Pergamon

0031-9422(95) 00 566-8

Phytochemistry, Vol. 41, No. 1, pp. 317–321, 1996
Elsevier Science Ltd
Printed in Great Britain.
0031-9422/96 $15.00 + 0.00

MILLINGTONINE, AN UNUSUAL GLUCOSIDAL ALKALOID 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

(*Received in revised form* 19 *June* 1995)

Key Word Index—*Millingtonia hortensis*; Bignoniaceae; flower buds; millingtonine; alkaloid; phenylethanoid; glucoside.

Abstract—From the flower buds of *Millingtonia hortensis*, an unusual glucosidal alkaloid was isolated in diastereomeric form. Its structure has been established by chemical and spectroscopic methods.

INTRODUCTION

From the flower buds of *Millingtonia hortensis*, one of the important herbal medicines used for the treatment of asthma, sinusitis and as a cholagogue and tonic in Southeast Asia [1], we have isolated two phenylpropanoid glucosides, four phenylethanoid glycosides and 15 cyclohexylethanoids including seven glucosides, and the biogenetic relationship between these compounds has been discussed [2]. However, some of the isolated glucosides were non-separable diastereomers caused by the presence of a racemic aglycone. In the continuation of this study, an unusual glucosidal alkaloid was isolated from the same species. In this paper, the structural elucidation of this compound is presented. The biogenetic relationship between this alkaloid and the previously isolated cyclohexylethanoids is also considered.

RESULTS AND DISCUSSION

Successive column chromatography followed by HPLC of a hot methanolic extract of the flower buds of *M. hortensis* yielded compound **1**. It had a molecular formula of $C_{32}H_{45}NO_{14}$ (elemental analysis and HR-FAB-mass spectrum) and was a non-separable mixture of diastereomeric glucosides judging from the appearance of a close set of dual peaks in the ^{13}C NMR spectrum (Table 1). The same phenomenon was observed in the co-existing glucosides [2]. The ^{13}C NMR data suggested the existence of two β-glucopyranosyl units, one of which constituted a glucoside of a *p*-substituted phenethyl alcohol (C-1′–C-8′) moiety. Enzymatic hydrolysis of **1** with β-glucosidase liberated two moles of glucose and afforded two aglycones, **2** and **3**. Both of the resulting aglycones were no longer diastereomeric, judging from the disappearance of dual signals in the ^{13}C NMR spectra.

Table 1. ^{13}C NMR of compound **1** (in pyridine-d_5)

C	δ		Mult.
aglycone			
1	82.01, 81.97		*s*
2	46.06, 45.93		*d*
3	37.64, 37.58		*t**
4	197.53, 197.47		*s*
5	128.65, 128.53		*d*
6	152.44, 152.34		*d*
7	37.40, 37.26		*t**
8	65.19, 64.92		*t*
1′	127.74, 127.72		*s*
2′, 6′	129.93, n†		*d*
3′, 5′	114.04, 114.01		*d*
4′	144.55, 144.53		*s*
7′	35.88, 35.86		*t*
8′	71.24, 71.22		*t*
1″	92.27, 92.19		*d*
2″	48.97, 48.93		*d*
3″	28.31, 28.22		*t*
4″	45.61, 45.53		*t*
glucosyl			
1	104.66, n	104.56, 104.26	*d*
2	75.14, n	74.99, n	*d*
3	78.58, n	78.52, n	*d**
4	71.66, n	71.53, 71.49	*d*
5	78.46, n	78.28, n	*d**
6	62.79, n	62.65, 62.61	*t*

*Assignment of a set of signals may be reversed.
†n = Not resolved with signal shown on left.

* Author to whom correspondence should be addressed.

