THE STRUCTURE OF NEO-ENACTIN A, A NEW ANTIFUNGAL ANTIBIOTIC POTENTIATING POLYENE ANTIFUNGAL ANTIBIOTICS

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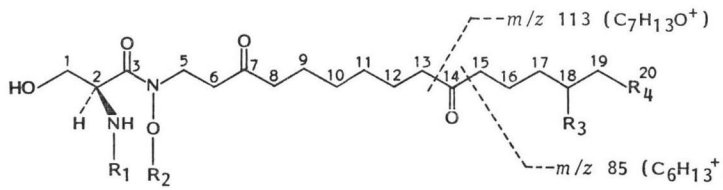
Neo-enactin (NE) produced by *Streptoverticilium olivoreticuli* subsp. *neoenacticus* is the antifungal antibiotic potentiating the activities of polyene antifungal antibiotics and antitumor agents, such as bleomycins and vincristines[1~3]. Physico-chemical and biological properties of NE are closely related to those of antibiotic H 646-SY3 (later designated as enactin)[4]. Both antibiotics contain L-serine as the common constituent and are classified into the enactin group antibiotics. Lipoxamycin[5,6] and serinomycin[7] can also be thought to belong to the same group of antibiotics. Later, NE has been revealed to be a mixture of several congeners and separated into NE-A ($C_{16}H_{36}N_2O_5$, the main component), NE-B_1 ($C_{20}H_{36}N_2O_4$) and NE-B_2 ($C_{20}H_{38}N_2O_5$) as their sulfates by HPLC[8] and all of the components are monoacidic bases. Neo-enactin A (NE-A) has the same molecular formula as that of lipoxamycin. Nevertheless, minor but significant differences in mp of the sulfates and ^{1}H NMR spectra of both antibiotics led us to study the structure of NE-A.

The present paper describes the structure elucidation of NE-A (**Ia**).

Structural Characterization

The existence of the hydroxamic acid-type structure in NE-A was shown by the positive $FeCl_3$ test (red); lipoxamycin also is positive to the $FeCl_3$ test. ^{1}H NMR (400 MHz, $CDCl_3$) of the free base of NE-A showed signals at δ 0.88 (3H, t, J=6.8 Hz, CH_2CH_3), 1.28 (10H, m, $(CH_2)_5$), 1.55 (6H, m, $CH_2\overset{|}{\underset{|}{C}}CO$), 2.38 (4H, t, J=7.2 Hz, CH_2CH_2CO) and unresolved signals at δ 2.73, 3.72 and 3.75~4.45. The signal at δ 0.88 indicated the presence of a methylene-adjacent methyl group in NE-A, while lipoxamycin (**II**) would show the signal due to the methine-adjacent geminal methyl groups. However, NE-A was gradualy decomposed to precipitate L-serine during the overnight measurement of ^{13}C NMR. Therefore, NE-A was converted to the stable 2,4-dinitrophenyl (DNP)

Fig. 1. Structures of neo-enactin A, DNP-derivatives of neo-enactin A and lipoxamycin.



	R_1	R_2	R_3	R_4
Ia	−H	−H	−H	$-CH_3$
Ib	2,4-dinitrophenyl	−H	−H	$-CH_3$
Ic	2,4-dinitrophenyl	2,4-dinitrophenyl	−H	$-CH_3$
II	−H	−H	$-CH_3$	−H

Table 1. ^{1}H NMR data (400 MHz, $CDCl_3$, 50°C) of mono- and bis-DNP derivatives of neo-enactin A.

