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TWO NEW NEOFLAVONOIDS AND *C*-GLYCOSYLFLAVONES FROM *PASSIFLORA SERRATODIGITATA*

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Key Word Index—*Passiflora serratodigitata*; Passifloraceae; 5,7-dihydroxy-4-phenylcoumarin (serratin); serratin 7-β-glucoside; C-glycosylflavones.

Abstract—The aerial parts of *Passiflora serratodigitata* yielded 5,7-dihydroxy-4-phenylcoumarin, its 7-β-glucoside and the known *C*-glycosylflavones 2″-xylosylvitexin, 2″-xylosylisovitexin, vitexin, isovitexin, a vicenin, and orientin. The known flavone chrysin was also isolated. This is the first report of neoflavonoids in the family Passifloraceae.

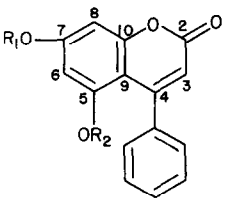
INTRODUCTION

In our continuing chemical investigation of the genus *Passiflora* [1–4] we analysed the leaves of *Passiflora serratodigitata* and report here the isolation and identification of two new neoflavonoids, namely 5,7-dihydroxy-4-phenylcoumarin (serratin, **1a**) and its 7-β-glucoside (**1b**). The structures of these compounds were determined by physical methods (mass spectrometry, UV, IR and ^1H and ^{13}C NMR) and structure **1a** was confirmed by synthesis. This is the first report of neoflavonoids from the Passifloraceae. Previously, members of this class of flavonoids were reported from the subfamily Lotoideae of the Leguminosae (e.g. the well-known dalbergin), Guttiferae and the Rubiaceae [5, 6]. Typically, the neoflavonoids of the Lotoideae are oxygenated at the 6- and 7-positions [7, 8], with infrequent additional oxygenation at the 5- or 8-positions [9, 10]. On the other hand, neoflavonoids from the Guttiferae have oxygen substituents at the 5- and 7-positions, as do the compounds reported here. However, the Guttiferae compounds are also alkylated at the 6- and 8-positions [5]. The one compound isolated from the rubiacious genus *Exostemma* has a 5,7,8 oxygenation pattern [6].

In addition to **1a** and **1b** and the known flavone chrysin, *P. serratodigitata* yielded the known *C*-glycosylflavones 2″-xylosylvitexin, 2″-xylosylisovitexin, vitexin, isovitexin, orientin and a vicenin.

RESULTS AND DISCUSSION

The new neoflavonoid serratin (**1a**), mp 213°, was shown to have the formula $C_{15}H_{10}O_4$ by elemental analysis and high resolution mass spectrometry. It gave a positive Shinoda colour reaction [11] and a violet colour with the $NH_2OH{\cdot}HCl + FeCl_3$ test. The UV spectrum in methanol of **1a** exhibited maxima at 333 (log ε 3.91), 266(3.9) 254(sh), and 208(5.1) nm. These data, in addition to its fluorescent blue colour under UV light, suggested a coumarin skeleton for **1a**. A



Serratin (**1a**) Dalbergin (**2**)

1a $R_1 = R_2 = H$
1b R_1 = glucose; $R_2 = H$
1c R_1 = COMe; $R_2 = H$
1d $R_1 = R_2 = Me$

67 nm bathochromic shift of the band at 333 nm with 0.1 N potassium hydroxide indicated that **1a** possessed at least one phenolic hydroxyl group. The dark-blue colour given by **1a** with ferric chloride gave further support for its phenolic nature. The preparation of a dimethyl ether (**1d**) from **1a** indicated that two phenolic groups were present in the molecule. The IR spectrum of **1a** was similar to that of other 4-phenylcoumarins. A sharp absorption at 1660 cm^{-1} was supportive of a neoflavonoid carbonyl; on methylation this peak gave a characteristic shift to 1710 cm^{-1}. A similar shift was observed for dalbergin (**2**) after methylation [8]. Further support for the 4-phenylcoumarin skeleton of **1a** was given by the mass spectrometry. The M^+ at *m/z* 254 (100) suggested a neoflavonoid structure with two hydroxyl substituents. Prominent peaks at *m/z* 226 $[M-CO]^+$ (95) and 197 $[M-CO-COH]^+$ (47) were consistent with the proposed structure **1a**. The ^1H NMR of serratin further clarified its structure. A five-proton broadened singlet which appeared at δ 7.4 is typical of an unsubstituted B-ring in neoflavonoids. An additional singlet at δ 5.8 (H-3) and two *meta*-coupled doublets

($J = 2$ Hz) at δ 6.3 and 6.25 for H-8 and H-6 were also observed. Synthesis of **1a** from 2,4,6-trihydroxybenzophenone confirmed that the new compound was hydroxylated at the 5- and 7-positions. Compound **1a** yielded only a monoacetate (**1c**) in accordance with the 5-position being sterically hindered by the 4-phenyl group. Finally, comparison of the ^{13}C NMR of **1a** and dalbergin (**2**) provided further support for the structure **1a** (Table 1).

