A part of this crude extract (6.51 g) was partitioned between dichloromethane and water. The dichloromethane layer, upon concentration, gave 1.52 g of material, which was then partitioned between hexane and 10% aqueous methanol. The hexane layer was concentrated to give 1.33 g of material which was subjected to flash chromatography, HPLC on silica gel, and reverse-phase HPLC in nearly the same manner as described above to give more naurol A and B.

From the combined separations 505 mg of naurol A (1), 61.4 mg of naurol B (2), and 10.1 mg of 3 were obtained.

Naurol A (1): white solid; mp 97 °C; $[\alpha]$ = +6.21° (c = 2.64, MeOH); UV λ_{max} 286 nm (ϵ_{max} 49 131); IR (thin film) 3416 cm^{-1}; ^1H NMR, see Table I; ^{13}C NMR, see Table I; LRMS (12 eV) m/z (relative intensity) 314.1 (10.3), 278.0 (26.1), 228.0 (10.2), 214.0 (25.5), 192.1 (18.2), 180.0 (45.8), 177.8 (100.0), 164.0 (16.7), 84.8 (91.6), 82.9 (98.9); FAB MS with added Na^+ $(m + Na)^+$ 421; FAB MS $(m + H)^+$ 399; FAB MS with added Li^+ $(m + Li)^+$ 405.

Naurol B (2): white solid; $[\alpha]$ = +12.63° (c = 0.19, MeOH); UV λ_{max} 292.4 nm (ϵ_{max} 44 236); IR (neat) 3466 cm^{-1}; ^1H NMR see Table I; ^{13}C NMR, see Table I; LRMS (12 eV) m/v (relative intensity) 314.1 (5.8), 278.0 (10.3), 228.0 (7.9), 213.9 (12.0), 192.0 (11.9), 180.0 (38.5), 177.9 (100.0), 164.0 (12.6), 85.0 (46.3), 83.0 (56.9).

Compound 3: white film; ^1H NMR, see Table I; ^{13}C NMR, see Table I; LRMS (70 eV) m/z (relative intensity) 389.1 (11.3), 387.1 (23.4), 385.1 (17.3), 314.0 (12.0), 214.0 (16.9), 179.9 (38.1), 177.9 (100.0).

Acknowledgment. This work was supported by Department of Commerce, NOAA Sea Grant Project NA86AA-D-SG074. We thank Mr. Charles Arneson for specimen collection and Dr. Pat Bergquist, University of Auckland, New Zealand, for sponge identification. We gratefully acknowledge NSF Grant CHE 8113507 and the University of Oklahoma Research Fund for funds to purchase a high-field NMR spectrometer.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra of naurol A and B (4 pages). Ordering information is given on any current masthead page.

Sterols of Marine Invertebrates. 63.[1] Isolation and Structure Elucidation of Sutinasterol, the Major Sterol of the Marine Sponge *Xestospongia* sp.

Russell G. Kerr,[†] Sutinah L. Kerr,[†] George R. Pettit,[‡] Delbert L. Herald,[‡] Thomas L. Groy,[‡] and Carl Djerassi*,[§]

Hopkins Marine Station, Stanford University, Pacific Grove, California 93950, Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85287, and Department of Chemistry, Stanford University, Stanford, California 94305

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A new sterol, sutinasterol ((24*R*)-24-ethyl-26,26-dimethyl-3β-hydroxycholesta-7,25(27)-diene) with a side chain arising from quadruple biomethylation has been isolated from the marine sponge *Xestospongia* sp. Since it represents the bulk (94%) of the sterol fraction, it presumably plays a biological role in membrane function. Four minor sterols were also characterized, one of which appears to be a biosynthetic intermediate of sutinasterol. A second trace sterol contains a side chain that is the result of five biomethylations and is the largest sterol isolated from natural sources to date. The structures of these sterols were deduced from spectral data (^1H and ^{13}C NMR and MS). A crystal structure study of sutinasterol was performed to determine the stereochemistry of the C_{24} ethyl group.

Introduction

The occurrence of a wide variety of novel sterols in sponges has been well documented.[2] This includes unconventional sterol nuclei as well as sterol side chains with cyclopropanes, cyclopropenes, and ones with high degrees of alkylation. Recently, the main emphasis in our laboratory has been on elucidating the biosynthesis of these compounds.[3] It is most interesting to examine unusual sterols that are present in large quantities, as these very likely play a functional (rather than metabolic) role in cell membranes.[4] Unconventional sterols often co-occur with conventional ones and are sometimes present in small amounts.[2] It is therefore particularly interesting when a sponge is found with an unusual sterol as the overwhelmingly predominant one.

