

On the Occurrence of Exudate Flavonoids in the Borage Family (Boraginaceae)

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Externally accumulated flavonoid aglycones have been found for the first time in *Nonea* species. They exhibit only flavones, one of them being the rare tricetin-4'-methyl ether. Within the subfamily Boraginoideae, exudate flavonoids appear to be a rare character.

Introduction

Reports on the occurrence of flavonoid aglycones in Boraginaceae are scarce so far. In the scope of ongoing research on the distribution of flavonoid aglycones on plant surfaces we have now studied some members of this family. We found exudate flavonoids in three European *Nonea* species and in two *Heliotropium* species from Chile. Several indigeneous species of the subfamily Boraginoideae were found devoid of exudate flavonoids.

Material and Methods

Nonea lutea Bory & Chaub. and *N. rosea* Link were cultivated in the Botanical Garden of the University of Darmstadt. Aerial parts were collected in bulk from flowering plants (*N. lutea*: May, 1999; *N. rosea*: July 2000, August 2001. Vouchers: Botanischer Garten der TU Darmstadt). Fresh material was briefly rinsed with acetone to dissolve the lipophilic material accumulated on leaf and stem surfaces. Some plants of *N. pulla* (L.) DC. were collected at natural sites near the Kyffhäuser (Germany; coll. H. Dietrich, July 1999; voucher at HAL) and in N-Burgenland (Hackelsberg, Lake Neusiedl, Austria; coll. K. Vetschera, June 2001; voucher at WU), respectively. The air-dried samples were also briefly rinsed with acetone. Aerial parts of *Heliotropium pycnophyllum* Phil. were collected in Quebrada Botija (24°31'S, 70°33'W.), Prov. Antofagasta, Chile (Dillon 5355). *Heliotropium stenophyllum* Hook et Arn. was also

collected in Chile, near La Serena, Prov. De Elqui (P. López 2471). In each case, concentrated solutions were defatted (MeOH, –10°, centrifugation) and passed over Sephadex LH-20, eluted with MeOH, to separate the flavonoids from the terpenoids. The flavonoid portions of *N. lutea* were further chromatographed over polyamide DC-11, eluted with toluene and increasing quantities of methylethyl ketone and methanol. Fractions were monitored and comparisons with markers were done by TLC on polyamide (DC 11, Macherey-Nagel) with the solvents a) petrol_{100–140}–toluene–MeCOEt–MeOH 12:6:1:1 v/v/v/v, b) toluene–petrol_{100–140}–MeCOEt–MeOH 12:6:2:1 v/v/v/v, and c) toluene–MeCOEt–MeOH 12:5:3 v/v/v, and on silica with solvent d) toluene–MeCOEt 9:1 v/v. Chromatograms were viewed under UV (366 nm) before and after spraying with “Naturstoffreagenz A” (1% of diphenyl-boric acid-ethanolamine complex in MeOH). Authentic samples of flavonoids were available in E. W.’s lab. – Atmospheric pressure chemical ionization (APCI) mass spectra were recorded on a PE Sciex API III Plus triple-quadrupole instrument. NMR spectroscopy was run on a Bruker DRX 600 instrument at 600 MHz (¹H) in DMSO-*d*₆.

Compound **1** was isolated from some combined fractions of *N. lutea* by prep. TLC on silica with solvent e) toluene–dioxane–HOAc 18:5:1 v/v/v. It was further purified by HPLC on a 10 µm Econosil RP-18 column (250 × 10 mm) using a linear gradient from 40% to 100% MeCN in 1% aq. HCOOH over 30 min at 5.6 ml/min. The peak fraction con-

