Antiallergic Agent from Natural Sources. 2.[1] Structures and Leukotriene Release-Inhibitory Effect of Torososide B and Torosachrysone 8-*O*-6″-Malonyl β-Gentiobioside from *Cassia torosa* Cav.

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Two new anti-allergic compounds, torososide B and torosachrysone 8-*O*-6″-malonyl gentiobioside were isolated from the seeds of *Cassia torosa* Cav., and their structures were established as physcion 8-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside and torosachrysone 8-*O*-β-D-glucopyranosyl-(1→6)-6-malonyl β-D-glucopyranoside on the basis of spectral and chemical evidence.

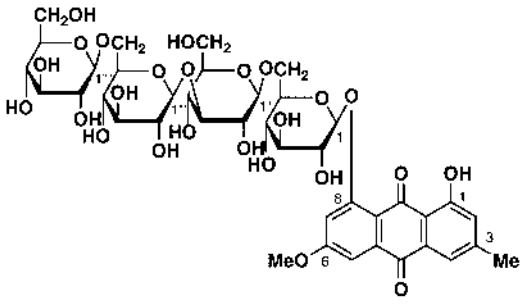
Torososide B and torosachrysone 8-*O*-6″-malonyl gentiobioside were found to inhibit the release of leucotrienes from rat peritoneal mast cells induced by calcium ionophore A 23187.

Key words *Cassia torosa*; anthraquinone glycoside; torososide B; torosachrysone 8-*O*-6″-malonyl β-gentiobioside; anti-allergic agent; leucotriene release inhibitor

Leukotrienes (LTs) are formed by several enzymes, *e.g.* 5-lipoxygenase, LTC_4 synthetase and LTA_4 hydrase, from arachidonic acid released from the phosphatide of the cell membrane such as mast cells or neutrophils by phospholipase A_2. A mixture of sulphidopeptides LTC_4, LTD_4 and LTE_4 is known as the slow reacting substance of anaphylaxis (SRS-A) and when combined they induce bronchospasm, mucus secretion, and enhanced vascular permeability, while LTB_4 is a very potent chemoattractant for neutrophils, eosinophils and mononuclear phagocytes. Therefore, we have researched inhibitors of LT release from mast cells from natural sources.

The seeds of *Cassia torosa* Cav. (Leguminosae) have been used as a Japanese folk medicine to help digestion, as an antidote and as a tonic. We have previously reported the isolation of anthraquinone, anthrones, hydroanthracenes, naphthalenic lactones, and flavonoids from ripe and unripe seeds, seedlings, roots, and leaves of *Cassia torosa* Cav.[2—17] During our search for antiallergic compounds from a natural source, two new phenolic glycosides, torososide B (**1**) and torosachrysone malonylgentiobioside (**2**), isolated from seeds of *Cassia torosa* Cav., were found to inhibit the release of LTs from rat peritoneal mast cells induced by calcium ionophore A 23187 (A 23187).

Torososide B (**1**), yellow powder, mp 208—209 °C, $[\alpha]_D$ −71.3°, showed a red coloration in the methanolic sodium hydroxide test and in the magnesium acetate test.[18] The UV spectrum showed maxima at 223, 271 and 418, and the IR spectrum exhibited bands due to hydroxyls (3412 cm^{-1}), a non-chelated quinone (1670 cm^{-1}), a chelated quinone (1630 cm^{-1}) and aromatic rings (1596, 1485 cm^{-1}). Torososide B (**1**) was presumed to be an anthraquinone glycoside



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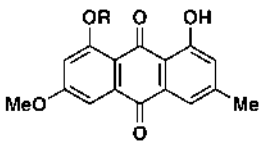
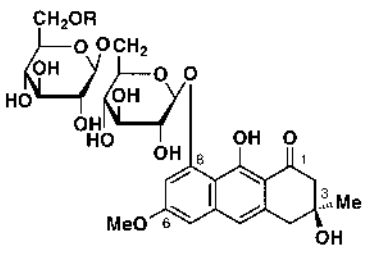


1a: R =H
2b: R = gentiobiosyl



2: R = -OOCCH$_2$COOH
2a: R = H

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from its characteristic color reaction and spectral properties. The ^{1}H-NMR spectrum of **1** indicated the presence of one aromatic methyl group (δ 2.38), one methoxyl group (δ 3.93), four aromatic protons (δ 7.15, 7.18, 7.34, 7.45), one chelated hydroxyl (δ 13.09), and four anomeric protons (δ 4.18, 4.31, 4.33, 5.13) (Table 1). The field desorption (FD) MS of **1** observed at *m/z* 955 $[M+Na]^+$ and 284 $[M-hexose\times4]^+$ suggested that **1** is an anthraquinone tetrahexoside.

