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FLAVONOID AGLYCONES OF *HOLOCARPHA OBCONICA**

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Key Word Index—*Holocarpha obconica*; Asteraceae; Heliantheae; flavonoid aglycones; flavanones; flavones.

Abstract—Eleven flavonoid aglycones were isolated from the dichloromethane leaf-wash of the tarweed, *Holocarpha obconica*. Included within the extract were three novel flavanones, 7,4′-dimethoxyflavanone, 5,6,3′,4′-tetrahydroxy-7-methoxy-flavanone, and 3′,4′-dihydroxy-3,7-dimethoxyflavanone.

INTRODUCTION

The genus *Holocarpha* (DC.) Greene (Heliantheae: Madiinae) consists of four species of summer-flowering, self-incompatible annuals restricted to central and southern California [1]. Its species possess low chromosome numbers ($N = 4$ and 6) and highly specialized leaf and trichome anatomy [1–3], suggesting an advanced evolutionary disposition for the genus within the subtribe. These tarweeds are copiously glandular, and exude considerable amounts of aromatic resin. No previous reports of flavonoids from *Holocarpha* have appeared. Related tarweeds in the genus *Hemizonia* contain mixtures of flavanones, flavones, and flavonols in their leaf-wash extracts [4]. Emerson *et al.* [5] found a series of 8-methoxylated flavonols in the tarweed, *Calycadenia ciliosa* Greene, a structural type also found in *Hemizonia* [4]. However, flavanones were not among the constituents of *C. ciliosa*.

Holocarpha obconica (Clausen and Keck) Keck contains a complex mixture of flavonoids, including flavanones, flavones, and flavonols. Three of the flavanones, 7,4′-dimethoxyflavanone, 5,6,3′,4′-tetrahydroxy-7-methoxyflavanone, and 3′4′-dihydroxy-3,7-dimethoxyflavanone, appear to be new natural products.

RESULTS AND DISCUSSION

Eleven flavonoids were isolated from the dichloromethane leaf-wash of *Holocarpha obconica*. Five flavanones (**1–5**), two flavones (**6**, **11**), and four flavonols (**7–10**) were present in the dichloromethane leaf-wash.

The flavanones (**1–4**) appeared as faint dark spots under $UV_{366\,nm}$. Compound **1** was found in relatively small quantities. It has the highest R_f of all the flavonoids in *H. obconica*. It became pale green after spraying with Naturstoffreagenz A (NA). Its UV spectrum was typical of a flavanone, with a shoulder at 332 nm and a peak at 286 nm. Owing to persistent contamination from compound **11**, reactions of **1** with the shift reagents were inconclusive. The MS showed a parent ion at $m/z = 284$, indicating the presence of two methoxyl and no hydroxyl groups [6]. The lack of hydroxyl groups, particularly at C-5, is unusual, but not unprecedented [7], in the composites. Crawford and Stuessy [8] point out that resorcinol precursors are used in the biosynthetic pathways of members of the tribe Heliantheae. Thus, the synthesis of 5-unsubstituted compounds by tarweeds could be expected. The 7,4′-dihydroxyl counterpart of **1**, liquiritigenin, has been found in various plants [7], and 6-*C*-methyl-7,4′-dimethoxyflavanone is known from *Adina* [9].

Compound **2** is similar to **1**, with the addition of a hydroxyl group at C-5. It gave a bluish-green colour with NA and its UV and MS properties conform closely to those reported by Lam and Wrang [10].

Compound **3** became bright red after spraying with NA, suggesting an eriodictyol derivative. Sodium acetate fails to produce a shift relative to its methanol UV spectrum, placing a methoxyl group at C-7. Other shift reagents produce reactions similar to those with eriodictyol [11]. The MS data are in accord with the structure of this compound as 7-methyleriodictyol ($M^+ = 302$; A-ring fragment = 167; B-ring fragments = 109 and 136).

Compound **4** became yellow-brown after spraying with NA. A large sodium methoxide shift, lack of a sodium acetate shift, and a slightly acid-labile aluminium chloride shift relative to the UV spectrum in methanol suggest that this flavanone has free hydroxyl groups at C-5, C-3′, and C-4′, and a methoxyl group at C-7. Its mass spectrum (parent ion, $m/z = 318$) indicated that a fourth hydroxyl group is located on the A-ring, either at C-6 or C-8. Given the structures of the other compounds characterized in *H. obconica*, and on the basis of biosynthetic considerations, the most likely structure for **4** is 5,6,3′,4′-tetrahydroxy-7-methoxyflavanone. Insufficient material was available for NMR analysis.

