Pergamon

PII: S0031–9422(98)0018–00

Phytochemistry, Vol. 49, No. 5, pp. 1421–1424, 1998

Printed in Great Britain
0031–9422/98/$—see front matter

CHROMONES AND FLAVANONES FROM *ARTEMISIA CAMPESTRIS* SUBSP. *MARITIMA*

JOÃO M. J. VASCONCELOS, ARTUR M. S. SILVA* and JOSÉ A. S. CAVALEIRO

Department of Chemistry, University of Aveiro, 3810 Aveiro, Portugal

(*Received in revised form* 2 *February* 1998)

Key Word Index—*Artemisia campestris* subsp. *maritima*; Compositae; chromones; flavanones; 1D selective INEPT.

Abstract—From the acetone extract of *Artemisia campestris* subsp. *maritima* six flavanones, two chromones and the coumarin scopoletin were isolated. 5-Hydroxy-7-methoxychromone and 5,7-dimethoxychromone are new compounds, while the flavanone eriodictyol-7,3′-dimethyl ether is reported for the first time in this species. The structures were elucidated by 1D and 2D NMR techniques. The unequivocal assignments of carbon resonances, mainly made by using 1D selective INEPT and 2D HETCOR experiments, allowed us to correct some ^{1}H and ^{13}C resonances of the isolated flavanones and also to differentiate between the flavanone isomers sakuranetin/isosakuranetin and eriodictyol-7,3′-dimethyl ether/eriodictyol-7,4′-dimethyl ether.

INTRODUCTION

There has been an increasing interest in the genus *Artemisia* L. (Compositae) since the discovery of artemisinine, obtained from *A. annua*, and its successful clinical trials as an antimalarial drug [1]. Several phytochemical studies on the genus *Artemisia* have been published; however, only a few of them are concerned with *A. campestris* L. [2–13]. To the best of our knowledge, in the case of *A. campestris* L. subsp. *maritima* Arcangeli only five phytochemical studies have been carried out. These studies are concerned with the elucidation of their acetylene, flavonoid and other phenolic components [2, 9, 11, 13] and also on their essential oil contents [3].

Pursuing our studies on the chemical composition of *A. campestris* L. subsp. *maritima* Arcangeli [9], growing on the beach sands near Aveiro, we have now considered the acetone extracts of this plant. As a result, six flavanones **1–6**, two chromones **7,8** and one coumarin **9** have been isolated and characterised.

RESULTS AND DISCUSSION

The aerial parts of *Artemisia campestris* were ground and extracted successively with hexane and acetone. The acetone extracts were subjected to preparative silica gel thin layer chromatography, as described in the Experimental, affording **1–9** as pure compounds.

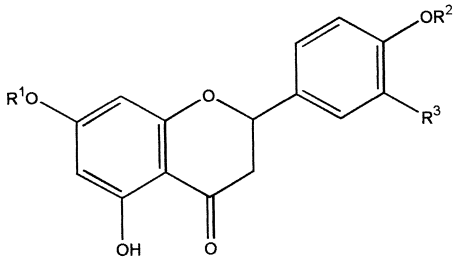
The ^{1}H assignments in the NMR spectra of all compounds **1–9** were based on COSY experiments; however, for the ^{13}C atoms, HETCOR and 1D selective INEPT experiments [14] were carried out. All the assignments revealed the presence of six flavanones **1–6**, two chromones **7,8** and scopoletin **9**.

By using 1D selective INEPT experiments for compounds **2–6**, upon irradiation of the O*H*-5 resonance, enhancements on C-5, C-6 and C-10 signals were observed (Table 1). These results together with the direct ^{1}H-^{13}C correlation spectra (HETCOR) allowed us to conclude that the H-6 resonances appear at higher frequency values than those of H-8. These results indicate that it is necessary to correct certain literature data about the H-6 and H-8 chemical shifts [9, 11, 15–20].

