THE STRUCTURAL IDENTIFICATION OF SOME NATURALLY OCCURRING BRANCHED CHAIN FATTY ALDEHYDES

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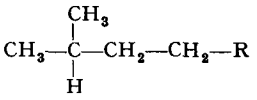
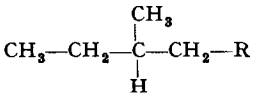
A systematic study of the distribution patterns of the fatty acids and fatty aldehydes of the lecithin- and kephalin-phospholipid fractions isolated from selected animal tissues[1] has revealed the complex nature of some of the mixtures of aldehydes. The aldehydes were separated and identified by gas–liquid chromatography[2]. A large proportion of the aldehyde fraction isolated from ox-spleen and from ox-liver was composed of compounds which, on the basis of their chromatographic behaviour[2], were identified as branched chain aldehydes.

Branched chain acids were first shown to be present in animal fats by SHORLAND[3] and co-workers and were identified as belonging to the iso-(I) and (+)-anteiso-(II) series of saturated branched chain compounds.



(I)



(II)

$R = (CH_2)_nCOOH$

It is reasonable to suppose that the branched chain aldehydes would also have the same iso- or anteiso-structure since the biosynthesis of both fatty acids and fatty aldehydes probably proceeds initially from the same set of carbon "fragments".

The gas chromatographic behaviour of methyl esters of authentic members of the iso- and anteiso-series of fatty acids has been studied and reliable retention data on different stationary phases have been published[4,5]. The branched chain aldehydes were therefore oxidized to the corresponding acids and the chromatographic behaviour of the methyl esters of these acids compared with that of authentic iso- and anteiso-markers. The results showed that the behaviour of the methyl esters of the acids derived from the aldehydes was identical to that of the authentic iso- and anteiso-markers on both polar and non-polar stationary phases. On the basis of the evidence the naturally occurring branched chain aldehydes belong therefore to the iso- and anteiso-series.

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EXPERIMENTAL

Materials

The methyl esters of 12-methyltridecanoic acid, 12-methyltetradecanoic acid, 14-methylpentadecanoic acid, 16-methylheptadecanoic acid and 16-methyloctadecanoic acid were prepared from authentic samples kindly donated by Dr. A. H. MILBURN.

The aldehydes were isolated from the choline plasmalogen fraction of ox-spleen and converted to their dimethyl acetal derivatives by methods described previously[1,2].

The quantitative oxidation of the aldehydes to the corresponding acids was carried out by adapting the excellent method of POLLARD, CHIBNALL AND PIPER[6] for the oxidation of long chain alcohols to acids. The aldehyde dimethyl acetals (0.1 ml) were dissolved in 4.0 ml 90% acetic acid, 0.25 ml 5 *N* anhydrous methanolic HCl was added and the solution was heated for 1 h at 90–100°. The solution was diluted with one volume of water and extracted with (4 ×) 1.5 ml light petroleum

