The Geological Fate of Chlorophyll: the Absolute Stereochemistries of a Series of Acyclic Isoprenoid Acids in a 50 Million Year Old Lacustrine Sediment

By R. E. Cox, J. R. Maxwell,* G. Eglinton, and C. T. Pillinger
(*Organic Geochemistry Unit, School of Chemistry, University of Bristol,* BS8 1TS)
and R. G. Ackman and S. N. Hooper
(*Fisheries Research Board of Canada, Halifax Laboratory, Halifax, Nova Scotia*)

Summary Determination of the absolute stereochemistries of the major acyclic isoprenoid-type acids in a sediment of Eocene age (the Green River Shale, Colorado, *ca.* 50 $\times 10^6$ years) by g.l.c. analysis of the methyl and (−)-menthyl esters, indicates that the phytyl side chain of chlorophyll is the major ultimate precursor of the acids.

Biolipids and geolipids with acyclic isoprenoid-type† carbon skeletons are widely distributed throughout the biosphere and geosphere, respectively.[1,2] Three acids of this type, *viz.* 3,7,11,15-tetramethylhexadecanoic acid (I), 2,6,10,14-tetramethylpentadecanoic acid (II), and 4,8,12-trimethyltridecanoic acid (III) occur both in some contemporary organisms, albeit in relatively small amounts,[3] and in ancient sediments.[1,4]

Information about relative and absolute stereochemistries of naturally-occurring acids of this type, *e.g.* (I) and (II), has been obtained by g.l.c. analysis of their methyl and (−)-menthyl esters,[4,5] respectively. The present work extends this method, principally to (III) and (IV) whose elution order of stereoisomers has been elucidated.

In addition to the standards of known stereochemistry obtained from natural phytol (VII),[4] other standards were obtained as follows:

Acids (I)—(VI): All stereoisomers of each acid from synthetic[6] ethyl 3,7(*RS*),11(*RS*),15-tetramethylhexadec-2-enoate (ethyl phytenoate).

Acid (III): the 4(*S*),8(*R*),12-stereoisomer by Arndt–Eistert chain extension of the 3(*S*),7(*R*),11-stereoisomer of (IV).

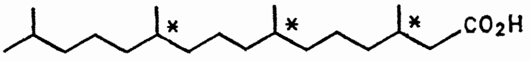
Acids (III) and (IV): The *RR*- and *SS*-stereoisomers of both by dichromate oxidation of synthetic[7] *meso*-2,6,10,14-tetramethylpentadecane (VIII).

Acids (II) and (III): The 2(*RS*),6(*S*),10(*S*),14-isomers of (II) and the 4(*S*), 8(*S*),12-isomer of (III) by microbial oxidation[8] of *meso*-2,6,10,14-tetramethylpentadecane (VIII).

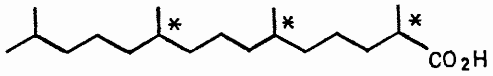
We have previously examined the relative stereochemistries of certain of the isoprenoid-type acids in the total acid fraction of the Green River Shale.[4] In the present study of absolute stereochemistries, the free acids (I—VI) were isolated as a mixture (40 mg) from a sample (420 g) of the Green River Shale from Sulfur Creek by solvent extraction without sediment demineralisation. The (−)-menthyl esters were prepared by reaction of the acid chlorides with (−)-menthol, and their identity confirmed by g.c.–m.s.

Stereoisomeric identities were established on open tubular capillary columns (*ca.* 40,000 plates) coated with butanediol succinate (BDS) by coinjection and/or comparison of retention data with the standards. The mixture of 4 stereoisomeric esters of all-synthetic (IV) appeared as a symmetrical triplet in the ratio 1:2:1. Comparison with the other standard (−)-menthyl esters of (IV) showed that the elution order was 3(*S*),7(*R*),11- ; 3(*R*),7(*S*),11- + 3(S),7(*S*),11- ; 3(*R*),7(*R*),11-. Coinjection (Figure) of the geological (−)-menthyl ester (Figure) with the all-synthetic triplet shows that the absolute stereochemistry is 3(*R*),7(*R*),11- (75%) with a lesser amount (25%) of the 3(*S*),7(*R*),11-isomer. The stereoisomeric composition of (III) was similarly determined (Table). Only one of the two isomers of (V) could be assigned from the standards available (Table).

