208. Ergosta-5,7,9(11),22-tetraen-3β-ol and its 24ξ-Ethyl Homolog, Two New Marine Sterols from the Red Sea Sponge *Biemna fortis*[1])

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Summary

The steroidal components of a Red Sea sponge, *Biemna fortis*, were fractionated through reversed phase HPLC. and analyzed by a combination of physical methods, including high resolution GC./MS. and 360 MHz ^{1}H-NMR. The sponge contains five conventional Δ^5-sterols, **1a-c**, **1e**, **1g**, which comprise about 25% of the mixture and 2,5% of gorgosterol (**1h**), a sterol never found before in Porifera. Three $\Delta^{5,7,22}$-sterols were also present as major components in the mixture (~70%): cholesta-5,7,22-trien-3β-ol (**2a**), ergosta-5,7,22-trien-3β-ol (**2c**) and (24R)-ethylcholesta-5,7,22-trien-3β-ol (**2e**) whereas two new tetra-unsaturated sterols were identified in minor amounts (2%): ergosta-5,7,9(11),22-tetraen-3β-ol (**3c**) and 24ξ-ethylcholesta-5,7,9(11),22-tetraen-3β-ol (**3e** or **3f**). NMR. spectroscopy made possible the assignment of a 24R configuration for all the C(24) substituted sterols isolated in sufficient amount from the mixture. The possible symbiotic, dietary or biosynthetic origins of these sterols are discussed.

Introduction. – Marine organisms such as sponges constitute a rich source of sterols possessing unusual side-chains or unconventional ring systems [2–6]. Besides their chemical structure elucidation such sterols raise interesting questions in terms of biosynthesis and biological function [7]. Against this background and in our continuing search for key biosynthetic intermediates among the minor and trace components of marine sterol mixtures [1], we report the composition of the Red Sea sponge *Biemna fortis*.

Results. – The gas-liquid chromatographic (GC.) and combined gas chromatographic-mass spectrometric (GC./MS.) analyses of the free sterol mixture allow the resolution of the number, the identity, and the amount of the components. These data are corroborated by the GC./MS. analysis on capillary columns of the trimethylsilyl ethers of the mixture. Further separations of the free sterols by high

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[1]) Part XIV in the Stanford series 'Minor and Trace Sterols in Marine Invertebrates'. Part XIII: [1].

Table 1. *Sterols identified in* Biemna fortis

Relative GC. retention time[a])	M^+	Structure	Percent in the sterol mixture[b])	Enriched HPLC. fraction no.
0.67	376	**4c** or **5c** or **6c**	0.1	–
0.73	390	**4e** or **5e** or **6e**	0.1	–
0.95	384	**1a**	1.2	4
1.00	386	**1b**	0.3	8
1.10	382	**2a**	~ 1 ⎱ mixt. 16.3	3
1.14	398	**1c**	~ 15 ⎰	6
1.35	394	**3c**	⩽ 1 ⎱	1
1.38	396	**2c**	~ 37 ⎬ mixt. 48.2	5
1.42	412	**1e**	~ 10 ⎰	9
1.60	414	**1g**	⩽ 0.2 ⎱	10
1.68	408	**3e** (or **3f**)	⩽ 1 ⎬ mixt. 31.2	2
1.72	410	**2e**	~ 30 ⎰	7
2.21	426	**1h**	2.5	11

[a]) Retention time (relative to cholesterol = 1.00) measured using OV 25-3% on Gas Chrom Q at 265°.

[b]) The averaged percentage for three different collections is listed and estimated from the GC. peaks of the mixtures before and after HPLC. fractionation. The relative proportion of the sterols varies only slightly from one collection to another. However, one of the sterol mixture contained more of **1c** (22%) than the two others (13%).

