mol) of dry pyridine in 10 mL of dry benzene. To this solution was added 1.0 g (5 mmol) of the phosphine oxide **22**. The mixture was refluxed for 1.5 h and allowed to stir for 2 h at room temperature. It was cooled in an ice bath and then treated slowly with 50 mL of 30% sodium hydroxide. The organic layer was separated and dried ($MgSO_4$). The solvent was removed, leaving 0.55 g (60%) of **28** as a clear oil; ^{31}P NMR ($CDCl_3$) δ +25.5; ^{13}C NMR ($CDCl_3$)[23] δ 7.38 (k, 35.2 Hz), 14.18 (i or j, 3.3 Hz), 16.18 (i or j, 5.5 Hz), 21.81 (g), 30.29, 31.46, 32.20 (b, f, or h), 40.29 (e, 4.4 Hz), 43.44 (a, 3.3 Hz), 118.13 (c, 12.1 Hz), 144.36 (d, 5.5 Hz).

(23) Carbon designations are those used for **22** in Table I; values in parentheses are $^{13}C^{31}P$ coupling constants (hertz).

The phosphine was dissolved in hexane and excess methyl iodide was added to form the phosphonium salt **28**. The salt was isolated as a white solid; mp 204-206 °C dec; ^{31}P NMR ($CDCl_3$) + 41.1. Anal. Calcd for $C_{12}H_{22}IP$: C, 44.46; H, 6.84; P, 9.55. Found: C, 44.25; H, 6.85; P, 9.99.

Registry No. **5**, 73376-41-7; **6**, 76549-55-8; **7**, 76505-31-2; **8**, 87432-88-0; **9**, 87432-89-1; **10**, 87432-90-4; **11**, 87432-91-5; **13**, 87450-35-9; **14**, 87432-92-6; **15**, 87432-93-7; **16**, 16083-91-3; **17**, 87432-94-8; **18**, 34136-10-2; **19**, 87432-95-9; **20**, 87432-96-0; **21**, 57377-76-1; **22**, 87507-29-7; **23**, 87432-97-1; **26**, 87432-98-2; **27**, 87432-99-3; **28**, 87433-00-9; *m*-chloroperbenzoic acid, 937-14-4; trifluoroperoxyacetic acid, 359-48-8.

Sterols of Marine Invertebrates. 44.[1a] Isolation, Structure Elucidation, Partial Synthesis, and Determination of Absolute Configuration of Pulchrasterol. The First Example of Double Bioalkylation of the Sterol Side Chain at Position 26

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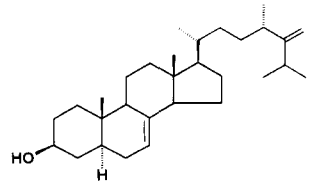
Received April 26, 1983

A new C_{30} marine sterol, (24*S*)-24,26,26-trimethylcholesta-7,25(27)-dien-3β-ol (pulchrasterol), has been isolated as the major component from the sterol fraction of the New Zealand deep sea sponge *Aciculites pulchra*. Its general structure was deduced from spectral data (360-MHz ^1H NMR and MS) and partial synthesis of its Δ^5 analogue, while its stereochemistry was determined by correlating spectral data (300-MHz ^1H NMR and CD) from synthetic steroids of similar structure and known configuration. The side chain of pulchrasterol is doubly biomethylated at position 26, which is discussed with respect to its possible biosynthetic origin and its unexpected 24*S* absolute configuration.

Introduction

It frequently occurs that the *trace* sterol components of a marine invertebrate possess unique hydrocarbon side chains unlike any found in terrestrial plants or animals.[2,3] The biosynthetic pathways that produce these novel side chains are currently under intense investigation.[2,3] When a *major* sterol component is discovered to possess a previously unknown side chain, then, aside from its biosynthetic novelty, the question of its biological function as an active membrane constituent becomes relevant. Herein, we report the isolation, structure elucidation, partial synthesis, and determination of absolute configuration of pulchrasterol (1), the major sterol component of *Aciculites pulchra*, which constitutes the first example of double biomethylation of the sterol side chain at position 26 and which probably serves as a membrane constituent.



1

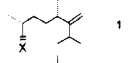
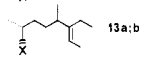
Pulchrasterol

(1) (a) For Part 43 in this series, see: Popov, S.; Carlson, R. M. K.; Djerassi, C. *Steroids*, submitted for publication. (b) Visiting investigator at Stanford University from the Shenyang College of Pharmacy and Liaoning Institute of Materia Medica, China. (c) Department of Zoology, University of Auckland, Auckland, New Zealand.

(2) (a) Djerassi, C. *Pure Appl. Chem.* **1981**, *53*, 873-890. (b) Djerassi, C.; Theobald, N.; Kokke, W. C. M. C.; Pak, C. S.; Carlson, R. M. K. *Ibid.* **1979**, *51*, 1815-1828.

(3) Catalan, C. A. N.; Thompson, J. E.; Kokke, W. C. M. C.; Djerassi, C. *Tetrahedron*, submitted for publication.

Table I. Sterols of *Aciculites pulchra*

structure	rel retent times[a] HPLC RRT	rel retent times[a] GC RRT	M^+ *m/z*	% of total sterol fraction
	1.00	1.00	386	10.6
 1	1.07	2.24	426	73.7
 13a;b	1.07	2.53	426	15.7

[a] Cholesterol was used as the standard (1.00) for both HPLC RRT and GC RRT. The conditions for HPLC were 100% methanol on a Whatman ODS-2 column at ambient temperatures. The conditions for GC were 260 °C on a 3% OV-17 column. (See Experimental Section for further details.)

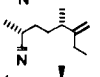
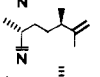
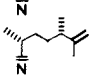
Isolation and Structure Elucidation

The 4-demethyl sterol fraction (15 mg) of the New Zealand deep sea sponge *Aciculites pulchra* was isolated by column chromatography, checked by GC for composition (three components, see Table I), and subjected to HPLC. Analysis by GC/MS revealed cholesterol (M^+ = 386) as one of the minor components and two other sterols both having M^+ = 426 daltons, which indicated an empirical formula of $C_{30}H_{50}O$ and two degrees of unsaturation in the nucleus and/or the side chain. The 246-dalton peak corresponding to loss of side chain and part of ring D of each of the unidentified sterols was used to assign a Δ^7 unsaturation,[4] which was confirmed by ^1H NMR analysis[5]

(4) Patridge, L. G.; Midgley, I.; Djerassi, C. *J. Am. Chem. Soc.* **1977**, *99*, 7686-7692.

