

## Phenolic Constituents of Cassia Seeds and Antibacterial Effect of Some Naphthalenes and Anthraquinones on Methicillin-Resistant *Staphylococcus aureus*

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Thirteen phenolic glycosides including six new compounds were isolated from seeds of *Cassia tora* (Leguminosae). The structures of the new compounds, rubrofusarin triglucoside (7), nor-rubrofusarin gentiobioside (9), demethylflavasperone gentiobioside (10), torachryson gentiobioside (11), torachryson tetraglucoside (12) and torachryson apioglucoside (13), were elucidated on the basis of spectroscopic and chemical evidence. The effects of the phenolic glycosides, their aglycones and several other compounds structurally related to them on *Escherichia coli* K12, *Pseudomonas aeruginosa* PAO1 and some strains of *Staphylococcus aureus* were then examined. Among them, torachryson (15), toralactone (16), aloe-emodin (18), rhein (19) and emodin (20) showed noticeable antibacterial effects on four strains of methicillin-resistant *Staphylococcus aureus* with a minimum inhibitory concentration of 2–64 µg/ml. On the other hand, the phenolic compounds tested did not show strong antibacterial effects on *E. coli* and *P. aeruginosa*.

**Key words** *Cassia tora*; naphthalene; anthraquinone; methicillin-resistant *Staphylococcus aureus*; antibacterial effect; Leguminosae

Phenolic constituents of plants of the family Leguminosae have been found to have various biological or pharmacological actions including radical-scavenging effects, inhibitory effects on enzymes and antimicrobial effects.<sup>1–6</sup> Recently we reported the isolation of new compounds related to condensed tannins from *Cassia nomame* and the inhibitory effects of phenolic constituents of *C. nomame* on lipase.<sup>6</sup>

In our continuing study on the bioactive phenolics of medicinal plants of Leguminosae, we isolated six new phenolic glycosides from seeds of *Cassia tora*, which have been used as a traditional medicine for eye diseases and intestinal disorders in Asian countries.<sup>7</sup> Antibacterial assay of phenolic compounds of the *C. tora* seeds and structurally related compounds revealed that several naphthalenes and anthraquinones have antibacterial effects on strains of methicillin-resistant *Staphylococcus aureus* (MRSA). This paper deals with the structures of the new compounds and the antibacterial effects of the phenolic compounds.

### Results and Discussion

Phenolic glycosides 1–13 were isolated from the polar fractions of the MeOH extract of the *C. tora* seeds by column chromatography on Diaion HP-20, Toyopearl HW-40, MCI-gel CHP-20P and YMC-gel ODS-AQ and/or droplet counter-current chromatography (DCCC). Six compounds from among these were identified as rubrofusarin 6-*O*-β-gentiobioside (1),<sup>8,9</sup> cassiaside C (2),<sup>9</sup> cassiaside (3),<sup>4,10</sup> chrysophanol 1-*O*-β-tetraglucoside (4),<sup>11</sup> torosachryson 8-*O*-β-gentiobioside (5),<sup>12</sup> cassiaside C<sub>2</sub> (6),<sup>13</sup> respectively.

**Structure of Rubrofusarin Glycosides** Compound 7 was obtained as colorless needles. The electrospray-ionization MS (ESI-MS) of 7 showed an [M+H]<sup>+</sup> ion peak at *m/z* 759 and [M+NH<sub>4</sub>]<sup>+</sup> ion peak at *m/z* 776, and high-resolution ESI-MS indicated its molecular formula to be C<sub>33</sub>H<sub>42</sub>O<sub>20</sub>. The UV spectrum of this compound which showed absorption maxima at 223, 258 (sh), 276, 322 and 390 nm was simi-

lar to those of γ-naphthopyrone derivatives such as 1. The <sup>1</sup>H-NMR spectrum showed two 1H singlets [δ 7.18 (H-10), δ 6.18 (H-3)], two *meta*-coupled doublets [δ 6.93, δ 6.78 (1H each, *J*=2 Hz, H-7, H-9)] due to protons on the naphthopyrone structure, along with the signal of a chelated hydroxyl group at δ 14.86, and two 3H singlets at δ 3.86 (OCH<sub>3</sub> at C-8) and 2.38 (CH<sub>3</sub> at C-2). The spectrum also showed three anomeric protons from its sugar residues [δ 5.04, 4.31, 4.28 (1H each, all d, *J*=7.5 Hz)]. These signals suggested that 7 is a trisaccharide derivative of rubrofusarin (14).<sup>8</sup>

The <sup>13</sup>C-NMR spectrum of 7 showed eighteen signals assignable to three glucose moieties along with those of the rubrofusarin residue as shown in Table 1. Chemical shifts of the <sup>13</sup>C signals of the aglycone of 7 were practically the same as those of 1,<sup>9,13</sup> indicating that the hydroxyl group at C-6 on the rubrofusarin structure was substituted by an oligosaccharide composed of three glucose residues. Among the carbon signals of the three glucose residues, the signals of the glucose residue directly attached to the aglycone moiety [*i.e.* glucose (Glc-I in 7, see the formula)] and the terminal glucose residue (Glc-III in 7) showed chemical shifts nearly identical to those of the corresponding signals (of Glc-I and Glc-II, respectively) of 1, while a signal assignable to C-3 of the remaining glucose residue (Glc-II in 7) showed a noticeable downfield shift (δ 88.3), indicating the presence of a 1→3 linkage between Glc-III and Glc-II (see Table 1). In fact, the chemical shifts of the sugar carbons of 7 are comparable with the reported ones for the corresponding carbons of chrysophanol triglucoside which has 1→3 and 1→6 sequences on the triglucoside moiety.<sup>11</sup> The β-glycosidic linkages for all of the three glucose residues were also indicated by coupling constants for the anomeric protons (7.5 Hz) in the <sup>1</sup>H-NMR spectrum of 7, and <sup>13</sup>C chemical shifts of the three anomeric carbons [δ 101.0 (Glc-I), 102.8 (Glc-II) and 104.3 (Glc-III)]. The connectivity of the three glucose residues was substantiated by the <sup>1</sup>H detected heteronuclear

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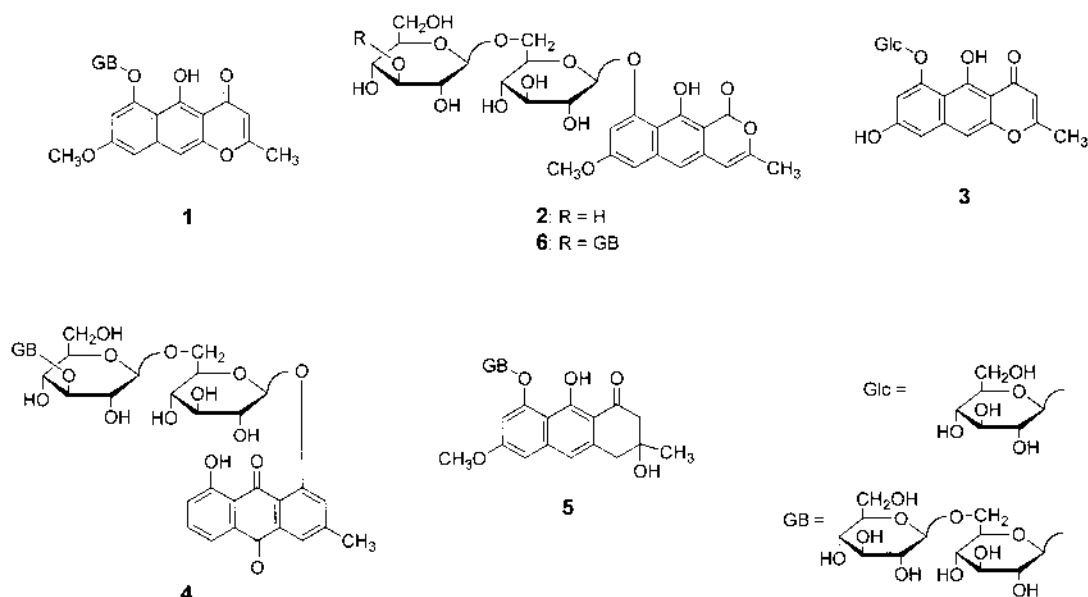


