Note

Optical Resolution of 1-(Methyl-sulfinyl)-propyl Alk(en)yl Bisulfides, Inhibitors of Platelet Aggregation Isolated from Onion

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The Allium species are strong appetizers and well-known for their pharmaceutical effects. For instance, garlic (Allium sativum L.) and onion (Allium cepa L.) have been reported to inhibit platelet aggregation,1,2 and some anti-platelet compounds have been isolated.3-5 Recently, we have found potent inhibitors of human platelet aggregation from a methanolic extract of crushed onion.6 These compounds were identified as l-(methylsulfmyl)-propyl alk(en)yl disulfides, and two diastereoisomers were separated by using normal-phase HPLC,7 which were termed AC-1a, b, AC-11a, b and AC-12a, b (Fig. 1). This report is concerned with the optical resolution of diastereoisomers AC-1b, AC-11a and AC-11b by using chiral-phase HPLC, and a comparison of the inhibitory activity of these enantiomers for human platelet aggregation.

Diastereoisomers AC-11a and AC-11b were separated by chiral-phase HPLC into two enantiomers respectively termed AC-11a-(1) and -(2) and AC-11b-(1) and -(2) (Fig. 2). These enantiomers were repurified before CD analyses and the test for platelet aggregation. AC-1b was also separated into two enantiomers termed AC-1b-(1) and -(2), but other diastereoisomers could not be separated by chiral-phase HPLC into the enantiomers because their individual peaks overlapped. The CD spectra of diastereoisomers AC-11a and AC-11b were flat lines (data not shown). After separating, the CD spectra of their enantiomers were symmetrical with respect to the base line (Fig. 3), but an assignment of the absolute configuration of the AC-11 series was difficult, since there was no regular relationship between the absolute configuration of sulfinyl groups and positive or negative signals of their CD spectra.

Fig. 2. Chiral-Phase HPLC Chromatograms of AC-11a and AC-11b.
Column, chiralcel OB (4.6 x 250mm); mobile phase, n-hexane-2-propanol = 9:1 (v/v); flow rate, 0.5 ml/min; detection, UV 270 nm.

Fig. 3. CD Spectra of the Enantiomers of AC-11a and AC-11b.
(A): ---, AC-11a-(1); -----, AC-11a-(2).
(B): ---, AC-11b-(1); -----, AC-11b-(2).
In each case, the solvent was methanol.
Table I. Inhibitory Effect on Human Platelet Aggregation Induced by Collagen

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (ȝM)</th>
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<tbody>
<tr>
<td>AC-1b</td>
<td>18.4</td>
</tr>
<tr>
<td>AC-1b-(1)</td>
<td>23.2</td>
</tr>
<tr>
<td>AC-1b-(2)</td>
<td>16.4</td>
</tr>
<tr>
<td>AC-1la</td>
<td>48.9</td>
</tr>
<tr>
<td>AC-1la-(1)</td>
<td>49.4</td>
</tr>
<tr>
<td>AC-1la-(2)</td>
<td>23.7</td>
</tr>
<tr>
<td>AC-1lb</td>
<td>11.7</td>
</tr>
<tr>
<td>AC-1lb-(1)</td>
<td>9.1</td>
</tr>
<tr>
<td>AC-1lb-(2)</td>
<td>22.9</td>
</tr>
</tbody>
</table>

Subsequently, the isolated enantiomers were tested for their inhibition of human platelet aggregation, and IC₅₀ values were determined (Table I). These anti-platelet compounds would inhibit cyclooxygenase and/or lipooxygenase on human platelets. Generally, inhibition of the enzymatic reaction is caused by stereospecific recognition, and only the most suitable configuration of an inhibitor may exhibit inhibitory activity. However, there is a 2- to 4-fold difference among the IC₅₀ values of the AC-11 series in Table I. A reasonable explanation of this result cannot be given at the present time, although it will be understandable if the real action of the AC series on the inhibition of platelet aggregation is proved, concerning the interaction between these enantiomers and the reaction sites of cyclooxygenase and/or lipooxygenase.

Experimental

Chiral-phase HPLC separation was done with a chiralcel OB column (4.6 x 250 mm, Daicel Chemical Industries) and a solvent of n-hexane-2-propanol (9 : 1) for each AC series (details in Fig. 1).

Ultraviolet spectroscopy. A Hitachi model 200-10 spectrophotometer was used, samples in methanol being scanned from 300 nm to 210 nm with a quartz cell. UV λₘₐₓ (MeOH) nm (ε): AC-1la-(1) and -(2), 212 (2100); AC-1lb-(1) and -(2), 212 (2320).

Circular dichroism. A Jasco J-40A automatic recording spectropolarimeter was used to obtain CD measurements. Samples in methanol were scanned from 300 nm to 200 nm in a 0.5 ml sample holder with a 1 mm path length. CD λₑₓₓ (MeOH) nm (Δε): AC-1la-(1), 202 (-63.2), 235 (-27.5); AC-1la-(2), 202 (+95.9), 235 (+42.0); AC-1lb-(1), 213 (-10.2), 234 (+11.1); AC-1lb-(2), 213 (+7.5), 234 (-13.6).

Preparation of platelets and aggregation studies. Venous blood was obtained from healthy donors, platelet-rich plasma (PRP) being prepared by centrifuging the blood as detailed elsewhere. Platelet aggregation was measured turbidimetrically by the method of Born et al. using a dual-channel aggregometer (Hematracer I, NKK). For all experiments, PRP (200 μl) and the methanolic test sample solution (1 μl) were incubated, and aggregation was then induced by adding collagen (0.2 μg). The IC₅₀ values were determined as the concentration of the compound that gave 50% inhibition compared with the blank test.

References