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TWO NEW FLAVONE GLYCOSIDES FROM *CATALPA OVATA*

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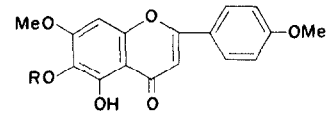
Key Word Index—*Catalpa ovata*; Bignoniaceae; flavone glycosides; 5,6-dihydroxy-7,4′-dimethoxyflavone-6-*O*-sophoroside; 5,6-dihydroxy-7,4′-dimethoxyflavone-6-*O*-glucoside.

Plant. *Catalpa ovata* G. Don (Bignoniaceae). Collected in October at Kyoto University campus, Kyoto, Japan. *Uses.* Official diuretic in Japan. *Previous work.* On fruits [1], woods [2,3], leaves [4,5] and barks [5]. *Present work.* Two new flavone glycosides, **1**, mp 240° (dec.) and **2**, mp 217–219°, were isolated by polyamide column chromatography of the water-soluble fraction of the seeds extract. Acid hydrolysis of **1** and **2** yielded glucose and aglycone **3** which was identified with a flavonoid previously obtained from the seeds [1]. This flavonoid has been characterized as 5,6-dihydroxy-7,4′-dimethoxyflavone which had been synthesized [6] and isolated only from *Nepeta hindustana* [7] and *Galeopsis ladanum* [8], although mp in ref. [8] (310–313°) differs greatly from **3**. **3** Formed a diacetate, $C_{21}H_{18}O_8$, mp 234–236°, and by the reaction with CH_2N_2, a methyl ether, $C_{18}H_{16}O_6$, mp 185–186°, which was identified with 7-*O*-methylpectolinarigenin. UV analysis (Table 1) and NMR spectra were also in complete agreement with structure **3**.



(1) R = β-Sophorosyl
(2) R = β-D-Glucosyl
(3) R = H

The NMR spectrum of per-TMS ether of **1** showed two anomeric proton signals at δ 4·72 (*d*, *J* 7 Hz) and δ 5·56 (*d*, *J* 6 Hz). An octaacetate of **1** was obtained as a white amorphous powder, whose NMR spectrum exhibited a phenolic Ac signal at δ 2·44 and seven aliphatic OAc signals at δ 2·1–1·9. These data, coupled with the result of acid hydrolysis, indicate that **1** is a glucosyl glucoside of **3**. Partial acid hydrolysis of **1** liberated a disaccharide and glucose (PC and GLC), and the former was identified as sophorose. The location of sophorose in **1** was shown to be at C-6 by the UV analysis: (1) the presence of a free hydroxyl group at C-5 and a substituent at 6-*O* was indicated by a bathochromic shift [9] of 19 nm for the band I upon addition of $AlCl_3$–HCl; (2) the band II maximum in MeOH is at 275 nm (as opposed to 286 nm, typical of a free 6-hydroxy group) [10].

Table 1. UV* and chromatographic† data for flavones of *Catalpa ovata*

Compound	Spectral maxima MeOH	+$AlCl_3$	+$AlCl_3$–HCl	+NaOAc	R_f TBA	15% HOAc	Color test UV ↓ UV/NH_3
(1)	275	300	300	277	0·59	0·71	dark purple ↓ no change
	331	347	350	328			
(2)	275	297	297	275	0·65	0·35	
	328	352	350	328			
(3)	285	304	303	285	0·81	0·08	
	332	360	357	330			

* All UV spectra were recorded using standard procedure [15].

† 2-D chromatograms were developed on Toyo-Roshi No. 51 paper with TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) and 15% HOAc.

The sophorosyl linkage in **1** is presumed to be β on the basis of the J value (6 Hz) [11] of the anomeric proton at δ 5·56 in the NMR spectrum and the comparison of the direction of its specific rotation (−30°) with those reported for quercetin-3-*O*-β-sophoroside (−43·6°) and kaempferol-3-*O*-β-sophoroside (−46·15°) in 90% EtOH [12]. Upon hydrolysis with excess emulsin for 4 weeks, only the terminal glucose was liberated, a finding consistent with earlier reports that β-sophorosides are very slowly hydrolyzed by emulsin [12,13].

Glycoside **2** showed similar UV spectrum to glycoside **1**, but its low R_f value on PC in 15% HOAc, and the NMR spectrum of the TMS ether indicated it to be monoglucoside of **3**. The position of the glucosyl linkage in **2** was shown to be at C-6 by the UV analysis (Table 1), and this linkage was shown to be β by the J value (6 Hz) of the anomeric proton signal at δ 5·30 in the NMR spectrum of per-TMS ether and also by the direction of the specific rotation. Glycoside **2** is thus the 6-*O*-β-D-glucoside of **3**.

