Structure Elucidation of (22*E*,24*R*,25*R*)-24-Methyl-5α-cholest-22-ene-3β,4β,5,6α,8,14,15α,25,26-nonaol and (22*E*,24*S*)-24-Methyl-5α-cholest-22-ene-3β,4β,5,6α,8,14,15α,25,28-nonaol, Minor Marine Polyhydroxysteroids Isolated from the Starfish *Archaster typicus*

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The structures of two minor polyhydroxysteroids isolated from the starfish *Archaster typicus* were determined as (22*E*,24*R*,25*R*)-24-methyl-5α-cholest-22-ene-3β,4β,5,6α,8,14,15α,25,26-nonaol (**4**) and (22*E*,24*S*)-24-methyl-5α-cholest-22-ene-3β,4β,5,6α,8,14,15α,25,28-nonaol (**5**).

The stereochemistry at the C-24 and C-25 positions in compound (**4**) was determined by asymmetric synthesis of 2,3-dimethylpentane-1,2-diols as models and comparison of the spectral data of their 1-(+)-MTPA esters with those of the 26-(+)-MTPA ester of the 22,23-dihydro derivative of the natural material. Similarly the stereochemistry at the C-24 position in compound (**5**) was proposed by comparison of the spectral data with those of a model compound.

The starfish *Archaster typicus*, collected off Nouméa, New Caledonia, contains large amounts of highly hydroxylated steroids. We have hitherto identified seven compounds having cholestane [*e.g.* (**1**)] and 27-norcholestane skeletons [*e.g.* (**2**) and (**3**)]. A common functionality of these compounds is the 3β,6α,8,14,15α-pentahydroxy moiety, other hydroxylation positions in the nucleus being 4β, 5α, and 7β. Some of these steroids have a sulphate group located at C-6 or C-15. Their structures were deduced from spectral data [1] and very recently the structure of (24*R*)-27-nor-5α-cholestane-3β,4β,5,6α,7β,8,14,15-α,24-nonaol (**2**) was confirmed by a single-crystal *X*-ray study.[2]

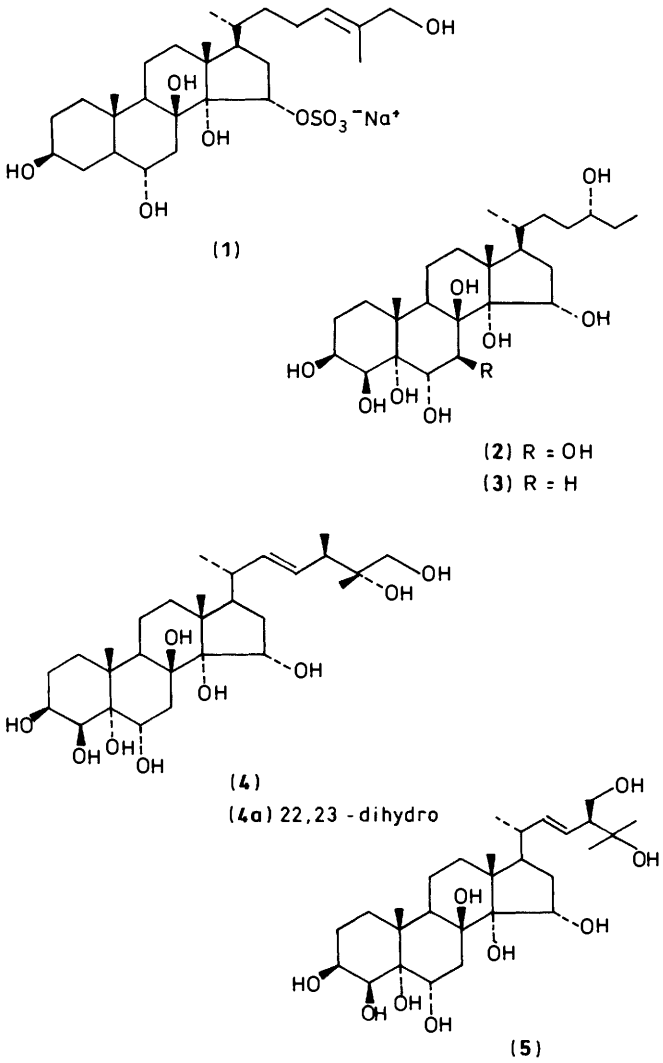
In the present paper we report the structures of two new polyhydroxysteroids (**4**) and (**5**) having a Δ^{22} 24-methylcholestane skeleton.

