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FLAVONOID AGLYCONES EXCRETED BY THREE *CALCEOLARIA* SPECIES

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Key Word Index—*Calceolaria*; Scrophulariaceae; leaf exudate; rare 8-methoxy flavonoids; gossypetin 7,8,3′-trimethyl ether; isoscutellarein 7,8,4′-trimethyl ether and 8,4′-dimethyl ether; herbacetin 7,8,4′-trimethyl ether.

Abstract—The lipophilic exudate produced by *Calceolaria irazuensis* leaf glands was shown to contain small amounts of three rare 8-methoxy flavonoids and one new one: gossypetin 7,8,3′-trimethyl ether. The leaf exudates of *C. chelidonioides* and *C. scabiosifolia* also contain trace amounts of flavonoid aglycones.

INTRODUCTION

One of the glandular species of *Calceolaria* (Scrophulariaceae), *C. glandulosa*, has been recently reported to contain a new primarane diterpene as one of its major natural products [1]. We assume that this diterpene, 'extracted' from aerial parts with chloroform, is in fact a constituent of the glandular excretion. We have now analysed three species of *Calceolaria* for the presence and identity of externally accumulated flavonoids.

RESULTS AND DISCUSSION

The leaf exudate of *Calceolaria irazuensis* Donn. Sm., obtained by rinsing freshly collected aerial parts with acetone, was processed by column chromatography in the usual manner. It appeared to consist mostly of terpenoid material which was not studied further. However, the exudate also contained 10 flavonoid aglycones, two of which were isolated in amounts just sufficient to allow MS and NMR spectra to be obtained. The occurrence of the ion [M − 15] as base peak in the MS of both products pointed to 8-methoxy flavonoids. The ^{1}H NMR spectrum of compound **1** (dark spot on polyamide, remaining dark with NA) showed the characteristic low field C-5 hydroxy signal, three methoxy signals, two aromatic one-proton singlets, and a pair of aromatic two-proton *ortho* coupled doublets. The latter indicate a symmetrical mono-substituted B-ring. The carbon spectrum compares well with some spectra of similar flavones reported in Markham's review [2] of flavonoid ^{13}C NMR (e.g. salvigenin and pectolinarigenin) if one makes allowance for C-8 rather than C-6 substitution. The structure of **1** is thus deduced to be 5-hydroxy-7,8,4′-trimethoxyflavone (isoscutellarein-7,8,4′-trimethyl ether). The proton spectrum of **2** (dull orange spot on polyamide in UV, brownish with NA) is very similar to that of **1** except that one aromatic singlet has disappeared (C-3–H) and a new low field broad singlet (C-3–OH) is present. The ^{13}C NMR spectrum clearly shows that C-2, C-3 and C-4 belong to a flavonol rather than a flavone. Except for minor shift differences most of the other signals are very similar to those of **1**. The structure of **2** is thus established as 3,5-dihydroxy-7,8,4′-trimethoxyflavone (herbacetin 7,8,4′-trimethyl ether). Its identity with tambulin was further confirmed by direct TLC comparison with a marker. With the identification of these compounds as trimethyl derivatives of isoscutellarein and of herbacetin, respectively, it seemed reasonable to compare some of the more polar compounds with the available dimethyl derivatives of these flavonoids. Thus, isoscutellarein 8,4′-dimethyl ether and herbacetin 8,4′-dimethyl ether were identified and gossypetin-7,8,3′-trimethyl ether was also found. Further flavonoids present in this exudate material were the 7-methyl ether, the 4′-methyl ether and the 7,4′-dimethyl ether of kaempferol and apigenin 4′-methyl ether.

Only a small amount of *C. scabiosifolia* Roem. et Schult was available. Its lipophilic exudate was passed over Sephadex and the flavonoids present were identified by direct comparison with markers to be gossypetin 3,8,3′-trimethyl ether, quercetin 3-methyl ether, quercetin 3,3′-dimethyl ether and kaempferol 3-methyl ether. Quercetin 3-methyl ether and quercetin 3,3′-dimethyl ether were also present in the exudate of *C. chelidonioides* H.B.K., but only a faint trace of kaempferol 3-methyl ether was detected and the presence of gossypetin 3,8,3′-trimethyl ether remains doubtful.

Isoscutellarein 7,8,4′-trimethyl ether (**1**) has been isolated as a natural product only once before, from fruit peel of *Citrus reticulata* [3]. The first report of isoscutellarein 8,4′-dimethyl ether had to be revised after synthesis; it is known to date only as the aglycone (bucegin) obtained from a glycoside found in the liverwort, *Bucegia romanica* [cf. 4]. Herbacetin 7,8,4′-trimethyl ether (**2**, tambulin) was first found in *Zanthoxylum acanthopodium*, and was erroneously reported as the 3,8,4′-trimethyl derivative. After re-isolation from the same source the revised structure was later confirmed by synthesis [5]. From what we now know about the exudate flavonoids of *Achillea* [cf. 6, 7], a recent report on the occurrence of tambulin in *A. depressa* [8] is probably erroneous. Gossypetin 3,8,3′-trimethyl ether was first isolated from *Cyanostegia angustifolia* [9], and later from four further plant species, three of which are Asteraceae [cf. 4]. The report of gossypetin 7,8,3′-trimethyl ether

from *Heteromma simplicifolium* was not substantiated by synthesis [10]. Ours is the first report, therefore, of its occurrence as a natural flavonol.

