Isolation and structure elucidation of glucosylated colchicinoids from the seeds of Gloriosa superba by LC-DAD-SPE-NMR

Isolation and Structure Elucidation of Glucosylated colchicinoids from the Seeds of *Gloriosa superba* by LC-DAD-SPE-NMR

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ABSTRACT: Four new colchicinoids were isolated from the seeds of *Gloriosa superba* L. (Colchicaceae) together with the known compounds colchicoside (**4**) and 3-de-*O*-methylcolchicine-3-*O*-*β*-D-glucopyranosyl-(1→4)-3-*O*-*β*-D-glucopyranoside (**6**), by means of conventional column chromatography and LC-DAD-SPE-NMR. The new compounds were identified as *N*-deacetyl-*N*-formyl-3-de-*O*-methylcolchicine-3-*O*-*β*-D-glucopyranoside (**1**), 3-de-*O*-methylcolchicine-3-*O*-*β*-D-glucopyranosyl-(1→6)-3-*O*-*β*-D-glucopyranoside (**2**), *N*-deacetyl-*N*-formyl-3-de-*O*-methylcolchicine-3-*O*-*β*-D-glucopyranosyl-(1→6)-3-*O*-*β*-D-glucopyranoside (**3**) and 3-de-*O*-methylcolchicine-3-*O*-*β*-D-glucopyranosyl-(1→3)-3-*O*-*β*-D-glucopyranoside (**5**). The structure elucidation was performed by means of NMR (COSY, HSQC, and HMBC), HRESIMS/MS, and GCMS data analysis.

Gloriosa superba L., also known as glory lily, flame lily, climbing lily, or creeping lily belongs to the family of the Colchicaceae. It is native to tropical Africa, south-eastern Asia, and India, but nowadays it is widely cultivated throughout the world as an ornamental plant. *G. superba* has traditionally been used for the treatment of various diseases. Medicinal uses of *G. superba* are closely linked to the main secondary metabolites, tropolone-type alkaloids, present in the seeds and tubers.[1] Colchicine, gloriosine, lumicolchicine, 3-de-*O*-methyl-*N*-deformyl-*N*-deacetylcolchicine, 3-de-*O*-methylcolchicine, and *N*-formyl-*N*-deacetylcolchicine have been isolated from this species[1] as well as some glucosides like colchicoside[2] and 3-de-*O*-demethylcolchicine-3-*O*-α-D-glucopyranoside.[3] The increased interest in glycosylated colchicine derivatives is based on the fact that these glycosides may have more beneficial effects and a better toxicity profile in comparison to aglycones such as colchicine. Colchicoside acts as a prodrug which releases active metabolites after oral administration. Extracts from *G. superba* rich in colchicoside, but poor in colchicine, have been shown to exhibit antitumoral activity in vivo in an animal model for pancreatic cancer.[4,5] Some glycosylated colchicine derivatives have been obtained using biotechnological approaches. *β*-1,4-Galactosyltransferase-catalyzed galactosylation of colchicoside was described by Sergio Riva *et al*.,[6] while Pišvejcová *et al*. described the influence of different parameters on the activity of the *β*-1,4-galactosyltransferase from bovine milk and optimization of the reaction conditions leading to the production of galactosyl-colchicoside and glucosylcolchicoside.[7] In the present study the isolation and structure elucidation of four new glucosylated colchicinoids together with colchicoside and 3-de-*O*-methylcolchicine-3-*O*-*β*-D-glucopyranosyl-(1→4)-3-*O*-*β*-D-glucopyranoside, isolated for the first time from a plant source, are described.

The seeds of *G. superba* were extracted with 70% ethanol and the crude extract was defatted by liquid-liquid partitioning with *n*-hexane followed by fractionation using column chromatography. The isolation of single compounds was performed with LC-DAD-SPE-NMR and in this way six tropolone-type alkaloids (**1-6**) were obtained. Their structures were elucidated by 1D (^{1}H, ^{13}C) and 2D NMR experiments (COSY, HSQC, HMBC), GCMS data analysis and confirmed by HRESIMS.

