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Short Report

C-24 Stereochemistry of Marine Sterols: (22*E*)-24-Ethyl-24-methylcholesta-5,22-dien-3β-ol and 24-Ethyl-24-methylcholest-5-en-3β-ol

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As configurações dos carbonos C-24 nos compostos (22*E*)-24-etil-24-metilcolesta-5,22-dien-3β-ol (**1**) e 24-etil-24-metilcolest-5-en-3β-ol (**2**), isolados da esponja do caribe colombiano *Topsentia ophiraphidites* foram determinadas como *R* e *S*, respectivamente, com base na comparação dos seus dados de RMN com aqueles de amostras de configurações já definidas 24*R* e 24*S*, as quais foram sintetizadas em rotas envolvendo ortoésteres provenientes do rearranjo de Claisen de álcoois Δ^{23}-22 alílicos. Este é primeiro estudo de síntese, em que o rearranjo de Claisen é utilizado para introduzir um centro quaternário em C-24, de maneira estereoespecífica e com rendimento aceitável. Análises por difração de raio X de **1** confirmaram essas atribuições estereoquímicas.

The C-24 configurations of (22*E*)-24-ethyl-24-methylcholesta-5,22-dien-3β-ol (**1**) and 24-ethyl-24-methylcholest-5-en-3β-ol (**2**), isolated from the Colombian Caribbean sponge *Topsentia ophiraphidites*, were determined to be *R* and *S*, respectively, by comparing their NMR data with those of stereodefined (24*R*)- and (24*S*)-samples that were synthesized in routes involving the orthoester Claisen rearrangement of Δ^{23}-22-allylic alcohols. This is the first synthetic study where the Claisen rearrangement is used to introduce a C-24 quaternary center in a stereospecific manner with acceptable yield. X-ray analysis of **1** confirmed these stereochemical assignments.

Keywords: 24-ethyl-24-methylcholesterol, 24-ethyl-24-methylcholesta-5,22-dien-3β-ol, *Topsentia ophiraphidites*, marine sterol, orthoester Claisen rearrangement

Introduction

It is well known that marine organisms such as sponges and corals are rich sources of uniquely modified sterols in the side-chain.[1] Among these sterols, C-24 dialkylated sterols are relatively limited. Djerassi's group reported the isolation of (22*E*)-Δ^{22}-24-ethyl-24-methylcholesterol (**1**), (22*E*)-Δ^{22}-24-ethyl-24-methylcholestanol, 24-ethyl-24-methylcholesterol (**2**), and (22*E*)-$\Delta^{22,25}$-24-ethyl-24-methylcholesterol from a sponge, *Pseudoaxinyssa* sp.[2,3] Although they briefly mentioned the C-24 configurations of these sterols, details of the stereochemical assignment have not been reported.[3] They also reported the preparation of stereochemically unestablished C-24 diastereomers of **1**[2] and stereochemically defined, C-24 diastereomers of 24-ethyl-24-methylcholestanols.[4] Recently, sterol **1** was isolated from the marine sponge *Psammocinia bulbosa* and its C-24 configuration was inferred as *R* without any discussion on the stereochemistry.[5] Furthermore, 24-ethyl-24-methylcycloartane triterpenoids were isolated from higher plants, *Coelogyne uniflora* (Orchidaceae)[6] and *Skimmia wallichii* (Rutaceae).[7] In a previous paper we reported the isolation of a number of multiply alkylated sterols, including compounds **1** and **2**, from the Colombian Caribbean sponge, *Topsentia ophiraphidites*.[8] In particular, compound **1** was a major sterol constituent (*ca.* 40% of the total sterol). With sizeable amounts of compounds **1** and **2** in hand, we undertook a study to elucidate their C-24 configurations. In this paper we report a stereochemical determination at C-24 of 24-ethyl-24-methylsterols **1** and **2** (Figure 1), along with the ^{13}C NMR data for the stereodefined C-24 epimers of **1** and **2**.

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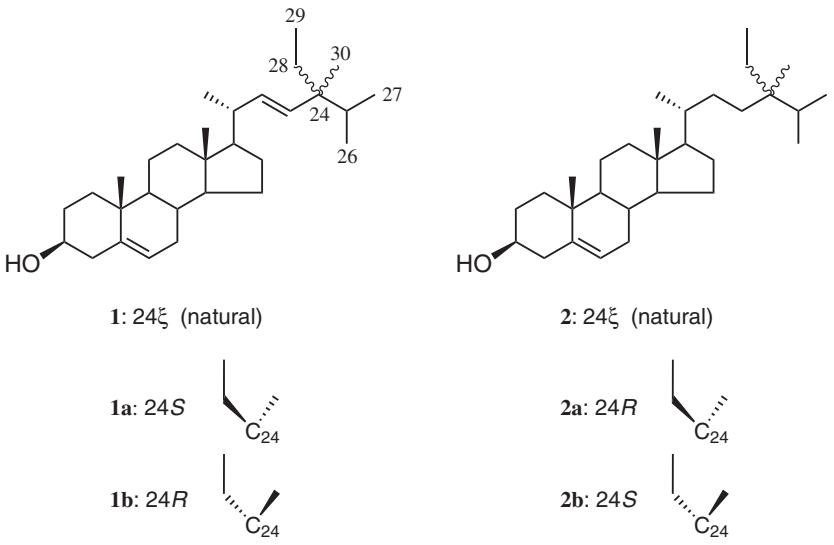


