Notes

Four New Compounds from the Seeds of *Cassia fistula*

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Received January 11, 2002

Four new compounds, 5-(2-hydroxyphenoxymethyl)furfural (**1**), (2′*S*)-7-hydroxy-5- hydroxymethyl-2-(2′-hydroxypropyl)chromone (**2**), benzyl 2-hydroxy-3,6-dimethoxybenzoate (**3**), and benzyl 2*β*-*O*-D-glucopyranosyl-3,6-dimethoxybenzoate (**4**), together with four known compounds, 5-hydroxymethylfurfural, (2′*S*)-7-hydroxy-2-(2′-hydroxypropyl)-5-methylchromone, and two oxyanthraquinones, chrysophanol and chrysophanein, were isolated and identified from the seeds of *Cassia fistula*. The structures of **1**−**4** were determined on the basis of spectral data explanation, and the synthesis of compound **1** was carried out.

There are 31 species of *Cassia* (Leguminosae) grown in Taiwan, but only six species are indigenous. *Cassia fistula* L. is an introduced species and is a popular ornamental tree with attractive yellow flowers. This plant is widely distributed in India, the Philippines, and the southern area of mainland China. Its seed is used in folk medicine to treat diarrhea and gastritis; it is also an insecticide.[1] Many chemical components of the seed have been found previously, including sugars,[2] unsaturated fatty acids,[3] cyclopropenoid fatty acids,[4] and triacylglycerols.[5]

To investigate the potential antidiarrheal components of this plant species, we have studied the chemical composition of the seeds. A methanolic extract of the seeds of *C. fistula* was concentrated to obtain a residue, which was suspended in H_2O and partitioned with *n*-BuOH. The organic layer was subjected to silica gel chromatography to afford four new compounds, 5-(2-hydroxyphenoxymethyl)furfural (**1**), (2′*S*)-7-hydroxy-5-hydroxymethyl-2-(2′-hydroxypropyl)chromone (**2**), benzyl 2-hydroxy-3,6-dimethoxybenzoate (**3**), and benzyl 2*β*-*O*-D-glucopyranosyl-3,6-dimethoxybenzoate (**4**), together with four known compounds, 5-hydroxymethylfurfural,[6] (2′*S*)-7-hydroxy-2-(2′-hydroxypropyl)-5-methylchromone,[7] and two oxyanthraquinones, 1,8-dihydroxy-3-methylanthraquinone (chrysophanol)[8] and 8-*O*-D-glucopyranosyl-1-hydroxyl-3-methylanthraquinone (chrysophanein).[9] In this paper, we describe the structural elucidation of compounds **1**−**4**.

Compound **1** had HREIMS and ^{13}C NMR data consistent with the molecular formula $C_{12}H_{10}O_4$. Absorptions in the IR spectrum were attributed to hydroxy (3351 cm^{-1}), conjugated ketone (1662 cm^{-1}), and benzene ring (1601, 1510 cm^{-1}) functional groups. The UV spectrum (λ_{max} 278 nm) was similar to that of 5-hydroxymethylfurfural (λ_{max} 281 nm). The signals for an aldehyde proton (δ 9.64, s), two furan β protons [δ 6.59 and 7.21 (each 1H, d, J = 3.6 Hz)], and a methylene proton (δ 5.14, s) were also similar to 5-hydroxymethylfurfural, except that the methylene protons of 5-hydroxymethylfurfural resonated at higher field (δ 4.65, s) than H-7 in **1**. The ^{13}C NMR data of the 5-methylenefurfural moiety of **1** were also like those of 5-hydroxymethylfurfural. Besides the signals of the 5-methylenefurfural moiety, the remaining signals of **1** belonged to phenyl and phenolic protons. An exchangeable signal at δ 5.59 was proposed as a phenolic proton. Detection of two oxygenated benzene ^{13}C NMR signals at δ 145.0 and 146.1 led to the assignment of a 1,2-dioxygenated benzene, with one of the phenoxy oxygens being attached to a methylene group (C-7), causing two protons to shift to lower field (δ 5.14). Four phenyl protons exhibited multiple resonances centered at δ 6.77 and 6.83. The total synthesis of **1** was carried out using 5-chloromethylfurfural (**5**),[10] prepared from fructose and concentrated HCl, which was reacted with catechol and K_2CO_3 in acetone solution under reflux. The product was identical to **1** by comparison of its physical and spectroscopic data. On the basis of all of the above evidence, the structure of **1** was established as 5-(2-hydroxyphenoxymethyl)furfural.

