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C_{26}-C_{29} TRIAROMATIC STEROID DERIVATIVES IN SEDIMENTS AND PETROLEUMS.

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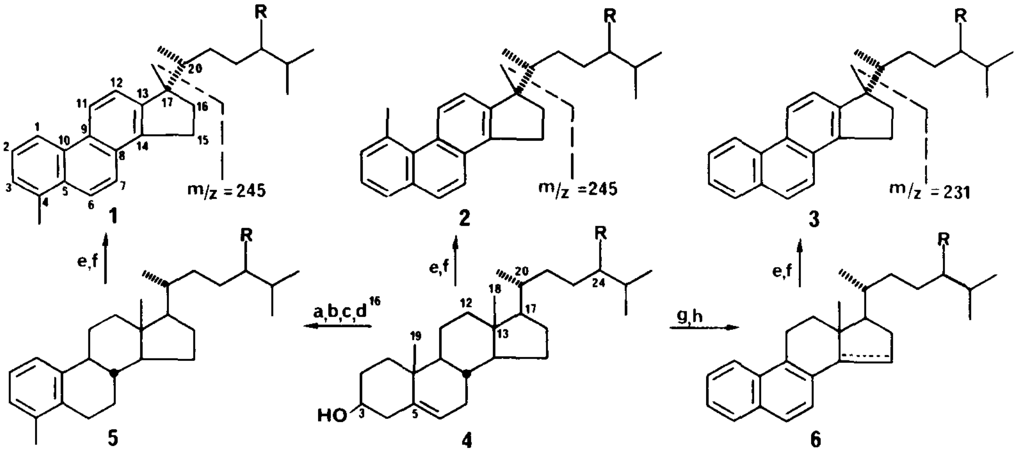
Summary : The C_{26}-C_{29} cyclopentenophenanthrenes 1 - 3 *have been identified as widespread constituents of sediments and petroleums. They are long term degradation products of sterols in the subsurface.*

Aromatisation is a commonly occurring process in the subsurface[1,2]. Some of the aromatisation reactions seem to be induced by microorganisms at a very early stage of diagenesis[3,4]. In a later stage of maturation, including the principal zone of petroleum formation, aromatisation continues mostly *via* hydrogen transfer reactions in the sediments which act as a closed system [1]. Although several studies have enabled the elucidation of various aspects of the early geochemical transformations of steroids[5-7], little attention has been paid to the aromatic degradation products of these molecules in the subsurface since the identification of Diels' hydrocarbon in petroleum[8]. Preliminary studies, mostly based on data obtained by mass spectrometry or coupled gas chromatography-mass spectrometry from the complex aromatic fractions of sediments and crude oils, have shown the presence of major series of mono-, di- and triaromatic steroid molecules probably derived from precursor sterols of living organisms such as phytoplankton[9-14]. The distribution of these geochemical markers has been increasingly used as a fingerprint for the study of the maturation of the organic matter of sediments[15], as well as for the correlation of crude oils and source rocks[12] (no proof of structures).

We have now elucidated the structure of several series of triaromatic steroids 1 - 3 in sediments and crude oils by comparison with authentic standards.



The aromatic hydrocarbons of sediment extracts and crude oils were separated by column chromatography and/or thin layer chromatography (SiO_2)[17]. The mono- + di-, tri-, tetra- and pentaaromatic fractions were obtained by high performance liquid chromatography (HPLC ; 10µ SiO_2; heptane), based on the retention times of references (naphthalene, phenanthrene, chrysene and 1,2,6,7-dibenzanthracene). As an example, 250 g of shale from the Lower Toarcian formation of the Paris Basin (Creveney) yielded 125 mg of total aromatic hydrocarbons, which were subdivided into 38% mono- + di-, 29% tri-, 23% tetra- and 10% pentaaromatic compounds. Further separations of the triaromatic fractions were carried out in several cases by reverse phase HPLC (RP 18, Merck, 10µ ; CH_3OH/H_2O gradient) in which the retention times of the authentic standards were used to collect purified fractions from the geological samples. This procedure was quite useful for obtaining good mass spectra of the geological compounds.

