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Gas-Liquid Chromatographic Separation of Aldose Enantiomers as Trimethylsilyl Ethers of Methyl 2-(Polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylates¹⁾

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Pairs of enantiomers of nine aldoses were separated by gas-liquid chromatography on an OV-17 capillary column as the trimethylsilyl ethers of methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylates, which were obtained by the reaction of aldoses with L-cysteine methyl ester. This method was applied to the determination of the absolute configurations of the component monosaccharides of a *Thladiantha* saponin.

Keywords—sugar enantiomer separation; GLC; OV-17 capillary column; L-cysteine methyl ester; thiazolidine derivative; methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylate

In the field of saccharide and glycoside chemistry, it is necessary to determine the absolute configurations (D or L) of the component monosaccharides. This has been a serious problem in the determination of glycoside structures, especially in cases where the amount of the sugar available is small, and the sugar moiety is composed of several kinds of monosaccharides, because the ordinary determination by measurement of the optical rotation requires considerable amounts of pure sugar samples.

Several investigators have achieved the separation of sugar enantiomers at the microgram level by employing gas-liquid chromatography (GLC) or high-performance liquid chromatography (HPLC). Some have separated enantiomers by GLC on chiral stationary phases.²⁾ However, application of these methods is still not widespread because of poor availability and high cost of materials. Other workers have tried separation by GLC or HPLC after conversion of sugars to chiral derivatives. The chiral derivatives so far reported for separation of enantiomers include glycosides of (–)-2-butanol and (+)-2-octanol,³⁾ bis[(+)-1-phenylethyl]dithioacetals⁴⁾ and 1-deoxy-1- α -methylaminoalditols⁵⁾ (obtained by the reaction of aldoses with chiral α -methylbenzylamine in the presence of sodium cyanoborohydride). Among these chiral derivatives, the last one seemed to be preferable for sugar analysis of glycosides having several kinds of component sugars because of the commercial availability and stability of the chiral reagent, the ease of derivation and the simplicity of the chromatogram. Recently, this method has been effectively employed by Kasai *et al.*⁶⁾ for determination of the absolute configurations (D/L) of the component sugars of several glycosides.

We have also tried to use this method, but found that it is still unsatisfactory in some cases for separation of enantiomers and differentiation of sugar species. This finding has led us to search for other chiral derivatives that might allow clear separation of sugars and determination of the absolute configuration of each sugar.

Bognár *et al.*⁷⁾ have reported that aldoses react quantitatively with L-cysteine or its methyl ester to give thiazolidine derivatives. They suggested the applicability of these derivatives for separation of sugars and their enantiomers.

Chart

This report deals with the GLC separation of enantiomers of nine aldoses on an OV-17 capillary column after converting the sugars to the trimethylsilyl (TMS) ethers of the corresponding methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylates.

Experimental

Materials—The following sugar samples were commercially obtained; D-xylose and L-arabinose (Wako Pure Chemicals, Co., Ltd., Tokyo), L-xylose and L-rhamnose (Aldrich Chemical Co., Inc., Wisconsin), D-arabinose (Katayama Chemical Co., Ltd., Osaka), D-lyxose and D-ribose (Tokyo Kasei Kogyo Co., Ltd., Tokyo), D-fucose, L-lyxose, L-fucose, L-glucose, L-ribose and L-galactose (Sigma Chemical Co., Ltd., St. Louis), D-glucose and D-mannose (Yoneyama Yakuhin Kogyo Co., Ltd., Osaka), D-galactose (Ishizu Pharmaceutical Co., Ltd., Osaka). D-Rhamnose was synthesized by catalytic hydrogenation of methyl 6-deoxy-6-iodo- α -D-mannopyranoside followed by hydrolysis.⁸⁾ L-Cysteine methyl ester hydrochloride and TMS-HT kit (hexamethyldisilazane-trimethylchlorosilane (HMDS-TMCS)) were obtained from Wako Pure Chemicals Co., Ltd., and Tokyo Kasei Kogyo Co., Ltd., respectively.

