Flavonoids from the Leaf Resin of Snakeweed, *Gutierrezia sarothrae*

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The resinous material excreted by aerial parts of snakeweed, *Gutierrezia sarothrae,* was analyzed for flavonoid aglycones. Six major flavonoids were characterized by their spectral data; nine minor flavonoids were identified by direct comparisons with markers. The major flavones form three pairs of isomeric products, five of which are 6,8-methoxylated.

Introduction

Snakeweed, *Gutierrezia sarothrae* (Pursh) Britton (Asteraceae, Astereae), also called broomweed, is a bushy erect suffrutescent shrub of low stature, growing in the western United States and in northern Mexico, flowering from midsummer until autumn. It is an aggressive weed that invades livestock ranges particularly after overgrazing and provides limited food for sheep and horses when better forage is lacking. Its linear leaves are glabrous and markedly resinous-glandular [1]. The resinous herbage attracted our interest in the course of continuing studies on the occurrence and distribution of free flavonoids accumulated on plant surfaces [2]. To our knowledge aerial parts of this species have been studied three times before. Common mono- and sesquiterpenes were reported from essential oils [3], and a series of diterpenes was found in the extract of ground aerial parts [4]. A 1976 doctoral thesis reported terpenoids and described the new compound sarothrin, 5,7,4′-trihydroxy-3,6,8-trimethoxy flavone, as the sole flavonoid thus far known for this species [5]. The following report describes the flavonoid aglycones that we found in the leaf resin of *Gutierrezia sarothrae*.

Materials and Methods

Aerial parts of *Gutierrezia sarothrae* were collected from chapparal vegetation in San Diego County, CA (July 28, 1984; G. Yatskievych and K. McCrary, 84–108; voucher specimens deposited at ARIZ and in E. W.'s herbarium in Darmstadt). After evaporation of the solvent, the acetone wash of 492 g of air-dried leaf and stem material yielded 29.82 g of exudate, *i.e.* ca. 6% of leaf dry weight. 10 g of this resin were passed over Sephadex LH-20, eluted with MeOH and the flavonoid portion obtained was further purified by column chromatography on silica and on polyamide as described previously [6]. Some fractions were further separated by preparative TLC on silica. Control of fractions and TLC comparisons with markers were done on polyamide and on silica and spots were visualized by spraying with Naturstoffreagenz-A (NA) and with a $MnCl_2$ reagent, respectively (*c.f.* [6, 7]). Mass spectra were recorded on a Varian MAT 311. ^{1}H NMR spectra were recorded on a Nicolet NTC 200 FT at 200 MHz; ^{13}C NMR spectra were obtained on the same instrument at 50 MHz. For ^{13}C NMR data see Table I. Melting points are uncorrected.

Compound **1**. Melting point not determined. UV λ_{max}^{MeOH} 350, 278, 260; $AlCl_3$ 378, 285, 270; +HCl 374, 289, 270; NaOH 416, 346, 280; NaOAc 367, 283; +H_3BO_3 325, 282. MS *m*/*z* (rel. int.) 390 (89%, M^+), 375 (100%, M-15).

Compound **2**. M.p. 168–170 °C (no lit. data). UV λ_{max}^{MeOH} 350, 281, 262; $AlCl_3$ 375, 288, 270; +HCl

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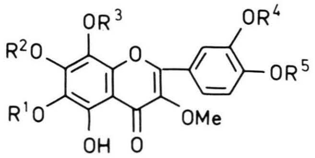
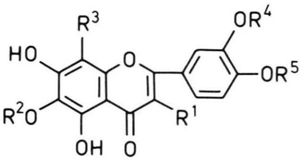
Table I. ^{13}C NMR spectra (DMSO-d_6).

Carbon	**2**	**3**	**6**
2	153.9	155.7	163.4
3	137.4	137.9	102.8
4	177.4	178.6	182.3
5	147.9	148.0	148.0
6	132.6	135.4	131.4
7	150.0	152.3	150.8
8	128.6	132.4	127.9
9	144.9	144.3	145.4
10	101.1	106.7	102.9
1′	122.9	122.2	121.6
2′	114.8	114.8	109.9
3′	146.5	146.4	148.4
4′	150.0	150.4	150.8
5′	112.0	112.0	115.9
6′	120.0	120.4	120.1
C3-OMe	59.7	59.6	–
C6-OMe	59.7	60.5	60.2
C7-OMe	–	61.8	–
C8-OMe	60.7	61.4	61.2
C4′-OMe	55.6	55.4	–
C3′-OMe	–	–	55.8



1 : $R^1=R^3=R^4=Me$, $R^2=R^5=H$
2 : $R^1=R^3=R^5=Me$, $R^2=R^4=H$
3 : $R^1=R^2=R^3=R^5=Me$, $R^4=H$
4 : $R^1=R^2=R^3=R^4=Me$, $R^5=H$



5 : $R^1=OMe$, $R^2=R^4=Me$, $R^3=R^5=H$
6 : $R^1=R^5=H$, $R^2=R^4=Me$, $R^3=OMe$

370, 290, 268; NaOH 398 (305), 278 (268); NaOAc 380, 324, 283; +H_3BO_3 365, 324, 280. MS *m/z* (rel. int.) 390 (80%, M^+), 375 (100%, M-15). ^1H NMR (DMSO-d_6) δ (ppm) 12.52 (1H, s; OH-5), 8.32 (1H, s; OH), 7.59 (1H, d, J = 2 Hz; H-2′), 7.57 (1H, dd, J = 2, 8 Hz; H-6′), 7.12 (1H, d, J = 8 Hz; H-5′), 3.86, 3.80, 3.78, 3.74 (3H each, s; 4 × OCH_3).