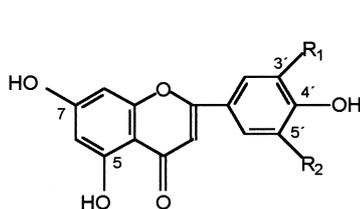
taining compound **1** was collected and taken to dryness by rota-evaporation and lyophilization. Its melting point is reported here for the first time: 274 °C (dec.). UV (MeOH) λ_{\max} 331 and 268 nm; +NaOH: 373, 316 and 274 nm; +AlCl₃: 346, 301 and 277 nm, unchanged on addition of HCl (Lit. (Ulubelen *et al.*, 1984): MeOH (331 nm), +NaOH (373 nm)). The ¹H spectrum showed three phenolic OH signals (δ_{H} 12.87 (OH-5), 10.92 and 9.57), two equivalent B-ring protons (δ_{H} 6.96, s, 2H, H-2' and H-6'), a singlet at δ_{H} 6.61 typical of a flavone H-3, two meta-coupled A-ring protons (δ_{H} 6.42 and 6.20, *J* = 1.8 Hz, H-8 and H-6), and one OMe singlet (δ_{H} 3.76, 4'-OMe). From the MS (MH⁺, *m/z* 317) and ¹H spectrum it was deduced that compound **1** was a 5,7,3',4',5'-pentahydroxyflavone monomethyl ether. The O-methyl group was placed at position 4' for two reasons, (1) the A-ring proton resonances were not shifted downfield compared to 5,7-dihydroxy flavones (Markham and Geiger, 1994; 7-O-methylation causes a downfield shift of the H-6 and H-8 doublets by about 0.2–0.4 ppm), and (2) the B-ring H-2' and H-6' protons gave rise to a 2H singlet indicating B-ring symmetry which is only pos-

sible when the hydroxy group at C-4' carries the methyl substituent. Compound **1** was therefore identified as 5,7,3',4',5'-pentahydroxyflavone-4'-O-methyl ether.

Samples of eight further species of Boraginaceae were collected in the field around Darmstadt or in the Botanical Garden of the University of Darmstadt: *Anchusa azurea* Mill., *A. sempervirens* L., *Borago officinalis* L., *Cerinth minor* L., *Cynoglossum officinale* L., *Echium vulgare* L., *Myosotis arvensis* (L.) Hill, and *Symphytum officinale* L. They were also rinsed with acetone and the concentrated solutions were checked for flavonoid aglycones by TLC.

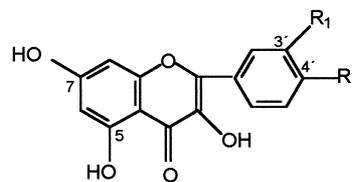
Results and Discussion

Nonea lutea, *Nonea pulla*, and *N. rosea* were found to exhibit exclusively flavones (Table I). Some of these are rather rare natural products. Tricetin-4'-methyl ether was reported only once before as an aglycone, namely from the leaf of *Passiflora palmeri* (Ulubelen, 1984.). Tricetin-3',4'-dimethyl ether (apometzgerin) was known from the grass *Poa hueca* (Rofi and Pomilio, 1985



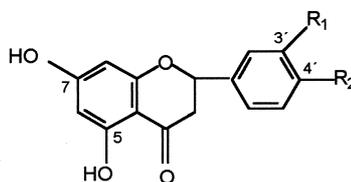
Flavones:

- R₁ = R₂ = H: Apigenin
- R₁ = OH, R₂ = H: Luteolin
- R₁ = R₂ = OH: Tricetin



Flavonols:

- R₁ = R₂ = H: Galangin
- R₁ = H, R₂ = OH: Kaempferol
- R₁ = R₂ = OH: Quercetin



Flavanones:

- R₁ = R₂ = H: Pinocembrin
- R₁ = H, R₂ = OH: Naringenin
- R₁ = R₂ = OH: Eriodictyol

Table I. Flavonoid patterns of 3 *Nonea* species.

Flavones	<i>N. lutea</i>	<i>N. pulla</i>	<i>N. rosea</i>
Apigenin	x	x	(x)
Ap-7-Methyl ether	x	x	x
Luteolin	x	(x)	
Lut-7-Me	x	x	
Lut-3'-Me			(x)
Lut-4'-Me			(x)
Lut-7,3'-diMe	x	(x)	
Lut-7,3',4'-triMe		x	
Tricetin-4'-Me	x	x	
Tric-3',4'-diMe		x	
Tric-3',4',5'-triMe		x	

[and as a C-glycoside from the liverwort *Apo-metzgeria pubescens* (Theodor *et al.*, 1980)]. Tricetin-3',4',5'-triMe had so far been reported only as a constituent of 4 Gramineae (Kaneta and Sugiyama, 1973). Recently it was also encountered on aerial parts of a Scrophulariaceae (Wollenweber, unpubl.). *Nonea* appears to be the first genus in which these tricetin derivatives are accumulated externally.

