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FLAVONOIDS OF *BALSAMORHIZA* AND *WYETHIA*

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Key Word Index—*Balsamorhiza hookeri*; *B. sagittata*; *Wyethia mollis*; Compositae; Heliantheae; flavonoids; isoflavones; flavanones; flavonols; prenylated flavonoids.

Abstract—The leaf exudate of *Balsamorhiza sagittata* was shown to contain a single major flavonoid, 6-hydroxykaempferol 7-methyl ether. The leaf exudate of *B. hookeri* contained quercetin 3-methyl ether, quercetin 3,3′-dimethyl ether and quercetagetin 3,6-dimethyl ether and possibly an 8-hydroxylated flavone. The occurrence of exudate flavonoids in *Balsamorhiza* is briefly reviewed. Further studies of *Wyethia mollis* showed the presence of several prenylated and/or *O*-methylated derivatives of the flavanones naringenin and eriodictyol including both 6,7-and 7,8-dihydrooxepinoeriodictyol. Several *O*-methylated derivatives of 5,7,3′,4′-tetrahydroxyisoflavone (orobol) were isolated including 6-*C*-prenylorobol 3′-methyl ether. This flavonoid profile is very similar to that of *W. angustifolia*.

INTRODUCTION

Published studies of *Balsamorhiza* and *Wyethia*, from this and other laboratories [1–8], have shown a rich array of flavonoids in the leaf exudates of all species so far examined. The pigments include simple and *C*-prenylated flavanones, isoflavones and *O*-methylated flavones and flavonols. To date, 11 species of *Wyethia* have been examined to greater or lesser extent, but only two species of *Balsamorhiza* have been studied; 14 species of *Wyethia* and seven species of *Balsamorhiza* are recognized. It is the purpose of the present paper to describe the exudate flavonoids of two additional species of *Balsamorhiza*, *B. sagittata* and *B. hookeri*, and to give a more detailed account of the flavonoids from the exudate of *Wyethia mollis*, preliminary observations on which appeared in an earlier publication [5].

RESULTS AND DISCUSSION

Balsamorhiza sagittata afforded the lowest yield of external flavonoids so far observed. Despite having several kilograms of plant material, we were able to isolate only a single flavonoid from the dichloromethane leaf wash. The compound was shown to be 3,5,6,4′-tetrahydroxy-7-methoxyflavone (**1**). A second phenolic compound was obtained but its mass and UV spectral data are not consistent with a flavonoid structure. An additional, very faint, spot was observed on TLC plates suggesting the presence of a highly *O*-methylated compound, possibly a flavone. Further work is underway on these compounds.

Balsamorhiza hookeri yielded four *O*-methylated flavonols. The three principal compounds identified were: quercetin 3-methyl ether (**2**), quercetin-3,3′-dimethyl ether (**3**), and quercetagetin 3,6-dimethyl ether (axillarin) (**4**). A small amount of a compound was isolated whose spectral characteristics are consistent with 5,8,4′-trihydroxy-3,6,7-trimethoxyflavone (**5**). Since this would be the first appearance of a flavonoid with 8-oxygenation in these genera, further work is required for corroboration of its structure.

With the exception of the putative 8-hydroxyflavonol, the compounds from *B. hookeri* and *B. sagittata* agree well with the flavonoids reported for *B. deltoidea* [5] and *B. macrophylla* [2]. 3,5,6,4′-Tetrahydroxy-7-methoxyflavone (**1**), the major pigment from *B. sagittata*, was reported earlier from *B. deltoidea* [5]. *Balsamorhiza deltoidea*, *B. hookeri* and *B. macrophylla*, have two compounds in common, quercetin 3-methyl ether (**2**) and quercetagetin 3,6-dimethyl ether (**4**). *Balsamorhiza deltoidea* and *B. macrophylla* have four compounds in common. The occurrence of flavonols in the four species of *Balsamorhiza* is summarized in Table 1.

Our earlier studies of *Wyethia* [1–5] showed a rich array of leaf-exudate flavonoids including isoflavones, flavanones, flavones and flavonols. In many instances the compounds exhibited *C*-prenylation and/or *O*-methylation. These reports are in agreement with observations on *Wyethia* from other laboratories [6–8]. A few of the more prominent compounds present in the leaf-wash of *W. mollis* were reported earlier [5]. Further work has resulted in the isolation and identification of additional pigments.

