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FLAVONOID AGLYCONES FROM LEAF RESINS OF TWO SPECIES OF *HETEROTHECA* (COMPOSITAE)*

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Key Word Index—*Heterotheca* species; Compositae; leaf resin; flavonoid aglycones.

Abstract—Twenty-nine flavonoid aglycones have been identified from two populations each of *Heterotheca grandiflora* and *H. psammophila*. Considerable qualitative variation was found between populations of the same species. Overall, *H. grandiflora* is more complex in its flavonoid profile, accumulating a total of 24 compounds based on eight skeletal types, compared with 13 compounds based on four skeletal types in *H. psammophila*.

INTRODUCTION

The objectives of the present study were to determine the flavonoid aglycone composition and potential variation in the external leaf and stem wash of *Heterotheca grandiflora* Nutt. and *H. psammophila* Wagenkn. This study is part of a continuing project to seek out chemosystematic trends and evaluate the usefulness of flavonoid characters within and among genera of the tribe Astereae [1, 2]. Although the resin of *Heterotheca* species has been examined chemically, previous studies have been primarily concerned with terpenes [3–9]. We report here the relatively high diversity of flavonoids found in these resins as well.

RESULTS AND DISCUSSION

Table 1 summarizes the types of flavonoid aglycones found in these two species and their distribution among the populations examined in this study. Twenty-nine compounds were identified, the majority of which were flavonols. This overall pattern is similar to those found so far in *Hazardia* [1] and *Ericameria* [2].

Two systematic trends can be observed in the data presented here if all of the compounds from both populations of *H. grandiflora* are compared with those of both populations of *H. psammophila*. The first is that *H. grandiflora* accumulates the greater number of aglycones, 24 versus 13. The second is that *H. grandiflora* also yields the greater diversity of skeletal types. Methyl ethers of eight parent flavonoids (i.e. kaempferol, 6-hydroxykaempferol, quercetin, quercetagetin, scutellarein, luteolin, 6-hydroxyluteolin and eriodictyol) are found in *H. grandiflora*. In contrast, *H. psammophila* was found to accumulate only derivatives of kaempferol, 6-hydroxykaempferol, quercetagetin and scutellarein. This pattern may reflect relative phylogenetic advancement, whereby the simpler flavonoid profile of *H. psammophila* represents the more derived condition, as has been suggested for the segregate genera of *Haplopappus* [10].

This study also shows the potential for qualitative variation in flavonoid chemistry within a species. As in the case of *Ericameria* [11], *Heterotheca* flavonoids vary considerably from one population to another. Of the 24 compounds found in *H. grandiflora* overall, only seven (29%) were shared by the two populations sampled. Likewise, in *H. psammophila*, only two of the total 13 compounds (15%) occurred in both populations. Interestingly, these two compounds **10** and **11** were also the only substances identified from both of the populations of each of the species examined in this study. The variability of flavonoid composition in *Heterotheca* species reported here suggests that further population studies are required before the observed systematic trends can be confirmed with confidence.

EXPERIMENTAL

Plant material. Leaves and stems of *H. grandiflora* were collected on the campus of the University of California at Irvine (pop. A) in September, 1982, and in Cochise Co., Arizona (pop. B) in September, 1983. Aerial parts of *H. psammophila* were collected in Arizona, in Cochise Co. (pop. A) in March, 1982, and in Yavapai Co. (pop. B) in December, 1981. Vouchers for *H. grandiflora* are deposited in ASU, for *H. psammophila* in ARIZ (pop. A) and ASU (pop. B).

Fractionation and identification of flavonoids. Leafy stem material of *H. grandiflora* (pop. A, 117 g; pop. B, 485 g) were immersed in CH_2Cl_2 or Me_2CO for *ca* 3 min to dissolve the external resin components. The solvent was quickly poured off, filtered and evaporated to a thick syrup (pop. A, 5.7 g; pop. B, 22 g). Each syrup was dissolved in warm MeOH and applied directly to a column (4 × 50 cm) poured with Sephadex LH-20 (Sigma) and eluted with MeOH for preliminary fractionation. Further purification of these fractions included polyamide CC (Polyamid SC-6, Macherey–Nagel) and prep. TLC on silica.

The samples of *H. psammophila* (pop. A, 580 g; pop. B, small test sample only) were first rinsed in Me_2CO. Pop. A yielded

*Part 3 in a series "Flavonoid Aglycones of the Tribe Astereae"; for part 2 see ref. [2].

