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FREE FLAVONOID AGLYCONES FROM LEAVES OF *MENTHA PULEGIUM* AND *MENTHA SUAVEOLENS* (LABIATAE)

FAROUK ZAIDI, BERNARD VOIRIN,† MAURICE JAY† and MARIE ROSE VIRICEL†

Unité de Recherche de Chimie, Université de Constantine, Route d'Ain-El-Bey, 25000 Algérie; † Laboratoire de Biologie micromoléculaire et Phytochimie, UFR de Biologie, Université Lyon 1, 43 Bd du 11 novembre 1918 69622 Villeurbanne, France

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Abstract—Methoxyflavone aglycones were isolated from the leaf surface of *Mentha suaveolens* and *M. pulegium*. 5,7-Dihydroxy-6-methoxy- and 5,6-dihydroxy-7-methoxyflavones with a substituted B-ring and 5,6-dihydroxy-7,8-dimethoxyflavones, characteristic flavonoid constituents of the subfamily *Nepetoideae* and of the tribe *Saturejeae*, respectively, were observed in both species. *Mentha suaveolens* showed considerable chemical variation between samples from Spain and Algeria. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In the past ten years, there have been several studies on free flavone aglycones from the Labiatae [1–4]. However, only a few dealt with the genus *Mentha* and most of them were focused on specific compounds. Thus, Tomas-Barberan *et al.* [2] reported on the distribution of 5,6-dihydroxyflavones in leaf exudates of five taxa: *M. longifolia*, *M. suaveolens*, *M. spicata*, *M.* × *piperita* and *M. aquatica*. Salvigenin, 5-*O*-desmethylangeritlin and 5,6,7,4′-tetramethoxyflavone were isolated from *M.* × *citrata* leaves [6], diosmetin from *M. spicata* [7], acacetin from *M. longifolia* [8] and apigenin, acacetin and luteolin from *M. aquatica* [9]. Only one relatively exhaustive paper described the free aglycones from *M.* × *piperita* [5].

The present work deals with a precise screening of free flavone aglycones from leaves of two *Mentha* species: *M. pulegium* L. (section *Pulegium*) and *M. suaveolens* Ehrh. (section *Mentha*) from wild populations located in Petite Kabylie, Algeria.

RESULTS AND DISCUSSION

The free flavone profiles from leaves of *M. pulegium* and *M. suaveolens* are presented in Table 1 together with bibliographic data for a further five species or hybrids. All the main aglycones isolated from both *Mentha* species were identified using spectrophotometric techniques [11–12] and comparative TLC with authentic markers (Table 2).

The flavone, jaceosidin is reported for the first time, in the genus *Mentha*, in both *M. pulegium* and *M. suaveolens*. However, *M. pulegium* specifically accumulated pectolinarigenin, pedalitin, 5-hydroxy-6,7,3′,4′-tetramethoxyflavone and chrysoeriol while hispidulin, nodifloretin and genkwanin were characteristic of *M. suaveolens*. All these flavones are new citations for the genus *Mentha*, even for *Mentha* × *piperita* for which the flavonoids are best known [5].

Another interesting find comes from comparison of the flavonoid profile of *M. suaveolens* collected in Spain by Tomas-Barberan *et al.* [2] with the present samples harvested in the Algerian mountains. Thus, a clear difference can be discerned in the 5,6-dihydroxyflavone derivatives in that thymusin, 5,6-dihydroxy-7,8,3′,4′-tetramethoxyflavone, ladanein, sorbifolin, nodifloretin were isolated only from Algerian samples while 5,6,4′-trihydroxy-7,3′-dimethoxyflavone was reported only from leaves of Spanish samples. On the other hand, both samples accumulated thymonin.

This difference in flavonoid profile could be explained by geographical and climatic differences (Spain, Algeria) combined with efficient genetic isolation. In fact, Harley and Brighton [10] have described a "high-altitude" topodeme of *M. suaveolens* in Morocco, named subsp. *timija* (Cosson ex Briq.) Harley. Some individual plants of this diploid topodeme have remained interfertile with more typical *M. suaveolens*. Moreover, the population of the subsp. *timija* were morphologically very variable with some of the plants showing a great similarity to lowland *M. suaveolens*.

