3β-Carboxysteranes, a Novel Family of Fossil Steroids

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Two 3β-carboxy-24-ethylcholestanes (24*R* and 24*S*) have been isolated as a mixture from a carbonated sediment and characterised by comparison of their mass spectrometric and ^{1}H NMR spectroscopic data with those of synthetic standards; their origin is still unknown, since their carbon skeleton has not yet been reported in living organisms.

The steroid skeleton occurs widely in sediments and crude oils; as a result of the information content inherent in structures and their often specific occurrence in organisms, steroids are extremely useful for geochemical purposes, in particular for correlating petroleums with their source-rocks. They also yield interesting information on the origin of geological organic matter and its thermal maturation, as well as on paleoenvironmental conditions and paleoecological relationships.[1—3] Among the methyl steroid group, the 4α-methyl compounds, which originate mainly from dinoflagellates,[4—5] are the most commonly encountered components in geological samples. Recently, two novel series of methyl steroid hydrocarbons, the 2α- and 3β-methyl steranes, have been identified in sediments and petroleums,[6] but their origin and their precursors are still unknown. We wish to report here the first characterisation of two 3β-carboxy-24-ethylcholestanes (**1**) and (**2**) (Figure 1), isolated from an ancient, immature, carbonated sediment (marl; Eocene–Oligocene) that belongs to the potash basin in south Alsace, France.

The organic matter was extracted from the pulverised rock (1500 g; 1.9% total organic carbon) with a mixture of chloroform : toluene : methanol (3 : 3 : 2). The acid fraction of the extract was obtained by separation from the neutral and the polar fractions on a potassium hydroxide impregnated silica gel column[7] and then esterified with CH_2N_2. Analysis of the ester fraction by gas chromatography–mass spectrometry revealed the presence of a prominent peak which, from its MS fragmentation pattern, could be tentatively attributed to a C_{29} sterane skeleton bearing an extra carboxylic group on ring A or B (only minute amounts of lower homologues could be detected). In order to isolate and characterise this novel component, the ester fraction was further fractionated by TLC and then by reverse-phase HPLC [RP-18; elution with methanol : chloroform (85 : 15)]. By using this procedure we obtained a small fraction (0.5 mg) which displayed one homogeneous peak in HPLC and GC.

The mass spectrum exhibited an important molecular ion at m/z = 458 corresponding to $C_{31}H_{54}O_2$ and a base peak at m/z = 275, with another important peak at m/z = 207. The ^{1}H

