Separation of ether homologues by adsorption thin-layer chromatography

Adsorption chromatography is widely used for the resolution of complex mixtures of lipids into classes according to type and number of functional groups. The homologues within a class usually are separated by reversed-phase partition chromatography, although separation of even numbered fatty acid (C_8 to C_{24}) and even numbered fatty alcohol (C_8 to C_{26}) homologues by phase partition chromatography between water and cyclohexane on diatomaceous earth has been described[1]. The present communication discusses separation of such homologues of a number of short-chain alcohol acetals of hexadecanal and alkyl hexadecyl ethers by adsorption thin-layer chromatography (TLC).

Experimental

The acetals were prepared by reacting an aldehyde with an alcohol in the presence of 100 % sulfuric acid at —30°, and subsequent neutralization of the mixture with alcoholic potassium hydroxide as described previously[2]. The short-chain alkyl hexadecyl ethers were obtained by gas–liquid chromatographic decomposition of acetals to vinyl ethers[3], followed by hydrogenation using platinum oxide as catalyst. The products were purified by preparative TLC and their purity tested by TLC and infrared spectroscopy[2]. The following compounds were prepared for study of their chromatographic properties on silica gel layers.

(*A*) *Acetals.* (1) Dimethyl acetals of dodecanal, hexadecanal and *cis,cis,cis*-9,12,15-octadecatrienal, and (2) diethyl, dipropyl, diisopropyl, diallyl, dibutyl, diisobutyl, dipentyl and diisopentyl acetals of hexadecanals.

(*B*) *Alkyl ethers.* (1) Dodecyl, hexadecyl and octadecyl methyl ethers and (2) ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, and isopentyl hexadecyl ethers.

The TLC separation of acetals, and of ethers, was attained on activated Silica Gel G plates[2] with toluene as the developing solvent. The compounds were made visible by spraying the plates with 50 % sulfuric acid, followed by charring in an air oven at 160° for 10 min.

Results

Separation of acetals. The separation is shown in Fig. 1, and for easy interpretation the separations are classified in three groups: (1) Dimethyl (a), diethyl (b), dipropyl (d), dibutyl (f) and dipentyl (h), acetals of hexadecanal separate from one another. (2) Diisopropyl acetal of hexadecanal (c) has a lower R_F value than its normal homologue—dipropyl acetal of hexadecanal (d), while diisobutyl acetal of hexadecanal (g) and diisopentyl acetal of hexadecanal (i) have higher R_F values than their normal homologues dibutyl acetal of hexadecanal (f) and dipentyl acetal of hexadecanal (h), respectively. (3) The following four critical pairs are formed: (i) dimethyl acetals of dodecanal (j), hexadecanal (a) and *cis,cis,cis*-9,12,15-octadecatrienal (k); (ii) diisopropyl (c) and diethyl (b) acetals of hexadecanal; (iii) dipropyl (d) and diallyl (e) acetals of hexadecanal partially overlap; and (iv) diisobutyl (g) and diisopentyl (i) acetals of hexadecanal partially overlap. The critical pairs are separated by a combination of TLC techniques. For example, the first critical pair is separated by reversed-phase partition chromatography on TLC[4]. The second critical pair is separated by adsorption TLC after converting them to alkyl ethers as described later in this communication. The

third critical pair is separated by argentation adsorption TLC with toluene as the developing solvent. The last critical pair is separated by double development in the same TLC system.

Separation of alkyl ethers. The separation is shown in Fig. 2, which indicates that methyl (a), ethyl (b), isopropyl (c), propyl (d), butyl (e) and pentyl (f) hexadecyl ethers separate well from each other, and isobutyl (g), pentyl (f) and isopentyl (h) hexadecyl ethers have very close R_F values. The dodecyl (i), hexadecyl (a) and octadecyl (j) methyl ethers have the same R_F values but are, however, easily separable in a reverse phase system (Kieselguhr impregnated with silicone, and developed with 90 % aqueous acetone previously saturated to 80 % with silicone—not shown in figure).

Discussion

The separations of acetals and ethers shown in Figs. 1 and 2 indicate that even the smallest variations in the chain length of their short-chain alkyl moieties can be distinguished easily by adsorption TLC, even when the other alkyl moiety has a long chain. The introduction of branching in the short-chain alkyl moiety has differing effects. The presence of branching near the ether group has a reducing effect on the R_F value of a component when compared with its homologue of same carbon number. This is readily observed when one has one isopropyl group in an ether and two in acetals. In ethers the R_F value of isopropyl hexadecyl ether lies halfway between ethyl hexadecyl ether and propyl hexadecyl ether, whereas in acetals this reducing effect is doubled, and thus the diisopropyl acetal of hexadecanal has the same R_F value as that of diethyl acetal of hexadecanal. When the branching is farther from the ether group, the R_F value is higher for the branched homologue than the straight-chain homologue as found in the case of butyl, isobutyl, pentyl and isopentyl hexadecyl ethers. Appreciable difference relative to chain length (above twelve carbon atoms) in either the aldehyde moieties of the acetals or the alcohol moieties of the ethers does not influence the adsorptive characteristics of these compounds.

This separation technique is useful in obtaining simultaneously a number of acetals, symmetrical as well as asymmetrical. For example, a mixture of dimethyl, dibutyl and methyl butyl acetals of hexadecanal was made by reacting hexadecanal with 100 % sulfuric acid in the presence of a 1:1 mixture of methanol and butanol by a technique described previously[2]. This resulted in the formation of two symmetrical acetals (with total alcohol carbon numbers 2 and 8) and one asymmetrical acetal (with total alcohol carbon number 5) of hexadecanal which were easily separated. Even the dipropyl acetal of hexadecanal (with total alcohol carbon number 6) could be separated easily from the asymmetrical methyl butyl acetal of hexadecanal (with total alcohol carbon number 5). This technique was found useful in obtaining a number of symmetrical and asymmetrical acetals for their mass spectral and infrared spectral characterization[5].

