INHIBITORS OF PROSTAGLANDIN BIOSYNTHESIS FROM DALBERGIA ODORIFERA

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From the heartwood of Dalbergia odorifera T. Chen, a Chinese medicinal drug (Japanese name "Koshinko") used for the stagnation of disordered blood, five new phenolic compounds, isoumeconustyrene (2) and hydroxybutustyrene (3) (cinnamylphenol), (†)-isoduvartin (6) (isoflavan), odoriflavene (7) (isoflav-3-ene) and (-)-odoricarpan (15) (pterocarpan) were isolated and their structures were elucidated on the basis of chemical and spectroscopic methods. Of the fifteen compounds isolated, the cinnamylphenols, isoflavans, isoflav-3-ene and a benzoic acid derivative inhibited prostaglandin biosynthesis more than the pterocarpan and isoflavone compounds.

KEYWORDS—Dalbergia odorifera; Leguminosae; stagnant blood; cinnamylphenol; isoflavonoid; isoflavan; isoflav-3-ene; pterocarpan; prostaglandin biosynthesis inhibitor; platelet aggregation inhibitor

In the course of our screening works to find biologically active principles contained in medical drugs used in traditional medicines, the hot aqueous extract of the heartwood of Dalbergia odorifera T. Chen (Japanese name "Koshinko"), which has been used as a Chinese medical drug to treat the stagnation of disordered blood, was found to inhibit prostaglandin (PG) biosynthesis (98% inhibition at 0.75 mg/ml). It has been recognized that the balance between thromboxane A2 and PG I2 has a very important role in the regulation of blood flow. The isolation of inhibitors of PG biosynthesis from this medical plant provides a substantial support for its usage.

The heartwood of D. odorifera purchased in the Taipei market was extracted with methanol and the methanol extract was fractionated into n-hexane-, chloroform- and water-soluble fractions by the usual procedure. A benzene-soluble fraction obtained from the chloroform-soluble fraction had the most potent inhibitory effect to PG biosynthesis.1) Separation and purification by repeated column chromatography, along with testing the inhibitory effects, gave obtustyrene (1), isoumeconustyrene (2), hydroxybutustyrene (3), (†)-mucronulatol (4), (†)-vestitol (5), (†)-isoduvartin (6), odoriflavene (7) and methyl 2-hydroxy-3,4-dimethoxybenzoate (8), as active principles. In the course of separation of the
active compounds, this fraction also afforded formononetin (9), (+)-duartin (10),
(±)-medicarpin (11), (-)-methylpissoinol (12), (-)-mellitocarpan C (13), (-)-
mellitocarpan D (14) and odoricarpan (15), which had no significant effects. Of
the compounds isolated 2, 3, 6, 7 and 15 are newly identified natural products.

Isomucronustyrone (2), a colourless oil, and hydroxyotustyrene (3),
colourless plates mp 76-76.5°C (n-hexane/ether), showed IR, MS and 1H-NMR spectra
of typical cinnamylphenols2 and were finally identified as E-1-(3-hydroxy-2,4-
dimethoxybenzyl)-2-phenylethylene and E-1-(3,4-dihydroxy-2-methoxybenzyl)-2-
phenylethylene by direct comparison with synthetic samples which had been prepared
from corresponding phenols and trans-cinnamyliccohol according to the method of
Mageswaran et al.3

(±)-Isoduartin (6, 4) a colourless oil, gave the spectral data very similar
to those of duartin (10, 5) a 7,8,2',3',4'-substituted isoflavan, except for the
chemical shift values of 5'-H (δ 6.44) and 6'-H (δ 6.79) signals in the 1H-NMR
spectrum. The MS spectrum and nuclear Overhauser effect (NOE) experiments showed
that isoduartin (6) had 7-hydroxyl and 8,4'-dimethoxyl groups. It was suggested
that the difference in the structures of duartin (10) and isoduartin (6) was the
substitution at C-2' and -3'. Isoduartin (6) was therefore identified as 7,2'-
dihydroxy-8,3',4'-dimethoxyl isoflavan.

The spectral data of odoriflavene (7, 6) colourless prisms mp 177.5-179°C
(n-hexane/AcOEt), showed that it had a 7-hydroxyisoflav-3-ene structure with an
other hydroxyl and two methoxyl groups at C-2', -3' and -4'. A compound
obtained by the catalytic reduction of odoriflavene (7) gave the same 1H-NMR and
MS spectra as those of (-)-isomucronulatol which was prepared by the catalytic
reduction of the corresponding pterocarpan, (−)-methylnissolin (12). Therefore, the structure of odoriflavene (7) was determined to be 7,2′-dihydroxy-3′,4′-dimethoxysiroflav-3-ene.

