/z* 591 (*M* + Na) and 569 (*M* + H, major). *M* is the molecular weight of the sodium salt. Upon solvolysis with dioxane–pyridine, sulphate (**5a**) was desulphated to compound (**5b**) of lower polarity. The FAB mass spectrum of this product showed the peak of highest mass at *m/z* 449 (*M* + H − H_2O), and the molecular formula $C_{27}H_{46}O_6$, which corresponded to a mono-unsaturated cholestanehexaol, was essentially determined by DEPT ^{13}C n.m.r. spectrometry. The ^{13}C n.m.r. spectrum also showed the presence of a trisubstituted double bond and indicated that of the six hydroxy groups, three were secondary, two tertiary, and one primary. The ^1H n.m.r. spectrum of the hexaol (**5b**) (Table 2) contained one double doublet at δ_H 4.45 (*J* 5, 9.5 Hz) already seen in the spectra of compounds (**1**)—(**4**) and assigned to the 15β-hydroxymethine proton. This suggested an 8β,14α,15α,-trihydroxy structure for (**5b**). In agreement with such a structure were the chemical shifts of the C-18 methyl protons at δ_H 1.15 [δ_H 1.14 in (**3**)—(**4**)] and the shifts of the resonances for C-8 and C-11 to C-18, virtually identical with those of the corresponding signals of compounds (**3**) and (**4**).

In the ^1H n.m.r. spectrum of (**5a**) the C-15 hydroxymethine signal was found to be shifted downfield to δ_H 4.97, thus indicating the location of the sulphate group at C-15. In confirmation the resonance for C-15 was observed to be shifted

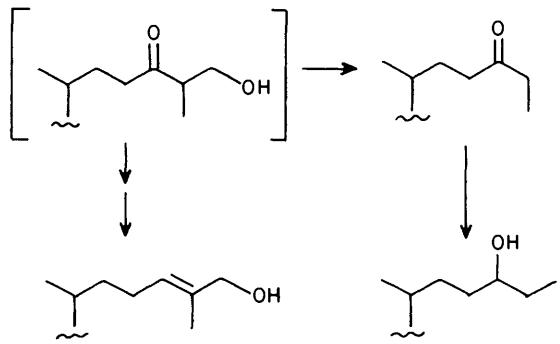
downfield, while that for C-16 was shifted upfield in the ^{13}C n.m.r. spectrum of sulphate (**5a**) relative to that of the desulphate analogue (**5b**). We note that the removal of sulphate also resulted in a large downfield shift of the resonance of C-12 (Table 3). The remaining two secondary hydroxy groups were located at the 3β- and 6α-positions; in the ^1H n.m.r. spectrum of sulphate (**5a**) the seven-line multiplet at δ_H 3.52 is typical for the 3α-proton of 3β-hydroxy steroids, and the double triplet (*J* 4, 11 Hz) at δ_H 3.67 is characteristic of the axial proton associated with a 5α-hydroxy group.[15] The spectrum also contained a one-proton double doublet (*J* 12.5, 4 Hz) at δ_H 2.31, coupled by 4 Hz with the 6β-proton. This allowed the assignment of this peak to the equatorial proton at C-7 and confirmed the location of one tertiary hydroxy group (at C-8). ^{13}C N.m.r. data also indicated a steroid structure with hydroxy groups at the 3β- and 6α-positions[16] (Table 3). Continuing the analysis of the ^1H n.m.r. data for (**5a**), the one-proton triplet at δ_H 5.40 (*J* 6.5 Hz, 24-H) together with the methyl singlet at δ_H 1.67 (27-H_3) and the hydroxymethylene singlet at δ_H 3.94 (26-H_2), suggested a 26-hydroxy-Δ^{24} side-chain. This was confirmed by ^{13}C–^1H correlation spectroscopy (direct and long range) which showed that the C-27 methyl singlet correlates with the carbon resonances for C-24, C-25, and C-26. The n.O.e. between the 24-H and 26-H_2 indicated *E* stereochemistry for the double bond. The methyl group ^{13}C n.m.r. chemical shift observed for (**5a**) (δ_C 13.4 p.p.m.) also sustantiated the *E* stereochemistry.[6c]

