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REVISED STRUCTURE FOR HORTENSIN, A FLAVONOID FROM *MILLINGTONIA HORTENSIS*

TAKESHI HASE, KAZUHIRO OHTANI, RYOJI KASAI, KAZUO YAMASAKI* and CHAYAN PICHEANSOONTHON†

Institute of Pharmaceutical Sciences, Hiroshima University, School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan; †Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand

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Key Word Index—*Millingtonia hortensis*; Bignoniaceae; flavonoid; hortensin; cirsimaritin; pectolinarigenin.

Abstract—The original structure of hortensin, from *Millingtonia hortensis*, and analysed as 4'-hydroxy-6,7-dimethoxyflavonol, has been changed once to 5,4'-dihydroxy-6,7-dimethoxyflavone (cirsimaritin). A further reinterpretation of the NMR data of newly isolated 'hortensin' showed that it is 5,7-dihydroxy-6,4'-dimethoxyflavone (pectolinarigenin). Several related flavonoids from the same plant were also identified.

INTRODUCTION

Hortensin [1], a methoxylated flavone possessing anticancer properties, was isolated from the flower of *Millingtonia hortensis* and given the structure 4'-hydroxy-6, 7-dimethoxyflavonol (**1**). However, re-examination of the published data and comparisons of hortensin with isolated and authentic samples suggested that it was identical with 5,4'-dihydroxy-6,7-dimethoxyflavone (cirsimaritin, **2**). In addition, hortensin was different from a sample of synthetic 4'-hydroxy-6,7-dimethoxyflavonol. Thus, the structure of hortensin was revised to cirsimaritin (**2**) [2].

In the course of our study on the constituents of the flower of *M. hortensis*, we isolated a compound **3**, whose melting point and NMR data were the same as those of hortensin [1]. The NMR spectral data of **3**, including NOE evidence, revealed that its structure was identical with neither 4'-hydroxy-6,7-dimethoxyflavonol (**1**) nor 5,4'-dihydroxy-6,7-dimethoxyflavone (cirsimaritin) (**2**). Instead, **3** was identical with 5,7-dihydroxy-6,4'-dimethoxyflavone (pectolinarigenin, **3**) [3, 4]. In this paper, the correction of the structure of 'hortensin' and the identification of four other known flavonoids (**4**–**7**) from this plant are presented.

RESULTS AND DISCUSSION

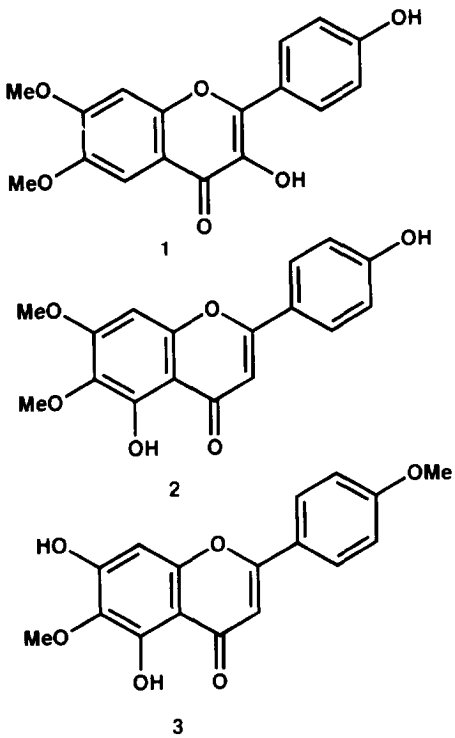
From the hexane-soluble portion of the hot methanolic extract of the dried flowers of *Millingtonia hortensis* (Thai origin), a flavonoid (**3**) was isolated. The melting point (210–211°, lit. [1]: 212–213°) and the ^{1}H and ^{13}C NMR data were essentially the same (with an additional ^{1}H NMR signal at δ13.00) as the reported data of hortensin, which was isolated from the same part of the same plant collected in the same country, and characterized as 4'-hydroxy-6,7-dimethoxyflavonol (**1**) through its spectroscopic properties, including CSCM 1D and selective INEPT experiments [1] (Table 1).

However, the ^{1}H and ^{13}C NMR data of **3** (Tables 1 and 2) could not satisfy the proposed structure for (**1**), but were identical with those of 5,7-dihydroxy-6,4'-dimethoxyflavone (pectolinarigenin, **3**) [3, 4]. The structure was confirmed with NOE experiments. When the signal for H-2' and H-6' (δ8.03, *d*, J = 9 Hz) was irradiated, NOE was observed at H-3 (δ6.87, *s*; 8%) and H-3' and H-5' (δ7.10, *d*, J = 9 Hz; 9%). With irradiation on 4'-OMe (δ3.75, *s*) NOE was observed at H-3' and H-5' (5%), while the irradiation of 6-OMe (δ3.86, *s*) resulted in no NOE.

Therefore, the structure of our compound **3**, which was previously named hortensin [1], is 5,7-dihydroxy-6,4'-dimethoxyflavone (pectolinarigenin, **3**), and the name 'hortensin' should be deleted.

