ISOLATION, STRUCTURAL ELUCIDATION AND BIOLOGICAL PROPERTIES OF NEOENACTINS B_1, B_2, M_1 AND M_2, NEOENACTIN CONGENERS

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The structures of three neoenactin congeners, designated as neoenactins B_1, B_2 and M_2 were elucidated by ^1H and ^{13}C NMR and mass spectroscopic studies. Another minor component, neoenactin M_1, isolated from the crude mixture, was identified as lipoxamycin. All three new compounds were structurally related to neoenactin A. While B_1 was proved to be a positional isomer of B_2, M_2 was found to be a dihydro derivative of A. All the compounds were active against yeasts and fungi and potentiated the activities of polyene antifungal antibiotics such as trichomycins A and B and amphotericin B.

Neoenactin (NE) is an antibiotic complex produced by *Streptomyces olivoreticuli* subsp. *neoenacticus* and potentiates the activities of polyene antifungal antibiotics and antitumor agents such as bleomycins and vincristine[1~3]. Physico-chemical and biological properties of NE were closely related to those of antibiotic H 646-SY3, later designated as enactin (EN)[4]. Thus, EN and NE were classified

Fig. 1. HPLC profile of crude neoenactin mixture.
Column: Radial Pak Nova Pak C_{18} (8×100 mm), mobile phase: MeOH-0.05 M KH_2PO_4 (3:2, pH 2.8), flow rate: 2.0 ml/minute, detector: UV 214 nm.

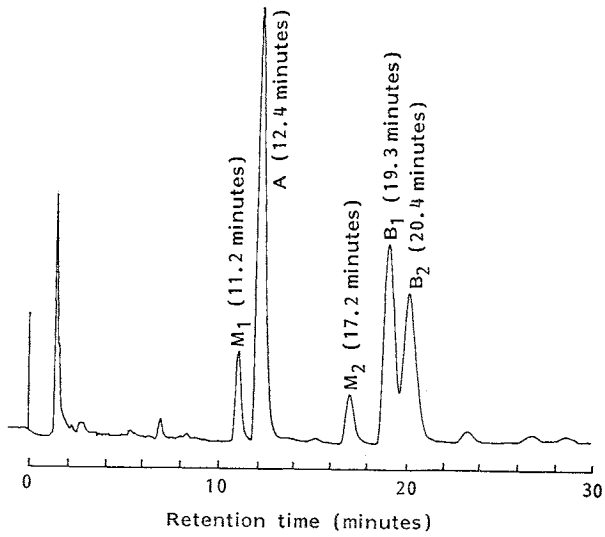
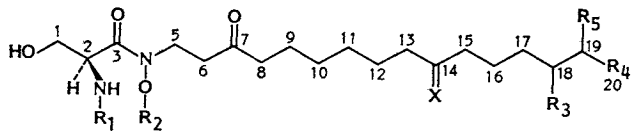
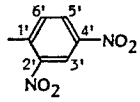
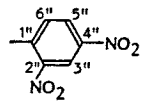


Fig. 2. Structures of neoenactin congeners and their bis-DNP-derivatives.



	R_1	R_2	R_3	R_4	R_5	X
NE-A	H	H	H	CH_3	H	=O
DNP-A	 (DNP)	 (DNP)	H	CH_3	H	=O
NE-M_1	H	H	CH_3	H	H	=O
NE-B_1	H	H	CH_3	CH_3	H	=O
DNP-B_1	DNP	DNP	CH_3	CH_3	H	=O
NE-B_2	H	H	H	CH_3	CH_3	=O
DNP-B_2	DNP	DNP	H	CH_3	CH_3	=O
NE-M_2	H	H	H	CH_3	H	
DNP-M_2	DNP	DNP	H	CH_3	H	