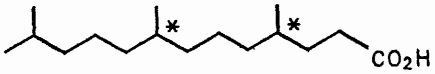
(I)



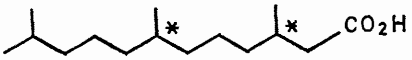
(II)



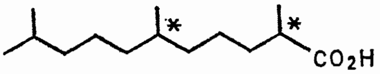
(III)



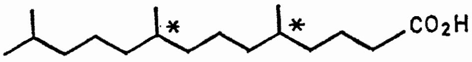
(IV)



(V)

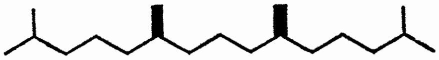


(VI)



(VII)

(VIII)



Separation of all 8 stereoisomers of (I) and (II) was not possible on the columns used. Acids (I) and (II) were isolated as methyl esters from the Green River Shale acid fraction by preparative-scale g.l.c., and the mixture

---

† Defined herein as possessing at least 2 isoprene skeletal units linked head-to-tail as part of the skeleton.

hydrolysed and oxidised with permanganate.[9] G.l.c. analysis of the major acid products, (III) and (IV) as methyl esters (Table), limited[4] the stereochemistry of the 7 and 11 positions in (I) and the 6 and 10 positions in (II) to the *RR* (and/or *SS*) configuration. This evidence, taken with the g.l.c. analysis of the (—)-menthyl esters of (I) and (II) and of all of the available standards of (I) and (II) confirmed the stereochemistries shown (Table).

No separation of the (—)-menthyl ester of the all-synthetic sample of (VI) could be obtained. However, relative stereochemistry of the geological acid was assigned by comparison of the chromatogram of the methyl ester with those of the methyl esters of the all-synthetic sample and the 5(*R*),9(*R*)-standard.

The diterpenoid side chain of chlorophyll, phytol (VII), has been shown[7] to have the 7(*R*),11(*R*)-configuration.

Stereoisomeric composition of Green River Shale isoprenoid acids by g.l.c.

	No. of peaks observed			
Acid	(—)-Menthyl ester	Methyl ester	Stereoisomeric assignment	
(I)	2	2[a]	3(*S*),7(*R*),11(*R*)	(45%)
			3(*R*),7(*R*),11(*R*)	(55%)
(II)	2	2	2(*S*),6(*R*),10(*R*)	(50%)
			2(*R*),6(*R*),10(*R*)	(50%)
(III)	1[b]	2	4(*R*),8(*R*)	(95%)
			4(*S*),8(*R*)	(5%)
(III)[c]	—	1	4(*R*),8(*R*)	(100%)
(IV)	2	2	3(R),7(R)	(75%)
			3(*S*),7(*R*)	(25%)
(IV)[c]	—	1	3(*R*),7(*R*)	(100%)
(V)	2	2	2(*R*),6(*R*)	(55%)
			another[d]	(45%)
(VI)[e]	1	1	5(*R*),9(*R*) and/or 5(*S*),9(*S*)	

[a] A minor peak (*ca.* 5%) within the elution time of all-synthetic (I) was observed but was not seen in the (—)-menthyl ester.

[b] 4(*R*), 8(*R*)- and 4(*S*),8(*R*)-(—)-menthyl esters are almost co-incident, but separable as methyl esters.

[c] Derived from permanganate oxidation of geological acids (I) and (II).

[d] Possibly 2(*S*),6(*R*). Studies on this stereoisomer are continuing.

[e] Relative stereochemistry alone established since no separation possible of all-synthetic (—)-menthyl ester.

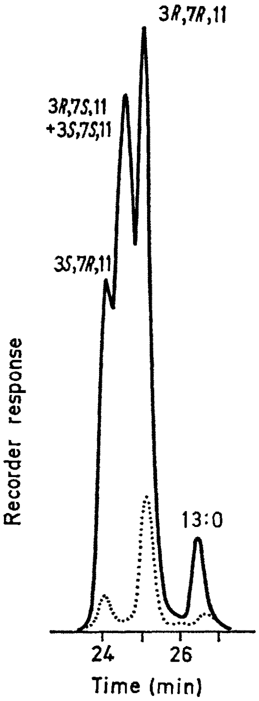


FIGURE. *Gas chromatograms of the (—)-menthyl esters of* 3,7,11-*trimethyldodecanoic acid* (IV) *showing Green River Shale sample* (. . . .) *and Green River Shale sample co-injected with all-synthetic* (IV) (————). *n-Tridecanoic acid (—)-menthyl ester as reference. Conditions: isothermal at* 170° *on* 150 *ft.* × 0·01 *in. stainless steel coated with BDS; flow rate* 5 *ml/min* He; *Perkin–Elmer* 900.

