Synthesis and Biological Evaluation of Cytogenin Derivatives

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To enhance the stability in vivo, new derivatives of cytogenin were synthesized, and their biological activity and stability in mice were estimated. 2-(8-Hydroxy-6-methoxy-1-oxo-1H-2-benzopyran-3-yl)propionic acid (NM-3) was found to be the most stable among them. It modified collagen-induced arthritis in mice. It also showed potent anti-angiogenic activity in a mouse dorsal air sac assay.

Cytogenin 1 (Fig. 1) was first found as an immunomodulative antitumor substance produced by Streptoversicillum eurocidium1). Later, it was found that cytogenin had an anti-inflammatory activity against collagen-induced arthritic model in mice2,3). However, cytogenin readily transformed to the less active sulfate or glucuronide, or was oxidized to the 3-carboxylic acid (MC-1) in the biological assessment in mice. These metabolic conversions seemed to reduce efficacy of cytogenin. So we prepared O-acyl and O-alkyl derivatives of cytogenin to prevent glucuronide or sulfate conjugation, and the 3-side chain modified analogues to avoid oxidation. Among these derivatives, NM-3 (2a in Fig. 1) showed the best pharmacological properties. In this paper, we report the syntheses, stability in vivo, and the pharmacological activities of the cytogenin derivatives.

Materials and Methods

O-Acyl Derivatives of Cytogenin

As shown in Scheme 1, O-acyl derivatives 4-11 were prepared simply by acylation with acylating agent in the presence of base (method A), or by nucleophilic displacement of the chloride 3, which was easily prepared by chlorination of cytogenin with PPh3-CCl3, with the corresponding nucleophilic reagent (method B).

Fig. 1. Structures of cytogenin and NM-3.
Scheme 1.  O-Acyl derivatives of cytogenin.

**method A**

\[
\begin{array}{cccc}
\text{Compound} & R^1 & R^2 & \text{acylating reagent, base} & \text{yield (%)} \\
4 & H & Ac & \text{Ac}_2\text{O (1.2 eq), pyridine} & 51 \\
5 & Ac & Ac & \text{Ac}_2\text{O (12 eq), pyridine} & 53 \\
6 & H & \text{CO(CH}_2\text{)}_2\text{CO}_2\text{H} & \text{succinic anhydride, none} & 74 \\
7 & H & \text{CO(CH}_2\text{)}_3\text{CO}_2\text{H} & \text{glutamic anhydride, none} & 77 \\
8 & H & \text{COCH}_2\text{Ph} & \text{phenacyl chloride, pyridine} & 74 \\
\end{array}
\]

In the method A, acylation generally occurs at the 9-position first, and acylation at the 8-position was slow because of the hydrogen bond with the 1-carbonyl group. In the method B, after the nucleophilic displacement, the Boc group was deprotected by TFA, if necessary.

**O-Alkyl Derivatives of Cytogenin**

O-Alkyl derivatives 12, 13, 14 and 16 were prepared as shown in Scheme 2. Treatment of cytogenin with excess methyl iodide and sodium hydride gave the 6,8,9-O-trimethyl derivative 12. Compound 13 was obtained after the selective demethylation of 12 at 8-position by boron trichloride in dichloromethane. Treatment of cytogenin with \(t\)-butylbromoacetate and \(K_2\text{CO}_3\) in dimethylformamide gave selective O-alkylation at the 8-position. Removal of the \(t\)-butyl group by TFA gave the 8-O-carboxymethyl ether 14.

On the other hand, the 9-O-carboxymethyl derivative 16 was prepared as follows. The intermediate, 8-monomethoxymethyl cytogenin 15, was prepared successively by methoxymethylation of 3, nucleophilic displacement of a 9-chlorine atom with sodium formate, and hydrolysis of the formate. After alkylation of 15 with \(t\)-butylbromoacetate, the protective groups was removed, affording the 9-O-carboxymethyl ether 16.

**Oxidized Derivative of Cytogenin (MC-1)**

Oxidation of cytogenin with Jones' reagent provided the 3-carboxyisocoumarine, MC-1, in 62% yield (Scheme 2), which was identical with one of the metabolites of cytogenin in mice.

**Modification of the Side Chain**

We designed the homologue of MC-1 not to be subjected to decarboxylation. The reaction of 3 with sodium cyanide in dimethylsulfoxide afforded the nitrile 17. The desired 9-
Scheme 2. O-alkyl derivatives of cytogenin.

![Scheme Diagram]

C-Alkyl Derivative of 2

The preparation of C-alkyl derivatives are shown in Scheme 3. First, the 8-hydroxyl group in 17 was protected as the tert-butyldimethylsilyl ether 18. Usual alkylation procedures such as sodium hydride and alkyl halide gave the dialkyl product as a main product. So we employed the two phase system of aqueous sodium hydroxide and dichloromethane in the presence of tetrabutyl ammonium bromide as a phase transfer catalyst to obtain the monoalkyl product. Even under these conditions, the reaction should be stopped generally within an hour before the dialkyl compound become a major product. Thus, mono-alkyl compounds 19a–c were prepared along with the recovery of about a half the amount of the starting material 18.

Mono alkyl nitriles 19a–c were hydrolyzed to 2a (NM-3), 2b and 2c, respectively.

Dialkyl derivatives 20a–c were obtained by using sodium hydride and alkyl halide, concomitant with partial desilylation as a side reaction. The 9,9-dimethylnitrile 20a was hydrolyzed to give the carboxylic acid 21a in 45% yield. However, the complete hydrolysis proved more resistant on the more hindered dialkyl compounds 20b and 20c under the same or even more drastic conditions, affording only the carbamoyl compounds 21b and 21c, respectively.

