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SEPARATION, BY G.L.C., OF ENANTIOMERIC SUGARS AS DIASTEREOISOMERIC DITHIOACETALS

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ABSTRACT

The reaction between (+)-1-phenylethanethiol and each of a pair of enantiomeric sugars produced two acyclic, diastereoisomeric dithioacetals. These diastereomers were then separated by gas–liquid chromatography. This method was applied to separate the following pairs of enantiomers: D- and L-arabinose, D- and L-lyxose, D- and L-fucose, D- and L-rhamnose, D- and L-galactose, D- and L-glucose, and D- and L-mannose.

INTRODUCTION

Routine, gas–liquid chromatographic analysis of carbohydrates cannot distinguish between enantiomers of the same sugar. Only when quantities of material large enough for polarimetric measurements (or, in some cases, enzymic studies) are isolated can the absolute configuration of a sugar be designated. There is thus considerable potential for an analytical method by which such assignments can be made. This is particularly important in glycoprotein, glycolipid, and polysaccharide research, where only small quantities of material can usually be isolated.

A convenient approach taken to separate enantiomers is the conversion of a racemic pair into diastereoisomeric compounds. These derivatives, formed by reaction of each enantiomer with an enantiomer of another optically active compound, can be separated by ordinary chemical means. A number of workers have taken this approach to separate D and L sugar pairs. For example, Pollock and Jermany[1] converted aldoses into aldonic acids, and separated a series of sugars as diastereoisomeric esters, but this method is laborious and cannot be extended to the common hexoses. Gerwig *et al.*[2] and Leontein *et al.*[3] used a reaction with optically active alcohols, and separated enantiomeric sugars as their diastereoisomeric glycosides. However, the resulting chromatograms were complicated by the presence of both anomers for each of two cyclic sugars.

Direct resolution on a chiral, stationary phase is an alternative approach to the separation of enantiomers. König *et al.*[4] have used capillary, glass columns coated with chiral stationary phases to separate sugar enantiomers, but this approach also

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produces a chromatogram in which four forms are present for each sugar. Thus, as many as eight peaks for isomers may be expected from a mixture containing only a single pair of enantiomers.

The synthesis of acyclic-sugar diastereomers results in a simplification of the chromatographic pattern. Several procedures have been reported[5-7] that give rise to these derivatives, but none has been shown to be practicable. Herein is reported the use of (+)-1-phenylethanethiol to convert pairs of sugar enantiomers into acyclic, diastereoisomeric dithioacetals. Separation of these diastereomers as volatile, acetylated and (trimethylsilyl)ated derivatives is achieved by g.l.c. on capillary columns of fused silica. This method is a simple and rapid procedure by which small amounts of enantiomeric sugars can be identified, and their relative quantities measured.

RESULTS AND DISCUSSION

Reaction conditions. — Sugars react rapidly with thiols in the presence of an acid catalyst to afford the acyclic dithioacetal in high yield. (For a review on sugar dithioacetals, see ref. 8.) A microanalytical method for converting simple sugars into diethyl dithioacetals has been developed by Honda *et al.*[9]. By similar methods, it has now been found that an optically active thiol, (+)-1-phenylethanethiol, reacts with sugars. Fig. 1 illustrates the course of the reaction between D-arabinose and (+)-1-phenylethanethiol in the presence of trifluoroacetic acid. As may be seen, at room temperature, the maximum yield of the dithioacetal occurs at 30 min. After this time, only one spot is detectable on a thin-layer plate. Extended reaction-times result in a significant degradation of the dithioacetal. A similar course was observed when the thiol was allowed to react with L-arabinose. Based on these observations, a reaction period of 30 min at room temperature was chosen for the micro-scale reactions.

