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FLAVONOIDS OF *ADENOTHAMNUS VALIDUS**

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IN MEMORY OF TONY SWAIN, 1922–1987

Key Word Index—*Adenothamnus validus*; Asteraceae; Heliantheae; flavonoid aglycones; flavonoid glycosides; flavanones; dihydroflavonols; flavones; flavonols.

Abstract—Twelve flavonoid aglycones and six flavonoid glycosides were isolated from the tarweed, *Adenothamnus validus*. 6-*O*-Methylation occurs in several of its constituents. Two rare glycosides, homoplantaginin and jaceoside, are among its polar flavonoids.

INTRODUCTION

The genus *Adenothamnus* (Asteraceae: Heliantheae: Madiinae) is a monotypic endemic of northern Baja California, Mexico [1]. Its perennial habit and relatively simple glandular trichomes suggest that it is a primitive member of the subtribe Madiinae [2], but its chromosome number ($n = 14$) [3] provides contradictory evidence, indicating that it is a polyploid derivative. Although the relationships of *A. validus* (Brandegee) Keck with other North American tarweeds requires clarification, it has been considered the most likely extant relative to the ancestor of the Hawaiian tarweeds, on ecological, morphological, and cytological grounds [4]. From this standpoint, an examination and comparison of its flavonoids and those of the Hawaiian tarweeds could provide further evidence on this hypothesis. This paper describes the flavonoids isolated from dichloromethane and methanol extracts of *A. validus*.

RESULTS AND DISCUSSION

Five flavanones (**1**–**5**), one dihydroflavonol (**6**), three flavones (**7**–**9**), and three flavonols (**10**–**12**) comprise the major constituents in the non-polar flavonoid profile of *Adenothamnus validus*. Three naringenin derivatives (naringenin, **1**, 4′-methylnaringenin, **2**, and 7,4′-dimethylnaringenin, **3**) absorb UV_{366nm} and become green after being sprayed with Naturstoffreagenz A (NA). Compound **3** gives a characteristic bright blue-green colour. Chromatographic and spectrophotometric comparisons of compounds **1** and **3** with standards and with previous reports [5–7] confirm their identities. Compound **2** exhibits a strong sodium acetate peak at 328 nm, in contrast to its UV spectrum in MeOH, which shows a shoulder at 338 nm and a peak at 292 nm, indicating the presence of a hydroxyl group at C-7. Mass spectral fragments at m/z 153 $[A]^+$ and m/z 123 $[B]^+$ confirm its structure as that of isosakuranetin (4′-methylnaringenin). Two eriodictyol derivatives (7-methyleriodictyol, **4**, and persicogenin, **5**) are also found in the non-polar leaf wash. Compound **4** produces a brilliant red colour after spraying with NA, and compound **5** is initially olive after spraying, but becomes brick red upon standing, as described in ref. [8]. The UV, mass spectrum (MS), and R_f of compound **4** correspond exactly with those previously determined [9] for 7-methyleriodictyol in *Holocarpha obconica* (Clausen and Keck) Keck. It is also known from several species in another tarweed genus, *Hemizonia* [10]. Persicogenin (7,4′-dimethyleriodictyol, **5**) has not been recorded from within the Madiinae previously. Its UV, MS, and colour reaction with NA correspond to characterizations of this compound from *Notholaena* [8] and *Prunus* [11].

The dihydroflavonol padmatin (7-methyldihydroquercetin, **6**) absorbs UV weakly, and produces an intense dark spot encircled by an orange halo when sprayed with NA. Its UV spectral characteristics are very similar to those of 7-methyleriodictyol (**4**). However, a mass peak at m/z 318 indicates the presence of four hydroxyl and one methoxyl group on the molecule [12], and the large peak at m/z 167 is characteristic of a C-5 hydroxyl, C-7 methoxyl substitution pattern on the A-ring. The nature of the colour reaction with NA and the absence of an $[M-1]^+$ ion indicate that the compound is a dihydroflavonol, with a hydroxyl at C-3. Mass fragments at m/z 138 and 122 correspond to B-ring fragments with *ortho*-dihydroxyl substitution.

The flavone aglycones produced by *A. validus* are 7-methylluteolin (**7**), eupafolin (**8**), and hispidulin (**9**). Compounds **7** and **8** are methylated luteolin derivatives which absorb UV and provide yellow colour reactions with NA. Eupafolin (6-methoxyluteolin, **8**) becomes greenish-yellow when fumed with ammonia. The structure of compound **7** was confirmed by its MS, with a mass peak at m/z 300 (three hydroxyl and one methoxyl groups on the flavone nucleus [12]), and major ions at m/z 167 $[A]^+$ and m/z 136 $[B]^+$, and its UV spectrum, which showed a partially acid labile aluminium chloride shift in Band I and no significant sodium acetate shift in Band II [13]. The UV and MS results obtained for compound **8** are in complete agreement with published data for eupafolin

*Part 3 in the series, 'Chemosystematic Studies of the Tarweeds (Asteraceae: Heliantheae: Madiinae)'. For part 2, see *Phytochemistry* **26**, 2128.

