

6,8-DI-C-METHYLDIHYDROMYRICETIN FROM *ALLUAUDIA HUMBERTII*

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Key Word Index—*Alluaudia humbertii*; Didiereaceae; 3,5,7,3',4',5'-hexahydroxy-6,8-di-C-methylflavanone; new natural compound.

Abstract—A novel flavanonol isolated from bark and spines of *Alluaudia humbertii* has been identified as 6,8-di-C-methyldihydromyricetin, by means of UV, mass and ¹H NMR spectra.

The UV spectrum in methanol, λ_{\max} Band I 299 nm (100%) and Band II 350 nm (20%) indicated that this flavonoid was a flavanone or a 3-hydroxyflavanone [1, 2]. In the ¹H NMR spectrum, the presence of a pair of doublets at δ 4.86 and 4.33, characteristic of AB system of vicinal protons at C-2 and C-3, proved that this compound was a flavanonol [3, 4]. The mass spectrum exhibited a molecular peak at m/z 348 (48%) in accord with a flavanonol containing six hydroxyl and two methyl groups ($C_{17}H_{16}O_8$ calc. 348.0841, found 348.0842). As the ¹H NMR spectrum showed two singlets at δ 1.95 (3H) and 1.97 (3H), the compound was substituted by two aryl methyl groups. In the mass spectrum, the presence of peaks at m/z 181 (5) ($A_1 + H$, 100%, $C_9H_9O_4$ calc. 181.0500, found 181.0496) and 180 (A_1 , 23%, $C_9H_8O_4$ calc. 180.0423, found 180.0422) indicated that the A ring was substituted by two methyl and two hydroxyl groups. The presence of free hydroxyls at C-5 and C-7 was confirmed by the bathochromic shift (42 nm) observed on addition of sodium acetate in the UV spectrum. The trihydroxylation of B ring was showed by the fragment at m/z 139 (B_4 , 37%, $C_7H_7O_3$ calc. 139.0395, found 139.0394) in the mass spectrum and by the presence, in the ¹H NMR spectrum, of a singlet at δ 6.41 (H-2' and H-6' isochrone). This new natural compound is thus identified as 3,5,7,3',4',5'-hexahydroxy-6,8-di-C-methylflavanone or 6,8-di-C-methyldihydromyricetin.

Different C-methyldihydroflavonols have been previously reported [4, 6–8]. Nevertheless all these compounds were mono-C-methylated at C-6 or C-8. It is of interest to note that we have observed as did Agrawal [4] in the mass spectrum, the presence of a peak at $[M - 2]^+$ (346) indicating a structural similarity among these compounds. Finally, it is the first time that a di-C-methyldihydroflavonol has been identified from a species

of Didiereaceae; other compounds known in the family with such substitution in the A ring are either flavonols [9–11] or flavanones [12].

EXPERIMENTAL

Alluaudia humbertii was collected in the South of Madagascar in July 1981. Spines and bark were ground and extracted in Et₂O. After evaporation of this solvent, the residue was dissolved in MeOH and chromatographed on a polyamide column (SC 6 Macherey Nagel) according to a technique previously described [13]. Among the 31 fractions collected, the compound was isolated in fractions 29 and 30. The purification was achieved by CP (Whatman 3, 30% HOAc) and Sephadex LH 20 (MeOH). UV fluorescence: purple. R_f ($\times 100$) TLC Cellulose Merck 30% HOAc: 67. λ_{\max}^{MeOH} nm: 299, 346 sh; + NaOAc: 300, 341; + NaOAc + H₃BO₃: 298, 340; + AlCl₃: 318; + NaOH: 341. MS 70 eV, m/z (%): 348 (48), 346 (22), 330 (17), 181 (100), 180 (23), 166 (39), 139 (37). ¹H NMR (Cameca 350 MHz, DMSO-*d*₆, 50°): δ 6.41 (2H, s, H-2', H-6'), 4.86, 4.33 (1H each, *d*, *J* = 10.4 Hz, H-2, 3), 1.95 (3H, s, C-Me), 1.91 (3H, s, C-Me), 12.15 (sharp s, 1H, OH-5), 8.80 (3OH, *m*), 8.15 (1OH, *s*), 5.65 (1H, large *s*, OH-3).

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A CHEMOTAXONOMIC STUDY OF FLAVONOIDS IN *THYMBRA CAPITATA*

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Key Word Index—*Thymbra capitata*; Labiatae; 6-hydroxyflavones; chemotaxonomy.

Abstract—5,6,4'-Trihydroxy-7,3'-dimethoxyflavone, 5,6-dihydroxy-7,3',4'-trimethoxyflavone, luteolin, diosmetin, vicenin-2 and luteolin 7-rutinoside have been isolated and identified from *Thymbra capitata*. The occurrence of these compounds supports the inclusion of this plant in the genus *Thymbra*, rather than *Thymus* or *Corydorthymus*.

Thymbra capitata (L.) Cav. Lag. Griseb. [= *Thymus capitatus* (L.) Hoffmann and Link = *Corydorthymus capitatus* (L.) Reicheb. fil.] is a Mediterranean plant that grows mainly in southern Spain and occasionally in eastern Spain (Alicante) and the Balearic islands. This plant constitutes a taxonomic problem that has been extensively discussed [1–3]. Several authors [4–6] have considered that there are two systematic groups sufficiently differentiated to sustain the existence of two separate genera, *Corydorthymus* Reicheb. fil. and *Thymus* L. Other workers [2, 3, 7, 8] have considered that there is geobotanical, morphological, cytological and chemotaxonomic evidence to include *Thymus capitatus* in *Thymus*, but separating it as a distinct subgenus *Corydorthymus*. Recently it has been suggested in agreement with Cavanilles, Lagasca and Grisebach, that it should be included in the genus *Thymbra*, on the basis of morphological and genetic similarities with *Thymbra spicata* [1] and has been renamed *Thymbra capitata*.

In this work we have studied the flavonoid compounds of *T. capitata* in order to establish a chemotaxonomic approach to this problem. The flavonoid aglycones 5,6,4'-trihydroxy-7,3'-dimethoxyflavone, 5,6-dihydroxy-7,3',4'-trimethoxyflavone, luteolin and diosmetin and the glyco-

sides vicenin-2 and luteolin 7-rutinoside, have been identified by UV and MS standard procedures [9–11].

5,6,4'-Trihydroxy-7,3'-dimethoxyflavone and 5,6-dihydroxy-7,3',4'-trimethoxyflavone have been recently isolated as new naturally occurring compounds from *Thymbra spicata* [12], this being the second report in which the former flavonoid has been found in nature. The present chemical evidence thus supports the inclusion of the problematic plant in the genus *Thymbra* as *Thymbra capitata*. Also, these unusual methylated flavones have not been found in *Thymus* species *sensu lato* [13] (35 species studied). However, 5,6-dihydroxy-7,3',4'-trimethoxyflavone has been identified in *Thymus piperella* (section *Piperella*) [14], a peculiar thyme which contains unusual flavones compared with other *Thymus* species, and which lacks the typical *Thymus* flavones, thymonin and thymusin [13].

EXPERIMENTAL

Plant material. Plants of *Thymbra capitata* were collected near Lanjarón (Granada) and Moraira (Alicante), and voucher specimens were deposited in the Herbarium of the Facultad de Ciencias de Murcia.