Analysis of a demosponge, *Xestospongia* sp., from Puerto Rico showed that one sterol, designated sutinasterol, composed 94% of the sterol mixture. Sutinasterol has not previously been isolated and was shown by MS analysis to have a C_{12} side chain, presumably the product of quadruple bioalkylation. This information, coupled with ^1H and ^{13}C NMR analysis, suggested that sutinasterol had the structure shown in Figure 1. An X-ray crystal structure study was performed to confirm this structure

[†] Hopkins Marine Station, Stanford University.
[‡] Arizona State University.
[§] Department of Chemistry, Stanford University.

(1) For part 62 in this series, see: Tam Ha, T. B.; Djerassi, C. *Steroids* **1989**, *53*, 356.

(2) For reviews, see: (a) Djerassi, C. *Pure Appl. Chem.* **1981**, *53*, 873. (b) Ikekawa, I. *Sterols and Bile Acids*; Danielson, H., Sjovall, J., Eds.; Elsevier: Amsterdam, 1985; Chapter 8. (c) Withers, N. *Marine Natural Products*; Scheuer, P. J., Ed.; Academic Press: New York, 1983; Vol. 5, p 87. (d) Djerassi, C. *Alfred Benzon Symposium 20: Natural Products and Drug Development*; Krogsgaard-Larsen, P., Brøgger Christensen, S., Kofod, H., Eds.; Munksgaard: Copenhagen, 1984; p 164. (e) Reference 1 and earlier papers in this series.

(3) Inter alia: (a) John, V.; Stoilov, I. L.; Djerassi, C.; Karuso, P.; Poiner, A.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 1642. (b) Kerr, R. G.; Stoilov, I. L.; Thompson, J. E.; Djerassi, C. *Tetrahedron* **1989**, *45*, 1893. (c) Cho, J.-H.; Thompson, J. E.; Stoilov, I. L.; Djerassi, C. *J. Org. Chem.* **1988**, *53*, 3466. (d) Stoilov, I. L.; Back, T. G.; Thompson, J. E.; Djerassi, C. *Tetrahedron* **1986**, *42*, 4156. (e) Doss, G. A.; Margot, C.; Sodano, G.; Djerassi, C. *Tetrahedron Lett.* **1988**, *29*, 6051. (f) Doss, G. A.; Silva, C. J.; Djerassi, C. *Tetrahedron* **1989**, *45*, 1273. (g) Stoilov, I. L.; Thompson, J. E.; Cho, J.-H.; Djerassi, C. *J. Am. Chem. Soc.* **1986**, *108*, 8235.

(4) (a) Carlson, R. M. K.; Tarchini, C.; Djerassi, C. *(IUPAC) Frontiers of Bioorganic Chemistry and Molecular Biology*; Ananchenko, S. N., Ed.; Permagon Press: Oxford, 1980; p 211. (b) Bloch, K. E. *Crit. Rev. Biochem.* **1983**, *14*, 47. (c) Lawson, M. P.; Stoilov, I. L.; Thompson, J. E.; Djerassi, C. *Lipids* **1988**, *23*, 750. (d) Zimmerman, M. P.; Hoberg, M.; Ayanoglu, E.; Djerassi, C. *Lipids*. In press.

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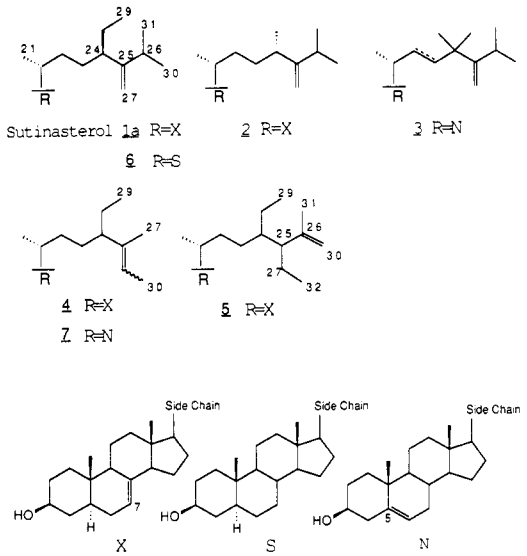


Figure 1.

as well as to determine the stereochemistry at C_{24}. In addition to the very unusual quadruple biomethylation[5] in the side chain, sutinasterol (**1a**) possesses a Δ^7 nucleus. This type of nuclear unsaturation is rare in sponges[2] but common in echinoderms.[6] The unusual structure of sutinasterol raises intriguing questions as to its biosynthesis.

