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METACOLCHICINE, A NEW COLCHICINOID FROM *SANDERSONIA AURANTIACA*

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Abstract – Metacolchicine is a new colchicinoid isolated from the tubers of *Sandersonia aurantiaca*. Its unique structure featuring an additional carbon unit at C-10 position was deduced from spectroscopic analyses.

Colchicine (**1**) is a well-known bioactive alkaloid that is used for the treatment of gout. It also acts as an antimetabolic compound by binding to tubulin.¹ The structure elucidation of the tubulin-colchicine complex by X-ray crystallographic study² has contributed to progress in structure-activity relationship studies that are geared toward the development of new antitumor agents based on colchicine (**1**). In our continuing studies on novel and bioactive alkaloids, new colchicinoids, including 4-oxygenated ones named gloriosamines A and B, were isolated from *Gloriosa rothschildiana*.³ Recently, we also found semi-synthetic 4-halo colchicine derivatives that exhibited higher activity against cancer cell lines than colchicine (**1**).⁴ *Sandersonia aurantiaca* Hook (Liliaceae) is known to produce some colchicinoids.⁵ Then, to discover new alkaloids, we investigated the alkaloidal constituents in the tubers of *S. aurantiaca* and isolated one new colchicinoid, metacolchicine (**2**). In this paper, we report the structure elucidation of this new colchicinoid.

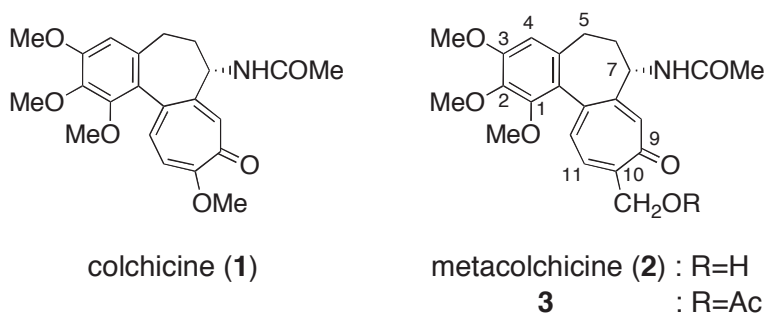


Figure 1. Structures of colchicine (**1**), metacolchicine (**2**), and **3**

From the MeOH extract of the tubers of *S. aurantiaca*, one new colchicinoid **2** (5.8 mg) was isolated together with six known alkaloids, colchicine (**1**, 44.4 mg), 2-demethylcolchicine (27.5 mg),⁶ 3-demethylcolchicine (126.8 mg),⁶ colchifoline (28.1 mg),⁷ demecolcine (7.0 mg),⁸ and 2-demethyldemecolcine (4.3 mg),⁹ by a combination of column chromatographies.

New alkaloid **2**, named metacolchicine, was found to have the same molecular formula $C_{22}H_{25}NO_6$ as colchicine (**1**) from HRFABMS [m/z 400.1752 (MH^+)]. Its 1H NMR spectrum (Table 1) showed signals assignable to three methoxy groups on the aromatic ring at δ_H 3.94 (2-OCH₃), 3.91 (3-OCH₃), and 3.67 (1-OCH₃) and a signal assignable to an acetyl group at δ_H 2.00, along with three proton signals on a tropolone ring at δ_H 7.30 (s, H-8), 7.50 (d, H-11), and 7.31 (d, H-12). These spectral data are similar to those of colchicine (**1**), the exceptions being the existence of signals of benzylic methylene protons of the aromatic alcohol at δ_H 4.72 (dd) and 4.60 (overlapped) instead of the signal for the 10-methoxy group, and chemical shift differences of H-8 and H-11 on the tropolone ring. In the ^{13}C NMR spectrum, a hydroxymethyl carbon signal at δ_C 65.8, as well as the higher-field-shifted C-10 (δ_C 150.5) and the lower-field-shifted C-9 (δ_C 186.9) and C-11 (δ_C 134.4) carbon signals compared with those of **1** (C-9, δ_C 179.5; C-10, δ_C 164.0; C-11, δ_C 112.9), was observed. HMBC cross-peaks between methylene protons at δ_H 4.72 and 4.60 and both carbons at δ_C 186.9 (C-9) and 134.4 (C-11) and between H-11 at δ_H 7.50 and carbon at δ_C 65.8 indicated that a hydroxymethyl group was attached to C-10. This was supported by the differential NOE correlations from H-11 to the protons of H-12 and the hydroxymethyl group.

