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HORTENSIN, AN UNUSUAL FLAVONE FROM *MILLINGTONIA HORTENSIS*

NUNTAVAN BUNYAPRAPHATSARA, GÁBOR BLASKÓ*† and GEOFFREY A. CORDELL*‡

Faculty of Pharmacy, Mahidol University, Bangkok, Thailand; *Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, U.S.A.

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Key Word Index—*Millingtonia hortensis*; Bignoniaceae; hortensin; 3,4'-dihydroxy-6,7-dimethoxyflavone.

Abstract—From the powdered flowers of *Millingtonia hortensis* hortensin, 3,4'-dihydroxy-6,7-dimethoxyflavone, has been isolated and characterized through its spectroscopic properties, including CSCM 1D and selective INEPT experiments.

INTRODUCTION

Millingtonia hortensis L. (Bignoniaceae) is a cultivated ornamental plant in Thailand, locally known as 'Peep', and is also used as a medicinal plant for the treatment of tuberculosis, sinusitis and asthma [1–5]. Previous phytochemical studies on this plant have reported the isolation of several flavonoids, e.g. scutellarein [6, 7], hispidulin [7, 8], dinatin [9] and their glycosylated derivatives [6, 10], together with carotene [9], lapachol [11, 12], poulownin [11] and β-sitosterol [11, 12]. We report here on the isolation and structure elucidation of an unusual new flavone, hortensin, 3,4'-dihydroxy-6,7-dimethoxyflavone, obtained from the flowers.

RESULTS AND DISCUSSION

Hortensin, $C_{17}H_{14}O_6$, mp 212–213°, $[\alpha]_D$ 0° (MeOH), gave a positive reaction with ferric chloride, and its UV spectrum, displaying λ_{max} 331, 276 and 216 nm, was typical for a flavonoid. Systematic studies of the UV spectrum [13,14] (see Experimental) established the presence of a phenolic group at position 4' and another at the 3-position of the flavone skeleton. These structural inferences were further confirmed by the ^{1}H NMR spectrum, obtained in DMSO-d_6 (see Table 1) which showed a four proton doublet pair at δ7.11 and 8.04 (J = 9 Hz) for the symmetrically substituted B ring protons, 3',5'-H_2 and 2',6'-H_2, respectively. Two singlet methoxy methyl groups were observed at δ3.76 and 3.86 and two aromatic singlets at δ6.62 and 6.88 from which the 6,7-dimethoxy substitution pattern could be established. The location of the methoxy groups and the assignment of the ^{1}H NMR spectrum was further supported by NOE experiments. Irradiation of the aromatic singlet at δ6.88 resulted in an area increase for the methoxy signal at δ3.86, and irradiation of the aromatic singlet at δ6.62 enhanced the methoxyl resonance at δ3.76. These spectral data were consistent with the substitution pattern indicated which was further established by unambiguous ^{13}C NMR measurements using APT, CSCM 1D [15] and selective INEPT [16] spectroscopic techniques. Details of selective INEPT experiments are shown in Table 2, and the complete assignment of the ^{13}C NMR spectrum is reported in Table 1.

Hortensin was evaluated in the P-388 lymphocytic leukemia and KB carcinosarcoma test systems *in vitro* according to established protocols [17, 18]. The isolate showed ED_{50} values of 6.3 and 8.5 μg/ml in the P-388 and KB assays, respectively. Compounds displaying an ED_{50} of 4 μg/ml are regarded as active.

Table 1. ^{1}H and ^{13}C NMR assignments for hortensin*

C	^{1}H	^{13}C
2	—	152.69
3	—	131.30
4	—	182.06
4a	—	104.08
5	6.88 (*s*)	103.00
6	—	163.24
7	—	162.21
8	6.62 (*s*)	94.23
8a	—	152.35
1'	—	122.80
2'/6'	8.04 (*d*, 9.0)	128.21
3'/5'	7.11 (*d*, 9.0)	114.49
4	—	157.23
6-OMe	3.86 (*s*)	59.90
7-OMe	3.76 (*s*)	55.50

*Recorded in DMSO-d_6. Proton chemical shifts are reported as δ values (ppm) from internal TMS at 300 MHz. Signal multiplicity and coupling constants (Hz) are shown in parentheses. Carbon chemical shifts are reported as δ values (ppm) at 90.8 MHz.

†On leave from the Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Hungary.

‡Author to whom correspondence should be addressed.

Table 2. Selective INEPT experiments on hortensin

Proton irradiated	δH	Carbon enhanced δC
2′-H/6′-H	8.04	128.21 (C-6′/C-2′), 152.69 (C-2)
3′-H/5′-H	7.11	114.49 (C-3′/C-5′), 122.80 (C-1′)
5-H	6.88	152.35 (C-8a), 162.21 (C-7), 182.06 (C-4)
8-H	6.62	104.08 (C-4a), 163.24 (C-6)
6-OMe	3.86	163.24 (C-6)
7-OMe	3.76	162.21 (C-7)

EXPERIMENTAL

Mp: uncorr. NMR spectra were measured using TMS as the internal standard. Chemical shifts are reported in δ values (ppm). Mass spectra were recorded on a Varian MAT 112S instrument operating at 70 eV.

Plant material. The dried flowers of *Millingtonia hortensis* L. were obtained from Bang-pa-in Palace, Ayudhaya Province and the specimen was identical with a herbarium specimen (Beusekom *et al.* 3427) deposited at the National Herbarium, Forestry Department, Ministry of Agriculture, Bangkok, Thailand.

Isolation of hortensin (*3,4′-dihydroxy-6,7-dimethoxyflavone*). The dried and powdered flowers of *Millingtonia hortensis* L. (2.74 kg) were exhaustively extracted with MeOH. The MeOH extract was successively partitioned with petrol, $CHCl_3$ and *n*-BuOH, to afford, petrol (51.9 g), $CHCl_3$ (223.3 g), *n*-BuOH (229.1 g) and (270.8 g) fractions. A sample of the petrol fraction (1 g) was submitted to CC on silica gel eluting with $CHCl_3$ to afford hortensin (**1**) (46.7 mg) as pale yellow crystals having the following physical and spectroscopic properties: mp 212–213°; UV, λ_{max} (log ε) (MeOH) 216 (4.36), 276 (4.10), 331 (4.20), (MeOH + NaOMe) 276 (4.23), 295 (4.14), 368 (4.01), (MeOH + NaOAc) 276 (4.29), 297 (4.10), 368 (4.03), (MeOH + $AlCl_3$) 284 (4.08), 300 (4.10), 355 (4.23), (MeOH + $AlCl_3$ + HCl) 284 (4.06), 299 (4.09), 354 (4.22); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; *m/z* (rel. int.) 314 (M^+, 32), 299 (26), 296 (21), 271 (24), 240 (2), 167 (9), 153 (4), 139 (15), 13 (18), 118 (5), 89 (12), 77 (8), 69 (100).

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