as the enactin group antibiotics. NISHIO *et al.*[5] revealed NE to be a mixture of several congeners and separated them into neoenactin A (NE-A), the main component, NE-B_1 and NE-B_2 as their sulfates by reverse-phase HPLC. We reported[6] the structural characterization of NE-A by ^1H and ^{13}C NMR and mass spectroscopic studies of the mono- and bis-dinitrophenyl (DNP) derivatives, whereupon NE-A was proved to be a positional isomer of lipoxamycin[7,8]. Recently, we isolated NE-B_1, NE-B_2 and two additional minor congeners, namely NE-M_1 and NE-M_2 (Fig. 1), from the crude mixture of NE by reverse-phase HPLC. However, NE-M_1 was identified as lipoxamycin. The present paper, therefore, deals with isolation, purification, physico-chemical and biological activities and structural identification of NE-B_1, NE-B_2, NE-M_1 and NE-M_2 (Fig. 2).

Isolation and Purification

The free base of each NE congener was separated from the crude mixture by preparative HPLC using 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) as the mobile phase. Further purification was achieved on a Radial Pak Nova Pak C_{18} cartridge (8×100 mm, Waters Assoc., Mass., U.S.A.) using MeOH - 0.05 M KH_2PO_4 (57:43, pH 2.8) as the mobile phase. Free base of each NE congener was recovered from the eluate by the following method. The eluate was adjusted to pH 5.6 to 6.0 by the addition of 1 N NaOH and concentrated *in vacuo* to remove MeOH. The concentrate was then adjusted to pH 8.0 and extracted three times with half-volume batches of $CHCl_3$. The aqueous layer was discarded; the extract was washed twice with half-volume batches of water, dried over anhydrous Na_2SO_4 and then evaporated *in vacuo* to furnish pure NE congener in the free base form. The NE sulfate was prepared by dissolving the free base in $CHCl_3$ (2 mg/ml), mixing with an equal volume of 0.1 N H_2SO_4, and allowing the mixture to stand at 5°C overnight. Filtration gave the crystalline NE sulfate in quantitative yield which was further purified by recrystallization from MeOH.

Physico-chemical Properties

The physico-chemical properties of NE congeners are listed in Table 1. NE-M_1 was identified with lipoxamycin by comparison studies (^{1}H NMR, admixture, Rf value on PEI-cellulose TLC and co-elution on HPLC) using authentic sample of lipoxamycin. NE-M_2 was found to be a new compound. Positive ion fast atom bombardment (FAB) and electron impact (EI) mass spectral data, *m/z* 387 $(M+1)^+$ led us to modify the molecular formula of NE-B_1 reported in a previous publication[5].

The bis-DNP-derivatives, namely DNP-B_1, DNP-B_2 and DNP-M_2 were prepared from the respective pure free bases by the method described in the previous paper[6]. Some of the physico-chemical properties of the bis-DNP-derivatives are presented in Table 2.

Biological Activity

The minimal inhibitory concentrations (MIC) were determined by the agar dilution method. The *in vitro* antimicrobial activities of all the NE congeners against a series of yeasts and fungi are

Table 1. Physico-chemical properties of neoenactin congeners.

	NE-M_1 (lipoxamycin)	NE-M_2	NE-B_1	NE-B_2
Molecular formula	$C_{19}H_{36}N_2O_5$	$C_{19}H_{38}N_2O_5$	$C_{20}H_{38}N_2O_5$	$C_{20}H_{38}N_2O_5$
MS*, *m/z* $(M+1)^+$	FD, 373	FD, 375	FAB (positive), 387	FAB (positive), 387
MP (dec) of sulfate (uncorrected, °C)	154~155	166	159	160
Rf on PEI-cellulose TLC** (of sulfate)	0.63	0.41	0.33	0.27

* Mass spectra were measured with Jeol JMS-HX110, or 01SG2 or D300 instrument.

** Pre-coated PEI-cellulose F plates: 0.1 mm thickness, E. Merck, Darmstadt; solvent: 0.1 M citrate - 0.2 M NaH_2PO_4 buffer, pH 4.0.

Table 2. Physico-chemical properties of bis-DNP-derivatives of neoenactin congeners.

