Synthesis of Cycleanine Mono-*N*-oxides

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Oxidation of cycleanine (**3**) with *m*-chloroperbenzoic acid gave two diastereomeric *N*-oxides (**1** and **2**), and their stereochemistry was unambiguously determined on the basis of spectroscopic evidence. The NMR spectra of synthetic cycleanine mono-*N*-oxides **1** and **2** were significantly different from those of the natural product previously reported to be cycleanine *N*-oxide.

In connection with the metabolic study of bisbenzyl-isoquinoline alkaloids, we prepared cycleanine *N*-oxides (**1** and **2**) as standard compounds. These synthetic compounds, however, gave significantly different NMR spectra from those of the natural product reported as cycleanine mono-*N*-oxide isolated from *Synclisia scabrida* by Ohiri et al.[1] and *Epinetrum villosum* by Parvez et al.[2] In this paper, we describe the preparation and structure determination of **1** and **2** and present evidence that the structure of the natural product previously reported to be cycleanine *N*-oxide may be in error.

Oxidation of cycleanine (**3**) with *m*-chloroperbenzoic acid (*m*-CPBA) gave two *N*-oxides (**1** and **2**) in yields of 8 and 9%, respectively. The separation of these compounds was readily achieved by column chromatography and preparative TLC. Reduction of **1** and **2** with sulfurous acid gave only **3**, indicating that skeletal change did not occur during the oxidation process (See Figure 1).

The molecular formula of *N*-oxide **1** was determined to be $C_{38}H_{42}N_2O_7$ by HRMS. The EIMS showed the molecular ion peak at *m/z* 638 (M⁺, 51%), base ion peak at *m/z* 622 (M⁺ −16), and strong ion peaks at *m/z* 312 (89%), 311 (36%), 204 (23%), and 190 (16%), suggesting that **1** is a compound with one oxygen atom (16 mass units) more than that of cycleanine (**3**). The ¹H-NMR spectrum (Table 1) was similar to that of **3**,[3] except that the signals for H-1, H-3, and an *N*-methyl proton were shifted downfield compared to that of **3**. The same tendency was shown in the ¹³C-NMR spectrum (Table 2), in which the signals for C-1, C-3, and an *N*-methyl carbon were observed at a lower magnetic field than that of **3**.[3] The downfield shifts of these specific protons and carbons suggested the presence of an *N*-oxide group having electron-withdrawing properties[4] and indicated that **1** is an *N*-oxide of **3**. This structure was also supported by COLOC experiments.

The stereochemistry of *N*-oxide **1** was determined as follows: in the NOESY experiments, the H-14 (δ_H 7.27) signal showed cross peaks to the H-1 (δ_H 4.73) and H-α (δ_H 4.60) signals, indicating that these protons were in close proximity as shown in Figure 2. Furthermore, in the ¹H-NMR spectrum, the H-α and H-14 signals were
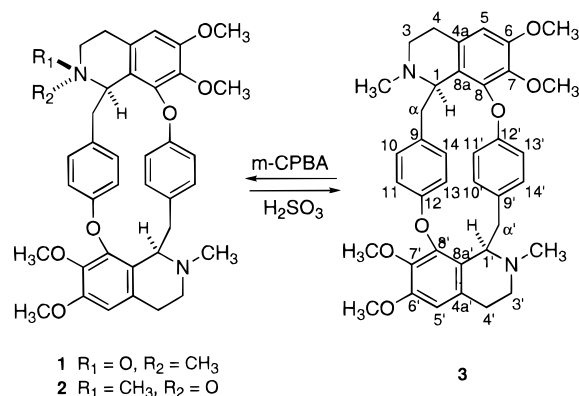

1 R₁ = O, R₂ = CH₃
2 R₁ = CH₃, R₂ = O
3

Figure 1. Structures of **1**, **2**, and **3**.

