Minor and Trace Sterols in Marine Invertebrates. Part 35.† Isolation and Structure Elucidation of Seventy-four Sterols from the Sponge *Axinella cannabina*

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Seventy-four 3β-hydroxy-sterols, of which twenty-four are new, with seven different nuclei [$\Delta^{5,7,9(11)}$, $\Delta^{5,7}$, Δ^{8}, Δ^{7}, Δ^{5}, 5α-saturated; and 5α-methoxy-$\Delta^{6,8(14)}$], and with eighteen different C_7—C_{10}-side chains, have been isolated by reverse-phase h.p.l.c. and argentic t.l.c. from the marine sponge *Axinella cannabina*. Characterization was accomplished by g.l.c.–m.s., high-resolution m.s., and 360-MHz ^{1}H n.m.r. and u.v. spectroscopy.

Sponges have been found to be the most diverse source of sterols.[1,2] An earlier Italian study of the sponge *Axinella cannabina* recorded the identification of three Δ^8-sterols, *viz.* 5α-cholest-8-en-3β-ol (3f), (24ξ)-24-methyl-5α-cholest-8-en-3β-ol (3k/3l), and (24ξ)-24-ethyl-5α-cholest-8-en-3β-ol (3q/3r), in addition to thirteen other sterols, using mainly g.l.c.-m.s.[3] Since the occurrence of Δ^8-sterols (lacking a 4-methyl group) in Nature is quite rare and since these three sterols are the only naturally occurring 4-demethyl-Δ^8-marine sterols reported in the literature, we considered it worthwhile as part of our continuing research on minor and trace sterols from marine invertebrates to reinvestigate the sterols of *A. cannabina* with the hope of isolating the Δ^8-sterols and detecting other novel sterols. We now report the isolation and structure elucidation of seventy-four sterols, of which twenty-four are new.

Results

Table 1 lists the seventy-four sterols isolated from the sponge *Axinella cannabina*, together with their molecular-ion peaks, relative retention times (r.r.t.) in h.p.l.c. (high-pressure liquid chromatography) and g.l.c., and percentage of the sterol components. The specific structures of the sterols are reproduced in the Figure. Of the seventy-four sterols, twenty-four are new ones, most of which were isolated from the crude mixture. Most of the sterols possessing a side chain with unknown configuration were isolated and their stereochemistry established by comparison with the 360-MHz ^{1}H n.m.r. spectra of known reference compounds.[4–10] The other sterols were identified on the basis of their r.r.t. values in both h.p.l.c. and g.l.c., and by their m.s. data.

The structural assignment of the novel sterols was achieved with the aid of high-resolution m.s., 360-MHs n.m.r. spectroscopy, and u.v. spectroscopic measurements as described below. Two side-chains, b and d, of some sterols have previously been synthesized,[10,11] but have never been found in natural compounds. The 360-MHz n.m.r. data of twenty-one sterols from among the twenty-four new ones are shown in Table 2.

Sterols with a $\Delta^{5,7,9(11)}$*-Nucleus* (1).—The u.v. spectra of the sterols with the nucleus (1) showed absorption peaks at 312, 324, and 338 nm, suggesting the presence of a $\Delta^{5,7,9(11)}$- or $\Delta^{5,7,14}$-conjugated double-bond system.[12] The m.s. displayed fragments characteristic of sterols with a tri-unsaturated nucleus:[13] *m/z* 269 ($C_{19}H_{25}O^+$) and 251 ($C_{19}H_{23}^+$) (loss of side chain and side chain plus water, respectively), and *m/z* 227 ($C_{16}H_{19}O^+$) and 209 ($C_{16}H_{17}^+$) (loss of ring D and ring D plus water, respectively). The two latter ions eliminated the possibility of Δ^{14}-unsaturation. The 360-MHz n.m.r. spectra showed nuclear olefinic proton signals (each 1 H) which are deshielded to δ *ca.* 5.68, 5.52, and 5.41, indicating a conjugated system. The chemical shifts of the C-18 and C-19 angular methyl signals, δ *ca.* 0.58 and 1.25, respectively, are typical of $\Delta^{5,7,9(11)}$-unsaturation.[14,15]

Of the thirteen sterols with the rare $\Delta^{5,7,9(11)}$-nucleus (1) characterized in the sterol mixture of *A. cannabina*, eleven (1a, c—e, g, i, k, n—p, and q/r) are new. The assignment of structures for compounds (1p) and (1q/1r) was based on chromatographic and m.s. data as described in the Experimental section. 24-Ethylcholesta-5,7,9(11),22-tetraen-3β-ol (1n/1o) has already been detected [15] in the sponge *Biemna fortis*, but no assignment of the C-24 stereochemistry was made.

Sterols with a $\Delta^{5,7}$*-Nucleus* (2).—Sterols with the nucleus (2) showed absorption peaks at 262, 271, 281, and 293 nm in their u.v. spectra, typical of $\Delta^{5,7}$-sterols.[12,16] These sterols afforded a fragment in the m.s. corresponding to $(M - C_3H_7O)^+$ (loss of part of ring A by cleavage of the 1–10 and 3–4 bonds), diagnostic for a $\Delta^{5,7}$-unsaturated nucleus,[17,18] The n.m.r. spectra showed the C-18 and C-19 angular methyl signals at δ *ca.* 0.63 and 0.94, respectively, indicating the sterols to be either $\Delta^{5,7}$-di-unsaturated [19,20] or Δ^8-mono-unsaturated.[20] However, the presence of two ring-olefinic signals deshielded to δ 5.39 and 5.57, indicating a conjugated system, eliminated the possibility of Δ^8-unsaturation.

