NEO-ENACTINS A, B_1 AND B_2, NEW ANTIFUNGAL ANTIBIOTICS POTENTIATING POLYENE ANTIFUNGAL ANTIBIOTICS

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Neo-enactin is a new antifungal antibiotic isolated from the cultured mycelia of *Streptoverticillium olivoreticuli* subsp. *neoenacticus*[1,2]. The antibiotic was thought to act on the cell membrane, because of its ability to potentiate the action of polyene antifungal antibiotics[1]. Further, neo-enactin markedly potentiated the action of the antitumor agents bleomycin and vincristine *in vitro*[3].

The free base of neo-enactin, showing a single spot on thin-layer chromatography[1], was changed to the sulfate and recrystallized from MeOH to give fine needles, mp 141~143°C. The elementary analysis calculated for $C_{19}H_{36}N_2O_5 \cdot \frac{1}{2} H_2SO_4$: C 54.14, H 8.85, N 6.65 and S 3.80%. Found: C 54.14, H 9.27, N 6.39 and S 3.96%. In addition to the molecular ion peak at *m/z* 372, another molecular ion peak at *m/z* 386 agreed with the coexistence of at least one other homologue having a molecular formula of $C_{20}H_{38}N_2O_5$. Subsequently, the existence of three homologues in crude neo-enactin was shown by HPLC, and these are named neo-enactins A, B_1 and B_2 according to their order of elution.

The isolation, physico-chemical and biological properties of neo-enactins A, B_1 and B_2 are described briefly in this paper.

The neo-enactins in the crude mycelial cake were extracted with MeOH and transferred into methylisobutyl ketone (MIBK) following the removal of MeOH *in vacuo* and adjustment of the pH to 8.0. The neo-enactins in the MIBK extract were re-extracted into the water phase after adjustment to pH 3.0 by the addition of dil. HCl. The water phase was concentrated *in vacuo* and lyophilized to give a crude powder containing

Fig. 1. HPLC profile of neo-enactins A, B_1 and B_2.

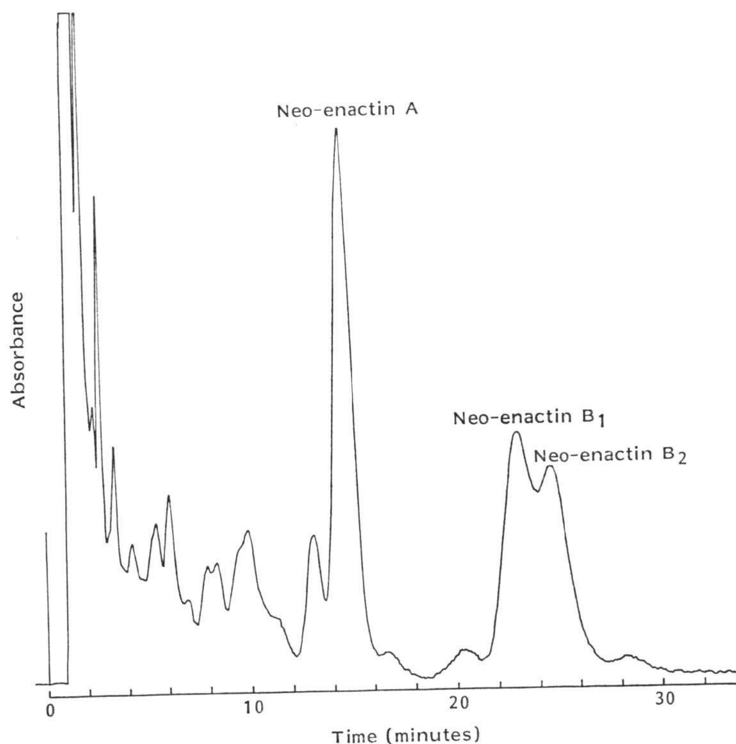
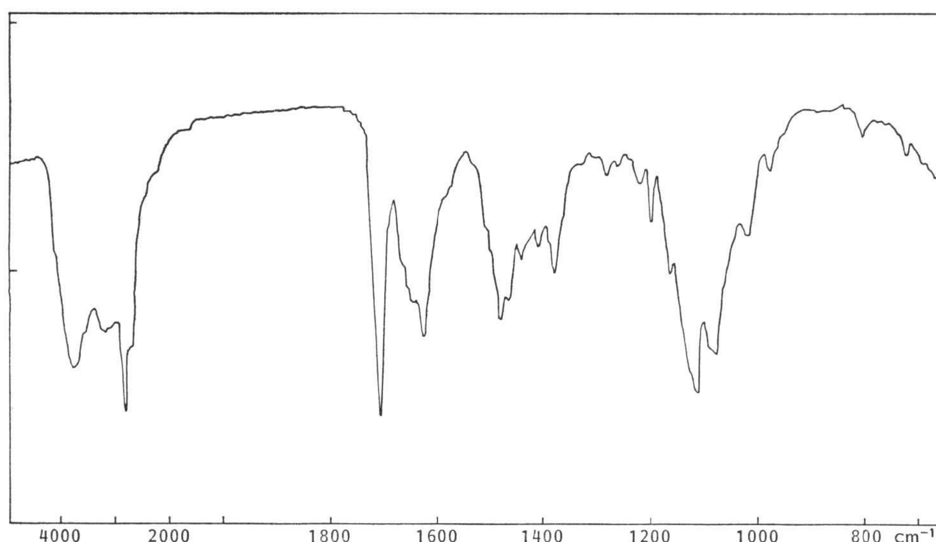


