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Torosachrysone and Physcion Gentiobiosides from the Seeds of *Cassia torosa*[1)]

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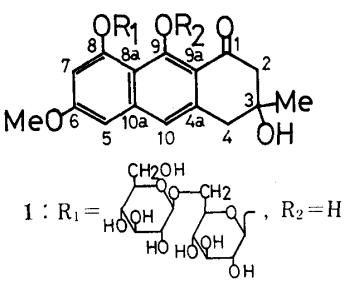
Torosachrysone 8-β-D-gentiobioside (**1**) and physcion 8-β-D-gentiobioside (**2**) were isolated along with protocatechuic acid from the unripe and ripe seeds of *Cassia torosa* CAVANILLES, respectively. The structure of **1** was established on the basis of chemical and spectroscopic findings. In addition,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

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

We previously reported the isolation of three anthraquinones, physcion,[2)] xanthorin and emodin, and a hydroanthracene, torosachrysone (**3**),[3)] from the ripe seeds and physcion-9-anthrone, physcion-10,10′-bianthrone, **3**, phlegmacins A_2 and B_2, anhydrophlegmacin B_2, torosanin and phytosterols[4)] from the unripe seeds of *Cassia torosa*. On the other hand, we have isolated several hydroanthracenes, germichrysone,[5)] germitorosone, and methylgermitorosone,[6)] as characteristic constituents of the seedlings together with dimeric hydroanthracenes, phlegmacins A_2 and B_2 and anhydrophlegmacin-9,10-quinones A_2 and B_2.[7)] The present paper deals with the isolation and characterization of the torosachrysone and physcion-glycosides from unripe and ripe seeds of this plant and the assignments of the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) signals.

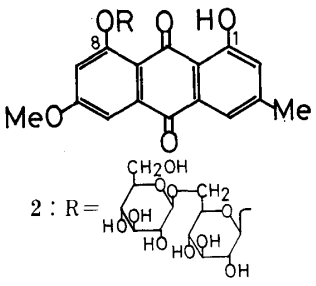
The methanolic extract of the unripe seeds was extracted with benzene, ethyl acetate and butanol successively. The ethyl acetate and butanol extracts afforded protocatechuic acid and torosachrysone 8-β-D-gentiobioside (**1**), respectively, whereas the butanol extract of the methanolic extract of ripe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

Compound **1**, yellow powder, mp 166—168 °C, $[\alpha]_D^{22}$ −43.6° (methanol), $C_{28}H_{36}O_{15}$, gave positive ferric chloride and Gibbs tests. The similarity of the chromophore of **1** to that of torosachrysone (**3**) was established by comparison of the ultraviolet (UV) spectra. The



1 : R_1 = , R_2 = H

3 : R_1 = H, R_2 = H
4 : R_1 = Me, R_2 = H
5 : R_1 = H, R_2 = Me



2 : R =

7 : R = H

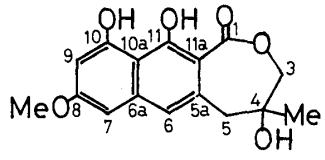


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Chart 1

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

	1	3	4	5
H-2	2.71 d	2.68 d	2.68 d	2.60 d
	2.91 d	2.88 d	2.90 d	2.78 d
	J=17.1 Hz	J=17.1 Hz	J=17.1 Hz	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	6.76 d	6.76 d
		J=2.4 Hz	J=2.4 Hz	J=2.4 Hz
OMe	3.86 s	3.84 s	3.87 s×2	3.83 s
				3.85 s
H-7	6.82 s	6.41 d	6.49 d	6.43 d
		J=2.4 Hz	J=2.4 Hz	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 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 (^{1}H-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 torosachrysone 8-β-D-gentiobioside.

The assignments of ^{13}C resonances to the carbon atoms of **3** were based on ^{1}H-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, $^3J_{CH}$=4.4 Hz) and 137.4 (t, $^2J_{CH}$=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 ($^3J_{CH}$=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, $C_{28}H_{32}O_{15}$, gave a red color with NaOH and $Mg(OAc)_2$ reagents.[11] This compound was a glycoside and upon β-D-glucosidase hydrolysis gave D-glucose and an aglycone $C_{16}H_{12}O_5$, 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, torosachrysone 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 (SiO_2) (Mallinckrodt). Thin-layer chromatography (TLC) was carried out using Merck silica gel G 60 with $CHCl_3$–MeOH–H_2O (14 : 6 : 1).