The high resolutions m.s. of sterol (2b) ($M^+ = C_{26}H_{40}O$) showed fragments $C_{23}H_{33}^+$ ($M - C_3H_7O)^+$, and $C_{19}H_{25}^+$ and $C_{19}H_{23}^+$ (loss of side chain and water without and with 2 H transfer) indicating the presence of a $\Delta^{5,7}$-di-unsaturated nucleus and a mono-unsaturated C_7-side-chain.[19,21] The n.m.r. spectrum displayed two methyl signals at δ 0.876 (3 H, t, *J* 7.3 Hz) and 1.029 (3 H, d, *J* 6.6 Hz) and one olefinic signal centred at δ 5.28 (2 H, m) arising from the side-chain protons, besides the above-described signals typical for the $\Delta^{5,7}$-nucleus. The methyl doublet and the olefinic multiplet signals are almost identical with those of the C-21 and C-22/C-23 signals, respectively, of compound (2e), a (22*E*)-$\Delta^{5,7,22}$-sterol. The possibility of (22*Z*) stereochemistry of the double bond was eliminated because such stereochemistry shifts the C-18 methyl [22] and C-22/C-23 olefinic [9,11] signals to lower field, and the C-21 methyl signal [9] to higher field. The remaining triplet methyl signal (δ 0.876) is ascribed to the terminal methyl group of an n-propyl moiety attached to the C-22/C-23 olefinic system. Thus the sterol (2b), (22*E*)-27-norcholesta-

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Table 1. Molecular ion (M^+), r.r.t.[a] (h.p.l.c. and g.l.c.) and composition (%) [b] of sterols present in *Axinella cannabina*

	Nucleus																							
	(1)				(2)				(3)				(4)				(5)				(6)			
		r.r.t.				r.r.t.				r.r.t.				r.r.t.				r.r.t.				r.r.t.		
Side chain	M^+ (*m/z*)	h.p.l.c.	g.l.c.	%	M^+ (*m/z*)	h.p.l.c.	g.l.c.	%	M^+ (*m/z*)	h.p.l.c.	g.l.c.	%	M^+ (*m/z*)	h.p.l.c.	g.l.c.	%	M^+ (*m/z*)	h.p.l.c.	g.l.c.	%	M^+ (*m/z*)	h.p.l.c.	g.l.c.	%
a	366	0.41	0.72	0.07	368	0.58	0.78	2.10	370	0.61	0.69	0.007	370	0.62	0.78	0.33	370	0.64	0.65	0.39	372	0.71	0.65	0.04
b					368	0.65	0.96	0.008																
c	380	0.48	0.99	0.32	382	0.68	1.05	6.62	384	0.71	0.95	0.07	384	0.75	1.06	0.34	384	0.75	0.90	0.20				
d	380	0.48	0.99	0.02	382	0.68	1.05	0.35																
e	380	0.50	1.03	0.12	382	0.71	1.09	9.96	384	0.73	0.99	0.10	384	0.79	1.10	0.34	384	0.79	0.93	0.87	386	0.91	0.93	0.08
f	382	0.63	1.10	0.09	384	0.91	1.17	7.81	386	0.92	1.06	0.01	386	1.00	1.18	0.68	386	1.00	1.00	3.83	388	1.10	1.01	0.16
g	394	0.55	1.26	0.34	396	0.78	1.34	18.77	398	0.79	1.35	0.54	398	0.86	1.35	0.54	398	0.86	1.14	1.50				
h	394	0.57	1.26	0.04	396	0.83	1.34	5.04					398	0.91	1.35	0.14	398	0.92	1.14	0.01				
i	394	0.58	1.50	0.04	396	0.83	1.59	5.74									398	0.85	1.35	0.04	400	0.95	1.36	0.02
j					396	1.15	1.55	0.30																
k	396	0.68	1.45	0.09	398	1.00	1.54	0.55	400	1.00	1.38	0.002 [c]	400	1.10	1.55	0.03	400	1.10	1.31	0.06				
l					398	1.00	1.54	4.91					400	1.10	1.55	0.24	400	1.10	1.31	0.24				
m																	412	1.10	1.36	0.008				
n	408	0.63	1.57	0.16	410	0.92	1.67	3.48	412	0.95	1.51	0.004	412	1.04	1.67	0.10	412	1.04	1.42	0.10 [c]				
o	408	0.63	1.57	0.15	410	0.92	1.67	8.13	412	0.95	1.51	0.004	412	1.04	1.67	0.02								
p	408	0.61	1.98	0.01	410	0.90	2.10	0.39					412	1.00	2.11	0.03	412	1.00	1.79	0.36				
q	410	0.73	1.78	0.08 [c]	412	1.06	1.89	0.71	414	1.06	1.68	0.002	414	1.18	1.89	0.08	414	1.18	1.61	0.18	416	1.25	1.62	0.002 [c]
r					412	1.06	1.89	6.43	414	1.06	1.68	0.006	414	1.18	1.89	0.20	414	1.18	1.61	0.07				

[a] Retention time relative to that of cholesterol. [b] Percentage in the sterol mixture estimated from h.p.l.c., argentic t.l.c., g.l.c., and 360 MHz ^{1}H n.m.r. data. [c] Configuration at C-24 remains undetermined.

In addition, (7g): *m/z* 426 (M^+); r.r.t. (h.p.l.c.) 0.39, (g.l.c.) 0.65; 0.02%. (7i): *m/z* 426 (M^+); r.r.t. (h.p.l.c.) 0.39, (g.l.c.) 0.77; 0.01%.

