Phytochemistry, Vol. 20, No. 8, pp. 1851–1852, 1981.
Printed in Great Britain.

0031–9422/81/081851–02 $02.00/0

FLAVONE *C*-GLYCOSIDES OF TWO *METZGERIA* SPECIES

RENATE THEODOR*, H. DIETMAR ZINSMEISTER*‡, RÜDIGER MUES* and KENNETH R. MARKHAM†

* Fachbereich 16-Botanik, Universität des Saarlandes, 6600 Saarbrücken, West Germany; † Chemistry Division, D.S.I.R., Petone, New Zealand

(*Received* 14 *November* 1980)

Key Word Index—*Metzgeria conjugata*; *Metzgeria leptoneura*; Metzgeriales; Hepaticae; flavones; tricin and apigenin di-*C*-glycosides; apigenin 6-*C*-arabinoside-8-*C*-(2″-*O*-feruloyl)glucoside.

Abstract—Six known tricin and apigenin di-*C*-glycosides, including 2″-*O*-feruloylisoschaftoside, have been identified in gametophytic material of *Metzgeria conjugata*. *M. leptoneura* contains a new di-*C*-glycoside, tricin 6-*C*-xyloside-8-*C*-hexoside. The chemotaxonomic relevance of the flavonoid patterns is briefly discussed.

INTRODUCTION

In an earlier paper [1] we reported the isolation and structures of eleven flavone di-*C*-glycosides from *Apometzgeria pubescens*. The present paper describes the flavonoids isolated from *Metzgeria conjugata* Lindb. and *M. leptoneura* Spruce (M. Lamata Lindb.) (see Table 1).

RESULTS

From extracts of air-dried gametophytic material of *Metzgeria conjugata* (30 g) and *M. leptoneura* (0.15 g) the compounds **1–6** (*M. conjugata*) and **7** and **8** (*M. leptoneura*) listed in Table 1 were isolated by combined column chromatography (CC), PC and preparative TLC.

The flavonoids of Metzgeria conjugata

The main component (18 mg) was **5**, **1–3** were present in less amounts and **4** and **6**, only in trace quantities. The colour reactions of **1–3** after fuming with ammonia (green fluorescence), spraying with NA (yellow fluorescence) and BR (green fluorescence) closely resemble those of the tricin derivatives found in *Apometzgeria pubescens* (Schrank) Kuwah. [1]. MS data for **1** were identical with those of the tricin 6-*C*-arabinoside-8-*C*-hexoside from *A. pubescens* and the presence of a permethylation by-product, the MS of which corresponds to PM 6-formyl-8-*C*-hexosyltricin (M^+ 618), confirms the presence of a 6-*C*-linked arabinose [2]. Co-chromatography of the PM derivative of **1** with the PM derivative of tricin 6-*C*-arabinoside-8-*C*-glucoside from *A. pubescens* confirms **1** as tricin 6-*C*-arabinoside-8-*C*-glucoside [3].

Table 1. Flavonoid *C*-glycosides isolated from gametophytic material of *Metzgeria conjugata* and *M. leptoneura*

Species	Compound	Structure
M. conjugata	1	Tricin 6-*C*-α-L-arabinopyranoside-8-*C*-β-D-glucopyranoside
	2	Tricin 6-*C*-glucopyranoside 8-*C*-arabinopyranoside
	3	Tricin 6,8-di-*C*-β-D-glucopyranoside
	4	Apigenin 6-*C*-α-L-arabinopyranoside-8-*C*-β-D-glucopyranoside (isoschaftoside)
	5	Apigenin 6-*C*-α-L-arabinopyranoside-8-*C*-β-D-(2″-*O*-feruloyl)glucopyranoside (feruloylisoschaftoside)
		Apigenin 6,8-di-*C*-β-D-glucopyranoside (vicenin-2)
M. leptoneura	7	Tricin 6-*C*-xyloside-8-*C*-hexoside

The PM derivative of **2** has the same mass as that of **1**, but the $M^+ - 175$ signal is higher than the $M^+ - 131$ signal, thus suggesting that **2** is a tricin 6-*C*-hexoside-8-*C*-pentoside [4]. The PM derivative of **2** co-chromatographed with the PM derivative of tricin 6-*C*-glucoside-8-*C*-arabinoside from *A. pubescens*.

Compound **3** is assigned the structure tricin 6,8-di-*C*-β-D-glucopyranoside on the basis of its chromatographic and MS data, which are identical with those of tricin 6,8-di-*C*-β-D-glucopyranoside from *A. pubescens*.