Apparatus—Gas chromatographic (GC) analysis was performed with a Shimadzu GC-8 gas chromatograph equipped with an H₂ flame ionization detector. The column was G-SCOT Silicone OV-17 on Silanox (0.3 mm i.d. $\times$ 50 m). Analytical conditions were as described in the caption of Fig. 3.

Proton nuclear magnetic resonance (¹H-NMR) spectra were taken in pyridine-*d*₅ solution with a JEOL JNM GX-400 spectrometer using tetramethylsilane as an internal standard. Fast-atom bombardment mass (FAB-MS) spectra were recorded with a JEOL JMS DX-300 spectrometer using a glycerol matrix containing NaI.

Derivation and Analytical Procedure—Pyridine solutions (100 μ l each) of the sugar (0.04 mol/l) and L-cysteine methyl ester hydrochloride (0.06 mol/l) were mixed, and warmed at 60 °C for 1 h. The trimethylsilylation reagent HMDS-TMCS (150 μ l) was added, and the warming at 60 °C was continued for another 30 min. The precipitate was centrifuged off, and the supernatant (1 μ l) was subjected to GLC analysis. When the sample was analyzed as an acetate, the HMDS-TMCS was replaced by acetic anhydride (150 μ l) and the mixture was warmed at 90 °C for 1 h. After evaporation of pyridine and acetic anhydride by air-blowing, the residue was dissolved in acetone (350 μ l) and the solution (1 μ l) was subjected to GLC.

Preparation of Methyl 2-(D- and L-arabino-Tetrahydroxybutyl)-thiazolidine-4(*R*)-carboxylates—The standard samples of thiazolidine derivatives of D- and L-arabinoses were prepared by the method reported by Bognár *et al.*⁹⁾ Thus, 0.58 g of L-cysteine methyl ester hydrochloride and 0.5 g of L-arabinose were dissolved in water (1 ml) and 0.3 ml of pyridine was added. The mixture was left to stand at room temperature overnight, then ethanol (2 ml) was added to complete the precipitation of the reaction product, and the precipitate was filtered off, washed with ethanol and dried *in vacuo*. The yield was 75%; amorphous powder, mp 155–156 °C, FAB-MS *m/z*: 290.068 ([M+Na]⁺) (Calcd for C₉H₁₇NNaO₆S: *m/z* 290.067). *Anal.* Calcd for C₉H₁₇NO₆S: C, 40.44; H, 6.41; N, 5.24. Found: C, 39.96; H, 6.37; N, 5.23.

D-Arabinose was treated in the same manner to give the corresponding derivative: crystalline powder, mp 156–159 °C, FAB-MS *m/z*: 290.067 ([M+Na]⁺). *Anal.* Found: C, 40.56; H, 6.44; N, 5.28.

Acetylation of Methyl 2-(L-arabino-Tetrahydroxybutyl)-thiazolidine-4(*R*)-carboxylate—The sample (0.1 g) was dissolved in pyridine (0.5 ml) and acetic anhydride (0.5 ml) was added. The reaction mixture was stirred at room temperature for 12 h. Water (3 ml) was added and the precipitate was filtered off and recrystallized from ethanol to give colorless needles, mp 126–127 °C, [α]_D²⁰ –49.1 ° (c = 1.12, CHCl₃). The melting point and optical rotation were in good agreement with those reported (127–128 °C, [α]_D²⁰ –50 °) for methyl 3-acetyl-2(*R*)-(L-arabino-1',2',3',4'-tetraacetoxybutyl)-thiazolidine-4(*R*)-carboxylate.⁹⁾

Determination of Absolute Configurations of Component Monosaccharides of a *Thladiantha* Saponin—The *Thladiantha* saponin (4 mg) was heated in 1 N HCl (0.5 ml) at 90 °C for 2 h. The precipitate that deposited on cooling was removed by centrifugation. The supernatant was neutralized with Ag₂CO₃. After centrifugation of the precipitate, the supernatant was bubbled through with H₂S and concentrated *in vacuo* to give a sugar fraction (ca. 1 mg). The derivation to the thiazolidine derivative and GC analysis were carried out according to the standard procedure. The result is shown in Fig. 3B.