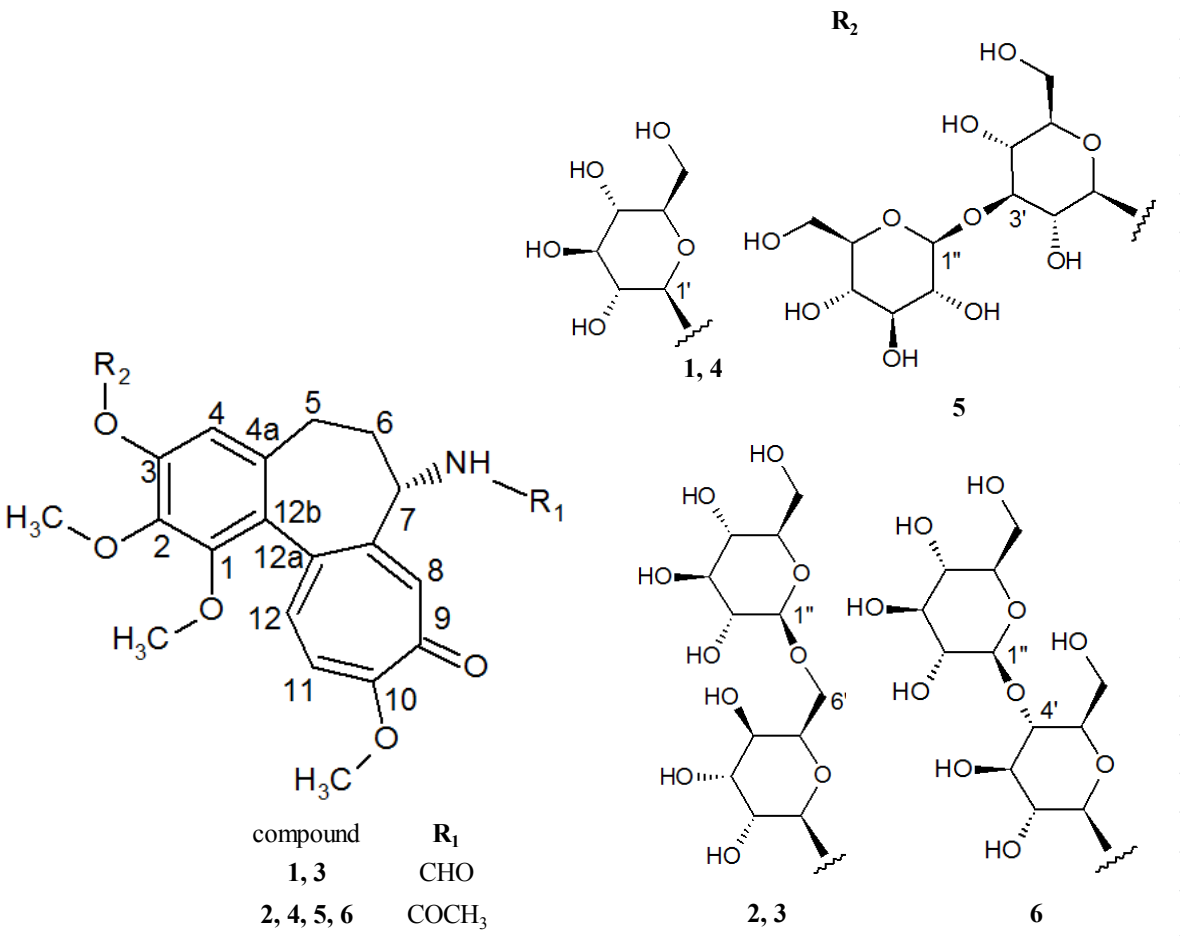

Fig. 1 Chemical structures of compounds **1**–**6**.

The NMR data of compound **4** were in agreement with the reported assignments for 3-de-*O*-methylcolchicine-3-*O*-*β*-D-glucopyranoside, trivial name, colchicoside.[2] ^{1}H and ^{13}C NMR

chemical shift assignments for compound **4** are listed in Tables 1 and 2, respectively. The molecular formula $C_{27}H_{33}NO_{11}$ was in agreement with the HRESIMS spectrum [a protonated molecule at *m/z* 548.2127 $[M+H]^+$ (calcd 548.2126) and a sodium adduct ion at *m/z* 570.1943 $[M+Na]^+$ (calcd 570.1946)].