pressure liquid chromatography (HPLC.) and subsequent GC. and GC./MS. analyses of the fractions made possible the identification of the sterols listed in the *Table 1*, with the specific structures reproduced in the *Figure*.
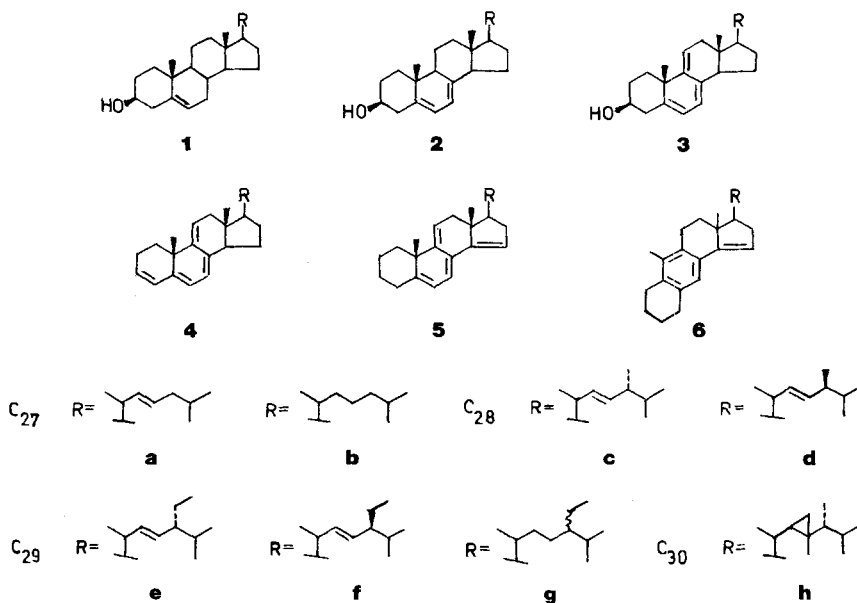

Figure. *Structure of the sterols*

The identification of known sterols was based on direct comparison of relative GC. retention times (including coinjection) and mass spectra (GC./MS.) with those of authentic samples. As far as possible, the sterols with a C(24) substituent were isolated from the crude mixture and purified by repeated HPLC. Their C(24) configurations were assigned by comparison of the 360 MHz proton nuclear magnetic resonance (NMR.) spectra with those of authentic stereochemically defined sterols.

Six Δ^5-3β-hydroxy-sterols, accounting for about 30% of the sterol mixture, were identified: cholesta-5,22-dien-3β-ol (1a), cholesterol (1b), (22E)-ergosta-5,22-dien-3β-ol (brassicasterol) (1c), (22E,24R)-24-ethylcholesta-5,22-dien-3β-ol (poriferasterol) (1e), 24ξ-ethylcholest-5-en-3β-ol (1g) and (22R,23R,24R)-22,23-methylene-23,24-dimethylcholest-5-en-3β-ol (gorgosterol) (1h). The two mono-unsaturated sterols 1b and 1g were detected in small amounts (less than 0,5% of the mixture) but are widely distributed among marine organisms [3]. The low concentration of 1g prevented its isolation and the attainment of an NMR. spectrum necessary for a secure stereochemical assignment. The occurrence in the mixture (2.5%) of the third mono-unsaturated sterol, gorgosterol 1h, is more intriguing. We found 1h in three separate collections of *Biemna fortis* and its ^{1}H-NMR. spectrum is identical with that of the authentic compound [8]. The presence of this sterol is reported in soft corals [3] [5] and in marine sediments [9] but to our knowledge this is its first identification in a sponge.

The di-unsaturated sterols 1a, 1c and 1e are widely distributed in the marine environment [3] and amount to more than 25% of the mixture. The first sterol (1a) has a slightly longer GC. retention time than 27-nor-24ξ-methylcholest-22-en-3β-ol [10]. The 360 MHz proton NMR. spectrum of the isolated 1c supports the proposed structure and configuration. Methyl signals are observed at 0.693 (s, H$_3$C(18)), 1.011 (s, H$_3$C(19)), 1.012 (d, J = 6.5 Hz, H$_3$C(21)), 0.833 (d, J = 6.5 Hz, H$_3$C(26)), 0.819 (d, J = 6.5 Hz, H$_3$C(27)) and 0.911 (d, J = 6.8 Hz, H$_3$C(28)). An authentic sample of brassicasterol 1c yielded the same ^{1}H-NMR. properties similar to those reported in [11] [12] and [13]²). Assignment for the C(24)R configuration is based on the chemical shift of the H$_3$C(21) signal shielded to 1.001 ppm in the case of the 24-S epimer [11] [12]. The 360 MHz proton NMR. spectrum of the isolated sterol 1e is identical to published poriferasterol spectra [12] [13]. Of particular importance for the determination of the C(24)R configuration is the chemical shift of H$_3$C(29) at 0.810 (t, J = 7.2 Hz, [13]: 0.809 ppm). This signal is moved upfield to about 0.800 ppm in the spectrum of the 24S epimer stigmasterol (1f) giving rise to a slightly different pattern [12] [13].

