Studies on the Constituents of the Leaves of *Cassia torosa* CAV. III.[1] The Structures of Two New Flavone Glycosides

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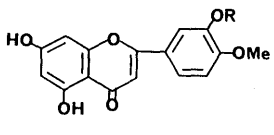
Two new flavone glycosides were isolated along with diosmetin, luteolin, and luteolin 7-*O*-glucoside, from the leaves of *Cassia torosa* CAV., and their structures were established as diosmetin 3′-*O*-*β*-D-glucopyranoside (1) and torosaflavone B 3′-*O*-*β*-D-glucopyranoside (2) (diosmetin 6-*C*-*β*-D-oliopyranosyl-3′-*O*-*β*-D-glucopyranoside) on the basis of spectroscopic and chemical evidence.

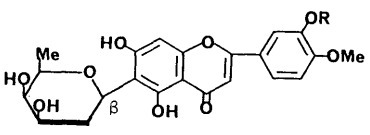
Keywords *Cassia torosa*; Leguminosae; flavone glycoside; oliose; 2,6-dideoxyglycoside; torosaflavone B 3′-*O*-*β*-D-glucopyranoside; diosmetin 3′-*O*-*β*-D-glucopyranoside

In previous papers,[1,2] we reported the isolation of two *C*-deoxyglycosylflavones, torosaflavones A and B, and two novel flavones, torosaflavones C and D from the ether-soluble fraction of methanolic extract prepared from the leaves of *Cassia torosa* CAV. We also reported the isolation[3–11] of anthraquinones, an anthrone, a dimeric anthrone, hydroanthracenes, dimeric hydroanthracenes, naphthalenic lectones, and sterols from the ripe and unripe seeds, the seedlings, and the roots of this plant.

In our present study, as part of our continuing investigation of the ether-, ethyl acetate-, and 1-butanol-soluble fractions of the methanolic extract of the leaves, we isolated two new flavone glycosides, called diosmetin 3′-*O*-*β*-D-glucopyranoside (**1**) and torosaflavone B 3′-*O*-*β*-D-glucopyranoside (**2**) together with diosmetin, luteolin, and luteolin 7-glucoside. We determined their structures by means of spectral and chemical evidence.



1: R = *β*-D-glucosyl
1a: R = H



2: R = *β*-D-glucosyl
2a: R = H

Chart 1

Compound **1**, pale yellow needles, mp 253—254 °C, $[\alpha]_D^{25}$ −32.4° (pyridine) gave a positive coloration in the $FeCl_3$ and the Mg–HCl tests. The ultraviolet (UV) spectrum showed maxima at 238, 245sh, 267, 290, 333 nm, and the infrared (IR) spectrum exhibited absorption bands due to the presence of hydroxyls (3392, 1075—1010 cm^{-1}), an α,β-unsaturated ketone (1656, 1609 cm^{-1}), and aromatic rings (1507—1360 cm^{-1}). The above evidence showed that **1** is a flavone glycoside. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** in dimethyl sulfoxide (DMSO)-d_6 showed an AMX system due to a 3′,4′-disubstitution in the B-ring, two doublets due to H-6 and H-8 attributed to an A-ring and a singlet due to H-3, a methoxyl, and a glycosyl anomeric proton signal at δ 5.08 (d, J = 6.8 Hz) (Table I). The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** gave sixteen carbon signals from the flavone nucleus and six carbon signals due to the sugar moiety (Table II). Acid hydrolysis of **1** with 2 N HCl yielded aglycone and glucose. The aglycone was identified as diosmetin (**1a**)[12] by UV, IR, and ^{13}C-NMR

