Rearranged Sterenes in a Shale : Occurrence and Simulated Formation

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The lipids extracted from a bituminous shale contained (20R)- and (20S)-5β,14β-dimethyl-18,19-dinor-8α,9β,10α-cholest-13(17)-enes [(I) and (II) ; R = H]. Also present were homologues carrying nuclear methyl groups and variously alkylated side chains.

Cholestanol has been treated under chemical and mineralogical conditions to yield these backbone-rearranged products. The value of these reactions as simulators of geological diagenesis, and the utility of these sterenes as biological markers in the geological environment is discussed.

THE polycyclic compounds which occur in geological materials, such as coal, shales, and crude oils, can give useful information as to the origin of the organic matter from these sources, as well as the chemical reactions undergone by the original biological material after burial in the geological environment.[1-3] As part of our investigations into the geological fate of natural products, we have studied the occurrence of unsaturated steroidal materials in a bituminous shale from the Paris Basin.

Material excavated from a site at Jouy-aux-Arches (France) is a marine clay-rich sediment deposited 180 million years ago. The shale contains 15% total carbon, and represents one of the more immature parts of the well studied Toarcian Formation.[4]

The non-aromatic unsaturated hydrocarbons extracted from this shale could be separated into two fractions, A and B. Fraction A was shown by g.l.c. to be composed of six components (Table 1), the first of which ran concurrently with synthetic (20R)-5β,14β-dimethyl-18,19-dinor-8α,9β,10α-cholest-13(17)-ene (I; R = H) in four different g.l.c. systems. A g.l.c.–mass spectrometric study of the mixture revealed for peak A1
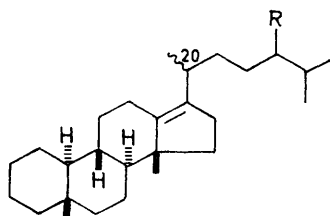
(I)   20 R

(II)   20 S

a fragmentation pattern identical with that of the synthetic material, but clearly different from those of the other synthetic sterene standards.

Peaks A2 and A4 gave analogous fragmentation patterns, with a characteristic base peak at m/e 257 for the tetracyclic nuclear fragment.[5] Peak A2, however, revealed a molecular ion at m/e 384 and an M^+ − 15 ion at m/e 369, and M^+ and M^+ − 15 ions for peak A4 appeared at m/e 398 and 383, respectively. These two components were thus tentatively identified as the side-

chain-alkylated homologues (I; R = Me or Et). Peaks A3, A5, and A6 exhibited mass spectral fragmentation patterns similar to those of peaks A1, A2, and A4 but with their respective M^+ and M^+ − 15 ions 14 mass

TABLE 1

G.l.c. and mass spectral data for fraction A

Peak	t_R *	Proportion (%)	M^+ (5%)	M^+ − side chain from C-17 (100%)	Formula
A1	0.454	16	370	257	$C_{27}H_{46}$
A2	0.575	13	384	257	$C_{28}H_{48}$
A3	0.608	11	384	271	$C_{28}H_{48}$
A4	0.696	17	398	257	$C_{29}H_{50}$
A5	0.774	16	398	271	$C_{29}H_{50}$
A6	0.929	22	412	271	$C_{30}H_{52}$
Minor components		5			

TABLE 2

G.l.c. and mass spectral data for fraction B

Peak	t_R *	Proportion (%)	M^+ (5%)	M^+ − side chain from C-17 (100%)	Formula
B1	0.387	22	370	257	$C_{27}H_{46}$
B2	0.473	14	384	257	$C_{28}H_{48}$
B3	0.530	10	384	271	$C_{28}H_{48}$
B4	0.576	20	398	257	$C_{29}H_{50}$
B5	0.562	15	398	271	$C_{29}H_{50}$
B6	0.776	14	412	271	$C_{30}H_{52}$
Minor components		5			

* Relative retention time on Apiezon L column at 275 °C (isothermal) (cholestane = 1.00).

units higher in each case. Furthermore the peaks at m/e 257 were absent, and were replaced by peaks at m/e 271 (100%). It is thought that this group represents a series of rearranged sterene homologues containing an additional methyl group in the tetracyclic nucleus. The six components of fraction A could not be separated preparatively. However, the n.m.r. spectrum of the mixture revealed resonances for protons on C-21, C-14β, and C-5β and the lack of olefinic proton signals characteristic of the rearranged sterenes of type (I).