Results and Discussion

Metabolite Profile

Orally administered cytogenin was rapidly metabolized in mice and excreted in urine mainly as the 8 (or 9)-O-
sulfonate (MCS), the 8 (or 9)-O-glucuronate (MCG) and the 3-carboxylic acid (MC-1). That was confirmed by the HPLC analysis of the metabolites before and after hydrolysis with sulfatase and/or glucuronidase. The rate of these compounds, MCS, MCG and MC-1, found in urine was: 26% (retention time=4 minutes), 22% (4.5 minutes) and 24% (18 minutes), respectively, only 7% cytogenin remained at 5.5 minutes. Another experiment showed that about 70% of cytogenin was conjugated in a plasma.

\[ \text{Scheme 3. Modification of the side chain.} \]

\[
\begin{align*}
\text{3} & \xrightarrow{a} \text{CH}_3\text{O} \quad \text{CH}_3\text{O} \\
& \quad \text{CN} \quad \text{CN} \\
\text{17} & \quad \text{y: 84%} \\
& \quad \text{CH}_3\text{O} \quad \text{CH}_3\text{O} \\
\text{17} & \quad \text{CN} \quad \text{CN} \\
& \quad \text{y: 75%} \\
& \quad \text{CH}_3\text{O} \quad \text{CH}_3\text{O} \\
\text{18} & \quad \text{y: 75%} \\
& \quad \text{CH}_3\text{O} \quad \text{CH}_3\text{O} \\
\text{19} & \quad \text{y: 46%} \\
& \quad \text{R = Me} \\
& \quad \text{b} \quad \text{R = Et} \\
& \quad \text{c} \quad \text{R = Bzl} \\
\text{2} & \quad \text{y: 80%} \\
& \quad \text{R = Me} \\
& \quad \text{b} \quad \text{R = Et} \\
& \quad \text{c} \quad \text{R = Bzl} \\
\text{20} & \quad \text{y: 92%} \\
& \quad \text{R = Me} \\
& \quad \text{b} \quad \text{R = Pr} \\
& \quad \text{c} \quad \text{R = Bzl} \\
\text{21} & \quad \text{y: 88%} \\
& \quad \text{R = Me, R, OH} \\
& \quad \text{b} \quad \text{R = Pr, R, NH}_2 \\
& \quad \text{c} \quad \text{R = Bzl, R, NF}_2 \\
\text{2} & \quad \text{y: 85%} \\
& \quad \text{R = Me} \\
& \quad \text{b} \quad \text{R = Et} \\
& \quad \text{c} \quad \text{R = Bzl} \\
\end{align*}
\]

a: NaCN/DMSO; b: HCl, AcOH; c: t-butyldimethylchlorosilane, imidazole/DMF; d: RX, NaOH, Bu$_4$NBr/CH$_2$Cl$_2$; e: RX, NaH/THF; f: H$_2$SO$_4$, AcOH

In vitro and in vivo stability are shown in Table 1. O-acyl derivatives 4, 5, 8, 9 and 10 were unstable in a plasma and deacylated to cytogenin. Although the O-acyl derivatives having a carboxyl group such as 6 and 7 were stable in a plasma, they were deacylated in vivo to cytogenin and gave the same metabolites as in the case of cytogenin. Based on these results and from the fact that MC-1 existed without conjugation, we assumed that other compounds having a carboxyl function in the molecule would be stable both in vitro and in vivo. Therefore, the stability of the carboxymethoxy derivative 16 and carboxylic acids 2, 2a–c and 21a were examined. They showed high stability and remained largely intact both in vitro and in vivo. On the other hand, 9,9-dialkyl carboxamides 21b and 21c were unstable. These findings showed that the carboxylic acid function is crucial for the in vivo stability.

**Pharmacological Activities**

Anti-arthritic activities of the derivatives with good in vivo stability were evaluated using type II collagen-induced arthritic mice at a dose of 20 mg/kg or 30 mg/kg, p.o. The inhibitory activities are shown in Table 2. The ED$_{50}$ values of NM-3 and cytogenin were 4.4 mg/kg/day and 44 mg/kg/day, respectively.

As indicated by the data in Table 2, the anti-arthritic activity was exhibited by only a few limited compounds, by oral administration. The –C$_9$–COOH linkage at C-3 is essential for exhibition of the activity. Substitution at the C-9 position was effective in strengthening the activity, and the methyl group was most efficacious. The bulkier
Table 1. Biological stability of cytogenin derivatives in plasma.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>in vitro (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>in vivo&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Conjugation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenin</td>
<td>98.0</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>MC-1</td>
<td>99.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>92.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2a (NM-3)</td>
<td>97.6</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2b</td>
<td>84.0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>2c</td>
<td>90.6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>n.t.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>n.t.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>95.4</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>92.9</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>n.t.</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0&lt;sup&gt;*&lt;/sup&gt;</td>
<td>n.t.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>41.1&lt;sup&gt;*&lt;/sup&gt;</td>
<td>n.t.</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>99.7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>95.3</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>99.2</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>101</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>21a</td>
<td>98.4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>21b</td>
<td>95.2</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>21c</td>
<td>80.3</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>

1): Stability in mouse plasma
2): Metabolism in mice (at 30 min after 25 mg/kg, po).
* : Hydrolized to cytogenin
n.t.: not tested

Table 2. Pharmacological activities of cytogenin derivatives.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Inhibiting activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-arthritic&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cytogenin</td>
<td>38.1</td>
</tr>
<tr>
<td>MC-1</td>
<td>32.6</td>
</tr>
<tr>
<td>2</td>
<td>3.7&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>2a (NM-3)</td>
<td>73.6</td>
</tr>
<tr>
<td>2b</td>
<td>6.7</td>
</tr>
<tr>
<td>2c</td>
<td>46.4</td>
</tr>
<tr>
<td>16</td>
<td>-1.9</td>
</tr>
<tr>
<td>21a</td>
<td>37.6</td>
</tr>
<tr>
<td>21b</td>
<td>18.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Inhibitory effect on collagen-induced arthritis at 20 mg/kg (*: at 30 mg/kg).
<sup>b</sup> Inhibitory effect on S-180-induced angiogenesis at 10 mg/kg (**: at 100 mg/kg).

Substituents such as ethyl for compound 2b or dimethyl for compound 21a showed less potent activity.

Anti-angiogenic activity has been found for cytogenin<sup>4</sup>. So a mouse dorsal air sac assay, as described in the previous report<sup>3</sup>, was also examined for cytogenin derivatives. As shown in Table 2, compounds 2a (NM-3) and 21a were more active than cytogenin. NM-3 showed 78% inhibitory activity against the angiogenesis induced by S-180 tumor cells in mice at a single dose of 10 mg/kg, p.o.

