6-METHOXYFLAVONOIDS FROM *DECACHAETA OVATIFOLIA*

DORIS H. DE LUENGO[1] and TOM J. MABRY

Department of Botany, University of Texas at Austin, Austin, Texas, 78713-7640

In a continuation of our chemotaxonomic studies in the tribe Eupatorieae (Compositae), we investigated *Decachaeta ovatifolia* (DC.) King and H. Robins.; early authors (1,2) placed this taxon in either the genus *Eupatorium* or *Ophryosporus*. A revision by King and Robinson (3) transferred this species to the genus *Decachaeta*, previously considered to be a monotypic genus. *D. ovatifolia*, along with five other species (3,4), was placed in a second subgenus, *Polydenia*.

The type species of the genus *Decachaeta haenkeana* DC. afforded a new quercetagetin derivative and its 3-potassium sulfate salt (5), and sesquiterpene lactones have been isolated from species of the subgenus *Polydenia; Decachaeta thieleana* (6,7), *D. ovatifolia* (8), and *Decachaeta scabrella* (Miski *et al.*, unpublished).

Three 6-methoxylated aglycones and three 6-methoxyflavonol glycosides were isolated from aerial parts (leaves and flowers) of *D. ovatifolia*. Among the aglycones identified were 6-methoxyapigenin, 6-methoxyacacetin, and 6-methoxyapigenin-7-methyl ether. The flavonol glycosides were patuletin-3-galactoside, patuletin-3-glucoside, and 6-methoxykaempferol-3-glucoside.

EXPERIMENTAL

PLANT MATERIAL.—Leaves and flowers of *D. ovatifolia* were collected in Michoacan, Mexico, 21 km south of Uruapan on 15 November 1983, by Fred Barrie (voucher Barrie, Ramamoorthy, and Martinez #533 on deposit at the University of Texas at Austin Herbarium). The plant material was air-dried prior to extraction.

EXTRACTION, ISOLATION, AND IDENTIFICATION OF COMPOUNDS.—Ground leaves and flowers of *D. ovatifolioa* (300 g) were extracted with 80% and 50% aqueous MeOH until the extract was colorless. The extracts were then combined and evaporated under reduced pressure until only H_2O remained. The aqueous layer was extracted successively with *n*-hexane, CH_2Cl_2, and EtOAc. The CH_2Cl_2 extract was concentrated and the residue adsorbed onto celite. After drying the resulting powder, the material was chromatographed over a Polyclar column packed in CH_2Cl_2. Flavonoids were eluted with a CH_2Cl_2/MeOH gradient with increasing amounts of MeOH until the column was finally eluted with MeOH. This column yielded 6-methoxyapigenin-7-methyl ether (6 mg) and 6-methoxyapigenin (8 mg). When the material from the EtOAc extract (4 g) was chromatographed over a Polyclar column using the same solvent system described for the material from the CH_2Cl_2 extract, three glycosides, patuletin-3-galactoside (85 mg), patuletin-3-glucoside (150 mg), and 6-methoxykaemferol-3-glucoside (80 mg), were obtained. When the external surfaces of leaves of *D. ovatifolia* (725 g) were washed with CH_2Cl_2, 5 mg of 6-methoxyapigenin and 7.5 mg of 6-methoxyacacetin were obtained.

All compounds were purified over Sephadex LH-20 using MeOH or 80% aqueous MeOH prior to spectral analyses by standard procedures [9,10]. The identities of all flavonoids were established by direct comparison (tlc, uv, ^{1}H nmr, ms) with authentic samples.

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LITERATURE CITED

1. B.L. Robinson, *Proc. Am. Acad. Arts. Sci.*, **41**, 275 (1905).
2. B.L. Robinson, *Contr. Gray Herb* (n.s.), **75**, 4 (1925).
3. R.M. King and H. Robinson, *Brittonia*, **21**, 275 (1969).
4. R.M. King and H. Robinson, *Phytologia*, **21**, 299 (1971).
5. M. Miski, D. Gage, and T.J. Mabry, *Phytochemistry*, (in press).
6. S. Alvarado, J.F. Ciccio, J. Calzada, V. Zabel, and W.H. Watson, *Phytochemistry*, **18**, 330 (1979).
7. V. Castro, F. Ciccio, S. Alvarado, F. Bohlmann, G. Schmeda-Hirschmann, and J. Jakupovic, *Liebigs Ann. Chem.*, 974 (1983).
8. D.H. de Luengo, M. Miski, D.A. Gage, T.J. Mabry, R.P. Kashyap, and W.H. Watson, *Phytochemistry*, (submitted).

[1]Permanent address: Universidad de Los Andes, Facultad de Ciencias, Departamento de Quimica, La Hechicera, Merida, Venezuela 5102.

9. J.B. Harborne, T.J. Mabry, and H. Mabry, (eds.), "The Flavonoids," Chapman Hall, London (1975).
10. T.J. Mabry, K.R. Markham, and M.B. Thomas, "The Systematic Identification of Flavonoids," Springer, New York (1970).

