(22*Z*,24*S*)-Stigmasta-5,22,25-trien-3β-ol and Other Novel Sterols from *Clerodendrum scandens*: First Report of the Isolation of a *cis*-Δ^{22}-Unsaturated Sterol from a Higher Plant

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(22*Z*,24*S*)-Stigmasta-5,22,25-trien-3β-ol was isolated from *Clerodendrum scandens* (Verbenaceae). It is only the second example of a naturally occurring *Z*-Δ^{22}-unsaturated sterol. Four other novel sterols were isolated from the same source, *viz.* (24*S*)-5α-stigmast-25-en-3β-ol, (22*E*,24*S*)-5α-stigmasta-22,25-dien-3β-ol, 24-methylergosta-5,25-dien-3β-ol, and (24*S*)-14α-methyl-5α-stigmasta-9(11),25-dien-3β-ol. All structures were determined by chemical and spectroscopic methods.

In a previous paper[1] we presented arguments to show that claims for the uniqueness of some marine sterols[2c] are misleading. In the last 15 years a large number (≈200) of new sterols with unusual side-chains or nuclear unsaturations have been isolated from marine sources because a major effort was made to find them.[2] In this period sterols from fresh† and brackish water‡ and terrestrial organisms received only scant attention.

When, more recently, modern techniques for separation and identification were applied to sterol mixtures from higher plants,§ unprecedented 14-methyl-(5β,19)-cyclosterols were obtained,[3] and also sterols with acetylenic side-chains,[1] 24-isopropyl and -isopropenyl substituents,[4] and additional methyl groups at C-24[5] and C-25.[6] These side-chains were supposed to be typical of marine sponge sterols.[2,¶]

In this paper we report the isolation of (22*Z*,24*S*)-stigmasta-5,22,25-trien-3β-ol (**2k**) and of four other novel sterols from *Clerodendrum scandens* (Verbenaceae). Compound (**2k**) is the double-bond isomer of one of the major sterols of this plant. Naturally occurring *Z*-Δ^{22}-unsaturated sterols are rare: (22*Z*,24ξ)-ergosta-5,22-dien-3β-ol (**2r**)[8] and (22*Z*)-cholesta-5,22-dien-3β-ol (**2q**)[9,‖] are the only other known members of this class.

Results

We have already published a comparison of the sterol patterns of five *Clerodendrum* spp.[10] Further work on the minor and trace sterols of *C. scandens* has resulted in the identification of five new sterols which were isolated as the acetates by argentic TLC and reverse-phase HPLC. Their isolated yields range from 0.1–0.3% of the sterol fraction of *C. scandens*. All identified sterols and their abundances are listed in Table 1.

Structure Elucidation of the New Sterols.—The mass spectrum of the acetate of compound (**2k**) showed a molecular ion at *m/z* 452 ($C_{31}H_{48}O_2$) and prominent fragment ions at *m/z* 392 (M^+ − HOAc), 255 (loss of side-chain and HOAc), and 253 (255 − 2 H), indicating that compound (**2k**) was an acetate of a C_{29} sterol with three degrees of unsaturation, one in the nucleus and two in the side-chain.[11] Comparison of its ^{1}H NMR data (Table 2) with those of (22*E*,24*S*)-stigmasta-5,22,25-trien-3β-ol (**2j**) acetate,[10] the main sterol of *C. scandens* (Table 1), left no doubt that compound (**2k**) had a regular sterol skeleton and a Δ^5-double bond. The NMR spectrum of (**2k**) acetate (Table 2) included two olefinic side-chain protons (dd) at δ 5.092 (*J* 9.8, 10.2 Hz) and 5.196 (*J* 10.7, 10.7 Hz). Irradiation of the former double doublet collapsed the latter into a doublet

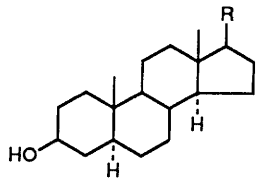
† As expected, freshwater dinoflagellates (unicellular algae, phylum Pyrrophyta) were found to contain uncommon sterols previously isolated from related marine species [N. Robinson, G. Eglinton, C. S. Brassell, and P. A. Cranwell, *Nature* (*London*), 1984, **308**, 439; N. Robinson, P. A. Cranwell, G. Eglinton, and G. H. M. Jaworski, *Phytochemistry*, 1987, **26**, 411; N. W. Withers, 'The Biology of the Dinoflagellates,' ed. F. J. R. Taylor, Blackwell, London, 1987, p. 316].

‡ There are two reports of the isolation of novel sterols from Black Sea sponges (J. Zielinski, H.-t. Li, T. S. Milkova, S. Popov, N. L. Marekov, and C. Djerassi, *Tetrahedron Lett.*, 1981, **22**, 2345; J. Zielinski, T. Milkova, S. Popov, N. Marekov, and C. Djerassi, *Steroids*, 1982, **39**, 675). The top 100 m layer of this sea consists of fresh or brackish water, depending on the depth and location (W. Anikouchine and R. Sternberg, 'The World Ocean. An Introduction to Oceanography,' 2nd edn., Prentice Hall, Ingelwood Cliffs, NJ, 1981).