Compound **2** was postulated to be based on a chromone skeleton from the UV absorption bands at λ_{max} 240, 250, and 290 nm.[7] It showed a molecular ion at *m*/*z* 250, and

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10.1021/np020003k CCC: $22.00
Published on Web 06/25/2002

HREIMS indicated a molecular formula of $C_{13}H_{14}O_5$. IR absorption bands at 3321, 1631, 1610, and 1495 cm^{-1} indicated hydroxyl and benzoyl groups. The ^1H NMR spectrum of **2** exhibited signals due to a secondary methyl (δ 1.27, d, J = 6.2 Hz), an allyl methylene (H-1′) [δ 2.57 (1H, dd, J = 14.4, 7.8 Hz) and 2.73 (1H, dd, J = 14.4, 5.1 Hz)], and a carbinyl methine (δ 4.19, m). It also displayed signals for an olefinic proton (δ 6.13, s), two aromatic protons [δ 6.75 and 7.06 (each 1H, d, J = 2.2 Hz)], and a hydroxymethyl group attached to an aromatic ring (δ 5.00, s). In the ^{13}C NMR spectrum, signals were attributed to a secondary methyl group (δ 24.0, q), two hydroxy-bearing carbons (δ 66.9, d; 65.1, t), and a methylene (δ 44.7, t) group. Comparison of the ^1H and ^{13}C NMR data of **2** with those of (2′*S*)-7-hydroxy- 2-(2′-hydroxypropyl)-5-methylchromone[7] suggested that **2** has an additional hydroxyl group at C-11. NOESY and HMBC techniques also confirmed the structure assigned, with the structure of **2** determined to be 7-hydroxy-5-hydroxymethyl-2-(2′-hydroxypropyl)chromone. By the application of the modified Horeau method, the absolute configuration of C-2′ in (2′*S*)-7-hydroxy-2-(2′-hydroxypropyl)-5-methylchromone ($[\alpha]_D$ +30.5°)[7] was determined with the *S* configuration. The stereocenter at C-2′ in compound **2** was also assigned an *S* configuration due to its positive specific rotation value similar to that of (2′*S*)-7-hydroxy-2-(2′-hydroxypropyl)-5-methylchromone.

Compound **3** was isolated as light yellow needles from ethanol, mp 134−136 °C. It was assigned a molecular formula of $C_{16}H_{16}O_5$ on the basis of its HREIMS and ^{13}C NMR spectral data. The IR spectrum showed the presence of a hydroxyl group with strong hydrogen bonding (2500−3300 cm^{-1}), aromatic ring (1615, 1487 cm^{-1}), and conjugated ester (1700 cm^{-1}) functionalities. A benzyl group attached to a benzoate unit was revealed by the following signals: δ_H 5.41 (2H, s, H-7′), 7.32 (3H, m, H-3′, -4′, -5′), 7.39 (2H, dd, J = 8.1, 1.8 Hz, H-2′, H-6′); δ_C 67.0 (C-7′), 127.6 (C-2′, -6′), 128.1 (C-4′), 128.5 (C-3′, -5′), 135.7 (C-1′). The H-7′ resonance exhibited a HMBC correlation with the benzoate carbonyl (δ 170.5), which caused the exchangeable phenolic proton signal to appear at δ 11.15 due to strong hydrogen bonding. The signals of two phenolic methyl groups [δ 3.83 and 3.78 (each 3H, s)] and two *ortho*-coupled phenyl protons [δ 6.31 and 6.94 (each 1H, d, J = 9.1 Hz)] were observed in the ^1H NMR spectrum of **3**. Irradiation of δ 6.31 resulted in the enhancement of the phenolic methyl signal at δ 3.83 by 21.0%, with the other phenolic methyl group at δ 3.78 being enhanced by 21.8% as a result of the irradiation of the signal at δ 6.94. This evidence confirmed the structure of **3** as benzyl 2-hydroxy-3,6-dimethoxybenzoate. EIMS fragments at *m/z* 91 (70%, benzyl cation) and *m/z* 180 (100%) supported the proposed structure. An interesting finding was the presence of a base peak at *m/z* 180 instead of a stable acylium ion [*m/z* 181 (0)].