Ultraviolet (UV.) analysis of the total sterol mixture revealed maxima at 262, 270, 281, 293 nm typical of $\Delta^{5,7}$-sterols, their extinction coefficients indicated that they amount to about 70% of the total sterols present. Three separate $\Delta^{5,7,22}$-sterols were identified, the first one being cholesta-5,7,22-trien-3β-ol (2a)

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²) The chemical shifts reported here and in [11–13] agree within experimental error except for H$_3$C(21) of brassicasterol and its acetate reported in [12] (1.001 and 1.003 ppm respectively). From [11], [13] and the present work it seems that these values have to be corrected by at least + 0.010 ppm. A closer look at the *Figure 5b* in [12] supports this conclusion.

encountered previously in other sponges [14–17]. The major component ($\sim 37\%$) of *B.fortis* was $(22E)$-ergosta-5,7,22-trien-3β-ol (ergosterol) (**2c**), one of the most widely distributed $\Delta^{5,7}$-sterols in marine organisms, notably the molluscs [3]. It has also been encountered in other sponges [14–17] but in these studies, as in most others, the C(24) configuration is not specified since the analytical techniques employed, usually GC. and MS., are not sufficient for the differentiation. On the other hand, the ^{1}H-NMR. analysis affords a secure assignment [18] and in the 360 MHz spectrum of the isolated **2c**, methyl signals occur at: 0.629 (s, H$_3$C(18)), 0.944 (s, H$_3$C(19)), 1.034 (d, $J=6.5$ Hz, H$_3$C(21)), 0.820 (d, $J=6.2$ Hz, H$_3$C(26 or 27)), 0.837 (d, $J=6.2$ Hz, H$_3$C(26 or 27)), and 0.915 (d, $J=7$ Hz, H$_3$C(28)). The attribution for the C(24)R configuration of **2c** is based on the chemical shift of H$_3$C(21) shielded to 1.024 ppm in the case of 24-*epi*-ergosteryl acetate (**2d**) [18][3]).

The third tri-unsaturated sterol of *B.fortis* was $(22E,24R)$-24-ethylcholesta-5,7,22-trien-3β-ol (**2e**). This component, again without specification of the C(24) configuration, has been reported to occur in other sponges [14–16]. As previously, high frequency ^{1}H-NMR. spectroscopy can be used for structural assignment. However, as far as we know, the spectrum of neither **2e** nor of its epimer **2f** has been published and the assignment has to be done by comparison with spectra of similar sterols. These data are collected in *Table 2*. For the isolated **2e**, methyl signals resonate at: 0.636 (s, H$_3$C(18)), 0.948 (s, H$_3$C(19)), 1.049 (d, $J=6.6$ Hz, H$_3$C(21)), 0.796 (d, $J=6.3$ Hz, H$_3$C(26 or 27)), 0.848 (d, $J=6.3$ Hz, H$_3$C(26 or 27)), and 0.818 (d, $J=7.2$ Hz, H$_3$C(29)). The difference between coupling constants in the H$_3$C(26) or H$_3$(27) and H$_3$C(29) signals [12][13] facilitates the assignments. Examination of the data in *Table 2* led to the following conclusions: a) the chemical shift differences between sterols and their corresponding acetates are within experimental error for H$_3$C(26), H$_3$C(27) and H$_3$C(29) (see also [12][13]); b) the chemical shift differences between H$_3$C(26) and H$_3$C(27) are independent of the C(24) stereochemistry: $\Delta_1 = -0.050\pm0.002$ ppm; c) by contrast the chemical shift differences between H$_3$C(26) and H$_3$C(29) are sensitive to the C(24) configuration: $\Delta_2 = -0.032\pm0.002$ ppm for 24R and $\Delta_2 = -0.043\pm0.002$ ppm for 24S-sterols; this last observation is valid for the chemical shift differences between H$_3$C(27) and H$_3$C(29): $\Delta_3 = +0.018\pm0.002$ ppm for 24R and $\Delta_3 = +0.006\pm0.003$ ppm for 24S-compounds. In the case of the sterol **2e** of *B.fortis* the parameters are $\Delta_1 = -0.052$ ppm, $\Delta_2 = -0.030$ ppm, $\Delta_3 = +0.022$ ppm suggesting a 24R configuration.