0022-3263/83/1948-4472$01.50/0

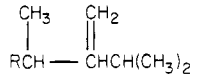
Table II. 300-MHz ^{1}H NMR Data for the Methyl Groups of the Free Hydroxy Dienes in $CDCl_3$ and C_6D_6 [a]

compound		abs config	chemical shift values (coupling constants) C-18	C-19	C-21	C-28	C-29; C-30	C-26 H
	1 [b,c]	(24*S*)	0.527	0.794	0.916 (6.54)	1.003 (NA)[d]	1.023; 1.023 (7.30)	4.685/4.750
			[0.603]	[0.746]	[1.011 (6.45)]	[1.095 (6.94)]	[1.067; 1.067 (6.82)]	[4.878/4.919]
	5a [c]	(24*R*)	0.668	1.004	0.907 (6.50)	0.996 (6.62)	1.020; 1.020 (7.01)	4.684/4.756
			[0.658]	[0.943]	[1.011 (6.42)]	[1.099 (6.85)]	[1.067; 1.067 (6.84)]	[4.868/4.919]
	5b [c]	(24*S*)	0.668	1.004	0.907 (6.50)	1.004 (6.62)	1.019; 1.019 (7.01)	4.682/4.745
			[0.668]	[0.944]	[1.011 (6.44)]	[1.094 (6.85)]	[1.066; 1.066 (6.84)]	[4.868/4.919]
	8a	(24*R*)	0.667	1.004	0.906 (6.58)	0.993 (6.70)	1.029 (7.36)	4.690/4.704
	8b	(24*S*)	0.667	1.004	0.906 (6.58)	0.999 (6.72)	1.029 (7.36)	4.684/4.702
	9	(24*R*)	0.667	1.004	0.906 (6.58)	0.992 (6.72)	1.028 (7.36)	4.688/4.703
	12a	(24*R*)	0.672	1.005	0.914 (6.50)	0.985 (6.87)	1.649	4.654/4.654
	12b	(24*S*)	0.672	1.006	0.910 (6.58)	0.991 (6.87)	1.635	4.657/4.660

[a] The chemical shift values are given in parts per million (ppm) and were referenced to $CDCl_3$ (7.260 ppm). The coupling constants are given in hertz and enclosed in the parentheses. [b] The NMR data reported for this compound were measured at 360 MHz. Compound 1 gave a broad singlet at 5.250 ppm. All other compounds show a doublet at 5.354 ppm. [c] Data in brackets were measured in C_6D_6. [d] NA means not accessible.

(see Table II). The 328- and 314-dalton peaks of these sterols are typical McLafferty rearrangements associated with a Δ^{25} double bond.[6] Sterols having molecular weights of 426 and unsaturations at positions 7 and 25 have previously not been isolated or synthesized.[2,7]

The 360-MHz data of the major sterol component displayed a broad singlet at 5.250 ppm in $CDCl_3$ (see Table II) characteristic of a Δ^7 vinyl proton.[7] The resonances assigned to the C-18 and C-19 methyl protons (0.527 and 0.794 ppm, respectively) confirmed the Δ^7 unsaturation.[5,7] Resonances at 4.685 and 4.750 ppm indicated a terminal methylene group[8] in accord with the aforementioned MS data. The doublet at 0.916 ppm was identified as the C-21 methyl group.[5] The resonances at 1.003 and 1.023 ppm (9 H) were interpreted as three overlapping methyl groups on the basis of the following: (1) the apparent coupling constants ($J \simeq 6.4$–6.8 Hz),[5,7,8] which are typical for a methyl group attached to a disubstituted carbon, (2) the chemical shifts,[5] and (3) the need to satisfy the empirical formula/structure relationship. Further interpretation of the $CDCl_3$ spectrum proved difficult. The 360-MHz ^{1}H NMR spectrum of this major sterol component was measured in C_6D_6 to observe any aromatic solvent shift (ASIS) of the resonances (see Table II). The resonances found to overlap in $CDCl_3$ near 1.000 ppm were well-resolved in C_6D_6 as a doublet (6 H) at 1.067 ppm and a doublet (3 H) at 1.095 ppm. In addition, a septet (1 H) and a broad doublet (1 H) appeared at 2.184 and 2.033 ppm, respectively. These resonances are typical of allylic protons.[9] The septet at 2.184 ppm implies that an allylic proton is coupled to two methyl groups forming an isopropyl unit. This arrangement is satisfied if an isopropyl group is attached to the unsaturated carbon at position 25. Selective irradiation of the septet at 2.184 ppm simplified the doublet at 1.067 ppm into a singlet, indicating that both methyls of the isopropyl group have identical resonances. Selective irradiation at 2.033 ppm simplified the doublet at 1.095 ppm, inferring that methyl group was allylic. Selective irradiation at 1.095 ppm simplified the bandshape at 2.033 ppm, while selective irradiation at 1.067 ppm reduced the septet to a singlet. The substructure of the side chain can then be defined as



From these data the structure (1) of the major sterol was deduced and given the trivial name pulchasterol. In retrospect, it was found that 25-dehydroaplysterol (9)[8] served as a good model for the comparison of 360-MHz ^{1}H NMR data. By compensating[5,10] the chemical shifts of the C-18 and C-19 methyl groups of 25-dehydroaplysterol for the difference in unsaturation ($\Delta^5 \rightarrow \Delta^7$), a close agreement was found for all chemical shifts for the comparison of 25-dehydroaplysterol with pulchrasterol. The configuration of pulchrasterol at C-24 could not be determined simply by comparing its 360-MHz ^{1}H NMR spectrum with that of known natural sterols. As described below the stereochemical assignment was derived from an extensive

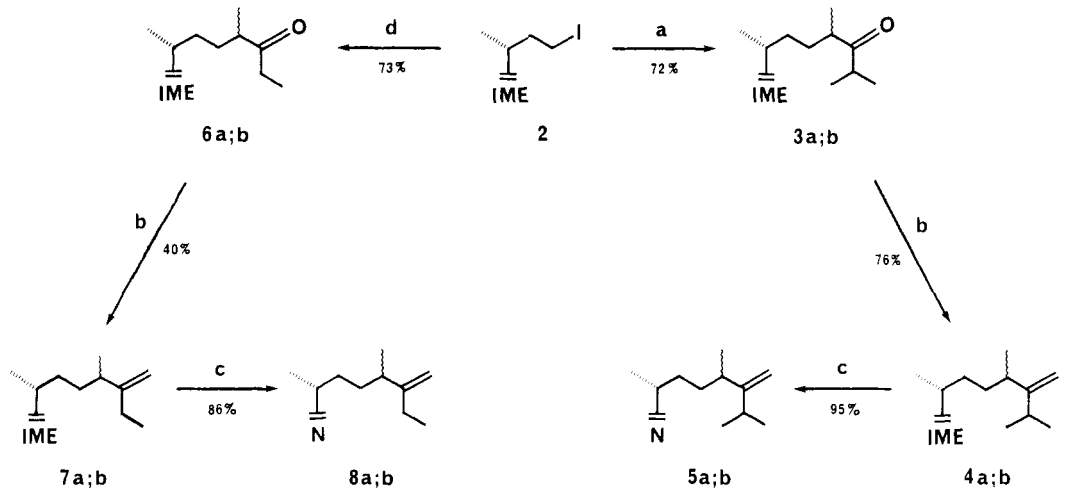
(5) Rubinstein, I.; Goad, L. J.; Clague, A. D. H.; Mulheirn, L. J. *Phytochemistry* **1976**, *15*, 195–200.
(6) Djerassi, C. *Pure Appl. Chem.* **1978**, *50*, 171–184.
(7) Our computerized data bank of ^{1}H NMR (300 MHz and 360 MHz), MS, GC RRT, HPLC RRT, and other data on over 200 sterols isolated from various marine invertebrates permits detailed comparisons to assign stereochemistry and general structure in our search for novel marine sterols.
(8) Kokke, W. C. M. C.; Pak, C. S.; Fenical, W.; Djerassi, C. *Helv. Chim. Acta* **1979**, *62*, 1310–1318.
(9) Pretsch, E.; Seibl, J.; Simon, W.; Clerc, T. "Tabellen zur Strukturaufklärung organischer Verbindeungen mit spektroskopischen Methoden"; Auflage, Springer, Verlag: Berlin, 1981; Vol. 2.
(10) Zürcher, R. F. *Helv. Chim. Acta* **1963**, *46*, 3054–3088.