Compound (**6a**), $[\alpha]_D$ +43.6° (c 1.0 in MeOH), is related to sulphate (**5a**) by the introduction of one hydroxy group at the 4β-position, which is a common feature among polyhydroxy-steroids from starfishes.[17] The FAB mass spectrum showed molecular-ion species at *m/z* 617 (*M* + Na) and 585 (*M* + H), shifted by sixteen mass units relative to (**5a**). Upon solvolysis compound (**6a**) was desulphated to (**6b**), FAB-m.s. *m/z* 483 (*M* + H).

The most significant features of the ^{13}C n.m.r. spectrum of sulphate (**6a**), which suggested the location of the hydroxy group at C-4β, were the upfield shift exhibited by C-2 (5.6 p.p.m.) and the downfield shift experienced by C-3 (1.6 p.p.m.), C-5 (3.3 p.p.m.), and C-19 (2.9 p.p.m.) relative to the corresponding signals in (**5a**). These shifts were close to the shifts observed in 4β-hydroxysteroids.[9,18] In the ^1H n.m.r. spectrum, the 4β-hydroxy group, revealed by a narrow signal at δ_H 4.28, caused the downfield shifts of 6-H_β to δ_H 4.13 [δ_H 3.67 in (**5a**)] and of 19-H_3 to δ_H 1.22 [δ_H 1.06 in (**5a**)]; the 3-H signal was observed as a double triplet (*J* 11, 4 Hz) at δ_H 3.48.

Compound (**7**) had $[\alpha]_D$ +47.4° (*c* 0.5 in MeOH). The FAB mass spectrum showed molecular-ion species at *m/z* 671 (M_K + K), 655 (M_{Na} + K), and 639 (M_{Na} + Na), which indicated a m.w. of 616 daltons for the sodium salt. An examination of its spectral data (Tables 2 and 3) immediately indicated that compound (**7**) shares the same 3β,4β,5α,7β,8β,14α,15α-heptahydroxy-6-sulphoxytetracyclic nucleus as compound (**2**) and contains the same 26-hydroxy-24-ene cholesterol side-chain as (**5a**) and (**6a**).

The detection of sterols with the 27-norcholestane side-chain, (**1**)—(**4**), is noteworthy. So far, such an unusual side chain-structure has been encountered only in a minor sterol from the marine sponge *Axinella cannabina*.[19] The occurrence of marine sterols with shortened side-chains, *i.e.* the C_{26} sterols possessing the unprecedented 24-norcholestane skeleton (or 24-methyl-26,27-dinorcholestane skeleton) and C_{27} sterols with the 24-methyl-27-norcholestane skeleton, found in nearly every marine phylum,[20] has raised many intriguing and yet unanswered biosynthetic questions.[21] The discovery of sterols with 24-hydroxy- or 24-keto-27-nor side-chains along with those with the 26-hydroxy-24-ene side-chain leads to the suggestion that these novel C_{26} sterols might be generated by a retro-aldol cleavage of a possible 24-keto-26-hydroxy precursor (Scheme).



Scheme. Possible biogenetic scheme to 27-norsteroids

The ability of the starfish to produce these C_{26} steroids needs to be tested.

Finally we note that the nonaols (**1**), (**2**), and (**7**) constitute, as far as we know, the most highly hydroxylated sterols isolated from a natural source.

