N-Glycosides of Amino Acid Amides from *Hemerocallis fulva* var. *sempervirens*

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As part of our search for sedative substances from natural sources, we isolated two novel amino acid amides connected with the fructopyranose, kwansonine A (1) and kwansonine B (2), together with three known amino acid amides, longitubanine A (3), longitubanine B (4), and pinnatanine (5), from *Hemerocallis fulva* L. var. *sempervirens* (Araki) M. Hotta. The structures of 1 and 2 have been determined on the spectroscopic evidences as N^2-(1-β-D-fructopyranosyl)-N^5-(2′,5′-dihydro-2′-furyl-3′-hydroxymethyl)-γ-hydroxyglutamine and N^2-(1-β-D-fructopyranosyl)-N^5-(2-hydroxymethylbutadienyl)-γ-hydroxyglutamine. This is the first report on the isolation of amino acid amide *N*-furctoside from *Hemerocallis* genus plant.

Key words amino acid amide; *Hemerocallis fulva* var. *sempervirens*; *N*-glycoside; kwansonine; longitubanine; pinnatanine

Daylilies (*Hemerocallis* spp.) have been utilized for thousands of years in eastern Asia. Many horticultural species are cultivated as ornamental plants in the U.S.A. and Europe due to their large flowers. In China, Korea, and Japan, the flowers or whole plants of some species are used as medicines (to treat depression and inflammation, for example) and as food items.[1] Daylilies have also been reported to act as antioxidants and to be active against the human pathogenic trematode *Schistosoma mansoni* Sambon.[2,3]

The edible flowers and leaves of *Hemerocallis fulva* L. var. *sempervirens* (Araki) M. Hotta (Liliaceae) are known to be taken as an aid to sleeping in Okinawa, Japan.[4] In our search for sleep-promoting substances from natural sources, we previously reported on the isolation and sedative activity of oxypinnatanine (OPT) from *H. fulva* var. *sempervirens*[5] and *H. fulva* L. var. *kwanso* Regal.[6] The current paper describes the structures of the glycosides of two novel amino acid amides, named as kwansonine A (**1**) and B (**2**), together with three known compounds, longitubanine A (**3**),[7] B (**4**),[7] and pinnatanine (**5**),[8] which were isolated from *H. fulva* var. *sempervirens*. A methanol extract of fresh leaves of *H. fulva* var. *sempervirens* was purified by ordinary and reversed-phase silica gel column chromatography followed by recycling HPLC to give kwansonine A (**1**, 0.2%), B (**2**, 0.0173%), longitubanine A (**3**, 0.029%), B (**4**, 0.0147%), and pinnatanine (**5**, 0.0051%) (Fig. 1).

Kwansonine A (**1**) was obtained as a white powder. The positive detection of kwansonine A (**1**), $[\alpha]_D$ −2.6° (c= 0.1, H_2O), by ninhydrin upon TLC revealed the presence of an amino group. The IR spectrum of **1** showed hydroxyl (3400—3200 cm^{-1}), carbonyl (1680 cm^{-1}), amide (1635 cm^{-1}), and trisubstituted olefin (819 cm^{-1}) groups. The positive FAB-MS spectrum of **1** showed a quasimolecular ion peak at m/z 423 $[M+H]^+$. The molecular formula of **1** was determined as $C_{16}H_{26}N_2O_{11}$ from the high-resolution (HR)-positive FAB-MS m/z 445.1430 $[M+Na]^+$ (Calcd for $C_{16}H_{26}N_2O_{11}Na$, 445.1434).

