CHROM 3371

PREDICTION OF RETENTION TIMES IN THE GLC OF DIASTEREOISOMERS OF METHYL-BRANCHED FATTY ACIDS

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SUMMARY

Examination of the order of elution of certain diastereoisomers of a number of multiple methyl-branched fatty acid esters from polar GLC columns suggests a general guide based on molecular rotations. Thus diastereoisomers having molecular rotations of the highest magnitude should elute first followed by others in order by decreasing order of magnitude of molecular rotation. In lieu of experimental molecular rotations which are lacking for most diastereoisomers an attempt has been made to correlate additive molecular rotations $\Sigma[M]_D$ with GLC data. It is believed that some discrepancies from predicted order of elution are due to complex interactions among asymmetric centers which modify one or more of the $[M]_D$ values assigned on the basis of data for fatty acids with a single methyl branch.

It has recently been noted in the gas–liquid chromatography (GLC) of the methyl esters of 2L,4L- and 2D,4L-dimethylhexanoic acids, and of methyl esters of four racemic 2,4,6-trimethyloctanoic acids, that the optical isomers with the same configurations at all of the asymmetric carbons had shorter GLC retention times than isomers where different configurations were present in any one molecule[1].

A different series of naturally occurring methyl-branched fatty acids, based on isoprenoid units, also exists. Recently resolution of methyl esters of certain diastereoisomers of some of these acids has been achieved with efficient polar (~ 45,000 theoretical plates) open tubular (capillary) GLC columns[2,3]. In the GLC of methyl esters of the saturated isoprenoid acids there is apparently a definite reversal of the above observation, with optical isomers having the same configurations at all of the asymmetric carbon atoms having lengthened retention times relative to some other diastereoisomers.

It is possible to calculate the retention time (as equivalent chain length) of esters of a number of methyl-branched fatty acids by the addition of increments of fractional chain length corresponding to the positions of individual methyl group substituents on the aliphatic chain[4,5]. When optically active centers occur in aliphatic chains it is often possible to add molecular rotations for individual asymmetric centers to obtain a reasonable approximation of the experimental molecular rotation for the whole molecule[1,6]. The analogy suggested that molecular rotations ($[M]_D$) might be usefully compared with GLC retention times.

Since experimental $[M]_D$ values were available for only a limited number of materials of interest this comparison was extended to include calculated molecular rotations ($\Sigma[M]_D$) derived from generally accepted $[M]_D$ values for individual optically active centers[6]. The latter, as employed below (Table I), are all to some

TABLE I

SIGNS AND MAGNITUDES OF INDIVIDUAL MOLECULAR ROTATIONS ASSIGNED TO MONOMETHYL SUBSTITUTED FATTY ACIDS (*cf.* ref. 6)

Methyl position	*Assigned* $[M]_D$ *for* D*-methyl substituent*
C_2	−28
C_3	+14
C_4	+ 0.4
C_5	0
ω-8	− 0.4
ω-7	− 0.8
ω-6	− 1.4
ω-5	− 2.0
ω-4	− 2.6
ω-3	− 3
ω-2	−14
ω-1	−12

degree "typical" rather than exact values. The precise point in a long chain at which the sign of rotation for a "D" methyl substituent alters from (+) to (—) is arbitrarily taken as near the C_5 carbon. The designations for carbon atoms relative to the terminal methyl group should be noted. In GLC the *iso* methyl substituent has a significant effect on retention time and was designated ω_1. In optical rotation studies the *iso* methyl substituent is inactive and following literature practices the *anteiso* substituent is designated ω-1. In agreement with GLC practice greater weight is given to the relation between centrally located substituents and the terminal methyl group than to the carboxyl group, excepting in the case of the C_2 and C_3 substituents of shorter chain acids. Since optical isomers totally opposite in configuration cannot be separated by GLC on optically inactive materials the magnitude of $[M]_D$ or $\Sigma[M]_D$ for only one of each diastereoisomeric pair of acids need be considered and sign of rotation is of no significance.

