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A Rare Glutamine Derivative from the Flower Buds of Daylily

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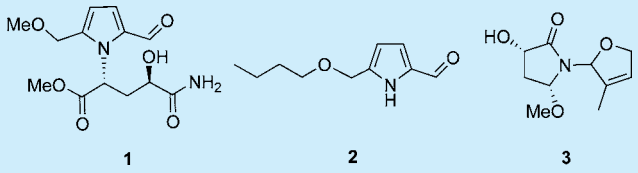
Supporting Information

ABSTRACT: A rare glutamine derivative, hemerocallisamine I (**1**), was isolated from the methanolic extract of the flower buds of daylily, together with a new pyrrole alkaloid hemerocallisamine II (**2**) and a new γ-lactam derivative, hemerocallisamine III (**3**). The chemical structures of the new compounds were elucidated on the basis of chemical and physicochemical evidence. For hemerocallisamine I (**1**), the absolute configuration was determined by Mo−Kα X-ray crystallographic analysis. This is the first report of a glutamine derivative with a pyrrole ring from natural plants.



Glutamine is the most abundant free amino acid, and it is essential for growth of normal and neoplastic cells.[1] In addition, glutamine and its derivatives have the important role of treating sleep disorders and other neurological conditions.[2,3] Despite the considerable attention, there are few reports about glutamine derivatives isolated from natural plants. Daylily (Liliaceae) has been widely cultivated as an ornamental plant in the United States and Europe. Its flowers or whole plant has been used as food or medicine in China, Korea, and Japan. The edible flowers of daylily are known to be taken as a sleeping aid in Okinawa, Japan.[4,5] From the leaves or aerial part of daylily, γ-lactam derivatives,[6] steroidal saponins,[7] and *N*-glycosides of amino acid amides were isolated and reported in a previous study. However, the chemical constituents of the flowers have not been characterized. In the course of our studies on the sedative amino acid derivatives from medicinal flowers,[8−12] surprisingly, we isolated a novel glutamine derivative, hemerocallisamine I (**1**), a new pyrrole alkaloid hemerocallisamine II (**2**), a new γ-lactam derivative, hemerocallisamine III (**3**), and five known compounds from the flower buds of daylily [(Chinese name: jin zhën cái) *Hemerocallis fulva* var. *kwanso*, *H. flava*, *H. minor*] (Figure 1). Herein, we describe the structural elucidation of the three new compounds.

Hemerocallisamine I (**1**) was isolated as a colorless platelet crystal (EtOH) with negative specific rotation ($[\alpha]^{25}_{D}$ −34.6, in MeOH). Its IR spectrum showed absorption bands at 3628, 3400, 1745, 1730, 1684, and 1074 cm^{-1} due to hydroxy, pyrrole, ester, formyl, amide, and ether functions. EIMS revealed a molecular ion $[M]^+$ at *m*/*z* 298, from which the molecular formula $C_{13}H_{18}N_2O_6$ was determined via HRMS and ^{13}C NMR data. The ^1H NMR (acetone-d_6 + D_2O) and ^{13}C NMR (Table 1) data of **1**, assigned via various NMR experiments, showed resonances assignable to a 4-hydroxyglutaminate moiety [a methine bearing a nitrogen function [δ 5.33 (br-s, H-2)], two methylene protons [δ2.42 (t-like, *J* = 12.1, 12.1 Hz, H-3a), 2.51(t-like, *J* = 12.1, 12.1 Hz, H-3b)], and a methine bearing an oxygen function[δ 3.30 (m, H-4)]], 2,5-disubstituted pyrrole moiety {two olefinic protons [δ 7.12 (d, *J*

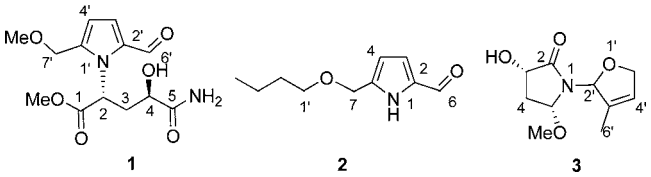


Figure 1. Structures of compounds isolated.

Table 1. ^{13}C NMR (125 MHz) and ^1H NMR (500 MHz) Spectroscopic Data for Compound 1 (Measured in Acetone-d_6 + D_2O)

	1	
position	δ C	δ H (*J* in Hz)
1	171.1	
2	56.2	5.33 (br-s)
3	37.3	2.42 (t-like, 12.1, 12.1)
		2.51 (t-like, 12.1, 12.1)
4	68.6	3.30 (m)
5	177.9	
2′	133.0	
3′	127.1	7.12 (d, 3.8)
4′	112.4	6.32 (d, 3.8)
5′	141.5	
6′	180.1	9.32 (s)
7′	65.7	4.41 (d, 13.1)
		4.72 (br-s)
1-OCH_3	52.7	3.58 (s)
7′-OCH_3	57.5	3.23 (s)

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= 3.8 Hz, H-3′) and 6.32 (d, J = 3.8 Hz, H-4′)]}, two methoxy protons [δ 3.58 (s, 1-OCH_3) and 3.23 (s, 7′-OCH_3)], and a formyl proton [δ 9.32 (s, H-6′)].