Table 1. Physico-chemical properties of neo-enactins A, B_1 and B_2.

	NE-A	NE-B_1	NE-B_2
Molecular formula	$C_{19}H_{36}N_2O_5 \cdot \frac{1}{2}H_2SO_4$ (MW 421.54)	$C_{20}H_{36}N_2O_4 \cdot \frac{1}{2}H_2SO_4$ (MW 417.55)	$C_{20}H_{38}N_2O_5 \cdot \frac{1}{2}H_2SO_4$ (MW 435.57)
m/z M^+	372($C_{19}H_{36}N_2O_5$)	368 ($C_{20}H_{36}N_2O_4$)	386 ($C_{20}H_{38}N_2O_5$)
λ_{max}^{MeOH} (ε)	211 (5,900)	211 (5,000)	211 (5,400)
$\lambda_{max}^{1N\ HCl - MeOH(1:9)}$ (ε)	211 (5,900)	211 (5,000)	211 (5,400)
$\lambda_{max}^{1N\ NaOH - MeOH(1:9)}$ (ε)	238 (5,900)	238 (5,800)	238 (6,000)
mp (dec.)	144~145°C	139.5~141°C	141~143°C

Abbreviations used are: NE-A, neo-enactin A sulfate, NE-B_1, neo-enactin B_1 sulfate, NE-B_2, neo-enactin B_2 sulfate.

Fig. 2. IR spectrum of neo-enactin A sulfate (KBr).



neo-enactin hydrochlorides.

The crude powder (3 mg) was separated by reverse phase HPLC using a Radial-PAK μBondapak C_{18} cartridge (distributed by Waters Associates, Mass.). The cartridge was eluted with MeOH - 0.05 M KH_2PO_4 (55: 45, pH was adjusted to 3.0 by the addition of H_3PO_4) at a flow-rate of 4.0 ml/minute. The eluate was monitored by a UV detector at 214 nm and the elution pattern is shown in Fig. 1. The antimicrobial activity against *Candida albicans* Yu 1200 was observed in the three peaks as seen in Fig. 1. The retention times of neo-enactins A, B_1 and B_2 were 15, 23 and 25 minutes, respectively. Each sulfate was precipitated from the MIBK extract by the direct addition of dil. H_2SO_4 and recrystallized from MeOH to give the pure sulfate of the homologue.

The physico-chemical properties of neo-enactins A, B_1 and B_2 sulfates are closely related to each other as seen in Table 1. The IR spectrum of neo-enactin A sulfate is shown in Fig. 2 and the existences of –NH and –OH groups (3400 cm^{-1}) and an amide bond (1700 and 1625 cm^{-1}) are suggested. Neo-enactin A sulfate is slightly soluble in MeOH, EtOH and H_2O but insoluble in ace-

Table 2. Rf values of neo-enactins A, B_1 and B_2 on PEI-cellulose TLC.

Solvent system	NE-A	NE-B_1	NE-B_2
0.2 M NaCl	0.52	0.28	0.19
0.1 M Citrate - 0.2 M Na_2HPO_4 buffer (pH 4.0)	0.56	0.33	0.27
0.2 M Pyridine	0.70	0.50	0.43
0.1 M AcONa - AcOH buffer (pH 4.0)	0.70	0.59	0.59

Same abbreviations are used as the Table 1.

Table 3. Antimicrobial spectra of neo-enactin A and trichomycin.