To our knowledge *Diplacus aurantiacus* was until now the only Scrophulariaceae known to exhibit flavonoid excretion [11, 12], since the localization of the highly methylated flavonol digicitrin from *Digitalis purpurea* [13] is unknown. The identification of exudate flavonoids in *Calceolaria* is thus another interesting result in our continuing study on the distribution of free flavonoid aglycones in higher plants [14, 15].

EXPERIMENTAL

Calceolaria chelidoniodes and *C. irazuensis* were cultivated in the Botanical Garden at Darmstadt and *C. scabiosifolia* in the Botanical Garden at Saarbrücken. Seeds of *C. irazuensis* were collected for this purpose in March 1986 in Costa Rica, Prov. San Jose, where it grows as a common decumbent perennial. The other two species have long been grown in Botanical Gardens and the origin of seeds is unknown. Voucher specimens of *C. irazuensis* are deposited at the Herbarium of Indiana University (IND) at Bloomington, Ind. and in E.W.'s personal herbarium in Darmstadt.

Freshly collected aerial parts were rinsed with Me_2CO to dissolve the exudate material. Concd solns were column chromatographed over Sephadex LH-20, eluted with MeOH, to separate the flavonoids from the large amount of terpenoid material. TLC analysis of fractions and comparisons with markers were done on polyamide DC-11 [solvents: petrol 100–140–toluene–MeCOEt–MeOH (12:6:1:1), toluene–petrol 100–140–MeCOEt–MeOH (12:6:2:1)] and toluene–dioxane–MeOH (8:1:1) and on silica gel [solvents: toluene–MeCOEt (9:1) and toluene–dioxane–HOAc (18:5:1)]. Chromatograms were evaluated in UV_{366} before and after spraying with Naturstoffreagenz A (NA). Authentic samples of synthetic herbacetin 7,8,4′-triMe and gossypetin 7,8,3′-triMe were provided by T. Horie [10], and synthetic isoscutellarein 8,4′-diMe was from M. Nakayama [16]. Natural gossypetin 3,8,3′-triMe was isolated from *Gutierrezia microcephala* [17]. The other flavonoid aglycones were available in E.W.'s lab.

Isoscutellarein 7,8,4′-trimethyl ether. R_f on polyamide DC-11 (petrol–toluene–MeCOEt–MeOH 12:6:1:1) 0.54 (kaempferol 7,4′-diMe has R_f 0.48 in this solvent). UV λ_{max}^{MeOH} nm 353 (sh), 302, 275; $+AlCl_3$ 349, 312, 277, unchanged with HCl; +NaOH 360 (sh), 276; no reaction with NaOAc and H_3BO_3. MS *m/z* (rel. int.) 328 ($[M]^+$, 60), 318 ([M − 15], 100), 298 (7), 181 (11), 153 (21). ^{1}H NMR (200 MHz, DMSO-d_6): 12.75 (1H, *s*; 5-OH), 8.05 (2H, *d*, *J* = 9 Hz; H-2′,6′), 7.16 (2H, *d*, *J* = 9 Hz; H-3′, 5′), 6.94 (1H, *s*; H-3), 6.60 (1H, *s*; H-6), 3.93, 3.88, 3.85 (3H each, *s*; 3 × OMe). ^{13}C NMR (50 MHz, DMSO-d_6): 163.5 (C-2), 103.3 (C-3), 182.2 (C-4), 156.7 (C-5), 96.0 (C-6), 158.4 (C-7), 128.5 (C-8), 148.8 (C-9), 103.9 (C-10), 122–9 (C-1′), 128.3 (C-2′), 114.8 (C-3′), 162.5 (C-4′), 114.8 (C-5′), 128.3 (C-6′), 56.5 (7-OMe), 61.1 (8-OMe), 55.6 (4′-OMe).

Herbacetin 7,8,4′-trimethyl ether. R_f on polyamide DC-11 (petrol–toluene–MeCOEt–MeOH 12:6:1:1) 0.64. UV λ_{max}^{MeOH} nm 380, 325, 272; $+AlCl_3$ 443, 358, 310, 275, unchanged with HCl; +NaOH 420, 327, 275; no reaction with NaOAc and H_3BO_3. MS *m/z* (rel. int.) 344 ($[M]^+$, 44), 329 ([M − 15] 100), 313 (11), 153 (16). ^{1}H NMR (200 MHz, DMSO-d_6): 12.26 (1H, *s*; 5-OH), 9.66 (1H, *br s*, 3-OH), 8.17 (2H, *d*, *J* = 9 Hz; H-2′, 6′), 7.16 (2H, *d*, *J* = 9 Hz; H-3′, 5′), 6.58 (1H, *s*; H-6), 3.90, 3.84, 3.81 (3H, each, *s*; 3 × OMe). ^{13}C NMR (50 MHz, DMSO-d_6): 155.9 (H-2), 136.1 (H-3), 176.5 (H-4), 155.9 (H-5), 95.3 (H-6), 157.9 (H-7), 128.3 (H-8), 146.7 (H-9), 103.5 (H-10), 123. 3 (H-1′), 129.3 (H-2′), 114.3 (H-3′), 160.7 (H-4′), 114.3 (H-5′), 129.3 (H-6′), 56.5 (7-OMe), 61.1 (8-OMe), 55.4 (4′-OMe).

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