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O- AND *C*-GLYCOSYLFLAVONES FROM *PASSIFLORA BIFLORA*

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Key Word Index—*Passiflora biflora*; Passifloraceae; *C*-glycosylflavones; luteolin 7-*O*-neohesperidoside; 4′-*O*-rhamnosylswertisin; 4′-*O*-rhamnosylswertiajaponin; 2″-*O*-rhamnosylisovitexin; 2″-*O*-rhamnosylisoorientin; swertisin; swertiajaponin.

Abstract—Six *C*-glycosylflavones including two new compounds, 4′-*O*-rhamnosylswertisin and 4′-*O*-rhamnosylswertiajaponin, were isolated from leaves of *Passiflora biflora*. Known *C*-glycosylflavones were swertisin, swertiajaponin, 2″-*O*-rhamnosylisoorientin and 2″-*O*-rhamnosylisovitexin. In addition, luteolin 7-*O*-neohesperidoside was isolated.

INTRODUCTION

As part of a biochemical systematic investigation of members of the genus *Passiflora* [1] we report the isolation and identification of flavonoids from *Passiflora biflora* Lam., a species placed by Killip in the subgenus *Plectostemma*, section *Decaloba*, series *Punctatae* [2]. *P. biflora* is widely distributed throughout Mexico and south into Columbia and Venezuela.

RESULTS AND DISCUSSION

4-*O*-*Rhamnosylswertiajaponin* (1)

The color of **1** on paper was purple with no change in the presence of either Naturstoffreagenz A or ammonia. The R_f values of 0.55 in TBA, 3:1:1, and 0.74 in 15% acetic acid indicated that the compound was a glycoside. After hydrolysis with 0.1 N trifluoroacetic acid, the compound appeared purple when viewed in the UV (366 nm) on paper changing to bright yellow when the spot was fumed with ammonia and turning orange when sprayed with Naturstoffreagenz A. These color changes indicated a luteolin-type flavonoid with a 4′ hydrolysable substituent. The color change to bright yellow rather than dull yellow with ammonia suggested a 7-OR substituent. The hydrolysable group was rhamnose and the R_f values after hydrolysis of 0.53 in TBA, 3:1:1, 0.48 in 15% acetic acid indicated that **1** was a *C*-glycosylflavone co-chromatographing with swertiajaponin. The UV spectrum in standard reagents supported a 4′-*O*-hydrolysable substituent, a 7-OR group and a luteolin-type B-ring. The ^{1}H NMR spectrum of the TMSi ether of **1** showed an overlapping set of signals for H-2′, H-5′ and H-6′ at δ 7.3 and two singlets for H-3 and H-8 at δ 6.3 and 6.5, respectively. A rhamnosyl H-1 appeared at δ 5.2 with a rhamnosyl methyl doublet at δ 1.2. The *C*-glucosyl H-1 appeared at δ 4.75. A three-proton singlet at δ 3.9 which shifted to δ 3.35 in deuterobenzene was assigned to OMe-7. The mass spectrum of the PM ether of **1** showed a very low intensity M^+ at m/z 734 (0.96) with the base peak (AH) at m/z 546, the latter corresponding to $[M-188]^+$ resulting from the loss of the 4′-*O*-rhamnosyl group. The T series of peaks at m/z 189, 157 and 125 also supported an *O*-linked rhamnose [3].

Luteolin 7-O-*neohesperidoside* (2)

Luteolin 7-*O*-neohesperidoside (**2**) appeared as a purple spot on paper under UV, turning bright yellow when fumed with ammonia vapors and orange when sprayed with Naturstoffreagenz A. Both color changes suggested a luteolin-type B-ring and a 7-OR substituent. R_f values on paper of 0.56 in TBA, 3:1:1, and 0.36 in 15% acetic acid indicated that **2** was a glycoside. Hydrolysis in 0.1 N trifluoroacetic acid yielded luteolin (R_f 0.68, TBA, 3:1:1, 0.05, 15% acetic acid) and rhamnose and glucose as the hydrolysable sugars. The UV spectrum of **2** confirmed a 7-OR substituent. Specifically the lack of band III in sodium methoxide and the relative shifts for band I in sodium methoxide and sodium acetate when compared to band I in methanol (392 and 410 nm, respectively) all supported a 7-OR. Shifts in aluminium chloride and aluminium chloride–hydrochloric acid supported a luteolin-type B-ring. The ^{1}H NMR of the TMSi ether of **2** recorded in carbon tetrachloride was in accord with luteolin 7-*O*-neohesperidoside. The position of the H-1 protons of the rhamnosyl [δ 4.88 (*d*)] and glucosyl [δ 5.12 (*d*)] moieties and of the rhamnosyl methyl [δ 1.2 (*d*)] supported the 7-*O*-neohesperidosyl group [4]. The luteolin skeletal protons appeared at δ 7.35 (*m*) for the H-2′ and the H-6′, 6.85 (*d*) for the H-5′, 6.67 (*d*) for the H-8, 6.35 (*d*) for the H-6 and 6.3 (*s*) for the H-3; 10 additional sugar protons appeared between 3.3 and 3.8. The mass spectrum of the PM derivative of **2** gave no $[M]^+$; however, a fragment for the aglycone appeared at m/z 328 and peaks assignable to the A_1, m/z 180 and B_1 m/z 162 were present. Fragments at m/z 189, 157 and 125 were assigned to the T series of the terminal rhamnose.