Test organisms	MIC (μg/ml)							
	NE-A		Trichomycin					
	⊖Serum	⊕Serum	⊖NE-A		⊕NE-A (0.025 μg/ml)		⊕NE-A (0.05 μg/ml)	
			⊖Chol.	⊕Chol.	⊖Chol.	⊕Chol.	⊖Chol.	⊕Chol.
Candida tropicalis NI 7495	1.56	1.56	0.20	0.39	0.05	0.10	0.05	0.10
C. pseudotropicalis NI 7494	0.20	0.20	0.20	0.20	0.05	<0.025	<0.025	<0.025
C. albicans 3147	1.56	1.56	1.56	3.13	0.78	1.56	0.39	1.56
C. albicans Yu 1200	3.13	3.13	1.56	3.13	0.78	1.56	0.39	0.78
C. albicans MTU 12013	0.78	0.39	0.05	0.20	<0.025	<0.025	<0.025	<0.025
C. krusei NI 7492	3.13	1.56	0.20	0.20	0.20	0.20	0.20	0.20
Saccharomyces cerevisiae	0.39	0.39	0.20	0.20	<0.025	<0.025	<0.025	<0.025
Alternaria kikuchiana	12.5	12.5	0.39	0.78	0.10	0.20	0.10	0.20
Glomerella cingulata	50	50	3.13	6.25	3.13	12.5	3.13	6.25
G. cingulata No. 3	0.78	1.56	0.39	0.78	0.39	0.39	0.39	0.39
Colletotrichum lindemuthianum No. 1	0.78	0.78	0.05	0.10	<0.025	<0.025	<0.025	<0.025
C. gloeosporioides Penzig	6.25	25	0.78	0.78	0.39	0.20	0.20	0.39
C. lagenarium	6.25	3.13	1.56	3.13	1.56	3.13	1.56	6.25
Gloeosporium laeticolor	50	50	0.78	6.25	1.56	6.25	0.78	3.13
Elsinoe fawcetti Bitancourt *et* Jenkins	0.20	0.39	0.20	0.10	0.05	0.05	<0.025	0.05
Trichophyton mentagrophytes (833)	50	12.5	6.25	25	6.25	12.5	3.13	12.5
T. asteroides 429	25	25	3.13	6.25	6.25	12.5	6.25	12.5
Aspergillus niger F-16	>50	>50	12.5	25	6.25	12.5	6.25	12.5
Pyricularia oryzae	0.78	0.39	0.78	0.78	0.39	0.78	0.39	0.78
Helminthosporium oryzae	6.25	1.56	0.78	0.10	0.20	0.05	0.10	0.05

Minimum inhibitory concentrations were determined on glucose nutrient agar at 27°C.
Abbreviations used are: NE-A, neo-enactin A sulfate;
Chol., cholesterol (4 μg/ml); ⊕, in the presence of; ⊖, in the absence of.

tone, MIBK, ether and EtOAc.

Each component of neo-enactins was separated from each other on PEI-cellulose thin-layer plates (distributed by E. Merck, Darmstadt) developed with several solvent systems as listed in Table 2.

The antimicrobial activity of neo-enactin A was not inactivated by the addition of 10% of calf serum, as seen in Table 3. Neo-enactin A potentiated the antimicrobial activity of trichomycin depending on the genera of microbes in the presence or absence of cholesterol as shown in Table 3. Potentiation between each of the neo-enactins and each polyene antifungal antibiotic was tested by the paper strip cross method using *C. albicans* Yu 1200 on glucose nutrient agar[4]. The neo-enactins potentiated the activity of trichomycin, amphotericin B and pimaricin but not potentiate that of nystatin and pentamycin.

References

1) KONDO, H.; H. SUMOMOGI, T. OTANI & S. NAKAMURA: Neo-enactin, a new antifungal antibiotic potentiating polyene antifungal antibiotics. I. Fermentation, extraction, purification and physico-chemical and biological properties. J. Antibiotics 32: 13~17, 1979

2) OTANI, T.; K. ISHIMARU, Y. KAWAKAMI, H. YOSHIYAMA, H. KONDO & S. NAKAMURA: Neo-enactin, a new antifungal antibiotic potentiating polyene antifungal antibiotics. II. Taxonomic studies of the producing microorganism and simultaneous production of bleomycin group and streptothricin-like antibiotics. Jpn. J. Antibiotics 32: 720~728, 1979

3) KASAI, S.; S. NAKAMURA, T. KUNIMOTO & K. NITTA: Action of a new antibiotic, neo-enactin, on the cell membrane and its application to combination treatment in a cultured tumor cell system. Gann 73: 649~655, 1982

4) FUKUDA, H.; Y. KAWAKAMI & S. NAKAMURA: A method to screen anticholesterol substances produced by microbes and a new cholesterol oxidase produced by *Streptomyces violascens*. Chem. Pharm. Bull. 21: 2057~2060, 1973