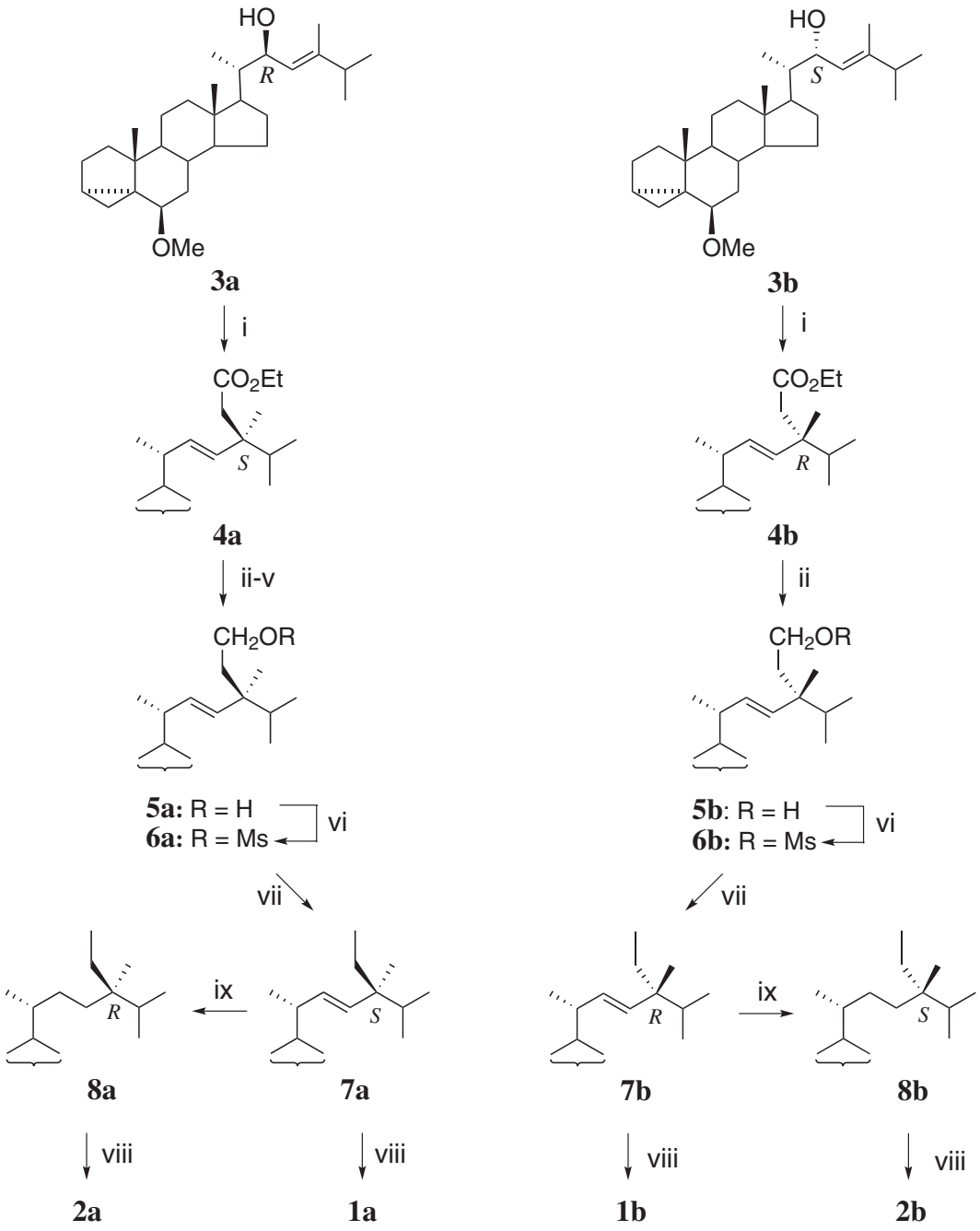
Figure 1. Chemical structures of C-24 epimers of (22*E*)-24-ethyl-24- methylcholesta- 5,22-dien-3β-ol (**1**) and 24-ethyl-24-methylcholest-5-en-3β-ol (**2**). C-24 configurations of **1** and **2** isolated from *Topsentia ophiraphidites* were not defined.

Results and Discussion

In order to determine the C-24 configuration of **1** and **2**, we decided to synthesize the stereochemically defined (24*S*)- and (24*R*)- (22*E*)-Δ^{22}-24-ethyl-24-methylcholesterols (**1a** and **1b**), and (24*R*)- and (24*S*)-24-ethyl-24-methylcholesterols (**2a** and **2b**). A method based on Claisen rearrangement appeared to be attractive for the stereoselective construction of the C-24 dialkyl side chains, although the application of this method to the construction of a C-24 quaternary center is unprecedented. The Johnson orthoester procedure and the Ireland variant of Claisen rearrangement are frequently employed for the synthesis of stereo-defined C-24 mono-alkylated sterol side-chains.[9-11] We also utilized the orthoester Claisen rearrangement for the stereoselective synthesis of (24*R*)- and (24*S*)- (22*E*)-24-isopropenyl-22-dehydrocholesterol and 24-isopropenylcholesterol, another multiply alkylated sterol of *T. ophiraphidites*.[12]