It is noteworthy that a seemingly biosynthetically related sterol, pulchrasterol (**2**), was isolated as the major component of the New Zealand sponge *Aciculities pulchra*.[7] Pulchrasterol has a side chain very similar to that of sutinasterol, the only difference being that the former has a (24*S*)-24-methyl group instead of the (24*R*)-24-ethyl group in sutinasterol. Also, both sterols have Δ^7 nuclei. It is indeed surprising that *A. pulchra*, a deep sea sponge from New Zealand, and *Xestospongia* sp. from a shallow reef in Puerto Rico should have such similar yet otherwise very rare sterols.

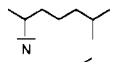
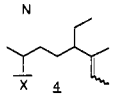
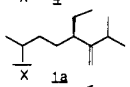
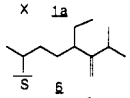
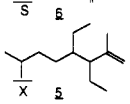
One other structurally related sterol (**3**) was recently isolated as the major sterol component in a *Halichondria* sp. from Vietnam.[8] It has the same 25(27)-dehydro-26,26-dimethyl side-chain substitution that is found in both sutinasterol (**1a**) and pulchrasterol (**2**). The possible biosynthetic relationships of these sterols (**1a**, **2**, and **3**) is discussed (vide infra).

Isolation and Structure Elucidation

Xestospongia sp. was collected at a depth of 7–8 m at Ahogado Reef, near La Parguerra, Puerto Rico, and immediately freeze-dried. The sponge was extracted with chloroform and the sterol fraction obtained by column chromatography. Normal-phase HPLC (6% ethyl acetate in hexanes as mobile phase) afforded two sterol fractions, the first present in trace amounts, while the second represented ca. 98% of the mixture. The major fraction was further purified by reversed-phase HPLC (methanol as mobile phase), to afford three fractions, with the second component comprising 94% of the mixture (see Table I).

The major fraction was shown to be a single sterol (**1a**) by capillary GC and was isolated as a colorless crystalline solid, mp 129–130 °C, that displayed a molecular ion at m/z 440.4039 ($C_{31}H_{52}O$). The empirical formula indicates that sterol **1a** has two degrees of unsaturation. A fragment ion at m/z 328 (7%) suggests the presence of a double bond at C_{25}. Such fragments are typical products of McLafferty rearrangements associated with this pattern of unsaturation.[9] A fragment ion at m/z 246 (17%) is characteristic of Δ^7 sterols[10] and represents the loss of the side chain and part of ring D.

Table I. Sterol Composition of *Xestospongia* sp.

sterol	Rel abundance	GC, RRT	M^+ m/z
	1.0	1.00	386
	1.2	1.83	426
	94	2.10	440
	1.0	1.95	442
	1.0	2.56	454

The ^1H NMR data for the methyl groups of sutinasterol, as well as the other minor sterols, are summarized in Table II. The resonance in the ^1H NMR spectrum of sutinasterol at 5.151 ppm is characteristic of a Δ^7 vinylic proton.[11] The ^{13}C NMR signals at 139.56 and 117.37 ppm are also in accord with those of C_8 and C_7, respectively, of a Δ^7 nucleus.[12] The singlets at 0.518 and 0.789 ppm agree with those expected for the C_{18} and C_{19} angular methyl groups of a Δ^7 sterol,[11] thus confirming the identity of the nucleus. ^1H NMR resonances at 4.809 and 4.657 ppm and the ^{13}C NMR resonances at 159.66 and 105.66 ppm are typical of a terminal methylene.[13] This is supported by the aforementioned MS data, which located the double bond at C_{25}. A septet, centered at 2.135 ppm (1 H), is typical of allylic hydrogens,[14] the splitting indicating that this signal is due to the methyne hydrogen of an isopropyl group. A broad doublet centered at 2.014 ppm (1 H) is also typical of allylic hydrogens.[14] It is now clear that the sterol side chain contains a terminal methylene at C_{25}–C_{27} with an isopropyl group attached to C_{25} and that C_{24} must be a tertiary center.

