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C-GLYCOSYLFLAVONOIDS FROM *PASSIFLORA COACTILIS*

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Key Word Index—*Passiflora coactilis*; Passifloraceae; *C*-glycosylflavonoids; 4′-*O*-glucosyl-2″-*O*-rhamnosylorientin; 4′-*O*-glucosyl-2″-*O*-rhamnosylvitexin.

Abstract—Eleven *C*-glycosylflavones were isolated from the leaves of *Passiflora coactilis*, including two new 4′-*O*-glucosides of 2″-rhamnosylorientin and 2″-rhamnosylvitexin.

INTRODUCTION

The genus *Passiflora* (Passifloraceae) contains *ca* 400 species and is distributed mainly in the New World [1]. *Passiflora coactilis* (Mast.) Killip, a member of the subgenus *Tacsonia*, is restricted to the western Andean slopes of Ecuador [2]. As part of a taxonomic study of all the species that comprise this Andean subgenus we here report the isolation and identification of 11 *C*-glycosylflavonoids from *P. coactilis*, including two new 4′-*O*-glucosides.

RESULTS AND DISCUSSION

Chromatographic separation of the aqueous methanol extract of *P. coactilis* afforded two novel compounds 4′-*O*-glucosyl-2″-*O*-rhamnosylorientin (**1a**) and 4′-*O*-glucosyl-2″-*O*-rhamnosylvitexin (**2a**), as well as the known flavone *C*-glycosides, vitexin, 4′-*O*-glucosylvitexin, isovitexin, isoorientin, 4′-*O*-glucosylorientin, 2″-*O*-rhamnosylorientin, scoparin, 2″-*O*-rhamnosylscoparin and 8-*C*-glucosyldiosmetin.

The R_f of **1a** on paper (TBA, 0.28; 15% acetic acid, 0.79) suggested that it could be a flavonoid triglycoside. The compound appeared purple on paper under UV light (365 nm) and did not change color upon fuming with ammonia. These data, along with the UV maxima at 336 and 272 nm and the shifts obtained with diagnostic reagents [3], indicated the compound was probably a flavone with free hydroxyl groups at the 5 and 7 positions but with the 4′ position substituted.

Additional information was obtained from the ^{1}H NMR spectrum of the TMSi ether of **1a**. The doublet at δ6.96 for H-5′ and the overlapping doublet and double doublet at 7.43 for H-2′ and H-6′ are characteristic for a 3′,4′ disubstituted B-ring. Two additional singlets at 6.30 and 6.16 can be assigned to H-3 and H-6, respectively. The multiplicity of the latter signal indicated that the C-8 position of **1a** is substituted. Finally, the triglycosyl nature of the compound was confirmed by the presence of a group of signals between δ3.06 and 3.87 integrating for 16 protons, as well as three overlapping one-proton signals between 4.28 and 5.07 corresponding to the H-1 of each of the three sugar moieties. A methyl doublet at 0.87 indicated that one of these sugars was a 6-deoxysaccharide such as rhamnose.

The position of attachment of the three sugar moieties to the flavone skeleton was determined by a series of hydrolysis experiments. Upon treatment with β-glucosidase **1a** yielded glucose and a glycosidic product **1b** (R_f TBA, 0.44; 15% acetic acid, 0.60) which was purple under UV light on paper. However, in contrast to **1a** fuming with ammonia caused **1b** to turn yellow. Again, in contrast to **1a**, **1b** reacted with NA (Naturstoffreagenz A) reagent to give an orange spot, indicating that a 3′,4′ *ortho*-dihydroxyl function is present in this hydrolysis product. Comparison of the aluminium chloride and aluminium chloride–hydrochloric acid spectra of **1b** (44 nm hypsochromic shift upon addition of hydrochloric acid) confirmed the presence of an *ortho*-dihydroxyl function in the B-ring. That a 4′-*O*-glucosyl moiety in **1a** had been hydrolysed by treatment with β-glucosidase was further supported by the shift of band I in the sodium methoxide UV spectrum of **1b** (57 nm bathochromic shift with increased intensity; **1a** gave a 42 nm shift but with a decrease in intensity).