The NMR spectra of compounds **1** and **3** showed similar chemical shifts to those of compound **4**, but the expected singlet between δ_H 1.98-2.01 (3H) typical for *N*-acetylation was replaced by a signal corresponding to an *N*-formyl group, *i.e.* a singlet at δ_H 8.12 (1H) for compound **1** and a singlet at δ_H 8.13 (1H) / δ_C 161.7 for compound **3**, respectively.[8] The absence of an *O*-methyl substituent at C-3 indicated glycosylation in this position, which was confirmed by the presence of an anomeric proton at δ_H 4.36 (*d*, *J* = 7.75 Hz)[9] for compound **1**, and two anomeric protons at δ_H 5.04 (*d*, *J* = 7.44 Hz) / δ_C 101.0 and at δ_H 4.37 (*d*, *J* = 7.70 Hz) / δ_C 103.7 for compound **3**, corresponding to a mono- and a di-glycosylated derivative, respectively, of *N*-deacetyl-*N*-formyl-3-de-*O*-methylcolchicine. HRESIMS data showed a protonated molecule *m/z* 534.1967 $[M+H]^+$ (calcd 534.1970), consistent with a molecular formula, $C_{26}H_{31}NO_{11}$ for compound **1**, and a protonated molecule *m/z* 696.2488 $[M+H]^+$ (calcd 696.2498) and sodium adduct ion *m/z* 718.2298 $[M+Na]^+$ (calcd 718.2318), consistent with a molecular formula $C_{32}H_{41}NO_{16}$ for compound **3**, respectively. Similar fragmentation patterns were observed for both compounds. Compound **1** displayed an ion at *m/z* 372.1438 $[M-C_6H_{10}O_5+H]^+$ corresponding to one hexosyl moiety, while compound **3** displayed *m/z* 534.1967 $[M-C_6H_{10}O_5+H]^+$ and 372.1438 $[M-C_6H_{10}O_5-C_6H_{10}O_5+H]^+$ ions indicating the subsequent loss of two hexosyl units. After acidic hydrolysis, trimethylsilylated monosaccharides of the hydrolysate of each compound were identified by GCMS data analysis. Comparison of the retention times with reference sugars after derivatization, revealed the monosaccharide moieties in both compound **1** and **3** to be

glucose, present in a *β*-configuration according to the anomeric proton coupling constants.[9] The D configuration is solely based on abundance in nature. ^{13}C NMR signals at δ_C 69.1 (C'-6) and 103.7 (C"-1) observed for compound **3**, indicated that the *β*-D-glucopyranosyl moieties were connected through a (1→6) linkage.[9] Therefore, the structure of compound **1** was elucidated as *N*-deacetyl-*N*-formyl-3-de-*O*-methylcolchicine-3-*O*-*β*-D-glucopyranoside. This compound, glorioside, is described here for the first time. Compound **3** was identified as *N*-deacetyl-*N*-formyl-3-de-*O*-methylcolchicine-3-*O*-*β*-D-glucopyranosyl-(1→6)-3-*O*-*β*-D-glucopyranoside. This is also a new compound and is named, gloriodiside.