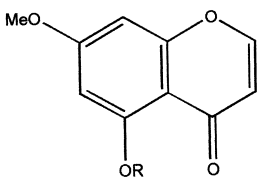
The irradiations of OCH_3, H-6 and H-8 resonances of **2**, using 1D selective INEPT experiments (Table 1), prompted us to conclude that the methoxyl group is at the 7-position. Compound **2** was then assigned as being the flavanone sakuranetin. In the case of **3** we could assign unequivocally the carbon resonances of C-2′,6′, C-1′ and C-4′, upon irradiation of OCH_3 and H-3′,5′ resonances (Table 1) and demonstrate that the methoxyl group is at the 4′ position; this compound **3** was then assigned as the flavanone isosakuranetin.

The unequivocal identification of each flavanone isomer **5** and **6** was also based on the assignment of all their ^{13}C resonances, by using 1D selective INEPT and HETCOR experiments. The key irradiation was

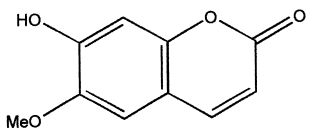
* Author to whom correspondence should be addressed.



1. $R^1 = R^2 = R^3 = H$
2. $R^1 = Me$; $R^2 = R^3 = H$
3. $R^2 = Me$; $R^1 = R^3 = H$
4. $R^1 = Me$; $R^2 = H$; $R^3 = OH$
5. $R^1 = Me$; $R^2 = Me$; $R^3 = OH$
6. $R^1 = Me$; $R^2 = H$; $R^3 = OMe$



7. R = H
8. R = Me



9

Table 1. Results obtained from 1D selective INEPT NMR experiments

Compound	Resonance irradiation (δ, ppm)	Signal Enhancement (δ, ppm)
2	OC*H*$_3$ (3.81)	C-7 (168.0)
	H-6 and H-8 (6.08 and (6.05)	C-10 (103.1); C-9 (162.1) and C-7 (168.0)
	O*H*-5 (12.03)	C-6 (95.1); (C-10 (103.1) and C-5 (164.1)
3	OC*H*$_3$ (3.84)	C-4′ (160.0)
	H-3′,5′ (6.95)	C-2′,6′ (127.7); C-1′ (130.3) and C-4′ (160.0)
	O*H*-5 (12.06)	C-6 (96.7); C-10 (103.0) and C-5 (164.3)
4	OC*H*$_3$ (3.84)	C-7 (168.7)
	H-6 and H8 (6.05 and 6.03)	C-10 (103.6); C-9 (164.1), C-5 (164.8) and C-7 (168.7)
	O*H*-5 (12.04)	C-6 (95.3); C-10 (103.6) and C-5 (164.8)
5	OC*H*$_3$ (3.81 and 3.94)	C-3′ (146.7) and C-7 (168.0)
	O*H*-4′ (5.72)	C-5′ (114.5); C-4′ (146.2) and C-3′ (146.7)
	O*H*-5 (12.04)	C-6 (95.1); C-10 (103.1) and C-5 (164.1)
6	OC*H*$_3$ (3.81 and 3.92)	C-4′ (147.0) and C-7 (168.0)
	O*H*-3′(5.73)	C-2′ (112.6); C-3′ (145.9) and C-4′ (147.0)
	O*H*-5 (12.03)	C-6 (95.1); C-10 (103.1) and C-5 (164.1)
7	OC*H*$_3$ (3.86)	C-7 (165.6)
	O*H*-5 (12.04)	C-6 (98.3); C-10 (106.7) and C-5 (162.3)
	OC*H*$_3$ (3.96)	C-6 (144.0)
	H-4 (7.60)	C-5 (107.4); C-9 (150.2) and C-2 (161.5)

that of the B ring O*H* resonance. In the case of compound **5**, enhancements were observed on the C-2′, C-3′ and C-4′ resonances (Table 1); such results are only in agreement with a 3′-OH substitution, and the structure then assigned to eriodictyol-7,4′-dimethyl ether. However, for **6**, enhancements were observed on the C-3′, C-4′ and C-5′ resonances (Table 1), thus showing a 4′-OH substitution. It was identified as being eriodictyol-7,3′-dimethyl ether.

