Phytochemistry, 1973, Vol. 12, pp. 2265 to 2268. Pergamon Press. Printed in England.

# FLAVONOLS OF *PARTHENIUM* SECTION BOLOPHYTUM*

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(*Received* 24 *January* 1973. *Accepted* 2 *April* 1973)

**Key Word Index**—*Parthenium*; Compositae; calciphiles; relicts; chemotaxonomy; 6-methoxyflavonoids; scopoletin.

**Abstract**—Casticin (V), 5,7,3′,4′-tetrahydroxy-3,6-dimethoxyflavone-7-glycoside (I), 5,7,4′-trihydroxy-6-methoxyflavone-7-glycoside (hispidulin 7-glycoside) (IV), 5,4′-dihydroxy-3,6,7,3′-tetramethoxyflavone (VII) and 5,7-dihydroxy-3,6,3′,4′-tetramethoxyflavone (XII) were found, with the coumarin scopoletin (IX), in various combinations in the three species of *Parthenium* section Bolophytum. No infraspecific variation was detected in these calciphilic relicts.

## INTRODUCTION

AS PART of a systematic study of *Parthenium* section Bolophytum, the flavonoids and coumarins were examined from the leaves, roots and underground stems of the three rare relictual species (*P. ligulatum*, *P. tetraneuris* and *P. alpinum*). Since no phenolics were detected in the roots and stems, only leaves (less than 5% of the dry weight of the organisms) were used. Even so, no phenols were detected in *P. alpinum* using standard techniques,[1] and phenolic compounds could only be obtained from freeze-dried leaves. *P. rollinsianum* Rzedowski, section Parthenichaeta, was also examined; the flavonoid complement in this species indicates the relatedness of Parthenichaeta to Bolophytum. One component of *P. rollinsianum*, rollinsianum-I, was used for comparison with minor components of the species of section Bolophytum.

## RESULTS

Every collection (Table 1) was chromatographed from several groups of specimens. No qualitative variation was observed for *P. ligulatum* and *P. tetraneuris*. Fresh and recently dried material gave identical 2-D PCs in each case. The chromatographic pattern for *P. tetraneuris* contained six compounds (*T* 1-6); material stored for more than 6 months however did not show spots of *T*1 and *T*2. *P. ligulatum* gave three unknowns *L* 1–3. Since the normal extraction procedure did not work for *P. alpinum*, it was not possible to check the qualitative variation in this case. The sole collection (JM-3013) which produced a pattern gave four unknowns, *A* 1–4.

Co-chromatography[2] has indicated the probable correspondence of *T*3 and *A*1; of *T*4,

* Part of a study presented in partial fulfillment of the requirements for the Ph.D. degree of the University of Texas at Austin.

[1] MABRY, T. J., MARKHAM, K. R. and THOMAS, M. B. (1970) *The Systematic Identification of Flavonoids*, Springer, New York.

[2] MEARS, J. A. (1970) *The Systematics of Parthenium section Bolophytum; Biochemical, Morphological and Ecological*, Dissertation, University of Texas at Austin.

*A2* and *L2*; of *T6*, *A4* and *L3*; and that *T5* and *A3* were similar to an unknown from *P. rollinsianum* (*R1*). The UV data of *T5* matched well with that of the *P. rollinsianum* compound. All these compounds appeared to be methylated 6-hydroxyflavones or flavonols.

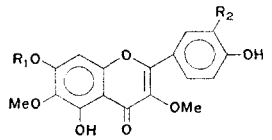
TABLE 1. SPECIMENS OF *Parthenium* EXAMINED*

| Name | Source | Coll. Nos. |
|---|---|---|
| *P. alpinum* | Alcova, Wyo. | JM-3010, JM-3013, JM-3045 |
| *P. ligulatum* | Uintah Co., Utah | JM-2878, JM-2874, JM-2929, JM-2930. |
| *P. ligulatum* | Moffat Co., Colo. | JM-2933 |
| *P. tetraneuris* | Florence, Colo. | JM-2731, JM-2738, JM-2741. |
| *P. rollinsianum* | Pozos de Santa Ana, San Luis Potosi, Mex. | *E. Rodriguez* |

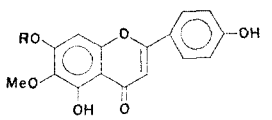
* All vouchers in the herbarium of the University of Texas at Austin.

