RODAPLUTIN, A NEW PEPTIDYLNUCLEOSIDE FROM *NOCARDIOIDES ALBUS*

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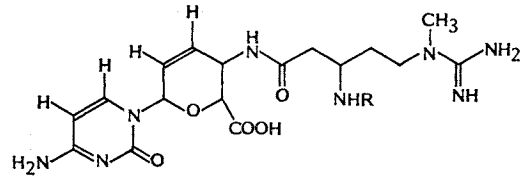
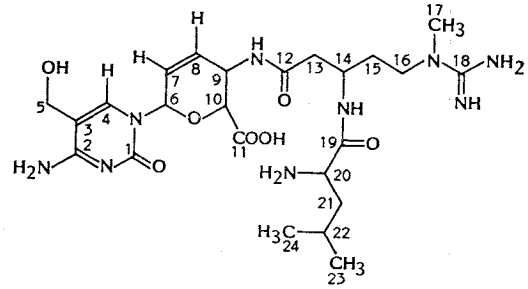
SETO *et al.*[1] reported leucylblasticidin S (**2**) to be a biosynthetic precursor in the formation of blasticidin S (**1**) by *Streptomyces griseochromogenes*. In this paper we report the isolation of **2** from *Nocardioides* strains[2]. These strains also produced the new antibiotic 5'-hydroxymethylleucylblasticidin S (**3**) which was named rodaplutin[3].



1 R=H
2 R=Leucine



3

Strains, Fermentation and Isolation

2 and **3** were produced by strains VN 14 (DSM 3177) and VA 10 (DSM 3176), characterized as *Nocardioides albus* based on their morphology, physiology, and cell wall composition[4,5]. VN 14 and VA 10 were isolated from a soil sample collected in Georgia, USSR on the south side of the Caucasus between Tiflis and Orschonikidse in the root area of hornbeams.

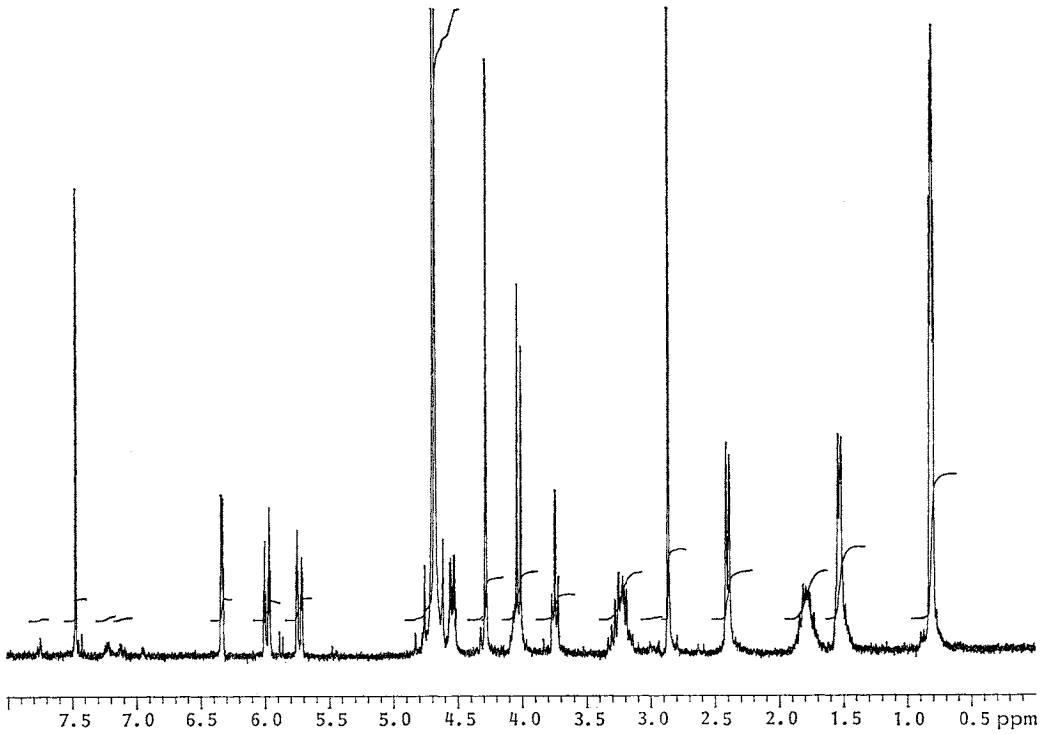
VN 14 or VA 10 were cultivated in a medium which contained in a total volume of 1 liter: Mannitol 40 g, arginine·HCl 5 g, $MgSO_4 \cdot 7H_2O$ 2 g, KH_2PO_4 0.5 g, NaCl 1 g, yeast extract (Difco) 0.5 g, 2 ml of trace element solution SL 4[6]. The medium components were dissolved in tap water, the pH value was adjusted to 7.0. The broth was heat sterilized at 121°C for 30 minutes. Cultivation was carried out in 1-liter shake flasks containing 120 ml of the medium on a rotary shaker (280 rpm) or in 200 liters fermentors (stirrer speed: 200 rpm; aeration rate: 1 v/v/m). Cultures were inoculated to 1 vol.% with a seed culture grown in the same medium for 48 hours. The fermentation temperature

Table 1. Chemical shifts of ^1H and ^{13}C NMR spectra of **3**.