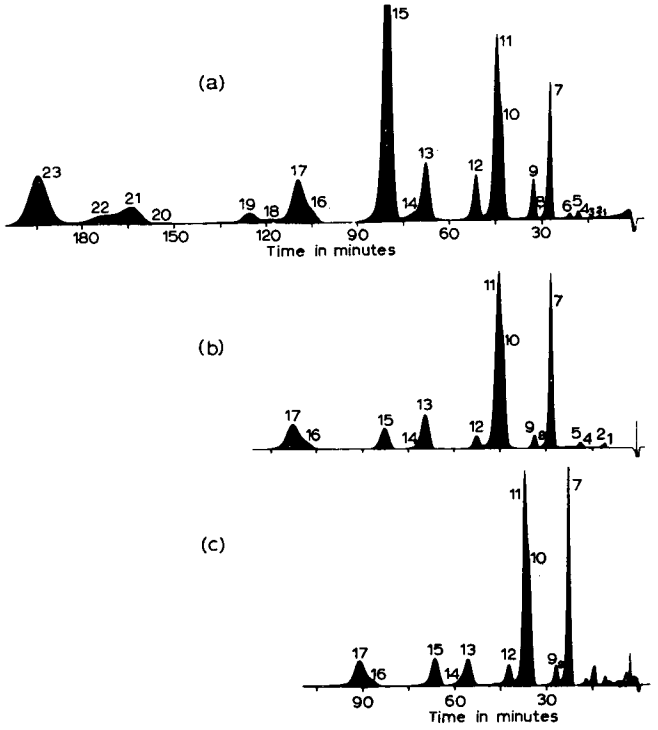

Fig. 1. 4 ft. column with Apiezon L grease as stationary phase at 190°. Argon pressure, column inlet, 103 cm Hg; outlet pressure, atmospheric. (a) Analysis of dimethyl acetals of aldehydes isolated from ox-spleen choline plasmalogen. (b) Analysis of dimethyl acetals of aldehydes isolated from ox-spleen choline plasmalogen after removing most of the straight chain saturated and unsaturated components. (c) Analysis of the same mixture as in (b) after the aldehyde dimethyl acetals had been converted by oxidation to the corresponding acid methyl esters. Peaks: (1) and (2) branched dodecanoic; (3) *n*-dodecanoic; (4) and (5) branched tridecanoic; (6) *n*-tridecanoic; (7) and (8) branched tetradecanoic; (9) *n*-tetradecanoic; (10) and (11) branched pentadecanoic; (12) *n*-pentadecanoic; (13) branched hexadecanoic; (14) branched hexadecanoic + monounsaturated hexadecanoic; (15) *n*-hexadecanoic; (16) and (17) branched heptadecanoic; (18) unknown; (19) *n*-heptadecanoic; (20) linoleic; (21) oleic; (22) isooleic; (23) *n*-octadecanoic.

(b.p. 40–60°). The light petroleum was removed under vacuum and the residue was dissolved in 2.5 ml glacial acetic acid. The solution was warmed slightly (approx. 40°) and small amounts of chromium trioxide in glacial acetic acid were added until excess was present (solution remained red-brown instead of gradually changing to green). The solution was then poured into water (2 volumes) and extracted with benzene. The benzene solution was washed with water, evaporated to dryness and the residue was dissolved in dry ether. Sodium methoxide was gradually added and a precipitate of the sodium salts of the fatty acids was formed. This was centrifuged, washed with a little light petroleum and dissolved in water. The acids were liberated with HCl, extracted with ether, dried over anhydrous sodium sulphate and finally converted to their methyl esters with anhydrous methanolic HCl.

Gas chromatographic analysis of dimethyl acetals of the aldehydes isolated from ox-spleen choline plasmalogen (Fig. 1(a)) indicated that the branched chain aldehydes were in the range C_{13}–C_{17} though there were considerable amounts of saturated and unsaturated straight chain C_{18} compounds and a small amount of saturated C_{20} compound[7]. In order to keep side products of the oxidation procedure to a minimum and make the identification of the branched chain compounds as easy as possible, all compounds of chain length above C_{17} and as much as possible of the straight chain saturated compounds C_{12}–C_{17} were removed prior to oxidation by passing a quantity of the whole aldehyde fraction through a preparative gas chromatographic column and collecting only the branched chain components. No attempt was made to separate all the saturated components as small amounts of these provide internal reference compounds for retention data. Fig. 1(b) shows the composition of the mixture after separation on the preparative column.

Columns

The gas–liquid chromatographic apparatus was a laboratory constructed model using the argon β-ray ionization detector. The amplifier was a commercial one supplied by W. G. Pye & Co. Ltd. All analytical columns were of glass 4 ft. long and 4 mm internal diameter. The stationary phases used were Apiezon L grease (1.8 g to 7 g celite) and Reoplex 400 (1.05 g to 7 g celite). The celite (100–120 mesh) was prepared by the method of JAMES AND MARTIN[8] and for use with Apiezon L grease was pretreated with alcoholic alkali.

The preparative column was of glass 5 ft. long and 13 mm internal diameter. The stationary phase was Apiezon L grease (18 g to 60 g celite) (80–100 mesh). A small bleeder unit was incorporated between the column and the argon detector so that only a controlled percentage of the gas outflow from the column, further diluted with pure argon, actually passed through the detector. Thus whatever the size of the sample loaded on the column no overloading of the detector occurred.

RESULTS

The composition of the mixture of aldehyde dimethyl acetals before oxidation of the

aldehydes to the corresponding acids is shown in Fig. 1(b). The interpretation of whether the recording of the chromatographic separation of the aldehyde mixture was showing the presence of small amounts of components (labelled 8 and 14) or excessive tailing of components 7 and 13 was checked by chromatographing the mixture on Apiezon L at 160°. The increase in the separation efficiency of the column at the lower temperature was sufficient to show that components 7 and 13 were not tailing and that components 8 and 14 were in fact real.