Four isoflavones were identified: orobol 7-methyl ether (santal) (**6**), orobol 3′-methyl ether (**7**), orobol 7,3′-dimethyl ether (**8**) and 8-*C*-prenylorobol 3′-methyl ether (**9**). This last mentioned compound is reported for the first time from *Wyethia* although the 6-*C*-prenyl isomer was reported from *W. angustifolia* [3]. We have identified 8-*C*-prenylnaringenin (**10**) and both 6- and 8-*C*-prenyleriodictyol (**11**, **12**), all previously reported from *Wyethia*. 8-*C*-prenyleriodictyol 3′- (or 4′)-methyl ether (**13**) and 8-*C*-prenylnaringenin 4′-methyl ether (**14**) were also obtained; neither has been reported from *Wyethia* before. Our report [5] of 8-*C*-prenyldihydroisorhamnetin (**15**) has been confirmed.

The most unusual compounds identified to date in our studies of these genera are the dihydrooxepino derivatives of eriodictyol, dihydroquercetin and 6′-hydrox-

Table 1. Summary of leaf exudate flavonoids of four species of *Balsamorhiza*

Flavonoid	Taxon*			
	delt	hook	macr	sagi
4′-Methylkaempferol	+			
6-Hydroxy-7-methylkaempferol	+			+
6-Methoxy-3-methylkaempferol	+			
3-Methylquercetin	+	+	+	
4′-Methylquercetin	+		+	
3,3′-Dimethylquercetin		+		
3,4′-Dimethylquercetin	+		+	
3′,4′-Dimethylquercetin	+			
7-Methylquercetagetin	+			
3,6-Dimethylquercetagetin	+	+	+	
6,3′-Dimethylquercetagetin			+	
3,6,7-Trimethylquercetagetin	+			
3,6,3′-Trimethylquercetagetin	+			
3,6,4′-Trimethylquercetagetin			+	

*delt = *B. deltoidea*; hook = *B. hookeri*; macr = *B. macrophylla*; sagi = *B. sagittata*.

ybutein all of which were obtained from *W. angustifolia* [3]. These compounds are formed by cyclization of the *C*-prenyl group through one of its terminal carbons to form a seven-membered ring. The eriodictyol derivative from *W. angustifolia* is based upon cyclization of the 8-prenyl group with the 7-hydroxyl group (**16**). Two compounds having very similar chromatographic and colour behaviour and mass spectral fragmentation patterns were isolated from *W. mollis*. One matched the compound from *W. angustifolia* (**16**) in all respects. The other failed to give a normal aluminium chloride/HCl shift in the UV spectrum suggesting that position-6 was substituted (**17**). Formation of such a compound can be rationalized by postulating ring closure of a prenyl group at position-6 with the hydroxyl group at position-7. Cyclization would likely occur with the sidechain in the 3-methyl-buten-3-yl form. Compounds with that sidechain have not been seen in *Wyethia* but their existence would not be surprising.

Samples of *W. mollis* were collected from four sites representing a major part of the range of the species. Four plants were taken from each site and the flavonoid pigment profiles determined for each individual. With the exception of two individuals from a population in north-central California (the 'Bridge' site, *Bohm*-1854*D*) the profiles were identical (given the overall complexity of the total leaf-exudate mixture). The difference between these two individuals and the others lay in the presence of 6,7-dihydrooxepinoeriodictyol (**17**) as a major component of the leaf-exudate profile of the 'Bridge' plants. In all other plants the 7,8-dihydrooxepino isomer (**16**) was a major component. It is not possible to rule out the existence of both isomers in all plants, owing to the complexity of the mixture, but the predominance of one isomer over the other in the two profiles was obvious.

The leaf-exudate flavonoid profile of *W. mollis* resembles that of *W. angustifolia* very closely. These species share orobol derivatives, *C*-prenylated as well as simpler flavanones, 8-*C*-prenyldihydroisorhamnetin and the rare seven membered prenyl cyclization products. These two species are more similar to each other, in terms of their exudate chemistry, than either is to any other species or group of species so far examined in these two genera. *Wyethia mollis* and *W. angustifolia* belong to section *Euwyethia* which consists of six species in all. The only other information available on the chemistry of this section is the report of 6-*C*-prenyleriodictyol in *W. arizonica* [8]. Further studies of *W. arizonica* and the remaining species of the section are needed before any discussion of relationships is warranted.

