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Acid catalysed backbone rearrangement of cholesta-2,4,6-triene: On the origin of ring A and ring B aromatic steroids in recent sediments

Patrick Schüpfer, Yvan Finck, Frédéric Houot, Fazil O. Gülaçar *

*Laboratory of Mass Spectrometry, Physical Chemistry, University of Geneva, Sciences I, Boulevard d'Ivoy 16,
CH-1211 Geneva 4, Switzerland*

Abstract

Rearrangement of cholesta-2,4,6-triene in the presence of *p*-toluenesulfonic acid in acetic acid at 70 °C leads to 4-methyl-19-*nor*-cholesta-1,3,5(10)-triene and 1(10 → 6)-*abeo*-14β-cholesta-5,7,9(10)-triene in less than 2 h. Postulated mechanisms of formation of these products are supported by molecular mechanics calculations of the relative stabilities of reaction intermediates. The results suggest that $\Delta^{5,7}$-sterols, the most common natural precursors of triunsaturated steroidal hydrocarbons in contemporary sediments, constitute another major source for monoaromatic A and B steroids in addition to Δ^5-sterols.
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1. Introduction

Steroidal biomarkers are commonly encountered in sediments. They are the result of numerous transformations, from their release as sterols from the biosphere to burial under high pressure and temperature conditions in the geosphere. The study of these processes is not only achieved through sediment analysis but also by laboratory simulation reactions reproducing the physicochemical conditions to which the sedimentary organic matter is subjected. Thus, for the study of early diagenetic processes, an anhydrous mixture of *p*-toluenesulfonic acid (TsOH) with acetic acid (AcOH) has been widely and successfully used (Kirk and Shaw, 1970; Wolff et al., 1986; Peakman et al., 1988; Peakman and Maxwell, 1988a; Peakman and Maxwell, 1988b; Peakman and Maxwell, 1988c; de Leeuw et al., 1989; Peakman et al., 1992). Schüpfer and Gülaçar (2000) showed via simulation reactions carried out on cholesta-3,5-diene that it yields ring A, B and C aromatic steroids (MAs, MBs and MCs, respectively), which are widespread in sediments. MA and MB steroids have been recently detected by our laboratory in immature sediment samples from the Marches-Ombrie basin (Central Italy). In all samples, the carbon number distributions of 1- and 4-methyl-MAs, 14α-and 14β-MBs, show a significant and systematic enrichment in C_{28}

* Corresponding author. Tel.: +41 22 3796395; fax: +41 22 3793213.
E-mail address: Fazil.Gulacar@chiphy.unige.ch (F.O. Gülaçar).

components, going from regular 5α,14α,17α-ster-anes to MAs and MBs (Fig. 1).

This observation, which cannot be explained on the basis of formation mechanism of MAs and MBs from $\Delta^{3,5}$-steradienes (Schüpfer and Gülaçar, 2000), suggests that another reaction pathway, starting from other sterol precursors containing higher proportions of C_{28} species, also operates. Our hypothesis was that $\Delta^{5,7}$-sterols with the most common members at C_{28} (e.g., ergosterol) may, via $\Delta^{3,5,7}$-steratrienes as their dehydration product, lead to MAs and MBs more quickly than do Δ^5-sterols. To test this hypothesis, we selected cholesta-3,5,7-triene as a model compound for a study of the acid-catalysed rearrangements mimicking geochemical processes. However, all attempts to synthesise cholesta-3,5,7-triene either by direct dehydration of cholesta-5,7-dien-3β-ol or via its tosylate derivative failed, leading to different backbone rearranged steratrienes and steratetraenes (Schüpfer, 2000). On the other hand, $NaBH_4/CeCl_3$ reduction of cholesta-3,5-diene-7-one led mainly to the

cholesta-2,4,6-triene (**1**) isomer, besides the corresponding sterol (Schüpfer, 2000). This is why this triene was finally chosen as the model steratriene to compare the acid catalysed rearrangement of steradienes and steratrienes in the presence of TsOH in AcOH at 70 °C. We present here results demonstrating the rapid rearrangement of cholesta-2,4,6-triene to 4- and 1-methyl-19-nor-cholesta-1,3,5(10)-trienes (**2a**, **b**) and 14α(H)- and 14β(H)-anthrasteroids (**3a**, **b**; Fig. 2).

2. Experimental

2.1. Materials and methods

Toluene (Fluka, Buchs, Switzerland, > 99%) was dried using P_2O_5 and stored over molecular sieve (4 Å). Et_2O was doubly distilled over Na metal from technical solvent. Cyclohexane (Fluka, > 99.5%), glacial acetic acid (Sigma–Aldrich, Buchs, Switzerland, 99%) and pyridine (Fluka, > 99%) were used without further purification. CH_2Cl_2, hexane and
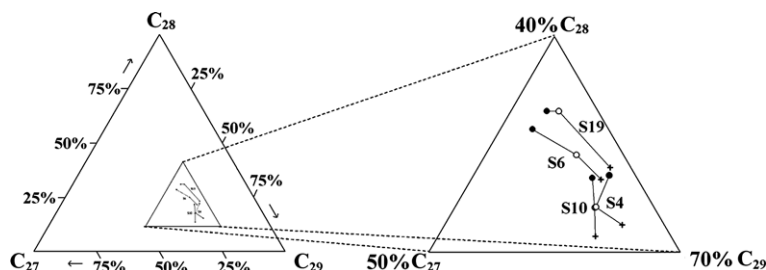

Fig. 1. Ternary diagram showing carbon number distribution of 5α,14α,17α steranes (crosses), ring A (MAs, open circles) and monoaromatic steroid hydrocarbons MBs (filled circles) in samples S4, S6, S10 and S19 of an immature sediment (middle Cretaceous) from the Selli level of the Marches-Ombrie Basin (Central Italy).

