Journal of Chromatography, 147 (1978) 524–527

CHROM. 10,356

Note

Thin-layer chromatographic fractionation of O-alkylglycerols according to chain length

HSIANG JU LIN and CLARA LAI HING LEE
Department of Pathology, University of Hong Kong, Queen Mary Hospital Compound (Hong Kong)
(First received May 31st, 1977; revised manuscript received June 27th, 1977)

Thin-layer chromatography (TLC) is widely used for the isolation of O-alkylglycerols, but it has not been applied to the fractionation of homologues within this lipid class[1,2]. This note describes a TLC method for the fractionation of alkylglycerols according to the number of carbon atoms in the aliphatic moiety, and describes the application of the method to a specimen of O-alkylglycerols with side-chains having C_{16}, C_{18}, C_{19}, C_{20}, C_{22} and C_{24} groups, which was prepared from human adrenal lipids[3].

EXPERIMENTAL

Materials

TLC was carried out using reagent-grade solvents and pre-coated silica gel G plates (0.25 mm, 20 cm long) from Macherey, Nagel & Co. (Düren, G.F.R.). Standard lipids were obtained commercially: 1-O-hexadecylglycerol and 1-O-octadecylglycerol from Analabs (North Haven, Conn., U.S.A.); 1-O-tetradecyl-, 1-O-octadec-9-enyl- and 1-O-eicosanylglycerol from Applied Science Labs. (State College, Pa., U.S.A.). Alkyldiacylglycerols were isolated from pooled adrenal glands (61 g, from adults and infants) with the aid of silicic acid column chromatography and TLC, and the alkylglycerols prepared therefrom by means of saponification, and TLC with diethyl ether–acetic acid (99:1). These procedures have been described elsewhere[3].

Recommended method for thin-layer chromatography

Samples are streaked on an activated plate by means of a Hamilton syringe, applying 20–25 μg of lipid per centimetre. The plate is developed at room temperature twice in the same direction, with chloroform–1-butanol (9:1, v/v). Immediately after air-drying, the lipids are made visible with the aid of 2,7-dichlorofluorescein. They can be recovered by elution with diethyl ether.

Gas chromatographic analysis

O-Alkylglycerols were treated with Sil-Prep (Applied Science Labs.) and analysed on a column of 1.5% OV-101 (152 × 0.32 cm) at 220° with a nitrogen flow-rate of 30 ml/min.

RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram of standard alkylglycerols obtained with the proposed method. The relative mobility of the individual O-alkylglycerols increased with the number of carbon atoms in the aliphatic moiety (lanes C–E and G). Octadecylglycerol (lane E) was usually closer to the origin than octadec-9-enylglycerol (lane F). Mixtures of alkylglycerols that differed by four or by six methylene groups chromatographed as two separate bands (lanes B, H and I) but a mixture of hexadecyl- and octadecylglycerols appeared as a single spot (lane A).

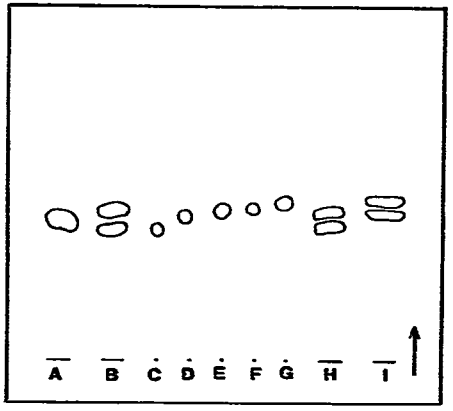

Fig. 1. Thin-layer chromatogram of standard alkylglycerols, developed with chloroform–1-butanol (9:1). (A) Mixture of 1-O-hexadecyl- and 1-O-octadecylglycerol; (B) mixture of 1-O-tetradecyl- and 1-O-eicosanylglycerol; (C) 1-O-tetradecylglycerol; (D) 1-O-hexadecylglycerol; (E) 1-O-octadecylglycerol; (F) 1-O-octadec-9-enylglycerol; (G) 1-O-eicosanylglycerol; (H) mixture of 1-O-tetradecyl- and 1-O-octadecylglycerol; (I) mixture of 1-O-hexadecyl- and 1-O-eicosanylglycerol. Arrow indicates direction of development.

The efficiency of such separations was tested with mixtures of equal weights of standard alkylglycerols (Table I). Gas chromatographic (GC) analysis was carried out on the alkylglycerol fractions recovered from the thin-layer plates. Tetradecyl- and eicosanylglycerol could be separated with very little cross-contamination. When binary mixtures of alkylglycerols that differed by four methylene groups were chromatographed, the lower homologue could be recovered at a purity of 99–100% as the band nearer the origin; the second band contained the higher homologue at 70–80% purity. Although TLC of a mixture of hexadecyl- and octadecylglycerol gave a single spot, the same bias was evident in the compositions of the upper and lower halves of the latter. Hexadecylglycerol was concentrated in the half closer to the origin and octadecylglycerol was found mainly in the upper half.

