# External Flavonoids of Three Species of *Viguiera*, Section *Hypargyrea* (Asteraceae)

Eckhard Wollenweber, Marion Dörr[a], James N. Roitman[b] and Edward Schilling[c]

[a] Institut für Botanik der Technischen Hochschule, Schnittspahnstrasse 3, D-64287 Darmstadt, Bundesrepublik Deutschland
[b] Western Regional Research Center, USDA-ARS, 800 Buchanan St., Albany, CA 94710, U. S. A.
[c] University of Tennessee, Department of Botany, 437 Hesler Biology Building, Knoxville, TN 37996–1100, U. S. A.

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The flavonoid aglycones excreted by aerial parts of three *Viguiera* species have been analyzed. One of them is a rare natural product. So far unpublished NMR data are reported for three flavones. Each species of *V.* section *Hypargyrea* exhibits a distinctive flavonoid profile based on different patterns of methoxylation, but the taxonomic section as a whole is not characaterized by any distinctive characters of its flavonoid chemistry.

## Introduction

In previous papers one of us has reported on external flavonoids found in various *Viguiera* species, belonging to the section *Maculatae* (Schilling *et al.*, 1988), to the series *Brevifoliae* (Schilling and Panero, 1988) and to the subgenus *Bahiopsis* (series *Viguiera*; Schilling, 1989). Flavonoid aglycones were found to be associated with the occurrence of small sessile or subsessile glandular trichomes sometimes called resin dots. In the present study the external flavonoids of the three species forming the section *Hypargyrea* have been analyzed.

## Materials and Methods

Leaves were collected and air-dried from plants at anthesis of each of the three species of *Viguiera* section Hypargyrea. Plants of *V. decurrens* were grown in a garden in Knoxville, Tennessee, from seed collected originally in Chihuahua, Mexico (Bye & Linares 14330). Material of *V. hypargyrea* was collected in Durango, Mexico (Schilling & Panero 88–12) and of *V. rosei* from two different sites in Aguascalientes, Mexico (Schilling & Panero, 88–18, 88–20). Vouchers have been deposited at the University of Tennessee Herbarium (TENN). The amounts of dry leaf material used in this study varied between 28 and 50 g. Dried leaves were briefly rinsed with acetone to dissolve externally accumulated lipophilic material. The concentrated exudate was defatted (MeOH, -10°) and passed over Sephadex LH-20, eluted with MeOH, to separate the flavonoids from the dominating terpenoids. In *V. decurrens* the flavonoids were identified by direct comparison with markers. In *V. decurrens* and in *V. rosei* the flavonoid fraction was subject to preparative TLC on silica, developed with toluene – dioxane – HOAc 18:5:1. The eluates were checked for purity and compared with markers by TLC on silica gel with the same solvent or with toluene – MeCOEt 9:1 and on polyamide DC-11 with toluene – dioxane – MeOH 8:1:1. In some cases we also used toluene-petrol$_{100-140°}$-MeCOEt-MeOH, 12:6:2:1; toluene-MeCOEt-MeOH 12:5:3, respectively. Chromatograms were viewed under UV before and after spraying with "Naturstoffreagenz A". Terpenoids were visualized by spraying silica plates with $MnCl_2$ reagent, followed by heating (Jork *et al.*, 1989). Flavonoid aglycones were identified by direct comparison with markers and/or by their spectral data. NMR spectra were recorded in DMSO-$d_6$ at 400 MHz ($^1$H) and 100 MHz ($^{13}$C) on a Bruker ARX-400 instrument. Melting points were not measured, due to the paucity of products.

To the best of our knowledge the $^{13}$C NMR spectra of onopordin, nevadensin and hymenoxin have not been published. We therefore report these data in Table I, along with those of sudachitin, for the sake of completeness and for comparison.

Onopordin (**1**). MS: *m/z* (rel. int.) 316 (54%, $M^+$, $C_{16}H_{12}O_7$), 301 (100), 273 (17), 167 (20), 139 (54), 135 (61). $^1$H-NMR: δ (ppm) 12.64(s, OH-5), 7.44 (m, H-2′/H-6′), 6.91 (d, $J$ = 8 Hz, H-5′), 6.68 (s, H-3), 6.27 (s, H-6), 3.85 (s, OMe).