Reverse-phase high-performance liquid chromatography (h.p.l.c.) of a polyhydroxysteroids fraction obtained from the aqueous extracts of *A. typicus* (7.5 kg, fresh weight) by chromatography on Amberlite XAD-2, followed by chromatography on Sephadex LH-60 and LH-20 and droplet countercurrent chromatography (DCCC), gave compound (**4**) (22 mg), m.p. 288—290 °C; $[\alpha]_D$ +33.3°; and compound (**5**) (3 mg), $[\alpha]_D$ +18.4°. An examination of their spectral data (^{1}H and ^{13}C n.m.r., Tables 1 and 2) indicated that the two minor compounds contained the same 3β,4β,5,6α,8,14,15α-heptahydroxytetracyclic nucleus as compound (**3**). Electron-impact mass spectrometry showed no molecular ions, but only very small fragments at *m/z* 492, 474, 456, and 438 corresponding to loss of two, three, four, and five molecules of water from M^+. The ^{13}C n.m.r. spectrum (Table 2) indicated that both compounds contained a total of 28 carbon atoms, and DEPT measurements revealed the presence in both compounds of a C_9 side-chain containing three methyl groups, two methines, together with one CH_2OH, one

-COH, and two =CH groups.

Continuing now with the analysis of ^{1}H n.m.r. data for compound (**4**) in CD_3OD (Table 1), an AB quartet (J_{AB} 11 Hz) at δ_H 3.40 and 3.49 indicated a CH_2OH group linked to a quaternary carbon. The spectrum also showed the signals of two secondary methyls at δ_H 0.95 (d, *J* 6 Hz) and 1.01 (d, *J* 7 Hz), one tertiary methyl (which is geminal to oxygen) at δ_H 1.12, and two well separated olefinic double doublets at δ_H 5.27 (dd, *J* 15, 7.5 Hz) and 5.44 (dd, *J* 15, 8 Hz) indicative of a Δ^{22E}-double bond.

Thus the structure of compound (**4**) was determined as (*E*)-24-methyl-5α-cholest-22-ene-3β,4β,5,6α,8,14,15α,25,26-nonaol.



The remaining features needed to establish the structure fully are the stereochemistries at C-24 and C-25. This required the synthesis of model compounds and we decided to synthesize the 2,3-dimethylpentane-1,2-diols and compare their spectral data

Table 1. Selected 250 MHz ^{1}H n.m.r. signals for steroids (**4**) and (**5**) in CD_3OD (*J* in Hz)

	(**4**)	(**5**)	Ref. compound (**6**)	Ref. compound (**3**[1])
3-H	4.07 m	4.06 m	3.52 m	4.06 m
4-H	3.96 d (4)	3.96 d (4)		3.96 d (6)
6-H	4.37 dd (5, 12)	4.37 dd (5, 12)	5.35 br d	4.37 dd (5, 12)
15-H	4.45 dd (4.2, 9.5)	4.44 dd (4.2, 9.5)		4.46 dd (4.2, 9.5)
18-H_3	1.16 s	1.17 s	0.77 s	1.14 s
19-H_3	1.33 s	1.33 s	1.05 s	1.33 s
21-H_3	0.95 d (6)	0.98 d (6.5)	1.08 d (6.5)	0.88 d (7)
22-H	5.44 dd (15, 8)	5.42 dd (15, 8)	5.42 dd (15, 8)	
23-H	5.27 dd (15, 8.5)	5.25 dd (15, 9)	5.24 dd (15, 9)	
26-$H_{2(3)}$	3.40—3.94 d (11)	1.18 s	1.18 s	
27-H_3	1.12 s	1.20 s	1.20 s	
28-$H_{3(2)}$	1.01 d (7)	3.62 dd (11, 7), 3.86 dd (11, 6.5)	3.60 dd (11, 7), 3.86 dd (11, 6.5)	

Table 2. ^{13}C N.m.r. shifts (δ_C) of steroids (**4**) and (**5**)[a]