The protocol just described for the unequivocal assignment of the ^{13}C resonances of compounds **2–5** prompted us to report some of them for the first time and also to elucidate some literature data related with the chemical shifts of C-6,8 and C-3′,4′ [11, 21].

Based on the ^{1}H and ^{13}C resonances, elucidated by 1D and 2D NMR techniques, and comparing with certain literature data [16–18, 20–24], the compounds **1, 4** and **9** were identified as naringenin, eriodictyol-7-methyl ether and scopoletin, respectively. However, in the case of scopoletin **9**, our results indicate that the literature assignments of H-5 and H-8 resonances must be reversed. In fact, by irradiation of the H-4 resonance, in a 1D selective INEPT experiment, enhancements were observed on the signals of C-2, C-5 and C-9 (Table 1). These results together with the analysis of the HETCOR spectra let us to assign the H-8 resonance at a higher frequency value than that of H-5.

Compounds **1–5** have already been reported from *Artemisia campestris* extracts [9–11], but to the best of our knowledge, eriodictyol-7,3′-dimethyl ether **6** is now reported for the first time as a component of this species. In addition to these compounds, two novel chromones **7,8** have also been isolated. Their IR and UV spectra are similar to those reported for the corresponding synthetic compounds [25].

Table 2. ^{1}H NMR data of flavanones **1–6**[a]

Protons	**1**	**2**	**3**	**4**[b]	**5**[b]	**6**[b]
H-2	5.36 *dd*, 3.2; 13.1	5.36 *dd*, 3.5; 13.5	5.36 *dd*, 3.1; 13.0	5.42 *dd*, 3.1; 12.8	5.33 *dd*, 3.1; 12.9	5.34 *dd*, 3.0; 13.1
H-3α	3.09 *dd*, 13.1; 17.2	3.10 *dd*, 13.5; 17.2	3.10 *dd*, 13.0; 17.2	3.17 *dd*, 12.8; 17.2	3.08 *dd*, 12.9; 17.2	3.11 *dd*, 13.1; 17.2
H-3*β*	2.78 *dd*, 3.2; 17.2	2.79 *dd*, 3.5; 17.2	2.78 *dd*, 3.1; 17.2	2.75 *dd*, 3.1; 17.2	2.78 *dd*, 3.1; 17.2	2.79 *dd*, 3.0; 17.2
H-6	6.00 *d*, 2.3	6.08 *d*, 2.3	6.00 *d*, 2.3	6.05 *d*, 2.3	6.07 *d*, 2.3	6.08 *d*, 2.3
H-8	5.98 *d*, 2.3	6.05 *d*, 2.3	5.98 *d*, 2.3	6.03 *d*, 2.3	6.05 *d*, 2.3	6.06 *d*, 2.3
H-2′	7.34 *d*, 8.6	7.34 *d*, 8.6	7.38 *d*, 8.7	7.04 *br s*	7.05 *d*, 2.0	6.94–6.98 *m*
H-3′	6.89 *d*, 8.6	6.89 *d*, 8.6	6.95 *d*, 8.7	—	—	—
H-5′	6.89 *d*, 8.6	6.89 *d*, 8.6	6.95 *d*, 8.7	6.86 AB, 8.1	6.88 *d*, 8.6	6.94–6.98 *m*
H-6′	7.34 *d*, 8.6	7.34 *d*, 8.6	7.38 *d*, 8.7	6.88 AB, 8.1	6.94 *dd*, 2.0; 8.6	6.94–6.98 *m*
O*H*-5	12.06 *s*	12.03 *s*	12.06 *s*	12.14 *s*	12.03 *s*	12.04 *s*
OCH_3		3.81 (7) *s*	3.84(4′) *s*	3.84 (7) *s*	3.81 (7) 3.92 (4′) 2 *s*	3.81 (7) 3.94 (3′) 2 *s*