§ All known nuclei and side-chains of plant sterols and steroidal triterpenoids which are only oxygenated in the 3-position are listed in a recent review article. See: T. Akihisa and T. Matsumoto, 'ISI Atlas of Science. Animal and Plant Sciences,' 1988, vol. 1, p. 100, Institute for Scientific Information, Philadelphia, PA. One side-chain having a 20(22) double bond and a 23-methyl group is not included. See also: A. S. R. Anjaneyulu, Y. L. N. Murthy, and L. R. Row, *Ind. J. Chem., Sect. B*, 1978, **16**, 650.

¶ References to marine sponge sterol papers: acetylenic sterols (E. Steiner, C. Djerassi, E. Fattorusso, S. Magno, L. Mayol, C. Santacroce, and D. Sica, *Helv. Chim. Acta*, 1977, **60**, 475; G. A. Doss and C. Djerassi, *J. Am. Chem. Soc.*, 1988, **110**, 8124; T. B. T. Ha and C. Djerassi, *Steroids*, 1989, **53**, 487), sterols with a 24-isopropyl- or -isopropenyl group (W. Hofheinz and G. Oesterhelt, *Helv. Chim. Acta*, 1979, **62**, 1307; N. T. Makarieva, L. K. Shubina, A. I. Kalinovski, V. A. Stonik, and G. B. Elyakov, *Steroids*, 1983, **42**, 267; T. B. T. Ha, W. C. M. C. Kokke, J. Proudfoot, C. Djerassi, and J. Thompson, *Steroids*, 1985, **45**, 263), an alkyl substituent at C-24[7] or C-25 (L. N. Li, U. Sjöstrand, and C. Djerassi, *J. Am. Chem. Soc.*, 1981, **103**, 115; see also ref. 7*c*). A 24-isopropyl sterol has also been found in a cultured marine alga (W. C. M. C. Kokke, J. N. Shoolery, W. Fenical, and C. Djerassi, *J. Org. Chem.*, 1984, **49**, 3742).

‖ Compound (**2q**) has been reported in many samples of marine origin. See ref. 9 for selected references.



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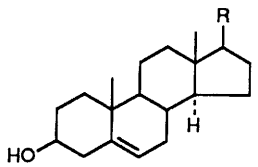

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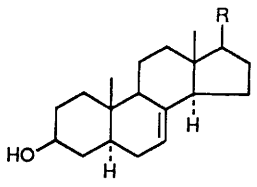

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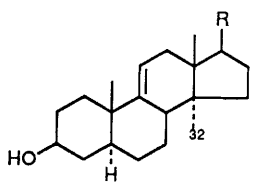

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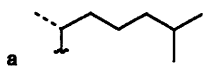

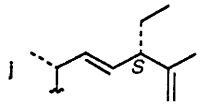

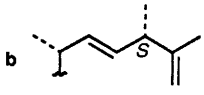

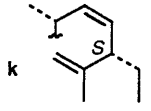

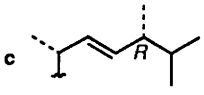

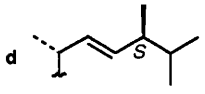

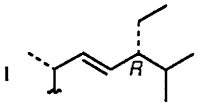

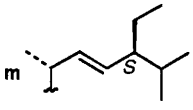

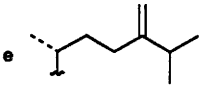

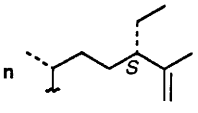

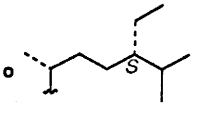

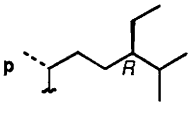

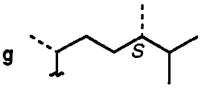

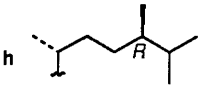

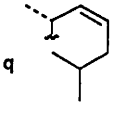

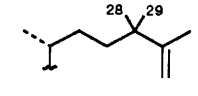

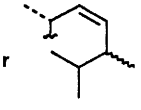

Structures of sterols in this paper. *Clerodendrum scandens* sterols have skeletons (**1**)–(**4**) and side chains (**a**)–(**p**).