2a: 4-methyl-19-nor-cholesta-1,3,5(10)-triene ; R=H
2b: 1-methyl-19-nor-cholesta-1,3,5(10)-triene ; R=H

3a: 14α(H)-anthrasteroid ; R=H
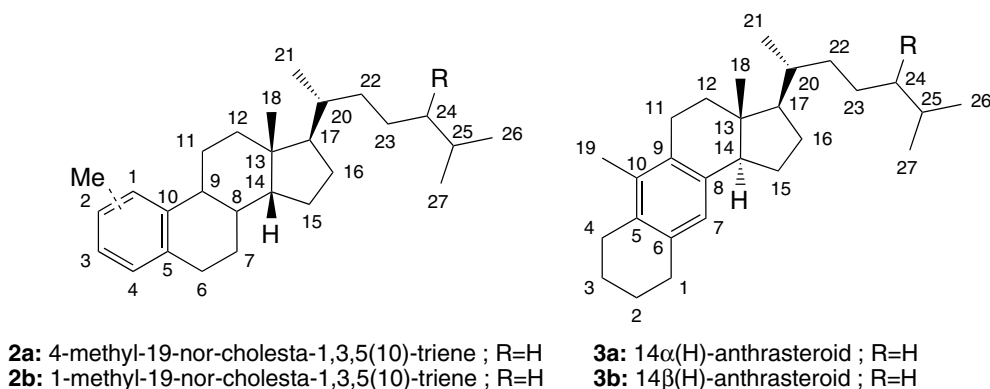**3b:** 14β(H)-anthrasteroid ; R=H

Fig. 2. Sedimentary ring A and ring B monoaromatic steroids.

acetone were doubly distilled from technical quality solvent prior to use. Reversed phase high performance liquid chromatography (HPLC) analysis was performed with a chromatographic system equipped with a Merck pump (Intelligent Pump L-6200A), a Merck L-4500 Diode Array Detector (180–800 nm) and a Merck RP-18 (10 × 250 mm, 7 μm) column. The mobile phase (MeOH:CH$_2$Cl$_2$, 9:1; HPLC grade solvents) was used in the isocratic mode. Gas chromatography–mass spectrometry (GC–MS) analysis was carried out with a Hewlett-Packard 5890 series II chromatograph coupled to a VG Masslab Trio-2 spectrometer, using a J&W SE-54 fused silica column (30 m × 0.25 mm, 0.25 μm film thickness). The oven temperature programme was as follows: 60 °C held for 1 min, heated to 270 °C at 20 °C/min, held 30 min. Injections were made in the splitless mode with a He head pressure of 0.80 MPa.

NMR spectra (^{1}H and ^{13}C, at 400 and 100 MHz, respectively) were recorded with a Brucker AMX 400 spectrometer at 25 °C; chemical shifts (δ) are in ppm relative to SiMe$_4$ ($\delta = 0$ ppm).

2.2. Synthesis of cholesta-2,4,6-triene (1)

Preparation of 24-ethylcholesta-2,4,6,22-tetraene has been described by Stoilov et al. (1994). The same methodology was applied to cholesterol (4)

in order to obtain cholesta-2,4,6-triene (1) in a three step synthesis (Fig. 3).

2.2.1. 5α,6α-Epoxycholestan-3β-ol (5)

CH$_2$Cl$_2$ (135 ml) containing 10 mmol (3.86 g) cholesterol (4) was cooled to 0 °C and 13 mmol (2.3 g) of *m*-chloroperoxybenzoic acid (MCPBA) was added in small portions for 20 min. The mixture was kept at 0 °C and stirred for 18 h. After filtration, the solution was first washed with saturated NaHCO$_3$ solution, then with water. The organic phase was dried over anhydrous MgSO$_4$, filtered and concentrated, leading to a white solid (4.1 g, 95%). MS: *m/z* (relative intensity) of TMS derivative: 474 (27, M$^{+\cdot}$), 459 [6, (M−Me)$^+$], 384 [35, (M−Me$_3$SiOH)$^{+\cdot}$], 369 (16), 366 (25), 356 (12), 271 (9), 229 (15), 145 (48), 95 (100), 75 (90). The spectrum matches well a published spectrum (Rontani and Aubert, 2004).

2.2.2. 5α,6α-Epoxycholestan-3β-yl mesylate (6)