The methyl group region of the ^1H NMR spectrum displayed a doublet at 1.017 ppm (J = 6.8 Hz, 6 H), assigned to the methyl groups of the isopropyl moiety. A second doublet at 0.911 ppm (J = 6.4 Hz, 3 H) is typical of C_{21} methyl groups.[7] The resonance at 0.819 ppm (t, J = 7.4 Hz, 3 H) suggests the presence of a methyl group attached to a CH_2 unit. Given the above spectral data, as well as the need to satisfy the empirical formula, it is evident that there is an ethyl group at C_{24}, thus leading to structure **1a** for sutinasterol.

An X-ray crystal structure was obtained to determine the stereochemistry of the C_{24} ethyl group as well as to

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(6) Goad, L. J.; Rubinstein, I.; Smith, A. G. *Proc. R. Soc. London B* **1972**, *180*, 223.
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(12) Tsuda, M.; Schroepfer, G. T., Jr. *J. Org. Chem.* **1979**, *44*, 1290.
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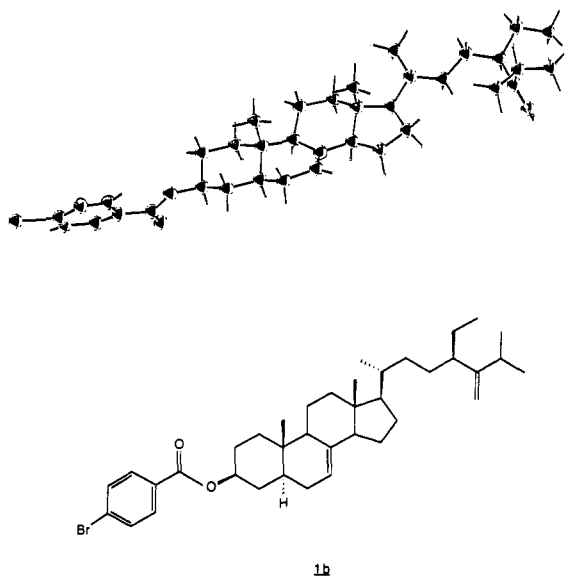
(14) Pretsch, E.; Seibl, J.; Simon, W.; Clerc, T. *Tabelle zur Strukuraufklarung Organischer Verbinde ungen mit spektroskopischen Methoden*; Springer: Berlin, 1981; Vol. 2.

Table II. ^{1}H NMR (400 MHz) Spectral Data[a] for the Methyl Groups of the Sterols of *Xestospongia* sp.

sterol (solvent)	C_{18}	C_{19}	C_{21}	C_{27}	C_{29}	C_{30}	C_{31}	C_{32}
1a ($CDCl_3$)	0.518 (s)	0.789 (s)	0.911 (d, 6.4)		0.819 (t, 7.4)	1.017 (d, 6.8)	1.017 (d, 6.8)	
4 ($CDCl_3$)	0.512 (s)	0.788 (s)	0.902 (d, 6.4)	1.578 (s)	0.740 (t, 7.4)	1.425 (s)		
5 ($CDCl_3$)	0.522 (s)	0.788 (s)	0.891 (d, 6.4)		0.794 (t, 7.4)		NA[b]	0.764 (t, 7.4)
5 (C_6D_6)	0.601 (s)	0.714 (s)	1.033 (d, 6.4)		0.896 (t, 6.8)		1.582 (s)	0.878 (t, 6.8)
6 ($CDCl_3$)	0.630 (s)	0.795 (s)	0.887 (d, 6.8)		0.814 (t, 7.4)	1.103 (d, 6.8)	1.013 (d, 6.8)	

[a] Chemical shifts are expressed in pm (δ); multiplicities and coupling constants (hertz) are given in parentheses. [b] This signal was masked by the DOH signal.



1b

Figure 2.

provide confirmation of the structural assignments based on the NMR spectra. Sutinasterol (**1a**) yielded a suitable heavy atom containing derivative, the 3-(*p*-bromobenzoate) **1b**, suitable for single-crystal X-ray crystallography. A computer-generated perspective drawing depicting the absolute stereochemical configuration of sutinasterol 3-(*p*-bromobenzoate) is shown in Figure 2.[15] On the basis of the absolute configuration of the well-established steroid nucleus, the sterochemical configuration of the C_{24} ethyl substituent was determined to be 24*R*. Sutinasterol (**1a**) can thus be designated as (24*R*)-24-ethyl-26,26-dimethyl-3β-hydroxycholesta-7,25(27)-diene.

In addition to this major component, five other sterols were isolated in trace quantities. In view of the unusual structure of sutinasterol (**1a**), these minor sterols were also analyzed as they could provide clues to the biosynthetic origin of sutinasterol. Due to the extremely small quantities, only four of the five sterols could be identified.

