THIN-LAYER CHROMATOGRAPHY OF SOME TRIMETHYLSILYLATED CARBOHYDRATES*

JACOB LEHRFELD
*Northern Regional Research Laboratory***, *Peoria, Ill. 61604 (U.S.A.)*
Received October 3rd, 1967)

Several notes on thin-layer chromatography of trimethylsilylated (TMS) hydroxyl-containing compounds appeared in 1966. Improved resolution of some mixtures and faster development of chromatograms, made possible by less polar solvent systems, have been reported. LINDGREN AND SVAHN[1] found that some trimethylsilylated sterols and terpenols were more easily resolved than the corresponding unsubstituted sterols and terpenols or their acetates. BROOKS AND CARRIE[2] separated some trimethylsilylated sterols by thin-layer chromatography. BROOKS AND HARRISON[3] resolved a mixture containing some TMS ethers of hydroxylated derivatives of cyclodiene insecticides by thin-layer chromatography. HANCOCK[4] separated adenosine from TMS-adenosine by thin-layer chromatography. KARKKAINEN, HAAHTI AND LEHTONEN[5] investigated thin-layer and column chromatography of some carbohydrates as TMS ethers with applications to mucopolysaccharide analysis.

TMS carbohydrates have been intensively investigated by gas–liquid chromatography[6]. In contrast, work on their thin-layer chromatographic behavior has been largely neglected because of their presumed hydrolytic lability. This paper reports the preparation of TMS carbohydrate samples for thin-layer chromatography, stability of these samples on thin layers of Silica Gel G***, and resolution of two mixtures: (1) TMS-α,β-D-glucose, methyl TMS-α- and β-D-glucopyranoside; (2) TMS-α,β-maltose.

EXPERIMENTAL AND RESULTS

Densitometer

A Photovolt TLC Densitometer, Model 530, equipped with a collimating slit apperture of 0.1 × 10 mm was used for quantitative work. The TLC stage was driven 1 in./min by a synchronous motor. The signal from the photometer was recorded on a 10-in. Beckman linear–log recorder on the log scale with a chart speed of 2 in./min. Peak areas (spot densities) were measured by cutting out and weighing and also by use of a K & E compensating polar planimeter.

---

* Presented at the 154th Meeting of the American Chemical Society, Chicago, Ill., Sept. 1967.
** This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.
*** Mention of firm names or trade products does not constitute endorsement over similar firms or products not mentioned by the U. S. Department of Agriculture.

Gas–liquid chromatography

Gas–liquid chromatographic analysis of some eluted spots was performed with an F & M Model 5751A-01 gas chromatograph equipped with a 10 % Carbowax 20M on 80–100 mesh Chromosorb W hexamethyldisilazane (HMDS) treated column (1/4 in. × 12 ft.).

Sample preparation

A sample (5–30 mg) was dissolved in 1 ml of dry pyridine (stored over KOH pellets)—solution A. TMS carbohydrates for thin-layer chromatography were prepared from solution A by four methods.

(1) To the dissolved mixture, 0.2 ml HMDS and 0.1 ml trimethylchlorosilane (TMCS) were added. The mixture was vigorously shaken and then allowed to stand at room temperature for 15 min before using[6].

(2) The suspension prepared above (1) was evaporated by blowing a stream of dry nitrogen over the surface. The residue was resuspended in hexane and then vigorously shaken for 2 min. The precipitate was centrifuged, and the hexane solution was removed. Hexane was again added to the residue; after the suspension was vigorously shaken for 2 min, and then centrifuged, the supernatant hexane solution was removed. Concentration of the TMS carbohydrate in hexane was increased to a convenient level by removing some hexane by blowing nitrogen over the surface[5].

(3) To solution A was added 0.9 ml HMDS and then 0.1 ml trifluoracetic acid. After the solution was vigorously shaken, it was allowed to stand at room temperature for at least 15 min before using[7].

(4) To solution A was added 0.4 ml N,O-bis-(trimethylsilyl)-acetamide (BSA) and 1 drop of TMCS. The solution was vigorously shaken and then allowed to stand at room temperature for at least 15 min before using.