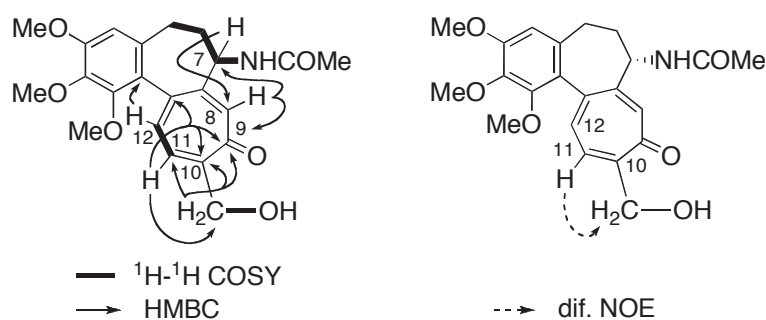


Figure 2. 1H - 1H COSY, selected HMBC, and NOE correlations of metacolchicine (**2**)

Acetylation of **2** with Ac_2O and pyridine gave acetate **3** quantitatively. Its NMR spectra showed signals due to an acetoxy group at δ_H 2.19, δ_C 170.4, and δ_C 21.0 and the lower-field-shifted methylene signals at δ_H 5.20 and 5.12 (each d), confirming the existence of a hydroxymethyl group in **2**. Therefore, the structure of metacolchicine was deduced to be that shown as formula **2**. The absolute configuration at C-7 was deduced to be the same as that of colchicine (**1**) by comparison of their CD spectra.

This is the first example of natural colchicinoid having an additional carbon unit at C-10 position.

Table 1. ^1H (500 MHz, J in Hz) and ^{13}C NMR (125 MHz) data for metacolchicine (**2**) in CDCl_3

Position	δ_{H}	δ_{C}
1	-	151.4
2	-	141.8
3	-	153.9
4	6.53 (s)	107.4
4a	-	134.0
5 α	2.43 (ddd, 13.0, 13.0, 6.5)	29.8
β	2.56 (dd, 13.0, 6.5)	
6 α	1.81 (ddd, 13.0, 13.0, 6.5)	36.4
β	2.24 (dddd, 13.0, 13.0, 6.5, 6.5)	
7	4.60 (overlapped)	52.2
7a	-	151.7
8	7.30 (s)	134.4
9	-	186.9
10	-	150.5
11	7.50 (d, 9.5)	134.4
12	7.31 (d, 9.5)	136.4
12a	-	143.2
12b	-	125.3
NH	6.83 (br-d, 6.7)	-
NCOCH_3	-	169.5
NCOCH_3	2.00 (3H, s)	23.0
10- CH_2OH	4.72 (dd, 14.3, 5.3)	65.8
	4.60 (overlapped)	-
10- CH_2OH	3.51 (br-dd, 5.3, 5.3)	-
1-OCH ₃	3.67 (3H, s)	61.6
2-OCH ₃	3.94 (3H, s)	61.4
3-OCH ₃	3.91 (3H, s)	56.1

EXPERIMENTAL

General Experimental Procedures

^1H and ^{13}C NMR spectra were recorded on a JEOL JNM A-500 at 500 MHz (^1H) and 125 MHz (^{13}C) and a JEOL JNM ECP-400 at 400 MHz (^1H), respectively. UV spectra were recorded on a JASCO V-560. EI-MS data were obtained on a JEOL GC mate. HR-FAB-MS data were acquired with a JEOL JMS-HX110 at the Chemical Analysis Center, Chiba University. Optical rotations were measured with a JASCO P-1020 polarimeter. CD spectra were measured with a JASCO J-720WI. Column chromatography was carried out over Silica gel 60 [Merck, 70-230 mesh (for open column chromatography)], Silica gel 60N [Kanto Chemical, 40-50 mm (for flash column chromatography)], and Chromatorex NH [Fuji Silysia Chemical, 100-200 mesh (for amino-silica gel open column chromatography)]. Medium pressure liquid column chromatography (MPLC) was carried out with C.I.G. prepacked column CPS-HS-221-05 (Kusano Kagakukikai, SiO_2).

Plant Material

The tubers of *Sandersonia aurantiaca* were provided by Mr. Eichi Seki from the Crop Breeding Institute, Chiba Prefectural Agriculture and Forestry Research Center.