Reprint requests to E. Wollenweber.
Telefax: 06151/166878.

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Table I. $^{13}C$ NMR spectra of the flavones **1–4** from *Viguiera*.

| Carbon No. | Onopordin (**1**) | Nevadensin (**2**) | Sudachitin (**3**) | Hymenoxin (**4**) |
|---|---|---|---|---|
| 2 | 163.7 | 162.9 | 163.4 | 163.0 |
| 3 | 102.6 | 103.0 | 102.7 | 103.3 |
| 4 | 181.7 | 182.1 | 182.3 | 182.3 |
| 5 | 156.1* | 148.4 | 148.0* | 148.3 |
| 6 | 98.9 | 131.8 | 131.5 | 131.6 |
| 7 | 157.1* | [a] | 150.8§ | 151.1 |
| 8 | 127.7 | 128.2 | 127.9 | 128.0 |
| 9 | 149.4§ | 145.5 | 145.3 | 145.5 |
| 10 | 103.4 | 102.5 | 102.9 | 102.8 |
| 1′ | 121.5 | 123.1 | 121.6 | 123.0 |
| 2′ | 113.2 | 128.1 | 110.0 | 109.2 |
| 3′ | 145.7 | 114.8 | 148.3* | 149.0 |
| 4′ | 149.8§ | 162.3 | 150.7§ | 152.1 |
| 5′ | 116.1 | 114.8 | 115.9 | 111.9 |
| 6′ | 118.8 | 128.1 | 120.1 | 119.8 |
| 6-OMe | – | 60.1 | 60.1 | 60.1 |
| 8-OMe | 61.0 | 61.1 | 61.1 | 61.1 |
| 3′-OMe | – | – | 55.8 | 55.7 |
| 4′-OMe | – | 55.6 | – | 55.7 |

[a] not detected; * and § values in any column may be interchanged.

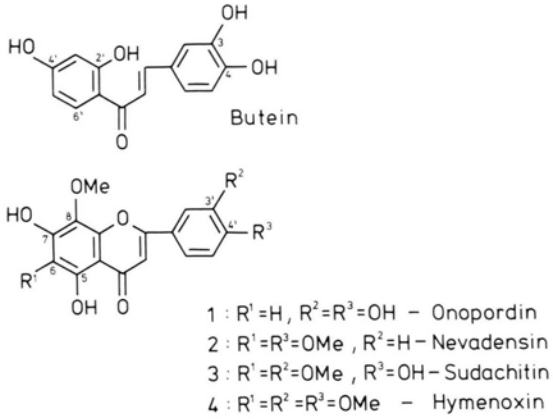


Fig. 1. Flavonoid aglycones from *Viguiera*.

Nevadensin (**2**). MS: *m/z* (rel. int.) 344 (68%, $M^+$; $C_{18}H_{16}O_7$). $^1$H-NMR: δ (ppm) 12.74 (s, OH-5), 8.02 (d, *J* = 9 Hz, H-2′/H-6′), 7.15 (d, *J* = 9 Hz, H-3′/H-5′), 6.84 (s, H-3), 3.86, 3.86, 3.76 (s, 3 x OMe).

Sudachitin (**3**). $^1$H-NMR: δ (ppm) 12.82 (s, OH-5), 7.58 (m, H-2′/H-6′), 6.98 (d, *J* = 8 Hz, H-5′), 6.94 (s, H-3), 3.89, 3.88, 3.80 (s, 3 x OMe).

Hymenoxin (**4**). MS: *m/z* (rel. int.) 374 (74%, $M^+$, $C_{19}H_{18}O_8$), 359 (100), 197 (14), 169 (20). $^1$H-NMR: δ (ppm) 12.76 (s, OH-5), 7.68 (dd, J = 2,8 Hz, H-6′), 7.57 (d, *J* = 2 Hz, H-2′), 7.18 (d, *J* = 8 Hz, H-5′), 6.98 (s, H-3), 3.89, 3.89, 3.86, 3.79 (s, 4 x OMe).