	DNP-B_1	DNP-B_2	DNP-M_2
Molecular formula	$C_{32}H_{42}N_6O_{13}$	$C_{32}H_{42}N_6O_{13}$	$C_{31}H_{42}N_6O_{13}$
MP (uncorrected, °C)	158	161	172
Rf on silica gel TLC*	0.59	0.59	0.58

* Pre-coated Kieselgel $60F_{254}$ plates; 0.5 mm thickness, E. Merck, Darmstadt; solvent: $CHCl_3$ - MeOH (6:1).

Table 3. Antimicrobial spectra (MIC, μg/ml) of neoenactin sulfates.

Test organisms	NE-A	NE-B_1	NE-B_2	NE-M_1	NE-M_2
Candida tropicalis NI 7495	0.05	0.095	0.095	0.19	0.39
C. pseudotropicalis NI 7494	0.095	0.05	0.095	0.19	0.095
C. albicans Yu 1200	0.78	0.39	1.56	0.78	1.56
C. albicans MTU 12013	0.39	0.19	0.39	0.78	0.78
Saccharomyces cerevisiae	0.19	0.095	0.095	0.39	0.39
Alternaria kikuchiana	1.56	1.56	1.56	1.56	1.56
Glomerella cingulata	0.39	0.095	0.05	0.095	0.095
Gloeosporium laeticolor	0.78	0.78	0.78	0.78	1.56
Trichophyton mentagrophytes (833)	3.125	1.56	1.56	6.25	3.125
Aspergillus niger F-16	>50	>50	>50	>50	>50
Pyricularia oryzae	0.19	0.095	0.05	0.39	0.19
Helminthosporium oryzae	0.19	0.095	0.05	0.39	0.05

Table 4. ^{1}H NMR data[a] (400 MHz, $CDCl_3$, 27°C) of bis-DNP-derivatives of neoenactin congeners.

Assignment (position)	(δ* ppm)			
	DNP-A	DNP-B_1	DNP-B_2	DNP-M_2
$CHCH_3$ (21)		0.85 (3H, d, J=6.5)		
$-CCH_3$ (20)	0.87	0.85 (3H, t, J=6.5)		0.87 (3H, t, J=6.8)
$-CH(CH_3)_2$ (20, 21)			0.75 (6H, d, J=6.5)	
CH_2 (18, 19)		1.12 (2H, m, H-19)	1.16 (2H, m, H-18)	
CH_2 (10, 11, 12, 13, 15, 16, 17, 18, 19) and $-CH$ (18, 19)	1.27 (10, 11, 17, 18, 19)	1.28 (7H, m, H-10, 11, 17, 18)	1.27 (7H, m, H-10, 11, 17, 19)	1.21~1.46 (18H, m, H-10, 11, 12, 13, 15, 16, 17, 18, 19)
CH_2CCO (9, 12, 16)	1.55	1.54 (6H, m)	1.54 (6H, m)	1.54 (2H, m, H-9)
CH_2CO (8, 13, 15)	2.41	2.40 (6H, m)	2.40 (6H, m)	2.42 (2H, t, J=7.5, H-8)
$NCCH_2CO$ (6)	2.84	2.84 (2H, t, J=6.0)	2.84 (2H, t, J=6.0)	2.83 (2H, t, J=6.0)
$CHOH$ (14)				3.58 (1H, m)
NCH_2CCO (5)	4.03, 4.26	4.02 (1H, ddd, J=12.0, 12.0, 15.0), 4.26 (1H, ddd, J=12.0, 12.0, 15.0)	4.01 (1H, ddd, J=12.0, 12.0, 15.0), 4.28 (1H, ddd, J=12.0, 12.0, 15.0)	4.00 (1H, ddd, J=12.0, 12.0, 15.0), 4.27 (1H, ddd, J=12.0, 12.0, 15.0)
$HOCH_2$ (1)	4.09, 4.15	4.09 (1H, ddd, J=8.6, 9.1, 11.5), 4.16 (1H, ddd, J=8.6, 9.2, 11.5)	4.09 (1H, ddd, J=9.8, 9.8, 15.5), 4.18 (1H, ddd, J=10.8, 10.0, 15.5)	4.07 (1H, ddd, J=5.2, 10.5, 11.5), 4.14 (1H, ddd, J=5.0, 10.5, 11.5)
$NHCHCO$ (2)	4.86	4.85 (1H, br m)	4.86 (1H, br m)	4.84 (1H, br m)
H-Ar (6′)	6.88	6.85 (1H, d, J=9.0)	6.88 (1H, d, J=9.0)	6.87 (1H, d, J=9.0)
H-Ar (6″)	7.60	7.57 (1H, d, J=9.0)	7.61 (1H, d, J=9.0)	7.59 (1H, d, J=9.0)
H-Ar (5′)	8.27	8.25 (1H, dd, J=2.8, 9.0)	8.26 (1H, dd, J=2.8, 9.0)	8.26 (1H, dd, J=2.8, 9.0)
H-Ar (5″)	8.52	8.50 (1H, dd, J=2.8, 9.0)	8.52 (1H, dd, J=2.8, 9.0)	8.51 (1H, dd, J=2.8, 9.0)
H-Ar (3′)	9.12	9.12 (1H, d, J=2.8)	9.11 (1H, d, J=2.8)	9.11 (1H, d, J=2.8)
H-Ar (3″)	8.95	8.95 (1H, d, J=2.8)	8.95 (1H, d, J=2.8)	8.94 (1H, d, J=2.8)
$-CNHAr$	9.16**	9.16** (1H, d)	9.18** (1H, d)	9.15** (1H, d)