Thin-layer chromatography

Qualitative thin-layer chromatography was carried out on glass plates (5 × 20 cm, 10 × 20 cm, and 20 × 20 cm) coated with either a 0.5 mm or 0.25 mm layer of Silica Gel G. A stable slurry containing 2.5 parts water to one part Silica Gel G was used to coat the plates[8]. Plates were activated by heating in an oven at 125° for 1 h. They were developed with benzene; benzene previously dried over Na·Pb for at least 24 h and 1 % ethylacetate (dried over Drierite for at least 24 h) in benzene (dried). Spots were visualized by spraying with 5 % sulfuric acid in ethanol (v/v) and then charring on a hot plate set at 150°.

For quantitative work, 20 × 20 cm plates coated with 0.5 mm Silica Gel G were used. After development they were left in a hood overnight, evenly sprayed with 5 % sulfuric acid in ethanol (v/v) until translucent, and then charred on a hot plate set at 124° for 40 min. The density of the charred spots was determined with a densitometer. Photodensitometric readings were made on the same day and in the same direction as the developing solvent flow. If necessary, they can be taken on succeeding days, but the density of the spots will decrease (9–40 % depending on the length of time after heating).

TMS carbohydrate stability on Silica Gel G plates

Calibration curve. A solution containing 20.8 mg/ml TMS-α,β-D-glucose, 25.2

mg/ml methyl TMS-α-D-glucopyranoside, and 17.9 mg/ml methyl TMS-β-D-glucopyranoside was prepared. Aliquots were diluted with an equal volume, 1.5 times its volume, and two times its volume with hexane.

Each of the diluted solutions (3 μl from a 10-μl Hamilton syringe) was spotted in duplicate, 1.4 cm from the edge of the adsorbent, on a 20 × 20 cm unactivated plate. The spotting was completed in 2.5 min. The plate was developed (17 cm with 1 % ethylacetate–benzene), left in a hood overnight, sprayed with sulfuric acid, and charred by heating on a hot plate. Three well-resolved spots were visible: TMS-α,β-D-glucose, R_F 0.75; methyl TMS-β-D-glucopyranoside, R_F 0.61; and methyl TMS-α-D-glucopyranoside, R_F 0.42. The density of the charred spots was determined 1 h after the plate cooled. Maximum variation between duplicate spots was 8 %. Fig. 1 shows that the density of the spot as measured by densitometry is a reasonably linear function of the amount of material in the charred spot.

Tank saturated with benzene. A 20 × 10 cm plate of Silica Gel G (0.25 mm not activated) was spotted in duplicate with 2 ml of a solution containing 8.5 mg/ml TMS-α,β-D-glucose. The plate was then left in a chromatographic tank saturated with benzene for 140 min. The plate was removed, respotted in duplicate with 2 μl of the same sample, and then developed with 1 % EtOAc in benzene. This last pair of spots serves as an internal standard. They should have the same density as the first pair of spots at zero time. Therefore, a comparison between the two pairs of spots will give the amount of TMS-α,β-D-glucose at zero time and the amount of TMS-α,β-D-glucose after 140 min. The plate was then sprayed and charred; relative densities were determined with the densitometer.

The same procedure was repeated with an activated plate.

The density of the spots is a linear function of the amount of material in the spots (Fig. 1). Therefore, the decrease in density of the charred spot TMS-α,β-D-glucose is a measure of the amount of material degraded during that 140-min period. Data are summarized in Table I.

Stability when exposed to air. The same 1:1 dilution as discussed under "Calibration curve" was used to study relative stability of TMS-α,β-D-glucose, methyl TMS-α-D-glucopyranoside and methyl TMS-β-D-glucopyranoside on plates of Silica Gel G. Two spots containing 3 μl each were placed on a 20 × 20 cm plate (0.5 mm Silica Gel G) 1.4 cm from the edge of the adsorbent. Succeeding pairs of spots were placed on the plate after 30, 45 and 60 min. After the fourth pair of spots, the plate was imme-

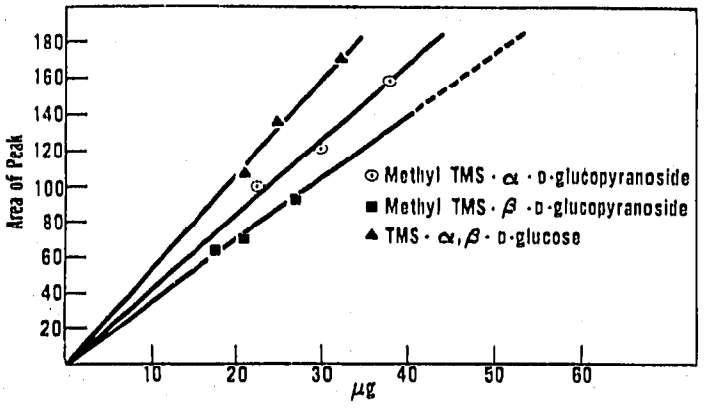

Fig. 1. A plot of peak area *vs.* weight (μg) in spot.

