signal for the rhamnose methyl was present upfield at δ0.82 while the remaining sugar protons occurred between δ3.3 and 4.2.

Acknowledgements—This work was supported by the National Science Foundation (Grant DEB 8402017) and by the Robert A. Welch Foundation (Grant F-130). We also wish to thank Dr. Billie L. and Gayle Turner for the collection and identification of *Brickellia vernicosa*.

REFERENCES

1. Ulubelen, A., Timmermann, B. N. and Mabry, T. J. (1980) *Phytochemistry* **19**, 905.
2. Timmermann, B. N., Mues, R., Mabry, T. J. and Powell, A. M. (1979) *Phytochemistry* **18**, 1855.
3. Roberts, M. F., Timmermann, B. N. and Mabry, T. J. (1980) *Phytochemistry* **19**, 127.
4. Mues, R., Timmermann, B. N., Ohno, N. and Mabry, T. J. (1979) *Phytochemistry* **18**, 1379.
5. Timmermann, B. N., Graham, S. A. and Mabry, T. J. (1981) *Phytochemistry* **20**, 1762.
6. Timmermann, B. N. and Mabry, T. J. (1983) *Biochem. Syst. Ecol.* **11**, 37.
7. Norris, J. A. and Mabry, T. J., *J. Nat. Prod.* (in press).
8. Robinson, B. L. (1917) *Mem. Gray Herb.* **1**, 35.
9. Mabry, T. J., Timmermann, B. N., Heil, N. and Powell, A. M. (1981) *Plant Syst. Evol.* **137**, 137
10. Bulinska-Radomska, Z., Norris, J. A. and Mabry, T. (1985) *J. Nat. Prod.* **48**, 144.
11. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.

Phytochemistry, Vol. 25, No. 6, pp. 1502–1504, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00

FLAVONOIDS FROM *ARTEMISIA LANATA**

Mª D. ESTEBAN, I. GONZÁLEZ COLLADO, F. A. MACÍAS, G. M. MASSANET and F. RODRÍGUEZ LUIS

Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Apdo. 40, Puerto Real, Cádiz, Spain

(*Revised received* 10 *October* 1985)

Key Word Index—*Artemisia lanata*; Compositae; Anthemideae; flavonoids; 3,5-dihydroxy-7,8,3',4'-tetramethoxyflavone; 5-hydroxy-6,7,3',4'-tetramethoxyflavone; artemetin.

Abstract—A new flavonoid, gossypetin-7,8,3',4'-tetramethyl ether (3,5-dihydroxy-7,8,3',4'-tetramethoxyflavone) was isolated and its structure elucidated by chemical and spectroscopic methods. The known flavonoids 5-hydroxy-6,7,3',4'-tetramethoxyflavone and artemetin were also isolated. Chemical transformations led to the conclusion that the structure previously reported as '8,3',4'-trimethoxyizalpinin' (3,5-dihydroxy-7,8,3',4'-tetramethoxyflavone) must be the isomer quercetagetin 6,7,3',4'-tetramethyl ether (3,5-dihydroxy-6,7,3',4'-tetramethoxyflavone).

INTRODUCTION

The genus *Artemisia*, which comprises several morphologically different sections, is one of the largest and most widely distributed of the approximately sixty genera in the tribe Anthemideae (Compositae). This genus has received considerable attention from the point of view of sesquiterpene lactones content [1, 2].

Flavonoid compounds are another important class of secondary metabolites frequently isolated from *Artemisia* [3–5]. Earlier phytochemical studies have led to the isolation of sesquiterpene lactones of the guaiane type from *A. lanata* Willd [6]; a perennial plant found in the calcareous hills in the south-east and east of the Iberian Peninsula [7]. Continuing the phytochemical investigation of this species, further TLC screening revealed the presence of several flavonoids.

RESULTS AND DISCUSSION

Three flavonoids were isolated from the ethanolic extract of the aerial part of *A. lanata*: gossypetin 7,8,3',4'-tetramethyl ether (**1**), 5-hydroxy-6,7,3',4'-tetramethoxyflavone (**2**) and artemetin (**3**). The structures of the last two compounds were confirmed by comparing physical and

*Part 7 in the series "Structure and Chemistry of Secondary Metabolites from Compositae". For part 6 see González Collado, I., Macías, F. A., Massanet, G. M., Rodríguez Luis, F., Salvá, J. and Vergara, C. (1985) *Phytochemistry* **24**, 2447.

spectroscopic data as well as those of their acetylated derivatives, with data found in the literature [8].

A very small amount of **1** was obtained as yellow crystals, $C_{19}H_{18}O_8$, 374 $[M]^+$. From UV spectral analyses of **1** using standard shift reagents (see Experimental) the absence of a shift of band II (256, 270 nm), on addition of sodium acetate indicated that C-7 is substituted by a methoxyl group. The 33 nm bathochromic shift of band I (378 nm) with aluminium chloride and 25 nm with aluminium chloride + hydrochloric acid are consistent with the presence of free hydroxyls at C-3 and C-5 and the presence of a double peak for band II (IIa 270, IIb 256) indicated that C-3′ and C-4′ are substituted by two methoxyl groups [9].