Compound **3**. M.p. 166–168 °C (no lit. data). UV λ_{max}^{MeOH} 351, 281, 263; $AlCl_3$ 379, 290, 276; +HCl (412, infl.), 370, 290, 272; NaOH 400, 276; NaOAc 350, 278, 264; +H_3BO_3 349, 277, 260. MS *m/z* (rel. int.) 404 (89%, M^+), 389 (100%, M-15), 374 (8), 359 (8), 345 (2), 331 (6), 211 (8), 202 (9), 183 (6), 151 (8). ^1H NMR (DMSO-d_6) δ (ppm) 12.46 (1H, s; OH-5), 9.58 (1H, s; OH-3′), 7.62 (1H, dd, J = 2, 9 Hz; H-6′), 7.61 (1H, d, J = 2 Hz; H-2′), 7.15 (1H, d, J = 9 Hz; H-5′), 4.03, 3.90, 3.88, 3.82, 3.81 (3H each, s; 5 × OCH_3).

Compound **4**. M.p. 151–152 °C (lit. 8 169–171 °C). UV λ_{max}^{MeOH} 351, 282, 264; $AlCl_3$ 376, 285, 274; +HCl (410, infl.), 372, 287, 271; NaOH 417, 275; NaOAc (415, infl.), 352, 277 (264, infl.); +H_3BO_3 350, 278, 261. MS *m/z* (rel. int.) 404 (85%, M^+), 390 (100%, M-15), 374 (8), 359 (9), 345 (2), 331 (6), 211 (6), 202 (7), 183 (8), 151 (11).

Compound **5**. M.p. not determined. UV λ_{max}^{MeOH} 350, 272, 256; $AlCl_3$ 383, 260; +HCl 373 (280), 264; NaOH 412 (338), 273. MS *m/z* (rel. int.) 360 (100%, M^+), 345 (55%, M-15), 327 (6), 317 (26), 299 (12), 224 (6).

Compound **6**. M.p. not determined. UV λ_{max}^{MeOH} 349, 285; $AlCl_3$ 375, 289, 265; +HCl 367, 291 (269); NaOH 415 (345), 286; H_3BO_3 367, 285; +NaOAc 317, 285. MS *m/z* (rel. int.) 360 (69%, M^+), 345 (100%, M-15), 327 (6), 317 (10), 302 (5). ^1H NMR ($CDCl_3$/DMSO-d_6, ca. 4:1) δ (ppm) 12.70 (1H, s; OH-5), 7.48 (1H, dd, J = 2, 8.2 Hz; H-6′), 7.42 (1H, d, J = 2 Hz; H-2′), 7.04 (1H, d, J = 8.2 Hz; H-5′), 6.56 (1H, s; H-3), 3.99 (6H, s; 2 × OCH_3), 3.96 (3H, s; OCH_3).

Results and Discussion

Thin-layer chromatographic examination of crude leaf resin from *Gutierrezia sarothrae* showed a few flavonoid spots only. After elimination of the bulk of terpenoids by passage over Sephadex LH-20 it became obvious, however, that some twenty flavonoid aglycones were present in this material. The major components were isolated by column chromatography and characterized by their spectral properties. Most of the minor flavonoids were identified by direct comparisons with markers, partly after purification by preparative TLC. They are apigenin, luteolin, lut-3′-Me, lut-4′-Me, 6-methoxy luteolin, quercetin-3-Me, qu-3,3′-diMe, quercetagetin-6,3′-diMe, and trace of eriodictyol-7-Me. Some 4–6 further trace constituents that didn't match any of our markers remain unidentified.

The major flavonoids form three pairs of products, two compounds each having the same molecular mass and appearing as one spot in some TLC systems. Only one of these pairs (**3**/**4**) could be separated by fractional crystallization from ethanol with some benzene; the others were isolated by preparative TLC on silica.

