HETEROCYCLES, Vol. 83, No. 8, 2011, pp. 1903 - 1907. © The Japan Institute of Heterocyclic Chemistry
Received, 16th May, 2011, Accepted, 6th June, 2011, Published online, 9th June, 2011
DOI: 10.3987/COM-11-12260

METACOLCHICINE, A NEW COLCHICINOID FROM *SANDERSONIA AURANTIACA*

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

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 antimitotic compound by binding to tubulin.[1] The structure elucidation of the tubulin-colchicine complex by X-ray crystallographic study[2] 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.

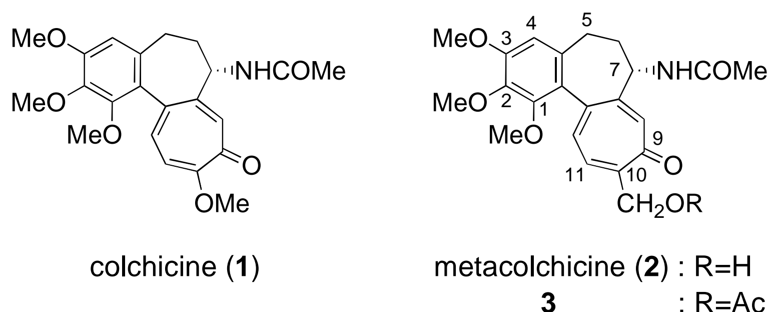

Figure 1. Structures of colchicine (**1**), metacolchicine (**2**), and **3**

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 ^{1}H NMR spectrum (Table 1) showed signals assignable to three methoxy groups on the aromatic ring at δ_H 3.94 (2-OCH_3), 3.91 (3-OCH_3), and 3.67 (1-OCH_3) 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.

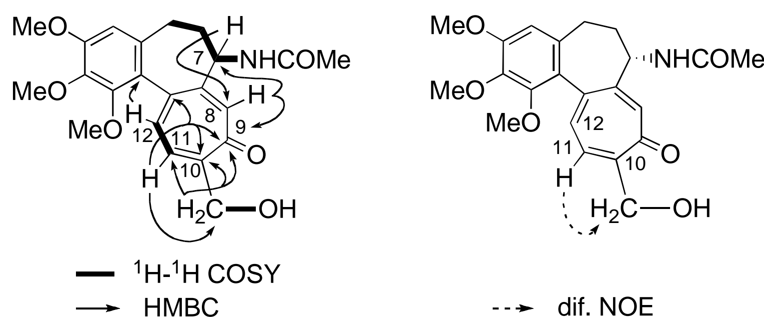

Figure 2. ^{1}H-^{1}H COSY, selected HMBC, and NOE correlations of metacolchicine (**2**)

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. ^{1}H (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
5α	2.43 (ddd, 13.0, 13.0, 6.5)	29.8
β	2.56 (dd, 13.0, 6.5)	
6α	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)	-
$N\underline{C}OCH_3$	-	169.5
$NCO\underline{C}H_3$	2.00 (3H, s)	23.0
$10\text{-}\underline{C}H_2OH$	4.72 (dd, 14.3, 5.3)	65.8
	4.60 (overlapped)	-
$10\text{-}CH_2O\underline{H}$	3.51 (br-dd, 5.3, 5.3)	-
$1\text{-}OCH_3$	3.67 (3H, s)	61.6
$2\text{-}OCH_3$	3.94 (3H, s)	61.4
$3\text{-}OCH_3$	3.91 (3H, s)	56.1

EXPERIMENTAL

General Experimental Procedures

^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM A-500 at 500 MHz (^{1}H) and 125 MHz (^{13}C) and a JEOL JNM ECP-400 at 400 MHz (^{1}H), 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/$CHCl_3$ and H_2O to afford the 10% MeOH/$CHCl_3$ extract (21.8 g). The extract was chromatographed on a silica gel column with MeOH/$CHCl_3$ gradient to give four fractions: fr. A, 0-5% MeOH/$CHCl_3$ (920 mg); fr. B, 5% MeOH/$CHCl_3$ (946 mg); fr. C, 5-30% MeOH/$CHCl_3$ (16.65 g); and fr. D, 30% MeOH/$CHCl_3$ and 10% H_2O/MeOH (2.98 g). Fr. C was separated by amino-silica gel column chromatography (0-30% MeOH/$CHCl_3$). The fraction that was eluted with 8% MeOH/$CHCl_3$ was purified by a combination of chromatographies [silica gel flash column (10-30% MeOH/AcOEt), MPLC (5% MeOH/AcOEt, and then 3-5% MeOH/$CHCl_3$)] 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, $CHCl_3$). UV (EtOH) λ_{max} nm: (log ε): 339 (3.95), 236 (4.29), 204 (4.35). ^1H and ^{13}C 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 $C_{22}H_{26}NO_6$ 400.1760). CD (*c* = 0.25 mmol/L, MeOH, 19 °C) $\Delta\varepsilon$ (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 SiO_2 open column chromatography (20% MeOH/$CHCl_3$) to give acetate **3** (1.8 mg, y. quant.).

