Gas–liquid chromatographic analysis of free long-chain aldehydes

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SUMMARY Quantitative results are reported for gas–liquid chromatography of mixtures of free tetra-, hexa-, and octadecanals and *cis*-9-octadecenal on polar and nonpolar liquid phases. Stability of the fatty aldehydes during chromatographic analysis and up to 1 year of storage appears to be related to the solvent (carbon disulfide) used. Analysis of the free aldehydes by gas–liquid chromatography eliminates the disadvantages associated with the preparation and analysis of derivatives for this lipid class.

SUPPLEMENTARY KEY WORDS synthesis · purification · quantitation · storage

GLYCERIDES and phosphoglycerides that contain an alk-1-enyl ether linkage, commonly called neutral plasmalogens and plasmalogens, respectively, occur in the lipids of normal rat tissues (1), transplantable rat and mouse tumors (2), human depot fat (3), and bacteria (4). A detailed review by Snyder (5), of lipids that contain an ether linkage, has recently appeared.

The percentage of alk-1-enyl glyceryl ethers in neutral lipids and phospholipids can be measured by quantitative photodensitometric thin-layer chromatographic analysis (1), whereas the nature of the alk-1-enyl ether hydrocarbon chain is usually measured by gas–liquid chromatographic (GLC) analysis of the dimethyl acetal (6, 7) or 1,3-cyclic acetal derivatives (8). The dimethyl acetals are contaminated with unreacted aldehydes (8) and artifacts formed during their preparation (9); some decomposition occurs during GLC analysis on some columns (10, 11) but not on others (12). Cyclic acetals have approximately three times the retention time of the corresponding free aldehyde and require purification before GLC analysis (8).

This communication describes the quantitative GLC analysis of free long-chain fatty aldehydes on polar and nonpolar liquid phases and the conditions for handling this class of compounds without any apparent decomposition or polymerization.

Aldehyde Synthesis. Long-chain aldehydes (14:0, 16:0, 18:0, and 18:1) were synthesized as follows: purified methyl esters were reduced to the corresponding alcohols with lithium aluminum hydride (13). Tosylates of the alcohols were prepared (14) and oxidized to the aldehydes according to the procedure of Mahadevan, Phillips, and Lundberg (15). The aldehydes were purified by TLC on Silica Gel G in hexane–diethyl ether 95:5. Complete oxidation of the tosylates, which usually occurred, allowed purification by silicic acid column chromatography. Chromatographic conditions were similar to those described previously (15) with the exception of the solvent system. We eluted a hydrocarbon fraction with 150 ml of hexane and the aldehydes with four 50-ml portions of hexane containing 0.5, 1.0, 1.5, and 2.0% diethyl ether, respectively. The IR spectrum of palmitaldehyde was practically identical with that previously reported (15). The aldehydes were stored in carbon disulfide at −20°C.

Gas–Liquid Chromatography. An Aerograph model 204 gas chromatograph (Varian Aerograph, Inc., Walnut Creek, Calif.) equipped with hydrogen flame ionization detectors was used for analyzing the aldehydes. A Pyrex column (5 ft. × 1/8 inch) packed with 15% ethylene glycol succinate–methyl silicone polymer (EGSS-X) coated on 100–120 mesh Gas-Chrom P (Applied Science Laboratories, State College, Pa.) and a stainless steel column (5 ft. × 1/8 inch) packed with 5% methyl silicone polymer (SE-30) coated on 60–80 mesh Chromosorb W were used. The column temperature of the polar liquid phase was manually programmed from 130 to 180°C at approximately 3°C/min, whereas the analyses on the nonpolar SE-30 column were carried out isothermally at 160°C. Injector and detector temperatures were maintained at 290 and 250°C, respectively. The flow rate of the carrier helium gas was 40–60 ml/min. Flow rates of hydrogen and oxygen were regulated to give maximum detector sensitivity. Peak areas were measured by triangulation and the reported values represent the mean of three determinations.

Materials. Purified methyl esters were purchased from The Hormel Institute, Austin, Minn. Spectroquality carbon disulfide and reagent grade diethyl ether were obtained from Matheson, Coleman and Bell and Fisher Scientific. Other solvents were glass distilled and were purchased from Burdick and Jackson Laboratories, Inc., Muskegon, Mich. Other chemicals and reagents were reagent grade or better and were used without further purification.

Results and Discussion. The flame ionization detector response of long-chain fatty aldehydes is equal to that obtained for methyl esters of the same chain-length, which indicates quantitative elution from the GLC column. This is illustrated in Fig. 1 *a* by the equal size of the peaks of 16:0 aldehyde and 16:0 methyl ester, which were present in the mixture in equal molar concentrations. The detector response of the 16:0 and 18:0 aldehydes, relative to methyl stearate, was linear over the concentrations used (from 0.1 to 20.0 μg).