*Flavonoid* T1

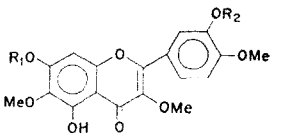
Demethylated and hydrolyzed *T1* compared well, co-chromatographically, with 3,5,6,7,-3′,4′-hexahydroxyflavone, obtained by demethylation of jaceidin. The $AlCl_3$ spectrum of *T1* indicates that both 3′ and 4′ hydroxyl groups are free, and the addition of HCl shows a free 5-hydroxyl adjacent to an oxygen moiety at $C_6$.[3] The lack of decomposition of either *T1* or its acid hydrolyzed product in base indicates a blocked oxygen function (for example, a methoxyl) at $C_6$ and $C_3$. On this basis, the proposed structure of *T1* is a flavonol glycoside (I). It is possible that the glycoside is a di- or tri-saccharide, since the $R_f$ is low in TBA (0·30) and high in HOAc (0·60). Since β-glucosidase effected hydrolysis, the sugar is probably glucose.



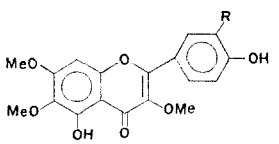
(I) $R_1$ = Gly; $R_2$ = OH
(III) $R_1$ = $R_2$ = H
(VI) $R_1$ = H; $R_2$ = OMe



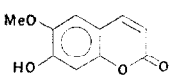
(II) R = H
(IV) R = Gly



(V) $R_1$ = Me; $R_2$ = H
(X) $R_1$ = $R_2$ = H
(XI) $R_1$ = Me; $R_2$ = H
(XII) $R_1$ = H; $R_2$ = Me



(VII) R = OMe
(VIII) R = H



(IX)

*Compound* T2

The $AlCl_3$–HCl spectrum of *T2* clearly indicates a free hydroxyl at $C_5$. The lack of decomposition in sodium methoxide of either *T2* or its acid hydrolyzed product indicates $C_6$ is probably methylated. The UV spectra of acid hydrolyzed *T2* corresponds reasonably well with the known flavonoid hispidulin (II). Another possible structure is 3,6-dimethoxy-5,7,4′-trihydroxyflavone (III), which has recently been reported as a glucoside from *Cen-*

[3] MEARS, J. A. and MABRY, T. J. (1972) *Phytochemistry* **11**, 411.

*taurea jacea*.[4] However, the spectra of hydrolyzed *T*2 are different from those of the *Centaurea* compound. *T*2 is therefore proposed to be hispidulin 7-*O*-glycoside (IV). Again, hydrolysis by β-glucosidase suggests that it is the 7-*O*-glucoside.

*Compound* T3

There was insufficient *T*3 to give complete structural information. The compound is probably a flavone or flavonol-3-methyl ether.

*Compounds* T4, A2 *and* L2

There was sufficient material only of *L*2 for analysis and the chromatographic and spectral data showed it had the structure of the known flavoid casticin (V). For comparison, casticin was extracted from seeds of *Vitex agnus-castus* and was also donated by Prof. H. Wagner.

*Compounds* T5, A3 *and* R1

Demethylation of *R*1 produced 3,5,6,7,3′,4′-hexahydroxyflavone as with *T*1. The spectra indicate a methoxyl at $C_6$ and $C_3$ and a free hydroxyl at $C_5$ and that one or both of the 3′,4′-dihydroxy group is substituted. There are two structures consistent with these data; one (VI) is jaceidin, which does not match with *R*1, and therefore the other (VII) is proposed as the correct structure for this compound. Comparisons of the spectra of *R*1 with the published data of the *Centaurea* flavonoid (III) and penduletin (VIII) support this identification.