Compounds **2**, **5**, and **6** displayed similar HRESIMS data. The estimated molecular formula of these compounds was $C_{33}H_{43}NO_{16}$ [protonated molecule *m/z* 710.2648 $[M+H]^+$ (calcd 710.2655) and sodium adduct ion 732.245 $[M+Na]^+$ (calcd 732.2474)]. MS^2 fragmentation of the protonated molecule at *m/z* 710.2648 $[M+H]^+$ revealed fragment ions at *m/z* 548.21 $[M-C_6H_{10}O_5+H]^+$ and *m/z* 386.16 $[M-C_6H_{10}O_5-C_6H_{10}O_5+H]^+$ corresponding to the subsequent net loss of two hexosyl units. Further investigation of the molecular ion, *m/z* 386.16 $[M+H]^+$ and the common aglycone observed for compound **2**, **5**, and **6** provided a product ion spectrum typical for de-*O*-methylcolchicine derivatives (*m/z* 344, 327, 295, 267).[10] The presence of 3-de-*O*-methylcolchicine as the aglycone of these three compounds was confirmed by comparison of the NMR chemical shifts with literature data and by investigation of the 2D NMR spectra (^1H and ^{13}C NMR chemical shift assignments for compounds **2**, **5**, and **6** are listed in Tables 1 and 2, respectively). GCMS analysis of the hydrolysate of compound **2**, **5**, and **6** indicated the presence of glucose as the unique monosaccharide. The D configuration of glucopyranosyl units is solely based on abundance in nature. The HMBC spectrum of compound **2** showed a cross-peak between the anomeric proton (H-1') at δ_H 5.02 (*d*, *J* = 7.52 Hz) / δ_C 102.2 and 151.3 (C-3), which

indicated the substitution of the aglycone at C-3. The coupling constant of the first and the second anomeric proton δ_H 4.35 (*d*, *J* = 7.57 Hz) / δ_C 104.9 confirmed the *β*-configuration of both glucosyl moieties. Similar to compound **3**, ^{13}C NMR signals at δ_C 70.3 (C-6') and δ_C 104.9 (C"-1) indicated a (1→6) connection between the *β*-D-glucopyranosyl moieties. Thus, compound **2** was identified as 3-de-*O*-methylcolchicine-3-*O*-*β*-D-glucopyranosyl-(1→6)-3-*O*-*β*-D-glucopyranoside. This compound, colchicodiside A, is reported here for the first time.

Coupling constants of the anomeric protons at δ_H 5.02 (*d*, *J* = 7.74) / δ_C 101.9 and δ_H 4.92 / δ_C 101.7 of compound **5** also indicated a *β*-configuration. Taking into account the ^{13}C NMR shifts at δ_C 85.0 (C'-3) and 101.7 (C"-1), the connection between the two *β*-D-glucopyranosyl moieties was established as (1→3).[9]

The expected *β*-configuration of the glucopyranosyl moieties of compound **6** was confirmed by the coupling constants of the anomeric protons at δ_H 5.04 (*d*, *J* = 7.14) / δ_C 102.0 and δ_H 4.43 (*d*, *J* = 8.07) / δ_C 104.5. The deshielded chemical shift of C'-4 (δ_C 85.0) and the chemical shift of C-1" at δ_C 104.5 were in agreement with a (1→4) connection between the *β*-D-glucopyranosyl moieties.[9] Therefore, compound **5** was identified as 3-de-*O*-methylcolchicine-3-*O*-*β*-D-glucopyranosyl-(1→3)-3-O-*β*-D-glucopyranoside, reported for the first time and named colchicodiside B, while the structure of compound **6** was elucidated as 3-de-*O*-methylcolchicine-3-*O*-*β*-D-glucopyranosyl-(1→4)-3-*O*-*β*-D-glucopyranoside, reported here for the first time from a plant source, and named colchicodiside C. Compound **6** had previously been reported as a biotechnologically-derived product.[7]

Table 1. ^{1}H NMR Assignments (400 MHz) for Compounds **1-6** (δ (ppm), Multiplicity (J in Hz))

