Exudate Flavonoids of Eight Species of *Ceanothus* (Rhamnaceae)

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Leaf glands of *Ceanothus* species excrete a lipophilic material that contains a variety of flavonoids. Most of these are aglycones, but some glycosides were also observed. Seven out of eight species exhibit flavonols, whereas flavones are excreted by only one species. Four species produce flavanones and dihydroflavonols; one excretes a remarkable quantity of flavonol glycosides. The exudate flavonoids thus form different patterns that might be characteristic for different *Ceanothus* species.

Key words: Ceanothus, Rhamnaceae, Exudates, Flavonoids

Introduction

Many species of the Rhamnaceae genus *Ceanothus* bear multicellular glandular trichomes on their leaves, in particular along the margins. Fig. 1 shows these glands on a leaf of *C. papillosus* and the typical shape of an individual *C. hearstiorum* gland, both observed with a scanning electron microscope. These glands produce a more or less lipophilic exudate of complex composition, which raised our interest. Several species were, therefore, analyzed for the flavonoids present in this externally accumulated resinous material.

The genus *Ceanothus* (Rhamnaceae) contains about 60 species. These are deciduous or evergreen shrubs or rarely small trees that are sometimes spiny. They occur chiefly in the Pacific coast region of North America from southwestern Canada to northern Mexico. Several species are cultivated as ornamentals, and are available from local nurseries. Almost all species in the genus can hybridize with others; thus *C.* × *veitchianus* Hook. is a hybrid between *C. griseus* (Trel.) McMinn., and *C. rigidus* Nutt. *C. dentatus* Torr. & A. Gray, *C. foliosus* Parry, *C. hearstiorum* Hoover & Roof, *C. impressus* Trel., *C. oliganthus* Nutt. ex Torr. & A. Gray, and *C. papillosus* var. *roweanus* Torr. &

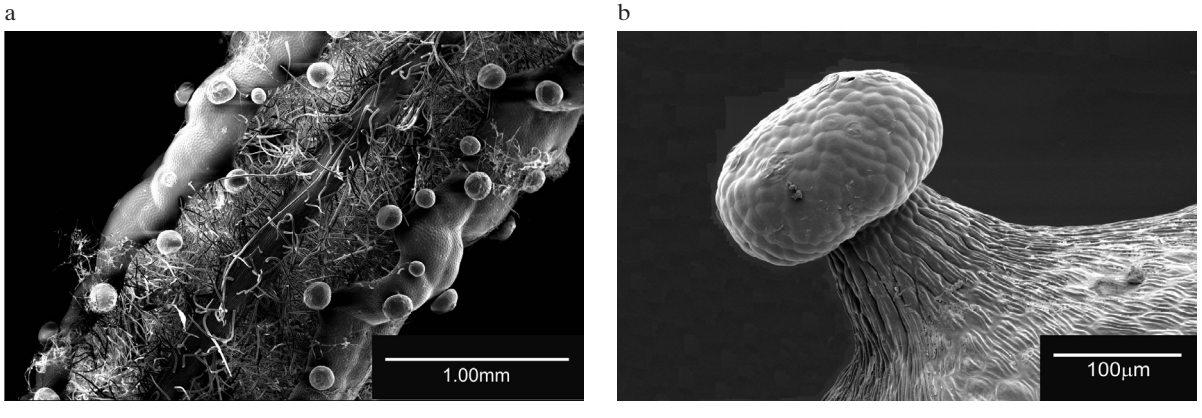

Fig. 1: SEM photos, showing a) multicellular glands on a leaf of *Ceanothus papillosus*, and b) a glandular trichome of *Ceanothus hearstiorum*.

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A. Gray are California natives. Among these, *C. hearstiorum* is an extremely rare species, known from less than 10 occurrences in San Luis Obispo County, CA. *C. velutinus* Douglas, on the other hand, covers a wide range from Colorado and Californian to British Columbia. In addition to these eight species that all exhibited exudate flavonoids, we also examined *C. americanus* L., *C. fendleri* A. Gray, and *C. thyrsiflorus* Eschsch.