Fraction B, similarly examined by g.l.c., g.l.c.–mass spectrometry, and n.m.r. and i.r. spectroscopy revealed

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a series of products (Table 2) corresponding to the analogous rearranged (20S)-sterenes (II) [5] the first of which, B1, corresponded to (20S)-5β,14β-dimethyl-18,19-dinor-8α,9β,10α-cholest-13(17)-ene (II; R = H). Traces of compounds containing a C_8H_{17} side chain, but isomeric with the cholestenes (I) and (II) (R = H),[6] and also with a $C_{11}H_{23}$ side chain (R = Pr),[7] were noted, but no compound containing a C_7H_{15} side chain (i.e. a C_{26} sterene) [8] was observed.

Since rearranged sterenes have not been reported to occur in living organisms, and have only been obtained previously by chemical synthesis in the laboratory,[5,9] it is probable that their occurrence in Jouy shale results from alteration of naturally occurring sterols in the geological environment. Stanols have been suggested as being the primary diagenetic products from naturally occurring Δ^5-sterols in young muds,[10] and stanols and Δ^2-sterenes have also been observed in several recent sediments.[11] It was thus of interest to study the chemical formation of the rearranged sterenes from stanols and sterenes in the hope of establishing a pathway for their geological formation from steroids known to occur in living organisms.

Turner et al.[12] have reported the rearrangement of cholest-5-ene and cholest-2-ene to form a ' Westphalen ' Δ^9-product. More recently, it has been shown that cholest-5-ene and cholest-4-ene undergo a backbone rearrangement in vitro in acidic medium to yield 5β,14β-dimethyl-18,19-dinor-8α,9β,10α-cholest-13(17)-ene,[9] as a mixture of the (20R)- (I) and (20S)- (II) isomers.[5] With cholest-2-ene under the same conditions we have found that the same two products are formed, i.e. the $\Delta^{13(17)}$ rather than the Δ^9-compounds as assigned by Turner et al.[12] The yields were low, typically 2—3%, owing to the addition of acetic acid across the 2,3-double bond.[12,13]

Similar treatment of cholestan-3β-ol produced the same (20R)- (I) and (20S)- (II) (R = H) rearranged sterenes, again in 2—3% yield, indicating that this rearrangement of cholestanol proceeded via the cholest-2-ene intermediate. Indeed, reducing the reflux time from 2 to 0.5 h made possible the isolation of some cholest-2-ene. This was not surprising, since the dehydration of 3β-hydroxy-sterols to Δ^2- and Δ^3-sterenes is extremely ready [14] and thence the rearrange-

ment may easily occur. The main disadvantage of this rearrangement of cholestanol was the low yield, attributed to the addition of acetic acid across the 2,3-double bond.