(−)-Odoricarpan (15), a colourless amorphous solid, gave spectral data very similar to those of mellilotocarpan C (13), a monohydroxy-trimethoxy 3,4,9,10-substituted pterocarpan, except for chemical shift values of 1-H (δ 7.34 in CDCl3) and 7-H (δ 6.77) signals in 1H-NMR. Two large solvent-induced methoxy-proton shifts in the 1H-NMR spectra measured in CDCl3 and d6-benzene and a positive Gibbs’ test indicated the presence of methoxyl groups at C-3 and -9. These data suggested that the difference in the structures of mellilotocarpan C (13) and odoricarpan (15) was the substituents at C-4 and -10. Odoricarpan (15) was therefore identified as 10-hydroxy-3,4,9-trimethoxy-pterocarpan. The comparison of an ORD curve with that of (−)-mellilotocarpan C (13) shows that (−)-odoricarpan (15) has the 6αR, 11αR-configuration.

Obstustyrene (1), (±)-vestitol (5), formomonetin (9), (±)-medicarpin (11), (−)-mellilotocarpan C (13) and (−)-mellilotocarpan D (14) were identified by direct comparison with authentic samples. (±)-Mucronulatol (9), (±)-duartin (10)5 and (−)-methylnissolin (12)13 were identified by comparing of their spectral data with those reported. Methyl 2-hydroxy-3,4-dimethoxybenzoate (8) were identified by its spectral data.14

IC50 (50% inhibitory concentration) values of 1–8 against PG synthetase were 7.7, 2.8, 9.2, 63, 47, 110, 4.8 and 23μM, respectively. The cinnamylphenols (1)-(3) and odoriflavene (7) inhibited PG synthetase as strongly as indomethacin which is a well-known potent inhibitor with an IC50 value of 4.9μM under the same assay condition. Inhibition of rabbit platelet aggregation induced by ADP, arachidonic acid and collagen were assayed for 2-5, 8-9 and 11-12,15 which were obtained in relatively high yields among the isolated compounds. 2, 3 and 8 were potent

Table I. Inhibitory Effects of ADP-, Arachidonic Acid-, and Collagen-Induced Platelet Aggregation

<table>
<thead>
<tr>
<th>Inducer</th>
<th>ADP (10μM)</th>
<th>Arachidonic acid (128μM)</th>
<th>Collagen (20μg/ml)</th>
<th>IC 50 values against PG-ase* (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>Adenosine (3.75μM)</td>
<td>Aspirin (11μM)</td>
<td>Aspirin (111μM)</td>
<td></td>
</tr>
<tr>
<td>Isomucronustyrene (2)</td>
<td>&gt;1000</td>
<td>52</td>
<td>370</td>
<td>2.8</td>
</tr>
<tr>
<td>Hydroxyobstustyrene (3)</td>
<td>&gt;1000</td>
<td>7.7</td>
<td>20</td>
<td>9.2</td>
</tr>
<tr>
<td>(±)-Mucronulatol (4)</td>
<td>&gt;1000</td>
<td>130</td>
<td>&gt;1000</td>
<td>63</td>
</tr>
<tr>
<td>(±)-Vestitol (5)</td>
<td>1000</td>
<td>92</td>
<td>1000</td>
<td>47</td>
</tr>
<tr>
<td>Methyl 2-hydroxy-3,4-dimethoxybenzoate (8)</td>
<td>&gt;1000</td>
<td>3.5</td>
<td>47</td>
<td>23</td>
</tr>
<tr>
<td>Formomonetin (9)</td>
<td>&gt;1000</td>
<td>190</td>
<td>&gt;1000</td>
<td>&gt;500</td>
</tr>
<tr>
<td>(±)-Medicarpin (11)</td>
<td>&gt;1000</td>
<td>370</td>
<td>&gt;1000</td>
<td>&gt;500</td>
</tr>
<tr>
<td>(−)-Methylnissolin (12)</td>
<td>&gt;1000</td>
<td>170</td>
<td>&gt;1000</td>
<td>&gt;500</td>
</tr>
</tbody>
</table>

Each figure indicates the concentration (μM) of test material which gave the same degree of inhibitory effect as positive control. * Prostaglandin synthetase.
inhibitors of PG synthetase and strongly inhibited platelet aggregation induced by arachidonic acid and collagen (Table I). Excessive platelet aggregation is considered to cause conditions of the blood hindrance. Therefore the inhibitors of PG biosynthesis isolated from D. odorifera seem to represent effective principles against the stagnation of disordered blood.