Chart 1

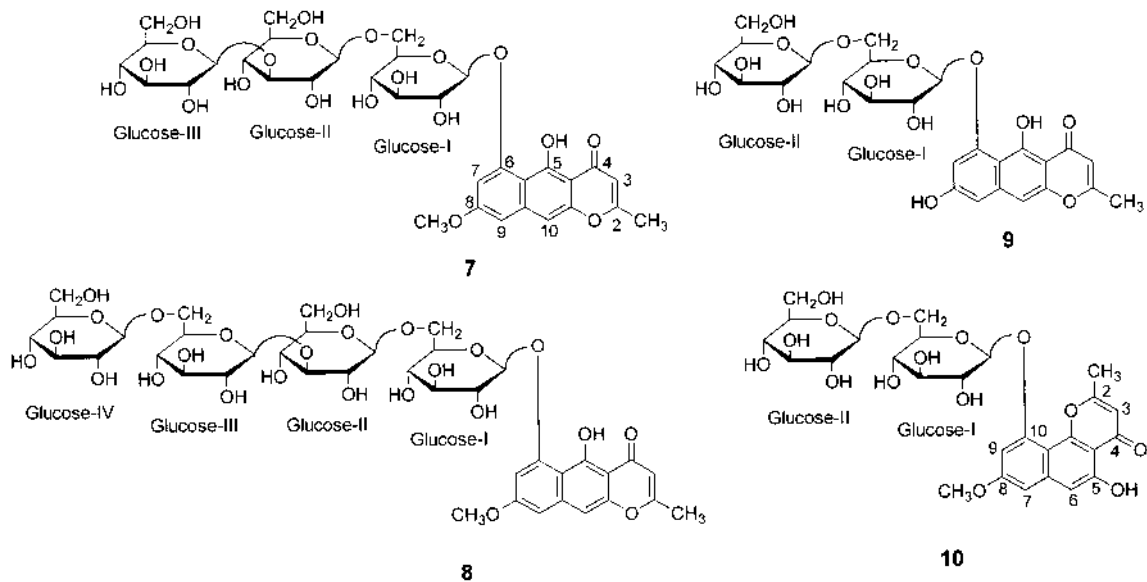


Chart 2

multiple bond connectivity (HMBC) spectrum of **7**, which showed correlations for the linkage Glc-II C-1→Glc-I C-6 [ $\delta_{\text{H}}$  4.07 (Glc-I H-6)– $\delta_{\text{C}}$  102.8 (Glc-II C-1) and  $\delta_{\text{H}}$  4.31 (Glc-II H-1)– $\delta_{\text{C}}$  68.7 (Glc-I C-6)] and the linkage Glc-III C-1→Glc-II C-3 [ $\delta_{\text{H}}$  3.28 (Glc-II H-3)– $\delta_{\text{C}}$  104.3 (Glc-III C-1) and  $\delta_{\text{H}}$  4.28 (Glc-III H-1)– $\delta_{\text{C}}$  88.3 (Glc-II C-3)]. The HMBC spectrum also showed a correlation between Glc-I H-1 ( $\delta_{\text{H}}$  5.04) and aglycone (Agl) C-6 ( $\delta_{\text{C}}$  157.8), indicating the location of the tetraglucoside moiety on O-6 of the aglycone.

The structure **7** thus assigned on the basis of the spectral data described above was supported by production of **1** and **14** upon the treatment of **7** with  $\beta$ -glucosidase. The nuclear Overhauser effect correlated spectroscopy (NOESY) for **7** showed correlations of the methoxyl signal with two *meta*-coupled protons (Agl H-7, H-9), and a correlation between Glc-I H-1 and Agl H-7, satisfied the locations of the sugar and the methoxyl group on the aglycone.

Compound **8** was obtained as pale-yellow needles. The ESI-MS showed an  $[\text{M}+\text{H}]^+$  ion peak at  $m/z$  921 and an  $[\text{M}+\text{Na}]^+$  ion peak at  $m/z$  943, which correspond to the molecular formula  $\text{C}_{39}\text{H}_{52}\text{O}_{25}$ . The UV,  $^1\text{H}$  and  $^{13}\text{C}$  spectral data (Experimental and Table 1) suggested that **8** is a rubrofusarin 6-*O*-tetraglucoside. Comparison of the  $^{13}\text{C}$  chemical shifts of its glucose carbons with those of the corresponding carbons of chrysophanol tetraglucoside which has the sequence of glucose-(1→6)-glucose-(1→3)-glucose-(1→6)-glucose,<sup>11)</sup> and its enzymatic hydrolysis which gave **7** and **1** led to structure **8** for this compound. This structure is identical with the reported one for cassiaside B<sub>2</sub>, which was recently isolated from *Cassia obtusifolia*,<sup>13)</sup> although the specific rotation of **8** ( $[-22.2^\circ]$ ) was different from the value of cassiaside B<sub>2</sub> ( $[\alpha]_{\text{D}} +11.3^\circ$ ) in the literature.<sup>13)</sup>

**Structure of Nor-rubrofusarin Gentiobioside** Compound **9** was obtained as a yellow powder. The ESI-MS

Table 1.  $^{13}\text{C}$ -NMR Spectral Data for Rubrofusarin Glycosides, **1**, **7** and **8**

Carbon	<b>1</b>	<b>7</b>	<b>8</b>
Aglycone			
C-2	169.0	169.0	169.0
C-3	106.9	106.9	106.9
C-4	184.0	184.0	183.9
C-4a	103.8	103.8	103.8
C-5	162.1	162.1	162.1
C-5a	107.9	107.9	108.0
C-6	157.8	157.8	157.8
C-7	101.3	101.5	101.6
C-8	161.3	161.3	161.2
C-9	99.9	100.0	100.0
C-9a	140.5	140.5	140.5
C-10	101.1	101.0	101.1
C-10a	152.6	152.6	152.6
CH <sub>3</sub> at C-2	20.4	20.4	20.4
OCH <sub>3</sub> at C-8	55.7	55.7	55.7
Glucose-I			
C-1	101.0	101.0	101.0
C-2	73.7	73.7	73.7
C-3	76.6	76.5	76.3
C-4	69.8	69.9	70.0
C-5	75.7	75.9	75.9 <sup>b)</sup>
C-6	68.9	68.7	68.8
Glucose-II			
C-1	103.8	102.8	102.7
C-2	73.7	72.4	72.1
C-3	76.9	88.3	89.0
C-4	70.3	68.5	68.8
C-5	77.1	76.3 <sup>a)</sup>	76.1 <sup>b)</sup>
C-6	61.2	60.9	60.7
Glucose-III			
C-1		104.3	104.2
C-2		74.0	73.7
C-3		77.1	77.0
C-4		70.3	70.1
C-5		76.5 <sup>a)</sup>	75.2
C-6		61.3	69.0
Glucose-IV			
C-1			103.0
C-2			74.0
C-3			76.5
C-4			70.6
C-5			76.1
C-6			61.1

