Stephaoxocanine, a Novel Dihydroisoquinoline Alkaloid from *Stephania cepharantha*

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Stephaoxocanine (**1**), a dihydroisoquinoline alkaloid bearing an oxocane ring, was isolated from the tubers of *Stephania cepharantha* cultivated in Japan, together with five known alkaloids. The structure was established on the basis of the spectroscopic data of **1** and its dihydro derivative (**2**), and the absolute configuration was determined by the modified Mosher's method. By comparison of the CD spectral data with that of **2**, the revised absolute stereochemistry of excentricine (**3**) was proposed as **3a**.

From the tubers of *Stephania cepharantha* Hayata (Menispermaceae), several types of alkaloids (e.g., bis-benzylisoquinoline, morphinane, hasubanane, and aporphine) have been isolated.[1−4] In our investigations of the alkaloid constituents of the same plant cultivated in Japan, we have obtained a new dihydroisoquinoline alkaloid, named stephaoxocanine (**1**), bearing an oxocane ring along with the five known alkaloids, corydine,[5,6] isocorydine,[7] reticuline,[8] *N*-methylcoclaurine,[9] and protosinomenine.[10] This paper reports the structure determination and absolute stereochemistry of **1**.

The MeOH extract of the tubers of *S. cepharantha* was fractionated, and the alkaloid-containing fraction was separated by a combination of column chromatography and preparative TLC to afford stephaoxocanine (**1**). Stephaoxocanine (**1**) exhibited the molecular formula of $C_{18}H_{21}NO_4$ as elucidated by HRMS. The IR spectrum suggested the presence of hydroxy (3400 cm^{-1}) and imino (1638 cm^{-1}) groups. The ^{1}H NMR spectrum exhibited signals for two methoxy groups (δ_H 3.90, 3.80), one aromatic proton (δ_H 6.63) as a singlet, and one olefinic proton (δ_H 6.20) as a double doublet. The ^{13}C NMR spectrum showed 18 signals, including one imino quaternary carbon (δ_C 162.29), two oxygen-connected tertiary carbons (δ_C 83.56, 71.20), and two methoxy carbons (δ_C 60.35, 56.00). The ^{1}H$-^1$H and ^{1}H$-^{13}$C COSY NMR experiments established the assignment of proton-bearing carbons and suggested the presence of the partial structures $-CH_2CH_2-$ and $=CHCH_2CH-$ $(-O-)CH_2CH_2CH(-O-)-$. In addition, COLOC NMR experiments permitted the construction of two possible partial structures **A** and **B** (Figure 1) as shown by the correlations among H-15 (δ_H 4.68), C-1 (δ_C 162.29), and C-9 (δ_C 145.55).

Treatment of **1** with NaBH$_4$ reduced the imino group to give 1,2-dihydrostephaoxocanine (**2**), which showed the H-1 signal at δ_H 4.33 as a doublet (J = 6.1 Hz) coupled with the H-15 signal. Furthermore, in the ^{13}C NMR spectrum, the C-1 signal of **2** was observed at δ_C 54.58. This spectroscopic evidence confirmed that **1** possesses the partial structure **A**, revealing the presence
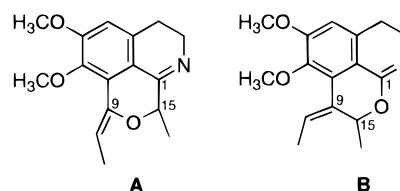
Figure 1. Possible partial structures proposed by COLOC experiments.
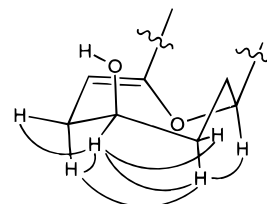
**Figure 2.** Main NOE observations for the oxocane ring of **1**.

of an eight-membered cyclic ether moiety (oxocane ring) in this molecule. Therefore, the structure of **1**, except for the stereochemistry, could be assigned as a 1,2-didehydro-14-dehydroxy derivative of excentricine (**3**), the sole tetrahydroisoquinoline alkaloid possessing an oxocane ring isolated from *Stephania excentrica* thus far.[11]

The relative stereochemistry of **1** was established by the NOESY NMR experiment, in which cross peaks were observed between the H-13 (δ_H 1.90) signal and the H-11 (δ_H 2.83) and H-15 (δ_H 4.68) signals, indicating that these protons are in the same orientation and are present in quasi-axial positions. Furthermore, the H-12 (δ_H 4.34) signal was related to both of the H-11 (δ_H 2.83, 2.38) and H-13 (δ_H 2.04, 1.90) signals, suggesting that H-12 has a quasi-equatorial orientation. This assigned stereochemistry was supported by the fact that the coupling constants of H-12 are less than 7 Hz. Therefore, as shown in Figure 2, the stereochemical relationship between H-12 and H-15 was concluded to be cis.

