Note

Spasmolytic Activity of Aurapten Analogs

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Seven coumaric compounds analogous to aurapten were synthesized. Their spasmolytic activity against Ba2+, acetylcholine and histamine was evaluated to investigate their structure-activity relationship. The results of the bioassay demonstrated the important roles of the cis type of double bond at C-2′ and the epoxide between C-6′ and 7′.

Key words: spasmolytic activity; aurapten; acetylcholine; histamine; barium ion

Some coumaric compounds such as aurapten (1),11 epoxaurapten,21 and marmín21 have been isolated from the peel of Citrus hassaku and revealed spasmolytic activity. This activity was measured by using small intestines removed from male guinea pigs. The authors are continuing their study of the relationship between chemical structure and spasmolytic activity.2–41 In order to compare the activity with those of the compounds previously reported, we synthesized a variety of monoterpenyl 7-O-substituted and 4-methyl-7-O-substituted coumarins as aurapten analogs (Fig. 1). The chemical shifts in 13C-NMR spectra of these compounds are shown in Table I.

In the present paper, we report the spasmolytic activity of aurapten analogs against three spasmodens, namely, the barium ion (Ba2+), acetylcholine, and histamine. The results are summarized in Table II. The epoxide of CMC at C-6′, 7′ (8, EP-CMC) showed the highest activity (77.1%) against Ba2+. It is noteworthy that the cis type at C-2′ such as NOC (3, 54.2%) and NMC (4, 27.1%) revealed more activity than that of the trans type such as GOC (1, aurapten, 5.7%) and GMC (2, 8.6%). The epoxide of GMC at C-6′, 7′ (6, EP-GMC) showed the highest activity (82.8%) against acetylcholine. However, GMC had only slight activity (6.4%). Against histamine, NOC (50.0%) and NMC (31.2%), which are of the cis type at C-2′, demonstrated more activity than those of the trans type such as GOC (25.0%)

Fig. 1. Structures of the Aurapten Analogs.

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Abbreviations: ACh, acetylcholine; Hist, histamine; GOC, 7-geranyloxycoumarin; NOC, 7-neroxycoumarin; GMC, 4-methyl-7-geranyloxycoumarin; NMC, 4-methyl-7-neroxycoumarin; CMC, 4-methyl-7-citronellyloxycoumarin; EP-GMC, 6,7-epoxy GMC; EP-CMC, 6,7-epoxy CMC; NMC diepoxide, 4-methyl-7-(2,3′-6′,7′-diepoxo-3,7′-dimethylfocantanyloxy)coumarin.
Spasmolytic Activity of Aurapten Analogs

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ba²⁺</th>
<th>ACh</th>
<th>Hist</th>
</tr>
</thead>
<tbody>
<tr>
<td>GOC (1)</td>
<td>5.7%</td>
<td>0.0%</td>
<td>25.0%</td>
</tr>
<tr>
<td>NOC (3)</td>
<td>54.2</td>
<td>0.0</td>
<td>50.0</td>
</tr>
<tr>
<td>GMC (2)</td>
<td>8.6</td>
<td>6.4</td>
<td>25.0</td>
</tr>
<tr>
<td>NMC (4)</td>
<td>27.1</td>
<td>2.1</td>
<td>31.2</td>
</tr>
<tr>
<td>CMC (5)</td>
<td>11.4</td>
<td>14.9</td>
<td>26.6</td>
</tr>
<tr>
<td>EP-GMC (6)</td>
<td>66.0</td>
<td>82.8</td>
<td>78.1</td>
</tr>
<tr>
<td>EP-CMC (8)</td>
<td>77.1</td>
<td>36.2</td>
<td>29.4</td>
</tr>
<tr>
<td>NMC diepoxide (7)</td>
<td>--</td>
<td>--</td>
<td>34.7</td>
</tr>
</tbody>
</table>

*Activity is presented by the inhibitory percentage (%) against the contraction induced by each spasmogen. The final concentration of each tested sample in a Tyrode solution was 5 x 10⁻² mol/liter.