[a] ^{1}H NMR spectra were recorded on Jeol GX 400 spectrometer. Assignments are based on two-dimensional correlated spectra and spin decoupling experiments.

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

** Temperature dependent.

Number of protons, multiplicity, coupling constants in Hz and position of protons where necessary are indicated in parenthesis.

listed in Table 3. All of them are almost equally active against a particular microorganism as compared to NE-A. All the NE congeners potentiated the activity of polyene antifungal antibiotics such as trichomycins A and B and amphotericin B when tested by the paper strip cross method[5,9] using *Candida albicans* Yu 1200 as the test microorganism on glucose - nutrient agar.

Structural Elucidation

As in the case of NE-A[5], the unstable free bases of NE-B_1, NE-B_2 and NE-M_2 were converted to the corresponding stable bis-DNP-derivatives. ^1H NMR data (400 MHz, $CDCl_3$) of DNP-B_1, DNP-B_2 and DNP-M_2 are listed along with those of DNP-A in Table 4. The ^{13}C NMR data of these derivatives are shown in Table 5. Structural elucidation of the NE was aided by comparing the ^1H

Table 5. ^{13}C NMR data† (100 MHz, $CDCl_3$) of bis-DNP-derivatives of neoenactin congeners.

Position	DNP-A (δ*, ppm)		DNP-B_1 (δ*, ppm)		DNP-B_2 (δ*, ppm)	DNP-M_2 (δ*, ppm)
	Exptl	Calcd	Exptl	Calcd	Exptl	Exptl
C-1	62.08 (t)		62.11 (t)		62.11 (t)	62.11 (t)
C-2	56.17 (d)		56.19 (d)		56.17 (d)	56.16 (d)
C-3	172.50 (s)		172.50 (s)		172.50 (s)	172.50 (s)
C-5	43.50 (t)		43.50 (t)		43.50 (t)	43.50 (t)
C-6	38.76 (t)		38.76 (t)		38.76 (t)	38.78 (t)
C-7	208.37 (s)		208.34 (s)		208.30 (s)	208.37 (s)
C-8	42.86[a](t)	43.7	42.86[a](t)	43.7	42.86[a](t)	42.96 (t)
C-9	23.36[b](t)	23.8	23.38[b](t)	23.8	23.36[b](t)	23.36 (t)
C-10	28.72 (t)	29.9	28.73 (t)	29.9	28.72 (t)	28.88[a](t)
C-11	28.72 (t)	29.9	28.73 (t)	29.9	28.72 (t)	29.17[a](t)
C-12	23.19[b](t)	23.8	23.20[b](t)	23.8	23.20[b](t)	25.63[b](t)
C-13	42.53[a](t)	43.7	42.53[a](t)	43.7	42.53[a](t)	37.24[c](t)
C-14	212.08 (s)		212.02 (s)		211.99 (s)	72.05 (d)
C-15	42.92 (t)	43.7	43.23 (t)	44.4	42.96 (t)	37.59[c](t)
C-16	23.84 (t)	23.8	21.47 (t)	21.0	24.14 (t)	25.26[b](t)
C-17	28.91 (t)	29.9	36.13 (t)	37.1	27.01 (t)	29.35[a](t)
C-18	31.58 (t)	31.9	34.27 (d)	34.6	38.72 (t)	31.83 (t)
