Examination of Coumarins, Flavonoids and Polysaccharopeptide for Antibacterial Activity

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ABSTRACT. 1. Coumarins, flavonoids and polysaccharopeptide were tested for antibacterial activity. 2. The bacteria used for this study included clinical isolates of Staphylococcus aureus, Shigella flexneri, Salmonella typhi, Escherichia coli and Pseudomonas aeruginosa. 3. Most of the coumarins tested failed to inhibit the bacteria at 25 mg/l. Edulgin at 128 mg/l inhibited 4 of the 8 P. aeruginosa strains and 1 of the S. aureus strains tested. O-acetylcolumbianetin and imperatorin did not inhibit any isolate, even at 128 mg/l. 4. When tested at the dose of 128 mg/l, the flavonoids (rutin, naringin and baicalin) inhibited 25% or less of P. aeruginosa and only baicalin was active against S. aureus. 5. Arbutin and 4-(β-D-glucopyranosylxoyl)-benzaldehyde inhibited 3 of the 8 P. aeruginosa strains when tested at 128 mg/l. 6. Polysaccharopeptide from the fungus Coriolus versicolor failed to inhibit any P. aeruginosa or S. aureus strain at 128 mg/l.

KEY WORDS. Coumarins, flavonoids, polysaccharopeptide, antibacterial activity

INTRODUCTION
Flavonoids consist of a variety of structures including flavone, flavone glycoside, flavonol, flavonol glycoside, flavone, flavonol, isoflavone, flavan-3-ol and flavlyum salt (Shibata and Saitoh, 1978). They possess a number of activities, including antioxidant and vitamin C-sparing activity (Middleton and Kandaswami, 1992). In addition, they inhibit histamine release (Sankawa and Chun, 1985) and the activities of 5-lipoxygenase (Yoshimoto et al., 1983), cyclo-oxygenase (Yoshimoto et al., 1983), protein kinase C (Ferruola et al., 1989) and tyrosine kinase (Graziani et al., 1983; Akiyama et al., 1987). Two anti-leukemic flavonoids, teicin and kaempferol-3-O-β-D-glucopyranoside, have been isolated from Wikstroemia indica (Lee et al., 1981). The fruit extract of Cnataea oxyantha, which contained flavonoids, was used as a mild vasodilator for the treatment of heart disease (Ammon and Handel, 1981a,b,c; Trnntzler and Schuler, 1962). The leaf extract of Cinchba bidenta, containing flavonoids, was used for improving blood circulation in brain varix and hemorrhoids (Tronnier, 1968). A number of isoflavones, with puerarin (B-glucosyldaidzein), daidzin (7-O-glucosyldaidzein) and daidzen as the main principles, were used for improving blood circulation (Tseng et al., 1974).

Five furanocoumarins, including imperatorin, isoorientin, bergapten, oxypeucedanin and isooxypeucedanin, have been isolated from Angelica koreana. They altered the hexobarbital-induced sleeping time with potencies comparable to that of SKF-525A, a widely used model drug-metabolizing-enzyme inhibitor. They were able to affect the serum hexobarbital concentration in mice in a way similar to that of SKF-525A (Woo, 1989). Some furanocoumarins exerted cytotoxic action (e.g. daphnoretin isolated from Wikstroemia indica significantly inhibited the growth of Ehrlich ascites tumor in mice, although the growth of the lymphocytic leukemia cell line P388 was not affected) (Lee et al., 1981). Li et al. (1989) isolated coumarins, including columbianetin, columbianetin acetate, columbianadin, osthol, isoorientin, bergapten, xanthotoxin and columbianetin-B-D-glucopyranoside, from Angelica pubescens Maxim. F. biserrata (Umbelliferae) and found that the first four and the last one potently inhibited ADP-induced platelet aggregation. Saqib et al. (1990) obtained from Zanthoxylum americanum berries five furanocoumarins, including criukidin, imperatorin, protoralen and xanthotoxin, the last 2 of which were toxic to brine shrimp larvae and human lung, breast and colon cancer cell lines. The polysaccharopeptide from the fungus Coriolus versicolor possesses immunomodulatory (Liu et al., 1993; Shi et al., 1993; Xu et al., 1993a; Yang et al., 1993) and antitumor (Xu et al., 1993b; Yang et al., 1993) activities. It has been used as an immunomodulatory and antitumor drug for cancer patients (Liao and Zhao, 1993; Shi et al., 1993). In addition, it exhibits hepatoprotective activity (Chiu et al., 1993).

In view of the diverse activities of flavonoids, coumarins and polysaccharopeptide, and the reports that these compounds may have antibacterial activity (Honda et al., 1984; Jiang and Xiao, 1986; Xu and Si, 1987; Xu, 1989), they were investigated for antibacterial activity in this study. The coumarins examined in the present study included O-acetylcolumbianetin, edulgin, enforin A, columbianadin and imperatorin. The flavonoids investigated comprised rutin, naringin and baicalin. Arbutin and 4-(β-D-glucopyranosylxyloxy)-benzaldehyde were also studied.

