

Four new colchicinoids, gloriosamines A–D, from *Gloriosa rothschildiana*

Mariko Kitajima, Akiko Tanaka, Noriyuki Kogure and Hiromitsu Takayama*

Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan

Received 17 October 2007; revised 10 November 2007; accepted 13 November 2007

Available online 17 November 2007

Abstract—Four new colchicinoids, gloriosamines A–D, were isolated from the aerial parts of *Gloriosa rothschildiana*. The structure of gloriosamine A, including the absolute configuration, was determined by chemical conversion from colchicine.

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Colchicine (**1**) is a well-known bioactive alkaloid that is used for the treatment of gout. It also acts as an antimitotic compound by binding to tubulin. The structure elucidation of the tubulin–colchicine complex by X-ray crystallographic study¹ has contributed to progress in structure–activity relationship studies geared toward the development of new antitumor agents based on colchicine (**1**). Colchicine (**1**) was first isolated from *Colchicum autumnale*² and later from other *Colchicum*, *Merendera*, and *Gloriosa* species belonging to Liliaceae. The existence of three colchicinoids, including colchicine (**1**) in tubers of *Gloriosa rothschildiana*, has been reported.³ To discover new colchicinoids, we investigated alkaloidal constituents in the aerial parts of *G. rothschildiana* and isolated four new colchicinoids, gloriosamines A (**2**), B (**3**), C (**4**), and D (**5**) (Fig. 1). We describe herein the structure elucidation of these new colchicinoids.

From the hot MeOH extract of the aerial parts of *G. rothschildiana*,⁴ four new colchicinoids, gloriosamines A (**2**), B (**3**), C (**4**), and D (**5**), were isolated together with four known alkaloids, colchicine (**1**), colchicine (**6**), colchifoline (**7**), and *N*-deacetyl-*N*-formylcolchicine (**8**), by a combination of column chromatographies.

New compound **2**, named gloriosamine A,⁵ exhibited $[\alpha]_D^{25} -35$ (*c* 0.10, CHCl₃). The HR-FAB-MS spectrum gave a protonated molecular ion peak at *m/z* 414.1564 ($[\text{MH}]^+$) that corresponded to the molecular formula

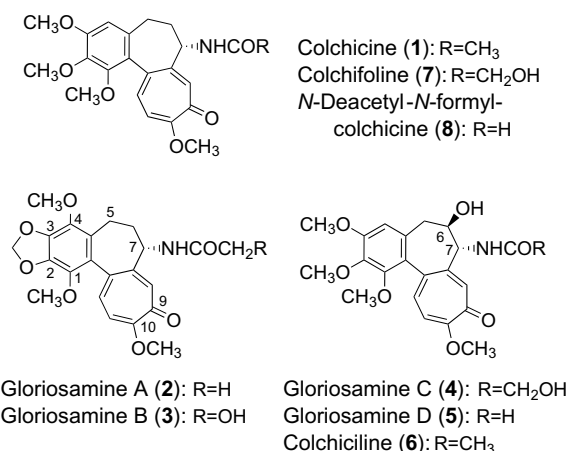


Figure 1. Structures of gloriosamines A–D (**2–5**) and known colchicinoids (**1, 6–8**).

C₂₂H₂₄NO₇ (*m/z* 414.1553). The UV absorption band at 351 nm was characteristic of a tropolone ring. The ¹H NMR spectrum (Table 1) showed signals assignable to three methoxy groups at δ 4.00 (10-OCH₃), 3.92 (4-OCH₃), and 3.68 (1-OCH₃), a methylenedioxy group at δ 6.03 and 6.02 (each 1H, d, *J* = 1.5 Hz), and an acetyl group at δ 2.01, as well as characteristic proton signals due to H-8 (δ 7.41), H-11 (δ 6.82, d), and H-12 (δ 7.24, d) on a tropolone ring. In the ¹³C NMR spectrum, signals for a carbonyl carbon (δ 179.4), an *N*-acetyl carbonyl carbon (δ 169.6), three methoxy carbons (δ 61.0, 60.5, 56.4), and a methylenedioxy carbon (δ 101.7) were observed. Therefore, **2** was deduced to be a colchicine derivative possessing two methoxy groups and one methylenedioxy group on the A ring. NOE correlation

Keywords: Alkaloid; Colchicine; *Gloriosa*; Structure elucidation; Chemical conversion.