Table III. 300-MHz ^{1}H NMR Data for the Methyl Groups of the Free Hydroxy Dienes in $CDCl_3$ [a, b]

compound		abs config	chemical shift values (coupling constants) C-18	C-19	C-21	C-28	C-29	C-30	C-26 H
X	13a [c]	(24R,25E) [c]	0.522	0.793	0.910 (6.42)	0.949 (6.96)	0.994 (7.34)	1.568 (NA) [d]	5.185
X	13b [c]	(24S,25E) [c]			0.914 (6.40)	0.959 (NA) [d]		1.559 (NA) [d]	
N	14a	(24R,25E)	0.668	1.006	0.908 (6.53)	0.947 (7.02)	0.993 (7.40)	1.586 (6.62)	5.179
N	14b	(24S,25E)	0.667	1.005	0.902 (6.37)	0.953 (NA) [d]	0.995 (7.57)	1.587 (6.62)	5.146
N	15	(24ξ,25Z)	0.665	1.004	0.899 (6.55)	0.964 (6.78)	0.959 (7.51)	1.586 (6.73)	5.142

[a] The chemical shift values are given in parts per million (ppm) and were referenced to $CDCl_3$ (7.260 ppm). The coupling constants are given in hertz and are enclosed in parentheses. [b] The data for the epimeric mixture of 13a and 13b are displayed in lines 1 and 2 of this table. [c] The assignments are reversible. [d] NA means not accessible.

Scheme I



[a] (a) KH, THF, 2-methylpentan-3-one; (b) $Ph_3PCH_3^+I^-$, KH, Me_2SO; (c) TsOH, dioxane, H_2O; (d) KH, THF, pentan-3-one.

spectroscopic (CD and NMR) analysis of epimeric pairs of synthetic sterols with established absolute configuration.

The 360-MHz ^{1}H NMR spectrum of the unidentified minor sterol component in $CDCl_3$ (see Table III) indicated a 2:1 mixture of epimers noted from the doubling of certain peaks. The resonances at 0.522 and 0.793 ppm assigned to the C-18 and C-19 methyl protons, respectively, inferred a Δ^7 unsaturation[5,10] as indicated by the MS spectral peak at m/z 246.[4] The doublets at 0.914 and 0.910 ppm are typical of C-21 methyl protons.[5] The remaining high-field NMR signals (0.939 → 1.015 ppm) overlapped and could not be readily interpreted. However, by comparing the $CDCl_3$ and C_6D_6 spectra, it was possible to infer the presence of a methyl triplet centered at 1.075 ppm in C_6D_6, which was not readily discerned in the $CDCl_3$ spectrum. A methyl doublet at 1.038 ppm was also distinguished in C_6D_6. Two closely spaced doublets centered at 1.657 and 1.611 ppm (2:1) were observed in C_6D_6 (traces of water had obscured this region in $CDCl_3$), indicating a vinyl methyl group. Interpretation of all the ^{1}H NMR data gathered for this minor component suggested the presence of an ethyl, an isolated methyl, and an ethylidene group as components of the sterol side chain. The aforementioned MS analysis indicated a Δ^{25} unsaturation, which was also consistent with the presence of an ethylidene group. After compensation for the difference[5,10] in unsaturation (Δ^5 vs. Δ^7), the ^{1}H NMR spectra of the minor sterol were essentially identical with those of verongulasterol (**14a**).[7] Therefore, we assume this minor sterol to consist of an epimeric mixture of the two Δ^7 sterols **13a** and **13b**.

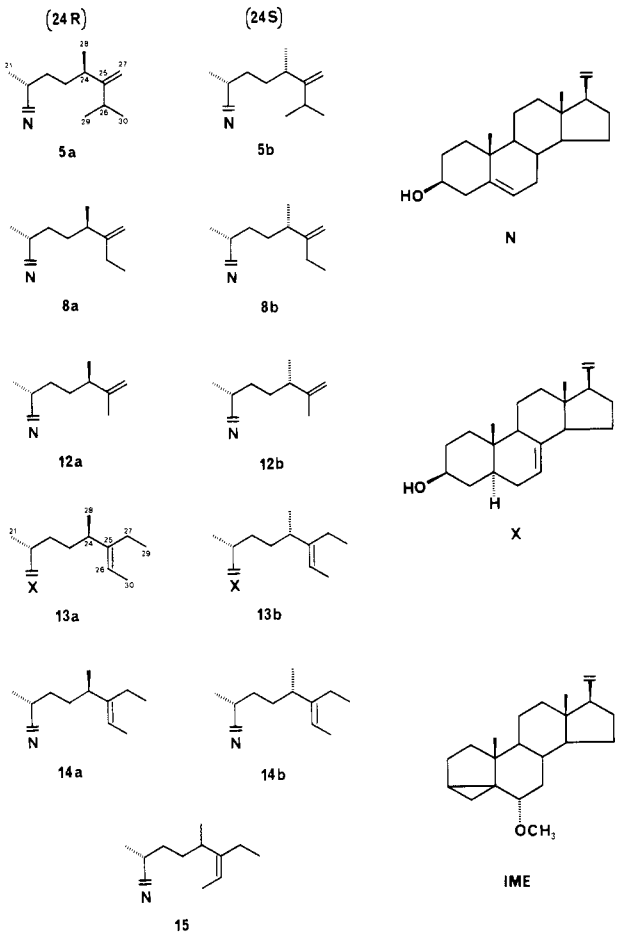
Synthetic Details

To provide support for the proposed structure of pulchrasterol (**1**), the two epimeric Δ^5 analogues **5a** and **5b** were synthesized as outlined in Scheme I. Iodide **2**[11] was treated at 0 °C with the potassium enolate of 2-methylpentan-3-one to afford a 72% yield of a 3:2 mixture of epimeric ketones **3a** and **3b**.[8,12] These epimers, separated by reverse-phase HPLC, were transformed at room temperature into the respective alkenes **4a** and **4b** by Wittig reaction with methylenetriphenylphosphorane in Me_2SO[12] and after removal of the *i*-methyl ether (IME) protecting group afforded free dienes **5a** and **5b** (Δ^5 analogues of pulchrasterol).

To assign the absolute configuration of pulchrasterol at C-24, it was necessary to synthesize the structurally related sterols **5a**, **5b**, **8a**, and **8b**. By substituting pentan-3-one in the above reaction of iodide **2**,[11] a 73% yield of a 3:2 mixture of epimeric ketones **6a** and **6b** was obtained (see Scheme I). These epimers, separated by reverse-phase HPLC, were converted at room temperature by the same process given above into **8a** and **8b**, respectively. As shown

(11) Theobald, N.; Wells, R. J.; Djerassi, C. *J. Am. Chem. Soc.* **1978**, *100*, 7677–7684.

(12) These conditions caused partial epimerization (20–50%) at C-24, which proved useful in the subsequent stereochemical assignments. A small amount of the positional isomer was also produced.



in Scheme II, 25-dehydroaplysterol (**9**), of known[8] absolute configuration (24*R*), was protected as IME **10** and reacted by controlled ozonolysis at −78 °C to afford, with retention of configuration at C-24, ketone **11**, a reference compound required for circular dichroism studies (vide infra).

