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# C-GLYCOSYLFLAVONES FROM *YEATESIA VIRIDIFLORA*

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ABSTRACT.—Three known di-C-glycosylflavones—vicenin-2, schaftoside, and isoschaftoside—one known and one new 7-O-glycosyl-6-C-glucosylflavone—saponarin and 7-O-rhamnosylisovitexin, respectively, and a known 6-C-glucosylflavone—isovitexin—were obtained from *Yeatesia viridiflora*. *Yeatesia* is a monotypic genus of the Acanthaceae with uncertain taxonomic affinities to other genera of the family.

As currently recognized, *Yeatesia* is a monotypic genus of the Acanthaceae occurring from east Texas through the Gulf Coastal States to southwestern Georgia and adjacent Florida (1). The generic placement of its single species has been in a state of flux for the past 125 years, yet the generic relationships of *Yeatesia* have remained uncertain (1-5). The genus is now the subject of a detailed taxonomic study (Hilsenbeck, in preparation), and in this connection, we report here the flavonoid constituents of the species. Coupled with flavonoid reports from other genera of the Acanthaceae, such as C-glycosylflavones in *Echolium* (6), the results of this investigation will help to assess the taxonomic affinities and relationships of *Yeatesia* within the family. *Yeatesia viridiflora* (Nees) Small was found to contain six C-glycosylflavones: schaftoside, isoschaftoside, vicenin-2, isovitexin, saponarin, and a compound here newly reported, 7-O-rhamnosylisovitexin.

## RESULTS AND DISCUSSION

Examination of an aqueous methanolic extract of stem and leaf material of *Y. viridiflora* by 2d-pc revealed four to five flavonoid glycosides. Further 2d-pc examination of a small aliquot of the crude extract subjected to prolonged acid hydrolysis revealed that the hydrolysis procedure had failed to produce an aglycone from any of the glycosides, suggesting that all were C-glycosylflavonoids (7,8). All compounds appeared purple when viewed on paper under uv and turned green upon fuming with $NH_3$, both before and after hydrolysis, indicating that all compounds had a free 5-OH group and a free 4′-OH group (9). The individual flavonoids were isolated by repeated one dimensional pc in three solvent systems. The flavonoid-containing bands were cut out and eluted with 50% aqueous MeOH. Identification was based on uv, pmr, and ms (9-11) coupled with two hydrolysis procedures and tlc of any liberated glycosyl moieties (8).

Compound **1** gave the uv spectrum of an apigenin-based compound with a free 7-OH group (9). Prolonged acid hydrolysis failed to liberate any glycoside moieties or to alter significantly the Rf values of **1**. Ms of the permethylated ether of **1** was that of isoschaftoside (10) with $[M]^+$ at *m/z* 704 followed by an intense $[M-15]^+$, *m/z* 689, peak and the base peak at $[M-31]^+$, *m/z* 673. Other diagnostic peaks for isoschaftoside were observed as previously reported (10). Identification was further verified by co-chromotography against a standard marker (see Experimental section).

Compound **2** gave uv spectra nearly identical to those of **1**, and pmr spectral data confirmed the apigenin B-ring (9) and also showed a singlet at δ 6.4, which was assigned as H-3. No signals for H-6 or H-8 were observed indicating substituents at these positions. Two signals characteristic of H-1s of C-linked glycosyls (9) were present, as were signals for the remaining protons for two sugars. The ms of the permethylated ether of **2** differed significantly from those of **1** in the relative intensities of the diagnostic fragments gh $[M-103]^+$, gp $[M-59]^+$, hh $[M-163]^+$, hp $[M-119]^+$, ih $[M-175]^+$, ip $[M-131]^+$, and jp $[M-145]^+$, confirming **2** as schaftoside, according to the results of

Bouillant *et al.* (10). Cochromotography against standards also confirmed the structure of **2**.

