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

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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.

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 study[1] 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**), colchiciline (**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, $CHCl_3$). The HR-FAB-MS spectrum gave a protonated molecular ion peak at *m*/*z* 414.1564 ($[MH]^+$) that corresponded to the molecular formula

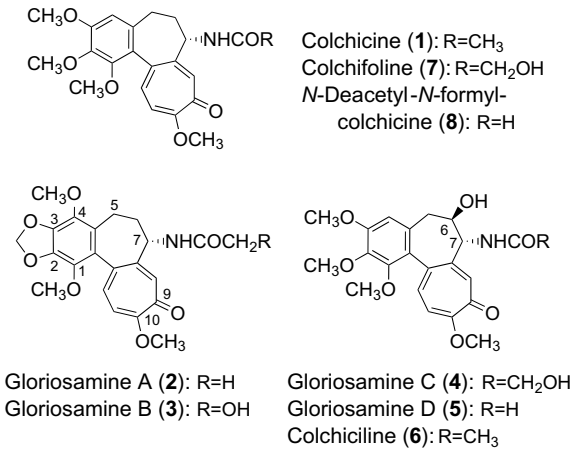

Figure 1. Structures of gloriosamines A–D (**2**–**5**) and known colchicinoids (**1**, **6**–**8**).

$C_{22}H_{24}NO_7$ (*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-OCH_3), 3.92 (4-OCH_3), and 3.68 (1-OCH_3), 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 ^{13}C 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.
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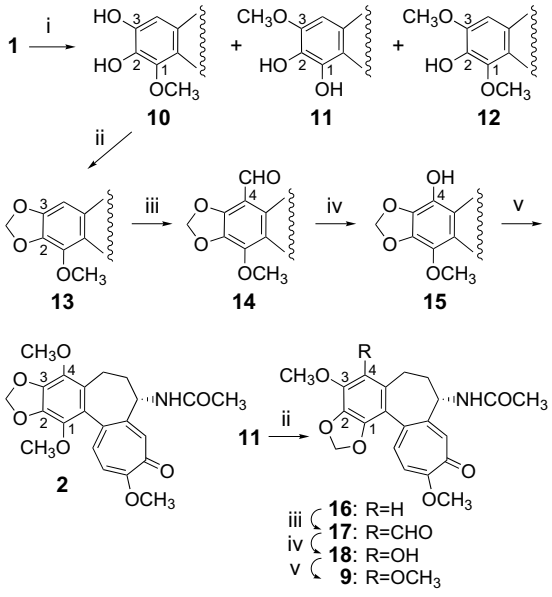
Table 1. ^{1}H and ^{13}C NMR data for gloriosamines A (**2**) and B (**3**) in $CDCl_3$

	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
5α	1.91 (ddd, 13.4, 13.4, 6.7)	21.3	1.95 (ddd, 12.6, 12.6, 6.3)	21.3
5β	3.11 (dd, 13.7, 5.8)		3.13 (dd, 13.7, 5.9)	
6α	1.74 (ddd, 11.6, 11.6, 5.8)	36.6	1.82 (ddd, 11.6, 11.6, 5.8)	36.7
6β	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)			
N*C*OCH$_3$		169.6		
NCO*C*H$_3$	2.01 (3H, s)	23.1		
N*C*OCH$_2$OH				
NCO*C*H$_2$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)		6.02 (d, 0.7)	
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-5β 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 MeOH/CH_2Cl_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) $BrCH_2CI$, 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_2CHOCH_3, $SnCl_4$, 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 ^{1}H 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 $[\alpha]_D^{25}$ −60 (*c* 0.10, $CHCl_3$). The molecular formula was established as $C_{22}H_{23}NO_8$ from the HR-FAB-MS spectrum (*m*/*z* 430.1493 $[MH]^+$), which indicated that **3** has an extra oxygen atom compared to gloriosamine A (**2**). The ^{1}H 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 ^{1}H 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 $[\alpha]_D^{24}$ −156 (*c* 0.09, $CHCl_3$). The molecular formula was established as $C_{22}H_{25}NO_8$ from the HR-FAB-MS spectrum (*m*/*z* 432.1683 $[MH]^+$), which indicated that **4** has an extra oxygen atom compared to known alkaloids colchiciline (**6**) and colchifoline (**7**). The UV spectrum was very similar to those of colchicine (**1**) and **6**. The ^{1}H 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 colchiciline (**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 colchiciline (**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 $[\alpha]_D^{25}$ −95 (*c* 0.06, $CHCl_3$). The molecular formula was established as $C_{21}H_{23}NO_7$ from the HR-FAB-MS spectrum (*m*/*z* 402.1568 $[MH]^+$). The ^{1}H and ^{13}C NMR spectra, which were very similar to those of colchiciline (**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. ^{1}H 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
5α	2.65 (dd, 14.6, 4.4)	38.6	2.62 (dd, 13.8, 4.1)	38.8
5β	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)			
N*C*OCH$_2$OH		173.3		
NCO*C*H$_2$OH	4.10 (d, 16.5)	62.3		
	4.01 (d, 16.5)			
N*C*HO			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 colchiciline (**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 μM (colchicine: IC_{50} 4.91 μM). 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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5. Gloriosamine A (**2**), amorphous, $[\alpha]_D^{25}$ −35 (*c* 0.10, $CHCl_3$). 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 $C_{22}H_{24}NO_7$ 414.1553). CD (*c* 0.45 mmol/L, MeOH, 25 °C) Δε (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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13. Compound **9**, amorphous, UV (EtOH) λ_{max} nm: 348, 238. ^1H NMR (400 MHz, $CDCl_3$): 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, –OCH_2O–), 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-OCH_3, 4-OCH_3, 10-OCH_3), 3.10 (1H, dd, $J = 13.5$, 6.0 Hz, H-5β), 2.23 (1H, dddd, $J = 12.4$, 12.4, 6.2, 6.2 Hz, H-6β), 2.03 (1H, ddd, $J = 13.6$, 13.6, 6.8 Hz, H-5α), 2.01 (3H, s, *N*-$COCH_3$), 1.76 (1H, ddd, $J = 12.4$, 12.4, 6.4 Hz, H-6α). EI-MS *m*/*z* (%): 413 (M^+, 67), 311 (100). HR-FAB-MS (NBA/PEG) *m*/*z*: 414.1567 (MH^+, calcd for $C_{22}H_{24}NO_7$ 414.1553). CD (*c* 0.23 mmol/L, MeOH, 25 °C) Δε (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).
14. Gloriosamine B (**3**), amorphous, $[\alpha]_D^{25}$ −60 (*c* 0.10, $CHCl_3$). 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 $C_{22}H_{24}NO_8$ 430.1502). CD (*c* 0.17 mmol/L, MeOH, 22 °C) Δε (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).
15. Gloriosamine C (**4**), amorphous, $[\alpha]_D^{24}$ −156 (*c* 0.09, $CHCl_3$). UV (EtOH) λ_{max} nm: 355, 245. IR ($CHCl_3$) 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 $C_{22}H_{26}NO_8$ 432.1658). CD (*c* 0.24 mmol/L, MeOH, 25 °C) Δε (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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17. Gloriosamine D (**5**), amorphous, $[\alpha]_D^{25}$ −95 (*c* 0.06, $CHCl_3$). 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 $C_{21}H_{24}NO_7$ 402.1553). CD (*c* 0.32 mmol/L, MeOH, 22 °C) Δε (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).