Treatment of **1** with β-glucosidase gave **1a**, the ^{1}H-NMR data of which were identical with those of physcion.[4] Compound **1** was thus presumed to be physcion tetraglucoside. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **1** revealed the presence of two methyl, four aromatic methine, eight tertiary aromatic carbon, twenty aliphatic methine, and four methylene signals (Table 1) . The ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectrum of **1** showed a ^{1}H–^{13}C long-range correlation between the anomeric proton at δ 5.13 (H-1′) and carbon signal at δ 161.0 (C-8) and also between a proton at δ 7.18 (H-7) and aromatic carbon signals at δ 161.0 (C-8) and 165.3 (C-6). So, the location of the sugar moiety in **1** was confirmed to be at C-8. The proton and carbon signals of the sugar moiety were assigned as shown in Table 1 by ^{1}H–^{1}H correlation spectrometry (COSY), heteronuclear multiple quantum coherence (HMQC) and HMBC analysis. The ^{1}H–^{13}C long-range correlations between δ 4.16 (H-1‴′) and δ 69.0 (C-6‴), between δ 4.33 (H-1‴) and δ 88.9 (C-3″), and between δ 4.29 (H-1″) and δ 68.9 (C-6′) were observed. Carbon signals due to the *O*-tetraglucoside moiety in the ^{13}C-NMR spectrum of **1** were found to be superimposable on those of the cassiasides B_2 and C_2.[1] Therefore, the structure of **1** was characterized as physcion 8-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

Torosachrysone 8-*O*-6″-malonyl β-gentiobioside (**2**), a yellow powder, $[\alpha]_D$ −20.9°, was fluorescent blue under UV light. The UV maxima at 304 and 314 nm showed that torosachrysone possesses a naphthalene nucleus, and the IR spectrum exhibited absorption bands due to hydroxyls (3406 cm^{-1}), a carbonyl ester (1729 cm^{-1}), a chelated carbonyl (1629 cm^{-1}) and aromatic rings (1610, 1590 cm^{-1}). The ^{1}H-NMR spectrum of **2** revealed the presence of a methyl group (δ 1.28), a methoxyl group (δ 3.86), two nonequivalent methylenes at δ 2.65 and 2.91 (each d, J=17.4 Hz) and δ 2.87 (d, J=16.9 Hz) and 3.10 (br d), an equivalent methylene group (δ 3.17), a pair of *meta*-coupled aromatic protons (δ 6.83 and 6.87), an aromatic proton (δ 6.96), a chelated hydroxyl (δ 14.87), and two anomeric protons at δ 4.22 (1H, d, J=7.3 Hz) and 5.18 (1H, d, J=7.3 Hz). Compound **2** is shown to be a derivative of torosachrysone biglycoside from its characteristic spectral properties.[5] The ^{13}C-NMR and DEPT spectra of **2** revealed the presence of sixteen carbons of the torosachrysone moiety, twelve carbons consist of two hexose molecules, an unassigned methylene and two carbonyl carbons containing two esters or a free carboxylic acid and an ester (Table 1). From analysis of the HMQC and HMBC spectra of **2**, the structure of the aglycone moiety was identified as torosachrysone.[7] Long-range correlations between an anomeric proton at δ 4.22 (H-1″) and a carbon at δ 69.2 (C-6′) and between the other anomeric proton at δ 5.18 (H-1′) and a carbon at δ 158.3 (C-8) were observed. The presence of a malonyl ester was estimated from a methylene carbon at δ 43.2 and two carbonyl groups at δ 167.9 and 168.2 in **2**. From this evidence and these observations at *m/z* 737 $[M+K]^+$ and 721 $[M+Na]^+$ in the FDMS, **2** was considered to be a malonate of torosachrysone 8-*O*-(1→6)-hexabioside.

Treatment of **2** with 1% Na_2CO_3/80% MeOH gave torosachrysone 8-*O*-β-gentiobioside (**2a**) and physcion 8-*O*-β-gentiobioside (**2b**).[5] The location of the malonyl ester in **2** was confirmed to be at the 6″-hydroxyl by comparison of the ^{13}C-NMR spectral data of **2** and **2a**. That is to say , the C-6″ (δ 64.2) signal in **2** shifted downfield by 4.0 ppm owing to

Table 1. NMR Spectral Data of Torososide A (**1**) and Torosachrysone 8-*O*-6″-Malonyl β-Gentiobioside (**2**) (400 Mz, Me_2CO-d_6, TMS as internal standard)