The ^{1}H-NMR spectrum (D_2O) of **1** displayed six methine protons, one of which was attached to the unsaturated carbon and oxygen or nitrogen atom (δ_H 6.26, 1H, br s), and five of which were attached to the hydroxyl or amino groups [δ_H 3.66 (1H, d, J=10.0 Hz), 3.75 (1H, dd, J=3.4, 10.0 Hz), 3.79 (1H, dd, J=3.8, 6.4 Hz), 3.87 (1H, dd, J=1.9, 3.4 Hz), 4.22 (1H, dd, J=3.8, 9.1 Hz)] (Table 1). The olefinic proton at δ 6.04 was observed as a broad singlet, which indicated the presence of trisubstituted olefin. A further five sets of methylene protons were present as follows: δ_H 2.17 (1H, ddd, J=3.8, 6.4, 15.8 Hz), 2.24 (1H, ddd, J=3.8, 9.1, 15.8 Hz); 3.15 (1H, d, J=12.9 Hz), 3.26 (1H, d, J=12.9 Hz); 3.63 (1H, dd, J=1.9, 12.4 Hz), 3.90 (1H, d, J=12.4 Hz); 4.04 (1H, br d, J=14.1 Hz), 4.10 (1H, br d, J=14.1 Hz) and 4.49 (1H, d, J=13.0 Hz), 4.59 (1H, m). In total, 16 signals, two carbonyl carbons (δ_C 172.2, 176.3), trisubstituted olefinic carbons (δ_C 126.8, 136.2), five methylene carbons (δ_C 32.4, 53.1, 56.6, 64.0, 74.2), six methine carbons (δ_C 61.3, 68.9, 69.4, 69.6, 70.5, 85.2), and one quaternary carbon (δ_C 95.2) signals were detected in the ^{13}C-NMR spectrum. The proton and carbon signals were assigned based on the measurements of two-dimensional (2D)-NMR spectra [^{1}H-detected heteronuclear multiple quantum coherency (HMQC) and ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC)] (Table 1). The gross structure of **1** was deduced from extensive analyses of the HMQC and HMBC spectra. The coupling constants of the ^{1}H-NMR spectrum revealed the connectivities of two partial structures, **a** (C-3″—C-6″) and **b** (C-2—C-4) (Fig. 2). The connectivities of C-2′—C-6′ were inferred from the HMBC correlations for H-6′ (δ_H 4.04, 4.10) and H-

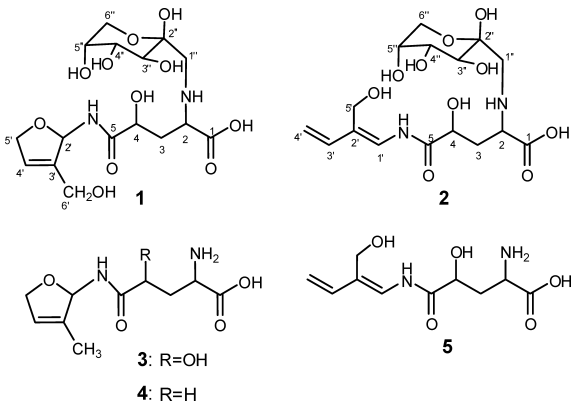

Fig. 1. Structures of Kwansonines A, B (**1**, **2**), Longitubanines A, B (**3**, **4**), and Pinnatanine (**5**)

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Table 1. NMR Spectroscopic Data of Kwansonine A, B (**1**, **2**) and Pinnatanine (**5**) (δ in ppm, J in Hz in D_2O)