6-Hydroxylation of the flavonoides has been shown to be a common structural feature in the Bignoniaceae [14]; the isolation of **1** and **2** from the seeds of *Catalpa ovata*, which are the first examples of scutellalein derivatives in this family, would reinforce this view. The glycosides **1** and **2** are also very unusual in that the sugar is linked to the 6-hydroxyl group.

EXPERIMENTAL

NMR spectra were obtained at 90 MHz on TMS ether [15] and chemical shifts are given in ppm relative to TMS as internal standard. GC–MS was carried out on a glass column (0·6 m × 3 mm i.d.) packed with 1·5% SE-30 on 60–80 mesh Chromosorb W at column temperature 240°. Temperature of ion source 250°, ion accelerating voltage 3·5 kV, ionizing potential 70 eV, trap current 60 μA. GLC was on columns of 1·5% SE-30 and 3% OV-17 on Chromosorb W, 2 m × 3 mm, with FID. TLC was carried out on Kieselgel G and PF_{254} developing with EtOAc–Me_2CO–HCOOH–H_2O (5:3:1:1). Silicic acid (100 mesh) and Polyamide C-200 were used for column chromatography. Sugars on PC were developed with *n*-BuOH–C_5H_5N–H_2O (6:4:3) (A) and (10:3:3) (B), and detected with *o*-aminobiphenyl hydrogen oxalate.

Isolation of glycosides **1** *and* **2**. The syrup (*ca* 40 g) of H_2O-soluble fraction was obtained from the seeds (1·5 kg) by the method of Ref. [1]. This syrup (10 g) was diluted with H_2O, treated with baker's yeast and filtered. Filtrate was concentrated and chromatographed on a polyamide (60 g) column, gradiently eluting with $H_2O \rightarrow$ MeOH. Evaporation of the H_2O eluant followed by recrystallization from MeOH–EtOH afforded crystals (118 mg), which showed two spots on TLC. Final purification was performed by prep. TLC followed by repeated recrystallization from MeOH–EtOH to give **1** as yellow crystals, mp 240° (dec), whose homogeneity was exhibited on TLC (R_f 0·4). $[\alpha]_D^{24}$ −30° (*c* 0·31, 90% EtOH), $[\alpha]_D^{24}$ −16° (*c* 0·33, MeOH). UV and PC (Table 1). IR (KBr): 3370, 1635, 1593, 1515, 1450, 1356, 1260, 1180, 1070 cm^{-1}. NMR (per-TMS ether, CCl_4): 3·85 and 3·98 (2 OMe), 4·72 (1H, *d*, *J* 7 Hz), 5·56 (1H, *d*, *J* 6 Hz), 6·37 (1H, *s*), 6·62 (1H, *s*), 6·97 (2H, *d*, *J* 9 Hz), 7·82 (2H, *d*, *J* 9 Hz). The octa-acetate was purified by column chromatography on silicic acid with $CHCl_3$ and obtained as a white amorphous powder. NMR ($CDCl_3$): 2·1–1·9 (7 OAc), 2·44 (OAc), 3·87 and 3·97 (2 OMe), 3·8–4·25 (6H, *m*), 5·16 (8H, *m*), 6·55 (1H, *s*), 6·95 (1H, *s*), 7·04 (2H, *d*, *J* 9 Hz), 7·82 (2H, *d*, *J* 9 Hz).

Evaporation of the 30% MeOH eluant gave crude **2** (17 mg), which was recrystallized from EtOH repeatedly to afford pure **2** (3·7 mg) as fine yellow needles, mp 217–219° (softening at 150°). $[\alpha]_D^{24}$ −20° (*c* 0·15, MeOH), TLC: R_f 0·61. UV and PC (Table 1). IR (KBr): 3400 (broad), 1660, 1604, 1565, 1500, 1450, 1357, 1248, 1180, 1118, 1035, 833 cm^{-1}. NMR (per-TMS ether, CCl_4): 3·85 (2 OMe), 5·30 (1H, *d*, *J* 6 Hz), 6·36 (1H, *s*), 6·53 (1H, *s*), 6·96 (2H, *d*, *J* 9 Hz), 7·81 (2H, *d*, *J* 9 Hz). (partial TMS ether [15]): 6·46 (1H, *s*), 6·40 (1H, *s*) (C_3—H and C_8—H), 12·86 (C_5—OH).