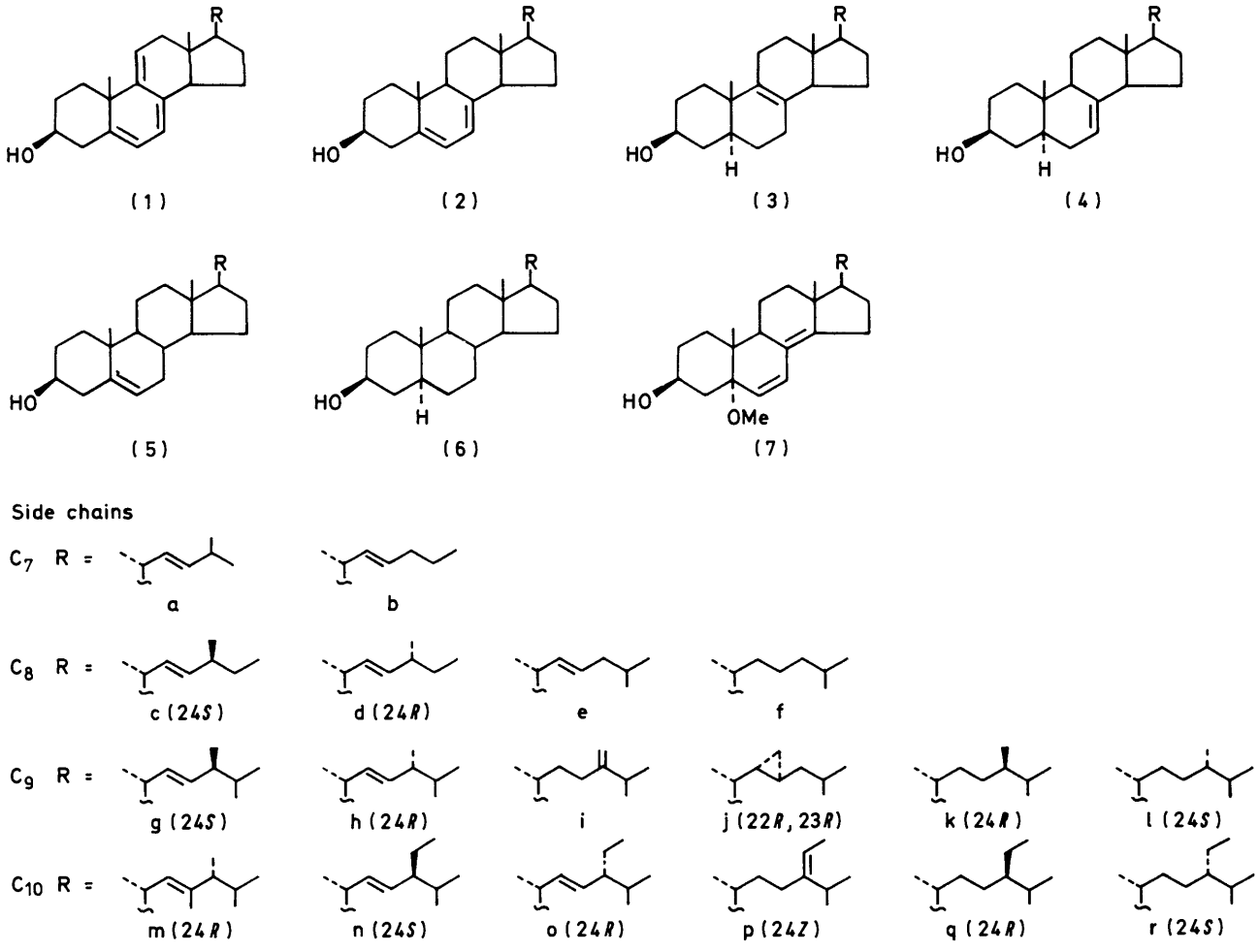


Figure. Sterols isolated from *Axinella cannabina*. All C-22–C-23 double bonds are *E*

5,7,22-trien-3β-ol, has an unusual side-chain structure which has so far not been encountered in Nature.[23]

Although most of the $\Delta^{5,7}$-sterols exhibited the C-18 methyl n.m.r. signal at δ *ca.* 0.63, the cyclopropane-containing sterol (22*R*,23*R*)-22,23-methylenecholesta-5,7-dien-3β-ol (2j) displayed it at far higher field (δ 0.557) owing to the presence of the C-22/23 cyclopropyl group in the side chain as already observed in its Δ^5-analogue.[7,9]

The $\Delta^{5,7}$-sterols make up the bulk (over 80%) of the sterol mixture of *A. cannabina*. Other than the unusual sterol (2b) described above, three sterols (2c, d, and j) are novel sterols among the seventeen $\Delta^{5,7}$-sterols characterized. The natural occurrence of compounds (2a) was reported recently in molluscs.[24]

Sterols with a Δ^8-(3) or Δ^7-(4) *Nucleus.*—Sterols with the rare Δ^8-unsaturated nucleus (3) exhibited fragments in the m.s. at *m/z* 246 ($C_{17}H_{26}O^+$, loss of side chain with part of ring D) typical for Δ^{7}-[13,25] or Δ^{8}-[25] sterols. The shorter g.l.c. retention times of Δ^8-sterols than of Δ^7-sterols made it possible to distinguish the two types.[26] The existence of Δ^8-unsaturation was confirmed by n.m.r. spectroscopy since sterols with nucleus (3) exhibited the C-18 and C-19 angular methyl signals at δ *ca.* 0.62 and 0.95, respectively, similar to those of $\Delta^{5,7}$-sterols, while signals due to nuclear olefinic protons were absent.

The chemical shift of the C-21 methyl doublet is a diagnostic measure for differentiating (24*R*)- and (24*S*)-epimers of (22*E*)-24-methyl-Δ^{22}-sterols [4,5] (side-chain g/h in the Figure) and their 27-nor-analogues (side-chain c/d).[10] The C-21 methyl doublet resonates at almost identical field in sterols possessing side-chains c/d and g/h, with the (24*R*)-epimers (d/h) displaying their signal at lower field ($\Delta \gtrsim 0.006$ p.p.m.) than their (24*S*) counterparts (c/g). On this basis, the (24*S*) configuration was assigned to the sterol (3c) because its C-21 methyl doublet (δ 1.012) is almost identical with that (δ 1.011) of compound (3g). In an identical manner, the configuration at C-24 of the Δ^7-sterol (4c) was assigned as *S*. In the case of this sterol the assignment was confirmed by direct n.m.r. comparison with authentic (4c) synthesized from compound (2c) by selective homogeneous catalytic hydrogenation.[27]

Among the ten Δ^8-sterols, seven (3a, c, g, n, o, q, and r) are newly characterized ones. 24-Ethyl-5α-cholest-8-en-3β-ol (3q/3r) has previously been found in the same sponge *A. cannabina*, but the stereochemistry at C-24 remained undetermined.[3]

Sterols with a 5α-*Methoxy*-$\Delta^{6,8(14)}$-*nucleus* (7).—The high-resolution m.s. of sterol (7g), *m/z* 426 (M^+, $C_{29}H_{42}O_2$), showed fragments $C_{28}H_{42}O^+$ and $C_{28}H_{40}^+$ (loss of methanol without and with water, respectively) indicating the presence of a methoxy-group and three double bonds. The fragments $C_{19}H_{23}^+$ and $C_{19}H_{21}^+$ (loss of the side chain and water without and with transfer of 2 H from the ion $C_{28}H_{42}O^+$) require that one double bond be located in the C_9-side-chain and the other two in the nuclues. The u.v. spectrum showed a maximum at 256 nm, typical of $\Delta^{6,8(14)}$-sterols.[12] Two n.m.r. doublets

Table 2. ^{1}H n.m.r. data [a] (360 MHz; $CDCl_3$) of some novel sterols from the sponge *Axinella cannabina*