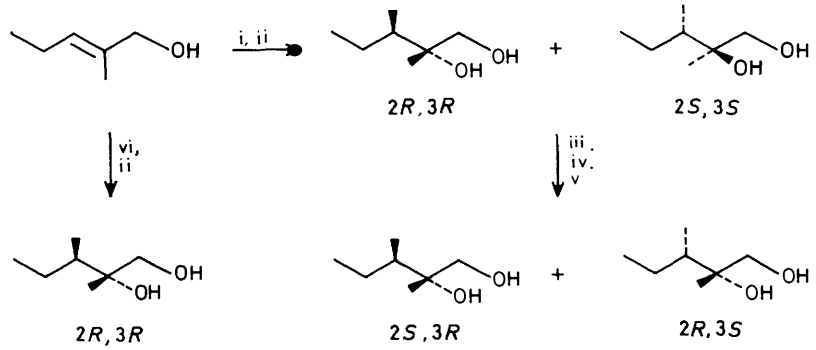
Carbon	(**4**)	(**5**)	Ref. compound (**6**)[b]	Ref. compound (**3**)[1]
1	32.8	32.9	37.5	32.8
2	26.7	26.8	32.0	26.8
3	68.5	68.5	72.7	68.5
4	72.5	72.6	43.0	72.6
5	78.1	78.2	141.7	78.1
6	65.8	65.8	121.0	65.8
7	40.4	40.4	32.2	40.0
8	78.8	78.8	32.1	78.8
9	41.1	41.1	50.5	41.1
10	39.7	39.8	36.7	39.8
11	18.0	18.0	21.2	18.0
12	39.7	39.8	39.9	39.8
13	47.8	47.9	42.5	48.0
14	84.6	84.6	57.0	84.4
15	69.1	62.1	24.4	69.1
16	34.8	34.9	28.6	35.0
17	50.9	50.7	55.8	51.1
18	17.3	17.4	12.2	17.2
19	17.3	17.4	19.4	17.4
20	39.7	40.0 (40.2)	40.3	
21	20.8	20.5 (20.6)	20.7	
22	137.7	140.0 (142.0)	142.2	
23	130.3	126.3 (126.8)	124.7	
24	44.2	55.5 (56.3)	55.4	
25	74.3	72.7 (72.7)	73.0	
26	68.7	26.1 (25.9)	26.3	
27	22.2	30.1 (29.7)	29.4	
28	15.5	64.2 (64.5)	64.0	

[a] Measured at 62.9 MHz in [$^2H_5$]pyridine; values in parentheses are shifts measured in CD_3OD; assignments were aided by the DEPT technique. [b] From CD_3OD solution.

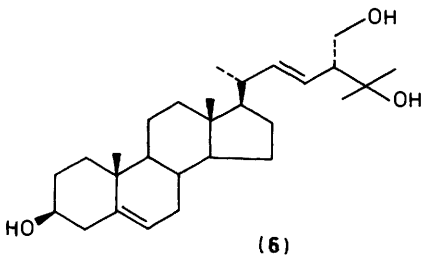
with those of the 22,23-dihydro derivative of the natural material (**4**) *i.e.* compound (**4a**). The syntheses are outlined in the Scheme. Epoxidation of (*E*)-2-methylpent-2-en-1-ol followed by reaction with lithium dimethylcuprate gave the (2*R*,3*R*)/(2*S*,3*S*)-2,3-dimethylpentane-1,2-diol enantiomeric pair, which was converted into the (2*S*,3*R*)/(2*R*,3*S*)-enantiomeric pair by tosylation, alkaline treatment, and opening of the resulting 1,2-epoxide with 0.05M-aq. sulphuric acid at room temperature for 3 h. In the ^{1}H n.m.r. spectrum of the dihydro derivative (**4a**), the 26-, 27-, and 28-protons resonated at δ 3.42—3.44 (2 × 1 H, each d, *J* 11 Hz), 1.04 (s, 27-H_3), and 0.88 (d, *J* 7 Hz, 28-H_3) in close accord with the values at δ 3.42—3.49 (2 × 1 H, each d, *J* 11 Hz,1-H_2), 1.04 (s), and 0.89 (d, *J* 7 Hz) measured for the enantiomeric pair (2*R*,3*R*)/(2*S*,3*S*)-2,3-dimethylpentane-1,2-diol as compared with the values at δ 3.45 (ABq, *J* 11 Hz), 1.07 (s), and 0.96 (d, *J* 7 Hz) measured for the other, (2*S*,3*R*)/(2*R*,3*S*)-enantiomeric pair.