EXPERIMENTAL

Plant material. Balsamorhiza sagittata was collected near Princeton, British Columbia (*Bohm*-1895); *B. hookeri* was collected near Weed, California, Siskiyou Co. (*Bohm*-1870); *Wyethia mollis* was collected from four sites in California: Lake Crawley, Rt. 395, Mono Co. (*Bohm*-1854*A*); near Doyle, Rt. 395, Lassen Co. (*Bohm*-1854*B*); near Susanville, Cal. Rt. 36, Lassen Co. (*Bohm*-1854*C*); and near Bridge Campsite, Cal. Rt. 89, Shasta Co. (*Bohm*-1854*D*).

Extraction and isolation procedures. Entire dried leaves were soaked for a few minutes in CH_2Cl_2 and then rinsed with fresh solvent. The combined washings were evapd to dryness and chromatographed on a column of Polyclar-AT using increasing concns of MeOH in CH_2Cl_2. More polar compounds were eventually eluted with Me_2CO. Individual compounds were purified by TLC using solvents described earlier [1].

Determination of structure. Standard UV [9] and mass spectral [10] methods were employed. Structures of compounds **1**, **2**, **4**–**12** and **15** were encountered earlier in this series. Their identities were established in the present study by comparing R_f and colour properties as well as UV and mass spectral data with information presented in our earlier papers.

The structure of 3,3′-dimethylquercetin (**3**) was readily established from ms data which showed *m/z* 330, and fragments corresponding to a 5,7-dihydroxy A-ring and a B-ring with one hydroxyl and one methoxyl group. UV behaviour was essentially identical to that of isorhamnetin 3-glycoside, i.e., substituted oxygen functions at positions-3 and 3′.

The UV spectrum of compound (**13**) suggested that it was a flavanone (290, *ca* 330sh); an $AlCl_3$ shift placed an hydroxyl group at position-5 and suggested that position-6 was unsubstituted. Mass spectral data (*m/z* 370) suggested that the compound was a prenylated flavanone having one methoxyl and three hydroxyl groups. Mass fragmentation placed the methoxyl function on the B-ring and the prenyl function on the A-ring. Since UV data suggested that position-6 was unsubstituted the prenyl function must be at position-8. Limited material prevented establishment of the precise location of the methoxyl group. The compound is thus 8-*C*-prenyleriodictyol 3'- (or 4')-methyl ether.

Compound **14** also exhibited UV behaviour suggesting a flavanone (289, *ca* 330sh) and gave a normal $AlCl_3$ shift showing 5-OH and an unsubstituted position-6. Mass spectral analysis gave *m/z* 354 which is consistent with a prenylated flavanone with one methoxyl and two hydroxyl groups. Fragmentation showed the prenyl group to be on the A-ring and the methoxyl group to be on the B-ring. We conclude that the compound is 8-prenylnaringenin 4'-methyl ether. Its UV and colour characteristics were essentially identical with those of naringenin 4'-methyl ether.

Compound **16** gave a UV spectrum with a major peak at 290 nm with a shoulder at *ca* 332 nm indicative of a flavanone. A peak at *m/z* 313 (*ca* 17% of M^+) was consistent with a flavanone fragmentation [11]. This compound had chromatographic and colour properties very similar to those of compound **15**, 7,8-dihydrooxepinoeriodictyol, which was described from *W. angustifolia* in an earlier paper [3]. Compound **16** failed to give a normal $AlCl_3$ shift in the UV spectrum, however. Flavanones with a 5-OH group and a proton at position-6 exhibit aluminium chloride shifts of approximately 20 nm. In the present instance compound **16** exhibited an $AlCl_3$ shift of *ca* 8 nm. This is taken to indicate a 5-OH group as well as the presence of a substituent at position-6. Mass spectral fragmentation showed that ring-B contained two hydroxyl groups and that a prenyl group, or some variant thereof, was present on the A-ring. Mass fragmentation did not show the typical breakdown pattern of a prenyl group. Rather, there was a large $[M-15]^+$ peak suggesting loss of a single methyl group. This is consistent with the pattern seen with 7,8-dihydrooxepinoeriodictyol [3] except that in the present case the $[M-15]^+$ peak is of greater abundance than $[M]^+$ by a factor of about two. These observations are consistent with compound (**16**) being 6,7-dihydrooxepinoeriodictyol.

Note added in proof: Recently Robson and McCormick [*Biochem. Syst. Ecol.* (1988) **16**, 411] reported six *O*-methylated flavonols from *B. sagittata* collected in California (Modoc Co.). This contrasts with the very sparse pattern described above from our British Columbia collection. An assessment of variation in this wide ranging species is clearly needed.

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