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Studies on the Constituents of the Seeds of *Cassia obtusifolia* LINN. The Structures of Two New Anthraquinone Glycosides[1)]

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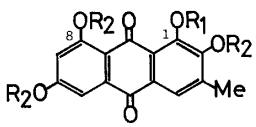
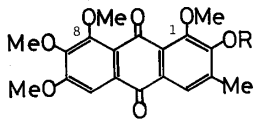
Two new anthraquinones, alaternin 1-*O*-*β*-D-glucopyranoside and chryso-obtusin 2-*O*-*β*-D-glucopyranoside, were isolated, along with physcion 8-*O*-*β*-D-glucopyranoside, from the seeds of *Cassia obtusifolia* LINN., and their structures were established on the basis of spectral and chemical evidence.

Keywords——*Cassia obtusifolia*; Leguminosae; anthraquinone glycoside; physcion 8-*O*-*β*-D-glucopyranoside; alaternin 1-*O*-*β*-D-glucopyranoside; chryso-obtusin 1-*O*-*β*-D-glucopyranoside

In previous papers,[2–7)] we reported the isolation of many anthraquinone, naphtopyrone, tetrahydroanthracene, and naphthooxepin derivatives from the seeds of *Cassia obtusifolia* LINN. In this paper, we wish to report the structural determination of two new anthraquinone glycosides, alaternin 1-*O*-*β*-D-glucopyranoside and chryso-obtusin 2-*O*-*β*-D-glucopyranoside, which have been isolated, along with physcion 8-*O*-*β*-D-glucopyranoside, from the seeds of this plant.

These compounds were obtained from the methanol extracts of the crushed seeds as described in Experimental.





2: R_1=glc, R_2=H
4: R_1, R_2=H
5: R_1, R_2=Ac

3: R=glc
6: R=glc-$(Ac)_4$
7: R=H

Chart 1

Compound **1** (yellow needles; mp 244—245 °C) was identified as physcion 8-*O*-*β*-D-glucopyranoside[8)] by direct comparison with an authentic sample.

Compound **2** (yellow needles; mp 254—255 °C; $C_{21}H_{20}O_{11} \cdot 1/2H_2O$) showed a red color in methanolic sodium hydroxide and magnesium acetate.[9)] The infrared (IR) spectrum showed that the compound is a glycoside (3300 and 1020—1100 cm^{-1}) and has two carbonyl groups (1630 and 1680 cm^{-1}), while the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **2** showed signals of an aromatic methyl group as a broad singlet (δ 2.39), aliphatic and hydroxyl protons (δ 2.80—5.25), two *meta*-coupled aromatic protons as a doublet (δ 6.58 and 7.11, each J=2.4 Hz), an aromatic proton as a broad singlet (δ 7.55), and a chelated hydroxyl proton (δ 12.09). The aromatic methyl protons at δ 2.39 ppm showed long-range coupling to a proton at δ 7.55. The hydrolysis of **2** with *β*-glucosidase (*β*-D-glucoside

glucohydrolase) gave glucose and the corresponding aglycone (**4**), mp >300 °C, $C_{15}H_{10}O_6$, which was identified as an anthraquinone derivative with vicinal hydroxyl groups showing a purple color in methanolic magnesium acetate.[9] The IR spectrum of **4** suggested the presence of a nonchelated carbonyl group and a chelated carbonyl group (1670 and 1620 cm^{-1} respectively). The ultraviolet (UV) maximum at 430 nm of **4** suggested a 1,8-dihydroxy structure;[10] both 1,4- and 1,5-dihydroxy structures are ruled out by the UV and IR evidence. On acetylation, compound **4** formed a tetraacetate (**5**); mp 229—230 °C, $C_{23}H_{18}O_{16}$. The structure of **4** was established as alaternin, 1,2,6,8-tetrahydroxy-3-methylanthraquinone, by a comparison of its spectral data (UV and IR) with those reported in the literature.[11] The position of the sugar in **2** was concluded to be at the C_1-OH of **4** based on a comparison of the color reactions in **2** and **4**. Therefore, the structure of **2** was established as alaternin 1-*O*-*β*-D-glucopyranoside, 1,2,6,8-tetrahydroxy-3-methylanthraquinone 1-*O*-*β*-D-glucopyranoside.