* Corresponding author. Tel./fax: +81 43 290 2901; e-mail: htakayam@p.chiba-u.ac.jp

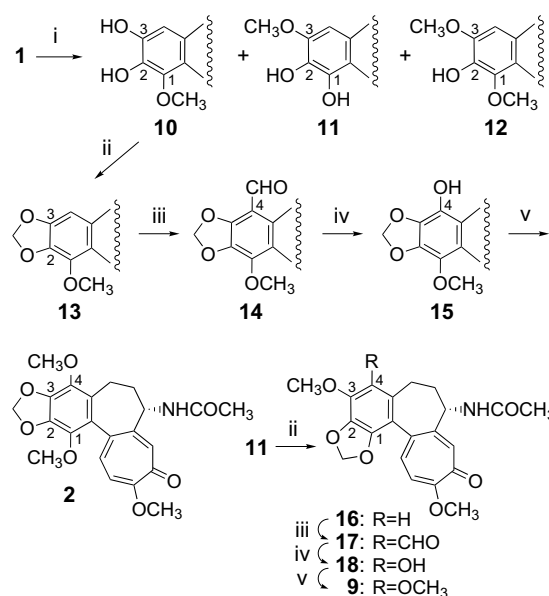
Table 1. ^1H and ^{13}C NMR data for gloriosamines A (**2**) and B (**3**) in CDCl_3

	Gloriosamine A (2)		Gloriosamine B (3)	
	δ_{H} (500 MHz)	δ_{C} (125 MHz)	δ_{H} (400 MHz)	δ_{C} (125 MHz)
1		136.5		136.5
2		139.0 ^a		139.0 ^a
3		138.7 ^a		138.7 ^a
4		136.2		136.2
4a		124.3		124.2
5 α	1.91 (ddd, 13.4, 13.4, 6.7)	21.3	1.95 (ddd, 12.6, 12.6, 6.3)	21.3
5 β	3.11 (dd, 13.7, 5.8)		3.13 (dd, 13.7, 5.9)	
6 α	1.74 (ddd, 11.6, 11.6, 5.8)	36.6	1.82 (ddd, 11.6, 11.6, 5.8)	36.7
6 β	2.17 (dddd, 12.4, 12.4, 6.2, 6.2)		2.19 (dddd, 12.6, 12.6, 6.3, 6.3)	
7	4.62 (ddd, 11.9, 6.4, 6.4)	52.2	4.68 (ddd, 14.0, 7.0, 7.0)	52.1
7a		151.2		150.9
8	7.41 (s)	130.5	7.42 (s)	130.6
9		179.4		179.4
10		164.1		164.1
11	6.82 (d, 10.7)	112.2	6.83 (d, 9.7)	112.1
12	7.24 (d, 10.7)	135.3	7.23 (overlapped)	135.2
12a		135.9		135.8
12b		126.5		126.5
NH	6.46 (br d, 6.4)			
NCOCH ₃		169.6		
NCOCH ₃	2.01 (3H, s)	23.1		
NCOCH ₂ OH				
NCOCH ₂ OH			4.17 (d, 16.8)	169.5 ^b
			4.06 (d, 16.8)	
1-OCH ₃	3.68 (3H, s)	61.0	3.69 (3H, s)	61.0
2,3-OCH ₂ O-	6.03 (d, 1.5)	101.7	6.03 (d, 0.7)	101.7
			6.02 (d, 1.5)	
4-OCH ₃	3.92 (3H, s)	60.5	3.93 (3H, s)	60.5
10-OCH ₃	4.00 (3H, s)	56.4	4.00 (3H, s)	56.3

^a Interchangeable.^b Undetected.

between H-5 β at δ 3.11 and methoxy protons at δ 3.92 indicated that one of the methoxy groups in the A ring was positioned at C-4. From the above data, two candidates, that is, **2** with a 2,3-methylenedioxy ring and **9** with a 1,2-methylenedioxy ring annulated to the A ring, were nominated for the structure of gloriosamine A. However, its structure could not be concluded by means of spectroscopic analyses alone. Therefore, syntheses of the two candidates, **2** and **9**, from colchicine (**1**) were performed.