Results and Discussion

As a preliminary test for the separation of sugar enantiomers, the TMS ethers of thiazolidine derivatives of arabinose enantiomers were checked on an OV-17 capillary column. The two enantiomers were clearly separated. Therefore, the conditions for derivation to thiazolidine derivatives were examined. Bognár *et al.*⁹⁾ obtained the thiazolidine derivatives by reaction in an aqueous medium or aqueous pyridine solution at room temperature. The yields were reported to be quantitative, but the reaction required more than 3 h for completion. If subsequent trimethylsilylation or acetylation and direct injection into the GLC column are required, an aqueous medium is not suitable for one-pot derivation of small amounts of sugar samples. Therefore, pyridine was used as the reaction medium and a suitable reaction temperature was examined. L-Arabinose, L-cysteine methyl ester hydrochloride and methyl 2-(D-arabino-tetrahydroxybutyl)-thiazolidine-4(R)-carboxylate (molar ratio, 1 : 1.5 : 1) were dissolved in pyridine and warmed at 20, 40 and 60 °C. Aliquots of the reaction solutions were taken out at intervals, trimethylsilylated and checked by GLC. Figure 1 shows the time courses of the reaction at the three temperatures. The ratio (D/L) of the peak areas of the TMS ethers of the product [methyl 2-(L-arabino-tetrahydroxybutyl)-thiazolidine-4(R)-carboxylate] and the internal standard (D-enantiomer) reached an almost constant value (*ca.* 0.8) after heating for 30 min at 60 °C, and this value was almost the same as the ratio of the peak areas of an equimolar mixture of thiazolidine derivatives of D- and L-arabinoses, indicating that the yield of the derivative is almost quantitative.

From these data, the conditions, warming at 60 °C for 1 h in pyridine, were employed for derivation of all other sugar samples.

To confirm the identity of the products with those obtained by Bognár *et al.*, ¹H-NMR spectra of the thiazolidine derivatives of L-arabinose and D-arabinose were compared with those of the products obtained by Bognár's method. The ¹H-NMR spectra of the products obtained by the two derivation methods showed identical chemical shifts of all proton signals, although the ratios of the two diastereoisomers were not quite the same. Figure 2 shows the ¹H-NMR spectrum of the product obtained from L-arabinose by Bognár's method.

Bognár *et al.*⁹⁾ claimed that L-arabinose reacted with L-cysteine methyl ester stereoselectively to give the 2(R) isomer. They might have concluded that the product was the 2(R) isomer because it gave methyl 2(R)-(L-arabino-1',2',3',4'-tetraacetoxybutyl)-thiazolidine-4(R)-carboxylate on acetylation in an acetic anhydride-pyridine mixture at room temperature. However, this does not represent unambiguous evidence for the configuration at C₂ of the original thiazolidine derivative because base-catalyzed isomerization of thiazolidine through a Schiff-base intermediate is a well-known process. Szilagyi *et al.*¹⁰⁾ reported that

Fig. 1. Time Course of the Reaction of L-Arabinose and L-Cysteine Methyl Ester

Fig. 2. ^1H -NMR Spectrum of Methyl 2-(L-arabino-Tetrahydroxybutyl)-thiazolidine-4(R)-carboxylate

The spectrum was measured in pyridine- d_5 on a JEOL JNM GX-400 Spectrometer. 1'-H(R) indicates a hydrogen on C-1' of methyl 2(R)-(L-arabino-tetrahydroxybutyl)-thiazolidine-4(R)-carboxylate.¹³⁾

Fig. 3. (A) Separation of Enantiomers of Arabinose and Galactose as TMS Ethers of Thiazolidine Derivatives; (B) Gas Chromatogram of Trimethylsilylated Thiazolidine Derivative of Component Monosaccharides of a *Thladiantha* Saponin

GLC conditions: column, G-SCOT OV-17 on Silanox (0.3 mm i.d. $\times$ 50 m); column bath temperature, 200°C; injection temperature, 270°C; carrier gas, He (0.8 ml/min); split ratio, 1/75; make-up gas, He (50 ml/min).

cysteine reacts with benzaldehyde to yield a mixture of the two C_2 -epimers of 2-phenylthiazolidine-4-carboxylic acid, which gave the 2,4-*trans* epimer of the N-acetate when treated with an acetic anhydride-pyridine mixture at room temperature, while it gave the 2,4-*cis* epimer when heated in 50% aqueous acetic anhydride.