(*J* 10.2 Hz). The coupling of *ca.* 11 Hz between the olefinic signals seemed to indicate the presence of a *Z* double bond.[1] The NMR spectrum of (**2k**) acetate also showed the presence of an ethyl group (δ 0.843, 3 H, t, *J* 7.5 Hz) and of an isopropenyl

Table 1. Identified sterol components of *Clerodendrum scandens* and their abundance (%).[a]

Systematic name	MW	%
5α-Cholestan-3β-ol (**1a**)	388	trace
(24ξ)-5α-Ergostan-3β-ol (**1g** or **h**)	402	trace
(22*E*,24*S*)-5α-Stigmasta-22,25-dien-3β-ol (**1i**)	412	0.2
(24*R*)-5α-Stigmast-22-en-3β-ol (**1l**)	414	0.2
(24*S*)-5α-Stigmast-22-en-3β-ol (**1m**)	414	1.3
(24*S*)-5α-Stigmast-25-en-3β-ol (**1n**)	414	0.3
(24ξ)-5α-Stigmastan-3β-ol (**1o** or **p**)	416	trace
Cholest-5-en-3β-ol (**2a**)	386	0.4
(22*E*,24*S*)-Ergosta-5,22,25-trien-3β-ol (**2b**)	396	0.3
(22*E*,24*R*)-Ergosta-5,22-dien-3β-ol (**2c**)	398	0.3
(22*E*,24*S*)-Ergosta-5,22-dien-3β-ol (**2d**)	398	0.2
(24*S*)-Ergost-5-en-3β-ol (**2g**)	400	4.8
(24*R*)-Ergost-5-en-3β-ol (**2h**)	400	3.3
24-Methylergosta-5,25-dien-3β-ol (**2i**)	398	0.2
(22*E*,24*S*)-Stigmasta-5,22,25-trien-3β-ol (**2j**)	410	30.9
(22*Z*,24*S*)-Stigmasta-5,22,25-trien-3β-ol (**2k**)	410	0.1
(22*E*,24*R*)-Stigmasta-5,22-dien-3β-ol (**2l**)	412	4.3
(22*E*,24*S*)-Stigmasta-5,22-dien-3β-ol (**2m**)	412	29.0
(24*S*)-Stigmasta-5,25-dien-3β-ol (**2n**)	412	22.2
(24*S*)-Stigmast-5-en-3β-ol (**2o**)	414	trace
(24*R*)-Stigmast-5-en-3β-ol (**2p**)	414	0.4
(22*E*,24*R*)-5α-Ergosta-7,22-dien-3β-ol (**3c**)	398	0.6
5α-Ergosta-7,24(28)-dien-3β-ol (**3e**)	398	0.2
(24ξ)-5α-Ergost-7-en-3β-ol (**3g** or **h**)	400	0.2
(24*S*)-5α-Stigmasta-7,25-dien-3β-ol (**3n**)	412	0.4
(24*S*)-14α-Methyl-5α-stigmasta-9(11),25-dien-3β-ol (**4n**)	428	0.2

[a] See Experimental section for chromatographic data of the acetates of the novel sterols (**1j**), (**1n**), (**2k**), (**2i**), and (**4n**), and of compound (**3e**). Chromatographic data of all other sterols are in a previous paper.[10b]

group [vinylic methyl group at δ 1.672 (d, *J* 1.5 Hz) and two olefinic protons at δ 4.693 (dt, *J* 4.4, 1.4 Hz) coupled to the vinylic methyl group]. The combined MS and NMR information confirmed that compound (**2k**) was (22*Z*)-stigmasta-5,22,25-trien-3β-ol. The stereochemistry at C-24 was shown to be *S* by partial hydrogenation to give (24*S*)-stigmast-5-en-3β-ol (**2o**) acetate.[12] Compound (**2k**) is the double-bond isomer of (22*E*,24*S*)-stigmasta-5,22,25-trien-3β-ol (**2j**), the main sterol of *C. scandens*. NMR data of compound (**2j**) acetate are included in Table 2 for comparison.

The shift of the signals for the angular methyl groups in the NMR spectra of the acetates of compounds (**1n**) and (**1j**) (Table 2) showed the presence of a normal (5α,14α) saturated sterol skeleton[10b,c] [expected for model compound (**1a**) acetate: 18-H_3 δ 0.650; 19-H_3 δ 0.825 on the basis of Zuercher's rules].[10c] Diagnostic peaks in the mass spectrum of (**1n**) acetate at *m/z* 456 (M^+, $C_{31}H_{52}O_2$), 396 (M^+ − HOAc), and 315 (loss of side-chain with 2 H transfer) showed that this sterol had a saturated skeleton with two additional carbons and a double bond in the side-chain.[12] The shifts of the side-chain protons (Table 2) were very similar to those of (24*S*)-stigmasta-5,25-dien-3β-ol (**2n**) (clerosterol) acetate,[10a] indicating that the new sterol (**1n**) and clerosterol (**2n**) had the same side-chain. That both sterols had indeed the same configuration at C-24 was confirmed by catalytic hydrogenation of (**1n**) acetate to give (**1o**) acetate, identified on the basis of its mass and ^{1}H NMR spectra.[10b,12a] Thus compound (**1n**) is (24*S*)-5α-stigmast-25-en-3β-ol.

Comparison of the NMR data of (**2j**) acetate with those of the acetate of the new sterol (**1j**) (Table 2) indicated that both sterols had the same side-chain. The configuration at C-24 of (**1j**) acetate was confirmed by catalytic hydrogenation which gave (24*S*)-5α-stigmastan-3β-ol (**1o**) acetate.[10b,12a] Thus the structure of compound (**1j**) is (22*E*,24*S*)-5α-stigmasta-22,25-dien-3β-ol.