When 25 mg/kg of NM-3 was orally administered, the maximal blood concentration (C<sub>max</sub>) reached 129.3 μg/ml (T<sub>1/2</sub>: 3.43 hour, T<sub>max</sub>: 0.141 hour), which was about 100 times higher than that of cytogenin. The area under the curve (AUC) value was 777 mg·hr/ml.

Furthermore, the acute toxicity test in mice showed that NM-3 had extremely low toxicity (the LD<sub>50</sub> value was more than 1 g/kg by p.o.). Consequently, NM-3 is the most promising candidate as an orally available therapeutic agent for the treatment of angiogenesis-associated diseases and rheumatoid arthritis.

Experimental

General

NMR spectra were recorded on a JEOL JNM-GSX400 spectrometer. Chemical shifts are expressed in δ-value down field from TMS as an internal standard. FAB-MS spectra were measured on a JEOL JMS-SX102A spectrometer using m-nitrobenzyl alcohol as a matrix. Silica gel column chromatography was carried out on silica gel 60 (Merck). IR and UV spectra were recorded on a JASCO FT-IR 5300 spectrophotometer and Hitachi model U-3210 spectrophotometer, respectively.

Metabolites of Cytogenin

Male Sprague-Dawley rats weighing 150~160 g (Japan SLC Inc, Shizuoka, Japan) were fasted overnight before and 8 hours after dosing. After oral administration of cytogenin (200 mg/kg), rats were kept separately in a glass metabolism cage. Urine was collected for up to 8 hours and analyzed before and after hydrolysis with sulfatase and/or α-glucuronidase. Briefly, 0.5 ml of urine sample was incubated with either 5 mg of sulfatase (Sigma, S-8629) in 2.0 ml of 0.2 M acetic acid buffer (pH 5.0), 2 mg of α-glucuronidase (Sigma, G-7646) in 2.0 ml of 0.1 M phosphate buffer (pH 6.8) or 5 mg of sulfatase containing α-
The column effluent was monitored by the ultraviolet absorption at 244 nm.

**Anti-arthritic Activity**
Males DBA/1J mice (Charles River Japan, Atsugi, Japan) were used at 8–12 weeks of age for the collagen-induced arthritis experiment. The animals were housed under standard laboratory conditions and were fed food and water *ad libitum*. All compounds used were dissolved or suspended in physiological saline or 0.5% carboxymethyl-cellulose (CMC) (Wako Pure Chemical Industries, Osaka, Japan). Bovine type II collagen (Cosmo-Bio, Tokyo, Japan) was dissolved in 0.01 M acetic acid at a concentration of 2 mg/ml before use. Mice were immunized by intradermal injection at a base of the tail with 100 μg of native collagen emulsified in an equal volume of Freund's complete adjuvant (Difco Labs., Detroit, MI, USA). Three weeks later, mice were boosted by i.p. injection with 100 μg of the same emulsified native collagen. Groups of 6 to 10 mice were daily treated p.o. with the compounds at a dose of 20 mg/kg/day for 3 weeks after the injection of booster. Groups of 3 and 6 to 10 mice were used for normal control (normal) and arthritis control (control), respectively. Normal and control mice received vehicle alone in the same manner. The mice were observed for clinical arthritis and were scored by grading each paw from 0 to 4 based on erythema and swelling of the joint (0 = no erythema or swelling; 1 = erythema or swelling of one toe; 2 = erythema or swelling of two or more of the toes; 3 = erythema and swelling of the entire paw; 4 = complete erythema and swelling of the entire paw and incapacity to bend the ankle). All four legs were scored; the highest score reached 16. Inhibitory percent on arthritis was calculated as \[ \frac{(C-T)}{C} \times 100 \], where C was the maximal score of control group and T was that of treatment group.

**Anti-angiogenic Activity**
Anti-angiogenic activity was evaluated using a mouse dorsal air sac assay. The assay was performed as described previously. The compound was administered orally once daily (10 or 100 mg/kg) for 5 consecutive days to ICR mice (Charles River Japan). The angiogenic response was assessed by determining the number of newly formed blood vessels, induced by implanted chamber containing S-180 tumor cells.

3-Chloromethyl-8-hydroxy-6-methoxy-1-oxo-1H-2-benzopyran (3)
To a solution of 1 (20.0 g, 90.09 mmol) in THF (100 ml), carbon tetrachloride (150 ml) and triphenylphosphine (40.3
g, 153.2 mmol) were added. The solution was refluxed for 2 hours and then concentrated. The residue was crystallized from ethanol to give 3 (18.9 g, 88% yield).

IR (KBr) cm⁻¹ 1685, 1165, 690; UV (MeOH) λ max nm (ε) 246 (47,900), 333 (7,700); ¹H NMR (CDCl₃) δ 10.98 (1H, s), 6.54 (1H, d, J=2.2 Hz), 6.52 (1H, s), 6.41 (1H, d, J=2.2 Hz), 4.34 (2H, s), 3.88 (3H, s); HR-FABMS m/z calcd for C₁₁H₁₀O₄Cl (M+H)+ 241.0268, found 241.0275.

8-Acetoxy-3-hydroxymethyl-6-methoxy-1-oxo-1H-2-benzopyran (4)

A mixture of 1 (500 mg, 2.25 mmol), pyridine (0.24 ml, 2.92 mmol) and acetic anhydride (0.26 ml, 2.70 mmol) in THF (2 ml) were stirred at room temperature for 15 hours. The reaction mixture was diluted with water and extracted with EtOAc (50 ml). The organic layer was washed with water and brine, dried over Na₂SO₄, and then concentrated. The residue was crystallized from methanol to give 4 (302 mg, 51% yield).

IR (KBr) cm⁻¹ 1735, 1680, 1245; UV (MeOH) λ max nm (ε) 244 (46,400), 330 (6,500); ¹H NMR (CDCl₃) δ 11.01 (1H, s), 6.53 (1H, d, J=2.2 Hz), 6.48 (1H, s), 6.40 (1H, d, J=2.2 Hz), 4.87 (2H, s), 3.88 (3H, s), 2.15 (3H, s); HR-FABMS m/z calcd for C₁₃H₁₃O₆ (M+H)+ 265.0712, found 265.0704.

8-Acetoxy-3-acetoxymethyl-6-methoxy-1-oxo-1H-2-benzopyran (5)

Compound 5 was prepared by use of a similar procedure analogous to that described for the preparation of 4 except for the amount of reagent (Ac₂O, 12 eq; pyridine, 12 eq).