In order to distinguish between the configuration 2*R*,3*R* and 2*S*,3*S* we synthesized, from (*E*)-2-methylpent-2-en-1-ol, the optically active (2*R*,3*R*)-2,3-dimethylpentane-1,2-diol by using the titanium tartrate-catalysed asymmetric epoxidation of allylic alcohols discovered by Katsuki and Sharpless,[3] followed by opening of the oxirane ring with lithium dimethylcuprate (Scheme). A (+)-(*R*)-MTPA (α-methoxy-α-trifluoromethylphenylacetate)(β,β,β-trifluoro-α-methoxy-α-phenyl(propionate) ester was then prepared from both the enantiomer (2*R*,3*R*)-2,3-dimethylpentane-1,2-diol and the (2*R*,3*R*)/(2*S*,3*S*)-enantiomeric pair. In the ^{1}H n.m.r. spectrum of the (+)-MTPA ester of the (2*R*,3*R*)-enantiomer the C-1 methylene protons appeared as an AB quartet at δ 4.16 (*J* 11 Hz) with the two central lines separated by 3.8 Hz, closely resembling the signal for the 26-protons of the (+)-MTPA ester of (**4a**) (AB quartet with the central lines separated by 3.8 Hz). In the spectrum of the (+)-MTPA ester of the (2*R*,3*R*)/(2*S*,3*S*)-enantiomeric pair the signals of the C-1 protons of the (2*S*,3*S*)-isomer appeared as two well separated doublets (*J* 11 Hz) at δ 4.05 and 4.27. On this basis the configurations of C-24 and C-25 of compound (**4a**) and accordingly of (**4**) are suggested to be 24*R*, 25*R*.

Coming back now to the ^{1}H n.m.r. analysis of compound (**5**) (Table 1), the signals at δ_H 5.25 (1 H, dd, *J* 15, 9 Hz) and 5.42 (1 H, dd, *J* 15, 8 Hz) indicated the presence of a Δ^{22E}-double bond. The spectrum also contained two one-proton double doublets at δ_H 3.62 (*J* 11, 7 Hz) and 3.86 (*J* 11, 6.5 Hz) associated with a 24-hydroxymethyl group, a signal for a secondary methyl group at δ_H 0.98 (d, *J* 6 Hz), and two three-protons singlets at δ_H 1.18 and 1.20 associated with tertiary methyls which are geminal to oxygen. Sequential decoupling, which also allowed the assignment of the signals for 20-H (m, δ 2.08) and 24-H (m, δ 2.18), established the sequence -(Me)CH–CH=CH–CH-(CH_2OH)-. The structural assignment of the side-chain of compound (**5**) received support from the direct comparison with 25,28-dihydroxy-7,8-dihydroergosterol [*i.e.* (22*E*,24*R*)-24-methylcholesta-5,22-diene-3β,25,28-triol(**6**)] obtained by stereospecific synthesis by Midland and Kwon[4] (a sample was generously given to us by Professor Midland). The ^{1}H n.m.r. signals for the side-chain protons of the synthetic material (**6**) were virtually superposable with those of the natural steroid (**5**) (Table 1), except that the signal for the 21-methyl protons in the natural product (**4**) was observed shifted upfield to δ 0.98 (δ 1.08 in the synthetic material, CD_3OD). A 0.05—0.1 p.p.m. upfield deviation of the chemical shift values for C-21 methyl protons has been observed in the spectra of all steroids of *A. typicus*[1] and is ascribed to the presence of the 14α,15α-glycol structure, after a single-crystal *X*-ray study on the steroid (**2**), which confirmed



Scheme. Synthesis of model 2,3-dimethylpentane-1,2-diols. *Reagents and conditions:* i, *m*-$ClC_6H_4CO_3H$; ii, $LiMe_2Cu$; iii, TsCl–pyridine; iv, Na_2CO_3; v, H_3^+O; vi, L-(+)-diethyl tartrate, Me_3CO-OH $Ti(OPr^i)_4$, CH_2Cl_2, −20 °C