The application of the method to glyceryl ethers of biological origin is illustrated by the fractionation of O-alkylglycerols derived from the alkyldiacylglycerols of the human adrenal gland (Table II). The specimen of alkylglycerols isolated by means of TLC with diethyl ether–acetic acid was divided into two portions, one of which was analysed (by means of GC) as the unfractionated sample. TLC (chloroform–1-butanol) of the other sample resulted in the appearance of a band 2.5 cm long

TABLE I

FRACTIONATION OF STANDARD ALKYLGLYCEROLS ON THIN LAYERS OF SILICA GEL

Mixture applied	*Fraction analysed (R_E)**	*Composition (%)*			
		*14:0***	*16:0*	*18:0*	*20:0*
14:0, 20:0**	0.90	99	—	—	1
	0.99	1	—	—	99
14:0, 18:0	0.92	99	—	1	—
	0.98	30	—	70	—
16:0, 20:0	0.94	—	100	—	0
	1.00	—	19	—	81
16:0, 18:0	0.93***	—	88	12	—
	0.98***	—	44	56	—

* R_E = mobility relative to the distance travelled by eicosanylglycerol.

** The numbers before and after the colon refer to the number of carbon atoms in the side-chain and the number of double bonds present therein, respectively. The composition of the separated fractions was calculated from the peak areas in the gas chromatograms.

*** The mixture migrated as a single spot, which was divided into upper and lower halves for GC analysis.

in the direction of development, which was divided laterally into fractions with R_E values of 0.90 (A), 0.98 (B) and 1.06 (C). Each fraction was eluted separately, subjected to silylation and studied by means of GC. Compared with the composition of the

TABLE II

THIN-LAYER CHROMATOGRAPHIC FRACTIONATION OF ALKYLGLYCEROLS PREPARED FROM ADRENAL ALKYLDIACYLGLYCEROLS

*Alkylglycerol**	*Unfractionated sample*	*Composition (%)*		
		Fraction A	*Fraction B*	*Fraction C*
16:1	t**	—	1	—
16:0***	18	60	15	—
17:1	—	t	—	—
17:0	1	7	t	—
18:1***	23	8	32	13
18:0***	17	13	28	2
19:1***	6	9	8	—
19:0	—	3	t	—
20:1***	2	t	2	2
20:0***	3	t	6	1
21:1	—	—	1	—
21:0	—	—	t	—
22:1***	9	—	3	20
22:0***	6	—	3	12
24:1***	15	—	1	45
24:0	t	—	—	5

* Identified on the basis of retention time.

** t = Trace component, amounting to less than 0.01 of the total peak area obtained in gas chromatograms.

*** Component previously identified by means of silver ion TLC and GC as one of the nine principal alkylglycerol moieties in human adrenal alkyldiacylglycerols[3].

unfractionated sample, fraction A was enriched with respect to hexadecylglycerol; it was devoid of alkylglycerols with C_{22} and C_{24} groups. Fraction B was enriched with respect to octadecyl- and octadecenylglycerols. Alkylglycerols with C_{22} and C_{24} groups comprised 82% of fraction C.

Different solvent systems were tested with respect to their ability to separate homologous O-alkylglycerols, but none was found to be superior to the specified chloroform–1-butanol. The relative mobilities of O-alkylglycerols chromatographed in solvent systems containing diethyl ether (diethyl ether–ammonia, 400:1; diethyl ether–water, 200:1; or diethyl ether–acetic acid, 99:1) were likewise found to increase with chain length (C_{14}–C_{20}). Methanol or ethanol (but not 1-propanol) could be used instead of 1-butanol without loss of resolving power. For the purpose of fractionation the optimal ratio of chloroform to alcohol was close to 9:1.

ACKNOWLEDGEMENTS

This study was supported by grants to H. J. L. from the Medical Faculty Research Grant Fund and from the Committee on Research Grants of the University of Hong Kong.

REFERENCES

1 F. Snyder, *Methods Cancer Res.*, 6 (1971) 399.
2 H. H. O. Schmid, P. C. Bandi and K. L. Su, *J. Chromatogr. Sci.*, 13 (1975) 478.
3 H. J. Lin, M. S. F. Lie Ken Jie, C. L. H. Lee and D. H. S. Lee, *Lipids*, 12 (1977) 620.