IR (KBr) cm⁻¹ 1765, 1740, 1720, 1225; UV (MeOH) λ max nm (ε) 246 (57,200), 300 (6,400); ¹H NMR (acetone-d₆) δ 6.72 (1H, d, J=2.2 Hz), 6.71 (1H, d, J=2.2 Hz), 6.46 (1H, s), 4.85 (2H, s), 3.90 (3H, s), 2.41 (3H, s), 2.14 (3H, s); HR-FABMS m/z calcd for C₁₅H₁₅O₇ (M+H)+ 307.0818, found 307.0812.

8-Hydroxy-6-methoxy-1-oxo-3-succinoyloxymethyl-1H-2-benzopyran (6)

To a solution of 1 (300 mg, 1.35 mmol) in toluene (5 ml) was added succinic anhydride (270 mg, 5.40 mmol). After refluxing for 8 hours, the solution was concentrated. The residue was crystallized from methanol to give 6 (320 mg, 74% yield).

IR (KBr) cm⁻¹ 1720, 1680, 1650, 1165; UV (MeOH) λ max nm (ε) 244 (52,200), 330 (7,100); ¹H NMR (CDCl₃) δ 10.99 (1H, s), 7.28~7.37 (5H, m), 6.51 (1H, d, J=2.2 Hz), 6.31 (1H, s), 6.30 (1H, d, J=2.2 Hz), 4.90 (2H, s), 3.87 (3H, s), 3.72 (2H, s); HR-FABMS m/z calcd for C₁₅H₁₅O₇ (M+H)+ 341.1025, found 341.1017.

8-Hydroxy-6-methoxy-3-phenylacetoxymethyl-1-oxo-1H-2-benzopyran (8)

By use of a procedure analogous to that described for the preparation of 4, compound 8 was prepared from 1 and phenacyl chloride (77% yield).

IR (KBr) cm⁻¹ 1745, 1680, 1165; UV (MeOH) λ max nm (ε) 244 (45,600), 330 (4,150); ¹H NMR (acetone-d₆) δ 10.99 (1H, s), 7.28~7.37 (5H, m), 6.51 (1H, d, J=2.2 Hz), 6.31 (1H, s), 6.30 (1H, d, J=2.2 Hz), 4.90 (2H, s), 3.87 (3H, s), 3.72 (2H, s); HR-FABMS m/z calcd for C₁₅H₁₅O₇ (M+H)+ 341.1025, found 341.1017.

8-Hydroxy-3- mandeloyloxymethyl-6-methoxy-1-oxo-1H-2-benzopyran (9)

To a solution of 3 (200 mg, 0.83 mmol) in DMF (1 ml), potassium carbonate (285 mg, 2.08 mmol) and D-L-mandelic acid (630 mg, 4.15 mmol) were added. After stirring at 60°C for 2 hours, the reaction mixture was diluted with water and extracted with EtOAc (30 ml). The organic layer was washed with water and brine, dried over Na₂SO₄, and then concentrated. The residue was purified by silica gel column chromatography to give 9 (234 mg, 79% yield).

IR (KBr) cm⁻¹ 1690, 1165; UV (MeOH) λ max nm (ε) 245 (52,300), 330 (7,300); ¹H NMR (CDCl₃) δ 10.93 (1H, s), 7.35~7.47 (5H, m), 6.51 (2H, d, J=2.2 Hz), 6.24 (1H, d, J=2.2 Hz), 6.09 (1H, s), 5.29 (1H, d, J=5.8 Hz), 4.96 (2H, s), 3.87 (3H, s), 3.31 (1H, d, J=5.8 Hz); HR-FABMS m/z calcd for C₁₅H₁₅O₇ (M+H)+ 577.0974, found 577.0949.

3-(2-Aminoisovaleryloxy)methyl-8-hydroxy-6-methoxy-1-oxo-1H-2-benzopyran (10)

A solution of 3 (300 mg, 1.35 mmol), potassium carbonate (285 mg, 2.08 mmol) and Boc-valine (580 mg, 2.70 mmol) in DMF (5 ml) was stirred at 80°C for 2 hours. The reaction mixture was diluted with water and extracted with...
EtOAc (50 ml). The organic layer was washed with water and brine, dried over Na₂SO₄, and then concentrated. The residue was purified by silica gel column chromatography to give N-Boc derivative of 10 (510 mg). To a solution of this compound in CH₂Cl₂ (10 ml) was added TFA (1 ml). After stirring at room temperature for 10 hours, the reaction mixture was concentrated. The residue was diluted with EtOAc (50 ml), washed successively with saturated sodium bicarbonate, water and brine. The organic layer was dried over Na₂SO₄ and concentrated to give 10 (237 mg, 55% yield).

IR (KBr) cm⁻¹ 1690, 1620, 1165; UV (MeOH) λ_max nm (ε) 244 (50,500), 330 (7,100); ¹H NMR (CD₃OD) δ 6.86 (1H, s), 6.70 (1H, d, J=2.2 Hz), 6.60 (1H, d, J=2.2 Hz), 4.99 (1H, d, J=13.2 Hz), 4.94 (1H, d, J=13.2 Hz), 3.87 (3H, s), 3.21 (1H, d, J=5.9 Hz), 1.84-1.92 (1H, m), 0.89 (3H, d, J=6.6 Hz), 0.84 (3H, d, J=6.6 Hz); HR-FABMS m/z calcd for C₁₆H₂₀NO₆ (M⁺H)+ 322.1291, found 322.1310.

3-(2,6-Diaminohexanoyloxy)methyl-8-hydroxy-6-methoxy-1-oxo-1H-2-benzopyran (11)

By use of a procedure analogous to that described for the preparation of 10, compound 11 was prepared from 1 and N,N' -di-Boc-lysine as trifluoroacetic acid salt (77% yield).

