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Minor and Trace Sterols in Marine Invertebrates. 8.[1] Isolation, Structure Elucidation, and Partial Synthesis of Two Novel Sterols—Stelliferasterol and Isostelliferasterol

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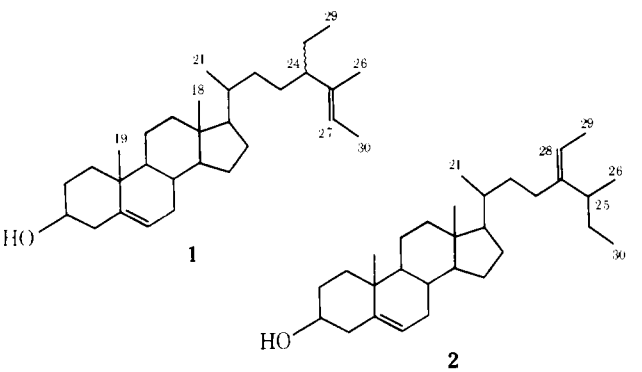
Abstract: The sterol constituents of the Australian sponge *Jaspis stellifera* consist of 12 3β-hydroxy-Δ^5-sterols and five stanols as shown by GC–MS and NMR spectroscopy. Among these were found two novel C_{30} sterols—stelliferasterol and isostelliferasterol—with unique side chains as depicted by structures **1** and **2**. Partial synthesis of these new sterols confirmed their structures and allowed the assignment of some stereochemical details of the sterol side chain. Attention is called to the significance of **1** and **2** in terms of sterol side chain biosynthesis.

The early predictions of Bergmann[2] in 1962 concerning the large diversity of marine sterols have been confirmed in the last 15 years by the discovery of more than 80 new sterols. Scheuer's review[3a] in 1973 and especially the most recent one by Schmitz[3b] showed again that marine organisms contain far more complex mixtures of sterols than terrestrial plants or animals. This amazing variety includes sterols with side chains that have no precedent in terrestrial sources such as cyclopropyl rings, alkylation at carbon atoms 22 and 23, a C_3 substituent at position 24, or an extra carbon at C-27. The diversity seems to be especially pronounced in primitive animals such as sponges and coelenterates. Therefore, our search for novel sterols has included a large number of sponges in an attempt to look for additional "missing links" which would shed some light on the interesting biochemical questions raised by the existence of these uniquely marine sterols.

From the Australian sponge *Jaspis stellifera* we obtained a sterol mixture with a fairly complex composition which forms the subject of this communication. By medium-pressure silver nitrate–silica gel chromatography of the sterol acetates and high-pressure liquid chromatography (LC) of the free sterols on reversed phase (C_{18}) columns we could identify 5 stanols and 12 3β-hydroxy-Δ^5-sterols (see Table I). Among these were found two novel C_{30} sterols which were named stelliferasterol and isostelliferasterol and whose structures were shown to be **1** and **2**.



The mass spectrum[4] of stelliferasterol showed the presence of an important peak at *m/e* 328 which has so far not been noted among marine sterols. By analogy to the well-known[5] McLafferty rearrangement of Δ^{24}-sterols to a mass 314 ion (Scheme I) we attribute the intense *m/e* 328 peak (a, Scheme I) to a similar rearrangement associated with a Δ^{25} double bond. The simultaneous occurrence of an *m/e* 314 peak (b) in the stelliferasterol spectrum can be rationalized by assuming a double bond migration (see Scheme I) in the mass spectrometer to the Δ^{24} position with subsequent McLafferty cleavage. A similar electron impact induced migration of a trisubstituted double bond to a tetrasubstituted position was

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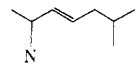
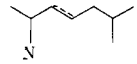
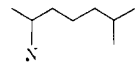
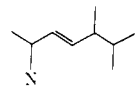
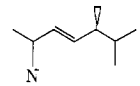
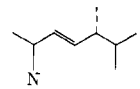
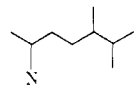
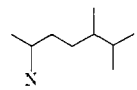
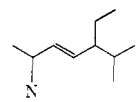
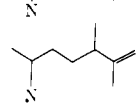
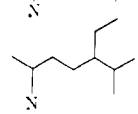
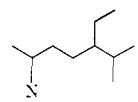
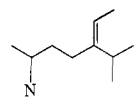
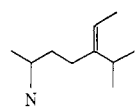
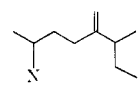
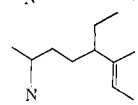
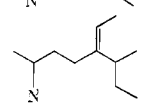
Table I. Sterols Identified in *Jaspis stellifera*

GC rel ret time	mol wt	fr no. col C	amount, %	structure of side chain: 3β-hydroxy-5α-androstane nucleus (N)	structure of side chain: 3β-hydroxy-Δ⁵-androstene nucleus (N)
0.94	384	15	1.5		
1.0	386	4-7	4.7		
1.0	388	3-4	0.7		
1.15	400	7-8	0.8		
1.15	398	9-15	5.0		 17
1.15	398	9-15	5.0		 18
1.3	400	4-7	3.8		
1.3	402	3-4	0.7		
1.42	412	7-9	2.8		
1.42	398	17-20	1.9		
1.6	414	4-7	6.6		
1.6	416	3-4	0.7		
1.78	412	15-16	3.3		 13
1.78	414	15	0.7		
1.78	412	16-20	54.9		 14
2.2	426	1013	5.6		 1
2.4	426	15	1.3		 2

recently observed in our laboratory[6] for Δ^7-sterols. Metastable defocusing experiments verified that both *m/e* 328 and 314 ions originate from the molecular ion. The nature of the steroid nucleus could easily be deduced from its mass spectrum since all the fragments typical[7] for a 3-hydroxy-Δ^5-sterol are present.