(6)

the common 20*R* configuration.[2,†] The lack of the synthetic (24*S*)-epimer of (**6**) did not allow us to assign the stereochemistry at C-24 of the natural product (**5**) with confidence. Even so, an accurate analysis of the ^{13}C n.m.r. data of the model compound (**6**) and the natural product (**5**) in CD_3OD (Table 2) showed small but significative differences especially at carbons near to the chiral centre (C-24). We assign these differences to the different configuration at C-24 and on this basis we suggest the 24*S*-configuration for the steroid (**5**).

Experimental

General Methods.—For general methods see ref. 1. Light petroleum refers to the fraction boiling in the range 40—70 °C.

Isolation of Steroids (**4**) *and* (**5**).—Extraction of *Archaster typicus* (7.5 kg, fresh) and chromatography, on a column of Amberlite XAD-2, of the aqueous extracts followed by two successive chromatographic purifications of the methanol eluate on columns of Sephadex LH-60 and Sephadex LH-20 to separate the sulphated 'asterosaponins' from the polyhydroxysteroids fraction was reported in ref. 1. The polyhydroxysteroid fraction was then submitted to DCCC with chloroform–methanol–water (7:13:8) in the ascending mode and 6-ml fractions were collected. Fractions 80—100 contained a mixture of mainly compounds (**4**) and (**5**) (35 mg), which were separated by h.p.l.c. on a μ-Bondapak C_{18} column with 70% aq. MeOH as eluant to give compound (**5**) (3 mg, elution time 16.5 min) as a glassy material, $[\alpha]_D$ +18.4° (*c* 0.4 in MeOH), and compound (**4**) (22 mg, elution time 22 min), m.p. 288—290 °C (from MeOH); $[\alpha]_D$ 33.3° (*c* 1.0 in MeOH). Spectral data are in Tables 1 and 2.

(2R,3R)/(2S,3S)-2,3-*Dimethylpentane*-1,2-*diol Enantiomeric Pair.*—A solution of (*E*)-2-methylpent-2-en-1-ol (2 g) in CH_2Cl_2 (30 ml) was treated with *m*-chloroperbenzoic acid (4 g) at 0 °C and the mixture was left at room temperature overnight. The solution was washed successively with saturated aq. $NaHCO_3$ and water, and the solvent was evaporated off to give a crude oil (1.5 g). The ^1H n.m.r. spectrum was consistent with the expected product, the *trans*-epoxide, δ_H ($CDCl_3$) 1.02 (3 H, t, *J* 7 Hz, *Me*CH_2), 1.29 (3 H, s, 2-Me), 1.59 (2 H, m, MeCH_2), 3.04 (1 H, t, *J* 6 Hz, 3-H), and 3.58—3.72 (2 × 1 H, each d, *J* 13 Hz, CH_2OH).

The crude product (0.7 g) was dissolved in dry ethyl ether (4 ml) and the solution was slowly added to a solution of lithium dimethylcuprate at 0 °C, prepared by adding, under nitrogen, 1.6M-methyl-lithium in hexane (36 ml) to a stirred suspension of copper(I) iodide (5.3 g) in diethyl ether (30 ml), and the mixture was allowed to react for 5 h at 0 °C. After warming at room temperature, the mixture was poured into a 2:1 mixture (200 ml) of saturated aq. ammonium chloride solution and 28% aq. ammonium hydroxide and extracted with diethyl ether (3 × 100 ml). The combined ethereal layers were washed with water, dried with sodium sulphate, and evaporated to yield a crude product (0.8 g). The product was purified by column chromatography [silica gel (30 g)] with chloroform and increasing amounts of methanol as eluant to afford, in the 10% methanol fractions, the (2*R*,3*R*)/(2*S*,3*S*)-2,3-dimethylpentane-1,2-diol enantiomeric pair (0.40 g); ^1H n.m.r. data are in the text; δ_C (CD_3OD) 13.1 (C-5), 14.1 (3-Me), 19.6 (2-Me), 24.2 (C-4), 42.6 (C-3), 69.3 (C-1), and 76.2 (C-2).