IR (KBr) cm⁻¹ 1690, 1620, 1200; UV (MeOH) λ_max nm (ε) 245 (40,800), 331 (5,600); ¹H NMR (DMSO-d₆) δ 10.89 (1H, s), 8.48 (2H, br), 7.73 (2H, br), 6.93 (1H, s), 6.71 (1H, d, J=2.2 Hz), 6.64 (1H, d, J=2.2 Hz), 5.14 (1H, d, J=13.2 Hz), 5.09 (1H, d, J=13.2 Hz), 4.15 (1H, br), 3.88 (3H, s), 2.76 (2H, br), 1.79 ~ 1.84 (2H, m), 1.33 ~ 1.58 (4H, m); HR-FABMS m/z calcd for C₁₇H₂₃N₂O₆ (M⁺H)+ 351.1556, found 351.1560.

6,8-Dimethoxy-3-methoxymethyl-1-oxo-1H-2-benzopyran (12)

To a solution of 1 (1.00 g, 4.50 mmol) in DMF (15 ml), sodium hydride (60%, in oil, 540 mg, 13.5 mmol) and methyl iodide (3.6 ml, 27.0 mmol) were added. After stirring at room temperature for 15 hours, the reaction mixture was diluted with water and extracted with EtOAc (100 ml). The organic layer was washed with water and brine, dried over Na₂SO₄, and then concentrated. Chromatographic separation followed by recrystallization from EtOH gave 12 (235 mg, 21% yield).

IR (KBr) cm⁻¹ 1755, 1695, 1210, 1090; UV (MeOH) λ_max nm (ε) 244 (53,500), 333 (7,100); ¹H NMR (DMSO-d₆) δ 6.74 (1H, d, J=2.2 Hz), 6.54 (1H, s), 6.46 (1H, d, J=2.2 Hz), 5.59 (1H, s), 4.86 (2H, s), 4.22 (1H, s), 3.85 (3H, s); HR-FABMS m/z calcd for C₁₃H₁₃O₅ (M⁺H)+ 281.0661, found 281.0662.

8-Hydroxy-6-methoxy-3-methoxymethyl-1-oxo-1H-2-benzopyran (13)

To a solution of 12 (431 mg, 1.72 mmol) in CH₂Cl₂ (10 ml) was added boron trichloride (1 m CH₂Cl₂ sol., 3.5 ml) at -35°C. After stirring at the same temperature for 30 minutes and further at room temperature for 1 hour, the reaction mixture was diluted with water and extracted with CH₂Cl₂ (10 ml). The organic layer was washed with water and brine, dried over Na₂SO₄ and then concentrated. The residue was recrystallized from EtOH to give 13 (231 mg, 57% yield).

IR (KBr) cm⁻¹ 1755, 1240, 1120; UV (MeOH) λ_max nm (ε) 245 (47,000), 326 (6,700); ¹H NMR (CDCl₃) δ 11.05 (1H, s), 6.50 (1H, d, J=2.2 Hz), 6.46 (1H, s), 6.38 (1H, d, J=2.2 Hz), 4.24 (2H, s), 3.87 (3H, s), 3.48 (3H, s); HR-FABMS m/z calcd for C₁₃H₁₃O₅ (M⁺H)+ 237.0763, found 237.0765.

3-Hydroxymethyl-8-carboxymethyloxy-6-methoxy-1-oxo-1H-2-benzopyran (14)

A solution of 1 (330 mg, 1.50 mmol), potassium carbonate (330 mg, 2.25 mmol) and t-butyl bromoacetate (1.1 ml, 7.50 mmol) in DMF (5 ml) was stirred at room temperature for 19 hours. The reaction mixture was diluted with water and extracted with EtOAc (100 ml). The organic layer was washed with water and brine, dried over Na₂SO₄, and then concentrated. The residue was purified by silica gel column chromatography to give t-butyl ester of 14 (501 mg). To a solution of the t-butyl ester (501 mg) in CH₂Cl₂ (5 ml) was added TFA (1 ml). After stirring at room temperature for 2 hours, the reaction mixture was concentrated. Crystallization from MeOH (5 ml) gave 14 (211 mg, 51% yield).

IR (KBr) cm⁻¹ 1755, 1240, 1120; UV (MeOH) λ_max nm (ε) 244 (53,500), 333 (7,100); ¹H NMR (CDCl₃) δ 6.47 (1H, d, J=2.2 Hz), 6.54 (1H, s), 6.46 (1H, d, J=2.2 Hz), 5.59 (1H, s), 4.86 (2H, s), 4.22 (1H, s), 3.85 (3H, s); HR-FABMS m/z calcd for C₁₃H₁₃O₅ (M⁺H)+ 281.0661, found 281.0662.
and brine, dried over \( \text{Na}_2\text{SO}_4 \), and then concentrated. To a solution of the residue in DMF (10 ml) was added sodium formate (10.3 mmol) and the solution was stirred at 80°C for 2 hours. The reaction mixture was diluted with water and extracted with EtOAc (100 ml). The organic layer was washed with water and brine, dried over \( \text{Na}_2\text{SO}_4 \), and then concentrated. The residue in acetonitrile was treated with sodium bicarbonate (10 ml) at 40°C for 17 hours. The reaction mixture was diluted with EtOAc (100 ml), which was washed with water and brine, dried over \( \text{Na}_2\text{SO}_4 \), and then concentrated. To a solution of 15 (526 mg, 88% yield).

IR (KBr) cm\(^{-1}\) 1700, 1160, 1050; UV (MeOH) \( \lambda_{\text{max}} \) nm (e) 244 (40,700), 321 (4,100); \( ^1\text{H NMR} \) (CDCl\(_3\)) \( \delta \) 6.77 (1H, d, \( J=2.2\) Hz), 6.46 (1H, d, \( J=2.2\) Hz), 6.37 (1H, s), 5.35 (2H, s), 4.44 (2H, d, \( J=6.6\) Hz), 3.88 (3H, s), 3.55 (3H, s), 2.22 (1H, t, \( J=6.6\) Hz). HR-FABMS m/z calcd for \( \text{C}_{13}\text{H}_{15}\text{O}_6 \) (M+H)+ 267.0869, found 267.0869.