NON-ISOPRENOID FATTY ACIDS

A number of these acids, of the type studied by ODHAM, occur in the preen glands of water birds and are all-"D" in configuration[1,7–9]. As an adjunct to work on these materials the simpler diastereoisomers 2L,4L- and 2D,4L-dimethylhexanoate were synthesized and separated by preparative GLC, the 2L,4L diastereoisomer appearing first when either polypropylene glycol or Versamid was the liquid phase[1]. Four diastereoisomeric pairs of 2,4,6-trimethyloctanoates have also been synthesized and separated by analytical GLC on a polar open-tubular column[7,9]. The order of elution is indicated as: 2D,4D,6D slightly before 2D,4L,6D, followed by a well-separated peak for 2D,4D,6L and finally by a well-separated peak for 2L,4D,6D.

In both instances the order of GLC elution is by decreasing order of magnitude

for $\Sigma[M]_D$ values. In the 2,4-dimethylhexanoates the difference in $\Sigma[M]_D$ values (Table II) is large, and the observed $[M]_D$ values show an even greater difference. This suggests a relation to the facile GLC separation. In the 2,4,6-trimethyloctanoates there is a rough correlation between $\Sigma[M]_D$ magnitudes ($43 > 37 \gg 19 \gg 13$) and observed component retention times in GLC. However, the data of Table II indicate that in this series of acids it is unusual for $[M]_D$ and $\Sigma[M]_D$ values to be in good agreement.

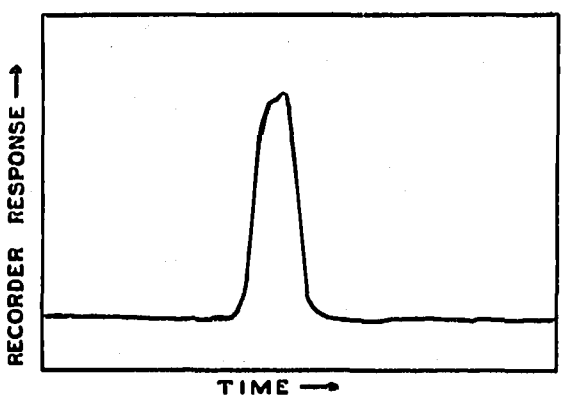

Fig. 1. Resolution of diastereoisomers of methyl esters of 3,7,11-trimethyldodecanoic acid (prepared from farnesol). Coupled columns (300 ft.) operated at 140° and 80 p.s.i.g. helium. Retention time 28 min.

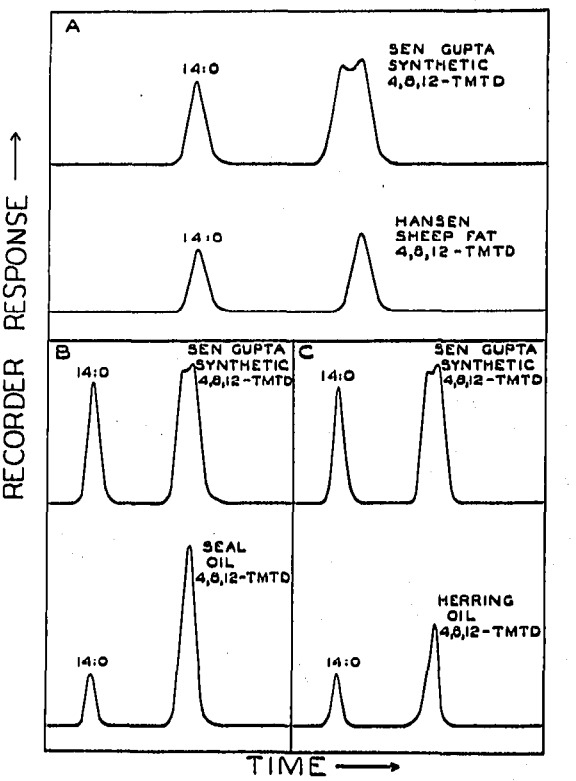

Fig. 2. Resolution of diastereoisomers of methyl esters of 4,8,12-trimethyltridecanoic acid (prepared from farnesol) compared with materials isolated from natural sources. Coupled columns (300 ft.). Operating conditions: (A) 140°, 80 p.s.i.g. helium; (B and C) 150°, 80 p.s.i.g. helium. Retention times: (A) 14:0, 47 min; 4,8,12-TMTD, 50 min. (B and C) 14:0, 32 min; 4,8,12-TMTD, 34 min.