Replacing the acetic acid–toluene-p-sulphonic acid reaction mixture by a solid-phase mineral catalyst obviated this problem, and yields in the region of 20% were noted. The catalyst chosen was the naturally occurring montmorillonite, a silicoaluminate which is frequently found in recent geological sediments, and has a demonstrable catalytic activity towards organic reactions.[15,16] This activity is thought to be due to ' active acid sites ' on the mineral surface.[17] Heating this activated clay with cholestanol for 16 h at 146 °C yielded 50% of unchanged starting material, along with significant amounts of both (20R)- and (20S)-5β,14β-dimethyl-18,19-dinor-8α,9β,10α-cholest-13(17)-ene [(I) and (II); R = H] isomers. Shorter periods of heating resulted in an increased proportion of intermediate cholestenes, identified as the Δ^2-, Δ^4-, and (tentatively) Δ^5-isomers. This indicated that the pathway of formation of the rearranged sterenes from cholestanol under mineral catalysis included dehydration and carbocation migration similar to that postulated in the liquid-phase reaction.[5,9] Increasing the temperature resulted in the appearance of larger amounts of polar materials, possibly sterol breakdown or polymerisation products and aromatic compounds. Saturated and aromatic cholestane analogues (tentatively identified) were always produced in the mineral-catalysed rearrangements of cholestanol, indicating the occurrence of a certain degree of dismutation simultaneous with the rearrangement (cf. ref. 18).

The thermal alteration of lipids in the presence and absence of catalysts has been extensively studied.[19] Heating of sediments and kerogens is known to cause the fragmentation of organic polymers, yielding low molecular weight hydrocarbons.[20] Simulated maturation experiments involving sterols and clays have indicated a variety of products, including petroleum-like hydrocarbons,[21] steranes,[22] and sterenes.[18] Rhead et al.[18] have shown that the heating of radioactively labelled cholesterol with Green River Shale produces steranes and Δ^4- and Δ^5-sterenes in high yields. No rearranged

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sterenes were reported, presumably because this shale was not as 'acidic' as montmorillonite or Jouy shale.

Since sterenes, accompanied by similar aromatic and saturated sterane analogues, have been observed to occur in a number of bituminous shales, including this sample from Jouy and also samples from Fecocourt and Aquitaine (France) and Green River Shale (U.S.A.),[23] it is possible that such simulated diagenesis experiments using mineral catalysts may give valuable information as to the geological fate of natural steroidal compounds.

Current investigations are being directed towards a closer analysis of the side-chain alkylations [24] and the nuclear methyl groups,[25] to determine the organisms responsible for the formation of the parent sterols. It is hoped that such a study of the steroidal material occurring in geological sources may be useful in the quest for a 'biological marker' system,[1] which may be used to trace the transformation and movement of bitumens and crude oils within the geological environment.

EXPERIMENTAL

T.l.c. was performed on silica gel G, with or without a 10% silver nitrate content, and with petroleum (b.p. 60—80°) as developing solvent. N.m.r. spectra were recorded by Mme E. Krempp on a Perkin-Elmer R12B apparatus (60 MHz) for solutions in deuteriochloroform with Me_4Si as internal standard. I.r. spectra were determined on a Perkin-Elmer 177 apparatus and u.v. spectra on a Varian Cary 118 instrument. M.p.s were determined on a Kofler hot-stage apparatus. G.l.c. was carried out on a modified Perkin-Elmer F17 (FID) machine equipped with all-glass 2 m × 2 mm columns packed with 1% Dexsil 300 or 1% SE 30 on Gaschrom Q. Helium carrier gas was maintained at a flow rate of 45 ml min^{-1}. Coinjections of natural and synthesised products were performed on this apparatus equipped with metal capillary columns (25 m × 0.5 mm) whose walls were coated with Apiezon L or OV 101. The carrier gas flow rate in these cases was 2 ml min^{-1}. Experiments with synthesised materials revealed no appreciable breakdown of the sterenes on the metal columns. G. Teller and R. Hueber recorded the mass spectra on an LKB 9000 machine, either by direct injection or by g.l.c.–mass spectrometer coupling (with the above columns). The ionisation voltage was 70 eV. All reagents and solvents were purified to exclude contaminating hydrocarbons (checked by analysis) before use.

Syntheses of 5β,14β-*Dimethyl*-18,19-*dinor*-8α,9β,10α-*cholest*-13(17)-*ene*.—Synthesis from cholest-5-ene by the method of Blunt *et al.*[9] gave a yield of 50%. From cholestan-3β-ol and cholest-2-ene, yields were 2—3%. Separation by t.l.c. (SiO_2-$AgNO_3$) gave about equal proportions of the (20R)- (I) and (20S)- (II) (R = H) isomers as viscous oils.

