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ANTHRAQUINONE GLYCOSIDES FROM THE SEEDS OF *CASSIA TORA**

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Key Word Index—*Cassia tora*; Leguminosae; anthraquinone glucosides; chrysophanol-triglucoside; chrysophanol-tetraglucoside; obtusifolin-monoglucoside.

Abstract—Three new anthraquinone glycosides, 1-[(β-D-glucopyranosyl-(1 → 3)-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl)oxy]-8-hydroxy-3-methyl-9,10-anthraquinone, 1-[(β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl-(1 → 3)-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl)oxy]-8-hydroxy-3-methyl-9,10-anthraquinone and 2-(β-D-glucopyranosyloxy)-8-hydroxy-3-methyl-1-methoxy-9,10-anthraquinone were isolated from the seeds of *Cassia tora*. Their structures were elucidated on the basis of chemical and spectral data. The first two compounds exhibited a weak protective effect on primary cultured hepatocytes against carbon tetrachloride toxicity.

INTRODUCTION

Cassia tora L. is widely distributed in tropical Asian countries. It is also commonly known as sicklepod. Various parts of the plant are reputed for their medicinal value [1, 2]. The seeds of *C. tora* have been used in Chinese medicine as aperient, antiasthenic and diuretic agents and also to improve visual acuity [3]. In Korea, the hot aqueous extract of the seeds of *C. tora* is taken orally for protection of the liver [4]. A weak antihepatotoxic activity in carbon tetrachloride treated mice was found when the drug was administered orally at a dose of 670 mg/kg to the animals [4]. Although the plant has been subjected to extensive phytochemical investigations [1, 3, 5–12], no study has been done to isolate and identify the hepatoprotective agent(s). We, therefore, investigated the methanol extract of the seeds of *C. tora* because a preliminary study had shown that this extract exhibited a significant protective effect on primary cultured hepatocytes against galactosamine cytotoxicity.

RESULT AND DISCUSSION

From the methanol extract of the seeds of *C. tora* three new anthraquinoneglycosides, **1**–**3**, were isolated. Compound **1** is a glycoside as suggested by the strong IR absorption bands at 3400 and 1120–1000 cm^{-1}, and the proton signals of a sugar unit in the region between δ3.0 and 5.25. GC analysis of the alditol acetate obtained from reduction and acetylation of the hydrolysates of **1** by the method of Blakeney *et al.* [13] indicated the presence of glucose only. Furthermore, hydrolysis of compound **1** with β-glucosidase gave glucose and the corresponding aglycone, which was identified as chrysophanol by direct comparison with an authentic sample. Hence compound **1** is a chrysophanol-β-D-glucoside.

The quasi molecular ion at *m/z* 747 $[M+Li]^+$ from positive ion FABMS suggested a molecular formula of $C_{33}H_{40}O_{19}$. In the negative ion FABMS spectrum, the fragment ions at *m/z* 578, 416 and 254 indicated a successive loss of three glucose units from the parent compound to give the molecular ion peak of the aglycone. The presence of three glucose units in compounds **1** was further confirmed by the three anomeric proton signals at δ5.28 (1H) and 4.43 (2H), the three anomeric carbon signals at δ100.4, 103.1 and 104.2, and the formation of an undeca-acetate derivative. Since only one hydrogen bonded proton signal at δ12.9 was observed in the ^1H NMR spectrum of **1**, the attachment of the sugar residue to the chrysophanol unit should be either at position 1 or 8. Comparison of the aromatic proton signals of compound **1** with those of chrysophanol revealed a downfield shift of the H-2 signal by 0.5 ppm and a practically unchanged H-7 signal. Therefore the triglucoside unit must be attached to position 1 of chrysophanol.

