Phytochemistry, 1973, Vol. 12, pp. 1451 to 1454. Pergamon Press. Printed in England.

5-*O*-XYLOSYLGLUCOSIDES OF APIGENIN AND LUTEOLIN 7- AND 7,4′-METHYL ETHERS FROM *OVIDIA PILLO-PILLO*

JUANA NÚNEZ-ALARCÓN*
Instituto de Quimica, Universidad Austral de Chile, Valdivia, Chile

and

ELOY RODRIGUEZ, ROLF D. SCHMID† and TOM J. MABRY
The Cell Research Institute and The Department of Botany, The University of Texas at Austin, Texas, TX 78712, U S.A.

(*Received* 16 *November* 1972. *Accepted* 11 *December* 1972)

Key Word Index—*Ovidia pillo-pillo*; Thymelaeaceae; flavone methyl ethers; 5-*O*-xylosylglucosides.

INTRODUCTION

IN CONNECTION with our biochemical systematic investigations of Chilean plants, one of us previously encountered luteolin 7,4′-dimethyl ether (I) in *Ovidia pillo-pillo* Meisner.[1] We now report from this same species the isolation and identification of 5-*O*-xylosylglucosides of apigenin and luteolin 7- and 7,4′-methyl ethers (II–V). The natural occurrences of flavone 5-*O*-glycosides are relatively rare in Nature.[2–4]

DISCUSSION

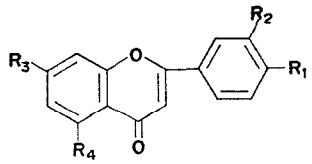
Paper chromatography of the new flavonoids indicated that they were 5-*O*-diglycosides by their high R_f values in 15% HOAc and bright light-blue fluorescence in UV light.[3,5] The NMR spectra of the trimethylsilylated derivatives of IJI–V exhibited the usual patterns for the B-ring protons of apigenin and luteolin methyl ethers (see Table 1 for NMR assignments). The C-6 and C-8 protons in compounds III–V overlapped to give a broad two-proton singlet. The anomeric H-1 of glucose in all three compounds exhibited a doublet (J 7 Hz) at 5·20. In benzene-d_6[7] the methoxyl groups at the 4′ and 7 positions exhibited the expected upfield shifts (see Table 1, Solvent Shifts). UV analysis of III–V confirmed that

* Most of this work was carried out by the senior author during a 1971 tenure at the University of Texas at Austin.

† Present address: Henkel & Cie Gmbh. Biochem. Abteilung, 0–4 Duesseldorf, Germany.

[1] NÚNEZ-ALARCÓN, J. (1971) *J. Org. Chem.* **36**, 3829.
[2] HARBORNE, J B. (1967) *Phytochemistry* **6**, 1569.
[3] GLENNIE, C. W. and HARBORNE, J B (1971) *Phytochemistry* **10**, 1325.
[4] ULUBELEN, A., CETIN, E. T., GURAN, A. and IYENGAR, M. I. (1970) *Lloydia* **33**, 258.
[5] MABRY, T. J., MARKHAM, K. R. and THOMAS, M. B. (1970) *The Systematic Identification of Flavonoids*, Springer, Heidelberg.
[6] KAGAN, J. and MABRY, T. J. (1965) *Anal. Chem.* **37**, 288.
[7] RODRIGUEZ, E., CARMAN, N. J. and MABRY, T. J. (1972) *Phytochemistry* **11**, 409.

their 5-hydroxyl groups were substituted since no bathochromic shifts were observed in $AlCl_3$ (see Table 2, UV data); Mild acid hydrolysis of these glycosides yielded luteolin 7- and 7,4′-methyl ethers and apigenin 7- and 7,4′-methyl ethers, respectively.



