METABOLIC PRODUCTS OF MICROORGANISMS. 254†

STRUCTURE OF THE NEW NIKKOMYCINS PSEUDO-Z AND PSEUDO-J

HOLGER HEITSCH and WILFRIED A. KÖNIG*

Institute of Organic Chemistry, University of Hamburg,
D-2000 Hamburg 13, FRG

HEINRICH DECKER, CHRISTIANE BORMANN, HANS-PETER FIEDLER and HANS ZÄHNER

Institute of Biology II, Department of Microbiology,
University of Tübingen,
D-7400 Tübingen, FRG

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Two new nikkomycins were isolated from the fermentation broth of *Streptomyces tendae* Tü 901/PF 53$^+$-3. These new metabolites, nikkomycins pseudo-Z (ψ-Z, **1**) and pseudo-J (ψ-J, **2**) differ from the corresponding nikkomycins Z and J by a *C*-glycosidic bond between C-5 of uracil and C-1′ of 5-amino-5-deoxy-D-*allo*-furanuronic acid instead of an *N*-glycosidic bond. The structure elucidation was achieved by two-dimensional NMR techniques and mass spectrometry.

As described before[2] the new metabolites were found to be members of the nikkomycins[3,4], a group of peptide-nucleoside antibiotics with antifungal and insecticidal properties. The biological activity of the new nikkomycins is markedly lower than that of nikkomycin Z[3] or X.

Materials and Methods

Mass Spectrometry

Mass spectra were recorded on a Hewlett-Packard instrument HP 5985 A (GC-MS), on a Finnigan MAT 311A (negative ion fast atom bombardment (FAB)) and a VG Instruments VG 70-250 S (positive ion FAB). Glycerol was applied as a matrix and xenon as collision gas.

Gas Chromatography

For GC-MS investigations a 25-m fused silica capillary column with OV 1, for determination of the configuration of amino acids a 35-m capillary with the chiral stationary phase XE-60-L-valine-(*S*)-α-phenylethylamide[5] was applied.

NMR Spectroscopy

The ^{1}H, ^{1}H-^{1}H correlation spectroscopy (COSY) and ^{1}H-^{13}C COSY NMR spectra were recorded on a Bruker WM 270 (270 MHz) and a WM 400 (400 MHz) spectrometer, using the DOH-resonance at δ 4.64 as an internal reference. ^{13}C spectra were recorded at 100.61 MHz on a Bruker WM 400 spectrometer using methanol (δ 3.30) as an internal standard.

Hydrolysis of Nikkomycins

Samples of about 1 mg were hydrolyzed in a screw cap vial in 0.5 ml of 3 N HCl for 2 hours at 100°C.

† Part 253: See ref 1.

Formation of Derivatives

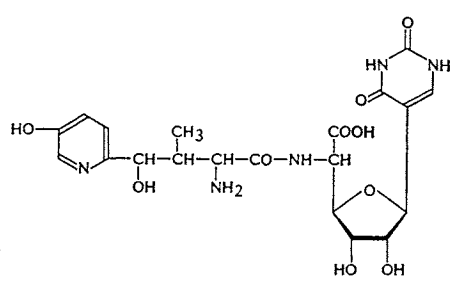
Trimethylsilyl derivatives of the hydrolysis products of nikkomycins were obtained by heating 0.5~1 mg of hydrolyzed nikkomycins with 100 μl of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide for 1 hour at 100°C in a screw cap vial. Isopropyl esters were prepared by heating the hydrolysate in 0.5 ml of 1.5 mol solutions of hydrogen chloride in 2-propanol. Trifluoroacetyl derivatives were obtained by heating the esterified samples in a mixture of 200 μl of dichloromethane and 50 μl of trifluoroacetic anhydride for 30 minutes at 100°C in a screw cap vial with Teflon lined cap.

Results and Discussion

Nikkomycin Pseudo-Z (ψ-Z)

From the very similar retention volume in HPLC a close structural relationship of nikkomycins Z (**3**) and ψ-Z (**1**) was concluded. This assumption was confirmed by FAB-MS. In the negative ion FAB spectrum a $(M-H)^-$ ion at *m/z* 494 showed the molecular mass of nikkomycin ψ-Z to be identical to that of nikkomycin Z. The absence of a fragment ion at *m/z* 111 of uracil as found in nikkomycin Z indicated a difference in the nucleoside residue of both nikkomycins. This assumption was confirmed by GC-MS investigation of the acid hydrolysate of nikkomycin ψ-Z. After trimethylsilylation only the *N*-terminal amino acid nikkomycin D (**4**)[6], however, no uracil (as in nikkomycin Z) was detected.