As described earlier, the normal-phase HPLC purification produced one major component (ca. 98%), which could be separated into three fractions by reversed-phase HPLC. The first of these represented 1.2% of the sterol mixture and was shown to be a single sterol by capillary GC. This sterol (**4**) was found to have a molecular formula of $C_{30}H_{50}O$ (m/z 426). The ^{1}H NMR spectrum of **4** was compared with those of other M^+ 426 sterols isolated in our laboratory. A very close match was found with stelliferasterol (**7**).[16] The only significant difference was that the ^{1}H NMR spectrum of **4** lacked the C_5-H signal of stelliferasterol at 5.35 ppm[16] and instead had a broad singlet at 5.15 ppm, consistent with a Δ^7 nucleus.[11] The chemical shifts for the C_{18} (0.512 ppm) and C_{19} (0.788 ppm) angular methyl groups were also consistent with this type of nucleus.[11] The spectral analysis indicated that sterol **4** is the Δ^7 isomer of stelliferasterol (**7**).

The second fraction isolated on HPLC was the previously described sutinasterol (**1a**), which composed 94% of the sterol mixture. The last fraction was another minor component, which represented 1% of the total sterol mixture and was shown to be a single sterol by capillary GC. A molecular formula of $C_{32}H_{54}O$ (m/z 454.4156) was obtained from the high resolution mass spectrum. Apparently five biomethylation steps occurred in the formation of this sterol (**5**). To our knowledge, this represents the most highly alkylated sterol side chain isolated to date. The structure was deduced from MS and ^{1}H NMR analyses. Unlike the mass spectrum of sutinasterol, the mass spectrum of **5** did not contain a fragment ion at m/z 328, indicative of a C_{25} double bond. A Δ^7 nucleus was assigned to **5** on the basis of the olefinic proton signal at 5.15 ppm[11] as well as the chemical shifts of the C_{18} and C_{19} angular methyl groups[10] (see Table II). Signals at 4.787 ppm (1 H) and 4.640 ppm (1 H) are typical of a terminal methylene.[13] These are very similar chemical shifts to those of the terminal methylene of sutinasterol (vide supra); however, due to the aforementioned differences in the mass spectra of **1a** and **5**, this terminal methylene cannot be located at C_{25}.

The ^{1}H NMR resonances for the methyl groups of **5** are summarized in Table II. The methyl-group region of the ^{1}H NMR spectrum contained a doublet (J = 6.4 Hz, 3 H) centered at 0.887 ppm, which was identified as the C_{21} methyl.[7] Two overlapping triplets at 0.794 and 0.764 ppm suggested the presence of two methyl groups attached to two CH_2 units. The remaining methyl signals in this region were assigned to the C_{18} and C_{19} angular methyl groups. A singlet located at 1.582 ppm[17] (3 H) is characteristic of olefinic methyl groups. With the exception of the complex CH_2 envelope, the only remaining signal of significance is the doublet of triplets (1 H) at 2.015 ppm, typical of allylic protons.[14] The splitting suggests that this allylic methyne is coupled with both a second methyne and a methylene. This arrangement can be satisfied with structure **5**. The stereochemistry of the two ethyl groups is unknown; the extremely small amounts of material prohibited an X-ray crystal structure determination. For biosynthetic reasons, the stereochemistry of the ethyl group at C_{24} is presumably the same as that in the other sterols.

The remaining sterol **6** was isolated from the first, small fraction in the normal-phase HPLC analysis (vide supra). The material from 22 injections was combined and further purified by reversed-phase HPLC (methanol). The first of two major fractions was identified as cholesterol by comparison of its ^{1}H NMR spectrum with that of an authentic sample. The later fraction was found to have a molecular formula of $C_{31}H_{54}O$ (m/z 442) and accounted for 1.0% of the sterol mixture. The ^{1}H NMR spectrum

(15) Johnson, C. K. ORTEPII. A FORTRAN Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations, ORNL-5138; Oak Ridge National Laboratory, Oak Ridge, TN.

