Die endständigen Hydroxylgruppen leisten somit nur einen geringen Beitrag zur Auftrennung.

Die Trimethylsilylester werden erheblich schneller eluiert als die Methylester. Die Trennfähigkeit der Säule ist bei diesen Verbindungen etwas geringer, gleichfalls der Trennfaktor (s. Tabelle I). Dies gilt auch noch, wenn durch Temperaturerniedrigung die Retentionsvolumina der Trimethylsilylester den der Methylester angeglichen werden.

In den letzten Spalten von Tabelle I ist ein Vergleich der quantitativen Bestimmungen von Stearinsäure, Ölsäure, Linolsäure und Linolensäure, aus Leinöl gewonnen, nach Methylierung und nach Silylierung zusammengestellt. Hieraus geht hervor, dass die gaschromatographische Bestimmung als Trimethylsilylester, was Reproduzierbarkeit und Richtigkeit angeht, durchaus mit den bischer beschriebenen Verfahren konkurrieren kann.

Diese Arbeit wurde unterstützt vom Kuratorium für die sportmedizinische Forschung.

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Eingegangen am 18. Juni 1969

J. Chromatog., 43 (1969) 490–492

CHROM. 4226

Gas chromatographic separation and determination of saturated and unsaturated fatty aldehydes

Long-chain fatty aldehydes occur in living tissues in both free and combined forms[1,2]. They are frequently analyzed by gas–liquid chromatography (GLC) after being converted to dimethyl acetals[3–5].

Recently it has been shown that the dimethyl acetals are decomposed to the corresponding alk-1-enyl methyl ethers during GLC, the decomposition being brought about by aluminum metal and aluminum containing solid supports[6,7]. The alk-1-enyl methyl ethers are further resolved into their *cis* and *trans* isomers during GLC[8]. Other

complicating factors in the use of dimethyl acetals are the concomitant formation and presence of aldehydes[9] and alk-1-enyl methyl ethers[10] during their preparation. To overcome these difficulties the use of cyclic acetals for GLC purposes has been suggested[9]. However, these derivatives have approximately three times the retention times of the free aldehydes.

Direct analysis by GLC of 2,4-dinitrophenylhydrazone derivatives has not been successful except in the case of short-chain aldehydes[11].

This communication describes the separation of long-chain saturated (C_{14}–C_{18}) and unsaturated (oleic, linoleic and linolenic) fatty aldehydes on polar and nonpolar liquid phases, as well as their quantitative analysis.

Experimental

Materials. Pure fatty aldehydes were prepared as described previously[12] and their purity was established by thin-layer chromatography and other techniques[12,13]. Solutions containing each of the acids in concentrations ranging from 25 ng/μl to 400 ng/μl were made in petroleum ether (30–60°) from a stock standard solution.

The following packing materials were used during this study: (1) Gas-Chrom R, 30–60 mesh + 20% β-cyclodextrin acetate (β-CDX); (2) Gas-Chrom P, 80–100 mesh + 20% ethylene glycol adipate (EGA); (3) Chromosorb W, 60–80 mesh + 20% Apiezon L; and (4) Gas-Chrom P 80–100 mesh + 15% ethylene glycol succinate (EGS). All solid supports and liquid phases were obtained from Applied Science Laboratories, State College, Pa., U.S.A..

Gas–liquid chromatography. Gas chromatographic analyses were performed with a dual column Hewlett-Packard Model 5750 instrument equipped with flame ionization detectors and a 1-MV span recorder (Hewlett-Packard). All results were determined isothermally. Aluminum columns (both 1/4 in. and 1/8 in. O.D.) were used, the length being 6 ft. except for the column containing β-CDX, which was 12 ft. long. Helium was employed as the carrier gas. The hydrogen and air flow rates were set so as to optimize the detector response. All sample injections were standardized at 1 μl using a Hamilton microsyringe of 10 μl capacity.