Compounds **1** and **2** have molecular mass 390, indicating flavone/flavonol with 3 OH- and 4 OMe-groups. The base peak at M-15 indicates 8-methoxy- or 6,8-dimethoxy substitution. Comparison of UV and MS data with those reported recently for flavonoids found in other *Gutierrezia* species [8, 9] revealed that they agreed well with those of 5,7,4′-triOH-3,6,8,3′-tetraOMe flavone (compound **2** in ref. [8]) and with 5,7,3′-triOH-3,6,8,4′-tetraOMe flavone (compound **4** in ref. [9]), respectively. Direct comparison of compound **1** with an authentic sample of 5,7,4′-triOH-3,6,8,3′-tetraOMe flavone proved its identity. The ^{13}C NMR spectrum of compound **2** had A and C ring resonances identical to those reported for compound **1** [8] but its B ring resonances indicated that the OMe was located at C4′ and the OH at C3′ establishing its structure as 5,7,3′-trihydroxy-3,6,8,4′-tetramethoxy flavone, a compound recently reported from *G. grandis* [9].

Compounds **3** and **4** have molecular mass 404, suggesting flavone/flavonol with 2 OH- and 5 OMe-groups. The similarity in their chromatographic behaviour and their UV and MS spectra led us to assume that they were closely related isomers, too, probably varying in methylation of positions 3′ and 4′, just as the former isomeric products. Compound **4** had spectral properties which suggested it was 5,4′-dihydroxy-3,6,7,8,3′-pentamethoxy flavone (calycopterin). This was confirmed by direct comparison with an authentic specimen isolated from *G. microcephala* [8]. The ^{13}C NMR spectrum of the isomeric compound **3** showed A and C ring resonances identical to those of calycopterin [8] but B ring signals indicative of 3′-OH and 4′-OMe establishing **3** as 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxy flavone (also reported recently from *G. microcephala* [10]).

Compounds **5** and **6** are more polar than the other four. Their molecular mass 360 shows that they have three OH-groups and three OMe-groups. From their different colour reactions with NA (compound **5** turns yellow-brown, compound **6** remains dark) as well as from the different M/M-15 ratios in the MS it is obvious that although they too are isomers they are less similar to one another than the two previous pairs of products. Compound **5** was readily identified as quercetagetin-3,6,3′-trimethyl ether (jaceidin) by direct comparison with an authentic sample. The ^{1}H NMR spectrum of compound **6** exhibits three methoxyl signals and a low field singlet indicating free 5-OH, three aromatic coupled signals corresponding to protons in 3′,4′-disubstituted flavonoid B-rings, and one further aromatic singlet that could be ascribed either to H-8 or H-3. From the ^{13}C NMR spectrum it becomes clear that it must be ascribed to H-3 rather than to H-8 as the C-ring resonances of C-2, C-3 and C-4 are characteristic of flavones. The ^{13}C NMR data agree with those reported for sudachitin in ref. [11], apart from the fact that some assignments reported in [11] should be interchanged. Our compound **6** is thus identical with 5,7,4′-trihydroxy-6,8,3′-tetramethoxy flavone (sudachitin).

Compound **5**, jaceidin (quercetagetin-3,6,3′-trimethyl ether), is a trivial compound, encountered rather often now as the aglycone, mostly in plant exudates. It is also present in *G. microcephala* [8]. Compound **6**, sudachitin, is long known as a constituent of *Citrus* fruit peel. This flavone had also been described as "majoranin" from *Majorana hortensis* [12], but the structure of majoranin was revised recently to the isomeric thymonin [13]. Sudachitin has been found in an Asteraceae once before, in aerial parts of *Helianthus strumosus* [14]. Compound **4**, 5,4′-diOH-3,6,7,8,3′-pentaOMe flavone, was first described some fifty years ago from leaves of *Calycopteris floribunda* and reported recently from *Baccharis incarum* [15], from *Gut. resinosa* [16], from *G. microcephala* [8] and from *Calycadenia ciliosa* [17], all in the Asteraceae. Compound **1**, 5,7,4′-trihydroxy-3,6,8,3′-tetraOMe flavone, has first been described from leaves of *Chrysothamnus viscidiflorus* [18] and was reported recently from *Gut. microcephala* [8, 10]. Compound **2**, 5,7,3′-triOH-3,6,8,4′-tetraOMe flavone, is so far only known from *G. grandis* [9] and *G. microcephala* [10] while compound **3**, 5,3′-diOH-3,6,7,8,4′-pentaOMe flavone, was only found in *G. microcephala* [10]. Although it was not mentioned in any of these papers, it may be assumed that in most or even in all cases the highly methylated flavonoids are accumulated externally on the plant surfaces, at least as far as Asteraceae are concerned.

The flavone sarothrin (5,7,4′-triOH-3,6,8-OMe-flavone), reported previously as the only, hence presumably the major flavonoid from *Gutierrezia sarothrae* [5] was not detected in the plant material used in this study. This is likely to be due to the different origin of the plant material used, obviously belonging to different populations, different chemical races. In our plants, compounds **1** and **2** are the major flavonoids of the leaf resin. The remarkable difference in the flavonoid aglycone patterns reported for *G. microcephala* in [8] and [10] is possibly also due to the existence of chemotypes.

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Note added in proof: 5,3′,4′-triOH-3,6,7,8-tetraOMe flavone and 5,4′-diOH-3,6,7,8,3′-pentaOMe flavone (= our compd. **4**) were recently reported as constituents of *Gutierrezia resinosa* [19].

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