According to the literature,⁶ acid hydrolysis of the methoxy groups in **1** was carried out by heating with concd H_2SO_4 to give 2,3-*O*-didemethylcolchicine (**10**, y. 4%),^{6,7} 1,2-*O*-didemethylcolchicine (**11**, y. 28%),^{6,7} and 2-*O*-demethylcolchicine (**12**, y. 19%) (Scheme 1).^{8,9} 2,3-*O*-Didemethyl compound **10** was treated with bromochloromethane in the presence of K_2CO_3 in CH_3CN to afford 2,3-methylenedioxy derivative **13** in 48% yield.⁶ A formyl group was introduced onto C-4 position in 65% yield by reacting **13** with Cl_2CHOMe and SnCl_2 .^{10,11} Baeyer–Villiger oxidation of **14** with magnesium bis(monoperoxyphthalate) (MMPP)¹² in $\text{MeOH}/\text{CH}_2\text{Cl}_2$ gave **15** in 49% yield. Finally, methylation of the hydroxyl group in **15** with MeI and K_2CO_3 in acetone afforded **2** with a 2,3-methylenedioxy ring in 61% yield. By the same sequential treatment, candidate **9** with a 1,2-methylenedioxy ring was obtained from



Scheme 1. Reagents and conditions: (i) concd H_2SO_4 , 55 °C, 3 h, 87 °C, 2 h, **10**: y. 4%, **11**: y. 28%, **12**: y. 19%; (ii) BrCH_2Cl , K_2CO_3 , CH_3CN , rt, 23 h, 70 °C, 4 h, y. 48% for **13**, 70 °C, 9 h, rt, 13 h, y. 71% for **16**; (iii) Cl_2CHOMe , SnCl_4 , CH_2Cl_2 , 0 °C, 30 min, rt, 20 h, y. 65% for **14**, 0 °C, 30 min, rt, 23 h, y. 27% for **17**; (iv) MMPP, MeOH , CH_2Cl_2 , rt, 72 h, y. 49% for **15**, rt, 71 h, y. 40% for **18**; (v) MeI , K_2CO_3 , acetone, 50 °C, 5 h, y. 61% for **2**, 70 °C, 7 h, y. 28% for **9**.

1,2-*O*-didemethyl compound **11**. Comparison of the ^1H NMR spectra of natural gloriosamine A with those of synthetic compounds **2** and **9**¹³ led to the unambiguous determination of the structure of gloriosamine A, shown as **2** having the methylenedioxy group at C-2 and C-3 positions. The CD spectrum of natural **2** was identical to that of the synthetic one, indicating that the absolute configuration was the same as that of colchicine (**1**).

New compound **3**, named gloriosamine B,¹⁴ exhibited $[\alpha]_{\text{D}}^{25} -60$ (c 0.10, CHCl_3). The molecular formula was established as $\text{C}_{22}\text{H}_{23}\text{NO}_8$ from the HR-FAB-MS spectrum (m/z 430.1493 $[\text{MH}]^+$), which indicated that **3** has an extra oxygen atom compared to gloriosamine A (**2**). The ^1H NMR spectrum was very similar to that of **2** except for the lack of signals assignable to methyl protons of the acetoamide group and the existence of signals for protons of methylene bearing a hydroxyl group at δ 4.17 and 4.06 (each 1H, d), as in the case of known alkaloid colchifoline (**7**) that possesses a hydroxymethyl group on the 7-amide side chain. Furthermore, signals assignable to methylenedioxy protons were observed at δ 6.03 and 6.02 (each 1H, d). In general, protons of the methylenedioxy group at C-2/C-3 positions were observed as signals with similar chemical shift in the ^1H NMR spectra. In contrast, protons of the C-1/C-2 methylenedioxy group were observed as signals having different chemical shifts.⁶ From these data, the structure of gloriosamine B was deduced to be that shown as formula **3**. Gloriosamines A (**2**) and B (**3**) are the first examples of natural colchicinoids possessing a substituent at C-4 position.

New compound **4**, named gloriosamine C,¹⁵ exhibited $[\alpha]_{\text{D}}^{24} -156$ (c 0.09, CHCl_3). The molecular formula was established as $\text{C}_{22}\text{H}_{25}\text{NO}_8$ from the HR-FAB-MS spectrum (m/z 432.1683 $[\text{MH}]^+$), which indicated that **4** has an extra oxygen atom compared to known alkaloids colchicine (**6**) and colchifoline (**7**). The UV spectrum was very similar to those of colchicine (**1**) and **6**. The ^1H and ^{13}C NMR spectra (Table 2), which showed four aromatic signals, four methoxy signals, and an oxymethine signal, were very similar to those of colchicine (**6**) except for the lack of signals indicative of the methyl group of acetoamide and the existence of signals assignable to the hydroxymethyl group [δ_{H} 4.10 and 4.01 (each 1H, d), δ_{C} 62.3]. HMBC correlation between methylene protons at δ 4.10 and 4.01 and amide carbonyl carbon at δ 173.3 indicated that the methyl group of acetoamide in **6** was oxidized to hydroxymethyl, similar to gloriosamine B (**3**). The relative stereochemistry of the antiperiplanar relationship between methine protons at C-6 and C-7 was determined from the coupling constant of H-6/H-7 ($J_{6,7} = 8.9$ Hz), which was similar to that of colchicine (**6**).¹⁶ Therefore, the structure of gloriosamine C was deduced to be that shown as formula **4**.

