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FLAVONOIDS FROM THE GENUS *TRAGOPOGON* (COMPOSITAE)

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Abstract—The known flavonoids apigenin, luteolin, luteolin 7-O-β-D-glucoside, quercetin 3-O-β-D-glucoside, vitexin, isovitexin, vicenin-1, vicenin-2, swertisin, orientin, isoorientin, lucenin-1, lucenin-2, and swertiajaponin were identified as constituents of one or more of the five North American *Tragopogon* species. The distribution of these compounds is consistent with the genetic relationships between the species. A new C-glycosyl flavone, O-xylosylisovitexin, was detected in *T. dubius*.

INTRODUCTION

CONSIDERABLE genetic information has been accumulated for the five North American species of the genus *Tragopogon* (Compositae) during the past two decades.[1–5] This genetic information has indicated that *T. mirus* is derived from hybrids between *T. porrifolius* and *T. dubius* and that *T. miscellus* had resulted from hybridization between *T. dubius* and *T. pratensis*. In view of the established chemotaxonomic value of plant flavonoids, it was of interest to study the flavonoid chemistry of these taxa.

STRUCTURE DETERMINATIONS

Known Flavonoids

Apigenin (I), luteolin (II), luteolin 7-O-β-D-glucoside (III), quercetin 3-O-β-D-glucoside (IV), vitexin (V), isovitexin (VI), swertisin (X), orientin (XI), isoorientin (XII), lucenin-1 (XIII) and swertiajaponin (XV) were each isolated from the appropriate two-dimensional paper chromatograms (see Table 1 for the distribution of these compounds) and subsequently shown to be identical with authentic samples by co-chromatography and u.v. spectral analyses;[6,7] for each compound, one spectrum was determined in methanol alone and five were

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recorded in methanol plus one of the diagnostic reagents: sodium methoxide, sodium acetate, sodium acetate plus boric acid, aluminum chloride, and aluminum chloride plus hydrochloric acid.[8] The two O-glycosides (III and IV) upon treatment with β-glucosidase yielded the expected aglycones. None of the C-glycosylflavones (V, VI, X, XI, XII, XIII and XV) could be hydrolyzed by either acidic or enzymatic treatments although with acid reflux vitexin (V) and isovitexin (VI) were shown to be readily interconvertable.

TABLE 1. THE DISTRIBUTION AND RELATIVE CONCENTRATIONS* OF FLAVONOIDS ISOLATED FROM THE FIVE NORTH AMERICAN SPECIES OF *Tragopogon*

Flavonoids	*Tragopogon* species				
	porrifolius (2 N)†	*mirus* (4 N)‡	*dubius* (2 N)†	*miscellus* (4 N)‡	*pratensis* (2 N)†
Orientin (XI)	++++	++++	++	++	++++
Isoorientin (XII)	+++	++++	++++	++++	++++
Isovitexin (V)	++	+	+++	+	++
Vicenin-2 (VIII)	++	+++	+	++	+++
Luteolin (II)	++	++	++	+	++
Quercetin 3-O-β-D-glucoside (IV)	++	++	+++	+	+++
Swertiajaponin (XV)	−	+	+++	+++	+++
Swertisin (X)	−	+	+	+	+
Luteolin 7-O-β-D-glucoside (III)	−	+++	++	+++	++
Vicenin-1 (VII)	++	+++	+++	++	−
Lucenin-1 (XIII)	++	+++	++	+	−
O-xylosylisovitexin (IX)	−	−	+	−	−
Apigenin (I)	−	−	−	−	++
Lucenin-2 (XIV)	−	−	−	+	++
Vitexin (V)	++	++	−	−	−

* Relative concentrations: ++++ = high; + = low; − = absent.
† Diploid, $n = 12$.
‡ Amphidiploid, $n = 24$. The two amphidiploid species, *T. mirus* and *T. miscellus*, are arranged in the chart between the two species from which they are considered to be derived.

Swertisin (X) and swertiajaponin (XV), which were recently reported for the first time in *Swertia japonica*,[9] are the 7-O-methyl ethers of isovitexin and isoorientin, respectively. The structures of X and XV were proven by synthesis: isovitexin and isoorientin being converted into X and XV, respectively, by selective methylations.[10,11]

Vicenin-1 (VII), Vicenin-2 (VIII), and Lucenin-2 (XIV)

Although authentic samples of three di-C-glycosylflavones, vicenin-1, -2, and lucenin-2, were not available, the chromatographic and spectral properties of the compounds isolated in the present study were in accord with the appropriate published values.[6,7,12]

[8] K. R. MARKHAM and T. J. MABRY, *Phytochem.* **7**, 1197 (1968).
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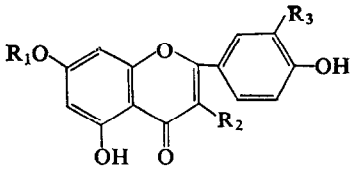
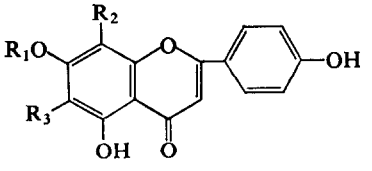
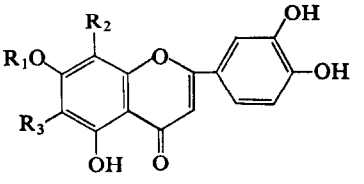
[11] K. S. PANKAJAMANI and T. R. SESHADRI, *J. Indian Chem. Soc.* **31**, 565 (1954).
[12] M. K. SEIKEL, J. H. S. CHOW and L. FELDMAN, *Phytochem.* **5**, 439 (1966).