TABLE I. ^{1}H-NMR Data[a] of **1**, **1a**, **2**, and **2a**

	1	1a	2	2a
Aglycone moiety				
H-3	6.91 (s)	6.75 (s)	6.93 (s)	6.80 (s)
H-6	6.21 (d, J = 2.2 Hz)	6.20 (d, J = 2.2 Hz)		
H-8	6.55 (d, J = 2.2 Hz)	6.47 (d, J = 2.2 Hz)	6.62 (s)	6.56 (s)
H-2′	7.72 (d, J = 2.2 Hz[b])	7.43 (d, J = 2.2 Hz)	7.73 (d, J = 2.2 Hz)	7.45 (d, J = 2.2 Hz)
H-5′	7.17 (d, J = 8.8 Hz)	7.09 (d, J = 8.1 Hz)	7.17 (d, J = 8.8 Hz)	7.10 (d, J = 8.8 Hz)
H-6′	7.73 (dd, J = 8.8, 2.2 Hz)	7.54 (dd, J = 8.1, 2.2 Hz)	7.71 (dd, J = 8.8, 2.2 Hz)	7.57 (dd, J = 8.8, 2.2 Hz)
5-OH	12.93 (s)	12.93 (s)	13.46 (s)	13.47 (s)
OMe	3.87 (s)	3.87 (s)	3.87 (s)	3.87 (s)
Sugar moiety				
Oliosyl				
H-1″			5.01 (dd, J = 11.7, 2.9 Hz)	5.00 (dd, J = 11.7, 3.2 Hz)
H-2″$_{ax}$			2.05 (q, J = 11.7 Hz)	2.06 (q, J = 11.7 Hz)
H-2″$_{eq}$			1.61 (ddd, J = 11.7, 3.8, 2.9 Hz)	1.61 (ddd, J = 11.7, 5.2, 3 Hz)
H-3″			3.73 (dd, J = 11.7, 3.8 Hz)	3.71 (ddd, J = 12.7, 5.2, 2.4 Hz)
H-4″			3.49 (br s)	3.47 (d, J = 2.4 Hz)
H-5″			3.66 (q, J = 6.4 Hz)	3.66 (q, J = 6.4 Hz)
5-Me			1.19 (d, J = 6.4 Hz)	1.19 (d, J = 6.4 Hz)
Glucosyl				
H-1‴	5.08 (d, J = 7.8 Hz)		5.08 (d, J = 7.8 Hz)	

a) Measured in DMSO-d_6 at 400 MHz, with tetramethylsilane (TMS) as the internal standard. The following abbreviations are used: s, singlet; d, doublet; q, quarter; br, broad. *b*) The underlined values are recognized for the glycosylation shifts.

Table II. ^{13}C-NMR Data[a] of **1**, **1a**, **2**, and **2a**

	1	1a	2	2a[2]
Aglycone moiety				
C-2	163.1	163.4	162.3	162.3
C-3	103.7	103.4	103.4	103.4
C-4	181.6	181.6	181.9	181.9
C-4a	103.7	103.6	103.7	103.4
C-5	161.3	161.4	157.2	157.4
C-6	98.8	98.8	110.0	110.0
C-7	164.1	164.1	163.4	163.7
C-8	94.1	93.8	94.9	94.6
C-8a	157.2	157.2	156.1	156.1
C-1′	122.8	122.9	122.7	122.8
C-2′	113.0	112.8	112.3	112.9
C-3′	146.5	146.7	146.5	146.7
C-4′	152.1 (+1.1)[b]	151.0	152.2 (+1.0)	151.2
C-5′	112.3	112.1	113.0	112.1
C-6′	121.0 (+2.4)	118.6	121.2 (+2.4)	118.8
OMe	55.8	55.7	55.8	55.7
Sugar moiety				
Oliosyl				
C-1			70.2	70.1
C-2			32.4	32.3
C-3			68.5	68.4
C-4			69.6	69.5
C-5			74.5	74.4
C-6			17.5	17.4
Glucosyl				
C-1	99.9		99.9	
C-2	73.2		73.2	
C-3	76.8		76.9	
C-4	69.7		69.9	
C-5	77.1		77.2	
C-6	60.8		60.8	

a) Measured in DMSO-d_6 at 100 MHz, TMS as the internal standard. *b*) Values in parentheses indicate glycosylation shifts.

spectra. The bathochromic shift[13] of UV bands of **1** with $AlCl_3$ (λ_I=19 nm) and AcONa (λ_{II}=8 nm) suggested the presence of free 5- and 7-hydroxyls, respectively. Therefore, the location of glucosyl moiety in **1** was estimated as the C_3′-position by shift experiments in the UV spectra and was confirmed by glycosylation shifts[14] in the ^{13}C-NMR data. On going from **1a** to **1**, the carbon signals of C-4′ and C-6′ in the B-ring shifted downfield by 1.1 and 2.4 ppm, respectively, while the carbon signals arising from the A- and C-rings remained unchanged.

