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NON-POLAR FLAVONOIDS OF *PERITYLE VASEYI* (ASTERACEAE)*

BRUCE A. BOHM, JOHN E. AVERETT† and A. MICHAEL POWELL‡

Department of Botany, University of British Columbia, Vancouver, B. C., Canada V6T 1W5; †Department of Biology, University of Missouri-St. Louis, St. Louis, MO 63121, U.S.A.; ‡Department of Biology, Sul Ross State University, Alpine, TX 79830, U.S.A.

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Abstract—Ten flavonoids have been isolated from a dichloromethane leafwash of *Perityle vaseyi*. Trace amounts of 7,4′-dimethylnaringenin were observed along with nine *O*-methylated flavonols. Two kaempferol, four 6-hydroxykaempferol and three 6-hydroxyquercetin *O*-methylated derivatives were identified. 5,7,4-Trihydroxy-3,6-dimethoxyflavone and 5,4′-dihydroxy-3,6,7-trimethoxyflavone were the major components.

INTRODUCTION

The genus *Perityle* Benth. comprises 55 species [1–5] of primarily rock-dwelling members of the Asteraceae. The tribal placement of *Perityle* has been the subject of recent discussions: Powell and Turner [6] have considered its relationships with members of the Senecioneae while Robinson [7] argued for relationship within the Heliantheae. The genus occurs in the southwestern United States and northwestern Mexico including Baja California with one species also known from South America.

Two-dimensional paper chromatographic analyses of most of the species in sections *Pappothrix* [1], *Laphamia* [2] and *Perityle* [3] have been performed. Although no structural analyses were recorded in these studies clearcut differences were noted in the occurrence of chromatographic spot patterns. The only detailed work on flavonoid constituents of *Perityle* is the work of Southwick *et al.* [8] who described the presence of penduletin 4′-methyl ether in *P. vaseyi* Coult. Studies of sesquiterpene lactones from *P. vaseyi* have recently been published [9] and these authors also reported isorhamnetin.

The present paper describes the results of our analysis of the non-polar flavonoids of *P. vaseyi*. This is the first in a series of papers devoted to the analysis of flavonoids in the genus *Perityle* undertaken with the aim of using chemical data as an additional set of markers to study relationships within *Perityle* and between *Perityle* and supposedly related genera.

RESULTS

Ten flavonoids were isolated from the dichloromethane leafwash of *P. vaseyi*. Their structures were established using UV and mass spectral analysis in all cases plus comparison with known compounds in a few. Nine of the compounds were flavones methoxylated at position 3. The remaining compound was identified as a flavanone (**1**). It showed the typical spectrum of a flavonoid with reduced C-ring and gave a positive colour with magnesium–HCl. Mass spectral analysis gave *m/z* 300 which requires one hydroxyl and two methoxyl groups. Mass fragmentation suggested that the methoxyl functions are at positions 7 and 4′. The compound, which was seen only as a trace constituent, was tentatively identified as 5-hydroxy-7,4′-dimethoxyflavanone (naringenin 7,4′-dimethyl ether).

The remaining nine compounds showed up as dark-absorbing spots on chromatograms suggesting the presence of 3-methoxyl groups. This was confirmed by the presence of $M^+ - 43$ peaks in the mass spectra of all compounds [10]. These flavonoids can be separated into two groups depending on whether the compounds have 6-methoxyl groups or not. Two compounds (**2** and **3**) lack 6-methoxyl groups as shown by the absence of a major fragment peak at $M^+ - 15$ in the mass spectrum [11]. Compound **2** gave a *m/z* of 300 which requires one methoxyl and three hydroxyl groups. The UV spectrum was essentially that of kaempferol 3-glucoside and the mass spectral fragmentation agreed with a 5,7-dihydroxyl pattern in the A-ring and a single hydroxyl group in the B-ring. The methoxyl must therefore be at the 3-position and **2** is kaempferol 3-methyl ether. Compound **3**, with *m/z* 314, gave a fragmentation pattern suggesting *O*-methylation at positions 3 (see above) and 4′. UV absorption data agreed establishing the structure as 3,4′-di-*O*-methylkaempferol (ermanin).