On acetylation of the thiazolidine derivative of L-arabinose obtained by Bognár's method, it gave a mixture (ratio, *ca.* 4 : 1, checked by GLC)¹¹⁾ of two acetates, from which the predominant acetate was obtained by crystallization from ethanol. The melting point and optical rotation were in good agreement with those of methyl 3-acetyl-2(R)-(L-arabino-1',2',3',4'-tetraacetoxybutyl)-thiazolidine-4(R)-carboxylate reported by Bognár *et al.*⁹⁾

A typical gas chromatogram is shown in Fig. 3A, and the results of analysis of nine pairs of aldose enantiomers are listed in Table I.

Each enantiomer gave single peak, and no interfering peak was observed. The peaks were rather broad even though separation of the enantiomers was clear-cut, and the faster-eluting enantiomer gave a broader peak than the other enantiomer, contrary to the common GLC feature that the faster-eluting peak is sharper than those that follow. This tendency was observed in all sugar species examined. The broadness of the peak is probably due to the co-existence of the two epimers at C_2 .

The separation of enantiomers of all sugar species was quite clear, though the derivatives

TABLE I. Retention Times, Separation Factors and Resolutions of TMS Ethers of Methyl 2-(Polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylates Derived from Aldoses

Sugars	t_R		γ	Rs
	D-	L-		
Xylose	11.05 (<i>R</i>)	12.01 (<i>S</i>)	1.087	1.66
Arabinose	12.02 (<i>S</i>)	10.89 (<i>R</i>)	1.104	2.05
Lyxose	11.71 (<i>S</i>)	11.32 (<i>R</i>)	1.034	0.98
Ribose	11.25 (<i>R</i>)	12.45 (<i>S</i>)	1.107	3.16
Fucose	13.06 (<i>R</i>)	14.48 (<i>S</i>)	1.109	3.16
Rhamnose	12.60 (<i>S</i>)	12.14 (<i>R</i>)	1.038	1.10
Glucose	17.87 (<i>R</i>)	19.19 (<i>S</i>)	1.074	1.65
Galactose	19.51 (<i>R</i>)	21.73 (<i>S</i>)	1.114	3.31
Mannose	18.03 (<i>S</i>)	17.51 (<i>R</i>)	1.030	1.04

t_R , retention time (min); γ , separation factor (t_{R2}/t_{R1}); Rs , resolution [$2(t_{R2} - t_{R1})/(W_1 + W_2)$] (*R* or *S* in parentheses is the configuration of C-1').

of some different sugar species (e.g. D-xylose and L-arabinose; L-xylose and D-arabinose; L-lyxose and D-ribose) gave similar t_R values and were difficult to separate.

It seemed that the absolute configuration (D or L) of the original sugar species had no correlation to the t_R value: the derivatives of D-enantiomers have smaller t_R values than those of L-enantiomers in some cases, while in other cases, the L-enantiomers were eluted first. However, when the t_R values and the structures of the products were examined in detail, it became apparent that the absolute configuration at C-1' had a significant influence on the elution order. A sugar derivative which has the *R*-configuration at C-1' has a smaller t_R value than the counterpart, without exception. The influence of the configuration of other carbons could not be separated because of the limited number of sugars investigated, but the small separation factors (γ) for lyxose, rhamnose and mannose, which have the same absolute configurations at C-1' and C-2', and the large separation factor for ribose, which has the same absolute configurations at C-1', -2' and -3', imply that the absolute configurations of carbons of the polyol moiety or the steric relationship of the trimethylsilylated hydroxyl groups may have a significant influence on the separation of a pair of enantiomers.