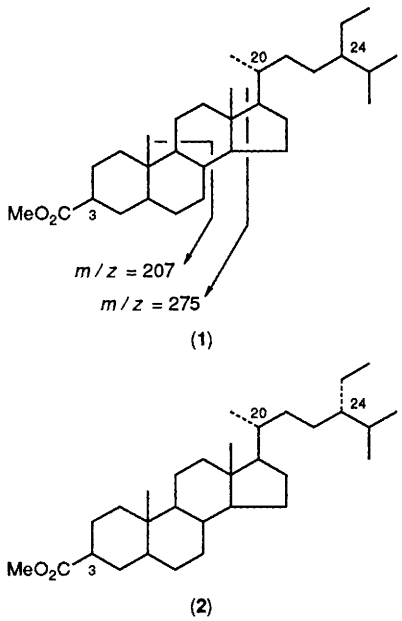


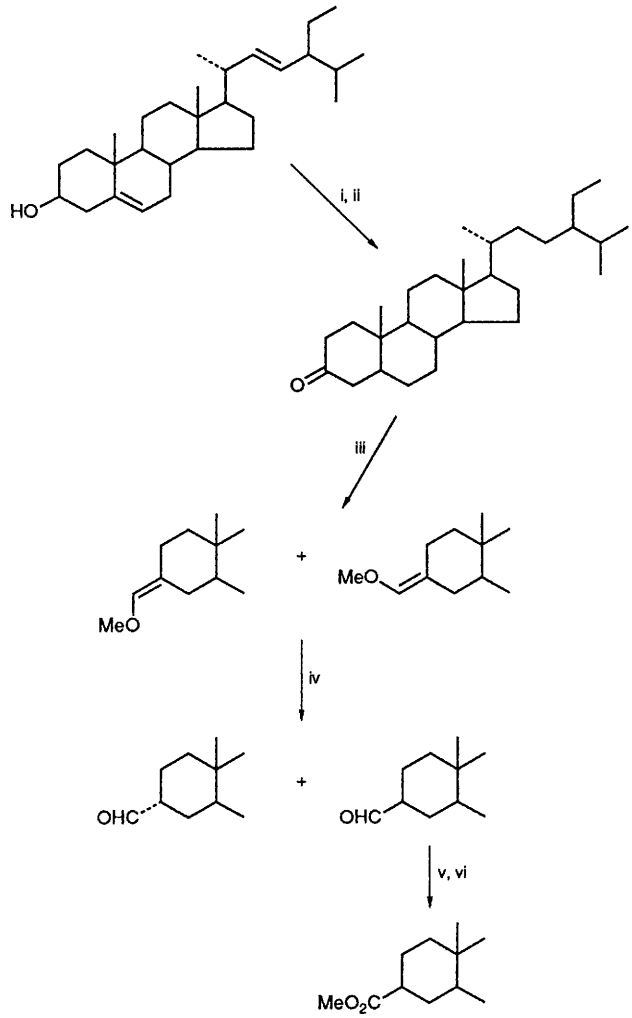
Figure 1. (24*R*)- and (24*S*)-3β-carboxy-24-ethylcholestanes [as their methyl esters (**1**) and (**2**)] occurring in carbonated sediments.

NMR spectrum showed a singlet at 3.65 ppm (methoxy group) and a multiplet at 2.31 ppm, which was consistent with the presence of a methyl ester function. Comparison of the spectral data and of the chromatographic retention times with those obtained previously on a series of 3β-carboxysteranes formed by oxidation of petroleum asphaltenes with ruthenium tetroxide (C_{27} homologue conclusively identified by synthesis)[8], indicated that the structure most probably corresponded to a 3β-carboxy-24-ethylcholestane. A detailed examination of the chemical shifts of the methyl groups, along with literature[9] data on 24*R* and 24*S* steroids inferred, however, the presence of a mixture of two compounds likely to be the 24*R* and 24*S* isomers. We, therefore, synthesised the (24*R*)- and (24*S*)-3β-carboxy-24-ethylcholestanes (**1**) and (**2**) starting from (24*S*)-24-ethylcholesta-5,22-dien-3β-ol (stigmasterol) and (24*S*)-24-ethylcholest-5-en-3β-yl acetate (clionasteryl acetate) respectively, and following Scheme 1. A comparison of the three ^{1}H NMR spectra (two synthetic products† and the isolated mixture) indicated that we had a mixture of the (24*S*)- and (24*R*)-3β-carboxy-24-ethylcholestane isomers in a ratio of ~3 : 1.

The two synthetic products and the isolated mixture are inseparable on gas chromatography columns of varying polarities [*e.g.*, DB5 J&W, 30 m × 0.25 mm × 0.1 μm, H_2; OV31OH, 30 m × 0.3 mm × 0.25 μm, H_2; Supelcowax 10, 60 m × 0.25 mm × 0.25 μm, H_2]. Furthermore their mass spectra‡ show no significant differences.

† *Spectroscopic data* for: ^{1}H NMR, 400 MHz, ($CDCl_3$), (**1**) δ_H 0.642 (s, 3 H), 0.796 (s, 3 H), 0.806 (d, *J* 7.2 Hz, 3 H), 0.825 (d, *J* 7.2 Hz, 3 H), 0,840 (t, *J* 7.4 Hz, 3 H), 0.900 (d, *J* 6.5 Hz, 3 H), 2.310 (m, 1 H), 3.650 (s, 3 H). (**2**) δ_H 0.643 (s, 3 H), 0.796 (s, 3 H), 0.806 (d, $J \approx 7$ Hz, 3 H), 0.825 (d, $J \approx 7$ Hz, 3 H), 0.850 (t, *J* 7.4 Hz, 3 H), 0.906 (d, *J* 6.5 Hz, 3 H), 2.309 (m, 1 H), 3.650 (s, 3 H).