The composition of the mixture of methyl esters of the acids obtained by oxidation of the aldehydes is shown in Fig. 1(c) and a comparison with Fig. 1(b) shows the marked difference in retention volumes of acetals and esters of the same carbon number. The appearance of a number of small fast-running components after oxidation was probably the result of breakdown across the double bond of some palmitoleic aldehyde present in the mixture. A decrease in the height of the peak representing components 13 and 14 (Fig. 1(c)) on Apiezon L and palmitoleic aldehyde on Reoplex 400 after oxidation as compared with the peak heights of other components indicated that the unsaturated components do suffer some breakdown during the oxidation procedure.

A number of samples of the mixture of acid methyl esters, each plus a different authentic branched chain marker, were chromatographed on Apiezon L (Fig. 2) and Reoplex 400 stationary phases. The carbon numbers of the acid esters were

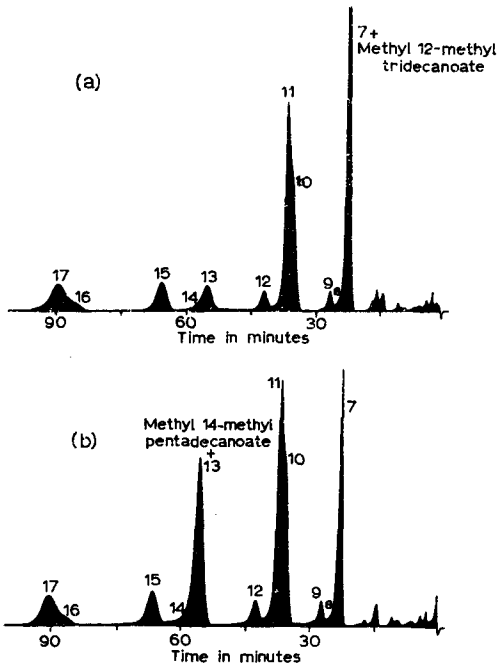

Fig. 2. Analysis of the methyl esters of acids (Fig. 1(c)) obtained by oxidizing a mixture of aldehydes (Fig. 1(b)) isolated from ox-spleen choline plasmalogen. Column conditions as for Fig. 1. (a) Mixture plus methyl 12-methyltridecanoate. (b) Mixture plus methyl 14-methylpentadecanoate. Peak identification as for Fig. 1.

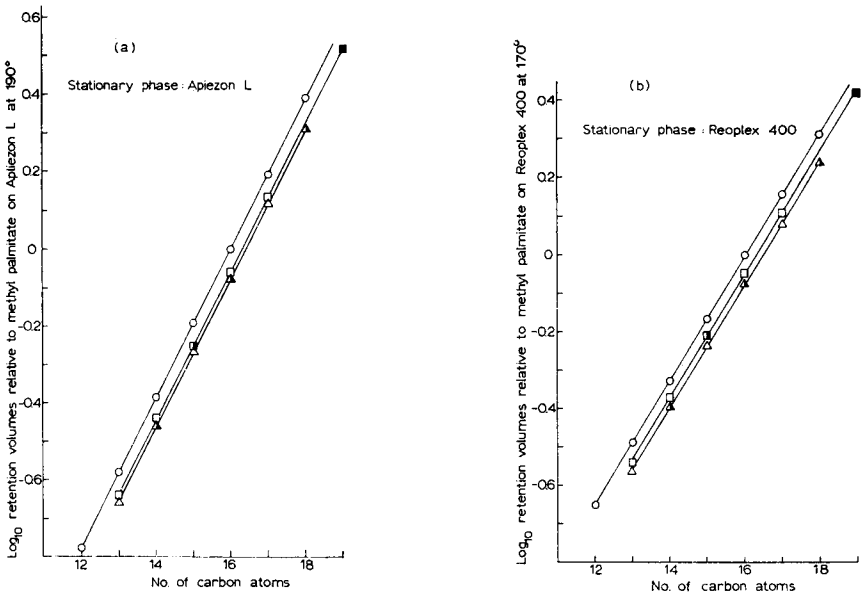

Fig. 3. Retention volumes of some saturated straight chain and branched chain fatty acids relative to methyl palmitate. Straight chain saturated acids ○ ; pure synthetic branched chain acids of the iso-series ▲ ; pure synthetic branched chain acids of the anteiso-series ■ ; branched chain acids obtained by oxidation of branched chain aldehydes isolated from ox-spleen choline plasmalogen △, □ ; synthetic and isolated compounds with identical retention volumes ◭, ◨.

