3-*EPI*-DEOXYNEGAMYCIN AND LEUCYL-3-*EPI*-DEOXYNEGAMYCIN PRODUCED BY *STREPTOMYCES*

Sir:

Two hydrazide antibiotics related to negamycin[1~3] have been isolated from the culture broth of *Streptomyces* No. MD967-A2, a strain of *S. goshikiensis*[4]. The antibiotic (I) was assayed by the cylinder plate method using both *Bacillus subtilis* PCI219 and *Pseudomonas fluorescens* as test organisms and was identified to be 3-*epi*-deoxynegamycin[5,6] which had been synthesized in our studies on negamycin analogs. Another antibiotic (II) which weakly inhibited the growth of *Pseudomonas fluorescens* was a leucyl derivative of 3-*epi*-deoxynegamycin. In this communication, we report the isolation, properties and structural elucidation of 3-*epi*-deoxynegamycin and leucyl-3-*epi*-deoxynegamycin.

The antibiotics were produced in a reciprocal shaken culture of strain No. MD967-A2 at 28°C in a medium containing 2.0% starch, 2.0% corn gluten meal, 1.0% glucose, 1.0% corn steep liquor, 0.6% $CaCO_3$, 0.3% NaCl and 0.25% NH_4Cl. After 5~9 days culture, 100~300 μg/ml of the antibiotics was present when assayed using antibiotic **I** as standard. The antibiotics in the culture filtrate (pH 8.0, 7,160 ml) were adsorbed on a column of Amberlite IRC-50 (70% NH_4^+ form) and eluted with 1 N ammonia. The active eluate was concentrated to dryness yielding a crude powder (1,073 mg). An aqueous solution of the crude powder was passed through a column of Amberlite CG-50 (Type I, NH_4^+ form) and the two antibiotics were separated by elution with water and 0.2 N ammonia. Lyophilization of the active fraction eluted with water followed by rechromatography on Amberlite CG-50 (NH_4^+ form) using water for elution gave a purified powder (23 mg) of **II**. Lyophilization of the active fraction eluted with ammonia followed by rechromatography on Amberlite CG-50 (NH_4^+ form) using 0.01 N ammonia for elution gave a purified powder (141 mg) of **I**. Further purification of **I** and **II** was accomplished by silica gel column chromatography developed with methanol - chloroform - 17% ammonia (8: 2: 1 in volume) and 1-butanol - ethanol - chloroform - 17% ammonia (4: 5: 2: 1 in volume), respectively.

The antibiotic **I** is a colorless hygroscopic powder melting at 93~105°C with decomposition. $[\alpha]_D^{24}+5°$ (*c* 2.0, water). Anal. Calcd. for $C_9H_{20}N_4O_3\cdot\frac{1}{2}H_2O$: C 44.80, H 8.77, N 23.22, O 23.21. Found: C 45.00, H 8.41, N 22.68, O 23.01. MS of the di-N-acetyl methyl ester (CI using methane): *m/e* 331 (MH^+ for $C_{14}H_{26}N_4O_5$). The antibiotic shows pKa′: 3.5, 8.4 and 10.3; UV: end absorption; IR (KBr): 3420, 3200, 3050, 2900, 1660, 1580, 1450, 1400, 1315, 1130, 1040, 960, 885, 820 and 710 cm^{-1}; PMR (D_2O, TMS as the external reference): δ 2.0 (m, $CH_2\times2$), 2.76 (m, CH_2–CO), 3.03 (s, N–CH_3), 3.41 (t, N–CH_2), 3.7 (m, N–CH) and 3.79 (s, N–CH_2–CO). The antibiotic **I** was confirmed to be identical with synthetic 3-*epi*-deoxynegamycin[5] which was prepared from L-β-lysine and 1-methylhydrazinoacetic acid in all respects including optical rotation and biological activity.

Antibiotic **II** is a colorless hygroscopic powder melting at 120~130°C with decomposition. $[\alpha]_D^{24}-4°$ (*c* 1.0, water). Anal. calcd. for $C_{15}H_{31}N_5O_4\cdot\frac{1}{2}H_2O$: C 50.83, H 9.10, N 19.76, O 20.31. Found: C 50.70, H 8.91, N 19.70, O 20.05. MS of the di-N-acetyl methyl ester (CI using methane): *m/e* 444 (MH^+ for $C_{20}H_{37}N_5O_6$). The antibiotic shows pKa′: 3.6, 7.5 and 10.0; UV: end absorption; IR (KBr): 3420, 3250, 3050, 2950, 1660, 1580, 1470, 1450, 1400, 1320, 1200, 1170, 1130, 1030, 970, 920, 890, 820 and 715 cm^{-1}; PMR (D_2O): δ 1.34 (dd, C–$(CH_3)_2$), 2.0 (m, $CH_2\times3$, CH), 2.80 (m, CH_2–CO), 3.02 (s, N–CH_3), 3.43 (t, N–CH_2), 3.78 (s, N–CH_2–CO), 3.89 (t, N–CH–CO) and 4.7 (m, N–CH).

Thin-layer chromatography of **I** and **II** using Silica gel G (Merck, Art. 5715) with 1-butanol - ethanol - chloroform -17% ammonia (4: 5: 2: 5 in volume) as a developing solvent showed Rf 0.13 and 0.38, respectively. By high-voltage paper electrophoresis with 3,500 V for 15 minutes in formic acid - acetic acid - water (1: 3: 36 in volume), **I** and **II** moved to the cathode with Rm (relative mobility to alanine) 1.58 and 1.20, respectively.