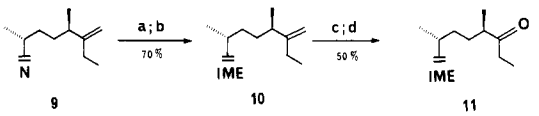
While the respective epimeric mixtures of ketones **3a/3b** and **6a/6b** were readily separable by reverse-phase HPLC, it was either impossible or very difficult to separate the epimeric mixtures of alkenes **4a/4b**, **5a/5b**, **7a/7b**, and **8a/8b**.

Determination of Absolute Configuration

The stereochemistry of pulchrasterol (**1**) at C-24 was related by spectral means to the known 24*R* stereochemistry of the structurally similar 25-dehydroaplysterol (**9**).[8] This was accomplished by (a) comparing the 300-MHz ^{1}H NMR data of the free hydroxy alkenes in $CDCl_3$ (see Table II) and in C_6D_6 (see Table II), (b) comparing the 300-MHz ^{1}H NMR data of the IME alkenes in $CDCl_3$ (see Table III), and (c) comparing the room-temperature and low-temperature chiroptical data of the IME ketones in EPA[13] (see Table IV).

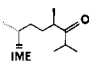
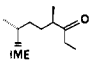
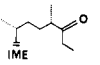
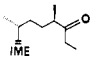
The 0.992-ppm resonance assigned to the C-28 methyl protons of **9** in $CDCl_3$ (see Table II) was attributed to its 24*R* stereochemistry.[8] This correlation served to elucidate the absolute configuration at C-24 for the structurally related sterols **8a**, **8b**, **5a**, **5b**, and **1** in that order. The 300-MHz ^{1}H NMR spectrum of **8a** was identical with that of **9** (see Table II), which demonstrated that **8a** possessed the 24*R* absolute configuration. The 0.999-ppm resonance assigned to the C-28 methyl protons of **8b** was directly

Scheme II



[a] (a) TsCl, pyridine; (b) KOAc, CH_3OH; (c) O_3, CH_2Cl_2; (d) CH_3SCH_3, CH_2Cl_2.

Table IV. Chiroptical Data of *i*-Methyl Ether Ketones in EPA

compd	[θ] (λmax) 25 °C	[θ] (λmax) −196 °C
 3a	−987 (292 nm)	+2884 (293 nm)
 3b	−1124 (292 nm)	−12972 (292 nm)
 6a	−1124 (288 nm)	−50 (281 nm)
 6b	−640 (290 nm)	−8339 (288 nm)
 11	−900 (288 nm)	−28 (282 nm)

attributed to its 24*S* absolute configuration. The 300-MHz ^{1}H NMR data for **8a** were nearly identical with the data found for **8b**, thus confirming that they are indeed epimers. The 0.006 difference in chemical shift values in the C-28 methyl resonances of **8a** and **8b** is consistent with opposing stereochemistries at C-24.[5,7,14] The 0.008 chemical shift difference for the C-28 methyl protons of **5a** (0.996 ppm) vs. **5b** (1.004 ppm) was used to assign a 24*R* absolute configuration to **5a** and a 24*S* absolute configuration to **5b** in accord with the relative difference observed between **8a** (24*R*) and **8b** (24*S*). Comparison of the NMR properties of pulchrasterol (**1**) with those of **5a** and **5b** led to a 24*S* absolute configurational assignment for pulchrasterol (**1**). This was based on the nearly identical values of the C-28 methyl resonances of **1** (1.003 ppm) and **5b** (1.004 ppm). (The difference in nuclear unsaturation, i.e., Δ^7 vs. Δ^5, between pulchrasterol (**1**) and **5b** was assumed to have no significant influence on the chemical shift value of the far removed C-28 methyl group.[5,10])

In C_6D_6 the C-28 methyl protons of pulchrasterol displayed a doublet centered at 1.095 ppm (see Table II) in close agreement with the doublet observed at 1.094 ppm for the C-28 methyl protons of **5b** (24*S* absolute configuration). This agreement gave further support for assigning a 24*S* absolute configuration to pulchrasterol (**1**).

Additional confirmation for the correctness of the assigned absolute configuration of **5a**, **5b**, **8a**, and **8b**, which were used to assign the stereochemistry of pulchrasterol, was drawn from (a) comparing the 300-MHz ^{1}H NMR data for **4a**, **4b**, **7a**, and **7b** with the data for **10** in $CDCl_3$ (see Table V) and (b) comparing the chiroptical data for **3a**, **3b**, **6a**, and **6b** with the data for **11** in EPA measured at 25 and −196 °C (see Table IV). With respect to the NMR

(13) EPA is a 5:5:2 v/v solution of diethyl ether/isopentane/ethanol, which forms a transparent glass at low temperatures.

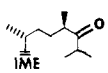
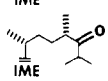
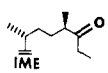
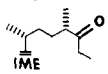
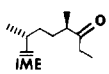
(14) The vinyl proton resonances (see Table II) also reflect the difference in stereochemistry.

Table V. 300-MHz ^{1}H NMR Data for the Methyl Groups of the *i*-Methyl Ether Alkenes in $CDCl_3$[a]

compound		abs config	chemical shift values (coupling constants) C-18	C-19	C-21	C-28	C-29; C-30	C-26 H
	4a	(24*R*)	0.710	1.021	0.907 (6.40)	1.001 (6.99)	1.024; 1.024 (6.97)	4.686/4.751
	4b	(24*S*)	0.708	1.021	0.906 (6.50)	1.009 (6.75)	1.026; 1.025 (6.81)	4.687/4.757
	7a	(24*R*)	0.704	1.017	0.900 (6.46)	0.993 (7.07)	1.031 (7.45)	4.688/4.704
	7b	(24*S*)	0.704	1.017	0.900 (6.46)	0.999 (7.09)	1.031 (7.45)	4.691/4.704
	10	(24*R*)	0.707	1.019	0.903 (6.51)	0.994 (6.82)	1.030 (7.40)	4.688/4.704

[a] The chemical shift values are given in parts per million (ppm) and were referenced to $CDCl_3$ (7.260 ppm). The coupling constants are given in hertz and are enclosed in parentheses.

Table VI. 300-MHz ^{1}H NMR Data for the Methyl Groups of the *i*-Methyl Ether Ketones in $CDCl_3$[a]

compound		abs config	chemical shift values (coupling constants) C-18	C-19	C-21	C-28	C-29	C-30
	3a	(24*R*)	0.706	1.019	0.909 (6.49)	1.037 (7.40)	1.066[b] (6.87)	1.077[b] (6.91)
	3b	(24*S*)	0.703	1.018	0.913 (6.49)	1.050 (6.90)	1.064[c] (6.84)	1.079[c] (6.90)
	6a	(24*R*)	0.702	1.015	0.907 (6.47)	1.049 (6.70)	1.040 (7.48)	
	6b	(24*S*)	0.698	1.013	0.907 (6.48)	1.056 (6.90)	1.039 (7.29)	
	11	(24*R*)	0.702	1.016	0.907 (6.50)	1.050 (6.72)	1.041 (7.59)	

[a] The chemical shift values are given in parts per million (ppm) and were referenced to $CDCl_3$ (7.260 ppm). The coupling constants are given in hertz and are enclosed in parentheses. [b,c] These data are interchangeable.

data, the transformation of **9** into **10** was expected to proceed with retention of configuration of C-24 (compare data in Table V for **10** with those of **7a** and **7b**). The ^{1}H NMR data for the C-28 methyl group of **10** were used to assign the stereochemistry at C-24 for the IME alkenes **7a** and **7b**, which in turn led to the stereochemistry at C-24 for the IME alkenes **4a** and **4b**. The transformation of **10** into **11** under neutral workup conditions was also expected to proceed with retention of configuration at C-24 (compare the data for **11** in Table VI with the data for **6a** and **6b**). The ^{1}H NMR data for the C-28 methyl group of **11** were used to assign the stereochemistry at C-24 for the IME ketones **6a** and **6b**, which in turn were used to assign the stereochemistry for the IME ketones **3a** and **3b**. In this manner the absolute configuration of each pure ketone, **3a** and **3b**, was determined before transforming each into its respective Δ^5 analogue of pulchrasterol.