The UV. spectrum of the crude sterol mixture exhibited also small absorption near 310, 324 (maximum) and 340 nm. These data suggested the presence of $\Delta^{5,7,9(11)}$- or $\Delta^{5,7,14}$-sterols in the mixture [19]. Their small extinction coefficients indicated that they amount to 1–2% of the total sterols present. GC./MS. analysis of the sterol mixture enabled the detection of two of the components. The first was identified on the basis of its spectroscopic data as $(22E)$-ergosta-5,7,9(11),22-

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3) This value is corrected by -0.005 ppm, a systematic difference observed between [12], our experimental data and [18]. Chemical shift differences between sterols and their corresponding acetates are within experimental error for H$_3$C(21) [12][13].

Table 2. ^{1}*H-NMR. chemical shifts of* $H_3C(26)$, $H_3C(27)$ *and* $H_3C(29)$ *methyl groups in* Δ^{22}*-24-ethyl-steroids (ppm/TMS, [12] [13])*[a].

Steroid	Δ_2
	$H_3C(26) \xleftrightarrow{\Delta_1} H_3C(27) \xleftarrow{\Delta_3} H_3C(29)$
(22E,24R)-24-ethylcholesta-5,22-dien-3β-ol	-0.032
	$0.841 \xleftarrow{-0.050} 0.791 \xleftarrow{0.018} 0.809$
(22E,24S)-24-ethylcholesta-5,22-dien-3β-ol	-0.041
	$0.845 \xleftarrow{-0.050} 0.795 \xrightarrow{0.009} 0.804$
(22E,24R)-24-ethylcholesta-5,22-dien-3β-yl acetate	-0.034
	$0.842 \xleftarrow{-0.051} 0.791 \xleftarrow{0.017} 0.808$
(22E,24S)-24-ethylcholesta-5,22-dien-3β-yl acetate	-0.043
	$0.842 \xleftarrow{-0.051} 0.791 \xrightarrow{0.008} 0.799$
(22E,24R)-24-ethylcholesta-7,22-dien-3β-yl acetate	-0.030
	$0.850 \xleftarrow{-0.050} 0.800 \xrightarrow{0.020} 0.820$
(22E,24S)-24-ethylcholesta-7,22-dien-3β-yl acetate	-0.045
	$0.853 \xleftarrow{-0.048} 0.805 \xrightarrow{0.003} 0.808$
(22E,24ξ)-24-ethylcholesta-5,7,22-trien-3β-ol isolated from *B. fortis*	-0.030
	$0.848 \xleftarrow{-0.052} 0.796 \xleftarrow{0.022} 0.818$

[a] A negative sign of the chemical shift differences Δ between methyl groups indicates a deshielding.

tetraen-3β-ol (3c). The high resolution mass spectrum ($M^+ = C_{28}H_{42}O$) displayed fragments characteristic of a sterol with a tri-unsaturated nucleus and an unsaturated side chain [20] [21] corresponding to $C_{19}H_{25}O^+$, $C_{19}H_{23}O^+$ (loss of side chain without and with 2 H transfer respectively), $C_{19}H_{23}^+$, $C_{19}H_{21}^+$ (loss of side chain and water without and with 2 H transfer), $C_{16}H_{19}O^+$ and $C_{16}H_{17}^+$ (loss of D ring and ring D plus water respectively). The two last ions eliminated the possibility of Δ^{14} unsaturation, while the presence of a Δ^{22} double bond could be deduced from the existence of the ion $C_{25}H_{33}^+$ (loss of the terminal isopropyl group and water) [20]. The 360 MHz ^{1}H-NMR. spectrum of this sterol revealed 5 olefinic protons: the ring olefinic ones (3 H) are deshielded to 5.655, 5.496 and 5.385 ppm indicating a conjugated system; a second order olefinic signal (2 H) centered at 5.182 ppm confirms the presence of the Δ^{22} side-chain double bond. The chemical shift of $H_3C(18)$ and $H_3C(19)$, 0.562 and 1.227 ppm respectively, are typical of $\Delta^{5,7,9(11)}$ unsaturation [22]. Other methyl signals are observed at 1.001 (d, $J = 6.5$ Hz, $H_3C(21)$), 0.805 (d, $J = 6.0$ Hz, $H_3C(26$ or 27)), 0.822 (d, $J = 6.0$ Hz, $H_3C(26$ or 27)) and 0.899 (d, $J = 7.0$ Hz, $H_3C(28)$), these assignments being based on the analogy with the spectrum of ergosterol.