The aromatic fractions from sediments and petroleums are quite complex and require for their analysis the use of computerized gas chromatography - mass spectrometry. Extensively occurring series derived from steroid precursors display major fragmentations at m/z = 245 and 231 corresponding to the loss of the side chain of the steroid molecules at a benzylic position[13,15]. These specific cleavages can then be used for the study of the distribution of the two classes of compounds in the complex aromatic mixtures by mass fragmentography monitoring the 245 and 231 fragment ions (Figure 1).

The cyclopentenophenanthrenes 1 and 2 (R = H ; C_2H_5), as well as 3 (R = H) were prepared following previously described procedures. Compounds 1 (R = H ; C_2H_5) were obtained from cholest-5-en-3β-ol (cholesterol) 4 (R=H) and 24(R)-ethyl-cholest-5-en-3β-ol (sitosterol) 4 (R = C_2H_5) *via* the ring A monoaromatic components 5[18,19], which were further dehydrogenated with chloranil in refluxing xylene[20]. Purification by chromatography (SiO_2), followed by reduction of the 15(16) double bond (Pd/C) led to the C_{27} and C_{29} steroid derivatives 1. Compounds 2 (R = H ; C_2H_5) were obtained by direct dehydrogenation of cholesterol and sitosterol with chloranil in refluxing anisole, followed by chromatographic purifications (Al_2O_3 ; SiO_2) and subsequent hydrogenation of the 15(16) double bond[21]. Compound 3 was prepared in two stages. In the first step cholesta-3,5-diene was oxidised with phenanthrenequinone in refluxing anisole. This reaction led to a mixture of diaromatic compounds 6 having lost the C-19 angular methyl group[22]. Further oxidation of these intermediates with chloranil in refluxing xylene led to a cyclopentadienophenanthrene[21], which was purified by chromatography (SiO_2); subsequent hydrogenation of the 15(16) double bond led to 3. It is noteworthy that position 20 in the steroid skeleton is not epimerised in these oxidation reactions[23].

The data for the final compounds were in agreement with those of the literature (1 and 2, R = H[20,21]). The final structures were confirmed by proton magnetic resonance and mass spectrometry.

1 R=H Nmr (250 MHz; $CDCl_3$; δppm, J Hz) : 0.73(3H,d,J=6.8); 0.87(6H,d,J=6.5); 1.34(3H,s); 2.31(2H,m); 2.75(3H,s); 3.29(2H,m); 7.50-8.57(7H,m). MS (70eV): m/z = 358 (M^+,4%); 245($M^+-C_8H_{17}$,100%); 230($M^+-C_8H_{17}-CH_3$, 8%); 215($M^+-C_8H_{17}-2CH_3$,8%).

R=C_2H_5 Nmr (90 MHz, $CDCl_3$) : 0.75(3H,d,J=6.5); 0.86(3H,t,J=6.5); 0.87(6H,d,J=6.5); 1.33(3H,s), 2.72(3H,s); 3.30(2H,m); 7.30-8.52(7H,m) MS (70eV) : m/z = 386(M^+,4%); 245($M^+-C_{10}H_{21}$, 100%); 230($M^+-C_{10}H_{21}-CH_3$,7%); 215($M^+-C_{10}H_{21}-2CH_3$,7%).

2 R=H Nmr (250 MHz, $CDCl_3$): 0.78(3H,d,J=6.8); 0.91(6H,d,J=6.8); 1.38(3H,s); 2.34(2H,m); 3.17 (3H,s); 3.29(2H,m); 7.50-8.72(7H,m). MS : m/z = 358(M^+,4%); 245($M^+-C_8H_{17}$,100%); 230($M^+-C_8H_{17}-CH_3$,10%); 215($M^+-C_8H_{17}-2CH_3$, 10%).

$R=C_2H_5$ Nmr (250 MHz, $CDCl_3$): 0.75(3H,d,J=6.8); 0.84(6H,d,J=6.8); 0.86(3H,t,J=7.6); 1.35(3H,s); 2.31(2H,m); 3.13(3H,s); 3.28(2H,m); 7.44-8.78(7H,m). MS : m/z = 386(M^+,3%); 245(M^+ $-C_{10}H_{21}$,100%); 230($M^+-C_{10}H_{21}-CH_3$,7%); 215($M^+-C_{10}H_{21}-2CH_3$,7%).