Gas–liquid chromatography. — Separation of diastereoisomeric dithioacetals was

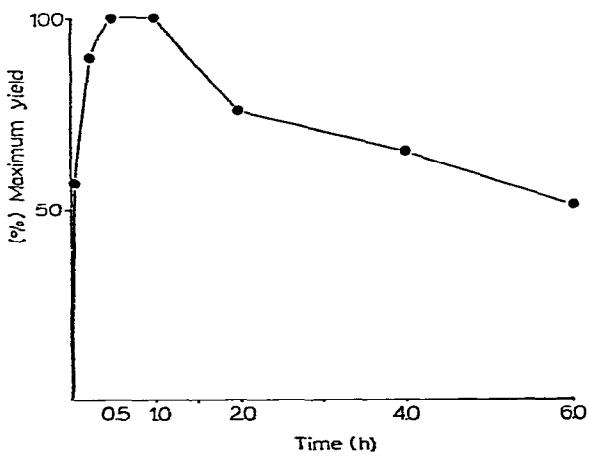

Fig. 1. Course of the reaction between (+)-1-phenylethanethiol and D-arabinose. (Maximum yield of D-arabinose bis[(+)-1-phenylethyl] dithioacetal is found after 30 min.)

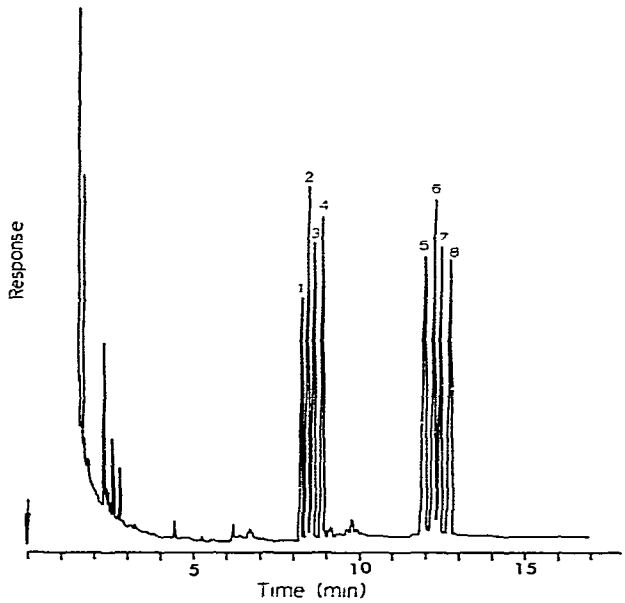

Fig. 2. Gas–liquid chromatogram of some acetylated sugar dithioacetals on a capillary column of SE-54–fused silica. (1, D-Rhamnose; 2, D-arabinose; 3, L-rhamnose; 4, L-arabinose; 5, D-mannose; 6, L-galactose; 7, L-mannose; and 8, D-galactose. Column temperature, 275°, programmed at 2°/min to 300°; inlet temperature, 280°.)

TABLE I

SUMMARY OF G.L.C. DATA FOR ACETYLATED AND (TRIMETHYLSILYL)ATED SUGAR DITHIOACETALS ON SE-30 AND SE-54, FUSED-SILICA, CAPILLARY COLUMNS

Sugar	*SE-30 (280°)*				*SE-54 (280°)*			
	Acetylated		*Silylated*		*Acetylated*		*Silylated*	
	k^a	α^b	k	α	k	α	k	α
D-Ara	6.0	1.066	4.8	1.063	8.8	1.075	5.6	1.070
L-Ara	6.4		5.1		9.4		6.0	
D-Lyx	6.1	1.059	4.9	1.051	8.9	1.066	5.8	1.056
L-Lyx	6.4		5.2		9.5		6.1	
L-Fuc	6.1	1.051	6.5	1.057	8.9	1.042	7.7	1.065
D-Fuc	6.4		6.9		9.2		8.2	
D-Rha	5.9	1.053	6.5	1.047	8.5	1.060	7.6	1.041
L-Rha	6.2		6.8		9.0		8.0	
L-Glc	9.6	1.079	9.6	1.071	14.4	1.088	11.3	1.082
D-Glc	10.4		10.3		15.7		12.3	
D-Man	9.9	1.056	10.2	1.000	14.8	1.060	12.0	1.000
L-Man	10.4		10.2		15.7		12.0	
L-Gal	10.2	1.055	9.7	1.053	15.2	1.063	11.4	1.062
D-Gal	10.8		10.2		16.2		12.1	

