# Antiallergic Agent from Natural Sources. Structures and Inhibitory Effect of Histamine Release of Naphthopyrone Glycosides from Seeds of *Cassia obtusifolia* L.

Susumu KITANAKA,* Toshimitu NAKAYAMA, Takeshi SHIBANO, Emika OHKOSHI, and Michio TAKIDO

*College of Pharmacy, Nihon University, 7–1 Narashinodai, 7-Chome, Funabashi-shi, Chiba 274–8555, Japan.*
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**Two new naphthopyrones, cassiasides $B_2$ (1) and $C_2$ (2), were isolated from the seeds (Cassiae Semen) of *Cassia obtusifolia* L. The structures of the two new compounds 1 and 2 were established as rubrofusarin 6-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranoside and toralactone 9-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranoside, respectively, on the basis of spectral and chemical evidence.**

**Compound 2 was found to inhibit the histamine release from rat peritoneal exudate mast cells induced by antigen–antibody reaction.**

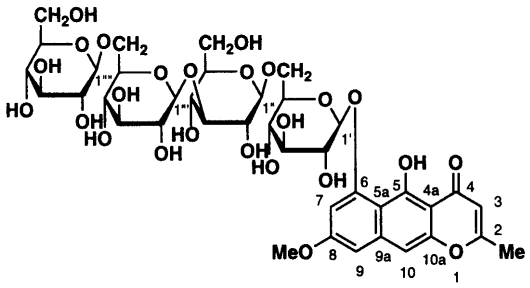
**Key words** *Cassia obtusifolia*; naphthopyrone glycoside; cassiaside $B_2$; cassiaside $C_2$; antiallergic agent; histamine release inhibitor

The seeds (Cassiae Semen) of *Cassia obtusifolia* L. (Leguminosae) have been used as a traditional medicine for constipation, asthenic, eye disease, hepatitis and diuretic agents and also to improve visual acuity in Chinese medicine. As chemical constituents of *Cassia obtusifolia*, we previously reported the isolation of anthraquinone, anthrones, hydroanthracenes, and naphthalenic lactones.[1—8] Repeated chromatographic purification guided by inhibitory effect against the histamine release from rat peritoneal mast cells induced by antigen–antibody reaction led to the isolation of the phenolic glycosides, cassiasides $B_2$ (**1**) and $C_2$ (**2**). The inhibitory effects of extract and each fraction are shown in Experimental.