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography. Aldehydes and acyl radicals are designated by chain length:number of C=C double bonds.

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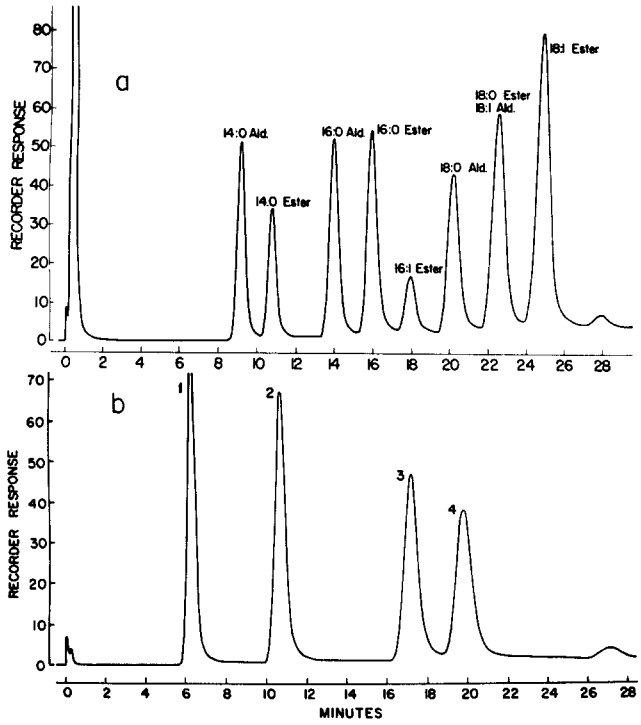

FIG. 1. Representative chromatograms (GLC) obtained on EGSS-X (130–180°C at approximately 3°C/min), showing the retention time of free aldehydes relative to methyl esters (*a*) and the resolution of an aldehyde standard mixture (*b*). The numbered peaks are (*1*) myristoyl; (*2*) palmitoyl; (*3*) stearoyl; and (*4*) oleoyl aldehydes. The small peak with a retention time of 27 min is a contaminant that was not removed from the synthetic oleoyl aldehyde. Differences in the retention times on the two chromatograms are due to differences in initial manual temperature program rates.

Analysis of long-chain fatty aldehydes by GLC yields quantitative values on both polar and nonpolar liquid phases. Experimentally determined values obtained for an aldehyde standard mixture are compared with known percentages in Table 1. Good agreement was obtained between experimental and known values for both the saturated and unsaturated aldehydes. Farquhar (6) has previously chromatographed free aldehydes on ethylene glycol adipate, but gave no quantitative data and uttered a caution about their instability. He was not able to elute free aldehydes from an Apiezon-M column (6), but we encountered no such difficulty with the nonpolar SE-30 column.

The aldehydes were eluted at relatively low temperatures and gave symmetrical peaks on both polar and nonpolar liquid phases. The saturated C_{18} aldehyde was well resolved from the corresponding monounsaturated aldehyde on the EGSS-X column (Fig. 1 *b*); they were also sufficiently resolved on the SE-30 column to allow quantitation. The retention times of aldehydes are shorter than methyl esters of the same chain length (Fig. 1 *a*); thus, olealdehyde elutes with methyl stearate on the EGSS-X liquid phase (Fig. 1 *a*). Dimethyl acetals of saturated aldehydes (or their decomposition products), which have slightly longer retention times than the corresponding free aldehydes (6), elute from polar columns in the approximate region of odd-chain monounsaturated esters and undoubtedly have been erroneously reported as such.

TABLE 1 QUANTITATION OF FREE ALDEHYDES BY GLC

	Known Weight %	Known Mole %	Area % Found* EGSS-X	Area % Found* SE-30
Tetradecanal	20.6	23.9	24.1 ± 1.0	22.4 ± 0.7
Hexadecanal	27.0	27.7	26.4 ± 0.8	27.6 ± 0.2
Octadecanal	28.3	26.1	25.7 ± 0.9	26.8 ± 0.3
cis-9-Octadecenal	24.1	22.4	23.8 ± 0.7	23.7 ± 0.6

* Each of the percentages represents the mean of three determinations ± SD.

The identity of the compounds eluting from the columns was not determined, but we assumed that they were aldehydes since aldehydes elute unchanged under preparative conditions (10).

Despite a common belief that free aldehydes are unstable, decompose, and polymerize (6, 7), we observed no detectable qualitative or quantitative change during TLC or GLC of the standard aldehyde mixtures that included the unsaturated olealdehyde even after storage for more than 1 yr at −20°C in carbon disulfide. The difference between our success and the failure of others to store, handle, and analyze long-chain aldehydes without any apparent decomposition must be attributed to the antioxidant properties of carbon disulfide. This method has been applied to the determination of aldehydes liberated from alk-1-enyl ethers isolated from insects (16), tumor tissue (17), and a number of normal rat tissues (18).

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