position	***1***	***2***	***3***	***4***	***5***	***6***
	δ_H,	δ_H	δ_H	δ_H	δ_H	δ_H
4	6.96, s	6.98, s	6.99, s	6.90, s	6.91, s	6.89, s
5	n.o.	2.69, m	2.71, m	2.66, m	2.60, m	2.66, m
		2.21-2.40, m	2.25-2.31, m	2.35, m	2.32, m	2.36, m
6	n.o.	1.94, m	1.93, m	1.93, m	1.88, m	1.95, m
		2.21-2.40, m	2.25-2.31, m	2.24, m	2.19, m	2.23, m
7	4.47, m	4.51, m	4.61, m	4.47, m	4.47, m	4.47, m
8	7.41, s	7.40, s	7.42, s	7.37, s	7.37, s	7.37, s
11	7.23, d (10.9)	7.22, d (11.0)	7.24, d (11.1)	7.20, d (10.8)	7.20, d (11.1)	7.20, d (11.0)
12	7.43, d (10.8)	7.43, d (10.9)	7.44, d (10.7)	7.40, d (10.8)	7.40, d (10.9)	7.40, d (10.9)
OCH3(C-1)	3.64, s	3.63, s	3.65, s	3.59, s	3.59, s	3.59, s
OCH3(C-2)	3.96, s	3.96, s	3.98, s	3.93, s	3.93, s	3.93, s
OCH3(C-10)	4.03, s	4.03, s	4.04, s	4.00, s	3.99, s	4.00, s
N-COCH3	-	2.02, s	-	1.98, s	1.98, s	1.98, s
N-CHO	8.12, s	-	8.14, s	-	-	-

Glc-1'	4.36, d (7.75)	5.02, d (7.52)	5.04, d (7.44)	4.99, d (7.52)	5.02, d (7.74)	5.04, d (7.14)
2'	n.o.	3.53, m	3.57, t	3.52, m	3.67, m	3.59, m
3'	n.o.	3.49, m	3.51, t	3.87, m	3.74, m	3.60, m
4'	n.o.	3.42, m	3.43, t	3.38, m	3.51, m	3.63, m
5'	n.o.	3.76, m	3.78, t	3.47, m	3.25, m	3.64, m
6'	n.o.	3.85/4.18, m	3.88, s/4.2, dd	3.88/3.67, m	n.o.	3.89/3.66, m
Glc-1"	-	4.35, d (7.57)	4.37, d (7.70)	-	4.92b	4.43, d (8.07)
2"	-	3.20, m	3.23, t	-	4.02, m	3.24, m
3"	-	3.32, m	3.36, m	-	3.46, m	3.37, m
4"	-	3.26, m	3.29, d (2.77)	-	3.53, m	3.30, m
5"	-	3.28, m	3.30, d (1.89)	-	3.51, m	3.34, m
6"	-	3.88/3.66, m	3.67/3.90, m	-	3.67/3.88, m	3.87/3.64, m

[a]Spectra were recorded in methanol-d_4, n.o.: not observed. [b]Overlapping with residual solvent signal.

Table 2. ^{13}C NMR Assignments (100 MHz) for Compounds **1-6** (δ (ppm), Multiplicity (J in Hz))

position	***1***	***2***	***3***	***4***	***5***	***6***
	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type
1	n.o.	152.5, s	151.1, s	151.2, s	150.9, s	151.1, s
2	n.o.	143.4, s	142.2, s	142.1, s	142.1, s	142.2, s
3	n.o.	152.6, s	151.5, s	152.6, s	152.5, s	152.7, s
4	113.2, d	113.1, d	111.7, d	112.9, d	112.8, d	113.0, d
4a	n.o	n.o	n.o.	n.o.	n.o.	n.o.
5	n.o.	30.4, t	29.1, t	30.4, t	30.3, t	30.4, t
6	n.o.	37.0, t	36.4, t	37.1, t	37.1, t	n.o.
7	53.6, d	53.7, d	51.1, d	53.8, d	53.6, d	53.8, d
7a	n.o.	154.3, s	152.2, s	154.3, s	153.9, s	154.4, s
8	131.2, d	131.0, d	129.9, d	131.2, d	131.2, d	131.3, d
9	n.o.	180.7, C=O	179.3, C=O	180.9, C=O	180.6, C=O	180.9, C=O
10	164.2, s	165.4, s	164.2, s	164.3, s	164.2, s	164.0, s
11	n.o.	115.1, d	114.9, d	115.1, d	115.0, d	115.2, d
12	n.o.	137.6, d	136.6, d	137.9, d	137.6, d	137.8, d
12a	n.o.	138.5, s	136.9, s	138.4, s	138.1, s	138.6, s