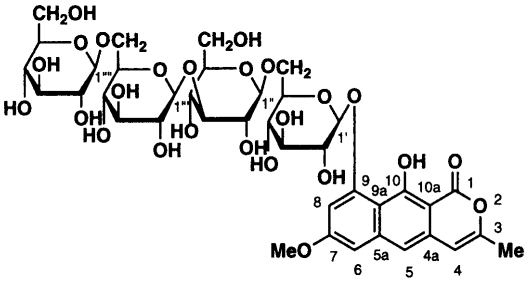
Cassiaside $C_2$ (**2**), yellow needles, mp 195.5—197 °C, $[\alpha]_D$ +5.2°, showed a deep yellow color in sodium hydroxide solution and a strong blue fluorescence under ultraviolet light. The UV spectrum showed maxima at 218, 279, 384 nm, and the IR spectrum exhibited absorption bands due to hydroxyls (3385 $cm^{-1}$), α-pyrone (1660 and 1626 $cm^{-1}$) and aromatic ring (1587 and 1415 $cm^{-1}$). Fragmentation of the FAB-MS of **2** observed at *m/z* 921 $[M+H]^+$ and 273 $[M+H-hexose\times4]^+$ was indicated to be a naphthopyrone tetraglucoside from the spectral properties and its hydrolysis with β-glucosidase. $^1$H-NMR spectrum indicated the presence of one aromatic methyl at δ 2.21 (3H, s, Me-3), one methoxyl at δ 3.88 (3H, s, OMe), a pair of *meta*-coupled protons at δ 6.86 (1H, d, *J*=2.2 Hz, H-8) and 6.90 (1H, d, *J*=2.2 Hz, H-6), two aromatic protons at δ 6.48 (1H, s, H-4) and 7.13 (1H, s, H-5), a chelated hydroxyl proton at δ 12.58 (1H, s, OH-10) and four anomeric protons at δ 4.17 (1H, d, *J*=8.1 Hz, H-1′′′′), 4.31 (1H, d, *J*=7.3 Hz, H-1′′′), 4.32 (1H, d, *J*=8.1 Hz, H-1′′) and 5.09 (1H, d, *J*=8.1 Hz, H-1′). The $^{13}$C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **2** revealed the presence of one methyl, one methoxyl, four methylenes, twenty methines, four aromatic methine groups, and eight quaternary aromatic carbons and one lactonic carbonyl carbon (C-1) (Table 1). On enzymatic hydrolysis with β-glucosidase, **2** afforded toralactone (**2a**)[9] as an aglycone and two progenins, **2b** and **2c**. Compound **2c** was identified as cassiaside C, toralactone 9-*O*-gentiobioside,[7] by direct comparison with an authentic sample. Therefore **2** is a toralactone β-D-tetraglucoside. The attachment of the sugar to the aglycone unit in **2** was confirmed as hydroxyl at position 9 by the existence of a hydrogen bonded proton signal at δ 12.58 (OH-10). In the $^{13}$C-NMR spectrum of **2**, two of the C-6 carbon signals of the glucose appeared at δ 68.6 and 68.9. These two signals together with the C-3 carbon signal of the glucose molecule at δ 88.9 revealed the presence of two 1→6 and one 1→3 linkages among the four glucose molecules in **2**. Compound **2b** exhibited three anomeric protons at δ 4.31, 4.33 and 5.13. In the heteronuclear multiple bond connectivity (HMBC) experiment of **2b**, $^1$H–$^{13}$C long-range correlations were observed between an anomeric proton at δ 5.13 (H-1′) and a carbon at δ 157.5 (C-9), an anomeric proton at δ 4.33 (H-1′′) and a carbon at δ 68.5 (C-6′), and an anomeric proton at δ 4.31 (H-1′′′) and a carbon at δ 88.6 (C-3′′). From these findings, the structure of **2b** was characterized as toralactone 9-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranoside. As the chemical shifts of the carbon signals of the first three glucose units in compound **2**, with the exception of C-5′′′ and C-6′′′, were compatible with those of **2**, the arrangement of these three glucose units should be the same in both compounds. A downfield shift of the C-6′′′ signal to δ 68.9 and an upfield shift of C-5′′′ signal to δ 75.0 clearly confirmed a 1→6 linkage between the terminal and the third glucose units. Therefore, the structure of **2** was elucidated as toralactone 9-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranoside.

Cassiaside $B_2$ (**1**), mp 200—203 °C, $[\alpha]_D$ +11.3° showed its pseudo molecular ion peak at *m/z* 921 in the FAB-MS. The UV spectrum showed maxima at 225, 278, 394 nm and the IR spectrum exhibited absorption bands due to hydroxyl (3392 $cm^{-1}$), γ-pyrone (1680 and 1625 $cm^{-1}$) and aromatic ring (1414 and 1370 $cm^{-1}$). Enzymatic hydrolysis of **1** with β-glucosidase afforded glucose and rubrofusarin (**1a**) as an aglycone. The $^1$H-NMR and $^{13}$C-NMR spectra of **1** displayed signals due to a rubrofusarin unit and four glucosyl units, which were indicated by the signals of an aromatic methyl at

* To whom correspondence should be addressed.



1



2

Chart 1

Table 1. $^{13}$C-NMR Data for Cassiaside $B_2$ (**1**), Cassiaside $C_2$ (**2**), **2c** and **2b** (in DMSO-$d_6$/$D_2O$, 5%)