126 MHz, in DMSO-*d*<sub>6</sub> at 27 °C. *a, b*) Values with the same superscript may be interchanged.

showed an  $[\text{M}+\text{H}]^+$  ion peak at  $m/z$  583 and an  $[\text{M}+\text{Na}]^+$  ion peak at  $m/z$  605, corresponding to the molecular formula  $\text{C}_{26}\text{H}_{30}\text{O}_{15}$ , which is 14 mass units lower than that of **1**. The UV spectrum of **9** suggested that this compound also has a  $\gamma$ -naphthopyrone chromophore like that in **1**. The  $^1\text{H}$ -NMR spectrum showed two 1H singlets at  $\delta$  7.04 (H-10) and  $\delta$  6.14 (H-3), two *meta*-coupled doublets at  $\delta$  6.77 and  $\delta$  6.71 (1H each,  $J=2$  Hz, H-7 and H-9), a broad singlet due to a chelated hydroxyl group at  $\delta$  14.90, and a 3H singlet at  $\delta$  2.35 (CH<sub>3</sub> at C-2), suggesting that the aglycone has a structure closely related to rubrofusarin (**14**). However, a 3H singlet due to the methoxyl group, which was observed in the  $^1\text{H}$ -NMR spectrum of **1**, was absent in that of **9**. The spectrum also showed two doublets of anomeric protons at  $\delta$  4.95 and  $\delta$  4.23 (1H each,  $J=7.5$  Hz) of the sugar moiety. The  $^{13}\text{C}$ -NMR spectrum of **9** showed similarity in the chemical shifts of the sugar carbons to the corresponding carbons of **1**, indicating that the sugar is gentiobiose (see Experimental).

Treatment of **9** with diazomethane gave **1**. The structure of nor-rubrofusarin 6-*O*-gentiobioside was therefore assigned for this compound.

**Structure of Demethylflavasperone Gentiobioside** Compound **10** was obtained as a yellow powder. Its molecular formula  $\text{C}_{27}\text{H}_{32}\text{O}_{15}$ , isomeric to **1**, was indicated by the  $[\text{M}+\text{H}]^+$  ion peak at  $m/z$  597 and the  $[\text{M}+\text{Na}]^+$  ion peak at  $m/z$  619 in its ESI-MS. The  $^1\text{H}$ -NMR spectrum of **10** showed two 1H singlets ( $\delta$  6.91,  $\delta$  6.46), two *meta*-coupled doublets [ $\delta$  6.89 and  $\delta$  6.79 (1H each,  $J=2$  Hz)], a broad singlet due to a chelated hydroxyl group ( $\delta$  12.90), and two 3H singlets [ $\delta$  3.86 (OCH<sub>3</sub>),  $\delta$  2.52 (CH<sub>3</sub>)]. The spectrum also showed two doublets [ $\delta$  5.12, 4.18 (1H each,  $J=8$  Hz)] attributable to the anomeric protons of its gentiobiose residue, and the presence of the gentiobiose residue in the molecule was substantiated by its  $^{13}\text{C}$ -NMR spectrum (Experimental).

Although the coupling patterns for the aglycone proton signals are analogous to the corresponding signals of **1**, two singlets at lower field and the singlet from the chelated hydroxyl group showed distinctive changes in their chemical shifts [ $\delta$  6.91, 6.46 (**10**) $\leftarrow$  $\delta$  7.16, 6.17 (**1**);  $\delta$  12.90 (**10**) $\leftarrow$  $\delta$  14.85 (**1**)]. These changes suggest an "angular" ring system for **10**. The NOESY spectrum of **10** showed two sets of sequence,  $\delta$  2.52 (CH<sub>3</sub> at C-2) $\rightarrow$  $\delta$  6.46 (H-3) and  $\delta$  12.90 (OH at C-5) $\rightarrow$  $\delta$  6.91 (H-6) $\rightarrow$  $\delta$  6.89 (H-7) $\rightarrow$  $\delta$  3.86 (OCH<sub>3</sub> at C-8) $\rightarrow$  $\delta$  6.79 (H-9) (see formula), which supported the "angular" structure for the aglycone. In fact, the chemical shifts of the aglycone protons are comparable with those of demethylflavasperone apioglucoside.<sup>14)</sup> The location of the gentiobiosyl residue at C-10 on the aglycone was also indicated by a correlation  $\delta$  6.79 (Agl H-9) $\rightarrow$  $\delta$  5.12 (H-1 of Glc-I at C-10) in this NOESY spectrum.

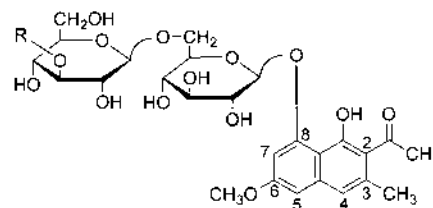
The structure **10** thus assigned for this compound was verified by HMBC correlations shown in Table 2. The correlation between  $\delta_{\text{H}}$  5.12 (H-1 of Glc-I) and  $\delta_{\text{C}}$  156.3 (Agl C-10) observed in the HMBC spectrum also satisfied the location of the sugar residue on the aglycone.

**Structure of Torachryson Glycosides** Compound **11** was obtained as colorless needles. The ESI-MS showed an  $[\text{M}+\text{H}]^+$  ion peak at  $m/z$  571 and an  $[\text{M}+\text{NH}_4]^+$  ion peak at  $m/z$  588, which correspond to the molecular formula  $\text{C}_{26}\text{H}_{34}\text{O}_{14}$ . The UV maxima at 236, 263, 310, 324 and 339 nm suggest that this compound has a naphthalene such as torachryson (**15**).<sup>15)</sup> The presence of the torachryson structure in the molecule of **11** was shown by an aromatic singlet [ $\delta$  7.07 (1H, H-4)], two doublets [ $\delta$  7.05, 6.88 (1H each,  $J=2$  Hz, H-7, H-5)], three 3H singlets [ $\delta$  3.82 (OCH<sub>3</sub> at C-6), 2.49 (COCH<sub>3</sub> at C-2), 2.21 (CH<sub>3</sub> at C-3)] and a broad singlet of chelated hydroxyl group [ $\delta$  9.45 (br s, OH at C-1)] in the  $^1\text{H}$ -NMR spectrum. The spectrum also showed two doublets of anomeric protons [ $\delta$  5.09, 4.20 (1H each,  $J=8$  Hz)] in the sugar residue. Although these  $^1\text{H}$ -NMR spectral data were similar to those for torachryson xyloglucoside,<sup>16)</sup> the chemical shifts of the sugar carbons in the  $^{13}\text{C}$ -NMR spectrum of **11** were comparable with the corresponding carbons of **1**, rather than those of the xyloglucoside. Therefore the structure of torachryson 8-*O*-gentiobioside (**11**) was assigned for this compound. The location of the gentiobiose moiety on the aglycone was confirmed by its HMBC spectrum which showed a correlation between H-1 of glucose I ( $\delta_{\text{H}}$  5.09) and C-8 of the torachryson moiety ( $\delta_{\text{C}}$  155.4).