No.	**1**		**2**		**5**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		172.2		172.2		173.2
2	3.79 (dd, 3.8, 6.4)	61.3	3.80 (dd, 4.0, 6.0)	61.1	3.79 (dd, 3.7, 7.5)	52.7
3	2.17 (ddd, 3.8, 6.4, 15.8)	32.4	2.22 (m)	32.5	2.09 (m)	33.9
	2.24 (ddd, 3.8, 9.1, 15.8)				2.19 (m)	
4	4.22 (dd, 3.8, 9.1)	69.6	4.35 (dd, 4.3, 8.5)	68.9	4.32 (dd, 3.0, 9.3)	69.1
5		176.3		172.9		174.9
1′			6.61 (br s)	120.8	6.61 (br s)	122.2
2′	6.26 (br s)	85.2		122.9		124.3
3′		136.2	6.51 (dd, 11.4, 17.7)	128.6	6.51 (dd, 11.3, 17.8)	130.2
4′	6.04 (br s)	126.8	5.18 (br d, 11.4)	116.1	5.18 (dd, 1.1, 11.3)	117.7
			5.32 (br d, 17.7)		5.32 (dd, 1.1, 17.8)	
5′	4.49 (d, 13.0)	74.2	4.17 (s)	61.1	4.18 (s)	62.6
	4.59 (m)					
6′	4.04 (br d, 14.1)	56.6				
	4.10 (br d, 14.1)					
1″	3.15 (d, 12.9)	53.1	3.13 (d, 13.0)	52.8		
	3.26 (d, 12.9)		3.27 (d, 13.0)			
2″		95.2		95.2		
3″	3.66 (d, 10.0)	70.5	3.65 (d, 10.0)	70.5		
4″	3.75 (dd, 3.4, 10.0)	69.4	3.74 (dd, 2.8, 10.0)	69.4		
5″	3.87 (dd, 1.9, 3.4)	68.9	3.86 (m)	68.9		
6″	3.63 (dd, 1.9, 12.4)	64.0	3.61 (br d, 13.3)	64.0		
	3.90 (d, 12.4)		3.88 (d, 13.3)			

The assignments were based on HMQC and HMBC experiments.

5′ (δ_H 4.49, 4.59) to C-2′ (δ_C 85.2), H-5′ to C-4′ (δ_C 126.8) and C-3′ (δ_C 136.2), and H-4′ (δ_H 6.04) to C-3′ and C-6′ (δ_C 56.6), which indicated the presence of an unsaturated furan ring (partial structure **c**) attached to the hydroxymethyl group (C-2′—C-6′). The connectivities of the carbonyl carbons C-1 and C-5, and the partial structure **b** were inferred from the HMBC correlations for H-3 (δ_H 2.17, 2.24) to C-1 (δ_C 172.2) and H-4 (δ_H 4.22) to C-5 (δ_C 176.3). From the above mentioned data and the presence of an amide group shown by the IR spectrum, OPT[9] as a partial structure of **1** were deduced.

The connections among partial structure **a**, C-2″, and C-1″ were inferred from the HMBC correlations of H-1″ (δ_H 3.15, 3.26) to C-2″ (δ_C 95.2) and C-3″ (δ_C 70.5), and H-6″ (δ_H 3.63, 3.90) to C-2″. This evidence, along with the coupling constants of each proton H-1″ and H-3″—H-6″, and comparisons with the reference data for the ^{13}C-NMR spectra,[10,11] indicated that kwansonine A (**1**) possessed a β-D-fructopyranose sugar moiety.

The proton and carbon signals of the ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of OPT,[9] except for some signals of C-1 (δ_C 172.2), C-2 (δ_C 61.3), C-3 (δ_C 32.4), and H-3 (δ_H 2.17, 2.24), and the signals of the sugar moiety. These data, and the HMBC correlations of H-1″ to C-2 at δ_C 61.3 and H-2 (δ_H 3.79) to C-1″ at δ_C 53.1, led us to conclude that the C-1″ of the β-D-fructopyranosyl moiety was connected *via* a nitrogen atom attached to the C-2 of OPT. These observations suggested that kwansonine A (**1**) had a unique glycoside structure as *N*-glycosylation. Additionally, the *N*-gylcosylation fused with C-1″ of D-fructopyranosyl, it was not with the anomeric carbon C-2″.

Consequently the structure of kwansonine A was established to be N^2-(1-β-D-fructopyranosyl)-N^5-(2′,5′-dihydro-2′-furyl-3′-hydroxymethyl)-γ-hydroxyglutamine (**1**).