3-Carboxymethyl-8-hydroxy-6-methoxy-1-oxo-1'-\( \gamma \)-2-benzopyran (16)

To a solution of 15 (300 mg, 1.13 mmol) in DMF (10 ml), sodium hydride (136 mg, 3.40 mmol) and bromoacetic acid f-butyl ester (0.83 ml, 5.66 mmol) were added at 0°C. After stirring at room temperature for 2 hours, the reaction mixture was diluted with water and extracted with EtOAc (100 ml). The organic layer was washed with water and brine, dried over \( \text{Na}_2\text{SO}_4 \), and then concentrated. After chromatographic purification, the ester was dissolved in acetonitrile (10 ml) and was treated with 1 M HC1 (10 ml) at 40°C for 1 hour. The reaction mixture was diluted with EtOAc (50 ml), which was washed with water and brine, dried over \( \text{Na}_2\text{SO}_4 \), and then concentrated. To a solution of the residue in CH\(_2\)Cl\(_2\) (5 ml) was added TEA (1 ml), and the solution was stirred at room temperature for 2 hours. The reaction mixture was added water and extracted with EtOAc (50 ml). The organic layer was washed with water and brine, dried over \( \text{Na}_2\text{SO}_4 \), and then concentrated. Crystallization from EtOAc (10 ml) gave 16 (140 mg, 44% yield).

IR (KBr) cm\(^{-1}\) 1730, 1680, 1165, 1130; UV (MeOH) \( \lambda_{\text{max}} \) nm (e) 244 (44,700), 329 (6,600); \( ^1\text{H NMR} \) (DMSO-d\(_6\)) \( \delta \) 10.89 (1H, s), 7.59 (1H, s), 6.96 (1H, d, \( J=2.4\) Hz), 6.71 (1H, d, \( J=2.4\) Hz), 3.88 (3H, s), 3.55 (3H, s), 3.22 (1H, t, \( J=6.6\) Hz). HR-FABMS m/z calcd for \( \text{C}_{13}\text{H}_{13}\text{O}_7 \) (M+H)+ 281.0661, found 281.0637.

3-Carboxy-8-hydroxy-6-methoxy-1-oxo-1'-\( \gamma \)-2-benzopyran (MC-1)

To a solution of 1 (3.6 g, 16.2 mmol) in acetone (100 ml) was added Jones' reagent (14 ml) at 0°C. After stirring at the same temperature for 10 minutes, the mixture was diluted with water (500 ml) and extracted with EtOAc. The organic layer was washed with 20% aq. NaCl (200 ml x 3). The extract was back-extracted with 5% NaHCO\(_3\) (300 ml x 4). The volume of the aqueous solution was adjusted to 1400 ml by addition of water, and acidified to pH 3 with conc. HCl. The white precipitate (2.36 g) formed was obtained by filtration, while 150 mg of MC-1 was recovered from EtOAc extract of the mother liquid. The combined solid was washed with hot 90% aq. MeOH (40 ml) to give pure MC-1 (2.36 g, 62% yield).

IR (KBr) cm\(^{-1}\) 3436, 1723, 1692, 1173; \( ^1\text{H NMR} \) (DMSO-d\(_6\)) \( \delta \) 10.89 (1H, s), 7.59 (1H, s), 6.58 (1H, s), 6.42 (1H, d, \( J=2.4\) Hz), 6.30 (1H, d, \( J=2.4\) Hz), 3.88 (3H, s); \( ^1\text{C NMR} \) (DMSO-d\(_6\)) \( \delta \) 56.0, 100.9, 102.7, 104.5, 112.6, 137.1, 143.2, 160.7, 162.5, 163.8, 166.3; FAB-MS m/z 237 (M+H)+.

3-Cyanomethyl-8-hydroxy-6-methoxy-1-oxo-1'-\( \gamma \)-2-benzopyran (17)

To a solution of 3 (5.00 g, 20.8 mmol) and sodium cyanide (4.09 g, 83.3 mmol) in DMSO (250 ml) was stirred at room temperature for 1 hour under a nitrogen atmosphere. The reaction mixture was diluted with water and extracted with EtOAc (500 ml x 3). The organic layer was washed with 20% brine (400 ml x 3), dried over \( \text{Na}_2\text{SO}_4 \), and then concentrated. The residue was dissolved in hot EtOH (50 ml) and the solution was stirred at room temperature for 1 hour. The precipitate was collected by filtration, dried in vacuo to give 17 (4.02 g, 84% yield).

IR (KBr) cm\(^{-1}\) 2260, 1680, 1195; UV (MeOH) \( \lambda_{\text{max}} \) nm (e) 244 (44,700), 329 (6,600); \( ^1\text{H NMR} \) (DMSO-d\(_6\)) \( \delta \) 10.82 (1H, s), 6.58 (1H, s), 6.54 (1H, d, \( J=2.2\) Hz), 6.42 (1H, d, \( J=2.2\) Hz), 3.89 (3H, s), 3.65 (2H, s); HR-FABMS m/z calcd for \( \text{C}_{13}\text{H}_{10}\text{NO}_4 \) (M+H)+ 232.0610, found 232.0598.

(8-Hydroxy-6-methoxy-1-oxo-1'-\( \gamma \)-2-benzopyran-3-yl)-acetic acid (2)

To a solution of 17 (1.50 g, 6.49 mmol) in AcOH (12 ml) was added cone. HCl (12 ml) and stirred at 80°C for 8 hours. Water (9 ml) was added to the solution and stirred at room temperature for 15 hours. The precipitate was collected by filtration, washed with water, and then dried in vacuo to give crude 2. The crude 2 was dissolved in EtOH (30 ml) and added charcoal (1.00 g). The solution was stirred at 60°C for 1 hour and concentrated after filtration. Recrystallization from acetone-hexane (1:5) gave 2 (1.12 g, 69% yield).

IR (KBr) cm\(^{-1}\) 1720, 1690, 1240; UV (MeOH) \( \lambda_{\text{max}} \) nm (e) 245 (47,200), 329 (6,300); \( ^1\text{H NMR} \) (DMSO-d\(_6\)) \( \delta \) 10.93 (1H, s), 6.66 (1H, s), 6.63 (1H, d, \( J=2.2\) Hz), 6.56
(1H, d, \(J = 2.2\) Hz), 3.86 (3H, s), 3.62 (2H, s). HR-FABMS 
\(m/z\) calcd for \(C_{12}H_{11}O_6\) (M+H)+ 251.0556, found 
251.0557.