Table 2. ^{1}H NMR data (400 MHz; $CDCl_3$) (*J* values in parentheses) of the acetates of five new sterols and of a reference compound (**2j**) isolated from *Clerodendrum scandens*.[a]

	(**1j**) acetate	(**2j**) acetate	(**1n**) acetate	(**2i**) acetate	(**2k**) acetate	(**4n**) acetate
3-H	4.680 (m)	4.601 (m)	4.682 (m)	4.602 (m)	4.603 (m)	4.674 (m)
6-H		5.370 (br d) (4.9)		5.370 (br d) (4.9)	5.373 (br d) (4.9)	
11-H						5.281 (dt) (5.7, 1.6)
18-H_3	0.661 (s)	0.691 (s)	0.638 (s)	0.664 (s)	0.702 (s)	0.650 (s)
19-H_3	0.818 (s)	1.012 (s)	0.813 (s)	1.015 (s)	1.025 (s)	0.976 (s)
20-H					2.481 (m)	
21-H_3	0.995 (d) (6.8)	1.011 (d) (6.8)	0.887 (d) (6.4)	0.907 (d) (6.8)	0.975 (d) (6.8)	0.867 (d) (6.2)
22-H	5.234 (dd) (7.8, 15.1)	5.243 (dd) (7.3, 15.1)			5.196 (dd) (10.7, 10.7)	
23-H	5.162 (dd) (7.3, 15.1)	5.171 (dd) (7.3, 15.1)			5.092 (dd) (9.8, 10.2)	
24-H	2.417 (q) (7.1)	2.420 (q) (7.3)			2.887 (dt) (8.3, 7.8)	
26-H_2	4.691 (m)	4.694 (m)	4.637 (1 H, d) (2.4) 4.724 (1 H, dt) (3.9, 1.5)	4.652 (1 H, d) (1.9) 4.717 (1 H, dd) (1.7, 1.7)	4.693 (dt) (4.4, 1.4)	4.645 (1 H, d) (1.2) 4.730 (1 H, dt) (2.6, 1.0)
27-H_3	1.644 (d) (1.6)	1.646 (d) (1.0)	1.563 (d) (1.0)	1.682 (d) (1.0)	1.672 (d) (1.5)	1.570 (d) (1.6)
28-H_3				1.006 (s)		
29-H_3	0.831 (t) (7.5)	0.833 (t) (7.3)	0.799 (t) (7.3)	1.010 (s)	0.843 (t) (7.5)	0.805 (t) (7.3)
32-H_3						0.741 (s)
Ac	2.017 (s)	2.030 (s)	2.017 (s)	2.032 (s)	2.032 (s)	2.033 (s)

[a] Shifts are δ-values. Internal standard Me_4Si.

The MS data of (**2i**) acetate [*m/z* 454 (M^+, $C_{31}H_{50}O_2$), 394, (M^+ − HOAc), 255 (M^+ − side-chain − HOAc), 253 (255 − 2 H)] supported a structure with one double bond in the skeleton, and one double bond and two additional carbons in the side-chain.[11] The ^{1}H NMR data (Table 2) showed that compound (**2i**) was a Δ^5-sterol. The NMR spectrum also showed the presence of an isopropenyl group and of two additional methyl singlets. The combined spectral data suggested that the structure of (**2i**) acetate was 24-methylergosta-5,25-dien-3β-yl acetate. This was confirmed by comparison with the NMR spectrum of 24-methyl-5α-ergosta-7,25-dien-3β-ol (**3i**) acetate which is a known compound.[5a]

The MS of (**4n**) acetate included a molecular ion at *m/z* 468 ($C_{32}H_{52}O_2$) and diagnostic fragment ions at *m/z* 453 (M^+ − CH_3), 393 (M^+ − HOAc − CH_3), and 327 (loss of side-chain with 2 H transfer), indicating that it was an acetate of a C_{30} sterol with one additional carbon and one double bond in the ring system, and two additional carbons and one double bond in the side-chain.[11,13] The MS also included ions having *m/z* 287 (loss of side-chain and ring D) and 273 (287 − CH_2) which are characteristic of acetates of 14α-methyl sterols.[13] NMR comparison with 14α-methyl-5α-ergosta-9(11),24(28)-dien-3β-ol (**4e**) acetate[14a] showed the structure of the skeleton (**4**). The side-chain NMR signals of (**4n**) acetate were very similar to those of the acetate of another new sterol (**1n**) from the same plant (see Table 2). Thus the proposed structure of compound (**4n**) is (24*S*)-14α-methyl-5α-stigmasta-9(11),25-dien-3β-ol. The 24*S*-configuration was confirmed by catalytic hydrogenation to give compound (**4o**).

Discussion

The only other known naturally occurring *Z*-Δ^{22}-unsaturated sterols seem to be (22*Z*)-ergosta-5,22-dien-3β-ol (**2r**), which was isolated from the clam *Tapas philippinarum* by Teshima *et al.*[8], and (22*Z*)-cholesta-5,22-dien-3β-ol (**28**).[9] However, these authors failed to identify these sterols properly by chemical correlation with known sterols.

