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FLAVONOIDS FROM THE EXTERNAL LEAF RESINS OF FOUR *HEMIZONIA* SPECIES (ASTERACEAE)

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Key Word Index—*Hemizonia fasciculata*; *H. increscens* subsp. *villosa*; *H. lobbii*; *H. pentactis*; Asteraceae; methylated flavonoids; 6-methoxy-5,7,8,3′,4′-pentahydroxyflavone; leaf resin.

Abstract—The external leaf resins of four *Hemizonia* species afforded nine methylated flavonoids, including flavanones, flavones and flavonols. Besides the rare compounds 7-methyleriodictyol and 3,6,8-trimethoxy-5,7,3′,4′-tetrahydroxyflavone the new 6-methoxy-5,7,8,3′,4′-pentahydroxyflavone was isolated and identified by spectroscopic means.

INTRODUCTION

The composite genus *Hemizonia* (*Heliantheae*: *Madiinae*) comprises 31, mostly annual species, restricted to the valleys and foothills of California, Baja California Norte, Mexico, and their respective offshore islands [1, 2]. The common name tarweed reflects the viscid-glandular herbage that is characteristic for most *Hemizonia* species.

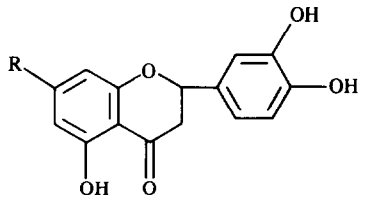
Little is known about the chemistry of the genus at present, but recently, several labdane type diterpenes and chromene derivatives have been reported from *Hemizonia fitchii* (section *Centromedia*), *H. lutescens* (section *Hemizonia*) and *H. congesta* (= *H. leucocephala*, section *Hemizonia*) [3, 4]. In continuation of our studies on resin secreting plants from California and Mexico [5–7], we have analysed the composition of the external leaf resins of *Hemizonia fasciculata* (DC.) Torr. et Gray, *H. increscens* (Hall ex Keck) Tanowitz subsp. *villosa* Tanowitz, *H. lobbii* Greene and *H. pentactis* (Keck) Keck (all section *Madiomeris*). We have found methylated flavonoids to be among the characteristic components. In this study, we report the identification of nine flavonoid aglycones, including the new 6-methoxy-5,7,8,3′,4′-pentahydroxyflavone.

RESULTS AND DISCUSSION

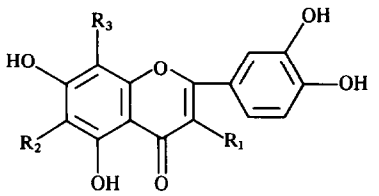
The viscid-resinous leaves and stems of *Hemizonia fasciculata*, *H. increscens* subsp. *villosa*, *H. lobbii* and *H. pentactis* were dipped in methanol to remove those compounds present as epicuticular components. The resulting extracts were dark yellow in colour indicating the presence of phenolic compounds. Chromatography of the crude extracts on Polyamide DC 6 TLC plates revealed several flavonoid aglycones. All the compounds visible under $UV_{366\,nm}$ showed dark absorbance, except two which were yellow. Spraying the developed plates with Naturstoffreagenz A turned most of these constituents to an orange colour, suggesting the presence of 3′,4′-dihydroxy systems [8]. In addition, *Hemizonia increscens* subsp. *villosa*, *H. lobbii* and *H. pentactis* showed two spots that turned red in visible light after the sprayed plate had been exposed for *ca* 1 hr. The spectral data of these two compounds and cochromatography with authentic standards, clearly indicated that they were the flavanones eriodictyol (**1**) and the rare 7-methyleriodictyol (**2**). The latter has been reported only once previously from a farinaceous fern [9]. By column chromatography on Sephadex LH-20 and by preparative thin layer chromatography on Polyamide DC 6, seven additional flavonoids were isolated from *Hemizonia increscens* subsp. *villosa*, *H. lobbii*, and *H. pentactis*. These species yielded luteolin (**3**)‡, quercetin (**4**), 3-methylquercetin (**5**) and patuletin (**6**). Two of the three remaining dark compounds (**7** and **8**) had similar UV data, especially with regard to the $AlCl_3$–HCl shifts of Band I in the range of +15–18 nm relative to the methanol spectrum. Shifts of this magnitude are indicative of 5-hydroxyflavones and 5-hydroxy-3-substituted flavonols with methoxy groups at C-6 [10]. From the spectral data and colour reactions on the TLC plate after spraying with Naturstoffreagenz A these two compounds must have additional hydroxy groups at the C-7,C-3′ and C-4′ positions and were identified as 3,6-dimethoxy-5,7,3′,4′-tetrahydroxyflavone (**7**) and the rare 3,6,8-trimethoxy-5,7,3′,4′-tetrahydroxyflavone (**8**), known only from *Pluchea saggitalis* (Asteraceae) [11].