The chromatographic (fuming with NH_3: olive; NA: green; BR: olive) and MS data of compounds **4–6** indicate that these are apigenin *C*-glycosides. The MS of PM-**4** is identical with that of authentic PM-isoschaftoside and co-chromatography of the permethyl ethers confirms this identity. **5** was separated from the other compounds by preparative TLC and PC (BAW) and was crystallized from MeOH–H_2O. The hydrolysation products were ferulic acid and isoschaftoside [1]. **5** co-chromatographed with feruloylisoschaftoside from *A. pubescens* and ^{13}C NMR spectroscopy revealed that the feruloyl moiety is attached to the 2-hydroxyl of the glucose. Thus, a comparison of the spectrum of **5** with that previously published [5] for isoschaftoside reveals that the arabinosyl carbon resonances are the same. However, upfield shifts are evident in the glucose C-1 and C-3 signals of 2.4 and 2.5 ppm respectively, together with a downfield shift of 1.7 ppm in the C-2 signal (see Experimental). Such shifts are in accord with those previously observed for 2″-*O*-acylated glycosides [6]. The PM product of **6** was chromatographically identical with authentic PM-vicenin-2.

‡ To whom correspondence should be addressed.

The flavonoids of Metzgeria leptoneura

Two compounds were isolated from *M. leptoneura* (**7** and **8**). The main compound is **7**, **8** being present in only very small amount. Two other compounds which appeared as extremely faint spots only after spraying with NA may possibly be tricetin derivatives. **7** (hR_f values: BAW-upper phase: 23, 15% HOAc: 26, UV: purple, UV + NH_3: green, UV + NA: yellow, UV + BR: green) showed the chromatographic and colour properties of a tricin derivative and this was confirmed by UV spectroscopy (λ_{max} in MeOH 350 nm, bathochromic shift of 70 nm in NaOMe with increasing peak intensity and a bathochromic shift of 24 nm in $AlCl_3$, stable after treatment with HCl). The MS of the PM derivative shows ions at *m/z* 764 (M^+), 749, 733, 717, 703, 645, 633, 619, 601, 589. Visible fragment ions at *m/z* $M^+ - 119$ (−126)*, $M^+ - 131$ (−137), $M^+ - 145$ (−154) indicate the presence of a pentose in the molecule, whereas $M^+ - 163$ and $M^+ - 175$ (PM) and $M^+ - 173$ and $M^+ - 184$ (PDM) reveal a hexose [4]. Since the intensity of $M^+ - 119$ (−126) is higher than $M^+ - 131$ (−137) and that again higher than $M^+ - 145$ (−154), the pentose is xylose rather than arabinose [2] at C-6. The higher peak at $M^+ - 131$ (−137) than $M^+ - 175$ (−184) indicates that the hexose is at C-8. From these data, **7** is assigned the structure tricin 6-*C*-xyloside-8-*C*-hexoside. There was not sufficient material for further analysis of **8**.

DISCUSSION

The present results are consistent with the previous suggestion that flavone-*C*-glycosides are the predominant flavonoids in the Metzgeriales [1, 7]. Comparing the flavonoid patterns of the three species *Apometzgeria pubescens*, *Metzgeria conjugata* and *M. leptoneura*, a number of both qualitative and quantitative differences may be observed. Thus, *M. leptoneura* synthesizes two main flavonoids, the major one being the new compound tricin 6-*C*-xyloside-8-*C*-hexoside, which has not been detected in any other species of the Metzgeriaceae. On the other hand, *M. conjugata* and *A. pubescens* produce some different flavonoids and differ in the number and amount of flavone *C*-glycosides present. From *M. conjugata* the above-described tricin and apigenin di-*C*-glycosides were isolated but no tricetin and apometzgerin di-*C*-glycosides were found in this species. Also a quantitative difference was observed in the amount of ferulylisoschaftoside, the main flavonoid of *M. conjugata*, which was found only as a minor component in *A. pubescens*. These differences in flavonoid pattern between *M. conjugata*, *M. leptoneura* on one hand and *A. pubescens* on the other, lend some support to Kuwahara's [8] separation of *Apometzgeria* as an individual genus but further investigations of other species of the Metzgeriaceae are necessary before a final decision can be made.