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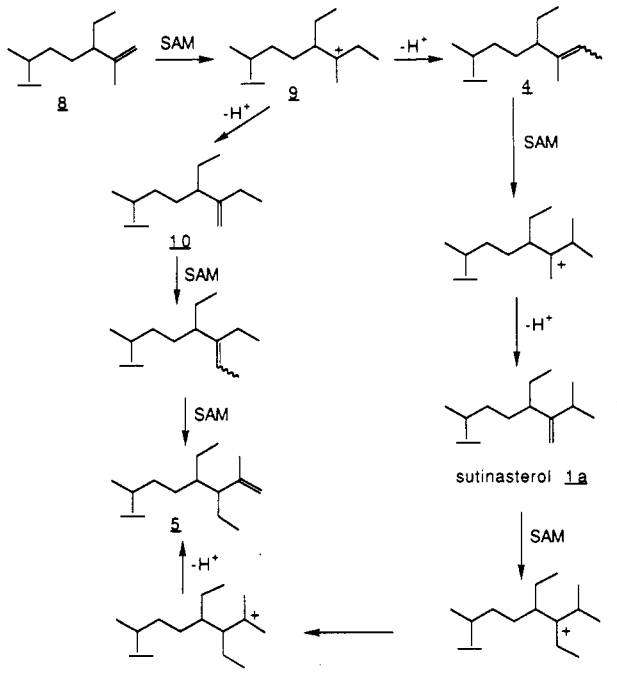
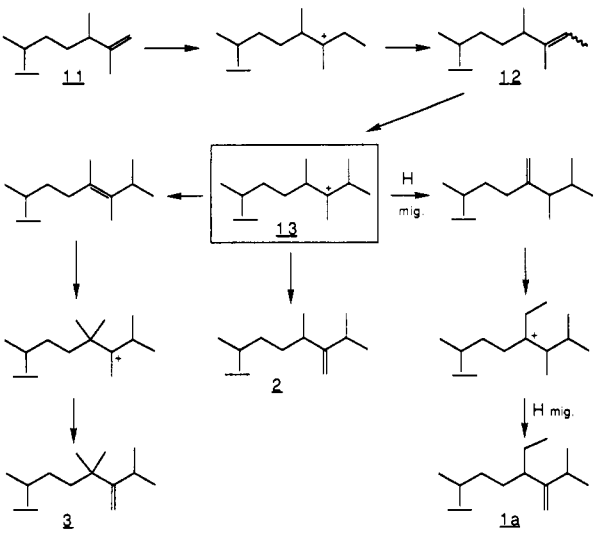
(17) This signal was only observed in the C_6D_6 spectrum of **5** as the DOH signal masked this region in the $CDCl_3$ spectrum (see Table II).

Scheme I



Scheme II



of this sterol (**6**) was nearly identical with that of sutinasterol, except that the ^1H NMR spectrum of **6** lacked the C_7-H signal at 5.15 ppm. Also, the resonances for the C_{18} and C_{19} angular methyl groups were different from those of sutinasterol (see Table II) and correspond to those of 5α-cholestanol. We conclude that this trace sterol, therefore, is 24-ethyl-26,26-dimethyl-3β-hydroxycholest-25-ene.

Biosynthetic Considerations

The unusual structures of sutinasterol as well as of the other minor sterols raise intriguing questions as to their biosynthesis. The origin of sterols with four and five biomethylations in the side chain have so far not been investigated.[2,3] There is also the question of when the unusual Δ^7 unsaturation appeared in the biosynthetic pathway.

The biosynthesis of Δ^7 nuclei has been discussed in our earlier report.[18] In this investigation, it was demonstrated that Δ^7 sterols can be produced from Δ^5 sterols via the $\Delta^{5,7}$ nucleus. Also, in the de novo biosynthesis of sterols[3b] the Δ^7 nucleus is formed directly from Δ^8 sterol precursors. Sterol **6** presumably arises from the hydrogenation of the C_7 double bond of sutinasterol (**1a**).

A priori, the most direct route to the novel side chain of sutinasterol would be from the Δ^7 isomer of clerosterol (**8**), a known marine sterol (see Scheme I). SAM (*S*-adenosylmethionine) biomethylation[5] of **8** could produce cation **9**, which after loss of a proton would yield **4**. A second SAM biomethylation at C_{26} of **4**, followed by loss of a proton, should afford sutinasterol (**1a**). This mechanism is supported by the isolation of intermediate **4** as 1% of the sterol mixture of the sponge.