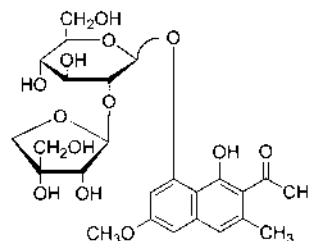
Table 2.  $^{13}\text{C}$  Chemical Shifts and Protons Correlated with the Carbon Signals in the  $^1\text{H}$  Detected Heteronuclear Multiple Quantum Coherence (HMQC) and HMBC Spectra of **10**

Carbon	$\delta_{\text{C}}$	Proton coupled via one bond ( $\delta_{\text{H}}$ )	Proton coupled via two or three bonds
<b>Aglycone</b>			
C-2	168.6		Agl H-3, $\text{CH}_3$ at C-2
C-3	109.6	6.46	$\text{CH}_3$ at Agl C-2
C-4	182.6		Agl H-3
C-4a	108.3		Agl H-3, H-6, OH at C-5
C-5	155.8		Agl H-6, OH at C-5
C-6	105.2	6.91	Agl H-7, OH at C-5
C-6a	140.5		Agl H-6
C-7	99.8	6.89	Agl H-6, H-9
C-8	161.4		Agl $\text{OCH}_3$ at C-8, H-7, H-9
C-9	100.3	6.79	Agl H-7
C-10	156.3		Agl H-9, Glc-I H-1
C-10a	104.4		Agl H-6, H-7, H-9
C-10b	155.4		
$\text{CH}_3$ at C-2	20.1	2.52	Agl H-3
$\text{OCH}_3$ at C-8	55.7	3.86	
<b>Glucose-I</b>			
C-1	100.6	5.12	
C-2	73.9	3.48	Glc-I H-1
C-3	77.0	3.33	Glc-I H-1
C-4	69.8	3.26	Glc-I H-3
C-5	75.7	3.73	Glc-I H-1
C-6	68.9	3.97	Glc-II H-1
<b>Glucose-II</b>			
C-1	103.8	4.18	Glc-I H-6, Glc-II H-2
C-2	73.8	2.96	Glc-II H-1
C-3	76.9	3.11	Glc-II H-1
C-4	70.3	3.02	Glc-II H-3
C-5	77.2	3.03	Glc-II H-1
C-6	61.3	3.38, 3.64	

500 MHz for  $^1\text{H}$ , and 126 MHz for  $^{13}\text{C}$ , in  $\text{DMSO}-d_6$  at  $27^\circ\text{C}$ .



**11**: R = H  
**12**: R = GB



**13**  
Chart 3

able to an apiofuranose residue [ $\delta$  108.7 (C-1), 77.3 (C-2), 79.5 (C-3), 74.2 (C-4), 64.6 (C-5)], along with glucopyranose carbons [ $\delta$  98.0 (C-1), 77.5 (C-2), 75.8 (C-3), 70.2 (C-4), 76.3 (C-5), 60.8 (C-6)]. These chemical shifts were almost the same as those of the apioglucoside moiety of isoliquiritin apioside.<sup>17)</sup> The structure of torachryson apioglucoside (**13**) was therefore assigned for this compound.

**Antibacterial Activity of Phenolic Glycosides and Related Compounds of Cassia Seeds** Nosocomial infection due to MRSA is a serious problem in hospitals, and development of new antibacterial substances against MRSA is important. Since the antibacterial activity of the phenolic constituents of *Cassia obtusifolia* was reported,<sup>2)</sup> the effects of the phenolic glycosides isolated from *Cassia tora*, their aglycones, and some other phenolics with related structure including those reported as constituents of *Cassia* species on MRSA were examined.

The results are summarized in Table 3. Their effects on two other bacterial species including *Pseudomonas aeruginosa*, which often cause opportunistic infection, are also shown.

Although the tested glycosides isolated from the *Cassia tora* seeds showed negligible antibacterial effects on the bacterial strains, unglycosylated naphthalenes (including naphthopyrones), torachryson (**15**) and toralactone (**16**), and anthraquinones, aloe-emodin (**18**), rhein (**19**) and emodin (**20**), showed significant antibacterial activity against four strains of MRSA and also a strain of methicillin-sensitive *Staphylococcus aureus* (MSSA). Physcion (**21**) showed no effects on MRSA and MSSA at  $128\ \mu\text{g}/\text{ml}$ . Among the tested compounds, aloe-emodin (**18**), which is also a constituent of *Cassia* species,<sup>2)</sup> showed the most potent antibacterial effects on the strains of MRSA and MSSA [minimum inhibition concentration (MIC),  $2\ \mu\text{g}/\text{ml}$ ]. On the other hand, they showed no effects on *Pseudomonas aeruginosa* PAO1 at the concentrations examined. Torachryson (**15**) and rhein (**19**) showed

Compound **12** was obtained as a pale-yellow powder. The UV spectrum of **12**, which was similar to **11**, was attributable to the torachryson structure in the molecule. The presence of torachryson (**15**) in the molecule was evidenced by acid hydrolysis of **12** which gave **15** along with glucose. The molecular mass 894 of **12**, which is shown by an  $[\text{M}+\text{H}]^+$  ion peak at  $m/z$  895 on the ESI-MS, is 324 mass units higher than that of **11**, suggesting that **12** is a tetraglycoside of torachryson. The  $^1\text{H}$ -NMR spectrum of **12** showed four anomeric protons [ $\delta$  5.09, 4.34, 4.33, 4.17 (1H each,  $J=7.5\ \text{Hz}$ )], along with the signals of the torachryson residue [ $\delta$  7.07 (1H, s, H-4), 7.04, 6.88 (1H each, d,  $J=2\ \text{Hz}$ , H-7, H-5), 3.82 (3H, s,  $\text{OCH}_3$  at C-6), 2.49 (3H, s,  $\text{COCH}_3$  at C-2), 2.21 (3H, s,  $\text{CH}_3$  at C-3), 9.45 (br s, OH at C-1)]. The chemical shifts of the sugar carbon signals in the  $^{13}\text{C}$ -NMR spectrum of **12** were in agreement with those of the corresponding carbons of **8**. The structure of torachryson tetraglycoside (**12**) thus assigned for this compound was substantiated by formation of **11** upon the treatment of **12** with  $\beta$ -glucosidase.

