Communications to the Editor

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A New Alkaloid, Croomine, from *Croomia heterosepala* Okuyama

A new main alkaloid, croomine (I), was isolated from the roots and rhizomes of *Croomia heterosepala* Okuyama (Stemonaceae). The structure was established by chemical and spectral evidences and X-ray analysis.

Keywords——alkaloid; croomine; *croomia heterosepala*; Stemonaceae; dehydrogenation; Grignard reaction; picrate; methiodide; X-ray analysis; ^{13}C-NMR

Stemonaceae has two genera, *Stemona* and *Croomia*. The structures of tuberostemonine[1] and nine other alkaloids of *Stemona* spp. were determined, but the constituents of *Croomia* spp. had not been investigated. So this work was started from the point of chemotaxonomy. In this paper, the authors wish to report the isolation and structure of a new alkaloid named as croomine (I) from *Croomia heterosepala* Okuyama (Stemonaceae).

The basic fraction of methanol extract of the roots and rhizomes was fractionated by column chromatography on silica gel. The main alkaloid fraction eluted with chloroform was submitted to preparative thin-layer chromatography on silica gel to give croomine (I) (0.2% of dry plants): bp 210—215° (2×10^{-3} mmHg); $[\alpha]_D^{18}$ +9.8 (c=0.11, $CHCl_3$); molecular formula, $C_{18}H_{27}NO_4$, was confirmed by elemental analyses and mass spectra of I, the picrate and the methiodide of I; IR (liquid film) 2940, 2860, 1765, 1460, 1195, 1170, 1005, 945, 760 cm^{-1}; UV (EtOH) transparent above 210 nm; ^{1}H-NMR ($CHCl_3$+TMS) δ: 1.23 (3H, d, J=6.5 Hz), 1.28 (3H, d, J=7.0 Hz), 1.4—2.2 (11H, m), 2.2—2.9 (5H, m), 3.10 (2H, m), 3.35 (1H, dd, J=6.5, 7.0 Hz), 3.48 (1H, t, J=6.5 Hz), 4.32 (1H, octet, J=5.0, 6.5, 10.0 Hz); ^{13}C-NMR ($CHCl_3$+TMS) δ: 14.85(q), 17.85(q), 22.07(t), 26.29(t), 26.86(t), 27.67(t), 34.81(C×2), 35.87(d), 37.41(t), 40.82(t), 48.61(t), 66.87(d), 68.82(d), 80.43(d), 89.27(s), 179.28(s, CO×2); EMM[2] m/e: 321.192(M$^+$, Calcd. for $C_{18}H_{27}NO_4$: 321.194); MS m/e (relative intensity): 321(M$^+$, 12), 223(61), 222(100), 194(42), 178(24), 124(77), 110(71), 83(35), 68(31), 55(60), 41(61).