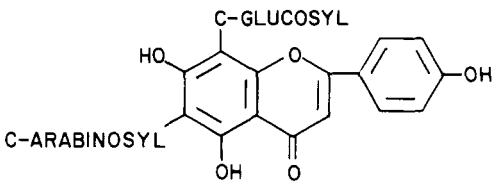
Compound **3** also gave uv spectra of an apigenin-type with a free 7-OH. The pmr again confirmed the apigenin B-ring, exhibiting a singlet for H-3 and no signals for H-6 or H-8. As in **2**, the presence of two C-linked glycosyl H-1s and signals for two sugars were observed in the pmr, indicating C-linked sugars at the 6 and 8 positions. The ms of the permethylated ether of **3** gave the molecular ion, $[M]^+$, at *m/z* 748 with $[M-15]^+$ at *m/z* 733 and the base peak $[M-31]^+$ at *m/z* 717. Other diagnostic peaks as reported by Bouillant *et al.* (10) and cochromatography against standards confirmed the structure of **3** as vicenin-2 (6,8-di-C-glucosylapigenin).

Prior to hydrolysis, **4** gave uv spectra with diagnostic shift reagents (9) of an apigenin-type with a substituted 7 position (7-OR). After prolonged hydrolysis of **4**, the uv spectra showed the 7 position to be free (7-OH), and glucose, vitexin, and isovitexin were detected in the hydrolysate. This suggested that **4** was saponarin. Pmr supported this conclusion, demonstrating an apigenin B-ring pattern, singlets for H-3 and H-8, and additional signals for sugar protons as reported for saponarin (9). Ms of the permethylated ether of **4** confirmed its structure as saponarin, with $[M]^+$ at *m/z* 734 followed by two homologous series of peaks (11): the first series relating to the fragmentation of a permethylated 6-C-glycosylflavone with a base peak at $[M-31]^+$ (*m/z* 703) and the second series relating to the "aglycone" peak AH, with AH at $[M-218]^+$ (*m/z* 516) with a second base peak at $[AH-31]^+$ (*m/z* 485). These diagnostic peaks allowed for assignment of glucose as the O-linked sugar at the 7 position and as the C-linked sugar at the 6 position as well (11).

The new compound, (**5**), had uv spectra nearly identical to those of **4**, indicating an apigenin-type with a 7-OR. Since rhamnose, vitexin, and isovitexin were produced from **5** on prolonged acid hydrolysis and because the uv of the flavonoid mixture in the hydrolysate showed the 7 position now to be free, **5** must be a 7-O-rhamnoside. The high Rf value of **5** (TBA 0.37, 15% HOAc 0.78) suggested that it was the 7-O-rhamnoside of isovitexin, not of vitexin. Pmr confirmed this conclusion, showing an apigenin B-ring, an H-8 singlet at $\delta$ 6.8, an H-3 singlet at $\delta$ 6.4, and other signals consistent with a C-linked sugar at the 6 position (9). The identity of **5** as 7-O-rhamnosylisovitexin was confirmed by the ms of the permethylated ether, which, as in **4**, gave two homologous series of peaks relating to fragmentation of a permethylated 6-C-glycosylflavone and the loss of the O-linked sugar. The molecular ion $[M]^+$ of **5** was at *m/z* 704 with $[M-15]^+$, *m/z* 689, and the first base peak at $[M-31]^+$, *m/z* 673. Fragment AH was at $[M-189]^+$, *m/z* 516, with the second base peak $[AH-31]^+$ at *m/z* 485. Glucose was determined to be the 6-C-linked sugar by comparison of AH with i (AH) or 516-341=175, which indicates glucose as the C-linked sugar at the 6 position of an apigenin residue (11).

Compound **6** was present in only trace amounts but was determined to be isovitexin by Rf values, uv, hydrolysis, and cochromatography.

The presence of these C-glycosylflavones in *Yeatesia* as well as morphological and cytological evidence (Hilsenbeck, in preparation) suggest a relationship between *Yeatesia* and *Echolium*, a genus restricted to the Old World. Nair *et al.* (6) reported the presence of four C-glycosylflavones from *Echolium linneanum*: vitexin, isovitexin, orientin, and isoorientin. Two of these compounds, like all of those found in *Yeatesia*, are apigenin-based C-glycosylflavones. A recent discovery that O-methoxylated C-glycosylflavones are present in the genus *Siphonoglossa*, also of the Acanthaceae (Hilsenbeck and Mabry, in preparation)—a genus not closely associated with *Yeatesia* or *Echolium*—suggests that C-glycosylflavones are more widespread than previously suspected in this relatively advanced family.