With respect to the chiroptical data, the circular dichroism (CD) spectra of ketone **11**, with its known 24*R* absolute configuration, was measured at 25 and −196 °C (see Table IV). By comparison of the temperature dependency of the molecular ellipticity of ketone **11** with that of ketones **6a** and **6b** (see Table IV), the absolute configurations of **6a** and **6b** were readily determined. The chiroptical behavior of ketones **3a**, **6a**, and **11** was in direct opposition to that of ketones **3b** and **6b** (i.e., relative to room-temperature data the molecular ellipticity of **3a**, **6a**, and **11** became *less* negative at low temperature, while that of **3b** and **6b** became dramatically *more* negative at low temperature; see Table IV). This unique and revealing phenomenon was attributed to subtle steric interactions between the C-28 and C-19 methyl groups.[15] *It is important to note that if only the room-temperature CD spectra of each ketone had been measured, the wrong stereochemical conclusion would have been reached.*

After assigning the stereochemistry of each epimer, it was found that the 24*S* epimer eluted before the 24*R* epimer whenever an epimeric mixture could be separated by reverse-phase HPLC.[16]

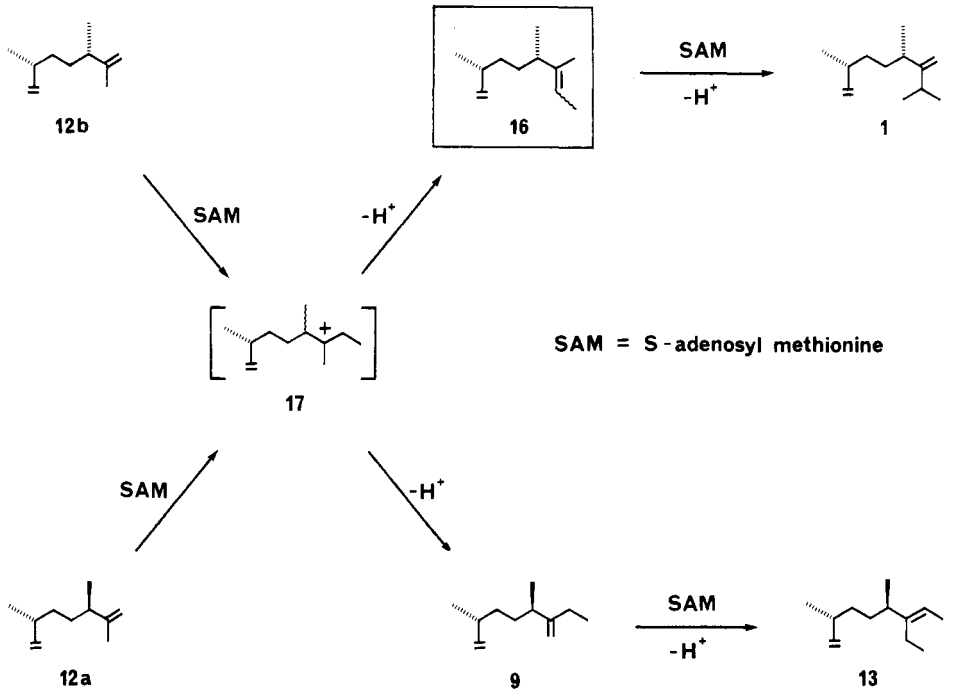
Biosynthetic Implications

Because pulchrasterol (1) is the major sterol component (73.7%) of *Aciculites pulchra*, it is likely to function as an active membrane constituent. With respect to the known marine sterols and known biosynthetic pathways there are two unprecedented features noted for the side chain of pulchrasterol that invite speculation. The double biomethylation of one of the terminal carbons (C-26 or C-27 as defined for the side chain or cholesterol) constitutes a new direction in the biosynthetic pathways of

(15) The steric interactions observed from Dreiding models of **3a** and **3b** indicated that the preferred conformation of **3a** would have its dominating perturber (the sterol nucleus) in a (+) front octant; in contrast, **3b** would have its dominating perturber (the sterol nucleus) in a (−) back octant, which is consistent with the observed low-temperature chiroptical data.

(16) Bohlin, L.; Sjöstrand, U.; Djerassi, C.; Sullivan, B. W. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1023–1028.

Scheme III. Proposed Biosynthetic Pathway to Pulchrasterol



marine sterols.[2,3] The unprecedented reversal of stereochemistry at C-24 (relative to that observed for structurally similar sterols[2,3] extracted from various marine invertebrates) is probably intertwined with the process of double biomethylation. It is tempting to speculate that codisterol (**12b**), which has the same stereochemistry as pulchrasterol (**1**) and is relatively common among marine invertebrates, undergoes biosynthetic transformation into pulchrasterol via the as yet unisolated intermediate **16** due to either a unique conformation enforced by the uncommon 24*S* stereochemistry or the presence of the Δ^7 unsaturation (see Scheme III). Unsaturation at position 7 is not a common feature for sponge sterols[17,18] and the question arises as to when this unsaturation appeared during the biosynthesis. Experimentally this question is difficult to answer because this sponge grows at depths of 100–200 m. It should be noted that sterols with the 24*R* configuration (e.g., **9**) are known[3] to arise by bioalkylation of *epi*-codisterol [(24*R*)-**12a**].

It is interesting to note that a variety of recently discovered C_{30} and C_{31} marine sterols[2,3] can be derived from the cationic aplysterol intermediate **17**, yet only two (**1** and the C-24 epimers of **13**) were actually found in this particular sponge.

Experimental Section

General Methods. High-performance liquid chromatography (HPLC) was used for the preparative-scale separation of the natural sterol fraction of *Aciculites pulchra* and epimeric sterol mixtures. We used Waters Associates HPLC system (comprised of M6000 pump, UK 6 injector, R403 differential refractometer) and two different reverse-phase systems: (i) a Whatman Partisil M9 10/50 ODS-2 column (9 mm i.d. × 50 cm) with absolute methanol as the mobile phase for the separation of the natural sterol mixture and (ii) two Altex Ultrasphere ODS 5-μm columns (10 mm i.d. × 25 cm) in series with methanol/water (95:5, v/v) as the mobile phase for the separation of epimeric sterol mixtures. Flow rates ranged from 3 to 5 mL/min; the sterol solutions were dissolved in a minimum volume of methanol.

Analytical gas–liquid chromatography (GLC) was performed on a Hewlett-Packard 402A gas chromatograph instrument with a flame-ionization detector and a U-shaped glass column (4 mm i.d. × 1.8 m) packed with 3% OV-17 on Gas Chrom Q (100–120 mesh). The oven temperature was 260 °C with helium as the carrier gas at a flow rate of ca. 90 mL/min.

