Exudate Flavonoids of Some *Salvia* and a *Trichostema* Species

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Salvia spp., *Trichostema lanatum,* Lamiaceae, Leaf and Stem Exudate, Flavonoid Aglycones

Seven species of *Salvia* and one species of *Trichostema* were analyzed for their exudate flavonoids. They were found to exhibit flavonoid aglycones in various numbers and different patterns. Most of these are flavones, in which 6-methoxy-substituted compounds predominate.

Introduction

It is now well known that exudate flavonoids occur in both subfamilies of the Lamiaceae, in subfamily Lamioideae as well as in subfamily Nepetoideae [1]. Within the large genus *Salvia,* they are present especially in species producing a resinous excretion or exhibiting an aromatic scent. The first species to be analyzed for externally accumulated flavonoid aglycones, *Salvia glutinosa* (sticky sage), was found to produce a series of more or less lipophilic methylated flavones and flavonols [1, 2]. In addition to the previously studied *Salvias* [1, 3] we have now analyzed seven further species, plus one species of the closely related genus *Trichostema.*

Materials and Methods

Plant material was collected at the following localities:

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Salvia columbariae Benth.: a) San Diego Co., CA, Campo exit (State Highway 94) off Interstate 8 (G. Yatskievych & E. Wollenweber 90-27; April 30, 1990); b) San Diego Co., CA, 1.8 road miles NE of State Highway 79 and 5.0 road miles S of Indian Flats Campground (G. Yatskievych & E. Wollenweber 90-42; May 1, 1990); c) Riverside Co., CA, along State Highway 74, *ca.* 2 mi E of Cleveland National Forest Boundary (G. Yatskievych & E. Wollenweber 90-54; May 2, 1990).

Salvia compressa Vent.: Province Khousestan, between Khoramabad and Andimeshk (A. Rustaiyan 08-266; June 1990).

Salvia dorrii (Kellogg) Abrams ssp. *dorrii:* San Bernardino Co., CA, along State Highway 18 at Forest Road 3 NO 3 junction (G. Yatskievych & E. Wollenweber 90-55; May 2, 1990).

Salvia hypoleuca Bth.: Elbruz mountains, 60 km north of Teheran (A. Rustaiyan 12-267; July 1989).

Salvia macrosiphon Boiss.: Province Kerman, between Saidabad and Tchah-Tchoghok (A. Rustaiyan 19-268; June 1990).

Salvia mirzayani Rech.: Province Baloutchestan, 10 km north of Saravan (A. Rustaiyan 20-268; June 1990).

Salvia stenophylla (Burch. ex) Benth.: Cultivated from seeds collected in the Tarkastad area of the Eastern Cape South Africa on the experiment farm of the University of Fort Hare in Alice, Ciskei.

Trichostema lanatum Benth.: San Diego Co., CA, near Campo exit (State Highway 94) off Interstate 8 (G. Yatskievych & E. Wollenweber 90-28; April 30, 1990).

Vouchers are kept in the Missouri Botanical Garden Herbarium (MO; Californian species) and in the Herbarium of the Department of Botany at Shahid Beheshty University in Teheran (Persian species).

Air-dried aerial parts were rinsed with acetone to dissolve the exudate material. The three samples of *S. columbariae* proved to be identical on thin-layer chromatographic comparison and were, therefore, combined. The resinous or sometimes solid residue obtained from each species was dissolved in boiling methanol and after cooling to room temperature kept in the freezer at −15 °C for 15 min. The precipitated fatty and waxy material was eliminated by centrifugation and the supernatant solution was

then passed over Sephadex LH-20. Elution with methanol separated the flavonoids from the bulk of terpenoids; fractions with similar flavonoid content were combined. All flavonoids except one were identified by comparison with markers. TLC was on polyamide DC-11 with the following solvents: A, petrol$_{100-140}$–toluene–MeCOEt–MeOH 12:6:6:1; B, toluene–petrol$_{100-140}$–MeCOEt–MeOH 12:6:2:1; C, toluene–dioxane–MeOH 8:1:1; D, toluene–MeCOEt–MeOH 12:5:3; on silica with solvents E, toluene–MeCOEt 9:1 and F, toluene–dioxane–HOAc 18:5:1. Chromatograms were viewed in UV_{366} before and after spraying with Naturstoffreagenz A. Markers were available from E. W.'s lab. Authentic scutellarein-7,4′-dimethyl ether isolated from *Pulicaria paludosa* was kindly provided by Prof. A. San Feliciano [4].

Mass spectra were recorded on a Varian MAT 311 at 70 eV by direct inlet. ^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Nicolet NT-WB 200 FT spectrometer at 200 and at 50 MHz, respectively.

Compound **1**, isolated from *Salvia hypoleuca*, formed fine yellow needles, m.p. 187–189 °C and had the following spectral properties: UV λ MeOH (nm): 334, 287; +$AlCl_3$ 360, 302, unchanged with HCl; +NaOH 315sh, 300sh; MS *m/z* (rel. int. %): 314 (M^+, 100), 296 (43), 285 (12), 268 (64). ^1H NMR δ (ppm): 12.62 (s, 5-OH); 8.74 (br. s, OH); 8.08 (d, J = 9 Hz; H-2′/H-6′); 7.13 (d, J = 9 Hz; H-3′/H-5′); 6.95 (s, H-6 or H-3); 6.91 (s, H-3 or H-6); 3.93 (s, OMe); 3.87 (s, OMe). ^{13}C NMR δ (ppm): 162.3* (C-2); 103.1 (C-3); 182.2 (C-4); 146.2 (C-5); 130.0 (C-6); 154.4 (C-7); 91.2 (C-8); 149.7 (C-9); 105.1 (C-10); 123.0 (C-1′); 128.2 (C-2′/C-6′); 114.6 (C-3′/C-5′); 163.3* (C-4′); 56.3 (OMe); 55.6 (OMe).