[a]k, the partition ratio, is obtained by dividing the corrected retention-time (t'_R) by the retention time found for methane (t_m). [b]α, the separation factor, is the ratio of the corrected retention-times for two components, such that $\alpha = t'_{R_2}/t'_{R_1}$, where $t'_{R_2} > t'_{R_1}$.

conducted by gas–liquid chromatography. Acetylated and (trimethylsilyl)ated sugar dithioacetals were analyzed on capillary columns of fused silica coated with either SE-30 or SE-54. Fig. 2 shows, from an SE-54 column, a gas–liquid chromatogram of the acetylated sugar dithioacetals obtained from a mixture of four pairs of sugar enantiomers. A complete summary of the retention data is given in Table I.

By this method, the separations of enantiomeric pairs of sugars were good. Separation factors greater than 1.05 were common. Of the enantiomeric sugar pairs tested, only the (trimethylsilyl)ated diastereomers of D- and L-mannose could not be resolved at the baseline. Those diastereomers were, however, well separated as acetylated derivatives.

In general, the acetylated diastereomers were better separated. By comparison, the trimethylsilyl derivatives showed greater volatility, and were more readily prepared, and for these reasons, they were considered preferable for routine analyses. However, the trimethylsilyl derivatives were found to be more susceptible to adsorption, or decomposition, or both, at the glass inlet. Thus, periodical deactivation of the glass inlet was necessary.

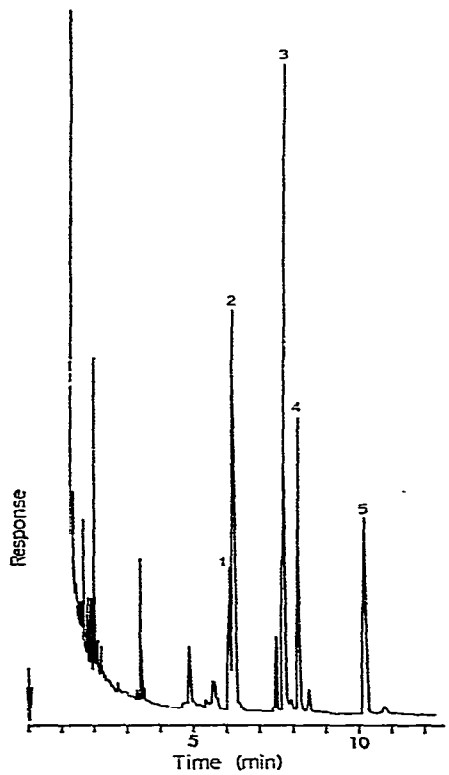

Fig. 3. Gas–liquid chromatogram of the (trimethylsilyl)ated sugar dithioacetals obtained from the analysis of flax-seed mucilage on an SE-54 capillary column. (1, L-Arabinose; 2, D-xylose; 3, L-rhamnose; 4, D-galacturonic acid; 5, L-galactose. Column temperature, 275°, programmed at 1°/min to 290°; inlet temperature, 280°.)

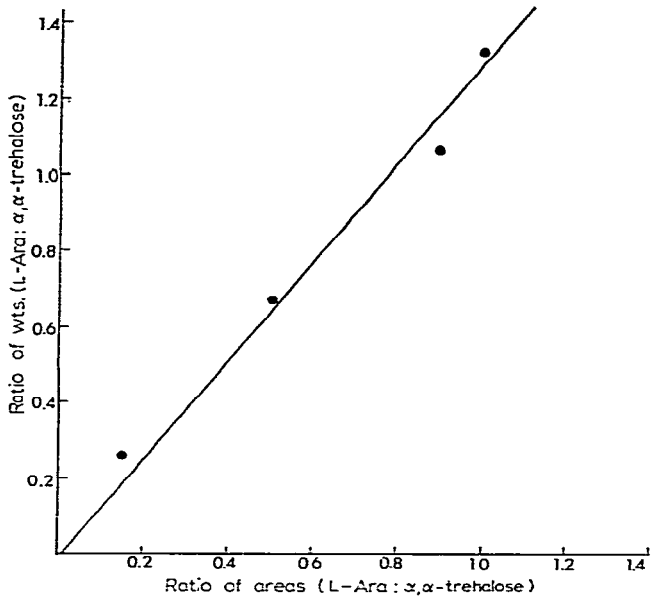

Fig. 4. Calibration curve obtained for L-arabinose with α,α-trehalose as the internal standard.