Prior to our correction of the structure of 'hortensin', Nair and Sivakumar [2] claimed the non-identity of hortensin with synthetic 4'-hydroxy-6,7-dimethoxyflavonol. Instead, they identified 'hortensin' as 5,4'-dihydroxy-6,7-dimethoxyflavone (cirsimaritin, **2**) based on comparisons of hortensin with their sample, which they isolated from the same plant, and with authentic samples of hortensin from Cordell and co-workers [1] and authentic cirsimaritin from *Flourensia ceruna*. However, the ^{1}H NMR data of their cirsimaritin [2] were neither identical with those of original hortensin [1], nor with those of cirsimaritin [5] recorded in the same solvent (Table 1). In addition, the melting point of their cirsimaritin was 258–260°, significantly different from that of hortensin

*Author to whom correspondence should be addressed.



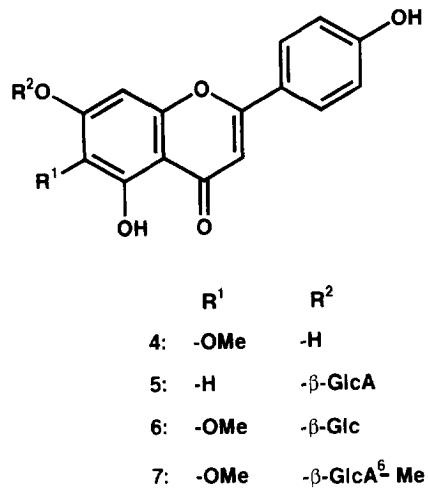


Table 1. ^{1}H NMR data of 'hortensin' and related compounds (in DMSO-d_6)

C	Original hortensin [1]*	This work 3	Revised 'hortensin' [2]†	Cirsimaritin 2 [5]	Pectolinarigenin 3 [3]
3		6.87 *s*	6.54 *s*	6.87 *s*	6.86 *s*
5	6.88 *s*				
8	6.62 *s*	6.62 *s*	7.34 *s*	6.75 *s*	6.63 *s*
2′/6′	8.04 *d*, 9.0	8.03 *d*, 9.0	7.82 *d*, 9.0	7.98 *d*, 9.0	8.03 *d*
3′/5′	7.11 *d*, 9.0	7.10 *d*, 9.0	7.01 *d*, 9.0	6.96 *d*, 9.0	7.12 *d*
6-OMe	3.86 *s*	3.86 *s*	3.90 *s*	3.79 *s*	3.86 *s*
7-OMe	3.76 *s*		3.95 *s*	3.96 *s*	
5-OH		13.00 *s*	12.95 *s*	12.9 *s*	13.00 *s*
4′-OH			9.95 *s*		
4′-OMe		3.75 *s*			3.77 *s*

*The structure (**1**) was proposed.
†The structure (**2**) was proposed.

(212–213°). They did not measure the ^{13}C NMR of their sample, but reassigned the reported data of hortensin [1] for cirsimaritin (Table 2).

Apart from **3**, four structurally related flavonoids (**4**–**7**) were isolated from the same plant, along with 21 phenylethanoids and related compounds [6]. Compounds **4**–**7** were identified as hispidulin [7, 8], apigenin 7-*O*-glucuronide [9], hispidulin 7-*O*-glucoside [10] and hispidulin 7-*O*-glucuronide methyl ester [11], respectively, by means of their NMR data. The NMR data of **4** also confirmed the structure of **3**.

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR (TMS as int. standard): 400 and 100 MHz, respectively, in DMSO-d_6.

Plant material. Millingtonia hortensis was collected at the suburb of Khon Kaen City, Thailand. A voucher specimen is deposited at the Herbarium of Khon Kaen University, Thailand.

Extraction and isolation. Dried flower (300 g) was extracted with MeOH at room temp. to give 149 g of extract, a part (51 g) of which was suspended in H_2O and

Table 2. ^{13}C NMR data of 'hortensin', in descending delta values and their assignments (in DMSO-d_6)

Original hortensin [1]*	C	Revised hortensin [2]† C	This work 3	C	Pectolinarigenin‡ 3[4]	C
182.1	4	4	182.1	4	181.3	4
163.2	6	2	163.3	2	163.0	2
162.2	7	4′	162.3	4′	162.0	4′
157.2	4′	7	157.4	7	157.0	7
152.7	2	5	152.7	5	153.4	5
152.4	9	9	152.4	9	152.1	9
131.3	3	6	131.4	6	131.2	6
128.2	2′/6′	2′/6′	128.3	2′/6′	127.9	2′/6′
122.8	1′	1′	122.9	1′	122.7	1′
114.5	3′/5′	3′/5′	114.5	3′/5′	114.3	3′/5′
104.1	10	10	104.1	10	103.9	10
103.0	5	3	103.0	3	102.8	3
94.2	8	8	94.3	8	94.0	8
59.9	6-OMe	6-OMe	59.9	6-OMe	59.6	6-OMe
55.5	7-OMe	7-OMe	55.5	4′-OMe	55.3	4′-OMe