Compound **13** was obtained as a pale-yellow powder. The ESI-MS showed an  $[\text{M}+\text{H}]^+$  ion peak at  $m/z$  541, corresponding to the molecular formula  $\text{C}_{25}\text{H}_{32}\text{O}_{13}$ . Although the chemical shifts of the  $^1\text{H}$ -NMR signals of the aglycone in the molecule of **13** (Experimental) were similar to those of **11**, the sugar protons showed a different signal pattern from that of **11**. The  $^{13}\text{C}$ -NMR spectrum of **13** showed signals attribut-

Table 3. Antibacterial Activity of Phenolic Glycosides Isolated from *Cassia tora* Seeds, Their Aglycones and Some Other Phenolic Compounds with Related Structures

Compound	MIC <sup>a)</sup> ( $\mu\text{g/ml}$ ) for bacterial species/strains						
	<i>Escherichia coli</i> K12	<i>Pseudomonas aeruginosa</i> PAO1	MSSA 209P	MRSA			
				OM481	OM505	OM584	OM623
Phenolic glycosides							
<b>1</b>	>1024	>1024	>1024	>1024	>1024	>1024	>1024
<b>2</b>	>1024	>1024	>1024	>1024	>1024	>1024	>1024
<b>4</b>	>1024	>1024	>1024	>1024	>1024	>1024	>1024
<b>7</b>	>1024	>1024	>1024	>1024	>1024	>1024	>1024
<b>11</b>	>1024	>1024	>1024	>1024	>1024	>1024	>1024
<b>12</b>	>1024	>1024	>1024	>1024	>1024	>1024	>1024
Naphthalenes/Naphthopyrones							
<b>14</b>	1024	>1024	256	256	256	256	256
<b>15</b>	512	>1024	32	32	32	32	32
<b>16</b>	>128	>128	64	64	64	64	64
Anthraquinones							
<b>17</b>	1024	>1024	512	256	256	256	256
<b>18</b>	>128	>128	2	2	2	2	2
<b>19</b>	128	>128	32	32	32	16	32
<b>20</b>	>128	>128	64	64	64	64	64
<b>21</b>	>128	>128	>128	>128	>128	>128	>128

a) Values with the sign ">" indicate the highest concentrations tested which did not inhibit the bacterial growth.

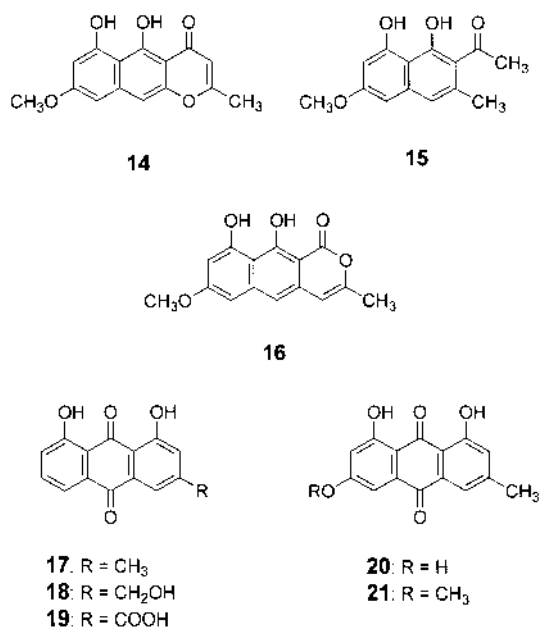


Chart 4

antibacterial activity against *Escherichia coli* K12 with MIC values of 512 and 128  $\mu\text{g/ml}$ , respectively.

The presence of phenolic glycosides in the seeds should not be neglected even if they did not show direct antibacterial effect, since phenolic glycosides are considered to be, at least in part, converted to their aglycones in the intestines on the medicinal use of cassia seeds. Further study on the effects of combination<sup>18)</sup> of antibiotics with phenolic compounds, to find candidates for lead compounds effective against MRSA, is now in progress.

#### Experimental

Optical rotations were measured on a JASCO DIP-1000 spectropolarimeter. UV spectra were recorded on a Hitachi U-2001 spectrometer. ESI-MS

were measured on a Micromass AutoSpec OA-Tof spectrometer, and 50% aqueous MeOH containing 0.1% sodium acetate as a solvent. NMR spectra were recorded on a Varian VXR-500 (500 MHz for <sup>1</sup>H and 126 MHz for <sup>13</sup>C) or a Varian VXR-200 (200 MHz for <sup>1</sup>H and 50.1 MHz for <sup>13</sup>C) instrument in dimethylsulfoxide (DMSO)-d<sub>6</sub> or DMSO-d<sub>5</sub> containing D<sub>2</sub>O at 27 °C, unless otherwise mentioned. Chemical shifts are given in  $\delta$  (ppm) values based on those of solvent signals ( $\delta_{\text{H}}$  2.48 and  $\delta_{\text{C}}$  39.7 for DMSO-d<sub>6</sub>). High-performance liquid chromatography (HPLC) was conducted on a YMC-pack ODS AQ-302 or on an A-302 column (4.6 $\times$ 150 mm) with 0.01 M H<sub>3</sub>PO<sub>4</sub>-0.01 M KH<sub>2</sub>PO<sub>4</sub>-MeCN (39:39:22) at 40 °C. The flow rate was 1.0 ml/min and a UV detector was set at 280 nm. Preparative HPLC was conducted on an Inertsil SIL 100-5 (10 $\times$ 250 mm, GL Science) using a mixture of hexane-MeOH-tetrahydrofuran (6:3:1) as a solvent with a flow rate of 3.0 ml/min. Gas liquid chromatography (GLC) was conducted on a column packed with 2% OV-17 on Chromosorb W (3 $\times$ 2050 mm) using He as a carrier gas (flow rate, 30 ml/min). Injection and column temperatures were set at 210 °C and 190 °C, respectively. DCCC was performed on an apparatus which consists of 95 glass tubes (3.2 $\times$ 1200 mm) connected with Junflon tubes. The solvent system used was BuOH-PrOH-H<sub>2</sub>O (4:1:5).

**Isolation of Phenolic Compounds from *Cassia tora* Seeds** Dried seeds of *Cassia tora* (cultivated in Kwan-si, China) were purchased from Tochimoto-tenkai-do (Osaka, Japan). Crushed seeds (500 g) were soaked in MeOH (500 ml $\times$ 3), and the MeOH solution was concentrated. The residue (30 g) thus obtained was partitioned between water and Et<sub>2</sub>O. The aqueous layer was then extracted with EtOAc and BuOH, successively. Each solvent was evaporated off to give Et<sub>2</sub>O (0.3 g), EtOAc (2.1 g), BuOH (8.5 g) and aqueous (19.8 g) extracts. A part (4.5 g) of the BuOH extract was subjected to column chromatography on Diaion HP-20 (Mitsubishi Chemical Industries) with increasing concentrations of MeOH in water, to separate 19 fractions. Combined fractions 12, 13 were further purified by column chromatography on Toyopearl HW-40F (Toso), MCI-gel CHP-20P (Mitsubishi) and YMC-gel ODS-AQ 120-S50 (YMC) to give rubrofusarin gentiobioside (**1**) (19.5 mg), cassiaside C (**2**) (10.1 mg), cassiaside (**3**) (4.0 mg), nor-rubrofusarin gentiobioside (**9**) (2.6 mg), demethylflavasperone gentiobioside (**10**) (2.6 mg), torachryson gentiobioside (**11**) (37.3 mg) and torachryson apiglucoside (**13**) (2.9 mg).