[a]For each proton resonance are shown the chemical shift, multiplicity and coupling constant.
[b]O*H* resonances of: **4** 8.21 (*br s*, 3′,4′); **5** 5.73 (*s*, 3′) and **6** 5.72 (*s*, 4′)

EXPERIMENTAL

General. Melting points are uncorrected and were determined on a Reichert Thermovar apparatus fitted with a microscope. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AMX 300 at 300.13 and 75.47 MHz, respectively; the chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as internal reference and the coupling constants J (^{1}H/^{1}H) are expressed in Hz. ^{1}H Assignments were made using 2D COSY experiments, while ^{13}C assignments were made using HETCOR experiments as well as one-dimensional selective INEPT (long-range C/H coupling constants were optimized to 7 Hz) [14]. Mass spectra were obtained at 70 eV electron impact ionization using a VG Autospec Q mass spectrometer. Preparative TLC were carried out on silica gel plates (Merck silica gel 60 F_{254}). Column chromatography were also performed on silica gel (Merck silica gel 60, 70–230 mesh).

Plant material Aerial parts of plants *A. campestris* L. subsp. *maritima* Arcangeli were collected on the Atlantic beach sands of Costa Nova, near Aveiro (Portugal), in June 1990.

Extraction and isolation The plant material was air-dried at room temperature, finely grounded and extracted (165 g), in a soxhlet apparatus, successively with hexane and acetone. The acetone extract (18.2 g) was fractionated on preparative Si 60 tlc into three frs (I–III), using a 1 : 1 mixture of CH_2Cl_2 : acetone as eluent. Each fr was further chromatographed using different mixtures of CH_2Cl_2 : acetone, giving the 1–9 compounds as follows: fr I–**4** (420 mg) and **1** (2 mg); fr II-mixt. of **2** and **3** (60 mg), **7** (12 mg) and **9** (15 mg); fr III-**8** (2 mg) and mixt. of **5** and **6** (40 mg). Very diluted $CHCl_3$ solutions containing mixtures of isomers **2,3** and **5,6** were also separated under the same conditions.

Naringenin **1** Mp 248–250° (from CH_2Cl_2/light petroleum, Lit. 250–251° [16]); ^{1}H and ^{13}C NMR (Tables 2 and 3).

Sakuranetin (5,4′-*dihydroxy*-7-*methoxyflavanone*) **2** Mp 151–153° (from CH_2Cl_2/light petroleum, Lit. 152° [26]); ^{1}H and ^{13}C NMR (Tables 2–3).

Isosakuranetin (5,7-*dihydroxy*-4′-*methoxyflavanone*) **3** Mp 180–182° (from CH_2Cl_2/light petroleum, Lit. 177–179° [9]); ^{1}H and ^{13}C NMR (Tables 2–3).

Eriodictyol-7-*methyl ether* (5,3′,4′-*Trihydroxy*-7-*methoxyflavanone*) **4** Mp 220–223° (from CH_2Cl_2/light petroleum, Lit. 221° [17]); ^{1}H and ^{13}C NMR (Tables 2–3).

Eriodictyol-7,4′-*dimethyl ether* (5,3′-*Dihydroxy*-7,4′-*dimethoxyflavanone*) **5** Mp 165–168° (from CH_2Cl_2/light petroleum, Lit. 163–164° [27]); ^{1}H and ^{13}C NMR (Tables 2–3).

Eriodictyol-7,3′-*dimethyl ether* (5,4′-*Dihydroxy*-7,3′-*dimethoxyflavanone*) **6** Mp 148–150° (from CH_2Cl_2/light petroleum, Lit. 149–150° [11]); ^{1}H and ^{13}C NMR (Tables 2–3).