Compd. [b]	18-H_3 [c]	19-H_3 [c]	21-H_3 [d]	26- and 27-H_3	28- or 29-H_3	22- and 23-H [e]
(1a)	0.577	1.245	1.007 (6.5)	0.948 (d, 6.6)		5.176 (dd, 15.8 and 8.3)
						5.298 (dd, 14.9 and 6.7)
(1c)	0.578	1.245	1.010 (6.6)	0.838 (t, 7.4)	0.933 (d, 6.7)	5.17
(1d)	0.578	1.245	1.022 (7.0)	0.838 (t, 7.1)	0.933 (d, 6.6)	5.16
(1e)	0.580	1.245	1.016 (6.6)	0.863 (d, 6.6)		5.26
				0.867 (d, 6.6)		
(1g)	0.580	1.245	1.010 (6.6)	0.824 (d, 6.6)	0.917 (d, 6.8)	5.18
				0.842 (d, 6.5)		
(1i)	0.569	1.246	0.955 (6.4)	1.026 (d, 6.8)	4.663 (s)	
				1.030 (d, 6.8)	4.720 (s)	
(1k)	0.566	1.246	0.917 (6.4)	0.807 (d, 6.8)	0.780 (d, 6.5)	
				0.855 (d, 6.7)		
(1n)	0.584	1.245	1.028 (6.5)	0.801 (d, 6.1)	0.809 (t, 7.2)	5.042 (dd, 14.9 and 8.5)
				0.853 (d, 6.3)		5.161 (dd, 15.5 and 9.0)
(1o)	0.583	1.246	1.030 (6.5)	0.795 (d, 6.0)	0.818 (t, 7.2)	5.050 (dd, 15.0 and 8.3)
				0.847 (d, 6.2)		5.167 (dd, 15.1 and 8.5)
(2b)	0.628	0.945	1.029 (6.6)	0.876 (t, 7.3)		5.28
(2c)	0.628	0.945	1.027 (6.7)	0.837 (t, 7.4)	0.932 (d, 7.1)	5.16
(2d)	0.629	0.945	1.033 (7.0)	0.833 (t, 7.4)	0.935 (d, 7.2)	5.18
(2j)	0.557	0.932	1.017 (6.7)	0.887 (d, 6.6)	0.39 (m)	0.23
				0.910 (d, 6.6)		
(3c)	0.620	0.951	1.012 (6.6)	0.834 (t, 7.3)	0.930 (t, 6.7)	5.15
(3g)	0.621	0.952	1.011 (6.6)	0.820 (d, 6.4)	0.913 (d, 6.9)	5.16
				0.838 (d, 6.5)		
(3n)	0.624	0.951	1.028 (6.6)	0.797 (d, 6.0)	0.805 (t, 7.0)	5.022 (dd, 14.8 and 8.7)
				0.847 (d, 6.4)		5.147 (dd, 14.9 and 8.3)
(3o)	0.622	0.949	1.029 (6.6)	0.790 (d, 6.2)	0.810 (t, 7.2)	5.025 (dd, 14.8 and 8.5)
				0.841 (d, 6.6)		5.152 (dd, 15.0 and 8.5)
(3q) [f]	0.605	0.947	0.924 (7.0)	0.807 (d, 6.8)	0.841 (t, 7.1)	
				0.827 (d, 6.6)		
(3r) [f]	0.605	0.947	0.930 (7.0)	0.807 (d, 6.8)	0.850 (t, 7.3)	
				0.827 (d, 6.6)		
(7g)	0.746	0.901	1.024 (6.6)	0.825 (d, 6.7)	0.912 (d, 7.5)	5.19
				0.840 (d, 6.6)		
(7i)	0.748	0.895	0.975 (6.6)	1.022 (d, 6.8)	4.661 (s)	
				1.028 (d, 6.8)	4.720 (s)	

[a] Given as δ values; multiplicities and *J* values (Hz) are shown in parentheses. [b] The following signals were also observed: δ 5.41 and 5.52 (each 1 H, m, 6- and 7-H), 5.68 (1 H, m, 11-H), and 3.61 (1 H, m, 3-H_α) for compounds (1a—o); δ 5.39 and 5.57 (each 1 H, m, 6- and 7-H) and 3.64 (1 H, m, 3-H_α) for compounds (2b—j); δ 3.62 (1 H, m, 3-H_α) for compounds (3c—r); δ 3.171 (3 H, s, OMe), 5.387 (1 H, d, *J* 10.0 Hz, 6- or 7-H), 6.306 (1 H, d, *J* 9.8 Hz, 7- or 6-H), and 3.92 (1 H, m, 3-H_α) for (7g); and δ 3.173 (3 H, s, OMe), 5.397 (1 H, d, *J* 9.9 Hz, 6- or 7-H), 6.319 (1 H, d, *J* 9.6 Hz, 7- or 6-H), and 3.92 (1 H, m, 3-H_α) for (7i). [c] Singlet. [d] Doublet. [e] Multiplet if not otherwise specified. [f] The epimeric pair (3q) and (3r) was not separated.

deshielded to δ 6.306 (*J* 9.8 Hz) and 5.387 (*J* 10.0 Hz), arising from the 6- and 7-protons, supported the existence of a conjugated system of two double bonds. Since the C-3 methine multiplet (δ 3,92) and C-19 methyl singlet (δ 0.901) were observed in the same regions as the corresponding signals (δ 3.90 and 0.91, respectively) [28] of (22*E*,24*R*)-5α,8α-epidioxy-24-methylcholesta-6,22-dien-3β-ol,[28,29] the methoxy-group (s, δ 3.171) can be reasonably located at C-5α. The other signals due to the side-chain protons are closely related to those of compound (2g), thus leading to (22*E*,24*S*)-5α-methoxy-24-methylcholesta-6,8(14),22-trien-3β-ol (7g) as the structure of this sterol.

The n.m.r. spectrum of the sterol (7i) afforded signals, due to the nuclear protons, which were almost identical with those of compound (7g), whereas the side-chain proton signals closely resembled those of compound (2i). We conclude that the structure of this sterol is 5α-methoxy-24-methylenecholesta-6,8(14)-dien-3β-ol (7i).