To a stirred solution of 5α,6α-epoxycholestan-3β-ol (5; 5 mmol, 2 g) in CH$_2$Cl$_2$ (15 ml) cooled to 0 °C, 14 mmol (2 ml) Et$_3$N was added, followed by 6.4 mmol (0.5 ml) methanesulfonyl chloride under N$_2$. The resulting yellow mixture was stirred for 2.5 h and 60 ml of cold water was added. The organic phase was separated and the aqueous phase was extracted twice with CH$_2$Cl$_2$. The combined
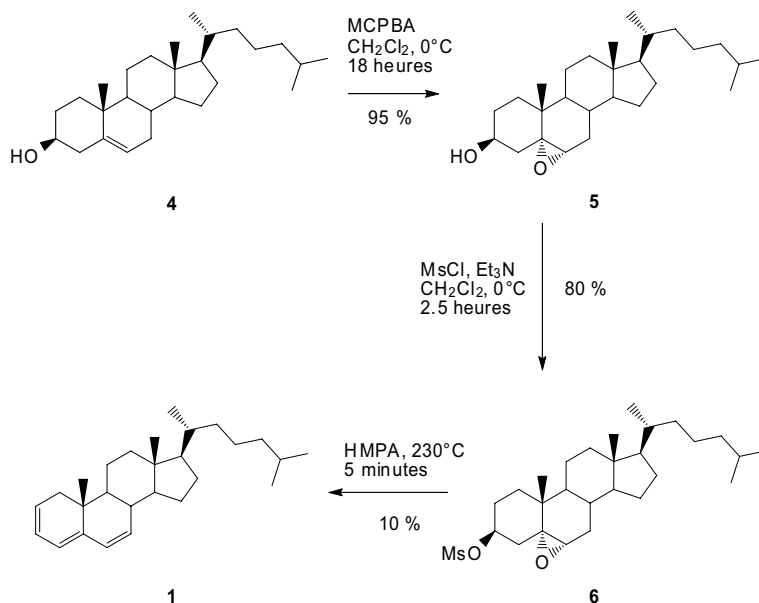

Fig. 3. Preparation of cholesta-2,4,6-triene (1). MCPBA = *meta*-chloroperbenzoic acid, MsCl = methanesulfonyl chloride and HMPA = hexamethylphosphoramide.

extracts were washed with water until pH 8. After drying over anhydrous $MgSO_4$, the extract was filtered and the solvent evaporated, yielding a pale yellow solid (2.2 g, 4.2 mmol, 84%). ^{1}H NMR: 4.820 (sept., $J \approx 5.5$, H-3) ; 2.985 (3H, s, 3-OSO_2Me); 1.075 (3H, s, H-19); 0.884 (3H, d, $J = 6.4$, H-21); 0.861 (3H, d, $J = 6.4$, H-27); 0.856 (3H, d, $J = 6.4$, H-26); 0.607 (3H, s, H-18). ^{13}C NMR (100 MHz, $CDCl_3$): 79.71 (3-OSO_2Me); 65.00 (C-5); 59.24; 56.75; 55.88; 42.44; 42.32 (C-13); 39.49; 39.32; 38.44; 37.36; 36.13; 35.73; 34.80; 32.20; 29.83; 28.68; 28.58; 28.03; 27.98; 24.02; 23.82; 22.78 (C-27); 22.52 (C-26); 20.57; 18.63 (C-21); 15.78 (C-19); 11.84 (C-18). ^{1}H and ^{13}C NMR chemical shifts of 5α,6α-epoxycholestan-3β-yl mesylate (**6**) are in agreement with data from Stoilov et al. (1994) for 5α,6α-epoxy-24-ethylcholest-22-en-3β-ol mesylate.

2.2.3. Cholesta-2,4,6-triene (1)

A solution of 4.2 mmol (2.2 g) 5α,6α-epoxycholesta-3β-mesylate (**6**) in hexamethylphosphoramide (HMPA; 15 ml) was heated to 230 °C for 5 min under N_2. After cooling to room temperature, the mixture was poured into 100 ml water and extracted three times with 100 ml hexane. The resulting organic phase was washed with saturated NaCl solution, dried over anhydrous $MgSO_4$ and purified on a silica gel column impregnated with 0.5% Et_3N. A colourless oil of cholesta-2,4,6-triene was obtained (11, 59 mg, yield 17%, purity > 98%). ^{1}H NMR: 5.987 (dd, $J = 9.8, 2.6$, H-7); 5.941 (ddd, $J = 9.1, 5.3, 3.2$, H-3); 5.715 (ddd, $J = 9.1, 6.3, 2.8$, H-2); 5.669 (dd, $J = 9.8, 1.5$, H-6); 5.612 (d, $J = 5.3$, H-4); 0.952 (3H, s, H-19); 0.926 (3H, d, $J = 6.4$, H-21); 0.878 (3H, d, $J = 6.4$, H-27); 0.874 (3H, d, $J = 6.4$, H-26); 0.722 (3H, s, H-18). ^{13}C NMR: 142.97 (C-5); 131.70 (C-7); 127.93 (C-6); 125.27 (C-2); 124.25 (C-3); 119.23 (C-4); 56.20; 54.69; 51.69; 43.19 (C-13); 39.98; 39.55; 37.06; 36.47; 36.23; 35.80; 35.67 (C-10); 28.20; 28.02; 23.91; 23.79; 22.79 (C-27); 22.55 (C-26); 20.97 (C-11); 18.71 (C-21); 15.38 (C-19); 11.90 (C-18). ^{1}H and ^{13}C NMR shifts for cholesta-2,4,6-triene (**1**) are in agreement with published data for 24-ethylcholesta-2,4,6,22-tetraene (Stoilov et al., 1994). The coupling pattern for the triene part [H–C(2)–H–C(7)] of (**1**) are in perfect accord with those reported by Stoilov et al. (1994) for 24-ethylcholesta-2,4,6,22-tetraene. MS: m/z (relative intensity): 366 (80, M$^{+\cdot}$), 351 (7, [M−Me]$^+$), 253 (10, [M−side

chain]$^+$), 247 (32), 211 (9), 157 (40), 143 (100), 135 (98), 119 (62), 105 (53), 91 (52), 81 (54).

