Isolation and Characterization of a Triterpenoid Alcohol from the Green River Shale

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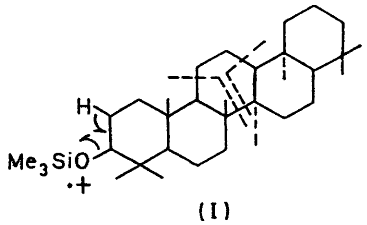
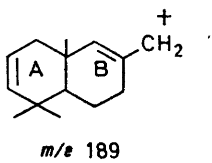
Summary A triterpenoid alcohol (tetrahymanol) has been isolated from the Green River Shale (60 million years).

SEVERAL publications have shown the presence of fully reduced steroids and triterpenoids (steranes and triterpanes) in the Green River Shale (Eocene, 60 × 10^6 years old).[1–3] The identities of many of these compounds have been established by capillary g.l.c., capillary g.c.–m.s.,[3] and by *X*-ray crystallography.[2] Although absolute stereochemistries in most cases have not been established, the following structural identifications have been made: cholestane, ergostane, stigmastane, gammacerane, and a hopane-type structure. The presence of these compounds in the Green River Shale has been taken as substantial proof that the original source of the organic content of the sediment was biological and probably dominantly algal.[4] It has been suggested that these molecules were derived from naturally occurring phytosterols, and higher plant and micro-organism triterpenoids which, through the processes of deposition, consolidation and maturation of the sediment, were reduced to the cycloalkanes described above. No evidence has been produced which would confirm this postulate by identifying the precursors of the cycloalkanes, *i.e.*, sterols and triterpenoid alcohols, from this sediment. However, it has been reported that the Messel Oil Shale (Eocene; with a milder geological history than the Green River Formation) does contain triterpenoid alcohols although the authors found no evidence for triterpenoid alcohols in the Green River Shale.[5,6] We now report the isolation and identification of a triterpenoid alcohol from the Green River Shale.

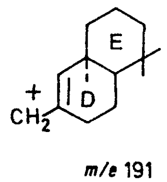
The sediment (350 g) from the Mahogany Ledge was broken into small pieces, pulverized in a disc mill (Angstrom Inc., Chicago, Illinois) and the powder extracted with a mixture of benzene–methanol (3:1 v/v) with ultrasonication. After centrifugation the supernatant solution was separated, evaporated to dryness, and the total extract chromatographed on a silica gel column eluting successively with n-heptane, mixtures of 5—40% diethyl ether in n-heptane, diethyl ether, and finally ethyl acetate. The fractions collected were monitored by analytical t.l.c. using a solvent mixture of n-heptane, diethyl ether, and methanol (18:6:1 v/v). By comparison of the extract fractions with the mobilities of reference compounds some of the fractions obtained using 40% diethyl ether in n-heptane showed constituents with the correct mobilities to be sterol or triterpenoid in character. The hydroxylated compounds were selectively removed from extraneous materials by preparative t.l.c. (n-heptane: benzene 1:1 v/v as mobile phase) in the form of trimethylsilyl ethers. After purification, fractions as trimethylsilyl ethers were examined by g.l.c. on two different liquid phases (*viz.* JXR and OV-17) and finally by g.c.–m.s.[7]



(I)



m/e 189



m/e 191

The mass spectra obtained showed that one of the main derivatives had a molecular ion (M) at m/e 500 and showed other ions at m/e 410 ($M - 90$), 395 ($M - 105$), and 73 characteristic of the trimethylsilyl ether of an alcohol. The two most abundant ions in the spectrum were found at m/e 191 and m/e 189, respectively, which were deduced to result from a fragmentation of a symmetrical nucleus such as is found in the pentacyclic triterpenoid alcohol tetrahymanol (I; shown here as the trimethylsilyl ether).

TABLE

G.l.c. retention data for extracts and reference compounds as trimethylsilyl ethers

Parent compound	I(JXR)[a]	I(OV-17)[b]
Alcohol from Green River Shale ..	3520	3725
Alcohol from *Tetrahymena pyriformis*	3520	3725
Authentic tetrahymanol	3520	3730

[a] 10 ft × $\frac{1}{8}$ in stainless-steel column packed with 3% JXR on 60–80 mesh Gas Chrom Q at 265°.

[b] 6 ft × $\frac{1}{8}$ in stainless-steel column packed with 3% OV-17 on 60–80 mesh Gas Chrom Q at 270°.

In order to confirm this tentative identification a sample of the protozoan, *Tetrahymena pyriformis* (from which tetrahymanol had previously been isolated and identified[8]) was obtained and cultured in this laboratory. Extraction and liquid–solid chromatography followed by t.l.c. of the trimethylsilyl ether fraction yielded a mixture of components dominated by tetrahymanol trimethylsilyl ether. Comparison of the g.l.c. retention indices of this component with those of the alcohol isolated from the Green River Shale showed them to be identical. Further confirmation was achieved by comparison of the retention data (Table) and mass spectrum of an authenticated sample of tetrahymanol trimethylsilyl ether with those of the compounds obtained from *Tetrahymena pyriformis* and the Green River Shale.

The triterpane gammacerane has already been identified in the hydrocarbon fraction from Green River Shale,[2,3] and this new evidence showing the presence of the corresponding saturated alcohol indicates that hydroxylated triterpenoids can exist intact over a significant period of geological time and provides information on the mechanisms of diagenesis and maturation of organic natural products in a sedimentary environment. Qualitative evidence indicates the presence of other, as yet unidentified, possible triterpenoid alcohols in the Green River Shale.

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