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Torosachryson and Physcion Gentiobiosides from the Seeds of *Cassia torosa*¹⁾

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Torosachryson 8- β -D-gentiobioside (**1**) and physcion 8- β -D-gentiobioside (**2**) were isolated along with protocathechuic acid from the unripe and ripe seeds of *Cassia torosa* CAVANILLES, respectively. The structure of **1** was established on the basis of chemical and spectroscopic findings. In addition, the ¹³C nuclear magnetic resonance spectra of torosachryson and related compounds are discussed.

Keywords—*Cassia torosa*; Leguminosae; torosachryson 8- β -D-gentiobioside; physcion 8- β -D-gentiobioside; ¹³C-NMR; long-range selective proton decoupling (LSPD)

We previously reported the isolation of three anthraquinones, physcion,²⁾ xanthorin and emodin, and a hydroanthracene, torosachryson (**3**),³⁾ from the ripe seeds and physcion-9-anthrone, physcion-10,10'-bianthrone, **3**, phlegmacins A₂ and B₂, anhydrophlegmacin B₂, torosanin and phytosterols⁴⁾ from the unripe seeds of *Cassia torosa*. On the other hand, we have isolated several hydroanthracenes, germichryson,⁵⁾ germitorosone, and methylgermitorosone,⁶⁾ as characteristic constituents of the seedlings together with dimeric hydroanthracenes, phlegmacins A₂ and B₂ and anhydrophlegmacin-9,10-quinones A₂ and B₂.⁷⁾ The present paper deals with the isolation and characterization of the torosachryson and physcion-glycosides from unripe and ripe seeds of this plant and the assignments of the ¹³C-nuclear magnetic resonance (¹³C-NMR) signals.

The methanolic extract of the unripe seeds was extracted with benzene, ethyl acetate and butanol successively. The ethyl acetate and butanol extracts afforded protocathechuic acid and torosachryson 8- β -D-gentiobioside (**1**), respectively, whereas the butanol extract of the methanolic extract of ripe seeds afforded physcion 8- β -D-gentiobioside (**2**).

Compound **1**, yellow powder, mp 166–168 °C, [α]_D²² –43.6° (methanol), C₂₈H₃₆O₁₅, gave positive ferric chloride and Gibbs tests. The similarity of the chromophore of **1** to that of torosachryson (**3**) was established by comparison of the ultraviolet (UV) spectra. The