The diol (20 mg) was treated with a solution of freshly distilled (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (β,β,β-trifluoro-α-methoxy-α-phenylpropionyl chloride) (50 μl) in dry pyridine (1 ml) for 2 h. Removal of solvent gave the diastereoisomeric (+)-MTPA esters mixture, δ_H($CDCl_3$) 0.75 (d, *J* 7 Hz, 3-Me of the 2*R*,3*R*-isomer), 0.78 (d, *J* 7 Hz, 3-Me of the 2*S*,3*S*-isomer), 0.82 (t, *Me*CH_2), 1.00 (s, 2-Me), 1.30 and 1.63 (each br m, 3-H and 4-H_2), 3.47 (s, OMe), 4.05—4.27 (each d, AB system, *J* 11 Hz, CH_2OMTPA of the 2*S*,3*S*-isomer), 4.13—4.18 (each d, AB system, *J* 11 Hz, CH_2OMTPA of the 2*R*,3*R*-isomer), and 7.37, 7.43, and 7.57 (Ph).

(2S,3R)/(2R,3S)-2,3-*Dimethylpentane*-1,2-*diol Enantiomeric Pair.*—(2*R*,3*R*)/(2*S*,3*S*)-2,3-Dimethylpentane-1,2-diol enantiomeric pair (180 mg) was treated with a solution of *p*-TsCl (350 mg) in dry pyridine (4 ml) at 0 °C. After being kept overnight at room temperature, the mixture was poured into 1M-HCl (50 ml) and extracted with diethyl ether (3 × 50 ml). The combined ethereal layers were washed successively with water, saturated aq. $NaHCO_3$, and water, and evaporated to yield the crude tosylester which, without further purification, was dissolved in dry ethanol and treated with Na_2CO_3 (50 mg). The mixture was stirred at room temperature for 3 h. Light petroleum was added and the mixture was washed with water. The organic layer was

† A 20*S*-configuration could be suspected because the proton shifts for the C-21 methyl group are reported as being shifted *ca.* 0.1 p.p.m. upfield in 20*S*-cholesterol relative to 'natural' 20*R*-cholesterol (W. R. Nes, T. E. Vorkey, and K. Krevitz, *J. Am. Chem. Soc.*, 1977, **99**, 250).

dried over anhydrous $MgSO_4$, and evaporated to give the (2*R*,3*R*)/(2*S*,3*S*)-2,3-dimethylpentane 1,2-epoxide enantiomeric pair (140 mg). The ^1H n.m.r. spectrum was consistent with the expected product, δ_H ($CDCl_3$) 0.89 (3 H, t, *J* 7 Hz, *Me*CH_2), 0.91 (3 H, d, *J* 7 Hz, 3-Me), 1.18 (3 H, s, 2-Me), 1.20 (2 H, m, MeCH_2), 1.58 (1 H, m, 3-H) and 2.49 (2 H, s, epoxide methylene protons).

The epoxide (50 mg) was then opened by treatment with 0.05M-aq. H_2SO_4 (5 ml) at room temperature for 3 h. The reaction mixture was then extracted with CH_2Cl_2 (×3). The combined extracts were washed successively with water, saturated aq. $NaHCO_3$, and water, dried over anhydrous Na_2SO_4, and evaporated to give the (2*S*,3*R*)/2*R*,3*S*)-2,3-dimethylpentane-1,2-diol enantiomeric pair (43 mg), which was purified as described before; ^1H n.m.r. spectral data are in the text; δ_C (CD_3OD) 13.0 (C-5), 13.2 (3-Me), 20.4 (2-Me), 25.2 (C-4), 42.8 (C-3), 69.0 (C-1), and 76.0 (C-2).