Compound **3** (yellow needles, mp 227—230 °C, $C_{25}H_{28}O_{12}$) is negative in the ferric chloride test and in the methanolic sodium hydroxide test. The similarity of the chromophore of **3** to that of chryso-obtusin (**7**) was shown by a comparison of the UV spectra, while the IR spectrum of **3**, with strong absorption bands at 3400 and 980—1120 cm^{-1}, suggested that the compound is a glycoside. The ^{1}H-NMR spectrum of **3** indicated the presence of a methyl group (δ 2.39) as a broad singlet, aliphatic and hydroxyl protons (δ 3.00—5.50 ppm) four methoxyl groups (δ 3.87, 9H and 3.99 ppm, 3H), and two aromatic protons (δ 7.50 and 7.76 ppm each as a broad singlet). Compound **3** formed, on acetylation, a tetraacetate (**6**); mp 99—101 °C, $C_{27}H_{26}O_{11}$. On *β*-glucosidase hydrolysis, **3** gave glucose and an aglycone ($C_{19}H_{18}O_7$; mp 224.5 °C), which was identified as chryso-obtusin (**7**) by comparison with an authentic sample. Therefore, **3**, was established to be chryso-obtusin 2-*O*-*β*-D-glucopyranoside.

Alaternin and chryso-obtusin have been isolated from *Rhamnus alaternus* LINN. and *Cassia* spp. (*C. obtusifolia* LINN. and *C. tora* LINN.) respectively. However, this is the first report of the isolation of their glycosides from natural sources.

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 NMR spectra were taken on a JEOL FX-100 instrument; the chemical shifts are given in ppm relative to internal tetramethylsilane (TMS). The mass spectra (MS) were obtained on a Hitachi RMU-7M spectrometer. Column chromatography was performed on Wako gel C-200 (Wako Pure Chemical Ind., Ltd.).

Extraction and Isolation——Crushed seeds (3 kg) of *C. obtusifolia* were extracted with 90% MeOH (3 × 4 l) under reflux. The MeOH extract was concentrated *in vacuo* to give a brown mass, which was then dissolved in H_2O (2.5 l). This solution was extracted with AcOEt and BuOH successively. The BuOH solution was concentrated *in vauco* to give a brown mass (20 g), which was then chromatographed on SiO_2 with $CHCl_3$–MeOH–H_2O (9 : 1 : 0.1) to give fractions 1—8. Fraction 2 (25 mg) was chromatographed on a Sephadex LH-20 column and eluted with MeOH to give **1** (2 mg). Fraction 4 was recrystallized from MeOH–H_2O to afford **3** (60 mg). Fraction 6 (525 mg) was chromatographed on a Sephadex LH-20 column and eluted with MeOH to give cassiaside and **2** (18 mg).

Physcion 8-*O*-*β*-D-Glucopyranoside (1)——Compound **1** was recrystallized from MeOH to yield yellow needles; mp 244—245 °C. UV λ_{max}^{MeOH} nm: 221, 245 sh, 269, 277 sh, 416, 436 sh. IR ν_{max}^{KBr} cm^{-1}: 3400, 1670, 1630, 1595. This compound was identified as physcion 8-*O*-*β*-D-glucopyranoside by direct comparison with an authentic sample.

Alaternin 1-*O*-*β*-D-Glucopyranoside (2)——Compound **2** was recrystallized from MeOH to yield yellow needles; mp 254—255 °C. UV λ_{max}^{MeOH} nm (log ε): 227 (4.46), 247 sh (4.16), 256 sh, (4.20), 276 (4.35), 310 (3.99), 436 (4.04), 455 sh (3.98). IR ν_{max}^{KBr} cm^{-1}: 3300, 1680, 1630, 1610, 1020—1100. ^{1}H-NMR (DMSO-d_6) δ: 2.39 (3H, br s, Me-3), 2.80—5.25 (13H, aliphatic H and OH), 6.58 (1H, d, J=2.4 Hz, H-7), 7.11 (1H, d, J=2.4 Hz, H-5), 7.55 (1H, br s, on irradiation at δ 2.39 changed to narrow s, H-4), 12.09 (1H, br s, OH). *Anal.* Calcd for $C_{21}H_{20}O_{11} \cdot 1/2H_2O$: C, 55.14; H, 4.63. Found: C, 54.88; H, 4.50.