Sterols with a quaternary carbon at C-24 were first isolated from marine sponges.[7] Their discovery was preceded by the isolation of steroidal triterpenoids having this type of side-chain from plants.[15] Our recent study revealed the presence of several 24,24-dimethyl sterols in the higher plants *Gynostemma pentaphyllum* (Cucurbitaceae)[5a–c] and *Clerodendrum inerme*.[5d]

Sea cucumbers are sources of sterols having a 14α-methyl-$\Delta^{9(11)}$-skeletal structure.[16] Several sterols with this skeleton have also been isolated from higher plants belonging to the Cucurbitaceae[14] and Solanaceae.[17]

The *C. scandens* sterols listed in Table 1 have four different skeletons (**1**)–(**4**) and sixteen different side-chains (**a**)–(**p**). It is interesting to note that we have managed to isolate only one epimer of each Δ^{25}-unsaturated sterol with a methyl or ethyl substituent at C-24. 24-Methylated or -ethylated sterols without a Δ^{25}-double bond occur as mixtures of epimers at C-24. The reason why some sterols are sterically pure at C-24 whereas others are mixtures of epimers is that in higher plants, such as *Clerodendrum* spp.[10] and members of the Cucurbitaceae,[12] sterol side-chain biosynthesis has become complicated. The 24-α and 24-β epimers are end products of different side chain alkylation pathways operating in the same plant.

Experimental

General Methods.—Crystallizations were performed from acetone–methanol. M.p.s were measured on a Yanagimoto micro m.p. apparatus and are uncorrected. Argentic TLC plates [silica gel–$AgNO_3$ (4:1)] were developed three times with CCl_4–CH_2Cl_2 (5:1). HPLC separations were performed using an Altex (= Beckman) Ultrasphere ODS column (5 μ; 10 mm i.d. × 25 cm; MeOH at 4 ml min^{-1}) and a refractive index detector. A SCOT OV-17 glass capillary column (30 m × 0.3 mm i.d.; 255 °C) was used for GLC. Cholesterol (**2a**) acetate was the standard for the determination of R_f-values in argentic TLC (R_f 1.00) and of relative retention times in both GLC and HPLC ($t_{R(rel)}$ 1.00). (Note: in this paper the symbol R_f means mobility relative to cholesterol acetate, not relative to the solvent front. Hence some R_f-values are greater than 1.00). The EI–MS spectra were recorded on a Hitachi M-80B double-focussing GC–MS machine (70 eV; direct probe for the high-resolution MS). The MS data do not include peaks with *m/z* < 200. ^{1}H NMR spectra were recorded on a JEOL JNM GX-400 spectrometer, and the ^{13}C NMR spectra (100.62 MHz) on a Bruker AM400 instrument. Carbon multiplicities were determined by DEPT experiments. When sample size permitted, the ratio of epimers at C-24 was determined by ^{13}C NMR analysis. Acetylation was performed in Ac_2O–pyridine at room temperature overnight. The aerial parts (leaves and stems) of *C. scandens* were collected locally in West Bengal (India). Experimental details of the extraction of plant material and the separation of sterol fractions have already been reported.[10b]

Isolation of Sterols.—The crude sterols of *C. scandens* were acetylated and the acetates (600 mg) were fractionated by argentic TLC. Seven bands (#1–7; #1 has the highest R_f-value) were scraped off and extracted.

Band #1 (R_f 1.04–1.10) afforded a mixture (8 mg) of the acetates of 5α-cholestan-3β-ol (**1a**), (24ξ)-5α-ergostan-3β-ol (**1g** or **h**), (24*R*)- and (24*S*)-5α-stigmast-22-en-3β-ol (**11**) and (**1m**), (24ξ)-5α-stigmastan-3β-ol (**1o** or **p**), and (24ξ)-5α-ergost-7-en-3β-ol (**3g** or **h**), from which were isolated (**11**) and (**1m**) acetates (as a mixture) (2.6 mg) by HPLC.

Band #2 (R_f 0.95–1.04) yielded a mixture (34 mg) of several steryl acetates. HPLC of this mixture gave the acetates of cholest-5-en-3β-ol (**2a**) (0.6 mg), (24*R*)- and (24*S*)-ergost-5-en-3β-ol (**2g**) and (**2h**) (as a mixture) (12.3 mg), and (24*R*)- and (24*S*)-stigmast-5-en-3β-ol (**2o**) and (**2p**) (as a mixture) (2.4 mg).

Band #3 (R_f 0.78–0.95) gave (22*E*,24*R*)- and (22*E*,24*S*)-stigmasta-5,22-dien-3β-ol (**2l**) and (**2m**) acetates (as a mixture) (92 mg).

HPLC of a mixture (17 mg) obtained from band #4 (R_f 0.56–0.78) afforded the acetates of (24*S*)-5α-stigmast-25-en-3β-ol (**1n**) (2.4 mg), (22*E*,24*R*)-ergosta-5,22-dien-3β-ol (**2c**) (0.4 mg) and its (24*S*)-epimer (**2d**) (0.3 mg), (22*E*,24*R*)-5α-ergosta-7,22-dien-3β-ol (**3c**) (0.7 mg), and (24*S*)-5α-stigmasta-7,25-dien-3β-ol (**3n**) (0.4 mg).