MATERIALS AND METHODS
Isolation of coumarins
The fruits of Cnidium monnieri (L.) Cuss. were collected from Xinmin County, the Province of Liaoning, China. They were extracted 3 times with 95% ethanol, each extraction lasting for 4 hr. The ethanolic extract was then defatted with petroleum ether and chromatographed on a column of silica gel. The column was eluted with...
a petroleum ether-ethyl acetate gradient. The fraction eluted by petroleum ether-ethyl acetate (V:V, 9:1) was subjected to preparative thin layer chromatography on silica gel to yield (O-acetylcolumbianetin (coumarin CC1), edulxin (coumarin CC2), conforin A (coumarin CC3), columbianadin (coumarin CC4) and imperatorin (coumarin CC5). The structures of the various coumarins were elucidated using spectroscopic methods. All except the linear coumarin imperatorin are angular coumarins.

**Isolation of arbutin**

The roots of Gerbera piloselloides Cass, collected from Yunnan, China, were extracted with 95% ethanol. The ethanolic extract was chromatographed on a silica gel column which was eluted with a gradient of petroleum ether-chloroform-methanol. Arbutin was recrystallized from the chloroform-methanol (v:v, 8:2) eluate of the column and its structure was elucidated spectroscopically.

**Isolation of baikalin**

The roots of Scutellaria baicalensis Georgi, collected from Gansu, China, were cut into thin slices and decocted twice with water (30 min per decoction). The pH of the combined aqueous solution was adjusted with HCL to 1–2. After standing at 80°C for 30 min, it was filtered. The precipitate was washed 2–3 times with 95% ethanol under reduced pressure and then dried at 60°C. To the ppt., 10 volumes of water were added, the pH was adjusted to 6.5–7.0 with 40% NaOH, and some active charcoal was added to the solution. After standing at 80°C for 30 min, an equal volume of ethanol was added before filtration was carried out under reduced pressure. The pH of the solution was adjusted to 1–2 by adding HCL. It was then allowed to stand at 80°C for 30 min and then at room temperature for 10 hr. The ppt. was collected by filtration and rinsed with ethanol 2–3 times under reduced pressure, dried at 60°C, and the resulting product was baikalin.

**Isolation of naringin**

The rhizomes of Drynaria fortunei (Kze.) J. Sm., collected from Gansu, China, were extracted with 60% ethanol. The ethanolic extract was concentrated and partitioned between water and butanol. The butanolic fraction was chromatographed on a polyamide column that was eluted stepwise with water containing an increasing concentration of methanol. From the eluate of water-methanol (v:v, 7:3), naringin was obtained and recrystallized.

**Isolation of rutin**

The flower buds of Sophora japonica L., collected from Hubei, China, were extracted with an ammonium bicarbonate solution in a boiling water bath. The pH of the alkaline extract was adjusted to 5 with HCl at 60–70°C and left to stand for 24 hr before it was filtered. The precipitate was recrystallized in water and crystalline rutin was obtained.

**Testing for antibacterial activity**

This was performed by an agar dilution method. Stock cultures of clinical isolates of Escherichia coli, Shigella flexneri, Salmonella typhi, Pseudomonas aeruginosa and Staphylococcus aureus were grown on nutrient agar at 37% overnight. The inoculum was prepared by emulsifying a few colonies in 1 ml normal saline so as to give a turbidity corresponding to that of a MacFarland 0.5 standard. A ten-fold dilution was made from this in normal saline. The medium used was iso-

**TABLE 1. Testing of coumarins for antibacterial activity**

<table>
<thead>
<tr>
<th>Expt</th>
<th>Bacteria</th>
<th>No. of strains tested</th>
<th>CC-2</th>
<th>CC-3</th>
<th>CC-4</th>
<th>CC-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staphylococcus aureus</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Shigella flexneri</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Salmonella typhi</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(No. of strains inhibited by 25 mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Staphylococcus aureus</td>
<td>16</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Coumarin CC-2, edulxin; CC-3, conforin A; CC-4, columbianadin; and CC-5, imperatorin.

