Phytochemistry, 1973, Vol. 12, pp. 2275 to 2278. Pergamon Press. Printed in England.

FLAVONE *C*-GLYCOSIDES FROM *CORONILLA VARIA*

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(*Received* 5 *March* 1973. *Accepted* 1 *April* 1973)

Key Word Index—*Coronilla varia*; Leguminosae; crownvetch; flavone *C*-glycosides; isovitexin; isoorientin; isoorientin 2″-*O*-rhamnoside; lutonarin, isovitexin 4′-glucoside; isoorientin 4′-glucoside.

Abstract—One new and 5 known flavone *C*-glycosides were isolated from leaves and stems of *Coronilla varia*. The new compound was shown to be isoorientin 2″-*O*-rhamnoside. The known compounds were isovitexin, isoorientin, isovitexin 4′-*O*-glucoside, isoorientin 4′-*O*-glucoside, and isoorientin 7-*O*-glucoside.

INTRODUCTION

CROWNVETCH, *Coronilla varia* L., a creeping, perennial legume, is planted on roadbanks and mine spoilbanks in the north-eastern U.S.A. It is occasionally used as livestock forage. In feeding quality trials, certain lots of dried hay forage are toxic to meadow voles, *Microtus pennsylvanicus*.[1,2] Partial characterization has indicated that the toxic factor is a flavonoid compound(s).[3,4] This prompted an examination of the flavonoid composition of crownvetch forage.

RESULTS AND DISCUSSION

Isovitexin (I), isoorientin (II), isovitexin 4′-*O*-monoglucoside (III), isoorientin 4′-*O*-monoglucoside (IV), isoorientin 7-*O*-glucoside (V), and a new natural product, isoorientin 2″-*O*-rhamnoside (VI) were identified in methanolic extracts from dried hay (stem and leaf) samples of crownvetch. Compounds I–IV were conspicuously present in 2-D PC of extracts from each of 9 samples of cultivars 'Penngift', 'Emerald' and 'Chemung' (3 replicated field plots/cultivar, harvested 3 times). Compounds V and VI occurred in one or another, but not all, of the samples of each cultivar. Other flavonoids were present in amounts too small to allow full characterization.

Compounds I and II were identified by UV spectra in 6 standard reagents,[5] fluorescence

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[2] BARNES, R. F., FISSEL, G. W. and SHENK, J. S. (1973) *Agron. J.* to be published.
[3] GUSTINE, D. L., BARNES, R. F. and FISSEL, G. W. (1972) *Agron. Abstr.* 69.
[4] GUSTINE, D. L., SHENK, J. S., MOYER, B. G. and BARNES, R. F. (1973) *Agron. J.* to be published.
[5] MABRY, T. J., MARKHAM, K. R. and THOMAS, M. B. (1970) *The Systematic Identification of Flavonoids*, Springer, New York.

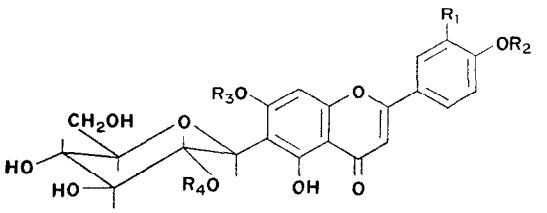
characteristics, 2-D co-chromatography with authentic samples from *Tragopogon* spp.,[6] and by rearrangement to the expected C-8 isomers in hot 6% HCl.[5]

TABLE 1. UV SPECTRA OF FLAVONE *C*-GLYCOSIDES FROM *Coronilla varia**

Compound†	MeOH	NaOMe	$AlCl_3$	$AlCl_3$–HCl	NaOAc	NaOAc–H_3BO_3
III	274 327	282 375	283, 301 345, 383	283, 300 338, 381	280, 293sh 375	276 332
IV	273 336	271 381	282, 295 356, 384	284, 295 348, 384	277 376	275 338
V	272 342	277, 328sh 408	279, 300sh 348, 430	280, 297sh 357, 386	270 407	265 375
VI	259, 271 350	280, 336 408	279, 304sh 340, 430	280, 300sh 363, 389	274, 328sh 393	268, 309sh 378, 430sh

* All UV spectra were recorded using standard procedures.[5]
† UV spectra for I and II agreed with published[5] spectra.