2.3. Simulation reactions with cholesta-2,4,6-triene (1)

Reactions were carried out using a mixture of 3% (w/v) anhydrous *p*-toluenesulfonic in acetic acid prepared as described by Schüpfer (2000). Cholesta-2,4,6-triene (**1**, 6.6 mg) in 1.2 ml of this mixture was heated to 70 °C under N_2 in a 3 ml vial capped with a Teflon seal. Aliquots were removed, neutralized with saturated $NaHCO_3$ solution and extracted using 3–4 portions of hexane. The extracts were dried over anhydrous $MgSO_4$ and analyzed using GC–MS.

2.4. Synthesis of 14α and 14β (H)-1(10 → 6)-abeo-cholesta-5,7,9(10)-trienes (3a, b)

2.4.1. Cholesta-5,7-dien-3β-ol tosylate (10)

Tosyl chloride (0.95 g, 5 mmol) was added to a solution of cholesta-5,7-dien-3β-ol (**9**; 1.92 g, 5 mmol) in anhydrous pyridine (20 ml) at 0 °C. The mixture was heated to reflux in the dark for 12 h under N_2. The tosylate was crystallized in ice water, filtered and recrystallized in acetone (2.16 g, 80%).

2.4.2. 1(10 → 6)-abeo-14α- and β-Cholesta-5,7,9(10)-trienes (3a, b)

The $CuSO_4/SiO_2$ catalyst was prepared by mixing silica gel (11.25 g, 230–400 mesh) and $CuSO_4$ pentahydrate (3.75 g, 15 mmol), heating at 100 °C to obtain a blue powder and activating the product at 240 °C for 1 h. A solution of cholesta-5,7-dien-3β-ol tosylate (**10**; 800 mg, 1.5 mmol), in a minimum of anhydrous toluene, was added dropwise to a mixture of anhydrous toluene (20 ml) and $CuSO_4/SiO_2$ (2.75 g, 3 mmol $CuSO_4$) and heated under reflux for 4 h. After cooling to room temperature the reaction mixture was filtered through a plug of silica (∅ = 4.2 cm, h = 1.6 cm) and concentrated under vacuum to yield a yellow oil (394 mg, 1.1 mmol, 71%). GC–MS showed that the mixture contained **3b** (62%) and its 14α(H) epimer (**3a**; 7%) which were separated using reversed phase HPLC. ^{1}H NMR analysis enabled distinction of the 14α(H) and 14β(H) MA epimers thanks to the proton shift at C-18. According to Steele et al. (1963), the values for 14α(H) and 14β(H) epimers are approximately 0.6 ppm and 1.05 ppm, respectively (identity con-

firmed by X-ray crystallography; Hanson and Truneh, 1988). (**3b**): ^{1}H NMR: 6.8413 (s, H-7); 1.0515 (s, H-18); 2.1834 (s, H-19); 1.0399 (d, $J = 6.64$, H-21); 0.9399 (d, $J = 6.20$, H-26); 0.9399 (d, $J = 6.20$, H-27). ^{13}C NMR: 138.29; 134.40; 133.74; 132.37; 132.23; 127.53 (C-7); 53.71 (C-17); 52.33 (C-14); 40.75 (C-13); 39.56 (C-24); 36.15 (C-12); 35.61 (C-22); 33.96; 33.91 (C-20); 30.15; 28.88; 28.03 (C-25); 27.26; 24.49 (C-23); 23.84; 23.82; 22.94; 22.82 (C-27); 22.65 (C-18); 22.59 (C-26); 19.72 (C-21); 14.47 (C-19). MS: m/z (relative intensity): 366 (40, M$^{+\cdot}$), 351 [2, (M−Me)$^+$], 253 [9, (M−side chain)$^+$], 212 (52), 211 (100), 199 (41), 197 (31), 169 (22), 159 (17), 157 (12), 143 (11), 91 (9), 73 (12), 55 (24). (**3a**): ^{1}H NMR: 6.646 (s, H-7); 0.608 (3H, s, H-18); 1.006 (3H, d, $J = 6.19$, H-21); 0.890 (3H, d, $J = 6.64$, H-26); 0.895 (3H, d, $J = 6.64$, H-27). ^{13}C NMR: 137.24; 134.08; 133.87; 132.31; 131.66; 124.39 (C-7); 55.24; 51.86 (C-14); 41.85 (C-13); 39.53 (C-24); 37.30 (C-12); 36.21 (C-20); 36.18 (C-22); 30.16; 28.94; 28.04 (C-25); 27.19; 24.23; 23.87; 23.84 (C-23); 22.96; 22.83 (C-27); 22.57 (C-26); 18.81 (C-21); 14.41 (C-19); 11.13. MS: m/z (relative intensity): 366 (40), 351 (2), 253 [9, (M−side chain)$^+$], 226 (36), 225 (9), 213 (33), 212 (52), 211 (100), 209 (8), 207 (21), 199 (41), 198 (13), 197 (31), 195 (9), 185 (11), 183 (12), 172 (12), 169 (22), 159 (17), 157 (12), 155 (21), 143 (11), 141 (8), 73 (12), 69 (12), 57 (14), 55 (24).

2.5. Molecular mechanics calculations

Geometry optimization and calculation of the enthalpy of formation of cholestatriene isomerswere performed using the AM1 semi-empirical method with Polak-Ribiere minimization (*Hyperchem*^{TM} software). Molecular mechanics [MM3, Tripos Inc. MM3 (95) software] and ab initio [DFT (B3LYP-6/31G), Gaussian 03, Revision B.01, Gaussian Inc. software] methods were also tested for optimization and enthalpy calculations of the compounds involved in the rearrangement of cholesta-2,4,6-triene. DFT calculations required considerably greater computer time but the enthalpy results were similar to those obtained with AM1 (within ±2 kcal/mol). Enthalpies of formation from MM3 calculations differed by up to 10 kcal/mol from those calculated with AM1, although the stability order of the different isomers remained the same. Therefore, only the results from AM1 calculations are reported.