Thus, the structure of **1** was established as diosmetin 3′-*O*-D-glucopyranoside.

Compound **2**, pale yellow needles, mp 212—213 °C, $[\alpha]_D^{24}$ +36.1° (pyridine), $C_{28}H_{32}O_{14}$ produced a positive coloration in the Mg–HCl test. The bathochromic shifts of the UV bands of **2** with AcONa (λ_{II}=8 nm) and $AlCl_3$ (λ_I=20 nm) showed the respective presence of 7- and 5-hydroxyls in a flavone skeleton, while the presence of *ortho*-phenol in the flavone nucleus was not revealed by the shift experiments, its UV and IR spectra suggested a flavone glycoside. The field desorption mass spectrum (FD-MS) gave peaks due to molecular ion+H (M^++H) at *m/z* 593 as base peak and M^++H−hexose ($C_6H_{10}O_5$) at *m/z* 431, M^++H−hexose−$C_6H_{10}O_3$ at *m/z* 300 and hexose ions at *m/z* 163, suggesting the linkage of a hexose and a dideoxyhexose.

Compound **2** showed a similar ^{1}H-NMR spectrum to that of torosaflavone B (**2a**) except for additional signals due to a glycosyl moiety (Table I).

Hydrolysis of **2** with both 1 N HCl and β-D-glucosidase yielded D-glucose and an aglycone (**2a**) which was identified by an authentic sample of torosaflavone B (diosmetin 6-*C*-2,6-dideoxy-β-D-lyxohexoside).[2]

The position of the linkage of glucose in **2** was confirmed as C_3′-position by comparing the ^{1}H-NMR and ^{13}C-NMR spectra of **2** with those of **2a**.

Thus, the structure of **2** was determined to be diosmetin 6-*C*-β-D-oliopyranosyl-3′-*O*-β-D-glucopyranoside.

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 IR spectra were on a JASCO IR A-2 spectrophotometer. The NMR spectra were taken on JEOL JNM-GX-400 instruments. The MS were obtained on a Hitachi RMU-7M spectrometer. Column chromatography was carried out with sillica gel (Wako gel C-200, Wako Pure Chemical Industry, Ltd.) or Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemical Co., Ltd.).

Extraction and Isolation The dried leaves (5.5 kg) were extracted with MeOH (77 l×3) under reflex. The MeOH extract was concentrated *in vacuo* to give a dark green mass, which was then suspended in H_2O. This suspension was successively extracted with Et_2O, AcOEt, and 1-BuOH. The Et_2O extract (11.5 g) was then chromatographed on SiO_2 with $CHCl_3$ to give fractions 1—3 and with $CHCl_3$–MeOH mixture to give fractions 4—12, respectively. Fractions 2 and 3 (120 mg) were chromatographed on Sephadex LH-20 to give torosaflavone C (35 mg) and diosmetin (15 mg), respectively. Fractions 6—8 (2.6 g) gave torosaflavone D (130 mg) from MeOH. Fractions 9, 10 (3% MeOH–$CHCl_3$) were chromatographed on Sephadex LH-20 to give torosaflavone A (67 mg) and luteolin (23 mg). The AcOEt extract (10 g) was chromatographed on SiO_2 with $CHCl_3$–MeOH mixture to give fractions 1—7. Fractions 4 and 5 (4% MeOH–$CHCl_3$) were each chromatographed on Sephadex LH-20 with MeOH to give torosaflavone B (11 mg), luteolin 7-glucoside (90 mg), and **1** (16 mg), respectively. The 1-BuOH extract (5 g) was chromatographed on SiO_2 with $CHCl_3$–MeOH (6% MeOH–$CHCl_3$) mixture to afford **2** (224 mg).