The structure of the side chain could be defined precisely by the 360-MHz NMR spectrum (Figure 1) in which all the methyl group signals are separated. The two singlets at 0.659 and 1.003 ppm and the doublet at 0.899 ppm can be assigned to C-18, C-19, and C-21 and are in good agreement with values reported in the literature.[8] If the above mass spectrometric evidence for a Δ^{25} double bond is accepted, then the two vinyl methyl signals must be associated with C-26 (singlet at 1.422

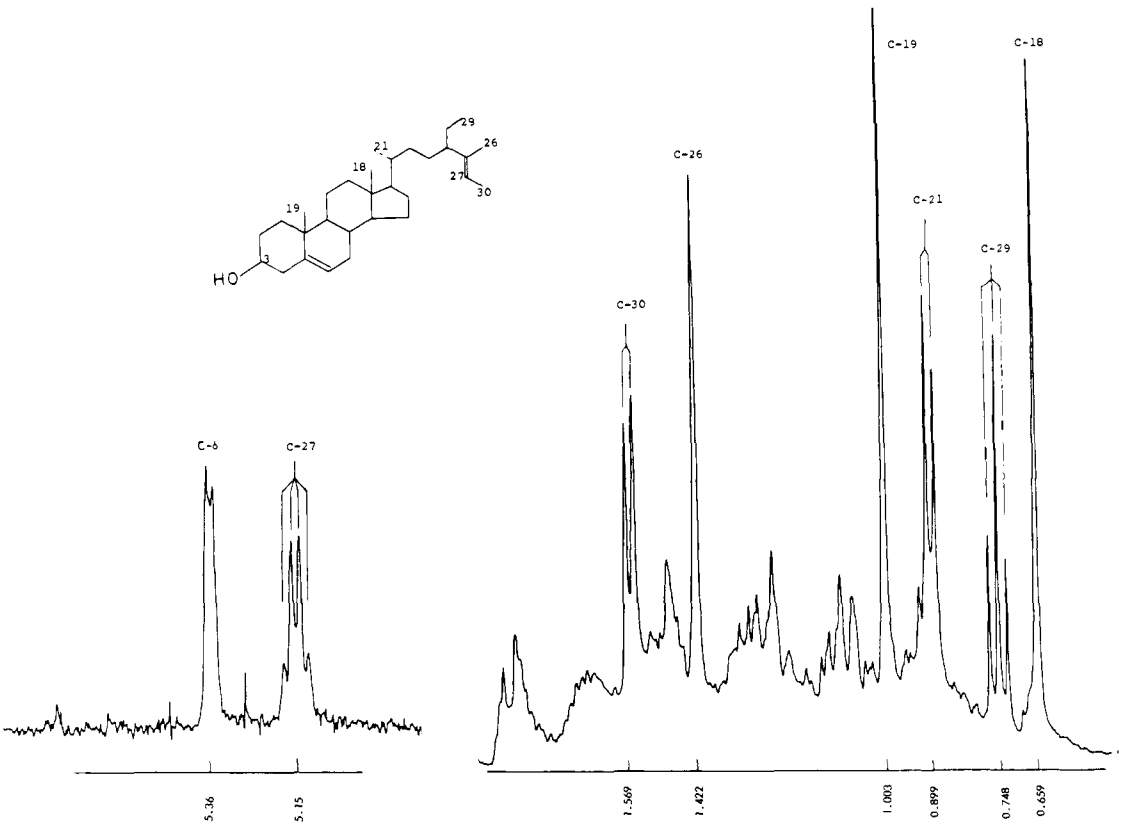
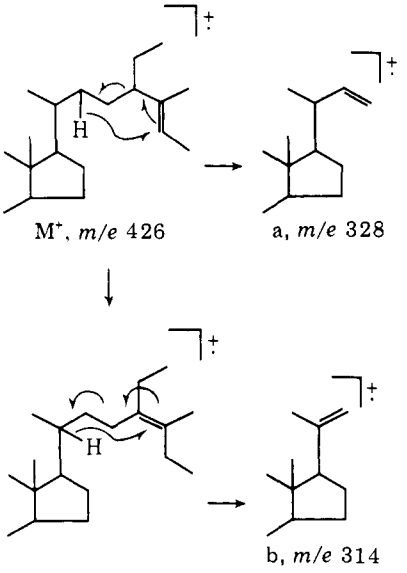


Figure 1. NMR spectrum (360 MHz) of stelliferasterol (**1**). The C-3 proton multiplet is not shown.

Scheme I



ppm) and C-30 (doublet at 1.569 ppm coupled with the olefinic proton at 5.15 ppm). The remaining triplet at 0.748 ppm can be assigned to a 24-ethyl group thus leading to structure **1** (except for stereochemical details) for stelliferasterol.

Since no such side chain had been encountered previously in nature, we embarked on an unambiguous synthesis which would also shed some light on the stereochemistry of the stelliferasterol side chain. As outlined in Scheme II, the synthesis started with methyl 3β-acetoxychol-5-enate (**3**), which was degraded to 3α,5-cyclo-6β-methoxy-23-iodonorcholane (**8**) and then subjected to an acetoacetic ester condensation to yield 3α,5-cyclo-6β-methoxy-24ξ-ethyl-27-norcholestan-25-one (**10**). Wittig condensation and acid cleavage of the ring A protecting group led to all four isomers (24*S*,*R* and 25*E*,*Z*)

Scheme II

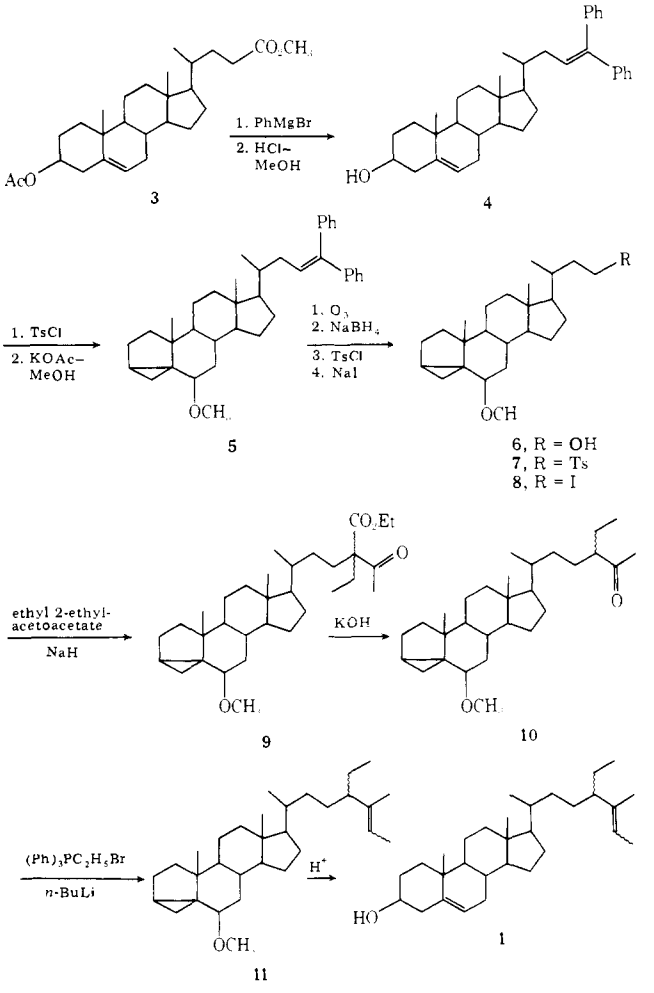
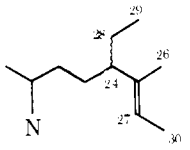


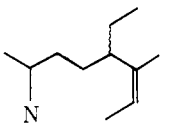
Table II. ^{1}H NMR Chemical Shifts (360 MHz) of Stelliferasterol and Its Isomers[a]

	stelliferasterol 24 epimer 25*E*		25 isomers 25*Z*	
C-18-Me	0.659	0.666	0.666	0.666
C-29-Me	0.748	0.756	0.780	0.785
C-21-Me	0.899	0.891	0.912	0.903
C-19-Me	1.003	1.005	1.005	1.005
C-26-Me	1.422	1.418	1.499[b]	1.495[b]
C-30-Me	1.569	1.570	1.553[b]	1.544[b]
C-27-H	5.153	5.158	5.303	5.303

[a] All values ±0.004 ppm. [b] Assignment could be reversed.