TABLE I

EFFECT OF ACTIVATION OF SILICA GEL G ON STABILITY OF TMS-α,β-D-GLUCOSE

Plate	*Loss in 140 min (%)*
Activated	14
Unactivated	43

TABLE II

STABILITY OF TMS CARBOHYDRATES ON UNACTIVATED SILICA GEL G PLATES

Material	*Starting material left (%)*		
	15 min	*30 min*	*60 min*
TMS-α,β-D-glucose	86	82	62
Methyl TMS-β-D-glucopyranoside	69	44	26
Methyl TMS-α-D-glucopyranoside	94	84	77

diately developed, 17 cm with 1 % EtOAc in benzene. The plate was dried overnight in a hood, sprayed, and charred. Densitometric readings were made 1 h after the plate reached ambient temperature. Data are summarized in Table II and Fig. 2.

Resolution of two TMS carbohydrate mixtures on Silica Gel G

Two samples, one containing TMS-α,β-D-glucose (25 mg/ml) and methyl TMS-α,β-D-glucopyranoside (22 mg/ml) and the other, 75 % TMS-α-maltose–25 % TMS-β-maltose (50 mg/ml total) were prepared by procedure 1. Three microliters were applied to each plate. Most of the excess pyridine in the spot was removed by holding the plate in a heated air stream for 20 sec or by storing in a heated vacuum desiccator (50° and about 80 mm Hg) for 10 min. Benzene was the developing solvent and the front was allowed to migrate 15.2 cm.

Unactivated plates readily separated mixture 1 into three components: TMS-α,β-D-glucose, methyl TMS-β-D-glucopyranoside, and methyl TMS-α-D-glucopyranoside; and mixture 2 into two: TMS-β-maltose and TMS-α-maltose. Acitvated plates in addition to lowering the R_F values resolved TMS-α-D-glucose and TMS-β-D-glucose. However, activated plates exposed to the air for as short a time as 7 min did not separate TMS-glucose anomers. The data are summarized in Fig. 3 and Table III.

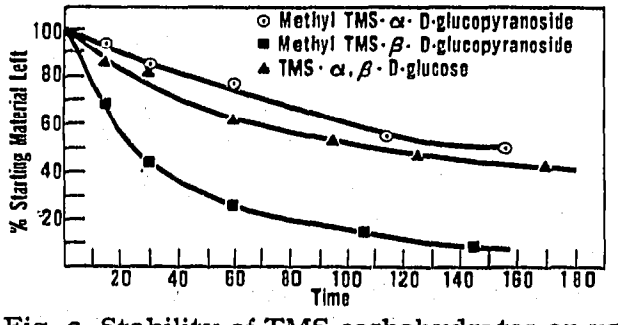

Fig. 2. Stability of TMS carbohydrates on unactivated Silica Gel G plates.