ISOPRENOID FATTY ACIDS

There is only partial resolution of the diastereoisomeric pairs of synthetic (from farnesol) 3,7,11-trimethyldodecanoic acid esters (Fig. 1). The $\Sigma[M]_D$ values of (C_3) + (ω-4) are respectively (—) 16.6 for the 3L,7D isomer and (+) 11.4 for the 3D,7D isomer. It is accordingly presumed that the decrease in $\Sigma[M]_D$ magnitude corresponds to the actual order of elution in GLC since the all-"D" component elutes last in several members of this series.

Somewhat more information is available for evaluation of data in the diastereoisomeric pairs of synthetic (from farnesol) 4,8,12-trimethyltridecanoic acid (Fig. 2). The $\Sigma[M]_D$ values of (C_4) + (ω-4) are respectively (—) 3.0 for the 4L,8D isomer

TABLE II

OBSERVED[1,7–9] AND CALCULATED MOLECULAR ROTATIONS FOR METHYL ESTERS OF DIASTEREOISOMERS OF NON-ISOPRENOID METHYL-BRANCHED FATTY ACIDS

Fatty acid methyl ester	*Molecular rotation* (°)		
	Experimental ($[M]_D$)	*Calculated* ($\Sigma[M]_D$)	
2L,4L-dimethylhexanoate	+50.9	(C_2) + (ω-1)	+40
2D,4L-dimethylhexanoate	−12.8		−16
2D,4D,6D-trimethyloctanoate	−60.4	(C_2) + (ω-3) + (ω-1)	−43
2L,4D,6D-trimethyloctanoate			+13
2D,4D,6L-trimethyloctanoate			−19
2D,4L,6D-trimethyloctanoate			−37
2D,4D,6D-trimethylnonanoate	−48.8	(C_2) + (ω-4) + (ω-2)	−44.6
2D,4D,6D,8D-tetramethyldecanoate	−80.6	(C_2) + (ω-5) + (ω-3) + (ω-1)	−44.4
2D,4D,6D,8D-tetramethylundecanoate	−59	(C_2) + (ω-6) + (ω-4) + (ω-2)	−46

TABLE III

$\Sigma[M]_D$ VALUES FOR DIASTEREOISOMERS OF PRISTANIC (2,6,10,14-TETRAMETHYLPENTADECANOIC) ACID

Substituent	*Diastereoisomer*			
	2D,6D,10D	2L,6D,10D	2D,6D,10L	2D,6L,10D
C_2	−28	+28	−28	−28
C_6 (ω-8)	− 0.4	− 0.4	− 0.4	+ 0.4
ω-4	− 2.6	− 2.6	+ 2.6	− 2.6
$\Sigma[M]_D$	−31	+25	−25.8	−30.2

TABLE IV

$\Sigma[M]_D$ VALUES FOR DIASTEREOISOMERS OF PHYTANIC (3,7,11,15-TETRAMETHYLHEXADECANOIC) ACID

Calculation	*Substituent*	*Diastereoisomers*			
		3D,7D,11D	3L,7D,11D	3D,7D,11L	3D,7L,11D
I	C_3	+14	−14	+14	+14
	C_7 (ω-8)	− 0.4	− 0.4	− 0.4	+ 0.4
	ω-4	− 2.6	− 2.6	+ 2.6	− 2.6
	$\Sigma[M]_D$	+11.0	−17.0	+16.2	+11.8
II	C_3	+14	−14	+16	+16
	C_7 (ω-8)	− 0.4	− 0.4	− 0.4	+ 0.4
	ω-4	− 2.6	− 2.6	+ 2.6	− 2.6
	$\Sigma[M]_D$	+11.0	−17.0	+18.2	+13.8

