[Chem. Pharm. Bull.]
36(10)3980—3984(1988)

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

SUSUMU KITANAKA* and MICHIO TAKIDO

College of Pharmacy, Nihon University, 7-1, Narashinodai 7-chome,
Funabashi-shi, Chiba 274, Japan

(Received March 23, 1988)

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, $[\alpha]_D^{25} -93.3^\circ$ (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 (1H -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 $\rightarrow$ 6)-*O*- β -D-glucopyranoside by a comparison of the chemical shifts with those of the known β -apiofuranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosylumbelliferone.⁹⁾ The field desorption mass spectrum (FD-MS) gave the molecular ions + Na ($M^+ + \text{Na}$) 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 ($M^+ - \text{apiose}$) and 272 ($M^+ - \text{apiose} - \text{glucose}$), 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 C₆-OH by comparison of the ^1H -NMR spectrum of **1** with that of **4**. That is to say, two chelated hydroxyl signals at δ 9.94 (C₅-OH) and 15.71 (C₆-OH) were seen in **4**, but only one at δ 14.87 (C₅-OH) in **1**. The coupling constants of the anomeric proton signal of apiose (δ 4.82, d, $J=2.9$ Hz) in the ^1H -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 $\rightarrow$ 6)-*O*- β -D-glucopyranoside.

Compound **2**, yellow needles, mp 235–237 °C, $[\alpha]_{\text{D}}^{25} -56.4^\circ$ (pyridine), C₂₇H₃₂O₁₅ exhibited a strong blue fluorescence and gave a positive Molisch test. The UV and IR, and ^1H -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. ^1H -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 br s	14.87 br s	15.71 br s	9-H	12.60 br s	12.49 br s
6-OH			9.94 br s	10-OH		10.54 br s
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; br s, 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 C₉-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 $\rightarrow$ 6)-*O*- β -D-glucopyranoside (toralactone 9-*O*- β -gentiobioside).

Compound **3**, yellow needles, mp 259–261 °C, C₂₇H₃₂O₁₅, appeared to be a naphthopyrone 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 C₆-OH by comparison of the ^1H -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 ^1H - 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:H₂O=3:1:1 (upper layer) and b) 1-BuOH:EtOH:H₂O=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 $\times$ 3) under reflux. The extract was concentrated *in vacuo* to give a brown mass which was then dissolved in H₂O (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 SiO₂ with CHCl₃–MeOH–H₂O mixture. Fraction 1 was eluted with CHCl₃–MeOH–H₂O (9:1:0.1), and fractions 2 and 3 with CHCl₃–MeOH–H₂O (8:2:0.2). Fraction 2 (1.5 g) gave cassiaside⁵¹ (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 H₂O gave **3** (50 mg) and further elution with 20% MeOH gave **2** (15 mg).

Cassiaside B (1)—Recrystallization (MeOH–H₂O) gave orange-yellow needles, mp 231–231.5 °C, $[\alpha]_{\text{D}}^{25}$ –93.3 (pyridine, c =0.85), *Anal.* Calcd for C₂₆H₃₀O₁₄·1/2H₂O: C, 54.26; H, 5.43. Found: C, 54.67; H, 5.30. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 202 sh (4.10), 221 (4.44) 252 sh (4.45), 274 (4.72), 320 (3.45), 392 (3.81). IR $\nu_{\text{max}}^{\text{KBr}}$ 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 ^1H - 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 0.2N 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 H₂O. The AcOEt extract (3 mg) was recrystallized from C₆H₆ 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, R_f =0.44; solvent b, R_f =0.43) and D-glucose (solvent a, R_f =0.25; solvent b, R_f =0.24) on Avicel SF TLC in comparison with authentic samples.

