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# FLAVONOIDS OF *GAURA TRIANGULATA*

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**Key Word Index**—*Gaura triangulata*; Onagraceae; Onagreae; chemotaxonomy; luteolin; *C*-glycosylflavones; mass spectra.

**Abstract**—Ten flavonoids were obtained from *Gaura triangulata* including luteolin and luteolin 7-glycoside derivatives which are atypical for the family Onagraceae. Apigenin-based *C*-glycosylflavones, previously reported from the Onagraceae in the monogeneric tribe Circaeeae, have also been identified. The flavonoid profile of *G. triangulata* is distinctive for the family since previous studies of the Onagraceae reported primarily flavonols.

## INTRODUCTION

As part of a comprehensive survey of the flavonoids of the Onagraceae, many species and genera have been surveyed for their flavonoid constituents [1–9]. The flavonoid chemistry of the family appears to be relatively homogeneous containing predominantly flavonols, particularly *O*-glycosides or methyl ethers of kaempferol, quercetin and myricetin. Most of the reports are from the tribe Onagreae, particularly *Oenothera*, but screening of additional taxa [5] suggest the occurrence of similar compounds throughout much of the family. *C*-Glycosylflavones have, however, been reported in the genus *Circaea* [5], which is assigned to a monogeneric tribe of the Onagraceae [10]. In contrast to some genera of the Onagraceae, *Gaura* has received relatively little attention [11]. For example, only one species of *Gaura*, *G. coccinea*, has been examined for flavonoids [2], and it was reported to contain only flavonols. In the present study we report the detailed flavonoid chemistry of *Gaura triangulata* Buckl. The flavonoid chemistry of this species is distinctive for the family and is of interest because both luteolin and its glycosides, and *C*-glycosylflavones, both reported for only the second time in the family have been detected.

## RESULTS AND DISCUSSION

Aqueous methanolic extracts of stem and leaf material of *Gaura triangulata* yielded a complex mixture of flavonoid glycosides and one aglycone. All compounds appeared purple on paper under UV light indicating that a free 5-hydroxyl group was present in each. Five of the compounds turned yellow upon fuming with ammonia under UV light indicating possible free 3′,4′-dihydroxyl groups, while the other five appeared green indicating a free 4′-hydroxyl [12]. Examination by two dimensional paper chromatography of the hydrolysate, obtained by prolonged acid hydrolysis of a small aliquot of the crude extract, revealed a total of seven compounds, four appeared to be aglycones, while three appeared to still be glycosides, i.e. *C*-glycosylflavonoids [13]. Flavonoids were isolated from the original extract by repeated one dimensional paper chromatography in two solvent systems. Subsequent analysis of the *O*-glycosides was by conventional UV and $^1$H NMR spectroscopy [12] coupled with mild acid hydrolysis [13]. For *C*-glycosylflavones, mass spectra of the permethyl ethers were recorded as well.

Following these methods, it was determined that the aglycone present in the original extract from 95 g of leaf and stem material was luteolin (15 mg). In addition, three 7-*O*-glycosides of luteolin were identified: luteolin 7-glucoside (28 mg), luteolin 7-galactoside (16 mg) and luteolin 7-rhamnoside (21 mg). Apigenin 7-glucoside was also present in small amounts (3 mg). In addition to these flavones, 3 mg of another flavone glucoside were obtained but it was not possible to fully characterize it with the amount of material available. The compound appeared purple on paper under UV light turning green, both before and after hydrolysis, upon fuming with ammonia indicating only a 4′-hydroxyl in the B-ring. The UV spectrum in methanol was of an apigenin-like compound (Band I at 332 nm) but with Band I of lower intensity than Band II, indicating the possible presence of tri-oxygenation in the A-ring. Glucose was liberated during the hydrolysis. Mass spectrometry of the PM ether of the glucoside gave an unusual spectrum with the molecular ion, $[M]^+$, at $m/z$ 546 (100%), with a second major peak $[M-218]^+$ at $m/z$ 328 (98%) indicating loss of a PM *O*-linked glucose [14]. The compound present in the highest concentration was a flavonol, quercetin 3-rhamnoglucosyl (rutin, 102 mg) which has previously been found in several taxa in the family [3, 7] including *Gaura coccinea* [2].

The identification of the three *C*-glycosylflavones was confirmed using the same standard methods as for the *O*-glycosides but with the additional data derived from mass spectra of their permethyl ethers [15]. The *C*-glycosylflavone in highest concentration (23 mg) gave the mass spectrum of a PM 6,8-di-*C*-glucosylapigenin (vicenin-2) with $[M]^+$ at $m/z$ 748 followed by $[M-15]^+$, $m/z$ 733, and the base peak $[M-31]^+$ at $m/z$ 717. Other diagnostic fragments were observed as previously reported [15]. $R_f$ values and hydrolysis data (no $R_f$ change after hydrolysis and no hydrolysable sugars) are consistent with this