Experimental

The following instruments were used: n.m.r. spectra, Bruker WM-250 or WM-400; mass spectra, Kratos MS-50 mass spectrometer equipped with kratos FAB or e.i. source; DCCC-A apparatus manufactured by Tokyo Rikakikai Co. equipped with 300 tubes; h.p.l.c., Waters model 6000A pump equipped with a U6K injector and a differential refractometer model 401; optical rotations, Perkin-Elmer model 141 polarimeter. The FAB mass spectra were obtained by dissolving the samples in a glycerol matrix and placing them on a copper probe prior to bombardment with Ar atoms of energy 2—6 kV. The ^1H n.m.r. spectra were measured for solutions in CD_3OD; the ^{13}C n.m.r. spectra were measured for solutions in [$^2H_5$]pyridine.

The DEPT experiments were performed using polarization transfer pulses of 90° and 135°, respectively, obtaining in the first case signals for the CH group only and in the other case positive signals for CH and CH_3 and negative ones for CH_2 groups. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. These delays were also applied for two-dimensional carbon–proton shift correlations on a 256 × 1 024 data matrix. An eight-step pulse cycle was used for P-type selection. In the case of long-range carbon–proton shift correlations, delays were adjusted to an average CH coupling of 5 Hz.

Extraction and Isolation.—Archaster typicus (7.5 kg, fresh weight) was collected off Nouméa, New Caledonia, and identified by Dr. Michel Jangoux of the Université Libre de Bruxelles. The animals were chopped and soaked in distilled water. The extraction was repeated four times. The aqueous extracts were passed through a column of Amberlite XAD-2, eluting with water (1 bed vol.) and then methanol. The methanol fraction was evaporated to give a crude extract (8 g) which was then chromatographed on a column of Sephadex LH-60 (4 × 80 cm) with methanol–water (2:1) as eluant, in two portions of 4 g each. In a typical experiment the initial 500 ml of eluate were discharged, and 12 ml fractions were then collected and analysed by t.l.c. on SiO_2 in butan-1-ol–acetic acid–water (60:15:25) or chloroform–methanol–water (80:18:2), detection being with cerium(IV) sulphate/H_2SO_4. Fractions 38—46 afforded a crude mixture of sulphated asterosaponins (330 mg); fractions 47—52 contained a mixture of asterosaponins and polyhydroxylated steroids (870 mg) which was further resolved on a column of Sephadex LH-20 (2.5 × 80 cm;

eluant methanol), while fractions 53—77 contained crude polyhydroxylated steroids (1.6 g).

All fractions containing polyhydroxylated steroids were combined and further purified, in portions of 1.3 g each, by DCCC with chloroform–methanol–water (7:13:8), ascending mode (the lower phase was the stationary one). In a typical separation a flow rate of 18 ml h^{-1} was used and 6 ml fractions were collected. Initial fractions contained mostly sulphated compounds (**2**), (**5a**), (**6a**) and (**7**) together with more polar material. Fractions 41—51 contained pure octaol (**3**) (80 mg) and fractions 59—71 gave pure nonaol (**1**) (100 mg). Fractions 93—102 were combined with corresponding fractions from subsequent separations and purified by h.p.l.c. on a μ-Bondapack C_{18} column (30 cm × 8 mm i.d.) with methanol–water (70:30) as eluant (5 ml min^{-1}) to give compound (**4**) (10 mg). Fractions containing sulphated compounds (1.5 g) were loaded again into the DCCC apparatus, this time with the lower phase as the stationary one. After about 2 l of mobile phase had passed through the columns, these were discharged by being pumped through the stationary phase and the eluate was collected in 6 ml fractions. Thus were obtained the sulphates (**5a**) (380 mg) and (**6a**) (350 mg) together with an intermediate mixture of both and a fraction containing compounds (**2**) and (**7**) which was further fractionated by reverse-phase h.p.l.c. (methanol–water; 50:50) to afford compound (**2**) (20 mg) and compound (**7**) (8 mg). The n.m.r. spectral data for compounds (**1**)—(**7**) are reported in the text and in Tables 1—3.