Cassiaside C (2)—Recrystallization (MeOH–H₂O) gave pale yellow needles, mp 235–237 °C, $[\alpha]_{\text{D}}^{25}$ –56.4° (pyridine, c =0.37), *Anal.* Calcd for C₂₇H₃₂O₁₅: C, 60.20; H, 5.05. Found: C, 60.08; H, 5.11. UV $\lambda_{\text{max}}^{\text{MeOH}}$ 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 $\nu_{\text{max}}^{\text{KBr}}$ cm^{–1}: 3400, 1675, 1645, 1585. FD-MS m/z : 619 (M^+ + Na), 597 (M^+ + 1), 435 (M^+ + 1 – glucose), 272 (M^+ – 2 $\times$ glucose). ^1H - 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 H₂O (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–C₆H₆ to afford the aglycone (**5**) as pale yellow needles, mp 252–254 °C. The aqueous layer was evaporated to dryness, and the residue showed the presence of D-glucose on Avicel SF TLC (solvent a, R_f =0.25).

Rubrofusarin 6-O- β -Gentiobioside (3)—Recrystallization (MeOH) gave yellow needles, mp 252.5–254 °C, *Anal.* Calcd for C₂₇H₃₂O₁₅: C, 60.20; H, 5.05. Found: C, 60.03; H, 5.13. UV $\lambda_{\text{max}}^{\text{MeOH}}$ 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 $\nu_{\text{max}}^{\text{KBr}}$ cm^{–1}: 3400, 1650, 1620, 1580, 1120–1020. FD-MS m/z : 619 (M^+ + Na), 597 (M^+ + 1), 596 (M^+), 272 (M^+ – 2 $\times$ glucose). ^1H - and ^{13}C -NMR data are shown in Tables I and II, respectively. A solution of **3** (5 mg) in H₂O was treated with β -glucosidase (2 mg) for 20 h at 37 °C. The mixture was diluted with H₂O and extracted with AcOEt. The AcOEt extract was recrystallized from C₆H₆ 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, R_f =0.25).

Acknowledgements The authors are grateful to Prof. J. Shoji, School of Pharmaceutical Sciences, Showa University, for his generous gift of a sample of apiin. Thanks are also due to Miss Y. Kimura of the Department of Pharmacy, Nihon University, for IR spectra, and Dr. T. Takido and Mr. M. Aimi of the Analytical Center, College of Science and Technology, Nihon University, for NMR spectra and MS.

References and Notes

- 1) Part XXII in the series "Studies on the Constituents of Purgative Crude Drugs." For Part XXI, see S. Kitanaka and M. Takido, *Yakugaku Zasshi*, **106**, 302 (1986).
- 2) M. Takido, *Chem. Pharm. Bull.*, **6**, 397 (1958).
- 3) M. Takido, *Chem. Pharm. Bull.*, **8**, 246 (1960).
- 4) M. Takido and S. Takahashi, *Shoyakugaku Zasshi*, **86**, 1087 (1966).
- 5) Y. Kimura, M. Takido, and S. Takahashi, *Yakugaku Zasshi*, **86**, 1087 (1966).
- 6) S. Kitanaka and M. Takido, *Phytochemistry*, **20**, 1951 (1981).

-
- 7) S. Kitanaka and M. Takido, *Chem. Pharm. Bull.*, **32**, 860 (1984).
 - 8) S. Kitanaka, F. Kimura, and M. Takido, *Chem. Pharm. Bull.*, **33**, 1274 (1985).
 - 9) P. Satyanarayana, P. Subrahmanyam, R. Kasai, and O. Tanaka, *Phytochemistry*, **24**, 1862 (1985).
 - 10) S. Sakuma and J. Shoji, *Chem. Pharm. Bull.*, **29**, 2431 (1981).
 - 11) A. D. Ezekiel, W. G. Overend, and N. R. Williams, *J. Chem. Soc., C.*, **1971**, 2907.
 - 12) S. Takahashi and M. Takido, *Yakugaku Zasshi*, **93**, 261 (1973).
 - 13) a) T. Usui, N. Yamaoka, K. Matsuda, K. Tsuzimura, H. Sugiyama, and S. Seto, *J. Chem. Soc., Perkin Trans. I*, **1973**, 2425; b) S. Kitanaka and M. Takido, *Chem. Pharm. Bull.*, **32**, 3436 (1984).
 - 14) M. Kaneda, E. Morishita, and S. Sibata, *Chem. Pharm. Bull.*, **17**, 458 (1967).