The synthetic route to **1a** and **1b**, and **2a** and **2b** is shown in Figure 2. The required starting materials, (22*R*)-allylic alcohol **3a** and (22*S*)-allylic alcohol **3b**, for the orthoester Claisen rearrangement were obtained stereoselectively from the corresponding enone[13-15] by reduction using DIBAL-H and L-selectride, respectively.[14] Treatment of **3a** with triethyl orthoacetate and propionic acid in refluxing xylene gave a mixture of the desired rearranged product **4a** (not isolated as such) and by-products containing the 22-propionate ester of **3a**. The C-24 epimer of **4a** was not formed in the rearrangement reaction, since careful TLC and NMR analysis revealed that compounds **4a** and **5a** were free from **4b** and **5b**, respectively. The 24*S* configuration of **4a** was assumed on the basis of the preferred transition state conformation for the rearrangement reaction (Figure S1) that is analogous to the conformation well established for the 3,3-sigmatropic reactions of steroidal 23-ene-22-allylic alcohols.[16] The 24*S* configuration of **4a** was ascertained by the eventual conversion of **4a** to the (24*S*)-diastereomer **1a** (*vide infra*). The product mixture containing **4a** was reduced with $LiAlH_4$ and then the resulting alcohol mixture was acetylated to facilitate the separation. Hydride reduction of the purified acetate gave the primary alcohol **5a** in 24% yield from **3a**. Deoxygenation of the C-29 hydroxy group of **5a** was achieved by super-hydride reduction of the corresponding mesylate **6a** to give the *i*-methyl ether **7a**. Removal of the *i*-methyl ether of compound **7a** furnished the (24*S*)-diastereomer **1a**. Hydrogenation of **7a** gave the saturated (24*R*)-diastereomer **8a** which was converted to the (24*R*)-diastereomer **2a** upon deprotection of the *i*-methyl ether. Application of the same sequence of reactions to the (22*S*)-allylic alcohol **3b**, furnished the (24*R*)-diastereomer **1b** *via* the intermediates **4b**, **5b**, **6b** and **7b**. Hydrogenation of **7b** gave **8b**, which furnished the (24*S*)-diastereomer **2b** by regeneration of the Δ^5-3β-hydroxy system.

The ^{1}H and ^{13}C NMR spectroscopic data of the synthetic diastereomers **1a** and **1b** are listed in Tables 1 and 2, respectively, together with those of the sterol **1** isolated from *T. ophiraphidites*. The ^{1}H and ^{13}C signals of **1a** and **1b** were unambiguously assigned on the basis of 2-D (H-H COSY, HMQC and HMBC) NMR spectra. For example, in the HMQC spectrum of **1a**, H-22 (δ 5.058) and H-23 (δ 5.144) were correlated with C-22 (δ 134.9) and C-23 (δ 134.6), respectively, while in the HMBC spectrum, correlation peaks were observed from 21-H_3 (δ 1.017) to



Reagents: i) $MeC(OEt)_3$, propionic acid, ii) $LiAlH_4$, iii) Ac_2O, Py, iv) separation, v) $LiAlH_4$, vi) MsCl, Py, vii) Super hydride, viii) *p*-TsOH/aq. dioxane, ix) H_2, Pd/C.

Figure 2. Synthesis of sterols **1a**, **1b**, **2a** and **2b**.

C-17, C-20 and C-22, H_3-26/H_3-27 (δ 0.785 and 0.803) to C-24 and C-25, H_3-29 (δ 0.739) to C-24 and C-28, and H_3-30 (δ 0.795) to C-23, C-24, C-25 and C-28. The ^{1}H NMR data of the epimers were closely similar to each other. However, careful comparison of the spectra revealed that **1a** or **1b** showed an appreciable difference in the chemical shifts of 29-H_3 ($\Delta\delta$ 0.011 ppm), and natural **1** seemed to be identical with the (24*R*)-diastereomer **1b** in this regard. As shown in Table 2, the ^{13}C data were much more diagnostic for the identification of the diastereomers **1a** and **1b**. The C-24 signal showed the largest difference (0.19 ppm) between the diastereomers and the C-29 and C-28 signals

Table 1. ^{1}H NMR spectroscopic data (400 MHz, in $CDCl_3$) for compounds **1**, **1a**, **1b**, **2**, **2a** and **2b**

No.	natural-**1**	(24*S*)-**1a**	(24*R*)-**1b**	natural-**2**	(24*R*)-**2a**	(24*S*)-**2b**
3	3.53 (m)	3.53 (m)	3.53 (m)	3.52 (m)	3.52 (m)	3.52 (m)
6	5.35 (m)	5.35 (m)	5.35 (m)	5.35 (m)	5.35 (m)	5.35 (m)
18	0.698 (s)	0.698 (s)	0.699 (s)	0.678 (s)	0.678 (s)	0.678 (s)
19	1.010 (s)	1.011 (s)	1.011 (s)	1.009 (s)	1.008 (s)	1.008 (s)
20	2.05 (tq, 8.4, 6.1)	2.05 (tq, 8.4, 6.1)	2.05 (tq, 8.3, 6.4)			
21	1.018 (d, 6.1)	1.017 (d, 6.1)	1.019 (d, 6.4)	0.925 (d, 6.6)	0.923 (d, 6.6)	0.925 (d, 6.6)
22	5.054 (dd, 15.7, 8.4)	5.058 (dd, 16.0, 8.4)	5.060 (dd, 15.9, 8.3)			
23	5.142 (d, 15.8)	5.144 (d, 16.0)	5.146 (d, 15.9)			
26	0.779 (d, 7.0) [a]	0.785 (d, 7.0) [a]	0.779 (d, 7.1) [a]	0.793 (d, 7.1) [a]	0.786 (d, 7.1) [a]	0.793 (d, 6.8) [a]
27	0.801 (d, 7.1) [a]	0.803 (d, 7.0) [a]	0.802 (d, 7.1) [a]	0.796 (d, 7.1) [a]	0.797 (d, 6.8) [a]	0.796 (d, 7.1) [a]
29	0.748 (t, 7.9)	0.739 (t, 7.6)	0.750 (t, 7.9)	0.755 (t, 7.6)	0.749 (t, 7.6)	0.755 (t, 7.6)
30	0.796 (s)	0.795 (s)	0.799 (s)	0.674 (s)	0.674 (s)	0.675 (s)

[a]An upfield resonance was tentatively assigned to H_3-26.

