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3-METHOXYFLAVONES AND COUMARINS FROM *ARTEMISIA INCANESCENS*

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Key Word Index—*Artemisia incanescens*; Compositae; Anthemideae; structure elucidation; flavonoids; 3-methoxyflavones; coumarins; ^{1}H NMR; ^{13}C NMR.

Abstract—Seven 3-methoxyflavones and three coumarins have been isolated from aerial parts of *Artemisia incanescens*. Their ^{1}H NMR spectra in DMSO-d_6 and $CDCl_3$ are compared and discussed. The hitherto unreported ^{13}C NMR spectra of some of these compounds are also discussed.

INTRODUCTION

Artemisia incanescens Jordan is a medium-sized herbaceous shrub with long, thin woody stems, which occurs scattered in Central and South Europe. In Spain, this species can be found in some north-eastern regions, especially in arid, mountain areas [1]. It belongs to an extensively studied genus from which many metabolites, mainly sesquiterpene lactones [2], have been isolated. Several 3-methoxyflavones [3–9] and coumarins [10] have already been found in *Artemisia* spp. We now report the isolation of seven 3-methoxyflavones **1**–**7** and three coumarins **8**–**10** from the ether-soluble portion of a methanolic extract of *A. incanescens*. Interestingly, coumarins **8**–**10** have been deemed to be responsible for the anticholeretic action of extracts of *A. abrotanum* [11].

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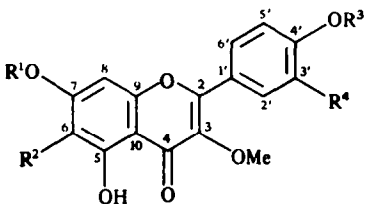
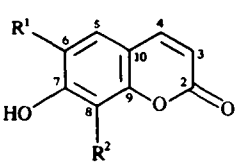


1	Santin	$R^1 = R^4 = H$, $R^2 = OMe$; $R^3 = Me$
2	Casticin	$R^1 = R^3 = Me$; $R^2 = OMe$; $R^4 = OH$
3	Penduletin	$R^1 = Me$; $R^2 = OMe$; $R^3 = R^4 = H$
4	Centaureidin	$R^1 = H$; $R^2 = OMe$; $R^3 = Me$; $R^4 = OH$
5	Quercetin 3,4′-dimethyl ether	$R^1 = R^2 = H$; $R^3 = Me$; $R^4 = OH$
6	Axillarin	$R^1 = R^3 = H$; $R^2 = OMe$; $R^4 = OH$
7	Quercetin 3-methyl ether	$R^1 = R^2 = R^3 = H$; $R^4 = OH$



8	Umbelliferone	$R^1 = R^2 = H$
9	Scopoletin	$R^1 = OMe$; $R^2 = H$
10	Isofraxidin	$R^1 = R^2 = OMe$

RESULTS AND DISCUSSION

The coumarins isofraxidin (**10**) and scopoletin (**9**) are the major constituents of the ether-soluble extract. Further we isolated the coumarin umbelliferone (**8**) and seven 3-*O*-methylated flavonols: santin (**1**), casticin (**2**), penduletin (**3**), centaureidin (**4**), axillarin (**6**), quercetin-3-methyl ether (**7**) and quercetin-3,4′-dimethyl ether (**5**). Except for the two quercetin derivatives, these flavonols are 6-methoxy compounds. Santin [12], casticin [13], centaureidin [14] and quercetin-3,4′-dimethyl ether [15] are reported for the first time in the genus *Artemisia*. It should be remarked that *A. incanescens* is included in the subgenus *Artemisia*, made up of the former subgenera *Abrotanum* and *Absinthium* [2], which are essentially confined to the Old World. It is a phylogenetically primitive taxon and that may be reflected by the fact that we have isolated 3-*O*-methylated flavonols but not flavones. The appearance of the latter type of substance, indeed, is considered an advanced feature from the phylogenetic point of view [16].