Results and Discussion

The exudate of *Salvia columbariae* contained scutellarein-6,7-dimethyl ether, scutellarein-6,7,4′-trimethyl ether, a small amount of 6-hydroxyluteolin-6,7-dimethyl ether and traces each of the 5,6-dimethyl ethers of 6-hydroxygalangin and of 6-hydroxykaempferol. The two latter products appear as brilliant yellow spots in UV_{366}, otherwise the minute amounts present would probably have escaped notice.

In *Salvia compressa*, only trace amounts of apigenin and of quercetin-3-methyl ether were identified. The predominant phenolic components of the exudate seemed to be rather polar caffeic acid derivatives, with TLC properties similar to those of chlorogenic acid and they have not yet been analyzed.

Salvia dorrii contained apigenin, apigenin-7-methyl ether, apigenin-7,4′-dimethyl ether, luteolin, luteolin-3′-methyl ether, and 6-hydroxyluteolin-6,7-dimethyl ether as minor constituents; the major constituents were scutellarein-6,7-dimethyl ether and scutellarein-6,7,4′-trimethyl ether. Small amounts of kaempferol and quercetin were also observed. In addition, the exudate contained unidentified phenolic products.

Salvia hypoleuca yielded scutellarein-7,4′-dimethyl ether (compound **1**) and scutellarein-6,7,4′-trimethyl ether as crystalline products. Its exudate contained, in addition, scutellarein-6,4′-dimethyl ether and small amounts of apigenin, apigenin-7,4′-dimethyl ether, luteolin, luteolin-7-methyl ether, and 6-hydroxyluteolin-6,7-dimethyl ether. In our previous work scutellarein-7,4′-dimethyl ether had never been isolated and was treated as an unknown. It was identified unambiguously by its spectral properties, in particular by NMR, and its identity was later confirmed by direct comparison with an authentic sample [4]. Although the m.p. of our product was lower than that reported in the literature, the identity of the material is certain.

The exudate of *Salvia macrosiphon* contained apigenin, apigenin-7-methyl ether, apigenin-7,4′-dimethyl ether, scutellarein-6,7-dimethyl ether, scutellarein-6,7,4′-trimethyl ether, and the 6,7-dimethyl, 6,7,4′-trimethyl and 6,7,3′,4′-tetramethyl ethers of 6-hydroxyluteolin. The 6,7,4′-trimethyl ether was obtained in crystalline form.

From *Salvia mirzayana*, we identified apigenin, scutellarein-6,7-dimethyl ether, scutellarein-6,7,4′-trimethyl ether, luteolin-3′-methyl ether, and the 6,7,4′-trimethyl and 6,7,3′,4′-tetramethyl ethers of 6-hydroxyluteolin. In this case, scutellarein-6,7-dimethyl ether and 6-hydroxyluteolin-6,7,4′-trimethyl ether were obtained in crystalline form.

Salvia stenophylla accumulates apigenin, apigenin-7-methyl ether, scutellarein-7,4′-dimethyl ether (obtained in crystalline form), luteolin, and 6-hydroxyluteolin-6,7-dimethyl ether. The major constituent of the exudate was an unidentified

* Interchangeable signals.

polar phenolic product (M^+ at m/z 286). Furthermore, *Salvia stenophylla* exhibited a minor nonpolar constituent also observed in *S. dorrii*.

The lipophilic exudate of *Trichostema lanatum* contained the flavones apigenin, apigenin-7,4′-dimethyl ether, scutellarein-6,7-dimethyl ether and scutellarein-6,7,4′-trimethyl ether, luteolin, the 6,7-dimethyl and the 6,7,4′-trimethyl ethers of 6-hydroxyluteolin. Moreover it contains the flavonol galangin and traces of the 5,6-dimethyl ethers of 6-hydroxygalangin and of 6-hydroxykaempferol.

To the best of our knowledge, none of these species has been previously analyzed for flavonoid aglycones. The flavonoid aglycones of 17 species of *Salvia* have been reported in 12 papers published between 1983 and 1990. The predominant products found were methyl derivatives of apigenin, scutellarein, luteolin and 6-hydroxyluteolin. Only *Salvia glutinosa* and *Salvia pedicellata* produced flavonol aglycones (14 in *S. glutinosa*, 3 in *S. pedicellata*). Two flavanones, pinostrobin and isosakuranetin, were reported for one species each; we are not aware of any reports of chalcones in *Salvia*. The species that we have now examined agree well with what is already known, since most of them produce the same type of flavones; *i.e.*, 6-methoxy flavones predominate, whereas flavonols are seldom present. The occurrence of two rare flavonols, namely 6-hydroxygalangin-5,6-dimethyl ether and 6-hydroxykaempferol-5,6-dimethyl ether in both *Salvia columbariae* and *Trichostema lanatum*, and the presence of galangin in the latter is noteworthy. In summary our results corroborate the earlier generalization that 5,7-dihydroxy-6-methoxyflavones with various B-ring substitution patterns are characteristic of the genus *Salvia* [1].

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