1-*E*



1-*Z*

of **1**. These isomers have the same GC retention time and identical mass spectra as natural stelliferasterol, but can be distinguished by their 360-MHz ^{1}H and ^{13}C NMR spectra. Attempts at separation were only partially successful since only one isomer could be separated completely by reversed phase LC from the other three; according to its ^{1}H NMR spectrum this is the 24 epimer of the natural sterol. By comparing the NMR spectra of the two pure compounds (natural stelliferasterol and its synthetic 24 epimer) with the spectra of the rest of the unresolved synthetic mixture (containing synthetic stelliferasterol and its two (24*R* and 24*S*)Δ^{25} double bond isomers) it was possible to assign most of the signals (Table II). Therefore, even though synthetic stelliferasterol could not be separated from its stereoisomers, we consider the synthetic structure proof unambiguous as all signals of the natural sterol could be detected accurately in the ^{1}H and ^{13}C spectra (Tables II and III) of the mixture.

The *E* or *Z* stereochemistry of the Δ^{25} double bond can be assigned by the ^{1}H NMR spectra on the basis of shielding arguments, as the chemical shifts of the C-26 and C-30 methyl groups and of the C-27 proton show quite distinct differences (Table II). The C-27 proton should be more shielded in the *E* isomer than in the *Z* isomer; hence the C-24 isomers with C-27 H signals at 5.15 ppm should have the *E* configuration, while the C-24 isomers with signals at 5.30 ppm must possess the *Z* configuration. The differences in the methyl signals are not as distinct, but nevertheless are in agreement with our assignment.

The C-26 methyl group should be affected more than its C-30 counterpart, as it is cis to C-30 in 1-*E* but trans to C-30 in 1-*Z*; therefore one would expect greater shielding in the *E* than in the *Z* isomer and this is indeed observed (Table II: 1.420 vs. 1.499 ppm). The C-30 methyl group has in both cases a cis carbon (either C-26 or C-24) so the shielding difference is only very small (see Table II) with $\Delta\delta = 0.02$ ppm; the upfield shift in the *Z* isomer can be rationalized by the bulkier cis substituent. Because of the small effect, this last comparison is only valuable when taken together with the above arguments. A stronger case for our *Z* assignments can be made by the small coupling constants of 2 Hz between C-26 and C-30, which can be observed only in the *Z* isomers with C-26 and C-30 in a trans position to each other. A decoupling experiment confirmed this coupling.

Final proof for our assignment was produced by the ^{13}C NMR spectra, where the differences in the C-26 shifts between the *E* and *Z* isomers are expected[9] to be of the order of −6.7 ppm. We found an upfield shift of 6.5 ppm (Table III) for the C-26 methyl signal in the *E* isomer. The other carbon resonances around the Δ^{25} double bond are listed in Table III and are all in complete accord with our assignment. The *E* stereochemistry of the Δ^{25} double bond in stelliferasterol is therefore proven. Epimers at C-24 have occasionally been distinguished by their ^{1}H NMR spectra[8] because of chemical shift differences of their C-21 and C-26 resonances; although small differences between our 24-epimers can be observed, a structure assignment by this technique is problematic as the influence of a Δ^{25} double bond on these small effects is as yet unknown.

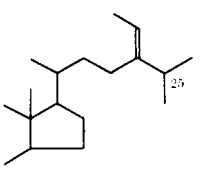
Table III. ^{13}C NMR Chemical Shifts of Stelliferasterol and Isomers

	stelliferasterol		
	natural	in synthetic mixture	25*Z* isomers
C-26	11.27	11.27	17.81
C-18	11.82		11.84
C-30	12.15		12.06
C-29	13.05		13.06
C-21	18.96	18.97[a]	18.97
			18.64
C-19	19.38		19.38
C-28	26.08	26.09	26.60
			26.25
C-23	29.69	29.77[a]	29.77
			29.56
C-22	33.97	33.98	33.80
			33.72
C-24	51.41	51.41	41.47
			41.05
C-20	55.94	55.97[a]	55.97
			56.13
C-27	119.35	119.36	120.62
			120.48
C-25	137.68		137.79
			137.47

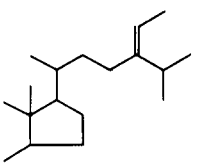
[a] *Z* signal enhanced.

The mass spectrum (see Experimental Section) of the second new sterol, called isostelliferasterol (M^+ 426), showed all fragments typical for a 3-hydroxy-Δ^5-sterol[7] with a double bond in the 24 position (i.e., McLafferty rearrangement leading to *m/e* 314[5]). However, no *m/e* 328 peak[4] was observed thus excluding the presence of a Δ^{25} double bond as in stelliferasterol. The well-resolved 360-MHz NMR spectrum (Figure 2) again allowed the assignment of the side chain in terms of structure **2**. The Δ^{24} double bond has only one methyl group which absorbs at 1.58 ppm and couples with the olefinic proton at 5.20 ppm (6 Hz). The methyl triplet at 0.817 ppm is indicative of an ethyl substituent, while the pseudotriplet at 0.955 ppm is caused by overlapping methyl doublets of C-21 and C-26. The singlets at 0.678 and 1.005 ppm are the normal C-18 and C-19 methyl signals.