Band #5 (R_f 0.38–0.56) gave (24*S*)-stigmasta-5,25-dien-3β-ol (**2n**) acetate (95 mg).

HPLC of a mixture (32 mg) recovered from band #6 (R_f 0.25–0.38) gave the acetates of (22*E*,24*S*)-5α-stigmasta-22,25-dien-3β-ol (**1j**) (2.4 mg), (22*E*,24*S*)-ergosta-5,22,25-trien-3β-ol (**2b**) (0.3 mg), 24-methylergosta-5,25-dien-3β-ol (**2i**) (0.3 mg), (22*Z*,24*S*)-stigmasta-5,22,25-trien-3β-ol (**2k**) (0.3 mg), 5α-ergosta-7,24(28)-dien-3β-ol (**3e**) (0.9 mg), and (14*S*)-14α-methyl-5α-stigmasta-9(11),25-dien-3β-ol (**4n**) (1.4 mg).

Band #7 (R_f 0.16–0.25) gave (22*E*,24*S*)-stigmasta-5,22,25-trien-3β-ol (**2j**) acetate (118 mg).

(22E,24S)-5α-*Stigmasta*-22,25-*dien*-3β-*ol* (**1j**) *Acetate.*—M.p. 145–147 °C, $t_{R(rel)}$ (GC) 1.53, $t_{R(rel)}$ (HPLC) 0.91, R_f 0.28; *m/z* (assignment, rel. int.) 454.3789 ($C_{31}H_{50}O_2$, M^+, 6%; Calc. *M*, 454.3808), 439.3520 ($C_{30}H_{47}O_2$, 2), 425.3789 ($C_{29}H_{45}O_2$, 6), 394.3622 ($C_{29}H_{46}$, 17), 379.3357 ($C_{28}H_{43}$, 2), 365.3179 ($C_{27}H_{41}$, 17), 344.2713 ($C_{23}H_{36}O_2$, 43), 329.2513 ($C_{22}H_{33}O_2$, 11), 315.2321 ($C_{21}H_{31}O_2$, 100), 285.2545 ($C_{21}H_{33}$, 17), 269.2280 ($C_{20}H_{29}$, 9), 257.2274 ($C_{19}H_{29}$, 74), 255.2131 ($C_{19}H_{27}$, 26), 229.1996 ($C_{17}H_{25}$, 4), and 215.1829 ($C_{16}H_{23}$, 15); δ_C($CDCl_3$)

36.75 (C-1), 27.47 (C-2), 73.78 (C-3), 34.02 (C-4), 44.65 (C-5), 28.59 (C-6),* 31.97 (C-7), 35.47 (C-8), 54.23 (C-9), 35.47 (C-10), 21.17 (C-11), 39.86 (C-12), 42.52 (C-13), 56.49 (C-14), 24.23 (C-15), 28.73 (C-16),* 55.97 (C-17), 12.14 (C-18), 12.26 (C-19), 40.23 (C-20), 20.76 (C-21), 137.26 (C-22), 129.98 (C-23), 51.99 (C-24), 148.66 (C-25), 109.50 (C-26), 20.22 (C-27), 25.70 (C-28), 12.22 (C-29), 21.48 (CO*Me*), and 170.74 (*C*OMe) (* denotes interchangeable assignments). The ^{13}C NMR data were assigned by comparison with those of the acetates of compound (**1l**)[10b] and (**2j**).[18] Catalytic hydrogenation of (**1j**) acetate afforded (24*S*)-5α-stigmastan-3β-ol (**1o**) acetate, $t_{R(rel)}$ (GC) 1.64, $t_{R(rel)}$ (HPLC) 1.49; *m/z* 458 (M^+, 56%), 443 (4), 398 (48), 383 (20), 290 (14), 276 (35), 275 (28), 261 (4), 257 (8), 230 (38), 215 (100), and 201 (20); δ_H 4.683 (tt, *J* 4.9, 11.5, Hz, 3-H), 0.645 (s, 18-H_3), 0.816 (s, 19-H_3), 0.907 (d, *J* 6.6 Hz, 21-H), 0.826 (d, *J* 9.7 Hz, 26-H_3), 0.807 (d, *J* 7.2 Hz, 27-H_3), 0.852 (t, *J* 7.1 Hz, 29-H_3), and 2.019 (s, Ac). The ^1H NMR data were assigned by comparison with published NMR data of (24*S*)-5α-stigmast-22-en-3β-ol (**1m**) acetate[10b] and (24*S*)-stigmast-5-en-3β-ol (**2o**).[12a]

