Identification of Steroids and Triterpenes from a Geological Source by Capillary Gas–liquid Chromatography and Mass Spectrometry

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RECENT reports have given tentative evidence for the occurrence of steroids and triterpenes in the saturated branched and cyclic fraction of the Green River shale (Eocene, *ca.* 60 × 10^6 years).[1,2] From the same shale, Hills *et al.*, using a large-scale isolation procedure followed by repeated recrystallisations, obtained a pure sample of gammacerane.[3]

We report results obtained from a 10 g. portion of Green River shale by techniques which allow identification of the carbon skeletons of these fossil natural products[†] on a micro-, rather than macro-scale.

The extraction and separation procedures employed to obtain the branched and cyclic alkane fraction (0·1% dry weight of rock) have been reported previously.[4,5] The complex mixture so obtained was analysed by g.l.c. using high-resolution capillary columns coated with pretreated Apiezon L[6] (Figure 1), and with seven-ring polyphenyl ether (7-PPE; 150 ft × 0·01 in.). Comparison of the g.l.c. retention data and the calculated Kovats indices[7] with those for authentic standards on those liquid phases of different polarity (Table) suggested the presence of 5β-cholestane (Figure 1, peak 1a), 5α-cholestane

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[†] *i.e.* compounds found in Nature, with the carbon skeletons almost certainly biologically formed. The cycloalkanes themselves have not been reported from biological sources and presumably result from the reduction and, in some cases, the rearrangement of the commonly encountered unsaturated and oxygenated counterparts during diagenesis and maturation.

TABLE

G.l.c. correlation of branched-cyclic alkane fraction from Green River shale with standard steroids and triterpenes.

Peak No. (Figure 1)	Abundance[a] (%)	Molecular[b] formula	Carbon[8] number (7-PPE)	Carbon[8] number (AP-L)	Co-injected standards[c]	Molecular formula
1a	0·7	—	30·07	29·58	5β-Cholestane	$C_{27}H_{48}$
1	2·3	$C_{27}H_{48}$	30·49	29·90	5α-Cholestane	$C_{27}H_{48}$
2	5·1	$C_{28}H_{50}$ and $C_{30}H_{56}$	30·73	30·30	—	—
7	6·6	$C_{28}H_{50}$	31·42	30·82	—	—
8	4·8	$C_{30}H_{56}$	31·52	30·94	—	—
—	—	—	32·05	31·23	Onocerane III	$C_{30}H_{54}$
—	—	—	32·36	31·35	Onocerane II	$C_{30}H_{54}$
—	—	—	33·38	31·42	Lupane	$C_{30}H_{52}$
14	11·6	$C_{20}H_{52}$	32·15	31·53	Stigmastane	$C_{29}H_{52}$
—	—	—	32·68	31·59	Onocerane I	$C_{30}H_{54}$
—	—	—	33·81	31·98	Moretane	$C_{30}H_{52}$
—	—	—	32·50	32·05	Lanostane	$C_{30}H_{54}$
—	—	—	33·22	32·43	Adiantane	$C_{29}H_{50}$
20	11·2	$C_{30}H_{52}$	33·51	32·60	—	—
—	—	—	34·46	33·72	Friedelane	$C_{30}H_{52}$
26	3·6	$C_{30}H_{52}$	35·20	34·06	Gammacerane	$C_{30}H_{52}$

[a] By peak areas from Figure 1; [b] by GC-MS; [c] commercially available and kindly provided by Syntex, Dr. K. H. Overton, and Mr. E. V. Whitehead.

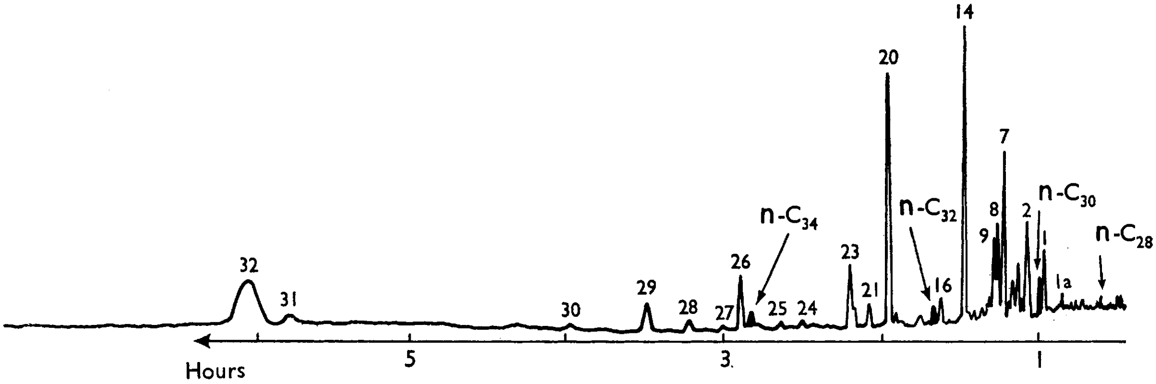

FIGURE 1. *Portion of the g.l.c. of the total branched and cyclic alkane fraction from the Green River shale with the reference* n-C_{28}, n-C_{30}, n-C_{32}, *and* n-C_{34} *alkanes added. Conditions*: 200 ft. × 0·01 in. *stainless steel capillary column coated with Apiezon L grease* (*AP-L*), 250°, *helium flow rate* 2 *ml./min.*