Table 1. Distribution of flavonoid aglycones in *Heterotheca*

Compound	*H. grandiflora*		*H. psammophila*	
	Pop. A	Pop. B	Pop. A	Pop. B
1. Kaempferol		+		
2. Kaempferol 3-Me ether		+		+
3. Kaempferol 7-Me ether		+		
4. Kaempferol 4′-Me ether		+		
5. Kaempferol 3,7-diMe ether	+	+		+
6. Kaempferol 3,4-diMe ether		+	+	
7. Kaempferol 3,7,4-triMe ether			+	
8. 6-OH-Kaempferol 6-Me ether		+		
9. 6-OH-Kaempferol 3,6-diMe ether		+		+
10. 6-OH-Kaempferol 6,7-diMe ether	+	+	+	+
11. 6-OH-Kaempferol 3,6,7-triMe ether	+	+	+	+
12. 6-OH-Kaempferol 3,6,4′-triMe ether	+	+		
13. 6-OH-Kaempferol 6,7,4′-triMe ether			+	
14. 6-OH-Kaempferol 3,6,7,4′-tetraMe ether		+	+	
15. Quercetin		+		
16. Quercetin 3-Me ether		+		
17. Quercetin 7,3′-diMe ether		+		
18. Quercetin 3,7,3′-triMe ether	+	+		
19. Quercetagetin 3,6-diMe ether	+			
20. Quercetagetin 6,3′-diMe ether		+		
21. Quercetagetin 3,6,7-triMe ether	+	+		+
22. Quercetagetin 6,7,4′-triMe ether	+			
23. Quercetagetin 3,6,7,4′-tetraMe ether			+	
24. Scutellarein 6,7-diMe ether	+	+		
25. Scutellarein 6,4′-diMe ether			+	
26. Scutellarein 6,7,4′-triMe ether			+	
27. Luteolin 7,3′-diMe ether	+			
28. 6-OH-Luteolin 6,7,3′-triMe ether	+			
29. Eriodictyol 7-Me ether	+			

20.4 g of syrup which was chromatographed over silica CC, followed by polyamide CC whenever necessary for further purification. The wash from pop. B was used directly for TLC comparisons.

The identities of flavonoids from the syrups of each species were confirmed according to ref. [12] using comparative TLC with authentic samples on polyamide (Polyamid DC-11, Macherey–Nagel), visualized under UV light (366 nm) both before and after spraying with Naturstoffreagenz A (β-aminoethyl ester of diphenyl boric acid, C. Roth). In addition, **10**, **11**, **21** and **24** were isolated from *H. grandiflora* (pop. A) and **14**, **23**, **25** and **26** from *H. psammophila* (pop. A) for further confirmation by the following spectral analyses: compound **10**, mp 290–292°; UV λ_{max}^{MeOH} nm: 366, 270; + $AlCl_3$ 422, 273 (unchanged with HCl); + NaOH 430, 276; + NaOAc 408, 383, 265; + H_3BO_3 367, 270; MS *m/z* (rel. int.): 330 (90), 329 (8), 315 (8), 312 (34), 287 (100), 121 (34), 93 (13), 69 (30). Compound **11**, mp 217–220°; MS *m/z* (rel. int.): 344 (100), 343 (31), 329 (66), 325 (21), 315 (12), 301 (15), 181 (16), 153 (27). Compound **14** (crystallized from EtOH), mp 172–174°; UV λ_{max}^{MeOH} nm: 343, 274; + $AlCl_3$ 370, 283; + NaOH 394, 275; + NaOAc 395, (350), 273; + H_3BO_3 337, 273. Compound **21**, mp 242–244°; UV λ_{max}^{MeOH} nm: 353, 256; + $AlCl_3$ 442, 345, 280; + HCl 381, 270; + NaOH 411, 273; + NaOAc 400, 268; + H_3BO_3 383, 265; MS *m/z* (rel. int.): 360 (100), 359 (55), 345 (79), 341 (33), 317 (15). Compound **23** (crystallized from EtOH), mp 187–189°; MS *m/z* (rel. int.): 374 (100), 359 (53), 331 (10), 181 (9), 153 (18), 151 (20), 135 (12), 69 (43); UV λ_{max}^{MeOH} 350, 259; + $AlCl_3$ 375, 269 (unchanged with HCl); + NaOH 383, 330, 273; + NaOAc 350, 259; + H_3BO_3 351, 259. Compound **24**, mp 252–255°; UV λ_{max}^{MeOH} nm: 335, 276; + $AlCl_3$ 363, 202, (290) (unchanged with HCl); + NaOH 389, 276; + NaOAc 390, 275; + H_3BO_3 337, 375; MS: 314 (98), 299 (100), 285 (24), 271 (38), 268 (23), 181 (28), 153 (51). Compound **25**, mp 215–217°; UV λ_{max}^{MeOH} nm: 381, 280; + $AlCl_3$ 355, 290 (unchanged with HCl); + NaOH 365, 280; + NaOAc 368, 280; + H_3BO_3 350, 277; MS *m/z* (rel. int.): 314 (100), 299 (100), 296 (76), 285 (32), 271 (38), 167 (22), 153 (38), 139 (21), 135 (18), 133 (42), 132 (23). Compound **26** (crystallized impure from MeOH, followed by prep. TLC on polyamide), UV λ_{max}^{MeOH} nm: 332, 278; + $AlCl_3$ 360, 355, 305 (unchanged with HCl); + NaOH 332, 290; + NaOAc 330, 227; + H_3BO_3 330, 277; MS *m/z* (rel. int.): 328 (100), 313 (87), 299 (26), 285 (28), 283 (20), 181 (21), 153 (60), 135 (21), 133 (30), 132 (18); ^1H NMR: δ 11.08 (OH-5), 8.06 (*dd*, 2H, H-2′/6′), 7.15 (*dd*, 2H, H-3′/5′), 6.95 (*s*, H-8), 6.90 (*s*, H-3), 3.96, 3.90, 3.76 (*s*, 3H each, 3 × OMe).