Compounds III and IV gave R_f (PC 3 solvents) and deep purple appearance in UV and UV/NH_3 in agreement with published[7] properties. Enzyme and acid hydrolyses yielded glucose and the expected aglycones. Published UV data[7] for III contained unexplained discrepancies, and that reported for IV was incomplete, hence we report here the data obtained for these compounds (Table 1). NMR spectra (Table 2) confirmed the predicted substitution patterns. Integration of the proton signals showed that these were mono-*O*-glucosides. Compound III was previously reported only from Gramineae[7] and Caryophyllaceae,[9] and IV was reported only from Gramineae.[7]



(I) $R_1 = R_2 = R_3 = R_4 = H$
(II) $R_1 = OH$, $R_2 = R_3 = R_4 = H$
(III) $R_1 = R_3 = R_4 = H$, $R_2 = Glc$
(IV) $R_1 = OH$, $R_2 = Glc$, $R_3 = R_4 = H$
(V) $R_1 = OH$, $R_2 = R_4 = H$, $R_3 = Glc$
(VI) $R_1 = OH$, $R_2 = R_3 = H$, $R_4 = Rha$

Compound V yielded glucose and isoorientin upon acid hydrolysis (10 min) and orientin, by re-arrangement,[5] upon longer hydrolysis (60 min). It was slowly hydrolysed by β-glucosidase. UV spectra (Table 1) suggested free 3′-, 4′- and 5-OH and substitution at C-7. The attachment of the sugar to the 7-position was corroborated by the downfield shift of the H-8 signal of the TMS ether[5] (Table 2). Integration of the proton signals indicated a mono-glucoside. The structure assigned compound V was previously assigned[10] to a compound from *Hordeum vulgare* (Gramineae) given the trivial name lutonarin. The usage of this

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[7] WILLIAMS, C. A. and MURRAY, B. G. (1972) *Phytochemistry* **11**, 2507.
[8] KOEPPEN, B. H. and ROUX, D. G. (1965) *Biochem. J.* **97**, 444.
[9] LITVINENKO, V. I., AMANMURADOV, K. and ABUBAKIROV, N. K. (1967) *Khim. Prir. Soedin.* **3**, 159.

name is ambiguous since it has also been used to designate orientin 7-*O*-glucoside[11,12] and isoorientin 4′-*O*-glucoside.[5] The PC behavior (4 solvents) and appearance (UV deep purple, UV/NH_3 bright orange–yellow) of V are identical to those in the original report[13] of lutonarin.

TABLE 2. NMR SPECTRA OF TMS ETHERS OF *C*-GLUCOSYL FLAVONES FROM *Coronilla varia**

Compound†	H-2′	H-6′	H-3′	H-5′	H-3	H-8	H-1″ C-Glu	H-1 *O*-Glycoside
II	7·32*d* (*J* 2·5)	7·46*dd* (*J* 2·5) (*J* 9·0)	—	6·92*d* (*J* 9·0)	6·47	6·47	4·74*m*	—
III	7·82*d* (*J* 9·0)	7·82*d* (*J* 9·0)	7·10*d* (*J* 9·0)	7·10*d* (*J* 9·0)	6·44	6·49	4·69*m*	4·90*m* (*J* 6·0)
IV	7·34*d* (*J* 2·5)	7·47*dd* (*J* 2·5) (*J* 9·0)	—	7·05*d* (*J* 9·0)	6·45	6·48	4·75*m*	5·07*dm* (*J* 6·0)
V	7·32*d* (*J* 2·5)	7·40*dd* (*J* 2·5) (*J* 9·0)	—	6·89*d* (*J* 9·0)	6·43	6·84	4·78*m*	5·25*dm* (*J* 6·0)
VI‡	7·31*d* (*J* 2·5)	7·38*dd* (*J* 2·5) (*J* 9·0)	—	6·90*d* (*J* 9·0)	6·38	6·47	4·75*m*	5·05*dm* (*J* 5·5)