Compound **2** had a molecular formula of $C_{20}H_{25}NO_4$ (HR-EI-mass spectrum). From the ^{13}C NMR data (Table 2), eight carbons (C-1′–C-8′) were attributed to a *p*-substituted phenethyl alcohol moiety by analogy with the corresponding signals from the same species [2]. Apart from these carbon signals, one carbonyl, δ208.4, four oxygen bearing carbons, δ95.0 (*d*), 90.8 (*s*), 81.6 (*d*) and 67.3 (*t*), five methylene carbons, δ47.0, 43.7, 41.3, 39.1 and 28.7, and two methine carbons, δ49.4 and 47.4, were observed. The ^1H–^1H and ^1H–^{13}C COSY NMR indicated the presence of partial structures of proton-bearing carbons shown in thick lines in Fig. 1. A HMBC experiment further clarified the connection of the quaternary carbons, δ208.4 (C-4) and δ90.8 (C-1), as shown by the dotted lines in Fig. 1; it also established the

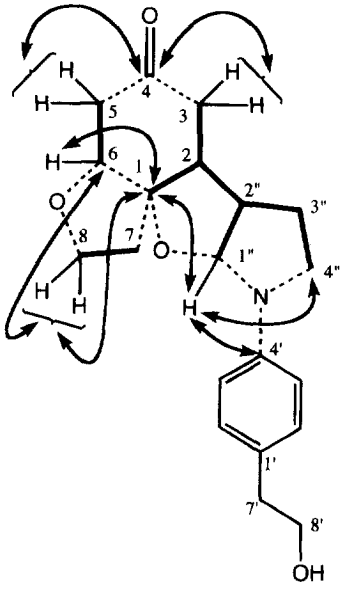

Fig. 1. Planar structure of compound **2**. Thick lines: partial structures deduced from ^1H–^1H and ^1H–^{13}C COSY. Arrows: diagnostically significant H–C long-range correlations. Dotted lines: bonds deduced from the HMBC experiment.

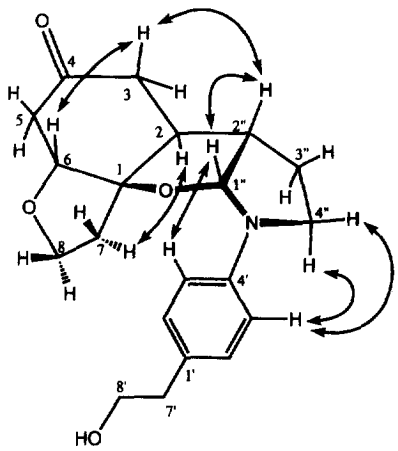

Fig. 2. Relative configuration of compound **2**. Arrows: diagnostically significant NOE.

Table 2. ^1H and ^{13}C NMR of compound **2** (in pyridine-d_5)

C	δC	Mult.	δH	Mult.	*J* in Hz
1	90.8	*s*	2.34	*dd*	4.5, 8.7
2	47.4	*d*	2.54	*dd*	4.5, 16.8
3	41.3	*t*	2.41	*dd*	8.7, 16.8
4	208.4	*s*			
5	43.7	*t*	2.60	*dd*	8.3, 17.2
			2.86	*dd*	4.7, 17.2
6	81.6	*d*	4.28	*dd*	4.7, 8.3
7	39.1	*t*	2.28	*ddd*	3.0, 6.6, 12.8
			1.98	*ddd*	8.2, 9.8, 12.8
8	67.3	*t*	3.91	*ddd*	3.0, 8.2, 8.5
			3.81	*ddd*	6.6, 8.5, 9.8
1′	129.4	*s*			
2′, 6′	130.1	*d*	7.35	*d**	8.7
3′, 5′	114.1	*d*	7.01	*d**	8.7
4′	145.2	*s*			
7′	39.7	*t*	3.05	*t**	7.1
8′	64.1	*t*	4.10	*t**	7.1
1″	95.0	*d*	5.70	*d*	6.2
2″	49.4	*d*	2.62	*ddd*	5.9, 6.2, 7.3
3″	28.7	*t*	1.80	*dddd*	5.9, 6.1, 7.3, 13.1
			2.01	*dddd*	5.9, 7.6, 8.1, 13.1
4″	47.0	*t*	3.48	*ddd*	5.9, 7.6, 9.3
			3.25	*ddd*	6.1, 8.1, 9.3

*Represented 2H, others are 1H.