Extraction and Isolation——Crushed unripe seeds (2.4 kg) were extracted with cold MeOH (3 × 5 l). The MeOH extract was concentrated *in vacuo* to give a yellow-brown mass, which was dissolved in H_2O (2 l) and extracted with C_6H_6, AcOEt and BuOH successively. The BuOH solution was concentrated *in vacuo* to give a brown mass (15.3 g), which was chromatographed on SiO_2 with the lower phase of $CHCl_3$–MeOH–H_2O (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 × 5 l). The MeOH extract was concentrated to give a brown mass, which was dissolved in H_2O (2 l) and extracted with C_6H_6, 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).

Torosachrysone 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 $C_{28}H_{36}O_{15} \cdot 2/3H_2O$: C, 53.84; H, 5.81. Found: C, 53.62; H, 6.27. UV λ_{max}^{MeOH} nm (log ε): 222 (4.47), 268 (4.76), 300 sh (3.77), 312 (3.90), 325 (3.85), 385 (4.04). IR ν_{max}^{KBr} cm^{-1}: 3400, 1625, 1610, 1000—1140. ^{1}H-NMR and ^{13}C-NMR data are shown in Tables I and II.

Enzymatic Hydrolysis of 1——A solution of **1** (3 mg) in H_2O was treated with β-D-glucosidase to give a yellow aglycone. The aglycone was recrystallized from C_6H_6 as yellow prisms, mp 203 °C, which were identified by direct comparison with an authentic sample of torosachrysone (**3**). The aqueous solution was evaporated *in vacuo* and glucose was identified by TLC.

Partial Methylation of Torosachrysone (3)——A solution of **3** (100 mg) in $CHCl_3$ (100 ml) was methylated with CH_2N_2 at room temp. for 10 h. The solution was evaporated to give a yellow residue, which was chromatographed on SiO_2 with C_6H_6–AcOEt (9 : 1) to afford 8-methyltorosachrysone (**4**) (15 mg) and 9-methyltorosachrysone (**5**) (40 mg).

8-Methyltorosachrysone (4)——Yellow needles (C_6H_6), mp 229—231 °C. UV $\lambda_{max}^{dioxane}$ 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 ν_{max}^{KBr} cm^{-1}: 3440, 1625, 1590, 1460, 1440, 1420. High resolution MS m/z: Calcd for $C_{17}H_{18}O_5$: 302.1152, Found: 302.1135. ^{1}H-NMR and ^{13}C-NMR data are shown in Tables I and II, respectively.

9-Methyltorosachrysone (5)——Yellow needles (C_6H_6), mp 180—182 °C. UV $\lambda_{max}^{dioxane}$ nm (log ε): 223 (4.29), 272 (4.77), 307 sh (3.53), 319 (3.67), 333 (3.66), 368 (3.84). IR ν_{max}^{KBr} cm^{-1}: 3350, 1675, 1635, 1600, 1560, 1500. High resolution MS m/z: Calcd for $C_{17}H_{18}O_5$: 302.1153. Found: 302.1161. ^{1}H-NMR and ^{13}C-NMR data are shown in Tables I and II, respectively.

Physcion 8-β-Gentiobioside (2)——Yellow powder, mp 221—223 °C. *Anal.* Calcd for $C_{28}H_{34}O_{17}$: C, 52.34; H, 5.23. Found: C, 52.58; H, 5.35. UV λ_{max}^{MeOH} nm (log ε): 221 (4.62), 245 sh (4.15), 268 (4.48), 280 sh (4.42), 296 sh (3.69), 3.95 sh (3.89), 416 (3.98), 435 sh (3.92). IR ν_{max}^{KBr} cm^{-1}: 3400, 1670, 1630, 1600, 1000—1140. ^{1}H-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). ^{13}C-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 H_2O (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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