4′-O-*Rhamnosylswertisin* (3)

4′-*O*-Rhamnosylswertisin (**3**) appeared purple on cel-

lulose plates when the plates were examined in UV, with no change with either ammonia vapors or Naturstoffreagenz A spray. After hydrolysis with 0.1 N trifluoroacetic acid, the resulting flavonoid co-chromatographing with swertisin was purple on a cellulose plate changing to yellow-green when fumed with ammonia and to yellow when sprayed with Naturstoffreagenz A. These changes suggested an apigenin 4′-*O*-glycoside. The UV spectra in methanol and with sodium methoxide before and after hydrolysis were in agreement with the color change data. There was a decrease in intensity in band I in sodium methoxide compared to band I in methanol before hydrolysis; after hydrolysis, spectra in the same solvents indicated a 4′ free hydroxyl (increase in intensity of band I in sodium methoxide when compared to band I in methanol). An OR-7 is supported by the lack of band III in sodium methoxide between 304 and 340 nm. The ^{1}H NMR of the TMSi ether of **3** in carbon tetrachloride was in accord with a 6-substituted apigenin. Signals for the B-ring protons occurred at δ 7.8 (*d*) for the H-2′ and H-6′ and at 7.1 (*d*) for the H-3′ and H-5′. A singlet at δ 6.5 was assigned to the H-8 and a singlet at 6.4, to the H-3. The H-1 of the *C*-glucosyl moiety appeared at δ 4.8. An additional H-1 signal appeared at δ 5.3 with the small coupling constant typical of a rhamnosyl H-1. A rhamnosyl methyl signal (*d*) was observed at δ 1.2. The methoxy singlet which appeared at δ 3.9 and shifted to δ 3.35 in C_6D_6 was assigned to the OMe-7. The mass spectrum of the PM ether of **3** was in accord with a 6-*C*-glycosylflavone [5]. The molecular $[M]^+$ ion occurred at *m/z* 704 and prominent $[M-15]^+$ and $[M-31]^+$ fragments were also observed. The fragment (AH) resulting from the loss of 188 from $[M]^+$ indicated an *O*-linked rhamnosyl group. This result was confirmed by the T series of fragments at *m/z* 189, 157 and 125 for a terminal rhamnosyl group.

EXPERIMENTAL

Plant material. Leaves of *Passiflora biflora* Lam. were collected from a plant grown from rootstock which had been cultivated at Duke University. The original collection was from Finca La Selva, OTS Field Station, on Rio Puerto Viejo, east of its junction with the Rio Sarapiqui, Province of Heredia, Costa Rica (collection by Steve Oberbauer; voucher MacDougal No. 464 deposited in Duke University and University of Texas Herbaria).

Air-dried leaf material (118.9 g) of *Passiflora biflora* was extracted with 80% aq. MeOH (3 × 4 l.). After evaporation under red. pres. the syrup (32.7 g) was partitioned against CH_2Cl_2 (yield: 3.9 g) and EtOAc (yield: 2.8 g). 2D-PC indicated no flavonoid compounds in the CH_2Cl_2 fraction. The EtOAc extracted material was chromatographed over a Polyclar column (27 × 5 cm) which was eluted with a modified Egger's solvent (CH_2Cl_2–MeOH–EtCOMe–Me_2CO, 20:10:5:1) with the polarity of the eluting solvent gradually increased by decreasing the amount of CH_2Cl_2. Fractions from this column were further separated by 1D-PC (15% HOAc or BAW 4:1:5, upper phase). The remaining syrup from partitioning was chromatographed over a Polyclar column (42 × 6 cm) in 50% aq. MeOH with the polarity of the solvent gradually decreased. Fractions were further separated on microcrystalline columns (15% HOAc) or 1D-PC (15% HOAc or BAW 4:1:5, upper phase). Some fractions were further separated on Polyclar columns (100% MeOH). All isolated compounds were cleaned over Sephadex LH-20 packed in MeOH prior to spectral analysis. Known compounds were identified by comparison of spectral data with published values [6–9].