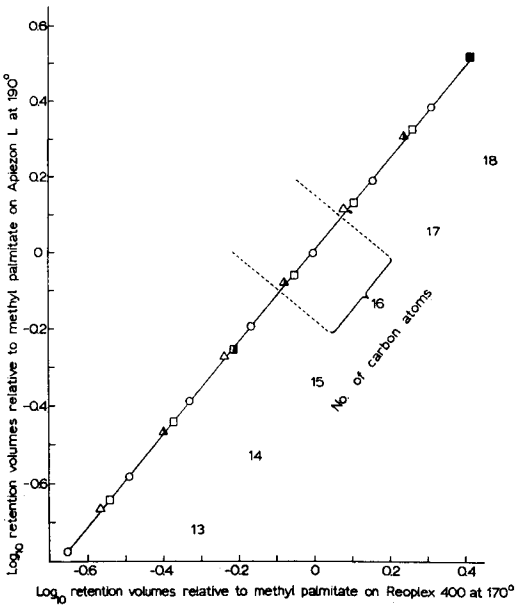

Fig. 4. Log_{10} relative retention volumes of saturated straight and branched chain methyl esters on Apiezon L plotted against log_{10} relative retention volumes on Reoplex 400. Symbols as for Fig. 3.

plotted against the logarithms of their retention volumes relative to methyl palmitate (Fig. 3).

The closed symbols represent the plots of synthetic branched chain acids of known structure and the half-closed symbols represent plots of the synthetic acid and of a component of the mixture with an identical retention volume. From a comparison of their chromatographic behaviour with that of the pure synthetic compounds it is apparent that all the branched chain acids derived from the aldehydes belong to either the iso- or anteiso-series of compounds. Additional confirmation of their saturated branched chain structure was obtained by plotting the logarithms of the relative retention volumes on Apiezon L stationary phase against those on Reoplex 400 stationary phase (Fig. 4).

These results provide sound evidence for stating that, like the naturally occurring branched chain acids, the naturally occurring branched chain aldehydes belong to the iso- and anteiso-series of compounds. The relative retention volumes and structures of a number of branched chain aldehydes found in ox-spleen and ox-liver are given in Table I. It was found that the retention volumes of the aldehyde dimethyl

TABLE I

RETENTION VOLUMES* OF SOME BRANCHED CHAIN ALDEHYDE DIMETHYL ACETALS RELATIVE TO PALMITALDEHYDE DIMETHYL ACETAL

Peak No. Fig. 1(a)	*Aldehyde*	*Shorthand designation*	*Stationary phase*	
			Apiezon L at 190°	*Reoplex 400 at 170°*
4	11-Methyldodecanal	iso-br. 13:0	0.218	0.270
5	10-Methyldodecanal	anteiso-br. 13:0	0.228	0.286
7	12-Methyltridecanal	iso-br. 14:0	0.348	0.400
8	11-Methyltridecanal	anteiso-br. 14:0	0.362	0.423
10	13-Methyltetradecanal	iso-br. 15:0	0.535	0.580
11	12-Methyltetradecanal	anteiso-br. 15:0	0.560	0.616
13	14-Methylpentadecanal	iso-br. 16:0	0.840	0.840
14	13-Methylpentadecanal	anteiso-br. 16:0	0.870	0.892
15	Hexadecanal	16:0	1.00	1.00
16	15-Methylhexadecanal	iso-br. 17:0	1.31	1.20
17	14-Methylhexadecanal	anteiso-br. 17:0	1.36	1.278

* All retention volumes were measured from the middle of the air peak.

acetals over the range C_{12} to C_{17} relative to palmitaldehyde dimethyl acetal were identical to the retention volumes of their corresponding acid methyl esters relative to methyl palmitate under the same chromatographic conditions.

ACKNOWLEDGEMENT

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SUMMARY

Branched chain aldehydes isolated from animal tissues have been shown to belong to either the iso- or anteiso-series of compounds by oxidising them to the corresponding acids and comparing the gas chromatographic behaviour of these acids with that of pure synthetic acids of known structure.

REFERENCES

[1] G. M. GRAY, *Biochem. J.*, 77 (1960) 82.
[2] G. M. GRAY, *J. Chromatog.*, 4 (1960) 52.
[3] F. B. SHORLAND, T. GERSON AND R. P. HANSEN, *Biochem. J.*, 61 (1955) 702.
[4] G. C. HAWKE, R. P. HANSEN AND F. B. SHORLAND, *J. Chromatog.*, 2 (1959) 547.
[5] A. T. JAMES, *J. Chromatog.*, 2 (1959) 552.
[6] A. POLLARD, A. C. CHIBNALL AND S. H. PIPER, *Biochem. J.*, 25 (1931) 2111.
[7] R. W. KEENAN AND B. H. MARKS, *Biochim. Biophys. Acta*, 39 (1960) 533.
[8] A. T. JAMES AND A. J. P. MARTIN, *Biochem. J.*, 50 (1952) 679.