*The structure (**1**) was proposed.
†Quoted from the data of lit. [1], structure **2** was proposed.
‡Measured at 50°.

extracted with hexane and EtOAc, successively. The hexane-soluble portion was concd and chromatographed on a column of silica gel eluted with CH_2Cl_2–MeOH, and on Sephadex LH-20 eluting with MeOH to afford **3** (33 mg) and **4** (120 mg). The first MeOH extract was chromatographed on Diaion HP-20 eluting with H_2O, 40% MeOH, 80% MeOH, MeOH and Me_2CO. From the 40% MeOH fr., compound **5** (360 mg) was isolated with silica gel and MPLC column (ODS). The 80% eluate was mixed with H_2O and extracted with EtOAc. The EtOAc extract was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O) followed by HPLC (Amide 80: 21.5 mmϕ × 300 mm) using MeOH–H_2O and/or MeCN–H_2O system at a flow rate of 6 ml min^{-1} to afford **6** (36 mg) and **7** (21 mg).

Pectolinarigenin (**3**). Crystals from MeOH, mp 210–211°, ^{13}C and ^{1}H NMR: see Table 1.

Hispidulin (**4**). Powder, ^{13}C NMR [7]: δ (from C-2 to C-10): 163.8, 102.4, 182.1, 152.8, 131.4, 157.3, 94.2, 152.4, 104.0; (from C-1′ to C-4′): 121.2, 128.4, 115.9, 161.2, 6-OMe: 59.9; ^{1}H NMR [8]: δ13.05 (1H, *s*, 5-OH), 7.90 (2H, *d*, *J* = 8.8 Hz, H-2′, H-6′), 6.90 (2H, *d*, *J* = 8.8 Hz, H-3′, H-5′), 6.75 (1H, *s*, H-3), 6.57 (1H, *s*, H-8), 3.73 (3H, *s*, 6-OMe).

Apigenin 7-O-glucoronide (**5**) [9]. Oil, $[\alpha]_D^{17}$ −30° (MeOH; *c* 0.47). ^{13}C NMR: δ (from C-2 to C-10): 164.3, 103.2, 182.0, 161.4, 99.4, 162.5, 94.7, 156.9, 105.4; (from C-1′ to C-4′): 121.0, 128.6, 116.0, 161.2, (from GlcA-1 to GlcA-6): 99.2, 72.7, 75.4, 71.2, 75.6, 170.0; ^{1}H NMR: δ12.98 (1H, *br s*, 5-OH), 10.42 (1H, *br s*, 6″-COOH), 7.96 (2H, *d*, *J* = 8.8 Hz, H-2′ H-6′), 6.94 (2H, *d*, *J* = 8.8 Hz, H-3′ H-5′), 6.87 (1H, *s*, H-3), 6.86 (1H, *d*, *J* = 2.2 Hz, H-8), 6.47 (1H, *d*, *J* = 2.2 Hz, H-6), 5.27 (1H, *d*, *J* = 7.1 Hz, GlcA-1), 4.05 (1H, *d*, *J* = 9.5 Hz, GlcA-5).

Hispidulin 7-O-glucoside (**6**) [10]. Oil, $[\alpha]_D^{17}$ −66° (MeOH; *c* 0.67). ^{13}C NMR: δ (from C-2 to C-10): 164.2, 102.5, 182.2, 152.3, 132.4, 156.3, 94.3, 152.0, 105.6; (from C-1′ to C-4′): 120.9, 128.5, 115.9, 161.3; 6-OMe: 60.2; (from Glc-1 to Glc-6): 100.1, 73.1, 77.1, 69.5, 76.6, 60.5; ^{1}H NMR: δ12.95 (1H, *br s*, 5-OH), 7.96 (2H, *d*, *J* = 8.9 Hz, H-2′, H-6′), 7.02 (1H, *s*, H-8), 6.95 (1H, *d*, *J* = 8.9 Hz, H-3′ H-5′), 6.85 (1H, *s*, H-3), 5.12 (1H, *d*, *J* = 7.4 Hz, Glc-1), 3.78 (3H, *s*, 6-OMe).

Hispidulin 7-O-glucuronide methyl ester (**7**) [11]. Powder, $[\alpha]_D^{17}$ −53° (MeOH; *c* 0.67). ^{13}C NMR: δ (from C-2 to C-10): 164.3, 102.7, 182.2, 152.6, 132.6, 155.8, 93.9, 152.0, 105.9; (from C-1′ to C-4′): 121.0, 128.5, 116.0, 161.3, 6-OMe: 60.2; (from GlcA-1 to GlcA-6): 99.5, 72.8, 75.6, 71.2, 75.3, 169.0; COOMe: 51.9; ^{1}H NMR: δ7.95 (2H, *d*, *J* = 8.8 Hz, H-2′, H-6′), 7.06 (1H, *s*, H-8), 6.95 (1H, *d*, *J* = 8.8 Hz, H-3′, H-5′), 6.85 (1H, *s*, H-3), 5.36 (1H, *d*, *J* = 7.4 Hz, GlcA-1), 3.77 (3H, *s*, 6-OMe), 3.67 (3H, *s*, COOMe).

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