12b	n.o.	128.7, s	127.2, s	128.6, s	128.5, s	128.8, s
OCH3(C-1)	61.7, q	61.8, q	60.5, q	61.9, q	61.7, q	61.9, q
OCH3(C-2)	62.2, q	62.1, q	60.9, q	62.1, q	62.1, q	62.3, q
OCH3(C-10)	57.1, q	56.9, q	55.7, q	57.1, q	57.0, q	57.1, q
NCOCH3	-	22.3, q	-	22.5, q	22.3, q	22.5, q
	-	172.8, C=O	-	172.9, C=O	172.5, C=O	172.7, C=O
NCHO	n.o.	-	161.9, C=O	-	-	-
Glc-1'	n.o.	102.2, d	100.9, d	102.0, d	101.9, d	102.0, d
2'	n.o.	74.7, d	73.5, d	74.7, d	74.6, d	74.6, d
3'	n.o.	77.8, d	76.5, d	61.6, d	85.0, d	76.8, d
4'	n.o.	71.2, d	70.0, d	71.2, d	69.7, d	80.2, d
5'	n.o.	77.0, d	75.9, d	78.0, d	78.4, d	76.4, d
6'	n.o.	70.3, t	69.1, t	62.3, t	62.5, t	62.4, t
Glc-1"	-	104.9, d	103.7, d	-	101.7, d	104.5, d
2"	-	74.9, d	73.7, d	-	72.3, d	74.9, d
3"	-	71.3, d	76.5, d	-	75.1, d	77.8, d
4"	-	71.4, d	70.2, d	-	68.3, d	71.3, d
5"	-	77.9, d	76.6, d	-	77.7, d	77.9, d
6"	-	62.7, t	61.34, t	-	62.5, t	62.40, t

[a]Spectra were recorded in methanol-d_4; n.o.: not observed; .

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-2000 spectropolarimeter at 20 °C (Easton, MD, USA) with Spectramanager software. NMR spectra were recorded on a Bruker DRX-400 NMR instrument (Rheinstetten, Germany) equipped with 3 mm inverse broad-band (BBI) probe, operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C. The spectra were processed with Topspin version 1.3. Mass spectra were recorded using a Thermo Scientific Q Exactive plus quadrupole - Orbitrap mass spectrometer coupled with a UPLC Dionex Ultimate 3 000 RSLC system equipped with a RP-18 Kinetex column (2.10 mm x 100 mm, 2.6 µm, Phenomenex Corporation, Torrence, CA, USA). Full scan data were recorded in (+) ESI mode from *m/z* 250 to 1000 at a resolution of 70000 (at *m/z* 200). Full scan dd-MS2 (top 5) was performed at a resolution of 17500 (at *m/z* 200), AGS target 1e5 with maximum IT 50 ms. For GCMS analysis of the glycosydic part of the molecule, compounds **1**-**6** (0.1 mg) were hydrolyzed by TFA (1.5 ml, 2 M) at a temperature of 120 ºC for 5 h. The reaction mixture was dried under N_2 and the residue was dissolved in pyridine (20 µL), treated with 80 µL *N*, *O*-bistrifluoraacetamide + 1 % TMSCl and sonicated for 1 h. The reference monosaccharides, D-galactose, D-glucose, D-mannose, L-rhamnose and L-fucose (100 µg) were also treated with pyridine and the trimethylsilylation reagent.[11] Gas chromatographic analysis was performed on a Trace 2000 GC with a Voyager quadrupole mass spectrometer equipped with an electron ionization (EI) source (Interscience) and column AT^{TM}-5MS/Rtx$^{®}$-5MS (30 m x 0.25 mm, 0.25 µm) (5% phenyl, 95% polysilphenylene siloxane) (Alltech Associates, Inc., Deerfield, Illinois, USA). Helium was used as a carrier gas with a constant flow rate of 1.3 mL/min and MS source temperature as well as injector temperature were maintained at 230 ºC and 200 ºC, respectively. 1 µL of every sample was analysed with a split ratio of 8. Elution of the compounds was

performed with the following temperature program: start at 65 °C – hold on 65 °C for 2 min – temperature increase of 6 °C/min till 300 °C – hold on 300 °C for 15 min. The MS was operated in the electron impact mode with ionization energy of 70 eV. Chromatograms were recorded using selected ion monitoring (SIM). Mass peaks at *m/z* 204 and 217 were used for detection of the saccharides. The monosaccharide units present in the hydrolyzed samples were elucidated by mean of retention time of the reference sugars. All data were recorded and processed using Xcalibur software, version 1.0 (Termo Fisher).