The second new compound **1b** is a glucoside of serratin, $C_{21}H_{20}O_9$, mp 166–168°, since acidic hydrolysis of **1b** yielded glucose and serratin. Although hydrolysis of **1b** with β-glucosidase was not successful, ^1H NMR indicated the linkage to be β (glucose H-1 doublet at δ 5.0 with $J = 6$ Hz). Other peaks in the spectrum were similar to those of **1a** with the exception of a broad group of signals at δ 3.1–3.7 integrating for six glucosyl protons. That **1b** is a 7-*O*- and not a 5-*O*-glucoside was suggested by the B-ring five-proton signal which appeared as a broadened singlet (at δ 7.52) rather than a more complex signal. The latter type of signal is observed when a 5-*O*-methyl ether group is present as in **1d**.

The known flavone chrysin and the *C*-glycosylflavones 2″-xylosylvitexin and 2″-xylosylisovitexin were also isolated and identified by their UV spectra with standard reagents[12], ^1H NMR (as TMSi ethers), mass spectrometry, hydrolysis with 0.1 N TFA and comparison with authentic samples. The 2″-*O*-linkage of the xylosyl residue in the two *C*-glycosylflavones was confirmed by ^1H NMR of their acetates. The absence of acetate methyl signals at δ 1.75 and 1.85, respectively, in the xylosylvitexin and xylosylisovitexin acetate derivatives indicated that in both cases the xylosyl moiety must be attached to the 2″-OH of the *C*-glucosyl group[13].

In addition to the above compounds four other *C*-glycosylflavones, a vicenin, orientin, vitexin and isovitexin were isolated in small quantities. The latter three compounds were identified by TLC comparison with authentic samples and vitexin and isovitexin were further characterized by UV using standard shift reagents.

Table 1. ^{13}C NMR of serratin (**1a**) and dalbergin (**2**)

C-2	163.00	160.3
C-3	96.05	111.3
C-4	140.00	135.5
C-5	163.88	111.3
C-6	100.40	148.3
C-7	158.87	143.6
C-8	111.00	111.3
C-9	102.40	110.5
C-10	158.03	151.9
C-1′	158.00	155.0
C-2′, C-6′	128.80	128.2
C-3′, C-5′	128.18	128.3
C-4′	128.18	129.3

EXPERIMENTAL

Plant material. Aerial parts of *P. serratodigitata L.* were obtained from the greenhouse collection of Dr. Larry Gilbert, Department of Zoology, University of Texas at Austin. The original plants were collected from Andrew Trace, Arima Pass, Trinidad. Voucher specimen Gilbert No. 70068 will be deposited in the University of Texas Herbarium.

General. MPs were uncorr. CC employed Si gel for the C_6H_6 and $CHCl_3$ extracts and Polyclar AT (GAF Corp.) for the EtOH extract. Pre-coated Si gel, cellulose (E. Merck) and Polyamide (Macherey–Nagel and Co.) plates were used for TLC. Prep. TLC was on 1 mm Si gel layers. The solvent systems used were: $CHCl_3$–EtOH (9 : 1), TBA (*t*-BuOH–HOAc–H_2O, 3 : 1 : 1), BAW upper phase (*n*-BuOH–HOAc–H_2O, 4 : 1 : 5), BMM (C_6H_6–MeCOEt–MeOH, 4 : 3 : 3), BPMM [C_6H_6–petrol (65–100°)–MeCOEt–MeOH, 60 : 26 : 7 : 7], CAA($CHCl_3$–Me_2CO–HCO_2H, 9 : 2 : 1) and BPA(C_6H_6–pyridine–HCO_2H, 36 : 9 : 5). Flavonoids were visualized on TLC plates with UV + NH_3 and by spraying with NA (Naturstuffreagenz-A in MeOH). Neoflavonoids were visualized by UV. All flavonoids were purified over Sephadex LH-20 (Pharmacia) using MeOH–H_2O as the eluent prior to ^1H NMR and UV analysis by standard procedures[12]. ^1H NMR was at 90 MHz and ^{13}C NMR at 22.61 MHz. MS were recorded by direct inlet at 70 eV.