Table 2. ^{13}C NMR spectroscopic data (100 MHz, in $CDCl_3$) for compounds **1**, **1a**, **1b**, **2**, **2a** and **2b**

No.	natural-**1**	(24*S*)-**1a**	(24*R*)-**1b**	natural-**2**	(24*R*)-**2a**	(24*S*)-**2b**
1	37.3	37.3	37.3	37.3	37.3	37.3
2	31.7	31.7	31.7	31.7	31.7	31.7
3	71.8	71.8	71.8	71.8	71.8	71.8
4	42.3	42.3	42.3	42.3	42.3	42.3
5	140.8	140.8	140.8	140.8	140.8	140.8
6	121.7	121.7	121.7	121.7	121.7	121.7
7	31.9	31.9	31.9	31.9	31.9	31.9
8	31.9	31.9	31.9	31.9	31.9	31.9
9	50.2	50.2	50.2	50.1	50.1	50.2
10	36.5	36.5	36.5	36.5	36.5	36.5
11	21.1	21.1	21.1	21.1	21.1	21.1
12	39.7	39.7	39.7	39.7	39.8	39.8
13	42.2	42.2	42.2	42.3	42.3	42.3
14	56.9	56.9	56.9	56.8	56.8	56.8
15	24.3	24.4	24.3	24.3	24.3	24.3
16	28.9	28.9	28.9	28.3	28.3	28.3
17	56.1	56.1	56.1	56.0	56.0	56.0
18	12.1	12.1	12.1	11.8	11.8	11.8
19	19.4	19.4	19.4	19.4	19.4	19.4
20	40.8	40.8	40.8	36.6	36.7	36.6
21	21.3	21.4	21.3	18.9	18.9	18.9
22	134.8	134.9	134.8	29.0	29.1	29.1
23	134.7	134.6	134.7	32.2	32.2	32.3
24	35.7	35.9	35.7	36.8	36.8	36.8
25	41.1	41.1	41.1	33.3	33.3	33.3
26	17.3[a]	17.3[a]	17.3[a]	17.1[a]	17.1[a]	17.2[a]
27	17.9[a]	18.0[a]	17.9[a]	17.2[a]	17.2[a]	17.2[a]
28	31.8	31.6	31.7	28.6	28.7	28.6
29	8.7	8.5	8.6	7.9	8.0	7.9
30	18.3	18.3	18.3	20.4	20.3	20.4

[a]An upfield resonance was tentatively assigned to C-26 (The C-26 and C-27 signals were not necessarily correlated to the H_3-26 and H_3-27 signals because of very close shifts of these signals).

were different by more than 0.10 ppm, and this much difference would be sufficient for the identification of the diastereomers. The ^{13}C data of natural **1** met with those of the (24*R*)-diastereomer **1b**. It was, therefore, concluded that the sterol **1** isolated from *T. ophiraphidites* has 24*R* configuration. The sterol **1** isolated from *Pseudoaxinyssa* sp.[3] was also identified as the (24*R*)-diastereomer **1b**, since the published ^{1}H NMR data (adjusted by the addition of 0.005 ppm) was much closer to those of **1b**, rather than those of **1a**. The sterol **1** isolated from *P. bulbosa* could be assigned as the (24*R*)-diastereomer **1b**, since the reported ^{13}C shifts of C-24, C-28 and C-29 resembled the values of **1b** rather than those of **1a**.

The ^{1}H and ^{13}C NMR spectroscopic data of the sterol **2** isolated from *T. ophiraphidites* and the synthetic 24-diasteromers **2a** and **2b** are listed in Tables 1 and 2, respectively. The ^{1}H and ^{13}C signals of **2a** and **2b** were unambiguously assigned as described for **1a** and **1b**. For example, in the HMBC spectrum of **2a**, H_3-21 (δ 0.923) was correlated with C-17, C-20 and C-22, H_3-26/H_3-27 (δ 0.786 and 0.797) with C-24 and C-25, H_3-29 (δ 0.749) with C-24 and C-28, and H_3-30 (δ 0.674) with C-23, C-24, C-25 and C-28. The ^{1}H NMR signals, in particular H_3-26 and H_3-29, of **2** were in excellent agreement with those of the (24*S*)-diastereomer **2b**, but not with those of the (24*R*)-diastereomer **2a**. This was confirmed by comparison of the ^{13}C NMR data. The C-30 signal showed the largest chemical shift difference ($\Delta\delta$ 0.11) between the diastereomers, and the C-20 and C-28 signals exhibited 0.09 and 0.07 ppm difference, respectively. Figure S2 illustrates a graphic representation of the systematic ^{13}C shift comparison. It is obvious from Figure S2 that the sterol **2** is identical with **2b**, rather than **2a**. The minor deviation observed in some signals of **2** and **2b** could be within an error level in the NMR measurements. Hence, the 24*S* configuration of the sterol **2** isolated from *T. ophiraphidites* was unequivocally established. Comparison of melting points and $[\alpha]_D$ values (see Experimental) also supported this stereochemical assignment. The C-24 configuration of 24-ethyl-24-methylcholesterol isolated from *Pseudoaxinyssa* sp.[2] was also assigned as *S*, since the reported ^{1}H NMR data (recorded in benzene-d_6) met with those (recorded in benzene-d_6) of (24*S*)-**2b**.