Although the above spectral data require the structure c or d for the side-chain, they do not allow differentiation between them. For the confirmation of the structure of this new marine sterol and for the unambiguous determination of its (24R) configuration, an authentic sample of (22E)-ergosta-5,7,9(11),22-tetraen-3β-ol was prepared by mercuric acetate dehydrogenation of ergosterol [23] [24]. This compound had identical GC. retention time, mass and NMR. spectral properties to those of the natural component 3c isolated from *B. fortis*.

The second tetra-unsaturated sterol present in the mixture was identified as 24ξ-ethylcholesta-5,7,9(11),22-tetraen-3β-ol (**3e** or **3f**) on the basis of the similarity of its mass spectrum (GC./MS.) to that of **3c** discussed above. Diagnostic fragments occur at $m/z = 408$ (M^+), 393 (loss of methyl), 390 (loss of water), 375 (loss of methyl and water), 347 (loss of the terminal isopropyl group and water), the same fragmentation as **3c** being observed at lower m/z units. This sterol was isolated by HPLC. but decomposed prior to ^{1}H-NMR. analysis. Its C(24) stereochemistry could therefore not be assigned, but between **3e** and **3f**, the former structure is more credible, since, in the mixture, all the other sterols with this type of side-chain (**1e**, **2e**) have a (24R) configuration.

Two low molecular weight penta-unsaturated olefins with shorter GC. retention time than cholesterol were also detected in the mixture. The MS. fragmentation of these components (GC./MS.; $M^+ = 376$ and 390) are essentially the same as those of **3c** and **3e** (or **3f**) except that no fragment due to loss of water was observed. Evidence supporting the absence of a hydroxyl group, is that the treatment of the crude sterol mixture with a trimethylsilyl reagent did not affect these components as shown by GC./MS. analysis. Pentaenes like **4c**, **5c**, **6c** (see *Figure*) are formed in yields of about 30% by action of a trace of hydrogen chloride on dehydro-ergosterol **3c** in chloroform solution at RT. [25] [26]. Solely based on their MS., the olefins found in *B.fortis* could be such pentaenes: **4c** or **5c** or **6c** for the one with $M^+ = 376$ and **4e** or **5e** or **6e** for the other with $M^+ = 390$. Further identification was not attempted because these components are probably artifacts arising from dehydration and (or) rearrangement of **3c** and **3e** during separation and storage of the sterols. This hypothesis is supported by the fact that the amount of these derivatives in the sterol mixture was not only small but variable for three different collections of the sponge (total of 0.01, 0.05 and 0.2%) and increased slightly upon storage of the crystalline mixture during 2 months.

Discussion. – The sponge *Biemna fortis* is a member of the order *Poecilo-sclerida* and of the family *Biemnidae* (*Hentschel*, 1923) within the class *Desmo-spongia*. As far as we know this is the first study on the sterols of sponges belonging to this family whereas pioneering work only has been reported on six other species of this order in which conventional C_{27}, C_{28}, C_{29} 5α-stanols, Δ^7 and Δ^5-sterols were found [3] [14]. Recently, reports on $\Delta^{5,7}$ sterols in sponges have appeared [14] [17]. They are the principal components of several *Spongidae* and this criterion has been initially proposed for isolating this family taxonomically [14]. It is obviously not possible to generalize too much in this direction since more recently [15–17], as in the present study of *B.fortis*, sponges of other families and other orders were also shown to contain major amounts of $\Delta^{5,7}$ sterols. The occurrence of such components certainly warrants further investigation since they are vitamin D precursors and may perhaps play a role in calcium metabolism as has been proposed for other marine organisms like molluscs [3] [19] [27]. The side-chain configuration of the C(24) substituted $\Delta^{5,7}$-sterols detected previously in sponges has never been specified [14–16]. This information is however of prime importance to study either the origins of the sterols in marine organisms or the possible modes of their biosynthesis [7]. The present work shows that the use of high frequency ^{1}H-NMR.

spectroscopy makes the assignment possible. Thus, in *B. fortis,* all the C(24) alkylated sterols isolated in sufficient amount for an NMR. analysis exhibited a (24R) configuration.