	R_1	R_2	R_3
(I)	H	H	H
(II)	H	H	OH
(III)	glu	H	OH
(IV)	H	O-glu	OH



	R_1	R_2	R_3
(V)	H	C-glu	H
(VI)	H	H	C-glu
(VII)	H	C-gly	C-gly ($R_2 \neq R_3$)
(VIII)	H	C-gly	C-gly ($R_2 = R_3$)
(IX)	H	H	O-xyl-C-glu
(X)	CH_3	H	C-glu



	R_1	R_2	R_3
(XI)	H	C-glu	H
(XII)	H	H	C-glu
(XIII)	H	C-gly	C-gly ($R_2 \neq R_3$)
(XIV)	H	C-gly	C-gly ($R_2 = R_3$)
(XV)	CH_3	H	C-glu

O-Xylosylisovitexin (IX)

The new C-glycosylflavone, O-xylosylisovitexin (IX), which was isolated from the two-dimensional paper chromatograms of *Tragopogon dubius*, yielded upon acid hydrolysis a mixture of vitexin, isovitexin and xylose. The identity of the sugar was established by gas chromatographic comparison of its penta-trimethylsilyl ether with that of authentic xylose.[13] It was evident from the u.v. spectra that the new C-glycosylflavone contained free hydroxyl groups at 4′, 5 and 7 positions; therefore, the xylose must be linked to the C-glucosyl moiety of either vitexin (V) or isovitexin (VI). Mild acid hydrolysis of the xyloside, however, yielded only isovitexin, indicating that the new flavone is O-xylosylisovitexin (IX). This is the first report of naturally occurring xylosylisovitexin from nature, although O-D-xylosylvitexin has been previously isolated from *Vitex lucens* and *Citrus sinensis*.[14]

DISCUSSION

The present investigation has extended the work of Brehm and Ownbey who used two-dimensional paper chromatographic patterns of flavonoids for the recognition of the North American *Tragopogon* species.[1] Moreover, the flavonoids detected in the amphidiploid species *T. mirus* corresponded (with the exception of xylosylisovitexin) to the combined flavonoid constituents of the two parent diploid species, *T. dubius* and *T. porrifolius* (see Table 1). Similarly, *T. miscellus* exhibited the combined flavonoids of its two parental species, *T. dubius* and *T. pratensis*. These results are in complete accord with the conclusions derived

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genetically, and provide further evidence of the value of flavonoids as chemotaxonomic markers.

EXPERIMENTAL

All of the genetically identified *Tragopogon* plant material was supplied by Dr. B. Brehm, Reed College, Portland, Oregon, and by Dr. M. Ownbey, Washington State University, Pullman, Washington. The u.v. spectral analyses were recorded on a Beckman DB Spectrophotometer using standard diagnostic reagents.[6,7] The 2-D paper chromatograms were prepared on Whatmann 3MM paper (46 cm × 57 cm) using first *t*-BuOH: HOAc: H_2O, 3:1:1, in the long direction and then 15% HOAc in the second dimension. The enzymatic and acidic hydrolyses were carried out by standard procedures.[6]

Extraction and Purification Procedures

In a typical extraction, dried and ground, flower, stem and leaf material (10 g) was preextracted for 24 hr with 300 ml of reagent-grade light petroleum, b.p. range 35–60°, at room temperature. The plant material was next extracted for 24 hr with 0·36% HCl in methanol. The flavonoids present in the latter extract were subsequently separated and isolated by standard 2-D paper chromatographic procedures.[6]

Selective Methylation of Isovitexin (VI) and Isoorientin (XII)

(*a*) *Isovitexin.* Isovitexin was methylated with Me_2SO_4 in the presence of $NaHCO_3$.[10] The three products, which were isolated by paper chromatography, were shown by their u.v. spectra to be 4′-O-methylisovitexin, 4′,7-di-O-methylisovitexin and 7-O-methylisovitexin. The latter was spectrally and chromatographically identical with compound X, swertisin.

(*b*) *Isoorientin.* Isoorientin was methylated with Me_2SO_4 in the presence of borax and NaOH according to Pankajamani and Seshadri,[11] and the three paper chromatographically separated products were shown to be 7,3′-di-O-methylisoorientin, 7,3′,4′-tri-O-methylisoorientin and 7-O-methylisoorientin, by u.v. spectroscopy. The latter compound was spectrally and chromatographically identical with compound XV, swertiajaponin.

Hydrolyses of Xylosylisovitexin (IX)

(*a*) When heated in 5% aq. methanolic HCl at 100° for 1 hr, xylosylisovitexin gave a mixture of two aglycones. These were paper chromatographically identical with authentic vitexin and isovitexin.

(*b*) When treated with 10% aq. HOAc at 20° for 18 hr, xylosylisovitexin yielded only one aglycone and this was paper chromatographically identical with isovitexin.

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