Introduction

Tropical plants have been proven to be useful sources of new anticancer agents. As part of a project program to discover new anticancer agents from plants and other organisms,1 an initial crude methanol-soluble extract of the stems of *Crauroxyllum cochinchinense*, collected in Vietnam, exhibited cytotoxicity toward human colon cancer (HT-29) cell line, and was selected as a target plant for further investigation. Previously detailed phytochemical studies of *C. cochinchinense* have resulted in the isolation of several xanthones,1-3 including some caged xanthones1,2 and -mangostin.4,5 The latter compound has shown cytotoxicity toward the human lung cancer (NCI-H1697) and human colon cancer (HT-29) cell lines,1,4,5 and acts in part by inducing caspase-3 dependent apoptosis.5 Using column chromatography, a new caged xanthone, a new prenylxanthone, and six known xanthones were isolated from the stems of *Crauroxyllum cochinchinense*. In addition, five new and seven known prenylated xanthone derivatives were synthesized from -mangostin and -cochinchinone A. Several of these substances were found to be cytotoxic toward HT-29 cell line, with the most potent being 3,6-di-O-acetylmangostin (8, IC50: 1.0 μM). Some compounds showed promising activities in NF-κB inhibitory and mitochondrial transmembrane potential (MTP) assays. This study describes the constituents of a tropical rainforest tree, *C. cochinchinense*, with both in vitro and in vivo activities germane to cancer.

Structures of Compounds from *C. cochinchinense*

The structures of the compounds isolated or produced by semi-synthesis were determined by analysis of their UV, IR, NMR, and MS spectra. The absolute configurations of new compounds, 1 and 2, were proposed from their detailed NOESY NMR and CD spectra.

Experimental Section

General Experimental Procedures. Specific rotation values were measured on a Perkin-Elmer 343 polarimeter. UV spectra were recorded on a UV-Hitachi U2910 spectrophotometer. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer. The CD spectra were performed using a JASCO J-810 spectropolarimeter. NMR data were recorded on a Bruker Avance DPX-300 or DRX-400 MHz spectrometer with TMS as internal standard. ESI-MS and HRESIMS were recorded on a LCT-TOF mass spectrometer.

Plant Material. A sample of the stems of *Crauroxyllum cochinchinense* was collected at Hon Ba Nature Reserve, Vietnam. The voucher herbarium specimen was identified as *Crauroxyllum cochinchinense* (Lou, Bl.). (Clust.) and has been deposited by John G. Searle Herbarum of the Field Museum of Natural History, Chicago, under accession number FM 2257439.

Extraction and isolation. The mixed stems of *Crauroxyllum cochinchinense* were extracted with MeOH at room temperature. The solvent was evaporated in vacuo. The dried MeOH extract was diluted with n-hexane and then extracted with CHCl3 and separated by repeated silica gel column chromatography, affording compounds 1-6, 9, and 19. Compounds 7, 8, 10-18, and 20 were semi-synthesized from -mangostin (3) and -cochinchinone A (8).

Cytotoxicity Assay. Cytotoxicity of the samples was screened against human colon cancer (HT-29) cell line by a previously reported procedure, with paclitaxel as the positive control.

Enzyme-Linked Immunosorbent Assay. A NF-κB inhibition assay was carried out with an E-Z-Detect Transcription Factor Assay System ELISA kit (Pierce Biotechnology, Rockford, IL), and rosiglitazone was used as a positive control.

MTP Assay. Changes in mitochondrial transmembrane potential were detected and quantified by a fluorescence cell-based assay, which was carried out from HT-29 cells, and staurosporine was used as a positive control.

In Vivo Hollow Fiber Assay. The in vivo hollow fiber assay was conducted as described previously.

Bioactivities of Compounds from *C. cochinchinense*

Compounds 1-6, 9, and 19 were isolated from complex 3, while compounds 7, 8, 10-18, and 20 were semi-synthesized from -mangostin (3) and -cochinchinone A (8), which were both isolated in relatively large quantities. All obtained compounds, 1-20, were screened in a cytotoxicity assay, and some compounds were tested in NF-κB inhibitory and mitochondrial transmembrane potential (MTP) mechanism assays. The bioactivities of active compounds are shown in Table 1, and all other compounds were inactive in these assay systems.

Table 1. Bioactivities of compounds obtained from *C. cochinchinense*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Cytotoxicity*</th>
<th>NF-κB inhibition p&lt;0.05</th>
<th>MTP**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.8</td>
<td>&gt;10</td>
<td>5.3</td>
</tr>
<tr>
<td>2</td>
<td>4.1</td>
<td>&gt;10</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>5</td>
<td>&gt;10</td>
<td>2.9</td>
<td>NT</td>
</tr>
<tr>
<td>7</td>
<td>8.8</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>&gt;10</td>
<td>1.8</td>
</tr>
<tr>
<td>10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>0.9</td>
</tr>
<tr>
<td>11</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>1.4</td>
</tr>
<tr>
<td>12</td>
<td>1.9</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>13</td>
<td>4.4</td>
<td>NT</td>
<td>&gt;10</td>
</tr>
<tr>
<td>14</td>
<td>4.4</td>
<td>NT</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>0.0001</td>
<td>0.075</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Positive control for cytotoxicity. **Positive control for NF-κB inhibition. MTP values expressed in μM. Measured at 48-h.

Inspection of Figure 1 and Table 1 shows that the cytotoxic compound -mangostin (8) possesses the highest potency in the MTP assay, 3,6-Di-acetylation (8) and 6-benzoyl (12) of -mangostin both increased its cytotoxicity toward HT-29 cells, and cyclization between the C-2 and C-3 positions retained such effect. However, both methylation and cyclization between the C-1 and C-2 positions reduced potency. Our preliminary SAR studies for -cochinchinone A (6) showed that acetylation, methylation, and benzoylation all decreased cytotoxicity toward HT-29 cells of this compound.

The most potently cytotoxic 3,6-Di-O-acetylmangostin (8), was tested further in an in vivo hollow fiber assay, but found to be inactive at the highest dose used (20 mg/kg).

References


Acknowledgment

This investigation was supported by grant P01 CA125586, funded by the National Cancer Institute, NIH, Bethesda, MD.