C-19	22.47 (t)	22.8	29.30 (t)	29.6	27.83 (d)	22.60 (t)
C-20	13.99 (q)	13.7	11.31 (q)	10.9	22.56 (q)	14.07 (q)
C-21			19.06 (q)	19.1	22.56 (q)	
C-1′	146.63[c](s)		146.62[c](s)		146.61[c](s)	146.61[d](s)
C-2′	131.53[c](s)		131.59[c](s)		131.59[c](s)	131.60[d](s)
C-3′	124.22 (d)		124.23 (d)		124.25 (d)	124.25 (d)
C-4′	137.10[c](s)		137.15[c](s)		137.16[c](s)	137.22[d](s)
C-5′	130.48 (d)		130.48 (d)		130.49 (d)	130.49 (d)
C-6′	113.97 (d)		113.94 (d)		113.93 (d)	113.90 (d)
C-1″	154.85 (s)		154.85 (s)		154.84 (s)	154.79 (s)
C-2″	137.28[c](s)		137.29[c](s)		137.31[c](s)	137.22[d](s)
C-3″	122.81 (d)		122.81 (d)		122.83 (d)	122.83 (d)
C-4″	143.01[c](s)		143.03[c](s)		143.03[c](s)	143.04[d](s)
C-5″	129.92 (d)		129.89 (d)		129.89 (d)	128.88 (d)
C-6″	115.43 (d)		115.40 (d)		115.37 (d)	115.33 (d)

† Spectra were recorded on Jeol GX 400 spectrometer.

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

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

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

Assignments are based on the two-dimensional (2D) ^{13}C-^1H correlation spectra and INEPT (Insensitive Nuclei Enhancement by Polarization Transfer) experiments.

and ^{13}C NMR data to the data for DNP-A.

Comparison of the molecular formula, ^{1}H and ^{13}C NMR data of DNP-B_1 with those of DNP-A revealed that NE-B_1 had similar structure to that of NE-A, consisting of the L-serine and the diketo-amine moieties connected to form the hydroxamic acid structure of NE-A, with an extra methyl substituent somewhere on the diketo-amine moiety.

The ^{1}H NMR data of DNP-B_1 suggested the presence of three β-methylene groups (δ 1.54 ppm, $-CH_2\dot{C}CO$), three α-methylene groups (δ 2.40 ppm, $-\dot{C}CH_2CO$), one non-equivalent methylene (not coupled with β-methylene protons) adjacent to methyl (δ 1.12 ppm, $-CH_2CH_3$), one methyl group adjacent to methine (δ 0.85 ppm, $-\dot{C}HCH_3$) and one methyl group adjacent to methylene (δ 0.85 ppm, $-CH_2CH_3$). Therefore, one of the two ketone groups, as observed in the ^{13}C NMR, could be placed on C-7, while the second ketonic function should be located between C-12 and C-15 and the extra methyl substituent between C-15 and C-18. Thus, ten structures were theoretically possible for NE-B_1, not taking chirality of the methine into account.