(24S)-5α-*Stigmast*-25-*en*-3β-*ol* (**1n**) *Acetate*.—M.p. 123–125 °C; $t_{R(rel)}$ (GC) 1.65, $t_{R(rel)}$ (HPLC) 1.08, R_f 0.75; *m/z* 456.3964 ($C_{31}H_{52}O_2$, M^+, 100%; Calc: *M*, 456.3964), 441.3725 ($C_{30}H_{49}O_2$, 15), 396.3756 ($C_{29}H_{48}$, 7), 381.3496 ($C_{28}H_{45}$, 15), 358.2855 ($C_{24}H_{38}O_2$, 33), 343.2653 ($C_{23}H_{35}O_2$, 37), 315.2311 ($C_{21}H_{31}O_2$, 98), 302.2238 ($C_{20}H_{30}O_2$, 22), 283.2417 ($C_{21}H_{31}$, 15), 257.2274 ($C_{19}H_{29}$, 48), 255.2101 ($C_{19}H_{27}$, 59), 229.1989 ($C_{17}H_{25}$, 22), and 215.1830 ($C_{18}H_{23}$, 85); $\delta_C(CDCl_3)$ 36.75 (C-1), 27.48 (C-2), 73.80 (C-3), 34.03 (C-4), 44.64 (C-5), 28.60 (C-6), 31.98 (C-7), 35.47 (C-8), 43.20 (C-9), 35.46 (C-10), 21.19 (C-11), 39.97 (C-12), 42.59 (C-13), 56.41 (C-14), 24.20 (C-15), 28.18 (C-16), 56.14 (C-17), 12.05 (C-18),* 12.22 (C-19), 35.56 (C-20), 18.60 (C-21), 33.65 (C-22), 29.40 (C-23), 49.53 (C-24), 147.59 (C-25), 111.38 (C-26), 17.77 (C-27), 26.52 (C-28), 12.08 (C-29),* 21.50 (CO*Me*), and 170.74 (*C*OMe)) (* denotes interchangeable assignments). The ^{13}C NMR data were assigned by comparison with those of (**1l**)[18b] and (**2n**).[18] Catalytic hydrogenation of (**1n**) acetate afforded (24*S*)-5α-stigmastan-3β-ol (**1o**) acetate, which was identified by direct comparison with (**1o**) acetate obtained from (**1j**) acetate by hydrogenation as described above.

24-*Methylergosta*-5,25-*dien*-3β-*ol* (**2i**) *Acetate*.—M.p. 146–148 °C; $t_{R(rel)}$ (GC) 1.69, $t_{R(rel)}$ (HPLC) 0.88, R_f 0.26; *m/z* 454.3846 ($C_{31}H_{50}O_2$, M^+, 1%. Calc: *M*, 454.3808), 394.3586 ($C_{29}H_{46}$, 100), 379.3323 ($C_{28}H_{43}$, 8), 310.2654 ($C_{23}H_{34}$, 10), 281.2303 ($C_{21}H_{29}$, 4), 273.2516 ($C_{20}H_{33}$, 4), 255.2115 ($C_{17}H_{27}$, 11), 253.1957 ($C_{19}H_{25}$, 10), 228.1901 ($C_{17}H_{24}$, 10), and 213.1665 ($C_{16}H_{21}$, 13).

(22Z,24S)-*Stigmasta*-5,22,25-*trien*-3β-*ol* (**2k**) *Acetate*.—$t_{R(rel)}$ (GC) 1.36, $t_{R(rel)}$ (HPLC) 0.69, R_f 0.33; *m/z* 452.3612 ($C_{31}H_{48}O_2$, M^+, 1%. Calc: *M*, 452.3651), 392.3408 ($C_{29}H_{44}$, 100), 377.3164 ($C_{28}H_{41}$, 6), 363.3053 ($C_{27}H_{39}$, 4), 255.2082 ($C_{19}H_{27}$, 34), 253.1913 ($C_{19}H_{25}$, 23), 239.1809 ($C_{18}H_{23}$, 4), 228.1897 ($C_{17}H_{24}$, 4), 213.1682 ($C_{16}H_{21}$, 11), 211.1511 ($C_{16}H_{19}$, 9), and 201.1643 ($C_{15}H_{21}$, 5). The size of the available sample did not allow us to obtain ^{13}C NMR data. Decoupling experiments: irradiation at δ 2.481 collapsed the methyl doublet at δ 0.975 into a singlet, and the methine double doublet at δ 5.196 into a doublet (*J* 10.8 Hz). Irradiation at δ 2.887 collapsed the methine double doublet at δ 5.092 into a doublet (*J* 10.7 Hz). Moreover, irradiation at δ 5.092 collapsed the methine double triplet at δ 2.887 into a triplet (*J* 7.1 Hz), and the methine double doublet at δ 5.196 into a doublet (*J* 10.2 Hz). These experiments allowed the assignment of the side-chain proton signals as δ 0.975 (21-H_3), 2.481 (20-H), 2.887 (24-H), 5.092 (23-H), and 5.196 (22-H). Catalytic hydrogenation of (**2j**) acetate afforded (24*S*)-stigmast-5-en-3β-ol (**2o**) acetate, $t_{R(rel)}$ (GC) 1.63, $t_{R(rel)}$ (HPLC) 1.26, which was identified on the basis of its mass and ^1H NMR spectra.[12]