8-\(\beta\)-Butyldimethylsilyloxy-3-cyanomethyl-6-methoxy-1-oxo-
1\/-2-benzopyran (18)

To a solution of crude 17, which was prepared from 21.6 
g of 3, in DMF (200 ml), imidazole (12.2 g, 180.2 mmol) 
and \(\beta\)-butyldimethylchlorosilane (21.8 g, 144.1 mmol) were 
added at 0°C and the solution was stirred at the same 
temperature for 2 hours. The reaction mixture was diluted 
with water and extracted with toluene (400 ml). The organic 
layer was washed with water and brine, dried over \(Na_2SO_4\), 
and then concentrated. The residue was crystallized from 
ethanol to give 18 (24.3 g, 75% yield).

IR (KBr) cm\(^{-1}\) 2260, 1740, 1170, 835; UV (MeOH) \(\lambda_{max}\) 
nm (e) 246 (55,000), 325 (6,200); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 
6.48 (1H, d, \(J = 2.2\) Hz), 6.45 (1H, d, \(J = 2.2\) Hz), 
3.87 (3H, s), 3.59 (2H, s), 1.05 (9H, s), 0.27 (6H, s); HR-
FABMS \(m/z\) calcd for \(C_{18}H_{24}NO_4Si\) (M+H)+ 346.1475, 
found 346.1454.

8-\(\beta\)-Butyldimethylsilyloxy-3-(1-cyanoethyl)-6-methoxy-
1\/-2-benzopyran (19a)

To a vigorously stirred solution of 18 (10.0 g, 29.0mmol) 
in two phase system of CH\(_2\)Cl\(_2\) (200 ml)- 1 m NaOH (200 
ml), tetra-w-butyl ammonium bromide (2.34 g, 7.25 mmol) 
and methyl iodide (7.2ml, 115.9mmol) were added and the 
solution was stirred at 0°C for 1 hour. The organic layer was 
separated, washed with water and brine, dried over \(Na_2SO_4\) 
and concentrated. The residue was purified by silica gel 
column chromatography to give 19a (4.79 g, 46% yield).

IR (KBr) cm\(^{-1}\) 2240, 1740, 1170, 840; UV (MeOH) \(\lambda_{max}\) 
nm (e) 244 (51,500), 323 (5,500); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 
6.48 (1H, s), 6.46 (1H, d, \(J = 2.2\) Hz), 6.45 (1H, d, \(J = 2.2\) Hz), 
3.87 (3H, s), 3.73 (1H, q, \(J = 7.0\) Hz), 1.69 (3H, d, \(J = 7.0\) 
Hz), 1.06 (9H, s), 0.28 (6H, s); HR-FABMS \(m/z\) calcd for 
\(C_{19}H_{26}NO_4Si\) (M+H)+ 360.1631, found 360.1617.

8-\(\beta\)-Butyldimethylsilyloxy-3-(1-cyano-2-phenyl)ethyl-6-
methoxy-1\/-2-benzopyran (19c)

By use of a procedure analogous to that described for the 
preparation of 19a, 19c was prepared from 18 and benzyl 
iodide (20% yield).

IR (KBr) cm\(^{-1}\) 2250, 1740, 1600, 1170, 840; UV 
(MeOH) \(\lambda_{max}\) nm (e) 246 (60,000), 324 (7,300); \(^1\)H NMR 
(CDCl\(_3\)) \(\delta\) 7.24-7.33 (5H, m), 6.45 (1H, d, \(J = 2.2\) Hz), 
6.39 (1H, d, \(J = 2.2\) Hz), 3.85 (3H, s), 3.39 (1H, dd, \(J = 5.1\) 
and 13.6 Hz), 2.11 (1H, dd, \(J = 8.8\) and 13.6 Hz), 1.07 (9H, s), 0.29 
(6H, s); HR-FABMS \(m/z\) calcd for \(C_{25}H_{30}NO_4Si\) (M+H)+ 
374.1788, found 374.1784.

8-\(\beta\)-Butyldimethylsilyloxy-3-(1-cyano-1-propyl)butyl-6-
methoxy-1\/-2-benzopyran (20b)

By use of a procedure analogous to that described for the 
preparation of 20a, 20b was prepared from 18 and ethyl 
iodide (82% yield).

IR (KBr) cm\(^{-1}\) 1735, 1600, 840; UV (MeOH) \(\lambda_{max}\) nm 
(e) 245 (54,500), 324 (6,100); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 
6.60 (1H, s), 6.45 (1H, d, \(J = 2.2\) Hz), 6.43 (1H, d, \(J = 2.2\) Hz), 
3.87 (3H, s), 2.04 (2H, dt, \(J = 5.2\) and 14.0 Hz), 1.82 (2H, 
dt, \(J = 5.2\) and 14.0 Hz), 1.49-1.59 (2H, m), 1.25-1.34 
(2H, m), 1.07 (9H, s), 0.95 (3H, t, \(J = 7.4\) Hz), 0.91 (3H, t, 
\(J = 7.4\) Hz), 0.29 (6H, s); FAB-MS \(m/z\) 430 (M+H)+

8-\(\beta\)-Butyldimethylsilyloxy-3-(1-cyano-2-phenyl)ethyl-6-
methoxy-1-oxo-1\/-2-benzopyran (20c)

By use of a procedure analogous to that described for the
preparation of 20a, 20c was prepared from 18 and benzyl bromide (86% yield).

IR (KBr) cm⁻¹ 2240, 1745, 1600, 1170, 840; UV (MeOH) λ_max nm (ε) 247 (55,700), 325 (6,500); ¹H NMR (CDCl₃) δ 7.23 (10H, s), 6.43 (1H, d, J = 2.2 Hz), 6.19 (1H, d, J = 2.2 Hz), 6.06 (1H, s), 3.77 (3H, s), 3.49 (2H, d, J = 13.6 Hz), 3.20 (2H, d, J = 13.6 Hz), 1.10 (9H, s), 0.32 (6H, s); HR-FABMS m/z calcd for C₃₂H₃₆NO₄Si (M+H)+ 526.2414, found 526.2391.

2-(8-Hydroxy-6-methoxy-1-oxo-1H-2-benzopyran-3-yl)-2-methyl-propionic Acid (21a)
To a solution of 20a (369mg, 0.99mmol) in AcOH (4 ml) was diluted with conc. HCl (4ml). After stirring at 70°C for 15 hours, the solution was added water (15ml) and stirred at room temperature for 3 hours. The precipitate was collected by filtration, washed with water and dried in vacuo to give the crude 21a. The crude 21a was decolorized with charcoal (100mg) in hot EtOH (10ml). Recrystallization from acetone-hexane (4:9) gave 21a (126mg, 45% yield).