Material and Methods

Plant material

Ceanothus dentatus and *C. foliosus* were cultivated as ornamentals in a private garden in Berkeley, CA. They were identified by Dr. John Strother, University of California Herbarium, Berkeley, CA. *C. hearstiorum, C. impressus, C. oliganthus* and *C. papillosus* var. *roweanus* were collected in the Tilden Park Botanic Garden of the East Bay Regional Parks District on June 18, 2003. *C. americanus, C. fendleri, C. thyrsiflorus* and *C.* × *veitchianus* were cultivated in the Botanischer Garten der Technischen Universität Darmstadt. *C. velutinus* Hook. var. *hookeri* Johnston was field-collected in June, 2001 by B. A. Bohm and L. R. Bohm south of Goldbridge, B.C., Canada.

Extraction and isolation

Leafy twigs of flowering plants were briefly rinsed with acetone to dissolve the exudate flavonoids. The solutions were evaporated to dryness, dissolved in MeOH, passed over Sephadex LH-20 and eluted with MeOH to separate the flavonoids from the prevailing terpenoids. Flavonoids from *Ceanothus dentatus* and *C. foliosus* exudates were purified to homogeneity by centrifugal preparative thin layer chromatography (Chromatotron; Harrison Research, Palo Alto, CA) with mixtures of chloroform and methanol. Their identities were established by ^{1}H and ^{13}C NMR. *C. velutinus* and *C.* × *veitchianus* flavonoids were purified as described recently, using standard procedures (Valant-Vetschera and Wollenweber, 2001). Individual compounds were identified by co-chromatography with markers available in E. W.'s lab, and in part by UV spectra. In some cases, MS data further supported the proposed structures. A trimethyl ether of tricetin was isolated by centrifugal preparative thin layer chromatography (Chromatotron, benzene/2-propanol 9:1 v/v) from a fraction of *C. velutinus* exudate. Its identity as the 7,3′,5′-trimethyl ether (7-Me-tricin) was determined from its NMR spectra and by comparison with spectra of genkwanin (Markham and Chari, 1982) and 5,7,4′-trihydroxy-6,3′,5′-trimethoxyflavone (available in J. N. R.'s lab). ^{1}H NMR (400 MHz, d_6-DMSO): δ = 7.05 (s, H-3), 6.38 (d, J = 2.2 Hz, H-6), 6.85 (d, J = 2.2 Hz, H-8), 7.37 (2H, s, H-2′ and H-6′), 12.96 (s, 5-OH), 9.32 (s, 4′-OH), 3.89 (s, 7-OMe), 3.90 (6H, s, 3′-OMe and 5′-OMe). – ^{13}C NMR (100 MHz, d_6-DMSO): δ = 163.9 (C-2), 103.7 (C-3), 181.9 (C-4), 161.1 (C-5), 98.0 (C-6), 165.1 (C7), 92.7 (C-8), 157.2 (C-9), 104.7 (C-10), 120.2 (C-1′), 104.5 (C-2′ and C-6′), 148.2 (C-3′ and C-5′), 140.0 (C4′), 56.0 (7-OMe), 56.4 (3′-OMe and 5′-OMe).

Results and Discussion

From eight species of *Ceanothus* that were found to accumulate flavonoids externally, we identified a series of aglycones of flavonols, flavones and flavanones as well as several flavonol glycosides. The exudate flavonoid profiles of these species are presented in Table I. Most of the flavonols and flavones encountered here are generally widespread compounds. Tricetin trimethyl ethers, however, are rather rare flavones that deserve some attention. Tricetin-7,3′,4′-trimethyl ether (tricetin-7,3′,4′-triMe) was previously found in leaves of *Lethedon tannaensis* (Thymeleaceae) and designated as Lethedocin (Zahir *et al.,* 1996). Tricetin-7,3′,5′-triMe was also reported from the same source, and also from aerial parts of the Lamiaceae *Betonica officinalis* (Kobzar and Nikonov, 1986) and the Asteraceae *Centaurea incana* (Akkal *et al.*, 1997). Tricetin-3′,4′,5′-triMe has been reported from four species of Gramineae (Kaneta and Sugiyama, 1973), and was found in the leaf and stem exudate of the Scrophulariaceae *Asarina procumbens* (Wollenweber, unpubl.).

Phytochemical studies on flavonoids in the genus *Ceanothus* are extremely rare. In 1970, Das *et al*. reported the isolation of luteolin-7,3′-dimethyl ether from leaves of *Ceanothus velutinus* and named it velutin. We are unaware of any further report on flavonoid aglycones in this genus, and surprisingly we also found only one paper dealing with glycosides: Pichon-Prum *et al*. (1984) reported the presence of kaempferol and quercetin *O*-glycosides in *C. americanus*, probably as leaf tissue constituents. In the present study, *C. americanus* L. seemed to accumulate traces of flavonoid

Table I. Exudate flavonoids of *Ceanothus* species (Me, methyl ether; OAc, acetate).