Another hypothesis for the difference between the Algerian and Spanish flavonoid profiles could be the

* Author to whom correspondence should be addressed

Table 1. Flavone aglycone distribution in *Mentha* species

Compound	Trivial name	Section *Mentha*							Section *Pulegium*	A-ring substitution patterns
		M. × *piperita* [2, 5]	*M.* × *citrata* [6]	*M. longifolia* [2, 8]	*M. spicata* [2, 7]	*M. aquatica* [2, 9]	*M. suaveolens* [2]	Algeria	*M. pulegium*	
5,6,4′-Trihydroxy-7,8,3′-trimethoxyflavone	Thymonin	+		+	+		+	+	+	
5,6,4′-Trihydroxy-7,8-dimethoxyflavone	Thymusin	+					−	+	−	5,6-diOH
5,6-Dihydroxy-7,8,3′,4′-tetramethoxyflavone		+					−	+	−	7,8-diOMe
5,6-Dihydroxy-7,8,4′-trimethoxyflavone	Pebrellin	+					−	−	−	
5,7,4′-Trihydroxy-6,3′-dimethoxyflavone*	Jaceosidin	−					−	+	+	5,7-diOH
5,7,4′-Trihydroxy-6-methoxyflavone*	Hispidulin	−					−	+	−	6-OMe
5,7-Dihydroxy-6,4′-dimethoxyflavone*	Pectolinarigenin	−					−	−	+	
5,6-Dihydroxy-7,4′-dimethoxyflavone	Ladanein	+					−	+	+	
5,6,4′-Trihydroxy-7-methoxyflavone	Sorbifolin	+					−	+	+	5,6-diOH
5,6,3′,4′-Tetrahydroxy-7-methoxyflavone*	Pedalitin	−					−	−	+	7-OMe
5,6,4′-Trihydroxy-7,3′-dimethoxyflavone		−		+	+		+	−	+	
5,6-Dihydroxy-7,3′,4′-trimethoxyflavone		+					−	−	+	
5-Hydroxy-6,7,3′,4′-tetramethoxyflavone*		−					−	−	+	5-OH
5-Hydroxy-6,7,4′-trimethoxyflavone	Salvigenin	+	+				−	−	−	6,7-diOMe
5,6,7,4′-Tetrahydroxy-3′-methoxyflavone*	Nodifloretin	−					−	+	−	5,6,7-triOH
5,4′-Dihydroxy-6,7,8-trimethoxyflavone	Xanthomicrol	+								
5-Hydroxy-6,7,8,4′-tetramethoxyflavone	Gardenin B	+	+				−	−	−	5-OH
5,3′,4′-Trihydroxy-6,7,8-trimethoxyflavone	Sideritoflavone	+					−	−	−	6,7,8-triOMe
5,3′-Dihydroxy-6,7,8,4′-tetramethoxyflavone	Gardenin D	+					−	−	−	
5-Hydroxy-6,7,8,3′,4′-pentamethoxyflavone	5-O-Desmethylnobiletin	+					−	−	−	
5,7,4′-Trihydroxyflavone	Apigenin	+				+	−	+	+	
5,7-Dihydroxy-4′-methoxyflavone	Acacetin	+		+		+	−	−	−	
5,7,3′,4′-Tetrahydroxyflavone	Luteolin	+				+	−	+	+	5,7-diOH
5,7,3′-Trihydroxy-4′-methoxyflavone	Diosmetin	−			+		−	−	−	
5,7,4′-Trihydroxy-3′-methoxyflavone*	Chrysoeriol	−					−	−	+	
5,4′-Dihydroxy-7-methoxyflavone*	Genkwanin						−	+	−	5-OH 7-OMe
5,6,7,4′-Tetramethoxyflavone		−	+				−	−	−	5,7-diOMe

* Compound reported for the first time in the genus *Mentha*; +: presence; −: absence; [see References]

Table 2. R_f values and spectral properties of the flavonoids isolated and identified from *Mentha suaveolens* and *M. pulegium*