While comparing the two-dimensional (2D) ^{13}C-^{1}H correlation spectra of DNP-B_1 and DNP-A, high field shifts (γ effect of methyl) were observed for one (C-16, $\Delta\delta_c$ 2.37 ppm) of the three β-carbons (C-9, C-12 and C-16) with respect to the two ketone groups (C-7 and C-14) and for the terminal methyl group (C-20, $\Delta\delta_c$ 2.68 ppm) on going from DNP-A to DNP-B_1. However no significant change in chemical shifts was observed for α-carbons (C-8, C-13 and C-15) to the ketone groups. On the other hand, substituent effects on ^{13}C chemical shifts over four bonds were generally negligible (<1 ppm) in aliphatic systems[10]. Hence the second ketone group was located on C-14, while the extra methyl substituent was on C-18. Additional evidence for this structure of DNP-B_1 was accumulated in the low field shifts (α and β effects of methyl) of C-17 ($\Delta\delta_c$ 7.22 ppm), C-18 ($\Delta\delta_c$ 2.69 ppm), and C-19 ($\Delta\delta_c$ 6.83 ppm) on going from DNP-A to DNP-B_1. Chemical shifts of carbons of the diketone chains (C-8 through C-21, except C-14) of DNP-A and DNP-B_1 were calculated using Lindeman-Adams Rule[10,11] and compared to the experimental values as seen in Table 5. Calculated chemical shifts of C-16, C-17, C-18, C-19 and C-20 were in excellent agreement with those of experimental origin, further supporting the above structural assignment for NE-B_1 (Fig. 2).

NE-B_2 has the same molecular formula as that of NE-B_1. The six hydrogen doublet at δ 0.75 ppm in the ^{1}H NMR of DNP-B_2 advocated the presence of a gem-dimethyl group in NE-B_2, which was further supported by the presence of a two-carbon quartet at δ 22.56 ppm in the ^{13}C NMR of DNP-B_2. Otherwise, similar ^{1}H NMR spectra of DNP-A and DNP-B_2 suggested very similar sturctures for NE-A and NE-B_2. Thus, placement of the extra methyl group on C-19 of NE-A gives rise to NE-B_2. Low field shifts (α and β effects of methyl) of C-18 ($\Delta\delta_c$ 7.14 ppm), C-19 ($\Delta\delta_c$ 5.36 ppm) and C-20 ($\Delta\delta_c$ 8.57 ppm) and high field shifts (γ effect of methyl) of C-17 ($\Delta\delta_c$ 1.9 ppm) on going from DNP-A to DNP-B_2 confirmed the above assignment. However, position of the second ketone group (C-14) was not unambiguously assigned by the ^{1}H NMR data alone, which could subsequently be located between C-12 and C-14. Calculated[10~12] spectra of the diketone chains of all the three possible isomers obtained by placing the second carbonyl group on C-12, C-13 and C-14 were found to be very similar (Table 6) to the experimental one. Hence, the position of the second ketone group still remained to be determined.

Careful examination of the ^{13}C NMR data of DNP-A, DNP-B_1 and DNP-B_2 (Table 5) revealed that C-1, C-2, C-3 and C-5 through C-14 and the DNP moieties had essentially the same chemical shifts (within experimental error range) for the above three compounds. Hence, those parts must

Table 6. Comparison of the experimental ^{13}C NMR spectrum of diketo-amine moiety (C-8 through C-21, except carbonyl carbon) of bis-DNP-neoenactin B_2 with those calculated for three possible isomers.

	Chemical shifts (δ_c ppm) of C-8 through C-21 except for ketone carbonyl							
DNP-B_2	42.86	23.36	28.72	24.14	27.01	38.72	27.83	22.56
(Exptl)	42.53	23.2	28.72					22.56
	42.96							
12 >C=0	43.1	23.9	29.7	23.7	27.4	39.2	28.0	22.3
(Calcd)	43.1	23.9	30.0					22.3
	43.6							
13 >C=0	43.7	23.6	30.6	23.8	27.1	39.3	28.2	22.4
(Calcd)	43.7	23.6	30.4					22.4
	43.8							
14 >C=0	43.7	23.7	29.7	24.0	27.7	38.9	28.1	22.4
(Calcd)	43.7	23.7	29.7					22.4
	43.6							

Table 7. Comparison of the experimental ^{13}C NMR spectrum of the keto-hydroxy moiety (C-8 through C-20) of bis-DNP-neoenactin M_2 with those calculated for four possible isomers.