Assignment (position)	**Ib** (δ*, ppm)	**Ic** (δ*, ppm)
—CCH$_3$ (20)	0.88 (3H, t, J=6.8 Hz)	0.87 (3H, t, J=6.8 Hz)
CH$_2$ (10, 11, 17, 18, 19)	1.26 (10H, m)	1.27 (10H, m)
CH$_2$CCO (9, 12, 16)	1.54 (6H, m)	1.55 (6H, m)
CH$_2$CO (8, 13, 15)	2.39 (4H, ddd, J=7.1, 7.8, 10.2 Hz) 2.51 (2H, ddd, J=15.8, 15.9, 20.8 Hz)	2.41 (6H, m)
HOC (1)	2.72** (1H, t)	2.56** (1H, t)
NCCH$_2$CO (6)	2.91 (2H, ddd, J=6.1, 7.9, 9.8 Hz)	2.84 (2H, t, J=5.6 Hz)
H (5)	3.87 (1H, ddd, J=10.9, 11.0, 19.8 Hz)	4.03 (1H, ddd, J=11.2, 11.4, 15.0 Hz)
NCCCO H (5)	3.99 (1H, ddd, J=11.0 Hz, partially overlapped with 4.03)	4.26 (1H, ddd, J=12.2, 13.0, 15.0 Hz)
HOC*H*$_2$C (1)	4.03 (2H, ddd, J=5.0, 5.4, 8.0 Hz)	4.09 (1H, ddd, J=11.0, 11.2, 11.8 Hz) 4.15 (1H, ddd, J=10.0, 10.2, 11.2 Hz)
NHC*H*CO (2)	5.09 (1H, ddd, J=7.6, 7.7, 7.8 Hz)	4.86 (1H, br m)
H-Ar (6′)	6.98 (1H, d, J=9.0 Hz)	6.88 (1H, d, J=9.0 Hz)
H-Ar (6″)		7.60 (1H, d, J=9.0 Hz)
H-Ar (5′)	8.25 (1H, dd, J=2.8, 9.0 Hz)	8.27 (1H, dd, J=2.8, 9.0 Hz)
H-Ar (5″)		8.52 (1H, dd, J=2.8, 9.0 Hz)
NOH (4)	8.80** (1H, br m)	
H-Ar (3′)	9.13 (1H, d, J=2.8 Hz)	9.12 (1H, d, J=2.8 Hz)
H-Ar (3″)		8.95 (1H, d, J=2.8 Hz)
—CN*H*Ar	9.28** (1H, d)	9.18** (1H, d)

* TMS (0 ppm) was used as an internal standard.

** Temperature dependent.

Number of protons, multiplicity and coupling constants are shown in parentheses.

derivatives, namely mono-DNP (**Ib**) and bis-DNP (**Ic**) NE-A. The ^{1}H NMR (400 MHz, $CDCl_3$) data of **Ib** and **Ic** are presented in Table 1 and the assignments are made on the basis of two-dimensional correlated spectroscopy (2-D COSY) and spin decoupling experiments. In case of **Ib**, the signal at δ 1.54 (6H) indicates the presence of three β-methylene groups to carbonyl groups (CH$_2$CCO) and the signals at δ 2.39 and 2.51 show the presence of three carbonyl-adjacent methylene groups (CH$_2$CO). These data account for the presence of one of the two carbonyl groups at C-7. However, the presence of two carbonyl groups are indicated by ^{13}C NMR of **Ib** as seen in Table 2. As none of protons of the three β-methylene groups is coupled with the terminal methyl protons, the second carbonyl group should be located between C-12 and C-16. On the other hand, mass spectroscopy of **Ib** shows fragments at m/z 85 ($C_6H_{13}^+$) and 113 ($C_7H_{13}O^+$) as seen in Fig. 1. Thus, the second carbonyl group proves to be located on C-14. The broad signal at δ 8.80 (–NOH) in the ^{1}H NMR of **Ib** is absent in that of **Ic**. Furthermore, **Ib** shows positive $FeCl_3$ test for the free hydroxamic acid moiety, but **Ic** fails the test. These two facts locate the position of the second DNP group in **Ic**. The ^{13}C NMR data of **Ib** and **Ic** are shown in Table 2. Assignments are based on the 2-D ^{13}C-^{1}H correlation spectrum of **Ib**. The signal at δ 156.9 (s) further supports the location of the second DNP group in **Ic**.

Table 2. ^{13}C NMR data (100 MHz) of mono- and bis-DNP derivatives of neo-enactin A.

Position	**Ib** ($CDCl_3$, δ*, ppm)	**Ic** (acetone-d_6, δ*, ppm)	Position	**Ib** ($CDCl_3$, δ*, ppm)	**Ic** (acetone-d_6, δ*, ppm)
C-1	62.9 (t)	63.1 (t)	C-18	29.2[d](t)	**
C-2	54.6 (d)	58.4 (d)	C-19	22.7[d](t)	24.1 (t)
C-3	167.0 (s)	172.4 (s)	C-20	14.3 (q)	15.3 (q)
C-5	44.7 (t)	45.8 (t)	C-1′	146.9[e](s)	148.4[e](s)
C-6	40.7 (t)	40.4 (t)	C-2′	131.1[e](s)	132.4[e](s)
C-7	213.6[a](s)	210.3[a](s)	C-3′	124.0 (d)	124.8[f](d)
C-8	43.1[b](t)	44.0[b](t)	C-4′	136.3[e](s)	137.7[e](s)
C-9	24.1[c](t)	25.4[c](t)	C-5′	130.0 (d)	131.2 (d)
C-10	29.0[d](t)	**	C-6′	114.3 (d)	116.8[g](d)
C-11	29.0[d](t)	**	C-1″		156.9 (s)
C-12	23.6[c](t)	25.2[c](t)	C-2″		138.5[e](s)
C-13	42.9[b](t)	43.8[b](t)	C-3″		123.5[f](d)
C-14	211.4[a](s)	208.6[a](s)	C-4″		144.0[e](s)
C-15	42.6[b](t)	43.7[b](t)	C-5″		131.2 (d)
C-16	23.6[c](t)	25.0[c](t)	C-6″		117.9[g](d)
C-17	31.8[d](t)	33.3 (t)			