Table II. Spasmolytic Activity* of the Aurapten Analogs

and GMC (25.0%) (Fig. 2). The same tendency was observed against Ba²⁺. Moreover, EP-GMC showed more activity (78.1%) than GMC (25.0%). However, the diepoxide of NMC (7, NMC diepoxide, 34.7%) revealed less activity than the mono-epoxide of GMC (EP-GMC). These findings suggest the importance of the cis type of double bond at C-2' and the O-function at C-6', 7' for spasmolytic activity.

Experimental

7-Nerylxylocoumarin (3, NOC). A solution of phosphorus tribromide (7.1 g) in hexane (80 ml) was added to a solution of nerol (6.6 g) in hexane (80 ml) at 0°C. After stirring for 1 hour at the same temperature, the reaction mixture was diluted with water and extracted with hexane. The extract was successively washed with a saturated NaHCO₃ solution and brine. Evaporation of the solvent gave 8.0 g of oily neryl bromide. A solution of 7-hydroxyxycoumarin (umbelliferone, 6.9 g) in DMF (70 ml) was added to a mixture of NaH (60%, 1.8 g) and dimethylformamide (DMF, 70 ml) under a nitrogen stream, before a solution of neryl bromide (8.0 g) in DMF (70 ml) was added. The mixture was stirred at room temperature for 6 hours, diluted with water and then extracted with dichloromethane. The extract was washed with water and dried over MgSO₄. Evaporation of the solvent yielded crude 7-nerylxylocoumarin (9.0 g), which was purified by column chromatography on silica gel with a solvent system of hexane-acetone (8:2, v/v) to afford 7-nerylxylocoumarin (NOC, 6.7 g) as an oil. UV λmax (EtOH) nm (log ε): 221, 323 (4.0); IR νmax (Film) cm⁻¹: 1730, 1610, 1275, 1230, 1120, 1000, 830. 1H-NMR (CDCl₃) δ: 1.61 (3H, s), 1.69 (3H, s), 1.82 (3H, s), 2.15 (4H, m), 4.56 (2H, d, J = 7.6 Hz), 5.12 (1H, m), 5.49 (1H, t, J = 5.5 Hz), 6.25 (1H, d, J = 9.5 Hz), 6.82 (1H, d, J = 2.5 Hz), 6.85 (1H, dd, J = 2.5, 8.5 Hz), 7.36 (1H, d, J = 8.5 Hz), 7.64 (1H, d, J = 9.5 Hz). MS m/z (%): 298 (M⁺, 17), 279 (4), 269 (4), 253 (5), 241 (4), 229 (4), 213 (29), 201 (6), 163 (90), 136 (100), 121 (20), 93 (56), 81 (86), 69 (65).

4-Methyl-7-geranylocoumarin (2, GMC). This title compound was prepared by a similar method to that for NOC. mp 54-57°C. UV λmax...
4-Methyl-7-neryl oxyloxy coumarin (4, NMC). This title compound was prepared by a similar method to that for EP-GMC. mp 70-72°C. UV λ_{max} (EtOH) nm (logs): 217 (4.3), 320 (4.2). IR ν_{max} (Nujol) cm\(^{-1}\): 1725, 1615, 1265, 1200, 1145, 1075, 850. \(^1\)H-NMR (CDCl\(_3\)): \(\delta\): 1.61 (3H, s), 1.76 (3H, s), 2.15 (4H, m), 2.40 (3H, s), 4.56 (2H, d, J = 6.8 Hz), 5.12 (1H, t, J = 7.3 Hz), 5.50 (1H, t, J = 6.8 Hz), 5.34 (1H, s), 6.82 (1H, d, J = 2.4 Hz), 6.86 (1H, dd, J = 2.4, 9.0 Hz), 7.49 (1H, d, J = 8.8 Hz). MS m/z (%): 312 (M\(^+\), 26), 243 (8), 227 (6), 201 (16), 177 (100), 148 (69), 121 (20), 95 (48), 81 (78), 67 (51), 54 (4), 41 (20), 27 (20).