The absolute configuration was deduced by the modified Mosher's method.[12] Treatment of **1** with *R*-(+)- and *S*-(−)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) in the presence of dicyclohexylcarbodiimide (DCC) in CH$_2$Cl$_2$ gave *R*- and *S*-MTPA esters (**1a** and **1b**), respectively. The chemical shift difference ($\Delta\delta$: δ_S

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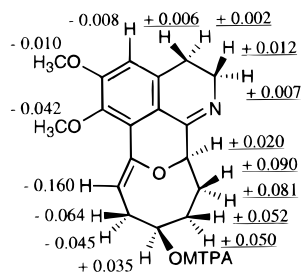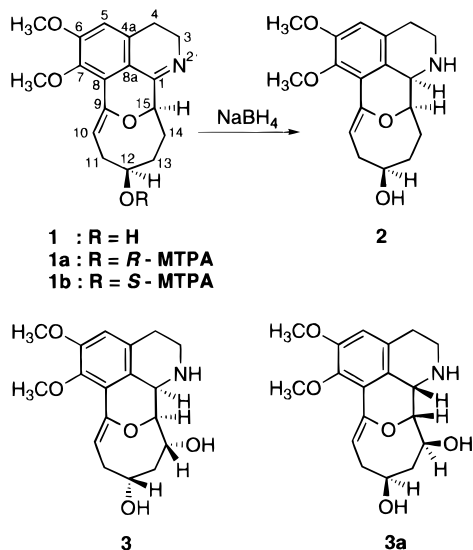
Figure 3. $\Delta\delta$ values for the (*R*)- and (*S*)-MTPA esters (**1a** and **1b**) (CDCl$_3$, 500 MHz).

$-\delta_R$) values of the individual protons of **1a** and **1b** are shown in Figure 3. The systematic arrangement of positive and negative $\Delta\delta$ values suggested that the absolute configuration of the C-12 is *R* (α-H) and, therefore, the C-15 has *R* (α-H) configuration. Thus, the structure of stephaoxocanine was established as **1**.

The absolute configuration of excentricine (**3**) was elucidated as 1*R* (α-H), 12*R* (β-H), 14*R* (β-H), and 15*S* (α-H) on the basis of the empirical helicity rule in regard to the styrene chromophore in the CD spectrum,[13,14] and the stereochemical relationship between H-1 and H-15 determined to be cis by the coupling constant of 6.2 Hz and, finally, by the reciprocal NOE observation. The H-1 and H-15 protons of 1,2-dihydrostephaoxocanine (**2**) were also deduced to have a cis relationship for the same reasons, namely, H-1 and H-15 are in an α orientation, which is the same as in **3**. However, the optical activity ($[\alpha]^{22}_D$ +261°) of **3** was opposite that of **2** ($[\alpha]^{24}_D$ −128°). Furthermore, in the CD spectrum, **3** showed positive Cotton effects at 260 ($\Delta\epsilon$ +14.4) and 306 ($\Delta\epsilon$ +0.8) nm, while **2** exhibited negative Cotton effects at 263 ($\Delta\epsilon$ −6.1) and 304 ($\Delta\epsilon$ −0.2) nm. These facts indicated that the stereochemistry of C-1 and C-15 of **3** is inverted from that of **2**. Therefore, the absolute configuration of **3** should be revised to be 1*S* (β-H), 12*S* (α-H), 14*S* (α-H), and 15*R* (β-H) as shown in **3a**.

1 : R = H
1a : R = *R* - MTPA
1b : R = *S* - MTPA

2

3　　　　**3a**

The identification of known alkaloids was accomplished by comparison of the spectroscopic data with published values.

Experimental Section

General Experimental Procedures. Melting points were measured on a Yanagimoto hot-stage melting point apparatus without correction. NMR spectra were taken on a JNM-α500 (JEOL) (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer in CDCl$_3$ with TMS as an internal standard. IR spectra were recorded on an FT/IR-5000 (JASCO) spectrometer as KBr pellets. UV spectra were measured on a Ubest-35 (JASCO) spectrometer in MeOH. MS were taken on JMS-D300 (JEOL) spectrometers at 30 eV. Optical rotations were determined on a DIP-1000 (JASCO) spectrometer in CHCl$_3$. CD spectra were measured on a J-600 (JASCO) spectrometer in MeOH. Column chromatography was performed on Wakogel C-200 (Wako Pure Chemical Industries, Ltd.). Preparative TLC was carried out on precoated Si gel 60 F$_{254}$ (0.25-mm thick) plates (Merck).