The stereochemistry of the Δ^{24} double bond in isostelliferasterol (**2**) could be established by comparing its NMR spectrum with those[10,11] of fucosterol (**12**) and isofucosterol



12



13

(**13**). A striking difference in those spectra is the chemical shift of the C-25 proton, which absorbs in isofucosterol (**13**, *Z* isomer) at 2.8 ppm compared to 2.2 ppm in fucosterol (**12**, *E* isomer). The sextet of the isostelliferasterol C-25 proton is observed at 2.56 ppm, which favors strongly the *Z* configuration as in isofucosterol (**13**). The *S* stereochemistry at C-25

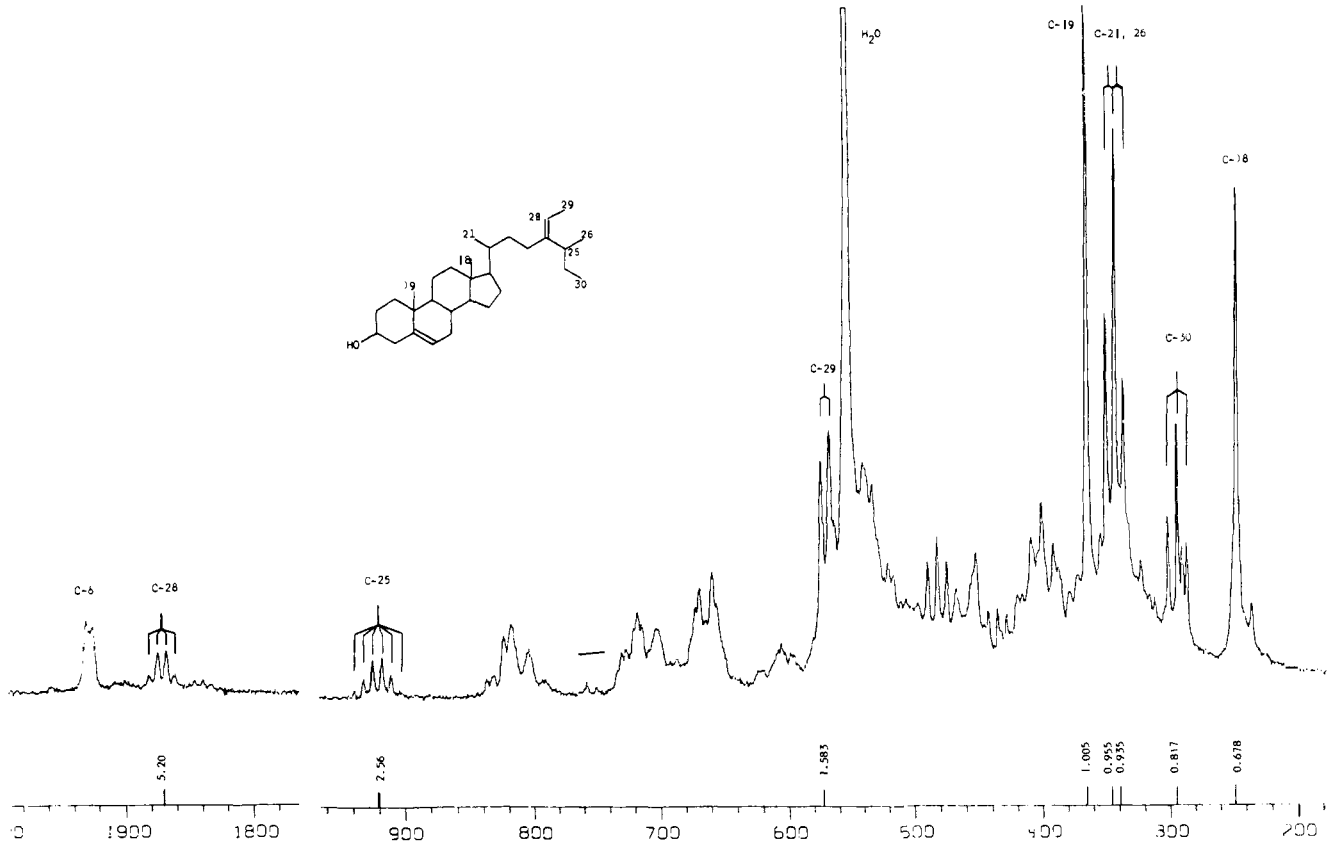


Figure 2. NMR spectrum (360 MHz) of isostelliferasterol (**2**). The C-3 proton multiplet at 3.53 ppm is not shown.

could be proved by synthesis (Scheme III) starting with natural dehydroaplysterol (**14**). Protection of the Δ^5-3β-hydroxy system via the *i*-methyl ether **15** followed by ozonolysis to the 24-ketone **16**, Wittig condensation, and regeneration of the Δ^5-3β-hydroxy system afforded isostelliferasterol (**2**), identical in all respects with the naturally occurring sterol. In constrast to the above synthesis (Scheme II) of stelliferasterol (**1**) only one double-bond isomer was formed and no epimerization occurred at the 25 position during the reaction sequence. Isostelliferasterol (**2**), therefore, possesses the same 25*S* stereochemistry as had been established earlier[12] for dehydroaplysterol (**14**).

The composition (Table I) of the sterols in *Jaspis stellifera* is biosynthetically quite interesting. The first notable point is in diversity of isomers which are due to the position or lack of double bonds and the co-occurrence of Δ^5-sterols and stanols. The second is the simultaneous content of 22-dehydro-24(*R*)- and 22-dehydro-24(*S*)-methylcholesterol (**17** and **18**). Most significant are the two new sterols stelliferasterol (**1**) and isostelliferasterol (**2**) since they are unique by possessing a side chain that formally is derived from biological methylation at positions 27 and 28. At least three biogenetic routes can be envisaged—all of them starting with unsaturated precursors since these are known to be required for biological C-alkylation of terrestrial plant and animal sterols.[13]

The first route (Scheme IV) assumes that 24-propylidenecholest-5-en-3β-ol (**19**), initially isolated in scallops,[14] but subsequently also found in sponges,[15] serves as the starting material. Loss of the C-27 methyl group, for which there is indirect evidence in the marine sterol field,[16] would lead to the as yet unknown C_{29} sterol **20** which would then undergo C-methylation at C-28 followed by double-bond migration to yield stelliferasterol (**1**). The route outlined in Scheme IV is easily distinguished from all others in that the origin of several of the side chain carbons is different from that of cholesterol. This could be easily verified if **19** radiolabeled at C-29 were fed to the sponge and found to be utilized in sterol synthesis.[17]

