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PHYTANIC ACID L-MENTHYL ESTERS

GAS-LIQUID CHROMATOGRAPHIC SEPARATION OF DIASTEREOISOMERS

R. G. ACKMAN AND S. N. HOOPER

Fisheries Research Board of Canada, Halifax Laboratory, Halifax, N.S. (Canada)

M. KATES

Chemistry Department, University of Ottawa, Ottawa, Ont. (Canada)

A. K. SEN GUPTA

Unilever Research Laboratories, Hamburg (G.F.R.)

G. EGLINTON

Organic Geochemistry Unit, School of Chemistry, University of Bristol, Bristol (Great Britain)

I. MACLEAN

Department of Chemistry University of Glasgow, Glasgow (Great Britain)

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SUMMARY

L-Menthyl esters of a naturally occurring (marine) phytanic acid, of an all-synthetic phytanic acid, and of a phytanic acid known to be of 3D, 7D, 11D configuration were compared by GLC. As in the pristanates, the LDD and DDD diastereoisomers were the farthest apart, but in the marine phytanate only two diastereoisomers, presumably LDD and DDD, could be detected. A general guideline is proposed that in the GLC of L-menthyl esters of isoprenoid fatty acids with 2-methyl and 3-methyl substituents the LDD and DDD diastereoisomers will be, respectively, the first and last to elute.

INTRODUCTION

The initial demonstration of the separation of diastereoisomers of phytol-derived pristanic (2,6,10,14-tetramethylpentadecanoic) and phytanic (3,7,11,15-tetramethylhexadecanoic) acids by gas-liquid chromatography (GLC) of the methyl esters gave only two GLC peaks in each case¹. It was inferred that if one peak in each case coincided with the corresponding ester of the authentic "all-D" isomer prepared from a bacterial (*Halobacterium cutirubrum*) lipid², then the other peak should be the other diastereoisomer (respectively, 2L,6D,10D-pristanate and 3L,7D,11D-phytanate) formed through the reduction of the double bond in phytol (3,7D,11D,15-tetramethyl-2-hexadecen-1-ol). Subsequently, a discrepancy was discovered between optical rotation and GLC data for a pristanic acid of marine origin. This led to an examination by GLC of the diastereoisomers of various samples of pristanic acids as their L-menthyl

esters, and the demonstration of the occurrence in the marine pristanic acid of lesser amounts of two new diastereoisomers in addition to those of the LDD and DDD configuration³. It was also shown that these particular diastereoisomers were essentially absent from two samples of pristanic acids isolated from ruminant fats; the latter were therefore considered to be closely linked to a direct origin from phytol.

The new diastereoisomers were believed to be the DLL and LLL diastereoisomers formed by oxidation of pristane (2,6,10,14-tetramethylpentadecane) in the marine lipid food web. This process could not occur with the phytol-derived hydrocarbon phytane (3,7,11,15-tetramethylhexadecane) since terminal oxidation either regenerates the common diastereoisomers of phytanic acid, or fatty acids of completely new and different structures. Only minor amounts of phytane occur, relative to pristane, in some marine organisms^{4,5}, but higher proportions are reported in others⁶. Phytane is well-known as a component of petroleum⁷⁻⁹ but not necessarily of petroleum precursor systems¹⁰. Small amounts are also found in terrestrial animal lipids^{6,11}. However, phytenic acids with double bonds in other than the 2-position are known to occur in biological systems¹², and various C₂₀ phytol-derived hydrocarbons with one or more double bonds in various positions have been reported as components of marine lipids¹³. Instead of terminal oxidation, the migration of a double bond along the chain might cause racemization of the asymmetric centers originally present in phytol (7D and 11D)¹⁴ and thus produce other diastereoisomers in addition to the expected LLD and DDD forms. It was therefore considered worthwhile examining the L-menthyl esters of the phytanic acid described above¹⁵ for the presence of these other diastereoisomers.

EXPERIMENTAL

The isolation of the marine phytanic acid has been described in detail¹⁵, as has the total synthesis of ethyl phytenate¹⁶ from which all-synthetic phytanic acid was prepared by hydrogenation and saponification. Farnesanoic (3,7,11-trimethyldodecanoic) acid containing four diastereoisomers was prepared from farnesol by procedures similar to those described elsewhere¹⁵. L-Menthyl esters were prepared by reacting the acid chlorides of the acids (generally prepared by refluxing with freshly distilled SOCl₂) with L-menthol in the presence of pyridine according to recognized procedures, strict attention being paid to anhydrous conditions. Gas-liquid chromatography was carried out as described elsewhere¹⁷ with high-efficiency open-tubular columns coated with butanediol-succinate polyester.

RESULTS AND DISCUSSIONS

The methyl esters of the marine pristanic and phytanic acids, analysed on a system of two columns coupled in series and capable of giving 80,000 theoretical plates, are partially resolved into two components as shown in Figs. 1 and 2. Analysis of these marine samples on a single column of half this efficiency has been illustrated elsewhere¹⁸. Analyses of the corresponding L-menthyl esters (single columns) are also shown in Figs. 1 and 2, including the results of coinjection of reference authentic all-DDD L-menthyl phytanate with the marine L-menthyl phytanate esters.

