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Isolation and Identification of Sterols from a Pleistocene Sediment

BECAUSE of their chemical stability, alkanes and fatty acids have been widely used as indicators of biological origin in studies of sediments[1-4], but their formation may often be attributable to abiogenic syntheses and transformations[5] so that their distribution patterns should be approached with caution. Little effort has been expended on analysis of sediments for relatively unaltered molecules which are biologically more significant, such as sterols which are present in sea water[6], bottom muds (both marine and non-marine)[7,8], surface soils[9], and an Eocene shale[10]. If such molecules are present in sediments representing a wide period of geological time, their significance with regard to depositional environment and contributory organisms would exceed any other biological marker in common use. We report some organic geochemical studies of a Pleistocene basin and a contemporary lake where we are attempting to correlate alkane, fatty acid, steroid, amino-acid and sugar distribution patterns with stratigraphy and biological environment.

Mono Lake is one of the strongly alkaline desert lakes of eastern California, and has a combination of high organic productivity with almost no scavengers. It is situated in a structural downwarp and has been continuously in existence for ~3 m.y. (refs. 11, 12). Sediments deposited in this lake show no clearly freshwater fauna but contain an abundance of algal debris, diatoms, ostracods and, locally, gastropods.

The sediment analysed contained alternate bands of algal silt and diatomite. It resides within a complete Pleistocene lacustrine sequence and its position within that sequence (that is, 80 m below the top of the Wilson Creek Formation, which is the latest Pleistocene sediment exposed in the Mono Basin) suggests an age ~130,000 yr.

We have isolated and identified the components of a complex mixture of steroids by gas-liquid chromatography (GLC) and combined gas chromatography–mass spectrometry (GCMS). Powdered sediment (9.95 g) was extracted using a mixture of benzene, acetone and methanol (2 : 1 : 1 v/v). The concentrated extract (6.75% w/w of sediment) was chromatographed on a silica gel column eluting successively with heptane, benzene and methanol. The methanol fraction was saponified with 5% NaOH and the non-saponifiables were dissolved in ether, chromatographed on a modified McCarthy-Duthie column[13] and the ether collected. The presence of sterols in this fraction was established on analytical thin-layer chromatographic plates (silica gel; 0.25 mm, using ceric sulphate and sulphuric acid spray reagent) by comparison with authentic sterols. Isolation of the sterol fraction was carried out by preparative thin-layer chromatography on silica gel with chloroform and ethyl acetate (3 : 1 v/v) as the mobile phase. The total fraction in pyridine solution was treated with *bis*-(trimethylsilyl) fluoroacetamide and trimethylchlorosilane (5 : 1 v/v). The resultant sterol trimethylsilyl ethers were analysed by GLC on a Perkin–Elmer Model 900 instrument using JXR, OV-17 and OV-210 as liquid phases. High and low voltage (70 and 16 eV) mass spectrometry (GEC-AEI MS-12) using a direct introduction solid probe yielded molecular ion data for the principal components of the complex mixtures. Single component identifications were achieved by GCMS using an Aerograph 204 gas chromatograph (flame ionization detector) coupled directly to a Dupont 492–1 mass spectrometer without a molecular separator or effluent splitting device.

Table 1 Identities of Sterols found in a Mono Lake Sediment

Peak	Retention index* (JXR)	Identity
1	3,120	
2	3,155	Cholesterol † TMS and cholestanol ‡ TMS
3	3,195	Brassicasterol † TMS
4	3,250	Campesterol † TMS
5	3,285	Stigmasterol † TMS and compound ‡ molecular weight 486
6	3,340	Stigmastanol † TMS and β-sitosterol TMS †
7	3,410	Compound of molecular weight 502 thought to be lanostanol ‡ TMS

* GLC retention indices from a 10 foot × 1/8 inch stainless steel column containing 3% JXR on 60–80 mesh (gas Chrom Q) at 265° C.