Kwansonine B (**2**) was assigned the molecular formula $C_{16}H_{26}N_2O_{10}$ on the basis of the negative HR-FAB-MS spectrum at m/z 405.1503 $[M-H]^-$ (Calcd for $C_{16}H_{25}N_2O_{10}$, 405.1509) and showed IR absorption bands at 1647 and 1690 cm^{-1} corresponding to amide and carbonyl groups, and a UV absorption band at 263 nm corresponding to a conjugated double bond. The ^{1}H-NMR spectrum (D_2O) of **2** indicated the presence of five sets of methylene protons, one of which exhibited low fields [δ 5.18 (1H, br d, J=11.4 Hz), 5.32 (1H, br d, J=17.7 Hz)] , whereas the others showed similar chemical shifts to those of kwansonine A (**1**). The ^{1}H- and ^{13}C-NMR spectra data resembled those of **1**, except for the presence of conjugated double-bond signals [δ_H 6.61 (1H, br s); δ_C 120.8, δ_C 122.9, δ_H 6.51 (1H, d, J=11.4, 17.7 Hz); δ_C 128.6, δ_H 5.18 (1H, br d, J=11.4 Hz), 5.32 (1H, br d, J=17.7 Hz); δ_C 116.1] instead of the absence of the resonance of an unsaturated furan ring in **1** (Table 1). HMBC correlations were observed from H-3′ (δ_H 6.51) to C-1′ (δ_C 120.8), H-5′ (δ_H 4.17) to C-2′ (δ_C 122.9), H-4′ (δ_H 5.18, 5.32) to C-2′ (δ_C 122.9), and H-1′ (δ_H 6.61) to C-5 (δ_C 172.9) (Fig. 2). These findings suggested that the C-1′ of the conjugated olefin attached to a nitrogen atom bearing C-5 and the hydroxymethyl group (C-5′) fused to C-2′. The aglycone part of **2** was assumed to be a pinnatanine[8] based on this evidence. In the ^{13}C-NMR spectrum of **2**, the glycosylation shifts (−1.0, +8.4, −1.4 ppm) were observed for C-1 (δ_C 172.2), C-2 (δ_C 61.1), and C-3 (δ_C 32.5) of pinnatanine, suggesting that the D-fructopyranosyl moiety was fused with the nitrogen atom bearing C-2; this was confirmed by the HMBC correlations from H-1″ (δ_H 3.13, 3.27) of D-fructopyranose to C-2 (δ_C 61.1) of pinnatanine (Fig. 2).

The structure of kwansonine B (**2**) was therefore established as N^2-(1-β-D-fructopyranosyl)-N^5-(2-hydroxymethylbutadienyl)-γ-hydroxyglutamine.

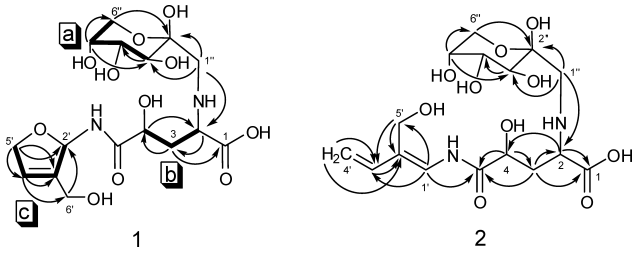

Fig. 2. Partial Structures **a**, **b** and **c** of Kwansonine A (**1**), and HMBC Correlations of **1** and Kwansonine B (**2**)

The three known compounds were identified as longitubanine A (**3**), longitubanine B (**4**), and pinnatanine (**5**), by comparing their physical and spectroscopic data with the reported literatures.[7—9] These substances were isolated for the first time from *H. fulva* var. *sempervirens*.