Finally, we carried out X-ray analysis for a crystalline sample of the sterol **1** isolated from *T. ophiraphidites*. The molecular structure and the C-24 stereochemistry of **1** were established as shown in Figure S3. The 24*R* configuration of **1** was inferred by assuming the absolute configuration of natural sterols. The results proved the 24*R* configuration of the rearranged product **4b** (therefore, **4a** should have 24*S* configuration) and correctness of the transition state conformation (Figure S1) proposed for the orthoester Claisen rearrangement employed for the stereoselective creation of the C-24 quaternary center.

Conclusions

The present study established the 24*R* configuration of the marine sterol **1**, isolated from *T. ophiraphidites, Pseudoaxinyssa* sp., *and Psammocinia bulbosa*, and the 24*S* configuration of the marine sterol **2**, isolated from *T. ophiraphidites and Pseudoaxinyssa* sp. The C-24 configurations for the sterols **1** and **2**, originally proposed by Djerassi's group,[3] was confirmed with full experimental details. The findings that sterols **1** and **2** possess the same C-24 orientation (24α_F for the methyl group) supported the view that the sterol **2** is a biosynthetic precursor of the corresponding Δ^{22}-sterol **1** in sponges. Djerassi and co-workers[3] suggested that stigmasta-5,24-dien-3β-ol could be a progenitor of the unique sterols, **1** and **2**. Stereochemical studies on the other stereochemically undefined multiply-alkylated sterols isolated from *T. ophiraphidites* are in progress in our laboratory.

Experimental

Melting points were determined on a Yazawa BY-1 hot-stage micro melting point apparatus and are uncorrected. NMR spectra were obtained on a JEOL JNM LA-400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) spectrometer in $CDCl_3$ solution (4-10 mg sample *per* test tube) with tetramethylsilane as an internal reference at *ca.* 25 °C. ^{13}C chemical shifts are referred to the solvent signal (δ 77.00). EI and FAB MS spectra were recorded with a JEOL JMS-AX700 spectrometer. Optical rotations were measured on a JASCO DIP-360 polarimeter.

*(22R,23E)-6β-Methoxy-3α,5-cyclo-5α-ergost-23-en-22-ol (**3a**) and (22S,23E)-6β- methoxy-3α,5-cyclo-5α-ergost-23-en-22-ol (**3b**)*

DIBAL-H (1.01 mol L^{-1} in toluene, 0.70 mL, 0.71 mmol) was added to a solution of 6β-methoxy-3α,5-cyclo-5α-ergost-23-en-22-one[14] (490 mg, 1.15 mmol) in dry THF (10 mL) at −78 °C under N_2, and the mixture was stirred at the same temperature for 30 min. Addition of ether and extractive work-up gave a crude product which was chromatographed on silica gel with hexane-EtOAc (15:1) to give the (22*R*)-alcohol **3a** (308 mg, 63%) as a white solid, mp 128-129 °C. ^{1}H NMR: δ 0.44 (dd, *J* 8.0, 5.1 Hz, 4α-H), 0.65 (dd, *J* 5.1, 4.4 Hz, 4β-H), 0.73 (s, H_3-18), 0.96 (d, *J* 6.4 Hz, H_3-21), 1.01 (d, *J* 6.8 Hz, H_3-26, H_3-27), 1.03 (s, H_3-19), 1.62 (d, *J* 1.2 Hz, H_3-28), 2.23 (sep, *J* 6.8 Hz,

H-25), 2.78 (brt, *J* 2.8 Hz, H-6), 3.33 (s, OMe), 4.47 (dd, *J* 7.8, 1.2 Hz, H-22), 5.33 (brd, *J* 7.8 Hz, H-23). ^{13}C NMR: δ 12.16, 12.29, 13.07, 14.16, 19.28, 21.27, 21.37, 21.46, 22.77, 24.18, 24.94, 27.92, 30.56, 33.34, 35.09, 35.23, 36.68, 40.20, 41.88, 42.73, 43.37, 47.97, 52.67, 56.38, 56.56, 70.54, 82.41, 124.85, 142.72. Analysis calc. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29. Found: C, 81.31; H, 11.56.

L-Selectride (1.0 mol L^{-1} in THF, 8.64 mL, 8.64 mmol) was added to a solution of 6β-methoxy-3α,5-cyclo-5α-ergost-23-en-22-one (1.23 g, 2.88 mmol) in dry THF (25 mL) at –78 °C under N_2, and the mixture was stirred at the same temperature for 5 h. Addition of ether and extractive work-up gave a crude product which was chromatographed on silica gel with hexane-ether (4:1) to give the (22*S*)-alcohol **3b** (784 mg, 64%) as a colorless oil. ^{1}H NMR: δ 0.43 (dd, *J* 8.1, 5.1 Hz, H-4α), 0.65 (dd, *J* 5.1, 4.4 Hz, H-4β), 0.74 (s, H_3-18), 1.00-1.02 (H_3-21, H_3-26, H_3-27, H_3-19), 1.70 (brs, H_3-28), 2.26 (sep, *J* 6.8 Hz, H-25), 2.77 (brt, *J* 2.3 Hz, H-6), 3.32 (s, OMe), 4.42 (dd, *J* 9.5, 3.7 Hz, H-22), 5.28 (brd, *J* 9.3 Hz, H-23). ^{13}C NMR: δ 12.20, 12.53, 13.04, 14.07, 19.25, 21.35, 21.43, 21.50, 22.72, 24.35, 24.92, 27.87, 30.45, 33.32, 34.97, 35.22, 37.04, 40.15, 42.04, 43.03, 43.33, 47.97, 53.24, 56.08, 56.52, 69.55, 82.34, 120.42, 146.45. Analysis calc. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29. Found: C, 81.44; H, 11.51.