Table 3. ^1H and ^{13}C NMR of compound **3** (in pyridine-d_5)

C	δC	Mult.	δH	Mult.	J in Hz
1	80.5	*s*			
2	42.3	*d*	2.59	*dddd*	1.3, 2.6, 2.6, 2.9
3	42.9	*t*	2.64	*dd*	2.9, 19.2
			2.26	*dd*	2.6, 19.2
4	211.4	*s*			
5	52.6	*d*	3.84	*dd*	3.6, 3.7
6	83.9	*d*	4.14	*dd*	1.3, 3.6
7	41.4	*t*	2.33	*ddd*	8.1, 10.3, 13.1
			2.06	*ddd*	2.6, 5.3, 13.1
8	66.0	*t*	3.78	m*	
1′	127.9	*s*			
2′, 6′	130.3	*d*	7.24	*d**	8.7
3′, 5′	113.5	*d*	6.73	*d**	8.7
4′	145.5	*s*			
7′	39.7	*t*	3.01	*t**	7.1
8′	64.0	*t*	4.09	*t**	7.1
1″	56.2	*d*	4.31	*dd*	3.7, 9.5
2″	42.6	*d*	2.81	*dddd*	2.6, 2.6, 9.5, 11.7
3″	29.2	*t*	2.69	*dddd*	2.6, 2.6, 7.9, 12.8
			2.05	*dddd*	8.2, 9.3, 11.7, 12.8
4″	50.6	*t*	4.01	*ddd*	7.9, 8.2, 8.4
			3.23	*ddd*	2.6, 8.4, 9.3

*Represented 2H, others are 1H.

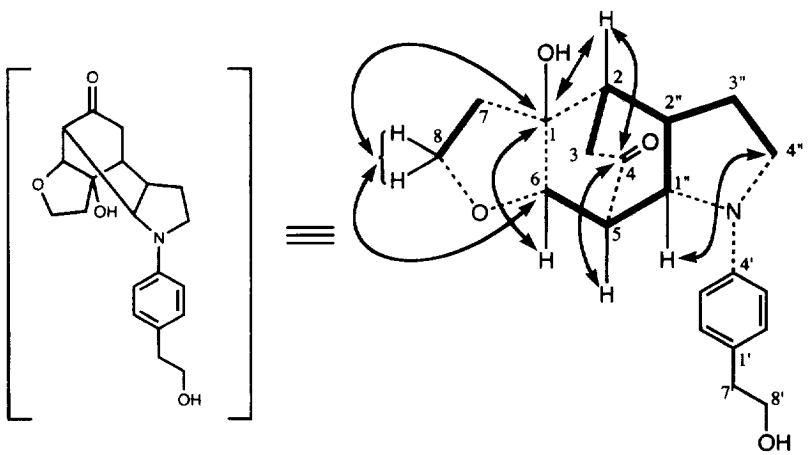

Fig. 3. Planar structure of compound **3**. Thick lines: partial structures deduced from ^1H–^1H and ^1H–^{13}C COSY. Arrows: diagnostically significant H–C long-range correlations. Dotted lines: bonds deduced from the HMBC experiment.

connection of C-6–O–C-8. From the δ value of C-1″(95.0), C-4″ (47.0) and C-4′ (145.2), they must be connected with nitrogen, and the correlation of C-4′ with H-1″ (δ5.70) confirmed the structure around the N-atom. Thus, the planar structure of **2** was characterized as shown (Fig. 1). The relative configuration of **2** was deduced by means of a NOESY experiment. The NOE correlation between the protons indicated by arrows in Fig. 2 confirmed the shown structure. Small but significant coupling was observed between H-2 and H-2″ in the ^1H–^1H COSY spectrum. This small coupling can be explained from the proposed structure, where the dihedral angle of the two protons is near 90°.

Compound **3** had the same molecular formula, $C_{20}H_{25}NO_4$, as **2**. The ^1H and ^{13}C NMR (Table 3) showed that it also had the same *p*-substituted phenethyl alcohol moiety (C-1′–C-8′) as in 2. Two partial structures (thick lines in Fig. 3) were deduced from ^1H–^1H and ^1H–^{13}C COSY NMR experiments. Aided by the HMBC experiment, and taking the chemical shifts into consideration, the remaining atoms could be connected with the dotted lines shown in Fig. 3, where only the diagnostically important HMBC relations are illustrated with arrowed lines. The long-range coupling (J = 1.3 Hz) between H-2 and H-6 also supported the structure. The NOESY experiment confirmed the position of the

phenethyl alcohol moiety, as evidenced by NOE between δ6.73 (H-3′ and H-5′) and δ3.23, 4.01 (two H-4″), as well as 3.84 (H-5) (Fig. 4). The relative configuration of **3** was deduced also from the NOESY experiment (Fig. 4).