The liquid chromatography-solid phase extraction-nuclear magnetic resonance spectroscopy (LC-SPE-NMR) configuration consisted of an Agilent 1200 series high performance liquid chromatography (HPLC) system, with degasser, quaternary pump, automatic injection sampler, and a UV/VIS variable wavelength detector (Agilent Technologies, Eindhoven, The Netherlands). Samples were collected via Bruker/Spark Solid Phase Extraction system into 10 x 2 mm HySphere Resin GP cartridges, mean particle size 12 µm and eluting analytes for NMR using Gilson Liquid Handler 215. The first steps of purification were performed on open glass columns for flash chromatography (40 x 450 mm) with different stationary phases such as MCI gel CHP20P (75 – 150 µm, Mitsubishi Chemical Corp.), Silica gel (40 – 63 µm, Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia Fine Chemicals AB, Uppsala, Sweden). TLC was performed on NP F_{254} plates (20 cm × 20 cm, Merck, Darmstadt, Germany) and the spots were observed under UV light (254 and 366 nm) and under visible light after spraying with vanillin / H_3PO_4.

EtOAc and MeOH, all of HPLC-quality were purchased from Fisher Chemical (Loughborough, UK). Acetonitrile, vanillin, H_3PO_4, TFA and pyridine (99+%), were supplied by Acros Organics (Geel, Belgium). D-galactose and D-mannose purchased from Janssen Chimica

(Beerse Belgium) and D-glucose, L-rhamnose, and L-fucose from Sigma-Aldrich (St.-Louis, USA), were used as monosaccharide standards. BSFTA containing 1% TMSCl was supplied by Thermo Fisher Scientific, Rockford, IL, USA). Methanol-d_4 (99.8% D) was from Sigma-Aldrich (Steinheim, Germany). Water for HPLC was prepared by a Milli-Q system, Millipore (Bedford, MA, USA).

Plant Material. The seeds of *Gloriosa superba* were purchased from Gaurav International INC - India (phytosanitary certificate PSC #52PC).

Extraction and Isolation. *G. superba* seeds (900 g) were exhaustively extracted with 70% EtOH (~30 L). After each portion of fresh solvent, the seeds were sonicated for 40 min, filtered through quartz sand, and the extract evaporated to complete dryness under reduced pressure and subsequently defatted with *n*-hexane (3L). The defatted extract (142 g) was applied onto a flash column filled with MCI gel (160 g). Gradient elution from H_2O to pure MeOH was performed. After TLC screening (mobile phase: EtOAc / formic acid / HOAc / H_20, (121:13:11:23); spraying with vanillin / H_3P0_4), fractions eluted with 30 – 38 % MeOH were combined into sub-fraction A. Dissolved in H_2O sub-fraction A was precipitated with cold MeOH and after centrifugation the precipitate was again subjected to MCI gel CC (160 g) using the same conditions as above. Compounds eluting between 36 and 38% MeOH were separated on Sephadex LH-20 (200 g), and 10 mL MeOH fractions were eluted. Based on TLC analysis, fractions 11-20 were combined (4.5 g) and repeatedly separated on silica gel 80 g, [EtOAc / MeOH / H_2O (100 : 27: 20)] to yield fraction B, containing compounds **1** to **6**.

Fraction B was subjected to LC-DAD-SPE-NMR. The system was operated with a C_{18} Xbridge column (250 mm x 5.0 mm, 5 µm, Waters, Ireland). As a mobile phase (A) H_2O + 5 mM ammonium formate, pH ~ 8.9 and (B) 10% MeOH in MeCN was used. Separation was affected using the following gradient: 0 to 1 min 5% B, 3 min 10.5% B, 33 min 12% B, 36 min 100% B. The flow rate was 1.0 mL/min and UV detection was performed at 254 nm. The compounds **1-6** were manually trapped based on the chromatographic profile with the following retention times (min) for the different compounds: **1** (12.80'), **2** (14.21'), **3** (14.91'), **4** (16.71'), **5** (17.62'), and **6** (19.15').