Hydrolysis of **1**. (1) *Complete acid hydrolysis.* **1** was refluxed with 6% HCl for 2 hr, insoluble yellow ppt. (aglycone) was filtered and recrystallized from MeOH–C_6H_6 to give yellow needles **3**, mp 215–216°. Color test: orange (Mg–HCl), yellow ($AlCl_3$), green [$Mg(OAc)_2$]. UV and PC (Table 1). NMR (per-TMS ether, CCl_4): 3·81, 3·85 (2 OMe), 6·31 (1H, *s*), 6·53 (1H, *s*), 6·90 (2H, *d*, *J* 9 Hz), 7·73 (2H, *d*, *J* 9 Hz). (C_6D_6 [16]): 3·22, 3·28 (4- and 7-OMe). (partial TMS ether, CCl_4 [13]): 6·38 (1H, *s*), 6·40 (1H, *s*) (C_3- and C_8-H), 12·48 (C_5-OH). Diacetate, colorless needles from EtOH, mp 234–236°, NMR ($CDCl_3$): 2·30 and 2·41 (2 OAc). (Found: C, 63·09; H, 4·64. $C_{21}H_{18}O_8$ requires: C, 63·31; H, 4·55%). Monomethyl ether, pale yellow plates, mp 185–186°. NMR ($CDCl_3$): 3·87, 3·91, 3·94 (3 OMe), 12·80 (C_5-OH). (Found; C, 66·05; H, 5·11. Calc for $C_{18}H_{16}O_6$: C, 65·85; H, 4·91%). This methyl ether was identified, by mmp and IR spectra, with 7-*O*-methylpectolinarigenin prepared by treating pectolinarigenin with CH_2N_2.

The filtrate from the hydrolyzate of **1** was neutralized with Amberlite IRA-410 (OH form), filtered and evaporated *in vacuo* to give a pale yellow syrup. PC (solvent A and B) and GLC (TMS ether, column temp 170°) showed glucose as the only sugar.

(2) *Partial acid hydrolysis.* **1** Was refluxed with 1% H_2SO_4 for 20 min, and deposited **3** was filtered. PC of the filtrate showed glucose and a disaccharide (solvent A: R_{Glc} 0·73, B: R_{Glc} 0·48) which, after purification by prep. PC (solvent B), yielded glucose (GLC) by the treatment with emulsin. This disaccharide was identified with synthetic sophorose [17] by co-chromatography (PC and GLC, column temp 220°) in the presence of laminaribiose, cellobiose, maltose, gentiobiose and isomaltose.

Alkaline degradation of **3**. A mixture of **3** (50 mg), KOH (1 g) and H_2O (0·5 ml) was heated at 200° for 40 min. The usual work up gave colorless crystals, mp 182–184°, identified as *p*-methoxybenzoic acid by mmp and IR spectra. (Found: C, 63·47; H, 5·52. Calc for $C_8H_8O_3$: C, 63·15; H, 5·30%).

Hydrolysis of **2**. Hydrolysis was carried out in analogous way as in the partial acid hydrolysis of **1**, to give aglycone **3** (PC, TLC, mmp and GC–MS of TMS ether). Chromatographic analysis of the sugar showed only glucose.

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PULVERIN, A NEW CHROMONE FROM THE FRUITS OF *NEOCHAMAELEA PULVERULENTA**

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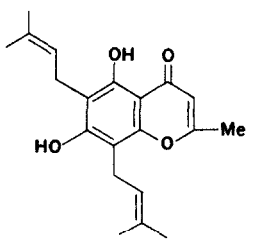
Key Word Index—*Neochamaelea pulverulenta*; Cneoraceae; leña santa; chromones; 3,3-dimethylallylspatheliachromene; pulverin; sitosterol; spatheliabischromene.

Plant. *Neochamaelea pulverulenta* *Erndt* (Vent) [1] (*Cneorum pulverulentum* Vent.) A voucher specimen is deposited in the Herbarium of the Botanical Department, University of La Laguna. *Source.* Guaza Mountain, Tenerife, Canary Islands, in August. *Uses* Medicinal. *Previous work.* Aerial parts [2,3].

Present work. Green fruits (300 g) were extracted with hot EtOH, concentrated *in vacuo* and chromatographed over SiO_2. Elution with C_6H_6, C_6H_6–EtOAc and EtOAc gave: 3,3-*dimethylallylspatheliachromene* [4,5], the new natural product *pulverin* (45 mg), *sitosterol* and *spatheliabischromene* [4,5].



(1)

Pulverin. (2-methyl-6,8-di-*C*-prenyl-5,7-dihydroxychromone) **(1)**, (Found: C, 73·29; H, 7·42; $C_{20}H_{20}O_4$ requires: C, 73·15; H, 7·37%) mp 147–149°, MS: *m/e* 328 (M^+), 313, 285, 273, 257, 229, 217 (100%) 205, 177, 128. UV λ_{max}^{EtOH} 214, 230, 265, 305(sh) nm. IR $\nu_{max}^{CHCl_3}$ 3350, 2960, 2900, 2850, 1660, 1600, 1420, 1100, 860 cm^{-1}. NMR ($CDCl_3$, τ) −2·96 (1H, phenolic proton at C_5), 3·62 (1H,

* Part 6 in the series *Chromenes and Chromones*. For Part V see González, A. G., Fraga, B. M. and Pino, O. (1975) *Rev. Real Acad. Ciencias* **69**, 347.