Bis-acetonide Formation from Nonaol (**1**).—Compound (**1**) (5 mg) was stirred for 16 h at 50 °C in a stoppered vial with dry acetone (2 ml) containing toluene-*p*-sulphonic acid (2 mg). $BaCO_3$ was then added; the reaction mixture was centrifuged and the supernatant was evaporated to dryness to give bis-acetonide (**9a**); *m/z* (e.i. m.s.) 582 (M^+) and 567; δ_H 4.45 (1 H, t, *J* 7.5 Hz, 15-H), 4.40 (1 H, d, *J* 5 Hz, 4-H), 4.30 (1 H, m, 3-H), 4.18 (1 H, d, *J* 9 Hz, 7-H), 3.98 (1 H, d, *J* 9 Hz, 6-H), 3.40 (1 H, m, 24-H), 2.42 (1 H, dd, *J* 4 and 10 Hz, 9-H), 1.51, 1.46, 1.45, and 1.39 (each 3 H, s, 4 × acetonide Me), 1.32 (3 H, s, 19-H_3), 1.18 (3 H, s, 18-H_3), 0.96 (3 H, t, *J* 7 Hz, 26-H_3), and 0.86 (3 H, d, *J* 7 Hz, 21-H_3).

A mixture of compound (**9a**) (2 mg) and an excess of acetic anhydride in pyridine (0.1 ml) was kept at room temperature overnight. The excess of reagent was removed under reduced pressure to give the diacetate (**9b**), δ_H 5.49 (1 H, d, *J* 10 Hz, 6-H), 4.75 (1 H, m, 24-H), 4.44 (1 H, d, *J* 10 Hz, 7-H), 4.13 (1 H, d, *J* 5 Hz, 4-H), 2.11—2.05 (3 H each, s, CH_3CO); the remaining signals were virtually identical to those of compound (**9a**).

Application of the Horeau Method to Compound (**9a**).—A solution of compound (**9a**) (10 mg) and racemic α-phenylbutyric anhydride (100 μl) in pyridine (300 μl) was left at room temperature for 16 h. Work-up as usual afforded crystalline α-phenylbutyric acid with positive rotation ($[\alpha]_D$ +0.010°). The neutral fraction contained exclusively (**9a**) 24-*O*-α-phenylbutyrate, δ_H 4.73 (24-H); remaining signals identical with those of compound (**9a**).

Solvolysis of Compounds (**2**), (**5a**) *and* (**6a**).—A solution of the sulphate (**2**) (5 mg) in a 1:1 mixture of dioxane–pyridine (0.5 ml) was kept at 140 °C in a stoppered reacti-vial for 3 h. The mixture was diluted with water, extracted with butan-1-ol, and the solvent was evaporated off under reduced pressure. The product obtained was identical with compound (**1**).

A solution of sulphates (**5a**) and (**6a**) (10 mg each) was treated as before at 120 °C for 4 h to give respectively: hexaol (**5b**), *m/z* (FAB m.s.) 449, 431, 413, and 395 (M + H − $n$$H_2O$) and heptaol (**6b**), *m/z* (FAB m.s.) 483 (M + H), 465, 447, 429, 411, and 393 (M + H − $n$$H_2O$). ^1H and ^{13}C N.m.r. spectral data are reported in Tables 2 and 3.

Acknowledgements

This research has been supported by M.P.I., Italy. We thank Messrs J. L. Menou, G. Bargibant, and P. Tirard of the Centre ORSTOM (Nouméa, New Caledonia) for collection of the animals. We are grateful to Professor E. Breitmaier (University of Bonn, FRG) for hospitality given to one of us (R. R.) and his invaluable advice in relation to 2D n.m.r. experiments. The mass spectra were provided by 'Servizio di Spettrometria di massa del C.N.R. e dell'Università di Napoli.' The assistance of the staff is gratefully acknowledged.