TABLE V

RELATIVE RETENTION DATA FOR METHYL ESTERS OF VARIOUS ISOPRENOID FATTY ACIDS COINJECTED WITH METHYL ESTERS OF ALIPHATIC FATTY ACIDS (BDS COLUMN NO. 10, 140°, 40 P.S.I.G. HELIUM)

Compound	*Bacterial "all-D" pristanate and phytanate*	*Phytol-derived phytanate*	*All-synthetic phytanate*	*Mixed all-synthetic + phytol-derived phytanate*	*Phytol-derived 4,8,12,16-TMHD*
14:0[a]	1.000	1.000	1.000	1.000	1.000
DDD-Pristanate	2.414	—	—	—	—
16:0	2.467	2.468	2.466	2.469	2.473
17:0	3.857	3.857	3.855	3.855	3.853
?-Phytanate	—	—	4.099	4.097	—
LDD-Phytanate	—	4.146	4.148	4.148	4.149
?-Phytanate	—	—	4.157	Not obvious	—
DDD-Phytanate	4.212	4.211	4.213	4.210	4.208
18:0	6.018	6.025	6.025	—	6.017
?	—	—	—	—	7.081
4L,8D,12D,16-TMHD (?)	—	—	—	—	7.161
4D,8D,12D,16-TMHD (?)	—	—	—	—	7.273

[a] Adjusted retention time 13 min.

and (—) 2.2 for the 4D,8D isomer. The 4,8,12-trimethyltridecanoic acids isolated from one terrestrial source and two marine sources[2,3] show only one peak on GLC and correspond to the synthetic component of longer retention time. There is strong evidence that these naturally occurring acids are derived from phytol (3,7D,11D,15-tetramethyl-2-hexadecen-1-ol) and are therefore all-"D" in configuration. The decrease in $\Sigma[M]_D$ magnitude is therefore probably in accord with the order of elution on GLC.

The pristanates (2,6,10,14-tetramethylpentadecanoates) isolated from natural sources show two GLC peaks[2,3] of which that with the longer retention time corresponds to the peak for a 2D,6D,10D isomer prepared from all-"D" phytanate. It is inferred that the other peak is the 2L,6D,10D isomer since both of these isomers should be formed when phytol is degraded to pristanic acid (see below). Unfortunately $\Sigma[M]_D$ values calculated for the four pristanic diastereoisomers (Table III) include respectively (—) 31 for the 2D,6D,10D isomer and (+) 25 for the 2L,6D,10D isomer. Decreasing $\Sigma[M]_D$ values are thus not in agreement with GLC retention time for the two isomers in question.

The phytanates (3,7,11,15-tetramethylhexadecanoates) isolated from natural animal sources also normally show two peaks. That of longer retention time coincides with the peak for 3D,7D,11D,15-phytanate prepared from the lipids of a halophilic bacterium[2,10]. Reduction of the double bond in phytol produces 3L and 3D asymmetric centers and hence the phytanate component of shorter GLC retention time is reasonably assumed to be the 3L,7D,11D isomer. Since the $\Sigma[M]_D$ values (Table IV) are respectively (—) 17.0 for the 3L,7D,11D isomer and (+) 11.0 for the 3D,7D,11D isomer the principle relating increasing GLC retention time and decrease in magnitude for $\Sigma[M]^D$ values appears to be operative for these particular isomers (see below).

Esters of 4,8,12,16-tetramethylheptadecanoic acids derived from phytol would have respective $\Sigma[M]_D$ values of (—) 3.4 for the 4L,8D,12D isomer and (—) 2.6 for the 4D,8D,12D isomer. Two GLC peaks are observed (Fig. 3; Table V) but as in the case of 3,7,11-trimethyltridecanoate there is no definite evidence that the component of longer retention time is the all-"D" isomer.