Methylation analysis of the sugar unit by GC-MS using the method of Hakomori [14] and Jansson *et al.* [15] indicated the presence of a 1:1:1 ratio of 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetyl-D-glucitol, 2,4,6-tri-*O*-methyl-1,3,5-tri-*O*-acetyl-D-glucitol and 2,3,4-tri-*O*-methyl-1,5,6-tri-*O*-acetyl-D-glucitol, which are formed from one 1-*O*-substituted glucose, a 1,3 and a 1,6-di-*O*-substituted glucose unit, respectively. A 1 → 6 and a 1 → 3 linkage between the three glucose units in compound **1** were further confirmed by the ^{13}C NMR spectrum in which a downfield shift of one of the C-6 signals of the glucose molecules to δ68.5 [16] and the appearance of one of the C-3 signals at δ88.2 [16] were observed. With the exception of C-3″ and C-2″, the carbon signals of the first two glucose units of compound **1** were nearly identical with those of the gentiobiosyl unit of physcion-8-β-D-gentiobioside [5] and torosachrysone-8-β-D-gentiobioside [5]. As a result, the linkage between the second and the first glucose unit in compound **1** should be β-(1 → 6) and that between the terminal and the second glucose unit should be β-(1 → 3). Therefore the structure of compound **1** was elucidated as 1-[(β-D-glucopyran-

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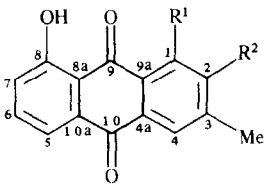

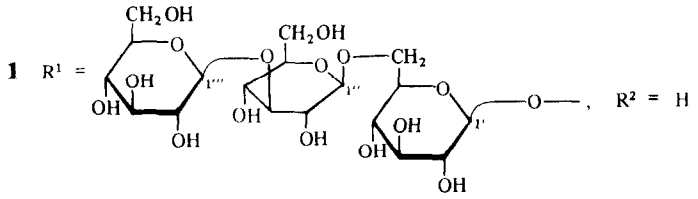

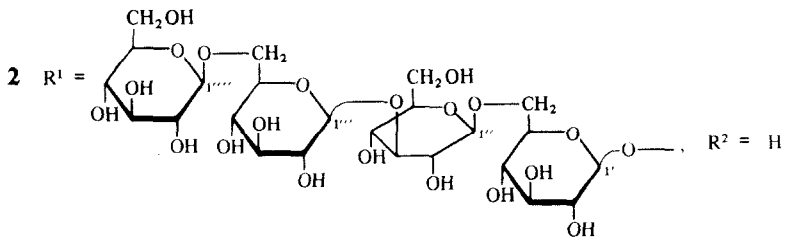

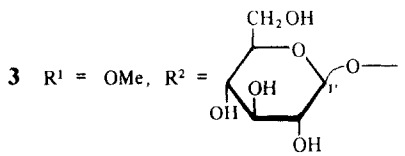

osyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl)oxy]-8-hydroxy-3-methyl-9,10-anthraquinone.

The close resemblance of the spectral data (IR, UV, ^{1}H NMR and ^{13}C NMR) of compounds **1** and **2** indicated that compound **2** is also a chrysophanol-*β*-D-glucoside. A molecular formula of $C_{39}H_{50}O_{24}$ was suggested by the pseudo-molecular ion at *m/z* 901 $[M-H]^-$ in the negative ion FABMS spectrum of compound **2**, indicating the presence of four glucose molecules. This is in close agreement with the four anomeric carbon signals observed at *δ* 100.6, 102.9, 103.0 and 104.1 and the four anomeric proton signals at *δ* 5.25, 4.40, 4.38, 4.24 in the NMR spectra of compound **2**. Therefore compound **2** is a chrysophanol-*β*-D-tetraglucoside. As in the case of compound **1**, the attachment of the sugar residue to the aglycone unit in compound **2** could be easily confirmed to be at position 1 by the hydrogen bonded proton signal at *δ* 12.96 and the H-2 and H-4 signals at *δ* 7.59 and 7.72, respectively. In the ^{13}C NMR spectrum of compound 2 (Table 1), two of the C-6 carbon signals of the glucose molecules appeared at *δ* 68.8 ppm and 68.9. These two signals together with the C-3 carbon signal of the glucose molecule at *δ* 88.9 revealed the presence of two 1 → 6 and one 1 → 3 linkages between the four glucose molecules in compound **2**. This is further confirmed by the methylation analysis of compound **2** in which 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetyl-D-glucitol from the terminal glucose, 2,4,6-tri-*O*-methyl-1,3,5-tri-*O*-acetyl-D-glucitol from a 1,3 di-*O*-substituted glucose and 2,3,4-tri-*O*-methyl-1,5,6-tri-*O*-acetyl-D-glucitol from the 1,6 di-*O*-substituted glucose units were obtained in the ratio of 1 : 1 : 2. As the chemical shifts of the carbon signals of the first three glucose units in compound **2**, with the exception of C‴-5 and C‴-6, were compatible with those of compound **1**, the arrangement of these three glucose units should be the same in both compounds. A downfield shift of the C‴-6 signal to *δ* 68.9 and an up field shift of C‴-5 signal to *δ* 75.1 clearly confirmed a 1 → 6 linkage between the terminal and the third glucose units. Therefore the structure of compound **2** was elucidated as 1-[(*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl)oxy]-8-hydroxy-3-methyl-9,10-anthraquinone.