The aqueous extract was chromatographed over Diaion HP-20 with aqueous MeOH to give 30 fractions. Combined fractions 11-15 (450 mg) was further purified by column chromatography on YMC-gel ODS-AQ 120-S50 to give torosachryson gentiobioside (**5**) (32.8 mg), rubrofusarin tetraglucoside (**8**) (6.3 mg) and torachryson tetraglucoside (**12**) (21.2 mg). DCCC of fractions 16-19 with the ascending mode followed by column chromatography on YMC-gel ODS-AQ 120-S50 and preparative HPLC gave rubrofusarin gentiobioside (**1**) (111.7 mg), cassiaside C (**2**) (7.2 mg), chryso-

phanol tetraglucoside (**4**) (16.0 mg), torachrysonone gentiobioside (**5**) (15.9 mg), cassiaside **C**<sub>2</sub> (**6**) (3.5 mg), rubrofusarin triglucoside (**7**) (19.4 mg), rubrofusarin tetraglucoside (**8**) (38.8 mg), nor-rubrofusarin gentiobioside (**9**) (9.0 mg), demethylflavasiperone gentiobioside (**10**) (11.2 mg) and torachrysonone tetraglucoside (**12**) (148.7 mg). Column chromatography of fractions 20–24 on Toyopearl HW-40 and YMC-gel ODS-AQ 120-S50 afforded rubrofusarin gentiobioside (**1**) (92.7 mg), cassiaside **C** (**2**) (52.5 mg), chrysophanol tetraglucoside (**4**) (16.6 mg) and rubrofusarin tetraglucoside (**8**) (60.0 mg).

**Rubrofusarin Triglucoside (7)** Colorless needles, mp 198 °C (from MeOH).  $[\alpha]_D^{20}$   $-16.6^\circ$  ( $c=0.1$ , 50% MeOH). ESI-MS  $m/z$ : 776 ( $[M+NH_4]^+$ ), 759 ( $[M+H]^+$ ), 597 ( $[M-Glc+H]^+$ ), 273 ( $[Agl+H]^+$ ). High-resolution ESI-MS  $m/z$ : 759.2301 ( $[M+H]^+$ ). Calcd for  $C_{33}H_{42}O_{20}+H$ , 759.2348. UV  $\lambda_{max}^{MeOH}$  nm (log  $\epsilon$ ): 223 (4.49), 258 (sh, 4.51), 276 (4.67), 322 (3.67), 390 (3.79).  $^1H$ -NMR  $\delta$ : 2.38 (3H, s, CH<sub>3</sub> at Agl C-2), 3.86 (3H, s, OCH<sub>3</sub> at Agl C-8), 4.07 (1H, d,  $J=10.5$  Hz, Glc-I H-6), 4.28 (1H, d,  $J=7.5$  Hz, Glc-III H-1), 4.31 (1H, d,  $J=7.5$  Hz, Glc-II H-1), 5.04 (1H, d,  $J=7.5$  Hz, Glc-I H-1), 6.18 (1H, s, Agl H-3), 6.78 (1H, d,  $J=2$  Hz, Agl H-7), 6.93 (1H, d,  $J=2$  Hz, Agl H-9), 7.18 (1H, s, Agl H-10), 14.86 (1H, br s, OH at Agl C-5).  $^{13}C$ -NMR, see Table 1.

**Rubrofusarin Tetraglucoside (8)** Pale-yellow needles, mp 211 °C (from MeOH).  $[\alpha]_D^{20}$   $-22.2^\circ$  ( $c=0.1$ , H<sub>2</sub>O). Anal. Calcd for  $C_{39}H_{52}O_{25} \cdot 3H_2O$ : C, 48.05; H, 5.95. Found: C, 47.94; H, 6.01. ESI-MS  $m/z$ : 943 ( $[M+Na]^+$ ), 921 ( $[M+H]^+$ ), 273 ( $[Agl+H]^+$ ). UV  $\lambda_{max}^{MeOH}$  nm (log  $\epsilon$ ): 223 (4.36), 257 (sh, 4.32), 276 (4.54), 322 (3.41), 394 (3.64).  $^1H$ -NMR  $\delta$ : 2.37 (3H, s, CH<sub>3</sub> at Agl C-2), 3.86 (3H, s, OCH<sub>3</sub> at Agl C-8), 3.98 (1H, d,  $J=10.5$  Hz, Glc-I H-6), 4.12 (1H, d,  $J=10.5$  Hz, Glc-III H-6), 4.16 (1H, d,  $J=7.5$  Hz, Glc-IV H-1), 4.31 (1H, d,  $J=7.5$  Hz, Glc-III H-1), 4.32 (1H, d,  $J=7.5$  Hz, Glc-II H-1), 5.04 (1H, d,  $J=7.5$  Hz, Glc-I H-1), 6.18 (1H, s, Agl H-3), 6.78 (1H, d,  $J=2$  Hz, Agl H-7), 6.92 (1H, d,  $J=2$  Hz, Agl H-9), 7.18 (1H, s, Agl H-10), 14.86 (1H, br s, OH at Agl C-5).  $^{13}C$ -NMR, see Table 1.

**Enzymatic Hydrolysis of Rubrofusarin Triglucoside (7) and Rubrofusarin Tetraglucoside (8) with  $\beta$ -Glucosidase** Compound **7** (0.5 mg) in water (0.5 ml) was treated with  $\beta$ -glucosidase (Sigma) for 5 h to give a yellow precipitate. The precipitate was identified as rubrofusarin (**14**) by its UV and  $^1H$ -NMR spectra. The supernatant was analyzed by HPLC, to show the formation of rubrofusarin gentiobioside (**1**) [retention time ( $t_R$ ), 8.8 min]. Compound **8** (10 mg) in water (2 ml) was treated with the enzyme in an analogous way, and the precipitate formed (0.5 mg) was identified as rubrofusarin (**14**) by its  $^1H$ -NMR spectrum. The supernatant was subjected to chromatography on SepPak C18 cartridge with H<sub>2</sub>O–MeOH, and the MeOH eluate was further purified by preparative HPLC to give rubrofusarin gentiobioside (**1**) (1.6 mg) and rubrofusarin triglucoside (**7**) (2.7 mg), which were identified by their  $^1H$ -NMR spectra.

**Acid Hydrolysis of Rubrofusarin Tetraglucoside (8)** A solution of **8** (4 mg) in 1% H<sub>2</sub>SO<sub>4</sub> (0.2 ml) in a sealed tube was heated on a boiling water bath for 1 h. The solution was then extracted with EtOAc, and concentration of the extract gave rubrofusarin (**14**) (1.2 mg), which was identified by its  $^1H$ -NMR spectrum. The aqueous layer was neutralized with Diaion SA-20AP (Mitsubishi Chemical), and evaporated. Treatment of the residue with a mixture of trimethylchlorosilane, hexamethyldisilazane and pyridine (1 : 2 : 10, by volume) followed by GLC analysis showed the presence of glucose in the residue.