*Compounds* T6, A4 *and* L3

The chromatographic evidence indicates this constituent is a coumarin, and the spectrophotometric and chromatographic data correspond exactly with scopoletin (IX).[5]

*Compound* L1

The $R_f$s in TBA and HOAc and the lack of spectral change upon acid hydrolysis suggest that this compound is an aglycone. Demethylation again produced 3,5,6,7,3′,4′-hexahydroxyflavone. The chromatographic and spectral data suggest the presence of methoxyls at $C_6$ and $C_3$, a hydroxyl at $C_5$ and no 3′,4′-dihydroxy grouping. The shift of +31 nm in sodium methoxide is much less than the usual +58 nm shift for the 4′-hydroxyl system.

Since *L*1 and *L*2 are of the same general structural type, and since the $R_f$ in TBA of *L*2 is greater, *L*2 should be the more highly methylated.* Neither centaureidin (X), artemetin (XI) nor casticin (V) compare with *L*1, which therefore must be (XII).

## DISCUSSION

The taxa of *Parthenium* section Bolophytum are characterized by a paucity of flavonoids and coumarins, a total absence of regular flavonoid types and a tendency to the aglycone condition. The absence of chemical variation (at least in *P. ligulatum* and *P. tetraneuris*) is correlated with a virtual absence of morphological variation[6] and may be related to the relictual status of these species.

* Within aglycones of a structural type, increasing methylation results in increasing $R_f$s in TBA.

[4] RÖSLER, H., STAR, A. E. and MABRY, T. J. (1973) *Phytochemistry* **12**, in press.

[5] BRACKENRIDGE, M. I. (1968) *The Ultra-Violet Spectral Analysis of Coumarins*, Thesis, The University of Texas at Austin.

[6] MEARS, J. A. (1973) *Proc. Acad. Nat. Sci. Phila.* in press.

Fresh samples of *P. alpinum* and *P. tetraneuris* might produce qualitatively identical patterns. Yet, quantitatively there is a major difference: *P. tetraneuris* yields much more phenolic material than does *P. alpinum*. The chromatographic pattern of *P. ligulatum* is distinct from the pattern of the other two species. Scopoletin (IX) and casticin (V) appear to be present in all three species.

All the flavonoids detected in *Parthenium* section Bolophytum are of an unusual type: penta- and hexa-hydroxylated flavonoids with methyls at $C_3$ and $C_6$. The development of a means of detecting 6-methoxyflavonoids on a microscale[3] has led to an increased frequency of reports of such compounds. Nonetheless, 6-methoxyflavonoids are apparently common only in certain taxa of the Compositae. Of even greater rarity is the absence of the usual apigenin, luteolin, kaempferol and quercetin derivatives. The presence of only one detectable coumarin is equally rare.

## EXPERIMENTAL

*Collection and preparation of material of* P. alpinum, P. tetraneuris, P. ligulatum *and* P. rollinsianum. Some bulk collections were ground and extracted before drying. Other bulk collections were air-dried and stored for 0·5–6 months before extraction. Samples (100 g) of fresh leaf and stem material of each collection of *P. alpinum*, *P. tetraneuris* and *P. ligulatum* were ground in 80% MeOH for 2 min in a Waring blender and left overnight. Concentrated extracts were examined by standard techniques.[1]

*Freeze-drying of* P. alpinum *leaves.* A sample (500 g) of stored dried leaves of *P. alpinum* (JM-3013) was ground and extracted as before, and the filtered extract was frozen under vacuum. The solid was triturated with hexane, $Et_2O$ and MeOH.