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structure as vicenin-2. $^1$H NMR data of the TMSi ether of this compound demonstrated the presence of an apigenin B-ring pattern and the presence of an H-3 proton. Signals for H-6 and H-8 were not observed indicating substituents at these positions. Signals characteristic for two *C*-glycosyl H-1's ($\delta$4.4–4.8) [12] were observed as were the remaining sugar protons between 3.85 and 3.2, confirming the other structural evidence. The two other *C*-glycosylflavones (14 mg and 11 mg) gave the mass spectra of PM schaftoside and isoschaftoside, respectively [15] both giving $[M]^+$ at *m/z* 704 followed by intense peaks of $[M-15]^+$, *m/z* 689, and the base peak of $[M-31]^+$ at *m/z* 673. Other diagnostic peaks were observed in accord with the scheme of Bouillant *et al.* [15]. The PM ethers of these compounds were co-chromatographed on silica gel plates against PM standard markers in chloroform–ethyl acetate–acetone (5:4:1 and 5:1:4), confirming their identity.

The presence of luteolin and luteolin 7-glycosides may indicate an advanced position of *Gaura* relative to the other genera of the Onagreae since flavones are considered to be characteristic of advanced or derived taxa [16] as compared to taxa containing flavonols (i.e. the other genera of the Onagreae examined to date). The presence of luteolin in *G. triangulata* may derive from the evolutionary history of the species, which is apparently a complex structural heterozygote that originated following hybridization between *G. suffulta* subsp. *suffulta* and *G. brachycarpa* [11]. However, these two species have fewer compounds (by 2D-PC) than *G. triangulata*; thus, this may represent an example of the hybrid elaborating compounds not found in either parent. The detailed examination of the flavonoid chemistry of these two putative parents, aimed at documenting the hybrid origin of *G. triangulata*, is currently in progress [R. A. Hilsenbeck and D. A. Levin, in preparation].

Several flavones, including unusual glycosides of luteolin, one of them new to nature, were recently reported by Williams *et al.* [17] from *Fuchsia* species and their hybrids. Their finding is the first report of flavone *O*-glycosides from the Onagraceae. They suggest that flavonoid chemistry is potentially useful for documenting the origin of hybrids in *Fuchsia*, as it is proving to be in *Gaura*. Published accounts of *C*-glycosylflavones from the Onagraceae are only from the genus *Circaea* in the monogeneric tribe Circaeeae, a very distinctive tribe, having no clear relationship to any other group within the family. The presence of apigenin-based *C*-glycosylflavones in *G. triangulata* may be indicative of a distant relationship between *Gaura*, in the Onagreae, and *Circaea*. However, because of the findings of Williams *et al.* [17] and those of Averett and Raven [9], who state that they have unpublished results of *C*-glycosylflavones from three other tribes of the family, it appears that more detailed flavonoid studies need to be completed before any definitive tribal relationships can be postulated based on flavonoid data.

## EXPERIMENTAL

*Plant material. Gaura triangulata* Buckl. was collected by Don A. Levin. A voucher specimen is deposited in the Herbarium of the University of Texas at Austin, Plant Resources Center (LL, TEX), D. A. Levin.

*Extraction and isolation.* Ground air-dried stem and leaf material (95 g) was extracted repeatedly with an excess vol. of 85% MeOH, followed by extraction with 50% MeOH. Extracts were combined and concd *in vacuo* to a thick syrup (*ca* 18 g). The crude extract was streaked on Whatman 3 MM paper developed by 1D-PC, first in *t*-BuOH–HOAc–$H_2O$, 3:1:1 (TBA), with the bands corresponding to flavonoids being cut out and eluted with 50% MeOH. The fractions were then concd and streaked on additional papers developed by 1D-PC in 15% HOAc (HOAc). The same procedure of cutting and eluting as described above was followed. Final purification of the compounds was on a Sephadex LH-20 (Pharmacia) column with 60% MeOH.

*Hydrolysis conditions.* (a) 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 hr. (b) 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 hr.

*Sugar analysis.* Sugars were recovered from the hydrolysed flavonoids after repeated evaporation *in vacuo* of the hydrolysis soln. The residue was taken up in $H_2O$ and the aq. soln 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.

*Derivatization techniques.* (a) Trimethylsilation: as described in ref. [12]. (b) Permethylation: as described in ref. [18].

*Spectroscopy.* (a) UV spectroscopy: as described in ref. [12]. (b) MS: by direct insertion of PM samples into the ion source, 70 eV, EIMS. (c) $^1$H NMR spectroscopy: as described in ref. [12], spectra recorded at 90 MHz.

*Data for the unknown flavone glucoside.* PC before hydrolysis: $R_f$ 0.71 (TBA), 0.43 (HOAc); PC after hydrolysis: 0.88 (TBA), 0.12 (HOAc). UV $\lambda_{max}^{MeOH}$ nm: 260 sh, 272, 332; + NaOMe 273, 395; + $AlCl_3$ 273, 300 sh, 355 sh, 397; + $AlCl_3$ + HCl 274, 298 sh, 355, 385. Permethyl ether: EIMS, 70 eV, *m/z* (rel. int.): 546 $[M]^+$ (100), 328 $[M-218]^+$ (98), 299 $[M-247]^+$ (57), 282 (14), 255 (12), 218 (51), 187 (69), 155 (50), 145 (28), 143 (28), 127 (46).

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