3. Results

Fig. 2 shows the GC–MS total ion chromatograms of the starting cholesta-2,4,6-triene (**1**) and the evolution of the acid-catalyzed rearrangement products with reaction time. The starting triene disappears almost completely after 30 min and is not detected after 60 min. After 4 min reaction time, a large portion of (**1**) (∼45%) is replaced by a later eluting (t_R = retention time = 23:36 min) compound (Fig. 4), exhibiting m/z 211 as base peak and abundant molecular ion, known to be characteristic of MAs and MBs (Hussler et al., 1981). From its GC t_R and its spectrum it was assigned as 4-methyl-19-*nor*-cholesta-1,3,5(10)-triene (**2a**). After 10 min, (**2a**) is the major compound (62% of total) in the mixture, along with several other minor components. That at t_R 20.40 min (∼3% of total) has a very similar mass spectrum to that of (**2a**) and has been recognized as its structural isomer, 1-methyl-19-*nor*-cholesta-1,3,5(10)-triene (**2b**) with a significantly shorter t_R (Hussler et al., 1981; Rontani and Aubert, 2004). The component eluting at t_R 21:28 min exhibits a spectrum with abundant ions at m/z 366, 351, 253 (base peak) and 199, closely resembling that of 3,5-cyclocholesta-6,8(14)-diene, previously isolated and characterized in our laboratory (Liu et al., 1996). However, its retention time is noticeably later than that of this cyclosteradiene (t_R 19.10 min). A mass spectral library search (NIST'02 Mass Spectral Database, National Institute of Standards and Technology, Gaithersburg, MD, USA) pointed to cholesta-4,6,8(14) triene, with a mass spectrum with the same characteristics. It should be noted that this component is detectable already after 6 min, reaches its maximum abundance (4.6%) at 10 min and then decreases smoothly in abundance. It is not present after 120 min. Although we are not able to isolate and thoroughly characterize this minor component we believe it to be 14β(H)-cholesta-4,6,8-triene for the reasons discussed in Section 4. The third minor component (∼2.5%) eluting at t_R 24.08 min is a tetraunsaturated steroidal hydrocarbon (M$^{+\cdot}$ m/z 364) and, on the basis of its spectrum [major peaks at m/z 251 (34%, loss of side chain), 209 (33%), 156 (49%), 155 (100%), 142 (61%)], it may be either an analogue of (**1**) with one additional double bond at C-1 or, more probably, an analogue of (**2a**), with a supplementary double bond at C-6. The maximum relative abundance of this component is also observed after 10 min.
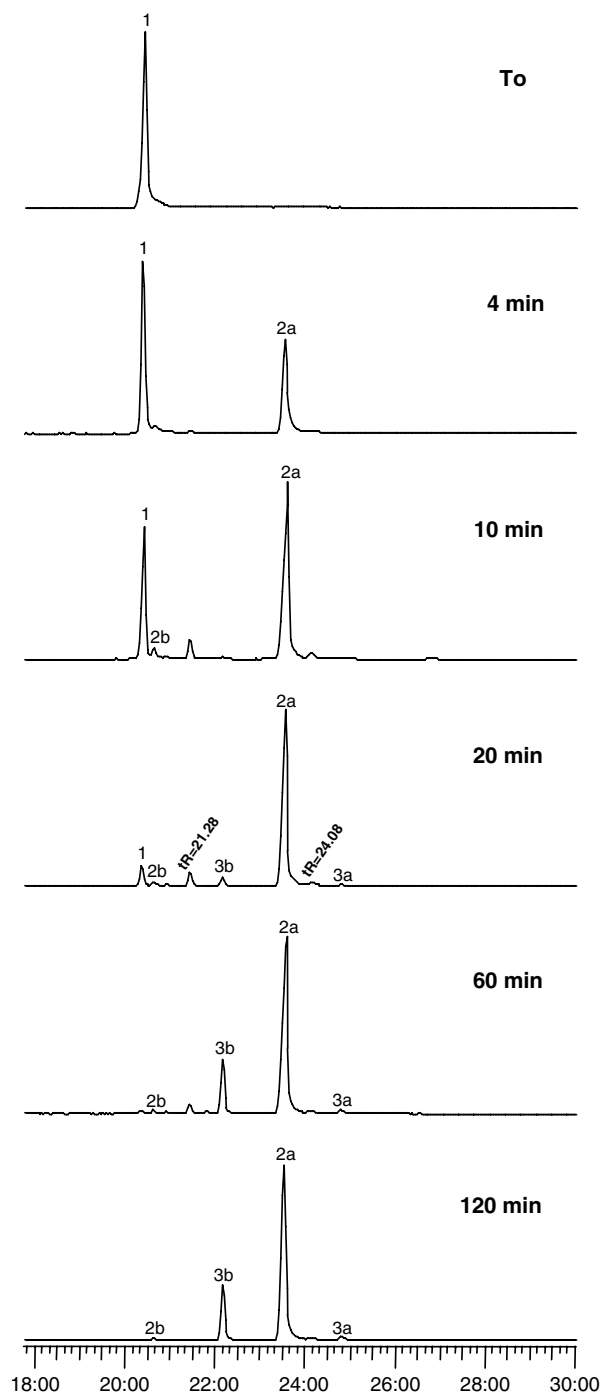
Fig. 4. Chromatograms of the product mixtures from acid catalysed rearrangement of cholesta-2,4,6-triene (**1**) over 4–120 min.