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1			161.9	203.9
2	7.15 br s	124.8	2.65 d (17.4)	51.5
			2.91 d (17.4)	
3		147.9		69.4
4	7.45 br s	120.1	2.87 d (16.9)	42.8
			3.10 br d	
4a		136.9		138.0
5	7.34 d (2.2)	107.0	6.83 d (2.2)	101.2
6		165.3		161.6
7	7.18 d (2.2)	108.2	6.87 d (2.2)	104.5
8		161.0		158.3
8a		115.1		109.4
9		187.0		164.4
9a		115.0		110.0
10		182.5	6.69 s	116.5
10a		132.6		141.0
3-Me	2.38 br s	28.0	1.28 br s	29.0
OMe	3.93 s	56.7	3.86 s	55.4
OH-1	13.09 s			
OH-9			14.87 s	
Sugar moiety				
1′	5.13 d (7.3)	100.6	5.18 d (7.3)	100.7
2′		73.3		73.5
3′		76.5		76.3
4′		69.9		69.9
5′		76.0		75.1
6′		68.9		69.2
1″	4.29 d (7.3)	102.9	4.22 d (7.3)	103.8
2″		72.0		73.0
3″		88.9		75.9
4″		69.0		69.8
5″		75.9		73.1
6″		60.7		64.2
1‴	4.33 d (7.3)	104.1		
2‴		73.6		
3‴		76.9		
4‴		71.1		
5‴		75.1		
6‴		69.0		
1⁗	4.16 d (7.3)	102.9		
2⁗		74.0		
3⁗		76.2		
4⁗		70.4		
5⁗		76.0		
6⁗		61.0		
Malonyl			3.17 s	43.2
				167.9
				168.2

Table 2. Inhibitory Effects of Torososide (**1**), Torosachrysone 8-*O*-6"-Malonyl Gentiobioside (**2**), Torosachrysone 8-*O*-Gentiobioside (**2a**), and Hydrocortisone on Leukotriene Release from Rat Peritoneal Mast Cells

Substance	Concentration (M)	*n*	Inhibitory ratio (%)			
			LTB_4	LTC_4	LTD_4	LTE_4
Torososide B (**1**)	10^{-4}	3	46.9	39.7	41.2	43.3
	10^{-5}	3	13.3	9.3	10.9	8.1
Torosachrysone 8-*O*-6″-malonyl	10^{-4}	3	35.4	30.9	29.3	32.3
β-gentiobioside (**2**)	10^{-5}	3	5.1	4.1	6.3	7.5
Torosachrysone 8-*O*-	10^{-4}	3	24.8	30.1	21.0	31.1
β-gentiobioside (**2a**)	10^{-5}	3	4.2	7.8	5.1	5.3
Hydrocortisone	2.5×10^{-4}	3	70.1	75.1	69.3	72.1

the acylation shift, compared with that of **2a**. On the basis of the evidence presented, the structure of **2** is torosachrysone 8-*O*-6″-malonyl *β*-gentiobioside.

The inhibitory effect of torososide B (**1**), torosachrysone 8-*O*-6″-malonyl *β*-gentiobioside (**2**), and torosachrysone 8-*O*-gentiobioside (**2a**) on LTs B_4, C_4, D_4 and E_4 released from rat peritoneal mast cells stimulated by calcium ionophore A23187 is shown in Table 2. Compound **1** strongly inhibited the release of LTs at a concentration of 10^{-4} M in the three compounds. Compounds **2** and **2a** exhibited somewhat weaker inhibition than **1**. This is the first example in which anthraquinone and tetrahydroanthracene derivatives showed a leukotriene release-inhibitory effect.

Experimental

General Procedures All melting points were measured with a Yanagimoto micro-melting-point apparatus and are uncorrected. The UV spectrum was obtained in MeOH with a Hitachi 200-10 spectrophotometer, and IR spectra were recorded on a JASCO IR A-2 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JNM GX-400 spectrometer using tetramethyl silane (TMS) as an internal standard. MS were obtained on a Hitachi M-80B spectrometer. Column chromatography was carried out on Wako gel C-200 (Wako Pure Chemical), Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemical), Diaion HP20 (Nippon Rensui), MCI gel CHP 20P (Mitsubishi Chemical) and Cosmosil 75 C_{18}-OPN (Nacalai Tesque). HPLC analysis was carried out on a JASCO apparatus (pump, Gulliver PU-980; ultraviolet spectrophotometric detector, Gulliver UV-970; peak area measurement, Shimadzu C-R 5A Chromatopak). HPLC reference standards, leukotrienes B_4, C_4, D_4 and E_4 and calcium ionophore A23187 were purchased from Wako Pure Chemical Industries, Ltd.