The order in which the diastereomers were eluted depended upon the stereochemistry at C-2 of the parent sugar. Those sugars having the (*S*) configuration at C-2 were eluted prior to those having the (*R*) configuration. It follows, then, that a D-arabinose derivative is eluted prior to its L enantiomer and that an L-galactose derivative is eluted prior to its D enantiomer.

Application. — A polysaccharide from flax-seed mucilage was hydrolyzed in acid, and the sugars released were subjected to analysis by this method. A chromatogram of the (trimethylsilyl)ated sugar dithioacetals is shown in Fig. 3. The absolute configurations of the following sugars were indicated: L-arabinose, L-rhamnose, and L-galactose. Definite assignments of the configuration of D-galacturonic acid and D-xylose could not be made, as the enantiomers of these sugars were not available for analysis.

Quantitation. — The split-injection technique inherently presents problems when quantitative analytical work is desirable. These problems are largely due to disproportionate splitting of the vaporized sample at the inlet and are particularly troublesome in the analysis of high-boiling components. For these reasons, reproducible measurements of the quantities of the sugars as dithioacetals were only realized when the analysis was performed at high split-ratios (100:1, or greater) and when peak areas were standardized against a high-boiling, internal standard (α,α-trehalose). Fig. 4 shows the linear calibration curve obtained for L-arabinose. Although the problems associated with the splitter can largely be overcome, it is expected that a splitless injection would simplify the quantitative work.

Herein is described the use of an optically active thiol to separate a number of enantiomeric sugar pairs, but this cannot be claimed to be a novel approach. Vetoček and Veselý[10] resolved diastereoisomeric sugar dithioacetals by fractional recrystallization in 1914. In addition, it had been shown that enantiomeric sugars could be resolved as diastereomeric dithioacetals by l.c. using (+)-2,3-dimercaptosuccinic acid[6], but the present author considers that (+)-1-phenylethanethiol is a more practical resolving reagent for the following reasons: (*1*) this thiol is readily synthesized in optical purity from inexpensive starting materials, (*2*) it reacts rapidly with various pairs of sugar enantiomers, and, (*3*) most important, it leads to the formation of acyclic diastereomers that can be readily resolved.

EXPERIMENTAL

General. — Micro-reaction vials (0.3 and 1.0 mL) having Teflon-lined rubber septa were purchased from Supelco, Inc. The (trimethylsilyl)ating reagents, *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and chlorotrimethylsilane (TMCS), were purchased from Pierce Chemical Co., and (−)-menthol and 4-(dimethylamino)-pyridine, from Aldrich Chem. Co. Racemic 1-phenylethyl bromide was prepared by the hydrobromination of styrene as described by Ashworth and Burkhardt[11]. L-Galactose was synthesized by Dr. P. A. Hebda in this laboratory from L-galactonolactone, as described by Frush and Isbell[12]. D-Rhamnose was synthesized by Dr. G. A. Barber, and crystallized by use of nucleating crystals of D-rhamnose kindly provided by Dr. N. K. Richtmyer. Dry pyridine was obtained by distillation from BaO. All other sugars and reagents were obtained from commercial sources.

Elemental analyses were made by Integral Microanalytical Laboratories (Raleigh, N.C.). Optical rotations were measured in a Perkin–Elmer Model 241 polarimeter. Thin-layer chromatography was performed on precoated layers (0.1 mm) of silica gel on poly(ethylene terephthalate) sheets (No. 13179, Eastman). The sheets were developed with 10:1 (v/v) chloroform–butanol, and sugar components were made visible with the alkaline, silver nitrate reagent. ^{1}H-N.m.r. spectroscopy was performed in a Varian T60 (60 MHz) spectrometer for solutions in Me_2SO-d_6 at 35°, using Me_4Si as the internal standard.

