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NEW 8-HYDROXYFLAVONOLS FROM *LARREA TRIDENTATA*

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Because *Larrea* (Zygophyllaceae) taxa are dominant elements in the two ecosystem sites in Argentina and Arizona which were selected for comparative study as part of the International Biological Program, all aspects of the chemistry and biology of members of *Larrea* are under investigation. *Larrea tridentata* Cav. is of special interest because it has three ploidy levels (*n* 13, 26, 39) in North America and is closely related to *L. divaricata* (n = 13) which occurs in South America.

Despite reports of flavonoids in *Larrea tridentata* as early as 1945 [1], the first account of a detailed structure elucidation of flavonoids in the genus was our 1972 description [2] of the structures and properties of 11 flavonoid aglycones in *Larrea cuneifolia* from Argentina. More recently, 10 flavonoids were identified from *L. tridentata*: kaempferol, its 3-methyl and 3,7-dimethyl ethers and 3-rhamnoglucoside, isorhamnetin, and quercetin and its 3-methyl and 3,7,3′-trimethyl ethers, 3-glucoside and 3-rhamnoglucoside [3,4].

Here, we report the isolation and structure determination of two new 8-hydroxyflavonols (**1** and **2**) from a hexaploid population of *L. tridentata* collected in Mojave Co., Arizona, July 1971. This is the first report of 8-hydroxyflavonols from the Zygophyllaceae.

The MS of compound **1** exhibited a molecular ion peak at *m/e* 330 as a base peak, corresponding to a dimethoxytrihydroxyflavone ($C_{17}H_{14}O_7$). Furthermore, an intense peak (39% intensity relative to the base peak) at *m/e* 287 corresponded to $(M–COMe)^{+\cdot}$, suggesting the presence of either a 3-, 6-, or 8-methoxy group [5–10]; however, a weak peak (2·3% relative intensity) at *m/e* 315 corresponding to $(M–Me)^{+\cdot}$ suggested a 3-methoxyl group rather than a 6- or 8- group since the loss of a methyl group from a 6- or 8-methoxyl function is a dominant fragmentation process for flavonols containing this group [5–12]. Peaks at *m/e* 105 and 121 (7% and 17% relative intensities respectively) suggested the presence of a *B*-ring with only one hydroxyl group [5].

An NMR spectrum of the TMS ether of **1** in CCl_4 showed a singlet at δ 3·90 (6H) for two methoxyl groups, a singlet at δ 6·27 (1H) for an isolated aromatic proton, and two doublets at δ 6·90 (*J* 8 Hz, 2H) and 8·13 (*J* 8 Hz, 2H) in accord with a *B*-ring substituted only at the 4′-position. The NMR spectrum of the TMS ether of **1** in C_6D_6 showed a large shift (Δ + 0·60 ppm) for one of the methoxyl groups, suggesting the presence of a 7-methoxyl group [13]; moreover, an MS peak at *m/e* 182 (6%) could correspond to an *A*-ring fragment containing a methoxyl function. In addition, the MS lacked peaks at *m/e* 119 and 135, indicating the absence of a 4′-methoxyl group; finally, the small NMR–benzene-induced shift observed for the second methoxyl group (Δ + 0·13 ppm) supported assigning it to the 3-position [13]. The chemical shift for the isolated aromatic proton was in accord with a proton at the 6- rather than the 8-position [15]; this interpretation was further supported by the absence of a color reaction with Sr^{+2}–NH_3 [16] (i.e. no green precipitate formed; a green precipitate is observed for flavonoids which contain a 5,6-dihydroxyl system).

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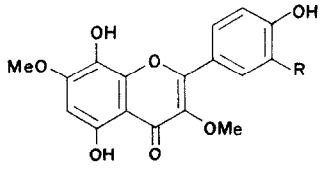
The UV spectra of **1** confirmed the presence of a 5-OH, and the absence of both an oxygen function at C_6 and a 3′,4′-*o*-dihydroxyl system [notably a large bathochromic shift of band I with both $AlCl_3$ (71 nm) and $AlCl_3$/HCl (62 nm) relative to the MeOH spectrum]. Therefore, the remaining hydroxyl group must be at the 8-position. These assignments were supported by NMR spectral data for the acetate of **1**; the spectrum exhibited signals for three acetate groups (with the C_5–OAc at δ 2·47) and two aromatic proton signals shifted to lower field: Δ − 0·40 ppm for the doublet for the 3′- and 5′- protons and Δ − 0·51 ppm for the singlet for the proton at the 6-position. Therefore, on the basis of the above data, compound **1** represents the 3,7-dimethyl ether of herbacetin (8-hydroxykaempferol).



(**1**) R = H
(**2**) R = OMe

Similarities in the UV spectra and color on paper under UV light (green) suggested that **2** had a hydroxylation pattern similar to **1**. The MS of **2** exhibited a molecular ion peak at *m/e* 360, corresponding to trihydroxytrimethoxyflavone ($C_{18}H_{16}O_8$). Furthermore, an intense peak (38% relative intensity) at *m/e* 317 corresponding to $(M-COMe)^+$, suggested the presence of a 3-methoxyl group. Peaks at *m/e* 135 and 151 (9% and 7%) indicated that the *B*-ring contained one hydroxyl and one methoxyl group [6].