Extraction and Isolation Plant material was obtained from the Drug Plant Garden of the College of Pharmacy, Nihon University. The seeds (1.0 kg) of *C. torosa* were extracted with 80% aqueous MeOH (2.5 l×3) in a sonicator. The MeOH extract was concentrated *in vacuo* into a dark mass (58.59 g) (LT release inhibitory effect (1000 μg/ml); LTB_4: 62%, LTC_4: 56%), which was suspended in H_2O and extracted with Et_2O. The H_2O layer (55.05 g) was separated on a Diaion HP20 column with H_2O and then with MeOH–H_2O mixtures to give frs. 2—7. Fraction 2 was applied to a Sephedex LH-20 (MeOH–H_2O as eluent), an ODS (MeOH–H_2O), a CHP 20P (MeOH–H_2O) and a silica gel ($CHCl_3$–MeOH–H_2O) column resulting in **2** (24.5 mg), **2a** (20.0 mg) and tryptophane (14.5 mg), respectively. Fraction 3 was applied to a Sephadex LH-20 (MeOH–H_2O) and silica gel ($CHCl_3$–MeOH–H_2O) column, resulting in the isolation of **2a** (20.2 mg) and **1** (14 mg), respectively.

Torososide B (1) Recrystallization (MeOH) gave a yellow powder, mp 208—209 °C, $[\alpha]_D^{14}$ −71.3° (pyridine; c=0.3). UV λ_{max}^{MeOH} nm (log ε): 223 (4.47), 271 (4.38), 418 (3.88). IR ν_{max}^{KBr} cm^{-1}: 3412, 2925, 1670, 1630, 1596, 1485, 1440, 1367, 1314, 1266, 1216, 1165, 1068, 906, 755. FDMS *m/z*: 955 [M+Na]$^+$, 284 [M-4 hexose]$^+$. ^{1}H-NMR and ^{13}C-NMR data: see Table 1.

Enzymatic Hydrolysis of Compound 1 A solution of **1** (1 mg) and *β*-glucosidase (1 mg) in H_2O (2 ml) was kept for 24 h at 37 °C. The resultant ppt. was recrystallized from methanol to afford **1a**, which was identified as physcion by comparison with an authentic sample (TLC and HPLC).

Torosachrysone 8-*O*-6″-Malonyl *β*-D-Gentiobioside (2) Recrystallization (MeOH–EtOAc) gave a yellow powder, $[\alpha]_D^{14}$ −20.9° (H_2O; c=0.3). UV λ_{max}^{MeOH} nm (log ε): 228 (4.16), 272 (4.44), 304 (3.44), 314 (3.68), 326 (3.63), 382 (3.78). IR ν_{max}^{KBr} cm^{-1}: 3406, 2934, 1729, 1629, 1610, 1590, 1455, 1413, 1377, 1292, 1246, 1204, 1171, 1062, 921, 833. FDMS *m/z*: 737 [M+K]$^+$, 721 [M+Na]$^+$. ^{1}H-NMR and ^{13}C-NMR data: see Table 1.

Alkaline Treatment of 2 A solution of **2** (2 mg) and 1% Na_2CO_3 in 80% methanol (5 ml) was kept for 5 min at 80 °C. The reaction mixture was neutralized with 10% HCl solution, which was identified as torosachrysone 8-*O*-*β*-D-gentiobioside (**2a**) and physcion 8-*O*-*β*-D-gentiobioside (**2b**) by comparison with an authentic sample (TLC and HPLC), respectively.

Preparation of Rat Peritoneal Mast Cells The preparation of mast cells was based on the method of Hirai *et al.*[19] with modifications. 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 gently massaged for 2 min and then the peritoneal exudate was collected. The peritoneal cavity fluid containing mast cells was suspended in PBS, then layered on BSA (d=1.068) in a test tube at room temp. 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. Cell viability was determined using trypan blue.

Assay of Inhibitory Activity on Leukotriene Release The cell suspension (10^7 cells/ml) and test sample solution were preincubated for 5 min at 37 °C. The cells or PBS were incubated with calcium ionophore A23187 (2 μM) for 10 min at 37 °C and the reaction was terminated by the addition of cold 0.001 N HCl. After removal of the cells by centrifugation at 2500×***g*** and 4 °C for 5 min, the supernatant was applied on an Isolute C_{18} cartridge (IST), which was quickly washed with successive Tyrode solution, 20% MeOH, MeOH and EtOAc. The MeOH and EtOAc eluents were combined and removed under a nitrogen stream at 30 °C, and the residue was dissolved in a small amount of methanol (70 μl). Samples thus obtained were analyzed by reversed phase HPLC[20—22] under the following operation conditions: Detection, UV 280 nm; injection volume, 20 μl; column; Capcell pak C_{18} UG120, (3 μm, 4.6×250 mm; Shiseido); column temperature, 40 °C; flow rate, 1.0 ml/min; mobile phase, 20% sodium acetate/acetonitrile/methanol/trifluoroacetic acid (55/30/22/0.04), *Rt* values (min): LTC_4 (14.7), LTD_4 (23.1), LTE_4 (26.4) and LTB_4 (31.8). The activity of the test substance on LT release from mast cells induced by A23187 was expressed as an inhibitory percentage.

References and Notes

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