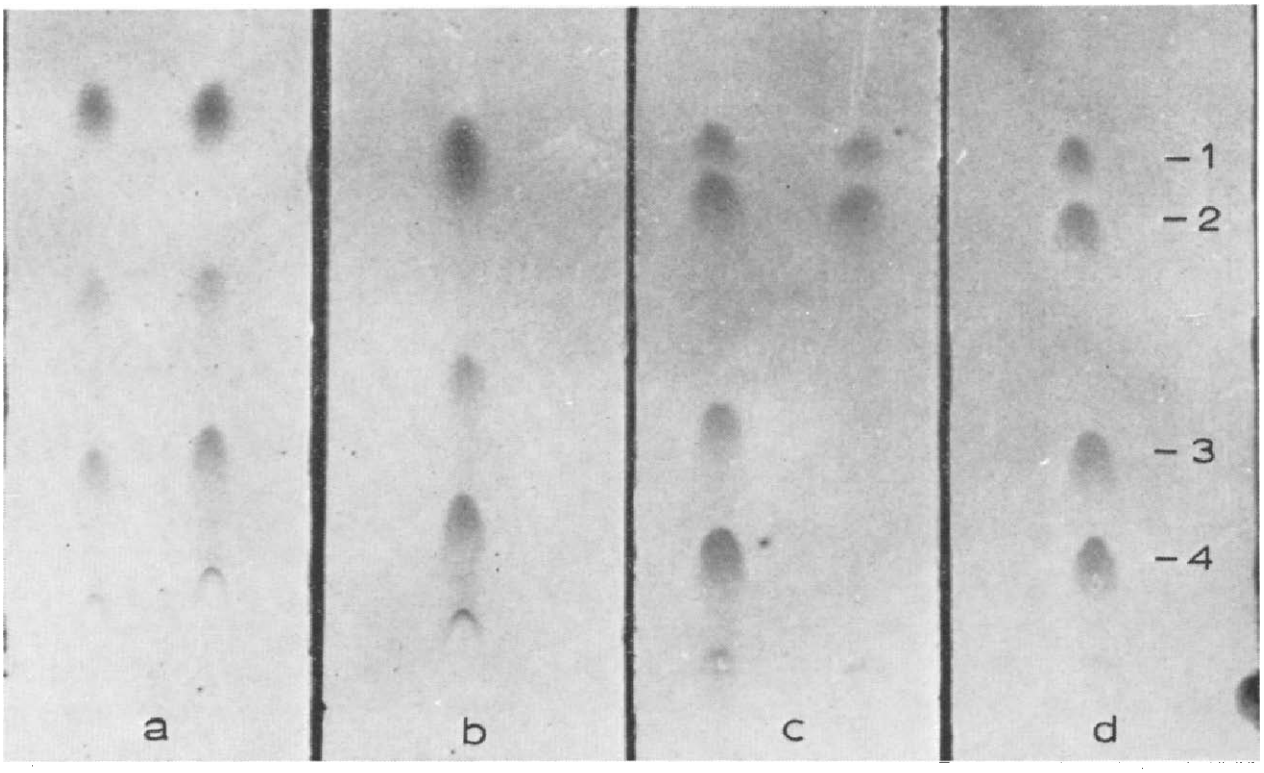

Fig. 3. Thin-layer chromatography of a mixture containing TMS-α-D-glucose (1), TMS β-D-glucose (2), methyl TMS-β-D-glucopyranoside (3), and methyl TMS-α-D-glucopyranoside (4) on Silica Gel G. Dry benzene was the developing solvent. (a) Plate not activated. (b) Plate activated at 130° for 1 h and then exposed to air for 7 min. (c) Plate activated at 130° for 1 h, cooled in heated vacuum desiccator for 10 min, spotted, replaced in vacuum desiccator for 10 min, and then developed. (d) Plate activated at 130° for 1 h, spotted while hot, and immediately developed.

TABLE III

R_F VALUES OF TMS CARBOHYDRATES OBTAINED BY THIN-LAYER CHROMATOGRAPHY ON SILICA GEL G[a]

Material	*Unactivated plate*	*Activated plate*	*Activated plate exposed to air for 7 min*
TMS-α-D-glucose	0.82 (large)	0.78	0.76 (large)
TMS-β-D-glucose		0.72	
Methyl TMS-β-D-glucopyranoside	0.57	0.42	0.47
Methyl TMS-α-D-glucopyranoside	0.35	0.26	0.30
TMS-β-maltose	0.92	0.72	
TMS-α-maltose	0.80	0.53	
Methyl TMS-β-maltoside	0.53		
Methyl TMS-α-maltoside	0.48		

[a] Layer on plate was 0.5 mm, eluting solvent was dry benzene.

DISCUSSION

Samples containing TMS-α,β-D-glucose and methyl TMS-α,β-D-glucopyranoside were prepared by procedures 1, 2, 3 and 4. All samples contained small amounts of material with low R_F value. Presumably the spots are carbohydrates that are only partially trimethylsilylated. A sample prepared by procedure 2 and purified by passage through a silica gel column contained no discrete spots with low R_F value. Removal of pyridine and trimethylsilylating reagents from the sample was unnecessary. They are immediately volatized or removed by spotting a hot thin-layer chromatographic plate, by blowing hot air over the plate for 10–20 sec, or by placing the plate in a heated vacuum desiccator. If pyridine is still present when plate development is initiated, a small increase in R_F occurs and the low R_F impurities are concentrated into a semicircular band. Slight tailing occurs when the spots were moderately loaded with material. The tailing is probably caused by the small amount of degradation occurring as the spot migrates.