Mild hydrolysis of **I** by refluxing with 1 N HCl for 3 hours gave two ninhydrin-positive spots, Rf 0.11 (β-lysine) and 0.15 (1-methylhydrazinoacetic acid) on thin-layer chromatography using Silica gel G developed with 1-butanol - acetic acid - water (4: 1: 2 in volume). Mild hydrolysis of **II** gave four ninhydrin-positive spots, Rf 0.11, 0.15, 0.29 (leucyl-β-lysine)

Table 1. Antimicrobial spectra

Test organisms	Minimum inhibitory concentrations (μg/ml)		
	Negamycin	3-*epi*-Deoxynegamycin	Leucyl-3-*epi* deoxynegamycin
Staphylococcus aureus FDA209P	12.5	25	>100
Staphylococcus aureus Smith	3.13	6.25	>100
Sarcina lutea PCI1001	50	100	>100
Micrococcus flavus FDA16	25	>100	>100
Bacillus subtilis NRRL B-558	25	25	>100
Mycobacterium smegmatis ATCC607	50	>100	>100
Escherichia coli NIHJ	3.13	100	>100
Escherichia coli K-12	3.13	50	100
Escherichia coli K-12 ML 1629	1.56	50	100
Salmonella typhi T-63	1.56	25	50
Proteus vulgaris OX-19	1.56	12.5	25
Proteus rettgeri GN311	3.13	100	>100
Proteus rettgeri GN466	3.13	50	100
Serratia marcescens	12.5	100	>100
Klebsiella pneumoniae PCI602	3.13	>100	>100
Pseudomonas fluorescens	0.78	6.25	12.5
Pseudomonas aeruginosa A3	12.5	100	>100
Pseudomonas aeruginosa No. 12	6.25	>100	>100

Minimum inhibitory concentrations were determined on a 0.5% peptone agar by incubation at 37°C for 17 hours.

and 0.46 (leucine). Furthermore, **II** was hydrolyzed to leucine and **I** by hog kidney leucine aminopeptidase at 37°C for 24 hours.

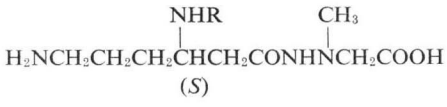
In the PMR spectrum of **II**, although the chemical shift assigned to the ϵ-methylene (δ 3.43) of the β-lysine was unchanged with that of **I** (δ 3.41), the chemical shift of the β-methine (δ 4.7) moved to the lower field than that of **I** (δ 3.7). It indicated that the β-amino group of **I** is acylated with leucine in **II**. The position of the leucyl group was thus shown to be different from that reported for leucylnegamycin[7].

The absolute structure of **II** was confirmed by the following synthesis. Antibiotic **I** was partially acylated with an equimolar amount of *tert*-butyloxycarbonyl (Boc) azide in a mixture of pyridine, water and triethylamine (10: 10: 1 in volume)[8] at room temperature for 6 hours followed by silica gel column chromatography developed with 1-butanol - ethanol - chloroform - 17% ammonia (4: 5: 2: 1 in volume) to give a colorless powder of N^{ϵ}-Boc-3-*epi*-deoxynegamycin (pKa' 3.6 and 9.1) in 70% yield. Condensation of N^{ϵ}-Boc-3-*epi*-deoxynegamycin with the N-hydroxysuccinimide ester of Boc-L-leucine in the presence of sodium bicarbonate in a mixture of dimethoxyethane and water at room temperature for 23.5 hours, followed by treatment with 90% trifluoroacetic acid at room temperature for 45 minutes and by column chromatography afforded N^{β}-L-leucyl-3-*epi*-deoxynegamycin in 54% yield. It was identical with the natural leucyl-3-*epi*-deoxynegamycin (**II**) in all respects.

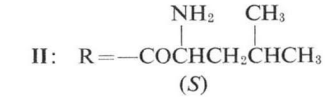
Two isomers of **II** were also synthesized. N^{β}-D-Leucyl-3-*epi*-deoxynegamycin, mp 106~118°C (dec.), $[\alpha]_D^{24}$ −23° (*c* 1.5, water), was prepared in 52% yield from N^{ϵ}-Boc-3-*epi*-deoxynegamycin and Boc-D-leucine by the method described above. N^{ϵ}-L-Leucyl-3-*epi*-deoxynegamycin, mp 123~138°C (dec.), $[\alpha]_D^{23}$ +15° (*c* 2, water), pKa' 3.5, 7.8 and 9.2, was synthesized in 55% yield from N^{β}-benzyloxycarbonyl-3-*epi*-deoxynegamycin which was prepared from N^{ϵ}-Boc-3-*epi*-deoxynegamycin. The N^{β}-D-leucyl and N^{ϵ}-L-leucyl isomers showed 7% and 21% of the activity of **II**, respectively, in the cylinder plate test using *Pseudomonas fluorescens*.

Minimum inhibitory concentrations of negamycin, **I** and **II** on a 0.5% peptone agar are shown in Table 1. Activity of **I** against most Gram-positive bacteria was about half that of negamycin, but **I** was far weaker against Gram-negative bacteria. A single intravenous injection

of 400 mg/kg of **I** or **II** into mice caused no toxicity.



I: R=H



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