When the enzymatic hydrolysis product **1b** was heated with 0.1 N TFA, rhamnose and an additional glycosidic product **1c** were obtained. The UV spectrum of **1c** was essentially identical to that of **1b**, suggesting that rhamnose was attached to the remaining glycosyl residue of **1c**. The *C*-glycosyl nature of **1c** was demonstrated by the Wessely–Moser rearrangement product produced by treatment of **1c** with 2 N hydrochloric acid. UV and authentic sample comparison confirmed that **1c** was orientin. The natural product **1a** must, therefore, be a 4′-*O*-glucosyl-rhamnosylorientin. The position of attachment of the rhamnosyl residue to the *C*-glucosyl unit was clearly shown by the ^{13}C NMR spectrum of **1b**. The downfield position of C-2″ (δ75.1) indicates that the rhamnose–glucose linkage is 1‴–2″. In addition, the chemical shifts of the remaining sugar carbons of **1b** are in close accord with those reported for 2″-*O*-rhamnosylvitexin [4].

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The second novel compound, **2a**, appeared to be very similar to **1a**. The R_f values (TBA, 0.35; 15% acetic acid, 0.84) and color reactions on a paper chromatogram (UV purple, UV–ammonia purple) were essentially identical. UV maxima at 323 and 270 nm suggested that **2a** could be monosubstituted in the B-ring. As with **1a**, the UV spectra in the presence of diagnostic reagents showed that the compound contains free hydroxyl groups at the 5 and 7 positions and a substituted 4′ hydroxyl group. Hydrolysis with β-glucosidase yielded glucose and a flavonoid glycoside **2b** (R_f: TBA, 0.55; 15% acetic acid, 0.62). The sodium methoxide UV spectrum indicated that **2b** contained a 4′ hydroxyl group. However, unlike **1b**, this compound turned green on paper under UV after exposure to ammonia vapor and did not react with NA reagent in accord with a 4′-monosubstituted B-ring structure for **2a**. Comparison by UV and co-TLC with an authentic sample showed that **2b** is 2″-rhamnosylvitexin. Further confirmation of this structure for **2b** was provided by hydrolysis. Treatment of **2b** with 0.1N TFA afforded rhamnose and a third glycoside **2c** (R_f: TBA, 0.45; 15% acetic acid, 0.30). The *C*-glycosyl nature of **2c** was demonstrated by the Wessely–Moser rearrangement products produced by hydrolysis with 2 N hydrochloric acid. UV and co-TLC with authentic samples showed that **2c** was vitexin. Therefore, the new glycoside **2a** was characterized as 4′-*O*-glycosyl-2″-rhamnosylvitexin.

The nine known *C*-glycosylflavonoids namely 4′-*O*-glycosylvitexin, 4′-*O*-glucosylorientin, 2″-*O*-rhamnosylorientin, 2″-*O*-rhamnosylscoparin, 8-*C*-glucosyldiosmetin, vitexin, isovitexin, isoorientin and scoparin were all identified by UV, hydrolysis and co-chromatography with authentic samples. In addition, the two 4′-*O*-glucosides of vitexin and orientin were further characterized by ^{1}H NMR (TMSi derivatives), UV and MS (perdeuteromethyl derivatives).

EXPERIMENTAL

Plant material. Leaves of *Passiflora coactilis* were collected in Cotopaxi Province, Ecuador during May 1979. Voucher specimen Albert de Escobar No. 1480 is deposited at the University of Texas Herbarium, Austin, Texas.

