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HIGHLY OXYGENATED FLAVONES FROM *MENTHA PIPERITA*

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Key Word Index—*Mentha piperita*; Labiatae; leaves; polymethoxyflavones; 5,6-dihydroxy-7,8,3′,4′-tetramethoxyflavone.

Abstract—Six highly oxygenated flavones have been isolated from the leaves of *Mentha piperita*. Five known compounds, 5-hydroxy-6,7,8,4′-tetramethoxyflavone, 5,4′-dihydroxy-6,7,8-trimethoxyflavone, 5,3′-dihydroxy-6,7,8,4′-tetramethoxyflavone, 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone and 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone, are reported for the first time in the genus *Mentha*. The sixth compound has been identified as 5,6-dihydroxy-7,8,3′,4′-tetramethoxyflavone by UV, NMR and mass spectra.

INTRODUCTION

From an alcoholic extract of the leaves of *Mentha piperita* L. var. *rubescens*, six highly oxygenated flavones have been separated by HPLC, PC and TLC. Four compounds have already been identified in other species of the Labiatae: 5,4′-dihydroxy-6,7,8-trimethoxyflavone (xanthomicrol) [1, 2]; 5,3′-dihydroxy-6,7,8,4′-tetramethoxyflavone (gardenin D), 5-hydroxy-6,7,8,3′,4′-pentamethoxyflavone (5-*O*-desmethylnobiletin) [3] and 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone [4]. 5-Hydroxy-6,7,8,4′-tetramethoxyflavone (gardenin B) is reported for the first time in this family. We report here the identification of a new natural compound, 5,6-dihydroxy-7,8,3′,4′-tetramethoxyflavone.

RESULTS AND DISCUSSION

The methanol spectrum of the new flavone (λ_{max} nm: 254 sh, 290, 340) and the spectra in the presence of neutral and acidic aluminium chloride (244 sh, 261, 303, 324 sh, 372; 240 sh, 256, 302, 322 sh, 363 nm) showed the same UV spectral characteristics as thymonin, 5,6,4′-trihydroxy-7,8,3′-trimethoxyflavone [5]. This indicates a tetra-substituted A-ring with 5,6-*O*-dihydoxy system and a 3′,4′-disubstituted B-ring [6]. The mass spectrum exhibited a molecular ion peak at *m/z* 374 ($C_{19}H_{18}O_7$) in accordance with a flavone containing two hydroxyl and four methoxyl groups, and this was confirmed by the ^{1}H NMR spectrum. While the two hydroxyl groups were located at C-5 and C-6, the remaining substituted carbons, 7,8,3′,4′, were methoxylated. Thus the new compound is 5,6-dihydroxy-7,8,3′,4′-tetramethoxyflavone or 4′-methoxythymonin.

Several aspects of the spectral analysis require further comment. In the presence of sodium methoxide, the UV spectrum of 5,6-dihydroxy-7,8,3′,4′-tetramethoxyflavone exhibited instantaneously a principal band at 326 nm, whereas that of thymonin exhibited band I at 392 nm. These results have to be compared with the data furnished by Misky *et al.* [7] for 5,6,4′-trihydroxy-7,3′-dimethoxyflavone and 5,6-dihydroxy-7,3′,4′-trimethoxyflavone. For the first compound, which possesses, as thymonin, a hydroxyl group at C-4′ and a methoxyl group at C-7, band I in the presence of sodium methoxide appears at 394 nm while, for the second, which has methoxyl groups at both C-4′ and C-7, a band appears at 316 nm. On the other hand, in the presence of sodium acetate, we have also noted differences in the spectra as a function of hydroxylation: in compounds which have only hydroxyl groups at C-5 and C-6, band I appears at 329–330 nm, while for 5,6,4′-trihydroxy derivatives it appears at 392–398 nm.

Thus 6,8-dimethoxylated flavonoids as described previously in other plants of the Labiatae are now shown to be present in the genus *Mentha*. Furthermore, the original substitution of A-ring, 6-hydroxylation 8-methoxylation, reported in *Thymus satureoides* and in some chemotypes of *T. vulgaris* [8], also occurs in *Mentha piperita*. Such compounds have a restricted distribution within *Sideritis* [1] and *Thymus* [2, 8], and presumably also within *Mentha* [9–15], although further work is still needed in the case of the latter group.