Results

Preliminary investigations were carried out by varying the different parameters such as column length, temperature and carrier gas flow rate in order to find out which one of the columns would give the best separation of the aldehydes.

Figs. 1, 2 and 3 show separations of the aldehydes on columns containing β-CDX, EGA and EGS, respectively. The retention time of each component was established by injecting it alone. Reproducible retention times were obtained in all cases. Of these three polar phases, well defined and symmetrical peaks of all the aldehydes, both saturated and unsaturated, were obtained with EGA and EGS columns. The retention times of the aldehydes on EGS columns were shorter than those obtained with EGA columns. All quantitative analyses, therefore, were performed with EGS columns. Fig. 4 shows the separations of the aldehydes on an Apiezon L column. In this non-polar phase separations of the aldehydes, according to chain length, were good but according to unsaturation were poor.

Detector response. The linearity of the detector response to the six aldehydes is illustrated in Fig. 5. The response of the detector to all the aldehydes is linear between

concentrations of 25 ng and 400 ng when 1 μl samples of the solutions were injected. The areas of the peaks were obtained with the aid of an electronic integrator (Infotronics).

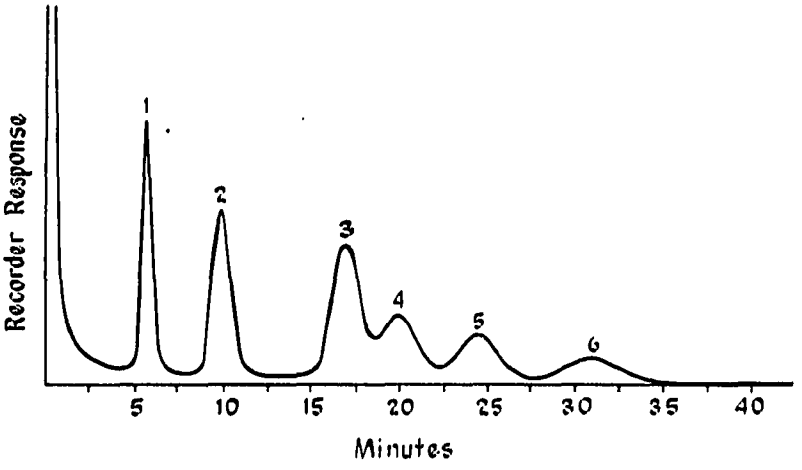

Fig. 1. Separation of myristaldehyde (1), palmitaldehyde (2), stearaldehyde (3), oleyl aldehyde (4), linoleyl aldehyde (5) and linolenyl aldehyde (6). Conditions: column, 12 ft. × 1/4 in. O.D. β-CDX. Temperature, 230°. Injection size 100 ng of each aldehyde in 1 μl petroleum ether.

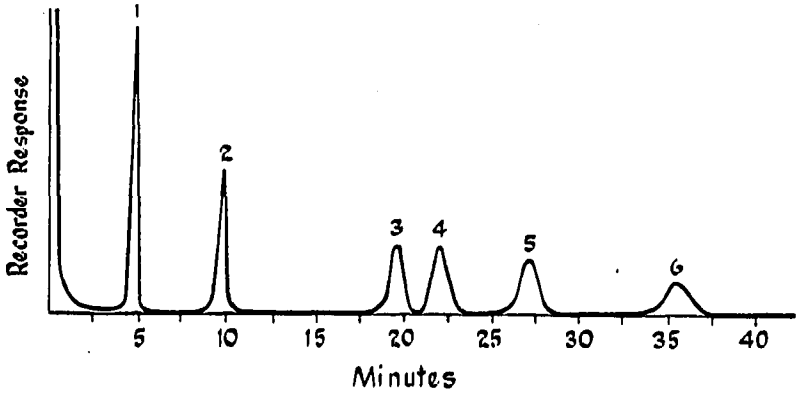

Fig. 2. Separation of myristaldehyde (1), palmitaldehyde (2), stearaldehyde (3), oleyl aldehyde (4), linoleyl aldehyde (5) and linolenyl aldehyde (6). Conditions: column, 6 ft. × 1/4 in. O.D. EGA. Temperature, 190°. Injection size 100 ng of each aldehyde in 1 μl petroleum ether.

