Facile Discrimination of Aldose Enantiomers by Reversed-Phase HPLC

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One-pot reactions of aldoses with L-cysteine methyl ester and o-tolyl isothiocyanate yielded methyl 2-(polyhydroxyalkyl)-3-(o-tolylthiocarbamoyl)-thiazolidine-4(R)-carboxylates. Direct HPLC analysis of the reaction mixture and UV detection at 250 nm discriminated D- and L-enantiomers of aldoses. The reaction was applied to the determination of absolute configuration the sugar residues of an aroma precursor.

Key words aldose; absolute configuration; HPLC; cysteine; isothiocyanate

Aldoses are one of the most important structural components of biomolecules, such as polysaccharides, nucleic acids, glycolipids and glycoproteins. In addition, numerous secondary metabolites in plants, such as terpenoids, steroids, and flavonoids, exist as glycosides, which conjugate with aldoses. Aldoses are optically active compounds, and confirmation of absolute configuration is required in natural product chemistry. Measurement of specific rotations of pure samples is the most reliable method, although this is impractical in many cases because only limited amounts of samples are available. Analysis using a column with a chiral stationary phase developed for the separation of enantiomers, or an HPLC system equipped with an optical rotation detector and a column specified for sugar analysis, may be applied. However, the latter method is not applicable to mixtures of D- and L-enantiomers. Identification of sugars with small optical rotation may be also difficult. Methods using capillary electrophoresis have also been developed, but these methods require specialized equipment or columns that are unfamiliar to most organic chemistry laboratories. Some methods based on conversion of aldose enantiomers to diastereomeric derivatives through coupling to an optically active reagent have been developed; however, there are not many methods applicable to the widely used HPLC systems equipped with a UV detector and C18 reversed-phase column. This paper describes a new method to discriminate between aldose enantiomers using a usual HPLC system.

Results and Discussion

Hara et al. developed an excellent method using gas chromatography, in which enantiomeric aldoses were converted to trimethylsilyl ethers of methyl 2-(polyhydroxyalkyl)-thiazolidine-4(R)-carboxylates. In order to apply this method to HPLC analysis, we converted the thiazolidine derivatives to arylthiocarbamate derivatives through coupling to an optically active reagent. However, there are not many methods applicable to the widely used HPLC systems equipped with a UV detector and C18 reversed-phase column. This paper describes a new method to discriminate between aldose enantiomers using a usual HPLC system.

Their structures were determined by 1H- and 13C-NMR spectra and FAB-MS analyses. Although the production of two diastereomers, which have an opposite configuration at the sugar C-1 position, was expected from each enantiomer, the 1H- and 13C-NMR spectra showed that one of the two possible diastereomers was produced preferably in the case of glucose.

When the reaction mixture was stored at room temperature for a few days, the derivatives decomposed to give thiohydantoin compounds by elimination of methanol. The derivative of l-glucose (4) was more unstable than that of D-glucose (3), and a hydantoin derivative (5) was produced. The resulting hydantoin derivatives of D- and L-enantiomers gave similar tR values (8.56, 8.57, respectively), indicating that the hydantoin derivatives were not suitable for the discrimination of enantiomers.

In order to find more stable thiocarbamoyl-thiazolidine derivatives, seven isothiocyanates (phenyl, benzyl, benzoyle, 3,4-dimethoxyphenyl, p-phenylethyl, p-tolyl, and o-tolyl isothiocyanates) were compared. Except for benzylisothiocyanate, all compounds yielded thiocarbamate derivatives, and the time differences (ΔtR) were enough to distinguish between D- and L-enantiomers. The smallest peak areas of the thiohydantoin derivatives were observed when o-tolyl isoth-
ioycanate was used. It is likely that its methyl group in the ortho position hindered thiohydanto in formation.

The \( f_R \) values of typical D- and L-aldo ses after treatment with cysteine methyl ester and \( \alpha \)-tolyl isothiocyanate are listed in Table 1.\(^8\) Thiohydanto in derivatives were estimated to represent less than 20% of all thio carbamoyl-thiazolidine derivatives, based on their peak areas. This method could not be applied to ketoses, such as fructose, which did not show any peaks on HPLC analysis. Interestingly, the migration order of D- and L-enantiomers depended on the structure of aldoses.