As an application, this method was utilized to determine the absolute configurations of component sugars of a quillaic acid glycoside isolated from the tuber of *Thladiantha dubia*.¹²⁾ The sugar moiety was previously determined to be composed of 1 mol each of arabinose, xylose, rhamnose, glucose and galactose by GLC. The hydrolysate of the glycoside was treated as described for the standard sugar samples and checked by GLC. The result is shown in Fig. 3B. By comparison of the data with the t_R values of the standard samples, the absolute configurations were determined to be D for xylose, glucose and galactose, and L for arabinose and rhamnose.

This method is superior to the methods hitherto reported for separation of enantiomers as chiral sugar derivatives in that (a) the derivation procedure is simpler and it is easier to get a quantitative yield of the derivative, and (b) a clear separation of enantiomers is obtained for almost all sugars under ordinary GLC conditions, even though it is still not possible to achieve the identification of all sugar species and determination of the absolute configuration of each sugar by a single GLC run.

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References and Notes

- 1) Preliminary communication: S. Hara, H. Okabe and K. Mihashi, *Chem. Pharm. Bull.*, **34**, 1843 (1986). In the preliminary communication, the analytical data obtained by using an SE-30 capillary column were given. After examination of other columns, the OV-17 capillary column was found to be better for separation of enantiomers. In this paper, the data obtained on an OV-17 column are presented.
- 2) W. A. König, I. Benecke and S. Sievers, *J. Chromatogr.*, **217**, 71 (1981); A. L. Leavitt and W. Sherman, *Carbohydr. Res.*, **103**, 203 (1982).
- 3) G. J. Gerwig, J. P. Kamerling and J. F. G. Vliegthart, *Carbohydr. Res.*, **77**, 1 (1979).
- 4) M. R. Little, *Carbohydr. Res.*, **105**, 1 (1982).
- 5) R. Oshima, J. Kumanotani and C. Watanabe, *J. Chromatogr.*, **259**, 159 (1983).
- 6) R. Kasai, M. Miyakoshi, K. Matsumoto, T. Morita and O. Tanaka, Symposium Papers of the 27th Symposium on the Chemistry of Natural Products, Hiroshima, 1985, p. 749.
- 7) R. Bognár, L. Somogyi and Z. Györgydeak, *Justus Liebigs Ann. Chem.*, **738**, 68 (1970).
- 8) Methyl 6-*O*-tosyl- α -D-mannopyranoside was treated with NaI and hydrogenated in the presence of Raney Ni to give methyl α -D-rhamnopyranoside, which was hydrolyzed. The procedure was essentially the same as that described for the preparation of α -D-fucose from D-galactose (O. Th. Schmidt, "Methods in Carbohydrate Chemistry," Vol. 1, ed. by R. L. Whistler and M. L. Wolfrom, Academic Press, Inc., New York, 1962, p. 191.).
- 9) R. Bognár, Z. Györgydeak, L. Szilagyi, G. Horváth, G. Czira and L. Radics, *Justus Liebigs Ann. Chem.*, **1976**, 450.
- 10) L. Szilagyi and Z. Györgydeak, *J. Am. Chem. Soc.*, **101**, 427 (1979).
- 11) GLC of acetates was conducted on the same column as used for TMS ethers. The column temperature was 250 °C and the injection temperature was 280 °C. t_R : 13.6 min (major), 14.0 min (minor).
- 12) The structures of the glycosides were reported at the 106th Annual Meeting of the Pharmaceutical Society of Japan, Chiba, April 1986.
- 13) The assignment of signals was based on the report by Szilagyi *et al.*¹⁰⁾ as well as measurement of the ^1H - ^1H shift correlation spectrum and the nuclear Overhauser effect (NOE) difference spectrum. On irradiation at the frequency of the signal at δ 5.43, a distinct NOE was observed at the proton signal at δ 4.14, while no NOE was recognized at the signal at δ 4.29 when the signal at δ 5.68 was irradiated. This spectral evidence led to the identification of the minor isomer as the 2(*R*)-epimer (2,4-*cis*) and the major one as the 2(*S*)-epimer (2,4-*trans*).