New compound **5**, named gloriosamine D,¹⁷ exhibited $[\alpha]_{\text{D}}^{25} -95$ (c 0.06, CHCl_3). The molecular formula was established as $\text{C}_{21}\text{H}_{23}\text{NO}_7$ from the HR-FAB-MS spectrum (m/z 402.1568 $[\text{MH}]^+$). The ^1H and ^{13}C NMR spectra, which were very similar to those of colchicine (**6**), indicated the existence of formamide group (δ_{H} 8.21, δ_{C} 162.0). In the HMBC spectra, correlations between protons of formamide and H-8 and methine carbon at δ 57.7 were observed. From the above data, the structure

Table 2. ^1H and ^{13}C NMR data for gloriosamines C (**4**) and D (**5**) in CDCl_3

	Gloriosamine C (4)		Gloriosamine D (5)	
	δ_{H} (600 MHz)	δ_{C} (150 MHz)	δ_{H} (400 MHz)	δ_{C} (125 MHz) ^a
1		151.1		151.1
2		141.9		141.9
3		153.6		153.6
4	6.68 (s)	109.1	6.65 (1H, s)	109.1
4a		131.2		131.1
5 α	2.65 (dd, 14.6, 4.4)	38.6	2.62 (dd, 13.8, 4.1)	38.8
5 β	2.69 (d, 14.6)		2.67 (br d, 13.8)	
6	4.21 (m)	75.4	3.99 (overlapped)	75.6
7	4.53 (dd, 8.9, 6.7)	59.3	4.55 (d, 8.8)	57.7
7a		149.6		149.2
8	7.52 (s)	131.4	7.37 (s)	131.1
9		179.2		179.4
10		164.1		164.1
11	6.92 (d, 11.0)	113.3	6.91 (d, 11.0)	113.2
12	7.45 (d, 11.0)	136.2	7.43 (d, 11.0)	136.1
12a		137.0		136.7
12b		125.3		125.3
NH	8.11 (br d, 6.6)			
NCOCH ₂ OH		173.3		
NCOCH ₂ OH	4.10 (d, 16.5)	62.3		
	4.01 (d, 16.5)			
NCHO			8.21 (s)	162.0
1-OCH ₃	3.66 (3H, s)	61.6	3.66 (3H, s)	61.7
2-OCH ₃	3.95 (3H, s)	61.4	3.96 (3H, s)	61.4
3-OCH ₃	3.93 (3H, s)	56.1	3.93 (3H, s)	56.1
10-OCH ₃	3.99 (3H, s)	56.5	4.01 (3H, s)	56.5

^a In CDCl_3 containing a few drops of CD_3OD .

of gloriosamine D was deduced to be that shown as formula 5. The CD spectra of gloriosamines C (4) and D (5) were similar to those of colchicine (1) and colchicine (6), indicating that their absolute configurations were the same as those of 1 and 6.

In conclusion, we have isolated and identified four new colchicinoids from the aerial parts of *G. rothschildiana*. Among them, gloriosamines A (2) and B (3) are the first examples of natural colchicinoids having a substituent at the C-4 position. Our preliminary biological evaluation of 4-substituted colchicines demonstrated that 4-methoxycolchicine has significant cytotoxic activity against human lung carcinoma cells A549 with an IC_{50} of 1.40 μ M (colchicine: IC_{50} 4.91 μ M). Biological activity of new colchicinoids, gloriosamines A (2) and B (3), which also have a methoxy function on the C-4 position, will be published in due course.