The *sterene* (I; R = H), R_F 0.20, showed $\nu_{max.}$ 2 930, 1 450, and 1 380 cm^{-1}; δ 0.85 (6 H, d, 26- and 27-H_3), 0.84 (3 H, s, 5β-Me), 0.90 (3 H, s, 14β-Me), and 0.95 (3 H, d, 21-H_3); m/e 370 (6%, M^+), 355 (21%, $M^+ - 15$), 285 (2%), and 257 (100%, $M^+ -$ side chain from C-17). The *sterene* (II; R = H), R_F 0.35, showed $\nu_{max.}$ 2 930, 1 450, and 1 380 cm^{-1}; δ 0.85 (6 H, d, 26- and 27-H_3), 0.84 (3 H, s, 5β-Me), 0.90 (3 H, s, 14β-Me), and 0.89 (3 H, d, 21-H_3); m/e 370 (7%, M^+), 355 (24%, $M^+ - 15$), 285 (2%), 257 (100%, $M^+ -$ side chain from C-17), and 206 (4%).

Other Standard Cholestenes.—The Δ^2-,[26] Δ^3-,[27] Δ^4-,[28] Δ^5-,[27] Δ^7-,[27] $\Delta^{8(14)}$,[29] Δ^{14}-,[30] and Δ^{24}-[31] cholestenes were synthesised, and all exhibited the expected physical and chemical characteristics, different from those of the above rearranged sterenes.

Extraction of Jouy Shale.—Jouy rock number 10 696 from the Institut Français du Pétrole (2.7 kg) was washed with chloroform, finely powdered, and extracted (Soxhlet) with petroleum (b.p. 60—80°). After decantation of sulphur (4.4 g), the oil (3.7 g) was chromatographed on a silica column (220 g). The hydrocarbons (1.3 g) were eluted in the first petroleum (2 l) fraction.

The hydrocarbons were separated on a SiO_2-$AgNO_3$ column (150 g). The first petroleum (1.5 l) fraction contained saturated material (800 mg); subsequent elution with diethyl ether (2 l) yielded unsaturated hydrocarbons (500 mg). The latter were subjected to preparative t.l.c. (SiO_2) which separated sterenes (90 mg; R_F 0.9—1.0) and aromatic materials (410 mg; R_F 0.0—0.9). Preparative t.l.c. ($AgNO_3$-SiO_2) of the sterenes resulted in only two bands, fractions A (50 mg) and B (40 mg). These were isolated as viscous oils, and could not be separated preparatively into their components. There was no u.v. absorption.

Fraction A corresponded to the (20R)-sterene mixture (I), R_F 0.20; $\nu_{max.}$ 2 930, 1 450, and 1 380 cm^{-1}; δ 0.85 (6 H, d, 26- and 27-H_3), 0.84 (3 H, s, 5β-Me), 0.90 (3 H, s, 14β-Me), 0.95 (3 H, d, 21-H_3), and 0.71, 0.76, and 0.87 (additional side chain and nuclear alkyl groups); m/e (g.l.c.–mass spectrum) 370, 384, 398, 412, or 426 (5%, M^+ for alkylated homologues), 355, 369, 383, 397, 411 (20%, $M^+ - 15$), 285 (2%), and 257 (100%, $M^+ -$ side chain from C-17; replaced by m/e 271 for nuclear methyl homologues) (Table 1).

