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Studies on the Constituents of the Seeds of *Cassia obtusifolia* L.[1] The Structures of Two Naphthopyrone Glycosides

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Two new naphthopyrones, cassiasides B (**1**) and C (**2**), were isolated from the seeds of *Cassia obtusifolia* L. along with rubrofusarin 6-*O*-gentiobioside. The structures of the two new compounds **1** and **2** were established as rubrofusarin 6-*O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside and toralactone 9-*O*-*β*-gentiobioside, respectively, on the basis of spectral and chemical evidence.

Keywords——*Cassia obtusifolia*; Leguminosae; naphthopyrone glycoside; cassiaside B; cassiaside C

In previous papers,[2-8] we reported the isolation of many anthraquinone, naphthopyrone, and tetrahydroanthracene derivatives from the seeds of *Cassia obtusifolia* L. In this paper, we wish to report the structural determination of two new naphthopyrone glycosides, cassiaside B (**1**) and cassiaside C (**2**), which have been isolated along with rubrofusarin 6-*O*-gentiobioside (**3**) from the seeds of this plant.

These compounds were obtained from the methanol extracts of the crushed seeds as described in Experimental.

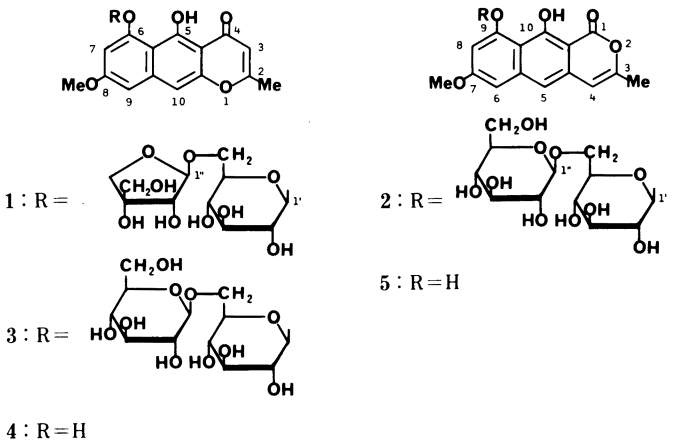

Chart 1

Compound **1**, yellowish orange needles, mp 231—231.5 °C, $[\alpha]_D^{25}$ −93.3° (pyridine), $C_{26}H_{30}O_{14} \cdot 1/2H_2O$ showed a yellow color in methanolic sodium hydroxide. The similarity of the chromophore of **1** to that of rubrofusarin (**4**) was established by comparison of the ultraviolet (UV) spectra, λ_{max} 202 sh, 221, 252 sh, 274, 320, 392. The infrared (IR) spectrum showed that **1** is a glycoside (3400 and 1030 cm^{-1}) and has a chelated carbonyl group (1660 cm^{-1}). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed the

presence of a methyl group, an aromatic methoxy group, an olefinic proton, three aromatic protons, and a chelated hydroxyl group in the aglycone moiety, and two sugar anomeric proton signals at δ 5.01 (d, J=7.8 Hz) and 4.82 (d, J=2.9 Hz) (Table I). The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** gave fifteen carbon signals due to the naphthopyrone skeleton and eleven carbon signals due to the sugar moiety, which was concluded to be a 6-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside by a comparison of the chemical shifts with those of the known β-apiofuranosyl-(1→6)-β-D-glucopyranosylumbelliferone.[9] The field desorption mass spectrum (FD-MS) gave the molecular ions+Na (M^++Na) at m/z 589, M^++1 at m/z 567 as the base peak, and M^+ at m/z 566, as well as fragments at m/z 434 (M^+−apiose) and 272 (M^+−apiose−glucose), confirmed apiose as the terminal sugar. Acid hydrolysis of **1** with 0.2 N HCl–dioxane (1 : 1)[10] yielded rubrofusarin (**4**), glucose, and apiose. The location of the sugar moiety in **1** was confirmed as C_6-OH by comparison of the ^{1}H-NMR spectrum of **1** with that of **4**. That is to say, two chelated hydroxyl signals at δ 9.94 (C_5-OH) and 15.71 (C_6-OH) were seen in **4**, but only one at δ 14.87 (C_5-OH) in **1**. The coupling constants of the anomeric proton signal of apiose (δ 4.82, d, J=2.9 Hz) in the ^{1}H-NMR spectrum of **1** suggested a β-linked glycoside structure.[11]

Therefore, the structure of cassiaside B (**1**) was shown to be rubrofusarin 6-*O*-β-D-apiofuranosyl-(1→6)-*O*-β-D-glucopyranoside.