The remaining seven compounds exhibited strong $M^+ - 15$ peaks in their mass spectra indicating the presence of 6-*O*-methoxylation. Four of these exhibited UV spectra consistent with singly substituted B-rings: **4**–**7**. Compounds **4** and **7** were the major compounds seen in the leafwash fraction; approximately 20 mg of each were isolated. Compound **4**, *m/z* 330, has methoxyl groups at positions 3 and 6 (see above) and hydroxyl groups at positions 5, 7 and 4′ as indicated by both UV and mass fragmentation data; its structure is 5,7,4′-trihydroxy 3,6-dimethoxyflavone. Compound **5**, with *m/z* 344, has methoxyl groups at positions 3, 6 and 7 as indicated by mass fragmentation and UV data. The suggested structure is

* Part 1 of "The flavonoids of *Perityle* Benth. (Asteraceae)".

5,4'-dihydroxy-3,6,7-trimethoxyflavone. Compound **5** agreed entirely in R_f, colour reaction with β-aminoethyl diphenylborate and spectral characteristics with an authentic sample of penduletin.

Compound **6**, with m/z 344, is an isomer of penduletin. Mass spectral analysis showed a B-ring fragment m/z 135, which places a methoxyl group at position 4' and an A-ring fragment concordant with a hydroxyl group at position 7. UV analysis supported these assignments. The structure for **6** is thus 5,7-dihydroxy-3,6,4'-trimethoxyflavone (santin).

Compound **7** gave m/z 358 which requires one hydroxyl group and four methoxyl groups. UV analysis showed only the 5-hydroxyl group to be free. Mass fragmentation allowed us to place methoxyl functions at positions 7 and 4' as well as at positions 3 and 6. This compound, 5-hydroxy-3,6,7,4'-tetramethoxyflavone, or penduletin 4'-methyl ether, was reported to be a constituent of *P. vaseyi* by Southwick *et al.* [8]. Our UV data agreed perfectly with their published results.

The last group of compounds has oxygenation at positions 3' and 4' as indicated by the complexity of Band-II spectra in each case. This was borne out by the presence of B-ring fragments of m/z 137 for **8**, m/z 151 for **9** and m/z 165 for **10**. Compound **8** gave the characteristic bright orange colour of the quercetin B-ring with β-aminoethyl diphenylborate as well as the expected borate complex shift of Band I in the UV spectra [12]. The final methoxyl function was located at position 7 on the basis of UV and MS data. The compound was identical with 5,3',4'-trihydroxy-3,6,7-trimethoxyflavone (chrysosplenol-D) which was isolated from *Chrysosplenium* in earlier studies [13]. Compound **9** was shown to be 5,7,4'-trihydroxy-3,6,3'-trimethoxyflavone (jaceidin) and **10** to be 5,7-dihydroxy-3,6,3',4'-tetramethoxyflavone (bonanzin) by characteristic UV and mass spectral data.

Small specimens (0.1–2.0 g) of eight additional species of *Perityle* were subjected to dichloromethane extraction and one-dimensional TLC (ethyl formate-based system). No compounds could be identified with certainty by this method but it was clearly evident that highly *O*-methylated flavonoids were present in each species.

DISCUSSION

Comments on relationships within *Perityle* and between *Perityle* and supposedly related genera are obviously unwarranted at this time, but our detailed observations on *P. vaseyi*, coupled with chromatographic comparison of several other species of *Perityle*, allow at least tentative remarks on the tribal affinities of the genus. The long-standing relationship of *Perityle* with the Senecioneae [6] has been re-examined with morphological [7] and sesquiterpene lactone data [9] presented in support of its placement in the Heliantheae. A comparison of the flavonoid chemistry of the two tribes is possible although the amount of information about these pigments in the Senecioneae is considerably smaller than that available for the Heliantheae. The non-polar flavonoids so far identified from *P. vaseyi* represent the types of pigments that have frequently been reported from members of the Heliantheae. Flavonoids with both extra hydroxylation in the A-ring and high levels of *O*-methylation, structural characters observed in the compounds from *Perityle* have been seen, for example, in members of *Helianthus* series *Angustifolii* [14], *Parthenium* [15], *Neurolaena* [16], *Hemizonia* [17] and *Desmanthodium* [18].