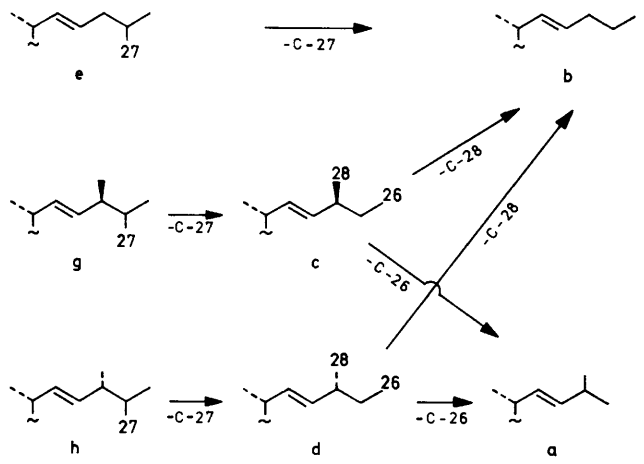
Uncharacterized Penta-unsaturated Sterols.—Three penta-unsaturated sterols (A—C) were also detected in trace amounts in the sterol mixture. The m.s. fragmentation patterns of these compounds in g.l.c.-m.s. [M^+ = 378 (A), 392 (B), and 406 (C)] are closely related to those of compounds (1c—e), (1g/h), and (1n/o), respectively, except for a two-unit shift to lower mass, and the presence of peaks at *m/z* 266 and 248. Thus, the three sterols A, B, and C have the same tetra-unsaturated nucleus with C-22 mono-unsaturated C_8-, C_9-, and C_{10}-side-chains, respectively. Further characterization of these sterols was not achieved because of their instability.

Biosynthetic Implications

The sterol fraction of *A. cannabina* has been shown to contain a complex mixture of more than seventy sterols with C_7—C_{10}-side-chains: $\Delta^{5,7}$-sterols predominate, but minor or trace amounts of $\Delta^{5,7,9(11)}$-, Δ^{8}-, Δ^{7}-, Δ^{5}-, 5α-saturated-, and 5α-methoxy-$\Delta^{6,8(14)}$-sterols are also present.

It has been pointed out earlier that there are four possible sources of sterols from invertebrates: (a) dietary origin without further chemical modification, (b) dietary origin of sterol(s) followed by chemical modification, (c) result of symbiotic relationship between host and symbiont (*e.g.*, algae, fungi, bacteria), and (d) *de novo* sterol biosynthesis *via* acetate, mevalonate, and squalene.[1,24,30]

The major $\Delta^{5,7}$-sterols may conceivably be of dietary origin since both fungi and some unicellular algae contain $\Delta^{5,7}$-sterols.[31] However, in view of the wide variety of side chains it



Scheme. Possible route of sterol side-chain dealkylation in the sponge *Axinella cannabina*

is more plausible that these sterols and their more highly unsaturated $\Delta^{5,7,9(11)}$-counterparts owe their formation to biochemical dehydrogenation of dietary Δ^5- or Δ^7-sterols by the sponge.[24] Unicellular protozoa such as *Tetrahymena pyriformis* [31] are known to perform such steps.

Whether the Δ^8-sterols are playing a role as biosynthetic intermediates for other sterols as is the case in higher animals ($\Delta^8 \longrightarrow \Delta^7 \longrightarrow \Delta^{5,7} \longrightarrow \Delta^5$) or whether they are only of dietary origin remains an open question. Potential food components, such as some unicellular marine red algae (*Porphyridium* sp.) have been shown to contain Δ^8-sterols (4α-methyl).[32]

The detection of sterols with the (22*E*,24*R*)-24-methyl-27-norcholestane side-chain d in *A. cannabina* indicates that this side chain might also participate in the sterol side-chain biodemethylation to side-chain a (h ⟶ d ⟶ a) as has been proposed for its (24*S*)-epimer c (g ⟶ c ⟶ a) [33] (Scheme).

There are three possible modes of biodemethylation for the formation of the novel (22*E*)-27-norcholestane side-chain b in this organism. One possibility is a one step demethylation, by loss of C-27, from side-chain e; the other two are two-step demethylations, by successive loss of C-27 and C-28 from side-chains g and h *via* c and d, respectively.

The two unusual 5α-methoxy-$\Delta^{6,8(14)}$-sterols (7g and i) may be artifacts, produced during the isolation procedure by reaction of methanol with the corresponding 5α,8α-epidioxysterols, the presence of which has been demonstrated in *A. cannabina*.[34]

Experimental

General.—G.l.c. was performed on a Hewlett Packard 402 A chromatograph equipped with a flame-ionization detector and a glass column (1.8 m × 2 mm i.d.) containing 3% OV-17/GCQ (carrier gas He; temperature 260 °C). Combined g.l.c.-m.s. analysis was performed on a Varian MAT-44 spectrometer system at 70 eV using a 3% OV-17 GCQ column (1.8 m × 2 mm i.d.), and a Ribermag g.l.c.-m.s. system using a fused silica capillary column and an SADR data system. High-resolution mass spectra were recorded on a Varian MAT-711 double-focusing spectrometer equipped with a PDP-11/45 computer. ^{1}H N.m.r. spectra were recorded on a Bruker HXS-360 (360 MHz) spectrometer in $CDCl_3$ solution with $SiMe_4$ as internal standard. Preparative h.p.l.c. was carried out on a Waters Associates HPLC system (M 6000 pump; R 403 differential refractometer), using two different reverse-phase columns: Whatman Partisil M9 10/50 ODS-2 (50 cm × 9 mm i.d.) with absolute methanol as the mobile phase, and Altex Ultrasphere ODS 5 μm (25 cm × 10 mm i.d., two columns in series) with methanol–water (95 : 5) as the eluant. U.v. spectra (ethanol) were recorded on a Cary-14 spectrophotometer.

Extraction, Sterol Isolation, and Fractionation.—The *Axinella cannabina* sponge, collected in the Bay of Taranto (Italy) in 1981, was extracted three times with methanol. The combined extracts were concentrated under reduced pressure and the resulting suspension was extracted several times with diethyl ether. The combined ethereal extracts were taken to dryness and the oily residue was chromatographed on a silica gel column (benzene, then diethyl ether as eluant) to provide the sterol mixture which was fractionated initially by h.p.l.c. on an ODS-2 column. G.l.c.-m.s. analysis of the separated fractions showed that most of them were still complex mixtures. The bulky, less polar fractions (r.r.t. > 0.7 on an ODS-2 column) were fractionated by argentic t.l.c.[35] as the acetate derivatives (pyridine–Ac_2O; room temperature); in those cases where a fraction still consisted of a mixture, fractionation was continued by h.p.l.c. on an Altex column, after hydrolysis (5% KOH in methanol; room temperature). The more polar, small fractions (r.r.t. 0.4—0.7 on an ODS-2 column) which contained the polyunsaturated sterols were fractionated by h.p.l.c. on an Altex column without prior subjection to argentic t.l.c. because some polyunsaturated sterols are suspected of undergoing chemical modification during the t.l.c. separation.