Gas–liquid chromatography. — G.l.c. was conducted in a Shimadzu Mini-2 gas chromatograph equipped with a flame-ionization detector and inlet-splitter assembly. Capillary columns (30 m × 0.25 mm) of fused silica respectively coated with SE-54 and SE-30 were obtained from J & W Scientific (Rancho Cordova, Cal.). Hydrogen carrier-gas velocity was maintained at 50 cm/s, with a split ratio adjusted to 100:1. The glass inlet was periodically removed, cleaned, and deactivated. Deactivation was achieved *in situ* by injecting a solution of 2:1 (v/v) BSTFA–TMCS into the sealed inlet. The inlet temperature during deactivation was set at 175°.

Preparation of (+)-1-phenylethanethiol. — (+)-1-Phenylethanethiol was synthesized as described by Isola *et al.*[13]. This procedure involves the reaction of sodium (−)-*O*-methyl dithiocarbonate with racemic 1-phenylethyl bromide, and

subsequent separation, by fractional recrystallization, of the diasteromers formed. The optically active thiol is released by solvolysis, and obtained in almost 100% optical purity. Samples were stored under N_2 at 5°.

Preparation of diastereoisomeric sugars. — A sample of sugar (10–100 μg) contained in a 0.3-mL reaction-vial was dried overnight in a desiccator containing KOH. Trifluoroacetic acid (5 μL) was added, and the vial was gently swirled until the solid dissolved. (+)-1-Phenylethanethiol (20 μL) was added, and the contents of the vial were mixed in a Vortex mixer. The reaction was allowed to proceed for 30 min at room temperature, with occasional mixing of the contents, and then cold pyridine (50 μL) was added to stop the reaction. For quantitative studies, a 0.01M solution (10 μL) of α,α-trehalose in pyridine was added prior to (trimethylsilyl)ation.

Formation of volatile derivatives. — Trimethylsilyl ethers were obtained by adding BSTFA (50 μL) and TMCS (25 μL) to the reaction vial and heating in an oven for 2 h at 65°. Samples (1 μL) of this mixture were analyzed by g.l.c.

Acetyl derivatives were obtained as follows. The excess of thiol and of pyridine were removed by placing the opened vial in a desiccator containing concentrated H_2SO_4, pellets of KOH, and a small beaker of activated charcoal impregnated with $CuSO_4$, and the vial was kept overnight *in vacuo*. To the pyridinium salt that remained was added a freshly prepared solution (100 μL) of 4-(dimethylamino)pyridine in acetic anhydride (2.5 mg/mL). The solution was heated for 2 h at 65°, cooled, and evaporated under a stream of dry N_2, and the residue was dissolved in CH_2Cl_2 (50 μL) for g.l.c. analysis.

Characterization of product. — D-Galactose bis[(+)-1-phenylethyl] dithioacetal was prepared as follows. A solution of D-galactose (60 mg) in warm trifluoroacetic acid (0.2 mL) was treated with (+)-1-phenylethanethiol (100 μL), and vigorously mixed for 10 min, and then small portions of cold water were added, to induce precipitation. The white precipitate was collected on a sintered-glass funnel, and recrystallized twice from EtOH–H_2O, to give a final yield of 38 mg; m.p. 152°, $[\alpha]_{589}^{25}$ +322° (*c* 0.9, EtOH) (further recrystallization effected no change in melting point or optical rotation); n.m.r. data: δ 1.45 (dd, 6 H), 7.2 (m, 10 H).

Anal. Calc. for $C_{22}H_{30}O_5S_2$: C, 60.24; H, 6.89; S, 14.61. Found: C, 60.14; H, 6.94; S, 14.74.

Hydrolysis of plant polysaccharide. — The polysaccharide from flax-seed mucilage was isolated as described by Araki and Arai[14]. The polysaccharide (5 mg) was hydrolyzed in 2M trifluoroacetic acid (1 mL) for 6 h at 100° in a sealed ampoule. A 0.4-mL aliquot was transferred to a 1-mL reaction-vial, concentrated under a stream of dry air, and dried overnight in a desiccator containing KOH. The (trimethylsilyl)ated dithioacetal derivatives were obtained as already described.

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