4′-O-*Rhamnosylswertiajaponin* (**1**): TLC (cellulose) R_f: 0.35 (TBA), 0.64 (15% HOAC). UV λ_{max}^{MeOH} nm: 340, 272, 250, 243; λ_{max}^{NaOMe} nm: 375 ↓, 271; $\lambda_{max}^{AlCl_3}$ nm: 385sh, 358, 296sh, 280, 269sh; $\lambda_{max}^{AlCl_3-HCl}$ nm: 385sh, 353, 296sh, 281, 259sh; λ_{max}^{NaOAc} nm: 339, 271; $\lambda_{max}^{NaOAc-H_3BO_3}$ nm: 341, 271. ^{1}H NMR (90 MHz, CCl_4): δ 1.2 (3H, *d*, *J* = 5.5 Hz, rhamnosyl Me), 3.9 (3H, *s*, OMe-7), 4.75 (1H, *d*, *J* = 10.0 Hz, glucosyl H-1), 5.2 (1H, *d*, *J* = 1.5 Hz, rhamnosyl H-1), 6.35 (1H, *s*, H-3), 6.5 (1H, *s*, H-8), 7.1–7.5 (3H, *m*, H-2′, H-5′, H-6′). ^{1}H NMR (90 MHz, C_6D_6): δ 3.35 (OMe-7). EIMS (70 eV) of PM ether, *m/z* (rel. int.): 734 $[M]^+$ (1), 546 $[M-188]^+$ (100), 500 (19), 189 $[T_1]^+$ (71), 157 $[T_1-MeOH]^+$ (27), 125 $[T_1-2MeOH]^+$ (17).

Luteolin 7-O-*neohesperidoside* (**2**): TLC (cellulose) R_f 0.44 (TBA), 0.22 (15% HOAc). UV λ_{max}^{MeOH} nm: 350, 266, 252; λ_{max}^{NaOMe} nm: 392 ↑, 266; $\lambda_{max}^{AlCl_3}$ nm: 430, 330sh, 266; $\lambda_{max}^{AlCl_3-HCl}$ nm: 388, 358, 296, 262; λ_{max}^{NaOAc} nm: 410, 268; $\lambda_{max}^{NaOAc-H_3BO_3}$ nm: 377, 260. ^{1}H NMR (90 MHz, CCl_4): δ 1.2 (3H, *d*, *J* = 8.0 Hz, rhamnosyl Me), 4.88 (1H, *d*, *J* = 1.5 Hz, rhamnosyl H-1), 5.12 (1H, *d*, *J* = 8.0 Hz, glucosyl H-1), 6.3 (1H, *s*, H-3), 6.35 (1H, *d*, *J* = 1.5 Hz, H-6), 6.67 (1H, *d*, *J* = 1.5 Hz, H-8), 6.85 (1H, *d*, *J* = 8.5 Hz, H-5′), 7.35 (2H, *m*, H-2′ and H-6′). EIMS (70 eV) of PM ether: 328 $[M-gly]^+$ (15), 189 $[T_1]^+$ (42.1), 157 $[T_1-MeOH]^+$ (19), 125 $[T_1-2MeOH]^+$ (10), 180 $[A_1]^+$ (1.3), 162 $[B_1]^+$ (1.3).

4′-O-*Rhamnosylswertisin* (**3**): TLC (cellulose) R_f 0.56 (TBA), 0.74 (15% HOAc). UV λ_{max}^{MeOH} nm: 328, 273; λ_{max}^{NaOMe} nm: 372, 291. ^{1}H NMR (90 MHz, CCl_4): δ 1.5 (3H, *d*, *J* = 5.5 Hz, rhamnosyl Me), 4.9 (3H, *s*, MeO-7), 4.8 (1H, *d*, *J* = 1 Hz, glucosyl H-1), 5.25 (1H, *d*, *J* = 2.0 Hz, rhamnosyl H-1), 6.41 (1H, *s*, H-3), 6.5 (1H, *s*, H-8), 7.10 (2H, *d*, *J* = 9.0 Hz, H-3′ and H-5′), 7.75 (2H, *d*, *J* = 9.0 Hz, H-2′ and H-6′). ^{1}H NMR (90 MHz, C_6D_6): δ 3.35 (OMe-7). EIMS (70 eV) of PM ether, *m/z* (rel. int.): 704 $[M]^+$ (28), 689 $[M-15]^+$ (16), 673 $[M-31]^+$ (90), 657 $[M-47]^+$ (7), 601 $[M-103]^+$ (4), 541 $[M-163]^+$ (7), 529 $[M-175]^+$ (39), 516 $[M-188]^+$ (49), 485 $[M-219]^+$ (5), 355 (17), 354 (38), 353 (3), 341 (15), 327 (6), 325 (6), 311 (4), 297 (3), 189 $[T_1]^+$ (100), 157 $[T_1-MeOH]^+$ (45), 125 $[T_1-2MeOH]^+$ (24).

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