Synthesis of Amuresterol (4c) *from the Triene* (2c) *by Selective Hydrogenation.*—A solution of the sterol (2c) (2 mg) in benzene (2 ml) containing chlorotris(triphenylphosphine)rhodium (6 mg) as catalyst was hydrogenated at atmospheric pressure and room temperature for 13 h. After filtration and evaporation, a solution of the residue in diethyl ether was filtered through Florisil and was then fractionated by h.p.l.c. on an Altex column to afford compound (4c), 30%, and unchanged triene (2c), 70%. All the chromatographic and spectral data of the synthetic compound (4c) were essentially identical with those of naturally occurring amuresterol (4c).

Physical Data of the New Sterols.—For g.l.c. and h.p.l.c. (on an ODS-2 column) r.r.t. values (cholesterol = 1.00) see Table 1. For the 360-MHz ^{1}H n.m.r. spectra of the newly characterized sterols see Table 2. The mass-spectral data [*m*/*z* (assignment, relative intensity)] of the new sterols, the 360-MHz ^{1}H n.m.r. data (*J* in Hz) of three other sterols (2e), (2i), and (4c), and some experimental data are given below. The following sterols were isolated.

(22E)-24-*Norcholesta*-5,7,9(11),22-*tetraen*-3β-*ol* (1a), *m*/*z* 366.290 72 (M^+, 37%. $C_{26}H_{38}O$, requires *M*, 366.292 24), 351.267 62 ($C_{25}H_{35}O$, 2), 348.282 37 ($C_{26}H_{36}$, 37), 333.257 85 ($C_{25}H_{33}$, 12), 277.195 26 ($C_{21}H_{25}$, 5), 269.191 92 ($C_{19}H_{25}O$, 4), 267.175 53 ($C_{19}H_{23}O$, 7), 251. 178 46 ($C_{19}H_{23}$, 100), 249.165 30 ($C_{19}H_{21}$, 18), 237.165 09 ($C_{18}H_{21}$, 12), 227.144 27 ($C_{16}H_{19}O$, 8), 225.164 40 ($C_{17}H_{21}$, 6), 209.134 07 ($C_{16}H_{17}$,21), 197.132 52 ($C_{15}H_{17}$, 17), and 195.117 53 ($C_{15}H_{15}$, 22); $\lambda_{max.}$ 312, 324, and 338 nm.

(22E,24S)-24-*Methyl*-27-*norcholesta*-5,7,9(11),22-*tetraen*-3β-*ol** (1c), *m*/*z* 380.307 69 (M^+, 72%. $C_{27}H_{40}O$ requires *M*, 380.307 89), 365.285 08 ($C_{26}H_{37}O$, 4), 362.298 28 ($C_{27}H_{38}$, 46), 347.274 56 ($C_{26}H_{35}$, 11), 277.194 49 ($C_{11}H_{25}$, 4), 269.187 90 ($C_{19}H_{25}O$, 9), 267.174 13 ($C_{19}H_{23}O$, 11), 251.179 36 ($C_{19}H_{23}$,

* Alternative name: (22E,24S)-27(25 ⟶ 24)abeo-*cholesta*-5,7,9(11),22-*tetraen*-3β-*ol*.

100), 249.164 88 ($C_{19}H_{21}$, 10), 237.163 63 ($C_{18}H_{21}$, 8), 235.147 60 ($C_{18}H_{19}$, 5), 227.143 02 ($C_{16}H_{19}O$, 10), 225.162 09 ($C_{17}H_{21}$, 5), 209.131 19 ($C_{16}H_{17}$, 23), 197.131 35 ($C_{15}H_{17}$, 24), and 195.117 03 ($C_{15}H_{15}$, 17); $\lambda_{max.}$ 312, 324, and 338 nm.

(22E,24R)-24-*Methyl*-27-*norcholesta*-5,7,9(11),22-*tetraen*-3β-*ol* * (1d), *m/z* 380.309 25 (M^+, $C_{27}H_{40}O$, 42%). The fragmentation pattern was essentially the same as that of the epimer (1c).

(22E)-*Cholesta*-5,7,9(11),22-*tetraen*-3β-*ol* (1e), *m/z* 380.306 51 (M^+, $C_{27}H_{40}O$, 27%), 365 (4), 362 (45), 347 (13), 319 (2), 277 (3), 269 (3), 267 (3), 251 (100), 249 (17), 237 (6), 235 (8), 227 (8), 225 (3), 209 (19), 197 (11), and 195 (16).

(22E,24S)-24-*Methylcholesta*-5,7,9(11),22-*tetraen*-3β-*ol* (1g), *m/z* 394.324 13 (M^+, 40% $C_{28}H_{42}O$ requires *M*, 394.323 54), 379 (4), 376 (57), 361 (11), 333 (3), 277 (3), 269 (2), 267 (4), 251 (100), 249 (18), 237 (5), 235 (6), 227 (6), 225 (5), 209 (12), 197 (11), and 195 (15).

24-*Methylenecholesta*-5,7,9(11)-*trien*-3β-*ol* (1i), *m/z* 394.321 96 (M^+, $C_{28}H_{42}O$, 73%), 379 (17), 376 (100), 361 (25), 310 (14), 277 (24), 269 (10), 267 (22), 251 (55), 249 (47), 237 (12), 235 (14), 227 (24), 225 (13), 209 (48), 197 (15), and 195 (35).

(24R)-24-*Methylcholesta*-5,7,9(11)-*trien*-3β-*ol* (1k), *m/z* 396 (M^+, 40%), 381 (11), 378 (100), 363 (36), 279 (7), 269 (3), 265 (3), 255 (31), 251 (67), 237 (18), 227 (29), 215 (17), 209 (68), 197 (51), and 195 (46).

(22E,24S)-24-*Ethylcholesta*-5,7,9(11),22-*tetraen*-3β-*ol* (1n), *m/z* 408.337 70 (M^+, 37%. $C_{29}H_{44}O$ requires *M*, 408.339 19), 393 (5), 390 (42), 375 (9), 347 (2), 227 (4), 269 (8), 267 (11), 251 (100), 249 (16), 237 (9), 235 (9), 227 (12), 225 (8), 209 (27), 197 (21), and 195 (21).