* TMS (0 ppm) was used as an internal standard.

** Signals overlapped with acetone-d_6 signals.

Multiplicity in the off-resonance spectrum is shown in a parenthesis.

[a~g] Values with identical superscript within a column may be interchanged.

Discussion

NE-A has been found to be a positional isomer of lipoxamycin. Both antibiotics are active against a variety of yeasts and fungi. While NE-A potentiates the action of polyene antifungal antibiotics as well as some antitumor agents, such as bleomycins and vincristines, there has been no such report on lipoxamycin.

It may be pointed out that the methylene protons on C-5 appear rather low field in the ^1H NMR of **Ib** and **Ic**, although C-5 is β-carbon with respect to the carbonyl group at C-7. This could be attributed to the attachment of C-5 to the nitrogen atom of the hydroxamic acid moiety. Moreover, significant differences in chemical shifts of the geminal protons on C-5 (0.12 and 0.23 ppm in **Ib** and **Ic**, respectively) might be due to steric reasons.

Experimental

All mp are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Jeol GX-400 spectrometer and mass spectra were measured with a Hitachi M80 spectrometer.

The free base of NE-A was separated from the crude mixture of neo-enactins by preparative HPLC on a YMC-Pack S-343 column (20×250 mm, Yamamura Chem. Lab. Co., Kyoto) and MeOH - 0.05 M KH_2PO_4 (17: 8, pH 2.8) was used as the mobile phase. Further purification was carried out on a NOVA PAK C_{18} cartridge (8×100 mm, Waters Assoc., Mass.) using MeOH - 0.05 M KH_2PO_4 (57: 43, pH 2.8) as the mobile phase.

Preparation of Mono- (**Ib**) and Bis- (**Ic**) DNP Derivatives of NE-A (**Ia**)

To a cooled solution of NE-A (25.0 mg/0.3 ml, 0.067 mmol) in dry MeOH was added 1% triethylamine solution (0.3 ml) in dry MeOH. To this was added 2,4-dinitrofluorobenzene (25.0 mg/1 ml, 0.134 mmol) in dry MeOH. Stirring was continued for one hour at room temp. The reaction mixture was diluted with EtOAc (12 ml), washed with brine (5 ml) and concentrated *in vacuo*. The residue was purified by preparative TLC (Kieselgel 60 F_{254} plates; 20×20 cm, 0.5 mm thickness, E. Merck, Darmstadt) using $CHCl_3$ - MeOH (28: 1) as the mobile phase. The two distinct yellow bands, visible at Rf 0.36 and 0.59, were eluted using $CHCl_3$ - MeOH (6: 1). The upper band furnished compound **Ic** (11.3 mg) while the lower band furnished compound **Ib** (3.6 mg). Compounds **Ib** and **Ic**, still contaminated with some minor components, were further purified by HPLC on a

μBondapak C_{18} column (8×100 mm) using mobile phases MeOH - H_2O (3: 1) in case of **Ib** and MeOH - H_2O (4: 1) in case of **Ic**. The flow rate was maintained at 2.0 ml/minute. The eluate was monitered by a UV detector at 254 nm. Retention times of **Ib** and **Ic** were 8.0 and 7.2 minutes, respectively. Appropriate fractions were pooled, concentrated *in vacuo* and kept in a refrigerator. Pure **Ib**, yellow needles, mp 116°C, MS (electron inpact) m/z 539 (MH^+) and pure **Ic**, yellow needles, mp 148°C, were obtained. Mass spectrometry (electron impact, chemical ionization and single ion monitoring spectra) failed to produce the molecular ion peak of compound **Ic**. However, the structure of **Ib** was assigned on the basis of its ^1H NMR and ^{13}C NMR data. Compound **Ib** was shown to be an intermediate in the formation of **Ic** from NE-A, because under the same reaction conditions **Ib** was converted to **Ic**.

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