Compound **5** is yellow under UV light which is characteristic of flavonols and 5-deoxy-flavanones [11], and became orange on spraying with NA. The methanol UV spectrum of this compound (shoulder at 332 nm and peak at 284 nm) suggests it as a 5-deoxyflavanone. It shows a large sodium methoxide shift, no sodium acetate shift, and an acid-labile aluminium chloride shift. This evidence supports the lack of C-5 substitution, the presence of *ortho*-dihydroxyl substitution on the B-ring, and the lack of a free hydroxyl group at C-7. Its mass spectrum (parent ion, $m/z = 316$) suggested the presence of two methoxyl and two hydroxyl groups. The fragmentation pattern of this compound agreed with the placement of a methoxyl group at C-3 [cf. 12 and 13]. Compound **5** is therefore

*Chemosystematic studies of the tarweeds (Asteraceae: Heliantheae: Madiinae) No. 2.

3',4'-dihydroxy-3,7-dimethoxyflavanone.

Compound **6** absorbs UV, and is yellow after spraying with NA. Its UV and MS characteristics indicate that it is luteolin 7-methyl ether. Compound **7** (3-methylkaempferol) absorbs UV and is bright green when sprayed with NA. Its UV and MS characteristics coincide with previously published reports [14, 15]. Compound **8** is yellow under UV, and is yellow-orange after spraying with NA. UV and MS data indicate that it is 3,5,3',4'-tetrahydroxy-7-methoxyflavone (rhamnetin). Compound **9** is dark under UV, and orange after spraying with NA. It is similar to **8** in its UV spectrum and reactions with shift reagents and its UV characteristics conform closely to those described by Urbatsch *et al.* [16] for 5,3',4'-trihydroxy-3,7-dimethoxyflavone. The MS data are also in accord with this structure. Compound **10** absorbs UV, and is orange after spraying with NA. Its UV and MS characteristics correspond closely to previously published reports [17, 18] of 5,6,3',4'-tetrahydroxy-3,7-dimethoxyflavone.

Compound **11** occurs at very low concentrations, and the proposed structure presented here is based largely on MS data. It was found as a contaminant with **1**, having a slightly lower R_f in the ethyl formate solvent system. The mass fragment structure reported for eupatorin [19] is in accord with fragments present in the MS of **11**.

EXPERIMENTAL

A voucher of *Holocarpha obconica* from Deer Valley Rd., California is deposited in UC (Ornduff 8917). Air-dried leaves and stems (360 g dry wt) were soaked in CH_2Cl_2. This extract was placed on a Polyclar AT column, and the constituent compounds eluted initially with 3:1 CH_2Cl_2–MeOH, and subsequently increasing proportions of MeOH in the eluent mixture. The compounds were viewed under $UV_{366\,nm}$ before and after spraying with Naturstoffreagenz A. Each flavonoid was purified using TLC on Polyamide 6.6 in ethyl formate–cyclohexane–*n*-butyl acetate–formic acid (50:25:23:2). UV spectra and MS were obtained for the purified compounds.

7,4'-*Dimethoxyflavanone* (**1**). UV λ_{max}^{MeOH} nm: 286, 332sh. MS *m/z*: 284 $[M]^+$, 177 $[A]^+$, 164 $[A-CH_2]$, 121 $[B+CH_2]$, 107 $[B]^+$.

5,6,3',4'-*Tetrahydroxy*-7-*methoxyflavanone* (**4**). UV λ_{max}^{MeOH} nm: 288, 336sh; NaOMe: 288, 354; NaOAc: 288, 336sh; $AlCl_3$: 315, 375sh; $AlCl_3$–HCl: 312, 375sh. MS *m/z*: 318 $[M]^+$, 289 $[M-CHO]$, 209 $[A]^-$, 196 $[A-CH]$, 179 $[A-OMe]$, 167 $[A-CH-OMe]$, 123 $[B+CH_2]$, 110 $[B]^+$.

3',4'-*Dihydroxy*-3,7-*dimethoxyflavanone* (**5**). UV λ_{max}^{MeOH} nm: 284, 332sh; NaOMe: 295, 369; NaOAc: 284, 332sh; $AlCl_3$: 293, 366; $AlCl_3$–HCl: 293, 366sh. MS *m/z*: 316 $[M]^+$, 315 $[M-H]$, 287 $[M-CHO]$, 273 $[M-C-OMe]$, 167 $[B+C_2H_2+OMe]$, 149 $[A]^+$, 137 $[B]^+$, 123 $[A-CO]$.

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