VALIDITY OF $\Sigma[M]_D$ VALUES FOR ISOPRENOID FATTY ACIDS

Experimental $[M]_D$ values for authentic all-"D" materials were respectively (—) 37.8° for methyl pristanate and (+) 10.8° for methyl phytanate (corrected for 3 % pristanate impurity)[10]. These compare with $\Sigma[M]_D$ values of (—) 31° and (+) 11°. It must be recognized that the $[M]_D$ values of Table I are adopted from literature values for acids[6], not esters, and that experimental values are sensitive to solvent and other factors. It is not possible, with present evidence, to rationalize interactions among the asymmetric centers of isoprenoid fatty acids which would modify the $\Sigma[M]_D$ values for the 2D,6D,10D and 2L,6D,10D pristanates. Possibly the discrepancy between decreasing magnitude of $\Sigma[M]_D$ values and increasing GLC retention times arises from the methyl substituent at C_2 in this case.

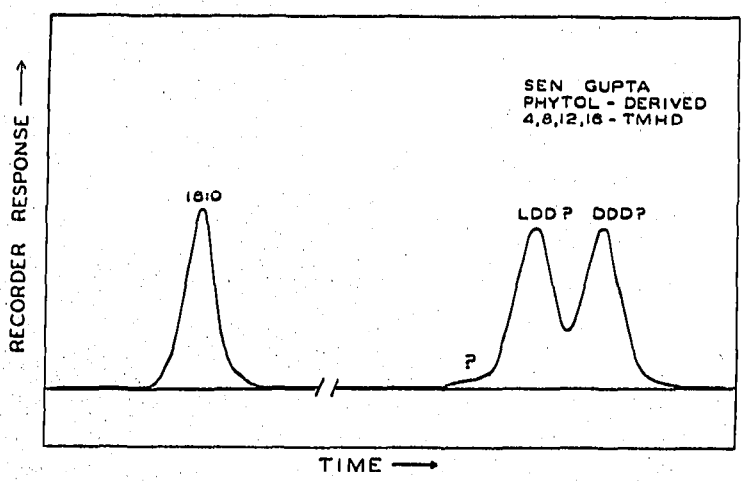

Fig. 3. Resolution of diastereoisomers of methyl esters of phytol-derived 4,8,12,16-tetramethylheptadecanoic acid. Single column (No. 10) operated at 140° and 40 p.s.i.g. helium. Retention data are given in Table V.

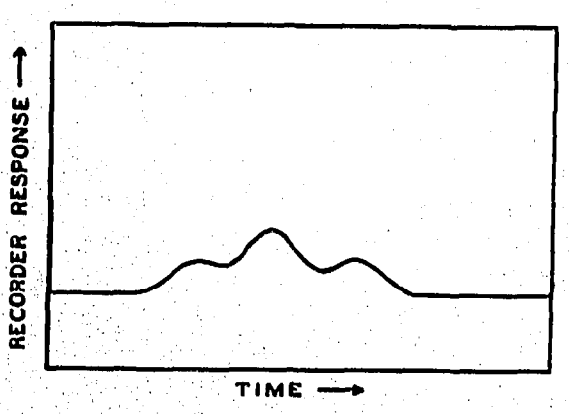

Fig. 4. Resolution of diastereoisomers in ethyl phytanates of completely synthetic origin. Single column (No. 7) operated at 150° and 40 p.s.i.g. helium. Retention time 64 min.