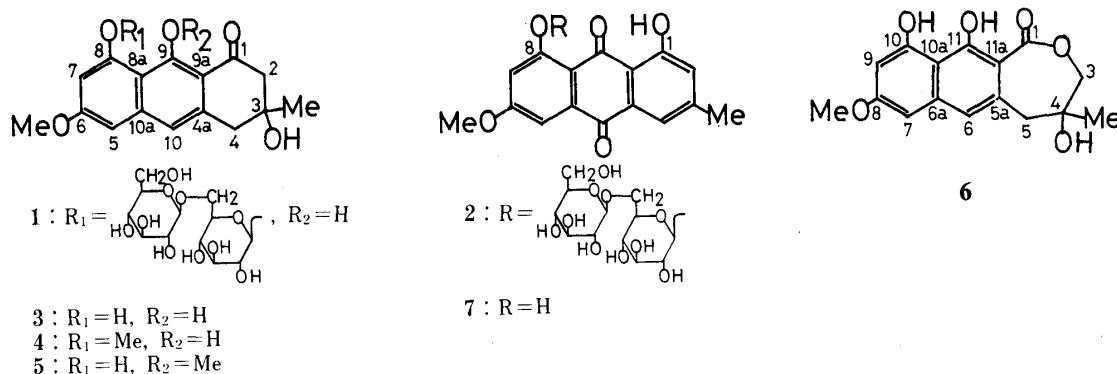


Chart 1

TABLE I. ^1H -NMR Data for **1**, **3**, **4** and **5**^{a)}

	1	3	4	5
H-2	2.71 d 2.91 d $J=17.1$ Hz	2.68 d 2.88 d $J=17.1$ Hz	2.68 d 2.90 d $J=17.1$ Hz	2.60 d 2.78 d $J=17.1$ Hz
Me-3	1.28 br s	1.29 br s	1.27 br s	1.26 br s
H-4	3.00 br s	2.96 br s	2.98 br s	3.04 br s
H-5	6.82 s	6.71 d $J=2.4$ Hz	6.76 d $J=2.4$ Hz	6.76 d $J=2.4$ Hz
OMe	3.86 s	3.84 s	3.87 s $\times$ 2	3.83 s 3.85 s
H-7	6.82 s	6.41 d $J=2.4$ Hz	6.49 d $J=2.4$ Hz	6.43 d $J=2.4$ Hz
OH-8		9.84 s		9.96 s
OH-9	14.89 s	15.78 s	14.86 s	
H-10	6.96 s	6.94 s	7.36 s	7.34 s
H-1'	5.04 br d $J=6.5$ Hz			
H-1''	4.23 br d $J=6.5$ Hz			

a) Measured in $\text{DMSO}-d_6$ at 100 MHz with TMS as an internal standard. s, singlet; br s, broad singlet; d, doublet.

infrared (IR) spectrum of **1** showed strong absorption bands at 3400 and 1000–1120 cm^{-1} , indicating the presence of sugar in **1**. Hydrolysis of **1** with β -D-glucosidase gave D-glucose and the corresponding aglycone, which was identified as **3** by direct comparison with an authentic sample. The proton nuclear magnetic resonance (^1H -NMR) spectral data are given in Table I. Two anomeric proton signals at δ 4.23 and 5.04 (each 1H, d, $J=6.5$ Hz) in the spectrum of **1** indicated the presence of two β -linkages. Two partial methyl ethers (**4** and **5**) obtained by diazomethane treatment of **3** were indicated by the signals at δ 14.86 (OH-9 in **4**) and δ 9.96 (OH-8 in **5**), while a chelated phenolic hydroxyl in **1** appeared at δ 14.89. Consequently, the position of the sugar was concluded to be OH-8 of **3**. The ^{13}C -NMR data (Table II) indicated that the sugar in **1** is gentiobiose, because the chemical shifts of the 6 and 5 positions in glucose were observed at δ 68.5 (C-6'), 60.0 (C-6''), 75.3 (C-5') and 76.4 (C-5'').⁸⁾ Therefore **1** was established to be torosachryson 8- β -D-gentiobioside.

The assignments of ^{13}C resonances to the carbon atoms of **3** were based on ^1H -noise and noise off-resonance decouplings, selective proton decoupling (SPD), long-range selective proton decoupling (LSPD)⁹⁾ and nondecoupling experiments, and comparisons with the results for substituted derivatives, **1**, **4**, **5** and cassialactone (**6**).

The SPD experiments with **3** showed clear enhancement. Based on irradiations of the proton resonances at δ 6.41 (H-7), 6.71 (H-5) and 6.94 (H-10) in **3**, the aromatic methine carbon signals were concluded to be at δ 100.3 (C-7, 95% enhancement), 99.1 (C-5, 21%) and 116.7 (C-10, 97%). The LSPD experiments on **3** showed that irradiations of the two hydroxyl protons at δ 9.84 (OH-8) and 15.78 (OH-9) and the methoxy protons at δ 3.84 produced signal enhancements at δ 158.6 (C-8, 75%), 164.4 (C-9, 134%) and 162.3 (C-6, 42%), which are strongly deshielded by the naphthalene skeleton,¹⁰⁾ respectively. Irradiation of H-4 at δ 2.96 caused large changes at both δ 108.3 (d, $^3J_{\text{CH}}=4.4$ Hz) and 137.4 (t, $^2J_{\text{CH}}=5.9$ Hz) among the quaternary carbon atoms (δ 107.3, 108.3, 137.4 and 141.7). The former signal changed into a sharpened doublet ($^3J_{\text{CH}}=7.3$ Hz) and showed increased peak height (209%), while the latter changed into a singlet and showed increased peak height (417%). Therefore, the signals were concluded to be due to C-9a and C-4a. Consequently, the carbon signals at δ 107.3 and 141.7