* Values in δ, J = coupling constant in Hz, spectra recorded on Varian A60-A in deuteriochloroform solutions containing tetramethylsilane as internal standard; abbreviations are *d*—doublet; *dd*—double doublet; *dm*—double multiplet; *m*—multiplet.

† NMR spectra for I[5] and for II in DMSO[8] have been reported.

‡ Signal for the rhamnose methyl group was present at δ1·06*d* (*J* 5H_z).

Isoorientin 2″-O-*rhamnoside* (VI)

The new compound VI yielded rhamnose (detected by GLC of the TMS ether) and isoorientin upon acid hydrolysis. Orientin was formed with longer hydrolysis times. UV spectral shift data (Table 1) indicated free phenolic functions at the 5,7,3′ and 4′ positions, hence the rhamnose must be attached to the C-glucosyl moiety in VI. The signals for the rhamnose methyl group and C-1 proton (Table 2) in the NMR spectrum of the *per*-TMS ether of VI were reminiscent of a neohesperidosyl unit[5] as opposed to a rutinosyl unit. The point of attachment of the rhamnose to the glucose at C-2″ was settled on the basis of the NMR spectrum of VI-peracetate, which contained an acetyl peak at δ 1·98 but no acetyl signals in the range δ 1·85–1·70.[5] Integration of the NMR spectrum as well as MS *m/e* 1014 (M^+), 699 (M^+-rhamnosyl, ketene), 273 (rhamnosyl) of VI-peracetate confirmed the presence of only one rhamnose unit in VI. Isoorientin 2″-*O*-rhamnoside migrated to R_f 0·40 on PC in TBA, 0·70 in 15% HOAc, and appeared deep purple under UV and green–yellow with UV/NH_3.

[10] McCLURE, J. W. and WILSON, K. G. (1970) *Phytochemistry* **9**, 763.
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EXPERIMENTAL

Isolation and purification of flavones. Hay samples were oven dried at 65° and milled. In a typical batch extraction, 30 g of powder was stirred in 1 l. 50% MeOH, brought to a boil for 3 min and steeped at room temp. for 3 days. The filtrate was concentrated to 30 ml, stirred with 4 vol. MeOH, refiltered, and reconcentrated for chromatography on polyamide. The column was eluted with H_2O followed by 5, 20, 40, 60, 80 and 100% MeOH. Fractions were purified by preparative 1-D chromatography on Whatman 3 MM paper with 6% HOAc followed by 1-D PC with TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) repeated until pure. Bands were eluted with MeOH and dried under high vacuum. 2-D PC of crude extracts from replicate field plots were run using the same solvent systems.

Hydrolyses. Acid hydrolyses were done by refluxing with 6% HCl at 100° for 10, 30 or 60 min. Enzyme hydrolyses were done with Worthington β-glucosidase in 0·2 M NaOAc, pH 5·0 at 37° for 16 hr.

Identification of sugars. TMS derivatives were prepared according to Mabry *et al.*[5] procedure III 2aA. GLC and co-GLC were performed with a Varian Aerograph series 200 gas chromatograph equipped with a FID and using N_2-air carrier gas in a column of 5% OV-1 on 60/80 mesh Chromosorb W programmed from 110° to 180° at 2°/min.[14]

NMR spectra. TMS ethers for NMR spectra were prepared according to Mabry *et al.*[5] procedure VIII-3c, using $CDCl_3$ as solvent.

Acknowledgements—We thank Mrs. Kathy Reich for competent technical assistance. This work was supported in part by PHS grant HE-12971 to MS.

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