EXPERIMENTAL

Plant material. Metzgeria conjugata Lindb. was collected in May 1979 near Brienz, Bernese Alps, Switzerland and *Metzgeria leptoneura* Spruce at Tongariro National Park, New Zealand. Voucher specimens are deposited in the Herbarium of the Fachrichtung Botanik (*M. conjugata*), Universität des Saarlandes, Saarbrücken and in Massey University Herbarium (*M. leptoneura*, No. MPN 17046).

Extraction and isolation. Air-dried plant material was extracted as described previously [9]. The isolation of the compounds was achieved by combined CC, PC and prep. TLC as outlined in ref. [1]. **5** was cryst. from MeOH–H_2O. Fractions containing **1**–**3** and **4** and **6**, respectively, were permethylated and the single compounds separated by TLC on Si gel.

Chromatography. CC: Cellulose microcrystalline for CC, Avicel, Merck (solvent: 3% HOAc); Sephadex LH 20, Roth (solvent: MeOH). PC: Whatman 3 MM (solvent: BAW). PLC: Cellulose MN 300, Macherey and Nagel (solvent: BAW). Prep. TLC: Si gel G 60, Merck (solvents: EtOAc, $CHCl_3$–EtOAc–Me_2CO, 5:1:4). TLC: Cellulose microcrystalline for TLC, Avicel, Merck; cellulose, plastic sheets F 1440, Schleicher & Schüll (solvents: H_2O, 15% HOAc, BAW, BEW); Si gel 60, plastic sheets, Merck (solvents: EtOAc, $CHCl_3$–EtOAc–Me_2CO, 5:1:4 and 5:4:1). Spray reagents: Naturstoffreagenz A (NA) [10] and Benedicts reagent (BR) [11].

UV spectroscopy. As described in ref. [12].

Mass spectrometry. Preparation of PM derivatives was performed according to ref. [13].

^{13}C *NMR of* **5** (DMSO-d_6, ppm from TMS). Sugar carbons (G refers to glucose and A to arabinose): 70.9 (G-1), 72.4 (G-2), 76.4 (G-3), 70.4 (G-4), 82.2 (G-5), 61.3 (G-6), 74.5 (A-1), 74.5 (A-2), 70.9 (A-3), 68.9 (A-4), 68.7 (A-5) ppm. Isoschaftoside [5]: 73.3, 70.7, 78.9, 70.5, 82.0, 61.4 (G); 74.3, 74.0, 70.9, 69.6, 68.6 (A) ppm.

Acknowledgements—We thank Prof. Dr. K. Pfleger, Universität des Saarlandes, FB 3, for running the MS of compounds **1**–**5** and Tate & Lyle Co. for MS of **7**. The help of Mrs. M. Ewerling in preparing the liverwort material, and Dr. E. O. Campbell, Massey University, New Zealand, in providing *M. leptoneura* is gratefully acknowledged. For authentic samples of vicenin-2 and isoschaftoside we thank Dr. B.-G. Österdahl, University of Uppsala, and Professor Dr. J. Chopin, Université Claude Bernard, Lyon, respectively.

REFERENCES

1. Theodor, R., Zinsmeister, H. D., Mues, R. and Markham, K. R. (1980) *Phytochemistry* **19**, 1695.
2. Bouillant, M.-L., Favre-Bonvin, J. and Chopin, J. (1975) *Phytochemistry* **14**, 2267.
3. Besson, E., Dombris, A., Raynaud, J. and Chopin, J. (1979) *Phytochemistry* **18**, 1899.
4. Bouillant, M.-L., Favre-Bonvin, J. and Chopin, J. (1974) *C. R. Acad. Sci. Sér. D* **279**, 295.
5. Österdahl, B.-G. (1979) Ph.D. Thesis, Act. Univ. Upsaliensis No. 516.
6. Markham, K. R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.
7. Markham, K. R. and Porter, L. J. (1978) in *Progress in Phytochemistry* (Reinhold, L., Harborne, J. B. and Swain, T., eds.) Vol. 5, p. 181. Pergamon Press, Oxford.
8. Kuwahara, Y. (1966) *Rev. Bryol. Lich.* **34**, 191.
9. Mues, R. and Zinsmeister, H. D. (1976) *Phytochemistry* **15**, 1757.
10. Neu, R. (1957) *Naturwissenschaften* **44**, 181.
11. Reznik, H. and Egger, K. (1961) *Z. Analyt. Chem.* **183**, 196.
12. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, Berlin.
13. Brimacombe, J. A., Jones, B. D., Stacey, M. and Willard, J. J. (1966) *Carbohydr. Res.* **2**, 167.

* Numbers in parentheses indicate PDM values.