When D-cysteine methyl ester was used, the \( f_R \) values of the D- and L-enantiomers were reversed. Using this method, the retention time of the derivatives of the enantiomeric sugars can be predicted. Therefore, only one of the enantiomers is required to estimate the \( f_R \) values of D- and L-aldo ses when L- and D-cysteine methyl esters are available. In our study, D-cysteine methyl ester was supplied in situ by the reduction of D-cysteine dimethyl ester with dithiothreitol. Indeed, the \( f_R \) values of D-rhamnose, D-apiose, L-glucuronic acid and N-acetyl-L-glucosamine in Table 1 were obtained by reaction of sugar enantiomers with D-cysteine methyl ester, because of a lack of authentic samples. A linear calibration curve of the derivative of D-glucose was obtained in the range of 0.2—5 mmol/l. However, the different sugars gave different peak areas (Table 1), indicating that the yields of the derivatization were unequal. A linear calibration curve of the values of D-rhamnose, D-apiose, L-glucuronic acid and N-acetyl-L-glucosamine in Table 1 were obtained by reaction of sugar enantiomers with D-cysteine methyl ester, because of a lack of authentic samples. A linear calibration curve of the derivative of D-glucose was obtained in the range of 0.2—5 mmol/l. However, the different sugars gave different peak areas (Table 1), indicating that the yields of the derivatization depended on the aldose structures. Therefore, estimation of the sugar molar ratio by comparing the peak area should be done carefully: calibration curves should be prepared for each sugar. Mannose, rhamnose and apiose showed large \( \Delta f_R \) values compared with other aldoses. This was probably caused by the difference in aldose C-1 configuration.

Next, we applied the method to structure determination of an aroma precursor isolated from the leaves of Illicium anisatum. The plane structure was determined to be 1-(3',4'-dihydroxyphenyl)-2-propene 3'-O-(4-O-\( \alpha \)-arabino pyranosyl)-\( \beta \)-glucopyranoside (6) by usual NMR techniques, including 2 dimensional NOESY spectrum, confirming the location of sugar moieties. In order to determine the absolute configuration, glycosides were hydrolyzed by heating in 0.5 m HCl and neutralized with Amberlite IRA400. After drying in vacuo, the residue was dissolved in pyridine and derivatized as described above. Direct HPLC analysis of the reaction mixture exhibited peaks at 19.58 and 17.52 min, which were coincided with derivatives of L-arabinose and D-glucose, confirming the absolute configuration of the sugar components.

Although the method described here is a modification of that developed for gas-liquid chromatography, C\( \alpha \)-reversed phase HPLC equipped with a UV detector is now a more common analytical system in laboratories worldwide. Our method is very simple and does not require any specialized columns or detectors, which are usually expensive. In addition, the reagents used for derivatization are inexpensive and readily available. Further optimization of the reaction conditions and application of the method to structure determination of natural products are now in progress.

### Experimental

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. \( ^1 \)H- and \( ^13 \)C-NMR spectra were recorded in pyridine-d\( _6 \) or D\( _2 \)OD with with a JEOL JMN-AL400 (JEOL Ltd., Japan) operating at 400 MHz for \( ^1 \)H and 100 MHz for \( ^13 \)C. Coupling constants were expressed in Hz, and chemical shifts were given on a \( \delta \) (ppm) scale with tetramethylsilane as an internal standard. MS were recorded on a JEOL JMS D-303 spectrometer, and glycerol was used as a matrix for FAB-MS measurement. Column chromato graphies were performed with Silica gel 60 (Merck) and Chromatorex ODS (100—200 mesh, Fuji Silysia Chemical Ltd.). TLC was performed on precoated Kieselgel 60 F\( _254 \) plates (0.2 mm thick, Merck) with chloro form–methanol–water (40 : 10 : 1 or 14 : 6 : 1, v/v). and spots were detected by ultraviolet (UV) illumination and by spraying 5% sulfuric acid reagent. Analytical HPLC was performed on a 250×4.6 mm i.d. Cosmosil 5C\( _18 \)-AR II column (Nacalai Tesque Inc.) at 35 \( ^\circ \)C with isocratic elution of 25% CH\( _3 \)CN in 50 m H\( _2 \)PO\( _4 \) for 40 min and subsequent washing of the column with 90% CH\( _3 \)CN at a flow rate 0.8 ml/min. Peaks were detected with a Jasco MD-910 photodiode array detector.