Compound **3** is also a glycoside as suggested by the strong IR absorption peaks at 3300 and 1020–1100 cm^{-1} and the proton signals in the region *δ* 2.8–5.35. Hydrolysis of **3** with *β*-glucosidase afforded glucose and the corresponding aglycone, which was identified as obtusifolin by direct comparison with an authentic sample. A molecular formula of $C_{22}H_{22}O_{10}$ was indicated by the molecular ion at *m/z* 447 $(M+H)^+$ in the positive ion CIMS spectrum of **3**. The aglycone ion fragment at *m/z* 285, which was formed by the loss of one glucose unit from the molecular ion, clearly indicated that compound **3** was obtusifolin-*β*-D-monoglucoside. In the ^{1}H NMR spectrum of compound **3**, an intramolecular hydrogen bonded proton signal (*δ* 12.80) and four aromatic proton signals were observed. Therefore the only possible position for sugar linkage is at 2-OH of obtusifolin. As a result, compound **3** is obtusifolin-2-*β*-D-glucoside, 2-(*β*-D-glucopyranosyloxy)-8-hydroxy-1-methoxy-3-methyl-9,10-anthraquinone.

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (90 MHz, DMSO-d_6)

C	A	1	2
1	161.4	161.4	161.4
2	119.2	116.8	116.8
3	146.9	147.6	147.6
4	124.1	124.4	124.4
4a	131.7	132.4	132.4
5	107.1	122.8	122.9
6	160.3	134.4	134.4
7	106.3	121.4	121.4
8	164.6	158.3	158.3
8a	114.1	118.3	118.3
9	186.2	187.7	187.7
9a	114.1	118.3	118.3
10	181.5	182.1	182.1
10a	136.0	136.2	136.2
Me	21.3	22.0	22.0
OMe	56.1	—	—
1′	103.6	103.1	103.0
2′	73.1	73.5	73.5
3′	76.7	76.5	76.5
4′	69.9	69.8	69.9
5′	75.5	75.8	75.8
6′	68.9	68.5	68.8
1″	100.3	100.5	100.6
2″	73.4	72.4	72.1
3″	76.2	88.2	88.9
4″	69.6	69.0	69.2
5″	76.2	76.1	76.0
6″	60.8	60.8	60.7
1‴		104.2	104.1
2‴		73.9	73.9
3‴		77.0	76.9
4‴		70.2	70.1
5‴		76.2	75.1
6‴		61.2	68.9
1⁗			102.9
2⁗			73.6
3⁗			76.1
4⁗			70.4
5⁗			76.2
6⁗			61.0

A: ^{13}C NMR data of physcion-8-β-gentiobioside were extracted from [5] and assigned for the comparison with compounds **1** and **2**.

Although Poethke *et al.* had suggested the presence of chrysophanol-diglucoside and chrysophanol-triglucoside by TLC analysis [17], they had never isolated these two compounds for structural investigation. The diglucoside was later isolated and identified as chrysophanol-β-gentiobioside [6]. Biogenetically, compound **1** and **2** should be closely related to chrysophanol-β-gentiobioside and the latter may be the precursor of compounds **1** and **2**.

The above three compounds showed no protective activity against galactosamine cytotoxicity in primary cultured hepatocytes. In the CCl_4-damaging hepatocyte model, however, the two chrysophanol glycosides **1** and **2** exhibited weak protective activity of 13.0 and 19.1%, respectively, at a dose of 1 mg/ml. Recently, in our laboratory, in evaluating the antihepatotoxic potential of anthraquinones in primary cultured hepatocytes, we found that aloe-emodin and cascaroside (from *Rhamnus purshianus*) showed similar antihepatotoxic activities as **1** and **2**. This is the first report of hydroxyanthraquinone derivatives as liver protective agents. It is of interest that a lot of herbal preparations for liver diseases contain hydroxyanthraquinones, compounds whose medicinal properties have been attributed to their laxative activities only.