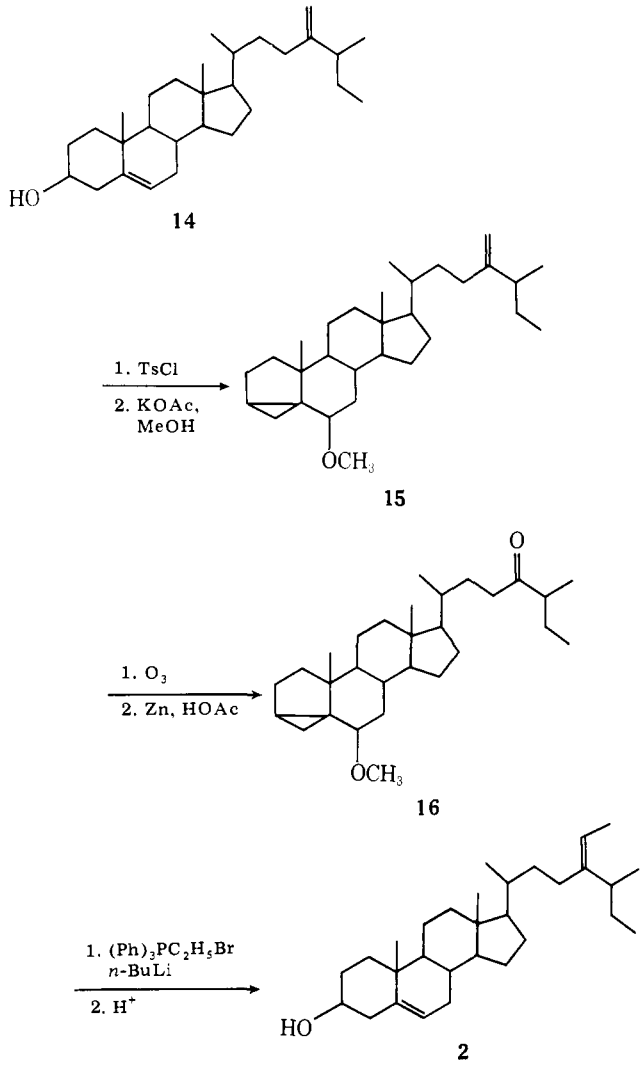
A second possible biosynthetic path (Scheme V) involves single C-methylation of the C_{29} sterol clerosterol (**21**) which has been found in the green alga *Codium fragile*.[18] If this biosynthetic route is operative, stelliferasterol (**1**) must possess the same (24*S*) configuration as clerosterol (**21**)—a stereochemical feature that has not yet been settled.

Based on the sterol composition (see Table I) of the sponge *Jaspis stellifera*, the most likely pathway (Scheme VI) would start with 24-dehydroaplysterol (**14**). This is not only the main sterol (54.9%) in *Jaspis stellifera* but the first methylation would yield directly isostelliferasterol (**2**). Double-bond isomerization, possibly by the reduction-dehydrogenation sequence established in Chrysophyte algae,[19] could then afford stelliferasterol (**1**). Appropriate radiolabel experiments would be required to distinguish among these alternative biosynthetic paths. In any event, the occurrence in nature of stelliferasterol (**1**) and isostelliferasterol (**2**) demonstrates the existence of a hitherto unknown alkylation sequence in sterol side chain biosynthesis and further substantiates the utility[20] of searching for new marine sterols in nature.

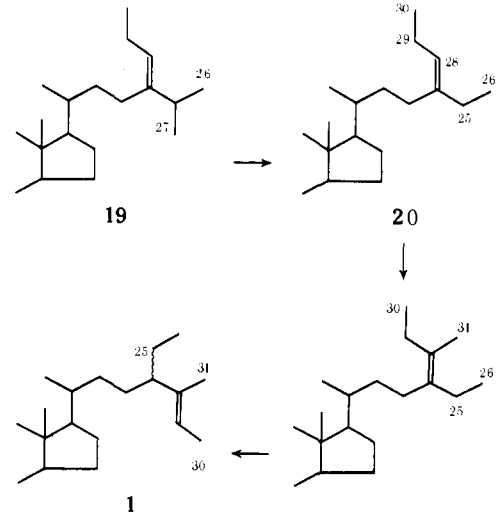
Experimental Section

General. GLC was performed using a Hewlett-Packard 402A chromatograph equipped with 4 mm × 6 ft "U"-shaped columns containing 3% OV-25 on Gas Chrom Q (GCQ) from Applied Science Inc. The oven temperature was 265 °C and helium was used as the carrier. The standard 402A flame ionization detector was used throughout the work. Combined GC–MS analysis was performed mainly on a Varian MAT 44 quadrupole spectrometer system using coiled GC columns (2 mm i.d. × 6 ft) containing 3% OV 17, OV 101, or SP 2525 on GCQ at a temperature of 270 °C. Other GC–MS analyses and high-resolution mass spectra were recorded by Annemarie Wegmann on a Varian MAT 711 system using the conditions reported earlier.[21] The mass spectra of pure compounds were obtained on an A. E. I. MS-9 or MAT 44 instrument using the direct inlet

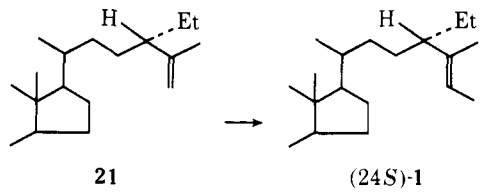
Scheme III



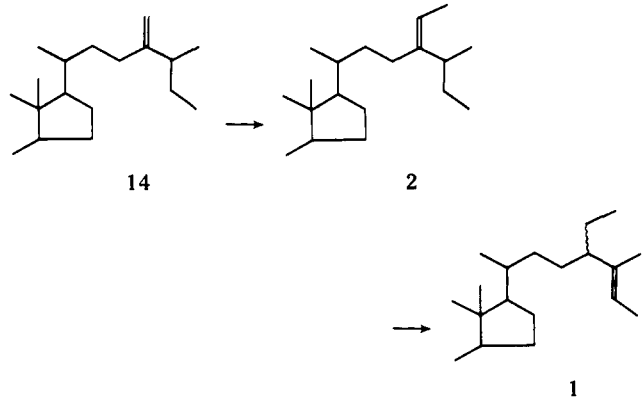
Scheme IV



Scheme V



Scheme VI



system. The metastable defocusing experiments were performed on the MS-9. ^{1}H NMR spectra were recorded on Varian A-60 (60 MHz) or XL-100 (100 MHz) spectrometers. The 360-MHz spectra were measured with the Bruker HXS 360 spectrometer of the Stanford Magnetic Resonance Laboratory. The 100- and 360-MHz spectra were recorded using the FT technique. In all instances, $CDCl_3$ was used as solvent and Me_4Si as internal standard. ^{13}C NMR spectra were obtained on a Varian FT-80 instrument at 20 MHz using capillary tubes (5 mg of sterol in 15 μL of $CDCl_3$). All melting points are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter, and IR spectra on a Perkin-Elmer 421 grating spectrometer in chloroform solutions. CD spectra were measured on a Jasco J-40 instrument.