**Nor-rubrofusarin Gentiobioside (9)** A yellow powder,  $[\alpha]_D^{20}$   $-45.0^\circ$  ( $c=0.1$ , pyridine). ESI-MS  $m/z$ : 605 ( $[M+Na]^+$ ), 583 ( $[M+H]^+$ ), 421 ( $[M-Glc+H]^+$ ), 259 ( $[Agl+H]^+$ ). High-resolution ESI-MS  $m/z$ : 583.1693 ( $[M+H]^+$ ). Calcd for  $C_{26}H_{30}O_{15}+H$ , 583.1663. UV  $\lambda_{max}^{MeOH}$  nm (log  $\epsilon$ ): 223 (4.13), 254 (4.15), 278 (4.33), 327 (3.26), 399 (3.45).  $^1H$ -NMR  $\delta$ : 2.35 (3H, s, CH<sub>3</sub> at Agl C-2), 4.01 (1H, dd,  $J=1.5, 12$  Hz, Glc-I H-6), 4.23 (1H, d,  $J=7.5$  Hz, Glc-II H-1), 4.95 (1H, d,  $J=7.5$  Hz, Glc-I H-1), 6.14 (1H, s, Agl H-3), 6.71 (1H, d,  $J=2$  Hz, Agl H-9), 6.77 (1H, d,  $J=2$  Hz, Agl H-7), 7.04 (1H, s, Agl H-10), 14.90 (1H, br s, OH at Agl C-5).  $^{13}C$ -NMR  $\delta$ : 20.3 (CH<sub>3</sub> at Agl C-2), 61.1 (Glc-II C-6), 68.4 (Glc-I C-6), 69.6 (Glc-I C-4), 70.2 (Glc-II C-4), 73.6 (Glc-I C-2), 73.8 (Glc-II C-2), 76.1 (Glc-I C-5), 76.5 (Glc-I C-3), 76.6 (Glc-II C-3), 77.0 (Glc-II C-5), 100.1 (Agl C-10), 101.4 (Glc-I C-1), 101.8 (Agl C-7), 102.8 (Agl C-9), 103.2 (Agl C-4a), 103.5 (Glc-II C-1), 106.7 (Agl C-3), 107.0 (Agl C-5a), 140.6 (Agl C-9a), 152.5 (Agl C-10a), 158.3 (Agl C-8), 159.9 (Agl C-6), 162.3 (Agl C-5), 168.8 (Agl C-2), 183.9 (Agl C-4).

**Methylation of Nor-rubrofusarin Gentiobioside (9)** Ethereal diazomethane (0.4 ml) was added to a solution of **9** (1.5 mg) in MeOH (0.1 ml). The solution was left to stand for 30 min, and the solvent was removed by N<sub>2</sub> stream. The residue was purified with SepPak C18 using H<sub>2</sub>O–MeOH to give rubrofusarin gentiobioside (**1**) (1.1 mg), which was identified by  $^1H$ -

NMR.

**Demethylflavasiperone Gentiobioside (10)** A yellow powder,  $[\alpha]_D^{20}$   $-14.0^\circ$  ( $c=0.1$ , pyridine). ESI-MS  $m/z$ : 619 ( $[M+Na]^+$ ), 597 ( $[M+H]^+$ ), 435 ( $[M-Glc+H]^+$ ), 273 ( $[Agl+H]^+$ ). High-resolution ESI-MS  $m/z$ : 597.1804 ( $[M+H]^+$ ). Calcd for  $C_{27}H_{32}O_{15}+H$ , 597.1819. UV  $\lambda_{max}^{MeOH}$  nm (log  $\epsilon$ ): 240 (4.49), 248 (sh, 4.39), 279 (4.35), 313 (sh, 3.84), 367 (3.50).  $^1H$ -NMR  $\delta$ : 2.52 (3H, s, CH<sub>3</sub> at Agl C-2), 3.86 (3H, s, OCH<sub>3</sub> at Agl C-8), 3.97 (1H, d,  $J=10.5$  Hz, Glc-I H-6), 4.18 (1H, d,  $J=8$  Hz, Glc-II H-1), 5.12 (1H, d,  $J=8$  Hz, Glc-I H-1), 6.46 (1H, s, Agl H-3), 6.79 (1H, d,  $J=2.5$  Hz, Agl H-9), 6.89 (1H, d,  $J=2.5$  Hz, Agl H-7), 6.91 (1H, s, Agl H-6), 12.90 (1H, br s, OH at Agl C-5).  $^{13}C$ -NMR, see Table 2.

**Torachrysonone Gentiobioside (11)** Colorless needles, mp 221 °C (from MeOH).  $[\alpha]_D^{20}$   $-18.7^\circ$  ( $c=0.1$ , MeOH). ESI-MS  $m/z$ : 588 ( $[M+NH_4]^+$ ), 571 ( $[M+H]^+$ ), 409 ( $[M-Glc+H]^+$ ), 247 ( $[Agl+H]^+$ ). High-resolution ESI-MS  $m/z$ : 571.2023 ( $[M+H]^+$ ). Calcd for  $C_{26}H_{34}O_{14}+H$ , 571.2027. UV  $\lambda_{max}^{MeOH}$  nm (log  $\epsilon$ ): 236 (4.38), 263 (4.19), 310 (3.59), 324 (3.59), 339 (3.59).  $^1H$ -NMR  $\delta$ : 2.21 (3H, s, CH<sub>3</sub> at Agl C-3), 2.49 (3H, s, COCH<sub>3</sub> at Agl C-2), 3.82 (3H, s, OCH<sub>3</sub> at Agl C-6), 4.00 (1H, d,  $J=12$  Hz Glc-I H-6), 4.20 (1H, d,  $J=8$  Hz, Glc-II H-1), 5.09 (1H, d,  $J=8$  Hz, Glc-I H-1), 6.88 (1H, d,  $J=2.0$  Hz, Agl H-5), 7.05 (1H, d,  $J=2.0$  Hz, Agl H-7), 7.07 (1H, s, Agl H-4), 9.45 (1H, br s, OH at Agl C-1).  $^{13}C$ -NMR  $\delta$ : 19.7 (CH<sub>3</sub> at Agl C-3), 32.4 (COCH<sub>3</sub> at Agl C-2), 55.6 (OCH<sub>3</sub> at Agl C-6), 61.3 (Glc-II C-6), 69.1 (Glc-I C-6), 70.1 (Glc-I C-4), 70.3 (Glc-II C-4), 73.5 (Glc-I C-2), 73.7 (Glc-II C-2), 76.1 (Glc-I C-5), 76.4 (Glc-I C-3), 76.9 (Glc-II C-3), 77.1 (Glc-II C-5), 101.3 (Agl C-5), 102.5 (Glc-I C-1), 103.3 (Agl C-7), 103.9 (Glc-II C-1), 108.8 (Agl C-9), 119.0 (Agl C-4), 123.4 (Agl C-2), 133.8 (Agl C-3), 137.0 (Agl C-10), 151.2 (Agl C-1), 155.4 (Agl C-8), 158.6 (Agl C-6), 204.7 (COCH<sub>3</sub> at Agl C-2).