Concurrently with the decrease in (**1**), a new component appeared at t_R 22.10 min in the 12 min reaction mixture (not shown in Fig. 2) and reached its maximum abundance after 120 min. This new com-

ponent could be fully identified as $1(10 \rightarrow 6)$-*abeo*-14β-cholesta-5,7,9(10)-triene (**3b**) by comparison with the data for the authentic standard and its 14α counterpart (**3a**), synthesized and characterized as described in Section 2. Close examination of the GC–MS data for products obtained for reaction times > 20 min revealed that compound (**3a**) is also present in the reaction mixture as a minor component (< 2%, t_R 24.48 min).

The acid catalysed rearrangement of (**1**) seems to reach an equilibrium composition after 120 min, since no noticeable modification was apparent on prolonged reaction times.

4. Discussion

4.1. Formation mechanism of 4-methyl-19-nor-cholesta-1,3,5(10)-triene (2) and $1(10 \rightarrow 6)$-abeo-14β-cholesta-5,7,9(10)-triene (3)

The acid treatment (*p*-TsOH/AcOH at 70 °C) of cholesta-2,4,6-triene (**1**) leads to a large proportion of a MA (**2a**) accompanied with significant amounts of a MB (**3b**). Based on previous work by Kirk and Shaw (1970) and Schüpfer (2000), the formation of these two compounds could be explained by the mechanisms shown in Figs. 5 and 6, respectively. The acid catalyzed isomerisation of cholesta-2,4,6-triene (**1**) occurs via allylic/tertiary carbocations and reversible protonation–deprotonation reactions, as observed with mono- and di-unsaturated steroids (Liu, 1995; Liu et al., 1996; Schüpfer, 2000; Schüpfer and Gülaçar, 2000).

Mechanisms in Figs. 5 and 6 are, however, only partly supported by the relative stabilities of the compounds involved in the rearrangements as calculated with the semi-empirical AM1 method. In Fig. 5, the small difference in ΔH_f^0 (0.95 kcal/mol) between cholesta-1,3,5-triene and cholesta-2,4,6-triene (**1**) can explain the easy interconversion of these trienes, whereas the thermodynamic driving force for the reaction is provided by the low ΔH_f^0 values of the aromatic end products (**2a**) and (**2b**). Indeed, even in pure CDCl₃, almost 15% of (**1**) rearranges to (**2a**) after one week of storage at room temperature, due to traces of HCl in 'aged' solvent. The preferential formation of (**2a**) rather than (**2b**) is consistent with the lower stability of the latter (Fig. 5).

In Fig. 6, the reaction pathway consistently goes to aromatic end products through structures of increasing stability. Epimerization at C-14 may be
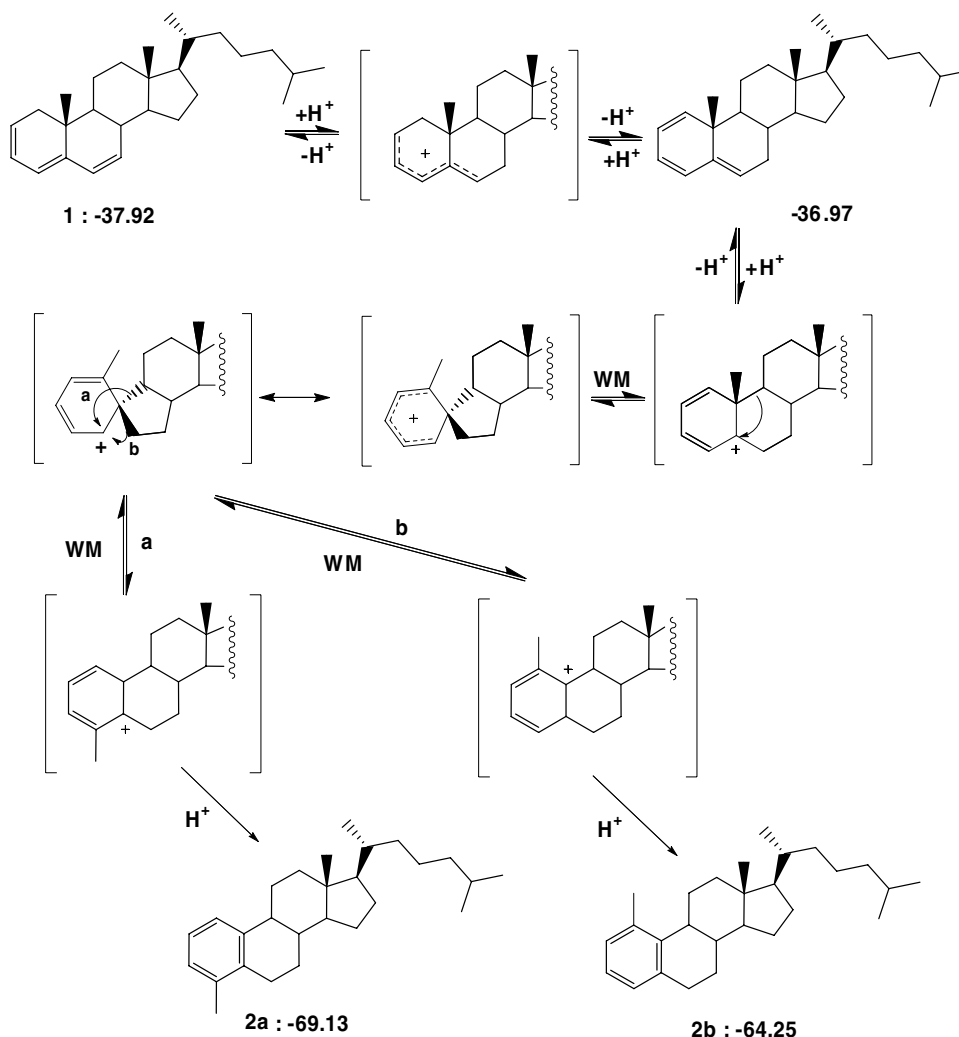
Fig. 5. Proposed formation mechanism of 4-methyl-19-nor-cholesta-1,3,5(10)-triene (**2a**) and 1-methyl-19-nor-cholesta-1,3,5(10)-triene (**2b**) from cholesta-2,4,6-triene (**1**). Calculated enthalpies of formation (in kcal/mol) are given below the structures.

