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The Occurrence of Nuclear Methylated Steranes in a Shale

By Ian Rubinstein and Pierre Albrecht*

(*Laboratoire de Chimie Organique des Substances Naturelles Associé au C.N.R.S., Institut de Chimie, Université Louis Pasteur, 1, rue Blaise Pascal, 67008-Strasbourg, France*)

Summary 4β-methyl steranes have been identified along with 4α-methyl and 4-demethyl homologues in a bituminous shale.

As part of our programme of investigations of the geological fate of natural products we have studied the occurrence of saturated steroidal hydrocarbons in a bituminous shale from the Paris Basin. Material excavated from a quarry at Jouy-aux-Arches (France) is a marine clay-rich sediment deposited 180 million years ago, and represents one of the more immature parts of the Toarcian Formation. The saturated hydrocarbons (800 mg from 2·7 kg of rock) were extracted as previously described.[1] After adduction into 5A molecular sieves and urea, the resulting oil (430 mg) was vacuum distilled (120 °C, 2 mm Hg) and the non-volatile fraction was treated with thiourea.[2]

The thiourea adduct (85 mg) could not be further separated. Analysis by g.l.c. and g.l.c.-m.s. revealed nine major components in this mixture (Table). Compounds (**1**), (**3**), and (**6**) were identified as cholestane, 24-methylcholestane and 24-ethylcholestane respectively, against authentic standards, by g.l.c. co-elution on three stationary phases (Apiezon L, Dexsil 300 and OV 101) and by comparison of m.s. fragmentation patterns.

Compounds (**2**) and (**4**) were identified as 4α-methyl and 4β-methyl cholestanes respectively by comparison with authentic standards synthesised from known 4α- and 4β-methylcholest-5-en-3β-yl acetates[3] by the following reaction sequence; hydrogenation over platinum oxide, saponification of the acetate, treatment of the alcohol with tosyl chloride and reduction of the tosylate with lithium aluminium hydride. The mass spectra of the natural compounds were superimposable on those of the synthetic materials, and clearly differentiable from the mass spectra of synthetic 1α-, 2β-, 3α-, 3β-, 6α-, 6β-, 7α- and 7β- methylcholestanes made for comparison.

Compounds (**5**) and (**8**), were identified as the 4α-methyl homologues of 24-methylcholestane and 24-ethylcholestane respectively from m.s. data, likewise compounds (**7**) and (**9**) as their 4β-derivatives, alkylation at C-24 being assumed on biogenetic grounds. Current investigations are being directed towards the determination of the absolute stereochemistry at C-24, which can give important information on the nature of the organisms responsible for the formation of the parent sterols.[4]

It was noted that the proportions of the side-chain unalkylated and 24-methyl steranes were similar to those found in the corresponding rearranged sterenes.[1] The 24-ethyl steranes were, however, present in smaller amounts relative to the 24-ethyl sterenes, perhaps reflecting the lower efficiency of adduction into thiourea of 24-ethyl steranes.

Sterols with 4-methyl substituents have been previously identified in a geological sample,[5] and nuclear methylated steroidal hydrocarbons have also been reported.[1,6] However, 4β-methyl tetracyclic compounds have not previously been observed. This occurrence of a large proportion of 4-methyl steroids relative to their 4-demethyl homologues, and in particular the thermodynamically less stable 4β-isomers, poses an interesting problem as to their origin.

Studies of present-day organisms have revealed the existence of large numbers of 4α-methyl sterols, but in small amounts compared to the quantities of 4-demethyl sterols.[7] 4β-Methyl sterols are rarely reported to occur in nature,[8] and their biosynthetic significance is in doubt.[9] Steranes are known to be formed by the diagenesis of naturally occurring sterols on clays and sediments.[1] We propose two possibilities to account for the occurrence of the

TABLE

G.l.c. and m.s. data for the sterane fraction of the bituminous shale from Jouy-aux-Arches (France).

Peak	R.r.t.[a]	% of total steranes	M^+	Ring D fragmentation[b]	Base peak	Formula	Compound
1	1·00	18	372 (30%)	217 (100%)	217	$C_{27}H_{48}$	cholestane
2	1·23	7	386 (30%)	231 (100%)	231	$C_{28}H_{50}$	4α-methylcholestane
3	1·32	12	386 (30%)	217 (100%)	217	$C_{28}H_{50}$	24-methylcholestane
4	1·39	12	386 (30%)	231 (75%)	123	$C_{28}H_{50}$	4β-methylcholestane
5	1·58	7	400 (30%)	231 (100%)	231	$C_{29}H_{52}$	4α,24-dimethylcholestane
6	1·59	6	400 (30%)	217 (100%)	217	$C_{29}H_{52}$	24-ethylcholestane
7	1·81	16	400 (30%)	231 (75%)	123	$C_{29}H_{52}$	4β,24-dimethylcholestane
8	2·13	5	414 (30%)	231 (100%)	231	$C_{30}H_{54}$	4α-methyl-24-ethylcholestane
9	2·22	14	414 (30%)	231 (75%)	123	$C_{30}H_{54}$	4β-methyl-24-ethylcholestane

Minor components, 3%.

[a] R.r.t. = relative retention time on Apiezon L column (25 m × 0·5 mm wall coated) at 275 °C isothermal. Cholestane = 1·00.
[b] Intensities relative to base peak.

4α- and 4β-methyl steranes in the Jouy shale; (a) the organisms responsible for the biosynthesis of the parent sterols at the time of deposition (180 million years ago) no longer exist at the present day or have remained hitherto undetected; (b) a selective loss of the 4-demethyl steroids has occurred, resulting in a relatively high concentration of the 4-methylated compounds.

It was noted that the rearranged sterenes found in this shale[1] contained only one nuclear methylated series. This is perhaps due to the formation of the more stable isomer during the course of the double bond migration in the diagenesis of the original sterols. It is also possible that 4β-methylsterenes do not backbone-rearrange, and hence build up in the remaining steranes, because the 4β-methyl–19-methyl interaction prevents either methyl from migrating on protonation of the Δ^5 bond.

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