(2R,3R)-2,3-*Dimethylpentane*-1,2-*diol*.—To dry dichloromethane (15 ml) cooled at −20 °C were added sequentially the following liquids: titanium tetraisopropoxide (Aldrich) (600 μl), L-(+)-diethyl tartrate (Aldrich) (400 μl), t-butyl hydroperoxide (freshly distilled (500 μl) in dry dichloromethane (15 ml), and finally a solution of (*E*)-2-methylpent-2-en-1-ol (980 mg) in dry dichloromethane (10 ml). The resulting homogenous solution was then stored overnight (*ca.* 18 h) in the freezer at *ca.* −20 °C. Then 10% aqueous tartaric acid (6 ml) was added at −20 °C while the mixture was stirred. After 30 min the cooling bath was removed and the mixture was stirred at room temperature for 2 h, according to the method of Katsuki and Sharpless.[3] The organic layer was washed with water, dried (Na_2SO_4), and evaporated to give a pale yellow oil, which was purified by column chromatography [silica gel (60 g)] with hexane and increasing amounts of diethyl ether to give 0.47 g of (2 *R*,3*R*)-2,3-epoxy-2-methylpentan-1-ol, $[\alpha]_D$ +11.1°, which was treated with lithium dimethylcuprate as described above to afford (2*R*,3*R*)-2,3-dimethylpentane-1,2-diol, $[\alpha]_D$ +6.7°. Analysis of this material as the (+)-MTPA ester gave an enantiomeric excess of 95%. The (+)-MTPA ester was prepared as described above; δ_H 0.75 (3 H, d, *J* 7 Hz, 3-Me), 0.82 (3 H, t, *Me*CH_2), 1.00 (3 H, s, 2-Me), 1.30 and 1.63 (each br m, 3-H and 4-H_2), 3.47 (3 H, s, OMe), 4.13—4.18 (2 × 1 H, d, *J* 11 Hz, CH_2O).

Hydrogenation of the Alkene (**4**) *to give Compound* (**4a**).—Hydrogenation of compound (**4**) (12 mg) was carried out at room temperature and atmospheric pressure in MeOH with 10% Pd/C for 24 h. Usual work-up afforded the saturated analogue (**4a**); *m/z* (e.i.m.s.) 494 ($M^+ - 2H_2O$), 476, and 458; δ_H (CD_3OD) data are in the text; δ_C(CD_3OD) C-1, 33.1; C-2, 26.4; C-3, 69.3; C-4, 72.7; C-5, 78.6; C-6, 66.6; C-7, 40.2; C-8, 79.2; C-9, 40.9; C-10, 40.1, C-11, 18.3; C-12, 39.4; C-13, 49.8; C-14, 85.2; C-15, 70.0; C-16, 35.7; C-17; 51.8; C-18, 17.3; C-19, 17.3; C-20, 36.2; C-21, 18.8; C-22, 35.5; C-23; 28.1; C-24, 41.7; C-25, 76.2; C-26, 19.8; C-27, 69.3; C-28, 14.7.

Nonaol (**4a**) (5 mg) was treated with a solution of freshly distilled (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 μl) in dry pyridine (0.5 ml) for 2 h. Removal of solvent gave a glassy residue, which was purified by passage through a Pasteur pipette filled with a slurry of silica gel in chloroform–methanol (95:5) to give the 3,6,26-tri-MTPA ester (6 mg), δ_H($CDCl_3$) 0.79 (3 H, d, *J* 6.5 Hz, 28-H_3), 0.83 (3 H, d, *J* 7 Hz, 21-H_3), 1.05 (3 H, s, 27-H_3), 1.07 (3 H, s, 18-H_3), 1.28 (3 H, s, 19-H_3), 3.52, 3.53, and 3.59 (each 3 H, s, OMe), 4.19 (1 H, d, *J* 3 Hz, 4-H), and 5.50 (2 H, m, 3- and 6-H).

Model Compound: (22E,24R)-24-*Methylcholesta*-5,22-*diene*-3β,25,28-*triol* (**6**).—A sample of 3,25-bis(dimethyl-t-butylsilyl)-protected (**6**) (20 mg) given to us by Professor M. M. Midland[4] (Department of Chemistry, University of California, Riverside), was desilylated using tetrabutylammonium fluoride [100 μl of a 1M solution in tetrahydrofuran (THF)] in THF (2 ml) at room temperature for 2 h.[5] Usual work-up and purification by passage through a Pasteur pipette filled with a slurry of silica gel in light petroleum–EtOAc (8:2) gave compound (**6**) (6 mg); *m/z* (e.i.m.s.) 430 (M^+); spectral data are in Tables 1 and 2.

Acknowledgements

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