| | 1 | 2 | 2c | 2b |
|---|---|---|---|---|
| 1 | — | 166.8 | 166.8 | 166.6 |
| 2 | 169.0 | — | — | — |
| 3 | 106.8 | 152.7 | 152.5 | 152.6 |
| 4 | 183.8 | 104.2 | 104.1 | 104.2 |
| 4a | 103.7 | 132.4 | 132.3 | 132.5 |
| 5 | 161.9 | 111.7 | 111.6 | 111.4 |
| 5a | 107.8 | 141.6 | 141.5 | 141.5 |
| 6 | 157.6 | 102.0 | 101.8 | 101.9 |
| 7 | 101.4 | 161.4 | 161.3 | 161.3 |
| 8 | 161.1 | 100.6 | 100.3 | 100.3 |
| 9 | 99.9 | 157.6 | 157.4 | 157.5 |
| 9a | 140.4 | 109.4 | 109.1 | 109.4 |
| 10 | 101.1 | 162.8 | 162.6 | 163.1 |
| 10a | 152.5 | 168.2 | 168.2 | 168.2 |
| Me | 20.3 | 18.8 | 18.8 | 18.7 |
| OMe | 55.6 | 55.6 | 55.4 | 55.5 |
| Sugar moiety | | | | |
| 1′ | 100.9 | 100.5 | 100.9 | 100.4 |
| 2′ | 73.6 | 73.5[a] | 73.5 | 73.3 |
| 3′ | 76.4 | 76.4 | 76.8 | 76.2 |
| 4′ | 69.8 | 69.8[b] | 70.1 | 69.8 |
| 5′ | 75.9[a] | 75.9[c] | 75.5 | 75.8 |
| 6′ | 68.7 | 68.6 | 68.8 | 68.5 |
| 1″ | 102.9 | 102.5 | 102.8 | 102.8 |
| 2″ | 72.0 | 72.3 | 73.5 | 72.2 |
| 3″ | 88.9 | 88.9 | 76.4 | 88.6 |
| 4″ | 68.7 | 68.9[b] | 69.7 | 68.9 |
| 5″ | 75.8[a] | 75.8[c] | 76.1 | 75.8 |
| 6″ | 60.6 | 60.5 | 61.6 | 60.8 |
| 1‴ | 104.0 | 104.1 | | 104.0 |
| 2‴ | 73.6 | 73.6[a] | | 73.7 |
| 3‴ | 76.9 | 76.9 | | 76.8 |
| 4‴ | 70.0 | 70.0 | | 70.0 |
| 5‴ | 75.0 | 75.0 | | 76.0 |
| 6‴ | 68.9 | 68.9 | | 61.0 |
| 1⁗ | 102.5 | 102.9 | | |
| 2⁗ | 73.9 | 73.9 | | |
| 3⁗ | 76.9 | 76.9 | | |
| 4⁗ | 70.4 | 70.4 | | |
| 5⁗ | 76.1 | 76.1 | | |
| 6⁗ | 61.0 | 61.0 | | |

*a*—*c*) Assignments may be interchanged in each column.

$\delta$ 2.30 (3H, s, Me-2), one methoxyl at $\delta$ 3.86 (3H, s, OMe), a pair of *meta*-coupled protons at $\delta$ 6.88 (1H, d, $J$=2.2 Hz, H-7) and 6.92 (1H, d, $J$=2.2 Hz, H-9), two aromatic protons at $\delta$ 6.17 (1H, s, H-3) and 7.18 (1H, s, H-10), a chelated hydroxyl proton at $\delta$ 14.86 (1H, s, OH-5) and four anomeric protons at $\delta$ 4.17 (1H, d, $J$=8.1 Hz, H-1⁗), 4.32 (1H, d, $J$=7.3 Hz, H-1‴), 4.33 (1H, d, $J$=8.1 Hz, H-1″), and 5.05 (1H, d, $J$=8.1 Hz, H-1′) and four anomeric carbons at $\delta$ 100.9, 102.5 102.9 and 104.0. A comparison of the $^{13}$C-NMR data of **1** and cassiaside $C_2$ (**2**) showed that chemical shifts for those sugar moieties were compatible. Further, those 2D-NMR spectra were found to have the same sugar structure. Thus, the structure of **1** was elucidated as rubrofusarin 6-*O*-$\beta$-D-glucopyranosyl-(1→6)-*O*-$\beta$-D-glucopyranosyl-(1→3)-*O*-$\beta$-D-glucopyranosyl-(1→6)-*O*-$\beta$-D-glucopyranoside.