## Results and Discussion

The lipophilic material obtained by rinsing dry leaves of three species of *Viguiera* was found to contain a series of flavonoid aglycones. *V. decurrens* A. Gray exhibits four flavonoids, one of which was readily identified as the trivial flavone luteolin. MS and NMR spectral studies allowed the identification of the second flavone (compound **1**) as onopordin (5,7,3′,4′-tetrahydroxy-8-methoxy flavone = hypolaetin-8-methyl ether). Two further compounds remain unidentified, due to the paucity of material. – In the exudate of *V. hypargyrea* Greenman we also detected four flavonoids. Three of these were unambiguously identified as luteolin, 6-methoxyluteolin (nepetin), and scutellarein-6-methyl ether (hispidulin). – In *V. rosei* Greenman nepetin and butein (2′,4′,3,4-tetrahydroxy chalcone) were identified by direct comparison with markers, while nevadensin (compd. **2**: 5,7-dihydroxy-6,8,4′-trimethoxy flavone), sudachitin (compd. **3**: 5,7,4′-trihydroxy-6,8,3′-trimethoxy flavone) and hymenoxin (compd. **4**: 5,7-dihydroxy-6,8,3′,4′-tetramethoxy flavone) were identified by their MS and NMR spectra.

The three species of *Viguiera* section *Hypargyrea* each exhibit distinctive flavonoid profiles characterized by different patterns of methoxylation. The 6-methoxylated type observed in *V. hypargyrea* is widespread in *Viguiera* and in subtribe Helianthinae. The 6,8,3′ and 6,8,4′ patterns of methoxylation characteristic of *V. rosei* are less common, and have been reported previously for *V.* sect. *Brevifoliae* and for *Helianthus*. The 8-methoxylated type in *V. decurrens* is a distinctive pattern that has not previously been reported for substribe Helianthinae. Flavonoid data would suggest a possible progression from *V. hypargyrea*, which exhibits a profile with generalized types of compounds, to *V. rosei* and *V. decurrens*, each of which exhibits a distinctive compound type. This is consistent with morphology, where *V. rosei* and *V. decurrens* share a distinctive type of large, coarse, ovate, and hispid leaf, in contrast to the smaller, lanceolate, pilose leaves of *V. hypargyrea*.

As a result of their distinctiveness at the species level, the species of *V.* section *Hypargyrea* do not collectively exhibit a common flavonoid profile that would characterize the section or clarify its phylogenetic placement. This is consistent with previous reports of flavonoid chemistry for *Viguiera*. For example, there is notable diversity within *V.* subgenus *Bahiopsis* (series *Viguiera*; Schilling 1989), *V.* section *Maculatae* (Schilling *et al.*, 1988) and *V.* series *Brevifoliae* (Schilling and Panero, 1988), as well as in *Tithonia* (La Duke, 1982), which may be phylogenetically close to *V.* section *Hypargyrea*. Each of these groups, including *V.* section *Hypargyrea*, has been suggested by molecular studies to be clearly monophyletic (Schilling and Panero, in press). Thus, flavonoid information appears to be most useful systematically in subtribe Helianthinae in characterizing and distinguishing individual species.

Onopordin (**1**) is a rather rare flavone. First isolated from *Onopordum acanthium*, it was later found in dikamali gum, in aerial parts of *Doronicum grandiflorum*, in the leaf exudate of *Wilkesia hobdyi* (c.f. Wollenweber, 1993) and recently in leaf and stem of *Centaurea chilensis* (Sepulveda *et al.*, 1994). It is thus far known only from Asteraceae. The chalcone butein has been encountered here for the first time in E. W.'s studies on exudate flavonoids (c.f. Wollenweber, 1993). It is known to occur in several Fabaceae, but rarely in Asteraceae. It is a distinctive compound for the subtribe Helianthinae where it usually occurs in glycosidic form in floral tissues (Crawford and Stuessy, 1981) and has only rarely been reported from extracts of foliar glands (Schilling and Panero, 1988). Nevadensin (**2**), on the other hand, has been found mostly in Asteraceae, including *Viguiera greggii* and *V. bicolor* (c.f. Wollenweber, 1993). Sudachitin (**3**) shows no preference for any plant family. Hymenoxin (**4**) is known from Asteracea, including *Viguiera greggii,* and from a Scrophulariacea (c.f. Wollenweber, 1993).

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