5α-*Ergosta*-7,24(28)-*dien*-3β-*ol* (**3e**) *Acetate*.—M.p. 138–141 °C; $t_{R(rel)}$ (GC) 1.60, $t_{R(rel)}$ (HPLC) 0.82, R_f 0.25; *m/z* 440 (M^+, 16%), 425 (14), 380 (5), 375 (6), 356 (39), 342 (9), 339 (3), 313 (100), 288 (5), 281 (5), 273 (7), 255 (22), 253 (11), 227 (16), and 213 (28); δ_H 4.695 (tt, *J* 4.4, 11.7 Hz, 3-H), 5.148 (dd, *J* 2.2, 4.7 Hz, 7-H), 0.537 (s, 18-H_3), 0.811 (s, 19-H_3), 0.954 (d, *J* 6.8 Hz, 21-H_3), 2.231 (sept., *J* 6.8 Hz, 25-H_3), 1.024 and 1.028 (each d, *J* 6.8 Hz, together 26- and 27-H_3), 4.652 and 4.715 (each 1 H, d, *J* 1.5 Hz, together 28-H_2), and 2.029 (s, Ac). This product was identified by comparison of its chromatographic behaviour, and mass and ^1H NMR data with those of the authentic compound isolated from Cucurbitaceous plants.[12]

(24S)-14α-*Methyl*-5α-*stigmasta*-9(11),25-*dien*-3β-*ol* (**4n**) *Acetate*.—M.p. 105–107 °C; $t_{R(rel)}$ (GC) 1.86, $t_{R(rel)}$ (HPLC) 0.85, R_f 0.32; *m/z* 468.3984 ($C_{32}H_{52}O_2$, M^+, 25%. Calc: *M*, 468.3954), 453.3690 ($C_{31}H_{49}O_2$, 45), 393.3462 ($C_{29}H_{45}$, 18), 370.2836 ($C_{25}H_{38}O_2$, 5), 369.2760 ($C_{25}H_{37}O_2$, 5), 355.2581 ($C_{24}H_{35}O_2$, 7), 327.2330 ($C_{22}H_{31}O_2$, 100), 309.2549 ($C_{23}H_{33}$, 3), 301.2204 ($C_{20}H_{29}O_2$, 3), 288.2070 ($C_{19}H_{28}O_2$, 15), 287.1980 ($C_{19}H_{27}O_2$, 8), 273.1806 ($C_{18}H_{25}O_2$, 12), 261.1858 ($C_{17}H_{25}O_2$, 12), 260.1755 ($C_{17}H_{24}O_2$, 8), 255.2066 ($C_{19}H_{27}$, 22), 227.1823 ($C_{17}H_{23}$, 17), 225.1700 ($C_{17}H_{21}$, 3), 215.1840 ($C_{16}H_{23}$, 7), and 213.1682 ($C_{16}H_{21}$, 10); δ_C 35.24 (C-1), 27.19 (C-2), 73.60 (C-3), 34.23 (C-4), 42.82 (C-5), 28.48 (C-6), 27.93 (C-7), 41.80 (C-8), 145.81 (C-9), 38.04 (C-10), 116.57 (C-11), 37.25 (C-12), 44.30 (C-13), 37.10 (C-14), 34.00 (C-15), 27.59 (C-16), 50.96 (C-17), 14.41 (C-18), 19.19 (C-19), 35.92 (C-20), 18.40 (C-21), 33.93 (C-22), 29.70 (C-23), 49.57 (C-24), 147.59 (C-25), 17.75 (C-26), 111.42 (C-27), 26.55 (C-28), 12.09 (C-29), 18.33 (C-32), 21.48 (CO*Me*), and 170.71 (*C*OMe). The ^{13}C NMR data were assigned by comparison with published NMR data of 14α-methyl-5α-cholest-9-(11)-en-3β-ol (**4a**) acetate[14b] and (**2n**) acetate.[18] Catalytic hydrogenation of (**4n**) acetate afforded (24*S*)-14α-methyl-5α-stigmast-9(11)-en-3β-ol (**4o**) acetate, $t_{R(rel)}$ (GC) 1.82, $t_{R(rel)}$ (HPLC) 1.18; *m/z* 470 (M^+, 31%), 455 (100), 410 (3), 395 (24), 330 (4), 302 (5), 287 (6), 273 (6), 269 (6), 261 (8), 255 (4), 227 (10), and 213 (8); δ_H 4.676 (tt, *J* 4.7, 11.4 Hz, 3-H), 5.286 (dt, *J* 6.0, 1.5 Hz, 11-H), 0.657 (s, 18-H_3), 0.980 (s, 19-H_3), 0.880 (d, *J* 6.6 Hz, 21-H_3), 0.836 (d, *J* 6.6 Hz, 26-H_3), 0.814 (d, *J* 6.6 Hz, 27-H_3), 0.858 (d, *J* 7.4 Hz, 29-H_3), 0.753 (s, 32-H_3), and 2.027 (s, OAc). Identification of (**4o**) acetate was aided by analysis of its mass and ^1H NMR spectra.[14b]

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