Acetate 3: Amorphous, $[\alpha]_D^{21}$ –95 (*c* 0.12, $CHCl_3$). UV (EtOH) λ_{max} nm: (log ε): 336 (3.77), 237 (sh, 4.20), 206 (4.40). ^1H NMR (400 MHz, $CDCl_3$) 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, -C$\underline{H_2}$OAc), 4.60 (1H, ddd, *J* = 13.1, 6.6, 6.6 Hz, H-7), 3.94, 3.90 (each 3H, s, 2-OCH_3, 3-OCH_3), 3.67 (3H,

s, 1-OCH_3), 2.56 (1H, dd, *J* = 13.2, 6.6 Hz, H-5β), 2.44 (1H, ddd, *J* = 12.7, 12.7, 6.9 Hz, H-5α), 2.20 (1H, m, H-6β), 2.19 (3H, s, -$CH_2OCOC\underline{H}_3$), 2.00 (3H, s, -$NHCOC\underline{H}_3$), 1.77 (1H, ddd, *J* = 11.6, 11.6, 6.1 Hz, H-6α). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 185.0 (C-9), 170.4 (-$CH_2O\underline{C}OCH_3$), 169.4 (-$NH\underline{C}OCH_3$), 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 (-$\underline{C}H_2OCOCH_3$), 61.7, 61.4, 56.1 (1-OCH_3, 2-OCH_3, 3-OCH_3), 52.0 (C-7), 36.5 (C-6), 29.7 (C-5), 23.1 (-$NHCO\underline{C}H_3$), 21.0 (-$CH_2OCO\underline{C}H_3$). 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 $C_{24}H_{28}NO_7$ 442.1866). CD (*c* = 0.25 mmol/L, MeOH, 20 °C) $\Delta\varepsilon$ (λ nm): –0.6 (399), –3.6 (340), –2.1 (296), –3.3 (253), 0 (240), +11.4 (217), +2.8 (206).

ACKNOWLEDGEMENTS

This work was supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science.

REFERENCES

1. T. Graening and H.-G. Schmalz, *Angew. Chem. Int. Ed.*, 2004, **43**, 3230.
2. R. B. G. Ravelli, B. Giant, P. A. Curmi, I. Jourdain, S. Lachkar, A. Sobel, and M. Knossow, *Nature*, 2004, **428**, 198.
3. M. Kitajima, A. Tanaka, N. Kogure, and H. Takayama, *Tetrahedron Lett.*, 2008, **49**, 257.
4. N. Yasobu, M. Kitajima, N. Kogure, Y. Shishido, T. Matsuzaki, M. Nagaoka, and H. Takayama, *ACS Med. Chem. Lett.*, 2011, **2**, 348.
5. J. F. Finnie and J. van Staden, *J. Plant Physiol.*, 1991, **138**, 691.
6. C. D. Hufford, C. C. Collins, and A. M. Clark, *J. Pharm. Sci.*, 1979, **68**, 1239.
7. P. Sedmera, H. Potěšilová, V. Malichová, V. Preininger, and F. Šantavý, *Heterocycles*, 1979, **12**, 337.
8. H.-G. Carpraro and A. Brossi, *Helv. Chim. Acta*, 1979, **62**, 965.
9. F. Q. Alali, T. El-Elimat, C. Li, A. Qandil, A. Alkofahi, K. Tawaha, J. P. Burgess, Y. Nakanishi, D. J. Kroll, H. A. Navarro, J. O. Falkinham, Ⅲ, M. C. Wani, and N. H. Oberlies, *J. Nat. Prod.*, 2005, **68**, 173.