The linkages of the two methoxy groups, aldehyde moiety, and 2,5-disubstituted pyrrole moiety as well as the structure of the methyl 4-hydroxyglutaminate moiety were confirmed on the basis of DQF COSY, HMBC, and NOESY experiments.

Namely, long-range correlations were observed between the following proton and carbon pairs: H-2 and C-1; H-3 and C-2, 4; H-4 and C-3, 5; H-3 and C-2′, 5′; H-4′ and C-7′; H-6′ and C-2′; H-7′ and C-5′; 1−OCH_3 and C-1; and 7′−OCH_3 and C-7′. NOESY experiments of **1** showed correlation between H-3 and H-6′, 7′ (Figure 2). Monoclinic crystals were obtained

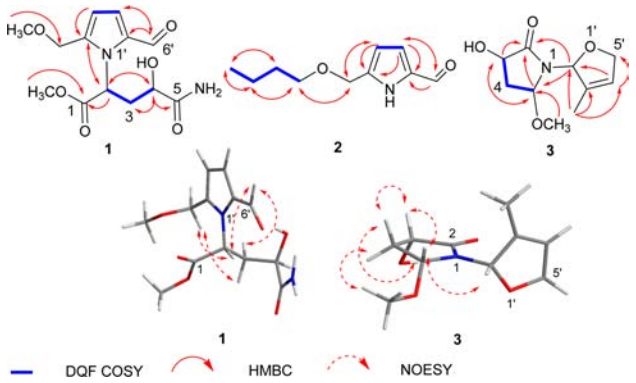

Figure 2. Key 2D NMR and NOESY correlations.

from EtOH solution, so the Mo−Kα X-ray diffraction was employed to determine the configuration of **1** (Figure 3).

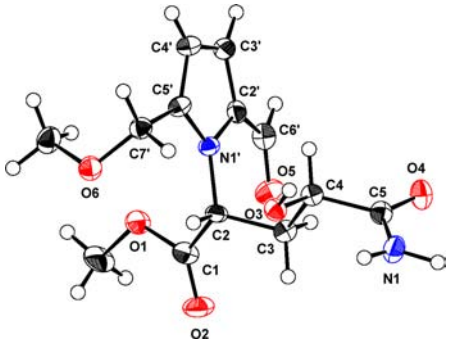

Figure 3. X-ray crystal structure of **1**.

Namely, the absolute configuration was assumed to be 2R,4R from the Flack parameter [absolute structure parameter = −0.0(16)].[13] On the basis of all this evidence, the chemical structure of hemerocallisamine I (**1**) was determined to be (2R,4R)-methyl-5-amino-2-(2-formyl-5-(methoxymethyl)-1H-pyrrol-1-yl)-4-hydroxy-5-oxopentanoate.

Hemerocallisamine II (**2**) was isolated as colorless oil. Its IR spectrum showed absorption bands at 3402, 1732, and 1074 cm^{-1} due to pyrrol, formyl, and ether functions. EIMS revealed a molecular ion $[M]^+$ at m/z 181, from which the molecular formula $C_{10}H_{15}NO_2$ was determined via HRMS and ^{13}C NMR data. The ^1H NMR (chloroform-d) and ^{13}C NMR (Table 2) data of **2**, assigned via various NMR experiments, showed resonances assignable to a 5-hydroxypyrrole-2-carbaldehyde moiety and a butoxy moiety. The proton and carbon signals in the ^1H and ^{13}C NMR spectra of the 5-hydroxymethylpyrrole-2-carbaldehyde moiety of **2** were superimposable on those of hemerocallisamine I (**1**), except for the signals around the 7-postion of **1**. The linkages of the buthoxy group and aldehyde moiety as well as the structure of the 2,5-disubstituted pyrrole moiety were confirmed based on DQF COSY, HMBC, and NOESY experiments. Namely, HMBC correlations were observed between the following proton and carbon pairs: H-3 and C-2, 5; H-4 and C-3, 5, 7; H-6 and C-2, 3; H-7 and C-5, 1′; H-1′ and C-7, 2′, 3′; H-4′ and C-2′, 3′. On the basis of all this evidence, the chemical structure of hemerocallisamine II (**2**) was determined to be 5-(butoxymethyl)-1H-pyrrole-2-carbaldehyde.