These results suggest that in the marine phytanic acid there are effectively only

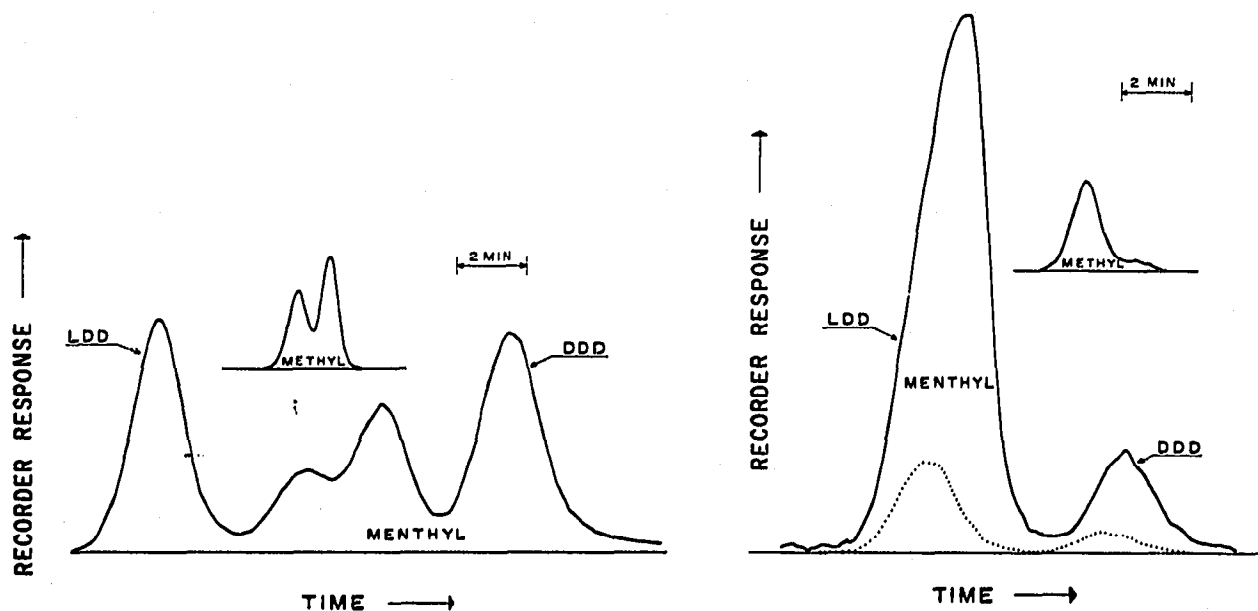


Fig. 1. Comparison of GLC analyses of methyl esters of marine pristanic acid (insert; analysis on coupled columns of approximately 90,000 theoretical plates) with menthyl esters (bottom, analysed on a single column of approximately 40,000 theoretical plates).

Fig. 2. Comparison of methyl (insert) and menthyl (solid line) esters of marine phytanic acid. GLC details as in Fig. 1. Dotted line shows peak area ratio when a small amount of authentic menthyl DDD phytanate was added to sample of marine origin.

two diastereoisomers present, the LDD predominating. The area percentages (Disc Instruments integrator) from the GLC analyses are:

	Methyl	Menthyl
LDD	89	89.2
DDD	11	10.8

The optical rotation measured for this sample (as the methyl ester) was $[\alpha]_D^{25} = -3.58^\circ$. Using the observed $[\alpha]_D^{25}$ values of $+3.78^\circ$ for the DDD diastereoisomer and -0.60° for phytanate derived from phytol (an equal mixture of LDD and DDD respectively^{2, 19, 20}, a value of -4.98° may be calculated for the LDD diastereoisomer. From these values of $[\alpha]_D^{25}$ for the LDD and DDD diastereoisomers it may then be calculated that the observed rotation of -3.58° would result from a mixture of 84 % LDD and 16 % DDD diastereoisomers; these calculated proportions may thus be taken as a reasonable confirmation of the GLC area results. The resolution of the LDD and DDD menthyl phytanates is generally inferior to that of the corresponding pristanates or to that which might be obtained on coupled columns, and this analysis might not disclose other phytanate diastereoisomers at a level of 2–3 % or less even should these occur between the LDD and DDD peaks. Previous results for diastereoisomer ratios in marine and terrestrial phytanates, and terrestrial pristanates, based on methyl esters^{1, 17, 18, 21} are in all probability correct, but published pristanate ratios for samples of marine origin^{1, 18} may be suspect.

The purely synthetic phytanic acid, previously examined by GLC as methyl

and ethyl esters²⁰, was also converted to L-menthyl esters. As shown in Fig. 3 this material gives a triplet peak system in ratios of 1:2:1, with the LDD and DDD diastereoisomers of marine origin coinciding with the first and last peaks. The shape of this triplet peak is similar in all of the known methyl, ethyl, and L-menthyl ester forms for purely synthetic acids, and very similar in aggregate to the composite quartet of peaks observed for synthetic farnesanoic (3,7,11-trimethyldodecanoic) acid in the form of the L-menthyl ester¹⁷.