**RESULTS**

None of the coumarins tested could inhibit the growth of Staphylococcus aureus, Shigella flexneri, Salmonella typhi, Escherichia coli or Pseudomonas aeruginosa, at 8 mg/L and 25 mg/L except for four strains of S. aureus, which were inhibited by 25 mg/L coumarin CC-3 (conforin A) (Table 1). At 128 mg/L the coumarin CC-2 (edulxin), but not CC-1 (O-acetylcolumbianetin) or CC-5 (imperatorin), exhibited slight antibacterial activity. CC-2 (edulxin) inhibited 4 of the 8 P. aeruginosa strains and 1 of the 16 S. aureus strains tested (Table 2). Of the three flavonoids examined, baikalin seemed to be the most potent in inhibiting the growth of Staphylococcus aureus: 11 of the 16 strains tested were inhibited at 128 mg/L. No inhibitory activity of rutin and naringin against S. aureus was observed at 128 mg/L. At the same concentration, naringin and baikalin inhibited 2 and rutin inhibited 1 of the 8 P. aeruginosa strains tested. The polysaccharopeptide from the fungus Coriolus versicolor did not exhibit any antibacterial activity at 128 mg/L (Table 2).

**DISCUSSION**

Honda et al. (1984) studied the antimicrobial activities of the coumarins osthol, imperatorin and isosinpinellin derived from the fruits of Cnidium species using various gram-positive bacteria, gram-negative bacteria and fungi. They showed that all three coumarins had very low activity against the gram-negative bacteria tested, including Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens and Citrobacter freundii. Osthol had the highest and imperatorin the lowest activity against the gram-positive bacteria tested, including Staphylococcus aureus, Sarcina lutea, Bacillus polymyxa and B. subtilis. Osthol also showed the highest activity against the fungal species tested: Trichophyton rubrum, T. mentagrophytes, T. tonsurans var. sulfurica, Microsporum gypseum, Sabouraudites canis and Epidermophyton floccosum. Imperatorin exhibited the lowest antifungal activity in all cases except when it was tested against T. rubrum and S. canis. In the present investigation, imperatorin showed no antibacterial activity even at 128 mg/L. This observation was in line with...
the report of Honda et al. (1984). The coumarins edulatin and ch riforin A appeared to have higher antibacterial activity than imperato rin as evidenced by their effect on Pseudomonas aeruginosa and Staphylococcus aureus, respectively.

Arbutin, baicalin and naringin have been reported to have antibacterial activity (Xu and Si, 1987; Xu, 1989). Rutin possesses anti-inflammatory and antiviral activities, but weak antibacterial activity (Jiang and Su, 1987), which was also shown in this study. In contrast, baicalin had the highest antibacterial activity and naringin, arbutin and 4-(β-D-glucopyranosyl)benzaldehyde had some antibacterial activity. The pharmacokinetics of these drugs remain to be investigated. If they can achieve a level of 128 mg/l or more in serum, there is a likelihood that they can be used clinically to treat infections.

Arbutin was reported to be isolated from the plants Arctostaphylos uva-ursi, Pyrethrum serrulatum, Saxifraga stolonifera, Sorbus sorbifolia, Pyrus calleryana, P. pyrifolia, Pyrola japonica, uva-ursi, Parhthia scandens, Saxifraga stolonifera, Saxifraga stolonifera, Pyrus calleryana, P. pyrifolia, Pyrola japonica, P. rotundifolia, Sorbaria sorbifolia, Scutellaria baicalensis, Arctostaphylos uva-ursi, and Arctostaphylos uva-ursi. The isolation of baicalin from the roots of Scutellaria baicalensis, rutin from Sophora japonica and coumarins from Cnidium monnicor and Arctostaphylos uva-ursi have been described (Honda et al., 1984; Jiang and Xiao, 1986).

We thank Doris Yeung and Mandy Chan for their expert secretarial assistance.

References


**TABLE 2. Testing of coumarins, flavonoids (rutin, naringin, baicalin) and polysaccharopeptide for antibacterial activity**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>No. of strains inhibited/No. of strains tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coumarin CC-1</td>
<td>32 mg/l</td>
<td>0/8</td>
</tr>
<tr>
<td>Coumarin CC-2</td>
<td>32 mg/l</td>
<td>0/8</td>
</tr>
<tr>
<td>Coumarin CC-5</td>
<td>128 mg/l</td>
<td>4/8</td>
</tr>
<tr>
<td>Rutin</td>
<td>32 mg/l</td>
<td>0/8</td>
</tr>
<tr>
<td>Naringin</td>
<td>32 mg/l</td>
<td>0/8</td>
</tr>
<tr>
<td>Baicalin</td>
<td>128 mg/l</td>
<td>2/8</td>
</tr>
<tr>
<td>Arbutin</td>
<td>32 mg/l</td>
<td>0/8</td>
</tr>
<tr>
<td>4-(β-D-glucopyranosyl)-benzaldehyde</td>
<td>32 mg/l</td>
<td>0/8</td>
</tr>
<tr>
<td>Polysaccharopeptide</td>
<td>32 mg/l</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>128 mg/l</td>
<td>0/8</td>
</tr>
</tbody>
</table>

**TABLE 2.** Testing of coumarins, flavonoids (rutin, naringin, baicalin) and polysaccharopeptide for antibacterial activity.