[14–16]. Compound **9** exhibits a dark green colour after spraying with NA, indicating the presence of a single hydroxyl group on the B-ring, at C-4′. Its UV and MS are identical to those of hispidulin (6-methoxyapigenin) [16, 17]. Eupafolin and hispidulin are known as free aglycones from a large number of angiosperms, including numerous composites [18].

The three flavonol aglycones characterized from the dichloromethane wash of *A. validus* are isokaempferide (**10**), 6-methoxykaempferol 3-methyl ether (**11**), and rhamnetin (**12**). Compounds **10** and **11** absorb UV and are green after spraying with NA. The published UV spectra for isokaempferide correspond closely to the results obtained from compound **10** [19], and the MS confirms this determination. This compound has also been found in *Holocarpha obconica* [9] and many other angiosperms [18]. The UV and MS of compound **11** correspond to those of a standard of 6-methoxykaempferol 3-methyl ether and to published data [20, 21]. Rhamnetin (7-methylquercetin, **12**) reflects UV and is yellow after spraying with NA. Its UV spectrum is in accord with that of ref. [22], and its MS corroborates this structure.

The MeOH extract of *A. validus* contains six flavonoid glycosides. Two of these glycosides are flavones (**13**, **14**), three are flavonols (**15**–**17**), and one is a flavanone (**18**). Compound **13** (homoplantaginin) shows UV and MS behaviour very similar to that of hispidulin (**9**), with the exception that the presence of glucose at C-7 prevents a Band II shift with sodium acetate. Compound **14** (jaceoside) also absorbs UV, but is brown after spraying with NA, suggesting that a methoxyl group is situated on the B-ring. The greater magnitude of the sodium methoxide Band I relative to Band I in MeOH places a hydroxyl group at C-4′. The shape of Band II in MeOH confirms the presence of two oxygen substitutions on the B-ring, and a major mass fragment at *m/z* 149 is also consistent with a 3′-OMe, 4′-OH substitution pattern on the B-ring. This rare luteolin dimethyl ether glycoside is also known from *Centaurea* [23]. Quercetin 3-*O*-glucoside (**15**) and patuletin 3-*O*-glucoside (**16**) are well known constituents of many angiosperms, including members of the Hawaiian tarweed genus, *Dubautia* [24]. Patulitrin (patuletin 7-*O*-glucoside, **17**) is also found in *A. validus*. It is yellow under UV and becomes bright yellow-orange after spraying with NA. The MS of the aglycone of compound **17** is in complete accord with the structure of patuletin, and its UV spectral properties indicate that the glucose moiety is at C-7. Compound **18** has not been fully characterized because of its low concentration in the MeOH extract. However, it is virtually colourless under UV, and becomes bright red after spraying with NA. This behaviour is characteristic of eriodictyol. Its R_f relative to the other glycosides indicates that it is a monoglycoside, and its colour reaction also indicates that the sugar is at C-7, rather than on the B-ring.

EXPERIMENTAL

An air-dried bulk sample (*ca* 200 g dry wt of leaves, stems, and inflorescences) of *Adenothamnus validus* from a roadside 2.2 miles from the La Lolla Maile park sign, Punta Banda, Baja California, Mexico (*Witter* 86–88, 89, 91, 93, 98, 99, 101, 104, 111) was washed in dichloromethane for 30 min and then extracted in 80% MeOH in dist. H_2O for 7 days. Vouchers are depositied in the herbarium at the University of California at Davis (UCD). The CH_2Cl_2 wash was evapd to dryness, taken up in a small volume of CH_2Cl_2–MeOH (3:1) and placed on a Polyclar AT column. Development of the column was performed with increasing proportions of MeOH in CH_2Cl_2, in the series 3:1, 2:1, 1:1, 1:2, followed by 100% MeOH. Compounds from this column were examined and purified using TLC (Polyamide 6.6) in HCO_2ET cyclohexane–*n*-butyl acetate–HCO_2H (50:25:23:2). The polar extract was chromatographed on an LH-20 column using the techniques described by Nicholls and Bohm [25], except that dichloroethane was used in place of benzene in the organic solvent for glycosides. UV analyses were performed according to the methods of Mabry *et al.* [22], and mass spectra were interpreted with the aid of information presented by Markham [13] and Wollenweber and Dietz [12]. Hydrolysis of glycosides with trifluoroacetic acid, and chromatography against standards, were also employed in the structural determinations.

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