The ^{1}H NMR spectrum of **1** (Table 1) showed a doublet at 7.61 and a double doublet at δ7.64 which correspond to the C-2′ and C-6′ protons and a doublet at δ6.93 (J = 8.5 Hz), assigned to C-5′. Furthermore a singlet (1H) at 12.57 showed the presence of a hydroxyl at the C-5 position. The singlets (3H) at δ3.84, 3.89, 3.93 and 3.96 were attributed to the methoxyl groups at C-3′, C-4′, C-7 and C-8. The singlet (1H) which appears at δ6.47 can be assigned to either C-6 or C-8 [10].

Acetylation of **1** yielded the diacetate **1a**, $C_{23}H_{22}O_{10}$. The ^{1}H NMR spectrum (Table 1) of **1a** showed the presence of two singlets at δ2.49 (C-5 OAc) and 2.34 (C-3 OAc); the H-6 is shifted to δ6.87 with a $\Delta\delta$ of +0.40 ppm, characteristic of a benzene proton with an *ortho* free hydroxyl group [11].

These data are consistent with the structure **1** proposed by Bohlmann and co-workers for '8,3′,4′-trimethoxyizalpinin' isolated from *Heteromma simplicifolium* [12]. However, the physical and spectroscopic constants described by these workers do not coincide with those obtained by us. For this reason, the preparation of **1** and **4** from artemetin (**3**), was carried out with HCl reflux treatment [13]. Two products were obtained: the first was identical to our natural product **1** and the other presented physical and spectroscopic constants (mp, ^{1}H NMR, UV) similar to those reported by Bohlmann *et al.* [12].

The presence of a methoxyl at C-6 in **4** was confirmed by treating artemetin (**3**) with aluminium chloride in dried diethyl ether, which by selective hydrolysis of the methyl ether in C-3 [13], gave rise to a product identical with **4**.

The structures of **1** and **4** were confirmed by the ^{1}H NMR study of their diacetylated derivatives: the diacetate of **1** (**1a**) presented a ^{1}H NMR spectrum identical with that exhibited by the diacetate derivative of our natural product (Table 1). The diacetate of **4** (**4a**) presented two singlets at δ2.40 (C-5 OAc) and 2.28 (C-3 OAc); in this case the chemical shift of H-8 (6.82, *s*) is practically the same as in **1a**, but the $\Delta\delta$ is of +0.25 ppm, characteristic of a benzene proton with a *para* free hydroxyl group [11].

These data are consistent with structure **1** for the product isolated from *Artemisia lanata*, described in this communication, and with structure **4** reported as '8,3′,4′-trimethoxyizalpinin', isolated from *Heteromma simplicifolium* [12]. Thus our compound **1** is gossypetin 7,8,3′,4′-tetramethyl ether, while Bohlmann's compound should be revised to quercetagetin-6,7,3′,4′-tetramethyl ether (3,5-dihydroxy-6,7,3′,4′-tetramethoxyflavone).

Compound **4** has previously been isolated from *Artemisia annua* by Djermanović *et al.* [14] and synthesized by Bhardwaj *et al.* [15] in 1977 and later, in 1979, by Stefanović *et al.* [16]. Data reported in ref. [15] are the same as those obtained by us for **4**, and as published by Bohlmann [12] for '8,3′,4′-trimethoxyizalpinin'.

Table 1. ^{1}H NMR spectral data for **1**, **1a**, **4** and **4a** (200 MHz, $CDCl_3$, TMS as internal standard)

	1	1a	4	4a
H-8	—	—	6.54 *s*	6.79 *s*
H-6	6.47 *s*	6.87 *s*	—	—
H-6′	7.64 *dd*	8.00 *dd*	7.70 *dd*	7.61 *dd*
H-5′	6.93 *d*	7.04 *d*	6.98 *d*	6.92 *d*
H-2′	7.61 *d*	7.76 *d*	7.64 *d*	7.57 *d*
OMe	3.96 *s*	3.95 *s*	4.00 *s*	3.90 *s*
	3.93 *s*	3.89 *s*	3.97 *s*	3.90 *s*
	3.89 *s*	3.83 *s*	3.97 *s*	3.87 *s*
	3.84 *s*	3.77 *s*	3.86 *s*	3.71 *s*
C-5 OH	12.57 *s*	—	12.40 *s*	—
C-5 OAc	—	2.49 *s*	—	2.40 *s*
C-3 OAc	—	2.34 *s*	—	2.28 *s*

J (Hz): 2′,6′ = 2; 5′,6′ = 8.5.

EXPERIMENTAL

Artemisia lanata Willd was collected in Algora (Guadalajara) on July 1981. A voucher is on deposit at the Herbarium of the Department of Botany (SEVF), Faculty of Pharmacy, Sevilla, Spain.

The air-dried material (10 kg) was extracted with hot EtOH and the resulting extract separated by CC (silica gel). The medium polar fraction contained a mixture of three flavonoids. CC and prep. TLC (silica) afforded **1** (30 mg), **2** (200 mg) and **3** (400 mg) ($CHCl_3$–*t*-BuOH, 95:5).