EXPERIMENTAL

Mentha piperita L. var *rubescens*, cultivated in La Begude de Mazenc (Drôme, France), was collected in 1983. Dried powdered leaves (320 g) were extracted with 50% aq. MeOH. After evapn of the solvent *in vacuo*, the residue was dissolved in boiling H_2O. After cooling, the aq. layer was partitioned with *n*-BuOH. The conc. alcoholic extract was dissolved in 20 ml DMF and chromatographed by HPLC (Waters Prep LC 500, column C 18, Prep Pak 500, 5.7 × 30 cm), using as solvents A = MeOH, B = H_2O, C = HOAc. Ten fractions of *ca* 1.5 l. were obtained using the following step-by-step gradient: fraction 1: A–B–C, 10:40:1; 2, 15:35:1; 3 and 4, 20:30:1; 5 and 6, 25:25:1; 7 and 8, 30:20:1; 9 and 10, 50:0:1. Fractions 8 and 9 contained the aglycones. These were further separated by PC on Whatman 3 in 15 and 40% HOAc. Each compound was purified by chromatography on Polyamide DC6, solvent H_2O–MeOH–MeCOEt–acetylacetone (7:3:3:1). Final purification was achieved on Sephadex LH-20. The previously known natural flavones, xanthomicrol, gardenins B and D, 5-*O*-desmethylnobiletin and 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone, were identified by UV and MS, and for the four latter by chromatographic comparisons with reference samples.

5,6-Dihydroxy-7,8,3′,4′-tetramethoxyflavone. UV spectral data λ_{max}^{MeOH} nm with relative A based on the highest peak as 100%

(1.00) of each spectrum given in parentheses: MeOH 254 sh, 290 (1.00), 340 (1.00); + NaOMe: 294 sh, 326 (1.00); + $AlCl_3$: 261 (0.63), 303 (0.83), 324 sh, 372 (1.00); + $AlCl_3$ + HCl: 256 (0.70), 302 (0.84), 322 sh, 363 (1.00); + NaOAc: 290 (1.00), 330 (0.94); + NaOAc + H_3BO_3: 289 (1.00), 336 (0.73). MS *m/z* (rel. int.): 374 $[M]^+$ (90) (found: 374.1002; calc. for $C_{19}H_{18}O_7$: 374.1005), 373 $[M-1]^+$ (14), 359 $[M-Me]^+$ (100), 197 $[A-15]^+$ (38) (found: 197.0086; calc. for $C_8H_5O_6$: 197.0085). ^1H NMR (350 MHz, DMSO-d_6): δ 7.69 (1H, *dd*, J = 8.4 and 2 Hz, H-6'), 7.58 (1H, *d*, J = 2 Hz, H-2'), 7.18 (1H, *d*, J = 8 Hz, H-5'), 6.98 (1H, *s*, H-3), 3.97 (3H, *s*, OMe), 3.94 (3H, *s*, OMe), 3.89 (3H, *s*, OMe), 3.87 (3H, *s*, OMe). R_f 0.50 on Polyamide DC6 (Merck), C_6H_6–petrol (bp 100–140°)–MeOH–MeCOEt (5:5:1:1) and 0.22 on cellulose in 30% HOAc.

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STRUCTURAL REVISION OF THE FLAVONE MAJORANIN FROM *MAJORANA HORTENSIS*

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Key Word Index—*Majorana hortensis*; Labiatae; majoranin; thymonin; 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone.

Abstract—Majoranin isolated from *Majorana hortensis* and characterized earlier as 5,7,4'-trihydroxy-6,8,3'-trimethoxyflavone was found to be different from sudachitin of the same structure. Its true structure has been established as 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone (thymonin) by spectral data and direct comparison.

INTRODUCTION

A trihydroxytrimethoxyflavone, majoranin, was isolated from the leaves of *Majorana hortensis* and assigned the structure 5,7,4'-trihydroxy-6,8,3'-trimethoxyflavone by Subramanian *et al.* [1]. Owing to the earlier occurrence of a flavone, sudachitin, with the same structure [2], Wollenweber [3] cited majoranin as a synonym. However, there exist subtle differences in the physical constants and UV spectra of the two compounds. This necessitated a re-examination of the structure of majoranin. Thus, since the precise differentiation of the positions of hydroxyl and methoxyl groups in flavonoid compounds can be accomplished by UV measurements [4], a re-examination of the structure of majoranin was undertaken and the results