(22E,24R)-24-*Ethylcholesta*-5,7,9(11),22-*tetraen*-3β-*ol* (1o), *m/z* 408.338 33 (M^+, $C_{29}H_{44}O$, 34%). The fragmentation pattern was essentially the same as that of the epimer (1n).

[24(28)Z]-24-*Ethylidenecholesta*-5,7,9(11)-*trien*-3β-*ol* (1p), *m/z* (>200 a.m.u.) 408 (M^+, 22%), 393 (8), 390 (36), 375 (9), 310 (8), 227 (29), 269 (3), 253 (46), 251 (56), 249 (40), 237 (63), 227 (43), 213 (77), and 209 (100). The characterization of this sterol was based upon m.s. and g.l.c. data. The presence of a $\Delta^{5,7,9(11)}$-conjugated double-bond system was deduced from the characteristic fragments for this system at *m/z* 269,251, 227, and 209.[14,15] The fragments at *m/z* 310 (McLafferty rearrangement by cleavage of the C-22–C-23 bond together with a one-hydrogen transfer from C-20) [36] and 277 (loss of methyl and water from ion with *m/z* 310) located the C-24 unsaturation.[13,21] In the absence of sufficient material for an n.m.r. determination of the stereochemistry of the ethylidene functionality, g.l.c. correlation of this sterol with other relevant sterols made it possible to assign the *Z* orientation to the side-chain double bond at C-24(28).[26]

(24ξ)-24-*Ethylcholesta*-5,7,9(11)-*trien*-3β-*ol* (1q/1r), *m/z* (>200 a.m.u.) 410 (M^+, 11%), 395 (4), 392 (57), 377 (12), 279 (4), 269 (2), 251 (75), 237 (17), 227 (28), 215 (17), and 209 (100). The structure of this sterol is based primarily on m.s. and g.l.c. data. It can be deduced from m.s. data that this sterol has a $\Delta^{5,7,9(11)}$-ring system (*m/z* 269, 251, 227, and 209) [14,15] with a saturated C_{10}-side-chain. By taking into consideration the g.l.c. mobility,[26] the title structure can be given tentatively to this sterol.

(22E)-27-*Norcholesta*-5,7,22-*trien*-3β-*ol* (2b), *m/z* 368.308 60 (M^+, 80%. $C_{26}H_{40}O$ requires *M*, 368.307 89), 353.286 20 ($C_{25}H_{37}O$, 5), 350.296 30 ($C_{26}H_{38}$, 13), 335.272 97 ($C_{25}H_{35}$, 81), 309.256 56 ($C_{23}H_{33}$, 38), 271.208 49 ($C_{19}H_{27}O$, 21), 253.196 42 ($C_{19}H_{25}$, 35), 251.182 03 ($C_{19}H_{23}$, 19), 237.163 79 ($C_{18}H_{21}$, 12), 227.177 23, ($C_{17}H_{23}$, 4), 225.163 55 ($C_{17}H_{21}$, 9), 213.125 83 ($C_{15}H_{17}O$, 4), 211.147 84 ($C_{16}H_{19}$, 19), 199.147 28 ($C_{15}H_{19}$, 10), 197.131 61 ($C_{15}H_{17}$, 19), and 55.054 89 (C_4H_7, 100); $\lambda_{max.}$ 262, 271, 281, and 293 nm.

(22E,24S)-24-*Methyl*-27-*norcholesta*-5,7,22-*trien*-3β-*ol* † (2c), *m/z* 382.324 65 (M^+, 100%. $C_{27}H_{42}O$ requires *M*, 382.323 54), 367.297 69 ($C_{26}H_{39}O$, 1), 364.311 15 ($C_{27}H_{40}$, 15), 349.286 73 ($C_{26}H_{37}$, 62), 323.272 13 ($C_{24}H_{35}$, 29), 271.206 32 ($C_{19}H_{27}O$, 15), 253.197 16 ($C_{19}H_{25}$, 38), 251.181 08 ($C_{19}H_{23}$, 4), 239.179 25 ($C_{18}H_{23}$, 8), 237.164 51 ($C_{18}H_{21}$, 5), 227.179 79 ($C_{17}H_{23}$, 6), 211.149 09 ($C_{18}H_{19}$, 13), 199.149 62 ($C_{15}H_{19}$, 9), and 197.132 99 ($C_{15}H_{17}$, 11); $\lambda_{max.}$ 262, 271, 281, and 293 nm.

(22E,24R)-24-*Methyl*-27-*norcholesta*-5,7,22-*trien*-3β-*ol* ‡ (2d), *m/z* 382.322 83 (M^+, $C_{27}H_{42}O$, 100%). The fragmentation pattern was essentially identical with that of the epimer (2c).

(22R,23R)-22,23-*Methylenecholesta*-5,7-*dien*-3β-*ol* (2j), *m/z* 396.337 21 (M^+, 100%. $C_{28}H_{44}O$ requires *M*, 396.339 19), 381 (2), 378 (11), 363 (50), 337 (16), 312 (2), 294 (5), 271 (6), 253 (14), 251 (4), 239 (3), 237 (3), 225 (4), 217 (4), 211 (10), 199 (12), and 197 (8).

(22E)-24-*Nor*-5α-*cholesta*-8,22-*dien*-3β-*ol* (3a), *m/z* 370 (M^+, 100%), 335 (59), 352 (3), 337 (10), 327 (5), 273 (38), 271 (49), 257 (24), 255 (26), 246 (34), 229 (32), and 213 (15). The fragment at *m/z* 246 indicated $\Delta^{7\text{-}13,25}$ or $\Delta^{8\text{-}25}$ unsaturation, whereas the two fragments at *m/z* 273 and 271 (loss of side chain without and with 2 H transfer) suggested the presence of a mono-unsaturated C_7-side-chain.[13,21] Taking into account the g.l.c. correlation with other sterols in Table 1, the title structure can be attributed to this sterol.

(22E,24S)-24-*Methyl*-27-*nor*-5α-*cholesta*-8,22-*dien*-3β-*ol* § (3c), *m/z* 384.341 68 (M^+, 100%. $C_{27}H_{44}O$ requires *M*, 384.339 19), 369.313 13 ($C_{26}H_{41}O$, 33), 336.324 89 ($C_{27}H_{42}$, 4), 351.301 29 ($C_{26}H_{39}$, 3), 299.237 36 ($C_{21}H_{31}O$, 5), 273.222 30 ($C_{19}H_{29}O$, 50), 271.206 07 ($C_{19}H_{27}O$, 40), 257.290 95 ($C_{18}H_{25}O$, 19), 255.212 35 ($C_{19}H_{27}$, 21), 246.198 79 ($C_{17}H_{26}O$, 44), 229.196 16 ($C_{17}H_{25}$, 36), and 213.165 81 ($C_{16}H_{21}$, 11).