Compound **4** was more polar than **3**, yet showed similar ^1H and ^{13}C NMR spectral data. It contained a benzyl group attached to an ester [δ_H 5.41, 5.24 (each 1H, d, J = 12.4 Hz, H-7′), 7.34 (3H, m), 7.42 (2H, dd, J = 8.0, 1.9 Hz), δ_C 68.0 (t, C-7′), 128.3 (d, C-2′, -6′), 128.4 (d, C-4′), 128.5 (d, C-3′, -5′), 143.5 (s, C-3)], two methoxy groups [δ_H 3.78, 3.82 (each 3H, s); δ_C 56.4, 56.7 (CH_3)], and two *ortho*-phenyl protons [δ 6.66, 6.91 (each 1H, d, J = 9.0 Hz, H-4, -5); δ_C 104.3, 114.3 (C-5, C-4)]. The IR spectrum exhibited signals for hydroxyl group (3411 cm^{-1}) and conjugated ester (1710, 1260 cm^{-1}) absorptions. The presence of an oxygenated benzoate ester group was revealed by UV absorption bands at λ_{max} 270, 300, and 360 nm and ^{13}C NMR signals at δ_C 166.6, 151.1, 145.6, and 143.5 (C-7, -6, -2, and -3). The NOE difference spectrum exhibited correlations between H-4/δ 3.78 and H-5/3.82 with 19.1% and 19.9% enhancements, respectively. The NMR data of **3** and **4** were found very similar. The only differences were the NMR signals of a glucopyranosyl unit [δ 4.63 (1H, d, J = 7.7 Hz, H-1″), 3.68−3.27 (6H, m, H-2″−H-6″); δ_C 107.9 (C-1″), 78.1 (C-5″), 76.4 (C-3″), 73.9 (C-2″), 69.7 (C-4″), 62.2 (C-6″)] in **4** instead of a strong hydrogen-bonded signal (δ 11.15) in **3**. Therefore, **4** was proposed as benzyl 2β-*O*-D-glucopyranosyl-3,6-dimethoxybenzoate.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. ^1H and ^{13}C NMR spectra were performed on a Bruker AM instrument at 300 MHz for protons and 75 MHz for carbons with tetramethylsilane (TMS) as internal standard. EIMS, FABMS, UV, and specific rotation were taken on JEOL JMS-HX 300, JEOL JMS-HX 110, and Hitachi S-3200 spectrometers and a JASCO DIP-180 digital polarimeter, respectively. Extracts were chromatographed on silica gel (Merk 70−230 mesh, 230−400 mesh, ASTM).

Plant Material. The seeds of *C. fistula* L. were collected in August 1996 on the campus of National Taiwan University. The plant material was identified by Mr. Muh-Tsuen Gun, formerly of the Department of Botany, National Taiwan University. A voucher specimen has been deposited at the Herbarium of the Department of Botany of the National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The pieces of seeds (3.5 kg) of *C. fistula* were extracted three times with methanol (20 L) at room temperature (5 days each). The methanol extract was evaporated in vacuo to leave a black residue, which was suspended in H_2O (700 mL) and then partitioned (3×) with 500 mL of *n*-BuOH. The *n*-BuOH fraction (99.4 g) was repeatedly chromatographed on Si gel using *n*-hexane, EtOAc, and MeOH of increasing polarity as eluent. Eight components, chrysophanol (30 mg) (10% hexane in EtOAc), **3** (15 mg) (20% hexane in EtOAc), 5-hydroxymethylfurfural (6 mg) (30% hexane in EtOAc), **1** (25 mg) (30% hexane in EtOAc), (2′*S*)-7-hydroxy-2-(2′-hydroxypropyl)-5-methylchromone (10 mg) (80% hexane in EtOAc), **2** (9 mg) (10% MeOH in EtOAc), **4** (16 mg) (10% MeOH in EtOAc), and chrysophanein (7 mg) (20% MeOH in EtOAc) were isolated.

5-(2-Hydroxyphenoxymethyl)furfural (1): mp 102−103 °C; UV (MeOH) λ_{max} (log ϵ) 278 (3.52) nm; IR (KBr) ν_{max} 3351, 1662, 1601, 1510, 1273, 1029, 816, 747 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.14 (2H, s, H-7), 5.59 (1H, br s, −OH), 6.59 (1H, d, J = 3.6 Hz, H-4), 6.77, 6.83 (4H, m, H-3′, -4′, -5′, -6′), 7.21 (1H, d, J = 3.6 Hz, H-3), 9.64 (1H, s, −CHO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 63.7 (t, C-7), 112.2 (d, C-4), 112.8 (d, C-6′), 115.4 (d, C-3′), 120.3 (d, C-4′), 121.7 (d, C-3), 122.9 (d, C-5′), 145.0 (s, C-1′), 146.1 (s, C-2′), 153.0 (s, C-2), 155.8 (s, C-5), 177.7 (d, C-6); EIMS *m/z* 218 $[M]^+$ (8), 110 (35), 109 (100), 81 (24), 53 (20); HREIMS *m/z* 218.0581 (calcd for $C_{12}H_{10}O_4$, 218.0579).