(I)	R_1 = OMe;	R_2 = OH;	R_3 = OMe;	R_4 = OH
(II)*	R_1 = OMe;	R_2 = OH;	R_3 = OMe;	R_4 = *O*-xyloglucosyl
(III)	R_1 = OH;	R_2 = OH;	R_3 = OMe;	R_4 = *O*-xyloglucosyl
(IV)	R_1 = OH;	R_2 = H;	R_3 = OMe;	R_4 = *O*-xyloglucosyl
(V)	R_1 = OMe;	R_2 = H;	R_3 = OMe,	R_4 = *O*-xyloglucosyl

The sugars in III–V were identified as xylose and glucose by comparison with known sugars by paper chromatography and GLC[6] (using trimethylsilyl ethers). No hydrolysis occurred with β-glucosidase, indicating that the glucose was not terminal and must therefore be attached directly to the flavonoid.

TABLE 1. NMR DATA FOR TRIMETHYLSILYL ETHERS OF FLAVONOIDS FROM *Ovidia pillo-pillo**

Compound†	H-2′	H-6′	H-3′	H-5′	H-3	H-6‡	H-8‡	Solvent shifts of OMe			
								CCl_4 (TMS)	C_6D_6 (TMS)	ppm	Position of OMe
III	7·43*d* (*J* 2·5)	7·50*dd* (*J* 2·5) (*J* 9)	—	6·96*d* (*J* 9)	6·50	6·68	6·68	3 98	3·53	+0·45	C-7
IV	7·96*d* (*J* 9)	7·96*d* (*J* 9)	6·97*d* (*J* 9)	6·97*d* (*J* 9)	6·50	6·64	6 64	3 96	3 53	+0 43	C-7
V	7·86*d* (*J* 9)	7·86*d* (*J* 9)	7 00*d* (*J* 9)	7·00*d* (*J* 9)	6·43	6 60	6·60	3 90 3·93	3·51 3·33	+0 39 +0·60	C-4′ C-7

* Spectra were recorded in CCl_4 on a Varian A60 spectrometer Values are given in ppm (δ-scale) relative to TMS as an internal standard, numbers in parenthesis denote couplings constants in Hz. Signals are singlets unless otherwise stated: *d*—doublet; *dd*—double doublet

† The chemical shift for the glucose anomeric proton appears at 5·20; all other protons in the xyloglucosyl group are between 3·04 and 4 02.

‡ In benzene-d_6 the C-6 and C-8 protons are well separated with the expected meta-coupling (*J* 2·5 Hz) observed.

MS analysis of a perdeuteriomethylated (PDM) mixture[8,9] of II, IV and V verified that xylose was the terminal sugar. Sequence peaks were observed at 527 (II), 497 (IV) and 494 (V) corresponding to the loss of 200 m.u. from the parent peak of each PDM 5-*O*-digylcoside (see Table 3). The loss of 200 m.u. can be assigned to the T series fragmentations (see Scheme 1), in which PDM xylose and the linkage oxygen are cleaved. Other *m/e* peaks at 183, 147 and 114 were consistent with xylose as the terminal sugar. That the interglycosidic

* Compound II was established only by MS and GC–MS of its derivatized aglycone, since there was not sufficient material for NMR; the data do not eliminate a 3′-methoxy, 4′-hydroxy system for this compound but the presence of pillion in the plant favors structure II.

[8] SCHMID, R. D. (1972) *Tetrahedron* **28**, 3259.

[9] SCHMID, R. D., MUES, R., MCREYNOLDS, J. H., VANDER VELDE, G., NAKATANI, N., RODRIGUEZ, E and MABRY, T. J. (1973) in preparation.

TABLE 2. UV* AND CHROMATOGRAPHIC† DATA FOR THE FLAVONE 5-*O*-XYLOGLUCOSIDES FROM *Ovidia pillo-pillo*

Compound	MeOH	$AlCl_3$	$AlCl_3$–HCl	NaOMe	NaOAc	NaOAc–H_3BO_3	R_fs TBA	R_fs HOAc	Colour test UV ↓ UV/NH_3
III	240	240 sh	275	240	250	250			fl. light blue
	250 sh	272	300	255	295	295			↓
		300 sh	360	295			0·10	0·75	
	345	325 sh	390	405	395	365			↓
		415‡							yellow
IV	260	260	275	265 sh	260 sh	265			fl. light blue
	330	330§	300	290	345		0·15	0·70	↓
			340						
			380	385	380	330			yellow–green
V	255 sh	263	265	265	255 sh	255 sh			fl. light blue
	265	275	275	330	265	265			↓
	330	305	300		330	330	0·18	0·67	
		330§	345						↓
			385						no change