Plant Material. *Stephania cepharantha* was cultivated at Yasato-machi, Ibaraki Prefecture, Japan, and collected in October 1987.

Extraction and Isolation. Dried and cut tubers of *S. cepharantha* (37.4 kg) were extracted twice with hot MeOH. The extract was concentrated *in vacuo*, and the residue was treated with 5% HCl. After removal of insoluble materials by filtration, the filtrate was extracted with Et$_2$O. The aqueous layer was adjusted with NH$_4$OH to pH 7 and extracted with Et$_2$O to yield fraction A (270.2 g). Next, the aqueous layer was basified with NH$_4$OH to pH 10 and extracted with Et$_2$O to yield fraction B (289.4 g). Fraction A was repeatedly subjected to Si gel column chromatography, using CHCl$_3$; 2%, 4%, and 8% MeOH−CHCl$_3$; and MeOH as eluents. The material eluted with 2% MeOH−CHCl$_3$ was further chromatographed, followed by preparative TLC, to afford corydine (82 mg) and isocorydine (106 mg). The material eluted with 4% MeOH−CHCl$_3$ gave stephaoxocanine (**1**, 54 mg). Fraction B was repeatedly chromatographed on Si gel, using 2%, 4%, 6%, 8%, and 50% MeOH−CHCl$_3$ as eluents. Further chromatography of the fraction eluted with 2% MeOH−CHCl$_3$ gave reticuline (410 mg). From the fraction eluted with 8% MeOH−CHCl$_3$, *N*-methylcoclaurine (52 mg) and protosinomenine (106 mg) were obtained in the same manner.

Stephaoxocanine (1): mp 160−162 °C (colorless fine needles from Me$_2$CO); $[\alpha]^{24}_D$ +60° (*c* 0.67, CHCl$_3$); UV (MeOH) λ max (log ϵ) 255 (4.48), 286 (4.15), 330 (sh, 3.65) nm; IR (KBr) ν max 3400, 1638, 1591, 1491, 1365, 1325, 1292, 1133, 1073, 1017 cm^{-1}; CD (MeOH) $\Delta\epsilon$ +2.5 (330), +11.4 (287), +9.2 (272), −28.0 (234) nm; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 3.62 (1H, ddd, *J* = 16.1, 11.0, 7.3 Hz, H-3), 3.89 (1H, ddd, *J* = 16.1, 7.0, 7.0 Hz, H-3), 2.64 (1H, ddd, *J* = 15.9, 7.3, 7.0 Hz, H-4), 2.77 (1H, ddd, *J* = 15.9, 11.0, 7.0 Hz, H-4), 6.63 (1H, s, H-5), 6.20 (1H, dd, *J* = 7.3, 7.3 Hz, H-10), 2.38 (1H, ddd, *J* = 13.4, 7.3, 6.7 Hz, H-11), 2.83 (1H, ddd, *J* = 13.4, 7.3, 1.2 Hz, H-11), 4.34 (1H, ddd, *J* = 6.7, 5.2, 1.2 Hz, H-12), 1.90 (1H, dd, *J* = 15.0, 10.7 Hz, H-13), 2.04 (1H, ddd, *J* = 15.0, 8.9, 5.2 Hz, H-13), 1.64 (1H, ddd, *J* = 15.3, 8.9, 4.0 Hz, H-14), 2.23 (1H, ddd, *J* = 15.3, 12.2, 10.7 Hz, H-14), 4.68 (1H, dd, *J* = 12.2, 4.0 Hz, H-15), 3.90 (3H, s, OCH$_3$-6), 3.80 (3H, s, OCH$_3$-7); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 162.29 (s, C-1), 46.95 (t, C-3), 25.18 (t, C-4), 133.20 (s, C-4a), 110.45 (d, C-5), 155.14 (s, C-6), 142.89 (s, C-7), 126.26 (s, C-8), 115.94 (s, C-8a), 145.55 (s, C-9), 117.13 (d, C-10), 31.52 (t, C-11), 71.20 (d, C-12), 33.76 (t, C-13), 23.21 (t, C-14), 83.56 (d, C-15), 56.00 (q, OCH$_3$-6), 60.35 (q, OCH$_3$-7); COLOC NMR C-1 → H-3, H-15, C-4 → H-5,

C-4a → H-3, H-4, C-6 → H-5, OCH$_3$-6, C-7 → H-5, OCH$_3$-7, C-8a → H-5, C-9 → H-11, H-15, C-10 → H-11, H-12, C-13 → H-14, C-14 → H-12, C-15 → H-13, H-14; EIMS (30 eV) m/z [M]$^+$ 315 (46), 300 (24), 287 (31), 286 (42), 272 (100), 268 (17), 259 (54), 258 (41), 244 (49), 231 (27), 230 (35), 216 (18); HRMS m/z 315.1491 (C$_{18}$H$_{21}$NO$_4$ requires 315.1471).