explained by the intermediate trienes with a double bond at C-14, such as cholesta-4,6,8(14)-triene and the spiro compound 1(10 → 5)-*abeo*-cholesta-6,8(14),9-triene. The greater stability of (**3b**) compared to (**3a**), as denoted by their respective enthalpies of formation, could explain why the latter appears only in trace amount in the end products. However, comparison of the thermodynamic data in Figs. 5 and 6 indicates that the formation of MAs and MBs from the acid-catalyzed rearrangement of (**1**) is the result of kinetic and not thermodynamic control, since the major end product (**2a**) is thermodynamically less stable than (**3b**). Examination of the reaction intermediates in Figs. 5 and 6 allows us to identify the rate determining steps as the [1, 2s] alkyl migrations in the cyclohexa-

dienyl cations. Migration of the primary (C-1)–(C-10) bond in the formation pathway of (**3b**) in Fig. 6, compared to the migration of the secondary (C-9)–(C-10) bond in the formation pathway of (**2a**) in Fig. 5, is expected to be less favoured. On this basis, the only intermediate steratriene which may accumulate sufficiently to be observed would be 14β(H)-cholesta-4,6,8-triene in the formation pathway of (**3b**).

4.2. Precursors of MAs and MBs in sediments

Monoaromatic ring A and ring B steroids have been detected in several shallow (Gagosian and Farrington, 1978; Simoneit et al., 1987; Farrington et al., 1988) and immature sediments (Hussler
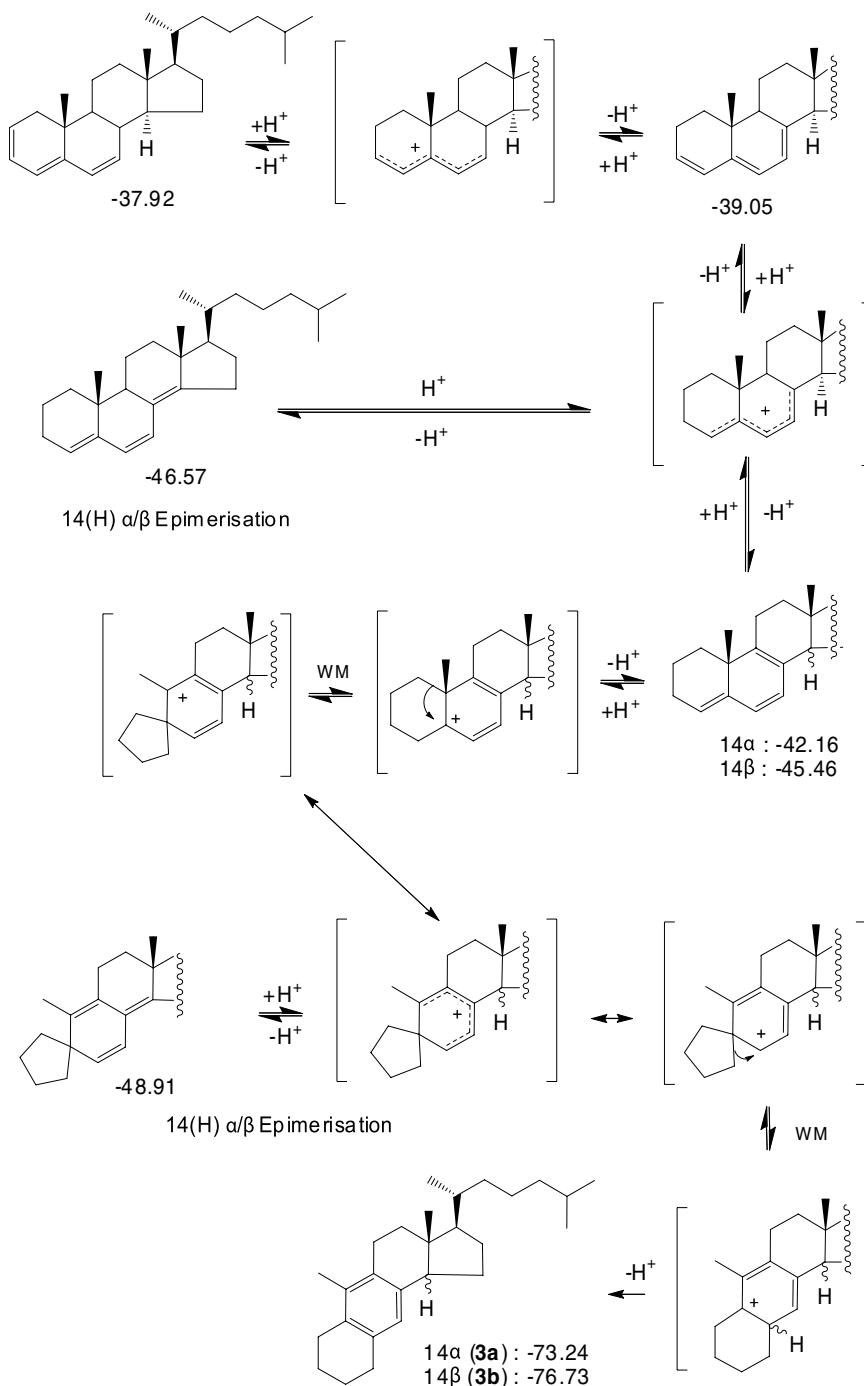
Fig. 6. Proposed formation mechanism of 1(10 → 6)-abeo-14β-cholesta-5,7,9(10)-triene (**3b**) and 1(10 → 6)-abeo-14α-cholesta-5,7,9(10)-triene (**3a**) from cholesta-2,4,6-triene (**1**). Calculated enthalpies of formation (in kcal/mol) are given below the structures.