Fourier transform nuclear magnetic resonance spectra (^1H) were recorded on a Bruker HXS-360 spectrometer equipped with a Nicolet TT 1010-A computer and operating at 360 MHz and also on a Nicolet Magnetics Corp. spectrometer equipped with a 1280 data system and operating at 300 MHz. All NMR spectra were referenced to either $CDCl_3$ (7.260 ppm) or C_6D_6 (7.150 ppm).

Low-resolution 70-eV mass spectra were obtained with a Finnigan MAT-44 spectrometer in its GC–MS mode, using a coiled glass column packed with 3% OV-17 on Gas Chrom Q and heated to 260 °C. High-resolution mass spectra were obtained with a Finnigan MAT-711 double-focusing spectrometer in its GC–MS mode and a PDP-11/45 computer for data acquisition and reduction.

Melting points are uncorrected and were determined on a Thomas-Hoover "Unimelt" capillary melting point apparatus. Optical rotations [α] were measured on a Perkin-Elmer Model 141 automatic polarimeter at 20 °C and at 589 nm with chloroform as the solvent.

Chiroptical data were obtained in EPA solution[13] on a JASCO J-40 automatic recording spectropolarimeter attached to a NOVA 840 computer (Data General Corp.) for data reduction. All chiroptical data have been corrected for solvent contraction. Ultraviolet data were likewise measured in EPA solutions, using a Hewlett-Packard 8450A UV/VIS spectrophotometer.

Dry THF was obtained by distilling from $LiAlH_4$ immediately prior to use. Dry Me_2SO was obtained by long-term (more than 2 months) contact with 4 Å molecular sieve spheres under an argon atmosphere. Potassium hydride was washed free of oil under a nitrogen atmosphere by mixing with hexane, vacuum filtering, and storing in a dessicator.

Extraction and Isolation of Free Sterols. An air-dried sample (150 g) of the deep sea sponge *Aciculites pulchra* was collected by dredging near Cape Reinga in June of 1981 at a depth of 100–200 m off the northeastern coast of New Zealand and extracted according to the method of Blegh and Dyer[19] with $CHCl_3/CH_3OH/H_2O$. The $CHCl_3$ layer was evaporated under reduced pressure and the crude residue chromatographed on a

(17) "Handbook of Marine Science: Compounds from Marine Organisms"; CRC Press: Boca Raton, FL, 1976; Vol. 1, pp 29–30.
(18) Bergquist, P. R.; Hofheinz, W.; Oesterhelt, G. *Biochem. Syst. Ecol.* **1980**, *8*, 423–435.
(19) Blegh, E. G.; Dyer, W. J. *Can. J. Biochem.* **1959**, *37*, 911–917.

column of silica gel (50 g) with hexane/diethyl ether (1:1, v/v). Fractions (100 mL) were collected and checked by TLC for sterol content. The yield of free sterols was 0.01% (0.015 g). GC analysis using 3% OV-17 at 260 °C revealed three well-resolved fractions. Fraction 1 (10.6%) was determined to be cholesterol by GC/MS analysis and by comparing its retention time with that of pure cholesterol (GC RRT = 1.00). Fractions 2 (73.7%, GC RRT = 2.24) and 3 (15.7%; GC RRT = 2.53) were also measured. Purification of the complete sterol mixture by revere-phase HPLC using an ODS-2 column with 100% methanol as the mobile phase gave only two fractions. Fraction 1 held only 7% of the total mixture, while fraction 2 held 93%, which included pulchrasterol (1) and Δ^7-verongulasterol (13). HPLC fraction 2 as purified by ODS-2 was separated into two components with use of a pair of reverse-phase Altex Ultrasphere ODS columns in series with CH_3OH/H_2O (98:2, v/v) as the mobile phase. GC/MS analysis of HPLC fraction 1 revealed cholesterol, thus defining its HPLC RRT as 1.00, which in turn inferred a 1.07 HPLC-RRT for fraction 2 as collected from the ODS-2 column.

(24*S*)-24,26,26-Trimethylcholesta-7,25(27)-dien-3β-ol (Pulchrasterol, 1). The data measured for pulchasterol are as follows: mp 130–131 °C (from CH_3OH); $[\alpha]^{20}_D$ +7.0° (*c* 0.2, $CHCl_3$); high-resolution GC–MS (70 eV), *m/z* (relative intensity, assignment) 426.3863 (63, $C_{30}H_{50}O$, M^+), 411.3569 (9, $C_{29}H_{47}O$), 408.3688 (1, $C_{30}H_{48}$), 393.3454 (1, $C_{29}H_{45}$), 383.3318 (1, $C_{27}H_{43}O$), 328.2774 (8, $C_{23}H_{36}O$), 314.2604 (6, $C_{22}H_{34}O$), 273.2172 (8, $C_{19}H_{28}O$), 271.2048 (100, $C_{20}H_{31}$), 255.2110 (19, $C_{19}H_{27}$), 246.1974 (14, $C_{17}H_{26}O$), 231.1755 (10, $C_{16}H_{23}O$), 213.1654 (7, $C_{16}H_{21}$). For ^1H NMR data, see Table II. The chromatographic data are reported in Table I.

(24*R*)- and (24*S*)-24,26,27-Trimethylcholesta-7,25(26)-dien-3β-ol (Δ^7-Verongulasterol, 13a and 13b). The low-resolution mass spectral data (GC–MS) at 70 eV were as follows: *m/z* (relative intensity) 426 (5, M^+), 411 (4), 408 (1), 328 (2), 314 (2), 300 (3), 299 (4), 271 (40), 255 (23), 246 (5), 231 (5), 213 (7), 55 (100). For ^1H NMR data, see Table II. The chromatographic data are reported in Table I.

General Procedures. (A) Generation of Wittig Reagent. Dry Me_2SO (40 mL) was added to oil-free powdered potassium hydride (800 mg, 19 mmol) at room temperature under an argon atmosphere. (One or two crystals of triphenylmethane had been added to serve as an indicator of excess base.) Frothing occurred immediately and the solution turned red. After 1 h the evolution of hydrogen had ceased and a solution of freshly dried methyltriphenylphosphonium bromide (9.5 g, 22 mmol) in dry Me_2SO (40 mL) was added through a syringe. A straw yellow color appeared; the solution was stirred 1 h more before using.

(B) Wittig Reaction. The procedure outlined for the transformation of the mixture of ketones **3a** and **3b** into the mixture of alkenes **4a** and **4b** was used to convert a small quantity (2–10 mg, ca. 0.01 mmol) of each pure ketone into its respective alkene. These reaction conditions caused partial epimerization (20–50%) at C-24. Preliminary purification before HPLC involved passing the crude alkene (after extraction) over a short column (4 mm × 2 cm) of silica gel, using hexane/diethyl ether (10:1, v/v).

(C) Removal of the *i*-Methyl Ether Group. The procedure outlined for the transformation of the mixture of IME alkenes **4a** and **4b** into the mixture of free hydroxy dienes **5a** and **5b** was used to convert the small quantities (0.5–5.0 mg) obtained for each IME alkene into its respective free hydroxy diene. Each free hydroxy diene was purified by reverse-phase HPLC. (The epimeric impurity introduced when each pure ketone was subjected to General Procedure A was carried over into General Procedure B because such mixtures of epimeric IME alkenes and free hydroxy dienes proved inseparable by reverse-phase HPLC.)