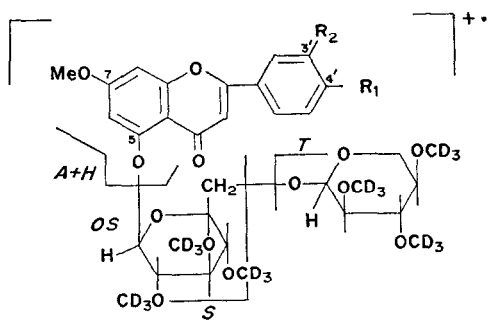
* All UV spectra were recorded using standard procedures.[5]

† Two-dimensional chromatograms on Whatman 3 MM paper were developed first in TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) and then in 15% HOAc.

‡ Hydrolysis of the 5-*O*-disaccharide linkage occurs prior to recording the spectrum.

§ After 5 min a bathochromic shift of about 50 nm were observed which indicated that hydrolysis of the 5-*O*-disaccharide linkage had occurred.

linkage was 1 → 6 was indicated when an S + 63 peak* was observed for all three PDM compounds (Table 3). GC–MS analysis of the ethylated perdeuteriomethylated aglycones (after hydrolysis) verified that the C_5 positions were glycosylated. (See Ref. 9 for R_ts and MS data).



(II) R_1 = OMe; R_2 = OCD_3
(IV) R_1 = OCD_3; R_2 = H
(V) R_1 = OMe; R_2 = H

* See Ref. 8, p. 3268 for a detailed discussion pertaining to the formation of the S + 63 fragment, as yet only observed for flavonoid diglycosides having a 1 → 6 interglycosidic linkage.

TABLE 3 MS DATA FOR PERDEUTERIOMETHYLATED FLAVONE GLYCOSIDES FROM *Ouidia pillo-pillo*

		M^+			S		A		OS
Compound	$M^{+}\cdot$	M^+-CD_3O	M^+-$2(CD_3O)+H$	S	S + H	S + 63	A + H (base peak)	A $+_{-}^{\cdot}$H–CO	
II*	727	693	658	527	528	590	331	303	396
V	694	660	625	494	495	557	298	270	396
IV	697	663	628	497	498	530	301	273	396

* The perdeuteriomethylation procedure was identical to that utilized in the previous studies [8,9]

EXPERIMENTAL

M.ps are uncorrected. Air-dried and ground material (leaves and stems) of *Ovidia pillo-pillo* (collected in December 1968, in Los Ulmos, about 10 km south of Valdivia, Chile—a voucher specimen is deposited in the Universidad Austral de Chile Herbarium, Valdivia, Chile) was extracted 3× with 6 l. EtOH at 50° for 12 hr. The EtOH extract was concentrated and mixed with H_2O; a white precipitate (4 g) was filtered off. The white powder (2 g) was chromatographed over polyamide[5] (120 g packed in MeOH), the column was eluted with MeOH and CCl_3 (4:1). The first fractions yielded 40 mg of apigenin 7,4′-dimethyl ether 5-*O*-xyloglucoside (V), m.p 206–209°. The next fractions afforded 60 mg of apigenin 7-methyl ether 5-*O*-xyloglucoside (IV), m p. 173–175°, while the final fractions contained the 5-*O*-xyloglucoside of luteolin 7-methyl ether (III), m.p 189–192°.

Pilloin (I) was detected in the ethanol fraction. Luteolin 7,4′-dimethyl ether 5-*O*-xyloglucoside (II) was obtained in one workup procedure as a mixture with compounds IV and V.

Acknowledgements—This work was supported by the Robert A. Welch Foundation (Grant F-130), The National Science Foundation (Grants GB-29576X and GB-27152). Contribution to the Origin and Structure of Ecosystems Integrated Research Program of the International Biological Program R.D.S. thanks the Deutsche Forschungsgemeinschaft for a fellowship.