WALDI[6] and GÄNSHIRT AND MORIANZ[7] reported the separation of volatile simple alcohols in the form of suitable derivatives—3,5-dinitrobenzoic acid *esters*[6] and *p*-hydroxybenzoic acid *esters*[7]—on Silica Gel G plates using a combination of non-polar and polar solvents (adsorption chromatography). Recently SCHWARTZ AND BREWINGTON[8] separated *esters* of a homologous series of primary, secondary and tertiary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone by partition TLC. Esters containing alcohols up to C_{11} were separated by a normal partition system while

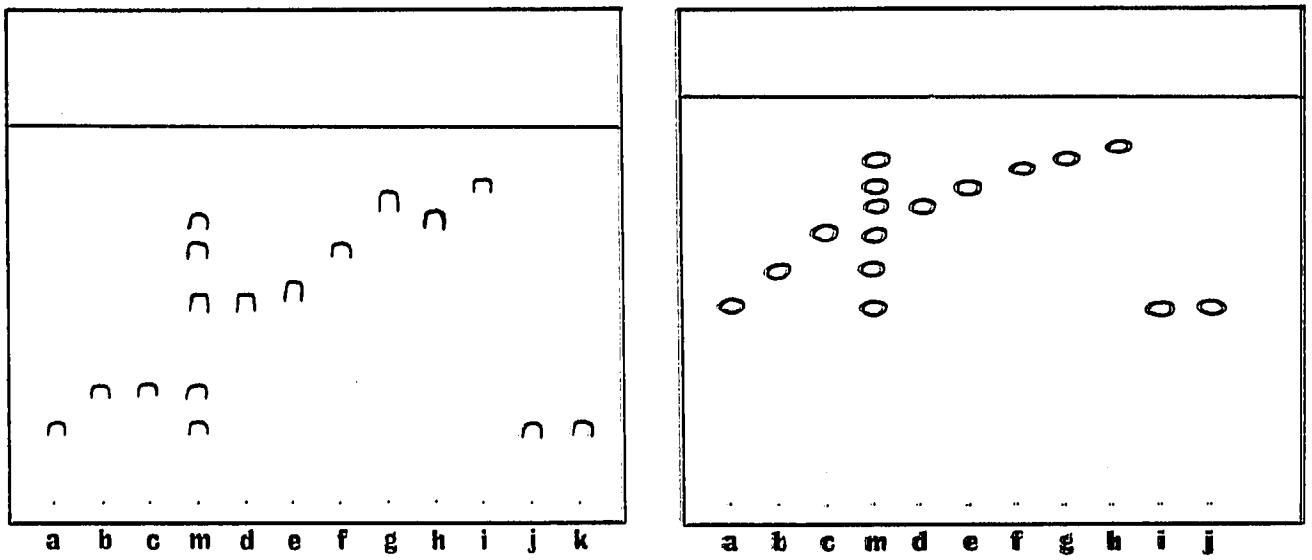

Fig. 1. Adsorption thin-layer chromatographic separation of acetals. a = Dimethyl acetal of hexadecanal; b = diethyl acetal of hexadecanal; c = diisopropyl acetal of hexadecanal; m = mixture of a, b, d, f and h; d = dipropyl acetal of hexadecanal; e = diallyl acetal of hexadecanal; f = dibutyl acetal of hexadecanal; g = diisobutyl acetal of hexadecanal; h = dipentyl acetal of hexadecanal; i = diisopentyl acetal of hexadecanal; j = dimethyl acetal of dodecanal; k = dimethyl acetal of *cis,cis,cis*-9,12,15-octadecatrienal.

Fig. 2. Adsorption thin-layer chromatographic separation of alkyl ethers. a = Methyl hexadecyl ether; b = ethyl hexadecyl ether; c = isopropyl hexadecyl ether; m = mixture of a, b, c, d, e and f; d = propyl hexadecyl ether; e = butyl hexadecyl ether; f = pentyl hexadecyl ether; g = isobutyl hexadecyl ether; h = isopentyl hexadecyl ether; i = methyl dodecyl ether; j = methyl octadecyl ether.

esters of long-chain alcohols between C_{12} to C_{19} were separated by a reversed-phase partition system. The present communication, on the other hand, reports the separation of *ether* derivatives of a wide variety of short-chain (including branched-chain) alcohols on Silica Gel G plates (adsorption chromatography), using a simple solvent system.

University of Minnesota, The Hormel Institute, Austin, Minn. 55912 (U.S.A.)

C. V. VISWANATHAN
F. PHILLIPS
W. O. LUNDBERG

1 S. J. PURDY AND E. V. TRUTTER, *J. Chromatog.*, 14 (1964) 62.
2 V. MAHADEVAN, C. V. VISWANATHAN AND W. O. LUNDBERG, *Lipids*, 1 (1966) 349.
3 V. MAHADEVAN, C. V. VISWANATHAN AND F. PHILLIPS, *J. Lipid Res.*, 8 (1967) 2.
4 V. MAHADEVAN, C. V. VISWANATHAN AND W. O. LUNDBERG, *J. Chromatog.*, 24 (1966) 357.
5 Manuscript in preparation.
6 D. WALDI (unpublished), referred in E. STAHL (Editor), *Thin-Layer Chromatography*, Academic Press, New York, 1965, p. 356.
7 H. GÄNSHIRT AND K. MORIANZ, *Arch. Pharm.*, 293 (1960) 1065.
8 D. P. SCHWARTZ AND C. R. BREWINGTON, *Microchem. J.*, 12 (1967) 1.

Received April 20th, 1967