The origins of the sterols in sponges are difficult to determine. They may arise from the sponge tissues, from dietary sources or from endosymbionts inhabiting the tissues of sponges, but the conventional methods of lipid extraction and analyses do not allow differentiation. As an illustration, *Spongia officinalis* has recently been reported [28] to contain exclusively Δ^5-sterols, a finding which is in contradiction to the earlier report [14] which states that $\Delta^{5,7}$-sterols predominate in that sponge. Porifera often house large numbers of symbiotic organisms such as fungi and algae in their tissues and cavities [29]. These organisms contain $\Delta^{5,7}$-sterols [3] and could be the origin of their occurrence in *B. fortis.* Their biosynthesis by the sponge or a dietary source is, however, not impossible. The same possibilities of biosynthetic, dietary or symbiotic origin appear for gorgosterol (**1h**) identified here for the first time in a sponge. On the one hand, the sterol mixture contains brassicasterol (**1c**), which possesses the same (24R) configuration, and has been proposed as its biogenetic precursor [7]. On the other hand, gorgosterol (**1h**) is more typical of soft corals [3] [5] and *Ciereszko et al.* [30] have even shown that it occurs in the zooxanthellae rather than the soft coral hosts with which these symbionts live. Although contamination of *B. fortis* by soft corals is not observed (they do not exist in the collection area) a symbiosis by other organisms carrying gorgosterol, or a diet of them by the sponge, must be considered. Although $\Delta^{5,7,9(11),22}$-sterols like **3c** and **3e** have often been synthesized in relation with vitamin D problems [19], they have never been identified as natural products. By contrast the correponding $5a,6a$-epidioxy-$\Delta^{6,9(11),22}$-sterols have recently been found in the sponge *Aplysinopsis* sp. [31]. The question whether such peroxides play an intermediate role in the biosynthesis of $\Delta^{5,7}$-sterols as discussed by *Tophan & Gaylor* in yeast [32] or whether they are converted from $\Delta^{5,7}$-sterols, as in fungi [33], has not been resolved. As already mentioned those tetra-unsaturated sterols are unstable in chloroform solution in the presence of traces of acid [25] [26] and decompose into several types of penta-unsaturated olefins *(Figure).* Such components are found in minor amounts in the sterol mixture of *Biemna fortis* but are probably artifacts.

Experimental Part

Three samples of a black sponge were collected during 1976 and 1977 in the Gulf of Eilat near Taba and in the Fijord (south of Eilat), Red Sea, Israel. This sponge was identified by Dr. *Zur-Namal,* Hebrew University, Jerusalem, as *Biemna fortis.* After removal from the sea the sponge was immediately frozen in dry ice then dried by freeze-drying and extracted (Soxhlet) with petroleum ether. Filtration through a silica gel column gave the sterol fraction.

Sterol characterization. GC. was performed using a *Hewlett Packard* 402A chromatograph equipped with a flame ionization detector; 1.80 m × 4 mm I.D. 'U'-shaped glass column containing 3% OV-25 on gas Chrom Q (*Applied Sci.* Inc.); temperature: 265°; carrier gas: He (100 ml/min). Combined GC./MS. analyses were performed on a *Varian* MAT 44 quadrupole spectrometer system with an electron ionization energy of 70 eV using, for the free sterols, a coiled GC. column (1,80 m × 2 mm I.D.) containing 3% SP-2250 on Supelcoport 100/120 (*Supelco* Inc.), temperature: 270° and, for the TMS derivatives, a coiled GC. capillary column (30 m) containing OV 101, temperature: 270°. Trimethyl-silyl ethers were prepared by warming for 15 min at 70° 5 mg of free sterol mixture with 0.5 ml of *N,O*-bis-(trimethylsilyl)-acetamide followed by evaporation of excess reagent. The high resolution mass spectra were recorded on a *Varian* MAT 711 system using the conditions reported [34]. The UV. spectra (MeOH) were recorded on a *Cary* 14 spectrophotometer and the optical rotations (CHCl$_3$) on a *Perkin Elmer* 241 polarimeter. 360 MHz ^{1}H-NMR. spectra (CDCl$_3$, TMS as internal reference, *J*Hz) were measured on a *Bruker* HXS 360 spectrometer at the Stanford Magnetic Resonance Laboratory. Melting points (m.p.) are uncorrected.