The exudate of *Heliotropium pycnophyllum* contains flavones and flavonols, whereas the exudate of *H. stenophyllum* contains flavonols and flavanones (Table II). The flavonoid pattern exhibited by our material of *H. stenophyllum* is rather different from what has been reported in literature previously. Neither did we see any trace of luteolin-7-methyl ether, nor was there any hint to the presence of a 5-methoxy flavonol (which would appear as a brilliant yellow fluorescent spot on tlc in UV light). In both studies, aerial parts were collected during the flowering period and dipped in dichloromethane or rinsed with acetone, respectively. The different flavonoid patterns observed might, therefore, point to the existence of varieties or of chemotypes in this species.

This is only the second time that exudate flavonoids have been found in members of the subfamily Boraginoideae. The first report was on flavonols from aerial parts of *Alkanna orientalis* (El Sohly *et al.*, 1997). It is striking that in *Nonea* exclusively flavones have been detected, whereas *Alkanna* exhibits 6 flavonols and lacks flavones. Eight further species belonging to this subfamily have also been checked for the presence of exudate flavonoids. The acetone leaf rinses of *Anchusa azurea*, *A. sempervirens*, *Borago officinale*,

Table II. Flavonoid patterns of 2 *Heliotropium* species.

Flavonoids	<i>H. pycnophyllum</i>	<i>H. stenophyllum</i>	<i>H. stenoph.</i> – Lit.*
Chrysin	x		
Chrys-7-Me	x		
Luteolin-7-Me			x
Galangin		x	x
Gal-3-Me	x	x	x
Gal-7-Me	x		
Gal-3,7-diMe	x		
Kaempferol		x	
Kae-3-Me	x		
Kae-7-Me		x	
Kae-3,7-diMe	x		
Kae-7,4'-diMe		(x)	
Kae-3,7,4'-triMe	x		
Quercetin		x	
Qu-3-Me		x	
Qu-7-Me		x	
Qu-3,7-diMe	(x)	x	
Qu-3,3'-diMe		(x)	x
Qu-5,3'-diMe			x
Qu-3,7'-diMe		x	
Qu-3,7,4'-triMe	x		
Pinocembrin		x	x
Naringenin		(x)	x
Nar-7-Me		x	x
Nar-7-Me-4'-Ac			(x)
Nar-7,4'-diMe		x	
Eriodictyol		x	
Eriod-7-Me		x	

* Urzua *et al.*, 2000.

Cynoglossum officinale, *Cerintho minor*, *Echium vulgare*, *Myosotis arvensis*, and *Symphytum officinale* were found devoid of flavonoids.

In the subfamily Cordioideae, there is only one report so far: *Cordia verbenacea* was found to exhibit three highly methoxylated flavonols (Van de Velde *et al.*, 1982; Lins *et al.*, 1990).

In the subfamily Heliotropioideae, seven species of *Heliotropium* were reported previously to exhibit exudate flavonoids (Urzua *et al.*, 2000). They produce several flavanones and dihydroflavonols (including natural acetates), two flavones, and a series of flavonols. *H. megalanthum* is outstanding in producing a flavonol and two flavanones with the relatively rare 3',4',5'-triO-substitution. We were unable to detect any flavonoid aglycones in *Heliotropium arborescens* (Botanical Garden, Darmstadt), so flavonoid excretion obviously is not a general character of the genus.

In a recent study on the leaf surface in Boraginaceae, *Nonea lutea*, *N. pulla*, *N. caspica* and other

taxa were reported to bear long glandular hairs, consisting of three or more stalk cells and an elongated secretory cell (Selvi and Bigazzi, 2001). Urzua and co-workers (2000) pointed out that, in *Heliotropium*, the presence of glandular trichomes is not necessarily a prerequisite for the presence of exudate flavonoids, but is correlated with the amount of resin produced. For two varieties of *H. chenopodiaceum*, on the other hand, they observed that the presence/absence of glandular trichomes had no influence on the flavonoid patterns observed in both varieties (Urzua *et al.*, 1998).

The genus *Nonea* comprises 35 Mediterranean species, *Heliotropium* comprises some 250 species, occurring in the tropic and temperate zones. In total, the borage family houses some 2000 species in 100 genera (Willis, 1973). It is thus obvious that the results presented here are not representative

and that many more species need to be checked in the future before any chemosystematic conclusions can be drawn.

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