3,5-*Dihydroxy*-7,8,3′,4′-*tetramethoxyflavone* (**1**). Yellow crystals, mp 178–180° (EtOAc). ^{1}H NMR: see Table 1. UV λ_{max}^{MeOH} nm: 256, 270, 345; ($AlCl_3$) 270, 279, 378; ($AlCl_3$ + HCl) 268, 281, 370; (NaOMe) 270, 390; (NaOAc) 256, 270, 345; (NaOAc + H_3BO_3) 258, 272, 348. MS *m/z* (rel. int.): 374.1038 $[M]^+$ (80), ($C_{19}H_{18}O_8$ requires 374.1020), 359 $[M-15]^+$ (100), 165 (3), 151 (13), etc.

Diacetate **1a**. Yellow crystals, mp 167–168° (EtOAc). ^{1}H NMR: see Table 1.

3,5-*Dihydroxy*-7,8,3′,4′-*tetramethoxyflavone* (**1**) *and* 3,5-*dihydroxy*-6,7,3′,4′-*tetramethoxyflavone* (**4**). Compounds **1** and **4** synthesized from artemetin (**3**). Conc. HCl (80 ml) was added to 200 mg of **3**. The reaction mixture was heated in a steam bath for 2 hr, poured into 75 ml of ice water and extracted twice with 100 ml portions of EtOAc. The extract was then dried and the solvent evaporated. The resulting products were chromatographed on a silica gel column; **1** (20 mg) and **4** (30 mg) were isolated.

3,5-*Dihydroxy*-6,7,3′,4′-*tetramethoxyflavone* (**4**). To 10 ml of dry Et_2O were slowly added 1 g anhydrous $AlCl_3$, and then 50 mg of artemetin (**3**). This mixture was refluxed for 24 hr, then H_2O was cautiously added and the Et_2O evaporated. The aq. layer was extracted with EtOAc, the extract dried and the solvent evaporated. The concd extract was chromatographed on a silica gel column; **3** (20 mg) and **4** (15 mg) were isolated. Compound **4** was isolated as yellow crystals, mp 210–212° (EtOAc). ^{1}H NMR, Table 1. UV λ_{max}^{MeOH} nm: 258, 278, 345; ($AlCl_3$) 260, 290, 378; ($AlCl_3$ + HCl) 268, 290, 370; (NaOMe) 290, 315 (sh), 342; (NaOAc) 278, 355. MS *m/z* (rel. int.): 374 $[M]^+$ (100), 373 [M

$-1]^+$ (53), 359 $[M-15]^+$ (40), 356 $[M-18]^+$ (7), 165 (9), 151 (3), etc.

Diacetate **4a**. Yellow crystals, mp 207–208° (EtOAc). ^{1}H NMR: see Table 1.

Acknowledgements—We wish to thank Professor S. Silvestre Domingo, Departamento de Botánica, Facultad de Farmacia, Universidad de Sevilla (Spain) for the identification of the plant material, Instituto de Productos Naturales Orgánicos (C.S.I.C.) La Laguna, Tenerife, for mass spectra and Paloma Peers for editorial assistance.

REFERENCES

1. Geissman, T. A. (1970) *Phytochemistry* **9**, 2377.
2. Geissman, T. A. and Irwin, M. A. (1970) *Pure Appl. Chem.* **21**, 167.
3. Brown, D., Asplund, R. O. and McMahon, V. A. (1975) *Phytochemistry* **14**, 1083.
4. Komiya, T., Naruse, Y. and Oshio, H. (1976) *Yakugaku Zasshi* **96**, 855.
5. Khafagu, S. M., El-Ghazooly, M. G. and Metwally, A. M. (1979) *Pharmazie* **34**, 748.
6. González, A. G., Bermejo, J., de la Rosa, A. D. and Massanet, G. M. (1976) *An. Quim.* **19**, 1761.
7. Polunin, O. and Senythies, B. E. (1977) *Guía de Campo de las Flores de España* (Omega S. A., ed.) p. 380. Barcelona.
8. González, A. G., Fraga, B. M., Hernández, M. G., Larruga, F., Luis, J. G. and Ravelo, A. G. (1978) *J. Nat. Prod.* **41**, 279.
9. Voirin, B. (1983) *Phytochemistry* **22**, 2107.
10. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, pp. 261–265. Springer, Berlin.
11. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, p. 272. Springer, Berlin.
12. Bohlmann, F. and Fritz, U. (1979) *Phytochemistry* **18**, 1080.
13. Tatum, J. H. and Berry, R. E. (1972) *Phytochemistry* **11**, 2283.
14. Djermanović, M., Jokić, A., Mladenović, S. and Stefanović, M. (1975) *Phytochemistry* **14**, 1873.
15. Bhardwaj, D. K., Jain, S. C. and Sharma, G. C. (1977) *Indian J. Chem.* **15B**, 860.
16. Stefanović, M., Krstić, L., Jokić, A., Rihter, B. and Mladenović, S. (1979) *Glas. Hem. Drus. Beograd* **44**, 497.