The NMR spectrum of the TMS ether of **2** in CCl_4 showed a singlet at δ 3·87 (9H) for three methoxyl groups, a singlet at δ 6·21 (1H) for a proton at C_6. Furthermore, a doublet at δ 6·83 (*J* 8 Hz, 1H), a double doublet at δ 7·63 (*J* 3, 8 Hz, 1H) and a doublet at δ 7·73 (*J* 3 Hz, 1H) for three *B*-ring protons suggested the presence of a 3′,4′-oxygenated system. The NMR spectrum of the TMS ether of **2** in C_6D_6 showed three singlets for methoxyl groups at δ 3·78 (Δ + 0·09 ppm; 3-methoxyl group [13]), δ 3·52 (Δ + 0·35 ppm; 3′-methoxyl group [13]), and δ 3·27 (Δ + 0·60 ppm; 7-methoxyl group [13] since the MS data indicated only one *B*-ring methoxyl group). The absence of a 6-hydroxyl group was supported by the absence of a color reaction with Sr^{+2}–NH_3 [16], i.e. no green precipitates. As with **1**, the UV spectra of **2** indicated a 5-OH and the absence of both a C_6 oxygen function (shift of 74 nm with $AlCl_3$ and 72 nm with $HCl/AlCl_3$) and a 3′,4′-ortho dihydroxy system. Therefore, on the basis of these data, compound **2** can be assigned to gossypetin 3,7,3′-trimethyl ether.

* Benzene–HOAc–H_2O (6:7:3) was mixed and the organic layer which formed was used as the developing solvent.

EXPERIMENTAL

Mps are not corrected. NMR spectra were measured at 60 MHz with tetramethylsillane as an internal standard. Air-dried and ground leaf material from a hexaploid population of *L. tridentata* (collected in Mojave Co., Arizona, July, 1971 by B.N.T.) was extracted with 85% aq MeOH and the extract was filtered and evaporated. The aq. soln was extracted with Et_2O. The ethereal extracts were combined, evaporated and taken to dryness *in vacuo*. Twelve g of the syrup were chromatographed over polyamide, 560 g packed in Egger's solvent ($CHCl_3$–MeOH–MeCOEt–2,4-pentanedione, 20:10:5:1); the compounds were also eluted from the column with Egger's solvent and fractions of about 20 ml each were collected. Rechromatography of Fraction 53–69 on another column of polyamide with MeOH gave orange needles (**2**) after recrystallization from MeOH. From Fractions 124–144, yellow fine needles of **1** were obtained in the same manner used to purify **2**.

Herbacetin 3,7-dimethyl ether (**1**): mp 247–8°; MS (*m/e*): 330 (M^+), 289 $(M-COMe)^+$; colors on paper: green (with UV, 366 nm): greenish yellow (with UV/NH_3); R_f values: 0·78 (TBA), 0·20 (15% HOAc), 0·27 (BeAW)* and 0·37 (25% AcOH). UV data λ_{max} (nm): MeOH 225, 278, 307, 328, 372; NaOMe 240, 388 (dec); $AlCl_3$ 240, 287, 320, 358, 443; $AlCl_3$–HCl 238, 286, 319, 351, 434; NaOAc 276, 305, 326, 380; H_3BO_3–NaOAc 276, 306, 326, 367.

Gossypetin 3,7,3′-trimethyl ether (**2**): mp above 255°; MS (*m/e*): 360 (M^+), 317 $(M-COMe)^+$; colors on paper: green (with UV); greenish yellow (with UV/NH_3); R_f values: 0·73 (TBA), 0·14 (15% HOAc), 0·65 (BeAW) and 0·29 (25% HOAc). UV data λ_{max} (nm): MeOH 257, 277, 305, 339, 365, sh; NaOMe 254, 403 (dec); $AlCl_3$ 274 sh, 287, 317, 368, 439; $AlCl_3$–HCl 272 sh, 286, 322, 360, 437; NaOAc 267, 336, 409; H_3BO_3–NaOAc 256, 276, 305, 337, 369 sh.

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THE CAROTENOID PATTERN IN *IRIS GERMANICA**

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Key Word Index—*Iris germanica*; Iridaceae; carotenoids; irones.

The rhizomes of different *Iris* species (*pallida, fiorentina, germanica*) synthesize considerable quantities of irones [1–4]. Since it is assumed that several naturally occurring 1,1,5-trimethylcyclohexyl derivatives are metabolic products of carotenoids [5,6], the occurrence of irone-like (2-methylated) carotenoids in irone producing plants might be expected. Both optically inactive [7] and active (2*R*,2′*R*)-2,2′-dimethyl-β,β-carotene [8], (2*R*,2′*R*, 6*R*,6′*R*)-2,2′-dimethyl-ε,ε-carotene [9] and (2*R*,2′*R*, 6*S*,6′*S*)-2,2′-dimethyl-ε,ε-carotene [9] have been prepared synthetically, but no 2-methylated carotenoids have so far been found in nature. However, C_{45}- and C_{50}-carotenoids with *iso*pentenyl substituents in 2,(2′)-positions occur in certain non-photosynthetic bacteria [10,11].

The grey-coloured roots of *Iris germanica* contain hardly any carotenoids. In the present work the following distribution pattern was found in the green parts of the plant:

β-carotene	7·1% (4·3 mg)
lutein	87·8% (53·2 mg)
neochrome (neoxanthin furanoxide)	3·5% (2·1 mg)
7 minor constituents	1·6% (1·0 mg)

Neochrome was isolated as a mixture of the two C-8′ epimers and is therefore considered an artefact, formed by rearrangement of neoxanthin during isolation.

The result demonstrates that the carotenoids present correspond to those occurring in most green plants. The carotene xanthophyll ratio is consistent with autumn values reported for green leaves by Willstätter and Stoll [12].

Presence of supernumerary methyl groups in the carotenoids isolated was excluded by mass spectrometry. It may consequently be inferred that irones are not likely to be formed by degradation of 2-methylated carotenoids.

* Part VIII in the series "Carotenoids of higher plants". For Part VII see (1974) *Acta Chem. Scand.* **28B,** 13.