TABLE II. ^{13}C -NMR Data for **1**, **3**, **4**, **5** and **6**^{a)}

	1	3	4	5		6
C- 1	203.7 S, t 5.9 ^{b)}	203.5 S, t 5.9	203.2 S	195.1 S	C- 1	170.1 S
2	51.3 T 129.4	50.6 T 129.4	51.5 T	55.0 T	3	66.3 T 144.1
3	69.3 S	69.5 S	69.3 S	69.4 S	4	84.6 S
4	42.6 T 128.0	42.3 T 128.0	42.8 T	43.8 T	5	33.5 T 129.4
4a	137.9 S, t 5.9	137.4 S, t 5.9	138.0 S	139.0 S	5a	133.1 S, t 5.9
10	116.3 D, d 162.5, 4.4	116.7 D, d 161.8, 4.4	116.4 D	122.3 D	6	116.5 D 164.7
10a	140.8 S, d 2.2	141.7 S	141.3 S	139.2 S	6a	140.6 S
5	100.5 D, t 4.4	99.1 D, t 163.2, 4.4	97.4 D	97.6 D	7	99.1 D, t 163.2, 4.6
6	161.2 q 3.7	162.3 S, q 3.7	161.6 S	160.6 S	8	161.9 S, d 2.9
7	101.2 D, t 159.6, 4.5	100.3 D, t 161.8, 5.9	98.9 D	101.4 D	9	101.1 D, d 160.3, 5.9
8	158.1 S, t 2.9	158.6 S, d 4.4	160.6 S	156.9 S	10	161.9 S, d 2.9
8a	109.3 S, m	107.3 S, m	109.2 S	117.7 S	10a	108.0 S, q 5.9
9	164.1 S, d 5.1	164.4 S	164.5 S	158.9 S	11	161.9 S
9a	109.5 S, q 6.6	108.3 S, d 4.4	109.4 S	111.8 S	11a	99.1 S, d 7.4
Me-3	28.8 Q 125.0	28.9 Q 125.0	28.7 Q	28.8 Q	Me-4	21.8 Q 125.0
OMe	55.3 Q 144.9	55.2 Q 144.7	55.2 Q	55.0 Q	OMe	55.4 Q 144.1
			55.7 Q	63.2 Q		
Sugar moiety						
C- 1'	103.4					
2'	73.3					
3'	76.7					
4'	69.9 ^{c)}					
5'	75.3					
6'	68.5					
1''	100.7					
2''	73.3					
3''	76.1					
4''	69.5 ^{c)}					
5''	76.4					
6''	60.0					

a) Measured in DMSO- d_6 at 25.2 MHz with TMS as an internal standard. S, s, singlet; D, d, doublet; T, t, triplet; Q, q, quartet (the small letters are splitting patterns with long-range couplings).

b) Coupling constants are given in Hz.

c) Values may be reversed.

which were unchanged were assigned to C-8a and C-10a, respectively. On the basis of the data for **3**, the ^{13}C -NMR data for the related compounds (**1**, **4**, **5** and **6**) were assigned as shown in Table II. Glycosylation of the 8-hydroxyl in **3** produced remarkable shifts. The signals of the *ortho* [C-7 (−0.9 ppm) and C-8a (−2.0 ppm)] and *para* [C-5 (−1.4 ppm)] carbons showed glycosylation shifts, and a marked effect was also seen in the signal of the C-9a (−1.2 ppm) carbon owing to the disruption of 8-hydroxy-9-hydroxy hydrogen-bonding.