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5,7,3′-TRIHYDROXY-4′,5′-DIMETHOXYFLAVONE AND OTHER PHENOLICS FROM *POA HUECU*

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Key Word Index—*Poa huecu*; Gramineae; 5,7,3′-trihydroxy-4′,5′-dimethoxyflavone; tricin; selagin.

Abstract—Whole plants of *Poa huecu* have yielded a new flavone characterized as 5,7,3′-trihydroxy-4′,5′-dimethoxyflavone as well as tricin, selagin, umbelliferone and scopoletin.

INTRODUCTION

In continuation of our research on *Poa huecu* Par. [1] (Gramineae), an Argentinian plant toxic to cattle, we now report the isolation and identification of the flavones: 5,7,3′-trihydroxy-4′,5′-dimethoxy flavone (**1**), 5,7,3′,4′-tetrahydroxy-5′-methoxyflavone (selagin, **2**), 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone (tricin, **3**) and the coumarins umbelliferone and scopoletin.

RESULTS

Upon column chromatography of the methanolic percolate of *Poa huecu* a fraction rich in flavonoids was obtained. Further chromatography on Sephadex LH-20 led to the five components mentioned above. Tricin was the main component. The UV spectrum of the new flavone **1** showed maxima at 271 and 329 nm. The presence of a chelated hydroxyl was supported by a bathochromic shift of 26 nm with aluminium chloride–hydrochloric acid in UV. The shift with sodium acetate ($\Delta\lambda$ 7 nm) indicated the presence of a hydroxyl at the 7-position. The aluminium chloride–hydrochloric acid as well as sodium acetate–boric acid spectra ruled out an *o*-dihydroxyl in the B-ring. The shift with sodium methoxide ($\Delta\lambda$ 21 nm) indicated the absence of a free hydroxyl group at the 4′-position. By contrast, tricin and selgin with the same agent, showed a shift higher than 50 nm, indicating a free hydroxyl group in that position.

The ^{1}H NMR spectrum of **1** showed two singlets at δ4.01 and 4.02 due to the presence of two methoxyl groups. The 5,7-disubstitution pattern of A-ring was indicated by the two doublets at δ6.27 (H-6) and δ6.59 (H-8) with a *meta* coupling constant of 2 Hz. The singlet at δ6.82 (H-3) integrating for one proton supported the flavone skeleton, whilst the singlet at 7.44 (2H) accounted for H-2′ and H-6′ that gave this signal instead of a doublet as has been also observed in selagin. The presence of a free hydroxyl at 5- position was confirmed by the signal at δ12.94.

A retro-Diels–Alder fragmentation pattern was observed in the MS leading to the fragments $A_1 + H$, B_2 and B_1. The results supported the presence of one hydroxyl and two methoxyl groups in ring B and two hydroxyls in ring A.

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