Compound **2**, yellow needles, mp 235—237 °C, $[\alpha]_D^{25}$ −56.4° (pyridine), $C_{27}H_{32}O_{15}$ exhibited a strong blue fluorescence and gave a positive Molisch test. The UV and IR, and ^{1}H-NMR spectra showed slightly different patterns from those of **1**. The aglycone moiety of **2** was considered to have an α-pyrone structure since the olefinic proton signal (δ 6.51) in **2** is observed downfield by 0.31 ppm, compared with that of **1**. Enzymatic hydrolysis of **2** with β-glucosidase yield glucose and the corresponding aglycone, which was identified as toralactone (**5**) by direct comparison with an authentic sample isolated from the seeds of *Cassia tora*.[12] Two anomeric proton signals at δ 4.20 and 5.09 (each 1H, J=7.8 Hz) in the spectrum of **2** indicated the presence of two β-linkages. The ^{13}C-NMR data indicated that the sugar in **2** is

TABLE I. ^{1}H-NMR Chemical Shifts[a]

	1	3	4		2	5
Aglycone moiety protons						
2-Me	2.40 s	2.40 s	2.41 s	3-Me	2.23 s	2.22 s
3-H	6.20 s	6.20 s	6.23 s	4-H	6.51 s	6.48 s
7-H	6.73 d	6.81 d	6.38 d	5-H	7.13 s	7.08 s
	J=2.1 Hz	J=2.1 Hz	J=2.1 Hz			
8-OMe	3.88 s	3.88 s	3.94 s	6-H	6.93 d	6.78 d
					J=2.1 Hz	J=2.1 Hz
9-H	6.95 d	6.94 d	6.81 d	7-OMe	3.89 s	3.85 s
	J=2.1 Hz	J=2.1 Hz	J=2.1 Hz			
10-H	7.20 s	7.20 s	7.16 s	8-H	6.88 d	6.43 d
					J=2.1 Hz	J=2.1 Hz
5-OH	14.87 brs	14.87 brs	15.71 brs	9-H	12.60 brs	12.49 brs
6-OH			9.94 brs	10-OH		10.54 brs
Anomeric protons						
api 1″-H	4.82 d	4.20 d		glc 1″-H	4.20 d	
	J=2.9 Hz	J=7.8 Hz			J=7.8 Hz	
glc 1′-H	5.01 d	5.07 d		glc 1′-H	5.09 d	
	J=7.8 Hz	J=7.8 Hz			J=7.8 Hz	

a) Measured in dimethyl sulfoxide-d_6 (DMSO-d_6) at 400 MHz with TMS as an internal standard. Abbreviations: s, singlet; brs, broad singlet; d, doublet.

TABLE II. ^{13}C-NMR Chemical Shifts for Compounds **1**—**3**[a]

	1	**3**		**2**
	Aglycone moiety			
C-2	168.6 (s)	168.2 (s)	C-1	162.6 (s)
C-3	101.1 (d)	101.3 (d)	C-3	166.8 (s)
C-4	183.5 (s)	183.5 (s)	C-4	104.1 (d)
C-4a	103.4 (s)	103.6 (s)	C-4a	132.3 (s)
C-5	161.5 (s)	161.7 (s)	C-5	111.6 (d)
C-5a	107.5 (s)	107.7 (s)	C-5a	141.5 (s)
C-6	160.8 (s)	160.9 (s)	C-6	100.3 (d)
C-7	100.7 (d)	103.6 (d)	C-7	157.4 (s)
C-8	157.5 (s)	157.6 (s)	C-8	103.6 (d)
C-9	99.6 (d)	99.7 (d)	C-9	161.3 (s)
C-9a	140.0 (s)	140.1 (s)	C-9a	109.1 (s)
C-10	106.5 (d)	106.6 (d)	C-10	152.5 (s)
C-10a	152.1 (s)	152.3 (s)	C-10a	98.4 (s)
Me	20.1 (q)	20.1 (q)	Me	18.8 (q)
OMe	55.4 (q)	55.4 (q)	OMe	55.4 (q)
	Sugar moiety			
C-1′	101.1 (d)	101.4 (d)		101.8 (d)
C-2′	75.5 (d)	73.4 (d)		73.5 (d)
C-3′	76.0 (d)	76.7 (d)		76.8 (d)
C-4′	69.9 (d)	70.0 (d)		70.1 (d)
C-5′	76.0 (d)	75.6 (d)		75.5 (d)
C-6′	67.7 (t)	68.8 (t)		68.8 (t)
C-1″	109.3 (d)	100.7 (d)		100.9 (d)
C-2″	76.3 (d)	73.4 (d)		73.5 (d)
C-3″	78.7 (d)	76.6 (d)		76.4 (d)
C-4″	73.3 (t)	69.6 (d)		69.7 (d)
C-5″	63.3 (t)	76.1 (d)		76.6 (d)
C-6″		61.1 (d)		61.6 (t)