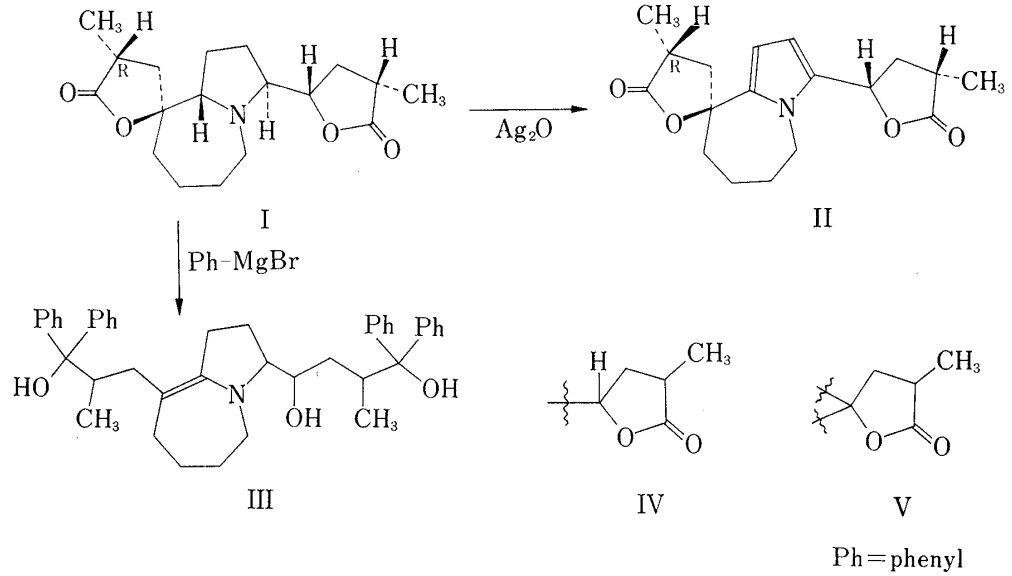

Chart 1

1) H. Harada, H. Irie, N. Masaki, K. Osaki, and S. Uyeo, *Chem. Commun.*, **1967**, 460.
2) Exact mass measurement.

The picrate of I, $C_{18}H_{27}NO_4 \cdot C_6H_3N_3O_7$; mp 125° (EtOH); IR (KBr) 1765 cm^{-1}; MS *m/e* (relative intensity): 321(M^+, 3), 229(picric acid, 16), 223(17), 222(100), 194(10), 178(5), 124(20), 110(12). The methiodide of I, $C_{18}H_{27}NO_4 \cdot CH_3I$; mp 188° (acetone); IR (KBr) 1770, 1740 cm^{-1}; MS *m/e* (relative intensity): 321(M^+, 2), 223(17), 222(100), 194(8), 182(21), 178(5), 142(CH_3I, 25), 124(19), 110(11).

Dehydrogenation of I with silver oxide gave tetradehydrocroomine (II), $C_{18}H_{23}NO_4$; EMM *m/e*: 317.162(M^+, Calcd. for $C_{18}H_{23}NO_4$: 317.163); MS *m/e* (relative intensity): 317(M^+, 29), 273(35), 218(92), 174(100), 148(34); ^{1}H-NMR($CHCl_3$+TMS) δ: 1.30(3H, d, J=6.5 Hz), 1.34(3H, d, J=7.0 Hz), 4.18(2H, m), 5.37(1H, dd, J=5.0, 10.0 Hz), 6.03(2H, m). II is positive for Ehrlich's pyrrole test. Grignard reaction of I with phenylmagnesium bromide gave tetraphenyl derivative (III), $C_{42}H_{49}NO_3$; mp 168° (benzene–hexane); IR (KBr) 3270, 2930, 1598, 1445 cm^{-1}; MS *m/e* (relative intensity): 615(M^+, 0.3), 361(18), 360(60), 342(14), 167(19), 166(100), 124(21), 105(13), 91(12), 84(11), 55(11); ^{1}H-NMR ($CHCl_3$+TMS) δ: 0.88 (3H, d, J=7.0 Hz), 1.05 (3H, d, J=7.0 Hz), 7.08—7.56 (20H, m).

The spectral data of I suggest that I is tertiary amine, and that I has two methynes (δ 3.35, 3.48; 66.87, 68.82) and one methylene (δ 3.10; 48.61) at the neighbor of the nitrogen. Pyrrole derivative II has an N-methylene-α,α'-substituted pyrrole structure in it, because the protons δ 6.03 are assigned for β-protons of pyrrole ring, and the protons δ 4.18 are assigned for the neighboring methylene of the nitrogen. So I must have N-methylene-α,α'-substituted pyrrolidine for partial structure. Comparison of the spectral data of I and III suggests that I has two α-methyl-γ-lactones. One of γ-lactone (IV) has one proton (δ 4.32) at the γ-position carbon (δ 80.43) which is jointing at the neighboring methyne (δ 3.35) of the nitrogen, and another γ-lactone (V) has no proton at the carbon (δ 89.27) of the γ-position. Degree of unsaturation of I is six. I has two α-methyl-γ-lactones and one N-methylene-α,α'-substituted pyrrolidine, but no C=C double bond. These components consume five degrees of unsaturation. So seven membered ring system must be built of these components and three remained methylenes, including spiro structure for I. The split pattern of the methyne proton (δ 3.48) of I suggests that γ-carbon of V is jointing to this methyne.