(22E,24S)-24-*Methyl*-5α-*cholesta*-8,22-*dien*-3β-*ol* (3 g), *m/z* 398.355 36 (M^+, 100% $C_{28}H_{46}O$ requires *M*, 398.354 84), 383 (24), 380 (2), 265 (2), 355 (4), 299 (4), 273 (51), 271 (30), 257 (9), 255 (16), 246 (29), 229 (27), and 213 (6).

(22E,24S)-24-*Ethyl*-5α-*cholesta*-8,22-*dien*-3β-*ol* (3n), *m/z* 412.368 96 (M^+, 100% $C_{29}H_{48}O$ requires *M*, 412.370 49), 397 (25), 394 (4), 379 (5), 369 (7), 314 (5), 299 (5), 273 (46), 271 (31), 257 (14), 255 (19), 246 (35), 229 (36), and 213 (11).

(22E,24R)-24-*Ethyl*-5α-*cholesta*-8,22-*dien*-3β-*ol* (3o), *m/z* 412.372 90 (M^+, $C_{29}H_{48}O$, 100%). The fragmentation pattern was essentially identical with that of the epimer (3n).

(24R)- (3q) *and* (24S)-24-*Ethyl*-5α-*cholest*-8-*en*-3β-*ol* (3r) (mixture), *m/z* 414.386 70 (M^+, 100%. $C_{29}H_{50}O$ requires *M*, 414.386 14), 399 (21), 396 (2), 273 (10), 255 (6), 246 (8), 231 (6), 229 (9), and 213 (6).

(22E,24S)-5α-*Methoxy*-24-*methylcholesta*-6,8(14),22-*trien*-3β-*ol* (7g), *m/z* 426.341 79 (M^+, 1%. $C_{29}H_{46}O_2$ requires *M*, 426.349 75), 394.323 25 ($C_{28}H_{42}O$, 47), 379.303 95 ($C_{27}H_{39}O$, 1), 376.315 16 ($C_{28}H_{40}$, 100), 361.290 00 ($C_{27}H_{37}$, 20), 333.257 47 ($C_{25}H_{33}$, 3), 324.279 97 ($C_{24}H_{36}$, 1), 314.260 00 ($C_{22}H_{34}O$, 2), 269.190 41 ($C_{19}H_{25}O$, 8), 268.182 01 ($C_{19}H_{24}O$, 12), 251.180 08 ($C_{19}H_{23}$, 32), 235.150 71 ($C_{18}H_{19}$, 11), 209.133 47 ($C_{16}H_{17}$, 12), and 197.132 22 ($C_{15}H_{17}$, 15); $\lambda_{max.}$ 256 nm.

(22*E*)-Cholesta-5,7,22-trien-3β-ol (2e), δ 0.631 (3 H, s, 18-H_3), 0.863 (3 H, d, *J* 6.5, 26- or 27-H_3), 0.866 (3 H, d, *J* 6.8, 27- or 26-H_3), 0.945 (3 H, s, 19-H_3), 1.033 (3 H, d, *J* 6.6, 21-H_3), and 5.26 (2 H, m, 22- and 23-H).

* Alternative name: (22E,24R)-27(25 ⟶ 24)abeo-*cholesta*-5,7,9(11),22-*tetraen*-3β-*ol*.

† Alternative name: (22E,24S)-27(25 ⟶ 24)abeo-*cholesta*-5,7,22-*trien*-3β-*ol*.

‡ Alternative name: (22E,24R)-27(25 ⟶ 24)abeo-*cholesta*-5,7,22-*trien*-3β-*ol*.

§ Alternative name: (22E,24S)-27(25 ⟶ 24)abeo-5α-*cholesta*-8,22-*dien*-3β-*ol*.

24-Methylenecholesta-5,7-dien-3β-ol (2i), δ 0.622 (3 H, s, 18-H_3), 0.943 (3 H, s, 19-H_3), 0.973 (3 H, d, *J* 6.5, 21-H_3), 1.023 (3 H, d, *J* 6.9, 26- or 27-H_3), 1.029 (3 H, d, *J* 6.8, 27- or or 26-H_3), and 4.661 and 4.719 (each 1 H, s, 28-H_2).

(22*E*,24*S*)-24-Methyl-27-nor-5α-cholesta-7,22-dien-3β-ol * (4c) (amuresterol), δ 0.543 (3 H, s, 18-H_3), 0.796 (3 H, s, 19-H_3), 0.834 (3 H, t, *J* 7.1, 26-H_3), 0.928 (3 H, d, *J* 6.7, 28-H_3), 1.007 (3 H, d, *J* 6.6, 21-H_3), 3.60 (1 H, m, 3-H_α), and 5.15 total (3 H, m, 7-, 22-, and 23-H).

The penta-unsaturated sterol A, *m/z* (>200 a.m.u.) 378 (M^+, 18%), 363 (3), 360 (5), 267 (20), 266 (25), 255 (11), 253 (30), 249 (100), 248 (51), 247 (12), 235 (32), 233 (34), 219 (24), 209 (47), and 207 (45); r.r.t. (h.p.l.c.) 0.47, (g.l.c.) 1.14.

The penta-unsaturated sterol B, *m/z* (>200 a.m.u.), 392 (M^+, 43), 377 (7), 374 (8), 359 (2), 331 (8), 303 (4), 275 (5), 267 (24), 266 (67), 265 (10), 253 (30), 249 (100), 248 (70), 247 (15), 233 (54), 219 (38), and 207 (41); r.r.t. (h.p.l.c.) 0.55, (g.l.c.) 1.40.

The penta-unsaturated sterol C, *m/z* (>200 a.m.u.) 406 (M^+, 23), 391 (3), 388 (4), 345 (3), 330 (10), 303 (6), 301 (8), 275 (4), 267 (15), 266 (36), 265 (12), 253 (11), 249 (100), 248 (40), 247 (13), 233 (24), 219 (15), and 207 (21); r.r.t. (h.p.l.c.) 0.59, (g.l.c.) 1.71.

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* Alternative name: (22*E*,24S)-26(25 ⟶ 24)*abeo*-5α-cholesta-7,22-dien-3β-ol. Side-chain numbering system is that shown in the Scheme.

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