a) Measured in DMSO-d_6 at 25.2 MHz with TMS as an internal standard. Abbreviations: s, singlet; d, doublet; t, triplet; q, quartet.

gentiobiose, because the chemical shifts of the 6 and 5 positions in the inner glucose showed downfield and upfield shifts (glycosylation shifts), respectively, in comparison with those of the outer glucose.[13] The position of sugar in **2** was concluded to be at the C_9-OH of **5** based on a comparison of the chemical shifts with the chelated hydroxy groups in **2** and **5**.

Therefore, the structure of cassiaside C (**2**) was established as toralactone 9-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside (toralactone 9-*O*-*β*-gentiobioside).

Compound **3**, yellow needles, mp 259–261 °C, $C_{27}H_{32}O_{15}$, appeared to be a nephthopyrone diglycoside from the spectral analysis. Enzymatic hydrolysis of **3** with *β*-glucosidase gave **4** and glucose. The ^{13}C-NMR spectrum of **3** indicated it to be rubrofusarin-gentiobioside, and the position of sugar was confirmed as C_6-OH by comparison of the ^{1}H-NMR spectrum of **3** with that of **4**. Thus, the structure of **3** was identified as rubrofusarin 6-*O*-gentiobioside. This compound has been isolated from the seed of *Cassia tora*.[14]

This is the first report of the isolation of cassiaside B (**1**), a naphtho-*γ*-pyrone glycoside containing apiose, and cassiaside C (**2**), a naphtho-*α*-pyrone glycoside.

Experimental

All the melting points were taken on a Yanagimoto micro-melting-point apparatus and are uncorrected. The UV

spectra were obtained on a Hitachi 200-10 spectrophotometer, and the IR spectra were recorded on a JASCO IR A-2 spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were taken on JEOL GL-400 and JEOL FX-100 spectrometers, respectively, using tetramethylsilane (TMS) as an internal standard. The mass spectra (MS) were obtained on a Hitachi M-80B spectrometer. Column chromatography was carried out with silica gel (Wako gel C-200, Wako Pure Chemical Industry Ltd.) or Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemical Co., Ltd.). Thin layer chromatography (TLC) for sugar was performed on Avicel SF cellulose plates (Funakoshi) [a) 1-BuOH : AcOH : H_2O = 3 : 1 : 1 (upper layer) and b) 1-BuOH : EtOH : H_2O = 52 : 32 : 16 as developing solvent systems; aniline hydrogen phthalate for detection].

Extraction and Isolation——Crushed seeds (10 kg) of *Cassia obtusifolia* were extracted with 90% MeOH (50 l × 3) under reflux. The extract was concentrated *in vacuo* to give a brown mass which was then dissolved in H_2O (8 l). This solution was extracted with AcOEt and 1-BuOH successively. The 1-BuOH solution was concentrated *in vacuo* to give a brown mass (148 g), which was then chromatographed on SiO_2 with $CHCl_3$–MeOH–H_2O mixture. Fraction 1 was eluted with $CHCl_3$–MeOH–H_2O (9 : 1 : 0.1), and fractions 2 and 3 with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2). Fraction 2 (1.5 g) gave cassiaside[5] (900 mg) from MeOH, and the mother liquid was chromatographed on Sephadex LH-20 with MeOH to afford **1** (100 mg). Fraction 3 (500 mg) was chromatographed on Sephadex LH-20; elution with H_2O gave **3** (50 mg) and further elution with 20% MeOH gave **2** (15 mg).