Diosmetin 3′-*O*-β-D-Glucopyranoside (1) Recrystallization (MeOH) gave pale yellow needles, mp 253—254 °C, $[\alpha]_D^{25}$ −32.4° (*c*=0.41, pyridine). UV λ_{max}^{MeOH} nm (log ε): 238 (4.15), 245sh (4.12), 267 (4.17), 290 (4.03), 333 (4.24); $\lambda_{max}^{MeOH+AcONa}$ nm: 275, 304sh, 358; $\lambda_{max}^{MeOH+AcONa+H_3BO_3}$ nm: 267, 333; $\lambda_{max}^{MeOH+AlCl_3}$ nm: 256, 275, 294, 349, 385sh; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 256, 274sh, 276, 292sh, 347, 385sh. IR ν_{max}^{KBr} cm^{-1}: 3392, 1656, 1609, 1507—1360, 1075—1010. The ^{1}H- and ^{13}C-NMR data are shown in Tables I and II, respectively.

Acid Hydrolysis of 1 A solution of **1** (5 mg) in 2 N HCl (4 ml) was heated in a boiling-water bath for 1 h. The reaction mixture was extracted with AcOEt, then the AcOEt layer was evaporated to dryness *in vacuo* after being washed with H_2O. The AcOEt extract was recrystallized from MeOH–H_2O to afford diosmetin (**1a**) (1.5 mg), which was identified by comparison with authentic sample (thin-layer chromatography (TLC) and IR). The aqueous layer was evaporated to dryness *in vacuo*, and the residue showed the presence of D-glucose on Avicel SF TLC (solvent, 1-BuOH : AcOEt : H_2O=3 : 1 : 1, *Rf*=0.24).

Torosaflavone B 3′-*O*-β-D-Glucopyranoside (2) Recrystallization (MeOH) gave pale yellow needles, mp 212—213 °C, $[\alpha]_D^{24}$ +36.1° (*c*=0.675, pyridine), *Anal.* Calcd for $C_{28}H_{32}O_{14}\cdot 3/2H_2O$: C, 54.18; H, 5.65. Found: C, 54.26; H, 5.76. UV λ_{max}^{MeOH} nm (log ε): 237sh (4.39), 244sh (4.34), 270 (4.43), 333 (4.45); $\lambda_{max}^{MeOH+AcONa}$ nm: 278, 304sh, 370; $\lambda_{max}^{MeOH+AcONa+H_3BO_3}$ nm: 270, 333; $\lambda_{max}^{MeOH+AlCl_3}$ nm: 258, 278, 353, 380sh; $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 255, 278, 347, 380sh. IR ν_{max}^{KBr} cm^{-1}: 3350, 1650, 1620, 1580, 1100—1000. FD-MS *m/z*: 593 (M^++H), 575 (M^++H−H_2O), 531 (M^+−H_2O−Me−CO), 499 [M^+−H_2O−Me−CH_2(OH)CHO], 431 (M^++H−$C_6H_{10}O_5$, 412 (M^+−H_2O−$C_6H_{10}O_5$), 300 (M^++H−$C_6H_{10}O_5$−$C_6H_{10}O_5$−$C_6H_{11}O_3$). The ^{1}H- and ^{13}C-NMR data are shown in Tables I and II, respectively.

Hydrolysis of 2 a) Acid Hydrolysis: A solution of **2** (30 mg) in 1 N HCl (5 ml) was heated in a boiling-water bath for 1 h. The reaction mixture was extracted with AcOEt, then the AcOEt layer was evaporated to dryness *in vacuo* after being washed with H_2O. The AcOEt extract was recrystallized from MeOH to afford torosaflavone B (**2a**) (10 mg), which was identified by comparison with authentic sample (TLC, IR,

and circular dichroism (CD)). The aqueous layer was neutralized with Amberlite IRA-45 (OH^- form), and evaporated to dryness *in vacuo*. The residue showed the presence of D-glucose on Avicel SF TLC (solvent, 1-BuOH : AcOH : H_2O = 3 : 1 : 1, Rf = 0.24).

b) Enzymatic Hydrolysis: A solution of **1** (3 mg) and β-glucosidase (5 mg) in H_2O (3 mg) was kept for 36 h at 37 °C, and aglycone precipitated. The precipitates were collected and recrystallized from MeOH to afford **2a** (1.4 mg). The filtrate was evaporated to dryness *in vacuo*. The residue show the presence of D-glucose on Avicel SF TLC (solvent, 1-BuOH–AcOH–H_2O = 3 : 1 : 1, Rf = 0.24).

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