Index of atoms	^1H NMR[a] (ppm)	^{13}C NMR[a] (ppm)
1	—	159.08
2	—	167.73
3	—	109.52
4	7.50	144.15
5	4.29	60.09
6	6.33	82.11
7	6.00	135.53
8	5.74	128.17
9	4.55	47.86
10	4.02	80.10
11	—	174.48
12	—	173.65
13	2.41	43.08
14	4.03	48.06
15	1.80	32.93
16	3.24	49.80
17	2.87	38.24
18	—	159.88
19	—	177.15
20	3.84	54.72
21	1.57	43.08
22	1.54	26.46
23	0.81	24.38
24	0.82	23.62

[a] Spectrometer: Bruker, AM 300; frequency: ^1H 300 MHz, ^{13}C 75.48 MHz; solvent: D_2O; internal standard: sodium salt of 3-(trimethylsilyl)propionic acid-d_4 (0 ppm).

Fig. 1. 300 MHz ^1H NMR spectrum of **3** in D_2O.



was 28°C; the broth was harvested after 144 hours when the product yield was highest.

The nucleosides **2** and **3** were separated from the culture supernatants by adsorption on a lipophilic resin (Lewatit OC 1031, Bayer AG) followed by desorption with methanol. The organic extracts were fractioned by ion exchange chromatography on CM-Sephadex (NH_4^+, Pharmacia Fine Chemicals) using a gradient from 0 to 25 mmol/liter ammonia solution to yield 1.77 g of a crude mixture of **2** and **3**, from which 120 mg of **2** and 950 mg of **3** could be isolated by chromatography on silica gel (Lobar Type C, E. Merck, FRG) using the eluent trichloromethane - methanol - 25% ammonia solution (2 : 2 : 1).

Properties and Structure of Rodaplutin

After lyophilization **3** ($C_{24}H_{39}N_9O_7$) forms a colorless amorphous powder. It is easily soluble in water and soluble in methanol and DMSO. UV spectrometry: UV $\lambda_{max}^{H_2O}$ nm ($E_{1cm}^{1\%}$) 271 (134). IR (KBr) exhibits absorptions at 3379, 2962, 1657, 1602, 1383, 1348, 1296, 1231, 1070, 829, 785 cm^{-1}.

The positive fast atom bombardment mass

Table 2. Antibiotic properties.
MIC in the agarplate assay.

Organism	MIC (μg/well)	
	Rodaplutin	Leucyl-blasti-cidin
Bacillus brevis[a]	>75	>75
B. cereus[a]	>75	>75
B. subtilis[a]	>75	>75
Escherichia coli 14[a]	70	75
E. coli A 261[a]	40	75
Micrococcus luteus[a]	75	75
Proteus vulgaris[a]	60	60
Pseudomonas aeruginosa[a]	>75	>75
Serratia marcescens[a]	>75	>75
Staphylococcus aureus 1756[a]	>100	>75
S. aureus 209P[a]	>75	>75
Pyricularia oryzae[b]	>100	>100
Aspergillus niger[b]	>100 (50[c])	>100 (25[c])
Mucor rouxii[b]	>100	>100

[a] Antibiotic medium 3 (Difco), 18 hours incubation time at 37°C.
[b] Potato - dextrose agar (Oxoid), 24 hours incubation time at 28°C.
[c] Inhibition of formation of aerial mycelium after 72 hours.

spectrum (FAB-MS) (matrix glycerol) of **3** shows a base peak at *m/z* 566. It was shifted to *m/z* 642 $(M+2K-H)^+$ by potassium cations, therefore confirming MW 565. Typical fragments appear at *m/z* 425 $(MH^+-C_5H_7N_3O_2)$ and *m/z* 301 $(M-C_{11}H_{10}N_3O_5)$.

Chemical shifts of the protons and carbon atoms in ^1H and ^{13}C NMR spectra are presented in Table 1. The structural assignment of signals were supported by spin decoupling experiments, 2D-H, H-, and 2D-H, C-COSY experiments, and by comparison with **1** and **2**. The 1D-H NMR spectrum of **3** is presented in Fig. 1.

The stereochemistry of 9-H and 10-H protons should be diaxial on the basis of a coupling constant of $J_{9,10}>10$ Hz. Acidic hydrolysis of **3** (6 N HCl, 110°C, 20 hours) gave leucine, identified by amino acid analysis. After hydrolysis in 2 N HCl (110°C, 2 hours) 2-hydroxymethylcytosine was identified by cochromatography with authentic samples.

Rodaplutin exhibits weak antimycotic and antibacterial activity (Table 2). Good insecticidal and acaricidal activities were for instance observed after treatment of food plants or diet with solutions of 0.4 mg/ml and less for *Phaedon cochlearia* (coleoptera), *Plutella maculipennis* (lepidoptera), *Dysdercus intermedius* (heteroptera), *Myzus persicae* (homoptera), and *Tetranychus urticae* (acari).

Addendum in Proof

After submission of this manuscript two reports were published that concerned Sch 36605, an antiinflammatory compound with physicochemical properties identical to those of rodaplutin[7,8].

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