In the Senecioneae extra hydroxylation has been reported in species of *Arnica* [19, 20] and *Lasthenia* [21]. *O*-Methylation is known also in *Arnica* [19, 20] and *Lasthenia* [21]. *O*-Methylation has also been reported in Egyptian species of *Senecio* [22] where the investigators found isorhamnetin, and in members of the *S. radicans* complex in Europe [23] where quercetin 3-methyl ether was identified in a few species. The *O*-methylated flavone diosmetin has been reported from *Hulsea* by Wilken [24]. The majority of compounds reported from members of the Senecioneae, however, are common glycosides of kaempferol, quercetin, apigenin and luteolin (not all species contain all glycosides) [19–26].

The presence of highly *O*-methylated flavonoids in *P. vaseyi*, as shown by our detailed study, and in several other species of the genus, as shown by TLC, is consistent with the placement of *Perityle* in the Heliantheae. Further detailed studies of *Perityle* and related genera are currently underway.

EXPERIMENTAL

Sources of plant material. Approximately 750 g (dry wt) of *P. vaseyi* Coult., collected in Brewster Co., Texas (Powell & Powell 5188), was used for the bulk extraction of flavonoids. Small samples of TLC comparison were obtained as follows: *P. bisetosa* (Torr. ex A. Gray) Shinners var. *scalaris* Powell, Brewster Co., Texas (Warnock 20604); *P. californica* Benth., Sonora, Mexico (Thomas *et al.* 12071); *P. cinerea* (A. Gray in Torr.) Powell, Pecos Co., Texas (Powell 1310); *P. emoryi* Torr., Sonora, Mexico (Felger 7117); *P. fosteri* Powell, Culberson Co., Texas (Powell 3365); *P. lemmonii* (Gray) Macbride, Graham Co., Arizona (Fletcher 7184); *P. rupestris* (Gray) Shinners var. *albiflora* Powell, Brewster Co., Texas (Powell & Powell 5182); and *P. villosa* (Blake) Shinners (Sikes 100). Voucher specimens have been deposited in the Herbarium of Sul Ross State University (SRSC).

Extraction and isolation of flavonoids. Plant material of *P. vaseyi* soaked in CH_2Cl_2 for 5 days and for a further 3 days in fresh solvent. The combined conc CH_2Cl_2 extract (*ca* 30 g of viscous oil) was taken up in 3:1 CH_2Cl_2–MeOH and run on a Polyclar AT column (*ca* 5 × 40 cm) and eluted successively with CH_2Cl_2–MeOH mixtures of 3:1, 2:1, 1:1 and 1:2 and finally with 100% MeOH. Very slow moving compounds were eluted with 1:1 MeOH and MeCOEt to which H_2O was added in increasing amounts. Fractions were collected to correspond with the major bands as visualized with UV 366 nm, evaporated to dryness and taken up in MeOH. The fractions were monitored by one-dimensional TLC using ethyl formate–cyclohexane–*n*-butyl acetate–HCOOH (50:25:23:2) on Polyamide 6.6 plates (homemade). Fractions with similar spot patterns were combined and purified by repeated prep TLC. Two pairs of compounds, **6** and **10** and **3** and **5**, however, were best resolved using toluene–cyclohexane–MeOH–MeCOEt (12:6:1:2). Compound **7**, which had the highest R_f in the ethyl formate system, was finally purified in cyclohexane–toluene–MeCOEt (12:1:1).

Structure determinations. UV analysis was done according to the standardized method of Mabry *et al.* [12]. MS were analysed with the assistance of information presented by Markham [10].

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