Extraction and isolation. Dried leaves (48 g) were ground and extracted with 85% aq. MeOH. The extract was concd and streaked across sheets of Whatman 3 MM chromatography paper. Descending development with TBA (t-BuOH–HOAc–H_2O, 3:1:1) produced several bands visible under UV which were subsequently cut out and eluted with 50–90% aq. MeOH depending upon their mobility in TBA. The most polar band was then chromatographed over a microcrystalline cellulose column eluted with 15% HOAc. Four bands were separated and collected. The least polar fraction from the cellulose column was then chromatographed on a Polyclar column. Compounds **1a** (93 mg) and **2a** (121 mg) were eluted with 100% MeOH. The nine known *C*-glycosylflavonoids were isolated and purified as necessary by similar procedures. Yields: vitexin, 30 mg; isovitexin, 30 mg; 4′-*O*-glucosylvitexin, 48.9 mg; 2″-*O*-rhamnosylvitexin, 50 mg; isoorientin, 30 mg; 4′-*O*-glucosylorientin, 45.7 mg; scoparin, 90 mg; 2″-*O*-rhamnosylscoparin, 30 mg; 8-*C*-glucosyldiosmetin, 70 mg. Prior to identification all compounds were purified on Sephadex LH-20 (Pharmacia) columns eluted with MeOH.

4′-O-*Glucosyl*-2″-O-*rhamnosylorientin* (**1a**). UV λ_{max}^{MeOH} nm: 336, 272, 250 sh, + NaOMe 378 ↓, 302 sh, 276, 266, 240; + $AlCl_3$ 384, 348, 292 sh, 276, 258 sh; + $AlCl_3$ + HCl 384, 346, 294 sh, 280, 256 sh; + NaOAc 374, 278, 271 sh; + NaOAc + H_3BO_3 336, 272. ^{1}H NMR: see text.

2″-O-*Rhamnosylorientin* (**1b**). UV λ_{max}^{MeOH} nm: 350, 268, 255; + NaOMe 407 ↑, 332 sh, 280 sh, 266; + $AlCl_3$ 430, 330, 304 sh, 274; + $AlCl_3$ + HCl 386, 356, 295, 274, 260; + NaOAc 400, 328 sh, 277 sh 269; + NaOAc + H_3BO_3 430 sh, 375, 261. ^{13}C NMR (22.6 MHz, DMSO-d_6) 182.1 (*s*, C-4), 164.2 (*s*, C-2), 162.4 (*s*, C-7), 160.7 (*s*, C-5), 155.9 (*s*, C-9), 149.7 (*s*, C-4′), 145.9 (*s*, C-3′), 122.0 (*s*, C-1′), 119.4 (*d*, C-6′), 115.8 (*d*, C-5′), 114.1 (*d*, C-2′), 104.4 (*s*, C-8), 104.2 (*s*, C-10), 102.4 (*d*, C-3), 100.4 (*d*, C-1‴ rhamnose), 98.3 (*d*, C-6), 81.9 (*d*, C-5″ glucose), 80.0 (*d*, C-3″), 75.1 (*d*, C-2″), 71.7 (*d*, C-3‴), 71.5 (*d*, C-1″), 70.9 (*d*, C-4‴, C-2‴), 70.3 (*d*, C-4″), 68.2 (*d*, C-5‴), 61.5 (*t*, C-6″), 17.6 (*q*, C-6‴). Hydrolysed sugars were identified by co-TLC with authentic samples.

4′-O-*Glucosyl*-2″-O-*rhamnosylvitexin* (**2a**). UV λ_{max}^{MeOH} nm: 323, 270; + NaOMe 372 ↓, 302 sh, 280; + $AlCl_3$ 386, 336, 302, 282, 258 sh; + $AlCl_3$ + HCl 386, 336, 302, 282, 258 sh; + NaOAc 364, 296 sh, 280; + NaOAc + H_3BO_3 323, 270.

2″-O-*Rhamnosylvitexin* (**2b**). UV λ_{max}^{MeOH} nm: 336, 296 sh, 268; + NaOMe 394 ↑, 328 sh, 279; + $AlCl_3$ 382, 346, 302, 276; + $AlCl_3$ + HCl 382, 346, 304, 277; + NaOAc 390, 330 sh, 280; + NaOAc + H_3BO_3 336, 270.

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