et al., 1981; Rullkötter et al., 1981; Rullkötter et al., 1982; Hussler and Albrecht, 1983; Rullkötter and Welte, 1983; Brassell et al., 1984; Curiale, 1987; Simoneit et al., 1987; Curiale, 1988; Sinninghe Damsté et al., 1989; Kenig et al., 1995). These

hydrocarbons have been considered as intermediates in the degradation of steroids, restricted to early diagenesis and leading to triaromatic steroids during further thermal maturation (Hussler et al., 1981; Hussler and Albrecht, 1983). Although some

organisms have been found to contain MB sterols (Koshino et al., 1989), they occur too rarely to be considered as possible precursors of sedimentary MBs. Dienes, i.e., $\Delta^{3,5}$-steradienes, have been proposed as potential precursors of MAs and MBs (Mackenzie et al., 1982; Brassell et al., 1984; and references cited therein). This was corroborated by careful examination of concentration profiles of these biomarkers in sediments (Brassell et al., 1984) and by laboratory simulation reactions (Schüpfer and Gülaçar, 2000).

The results we report here shed new light on the precursors of sedimentary MAs and MBs and explain the relative enrichment in sedimentary C_{28} MAs and MBs. MA (**2**) and MB (**3**) are formed from cholesta-2,4,6-triene (**1**) within 20 min reaction whereas they could be detected only after 5 h when cholesta-3,5-diene was treated under the same conditions (Schüpfer and Gülaçar, 2000). Furthermore, all the starting steratriene (**1**) is transformed mainly into MA (**2**) and MB (**3**) in less than 1 h, while cholesta-3,5-diene needs more than 14 days to disappear under the same conditions, with formation of several side products, including ring C aromatic steroids as major components (Schüpfer and Gülaçar, 2000). This shows that the formation kinetics of (**2**) and (**3**) are significantly faster starting from cholesta-2,4,6-triene (**1**) than starting from cholesta-3,5-diene. This is not surprising since the aromatization of steradienes requires an additional oxidation step. MAs and MBs should therefore originate much more rapidly from $\Delta^{5,7}$-sterols via intermediate diagenetic steratrienes than from Δ^5-sterols leading to $\Delta^{3,5}$-steradienes. This is supported by the fact that $\Delta^{5,7}$-sterols have never been reported in sediments presumably because of the instability of the 5,7-diene system. Moreover, ergosterol (24-methylcholesta-5,7,22-trien-3β-ol) in soils and surface sediments, is commonly used as an indicator of living fungal biomass (see, for example, Montgomery et al., 2000; Ruzicka et al., 2000; and references cited therein), based on the assumption that it is rapidly degraded after the death of fungal hyphae.

Finally, in surficial and shallow sediments where MAs and MBs have been detected (Gagosian and Farrington, 1978; Simoneit et al., 1987; Farrington et al., 1988) no MCs were reported. As the rearrangement of cholesta-3,5-diene leads to MA, MB, MC products in similar amounts, while cholesta-2,4,6-triene leads to MA (**2**) and MB (**3**) only, it is reasonable to conclude that in surficial and shallow sediments, MAs and MBs originate exclusively from steratriene precursors. Yet, the most common $\Delta^{5,7}$-sterols (precursors of $\Delta^{3,5,7}$-steratrienes) possess 28 carbon atoms. Thus, ergosterol, a major C_{28} $\Delta^{5,7}$-sterol common in most protozoa and fungi, is the most widespread $\Delta^{5,7}$-sterol (Nes and McKean, 1977; Patterson, 1994; Volkman, 2003; and references cited therein). As a result, sedimentary MAs and MBs will be enriched in C_{28} members compared to other steroidal biomarkers. It should be noted here that photooxidation/autoxidation products of Δ^5-sterols such as ster-5-en-3,7-diols and ster-4-en-3,6-diols, recently identified in sediment trap samples and surface sediments from Mediterranean Sea, have also been proposed as potential sources for sedimentary steratrienes (Rontani and Marchand, 2000; Marchand et al., 2005). In this case, sedimentary MAs and MBs might also be formed readily from Δ^5-sterols in the same way. However, if this process were to provide a significant pool of steratriene precursors, the aromatisation of such a pool would not lead to an enrichment in C_{28} MA and MB members.

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