From comparison of the NMR spectra of **1** and **2**, it was readily concluded that the two glucosyl units of **1** were located at C-8–O– and C-8′–O–; the former position must be cyclized during the enzymatic hydrolysis to **2** (Fig. 5). The simultaneous formation of **3** can be explained by the reaction shown in Fig. 5. This information allowed us to propose the structure shown for **1**. This is a very unusual skeleton and there are no precedents as far as we know.

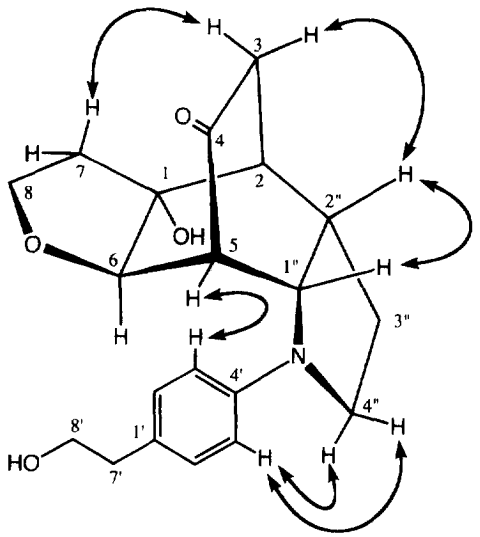

Fig. 4. Relative configuration of compound **3**. Arrows: diagnostically significant NOE.

Concerning the biogenesis of **1**, by analogy with coexisting compounds, the phenethyl alcohol (C-1′–C-8′) and cyclohexylethanoid moieties (C-1–C-8) can be derived from shikimic acid *via* cinnamic acid or its biological equivalent. The origin of the pyrrolidine unit (C-1″–C-4″) might be ornithine; however, the mechanism of insertion of this unit between the two C_6–C_2 units is unknown. It is also interesting that this glucosidal alkaloid is diastereomeric, that is, the β-D-glucoside of a racemic, not a diastereomeric aglycone. The small value of the optical rotation observed in the two aglycones (**2** and **3**) may be due to a small excess of one of the enantiomers, which was expected from the value of ^{13}C NMR dual peak ratio (*ca* 10:11) of **1**.

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR and ^{13}C NMR (TMS as int. standard): 400 and 100 MHz, respectively in pyridine-d_5. Digital resolution of the ^{13}C NMR was 0.002 ppm. EI-MS: 70 eV. FAB-MS: glycerol matrix.