Table 2. ^{13}C NMR (125 MHz) and ^1H NMR (500 MHz) Spectroscopic Data for Compound 2 (Measured in Chloroform-d)

	2	
position	δ C	δ H (J in Hz)
2	132.7	
3	121.4	6.90 (d, 3.8)
4	109.2	6.19 (d, 3.8)
5	138.2	
6	178.8	9.47 (s)
7	65.4	4.52 (s)
1′	70.9	3.47 (t, 8.3, 8.3)
2′	31.7	1.58 (m)
3′	19.3	1.36 (m)
4′	13.9	0.91 (t, 7.3, 7.3)

Hemerocallisamine III (**3**), obtained as colorless oil with a negative specific rotation ($[\alpha]^{25}_D$ −112.3 in MeOH), showed absorption bands at 3635, 1716, and 1105 cm^{-1} due to hydroxyl, amide, and ether functions. EIMS revealed a molecular ion $[M]^+$ at m/z 213 from which the molecular formula $C_{10}H_{15}NO_4$ was determined via HRMS and ^{13}C NMR data.

The ^1H (methanol-d_4) and ^{13}C NMR (Table 3) data of **3** showed resonances assignable to a 3,5-dihydroxy- γ-lactam moieties [two methylene protons [δ 1.75 (ddd, J = 3.2, 5.0, 8.0 Hz, H-4α), 2.57 (ddd, J = 6.4, 5.0, 8.5 Hz, H-4β)], a methine bearing an oxygen function [δ 4.18 (dd, J = 8.5, 5.0 Hz, H-3)], and a methine bearing an oxygen and a nitrogen function [δ 4.89 (dd, J = 3.2, 6.4 Hz, H-3)]], 2,5-dihydrofuryl moiety [two

Table 3. ^{13}C NMR (125 MHz) and ^1H NMR (500 MHz) Spectroscopic Data for Compound 3 (Measured in Methanol-d_4)

	3	
position	δ C	δ H (J in Hz)
2	176.9	
3	70.0	4.18 (dd, 8.5, 5.0)
4	35.8	a1.75 (ddd, 8.0, 5.0, 3.2)
		b2.57 (ddd, 8.5, 8.0, 6.4)
5	87.8	4.89 (dd, 6.4, 3.2)
2′	91.4	6.24 (br-s)
3′	136.4	
4′	127.8	5.76 (br-s)
5′	75.4	4.70 (m)
		4.53 (m)
6′	11.7	1.69 (s)
5-OCH_3	55.4	3.23 (s)

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methylene protons [δ 4.53 (m, H-5′β), 4.70 (m, H-5′α)], a methyl proton [δ 1.69 (s, H_3-6′)], a olefinic proton [δ 5.76 (br-s, H-4′)], and a methine bearing an oxygen and a nitrogen function [δ 6.24 (br-s, H-2′)]], and a methoxy proton [δ 3.23 (s, 5-OCH_3)]. The relative configuration was characterized by the observed NOE correlations between: H-3 and H-4β, 5-OCH_3; H-4β and 5-OCH_3; and between H-4α and H-5 (Figure 2). The absolute configuration of 3-position in **3** was characterized by the application of the modified Mosher's method (Figure 4). Treatment of **3** with (−)-2-methoxy-2-

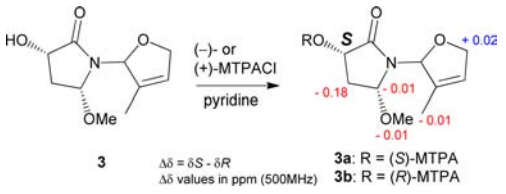

Figure 4. Determination of the absolute structure of 3-position in **3**.

trifluoromethylphenylacetyl choloride [(−)-MTPACl] in pyridine yielded the (*S*)-MTPA ester (**3a**). The (*R*)-MTPA ester (**3b**) was obtained from **3** using (+)-MTPACl in pyridine. As shown in Figure 4, the signals due to protons attached to the 4, 5, and 5-OCH_3 positions in **3a** were observed at higher fields compared with those of **3b** ($\Delta\delta$: negative), while the signals due to proton attached to the 5′ position in the (*S*)-MTPA ester (**3a**) were observed at lower field compared with those of the (*R*)-MTPA ester (**3b**) ($\Delta\delta$: positive). Thus, the absolute configuration at the 3-position in **3** was determined to be *S*. On the basis of all this evidence, the chemical structure of hemerocallisamine III (**3**) was determined as (3*S*,5*R*)-1-(3-methyl-2,5-dihydrofuran-2-yl)-3-hydroxy-5-methoxypyrrolidin-2-one.

In conclusion, a rare glutamine derivative, hemerocallisamine I (**1**), was isolated together with a new pyrrole alkaloid hemerocallisamine II (**2**) and a new γ-lactam derivative, hemerocallisamine III (**3**), from the methanolic extract of the flower buds of daylily. This is the first report of a glutamine derivative with a pyrrole ring from natural plants. The biological effects of the rare compound **1** should be studied.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details; UV, MS, HRMS, and ^{1}H and ^{13}C NMR spectra of new compounds; X-ray experimental details of compound **1**. This material is available free to via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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