Table 1. ¹H-NMR Data for **1**, **2**, and **3**[a]

position	1	2	3
1	4.73 d (11.0)	4.99 d (10.7)	4.26 d (10.4)
3	3.64 m	3.56 m	2.90 m
	3.86 m	3.76 m	3.25 m
4	3.19 m	2.93 m	2.91 m
	3.39 m	3.58 m	3.02 m
5	6.62 s	6.63 s	6.57 s
α	2.38 dd (12.2, 11.0)	2.59 dd (13.1, 10.7)	2.52 dd (12.8, 10.4)
	4.60 d (12.2)	3.38 d (13.1)	3.22 d (12.8)
10	6.27 dd (8.5, 2.1)	6.30 dd (8.5, 2.1)	6.28 dd (8.2, 2.1)
11	5.85 dd (8.5, 2.8)	5.91 dd (8.5, 2.8)	5.83 dd (8.2, 2.8)
13	6.63 dd (8.5, 2.8)	6.65 dd (8.5, 2.1)	6.60 dd (8.5, 2.8)
14	7.27 dd (8.5, 2.1)	7.06 dd (8.5, 2.1)	7.04 dd (8.5, 2.1)
N-CH₃	3.18 s	3.67 s	2.53 s
6-OCH₃	3.85 s	3.83 s	3.81 s
7-OCH₃	3.44 s	3.37 s	3.40 s
1′	4.26 d (11.0)	4.24 d (10.7)	4.26 d (10.4)
3′	2.92 m	2.92 m	2.90 m
	3.27 m	3.25 m	3.25 m
4′	2.90 m	2.91 m	2.91 m
	3.02 m	3.03 m	3.02 m
5′	6.58 s	6.59 s	6.57 s
α′	2.53 dd (12.5, 11.0)	2.50 dd (13.1, 10.7)	2.52 dd (12.8, 10.4)
	3.23 d (12.5)	3.22 dd (13.1)	3.22 d (12.8)
10′	6.30 dd (8.5, 2.1)	6.28 dd (8.2, 2.1)	6.28 dd (8.2, 2.1)
11′	5.81 dd (8.5,2.8)	5.83 dd (8.2, 2.8)	5.83 dd (8.2, 2.8)
13′	6.60 dd (8.5, 2.8)	6.62 dd (8.2, 2.8)	6.60 dd (8.5, 2.8)
14′	7.07 dd (8.5, 2.1)	7.04 dd (8.2, 2.1)	7.04 dd (8.5, 2.1)
N-CH₃	2.51 s	2.53 s	2.53 s
6′-OCH₃	3.82 s	3.82 s	3.81 s
7′-OCH₃	3.40 s	3.40 s	3.40 s

[a] Values in parentheses are coupling constants (Hz).

shifted downfield due to the anisotropic effect of *N*-oxide oxygen compared to those (δ_H 3.22, 7.04) of **3**, suggesting that the *N*-oxide should have an equatorial orientation
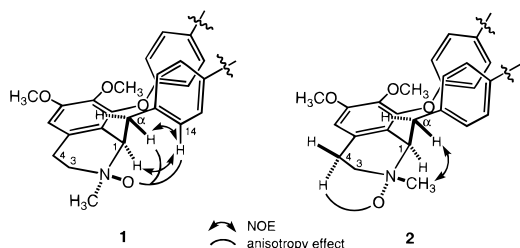
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Table 2. ^{13}C-NMR Data for **1**, **2**, and **3**

position	**1**	**2**	**3**
1	74.61	73.88	59.48
3	59.23	57.51	44.62
4	26.51	24.77	24.79
4a	125.26	126.48	129.73
5	108.70	108.41	109.18
6	153.30	152.55	151.80
7	139.96	139.13	138.87
8	143.60	144.03	143.64
8a	121.99	121.35	125.59
α	37.45	41.73	37.72
9	128.14	126.36	130.43
10	128.88	128.87	128.68
11	114.06	114.64	113.98
12	153.42	154.79	154.10
13	117.77	118.02	117.36
14	128.48	127.60	128.10
N-CH$_3$	56.51	58.45	42.37
6-OCH$_3$	56.02	55.94	55.99
7-OCH$_3$	60.26	60.09	60.01
1′	59.46	59.59	59.48
3′	44.62	44.63	44.62
4′	24.72	24.91	24.79
4a′	129.73	130.08	129.73
5′	109.24	109.38	109.18
6′	151.89	151.81	151.80
7′	138.87	138.73	138.87
8′	143.76	143.37	143.64
8a′	125.28	125.61	125.59
α′	37.95	37.56	37.72
9′	131.23	130.89	130.43
10′	128.64	128.55	128.68
11′	113.80	114.26	113.98
12′	154.43	153.38	154.10
13′	117.45	117.77	117.36
14′	128.47	128.14	128.10
N'-CH$_3$	42.36	42.41	42.37
6′-OCH$_3$	56.02	56.01	55.99
7′-OCH$_3$	60.06	60.09	60.01