5-*Hydroxy*-7-*methoxychromone* **7** Mp 98–101°. (from CH_2Cl_2/light petroleum). ^{1}H NMR: δ 3.86 (7-OCH_3, *s*, 3H), 6.22 (H-3, *d*, *J* 6.0 Hz), 6.37 (H-6, *d*, *J* 2.3 Hz), 6.39 (H-8, *d*, *J* 2.3 Hz), 7.75 (H-2, *d*, *J* 6.0 Hz), 12.58 (O*H*-5, *s*). ^{13}C NMR: δ 55.8 (7-O*C*H$_3$), 92.8 (C-8), 98.2 (C-6), 106.7 (C-10), 111.4 (C-3), 155.6 (C-2), 158.1 (C-9), 162.3 (C-5), 165.6 (C-7), 181.9 (C-4). EIMS *m*/*z*: 192 ($M^{+\cdot}$, 100), 167 (12), 164 (14), 149 (34), 138 (9), 121 (17).

5,7-*Dimethoxychromone* **8** Mp 127–130° (from CH_2Cl_2/light petroleum, Lit. 134–135° [28]). ^{1}H NMR: δ 3.93 (5-OCH_3, *s*, 3H), 3.96 (7-OCH_3, *s*, 3H), 6.30 (H-3, *d*, *J* 9.5 Hz), 6.85 (H-6, *d*, *J* 2.7 Hz), 6.86 (H-8, *d*, *J* 2.7 Hz), 7.63 (H-2, *d*, *J* 9.5 Hz). EIMS *m*/*z*: 206 ($M^{+\cdot}$, 94), 191 (64), 181 (5), 178 (63), 163 (85), 135 (70), 107 (49).

Scopoletin **9** Mp 205–207° (Lit. 203–205°C [24]). ^{1}H NMR: δ 3.96 (6-OCH_3, *s*, 3H), 6.17 (7-O*H*, *s*, 1H),

Table 3. ^{13}C NMR data of flavanones **2–6**

Carbons	**2**	**3**	**4**	**5**	**6**
C-2	80.0	78.9	80.0	79.0	79.4
C-3	43.2	43.1	43.4	43.2	43.4
C-4	196.0	196.1	197.7	196.0	196.0
C-5	164.2	164.3	164.8	164.1	164.1
C-6	95.1	96.7	95.3	95.1	95.1
C-7	168.0	164.9	168.7	168.0	168.0
C-8	94.2	95.5	94.5	94.2	94.3
C-9	162.9	163.2	164.1	162.8	162.8
C-10	103.1	103.0	103.6	103.1	103.1
C-1′	130.5	130.3	131.1	131.5	130.2
C-2′	128.0	127.7	114.6	112.6	108.7
C-3′	115.6	114.2	146.1	145.9	146.7
C-4′	156.1	160.0	146.5	147.0	146.2
C-5′	115.6	114.2	116.0	110.6	114.5
C-6′	128.0	127.7	119.1	118.2	119.6
OCH_3	55.7 (7)	55.4 (4′)	56.2 (7)	55.7 (7) 56.1 (4′)	55.7 (7) 56.0 (3′)

6.28 (H-3, *d*, *J* 9.5 Hz), 6.85 (H-5, *s*, 1H), 6.92 (H-8, *s*, 1H), 7.60 (H-4, *d*, *J* 9.5 Hz). ^{13}C NMR: δ 56.4 (6-O*C*H_3), 103.2 (C-8), 107.4 (C-5), 111.5 (C-10), 113.4 (C-3), 143.3 (C-4), 144.0 (C-6), 150.2 (C-7,9), 161.5 (C-2).

Acknowledgements—Thanks are due to the University of Aveiro and JNICT, Lisbon, for funding the Research Unit N° 62/94.