The ^{1}H NMR data compiled in Table 1 indicate that *O*-methylation, in spectra measured in DMSO-d_6, consistently produces a downfield shift of 0.15–0.30 ppm in protons in an *ortho*-position to the phenolic hydroxyl [17, 18]. For example, H-8 resonates at $\sim\delta$6.9 when there is a 7-methoxyl group and at $\sim\delta$6.6 when there is a 7-hydroxyl group. Furthermore, 4′-*O*-methylation induces a downfield shift of *ca* 0.2 ppm in the signal of H-5′. In spectra measured in $CDCl_3$ these effects are clearly smaller (less than 0.1 ppm) and erratic (Table 1). This different behaviour may be related to the known hydrogen-bonding properties of DMSO. On the other hand, in all compounds with a 3′-hydroxyl, 4′-methoxyl

Table 1. ^{1}H NMR spectral data for compounds 1–7*

Compound	H-6	H-8	H-2′	H-3′	H-5′	H-6′	OMe
1		6.58 *s*	7.98 *d* (8.9)	7.10 *d* (8.9)	7.10 *d* (8.9)	7.98 *d* (8.9)	3.83 *s* 3.76 *s* 3.73 *s*
1†		6.55 *s*	8.06 *d* (9.1)	7.02 *d* (9.1)	7.02 *d* (9.1)	8.06 *d* (9.1)	4.04 *s* 3.89 *s* 3.84 *s*
2		6.88 *s*	7.60–7.50 *m* AB part of an ABX system		7.10 *d* (9.3)	7.60–7.50 *m* AB part of an ABX system	3.91 *s* 3.85 *s* 3.79 *s* 3.72 *s*
2†		6.51 *s*	7.70–7.60 *m* AB part of an ABX system		6.97 *d* (8.6)	7.70–7.60 *m* AB part of an ABX system	3.99 *s* 3.96 *s* 3.92 *s* 3.87 *s*
3		6.89 *s*	7.97 *d* (8.9)	6.95 *d* (8.9)	6.95 *d* (8.9)	7.97 *d* (8.9)	3.90 *s* 3.78 *s* 3.72 *s*
3†		6.51 *s*	8.04 *d* (8.9)	6.97 *d* (8.9)	6.97 *d* (8.9)	8.04 *d* (8.9)	3.96 *s* 3.92 *s* 3.86 *s*
4		6.50 *s*	7.60–7.50 *m* AB part of an ABX system		7.08 *d* (9.3)	7.60–7.50 *m* AB part of an ABX system	3.84 *s* 3.76 *s* 3.72 *s*
4†		6.55 *s*	7.70–7.60 *m* AB part of an ABX system		6.97 *d* (9.2)	7.70–7.60 *m* AB part of an ABX system	4.04 *s* 3.99 *s* 3.86 *s*
5‡	6.17 *d* (1.9)	6.38 *d* (1.9)	7.60–7.50 *m* AB part of an ABX system		7.08 *d* (9.1)	7.60–7.50 *m* AB part of an ABX system	3.86 *s* 3.79 *s*
6		6.48 *s*	7.51 *d* (2.2)		6.90 *d* (8.5)	7.41 *dd* (8.5; 2.2)	3.78 *s* 3.76 *s*
7	6.17 *d* (1.9)	6.38 *d* (1.9)	7.52 *d* (2.2)		6.90 *d* (8.5)	7.42 *dd* (8.5; 2.2)	3.78 *s*

*At 200.13 MHz in DMSO-d_6, unless otherwise stated (room temp.). δ values are followed by multiplicity and below, in parentheses, coupling constants in Hz. The 5-hydroxyl originates in all cases a broad singlet at δ12.6.

†In $CDCl_3$.

‡See refs [15, 20, 21].