(24*R*)- and (24*S*)-24,26,26-Trimethyl-6β-methoxy-3α,5-cyclo-27-norcholestan-25-one (3a and 3b). Dry THF (10 mL) was added through a syringe to powdered potassium hydride (140 mg, 3.5 mmol) at 0 °C under an argon atmosphere. A solution of dry 2-methylpentan-3-one (300 μL, 2.5 mmol) in dry THF (2 mL) was added dropwise via syringe. After 1 h the evolution of hydrogen had ceased and a solution of iodide **2**[11] (235 mg, 0.5 mmol) in dry THF (2 mL) was added via syringe. The reaction mixture was stirred at 0 °C for 3 h and then allowed to warm to room temperature overnight (ca. 17 h). The reaction was quenched by carefully adding a saturated aqueous solution (1 mL) of ammonium chloride and stirring for 10 min. This mixture was partitioned between diethyl ether and water. The isolated organic layer was dried (Na_2SO_4) and evaporated. The resulting residue was chromatographed over a column (25 cm × 1 cm) of silica gel, using hexane/diethyl ether (30:1, v/v) as the eluent. Fractions 7–13 (25 mL/fraction) yielded 166 mg (72% yield) of ketones **3a** and **3b** as an oil. This mixture was separated by using reverse-phase HPLC with CH_3OH/H_2O (95:5, v/v) as the eluent. Pure ketone **3a** had the following: $[\alpha]^{20}_D$ +2.5° (*c* 0.34, $CHCl_3$); for ^1H NMR data, see Table VI; low-resolution mass spectrum (GC–MS), *m/z* (relative intensity) 442 (8, M^+), 427 (6), 410 (12), 387 (10), 289 (6), 255 (14), 71 (100); UV 285 nm (ϵ 60); for circular dichroism data, see Table IV. Pure ketone **3b** had the following: $[\alpha]^{20}_D$ +3.8° (*c* 0.24, $CHCl_3$); for ^1H NMR data, see Table VI; low-resolution mass spectrum (GC–MS), *m/z* (relative intensity) see data for **3a**; UV 284 nm (ϵ 58); for circular dichroism data, see Table IV.

(24*R*)- and (24*S*)-24,26,26-Trimethyl-6β-methoxy-3α,5-cyclocholest-25(27)-ene (4a and 4b). A straw yellow solution (10 mL, 5 equiv) of methylenetriphenylphosphorane (see General Procedure A) was added via syringe to the mixture of epimeric ketones **3a** and **3b** (44 mg, 0.1 mmol) in dry Me_2SO (1 mL) under an argon atmosphere. The reaction mixture was stirred 2.5 h, quenched with water, and partitioned between diethyl ether and water. The isolated organic layer was evaporated and the resultant residue chromatographed over a column (25 cm × 1 cm) of silica gel, using hexane/diethyl ether (95:5, v/v) as the eluent. Fractions 5–8 (30 mL/fraction) yielded 33.4 mg (76% yield) of alkenes **4a** and **4b** as an oil. This mixture was partially separated with difficulty by using reverse-phase HPLC with CH_3OH/H_2O (96:4, v/v) as the mobile phase. The mixture of epimers had the following: low-resolution mass spectrum (GC–MS), *m/z* (relative intensity) 440 (10, M^+), 425 (20), 408 (18), 385 (44), 342 (10), 314 (8), 218 (11), 55 (100); for ^1H NMR data, see Table V.

(24*R*)- and (24*S*)-24,26,26-Trimethylcholesta-5,25(27)-dien-3β-ol (5a and 5b). A solution of alkenes **4a** and **4b** (20 mg) and toluenesulfonic acid (2 mg) in aqueous dioxane (2 mL) was heated at reflux for 1 h and then partitioned between diethyl ether and water. The isolated organic layer was dried (Na_2SO_4) and evaporated. The residue (18 mg, 95% yield) was purified by HPLC to afford an inseparable mixture of **5a** and **5b**, which exhibited the following high-resolution mass spectrum (GC–MS, 70 eV): *m/z* (relative intensity, assignment) 408.3801 (100, $C_{30}H_{48}$), 393.3553 (5, $C_{29}H_{45}$), 310.2660 (3, $C_{23}H_{34}$), 296.2550 (8, $C_{22}H_{32}$), 283.2432 (12, $C_{21}H_{31}$), 253.1937 (10, $C_{19}H_{25}$), 228.1856 (3, $C_{17}H_{24}$), 213.1638 (5, $C_{16}H_{21}$), 97.1014 (50, C_7H_{13}); for ^1H NMR data, see Table II.

(24*R*)-24,26,26-Trimethyl-6β-methoxy-3α,5-cyclocholest-5(27)-ene (4a). Pure **3a** was converted into **4a** by General Procedure B. See synthesis of **4a** and **4b** for mass spectral data. The ^1H NMR data are reported in Table V.

(24*S*)-24,26,26-Trimethyl-6β-methoxy-3α,5-cyclocholest-25(27)-ene (4b). Pure **3b** was converted to **4b** by General Procedure B. ^1H NMR data are reported in Table V. MS data are reported for the mixture of **4a** and **4b**.

(24*R*)-24,26,26-Trimethylcholesta-5,25(27)-dien-3β-ol (5a). Alkene **4a** was converted to **5a** by General Procedure C. ^1H NMR data are reported in Table II. MS data are reported for the mixture of **5a** and **5b**.

(24*S*)-24,26,26-Trimethylcholesta-5,25(27)-dien-3β-ol (Δ^5-Pulchrasterol, 5b). Alkene **4b** was converted to **5b** by General Procedure C. ^1H NMR data are reported in Table II. MS data are reported for the mixture of **5a** and **5b**.

(24*R*)- and (24*S*)-24,26-Dimethyl-6β-methoxy-3α,5-cyclo-27-norcholestan-25-one[20] (6a and 6b). Dry THF (4 mL) was added via syringe to powdered potassium hydride (24 mg, 0.6 mmol) at room temperature under an argon atmosphere. A solution of dry pentan-3-one (43 μL, 0.5 mmol) in dry THF (1 mL) was added dropwise via syringe. After 1 h the evolution of hydrogen had ceased and a solution of iodide **2**[11] (117 mg, 0.25 mmol) in dry THF (2 mL) was added via syringe to the pale yellow enolate solution. The reaction mixture was stirred overnight (ca. 16 h) at room temperature. TLC indicated only 50% conversion; therefore, 2 more equiv. of the potassium enolate (48 mg, 1.2 mmol

(20) Kokke, W. C. M. C.; Fenical, W.; Pak, C. S.; Djerassi, C. *Tetrahedron Lett.* **1978**, 4373–4376.

of potassium hydride and 86 μL, 1.0 mmol of pentan-3-one) were added through a syringe at room temperature. After 3 h of additional stirring, TLC indicated 100% conversion to products. The reaction was quenched by carefully adding a saturated aqueous solution (1 mL) of ammonium chloride. This mixture was partitioned between diethyl ether and water. The isolated organic layer was dried (Na_2SO_4) and evaporated. The resulting residue was chromatographed by preparative TLC on silica gel (two 20 × 20 cm plates), using hexane/diethyl ether (4:1, v/v) as the eluent to afford 78 mg (73% yield) of a mixture of ketones **6a** and **6b** as an oil. This mixture was separated by using reverse-phase HPLC with CH_3OH/H_2O (95:5, v/v) as the mobile phase. Pure **6a** had the following: $[\alpha]^{20}_D$ +2.8° (*c* 0.80, $CHCl_3$); ^1H NMR data are reported in Table VI; low-resolution mass spectrum (GC–MS) at 70 eV, *m/z* (relative intensity) 428 (5, M^+), 413 (5), 396 (6), 373 (7), 275 (2), 255 (8), 229 (4), 213 (12), 57 (100); UV 280 nm (ε 55); circular dichroism data are reported in Table IV. Pure **6b** had the following: $[\alpha]^{20}_D$ +5.2° (*c* 0.46, $CHCl_3$); ^1H NMR data are reported in Table VI; low-resolution mass spectrum (GC–MS) at 70 eV, see data given for **6a**; UV 282 nm (ε 72); circular dichroism data are reported in Table IV.