Plates were prepared from a stable slurry of Silica Gel G in water[8] and also from a freshly prepared slurry (2 parts water to 1 part Silica Gel G)[9]. There were no significant differences in R_F, resolution, or stability. Spots developed on plates having a 0.5-mm layer of adsorbent had a slightly lower R_F. Resolution was not affected. Spots developed on activated plates had a lower R_F, less tailing, and improved resolution. TMS-α,β-D-glucose was resolved only on activated plates which were spotted and on which developing was started (dry benzene) within 3–4 min after removal from the oven. On the other hand, TMS-α,β-maltose was easily resolved on unactivated plates with either 1 % EtOAc in benzene or benzene alone.

The adsorbent activity was rapidly reduced on exposure to air. After 7 min an activated plate no longer resolved TMS-α,β-D-glucose (see Table III). Plate activity can be maintained over extended periods of time by storing in a vacuum desiccator or on a heated hot plate (about 65°). The second procedure was convenient when a large plate was being spotted with a large number of samples.

In the series of compounds reported here, the relative order of migration of the α- and β-anomers differed for different pairs. With TMS-glucose the α-anomer had the higher R_F value, whereas with TMS-maltose the β-anomer was the higher. In the glycoside series, the β-anomer of both methyl TMS-maltoside and methyl TMS-glucoside migrated faster. On gas–liquid chromatography, all the α-anomers had shorter retention times than the corresponding β-anomers.

Stability of TMS carbohydrates on Silica Gel is apparently a function of structure. Table II and Fig. 2 demonstrate the order of stability: methyl TMS-α-D-glucopyranoside > TMS-α,β-D-glucose > methyl TMS-β-D-glucopyranoside. The experiment described under stability was repeated a number of times, at different concentrations, with individual spots, and with Silica Gel G 0.25 mm. The results were always within 10 % of the values reported here. Even though there is a marked difference in the stability of the glucoside, no similar difference shows up with anomers of TMS-glucose. The α/β ratio of a sample of TMS-glucose was determined by gas–liquid chromatography. The sample was chromatographed on Silica Gel G, the region containing the spot was scraped off, and the sample eluted. Gas–liquid chromatographic analysis of the eluate indicated that about 50 % of the material was degraded. The α/β ratio remained the same.

The increased lability of methyl TMS-β-D-glucopyranoside may be due to its equatorial methoxyl group. Equatorial groups are sterically more accessible. The SiOH group on the surface of the adsorbent may hydrogen bond to oxygen of the methoxyl group and orient the molecule so that a second SiOH is placed in close proximity with a TMS group on the carbohydrate. Ester exchange can then occur. The axial methoxyl group on the α-anomer is less accessible. Therefore, there is less chance for it to hydrogen bond to the silanol group and be oriented into close proximity to another silanol group. Accessibility to the equatorial C-1 oxygen on TMS-β-D-glucose is sterically hindered by the bulky TMS group. Therefore no apparent difference is seen in the stability of these anomers.

SUMMARY

After thin-layer chromatography of some trimethylsilylated carbohydrates was performed, their stability on Silica Gel G plates was determined. Two mixtures were resolved on unactivated plates of Silica Gel G (1) TMS-α,β-D-glucose, methyl TMS-α-D-glucopyranoside, and methyl TMS-β-D-glucopyranoside, (2) TMS-α-maltose, TMS-β-maltose, methyl TMS-α-maltoside, and methyl TMS-β-maltoside. To minimize the appearance of low R_F spots caused by partial degradation, the time between sample spotting and development was kept at a minimum.

REFERENCES

1 B. O. LINDGREN AND C. M. SVAHN, *Acta Chem. Scand.*, 20 (1966) 1763.
2 C. J. W. BROOKS AND J. G. CARRIE, *Biochem. J.*, 99 (1966) 47P.
3 G. T. BROOKS AND A. HARRISON, *Chem. Ind. (London)*, (1966) 1414.
4 R. L. HANCOCK, *J. Chromatog.*, 4 (1966) 363.
5 J. E. KARKKAINEN, E. O. HAAHTI AND A. A. LEHTONEN, *Anal. Chem.*, 38 (1966) 1316.
6 C. C. SWEELEY, R. BENTLEY, M. MAKITA AND W. W. WELLS, *J. Am. Chem. Soc.*, 85 (1963) 2497.
7 K. M. BROBST AND C. E. LOTT, *Cereal Chem.*, 43 (1966) 35.
8 W. W. FIKE AND I. SUNSHINE, *J. Chromatog.*, 18 (1965) 405.
9 K. RANDERATH, *Thin-Layer Chromatography*, Academic Press, New York, 1963, p. 23.