Table 2. Inhibitory Effects of Cassiasides $B_2$ (**1**) and $C_2$ (**2**) Isolated from Seed of *Cassia obtusifolia* in Comparison with Indomethacin on Histamine Release from Mast Cells Induced by Antigen-Antibody Reaction

| Substance | Concentration (M) | *n* | Inhibitory ratio (%) |
|---|---|---|---|
| Cassiaside $B_2$ (**1**) | $10^{-4}$ | 4 | 17.2 |
| | $10^{-5}$ | 4 | 16.0 |
| Cassiaside $C_2$ (**2**) | $10^{-4}$ | 4 | 53.9 |
| | $10^{-5}$ | 4 | 16.0 |
| Indomethacin | $2.5\times10^{-4}$ | 4 | 46.6 |

We have examined the inhibitory activity of cassiasides $B_2$ (**1**) and $C_2$ (**2**) on the histamine release from rat peritoneal exudate cells induced by antigen-antibody reaction. As shown in Table 2, the inhibitory effect of **2** was much higher than that of a potent anti-inflammatory drug, indomethacin. It is interesting that the inhibitory effect on the histamine release is not found by naphtho-$\gamma$-pyrone tetraglucoside, cassiaside $B_2$ (**1**), and is shown only by naphtho-$\alpha$-pyrone tetraglucoside, cassiaside $C_2$ (**2**).

## Experimental

**General Procedures** All melting points were determined with a Yanagimoto micro melting point apparatus and were uncorrected. The UV spectra were recorded on a Hitachi 200-10 spectrophotometer. The NMR spectra were taken on a JEOL JNM GX-400 instrument (400 MHz for $^1$H-NMR); the chemical shifts were given in ppm relative to internal tetramethylsilane (TMS). The MS were obtained on a JEOL JMS-SX102 spectrometer.

**Extraction and Isolation** Crushed seeds (10 kg) of *Cassia obtusifolia* L. were extracted with 80% MeOH (10 l×3). The extract [inhibitory effect, 29.6% (300 $\mu$g/ml)] was concentrated *in vacuo* to give a brown mass (920 g), which was subjected to Diaion HP 20 column chromatography with MeOH–$H_2O$ (0→100) to give frs. A1 [63.0% (300 $\mu$g/ml)], A2 [35.0%], A3 [0%] A4 [100%] and A5 [65.1% (100 $\mu$g/ml)]. Fraction A4 (34.3 g) was chromatographed on silica gel using $CHCl_3$–MeOH–$H_2O$ (65 : 35 : 10, lower phase) to give frs. B1 [38.6% (300 $\mu$g/ml)], B2 [52.1%], B3 [4.9%], B4 [53.1%], B5 [73.7%] and B6 [24.5%]. Fraction B5 (1.25 g) was purified by reversed phase HPLC (Pegasil ODS) using aqueous MeOH to give compounds **1** (137 mg) and **2** (219 mg).

**Cassiaside $B_2$ (1)** Greenish yellow needles ($H_2O$–MeOH), mp 200—203 °C, $[\alpha]_D^{25}$ +11.3° ($c$=0.40, $H_2O$). FAB-MS $m/z$: 921 $[M+H]^+$. UV $\lambda_{max}^{H_2O}$ nm (log $\varepsilon$): 225 (3.08), 278 (3.39), 394 (2.43). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3392, 2893,

1680, 1625, 1414, 1370, 1239, 1206, 1167, 1068, 900, 595. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.30 (3H, s, Me-2), 3.86 (3H, s, OMe), 4.17 (1H, d, $J$=8.1 Hz, H-1''''), 4.32 (1H, d, $J$=7.3 Hz, H-1'''), 4.33 (1H, d, $J$=8.1 Hz, H-1''), 5.05 (1H, d, $J$=8.1 Hz, H-1'), 6.17 (1H, s, H-3), 6.88 (1H, d, $J$=2.2 Hz, H-7), 6.92 (1H, d, $J$=2.2 Hz, H-9), 7.18 (1H, s, H-10), 14.86 (1H, s, OH-5).

**Cassiaside $C_2$ (2)** Yellow needles, mp 195.5—197 °C, $[\alpha]_D^{18}$ +5.2° ($c$=0.5, $H_2O$). FAB-MS $m/z$: 921 $[M+H]^+$, 273 $[M+H\text{-hexose}\times4]^+$. UV $\lambda_{max}^{H_2O}$ nm (log $\varepsilon$): 218 (3.03), 279 (3.58), 384 (2.64). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3385, 1660, 1626, 1587, 1415, 1372, 1261, 1204, 1167, 1069, 907, 843, 578. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.21 (3H, s, Me-3), 3.88 (3H, s, OMe), 4.17 (1H, d, $J$=8.1 Hz, H-1''''), 4.31 (1H, d, $J$=7.3 Hz, H-1'''), 4.32 (1H, d, $J$=8.1 Hz, H-1''), 5.09 (1H, d, $J$=8.1 Hz, H-1'), 6.48 (1H, s, H-4), 6.86 (1H, d, $J$=2.2 Hz, H-8), 6.90 (1H, d, $J$=2.2 Hz, H-6), 7.13 (1H, s, H-5), 12.58 (1H, s, OH-10).