TABLE I

RATIOS OF PEAK AREAS OF ALDEHYDES RELATIVE TO PEAK AREA OF PALMITALDEHYDE OBTAINED BY GAS–LIQUID CHROMATOGRAPHY

Aldehyde	*Peak area ratios*[a]
Myristaldehyde	1.15
Palmitaldehyde	1.00
Stearaldehyde	1.00
Oleyl aldehyde	0.98
Linoleyl aldehyde	0.86
Linolenyl aldehyde	0.86

[a] Mean of six determinations.

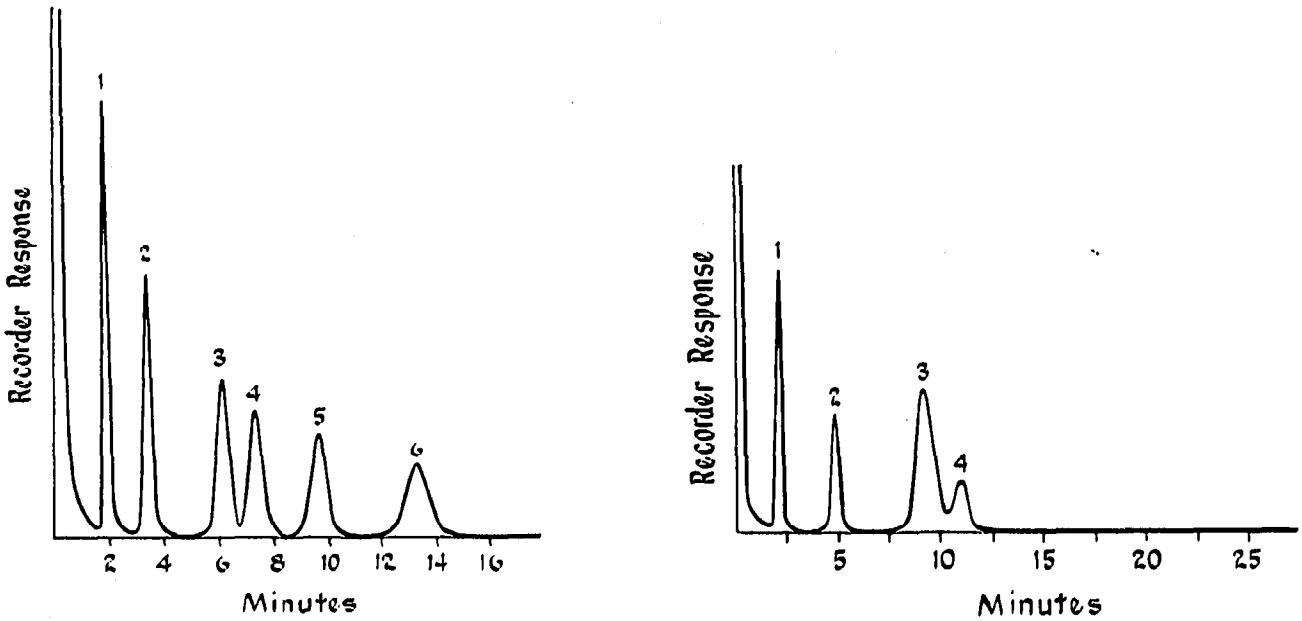

Fig. 3. Separation of myristaldehyde (1), palmitaldehyde (2), stearaldehyde (3), oleyl aldehyde (4), linoleyl aldehyde (5) and linolenyl aldehyde (6). Conditions: column, 6 ft. × 1/8 in. O.D. EGS. Temperature, 170°. Injection size 100 ng of each aldehyde in 1 μl petroleum ether

Fig. 4. Separation of myristaldehyde (1), palmitaldehyde (2), mixture of stearaldehyde, oleyl, linoleyl and linolenyl aldehydes (3 and 4). Conditions: Column, 6 ft. × 1/4 in. O.D. Apiezon L. Temperature, 230°. Injection size 100 ng of each aldehyde in 1 μl petroleum ether.