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ADDITIONAL PHTHALIDE DERIVATIVES FROM *MEUM ATHAMANTICUM*

MOURAD KAOUADJI and CORINNE POUGET

Laboratoire de Pharmacognosie, UFR de Pharmacie, Université Scientifique et Médicale de Grenoble, Domaine de La Merci, F-38700 La Tronche, France

In our earlier communications, we have reported the isolation and characterization of cinnamic acid esters (1, 2) and phthalides (3) from *Meum athamanticum* Jacq. (Umbelliferae) rhizomes. We now report the isolation of the seven hydroxylated phthalides listed below and their identification by standard spectral methods. Use of ^{1}H-nmr data of *Z*-3-butylidenephthalide, a common product isolated from the same source, was helpful for analysis of aromatic products. Extraction of the underground parts of *M. athamanticum* with *n*-hexane afforded 7-hydroxy-3-butylidenephthalide; on the other hand, the $CHCl_3$ extract yielded 4-hydroxy-3-butylidenephthalide, 5-hydroxy-3-butylidenephthalide, 3-(2-hydroxybutylidene)-phthalide, 9-hydroxyligustilide, and *cis*- and *trans*-6,7-dihydroxyligustilide. All of these compounds, found in the *Z*-form, are reported for the first time in the genus *Meum*. With the exception of 5-hydroxy- and 4-hydroxy-3-butylidenephthalide, each compound has been described in just one of two other Umbelliferous plants, *Ligusticum wallichii* Franch. [7-hydroxy-3-butylidenephthalide, *cis*- and *trans*-6,7-dihydroxyligustilide (4)], and *Cnidium officinale* Makino [9-hydroxy-3-butylidenephthalide, 3-(2-hydroxy butylidene)phthalide (5)]. 5-Hydroxy-3-butylidenephthalide is present in both of the above species (5, 6).

^{1}H-nmr data ($CDCl_3$) relative to 4-hydroxy-3-butylidenephthalide have been wrongly assigned to the 7-hydroxy isomer in *C. officinale* (5). Correction was made possible as a consequence of the isolation of both 4-hydroxy- and 7-hydroxy derivatives from *M. athamanticum*. The H-8 resonances in the two ^{1}H-nmr spectra, associated with the uv behavior of the related compounds in the presence of $AlCl_3$, clearly distinguished between the two isomers. Effectively, deshielding of H-8 at δ 5.95 ppm is observed when the hydroxyl group is located at the 4-position (γ-relationship), compared with the δ-value recorded at 5.68 ppm for this proton in the 7-hydroxy compound, as in *Z*-3-butylidenephthalide at δ 5.64 ppm. This was also shown and confirmed by the existence of a bathochromic uv shift (λ340 nm$\rightarrow\lambda$375 nm) after addition of $AlCl_3$ for the 7-hydroxy derivative, the 4-hydroxy compound being insensitive. Finally, ^{1}H-nmr and uv records were in agreement with chromatographic data (tlc and hplc), indicating that the 7-hydroxyphthalide was less polar than the 4-hydroxy isomer.

The same observation can be made for 6,7-dihydroxy-3-butylidenephthalide described as the 4,5-dihydroxy isomer in *L. wallichii* (6) since, in this case, H-8 which is not affected by deshielding induced by hydroxylation in the 4-position is recorded at δ 5.54 ppm (CD_3OD).

Finally, on the basis of the reported compounds, the three species, *C. officinale, L. wallichii,* and *M. athamanticum*, collected in the subtribe Seselinae in the family Umbelliferae, likely produce phthalides by the same biosynthetic pathways, probably from *Z*-ligustilide, the most accumulated phthalide in Umbelliferous plants (7).

EXPERIMENTAL

PLANT MATERIAL.—*M. athamanticum* rhizomes were collected from Col du Lautaret, France, at the beginning of the fruiting stage, as previously reported (1-3). A voucher specimen MAR-84 has been deposited at Laboratoire de Pharmacognosie de Grenoble, Domaine de La Merci, F-38700 La Tronche.

EXTRACTION AND ISOLATION OF PHTHALIDES.—The *n*-hexane extract (60 g) was subjected (2 g) to circular centrifugal thin layer chromatography (cctlc) on silica gel GF-254, with $CHCl_3$ as the solvent, affording thirteen fractions. Fraction 1 was used to obtain *Z*-3-butylidenephthalide (12 mg), by repeated cctlc on silica gel with increasing amounts of $CHCl_3$ in *n*-hexane; fraction 4, exhibiting a yellow fluorescence, was chromatographed on a column of polyamide and then purified by cctlc on silica gel with C_6H_6 affording *Z*-7-hydroxy-3-butylidenephthalide (3 mg). The $CHCl_3$ extract (15 g) was fractioned by SiO_2 cc to give nine fractions eluted by $CHCl_3$ up to MeOH. Fraction 2, exhibiting a bluish white fluorescence as with *Z*-ligustilide, was first treated by cctlc on silica gel (*n*-hexane-$CHCl_3$-*i*PrOH-MeOH, 36:2:1:1) and