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- The aerial parts (1944 g) of *Gloriosa rothschildiana* were extracted with hot MeOH to give the extract (81.6 g). After washing with *n*-hexane, the MeOH extract was chromatographed on a DIAION HP20. The fraction that was eluted with 80% MeOH/H₂O was purified by a combination of column chromatographies to afford four new alkaloids, gloriosamine A (2, 3.0 mg), gloriosamine B (3, 1.5 mg), gloriosamine C (4, 20.2 mg), and gloriosamine D (5, 1.0 mg).
- Gloriosamine A (2), amorphous, $[\alpha]_D^{25}$ –35 (*c* 0.10, CHCl₃). UV (EtOH) λ_{max} nm: 351, 235, 218. FAB-MS (NBA) *m/z*: 414 (MH⁺). HR-FAB-MS (NBA/PEG) *m/z*: 414.1564 (MH⁺, calcd for C₂₂H₂₄NO₇ 414.1553). CD (*c* 0.45 mmol/L, MeOH, 25 °C) $\Delta\epsilon$ (λ nm): 0 (396), –7.0 (346), –3.5 (294), –6.8 (262), 0 (251), +11.7 (237), +1.5 (216).
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- Compound 9, amorphous, UV (EtOH) λ_{max} nm: 348, 238. ¹H NMR (400 MHz, CDCl₃): 7.32 (1H, s, H-8), 7.29 (1H, d, *J* = 11.0 Hz, H-11), 6.78 (1H, d, *J* = 11.0 Hz, H-12), 6.03 and 5.90 (each 1H, d, *J* = 1.5 Hz, –OCH₂O–), 5.86 (1H, br d, *J* = 7.3 Hz, NH), 4.70 (1H, ddd, *J* = 12.0, 6.8, 6.8 Hz, H-7), 4.08, 3.97, 3.77 (each 3H, s, 3-OCH₃, 4-OCH₃, 10-OCH₃), 3.10 (1H, dd, *J* = 13.5, 6.0 Hz, H-5 β), 2.23 (1H, dddd, *J* = 12.4, 12.4, 6.2, 6.2 Hz, H-6 β), 2.03 (1H, ddd, *J* = 13.6, 13.6, 6.8 Hz, H-5 α), 2.01 (3H, s, *N*-COCH₃), 1.76 (1H, ddd, *J* = 12.4, 12.4, 6.4 Hz, H-6 α). EI-MS *m/z* (%): 413 (M⁺, 67), 311 (100). HR-FAB-MS (NBA/PEG) *m/z*: 414.1567 (MH⁺, calcd for C₂₂H₂₄NO₇ 414.1553). CD (*c* 0.23 mmol/L, MeOH, 25 °C) $\Delta\epsilon$ (λ nm): 0 (381), –5.3 (346), –1.2 (285), –3.9 (264), 0 (251), +6.6 (239), 0 (226), –4.5 (217), 0 (210).
- Gloriosamine B (3), amorphous, $[\alpha]_D^{25}$ –60 (*c* 0.10, CHCl₃). UV (EtOH) λ_{max} nm: 351, 235, 219. FAB-MS (NBA) *m/z*: 430 (MH⁺). HR-FAB-MS (NBA/PEG) *m/z*: 430.1493 (MH⁺, calcd for C₂₂H₂₄NO₈ 430.1502). CD (*c* 0.17 mmol/L, MeOH, 22 °C) $\Delta\epsilon$ (λ nm): 0 (393), –3.2 (347), –2.1 (316), –4.0 (291), 0 (252), +4.5 (232), 0 (219), –0.4 (215), 0 (212).
- Gloriosamine C (4), amorphous, $[\alpha]_D^{24}$ –156 (*c* 0.09, CHCl₃). UV (EtOH) λ_{max} nm: 355, 245. IR (CHCl₃) ν_{max} cm^{–1}: 3395, 2961, 2927, 2855, 1723. FAB-MS (NBA) *m/z*: 432 (MH⁺). HR-FAB-MS (NBA/PEG) *m/z*: 432.1683 (MH⁺, calcd for C₂₂H₂₆NO₈ 432.1658). CD (*c* 0.24 mmol/L, MeOH, 25 °C) $\Delta\epsilon$ (λ nm): 0 (396), –7.7 (353), –4.2 (318), –8.7 (289), –6.0 (262), 0 (247), +12.2 (234), 0 (225), –6.3 (217), 0 (208).
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- Gloriosamine D (5), amorphous, $[\alpha]_D^{25}$ –95 (*c* 0.06, CHCl₃). UV (EtOH) λ_{max} nm: 353, 244, 233. FAB-MS (NBA) *m/z*: 401. HR-FAB-MS (NBA/PEG) *m/z*: 402.1568 (MH⁺, calcd for C₂₁H₂₄NO₇ 402.1553). CD (*c* 0.32 mmol/L, MeOH, 22 °C) $\Delta\epsilon$ (λ nm): 0 (392), –4.2 (349), –2.2 (317), –5.0 (289), –3.6 (269), –3.7 (264), 0 (244), +5.7 (234), 0 (224), –3.3 (217), 0 (210).