Isolation and identification of compounds. Ground dried leaves (200 g) were extracted sequentially in a Soxhlet with petrol (30–60°), C_6H_6, $CHCl_3$ and EtOH yielding extracts of 6.9, 10.0, 13.8 and 13.5 g respectively. The petrol extract was not examined. When the C_6H_6 extract was chromatographed over a Si gel column, which was eluted with $CHCl_3$ containing increasing amounts of EtOH, chrysin (12 mg), mp 285°, was obtained. The $CHCl_3$ extract was chromatographed similarly; the first fraction ($CHCl_3$–EtOH, 9 : 1) yielded 35 mg **1a**. Later fractions contained mixtures of **1a** and **1b** which were separated by prep. TLC. Recrystallization yielded 350 mg **1a**. Found: C, 70.92; H, 3.92 for $C_{15}H_{10}O_4$, 254.05790; IR ν_{max}^{KBr} cm^{-1}: 3170, 1660, 1600, 1585, 1545, 1450, 1370, 1155, 1070, 940, 850, 820, 770 and 690. UV, MS, ^1H and ^{13}C NMR (DMSO-d_6, TMS) data are given in the text and Table 1. **1a** (25 mg) was acetylated by standard procedures, but only the monoacetate **1c** was obtained, yield 22 mg, mp 178° (after recrystallization from EtOH). MS *m/z* (rel. int.): 296 $[M]^+$(24), 254 $[M - MeCO]^+$(100), 226 $[M - 43 - CO]^+$(57), 197 (12); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1770 (acetate C=O), 1734 (lactone C=O), 1610, 1585, 1440, 1370, 1355, 1290, 1275, 1225, 1020, 895, 875, 780; NMR ($CDCl_3$, TMS): δ 2.32 (3H, *s*, acetyl Me), 6.2 (1H, *s*, H-3), 6.8 (1H, *d*, $J_{6,8} = 2$ Hz, H-6), 7.2 (1H, *d*, $J_{6,8} = 2$ Hz, H-8), 7.4 (5H, *m*, B-ring). **1a** was methylated with Me_2SO_4 to yield the dimethyl product **1d**, mp 167° after crystallization from EtOH, mmp 165–167° with synthetic product, mp 168–170° (Donelly, D.M.X., personal communication). MS *m/z* (rel. int.): 282 $[M]^+$(98), 267 $[M - Me]^+$(3), 254 $[M - 2Me]^+$(100), 239 (47), 224 (5), 196 (10); IR ν_{max}^{KBr} cm^{-1}: 1710 (lactone C=O), 1605, 1590, 1485, 1450, 1415, 1345, 1305, 1220, 1205, 1150, 1105, 1050, 950, 860, 820, 780, 750 and 700; ^1H NMR ($CDCl_3$, TMS): δ 3.43 (3H, *s*, OMe), 3.9 (3H, *s*, OMe) 6.0 (1H, *s*, H-3), 6.25 (1H, *d*, $J_{6,8} = 2$ Hz, H-6), 6.5 (1H, *d*, $J_{6,8} = 2$ Hz, H-8), 7.35 (5H, *m*, B-ring). Serratin 7-β-glucoside (**1b**) was separated from **1a** by prep. TLC. EtOH crystallization yielded beige-coloured needles (25 mg), mp 166–168°. Found: C, 60.62, H, 4.82. $C_{21}H_{20}O_9$ requires: C, 60.57 H, 4.80. UV λ_{max}^{MeOH} nm (log ϵ): 328 (3.9), 258 (4.1), 248 (sh), 208 (5.0); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1700 (lactone C=O), 1615, 1600, 1585, 1550, 1495, 1450, 1355, 1280, 1220, 1170, 1090, 1065, 1030, 940, 820, 700. ^1H NMR described in text. Hydrolysis of **1b** with 0.1 N HCl yielded **1a**, mp 213°; mmp and IR confirmed its identity. Glucose was identified by TLC comparison with authentic sugars. The EtOH extract was chromatographed as described above

using Egger's solvent ($CHCl_3$–MeOH–MeCOEt–Me_2CO, 4 : 2 : 0.5 : 0.1). The polarity was increased by gradual reduction in the proportion of $CHCl_3$. All *C*-glycosylflavones were eluted sequentially. 2″-Xylosylvitexin and 2″-xylosylisovitexin were obtained as a mixture and separated on a microcrystalline cellulose column.

Synthesis of serratin (**1a**). Synthetic serratin (**1a**) was prepared by refluxing 2,4,6-trihydroxybenzophenone (5 g) with fused NaOAc (2 g) and Ac_2O (5 ml) for 24 hr. When the mixture was treated with crushed ice, an orange ppt formed. The ppt contained a mixture of compounds from which **1a** was obtained in 5% yield by prep. TLC using $CHCl_3$–EtOH (9 : 1). Crystallization from EtOH yielded 250 mg of a product with mp 210°; mmp with serratin 209–210°. TLC comparison, NMR and IR showed the synthetic product to be identical to **1a**.

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