High-Pressure Liquid Chromatography (LC). LC was performed on our system as described by Popov et al.[21] The following columns and solvents were used: (A) two 25 cm × 4 mm i.d. prepacked 10 μ Bondapack C_{18} columns (Waters Associates), mobile phase methanol-water (92:8), flow rate 1.0-1.5 mL/min at 2000 psi, capacity 2-5 mg in 2 mL; (B) 50 × 0.8 cm i.d. prepacked 10 μ Partisil (C_{18}) column by Whatman, eluent absolute methanol, flow rate 6 mL/min at 1000 psi, capacity 20 mg in 5 mL; (C) two 1 m × 1 cm i.d. silver nitrate-silica gel columns, eluent hexane-ether (99.5:0.5 to 99:1), flow rate 6-8 mL/min at 400 psi, capacity 200-400 mg in 5 mL. The adsorbent (Porasil, 35-70 μ, Waters Associates) was coated as usual with 20% silver nitrate and dissolved in absolute acetonitrile, and the solvent was evaporated at 30-40 °C on a rotary evaporator at reduced pressure; the columns were dry packed.

Isolation and Separation of the Sterol Mixture from *Jaspis stellifera*. A 400-g sample of freeze-dried and milled *Jaspis stellifera*, collected on Wheeler Reef, northeast of Townsville, Australia, was percolated with petroleum ether (bp 40-60 °C) (4 L). Evaporation of the petroleum ether extract in vacuo gave 6 g (1.5%) of a yellow gum which was applied to a silica gel bed in hexane (Merck type H for TLC, 200 g) packed in a Buchner funnel and run by application of vacuum to the receiving flask. Sequential elution with hexane, dichloromethane-hexane mixtures, and dichloromethane (1 L each) gave a dichloromethane fraction (1.8 g) which was decolorized with charcoal and recrystallized from ethanol to yield 1.5 g of crude crystalline sterol mixture.

The crystalline sterol fraction (1.5 g) was acetylated as usual with pyridine-acetic anhydride (25 °C) and the sterol acetates were subjected to preliminary separation on $AgNO_3$-SiO_2 columns (LC column C) in batches of 200 mg. After eluting all but the sterols with a terminal methylene group the solvent system was changed from 0.5% to 1% absolute ether in hexane. Twenty fractions of 50 mL each were collected and monitored by GC. Fractions with approximately the same composition were combined and the acetates cleaved with $LiAlH_4$ in absolute ether. The free sterol mixtures were then chromatographed on the reversed phase column B, and the resulting fractions investigated by GC-MS. The unknown sterols and those sterols whose structures could not be assigned unambiguously by GC-MS were rechromatographed on column B or A until pure compounds were obtained.

2-(3β-Hydroxybisnorchol-5-enyl)-1,1-diphenylethylene (4).[22] A Grignard solution from 7.2 g (0.3 mol) of magnesium and 51.8 g (0.33 mol) of bromobenzene was formed in 150 mL of absolute ether. A solution of 8.56 g (0.02 mol) of methyl 3β-acetoxychol-5-enate (**3**) in 140 mL of dry benzene was added and the mixture heated under

reflux overnight. The Grignard complex was hydrolyzed with hydrochloric acid and ice and the water phase extracted twice with ether. The combined organic phases were washed with 5% hydrochloric acid, water, 5% NaOH, and water and dried over Na_2SO_4. After evaporation of the solvents, the residue was dissolved in 250 mL of methanol to which 4 mL of concentrated hydrochloric acid had been added. This mixture was stirred at 50 °C for 2 h, concentrated to about 50 mL, and cooled in the refrigerator. The product **4** was filtered and dried in vacuo: yield 7.2 g (73%); mp 179–181 °C (acetone); NMR (60 MHz) 7.3 (10, m), 6.1 (1, t, J = 7 Hz), 5.33 (1, m), 1.0 (3, s), 0.93 (3, d), 0.66 ppm (3, s); MS (MAT 711) *m/e* 494.353 21 (M^+, 1.44, calcd 494.354 86), 314 (2), 302 (2), 296 (4), 272 (10), 271 (27), 270 (9), 253 (10), 215 (5), 198 (14), 197 (100), 193 (22), 180 (39), 159 (11), 145 (10), 133 (12), 131 (10), 119 (10), 115 (16), 107 (14), 105 (48), 95 (15), 93 (12), 91 (19), 81 (20), 77 (23), 67 (10), 55 (17).

***i*-Methyl Ether 5.** The alcohol **4** (7.0 g) and 10 g of *p*-toluenesulfonyl chloride were dissolved in 100 mL of absolute pyridine and stirred at room temperature overnight. The solution was then poured into 500 mL of ice-cold 5% $KHCO_3$ solution, and the solid tosylate was filtered, washed with water, and dried in vacuo, yield 9.2 g (100%). The tosylate (8.8 g) was heated under reflux overnight with 10 g of fused KOAc in 400 mL of absolute MeOH. After evaporation of the solvent the residue was partitioned between water and ether. The ether phase was washed with 5% $KHCO_3$ and water and dried over $KHCO_3$: yield after evaporation 5.8 g (84%); mp 126–128 °C (methanol–ether); NMR (60 MHz) 7.22 (10, m), 6.12 (1, t, J = 7 Hz), 3.32 (3, s), 2.76 (1, t, J = 2 Hz), 1.04 (3, s), 0.72 (3, s), 0.3–0.8 ppm (m); MS (MAT 711) *m/e* 508 (M^+, 3), 476 (9), 306 (5), 296 (10), 285 (14), 283 (14), 254 (16), 253 (60), 198 (20), 197 (100), 193 (26), 180 (30), 167 (10), 159 (10), 133 (10), 115 (13), 107 (10), 105 (24), 91 (15), 81 (15), 66 (13). Anal. ($C_{37}H_{48}O \cdot CH_3OH$) C, H.

3α,5-Cyclo-6β-methoxy-23-hydroxynorcholane (6). A sample (4.0 g) of the *i*-methyl ether **5** was dissolved in 100 mL of dichloromethane and ozonized at −70 °C until a light blue color appeared. Sodium borohydride (4.5 g) in 100 mL of ethanol was added to the cold solution and the mixture stirred for 1 h below 10 °C. After 20 mL of acetic acid in 200 mL of ice water was added and the solution was stirred for 15 min the organic phase was separated and the aqueous phase extracted with ether. The combined organic phases were washed with water, 2% $NaHCO_3$, and water and dried over K_2CO_3. After the solvent was evaporated the residue was chromatographed on 200 g of SiO_2 (silica gel HF 254 + 366 for TLC, type 60, by E. Merck) in a 15 × 7.5 cm column, eluting with a hexane–ether gradient (3 L 90:10, 2 L 85:15, 2 L 80:20): yield 1.2 g (38%); mp 92–93 °C after recrystallization from methanol and ether; NMR (60 MHz) 3.72 (2, t, J = 6 Hz), 3.36 (3, s), 2.78 (1, t, J = 2 Hz), 1.06 (3, s), 1.00 (3, d), 0.79 (3, s); MS (MAT 44) *m/e* 360 (M^+, 9), 345 (15), 328 (19), 305 (35), 302 (9), 255 (9), 55 (100). Anal. ($C_{24}H_{40}O_2$) C, H.