Experimental

General Experimental Procedure IR spectra were recorded in KBr on a JASCO FT IR/6000 spectrophotometer and UV spectra were obtained with a Shimadzu UV-1700 spectrophotometer in water. ^{1}H- and ^{13}C-NMR spectra were measured in D_2O with a JEOL JEM-GSX 500 spectrometer operating at 500 MHz for ^{1}H and at 125 MHz for ^{13}C. FAB-MS and HR-FAB-MS spectra were recorded on a JEOL JMS-DX 300 spectrometer at an ion acceleration voltage of 5 kV. The mass marker was calibrated with perfluoroalkylphosphazine (ultra maker), and triethanolamine were used as the matrix. Column chromatography was performed using Diaion HP-20 (Mitsubishi Chemicals) and recycling HPLC was performed on a LC-9 (Japan Analytical Industry Co., Ltd.) with (GS-310×2).

Plant Material *Hemerocallis fulva* L. var. *sempervirens* (Araki) M. Hotta was collected from Nakijine, Okinawa, Japan, in June 2008 and identified by one of co-authors (T. K.). A voucher specimen (DWCLA-00153) has been deposited in Doshisha Women's College of Liberal Arts.

Extraction and Isolation The fresh leaves (2.5 kg) of *H. fulva* var. *sempervirens* were cut small pieces and extracted with MeOH in twice at room temperature for 24 h. The combined extracts were evaporated to yield a MeOH extract (140 g). The methanolic extract was subjected to a Diaion HP-20 column chromatography using H_2O–MeOH (100 : 0→0 : 100) to give six fractions (Ia—If). Fraction Ib (63.5 g), which eluted with H_2O, was repeatedly chromatographed on a Diaion HP-20 to obtain four fractions (IIa—IId). Fraction IIc (20 g) was further chromatographed on a Diaion HP-20 using H_2O–MeOH (100 : 0→0 : 100), to obtain four fractions (IIIa—IIId). Fraction IIIc (15.9 g) was purified on recycling HPLC with H_2O to give **1** (280 mg). Fraction Ie (2.9 g) was subjected to a Diaion HP-20 column chromatography , eluting with the step gradient H_2O–MeOH (100 : 0→0 : 100) to give six fractions (IVa—IVf). Fraction IVe (846 mg) was further chromatographed on a Diaion HP-20 using H_2O–MeOH step gradient (100 : 0→0 : 100) to give four fractions (Va—Vd). Fraction Vc (337.3 mg) was purified on a recycling HPLC eluted with H_2O to afford **2** (24.2 mg). Fraction Vb (94.5 mg) was separated by recycling HPLC with H_2O to afford **3** (40.6 mg), **4** (20.6 mg) and **5** (7.2 mg).

Kwansonine A (**1**): A white powder; $[\alpha]_D$ −2.6° (c=0.1, H_2O); IR (KBr) cm^{-1}: 3400—3200, 1680, 1635, 819; HR-FAB-MS m/z: 445.1430 $[M+Na]^+$ (Calcd for $C_{16}H_{26}N_2O_{11}Na$: 445.1434); FAB-MS (positive mode) m/z: 423 $[M+H]^+$; ^{1}H-NMR (D_2O, 500 MHz) and ^{13}C-NMR (D_2O, 125 MHz): see Table 1.

Kwansonine B (**2**): A white powder; IR (KBr) cm^{-1}: 3450—3200, 1690, 1647; UV λ_{max} (H_2O) nm (log ε): 263 (4.52); HR-FAB-MS m/z: 405.1503 $[M-H]^-$ (Calcd for $C_{16}H_{25}N_2O_{10}$: 405.1509); FAB-MS (negative mode) m/z: 405 $[M-H]^-$; ^{1}H-NMR (D_2O, 500 MHz) and ^{13}C-NMR (D_2O, 125 MHz): see Table 1.