*(22E,24S)-24-(2-Hydroxyethyl)-6β-methoxy-24-methyl-3α,5-cyclo-5α-cholest-22-ene (**5a**)*

A solution of the (22*R*)-alcohol **3a** (155 mg, 0.362 mmol), triethyl orthoacetate (0.23 mL, 1.25 mmol) and propionic acid (12 μL, 0.161 μmol) in xylene (5.0 mL) was heated at reflux under N_2 for 3 h. The mixture was directly subjected to silica gel column chromatography. Elution with hexane-EtOAc (15:1) gave the crude rearranged product (140 mg) which was mainly composed of the desired **4a** and 22-*O*-propionate side-product. The mixture was dissolved in dry THF (2.0 mL) and $LiAlH_4$ (24.0 mg, 0.632 mmol) was added and after 30 min stirring, it was worked up in the usual manner. The resulting mixture (62.4 mg) was mixed with Ac_2O (0.25 mL) and pyridine (0.50 mL) and allowed to stand overnight at room temperature. Extractive (ether) work-up gave a crude product which was chromatographed over silica gel with hexane-EtOAc (20:1) to give 29-acetate (40.5 mg) as an oil. $LiAlH_4$ (6.0 mg, 158 μmol) was added to a solution of the acetate in THF (1.0 mL) and the mixture was stirred for 10 min at room temperature. Extractive (ether) work-up gave the primary alcohol **5a** (36.0 mg, 24% from **3a**) as a colorless oil. ^{1}H NMR: δ 0.43 (dd, *J* 8.1, 5.1 Hz, H-4α), 0.65 (dd, *J* 5.1, 4.4 Hz, H-4β), 0.73 (s, H_3-18), 0.81 (d, *J* 7.1 Hz, H_3-26), 0.83 (d, *J* 7.1 Hz, H_3-27), 0.89 (s, H_3-30), 1.01 (d, *J* 6.8 Hz, H_3-21), 1.02 (s, H_3-19), 2.06 (m, H-20), 2.77 (m, H-6), 3.32 (s, OMe), 3.63 (m, H_2-29), 5.14 (dd, *J* 15.9, 8.6 Hz, H-22), 5.27 (d, *J* 15.9 Hz, H-23). ^{13}C NMR: δ 12.40, 13.06, 17.20, 17.73, 18.41, 19.26, 21.01, 21.43, 22.72, 24.21, 24.92, 29.02, 30.45, 33.32, 35.05, 35.20, 37.13, 40.14, 40.30, 40.73, 41.85, 42.68, 43.35, 48.01, 56.08, 56.52, 56.57, 60.28, 82.37, 134.77, 135.27. HREIMS *m/z*: 456.3967 calc. for $C_{31}H_{52}O_2$ [M$^+$]. Found: 456.4011.

*(22E,24S)-24-Ethyl-6β-methoxy-24-methyl-3α,5-cyclo-5α-cholest-22-ene (**7a**)*

MsCl (15 μL, 194 μmol) was added to a solution of **5a** (30.6 mg, 67 μmol) in pyridine (0.30 mL) and the mixture was stirred for 10 min at room temperature. Extractive (ether) work-up gave a crude mesylate **6a** as an oil. A solution of the mesylate in dry THF was treated with Super-hydride (1.0 mol L^{-1} in THF, 370 μL, 370 μmol) at room temperature under N_2 for 30 min. Extractive (ether) work-up gave a crude product which was chromatographed on silica gel with hexane-EtOAc (20:1) to yield the 22-ene **7a** (14.9 mg, 50%) as an oil. ^{1}H NMR: δ 0.43 (dd, *J* 8.1, 5.1 Hz, H-4α), 0.65 (dd, *J* 5.1, 4.4 Hz, H-4β), 0.73 (s, H_3-18), 0.74 (d, *J* 7.4 Hz, H_3-29), 0.79 (d, *J* 7.6 Hz, H_3-26), 0.80 (s, H_3-30), 0.81 (d, *J* 5.8 Hz, H_3-27), 1.01 (d, *J* 6.6 Hz, H_3-21), 1.03 (s, H_3-19), 2.07 (m, H-20), 2.77 (brt, 2.8 Hz, H-6), 3.32 (s, OMe), 5.06 (dd, *J* 15.7, 8.4 Hz, H-22), 5.14 (d, *J* 15.9 Hz, H-23). ^{13}C NMR: δ 8.52, 12.46, 13.08, 17.33, 17.99, 18.25, 19.30, 21.36, 21.50, 22.77, 24.26, 24.97, 29.03, 30.50, 31.62, 33.36, 35.09, 35.29, 35.87, 40.22, 40.87, 41.09, 42.68, 43.41, 48.09, 56.28, 56.55, 56.67, 82.45, 134.59, 134.93. HREIMS *m/z*: 440.4018 calc. for $C_{31}H_{52}O$ [M$^+$]. Found: 440.4067.