Compound	Trivial name	R_f values‡ Toluene 4 MeCOEt 3 MeOH 3	Toluene 60 petrol 26 MeCOEt 7 MeOH 7	λ_{max}^{MeOH} (nm)*		
5,6,4′-Trihydroxy-7,8,3′-trimethoxyflavone†	Thymonin	0.82	0.29	255 *sh*	290	344
5,6,4′-Trihydroxy-7,8-dimethoxyflavone†	Thymusin	0.82	0.26	256 *sh*	293	335
5.6-Dihydroxy-7,8,3′,4′-tetramethoxyflavone†		0.92	0.44	250 *sh*	291	339
5,7,4′-Trihydroxy-6,3′-dimethoxyflavone	Jaceosidin	0.81	0.30	252 *sh*	275	344
5,7,4′-Trihydroxy-6-methoxyflavone	Hispidulin	0.52	0.16		274	338
5,7-Dihydroxy-6,4′-dimethoxyflavone	Pectolinarigenin	0.8	0.28		276	333
5,6-Dihydroxy-7,4′-dimethoxyflavone	Ladanein	0.82	0.23		282	336
5,6,4′-Trihydroxy-7-methoxyflavone	Sorbifolin	0.66	0.2		285	335
5,6,3′,4′-Tetrahydroxy-7-methoxyflavone	Pedalitin	0.33	0.1	253 *sh*	283	342
5,6,4′-Trihydroxy-7,3′-dimethoxyflavone†		0.65	0.25	240 *sh*	285	343
5,6-Dihydroxy-7,3′,4′-trimethoxyflavone		0.85	0.65	253 *sh*	280	338
5-Hydroxy-6,7,3′,4′-tetramethoxyflavone		0.91	0.7	256 *sh*	277	340
5,6,7,4′-Tetrahydroxy-3′-methoxyflavone	Nodifloretin	0.68	0.18		285	342
5,7,4′-Trihydroxyflavone	Apigenin	0.56	0.02		268	333
5,7,3′,4′-Tetrahydroxyflavone	Luteolin	0.28	0.06	255 *sh*	267	350
5,7,4′-Trihydroxy-3′-methoxyflavone	Chrysoeriol	0.63	0.12	253 *sh*	269	341
5,4′-Dihydroxy-7-methoxyflavone	Genkwanin	0.71	0.28		268	339

* Spectral maxima measured in the presence of classical reagents were in accord with literature data

† EIMS recorded

‡ TLC on DC6 Polyamide

presence of true chemotypes without any morphological differences between them. Such a variability has been observed for *Thymus herba-barona* [13] and *M.* × *piperita* [14] both for terpenoids and for flavonoids, while, Hendriks and Van Os [15] and Lawrence [16] have described two and three terpenoid chemotypes of *M. suaveolens*, respectively. Thus, the question remains open whether the same applies to flavonoids. Tomas-Barberan *et al.* [17] have observed a similar "discrepancy" in the flavonoid patterns they obtained from the "cosmopolitan species *Lippia nodiflora* and those obtained earlier from an Indian population" [18]. They concluded that the "discrepancy cannot be readily explained unless there is marked infraspecific variation present, possibly determined by soil type". Thus further Algerian plants need to be sampled in both lowland and high altitude areas, before these differences can be explained.

The flavonoid patterns of *M. pulegium* and *M. suaveolens* also provide useful chemotaxonomic characters. Thus, thymonin alone or along with thymusin and 5,6-dihydroxy-7,8,3′,4′-tetramethoxyflavone give chemical support to the placement of *M. pulegium* and *M. suaveolens*, respectively in the tribe *Saturejeae*, which is characterized by 5,6-dihydroxy-7,8-dimethoxyflavone derivatives [3]. Moreover such a result confirms the previous suggestion that "thymonin is widespread in members of this tribe whereas thymusin and pebrellin have only a very limited distribution" [2]. Also the chemical characteristics of the subfamily *Nepetoideae* [3, 4] are found in both these two species namely 5,7-dihydroxy-6-methoxyflavones with a substituted B ring [3] (jaceosidin and pectolinarigenin in *M. pulegium* and jaceosidin and hispidulin in *M. suaveolens*) and 5,6-dihydroxy-7-methoxyflavone derivatives [4] (ladanein, sorbifolin in both species and pedalitin, 5,6,4′-trihydroxy-7,3′-dimethoxyflavone, 5,6-dihydroxy-7,3′,4′-trimethoxyflavone in *M. pulegium* only). By contrast, 5-hydroxy-6,7,8-trimethoxyflavone derivatives which "are quite common in members of subfamily *Nepetoideae*" [4] have not been identified in these two species.

EXPERIMENTAL

Plant material was collected in Petite Kabylie (Algeria) from wild populations and identified by Pr M. Jay (University of Lyon) and Pr N. Khalfalla (Université de Constantine). Voucher specimens of *M. pulegium* and *M. suaveolens* have been deposited in the Herbarium of Botanical Department of University of Constantine. Dried leaves (100 g) were extracted in 1 l of an alcoholic mixture (MeOH–EtOH 1:1) at room temp. for two days. The filtered concentrated extract was chromatographed on a Polyamide column using a gradient of MeOH into toluene. Forty-five fractions were collected and chromatographed on Polyamide DC6 (Macherey Nagel) using toluene–MeCOEt–MeOH 4:3:3 as solvent. Flavonoids of different frs were sepd by Polyamide DC6 using toluene–petrol (bp 100–140°)–MeCOEt–MeOH, 60:26:7:7. All the compounds were purified

by Sephadex LH 20 CC. The identification of flavonoids was based on chromatographic comparison with authentic samples and confirmed by comparison with published spectral data [5, 12, 13] and in some cases by EIMS (see Table 1).

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