**Torachrysonone Tetraglucoside (12)** A pale-yellow powder.  $[\alpha]_D^{20}$   $-9.0^\circ$  ( $c=0.1$ , pyridine). ESI-MS  $m/z$ : 917 ( $[M+Na]^+$ ), 912 ( $[M+NH_4]^+$ ), 895 ( $[M+H]^+$ ), 733 ( $[M-Glc+H]^+$ ), 571 ( $[M-2 \times Glc+H]^+$ ), 409 ( $[M-3 \times Glc+H]^+$ ), 247 ( $[Agl+H]^+$ ). High-resolution ESI-MS  $m/z$ : 895.3067 ( $[M+H]^+$ ). Calcd for  $C_{38}H_{54}O_{24}+H$ , 895.3083. UV  $\lambda_{max}^{MeOH}$  nm (log  $\epsilon$ ): 236 (4.48), 262 (4.24), 312 (3.64), 325 (3.65), 340 (3.65).  $^1H$ -NMR  $\delta$ : 2.21 (3H, s, CH<sub>3</sub> at Agl C-3), 2.49 (3H, s, COCH<sub>3</sub> at Agl C-2), 3.82 (3H, s, OCH<sub>3</sub> at Agl C-6), 4.01 (1H, d,  $J=10.5$  Hz, Glc-I H-6), 4.12 (1H, d,  $J=10.5$  Hz, Glc-III H-6), 4.17 (1H, d,  $J=7.5$  Hz, Glc-IV H-1), 4.33 (1H, d,  $J=7.5$  Hz, Glc-III H-1), 4.34 (1H, d,  $J=7.5$  Hz, Glc-II H-1), 5.09 (1H, d,  $J=7.5$  Hz, Glc-I H-1), 6.88 (1H, d,  $J=2.0$  Hz, Agl H-5), 7.04 (1H, d,  $J=2.0$  Hz, Agl H-7), 7.07 (1H, s, Agl H-4), 9.45 (1H, br s, OH at Agl C-1).  $^{13}C$ -NMR  $\delta$ : 19.7 (CH<sub>3</sub> at Agl C-3), 32.4 (COCH<sub>3</sub> at Agl C-2), 55.6 (OCH<sub>3</sub> at Agl C-6), 60.9 (Glc-II C-6), 61.1 (Glc-IV C-6), 68.9 (Glc-I C-6), 69.0 (Glc-III C-6, Glc-II C-4), 70.2 (Glc-I C-4), 70.3 (Glc-III C-4), 70.6 (Glc-IV C-4), 72.2 (Glc-II C-2), 73.6 (Glc-I C-2), 73.7 (Glc-III C-2), 74.0 (Glc-IV C-2), 75.2 (Glc-III C-5), 76.1.76.2 (Glc-I C-5, Glc-II C-5, Glc-IV C-5), 76.2 (Glc-II C-5), 76.3 (Glc-I C-3), 76.4 (Glc-IV C-3), 77.0 (Glc-III C-3), 89.0 (Glc-II C-3), 101.4 (Agl C-5), 102.6 (Glc-I C-1), 102.9 (Glc-II C-1), 103.5 (Agl C-7), 103.0 (Glc-IV C-1), 104.3 (Glc-III C-1), 108.8 (Agl C-9), 119.1 (Agl C-4), 123.4 (Agl C-2), 133.9 (Agl C-3), 137.0 (Agl C-10), 151.2 (Agl C-1), 155.4 (Agl C-8), 158.6 (Agl C-6), 204.8 (COCH<sub>3</sub> at Agl C-2).

**Hydrolysis of Torachrysonone Tetraglucoside (12)** 1) An aqueous solution (0.5 ml) of **12** (0.5 mg) was treated with  $\beta$ -glucosidase for 24 h, to give a pale-yellow precipitate, which was identified as torachrysonone (**20**) by its  $^1H$ -NMR spectrum. The supernatant was analyzed by HPLC, to show the presence of torachrysonone gentiobioside (**11**) ( $t_R$ , 11.4 min) in the reaction mixture.

2) Compound **12** (3.0 mg) in 1% H<sub>2</sub>SO<sub>4</sub> (0.2 ml) was heated on a boiling water bath for 15 min. The reaction mixture was extracted with EtOAc, to give torachrysonone (**20**) (0.7 mg), which was identified by its  $^1H$ -NMR spectrum. The remaining aqueous layer was neutralized with Diaion SA-20AP, and evaporated. The residue was trimethylsilylated and analyzed by GLC, to show the presence of glucose in the reaction mixture.

**Torachrysonone Apioglucooside (13)** A pale-yellow powder,  $[\alpha]_D^{20}$   $-23.4^\circ$  ( $c=0.1$ , pyridine). ESI-MS  $m/z$ : 563 ( $[M+Na]^+$ ), 541 ( $[M+H]^+$ ), 409 ( $[M-apiose (Api)+H]^+$ ), 247 ( $[Agl+H]^+$ ). UV  $\lambda_{max}^{MeOH}$  nm (log  $\epsilon$ ): 233 (4.41), 262 (4.09), 306 (3.58), 323 (3.59), 337 (3.64).  $^1H$ -NMR  $\delta$ : 2.21 (3H, s, CH<sub>3</sub> at Agl C-3), 2.50 (3H, s, COCH<sub>3</sub> at Agl C-2), 3.99 (3H, s, OCH<sub>3</sub> at Agl C-6), 5.03 (1H, d,  $J=7.5$  Hz, Glc H-1), 5.36 (1H, s, Api H-1), 6.63 (1H, d,  $J=2.0$  Hz, Agl H-5), 6.89 (1H, d,  $J=2.0$  Hz, Agl H-7), 7.00 (1H, s, Agl H-4), 9.68 (1H, br s, OH at Agl C-1).  $^{13}C$ -NMR, see text.

**Preparation of Unglycosylated Phenolics** Seeds of *Cassia tora* (8 kg) were successively extracted with hexane, benzene and MeOH at room temperature, and each solvent was evaporated off. A part (10 g) of the MeOH extract (460 g) was suspended in 5% H<sub>2</sub>SO<sub>4</sub> (100 ml), and heated on a boil-

ing water bath for 2 h. The reaction mixture was extracted with EtOAc (100 ml×3), and the solvent was evaporated off. A part (2.3 g) of the residue (2.5 g) was chromatographed over silica gel with benzene→EtOAc→MeOH, to give torachryson (20) (13.3 mg), chrysophanol (21) (33.4 mg) and a mixture containing rubrofusarin (14). This mixture was further purified by preparative HPLC to give 14 (11.2 mg). Toralactone (16) was obtained upon acid hydrolysis of cassiaside C (6). Chrysophanol (17), aloe-emodin (18), rhein (19) and physcion (21) were isolated from a commercial rhubarb. Emodin (20) was purchased from Sigma.

**Estimation of Antibacterial Activity** Bacterial strains used in this experiment were stored and subcultured in the laboratory of the Department of Microbiology. MICs were evaluated by a liquid dilution method. Mixtures containing aliquots of precultured bacterial solution (final concentration, 10<sup>4</sup> colony forming unit/well) and serial two-fold dilution of tested compounds were incubated at 32 °C for 24 h on 96-well plates. The lowest concentration of a tested compound which did not show visual turbidity due to bacterial growth was regarded as MIC.

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