	C. dentatus	*C. foliosus*	*C. hearstiorum*	*C. impressus*	*C. oliganthus*	*C. papillosus*	*C. × veitchianus*	*C. velutinus*
Quercetin-3,7,3′,4′-tetraMe	x	x	x	x	x	x	x	
Qu-7,3′,4′-triMe					x	x		
Qu-3,3′,4′-triMe					x			
Qu-3,7,4′-triMe	x	x	x	x	x	x	x	
Qu-3,7,3′-triMe	x	x	x	x	x	x	x	
Qu-7,3′-diMe		x			x			
Qu-3,3′-diMe	x	x	x	x	x	x	x	
Qu-3,7-diMe	x	x	x	x	x	x	x	
Qu-3′-Me	x		x	x	x	x		
Qu-7-Me			x		x			
Qu-3-Me	x	x	x	x	x	x	x	
Quercetin				x	x	x		
Kaempferol-3,7,4′-triMe			x		x		x	
Kae-7,4′-diMe					x			
Kae-3,4′-diMe							x	
Kae-3,7-diMe		x	x	x	x	x	x	
Kae-7-Me			x		x	x		
Kae-3-Me		x	x	x		x		
Tricetin-7,3′,4′-triMe								x
6-OH-Lut-6,7-diMe								x
Luteolin-7,3′,4′-triMe								x
Lut-7,3′-diMe								x
Apigenin-7,4′-diMe								x
Ap-7-Me								x
Taxifolin-7,3′-diMe-3-OAc	x			x	x			
Taxifolin-7-Me-3-OAc				x				
Eriodictyol-7,3′-diMe	x			x				
Eriodictyol-7-Me				x	x	x		
Naringenin-7,4′-diMe					x			
Naringenin-7-Me				x	x			
Glycosides:								
Myricetin-3-glucuside						x		
Myr-3-rhamnoside				x		x		
Quercetin-3-rh					x	x		
Qu-3-gluc				x	x	x		
Qu-3-rhgluc		x						
Kaempferol-3-rh		x		x		x		
Kae-3-gluc		x						
Kae-3-rhgluc		x						

glycosides externally, but no aglycones were detected. *C. fendleri* A. Gray and *C. thyrsiflorus* Eschsch. were also found to be devoid of exudate flavonoids. The three latter species were, therefore, not included in Table I.

The earlier paper on the occurrence of luteolin-7,3′-dimethyl ether in *C. velutinus* (Das *et al.*, 1970) reported its isolation from ground leaves. However, extraction was done with pentane, so it can be assumed that this flavone came, indeed, from externally accumulated material. As a matter of fact, we found it in the acetone leaf wash of this species, where it is accompanied by five further flavones, including one with the rare 3′,4′,5′-tri-*O*-substitution and the only case of 6-*O*-substitution. It is striking that *C. velutinus* exhibits exclusively flavones, whereas all other species studied exhibit flavonols, flavanones and flavanonols, but no flavones. The exudate flavonoid profiles of these species are dominated by quercetin derivatives: six of

the ten quercetin methyl ethers are present in all of them. Kaempferol methyl ethers are less abundant. The external accumulation of flavonol glycosides in four species, in particular the accumulation of rutin (quercetin-3-rhamnoglucoside) on *C. foliusus* leaves, is worth mentioning, since this phenomenon is observed rather rarely (see *e.g.* Wollenweber *et al*., 1997; Tattini *et al*., 2000). Five species exhibit flavanones and/or dihydroflavonols, the latter being natural 3-*O*-acetates. Some TLC-spots indicate the presence of eriodictyol-related flavanones in *C. velutinus*, but these could not be identified, due to lack of material. *C. oliganthus* exhibits the highest number of different flavonoids, and has many of them in common with *C. impressus* and with *C. papillosus*, respectively. These observations appear interesting from the chemotaxonomic point of view. The genus *Ceanothus* is fairly large, however, compared with the number of species we have studied thus far. Investigation of many additional species should show, therefore, the extent to which flavonoid excretion is distributed in the genus, and if the exudate flavonoid profiles sustain the characterization of individual taxa.

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