Reduction of 1 with NaBH$_4$. A solution of **1** (20 mg) in EtOH (10 mL) was treated with NaBH$_4$ (10 mg) for 30 min at room temperature. Workup of the product was in the usual manner and was followed by preparative TLC [with C$_6$H$_6$−diethylamine (9:1)] to afford **2** (10 mg) and **1** (8 mg).

1,2-Dihydrostephaoxocanine (2): mp 199−201 °C (colorless prisms from Me$_2$CO); [α]^{25}$_D$ −128° (c 0.20, CHCl$_3$); UV (MeOH) λ max (log ϵ) 228 (4.27), 264 (3.96), 300 (3.21) nm; IR (KBr) ν max 3400, 3260, 1593, 1485, 1379, 1352, 1323, 1263, 1133, 1065, 1029 cm^{-1}; CD (MeOH) $\Delta\epsilon$ −0.2 (304), −6.1 (263), −13.6 (220) nm; ^{1}H NMR (CDCl$_3$, 500 MHz) δ 4.33 (1H, d, J = 6.1 Hz, H-1), 3.14 (1H, ddd, J = 12.8, 11.3, 6.1 Hz, H-3), 3.39 (1H, ddd, J = 12.8, 7.3, 1.8 Hz, H-3), 2.72 (1H, ddd, J = 16.8, 6.1, 1.8 Hz, H-4), 2.88 (1H, ddd, J = 16.8, 11.3, 7.3 Hz, H-4), 6.55 (1H, s, H-5), 6.23 (1H, dd, J = 7.6, 7.6 Hz, H-10), 2.30 (1H, ddd, J = 13.4, 7.6, 5.5 Hz, H-11), 2.85 (1H, ddd, J = 13.4, 7.6, 1.2 Hz, H-11), 4.26 (1H, br dd, J = 5.5, 5.2 Hz, H-12), 1.79 (1H, ddd, J = 14.9, 10.1, 1.5 Hz, H-13), 2.09 (1H, ddd, J = 14.9, 9.5, 5.2 Hz, H-13), 1.43 (1H, ddd, J = 14.7, 9.5, 3.4 Hz, H-14), 1.96 (1H, ddd, J = 14.7, 12.2, 10.1 Hz, H-14), 4.41 (1H, ddd, J = 12.2, 6.1, 3.4 Hz, H-15), 3.84 (3H, s, OCH$_3$-6), 3.80 (3H, s, OCH$_3$-7); ^{13}C NMR (CDCl$_3$, 125 MHz) δ 54.58 (d, C-1), 44.16 (t, C-3), 27.99 (t, C-4), 130.07 (s, C-4a), 111.89 (d, C-5), 151.67 (s, C-6), 142.81 (s, C-7), 123.78 (s, C-8), 124.83 (s, C-8a), 146.88 (s, C-9), 115.49 (d, C-10), 31.28 (t, C-11), 71.77 (d, C-12), 34.79 (t, C-13), 17.89 (t, C-14), 81.53 (d, C-15), 55.94 (q, OCH$_3$-6), 60.50 (q, OCH$_3$-7); EIMS (30 eV) m/z [M]$^+$ 317 (82), 302 (20), 300 (39), 288 (46), 286 (81), 274 (62), 272 (25), 260 (30), 258 (19), 244 (18), 231 (39), 230 (100), 218 (30), 216 (50); HRMS m/z 317.1619 (C$_{18}$H$_{23}$NO$_4$ requires 317.1624).

(R)- and (S)-MTPA Esters of 1. A solution of R-(+)-MTPA (15 mg), DCC (18 mg), and 4-dimethylaminopyridine (6 mg) in CH$_2$Cl$_2$ (1 mL) was added to a solution of **1** (4 mg) in CH$_2$Cl$_2$ (0.5 mL) at room temperature. After 5 h, the solution was filtered, and the filtrate was concentrated. The residue was subjected to preparative TLC [with EtOAc−diethylamine (19:1)] to yield the R-MTPA ester (**1a**) (6 mg, 88%). The S-MTPA ester (**1b**) was prepared in the same manner.

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