3 R=H Nmr (250 MHz, CCl_4): 0.74(3H,d,J=6.5); 0.88(6H,d,J=6.5); 1.33(3H,s); 2.29(2H,m); 3.23 (2H,m); 7.43-8.58(8H,m).MS : m/z = 344(M^+,4%); 231($M^+-C_8H_{17}$,100%); 216($M^+-C_8H_{17}-CH_3$,8%)

Compounds 1, 2 (R=H ; C_2H_5) and 3 (R=H) were identified with those occurring in complex mixtures in geological samples[24] by following criteria : comparison of mass spectra and coelution, in single ion mass fragmentography, on two or three phases in all glass capillary column GC-MS (SE 30 or OV 1 ; SP 2250, Supelco, Pluronic F68, Fluka, 35 or 25 m x 0.25 mm). 1 and 2 ($R=CH_3$), and 3 ($R=CH_3$; C_2H_5) were tentatively identified on the basis of their mass spectra and retention times (Figure 1). The diastereomers at position 20 may contribute to the complexity of the fragmentograms, since steranes and sterenes undergo epimerization at this position during maturation in sediments[25-28]

Triaromatic steroid derivatives have not so far been detected in significant amounts in recent sediments, which indicates that their formation obviously takes place in sediments at a later stage of diagenesis *via* hydrogen transfer reactions. Although the mechanism of these reactions is not clear, clay minerals may play an important role as catalysts[28]. Aromatisation of the steroid skeleton may either start in ring A in immature samples[29] or, after a backbone rearrangement[28], in ring C, depending on the conditions prevailing in the sediments. Further aromatisation of monoaromatic steroids may then take place during the maturation of sediments and follow pathways A → C or C → A with rearrangement or loss of the C-19 methyl group to lead to compounds 1-3. 1 may also arise from 4-methyl steroids by loss of C-19 methyl

The identification of mono- and diaromatic intermediates, which are also widespread in sediments and petroleums[13], is in progress. From these results and from our preliminary data on monoaromatic steroids, it seems however clear that in the major series of ring C monoaromatic compounds the C-18 methyl group of the precursor sterols is displaced from position 13 to position 17, and not to position 12 on the aromatic ring, as previously indicated[14].

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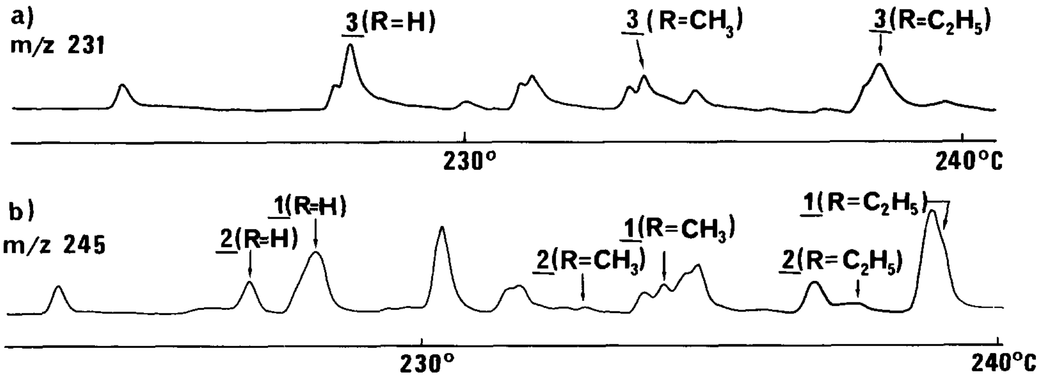

Figure 1 Single ion mass fragmentograms 231 and 245 from the triaromatic fraction of a Lower Toarcian shale (Créveney, Paris basin). GC-MS LKB 9000 S; SP 2250, 35m x 0.25mm ; 130-240°C, 2°C/min.(a) and 1°C/min (b), UV recorder 1 and 3 are major series.

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