The two anthraquinone glycosides **1** and **2** can only account for part of the antihepatotoxic activity of the methanol extract of the seeds of *C. tora*. Further phytochemical and pharmacological investigations by us have revealed that the naphthopyrone glycosides are the major antihepatotoxic principles in the seeds of *C. tora*. These findings will be published in a forthcoming paper.

EXPERIMENTAL

Mps: uncorr; IR: KBr; UV: MeOH; MS: AEI MS 30 and Kratos MS 80 RFA spectrometers: EIMS: 4 kV, 70 eV, 200°; CIMS: 120 eV, NH_3 as reactant gas; +ve FAB and −ve FABMS: Xe gun, 8 kV, glycerol as matrix. ^1H and ^{13}C NMR: TMS or solvents as internal standards.

Seeds of *Cassia tora* (500 g) purchased in Korea were ground and defatted with 2 l of petrol in a Soxhlet followed by extraction with 4 l of $CHCl_3$ and then 4 l of MeOH. After removal of the solvents *in vacuo*, 18.8 g of $CHCl_3$ extract and 93.7 g of MeOH extract were obtained.

Flash chromatography of the MeOH extract (50 g) over silica gel with EtOAc–MeOH–H_2O (20:6:3) afforded 10 fractions (2 l each). Fractions 5 and 7 were pooled and then subjected to MPLC over silica gel using EtOAc–MeOH–H_2O (10:2:1) to give 35 mg of compound **1** and 25 mg of compound **2**, which were further purified by crystallization from a mixture of MeOH and H_2O (50:1). Chromatography of fraction 2 by MPLC over silica gel using EtOAc–MeOH–H_2O (20:3:1) gave 6 mg of compound **3** which was purified by crystallization from MeOH.

1-[(-β-D-*glucopyranosyl*-(1 → 3)-O-β-D-*glucopyranosyl*-(1 → 6)-O-β-D-*glucopyranosyl*)*oxy*]-8-*Hydroxy*-3-*methyl*-9,10-*anthraquinone* (**1**). Yellow powder; mp 284–286°; UV λ_{max} nm: 224, 260, 286 (sh), 412; IR ν_{max} cm^{-1}: 3400, 2870, 1670, 1640, 1600, 1120–980; +ve FABMS *m/z*: 747 $[M+Li]^+$; −ve FABMS *m/z*: 739 $[M-H]^-$, 578 $[M-glu]^-$, 416 $[M-2\,glu]^-$, 254 $[M-3\,glu]^-$, 253 [aglycone − H]$^-$; ^1H NMR (80 MHz, DMSO-d_6) δ: 2.53 (3H, *br s*, Me-3), 3.0–5.25 (*m*, glucosyl protons), 7.35 (1H, *dd*, J = 2.0 and 7.6 Hz, H-7), 7.59 (1H, *br s*, H-2), 7.66 (1H, *dd*, J = 2.0 and 7.6 Hz, H-5), 7.72 (1H, *br s*, H-4), 7.76 (1H, *t*, J = 7.6 Hz, H-6), 12.90 (1H, *br* 1OH-8); ^1H NMR of the glycosyl protons after TFA exchange δ: 3.0–4.25 (18H, *m*), 4.43 (2H, *br d*, J = 6 Hz, H-1″ and H-2‴), 5.28 (1H, *br d*, J = 6 Hz, H-1′).