Cassiaside B (1)——Recrystallization (MeOH–H_2O) gave orange-yellow needles, mp 231—231.5 °C, $[\alpha]_D^{25}$ −93.3 (pyridine, c = 0.85), *Anal.* Calcd for $C_{26}H_{30}O_{14} \cdot 1/2H_2O$: C, 54.26; H, 5.43. Found: C, 54.67; H, 5.30. UV λ_{max}^{MeOH} nm (log ε): 202 sh (4.10), 221 (4.44) 252 sh (4.45), 274 (4.72), 320 (3.45), 392 (3.81). IR ν_{max}^{KBr} cm^{-1}: 3400, 1660, 1630, 1585, 1130—980. FD-MS *m/z*: 589 (M^+ + Na), 561 (M^+ + 1), 434 (M^+ − apiose), 272 (M^+ − apiose − glucose). The ^{1}H- and ^{13}C-NMR spectral data are shown in Tables I and II, respectively.

Acid Hydrolysis of 1——A solution of **1** (10 mg) in 0.2 N HCl–dioxane (1 : 1, 6 ml) was refluxed for 30 min. The reaction mixture was extracted with AcOEt, and the AcOEt layer was evaporated to dryness *in vacuo* after being washed with H_2O. The AcOEt extract (3 mg) was recrystallized from C_6H_6 to afford the aglycone (**4**) as dark red plates, mp 214 °C. The aqueous layer was neutralized with Amberlite IRA-45 (OH^- form), and evaporated to dryness *in vacuo*. The residue showed the presence of D-apiose (solvent a, Rf = 0.44; solvent b, Rf = 0.43) and D-glucose (solvent a, Rf = 0.25; solvent b, Rf = 0.24) on Avicel SF TLC in comparison with authentic samples.

Cassiaside C (2)——Recrystallization (MeOH–H_2O) gave pale yellow needles, mp 235—237 °C, $[\alpha]_D^{25}$ −56.4° (pyridine, c = 0.37), *Anal.* Calcd for $C_{27}H_{32}O_{15}$: C, 60.20; H, 5.05. Found: C, 60.08; H, 5.11. UV λ_{max}^{MeOH} nm (log ε): 224 sh (4.05), 247 sh (4.38), 257 sh (4.56), 267 (4.80), 277 (4.89), 287 sh (4.19), 308 (3.59), 322 (3.57), 347 (3.64), 381 (3.87), 410 sh (3.72). IR ν_{max}^{KBr} cm^{-1}: 3400, 1675, 1645, 1585. FD-MS *m/z*: 619 (M^+ + Na), 597 (M^+ + 1), 435 (M^+ + 1 − glucose), 272 (M^+ − 2 × glucose). ^{1}H- and ^{13}C-NMR data are shown in Tables I and II, respectively.

Enzymatic Hydrolysis of 2——A solution of **2** (4 mg) and β-glucosidase (2 mg) in H_2O (2 ml) was kept for 20 h at 37 °C. The reaction mixture was then extracted with AcOEt, and the AcOEt layer was evaporated to dryness *in vacuo*. The yellow residue was recrystallized from *n*-hexane–C_6H_6 to afford the aglycone (**5**) as pale yellow needles, mp 252—254 °C. The aqueous layer was evaported to dryness, and the residue showed the presence of D-glucose on Avicel SF TLC (solvent a, Rf = 0.25).

Rubrofusarin 6-*O*-β-Gentiobioside (3)——Recrystallization (MeOH) gave yellow needles, mp 252.5—254 °C, *Anal.* Calcd for $C_{27}H_{32}O_{15}$: C, 60.20; H, 5.05. Found: C, 60.03; H, 5.13. UV λ_{max}^{MeOH} nm (log ε): 223 (4.42), 254 sh (4.44), 276 (4.72), 307 (3.44), 332 (3.47), 340 (3.31), 393 (3.78). IR ν_{max}^{KBr} cm^{-1}: 3400, 1650, 1620, 1580, 1120—1020. FD-MS *m/z*: 619 (M^+ + Na), 597 (M^+ + 1), 596 (M^+), 272 (M^+ − 2 × glucose). ^{1}H- and ^{13}C-NMR data are shown in Tables I and II, respectively. A solution of **3** (5 mg) in H_2O was treated with β-glucosidase (2 mg) for 20 h at 37 °C. The mixture was diluted with H_2O and extracted with AcOEt. The AcOEt extract was recrystallized from C_6H_6 to afford the aglycone (**4**) as dark red plates, mp 214 °C. The aqueous layer was evaporated to dryness *in vacuo*, and the residue showed the presence of D-glucose on Avicel SF TLC (solvent a, Rf = 0.25).

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References and Notes

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