An opportunity to investigate possible asymmetric center interactions in the phytanates arose when a completely synthetic[11] sample of ethyl phytenate was obtained. This was hydrogenated to ethyl phytanate (Fig. 4) and subsequently converted to methyl phytanate (Fig. 5; Table V). The ethyl and methyl phytanates,

which have similar GLC peak patterns, each contain four diastereoisomeric pairs of phytanates. Careful GLC study suggests that these appear, on GLC, with the retention time and degree of separation as follows: unknown $\ll$ 3L,7D,11D $<$ unknown $\ll$ 3D,7D,11D. The original straightforward calculation I (Table IV) is correct only insofar as the $\Sigma[M]_D$ magnitudes indicate that 3L,7D,11D will precede 3D,7D,11D and that 3D,7D,11D will appear last. One example of the many possible interactions which might affect the magnitude, and possibly even the sign of a low $[M]_D$ value asymmetric center, such as the C_7 (ω-8), is given in calculation II, Table IV. Should the presence of opposite rotations at the C_7 (ω-8) and ω-4 asymmetric centers allow a slight increase in the $[M]_D$ value for the C_3 center in the 3D,7D,11L and 3D,7L,11D isomers, then the GLC retention times of the four diastereoisomers would be, in decreasing order of $\Sigma[M]_D$ magnitude, 3D,7D,11L $<$ 3L,7D,11D $<$ 3D,7L,11D$<$ 3D,7D,11D. This rudimentary Procrustean adjustment, although producing a reasonably attractive interpretation of the observed distribution of GLC peaks, is undoubtedly a gross oversimplification.

There are other aspects of these separations, such as the relatively poor separation among 3,7,11-trimethyldodecanoate and 4,8,12-trimethyltridecanoate diastereoisomers on comparison with 3,7.11,15-tetramethylhexadecanoate and 4,8,12,16-tetramethylheptadecanoate diastereoisomers, which are not easily explained.

OBSERVATIONS ON THE GLC HEIGHT RATIOS OF PHYTOL-DERIVED PHYTANATES

An additional minor component immediately preceded the 4,8,12,16-TMHD peaks (Fig. 3). This observation is of interest since it could be an additional diastereoisomer originating in the source phytol or as an artifact from the extensive chemical

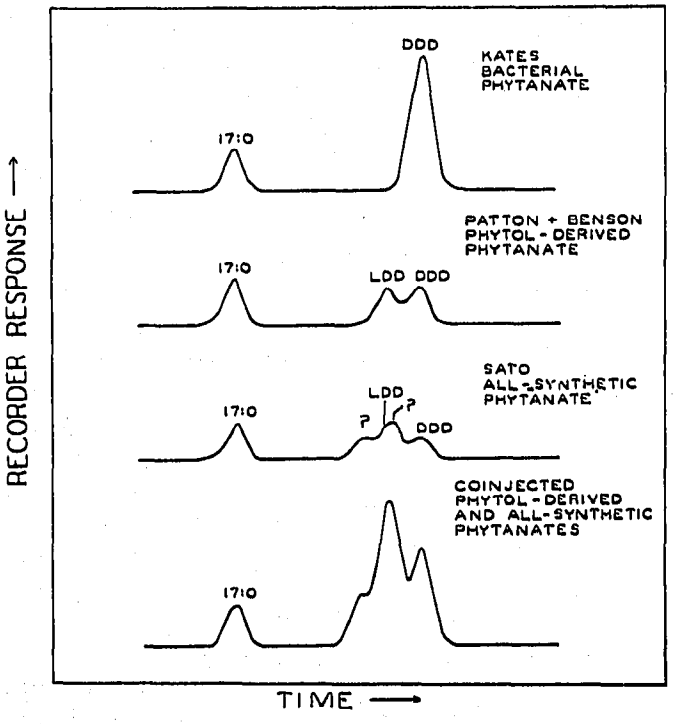

Fig. 5. Resolution of diastereoisomers in methyl phytanates of different degrees of complexity. Single column (No. 10) operated at 140° and 40 p.s.i.g. helium. Retention data are given in Table V.

treatment. If a fourth diastereoisomer were present, and if the order of elution were similar to the phytanates (Fig. 5), this would be superposed on the presumed 4L,8D, 12D,16-TMHD peak which it will be noted is of height equal to the presumed 4D,8D, 12D,16-TMHD peak.