3α,5-Cyclo-6β-methoxy-23-hydroxynorcholanyl Tosylate (7). A solution of 1.05 g of **6** in 10 mL of absolute pyridine was added dropwise to a cold solution of 2 g of tosyl chloride in 10 mL of absolute pyridine. The mixture was stirred at 0 °C for 3 h, a small piece of ice was added, and the mixture was stirred for another 1 h at room temperature. After the usual workup with ether–water, a quantitative yield of **7** was obtained: mp 114 °C after recrystallization from methanol and ether; NMR (60 MHz) 7.72 (2, d, J = 7 Hz), 7.28 (2, d, J = 7 Hz), 4.06 (2, m), 3.3 (3, s), 2.78 (1, t, J = 1 Hz), 2.44 (3, s), 1.0 (3, s), 0.6 ppm (3, s); MS (MAT 44) *m/e* 514 (M^+, 5), 499 (5), 482 (10), 459 (10), 361 (2), 360 (2), 310 (2), 255 (7), 228 (9), 213 (10), 91 (100), 55 (70). Anal. ($C_{31}H_{46}O_4S$) C, H.

3α,5-Cyclo-6β-methoxy-23-iodonorcholane (8). A solution of **7** (1.39 g) and 1.3 g of sodium iodide in 20 mL of acetone was heated under reflux for 1.5 h. After workup with water and ether a yield of 1.0 g (78.5%) was obtained: mp 104–105 °C after recrystallization from ether and methanol; NMR (60 MHz) 3.3 (3, s), 3.15 (1, t, J = 8 Hz), 2.75 (1, t, J = 2 Hz), 1.0 (3, s), 0.71 ppm (3, s); MS (MAT 44) *m/e* 470 (M^+, 6), 469 (9), 455 (9), 454 (4), 438 (14), 437 (6), 415 (20), 414 (11), 255 (6), 213 (7), 55 (100). Anal. ($C_{24}H_{39}IO$) C, H, I.

Acetoacetic Ester Condensation of 8. Sodium hydride (15 g, 57% in oil) was washed with anhydrous ether to remove the oil. Under ice cooling 7 mL of ethyl 2-ethylacetoacetate was added dropwise to the stirred NaH suspension in 30 mL of absolute DMF. After additional stirring for 0.5 h at room temperature, 700 mg of the iodide **8** was added and the mixture heated on an oil bath at 105 °C for 20 min. After cooling, the mixture was poured into 100 mL of ice-water and extracted three times with ether. The ether phase was washed with water, 1% hydrochloric acid, water, 2% $NaHCO_3$, and water. After drying over $MgSO_4$ and evaporation of the ether, the residue of **9** was kept at oil pump vacuum for 24 h: yield 650 mg of oil (87%); NMR (60 MHz) 4.22 (2, q, J = 7 Hz), 3.3 (3, s), 2.78 (1, t, J = 2 Hz), 2.1 (3, s), 1.26 (3, t, J = 7 Hz), 1.0 (3, s), 0.93 (3, d, J = 6 Hz), 0.70 (3, s), 0.2–0.75 ppm (m); MS (MAT 44) *m/e* 500 (M^+, 10), 485 (10), 468 (14), 453 (3), 445 (16), 442 (4), 423 (4), 310 (4), 255 (13), 213 (16), 148 (35), 55 (100). Anal. ($C_{32}H_{52}O_4$) C, H.

3α,5-Cyclo-6β-methoxy-24ξ-ethyl-27-norcholestan-25-one (10). The keto ester **9** (700 mg) was heated under reflux in a mixture of 120 mL of ethanol and 60 mL of aqueous 6 N KOH for 1 h. After the mixture was concentrated to one-half of its volume, 50 mL of water was added and the mixture was extracted with ether. The ether phase was washed with water, 5% hydrochloric acid, water, 1% $NaHCO_3$, and water, dried over $MgSO_4$, and evaporated. The residue was chromatographed on 60 g of SiO_2 (TLC grade) and eluted with a hexane–ether gradient of 500 mL 92:8, 250 mL 90:10, and 250 mL 150:100: yield 375 mg (62%) of oil; NMR (60 MHz) 3.3 (3, s), 2.78 (1, t, J = 2 Hz), 2.1 (3, s), 1.0 (3, s), 0.92 (3, d, J = 6 Hz), 0.7 (3, d), 0.3–0.7 ppm (m); MS (MAT 44) *m/e* 428 (M^+, 11), 413 (14), 396 (22), 373 (35), 255 (22), 213 (20), 55 (100). Anal. ($C_{29}H_{48}O_2$) C, H. CD: [θ] −419 (282 nm).

Stelliferasterol *i*-Methyl Ether and Isomers (11). A 2.4 M *n*-BuLi solution (4.16 mL, 10 mmol) was added under an atmosphere of nitrogen to a solution of 3.75 g (10 mmol) of triphenylethylphosphonium bromide in 100 mL of absolute THF. After the solution was stirred for 1 h at room temperature, 190 mg of **10** in 3 mL of absolute THF was added and the mixture was heated under reflux for 2 days. After workup with ether and water, the residue of the ether phase was chromatographed on 50 g of SiO_2 (TLC grade) eluting with benzene: yield 120 mg (62%) of oil; NMR (100 MHz) 5.3 (2, m), 3.3 (3, s), 2.7 (1, t, J = 2 Hz), 1.54 (s), 1.02 (3, s), 0.90 (3, d, J = 6 Hz), 0.7 ppm (3, s); MS (MAT 44) *m/e* 440 (M^+, 4), 425 (4), 408 (4), 385 (8), 313 (4), 277 (5), 255 (5), 201 (9), 55 (100). Anal. ($C_{31}H_{52}O$) C, H.