Enzymatic Hydrolysis of 2——A solution of **2** (5 mg) and *β*-glucosidase (5 mg, Sigma) in H_2O (10 ml) was incubated at 37 °C for 15 h. The reaction mixture was extracted with AcOEt, and the AcOEt layer was evaporated to dryness *in vacuo*. The yellow residue was recrystallized from MeOH–H_2O to afford compound **4** as brick-red needles;

mp 300 °C, UV λ_{max}^{EtOH} nm (log ε): 228 (4.30), 247 (4.06), 253 (4.07), 283 (4.37), 317 (3.95), 430 (3.95), 448 sh (3.93), IR ν_{max}^{KBr} cm^{-1}: 3400, 1665, 1615, 1570, ^{1}H-NMR (Me_2CO-d_6) δ: 2.36 (3H, br s, Me-3), 6.63 (1H, d, J=2.4 Hz, H-7), 7.26 (1H, d, J=2.4 Hz, H-5), 7.63 (1H, br s, H-4), 12.17 (2H, s, OH × 2), High-resolution MS m/z: Calcd for $C_{15}H_{10}O_6$: 286.0475, Found: 286.0448. The aqueous layer was evaporated to dryness, and glucose in the residue was detected by means of thin-layer chromatography (TLC) (Avicel SF; solvent, upper phase of BuOH–AcOH–H_2O (3 : 1 : 1); detection, aniline hydrogen phthalate; *Rf* 0.24).

Tetraacetate (5) of 4——Compound **4** (2 mg) was acetylated with Ac_2O–pyridine to give a tetaacetate (**5**), which was then recrystallized from MeOH to give yellow needles (2 mg); mp 229—230 °C. UV λ_{max}^{MeOH} nm (log ε): 208 (4.47), 235 sh (4.17), 269 (4.64), 277 sh (4.14), 335 (3.79). IR ν_{max}^{KBr} cm^{-1}: 1780, 1670, 1600. ^{1}H-NMR ($CDCl_3$) δ: 2.36 (6H, s, Me-3 and OAc), 2.38 (3H, s, OAc), 2.42 (3H, s, OAc), 2.43 (3H, s, OAc), 7.24 (1H, d, J=2.4 Hz, H-7), 7.95 (1H, d, J=2.4 Hz, H-5), 8.11 (1H, br s, H-4). High-resolution MS m/z: Calcd for $C_{23}H_{18}O_{10}$: 454.0899, Found: 454.0903.

Chryso-obtusin 2-β-D-Glucopyranoside (3)——Yellow crystals; mp 227—230 °C. UV λ_{max}^{MeOH} nm (log ε): 213 (4.29), 255 sh (3.92), 277 (4.47), 301 sh (3.87), 353 (3.65). IR ν_{max}^{KBr} cm^{-1}: 3400, 1670, 1580, 980—1120. ^{1}H-NMR (DMSO-d_6) δ: 2.39 (3H, br s, Me-3), 3.00—5.50 (11H, aliphatic H and OH), 3.87 (9H, s, OMe × 3), 3.99 (3H, s, OMe), 7.50 (1H, s, H-5), 7.76 (1H, br s, H-4). *Anal.* Calcd for $C_{25}H_{28}O_{12}$: C, 57.69; H, 5.42. Found: C, 57.99; H, 5.44.

Tetraacetate (6) of 3——Compound **3** (6 mg) gave a tetraacetate (**6**) upon acetylation with Ac_2O–pyridine; this product was recrystallized from MeOH to give a yellow powder (4 mg); mp 99—101 °C. UV λ_{max}^{MeOH} nm (log ε): 209 (4.46), 245 sh (4.12), 276 (4.61), 350 (3.80). IR ν_{max}^{KBr} cm^{-1}: 1755, 1670, 1580, 1340, 1230. ^{1}H-NMR ($CDCl_3$) δ: 1.98 (3H, s, OAc), 2.03 (3H, s, OAc), 2.04 (3H, s, OAc), 2.12 (3H, s, OAc), 2.37 (3H, br s, Me-3), 3.92 (3H, s, OMe), 3.99 (3H, s, OMe), 4.02 (3H, s, OMe), 4.03 (3H, s, OMe), 3.60—5.60 (7H, aliphatic H), 7.58 (1H, s, H-5), 7.86 (1H, br s, H-6). *Anal.* Calcd for $C_{33}H_{36}O_{16}$: C, 57.56; H, 5.27. Found: C, 57.38: H, 5.41.

Enzymatic Hydrolysis of 3——A solution of **3** (5 mg) and β-glucosidase (2 mg) in H_2O (5 ml) was incubated at 37 °C for 10 h. The reaction mixture was then extracted with AcOEt, and the AcOEt layer was evaporated to dryness *in vacuo*. The yellow residue was recrystallized from hexane–C_6H_6 to afford **7** as yellow needles (mp 224.5 °C); this product was identified as chryso-obtusin by comparison with an authentic sample. The aqueous layer was treated as above, and the residue was shown by TLC to contain glucose.

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References and Notes

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