Fraction B corresponded to the (20S)-sterene mixture (II), R_F 0.35; $\nu_{max.}$ 2 930, 1 450, and 1 380 cm^{-1}; δ 0.85 (6 H, d, 26- and 27-H_3), 0.84 (3 H, s, 5β-Me), 0.90 (3 H, s, 14β-Me), 0.89 (3 H, d, 21-H_3), and 0.70, 0.74, and 0.86 (additional side chain and nuclear alkyl groups); m/e (g.l.c.–mass spectrum) 370, 384, 398, 412, or 426 (5%, M^+ for respective alkylated homologues), 355, 369, 383, 397, 411 (20%, $M^+ - 15$), 285 (2%), 257 (100%, $M^+ -$ side chain from C-17, replaced by m/e 271 for nuclear methyl homologues), and 206 (4%) (Table 2).

Preparation of the Clay Catalyst.—Montmorillonite from Camp-Berteaux (Morocco) was purified as described by Sieskind and Ourisson [32] and activated at 120 °C.

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Solid-phase Mineral Catalytic Rearrangement of Cholestanol. —Blank tests were made under identical conditions but in the absence of clay. The sole product was the starting material. Freshly activated clay (100 mg) and purified cholestanol (30 mg) were ground together in a mortar, and sealed under vacuum in a 10 ml Pyrex vial. After heating at 146 °C for 16 h, the vial was cooled and opened, and the organic material extracted by agitation four times with benzene at room temperature. Starting material (15 mg) was removed by t.l.c., and the hydrocarbon material separated by t.l.c. (SiO_2–$AgNO_3$). The (20R)- (5 mg) and (20S)- (1 mg) 5β,14β-dimethyl-18,19-dinor-8α,9β,10α-cholest-13(17)-ene displayed t.l.c., g.l.c., and i.r., mass, and n.m.r. spectral characteristics identical with those of the authentic materials described above.

The remaining compounds were eluted from the t.l.c. plate, and identified as follows: 5α-cholest-2-ene (2 mg), R_F 0.21; m.p. and mixed m.p. 70—72°; $\nu_{max.}$ 2 930, 1 460, 1 380, 960, 825, and 800 cm^{-1}; δ 0.67 (3 H, s, 18-H$_3$), 0.77 (3 H, s, 19-H$_3$), 0.87 (6 H, d, 26- and 27-H$_3$), 0.93 (3 H, d, 21-H$_3$), and 5.64 (2 H, m, 2- and 3-H); m/e 370 (100%, M^+), 355 (36%, M^+ − 15), 316 (90%), 257 (22%, M^+ − side chain from C-17), and 215 (80%); cholest-4-ene (2 mg), R_F 0.35; m.p. and mixed m.p. 77—79°; $\nu_{max.}$ 2 930, 1 460, 1 380, and 955 cm^{-1}; δ 0.68 (3 H, s, 18-H)$_3$, 0.85 (6 H, d,

26- and 27-H$_3$), 0.89 (3 H, d, 21-H$_3$), 1.00 (3 H, s, 19-H$_3$), and 5.2—5.4 (1 H, m, 4-H); m/e 370 (100%, M^+), 355 (34%, M^+ − 15), 257 (22%, M^+ − side chain from C-17), and 215 (30%); cholest-5-ene (trace), R_F 0.40; m/e (g.l.c.–mass spectrum) 370 (100%, M^+), 355 (65%, M^+ − 15), 301 (24%), 275 (26%), 257 (36%, M^+ − side chain from C-17), and 215 (56%); 5α-cholestane (trace), R_F 1.0; m/e (g.l.c.–mass spectrum) 372 (36%, M^+), 357 (22%, M^+ − 15), 217 (100%), 151 (28%), and 149 (57%); [33] 5β-cholestane (trace), R_F 1.0; m/e (g.l.c.–mass spectrum) 372 (35%, M^+), 357 (25%, M^+ − 15), 217 (100%), 151 (38%), and 149 (27%); [33] aromatic products (trace), $\lambda_{max.}$ 205, 224, and 236 nm; m/e (g.l.c.–mass spectrum) 366 (80%, M^+), 351 (35%, M^+ − 15), 281 (20%), and 253 (60%, M^+ − side chain from C-17).

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