Compound **2**, yellow powder, mp 221–223 °C, $\text{C}_{28}\text{H}_{32}\text{O}_{15}$, gave a red color with NaOH and $\text{Mg}(\text{OAc})_2$ reagents.¹¹⁾ This compound was a glycoside and upon β -D-glucosidase hydrolysis gave D-glucose and an aglycone $\text{C}_{16}\text{H}_{12}\text{O}_5$, mp 213–215 °C, which was identified as physcion (**7**). The sugar moiety was proved to be gentiobioside by ^{13}C -NMR spectroscopy: δ 68.9 (C-6'), 60.8 (C-6''), 75.5 (C-5') and 76.2 (C-5''). A conversion of **1** to **2** confirmed the identity of **2** as physcion 8- β -D-gentiobioside. Recently, isolation of physcion 8- β -D-gentiobioside from rhubarb roots was reported.¹²⁾

In the present study we found an anthraquinone glycoside, physcion 8- β -D-gentiobioside, in the ripe seeds, together with a small amount of a tetrahydroanthracene glycoside, torosachrysone 8- β -D-gentiobioside (observed on thin layer chromatography). In the unripe

seeds, torosachryson 8- β -D-gentiobioside was found instead of physcion 8- β -D-gentiobioside. These findings suggest that the tetrahydroanthracene glycoside is dehydrated and oxidized to anthraquinone glycoside as the seeds mature.¹³⁾

Experimental

All 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 NMR spectra were taken on a JEOL FX-100 instrument, and the chemical shifts are given in ppm relative to internal tetramethylsilane (TMS). Mass spectra (MS) were obtained on a Hitachi RMU-7M spectrometer. Column chromatography was performed on silicic acid (SiO₂) (Mallinckrodt). Thin-layer chromatography (TLC) was carried out using Merck silica gel G 60 with CHCl₃-MeOH-H₂O (14:6:1).

Extraction and Isolation—Crushed unripe seeds (2.4 kg) were extracted with cold MeOH (3 $\times$ 5 l). The MeOH extract was concentrated *in vacuo* to give a yellow-brown mass, which was dissolved in H₂O (2 l) and extracted with C₆H₆, AcOEt and BuOH successively. The BuOH solution was concentrated *in vacuo* to give a brown mass (15.3 g), which was chromatographed on SiO₂ with the lower phase of CHCl₃-MeOH-H₂O (7:3:1) to afford a crude yellow mass. This was chromatographed on a Sephadex LH-20 column and eluted with MeOH to give a yellow powder (**1**) (70 mg).

Crushed ripe seeds (2.5 kg) were extracted with 90% MeOH (4 $\times$ 5 l). The MeOH extract was concentrated to give a brown mass, which was dissolved in H₂O (2 l) and extracted with C₆H₆, AcOEt and BuOH successively. The BuOH extract was concentrated *in vacuo* to 1 l. On addition of MeOH (1 l) a solid mass precipitated and was separated by filtration. The filtrate was evaporated to give a brown mass (15 g), which was recrystallized from MeOH to give **2** (150 mg).

Torosachryson 8- β -Gentiobioside (1)—Compound **1** was deposited from MeOH to yield a yellow powder, mp 166–168 °C, $[\alpha]_D^{22}$ –43.6° (*c* = 0.25, MeOH). *Anal.* Calcd for C₂₈H₃₆O₁₅ · 2/3H₂O: C, 53.84; H, 5.81. Found: C, 53.62; H, 6.27. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 222 (4.47), 268 (4.76), 300 sh (3.77), 312 (3.90), 325 (3.85), 385 (4.04). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3400, 1625, 1610, 1000–1140. ¹H-NMR and ¹³C-NMR data are shown in Tables I and II.

Enzymatic Hydrolysis of 1—A solution of **1** (3 mg) in H₂O was treated with β -D-glucosidase to give a yellow aglycone. The aglycone was recrystallized from C₆H₆ as yellow prisms, mp 203 °C, which were identified by direct comparison with an authentic sample of torosachryson (**3**). The aqueous solution was evaporated *in vacuo* and glucose was identified by TLC.