REFERENCES AND NOTES


2) C17H18O2 (M+: m/z 270.1285, Calcd: 270.1255). IR (Cap): 3510, 1615, 1598, 1492cm⁻¹. MS m/z (rel. int.%): 270(M⁺, 100), 255(16), 239(24), 167(13), 115(35), 91(33). ¹H-NMR (CDCl₃, δ ppm): 3.48(2H, m), 3.85, 3.88(each 3H, s), 5.52(1H, s), 6.32(2H, m), 6.55(1H, d, J=8.0Hz), 6.64(1H, d, J=8.0Hz), 7.04-7.30(5H, m).

3) C16H16O3 (M+: m/z 256.1137, Calcd: 256.1100). IR (KBr): 3400, 1600, 1509, 1486cm⁻¹. MS m/z (rel. int.%): 256(M⁺, 100), 225(15), 152(78), 115(41), 91(49). ¹H-NMR (d₆-benzene, δ ppm): 3.31(3H, s), 3.37(2H, d, J=5.4Hz), 4.91, 4.97(each 1H, s), 6.29(2H, m), 6.54(1H, d, J=8.4Hz), 6.69(1H, d, J=8.4Hz), 6.93-7.30(5H, m).


4) C17H16O4 (M+: m/z 332.1258, Calcd: 332.1259). MS m/z (rel. int.%): 332(M⁺, 63), 180(100), 169(42), 157(79), 165(32), 164(24), 153(31), 133(45). ¹H-NMR (CDCl₃, δ ppm): 2.90-3.05(2H, m), 3.30-3.75(1H, m), 3.85, 3.91, 3.92(each 3H, s), 4.09(1H, t, J=9.9Hz), 4.47(1H, m), 5.64, 6.00(each 1H, s), 6.44(1H, d, J=8.6Hz), 6.50(1H, d, J=8.4Hz), 6.74(1H, d, J=9.4Hz), 6.79(1H, d, J=8.6Hz).


6) C17H16O5 (M+: m/z 330.1008, Calcd: 300.0998). IR (KBr): 3245, 1610, 1495, 1460, 1430cm⁻¹. MS m/z (rel. int.%): 300(M⁺, 100), 299(29), 285(27), 180(20), 143(17). ¹H-NMR (d₆-acetone, δ ppm): 3.80(3H, s), 3.86(3H, s), 5.02(2H, d, J=1.3Hz), 6.32(1H, d, J=2.4Hz), 6.41(1H, d, J=2.4Hz, 7.9Hz), 6.57(1H, d, J=8.8Hz), 6.69(1H, br s), 6.95(1H, d, J=7.9Hz), 7.00(1H, d, J=8.8Hz), 8.10, 8.45(each 1H, s).

7) C18H18O6 (M+: m/z 330.1087, Calcd: 300.1102). [a]D -13° (c=0.245, CHCl₃). MS m/z (rel. int.%): 330(M⁺, 100), 315(67). ¹H-NMR (CDCl₃, δ ppm): 3.40-3.95(2H, m), 3.86, 3.88, 3.89(each 3H, s), 4.15-4.45(1H, m), 5.32(1H, s), 5.52(1H, d, J=6.0Hz), 6.48(1H, d, J=8.1Hz), 6.67(1H, d, J=8.7Hz), 6.77(1H, d, J=8.1Hz), 7.34(1H, d, J=8.7Hz). ¹H-NMR (d₆-benzene, δ ppm): 2.85-4.05(3H, m), 3.16, 3.33(each 3H, s), 3.83(3H, s), 5.11(1H, s), 5.27(1H, d, J=6.5Hz), 6.08(1H, d, J=7.7Hz), 6.33(1H, d, J=6.7Hz), 6.35(1H, d, J=8.7Hz), 6.35(1H, d, J=7.7Hz). ORD (c=0.018, dioxane, [a]D 232 (nm): -890°(295), -440°(286), -2110°(280), -4890°(249), -5330°(234), -7560°(222).


10) Authors are grateful to Dr. K. Takahashi (Meiji College of Pharmacy) for measurements of ORD spectra.

11) It was synthesized by the same method as 2. It was obtained by the reduction of 11, 9 and 11 were kindly supplied by Dr. T. Kinosita (University of Tokyo), 13 and 14 were kindly supplied by Dr. S. Fukushima (Shizuoka College of Pharmacy).


14) Colorless needles (L-BuOH), mp 80-87°C, IR (KBr): 3300, 1710, 1605, 1495, 1430, 1290, 1215cm⁻¹. ¹H-NMR (CDCl₃, δ ppm): 3.90(3H, s), 3.94(6H, s), 5.73(1H, s), 6.69(1H, d, J=9.0Hz), 7.46(1H, d, J=9.0Hz). MS m/z (rel. int.%): 212(M⁺, 26), 181(43), 180(31), 151(72), 137(100).

15) Details of assay methods will be reported elsewhere.

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