IR (KBr) cm⁻¹ 1725, 1685, 1170; UV (MeOH) A_max nm (ε) 244 (54,100), 329 (7,500); ¹H NMR (DMSO-d₆) δ 10.97 (1H, s), 6.74 (1H, s), 6.71 (1H, d, J = 2.2 Hz), 6.55 (1H, d, J = 2.2 Hz), 3.86 (3H, s), 1.45 (6H, s); HR-FABMS m/z calcd for C₁₄H₁₅O₆ (M+H)+ 279.0869, found 279.0872.

2-(8-Hydroxy-6-methoxy-1-oxo-1H-2-benzopyran-3-yl)-2-propyl-valeramide (21b)
To a solution of 20b (346mg, 0.81mmol) in AcOH (2 ml) was added sulfuric acid (2ml). After stirring at 70°C for 16 hours, the reaction mixture was diluted with water and extracted with EtOAc (50ml). The organic layer was washed with water and brine, dried over Na₂SO₄, and then concentrated. The residue was purified by silica gel chromatography to give 21b (210 mg, 78% yield).

IR (KBr) cm⁻¹ 1725, 1685, 1170; UV (MeOH) λ_max nm (ε) 244 (24,400), 330 (7,900); ¹H NMR (DMSO-d₆) δ 12.87 (1H, s), 10.90 (1H, s), 6.68 (1H, s), 6.67 (1H, d, J = 2.2 Hz), 6.56 (1H, d, J = 2.2 Hz), 3.86 (3H, s), 3.70 (1H, q, J = 7.4 Hz), 1.39 (3H, d, J = 7.4 Hz). HR-FABMS m/z calcd for C₁₉H₁₅NO₆ (M+H)+ 265.0712, found 265.0711.

2-(8-Hydroxy-6-methoxy-1-oxo-1H-2-benzopyran-3-yl)-2-phenylmethyl-3-phenyl-propionamide (21c)
By use of a procedure analogous to that described for the preparation of 20b, 21c was prepared from 20c (85% yield).

IR (KBr) cm⁻¹ 1720, 1690, 1240, 1170; UV (MeOH) λ_max nm (ε) 247 (53,000), 331 (7,500); ¹H NMR (CDCl₃) δ 10.96 (1H, s), 7.14~7.26 (10H, m), 6.51 (1H, d, J = 2.2 Hz), 6.25 (1H, s), 6.23 (1H, d, J = 2.2 Hz), 5.60 (2H, s), 3.84 (3H, s), 3.34 (2H, d, J = 14.0 Hz), 3.29 (2H, d, J = 14.0 Hz); HR-FABMS m/z calcd for C₂₆H₂₅NO₅ (M+H)+ 430.1654, found 430.1634.

2-(8-Hydroxy-6-methoxy-1-oxo-1H-2-benzopyran-3-yl)-3-phenyl-propionic Acid (2a, NM-3)
To a solution of 19a (14.27g, 39.75mmol) in AcOH (120 ml) was diluted with conc. HCl (120 ml). After stirring at 80°C for 8 hours, the mixture was added water (90ml), and stirred at room temperature for 15 hours. The precipitate was collected by filtration, washed with water and dried in vacuo to give crude 2a. The crude 2a was decolorized with charcoal (1g) in hot EtOH (10ml). Recrystallization from acetone-hexane (1:5) gave 2a (8.40 g, 80% yield).

IR (KBr) cm⁻¹ 1720, 1690, 1240, 1170; UV (MeOH) λ_max nm (ε) 244 (24,400), 330 (7,900); ¹H NMR (DMSO-d₆) δ 12.87 (1H, s), 10.90 (1H, s), 6.69 (1H, s), 6.67 (1H, d, J = 2.2 Hz), 6.56 (1H, d, J = 2.2 Hz), 3.86 (3H, s), 3.70 (1H, q, J = 7.4 Hz), 1.39 (3H, d, J = 7.4 Hz). HR-FABMS m/z calcd for C₁₉H₁₅NO₆ (M+H)+ 265.0712, found 265.0711.

2-(8-Hydroxy-6-methoxy-1-oxo-1H-2-benzopyran-3-yl)-butyric acid (2b)
By use of a procedure analogous to that described for the preparation of 2a, 2b was prepared from 19b (84% yield).

IR (KBr) cm⁻¹ 1715, 1690, 1165; UV (MeOH) λ_max nm (ε) 245 (47,200), 329 (6,300); ¹H NMR (DMSO-d₆) δ 10.90 (1H, s), 6.69 (1H, s), 6.67 (1H, d, J = 2.2 Hz), 6.56 (1H, d, J = 2.2 Hz), 3.86 (3H, s), 3.48 (1H, dd, J = 7.3 and 8.1 Hz), 1.88~1.96 (1H, m), 1.77~1.86 (1H, m), 0.91 (3H, t, J = 7.3 Hz). HR-FABMS m/z calcd for C₁₄H₁₅O₆ (M+H)+ 279.0869, found 279.0841.

2-(8-Hydroxy-6-methoxy-1-oxo-1H-2-benzopyran-3-yl)-3-phenyl-propionic Acid (2c)
By use of a procedure analogous to that described for the preparation of 2a, 2c was prepared from 19c (80% yield).

IR (KBr) cm⁻¹ 1685, 1165; UV (MeOH) λ_max nm (ε) 246 (51,900), 331 (7,000); ¹H NMR (DMSO-d₆) δ 10.86 (1H, s), 7.12~7.27 (5H, m), 6.59 (1H, d, J = 2.2 Hz), 6.58 (1H, s), 6.54 (1H, d, J = 2.2 Hz), 3.93 (1H, dd, J = 6.6 and 8.8 Hz), 3.83 (3H, s), 3.27 (1H, dd, J = 6.6 and 14.0 Hz), 3.10 (1H, dd, J = 8.8 and 14.0 Hz). HR-FABMS m/z calcd for C₁₉H₁₅NO₆ (M+H)+ 341.1025, found 341.1028.
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References