Longitubanine A (**3**): Colorless needles; mp 185 °C; ^{1}H-NMR (D_2O, 500 MHz) δ: 1.73 (3H, br s, H-6′), 2.22 (1H, ddd, J=2.9, 8.1, 12.8 Hz, H-3), 2.33 (1H, ddd, J=2.9, 6.3, 12.8 Hz, H-3), 3.95 (1H, dd, J=2.9, 6.3 Hz, H-2), 4.33 (1H, dd, J=2.9, 8.1 Hz, H-4), 4.55 (1H, ddd, J=2.0, 5.1, 10.6 Hz, H-5′), 4.66 (1H, ddd, J=2.0, 5.1, 10.6 Hz, H-5′), 5.89 (1H, ddd, J=2.0, 5.1, 6.5 Hz, H-4′), 6.21 (1H, m, H-2′); ^{13}C-NMR (D_2O, 125 MHz) δ: 13.6 (C-6′), 36.6 (C-3), 55.7 (C-2), 72.2 (C-4), 77.1 (C-5′), 90.3 (C-2′), 127.3 (C-4′), 135.8 (C-3′), 176.5 (C-1), 179.3 (C-5).

Longitubanine B (**4**): Colorless needles; mp 178 °C ; ^{1}H-NMR (D_2O, 500 MHz) δ: 1.73 (3H, br s, H-6′), 2.15 (2H, m, H-3), 2.43 (1H, ddd, J=6.2, 6.5, 13.4 Hz, H-4), 2.49 (1H, ddd, J=6.2, 6.5, 13.4 Hz, H-4), 3.78 (1H, dd, J=6.2, 13.4 Hz, H-2), 4.51 (1H, ddd, J=2.0, 4.1, 12.7 Hz, H-5′), 4.64 (1H, ddd, J=2.0, 4.1, 12.7 Hz, H-5′), 5.88 (1H, m, H-4′), 6.18 (1H, br s, H-2′); ^{13}C-NMR (D_2O, 125 MHz) δ: 13.6 (C-6′), 28.9 (C-3), 34.5 (C-4), 57.0 (C-2), 76.8 (C-5′), 90.3 (C-2′), 127.2 (C-4′), 135.8 (C-3′), 176.9 (C-1), 178.5 (C-5).

Pinnatanine (**5**): Colorless needles; mp 186 °C; ^{1}H-NMR (D_2O, 500 MHz) and ^{13}C-NMR (D_2O, 125 MHz): see Table 1.

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References

1) "Dictionary of Chinese Materia Medica," ed. by Shanhai Scientific Technological Publishers and Shogakukan, Shougakukan, Tokyo, 1985, p. 378.
2) Cichewicz R. H., Nair M. G., *J. Agric. Food Chem.*, **50**, 87—91 (2002).
3) Cichewicz R. H., Lim K. C., McKerrow J. H., Nair M. G., *Tetrahedron*, **58**, 8597—8606 (2002).
4) Uezu E., *Psychiatry Clin. Neurosci.*, **52**, 136—137 (1998).
5) Uchiyama N., Nakamura N., Wada M., Hung Z. L., Konishi T., Urade Y., *Sleep Biol. Rhythmus*, **5**, A78 (2007).
6) Inoue T., Iwagoe K., Konishi T., Kiyosawa S., Fujiwara Y., *Chem. Pharm. Bull.*, **38**, 3187—3189 (1990).
7) Yoshikawa K., Nagai M., Arihara S., *Phytochemistry*, **35**, 1057—1058 (1994).
8) Zhang Y., Nair M. G., Cichewicz R. H., *Life Sci.*, **75**, 753—763 (2004).
9) Grove M. D., Weisleder D., Daxenbichler M. E., *Tetrahedron*, **29**, 2715—2719 (1973).
10) Ciencialova A., Neubauerova T., Sanda M., Sindelka R., Cvacka J., Voburka Z., Budesinsky M., Kasicka V., Sazelova P., Solinva V., Mackova M., Koutek B., Jiracek J., *J. Pept. Sci.*, **14**, 670—682 (2008).
11) Nilsson M., Duarte I. F., Almeida C., Delgadillo I., Goodfellow B. J., Gil A. M., Morris G. A., *J. Agric. Food Chem.*, **52**, 3736—3743 (2004).