Table 2. ^{13}C NMR spectral data of compounds **1**, **4**–**7**, **9** and **10***

Carbon No.		1	1†	4	5	6	7	9‡	10†
2		155.19	154.98	155.13	155.10	155.58	155.85	162.83	160.77
3		137.59	138.82	137.63	137.94	137.34	137.92	112.23	113.29
4		178.16	179.21	178.09	177.81	178.09	178.13	144.47	143.93
5		152.33	152.26	152.23	161.21	152.37	161.53	108.55	103.24
6		131.26	130.00§	131.28	98.70	131.28	98.91	145.64	144.69
7		157.81	156.15	158.03	164.78	157.91	164.73	151.32	142.60
8		94.12	93.09	93.97	93.68	93.92	93.93	103.44	134.46
9		151.63	151.81	151.67	156.39	151.61	156.64	150.29	143.03
10		104.53	106.21	104.31	103.99	104.37	104.35	111.40	111.14
1′		122.24	122.78	122.36	122.31	120.85§	121.04§		
2′		129.95	130.19§	114.90	114.92	115.74‖	116.05‖		
3′		114.23	114.08	146.37	146.33	145.29	145.55		
4′		161.33	161.72	150.18	150.16	148.76	149.06		
5′		114.23	114.08	111.93	111.93	115.43‖	115.62‖		
6′		129.95	130.19§	120.30	120.27	120.54§	120.88§		
OMe	(3,6)	59.94	60.92	59.88	59.70	59.95	59.95	56.41 (6)	56.47 (6)
		59.78	60.16	59.72		59.68			
	(4′)	55.46	55.45	55.63	55.62				61.53 (8)

*At 50.32 MHz in DMSO-d_6, unless otherwise stated (room temp.).
†In $CDCl_3$.
‡In $CDCl_3$–CD_3OD (5:1).
§‖Assignments bearing the same superscript may be interchanged within the corresponding spectrum.

grouping (**2**, **4** and **5**), we always observed a complex, non first-order multiplet (AB part of a strongly coupled ABX system) for the hydrogens H-2′ and H-6′, in contrast to some earlier reports [15, 19–21].

As far as we know, the ^{13}C NMR spectra of flavonoids **1**, **4**, **6** and **7** and coumarins **9** and **10** have not been described in the literature so far. Although the ^{13}C NMR spectrum of **5** has already been reported [22], it is also included in Table 2 for comparison. Attribution of carbon signals has been made according to literature [22–24] and confirmed by examination of ^{1}H-coupled spectra. Experimental δ values of carbon atoms in scopoletin and isofraxidin (Table 2) coincide well with calculated ones [25] (maximum deviation $\pm 1.5\%$), with the exception of C-7 in isofraxidin (obs. 142.60, calc. 130.60), probably for steric reasons.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra of flavonoids were run on a Bruker AC-200 spectrometer at 200.13 and 50.32 MHz, respectively, in DMSO-d_6 at room temp., using the solvent signals at δ2.49 (^{1}H) and δ39.5 (^{13}C) as reference. Compounds **1**–**4**, but not **5**–**7**, were also sufficiently soluble in $CDCl_3$ for a good NMR measurement. The small amount available of compounds **2** and **3** did not allow ^{13}C NMR measurements. The ^{13}C NMR spectra of coumarins **9** and **10** were registered in $CDCl_3$–CD_3OD (5:1) and $CDCl_3$, respectively, with TMS as reference.

Plant material. Aerial parts of *A. incanescens* (stems, leaves and flowers) were collected in October 1984 at Arcos de las Salinas (Teruel, Spain) and authenticated by Prof. J. Mansanet of the Botany Department at the Faculty of Biology in Valencia. A voucher specimen has been deposited in the herbarium of the above-mentioned department.

Extraction and chromatography. The plant material was air-dried at room temp. during one month and finely ground. The obtained material (500 g) was extracted at room temp. under stirring with 80% aq. MeOH (5 l., 2 days) and then with 50% aq. MeOH (6 l., 3 days). Both extracts were combined, concd *in vacuo* to remove most MeOH, and successively extracted with Et_2O (4 l.) and EtOAc (7 l.). The ethereal extract was concd to dryness (7.7 g). The EtOAc extract is presently under study. The Et_2O extract was first chromatographed by CC on cellulose (elution with 15–40% aq. AcOH). The residue obtained (5 g) was chromatographed on Polyamide Macherey-Nagel SC6 (elution with H_2O to MeOH). Four main fractions I–IV were obtained and then further chromatographed on Sephadex LH-20 (elution with MeOH). Compounds **10** (128 mg) (fraction I), **9** (75 mg) and **8** (15 mg) (fraction II), **1** (20 mg), **2** (2 mg), **3** (2 mg) and **4** (10 mg) (fraction III), **5** (10 mg), **6** (10 mg) and **7** (8 mg) (fraction IV) were thus isolated and identified by their mps and spectral data.