(24*R*)- and (24*S*)-Dimethyl-6β-methoxy-3α,5-cyclocholest-25(27)-ene[20] (7a and 7b). An oven-dried three-necked flask equipped with a magnetic stirring bar, rubber septa, and a Gooch tube was filled with powdered potassium hydride (58 mg, 1.45 mmol, 14 equiv) and two crystals of triphenylmethane (red colored indicator for excess base) and charged with an atmosphere of argon. At room temperature dry Me_2SO (6 mL) was added. The resulting red froth was vigorously stirred for 1 h before adding solid methyltriphenylphosphonium iodide (700 mg, 1.73 mmol, 17 equiv): stored in the 50-mL flask attached via the Gooch tube. The now clear yellow solution (implying excess Wittig salt) was stirred 15 min before the mixture of ketones **6a** and **6b** (44 mg, 0.106 mmol) in dry THF (1 mL) was added. This solution was stirred overnight, quenched with water, and partitioned between diethyl ether and water. The isolated organic layer was evaporated and the resultant residue chromatographed over a column (10 cm × 1 cm) of silica gel, using hexane/diethyl ether (9:1, v/v) as the eluent. An oily mixture of alkenes **7a** and **7b** was isolated in 40% yield (17.5 mg) and could not be separated by reverse-phase HPLC. For ^1H NMR data on **7a** and **7b**, see Table V. The mixture was then converted to the free hydroxy dienes without further data processing.

(24*R*)- and (24*S*)-24,26-Dimethylcholesta-5,25(27)-dien-3β-ol[20] (25-Dehydroaplysterol, 8a and 8b). A solution of **7a** and **7b** (17.5 mg) and toluenesulfonic acid (ca. 2 mg) in aqueous dioxane (2 mL) was heated at reflux for 1 h, and then partitioned between diethyl ether and water. The isolated organic layer was dried (Na_2SO_4) and evaporated. The residue (15 mg, 86% yield) was purified by HPLC to afford an inseparable mixture of **8a** and **8b**. The physical and spectral data for this mixture have been reported previously.[20] For ^1H NMR data on each diene, see Table II.

(24*R*)-24,26-Dimethyl-6β-methoxy-3α,5-cyclocholest-25-(27)-ene[20] (7a). Pure **6a** was converted to **7a** by General Procedure B. ^1H NMR data are reported in Table V.

(24*S*)-24,26-Dimethyl-6β-methoxy-3α,5-cyclocholest-25-(27)-ene[20] (7b). Pure **6b** was converted to **7b** by General Procedure B. ^1H NMR data are reported in Table V.

(24*R*)-24,26-Dimethylcholesta-5,25(27)-dien-3β-ol[20] (8a). Alkene **7a** was converted to **8a** by General Procedure C. ^1H NMR data are reported in Table II.

(24*S*)-24,26-Dimethylcholesta-5,25(27)-dien-3β-ol[20] (8b). Alkene **7b** was converted to **8b** by General Procedure C. ^1H NMR data are reported in Table II.

(24*R*)-24,26-Dimethyl-6β-methoxy-3α,5-cyclo-27-norcholestan-25-one[20] (11). A solution of 25-dehydroaplysterol (40 mg, 0.09 mmol) taken from a natural source[8] and toluenesulfonyl chloride (50 mg, 0.26 mmol) in pyridine (20 mL) was stirred overnight at room temperature. Water (5 mL) was added and the mixture stirred for 1 h. The mixture was partitioned between diethyl ether and water, the isolated organic layer evaporated, and the residue dissolved in absolute methanol (30 mL) along with some potassium acetate (50 mg, 0.51 mmol) followed by heating at reflux overnight. The mixture was partitioned between diethyl ether and water, the isolated organic layer evaporated, and the residue purified by HPLC. The residue (16 mg) was dissolved in CH_2Cl_2 (1.0 mL) and added dropwise via syringe to 1 equiv of ozone in exactly 1.0 mL of CH_2Cl_2 at −78 °C.[21] After 5 min one drop of dimethyl sulfide was added and the solution stirred for 10 min. The solvent was evaporated and the residue purified by HPLC to afford **11** as an oil. The physical and spectral data for this compound have been reported previously.[20] For 300-MHz ^1H NMR data, see Table VI. For chiroptical data, see Table IV.

Acknowledgment. Financial support by the National Institutes of Health (GM-06840, RR-00612, and GM-28352) is gratefully acknowledged. In addition we are grateful for the use of the 360-MHz FT NMR spectrometer provided by the Stanford Magnetic Resonance Laboratory (NSF Grant No. GP-23633 and NIH Grant No. RR-0711) and for the mass spectral service provided by Annemarie Wegmann. We are indebted to W. C. M. C. Kokke for assistance in HPLC separations and to Ruth Records for measuring the chiroptical data.

Registry No. 1, 87307-28-6; 2, 69101-83-3; **3a**, 87307-29-7; **3b**, 87334-97-2; **4a**, 87307-30-0; **4b**, 87334-98-3; **5a**, 87307-31-1; **5b**, 87334-99-4; **6a**, 87391-81-9; **6b**, 70284-77-4; **7a**, 87307-32-2; **7b**, 87335-00-0; **8a**, 70284-75-2; **8b**, 70354-61-9; **12a**, 71486-08-3; **12b**, 52936-69-3; **13a**, 87307-33-3; **13b**, 87335-01-1; **14a**, 70284-74-1; **14b**, 70284-78-5; **15**, 87419-54-3; methylenetriphenylphosphorane, 3487-44-3; 2-methyl-3-pentanone, 565-69-5; 3-pentanone, 96-22-0.

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Synthesis of the Civet Constituent *cis*-(6-Methyltetrahydropyran-2-yl)acetic Acid

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Received June 1, 1983

Synthesis of the civet constituent *cis*-(6-methyltetrahydropyran-2-yl)acetic acid (**6b**) is described. In the key step, *trans*-2-chloro-6-methyltetrahydropyran (**3**) reacted with dimethyl sodiomalonate with inversion to afford *cis*-dimethyl (6-methyltetrahydropyran-2-yl)malonate (**4a**). Hydrolysis and decarboxylation of **4a** provided **6b**.

(+)-(*S*,*S*)-*cis*-(6-Methyltetrahydropyran-2-yl)acetic acid (**6b**) was recently identified as a minor constituent of civet, the costly glandular secretion of the civet cat, which is utilized in perfumery.[1] The structure was confirmed by

0022-3263/83/1948-4479$01.50/0