However, our earlier work[19] on the biosynthesis of strongylosterol suggests that the codisterol side chain (**11**)—which we have found to be the ubiquitous precursor to novel sterol side-chain-elongated sterols—may be the actual progenitor (Scheme II). An attractive feature of this scheme is that it invokes a common intermediate in the biosynthesis of sutinasterol (**1a**) as well as the two structurally related sterols pulchrasterol (**2**) and sterol **3**. SAM bioalkylation of codisterol (**11**) could proceed in an analogous fashion to that described for clerosterol in Scheme I, to produce 25(26)-dehydroaplysterol (**12**). As outlined in Scheme II, SAM biomethylation of **12** can give rise to carbonium ion **13**, which can act as a common intermediate to sutinasterol (**1a**), pulchrasterol (**2**), and sterol **3**.[8] A fundamental difference in the three pathways is that hydrogen migration occurs in the biosynthesis of sutinasterol (**1a**) and not in the formation of sterols **2** or **3**.

Two pathways to sterol **5** seem to be reasonable (Scheme I). First, SAM biomethylation of sutinasterol followed by hydrogen migration and subsequent loss of a proton would produce **5**. Alternatively, cationic intermediate **9** could give rise to strongylosterol[19] (**10**). The terminal methylene of **10** could then be transformed by two SAM biomethylations to the isopropenyl group of **5**. A similar conversion has been demonstrated in the Australian sponge *Pseudaxynissa* sp.[20] We hope to provide experimental verification of the biosynthetic pathways in this *Xestospongia* sp. in the future by synthesizing some of the most plausible precursors in radioactive form.

Experimental Section

General. HPLC separations were achieved by using normal-phase columns (Altex, Ultrasil-Si, 10 mm i.d. × 25 cm) with 6% ethyl acetate in hexanes as the mobile phase as well as two reversed-phase columns connected in series (Altex, Ultrasphere ODS, 10 mm i.d. × 25 cm) with absolute methanol as the mobile phase. In all cases, flow rates of 3 mL/min were used. Gas chromatography was carried out on an HP Ultra 2 capillary column (0.32 mm i.d. × 25 m with 0.52 μm film thickness). The temperature was 280 °C (1 min), 1°/min to 290 °C (40 min). Melting points are uncorrected.

^1H NMR chemical shifts are referenced to $CDCl_3$ (7.260 ppm) or C_6D_6 (7.150 ppm), while ^{13}C NMR spectra are referenced to the center peak of the $CDCl_3$ signal (77.000 ppm).

Extraction and Isolation of Sterols. Specimens of *Xestospongia* sp. were collected at a depth of 7–8 m from Ahogado Reef, 0.5 km from La Parguerra, Puerto Rico, and were immediately freeze-dried; 50 g of dry weight of this sponge was cut into cubes (ca. 1 cm^3) and extracted in chloroform for 3 days. This was filtered and concentrated to afford a dark green oil. The crude sterol fraction was obtained by flash chromatography over silica

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gel, using hexanes (two column volumes) followed by hexanes/ether (2:1, three column volumes). Fractions were checked for sterol content by TLC. The crude sterol mixture (150 mg) was purified by normal-phase HPLC using 6% ethyl acetate in hexanes as eluent. Two sterol fractions were isolated; the early one was present in trace quantities (2%) while the latter one represented 98% of the mixture. The first fractions from 22 separate injections were combined and further purified by reversed-phase HPLC (methanol as mobile phase) to afford cholesterol (0.10 mg) and **4** (0.15 mg). The major fraction from normal-phase HPLC was also purified by reversed-phase HPLC (methanol) in 22 separate injections. The fractions from these runs were combined and analyzed spectroscopically. The first fraction contained 0.20 mg of sterol **2**, the second contained 140 mg of sutinasterol (**1a**), and the third had 0.15 mg of sterol **3**.

(24*R*)-24-Ethyl-26,26-dimethyl-3β-hydroxycholesta-7,25(27)-diene (sutinasterol, 1a): mp 129–130 °C (methanol); high resolution MS (70 eV), *m/z* (relative intensity, assignment) 440.4039 (21, $C_{31}H_{52}O$, M^+), 425.3800 (12, $C_{30}H_{49}O$), 328.2779 (6, $C_{23}H_{36}O$), 271.2072 (100, $C_{19}H_{27}O$); ^1H NMR data are summarized in Table II; ^{13}C NMR ($CDCl_3$) 159.65, 139.56, 117.37, 105.66, 71.01, 55.96, 54.97, 49.37, 47.37, 43.31, 40.18, 39.48, 37.93, 37.08, 36.51, 34.14, 33.76, 32.89, 31.41, 31.20, 29.60, 27.87, 27.28, 22.91, 22.81, 22.78, 21.50, 18.91, 13.02, 11.99, 11.79.