Thus these results led to the planar structure of I for croomine. The absolute configuration of I was determined by X-ray analysis. The lattice constants and intensity data were measured on a Philips four-circle diffractometer using Cu$K\alpha$ radiation monochromated by a graphite plate. Crystal data are: croomine methiodide, $C_{18}H_{27}NO_4 \cdot CH_3I$, mol. wt.= 463.36. Orthorhombic, space group $P2_12_12_1$, Z=4. a=16.911(12), b=15.097(10), c=8.587(6) Å. 1818 *hkl* reflexions were used for the structure determination. The crystal structure was solved by the heavy atom method and refined by the method of block-diagonal least-squares. The final R value was 0.08 allowing for the anomalous dispersion correction for iodine atoms which was calculated on the basis of absolute configuration determined by comparison of diffraction intensities between the Friedel pairs.

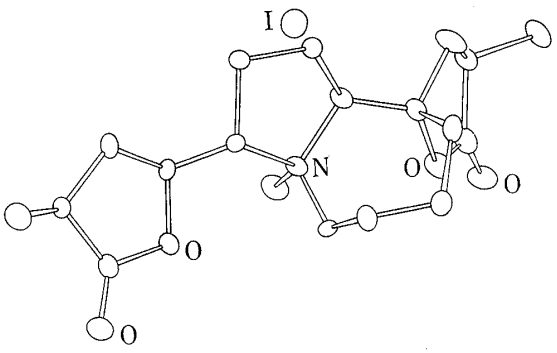

Fig. 1. A Stereoscopic View of Croomine Methiodide

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Chemical Modification of Lactose. XIII.[1] Synthesis of Lacto-N-tetraose

The protected tetrasaccharide (**6**) was synthesized in 77% yield by condensation of 1,6-anhydro-2,2′,3,4′,6′-penta-O-benzyl-β-lactose (**4**) with the oxazoline derivative of lacto-N-biose I (**5**). The protecting groups of **6** were removed by the following series of reaction to provide lacto-N-tetraose (**10**): debenzylation, acetylation, acetolysis, and de-O-acetylation. The synthetic product (**10**) was crystallized from aqueous ethanol as white needles, mp 225—228°, $[\alpha]_D^{21}$ +27° (4 min)→+21.3° (3 hr) (c=0.45, H_2O).

The homogeneity and the mobility of **10** were confirmed by the gel permeation chromatography using Bio-Gel P-4 column. The specific rotation and IR spectrum of **10** were similar to those of the natural material reported by Kuhn, Gauhe, and Baer [*Chem. Ber.*, **86**, 827 (1953)].

Keywords——human milk oligosaccharide; lactosan pentabenzylether; oxazoline; lacto-N-biose I; protected tetrassacharide; debenzylation; acetolysis; de-O-acetylation; Bio-Gel P-4 gel permeation chromatography; IR

Lacto-N-tetraose was the first aminodeoxy oligosaccharide shown to occur free in nature and that was isolated from human milk in crystalline form.[2] The methods employed to establish its structure, which is O-β-D-galactopyranosyl-(1→3)-O-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose, included partial hydrolysis and methylation.[3] Successive studies on the oligosaccharides in human milk have revealed that the sugar is the core structure of the more complex oligosaccharides such as lacto-N-fucopentaose I and II, lacto-N-difucohexaose I and II, LS-tetrasaccharide a and b, and disialyllacto-N-tetraose.[4]

In this communication, we wish to report a chemical synthesis of lacto-N-tetraose from lactose.

1,6-Anhydro-4′,6′-O-benzylidene-3′-O-tosyl-β-lactose (**2**), which was isolated in 15% yield by partial tosylation of 1,6-anhydro-4′,6′-O-benzylidene-β-lactose (**1**),[5] was catalytically de-

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2) R. Kuhn, A. Gauhe, and H.H. Baer, *Chem. Ber.*, **86**, 827 (1953).
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4) V. Ginsburg (ed.), "Methods in Enzymology," Vol. 28, Academic Press, New York, San Francisco, and London, 1972, p. 262; Vol. 50, 1978, p. 216.
5) T. Takamura and S. Tejima, *Chem.Pharm. Bull.* (Tokyo), **26**, 1117 (1978).