(peak 1), a stigmastane isomer (peak 14), and gammacerane (peak 26). Enhancement of these peaks on co-injection of this mixture and added authentic compounds confirmed the presence of these components. Further confirmation was obtained by combined capillary GC-MS (200 ft. × 0·01 in. column coated with Apiezon L).‡ The mass spectra of the compounds previously identified by their retention data on g.l.c. were identical with those of the authentic compounds (Figure 2). Furthermore, the mass spectra of other peaks allowed the provisional identification of carbon skeletons of the ambreane (peaks 2 and 8), ergostane (peak 7), and hopane (peak 20) types, and also indicated the presence of pentacyclic nortriterpenes, $C_{29}H_{50}$ (parent ion *m/e* 398; peak 16) and pentacyclic triterpenes of molecular formulae $C_{31}H_{54}$ (parent ion *m/e* 426; peaks 24 and 25). From the Table we can exclude the presence of other than trace quantities of onoceranes (I), (II),

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‡ LKB 9000 at the Chemistry Department, University of Glasgow. We thank Dr. C. J. W. Brooks for the use of this instrument.

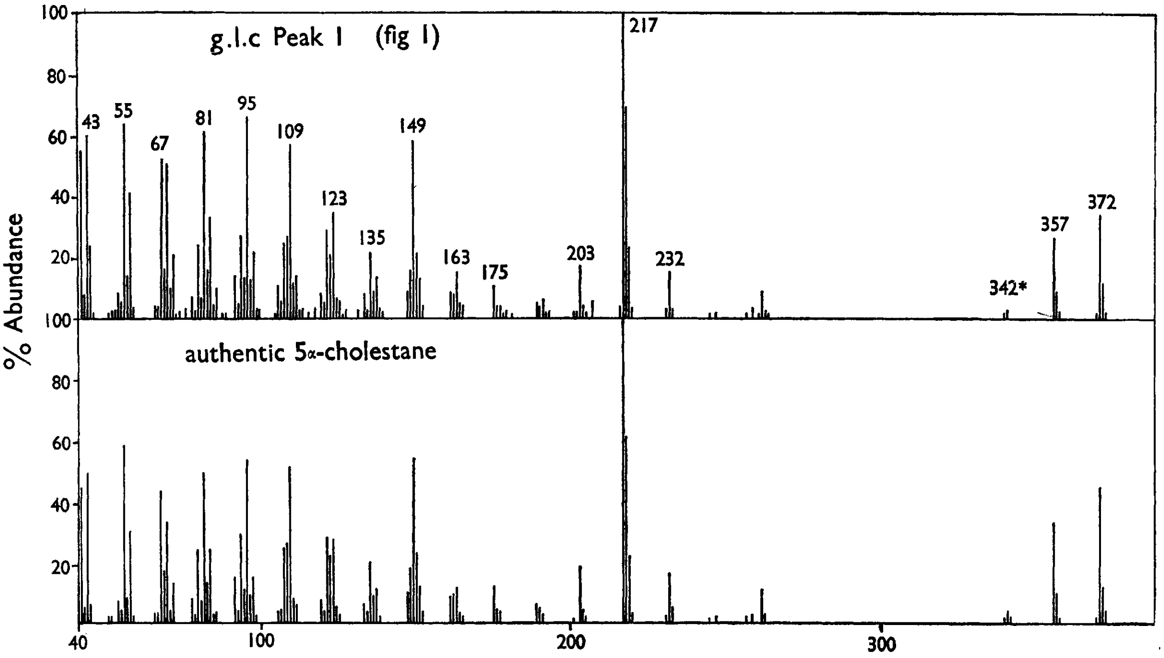

FIGURE 2. *Mass spectra of g.l.c. peak* 1 (Figure 1) *and of an authentic sample of* 5α-*cholestane.*

* Metastable peak.

and (III), lanostane, adiantane, lupane, moretane, and friedelane. The absence of a compound should prove just as significant paleochemotaxonomically as its presence.

These and other results[1-3] demonstrate that the branched and cyclic alkane fraction from this freshwater sediment is mainly composed of diterpenes, steroids, triterpenes, and tetraterpenes. The low abundance of cholestane implies plant rather than animal sources, and geological studies indicate contributions from algal and planktonic sources rather than higher plants.[8] The cycloalkane distribution pattern (*cf.* Figure 1) is different from that evident in the g.l.c. data published for the Nigerian[9] and the Mirando[10] crude oils though the isolation procedures were not the same.

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