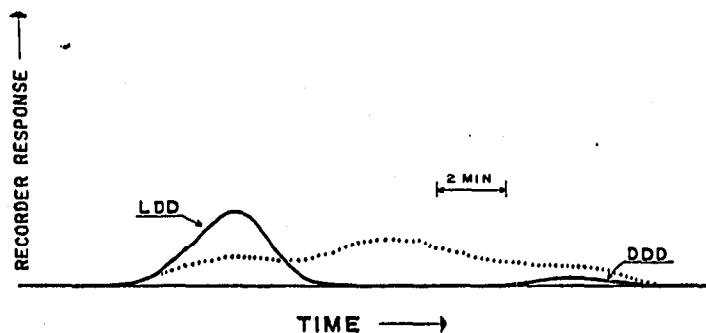


Fig. 3. Comparison of GLC analyses of menthyl esters of all-synthetic phytanic acid (dotted line) and the phytanic acid of marine origin. Single GLC column.

In Table I are listed three synthetic isoprenoid fatty acids (which are racemic at each appropriate methyl branch), the types of esters which have been examined by GLC, and the elution pattern observed on GLC. In the methyl esters enantiomeric pairs are present, but these become diastereoisomers when converted to L-menthyl esters. Pristanic acid of marine origin is included since four components of the eight are known. It is apparent that there is a possible extension to the L-menthyl esters of the guideline that optical isomers of isoprenoid fatty acids having the same configuration at all methyl-branched carbons will elute last when run as methyl esters by GLC²⁰. In the L-menthyl esters it is possible that the "all-D" configuration will definitely elute last, whereas in the methyl esters this guideline always included the enantiomeric "all-L" configuration. Not unexpectedly, in the four L-menthyl esters of pristanic acid (incomplete), and eight of phytanic acid (complete), the LDD diastereoisomer is known to elute first, since it is likely that the proximity of the first methyl branch removed from the carboxyl group gives it maximal interaction with the L-menthyl moiety. This also could be a general guideline. Such relationships have been studied for other ester systems^{22, 23}.

This view can be extended to argue that the modification of the volatility or polarity by the L-menthyl alcohol which confers diastereoisomeric properties to the enantiomeric isoprenoid acids will not be exact, but that subtle interactions will alter the nominally additive properties as tabulated for multiple-branched acids of another series²⁰. The resolution of the L-menthyl ester of synthetic 3,7,11-trimethyldodecanoate into four components by GLC indicates that a complete investigation of such effects through synthesis of the simpler related materials with only one asymmetric center in the fatty acid, and of the specific diastereoisomers with two asymmetric centers in the fatty acids, would be technically feasible, although a challenging problem. Extension of this to the materials with three asymmetric centers in the fatty

TABLE I

SUMMARY OF COMPARATIVE SEPARATIONS OF DIASTEREISOMERS OF ISOPRENOID FATTY ACIDS, AS METHYL AND L-MENTHYL ESTERS, WITH PARTICULAR REFERENCE TO THE ORDER OF ELUTION OF CERTAIN DIASTEREISOMERS WITH OPPOSITE CONFIGURATIONS AT THE FIRST METHYL SUBSTITUENT

Fatty acid and sources ^a	Methyl ester diastereoisomers			L-Menthyl ester diastereoisomers		
	Number expected	Observed format	Position of LD(D) DD(D)	Number expected	Observed format	Position of LD(D) DD(D)
2,6,10-Trimethylundecanoic, (probable), synthetic and natural	2	—	—	4	1:2:1 triplet	? last
3,7,11-Trimethyldodecanoic, synthetic and natural	2	1:1 doublet	first last	4	1:2:1 triplet ^b	? last
4,8,12-Trimethyltridecanoic, synthetic and natural	2	1:1 doublet	first last	4	—	—
2,6,10,14-Tetramethylpentadecanoic, natural	4	1:1 doublet	in first peak in last peak	8	four peaks	first last
3,7,11,15-Tetramethylhexadecanoic, synthetic and natural	4	1:2:1 triplet	second	8	1:2:1 triplet	in first peak in last peak

^a Natural includes chemical preparations from phytol and *H. cutirubrum*.

^b Center peak partially resolved¹⁷.

acid chain would be correspondingly more difficult, but the separations achieved for the natural marine L-menthyl pristanates suggest that the pristanates would be more promising than the phytanates as materials for study.

Exact GLC retention data is not relevant since there were minor variations in operating conditions (except in the instances of GLC runs shown in figures as superimposed); ECL values for L-menthyl esters (relative to methyl esters of normal aliphatic acids) also varied for a given column with age. Typical data have been listed elsewhere¹⁷ for most esters, but comparative ECL values of the four L-menthyl pristanates were 22.35, 22.43, 22.44 and 22.51 on a 150 ft. (50 m) BDS column operated at 150° and 40 p.s.i.g. helium, while the LDD and DDD L-menthyl phytanates had ECL values of 23.50 and 23.65 under different conditions of 170° and 50 p.s.i.g. on another BDS column. The prolonged retention times for L-menthyl esters preclude practical analysis on two high-efficiency columns coupled in series.

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