*(22E,24S)-24-Ethyl-24-methylcholesta-5,22-dien-3β-ol (**1a**)*

A solution of the 22-ene **7a** (7.3 mg, 16.6 μmol) in dioxane (0.5 mL) and H_2O (1.15 mL) containing *p*-TsOH·H_2O (12 mg, 63.1 μmol) was heated at 105 °C for 3 h. Extractive (ether) work-up gave a crude product which was crystallized from MeOH to yield the (24*S*)-22-ene **1a** (5.4 mg, 76%) as white needles, mp 159-161 °C. $[\alpha]_D^{25}$ –41.6° (c, 0.50, $CHCl_3$). ^{1}H and ^{13}C NMR spectroscopic data are listed in Tables 1 and 2, respectively. EIMS *m/z*: 426 [M$^+$, trace], 383 [M-isopropyl], 367, 273, 257, 231, 213. HRFABMS *m/z*: 409.3834 calc. for $C_{30}H_{49}$ [MH$^+$–H_2O]. Found: 409.3784.

*(24R)-24-Ethyl-6β-methoxy-24-methyl-3α,5-cyclo-5α-cholestane (**8a**)*

A solution of the 22-ene **7a** (10.5 mg, 23.8 μmol) in AcOEt (1.0 mL) was hydrogenated in the presence of 10% Pd/C (5.8 mg) overnight. Dilution with hexane and filtration through a pad of silica gel afforded an oily

saturated product **8a** (7.4 mg, 70%). ^{1}H NMR : δ 0.43 (dd, *J* 8.1, 5.1 Hz, H-4α), 0.65 (dd, *J* 5.1, 4.4 Hz, H-4β), 0.67 (s, H_3-30), 0.72 (s, H_3-18), 0.75 (t, *J* 7.6 Hz, H_3-29), 0.79 (d, *J* 7.1 Hz, H_3-26), 0.80 (d, *J* 7.1 Hz, H_3-27), 0.92 (d, *J* 6.6, H_3-21), 1.02 (s, H_3-19), 2.77 (brt, *J* 2.8 Hz, H-6), 3.33 (s, OMe). ^{13}C NMR: δ 7.95, 12.22, 13.07, 17.05, 17.15, 18.87, 19.29, 20.25, 21.50, 22.77, 24.21, 24.97, 28.37, 28.69, 29.05, 30.48, 32.26, 33.31, 33.36, 35.06, 35.30, 36.75, 36.82, 40.28, 42,77, 43.39, 48.03, 56.19, 56.54, 56.55, 82.45. HREIMS *m/z*: 442.4172 calc. for $C_{31}H_{54}O$ [M$^+$]. Found: 442.4156.

(24R)-24-Ethyl-24-methylcholest-5-en-3β*-ol (**2a**)*

Compound **8a** (5.9 mg, 13.3 μmol) was treated as described for the conversion of **7a** to **1a**. Crystallization of a crude product from MeOH gave the (24*R*)-epimer **2a** (4.7 mg, 82%) as white needles, mp 159-161 °C. $[\alpha]_D^{25}$ –30.8° (c, 0.42, $CHCl_3$). ^{1}H and ^{13}C NMR spectroscopic data are listed in Tables 1 and 2, respectively. EIMS *m/z*: 428 (M$^+$), 413, 410, 395, 385, 367, 343, 317, 273, 255, 231, 213. HREIMS *m/z*: 428.4018 calc. for $C_{30}H_{52}O$ [M$^+$]. Found: 428.4020.

*(22E,24R)-24-(2-Hydroxyethyl)-*6β*-methoxy-24-methyl-3*α*,5-cyclo-5*α*-cholest-22-ene (**5b**)*

Treatment of the (22*S*)-alcohol **3b** (414 mg) as described for **3a** gave a product (377 mg) which was composed of the desired **4b** and 22-*O*-propionate side-product. The mixture was reduced with $LiAlH_4$ (28.7 mg) in ether (5 mL) and the product was chromatographed on silica gel with hexane-ether (10:1) to give a primary alcohol **5b** (105 mg, 24% from **3b**) as an oil. ^{1}H NMR: δ 0.43 (dd, *J* 8.0, 5.1 Hz, H-4α), 0.65 (dd, *J* 5.1, 4.4 Hz, H_3-4β), 0.73 (s, H_3-18), 0.80 (d, *J* 7.6 Hz, H_3-26), 0.83 (d, *J* 7.6 Hz, H_3-27), 0.88 (s, H_3-30), 1.01 (d, *J* 6.6 Hz, H_3-21), 1.03 (s, H_3-19), 2.07 (m, H-20), 2.77 (brt, *J* 2.6 Hz, H-6), 3.32 (s, OMe), 5.13 (dd, *J* 15.6, 8.3 Hz, H-22), 5.25 (brd, *J* 15.6 Hz, H-23). ^{13}C NMR: δ 12.42, 13.06, 17.15, 17.61, 18.58, 19.28, 21.16, 21.47, 22.74, 24.25, 24.95, 28.99, 30.47, 33.35, 35.07, 35.26, 36.97, 40.14, 40.19, 40.78, 42.04, 42.71, 43.39, 48.05, 56.11, 56.55, 56.59, 60.41, 82.40, 134.62, 135.37. HREIMS *m/z*: 456.3967 calc. for $C_{31}H_{52}O_2$ [M$^+$]. Found: 456.3948.