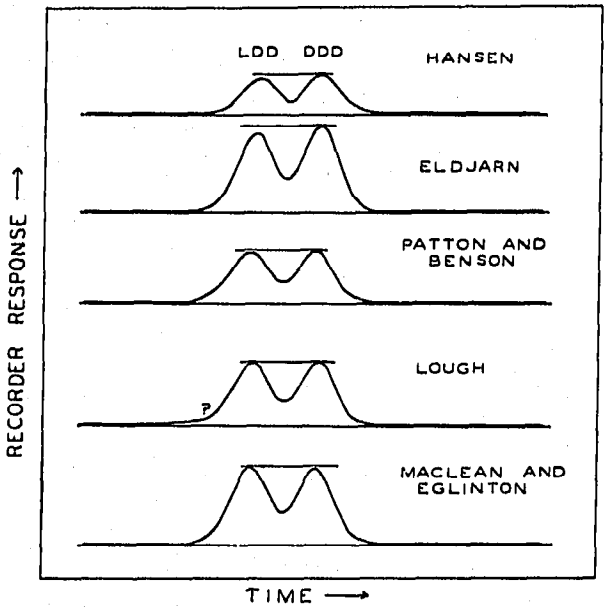

Fig. 6. Examination of peak height ratios for similar sized samples of phytol-derived phytanates prepared in different laboratories. All analyses were carried out with coupled column (300 ft.) operated at 150° and 80 p.s.i.g. helium. Retention time 108 min.

In the original publication on the resolution of diastereoisomers of phytanic acid a definite deviation from a 1:1 peak height relationship for phytanates derived from phytol was noted[2]. Since peak resolution was poor it was possible to explain this as due to tailing of the 3L,7D,11D-phytanate peak raising the peak height of the 3D,7D,11D-phytanate. Alternatively stereospecificity in chemical reactions was suggested (*cf.* refs. 1 and 7). During a study carried out with coupled columns samples of phytanate prepared in five different laboratories were examined under the same conditions (Fig. 6). It is evident that in respective samples prepared by HANSEN, ELDJARN and PATTON AND BENSON, the second or 3D,7D,11D-phytanate peak is higher than the 3L,7D,11D-phytanate peak. The sample prepared by LOUGH also possibly shows the same characteristics, but in the sample prepared by MACLEAN AND EGLINTON the peak heights are unequivocally equal.

The degree of resolution achieved in these analyses is such that it is difficult to consider that tailing of the earlier peak is responsible for the differences in peak height. If the phytol were not configurationally homogeneous then the additional diastereoisomers produced should be detectable as a separate peak well before the 3L,7D,11D-phytanate peak or should increase the height of this particular peak (*cf.* Fig. 5). Neither effect is observed in the majority of cases. The alternative is to assume stereospecificity in one or more of the chemical steps from phytol to methyl phytanate. It is, however, necessary to make the observation that inadvertent slight enrichment in one diastereoisomer could take place during preparative GLC of these materials on efficient packed columns coated with polar liquid phase, since an apparently homoge-

neous peak would actually be enriched in the 3L,7D,11D phytanate in the leading edge and correspondingly deficient in this diastereoisomer in the tailing edge.

EXPERIMENTAL

GLC operations were similar to those described previously[2,3]. Two butanediol–succinate coated open-tubular columns (Nos. 7 and 10) purchased from the Perkin-Elmer Corp., Norwalk, Conn., U.S.A., were used either singly or coupled in series. The latter combination achieved about 80,000 theoretical plates for methyl hexadecanoate at 150°. All retention data given are adjusted (measured from leading edge of solvent peak) and measured to the intercept of the tangent drawn at the leading edge of the peaks with the baseline.

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NOTE ADDED IN PROOF

Results of collaborative studies with I. MACLEAN AND G. EGLINTON (unpublished) have established that 3D,7D,11-trimethyldodecanoate and 4D,8D,12-trimethyltridecanoate coincide with the components of longer retention time in the respective esters of synthetic acids representing mixtures of diastereoisomers.

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