4-Methyl-7,2',3',6',7'-diep oxy-3',7'-dimethyloctan oxy coumarin (7, NMC diepoxide). This title compound was prepared by a similar method to that for EP-GMC. mp 70-72°C. UV λ_{max} (EtOH) nm (logs): 217 (4.3), 320 (4.2). IR ν_{max} (Nujol) cm\(^{-1}\): 1725, 1615, 1265, 1200, 1140, 1070, 850. \(^1\)H-NMR (CDCl\(_3\)): \(\delta\): 1.28 (3H, s), 1.31 (3H, s), 1.40 (3H, s), 1.72 (4H, m), 2.40 (3H, s), 2.73 (1H, dd, J = 3.7, 4.3 Hz), 3.19 (1H, t, J = 5.3 Hz), 4.14 (1H, d, J = 2.7 Hz), 4.26 (1H, dd, J = 4.3, 11.0 Hz), 6.15 (1H, s), 6.85 (1H, d, J = 3.7 Hz), 6.91 (1H, dd, J = 2.4, 8.5 Hz), 7.51 (1H, d, J = 9.2 Hz). MS m/z (%): 344 (M\(^+\), 42), 245 (14), 219 (9), 203 (4), 189 (17), 176 (61), 161 (13), 148 (44), 125 (74), 111 (38), 93 (49), 71 (94), 58 (11), 44 (100), 27 (20).

4-Methyl-7-citronel loxyloxy coumarin (5, CMC). This title compound was prepared by a similar method to that for NOC as an oil. UV λ_{max} (EtOH) nm (logs): 219, 321 (3.9). IR ν_{max} (Film) cm\(^{-1}\): 1720, 1615, 1390, 1280, 1200, 1150, 1075, 850. \(^1\)H-NMR (CDCl\(_3\)): \(\delta\): 0.97 (3H, d, J = 6.5 Hz), 1.61 (3H, s), 1.69 (3H, s), 1.10-2.10 (7H, m), 2.40 (3H, s), 4.05 (2H, m), 5.11 (1H, t, J = 7.5 Hz), 6.13 (1H, s), 6.81 (1H, d, J = 2.0 Hz), 6.85 (1H, dd, J = 2.5, 8.5 Hz), 7.49 (1H, d, J = 8.5 Hz). MS m/z (%): 314 (M\(^+\), 84), 259 (4), 245 (7), 229 (8), 203 (7), 189 (9), 176 (100), 162 (4), 148 (12), 123 (5), 109 (6), 83 (25), 69 (15).

4-Methyl-7,6'-epoxygena lloxy coumarin (6, EP-GMC). A solution of m-chloroperbenzoic acid (80%, 2.6 g) in ethyl ether (50 ml) was added to a solution of GMC (3.12 g) in ethyl ether (50 ml) at room temperature. The mixture was stirred for 15 hours, and then successively washed with a NaHCO\(_3\) solution and brine. Evaporation of the solvent gave an oil (3.2 g), which was chromatographed on silica gel by a solvent system of hexane-acetone (7:3, v/v) to afford 2.9 g of (±)-4-methyl-7,6'-epoxygenallyloxy coumarin (EP-GMC) as an oil. UV λ_{max} (EtOH) nm (logs): 211, 322 (4.0). IR ν_{max} (Nujol) cm\(^{-1}\): 1730, 1610, 1265, 1200, 1150, 1075, 850. \(^1\)H-NMR (CDCl\(_3\)): \(\delta\): 1.28 (3H, s), 1.30 (3H, s), 1.70 (2H, m), 1.79 (3H, s), 2.25 (2H, m), 2.40 (3H, s), 2.72 (1H, t, J = 6.2 Hz), 4.61 (2H, d, J = 6.6 Hz), 5.53 (1H, t, J = 6.6 Hz), 6.13 (1H, s), 6.82 (1H, d, J = 2.4 Hz), 6.87 (1H, dd, J = 2.7, 9.0 Hz), 7.49 (1H, d, J = 7.5 Hz). MS m/z (%): 328 (M\(^+\), 13), 227 (6), 201 (11), 176 (44), 153 (34), 135 (14), 109 (11), 95 (9), 81 (100), 67 (49), 44 (84), 28 (100).

Spasmolytic activity. Similar procedures to those previously reported were used to measure the spasmodic activity. Each coumarin dissolved in dimethyl sulfoxide was diluted in a Tyrode solution at a final concentration of 3 x 10^{-4} mol/liter, and then applied to the tube before adding BaCl\(_2\), acetylcholine, and histamine in a Tyrode solution at a final concentration of 3 x 10^{-4} mol/liter, respectively. The percentage reduction in the contraction induced by each coumarin is regarded as the percentage inhibition.

References