	Chemical shifts (δ_c ppm) of C-8 through C-20											
DNP-M_2	43.0	23.4	25.6	37.6	72.1	37.2	25.3	29.3	28.9	31.8	22.6	14.1
(Exptl)								29.2				
12-OH	44.2	24.0	25.4	37.8	71.1	38.1	25.1	30.0	29.8	32.4	23.0	14.2
(Calcd)								30.0				
13-OH	44.2	24.0	25.1	38.1	71.1	38.1	24.8	30.4	29.8	32.4	23.0	14.2
(Calcd)								30.1				
14-OH	44.2	24.0	25.1	38.1	71.1	38.1	25.1	30.4	29.8	32.4	23.0	14.2
(Calcd)								29.8				
15-OH	44.2	24.0	25.1	38.1	71.1	38.1	24.8	30.4	29.8	32.4	23.0	14.2
(Calcd)								30.1				

have the common structure in all of them. As DNP-A and DNP-B_1 have already been proved to contain the ketone group on C-14, therefore, DNP-B_2 was also suggested to contain the second ketone group at the same position (C-14).

The molecular formula of NE-M_2 was determined from its field desorption mass spectrum (FD-MS), m/z 375 $(M+1)^+$ and high resolution electron impact mass spectrum (HREI-MS), m/z 356 $(M-H_2O, C_{19}H_{36}N_2O_4)^+$ analyses.

Comparison of the ^1H NMR data of DNP-M_2 with those of DNP-A showed that they had almost the same structures except for one of the two ketone groups (C-7 and C-14) of the diketo-amine moiety of NE-A being reduced to a secondary alcoholic function in NE-M_2. The signal at δ 2.83 ppm in the ^1H NMR of DNP-M_2 clearly indicated that the carbonyl at C-7 was intact. Therefore, the second ketone group (C-14) must have been reduced.

While comparing ^{13}C NMR data (Table 5) of DNP-A and DNP-M_2, we observed high field shifts of only two (C-13, $\Delta\delta_c$ 5.29 ppm and C-15, $\Delta\delta_c$ 5.33 ppm) of the three α-carbons (C-8, C-13 and C-15) and low field shifts of only two (C-12, $\Delta\delta_c$ 2.44 ppm and C-16, $\Delta\delta_c$ 1.42 ppm) of the three β-carbons (C-9, C-12 and C-16) with respect to the two ketone groups on going from the former to the latter

compound. However, no appreciable shift was observed for γ-carbons (C-10, C-11 and C-17). Consequently, the hydroxyl group could be located between C-12 and C-15.

^{13}C Chemical shifts of the keto-hydroxy chains were calculated[10,13] for the four possible isomers obtained by placing the hydroxyl function on C-12 through C-15. All four calculated spectra (Table 7) were, however, very similar to the experimental one. Position of the hydroxyl group, therefore, remained ambiguous by this method.

EI-MS of NE-M_2 depicted fragments at m/z 271 $(M-H_2O-C_6H_{13})^+$ and 253 $(M-H_2O-C_6H_{13}-H_2O)^+$, a typical fragmentation pattern for secondary alcohols and thus provided strong evidence for the location of the hydroxyl function at C-14.

Discussion

NE-A, being a positional isomer of lipoxamycin, is eluted, as expected, right behind lipoxamycin on the HPLC column (Fig. 1). Very close retention times of the positional isomers NE-B_1 and NE-B_2 and their relatively high retention times compared to those of NE-A and even NE-M_2 are also justified in view of their relative molecular weights as well as structures.

Assignment of the individual ketone carbonyls (C-7 and C-14) in the ^{13}C NMR of DNP-A, DNP-B_1 and DNP-B_2 could be made from that of DNP-M_2.

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