*(22E,24R)-24-Ethyl-*6β*-methoxy-24-methyl-3*α*,5-cyclo-5*α*-cholest-22-ene (**7b**)*

The primary alcohol **5b** (100 mg) was converted to the 22-ene **7b** (47.5 mg, 49%) *via* the mesylate **6b** as described for the conversion of **5a** to **7a**. **7b**: an oil. ^{1}H NMR: δ 0.43 (dd, *J* 8.0, 5.1 Hz, H-4α), 0.65 (dd, *J* 5.1, 4.4 Hz, H-4β), 0.73 (s, H_3-18), 0.78 (d, *J* 7.1 Hz, H_3-26), 0.80 (s, H_3-30), 0.80 (d, *J* 6.8 Hz, H_3-27), 1.01 (d, *J* 7.1 Hz, H_3-21), 1.02 (s, H_3-19), 2.77 (brt, *J* 2.6 Hz, 6-H), 3.32 (s, OMe), 5.05 (dd, *J* 15.6, 8.0 Hz, H-22), 5.25 (brd, *J* 15.6 Hz, H-23). ^{13}C NMR: δ 8.67, 12.46, 13.08, 17.26, 17.88, 18.29, 19.29, 21.29, 21.49, 22.77, 24.24, 24.97, 28.96, 30.49, 31.76, 33.36, 35.09, 35.29, 35.70, 40.22, 40.84, 41.07, 42.68, 43.41, 48.09, 56.23, 56.54, 56.67, 82.44, 134.61, 134.88. HREIMS *m/z*: 440.4018 calc. for $C_{31}H_{52}O$ [M$^+$]. Found: 440.4014.

(22E,24R)-24-Ethyl-24-methylcholesta-5,22-dien-3β*-ol (**1b**)*

The 22-ene **7b** (25.8 mg) was treated as described for the conversion of **7a** to **1a**. The crude product was chromatographed on silica gel with hexane-EtOAc (7:1) and crystallized from MeOH to afford **1b** (15 mg, 60%) as white needles, mp 164-165 °C [mp of natural **1**, 165-166.5 °C (from methanol)[8]]. $[\alpha]_D^{25}$ –49.8° (c, 2.0, $CHCl_3$) (lit.[8] –45.4° for **1**). The EI-MS data were essentially identical with those of **1a**. ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2, respectively. Analysis calc. for $C_{30}H_{50}O$: C, 84.44; H, 11.81. Found: C, 84.68; H, 11.49.

*(24S)-24-Ethyl-*6β*-methoxy-24-methyl-3*α*,5-cyclo-5*α*-cholestane (**8b**)*

The 22-ene **7b** (24.8 mg) was hydrogenated as described for **8a** to afford the saturated compound **8b** (24.4 mg, 98%) as an oil. ^{1}H NMR: δ 0.43 (dd, *J* 7.8, 5.1 Hz, H-4α), 0.65 (dd, *J* 5.1, 4.4 Hz, H-4β), 0.67 (s, H_3-30), 0.71 (s, H_3-18), 0.75 (t, *J* 7.7 Hz, H_3-29), 0.77 (d, *J* 7.8 Hz, H_3-26), 0.79 (d, *J* 7.1 Hz, H_3-27), 0.92 (d, *J* 6.3 Hz, H_3-21), 1.02 (s, H_3-19), 2.77 (brt, *J* 2.7 Hz, H-6), 3.32 (s, OMe). ^{13}C-NMR: δ 7.94, 12.22, 13.07, 17.12, 17.15, 18.91, 19.29, 20.37, 21.50, 22.77, 24.21, 24.97, 28.38, 28.62, 29.03, 30.49, 32.27, 33.29, 33.36, 35.08, 35.31, 36.67, 36.81, 40.28, 42.78, 43.39, 48.05, 56.18, 56.55, 82.45. HRFABMS *m/z*: 443.4253 calc. for $C_{31}H_{55}O$ [MH$^+$]. Found: 443.4277.

(24S)-24-Ethyl-24-methylcholest-5-en-3β*-ol (**2b**)*

Compound **8b** (22.9 mg) was converted to **2b** as described for the conversion of **8a** to **2a**. Recrystallization of the product from MeOH gave **2b** (15 mg, 68%) as white needles, mp 147-148 °C (mp of natural **2**, 145-146 °C (from methanol)[8]), $[\alpha]_D^{25}$ –35.9° (c, 1.7, $CHCl_3$) (lit.[7] –34.6° for **2**). The EIMS data were essentially identical with those of **2a**. ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2, respectively. Analysis calc. for $C_{30}H_{52}O$: C, 84.04; H, 12.23. Found: C, 84.04; H, 12.53.

*X-ray study of natural sterol (**1**)*

Crystals for X-ray analysis were obtained by allowing to stand a warmed MeOH solution of **1** with slow

evaporation of the solvent through a small hole in the cap. $C_{30}H_{52}O{\cdot}1/2MeOH{\cdot}1/2H_2O$, orthorhombic, $P2_12_12_1$, $Z = 8$, $a = 70.8949(17)$, $b = 7.7590(2)$, $c = 10.0415(2)$ Å, $V = 5523.6(2)$ Å^3, T = 123 K, Dx = 1.086 gcm^{-3}, $\lambda(CuK\alpha) = 1.54184$ Å, $\mu = 0.490$ mm^{-1}. A number of 9298 reflections were collected by the oscillation photograph method using Rigaku R-AXIS RAPID Imaging Plate camera and Lp and semi-empirical absorption corrections were applied. The structure was solved by direct methods using SIR-97. The positional and anisotropic thermal parameters were refined by full-matrix least squares SHELXL-97. The positions of hydrogen atoms were calculated geometrically and refined using the riding model. Final R = 0.090 for 6737 unique reflections with $|I_o| > 2\sigma(I_o)$. One methyl group of the terminal isopropyl group has a disordered structure in both independent molecules. The occupancy factors are 0.60:0.40 and 0.52:0.48, respectively. Sterol molecules are linked by hydrogen bonding directly or via methanol and water molecules.

Supplementary Information

Supplementary data associated with this paper are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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