The remaining dark absorbing compound (**9**) from its mass spectrum had a molecular weight of 332, corresponding to a flavone with one methoxy and five hydroxy groups. The ^1H NMR spectrum showed the typical signals for a 3′,4′ substituted B-ring (see Experimental), one proton singlet at δ6.4 and the signal for a methoxy group at δ3.8. The UV data, the fragment at *m/z* 137 in the mass spectrum and the orange colour of the compound after spraying with Naturstoffreagenz A were indicative of two hydroxy groups at the 3′ and 4′-positions of the B-ring. The UV data as well as the dark absorbance

‡The structures of well known flavonoids are not illustrated.



1 R = OH
2 R = OMe



6 R_1 = OH; R_2 = OMe; R_3 = H
7 $R_{1,2}$ = OMe; R_3 = H
8 $R_{1,2,3}$ = OMe
9 R_1 = H; R_2 = OMe; R_3 = OH

of the compound under $UV_{366\,nm}$ led also to the assignment of two hydroxy groups at the C-5 and C-7 positions. The remaining hydroxy group and methoxy group had therefore to be assigned to either of the C-3, C-6 or C-8 positions. The shift of Band I in the $AlCl_3$–HCl spectrum, compared to the methanol spectrum, was + 17 nm, which suggests a 6-methoxy-5-hydroxyflavone [10]. This argument is supported by the intensities of the $[M-18]^+$ and $[M-43]^+$ fragments in the mass spectrum (see Experimental) that can be used to distinguish 6-methoxyflavones from 8-methoxyflavones [10]. Since the compound is not a flavonol from its UV spectrum and from its dark absorbance in UV light (366 nm), the remaining hydroxy group must be located at the 8-position. Thus we assign this new structure as 6-methoxy-5,7,8,3',4'-pentahydroxyflavone.

Hemizonia fasciculata displayed a simplified pattern (compounds **3**–**7**) compared to the other species (compounds **1**–**9**). The chemical data are highly consistent with morphological, cytological and ecogeographical data for these four species [12]. *Hemizonia increscens* subsp. *villosa*, *H. lobbii* and *H. pentactis* are considered a closely related phyletic group [13]. The external leaf flavonoid patterns underline the clear affinities within this group. The 6-methoxyflavones and -flavonols and eriodictyol flavanone derivatives may also prove significant in a broad chemotaxonomic survey of the section and possibly the genus.

EXPERIMENTAL

Vouchers of *Hemizonia fasciculata*, *H. increscens* subsp. *villosa*, *H. lobbii* and *H. pentactis* (identified by Dr. B. D. Tanowitz and Professor D. M. Smith) are deposited in the herbarium of the University of California, Santa Barbara. The air dried leaves and stems were dipped on MeOH for *ca* 1 min to extract the external resin components. The crude resin was fractionated by CC on Sephadex LH-20 with MeOH as eluent [5]. Further separation of resulting flavonoid mixtures was achieved by prep. TLC on Polyamide DC 6 (Macherey & Nagel, West Germany), solvent system C_6H_6–Me_2COEt–MeOH–H_2O (60:22:20:3). The compounds were viewed under $UV_{366\,nm}$ before and after spraying with Naturstoffreagenz A (1% soln of diphenylboric acid-2-amino ethyl ester in MeOH). Each flavonoid was purified by CC on Sephadex LH-20, MeOH as eluent, prior to UV analysis. UV spectra were recorded according to standard procedures [14]. Known compounds were identified by comparison of their spectra with published data and whenever possible by cochromatography with authentic standards.

6-Methoxy-5,7,8,3',4'-pentahydroxyflavone (**9**). UV λ_{max}^{MeOH} nm: 254, 265 sh, 350; NaOMe: 272, 340, 410; $AlCl_3$: 273, 304 sh, 340 sh, 430; $AlCl_3$–HCl: 268, 280 sh, 304 sh, 367, 405 sh; NaOAc: 272, 310 sh, 386; NaOAc–H_3BO_3: 260, 310 sh., 377. ^1H NMR (60 MHz, *d*-MeOH, TMS, underivatized compound): δ3.8 (3H, *s*, OMe/C-6), 6.4 (1H, *s*, C-3), 6.8 (1H, *d*, J = 9 Hz, C-5'), 7.5 (1H, *dd*, J = 9 Hz, 2 Hz, C-6'), 7.7 (1H, *d*, J = 2 Hz, C-2'). EIMS (Varian-MAT CH 7A; 70 eV, direct inlet) *m/z* (%): 332 $[M]^+$ (100), 317 $(M-Me]^+$ (20), 314 $[M-18]^+$ (45), 289 $[M-43]^+$ (78), 137 $[B_2]^+$ (15).

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