Table 3. ^{1}H-NMR Data for Cycleanine *N*-Oxide Reported by Ohiri et al.[1]

position	chemical shift[a]
N-CH$_3$	3.32 s
N'-CH$_3$	2.60 s
−OCH$_3$	3.84 s
−OCH$_3$	3.84 s
−OCH$_3$	3.42 s
−OCH$_3$	3.42 s
	4.31 d (10)
	4.31 d (10)
Ar-H	5.81 dd (8.5, 2.7)
Ar-H	5.81 dd (8.5, 2.7)
Ar-H	6.27 dd (8.5, 2.0)
Ar-H	6.27 dd (8.5, 2.0)
Ar-H	6.58 dd (8.5, 2.7)
Ar-H	6.58 dd (8.5, 2.7)
Ar-H	7.10 dd (8.5, 2.0)
Ar-H	7.10 dd (8.5, 2.0)

^{a} Values in parentheses are coupling constants (Hz).

related to the H-α (δ_H 3.38) signal, and furthermore, the chemical shift (δ_H 3.67) of the *N*-methyl proton of **2** was observed at a lower magnetic field than that (δ_H 3.18) of cycleanine β-*N*-oxide (**1**), indicating that the *N*-methyl group of **2** should have an equatorial orientation. These facts suggested that the *N*-oxide has an axial orientation (α-configuration). Therefore, **2** was confirmed to be cycleanine α-mono-*N*-oxide.

Although cycleanine (**3**) possesses two basic nitrogen atoms in the molecule, only two mono-*N*-oxides are theoretically possible, inasmuch as **3** is a symmetrical molecule consisting of the same two benzylisoquinoline moieties. The ^{1}H-NMR data (Table 3) of natural cycleanine mono-*N*-oxide reported by Ohiri et al. and Parvez et al.[5] were not identical with that of either synthetic cycleanine β-*N*-oxide (**1**) or α-*N*-oxide (**2**). In addition, their reported ^{1}H-NMR data seem to be inconsistent with the structure of cycleanine *N*-oxide. The ^{1}H-NMR data suggested that the compound has a symmetrical structure such as **3**, since the signals due to methoxy and aromatic protons, except for two different *N*-methyl proton signals, were observed as two sets of signal patterns (Table 3). Probably, on the basis of this symmetrical character, Ohiri et al. assigned this compound as cycleanine mono-*N*-oxide. However, cycleanine mono-*N*-oxide is not a symmetrical molecule, and therefore, it is not necessarily required that each pair of protons have the same chemical shift. Moreover, Ohiri et al. did not describe the signal corresponding to the H-1 proton, which should shift downfield from that of the H-1′ proton due to the electron-withdrawing effect of the *N*-oxide. The two proton signals at δ_H 4.31, though they were not assigned, are not attributable to either the H-1 or H-1′ proton because it is unreasonable that these protons have the same chemical shift. Ohiri et al. reported that cycleanine *N*-oxide was prepared by oxidation of cycleanine with hydrogen peroxide, and identification of the synthetic cycleanine *N*-oxide was performed only by comparison of TLC behavior and color reaction with the natural product.[1] However, the ^{1}H-NMR data (Table 3) of the natural product, if they are correct, are not consistent with the structure of cycleanine *N*-oxide as described above.

Figure 2. Stereochemistry of *N*-oxides **1** and **2**.

or β-configuration as shown in Figure 2. Therefore, **1** was determined to be cycleanine β-mono-*N*-oxide.

The molecular formula of the other *N*-oxide (**2**) was established as C$_{38}$H$_{42}$N$_2$O$_7$ by HRMS, and the EIMS showed a similar fragmentation pattern to that of cycleanine β-*N*-oxide (**1**). The ^{1}H- and ^{13}C-NMR spectra (Tables 1 and 2) were similar to those of cycleanine (**3**), except for the signals for H-1, H-3, an *N*-methyl proton, C-1, C-3, and an *N*-methyl carbon. These data and the COLOC experiments suggested that the structure of **2** is also consistent with an mono-*N*-oxide derivative of **3**.