Stelliferasterol (1) and Isomers. A 50-mg aliquot of the *i*-methyl ether mixture was dissolved in 20 mL of dioxane, 4 mL of water and 20 mg of *p*-toluenesulfonic acid were added, and the mixture was heated under reflux for 1 h. After workup with ether and water, 47 mg (97%) of the free sterols was obtained: mp 119–121 °C after recrystallization from methanol; MS (MS-9, 15 eV) *m/e* 426 (M^+, 100), 424 (7), 411 (12), 408 (12), 393 (6), 329 (13), 328 (48), 315 (12), 314 (25), 300 (14), 299 (35), 273 (10), 272 (15), 271 (31), 258 (10), 255 (8), 231 (7), 231 (5), 229 (7), 217 (8), 98 (45); $[\alpha]^{21}_D$ −50° ($CHCl_3$). Anal. ($C_{30}H_{50}O \cdot CH_3OH$) C, H. MS *m/e* 426.3870 (M^+, calcd 426.3861). The sterols were chromatographed on LC column B, using 2% water in methanol. By cutting unresolved peaks and rechromatographing them it was possible to obtain 0.5 mg of the pure 24 epimer of stelliferasterol while the three other isomers could not be resolved or enriched by this method. The appropriate NMR data are collected in Tables II and III. Natural stelliferasterol showed mp 121–122 °C, $[\alpha]^{21}_D$ −37° ($CHCl_3$).

Isostelliferasterol (2). Dehydroaplysterol (**14**)[12] is the major sterol (see Table I) in the sponge *Jaspis stellifera* and could easily be separated by $AgNO_3$–SiO_2 chromatography. Its *i*-methyl ether (**15**) was prepared from 148 mg of dehydroaplysterol (**14**) by the same procedure as reported for **5**: yield 142 mg (93%) of oil; NMR (60 MHz) 4.7 (2, s), 3.3 (3, s), 2.78 ppm (1, t, J = 2 Hz). A 70-mg sample of **15** was dissolved in 25 mL of CH_2Cl_2 and 5 mL of methanol and ozonized at −70 °C until a light blue color persisted. Zn dust (600 mg) and 5 mL of acetic acid were added and the reaction mixture was allowed to warm to room temperature. After stirring for 1.5 h the mixture was filtered and the filtrate freed from solvent by evaporation. The residue was poured into 75 mL of water and extracted twice with 50 mL of hexane. After washing (1% $NaHCO_3$ and water) and drying ($MgSO_4$), the solvent was evaporated and the residue (60 mg) was chromatographed on a preparative TLC plate, developing with hexane–ether (8:6). The fastest moving band was collected to yield 35 mg (50%) of the ketone **16**: mp 89–90 °C after recrystallization from methanol; CD [θ] −390 (290 nm); MS (MAT 44) *m/e* 428 (M^+, 4), 413 (5), 396 (6), 273 (10), 255 (7), 213 (9), 57 (100), 55 (45). Anal. ($C_{29}H_{48}O_2$) C, H.

The Wittig condensation was effected by adding 1.3 mL of 2.03 M *n*-BuLi in hexane in an atmosphere of nitrogen to a suspension of 1 g of triphenylethylphosphonium bromide in 30 mL of absolute THF. After 1 h 35 mg of the ketone **16** dissolved in 5 mL of absolute THF was added and the mixture heated under reflux for 2 days. After

workup with ether and water the ether phase was dried with $MgSO_4$ and evaporated. The residue was dissolved in 25 mL of dioxane, 5 mL of water and 20 mg of *p*-toluenesulfonic acid were added, and the mixture was heated under reflux for 1 h. Upon cooling colorless crystals appeared which were filtered and washed with water, yield 27 mg (77%). The nearly pure compound was purified further by LC (column B, absolute MeOH), the main peak (80%) yielding isostelliferasterol (**2**): mp 119–120 °C after recrystallization from methanol–water; NMR (360 MHz) 5.36 (1, m), 5.205 (1, q, J = 6 Hz), 3.53 (1, m), 2.56 (1, sextet, J = 6 Hz), 1.585 (3, d, J = 6 Hz), 1.011 (3, s), 0.960 (3, d, J = 6 Hz), 0.941 (3, d, J = 6 Hz), 0.816 (3, t, J = 6.5 Hz), 0.683 ppm (3, s); MS (MS-9) *m/e* 426 (M^+, 4), 361 (2), 343 (2), 314 (100), 299 (21), 281 (25), 271 (10), 255 (5), 229 (22), 213 (12), 55 (50); MS (MAT 711) *m/e* 426.3834 (M^+, calcd 426.3861); $[\alpha]^{20}_D$ −27° ($CHCl_3$).

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Des-*N*-tetramethyltriostin A and Bis-L-seryldes-*N*-tetramethyltriostin A, Synthetic Analogues of the Quinoxaline Antibiotics[1]

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Abstract: The synthesis of the des-*N*-tetramethyl analogue of the quinoxaline depsipeptide antibiotic triostin A has been accomplished, as also has the synthesis of the corresponding bis-L-serine analogue. The synthesis of des-*N*-tetramethyltriostin A (**2**) proceeded by coupling L-alanine β,β,β-trichloroethyl ester with *N*-benzyloxycarbonyl-D-serine to give dipeptide Z-D-Ser-Ala-OTce. Depsipeptide bond formation was effected using *N,N'*-dicyclohexylcarbodiimide in pyridine to provide tridepsipeptide **3**. Coupling of **3** with Boc-Cys(Acm)-OH gave tetradepsipeptide **4**, which, by removal of appropriate protective groups, was converted to tetradepsipeptides **5** and **6**. Fragment coupling of **5** and **6** furnished the octadepsipeptide **7** containing the amino acid sequence of the antibiotic. A sequence of deprotection and cyclization converted **7** to the cyclic octadepsipeptide **8**. Treatment of **8** with iodine in methanol effected formation of disulfide **9**. Removal of the *N*-benzyloxycarbonyl groups in **9** followed by N-acylation with 2-quinoxalinecarbonyl chloride gave des-*N*-tetramethyltriostin A (**2**). Analogue **2** was found to bind to DNA and, in common with the natural quinoxaline antibiotics, to do so as a bifunctional intercalating agent. The bis-L-serine analogue of **2** was prepared following the above procedure to furnish a quinoxaline antibiotic that showed no appreciable binding to DNA.

The quinoxaline antibiotics are a group of bycyclic depsipeptide antibiotics. Two families of these antibiotics, the quinomycins[2,3a] and the triostins,[3] are known. The structural features of the triostins are represented by triostin A (**1**). Triostin A is a symmetrical bicyclic octadepsipeptide composed of two units each of D-serine, L-alanine, *N*-methyl-L-cysteine, and *N*-methyl-L-valine. The depsipeptide bond occurs between the hydroxyl group of D-serine and the carboxyl group of *N*-methyl-L-valine, while a disulfide bridge exists between the two *N*-methyl-L-cysteine residues. A 2-quinoxalinecarbonyl (Qxc) moiety is attached to the amino group of each D-serine unit.

The quinoxaline antibiotics are active against gram-positive bacteria[4,5b] and against certain animal tumors.[5] It is known that the antibiotics bind to DNA and thereby function as potent inhibitors of RNA synthesis.[6] Of current interest, the

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