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

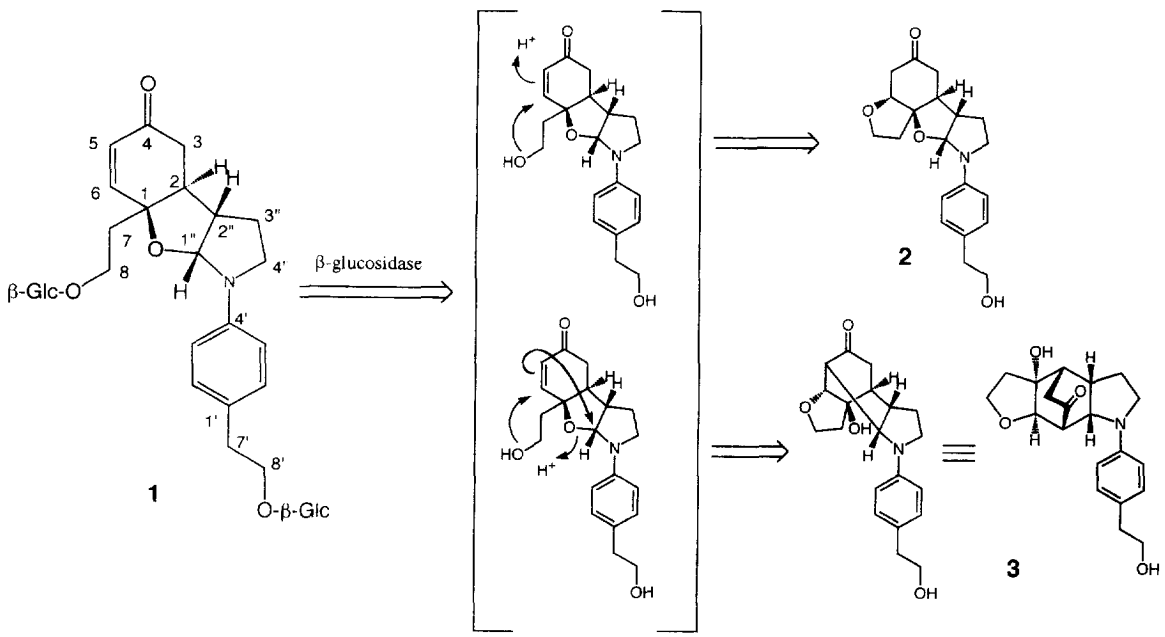

Fig. 5. Structure of **1** and products of its enzymatic hydrolysis. Structure of the aglycone part of **1** is shown by one of the enantiomers. Compounds **2** and **3** are partially racemic.

Extraction and isolation. Dried flower buds (300 g) were extracted with MeOH at room temp. to give 149 g of extract, a part (79 g) of which was suspended in H_2O and extracted with Et_2O to remove a nonpolar fr. (8.3 g). The aq. extract was chromatographed on a column of Diaion HP-20 eluted successively with H_2O, 40% MeOH, 80% MeOH, MeOH and Me_2CO. The 40% MeOH eluate was subjected to silica gel CC using $CHCl_3$–MeOH–H_2O to give frs 1–3. Fr. 2 was chromatographed on silica gel CC using the same solvent system as described above and further chromatographed on MPLC (ODS) to afford **1** (450 mg).

Millingtonine (**1**). Powder (Found: C, 55.75; H, 6.87; N, 2.07. $C_{32}H_{43}NO_{14}$ H_2O requires: C, 56.05; H, 6.91; N, 2.04%). HR-FAB-MS (neg.): $[M-H]^-$ *m/z* 666.2763, $C_{32}H_{42}NO_{14}$ requires 666.2761. ^1H NMR δ7.16/7.15 (*d*, $J = 7.9$ Hz, H-2′, 6′), 6.90/6.89 (*d*, $J = 7.9$ Hz, H-3′, H-5′ 5′), 6.78/6.70 (*d*, $J = 10.3$ Hz, H-6), 6.07/6.06 (*d*, $J = 10.3$ Hz, H-5), 5.38 (*d*, $J = 5.5$ Hz, H-1″), 4.89 (*d*, $J = 7.7$ Hz, Glc-1), 4.70 (*d*, $J = 7.7$ Hz, Glc-1). ^{13}C NMR: Table 1.

Enzymatic hydrolysis of **1**. An aq. soln of **1** (100 mg in 10 ml) was incubated with almond β-glucosidase (Sigma, 10 mg) at 37° for 4 days. After identification of glucose by TLC, the reaction mixt. was extracted with EtOAc and purified by HPLC (ODS, aq. MeOH) to afford **2** (12 mg) and **3** (5 mg).

Compound **2**. Powder. $[\alpha]_D^{28} - 12°$ (MeOH; *c* 1.4). HR-EI-MS $[M]^+$ *m/z* 343.1713, $C_{20}H_{25}NO_4$ requires 343.1784. EI-MS *m/z* 343 $[M]^+$, 312 $[M - CH_2OH]^+$, 298 $[M - CH_2CH_2OH]^+$. ^1H and ^{13}C NMR: Table 2.

Compound **3**. Powder. $[\alpha]_D^{28} - 27°$ (MeOH; *c* 0.27). HR-EI-MS $[M]^+$ *m/z* 343.1827, $C_{20}H_{25}NO_4$ requires 343.1784. EI-MS *m/z* 343 $[M]^+$, 312 $[M - CH_2OH]^+$, 298 $[M - CH_2CH_2OH]^+$. ^1H and ^{13}C NMR: Table 3.

Acknowledgements—We thank the Japan Society of the Promotion of Science (JSPS) and National Research Council of Thailand (NRCT) for the promotion of international collaborative research. Our thanks are also due to the Research Centre for Molecular Medicine, Hiroshima University School of Medicine, for the use of NMR.

REFERENCES

1. Anulakanapakorn, K., Bunyapraphatsara, N. and Satayavivad, J. (1989) *J. Sci. Soc. Thailand* **13**, 71.
2. Hase, T., Ohtani, K., Kasai, R., Yamasaki, K. and Picheansoonthon, C. (1995) *Phytochemistry* **39**, 235.