As to the stereochemistry of *N*-oxide **2**, the ^{1}H-NMR spectrum showed that the H-4 (δ_H 3.58) signal was shifted downfield from that (δ_H 3.02) of cycleanine (**3**), indicating that this downfield shift should be due to the anisotropic effect of the *N*-oxide oxygen. Thus, the H-4 and oxygen are in quasi 1,3-diaxial positions as shown in Figure 2. On the other hand, in the NOESY experiments, the *N*-methyl proton (δ_H 3.67) signal was cor-

Experimental Section

trometer in CHCl$_3$. IR spectra were recorded on an FT/IR-5000 (JASCO) spectrometer as KBr pellets, and data are given in cm^{-1}. UV spectra were measured on a Ubest-35 (JASCO) spectrometer in MeOH, and data are given as λ_{max} nm (log ϵ). 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, and the chemical shifts are given in δ values. MS were performed on JMS-D300 (JEOL) spectrometers at 30 eV, and data are given in *m/z* (rel int). Column chromatography was performed on Wakogel C-200 (Wako Pure Chemical Industries, Ltd.). Preparative TLC was done on precoated Si gel 60 F$_{254}$ (0.25-mm thick) plates (Merck).

Oxidation of 3 with *m*-CPBA. A mixture of **3** (500 mg) and *m*-CPBA (220 mg as 80% purity) in dry CH$_2$Cl$_2$ (20 mL) was stirred for 3 h at room temperature. The solution was extracted with CH$_2$Cl$_2$, washed with NaHCO$_3$ following H$_2$O, and dried over Na$_2$SO$_4$. After removal of the solvent *in vacuo*, the residue was chromatographed on Si gel using 5% MeOH−CHCl$_3$ as eluent to give **3** (295 mg, 59%). The material eluted with 10% MeOH−CHCl$_3$ was subjected to preparative TLC [with MeOH−CHCl$_3$ (2:3)] to yield **1** (42 mg, 8%) and **2** (48 mg, 9%).

Cycleanine β-*N*-oxide (1): amorphous powder; [α]^{24}$_D$ −17° (*c* 0.13); IR 1605, 1582, 1510, 1454, 1421, 1346, 1303, 1218, 1172, 1122; UV 283 sh (3.52), 276 (3.58); EIMS 638 (M$^+$, 52), 622 (100), 607 (29), 312 (89), 311 (36), 204 (23), 190 (16); HRMS 638.2993 (C$_{38}$H$_{42}$N$_2$O$_7$ requires 638.2992).

Cycleanine α-*N*-oxide (2): amorphous powder; [α]^{24}$_D$ −38° (*c* 0.15); IR 1607, 1584, 1510, 1454, 1421, 1352, 1299, 1220, 1172, 1118; UV 283 sh (3.48), 276 (3.53); EIMS 638 (M$^+$, 96), 622 (100), 607 (18), 312 (85), 311 (41), 204 (28), 190 (15); HRMS 638.2997 (C$_{38}$H$_{42}$N$_2$O$_7$ requires 638.2992).

Reductions of 1 and 2 with H$_2$SO$_4$. To 5% H$_2$SO$_4$ solution (20 mL), **1** (10 mg) was added and stirred for 8 h at room temperature. The solution was diluted with H$_2$O, basified with NH$_4$OH, and extracted with CHCl$_3$. The extract was washed H$_2$O and dried over Na$_2$SO$_4$. After removal of the solvent *in vacuo*, the residue was subjected to preparative TLC [with EtOAc−Et$_2$NH (9:1)] to afford **3** (7 mg, 70%), which is identical with an authentic sample of **3** by comparison of TLC behavior and IR spectrum. The reduction of **2** was performed in the same manner as that of **1**.

References and Notes

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(4) Phillipson, J. D.; Handa, S. S. *Phytochemistry* **1975**, *14*, 2683−2690.
(5) The spectroscopic data ([α], UV, and ^{1}H-NMR) reported by Parvez et al.[2] were completely in agreement with those of Ohiri et al.[1]

NP970362T