The results presented here provide a clear indication that this ubiquitous natural product is the major ultimate precursor of acid (I) and, by oxidative degradation, of acids (II)–(IV) [and possibly acids (V) and (VI)] in the Green River Shale. Acids (I) and (II), as might be expected from this postulate, are the major components of the acyclic isoprenoid-type acids in the sediment. Acids such as (I) and (II) may have entered the sediment directly from the biosphere at the time of deposition since they occur in biolipids.[3,4] Phytol, if not the major immediate precursor of the geological acids (I)—(IV) is almost certainly their major ultimate precursor. Other possible contributions, *e.g.* from high molecular weight isoprenoids such as carotenoids, squalene, *etc.*, abiologically modified over geological time, would appear negligible because of the specific stereochemistries found here. Certain other biolipids, *e.g.* vitamin K_1 and α-tocopherol have been shown[7] to possess a phytyl side chain with the same stereochemistry as that of phytol from chlorophyll. The relative proportions of these compounds in the biosphere in comparison with chlorophyll precludes a major contribution from them to a sediment such as the Green River Shale. The origin of the minor stereoisomers observed in acids (III) and (IV) is not certain at present. If the second isomer present in (V) is indeed the 2(*S*),6(*R*)-isomer, the stereoisomeric distribution possibly reflects epimerisation of the 2-position under geological conditions.

We thank the Natural Environment Research Council and the National Aeronautics and Space Administration for support. Two of us (R.E.C. and C.T.P.) thank the Science Research Council for a Research Studentship and a Research Fellowship, respectively. We are also grateful to Professor B. C. L. Weedon, Queen Mary College London, and Professor K. Sato, Yokohama National University for generous gifts of 3(*S*),7(*R*),11-trimethyldodecanoic acid and *meso*-2,6,10,14-tetramethylpentadecane, and ethyl 3,7.(*RS*),11(*RS*),15-tetramethylhexadec-2-enoate, respectively.

(*Received, October 16th*, 1970; *Com.* 1779.)

---

[1] G. Eglinton, A. G. Douglas, J. R. Maxwell, J. N. Ramsay, and S. Ställberg-Stenhagen, *Science*, 1966, **153**, 1133; "Organic Geochemistry: Methods and Results," eds. G. Eglinton and M. T. J. Murphy, Springer-Verlag, Berlin, Heidelberg and New York, 1969.

[2] T. Belsky, R. B. Johns, E. D. McCarthy, A. L. Burlingame, W. J. Richter, and M. Calvin, *Nature*, 1965, **205**, 446, and references therein.

[3] R. G. Ackman and S. N. Hooper, *Comparative Biochem. Physiol.*, 1968, **24**, 549; R. P. Hansen, F. B. Shorland, and J. D. Morrison, *J. Dairy Res.*, 1965, **32**, 21; R. P. Hansen, *Biochim. Biophys. Acta*, 1965, **106**, 304; A. K. Sen Gupta and H. Peters, *Fette, Seifen, Anstrichm.*, 1966, **68**, 349.

[4] I. MacLean, G. Eglinton, K. Douraghi-Zadeh, R. G. Ackman, and S. N. Hooper, *Nature*, 1986, **218**, 1019.

[5] R. G. Ackman, S. N. Hooper, M. Kates, A. K. Sen Gupta, G. Eglinton, and I. MacLean, *J. Chromatog.*, 1969, **44**, 256.

[6] K. Sato, S. Mizuno, and M. Hirayama, *J. Org. Chem.*, 1967, **32**, 117.

[7] J. W. K. Burrell, R. F. Garwood, L. M. Jackman, E. Oskay, and B. C. L. Weedon, *J. Chem. Soc.* (*C*), 1966, 2144, and references therein.

[8] R. E. Cox, J. R. Maxwell, R. G. Ackman, and S. N. Hooper, unpublished results.

[9] K. E. Murray, *Austral. J. Chem.*, 1959, **12**, 657.