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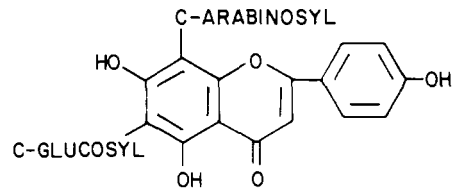


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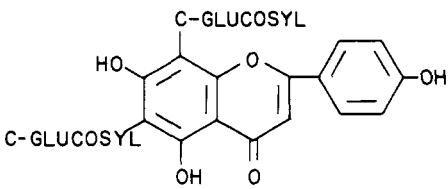


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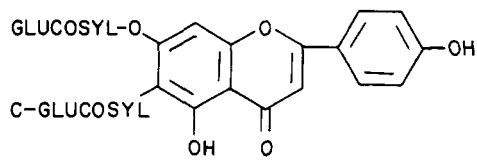


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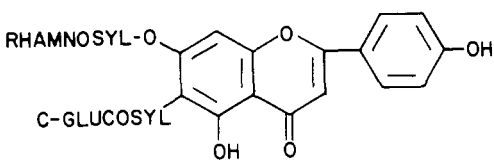


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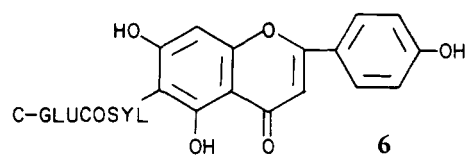


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## EXPERIMENTAL

PLANT MATERIAL.—*Y. viridiflora* was collected by the senior author in a mesic woodland adjacent to and in the flood plain of Cow Creek, off route 190, Newton County, TX. A voucher specimen is on deposit in the Herbarium of The University of Texas at Austin, Plant Resources Center (LL, TEX), Hilsenbeck 733.

EXTRACTION AND ISOLATION.—Ground, air-dried stem and leaf material (106 g) was extracted repeatedly with excess volumes of 85% aqueous MeOH followed by extraction with 50% aqueous MeOH. The combined extracts were concentrated *in vacuo*, and the aqueous syrup was partitioned against $CH_2Cl_2$. The remaining $H_2O$ fraction was streaked on Whatman 3 MM paper, and the papers were developed by one dimensional pc in one of three solvent systems: *t*-BuOH-HOAc-$H_2O$, 3:1:1 (TBA), *n*-BuOH-HOAc-$H_2O$, 4:1:5 (upper phase) (BAW), or 15% HOAc (HOAc). The bands corresponding to flavonoids were cut out and eluted with 50% aqueous MeOH. Final purification of compounds was on a Sephadex LH-20 (Pharmacia) column with 60% aqueous MeOH.

COCHROMATOGRAPHY.—Underivatized compounds were cochromatographed against authentic standards by one dimensional pc in the three solvents listed above. Permethylated compounds were co-

chromatographed against permethylated standards on Si gel plates in $CHCl_3$-EtOAc-$Me_2CO$, 5:4:1 and 5:1:4.

HYDROLYSIS CONDITIONS.—*Mild acid hydrolysis.*—A dry sample was dissolved in 0.1 N TFA; the flask was sealed and placed in a steam bath for 1.5 h.

*Prolonged acid hydrolysis.*—A dry sample was dissolved in 2 N HCl; the flask was sealed and placed in a steam bath for 2.5 h.

SUGAR ANALYSIS.—Sugars were recovered from the hydrolyzed flavonoids after repeated evaporations *in vacuo* of the hydrolysis solution. The residue was taken up in $H_2O$, and the aqueous solution was extracted with EtOAc. Sugars present in the $H_2O$ fraction were identified by tlc on cellulose against standard markers in pyridine-EtOAc-HOAc-$H_2O$ (36:36:7:21). Sugars were detected by spraying developed plates with aniline phthalate reagent (E. Merck).

DERIVATIZATION TECHNIQUES.—*Permethylation.*—This was done as described in reference (12).
*Trimethylsilylation.*—This was done as described in reference (9).

SPECTROSCOPY.—*Uv spectroscopy.*—This was done as described in reference (9).
*Ms.*—This was done by direct insertion of permethylated samples into the ion source, 70 eV, eims.
*Pmr.*—This was done as described in reference (9), spectra recorded at 90 MHz.

## ACKNOWLEDGMENTS

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