### Table 1. Retention Times of the Thiocarbamoyl-thiazolidine

<table>
<thead>
<tr>
<th>Aldoses</th>
<th>( t_R ) (min)</th>
<th>( \Delta t_R (\text{min}) )</th>
<th>Relative peak area (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexose D</td>
<td>17.48</td>
<td>1.33</td>
<td>1.0</td>
</tr>
<tr>
<td>Glucose D</td>
<td>16.15</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Mannose D</td>
<td>11.20</td>
<td>-6.21</td>
<td>1.1</td>
</tr>
<tr>
<td>Galactose D</td>
<td>17.41</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>Rhamnose D</td>
<td>15.95</td>
<td>-0.49</td>
<td>2.0</td>
</tr>
<tr>
<td>Apiose D</td>
<td>15.85</td>
<td>-13.61</td>
<td>0.5</td>
</tr>
<tr>
<td>Fucose D</td>
<td>29.47</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Glucuronic acid D</td>
<td>26.17</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>N-Ac-glucosamine D</td>
<td>17.64</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Pentose D</td>
<td>11.04</td>
<td>-3.89</td>
<td>0.2</td>
</tr>
<tr>
<td>Arabinose D</td>
<td>14.93</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Xylose D</td>
<td>20.83</td>
<td>1.31</td>
<td>1.7</td>
</tr>
<tr>
<td>Apiose D</td>
<td>19.52</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Ribose D</td>
<td>20.36</td>
<td>1.43</td>
<td>2.4</td>
</tr>
</tbody>
</table>

(a) Based on the peak area at 250 nm of D-glucose. b The \( t_R \) were obtained by using L-cysteine and dithiothreitol. c D-Apiose and L-apiose were synthesized from L- and D-riboses.
5.18 (1H, br s, glucose H-3), 4.64 (2H, br s, glucose H-4, 5), 4.60 (1H, br d, J=9.2 Hz, glucose H-2), 4.56 (1H, dd, J=11.0, 2.7 Hz, glucose H-6a), 4.38 (1H, dd, J=11.0, 5.1 Hz, glucose H-6b), 3.60 (5H, m, MeO, H-5a, 5b), 51C-NMR (100 MHz, pyridine-d$_5$) δ: 183.0 (C-8), 171.7 (COO), 141.4 (C-1’), 128.5 (C-3’), 5’), 125.1 (C-2’, 6’), 125.0 (C-4’), 75.9, 75.9, 72.7, 70.7, 70.3, 68.5, 65.0 (C-2, C-4, glucose-C), 52.6 (OMe), 31.4 (C-5).

i-Thiodyantoin Derivative (5) The derivative 5 (120 mg) was obtained as a by-product of 4 as a white powder, FAB-MS m/z: 401 [M+H]+, UV λ$_{max}$, 255, 273 nm, 1H-NMR (400 MHz, pyridine-d$_5$) δ: 3.70—3.74 (3H, m, C-2, 4, glucose-C), 3.84 (1H, dd, J=10.1 Hz, H-1), 8.3 Hz, glucose H-5), 4.03 (1H, dd, J=1.4 Hz, H-1’), 5.71 (1H, d, J=7.6 Hz, H-1’), 4.07 (1H, dd, J=1.4, 12.3 Hz, H-6’), 4.03 (1H, dd, J=1.4, 3.2 Hz, H-2’), 3.97 (1H, dt, J=3.3, 5.3 Hz, H-4’), 3.84 (1H, dd, J=3.2, 5.3 Hz, H-3’), 3.72 (1H, dd, J=3.2, 11.9 Hz, H-5’), 3.54—3.66 (3H, m, H-5’, 6’, 5’), 3.35—3.52 (3H, H-2’, 3’, 4’), 3.29 (2H, br d, J=6.7 Hz, H-7). 13C-NMR (CD$_2$OD) δ: 146.6, 146.5 (C-3, 4), 139.2 (C-8), 133.2 (C-1), 124.9 (C-6), 119.4, 117.0 (C-2, 5), 115.7 (C-9), 109.9 (C-1’), 104.5 (C-1’), 85.9 (C-2’), 83.2 (C-4’), 78.9 (C-3’), 77.5 (C-3’), 76.9 (C-5’), 74.9 (C-2’), 71.7 (C-4’), 67.9 (C-6’), 63.0 (C-5’), 40.5 (C-7). NOESY correlations: H-1’→H-2, H-1”→H-6’, H-2 and H-6→H-7.

**Determination of Sugar Configuration** The glycoside 6 (0.5 mg, 1.1×10$^{-4}$ mol) were hydrolyzed by heating in 0.5 m HCl (0.1 ml) and neutralized with Amberlite IRA400. After drying in vacuo, the residue was dissolved in pyridine (0.1 ml) containing i-cysteine methyl ester hydrochloride (0.5 mg) and heated at 60°C for 1 h. A 0.1 ml solution of o-tolylisothiocyanate (0.5 mg) in pyridine was added to the mixture, which was heated at 60°C for 1 h. The reaction mixture was directly analyzed by reversed-phase HPLC. The peaks at 19.58 and 17.52 min were coincided with derivatives of L-arabinose and d-glucose.

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**References and Notes**