(2′*S*)-7-Hydroxy-5-hydroxymethyl-2-(2′-hydroxypropyl)chromone (2): mp 208−210 °C; $[\alpha]^{28}_D$ +38.4° (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ϵ) 240 (407), 250 (4.50), 290 (4.28) nm; IR (KBr) ν_{max} 3321, 1631, 1610, 1495, 1150, 1054 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 1.27 (3H, d, J = 6.2 Hz, H-3′), 2.57 (1H, dd, J = 14.4, 7.8 Hz, H-1′), 2.73 (1H, dd, J = 14.4, 5.1 Hz, H-1′), 4.19 (1H, m, H-2′), 5.00 (2H, s, H-11), 6.13 (1H, s, H-3), 6.75, 7.06 (each 1H, d, J = 2.2 Hz, H-6, -8); ^{13}C NMR (CD_3OD, 75 Hz) δ 24.0 (q, C-3′), 44.7 (t, C-1′), 65.1 (t, C-11), 66.9 (d, C-2′), 103.0 (d, C-8), 112.8 (d, C-6), 114.9 (s, C-10), 115.1 (d, C-3), 147.2 (5, C-5), 162.1 (s, C-7), 164.5 (s, C-9), 168.3 (s, C-2), 182.3 (C-4); EIMS *m/z* 250 $[M]^+$ (100), 234 (16), 190 (80), 161 (20), 151 (32), 124 (12); HREIMS *m/z* 250.0837 (calcd for $C_{13}H_{14}O_5$, 250.0841).

Benzyl 2-hydroxy-3,6-dimethoxybenzoate (3): mp 134−136 °C; UV (MeOH) λ_{max} (log ϵ) 270 (1.8), 300 (2.4), 360 (4.1) nm; IR (KBr) ν_{max} 3300−2500, 1700, 1615, 1487, 1244, 1172, 1062 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.78, 3.83 (each 3H, s), 5.41 (2H, s, H-7′), 6.31, 6.94 (each 1H, d, J = 9.0, H-4, -5), 7.32 (3H, m, H-3′, -4′, -5′), 7.39 (2H, dd, J = 8.1, 1.8 Hz, H-2′, -6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.4, 56.8 (each q, 2× $-OCH_3$), 67.0 (t, C-7′), 101.3 (d, C-5), 104.7 (s, C-1), 117.1 (d, C-4), 127.6 (d, C-2′, -6′), 128.1 (d, C-4′), 128.5 (d, C-3′, -5′), 135.7 (s, C-1′), 142.6 (s, C-3), 152.9 (s, C-2), 154.3 (s, C-6), 170.5 (s, C-7); EIMS m/z 288 $[M]^+$ (60), 180 (100), 151 (20), 123 (20), 91(70); HREIMS m/z 288.0989 (calcd for $C_{16}H_{16}O_5$, 288.0997).

Benzyl 2*β*-*O*-D-glucopyranosyl-3,6-dimethoxybenzoate (4): amorphous; UV (MeOH) λ_{max} (log ϵ) 270 (1.7), 300 (3.0), 360 (4.2) nm; IR (KBr) ν_{max} 3411, 1710, 1632, 1483, 1260, 1059, 890 cm^{-1}; ^1H NMR (300 MHz, CD_3COCD_3) δ 3.27−3.68 (6 H, m, H-2″−H-6″), 3.78, 3.82 (each 3 H, s, 2× $-OCH_3$), 4.63 (1 H, d, J = 7.7 Hz, H-1″), 5.24, 5.41 (each 1H, d, J = 12.4 Hz, H-7′), 6.66, 6.91 (each 1H, d, J = 9.0 Hz, H-4, -5), 7.34 (3H, m, H-3′, -4′, -5′), 7.42 (2H, dd, J = 8.1, 1.9 Hz, H-2′, -6′); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 56.4, 56.7 (each q, 2× OCH_3), 62.2 (d, C-6″), 68.0 (t, C-7′), 69.7 (d, C-4″), 73.9 (d, C-2″), 76.4 (d, C-3″), 78.1 (d, C-5″), 104.3 (d, C-5), 107.0 (s, C-1), 107.9 (d, C-1″), 114.3 (d, C-4), 128.3 (d, C-2′, -6′), 128.4 (d, C-4′), 128.5 (d, C-3′, -5′), 135.4 (s, C-1′), 143.5 (s, C-3), 145.6 (s, C-2), 151.1 (s, C-6), 166.6 (s, C-7); FABMS m/z 473.4324 $[M + Na]^+$ (calcd for $NaC_{22}H_{26}O_{10}$, 473.4320).

Synthesis of Compound 1. 5-Chloromethylfurfural (**5**) (200.2 mg)[10] and catechol (182.8 mg) were dissolved in dry acetone (15 mL). An excess of K_2CO_3 (500 mg) was added to this solution, which was heated under reflux for 16 h. After removing the acetone in vacuo, 15 mL of water was added. The product was extracted by EtOAc and purified on SiO_2 to yield **1** (196.3 mg, 65%).

Acknowledgment. This research was supported by the National Science Council of the Republic of China.

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NP020003K