N-Deacetyl-N-formyl-3-de-O-methylcolchicine-3-O-β-D-glucopyranoside (glorioside) (**1**): white to yellowish powder (0.3 mg); $[\alpha]_D$ -33 (*c* 0.02, MeOH); UV λ_{max} 246, 348 nm; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 1; HRESIMS *m/z* 534.1967 $[M+H]^+$ (calcd. for $C_{26}H_{32}NO_{11}$, 534.1970)

3-De-O-methylcolchicine-3-O-β-D-glucopyranosyl-(1→6)-3-O-β-D-glucopyranoside (colchicodiside A) (**2**): yellowish powder (1.7 mg); $[\alpha]_D$ -2 (*c* 0.1, MeOH); UV λ_{max} 244, 350 nm; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 1; HRESIMS *m/z* 710.2649 $[M+H]^+$; 732.2457 $[M+Na]^+$ (calcd. for $C_{33}H_{44}NO_{16}$, 710.2655).

N-Deacetyl-N-formyl-3-de-O-methylcolchicine-3-O-β-D-glucopyranosyl-(1→6)-3-O-β-D-glucopyranoside (gloriodiside) (**3**): yellowish powder (1.6 mg); $[\alpha]_D$ -11 (*c* 0.11, MeOH); UV λ_{max} 244, 348 nm; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 1; HRESIMS *m/z* 696.2488 $[M+H]^+$ and 718.2298 $[M+Na]^+$ (calcd for $C_{32}H_{42}NO_{16}$, 696.2498).

Colchicoside (**4**): white powder (0.2 mg); $[\alpha]_D$ +55 (*c* 0.01 MeOH); UV λ_{max} 246, 350 nm; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 1; HRESIMS *m/z* 548.2127 $[M+H]^+$; 570.1943 $[M+Na]^+$ (calcd for $C_{27}H_{34}NO_{11}$, 548.2126).

3-De-O-methylcolchicine-3-O-β-D-glucopyranosyl-(1→3)-3-O-β-D-glucopyranoside (colchicodiside B) (**5**): white powder (0.9 mg); $[\alpha]_D$ +16 (*c* 0.1 MeOH); UV λ_{max} 244, 350 nm; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 1; HRESIMS *m/z* 710.2648 $[M+H]^+$; 732.2459 $[M+Na]^+$ (calcd for $C_{33}H_{44}NO_{16}$, 710.2655).

3-De-O-methylcolchicine-3-O-β-D-glucopyranosyl-(1→4)-3-O-β-D-glucopyranoside (colchicodiside C) (**6**): white powder (0.6 mg); $[\alpha]_D$ -10 (*c* 0.04 MeOH); UV λ_{max} 244, 352 nm; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 1; HRESIMS *m/z* 710.2649 $[M+H]^+$; 732.2456 $[M+Na]^+$ (calcd for $C_{33}H_{44}NO_{16}$, 710.2655).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

^{1}H-NMR, COSY, HSQC and HMBC spectra for compounds **1**-**6** (PDF).

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Notes

The authors declare no competing financial interest.

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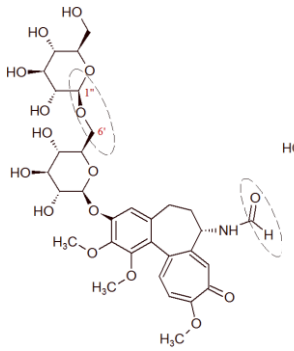
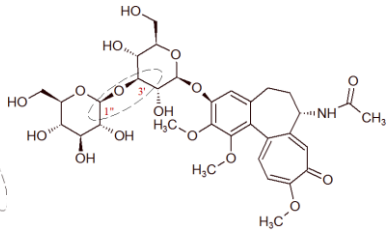
TABLE OF CONTENTS /ABSTRACT GRAPHIC



Gloriodiside



Colchicodiside B