REFERENCES

1. Butler, A. R. and Wu, Y.-L., *Chem. Soc. Rev.*, 1992, **21**, 85.
2. Bohlmann, F., Burkhardt, T. and Zdero, C., *Naturally Occuring Acetylenes*, Academic Press, 1973.
3. Stangl, R. and Greger, H., *Plant Syst. Evol.*, 1980, **136**, 125.
4. De Pascual-Teresa, J., Bellido, I. S., Gonzalez, M. S., Muriel, M. R. and Hernandez, J. M., *Phytochemistry*, 1980, **19**, 2781.
5. De Pascual-Teresa, J., Bellido, I. S., Gonzalez, M. S., Muriel, M. R. and Hernandez, J. M., *Phytochemistry*, 1981, **20**, 2417.
6. Hurabielle, M., Eberle, J. and Paris, M., *Planta Med.*, 1982, **46**, 124.
7. De Pascual-Teresa, J., Gonzalez, M. S., Muriel, M. R. and Bellido, I. S., *Phytochemistry*, 1983, **22**, 2587.
8. De Pascual-Teresa, J., Gonzalez, M. S., Muriel, M. R. and Bellido, I. S., *Phytochemistry*, 1984, **23**, 1819.
9. Cavaleiro, J. A. S., *Fitoterapia*, 1986, **57**, 278.
10. De Pascual-Teresa, J., Gonzalez, M. S., Muriel, M. R., Arcocha, A. D. and Bellido, I. S., *J. Nat. Prod.*, 1986, **49**, 177.
11. Rauter, A. P., Branco, I., Tostão, Z., Pais, M. S., Gonzalez, A. G. and Bermejo, J. B., *Phytochemistry*, 1989, **28**, 2713.
12. Wang, Y., Toyota, M., Krause, F., Hamburger, M. and Hostettmann, K., *Phytochemistry*, 1990, **29**, 3101.
13. Sanz, J. F., Garcia-Lliso, V., Marco, J. A. and Vallés-Xirau, J., *Phytochemistry*, 1991, **30**, 4167.
14. Bax, A., *J. Magn. Reson.*, 1984, **57**, 314.
15. Markham, K. R. and Mabry, T. J., in *The Flavonoids*, eds. J. B. Harborne, T. J. Mabry, and H. Mabry. Chapman and Hall, London, 1975, pp. 63.
16. Geissman, T. A., Mukherjee, R. and Sim, K. Y., *Phytochemistry*, 1967, **6**, 1575.
17. Wollenweber, E., *Z. Naturforsch.*, 1981, **36c**, 604.
18. Abe, N., Sato, H. and Sakumura, S., *Agric. Biol. Chem.*, 1987, **51**, 349.
19. Balza, F., Jamieson, L. and Towers, G. H. N., *J. Nat. Prod.*, 1985, **48**, 339.
20. Markham, K. R. and Geiger, H., in *The Flavonoids-Advances in Research Since* 1986, ed. J. B. Harborne, Chapman and Hall, London, 1994, pp. 458.
21. Agrawal, P. K., Thakur, R. S. and Bansal, M. C., in *Carbon*-13 *NMR of Flavonoids*, ed. P. K. Agrawal, Elsevier, Amsterdam, 1989, pp. 95.
22. Gonzalez, A. G., Reyes, R. E. and Velazquez, J. H., *An. Quim.*, 1975, **71**, 437.
23. Barberá, O., Marco, J. A., Sanz, J. F. and Sanchez-Parareda, J., *Phytochemistry*, 1986, **25**, 2357.
24. Sadavongvivad, C. and Supavilai, P., *Phytochemistry*, 1977, **16**, 1451.
25. Romussi, G. and Ciarallo, G., *J. Heterocycl. Chem.*, 1976, **13**, 211.
26. Agarwal, V. K., Thappa, R. K., Agarwal, S. G. and Dhar, K. L., *Phytochemistry*, 1984, **23**, 1342.
27. Wollenweber, E., Dietz, V. H., Schillo, D. and Schilling, G., *Z. Naturforsch.*, 1980, **35c**, 685.
28. Ciattini, P. G., Morena, E. and Ortar, G., *J. Heterocycl. Chem.*, 1982, **19**, 395.