† These identifications made by GLC and GCMS analyses.

‡ These identifications made by GCMS alone.

The results of the analysis of the purified sterol fraction in the form of trimethylsilyl ethers (TMS) by GLC is shown in Fig. 1. Comparison of the retention data obtained on two liquid phases (JXR, OV-17) with those of reference sterol derivatives suggested the following assignment of peak 2 to cholesterol TMS, peak 3 to brassicasterol TMS, peak 4 to campesterol TMS, peak 5 to stigmasterol TMS and peak 6 to β-sitosterol TMS. But analysis by GCMS indicated that some of the peaks contained more than one component. Peak 2 was shown to contain the TMS ethers of cholesterol and cholestanol, peak 5 contained a derivative of molecular weight 486 as well as stigmasterol TMS and peak 6 was chiefly stigmastanol TMS with a little β-sitosterol TMS. The assignments given to the chief peaks in Fig. 1 are also shown in Table 1. Further analyses by GLC on a more polar liquid phase (OV-210) confirmed the multicomponent nature of peaks 5 and 6 (Fig. 1).

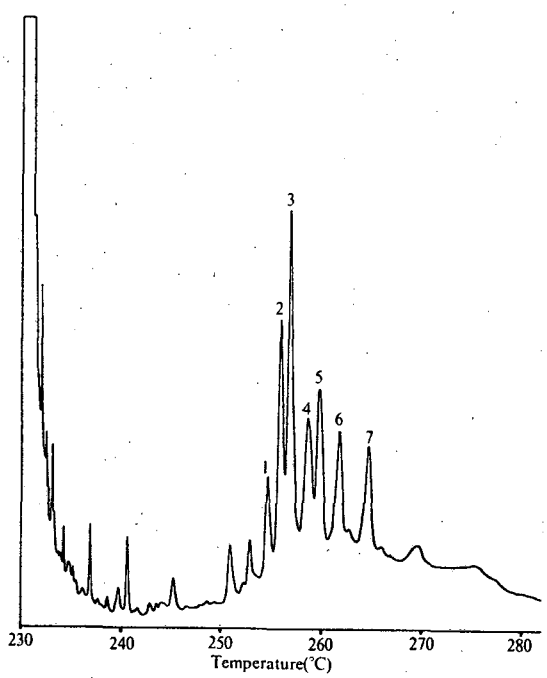

Fig. 1 Gas chromatogram of sterol trimethylsilyl ethers from a Mono Lake sediment obtained using a 10 foot × 1/8 inch stainless steel column packed with 3% JXR on 60–80 mesh ('Gas Chrom Q'). The column was programmed between 230° C and 280° C at 2° C min^{-1}

The types of sterol found in the sediment examined strongly suggest that they had an algal origin and underwent a certain amount of reduction during sedimentation and the initial stages of lithification. Indeed, cholesterol, stigmasterol and β-sitosterol are known components of fresh water algae[14,15] and cholesterol, brassicasterol, campesterol and β-sitosterol have been found in marine algae[16]. It may, therefore, not be possible to use sterol distribution patterns for identification of the deposition environment of a sediment (compare ref. 8).

Analyses of *in vitro* cultures of Mono Lake wild algae so far confirm the algal origin of the unsaturated sterols isolated from the sediment. There is no evidence for the presence of fully saturated sterols in the algae.

The identification of fully saturated counterparts of algal sterols, together with the unaltered compounds, is good evidence that at least partial reduction of naturally occurring molecules has taken place within the 130,000 yr since deposition. Simulated diagenesis experiments (using pressure, relatively mild temperatures and reducing conditions) seem to generate significant quantities of the fully reduced steroid hydrocarbons (steranes). Further work may not only allow chemotaxonomic correlations of organic compositions (alkanes, fatty acids, sterols, amino-acids and sugars) of sediments and contemporary organisms, but may also yield more specific information about the rates of the alteration of organic material in a geological environment and other parameters involved.

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