*UV spectrophotometric and chromatographic data. T*1: $R_f$ TBA–0·30, HOAc–0·60; color UV–purple, UV/$NH_3$–purple; $\lambda_{max}$ (all in MeOH): MeOH–260 nm, 278, 352; NaOMe–270, 389; $AlCl_3$–279, 308 (inflection), 342 (infl.), 434; $AlCl_3$/HCl–242 (infl.), 267, 290, 377; NaOAc–273, 404; NaOAc/$H_3BO_3$–268, 367. Hydrolyzed *T*1: color UV–purple, UV/$NH_3$–purple; $\lambda_{max}$: MeOH–246 nm (infl.), 278 (infl.), 355; NaOMe–281 (infl.), 300 (infl.) 406; $AlCl_3$–279 (infl.), 313 (infl.), 426; $AlCl_3$/HCl–296, 374.*

*T*2: $R_f$–0·45, –0·70; color UV–purple, UV ($NH_3$)–purple; $\lambda_{max}$: MeOH–236 nm (infl.), 279, 338; NaOMe–248 (infl.), 382; $AlCl_3$–240, 298, 373; $AlCl_3$/HCl–238, 294, 366; NaOAc–281, 338; NaOAc/$H_3BO_3$–238, 338. Hydrolyzed *T*2: color UV–purple, UV/$NH_3$–purple; $\lambda_{max}$: MeOH–255 nm (infl.), 337; NaOMe–250 (infl.), 300 (infl.), 387; $AlCl_3$–252 (infl.), 296 (infl.), 376; $AlCl_3$/HCl–255 (infl.), 300 (infl.) 367; NaOAc–297 (infl.), 335, 386 (infl.); NaOAc/$H_3BO_3$–285 (infl.), 330 (infl.).

*T*3: $R_f$–0·85, –0·35; color UV–purple, UV/$NH_3$–purple; insufficient material to obtain UV spectral information.

*T*5: $R_f$–0·69, –0·35; color UV–purple, UV/$NH_3$–purple; $\lambda_{max}$†: MeOH–254 nm, 269 (infl.), 346; NaOMe–272, 284 (infl.), 333, 402; $AlCl_3$–266, 277 (infl.), 296 (infl.), 377; $AlCl_3$/HCl–263, 275 (infl.), 296 (infl.), 373; NaOAc–253, 268 (infl.), 344, 412 (infl.); NaOAc/$H_3BO_3$–253, 268 (infl.), 346.

*T*6: $R_f$–0·85,–0·60; color UV–fluorescent blue, UV/$NH_3$–fluorescent blue; $\lambda_{max}$: MeOH–292, 340; NaOMe–280 (infl.), 395; $AlCl_3$–297 (infl.), 344; $AlCl_3$/HCl–292, 340; NaOAc–280 (infl.), 385; NaOAc/$H_3BO_3$–296, 350.

*L*1: $R_f$–0·61,–0·10; color UV–purple, UV/$NH_3$–purple; $\lambda_{max}$; MeOH–255 nm, 276, 344; NaOMe–267, 375 (slight decomposition); $AlCl_3$–245 (infl.), 271, 293 (infl.), 384; $AlCl_3$/HCl–242 (infl.), 264, 291 (infl.), 376; NaOAc–281, 338; NaOAc/$H_3BO_3$–280, 340.

*L*2: $R_f$–0·82, –0·25; color UV–purple, UV/$NH_3$–purple; $\lambda_{max}$: MeOH–258 nm, 270 (infl.), 347; NaOMe–273, 376; $AlCl_3$–268, 280 (infl.), 300 (infl.), 378, 404 (infl.); $AlCl_3$/HCl–265, 281, 295 (infl.), 367, 400 (infl.); NaOAc–257, 270 (infl.), 344; NaOAc/$H_3BO_3$–257, 271 (infl.), 350.

*Demethylation.* Each sample was treated with melted pyridinium hydrobromide for 5 min, diluted with $H_2O$ and extracted with EtOAc.

*Acknowledgements*—Tom J. Mabry and H. Rösler have contributed much to this research. My studies were supported by the National Institutes of Health Traineeship 5 TO 1 GM-00789. In addition, various funds were provided by NSF Grant GB-5548X. The final preparation of this paper has been during my receipt of NSF Grant GB-26276.

* Insufficient material for further analysis.

† Measured—from the *P. rollinsianum* constituent *R*1.