Extraction and Isolation

The tubers (4150 g, wet weight) of *S. aurantiaca* were extracted with MeOH (7.2 L, once at room temperature and twice under reflux) to give the extract (181.1 g). The MeOH extract was partitioned with 10% MeOH/CHCl₃ and H₂O to afford the 10% MeOH/CHCl₃ extract (21.8 g). The extract was chromatographed on a silica gel column with MeOH/CHCl₃ gradient to give four fractions: fr. A, 0-5% MeOH/CHCl₃ (920 mg); fr. B, 5% MeOH/CHCl₃ (946 mg); fr. C, 5-30% MeOH/CHCl₃ (16.65 g); and fr. D, 30% MeOH/CHCl₃ and 10% H₂O/MeOH (2.98 g). Fr. C was separated by amino-silica gel column chromatography (0-30% MeOH/CHCl₃). The fraction that was eluted with 8% MeOH/CHCl₃ was purified by a combination of chromatographies [silica gel flash column (10-30% MeOH/AcOEt), MPLC (5% MeOH/AcOEt, and then 3-5% MeOH/CHCl₃)] to afford metacolchicine (**2**, 5.8 mg). Colchicine (**1**, 44.4 mg) and colchifoline (28.1 mg) were obtained from fraction C. 2-Demethylcolchicine (27.5 mg), 3-demethylcolchicine (126.8 mg), and demecolcine (7.0 mg) were isolated from fractions C and D. 2-Demethyldemecolcine (4.3 mg) was obtained from fraction D.

Metacolchicine (2)

Amorphous, $[\alpha]_D^{21}$ -160 (*c* 0.39, CHCl₃). UV (EtOH) λ_{\max} nm: (log ϵ): 339 (3.95), 236 (4.29), 204 (4.35). ¹H and ¹³C NMR data, see Table 1. EI-MS *m/z* (%): 399 (M⁺, 41), 371 (33), 340 (22), 312 (100). HR-FAB-MS (NBA/PEG) *m/z*: 400.1752 (MH⁺, calcd for C₂₂H₂₆NO₆ 400.1760). CD (*c* = 0.25 mmol/L, MeOH, 19 °C) $\Delta\epsilon$ (λ nm): -0.9 (397), -6.6 (345), -3.9 (301), -6.6 (251), 0 (239), $+19.3$ (218), $+7.0$ (204).

Acetylation of Metacolchicine (2)

To a solution of metacolchicine (**2**, 1.2 mg, 0.003 mmol) in dry pyridine (0.1 mL) was added acetic anhydride (0.05 mL) and the mixture was stirred at room temperature for 3 h under Ar atmosphere. After removal of the volatiles, the residue was purified by SiO₂ open column chromatography (20% MeOH/CHCl₃) to give acetate **3** (1.8 mg, y. quant.).

Acetate 3: Amorphous, $[\alpha]_D^{21}$ -95 (*c* 0.12, CHCl₃). UV (EtOH) λ_{\max} nm: (log ϵ): 336 (3.77), 237 (sh, 4.20), 206 (4.40). ¹H NMR (400 MHz, CDCl₃) δ : 7.40 (1H, d, *J* = 10.3 Hz, H-11), 7.23 (2H, overlapped, H-8 and H-12), 6.52 (1H, s, H-4), 6.25 (1H, br-s, -NH), 5.20 and 5.12 (each 1H, d, *J* = 15.9 Hz, -CH₂OAc), 4.60 (1H, ddd, *J* = 13.1, 6.6, 6.6 Hz, H-7), 3.94, 3.90 (each 3H, s, 2-OCH₃, 3-OCH₃), 3.67 (3H,

s, 1-OCH₃), 2.56 (1H, dd, $J = 13.2, 6.6$ Hz, H-5 β), 2.44 (1H, ddd, $J = 12.7, 12.7, 6.9$ Hz, H-5 α), 2.20 (1H, m, H-6 β), 2.19 (3H, s, -CH₂OCOCH₃), 2.00 (3H, s, -NHCOCH₃), 1.77 (1H, ddd, $J = 11.6, 11.6, 6.1$ Hz, H-6 α). ¹³C NMR (125 MHz, CDCl₃) δ : 185.0 (C-9), 170.4 (-CH₂OCOCH₃), 169.4 (-NHCOCH₃), 153.8, 151.4, 150.6, 146.5, 142.8, 141.8 (C-1, C-2, C-3, C-7a, C-10, C-12a), 135.5, 134.4, 134.0, 133.1 (C-4a, C-8, C-11, C-12), 125.4 (C-12b), 107.3 (C-4), 63.8 (-CH₂OCOCH₃), 61.7, 61.4, 56.1 (1-OCH₃, 2-OCH₃, 3-OCH₃), 52.0 (C-7), 36.5 (C-6), 29.7 (C-5), 23.1 (-NHCOCH₃), 21.0 (-CH₂OCOCH₃). EI-MS m/z (%): 441 (M⁺, 48), 413 (33), 354 (39), 311 (83), 294 (100). HR-FAB-MS (NBA/PEG) m/z : 442.1842 (MH⁺, calcd for C₂₄H₂₈NO₇ 442.1866). CD ($c = 0.25$ mmol/L, MeOH, 20 °C) $\Delta\epsilon$ (λ nm): -0.6 (399), -3.6 (340), -2.1 (296), -3.3 (253), 0 (240), +11.4 (217), +2.8 (206).

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