1-[(β-D-*glucopyranosyl*-(1 → 6)-O-β-D-*glucopyranosyl*-(1 → 3)-O-β-D-*glucopyranosyl*-(1 → 6)-O-β-D-*glucopyranosyl*)*oxy*]-8-*Hydroxy*-3-*methyl*-9,10-*anthraquinone* (**2**): Yellow needles; mp 288–290°; UV λ_{max} nm: 224, 260, 286 (sh), 412; IR ν_{max} cm^{-1}: 3400, 2880, 1670, 1640, 1600, 1120–980; −ve FABMS *m/z*: 901 $[M-H]^-$, 740 $[M-glu]^-$, 578 $[M-2\,glu]^-$, 416 $[M-3\,glu]^-$, 254 $[M-4\,glu]^-$, 253 [aglycone − H]$^-$; ^1H NMR (360 MHz, DMSO-d_6) δ: 2.53 (3H, *br s*, Me-3), 2.8–5.4 (*m*, glucosyl-protons), 7.35 (1H, *dd*, J = 2.0 and 7.6 Hz, H-7), 7.59 (1H, *br s*, H-2), 7.66 (1H, *dd*, J = 2.0 and 7.6 Hz, H-5), 7.72 (1H, *br s*, H-4), 7.76 (1H, *t*, J = 7.6 Hz, H-6), 12.96 (*br s*, OH-8); ^1H NMR of the glucosyl protons after TFA exchange δ: 3.0–3.6 (18H, *m*), 3.72 (2H, *br d*, J = 12.2 Hz), 3.8 (2H, *m*), 4.10 (1H, *d*, J = 10.8 Hz), 4.22

(1H, *d*, $J = 8.5$ Hz), 4.24 (1H, *d*, $J = 7.2$ Hz, anomeric proton), 4.38 (1H, *d*, $J = 7.2$ Hz, anomeric proton), 4.40 (1H, *d*, $J = 7.2$ Hz, anomeric proton), 5.25 (1H, *d*, $J = 7.2$ Hz, H-1′).

2-(β-D-glucopyranosyloxy)-8-Hydroxy-1-methoxy-3-methyl-9,10-anthraquinone (**3**). Yellow needles, mp. 192–194°; UV λ_{max} nm: 218, 260, 288 (sh), 400; IR ν_{max} cm^{-1}: 3400, 2875, 1665, 1630, 1580, 1120–980; +ve CIMS *m/z*: 447 $(M + H)^+$, 331, 285 (aglycone + H^+); ^1H NMR (80 MHz, DMSO-d_6) δ: 2.45 (3H, *d*, $J = 1$ Hz, Me-3), 2.8–5.25 (*m*, glucosyl-protons), 3.90 (3H, *s*, OMe-1), 7.38 (1H, *dd*, $J = 3$ and 7.6 Hz, H-7), 7.70 (1H, *t*, $J = 7.6$ Hz, H-6), 7.89, 7.75 (1H, *dd*, $J = 3$ and 7,6 Hz, H-5), (1H, *d*, $J = 1$ Hz, H-4), 12.80 (1H, *br s*, OH-8); ^1H NMR of the anomeric proton after TFA exchange δ: 5.10 (1H, *br d*, $J = 6.4$ Hz, H-1′).

Enzymatic hydrolysis of compounds **1–3**. A soln of 3 mg of the compound and 3 mg of β-glucosidase (Sigma) in 5 ml of H_2O was kept at 37° for 15 hr and then extracted with EtOAc. The EtOAc was evapd to dryness to give the corresponding aglycone, which was further purified by recrystallization in MeOH and identified by comparison with an authentic sample. The aq layer was evaporated to dryness and glucose in the residue was identified by co-TLC with an authentic sample.

Sugar analysis of compounds **1–3** *by GC analysis*. 1 mg of the compound was hydrolysed with 1 ml 2M TFA in a sealed serum vial at 121° for 90 min. The serum vial was opened and internal standard (inositol) and 2 ml of MeOH were added. After evapn to dryness, the residue was redissolved in 0.1 ml 0.88 NH_3 followed by the addition of 1 ml DMSO containing 2% $NaBH_4$. The reaction mixture was kept in a water bath at 40° for 90 min. Excess $NaBH_4$ was destroyed by the addition of 0.1 ml HOAc. The resulting alditols were acetylated using 1-methylimidazole as catalyst and then analysed qualitatively by GC on a SP 2330 glass column (200° for 2 min and then increased to 250° at a rate of 2°/min.) together with the alditol acetates of authentic sugars according to the method of Blakeney *et al.* [13].

Methylation analysis of the sugar moieties of compounds **1** *and* **2**. Compounds **1** and **2** were methylated with methylsulphinyl-methanide and MeI and transformed into alditol acetates by the method of Hakomori [14]. The partially methylated alditol acetates were analysed by GC/MS according to Jansson *et al.* [15].

Determination of antihepatotoxic activity and effect on glutamate pyruvate transaminase activity. The methods for the evaluation of antihepatotoxic activity in primary cultured hepatocytes were the same as those reported earlier [18].

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