Note added in proof. Just prior to publication, we discovered that casticin had been isolated from *Artemisia annua*: Jeremic, D., Stefanovic, M., Dokovic, D. and Milosavljevic, S. (1979) *Glas. Hem. Drus. Beograd* **44**, 615; *Chem. Abstr.* (1980) **92**, 211806e.

REFERENCES

1. Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. and Webb, D. A. (eds) (1976) *Flora Europaea*, Vol. 4, p. 184. Cambridge University Press, Cambridge.
2. Kelsey, R. G. and Shafizadeh, F. (1979) *Phytochemistry* **18**, 1591.
3. Čekan, L. Z. and Herout, V. (1956) *Coll. Czech. Chem. Commun.* **21**, 79.
4. Chumbalov, T. K. and Fadeeva, O. V. (1969) *Khim. Prir. Soedin.* **5**, 439.
5. Lee, K., Simpson, R. and Geissman, T. A. (1969) *Phytochemistry* **8**, 1515.
6. Rodriguez, E., Carman, N. J., Vandervelde, G., McReynolds, J. H., Mabry, T. J., Irwin, M. A. and Geissman, T. A. (1972) *Phytochemistry* **11**, 3509.
7. Brown, D., Asplund, R. O. and McMahon, V. A. (1975) *Phytochemistry* **14**, 1083.

8. Oganesyan, E. T., Smirnova, L. P., Dzhumyrko, S. F. and Kechatova, N. A. (1976) *Khim. Prir. Soedin.* **12**, 599.
9. Khafagy, S. M., El-Ghazooly, M. G. and Metwally, A. M. (1979) *Pharmazie* **34**, 748.
10. Murray, R. D. H., Méndez, J. and Brown, S. A. (1982) *The Natural Coumarins*, pp. 481–484. John Wiley, Chichester.
11. Nieschulz, O. and Schwersahl, P. (1968) *Arzneim.-Forsch.* **18**, 1330.
12. Wollenweber, E. and Lebreton, P. (1971) *Biochimie* **53**, 935.
13. Belič, I., Bergant-Dolar, J. and Morton, R. A. (1961) *J. Chem. Soc.* 2523.
14. Farkas, L., Hörhammer, L., Wagner, H., Rösler, H. and Gurniak, R. (1964) *Chem. Ber.* **97**, 1666.
15. Kupchan, S. M. and Bauerschmidt, E. (1971) *Phytochemistry* **10**, 664.
16. Swain, T. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds) Chap. 20. Academic Press, London.
17. Batterham, T. J. and Highet, R. J. (1964) *Aust. J. Chem.* **17**, 428.
18. Hillis, W. E. and Horn, D. H. S. (1965) *Aust. J. Chem.* **18**, 531.
19. Bohlmann, F. and Zdero, C. (1967) *Tetrahedron Letters* 3239.
20. Urbatsch, L. E., Mabry, T. J., Miyakado, M., Ohno, N. and Yoshioka, H. (1976) *Phytochemistry* **15**, 440.
21. Nair, A. G. R., Ramesh, P., Subramanian, S. S. and Joshi, B. S. (1978) *Phytochemistry* **17**, 591.
22. Markham, K. R., Chari, V. M. and Mabry, T. J. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds.) Chap. 2. Chapman & Hall, London.
23. Calvert, D. J., Cambie, R. C. and Davis, B. R. (1979). *Org. Magn. Reson.* **12**, 583. Some assignments in this paper are not coincident with other data [22, 24].
24. Agrawal, P. K. and Rastogi, R. P. (1981) *Heterocycles* **16**, 2181.
25. Duddeck, H. and Kaiser, M. (1982) *Org. Magn. Reson.* **20**, 55.