‡ *Mass spectrometry data*: EI (70 eV), *m/z* (rel. int.) for: (**1**) 458 (M^+, 80%), 443(42), 290(27), 275(100), 276(61), 207(49), 166(16), 149(15), 135(16), 121(28), 107(49), 95(41), 81(37), 79(20); (**2**) 458(M^+, 100%), 443(41), 290(23), 275(98), 276(61), 207(40), 166(15), 149(11), 135(15), 121(24), 107(40), 95(33), 81(33), 79(15); isolated mixture: 458(M^+, 94%), 443(34), 290(29), 275(100), 276(65), 207(34), 166(15), 149(15), 135(15), 121(27), 107(50), 95(43), 81(40), 79(23).



Scheme 1. Synthesis of the (24*R*)-3β-carboxy-24-ethylcholestane starting from stigmasterol: i, H_2 Pd/C; ii, Jones; iii, $(C_6H_5)_3PClCH_2OCH_3$ Wittig; iv, $HClO_4$; v, Jones; vi, CH_2N_2. The 24*S* isomer was synthesised from (24*S*)-24-ethylcholest-5-en-3β-yl acetate (clionasteryl acetate) essentially following the same reaction sequence. Only 5α-isomer was obtained in i.

3β-Carboxysteranes have been obtained by oxidation of petroleum asphaltenes with ruthenium tetroxide. In this case it is probable that the carboxylic function was generated by oxidation of an aromatic entity attached to a sterane skeleton at position 3, although the release of carboxysteranes by hydrolytic cleavage from a polycondensed substrate should not be excluded.[8] However, in the present case an origin by cleavage from a macromolecular framework appears unlikely because carboxysteranes were not found when the polar fraction of the extract or the insoluble residue (kerogen) were submitted to the same oxidative conditions.

The origin of these compounds is still unknown since their carbon skeleton has not been observed to date in living organisms. Among the various hypotheses which can be considered are: (i) methylation at C-3 of the corresponding Δ^2-sterene (Δ^2-sterenes are commonly encountered in recent sediments[10]) followed by oxidation of the methyl group; (ii) addition of a carboxylic group to the corresponding Δ^2-sterene, mediated by micro-organisms.

The first hypothesis finds an analogy in the methylation pattern observed in hopanoid triterpenes recently identified in

some bacteria and cyanobacteria[11] (*e.g.*, 2β-methyl and 3β-methyl hopanoids), as well as in the occurrence in the alkane fraction of the sediment of 24-ethyl-3β-methylcholestane as the major component of the methylated steranes (the latter was identified by comparison with a synthetic product obtained by reduction of the corresponding 3β-carboxysterane). Another alternative, more consistent with the high degree of anoxicity in the environment of deposition, would be that the 3β-methylsteranes are formed in the sediment by reduction of the 3β-carboxysteranes. The latter could represent a yet unrecognised family of compounds occurring in living organisms, in which they could act as surrogates of sterols for the mechanical reinforcement of lipidic membranes. This situation would be quite similar to that of hopanoid triterpenes, which were first found in sediments and petroleums. Their ubiquitous occurrence subsequently led to the recognition, in bacteria, of polar counterparts that are essential membrane constituents.[12]

The predominance in our mixture of the 24*S* isomer probably indicates a marine planktonic origin for this compound, the 24*R* component appearing as a result of maturation.[13] Such an origin is corroborated by other molecular distributions observed particularly in the alkanes of the sediment (high predominance of C_{15} and C_{17} alkanes typical of an algal input). Therefore, a terrigenous origin appears unlikely for this compound although some higher plants do contain 24*S* steroids.[14]

It is noteworthy that 3β-carboxysteranes have also been observed in the extractable lipids of phosphatic sediments from Timahdit (Morroco)[15] and in a sediment from the Monterey formation[16] (California, USA). All these sediments are immature and have been deposited in a marine environment with a high primary input of microscopic algae. Further work aimed at the elucidation of the origin and formation of the 3β-carboxysteranes and their relationship to the corresponding 3β-methylsteranes is in progress.

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