**Enzymatic Hydrolysis of Cassiaside $C_2$ (2)** A solution of **2** (100 mg) in a phosphate buffer (pH 5.6, 30 ml) was treated with $\beta$-glucosidase (50 mg) at 37 °C for 6 h. The reaction mixture was extracted with BuOH, and the BuOH layer was evaporated *in vacuo* to obtain a residue, which was subjected to chromatography on ODS column and eluted with MeOH–$H_2O$ to afford **2b** (21.1 mg), **2c** (12.5 mg) and **2a** (1.2 mg). Compounds **2a** and **2c** were identified as toralactone and cassiaside C, toralactone 9-*O*-gentiobioside, by direct comparison with authentic samples. Compound **2b**, toralactone 9-*O*-$\beta$-D-glucopyranosyl-(1→3)-*O*-$\beta$-D-glucopyranosyl-(1→6)-*O*-$\beta$-D-glucopyranoside, was obtained as yellow powder, mp 205—207 °C $[\alpha]_D^{18}$ −24.5° ($c$=0.1, MeOH). FAB-MS $m/z$: 659 $[M+H]^+$. UV $\lambda_{max}^{H_2O}$ nm (log $\varepsilon$): 218 (3.03), 279 (3.58), 384 (2.64). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3385, 1660, 1626, 1587, 1415, 1372, 1261, 1204, 1167, 1069, 907, 843, 578. $^1$H-NMR (DMSO-$d_6$) $\delta$: 2.22 (3H, s, Me-3), 3.89 (3H, s, OMe), 4.31 (1H, d, $J$=7.3 Hz, H-1'''), 4.33 (1H, d, $J$=8.1 Hz, H-1''), 5.13 (1H, d, $J$=8.1 Hz, H-1'), 6.47 (1H, s, H-4), 6.86 (1H, d, $J$=2.2 Hz, H-8), 6.92 (1H, d, $J$=2.2 Hz, H-6), 7.10 (1H, s, H-5), 12.58 (1H, s, OH-10).

**Measurement of Histamine Release from Mast Cells** The preparation of mast cells and the assay of histamine release from mast cells were performed by the modified method of Hirai *et al.*[10] Male Wistar rats (Japan SLC, Shizuoka) weighing 180—200 g were exsanguinated and injected intraperitoneally with 10 ml of Tyrode solution. The abdominal region was massaged for 2 min and then the peritoneal exudate was collected. The peritoneal cavity fluid containing mast cells was suspended in phosphate buffered saline (PBS), then layered on bovine serum albumin (BSA) ($d$=1.068) in a test tube at room temperature for 20 min. After centrifugation at 300×***g*** and 4 °C for 10 min, the layer containing mast cells was pipetted out. The cells were washed three times with 4 ml of PBS (pH 7.0) and suspended in the same medium. The cell suspensions contained 85—90% or more viable mast cells. The peritoneal exudate cells were sensitized with diluted anti-dinitrophenyl (DNP) immunoglobulin E (IgE) (Yamasa Co.) (×100) at 37 °C for 1 h. The cell suspension ($1-2\times10^6$ cells/ml) and the test substances dissolved with dimethyl sulfoxide were preincubated for 15 min, and 5 $\mu$l of phosphatidyl-L-serine (100 $\mu$g/ml) and 10 $\mu$l of DNP-BSA (1 $\mu$g/ml) were added to the mast cell suspension in a final volume of 50 ml, then the mixture was incubated at 37 °C for 10 min. The reaction was terminated by cooling the mixture on ice. The mixture was centrifuged at 2000×***g*** and 4 °C for 5 min, then 30 $\mu$l of the supernatant fluid was taken to measure histamine released from the cells by high performance liquid chromatography.[11] The activity of the test substance on histamine release from mast cells induced by an antigen was expressed as the inhibitory percentage. Indomethacin was used as a standard drug.

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