24-Ethyl-26-methyl-3β-hydroxycholesta-7,25(26)-diene (4) (Δ^7-stelliferasterol): melting point was not determined due to insufficient material; low resolution MS (70 eV), *m/z* (relative intensity, assignment) 426 (19, $C_{30}H_{50}O$, M^+), 411 (22, $C_{29}H_{47}O$), 271 (56, $C_{19}H_{27}O$); ^1H NMR data are summarized in Table II.

24-Ethyl-26,26,27-trimethyl-3β-hydroxycholesta-7,26(30)-diene (5): melting point was not determined due to insufficient material; high resolution MS (70 eV), *m/z* (relative intensity, assignment) 454.4156 (36, $C_{32}H_{54}O$, M^+), 439 (26, $C_{31}H_{53}O$), 370.3257 (15, $C_{26}H_{42}O$), 271.2073 (100, $C_{19}H_{27}O$); ^1H NMR data are summarized in Table II.

24-Ethyl-26,26-dimethyl-3β-hydroxycholest-25(27)-ene (6): melting point was not determined due to insufficient material; low resolution MS (70 eV), *m/z* (relative intensity, assignment) 442 (22, $C_{31}H_{54}O$, M^+), 427 (4, $C_{30}H_{51}O$), 330 (3, $C_{23}H_{38}O$), 273 (26, $C_{19}H_{29}O$); ^1H NMR data are summarized in Table II.

Preparation of *p*-Bromobenzoate of Sutinasterol (1b). Sutinasterol (10 mg, 0.023 mmol), *p*-bromobenzoic anhydride (9 mg, 0.023 mmol), and 4-(dimethylamino)pyridine (3 mg, 0.025 mmol) were dissolved in 5 mL of warm benzene and stirred at room temperature for 5 h. The reaction mixture was extracted successively with 5% HCl, H_2O, $NaHCO_3$, and H_2O, dried ($MgSO_4$), and concentrated to afford a white solid, which was purified by column chromatography over silica (hexanes to 4% ether in hexanes) and recrystallized from hexanes.

X-ray Crystal Structure Determination of Sutinasterol *p*-Bromobenzoate (1b). A crystal (0.20 × 0.24 × 0.08 mm) of benzoate **1b**, $C_{38}H_{55}O_2Br$, formula weight 623.75, was mounted on a glass fiber and data were collected at 26 ± 1 °C on an Enraf-Nonius CAD-4 automatic X-ray diffractometer equipped with a graphite monochromator using Cu K radiation (λ = 1.541 84 Å). Unit cell dimensions (a = 11.371 (2) Å, b = 7.630 (1) Å, c = 39.467 (7) Å, α = 89.40 (1)°, β = 92.87 (1)°, γ = 90.98 (1)°, V = 3419.2 (7) Å^3) were obtained by least-squares analysis of 25 well-centered reflections. Density measurements were imprecise with variable crystal densities being observed for samples from the batch that provided the X-ray crystal. Best estimates of the number of molecules per unit cell suggested a Z of between 3 and 4. The crystal was finally assigned to the P1 space group with Z = 4. A full hemisphere of reflections (11 763) was collected with $2\theta < 130°$ using the ω–2θ scan technique. The total number of observed ($[F_o] > 2\sigma[F_o]$), unique reflections used in subsequent refinement was 5793. Data were corrected for absorption, Lorentz, and polarization factors. The structure was solved by direct-method solution using the MITHRIL[21] computing software. The coordinates for 164 non-hydrogen atoms (4 independent molecules) were determined. The structure was refined by full-matrix and block-diagonal least-squares methods using CRYSTALS[22] and SHELXTL-PLUS,[23] in which the quantity minimized was $w(|F_o| - |F_c|)^2$. Final refinements were performed on a model containing anisotropic non-hydrogen atoms and isotropic hydrogens. The coordinates of the latter were calculated at optimum positions; the thermal parameters and coordinates were then fixed for the final cycle of least-squares refinement. The model converged to residual of R = 0.094, R_W = 0.084. Tables of bond distances and angles and positional parameters for sutinasterol *p*-bromobenzoate (**1b**) are available in the supplementary material.

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Registry No. **1a**, 129620-23-1; **4**, 129620-24-2; **5**, 129620-25-3; **6**, 129620-26-4.

Supplementary Material Available: Tables of bond distances, angles, and positional parameters for compound **1b** (21 pages). Ordering information is given on any current masthead page.

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