Sterol fractionation. Reversed phase HPLC. [34] on a Partisil M9 10/50 ODS-2 column (*Whatman* Inc.), 50 cm × 8 mm O.D., was used at a pressure of 1200 psi. with absolute methanol as the mobile phase and sample loading of 15 mg sterol mixture in 5 ml of methanol. Three separations were made, 11 fractions were collected each time and evaporated under vacuum at RT. before further analysis.

(22E)-ergosta-5,7,9(11),22-tetraen-3β-ol (**3c**). A mixture of 6.5 g of mercuric acetate, 3.2 g of ergosterol, 75 ml of CHCl$_3$ and 120 ml of acetic acid was stirred vigorously for 20 h at RT. The filtered solution was washed free of acetic acid (H$_2$O with NaHCO$_3$, H$_2$O), dried and the solvent evaporated. After recrystallization of the residue from methanol, 1.25 g of **3c** was obtained as plates, m.p. 142–146°. Further purification by HPLC. gave a sample, m.p. 145–147° and $[a]_D^{20} = +147°$ ([23]: 147° and 149° respectively), sensitive to air and light exposure and which decomposes in CHCl$_3$ solution [23]. GC. relative retention time: 1.35 (OV25-3%, 265°). – UV.: max λ nm (ε): 311 (10,200), 324 (11,800), 339 (7400). – ^{1}H-NMR.: 5,655 (*m*, 1 H, olefinic ring H); 5,496 (*m*, 1 H, olefinic ring H); 5,385 (*m*, 1 H, olefinic ring H); 5,182 (*m*, 2 H, HC(22) and HC(23)); 3,600 (*m*, 1 H, HC(3)); 1,227 (*s*, 3 H, H$_3$C(19)); 1,001 (*d, J* = 6.5, 3 H, H$_3$C(21)); 0.899 (*d, J* = 7.0, 3 H, H$_3$C(28)); 0.822 (*d, J* = 6.0, 3 H, H$_3$C(26) or H$_3$C(27)); 0.805 (*d, J* = 6.0, 3 H, H$_3$C(26) or H$_3$C(27)); 0.562 (*s*, 3 H, H$_3$C(18)). – MS. characteristic fragmentations: 394.32216 (44, C$_{28}$H$_{42}$O$^+$); 379.30243 (4, C$_{27}$H$_{39}$O$^+$); 376.31346 (51, C$_{28}$H$_{40}{}^+$); 361.29020 (6, C$_{27}$H$_{37}{}^+$); 333.25666 (3, C$_{25}$H$_{33}{}^+$); 269.18866 (13, C$_{19}$H$_{25}$O$^+$); 267.171616 (4, C$_{19}$H$_{23}$O$^+$); 251.18092 (100, C$_{19}$H$_{23}{}^+$); 249.16433 (15, C$_{19}$H$_{21}{}^+$); 227.14418 (10, C$_{16}$H$_{19}$O$^+$); 225.16413 (6, C$_{17}$H$_{21}{}^+$); 225.12738 (4, C$_{16}$H$_{17}$O$^+$); 224.15693 (6, C$_{17}$H$_{20}{}^+$); 223.14827 (8, C$_{17}$H$_{19}{}^+$); 209.13220 (20, C$_{16}$H$_{17}{}^+$); 207.11635 (6, C$_{16}$H$_{15}{}^+$); 197.13122 (12, C$_{15}$H$_{17}{}^+$); 195.11577 (17, C$_{15}$H$_{15}{}^+$); 169.10160 (7, C$_{13}$H$_{13}{}^+$); 157.10175 (7, C$_{12}$H$_{13}{}^+$); 155.08711 (7, C$_{12}$H$_{11}{}^+$); 143.08539 (6, C$_{11}$H$_{11}{}^+$); 128.06186 (3, C$_{10}$H$_8{}^+$); 125.13334 (8, C$_9$H$_{17}{}^+$); 83.08630 (13, C$_6$H$_{11}{}^+$); 81.07093 (19, C$_6$H$_9{}^+$); 69.07058 (81, C$_5$H$_9{}^+$).

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