References

1 D. J. Burnell and J. W. ApSimon, in 'Marine Natural Products, Chemical and Biological Perspectives,' ed. P. J. Scheuer, Academic Press, New York, 1983, vol. 5, p. 287.
2 D. J. Faulkner, *Nat. Prod. Rep.*, 1984, **1**, 551.
3 L. Minale, R. Riccio, C. Pizza, and F. Zollo, Proceedings of the Naito Foundation International Symposium on Natural Products and Biological Activities, Tokyo, November 5th—7th, 1984 (in the press).
4 L. Minale, C. Pizza, R. Riccio, and F. Zollo, *Pure Appl. Chem.*, 1982, **54**, 1935.
5 M. R. Bendall, D. T. Pegg, D. M. Doddrell, and D. H. Williams, *J. Org. Chem.*, 1982, **47**, 3021; M. R. Bendall, D. M. Doddrell, D. T. Pegg, and W. E. Hull, 'DEPT-Bruker-Information Bulletin,' Bruker Analytishe Messtechnik, Karlsruhe, 1982; M. R. Bendall, D. T. Pegg, D. M. Doddrell, S. R. Johns, and R. I. Willing, *J. Chem. Soc., Chem. Commun.* 1982, 1138.
6 (*a*) J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972, p. 140; (*b*) *ibid.*, p. 146; (*c*) *ibid.*, p. 436.
7 N. Koizumi, Y. Fujimoto, T. Takeshita, and N. Ikekawa, *Chem. Pharm. Bull.*, 1979, **27**, 38.
8 A. A. Maudsley, L. Müller, and R. R. Ernst, *J. Magn. Reson.*, 1977, **28**, 463; G. Bodenhausen and R. Freeman, *ibid.*, p. 471; W. E. Hull, 'Two-Dimensional NMR,' Bruker Analytische Messtechnik, Karlsruhe, 1982.
9 R. Richarz and K. Wuthrich, *J. Magn. Reson.*, 1978, **30**, 147.
10 H. Eggert, C. L. VanAntwerp, N. S. Bhacca, and C. Djerassi, *J. Org. Chem.*, 1976, **41**, 71.
11 J. L. Gough, J. P. Guthrie, and J. B. Stothers, *J. Chem. Soc., Chem. Commun.*, 1972, 679.
12 W. R. Nes, T. E. Varkey, and K. Krevitz, *J. Am. Chem. Soc.*, 1977, **99**, 260.
13 H. Horeau, *Tetrahedron Lett.*, 1961, 506; 1962, 965.
14 J. McKenna and J. K. Norymberski, *J. Chem. Soc.*, 1957, 3889.
15 J. E. Bridgeman, P. C. Cherry, A. S. Clegg, J. M. Evans, E. R. H. Jones, A. Kasal, V. Kumar, G. D. Meakins, Y. Morisawa, E. E. Richards, and P. D. Woodgate, *J. Chem. Soc. C.*, 1970, 250.
16 J. W. Blunt and J. B. Stothers, *Org. Magn. Reson.*, 1977, **9**, 439.
17 R. Riccio, L. Minale, S. Pagonis, C. Pizza, F. Zollo, and J. Pusset, *Tetrahedron*, 1982, **38**, 3615; L. Minale, C. Pizza, R. Riccio, C. Sorrentino, F. Zollo, J. Pusset, and G. Bargibant, *J. Nat. Prod.*, 1984, **47**, 790; L. Minale, C. Pizza, F. Zollo, and R. Riccio, *ibid.*, 1983, **46**, 736.
18 C. L. Van Antwerp, H. Eggert, G. D. Meakins, J. O. Miners, and C. Djerassi, *J. Org. Chem.*, 1977, **42**, 789.
19 T. Itoh, D. Sica, and C. Djerassi, *J. Chem. Soc., Perkin Trans. 1*, 1983, 147.
20 L. J. Goad, in 'Marine Natural Products, Chemical and Biological Perspectives,' ed. P. J. Scheuer, Academic Press, New York, 1978, vol. 2, p. 75.
21 C. Djerassi, N. Theobald, W. C. M. C. Kokke, C. S. Pak, and R. M. K. Carlson, *Pure Appl. Chem.*, 1979, **51**, 1815.

Received 29*th July* 1985; *Paper* 5/1296