Partial Methylation of Torosachryson (3)—A solution of **3** (100 mg) in CHCl₃ (100 ml) was methylated with CH₂N₂ at room temp. for 10 h. The solution was evaporated to give a yellow residue, which was chromatographed on SiO₂ with C₆H₆-AcOEt (9:1) to afford 8-methyltorosachryson (**4**) (15 mg) and 9-methyltorosachryson (**5**) (40 mg).

8-Methyltorosachryson (4)—Yellow needles (C₆H₆), mp 229–231 °C. UV $\lambda_{\max}^{\text{dioxane}}$ nm (log ϵ): 227 (4.25), 269 (4.66), 302 sh (3.61), 312 (3.74), 324 (3.56), 381 (4.04), 395 sh (4.01). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3440, 1625, 1590, 1460, 1440, 1420. High resolution MS *m/z*: Calcd for C₁₇H₁₈O₅: 302.1152, Found: 302.1135. ¹H-NMR and ¹³C-NMR data are shown in Tables I and II, respectively.

9-Methyltorosachryson (5)—Yellow needles (C₆H₆), mp 180–182 °C. UV $\lambda_{\max}^{\text{dioxane}}$ nm (log ϵ): 223 (4.29), 272 (4.77), 307 sh (3.53), 319 (3.67), 333 (3.66), 368 (3.84). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3350, 1675, 1635, 1600, 1560, 1500. High resolution MS *m/z*: Calcd for C₁₇H₁₈O₅: 302.1153, Found: 302.1161. ¹H-NMR and ¹³C-NMR data are shown in Tables I and II, respectively.

Physcion 8- β -Gentiobioside (2)—Yellow powder, mp 221–223 °C. *Anal.* Calcd for C₂₈H₃₄O₁₇: C, 52.34; H, 5.23. Found: C, 52.58; H, 5.35. UV $\lambda_{\max}^{\text{MeOH}}$ nm (log ϵ): 221 (4.62), 245 sh (4.15), 268 (4.48), 280 sh (4.42), 296 sh (3.69), 395 sh (3.89), 416 (3.98), 435 sh (3.92). IR $\nu_{\max}^{\text{KBr}}$ cm⁻¹: 3400, 1670, 1630, 1600, 1000–1140. ¹H-NMR (DMSO-*d*₆) δ : 2.41 (3H, br s, Me), 3.97 (3H, s, OMe), 2.80–5.30 (21H, m), 7.17 (1H, br s, H-4), 7.22 (1H, d, *J* = 2.4 Hz, H-5), 7.34 (1H, d, *J* = 2.4 Hz, H-7), 7.47 (1H, br s, H-2), 13.10 (1H, s, OH-1). ¹³C-NMR (DMSO-*d*₆) δ : 21.3 (q), 56.1 (q), 60.8 (t), 68.9 (t), 69.6 (d), 69.9 (d), 73.1 (d), 73.4 (d), 75.5 (d), 76.2 (d), 76.7 (d), 100.3 (d), 103.6 (d), 106.3 (d), 107.1 (d), 114.1 (s), 119.2 (d), 124.1 (d), 131.7 (s), 136.0 (s), 146.9 (s), 160.3 (s), 161.4 (s), 164.6 (s), 181.5 (s), 186.2 (s).

Enzymatic Hydrolysis of 2—A solution of **2** (5 mg) and β -D-glucosidase in H₂O (3 ml) was kept at 37 °C for 10 h, then water was added and the reaction mixture was extracted with AcOEt. The extract was recrystallized from MeOH to afford yellow needles (**7**) (1 mg), mp 213–215 °C. Its spectrum was the same as that of physcion. The aqueous layer was evaporated to dryness and glucose was identified chromatographically.

Conversion of 1 to 2—A solution of **1** in 5% NaOH was kept at 4 °C for a week, then neutralized with 1% HCl, to give a yellow precipitate. The product was recrystallized from MeOH and identified as **2** by direct comparison.

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References and Notes

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