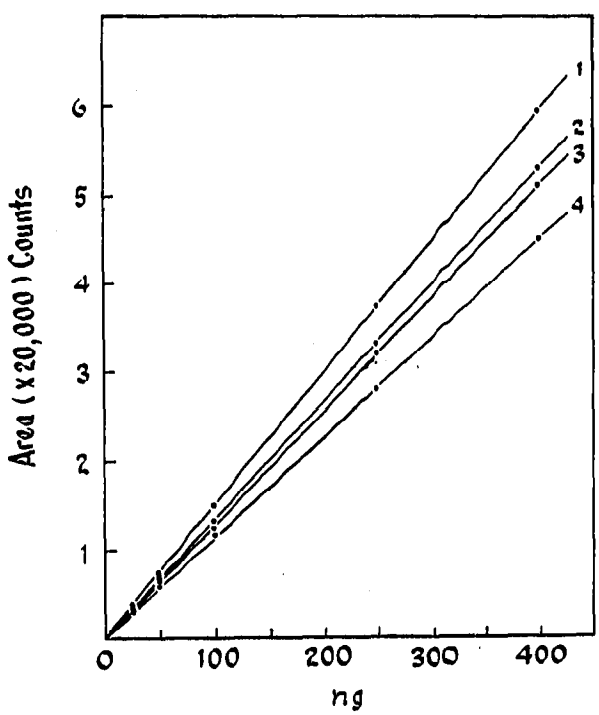

Fig. 5. Standard curves for myristaldehyde (1), palmitaldehyde and stearaldehyde (2), oleyl aldehyde (3), linoleyl aldehyde and linolenyl aldehyde (4) in the range of 25 to 400 ng.

The relationship of peak areas of individual aldehydes, relative to palmitaldehyde, is shown in Table I. In practice, when an unknown mixture of aldehydes is being studied, it will be desirable to use an odd-chain aldehyde, such as C_{15} or C_{17} aldehyde, as an interval standard. When a known amount of odd chain aldehyde is added as an interval standard, the aldehyde values in the unknown mixture can be obtained by peak area relationships as follows:

$$\frac{\text{wt. odd-chain aldehyde} \times \text{peak area aldehyde}}{\text{peak area odd chain aldehyde} \times \text{peak area ratio}} = \text{weight aldehyde in sample}$$

Discussion

Since aldehydes are known to undergo oxidation and polymerization reactions readily, their GLC analyses were usually performed as their dimethyl acetal derivatives[3–5]. That separations of the free aldehydes can be achieved by gas–liquid chromatography has since been shown by other investigators[9,12].

WHITE *et al.*[14] recently reported that higher aliphatic aldehydes are quite stable when stored under vacuum. Palmitaldehyde stored under vacuum at room temperature showed no oxidation in two weeks. The sample stored under nitrogen with refrigeration also showed very little alteration. The rate of acid formation decreased with increasing chain length of the aldehyde. In the present study the GLC responses of the solution of the aldehydes which were stored under nitrogen in the refrigerator for four weeks were identical to the original samples. Storage of the solutions for longer periods produced decreased responses from the polyunsaturated aldehydes.

Unlike the dimethyl acetals, the aldehydes do not undergo any decomposition or other side reactions during GLC[6] and lend themselves readily to separation and quantification by GLC on most of the liquid phases generally used for the GLC of methyl esters of fatty acids.

The technical assistance of JANICE M. OLSEN is gratefully acknowledged.

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Received May 22nd, 1969