The identity of the *N*-terminal amino acid of both nikkomycins was confirmed by ^{1}H and ^{13}C NMR investigations. The comparison of the ^{1}H-^{1}H COSY spectra of nikkomycin Z (Fig. 1) and nikkomycin ψ-Z (Fig. 2) clearly shows the difference in the type of bond between uracil and the sugar moiety, while the chemical shifts and coupling constants of the resonances of the *N*-terminal residue are very similar. In the spectrum of nikkomycin Z the uracil protons at C-5 and C-6 at δ 5.78 and



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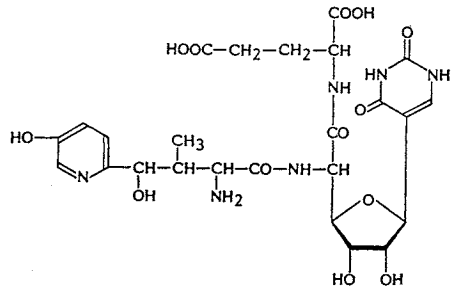

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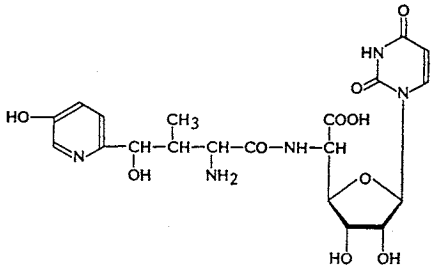

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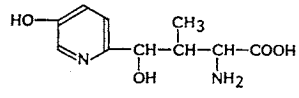

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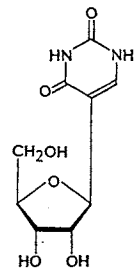

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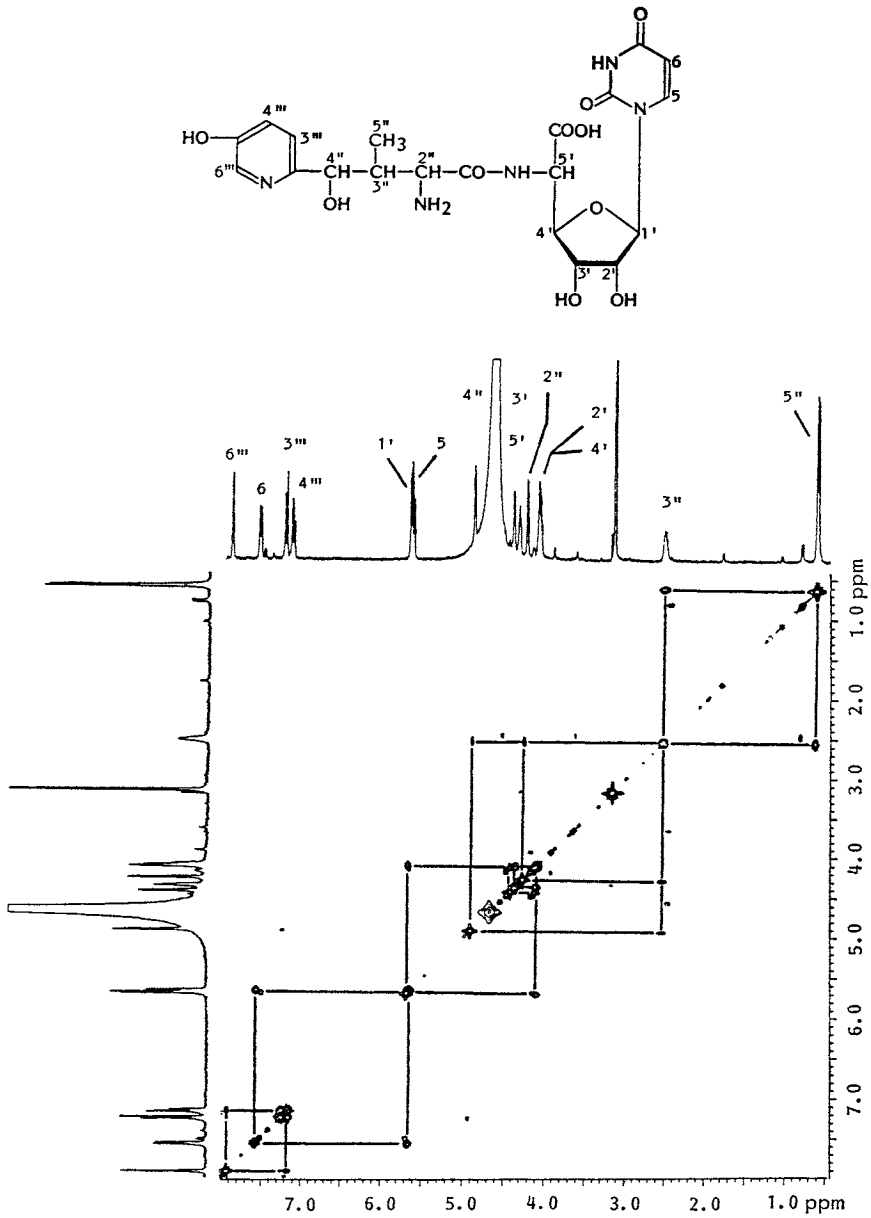
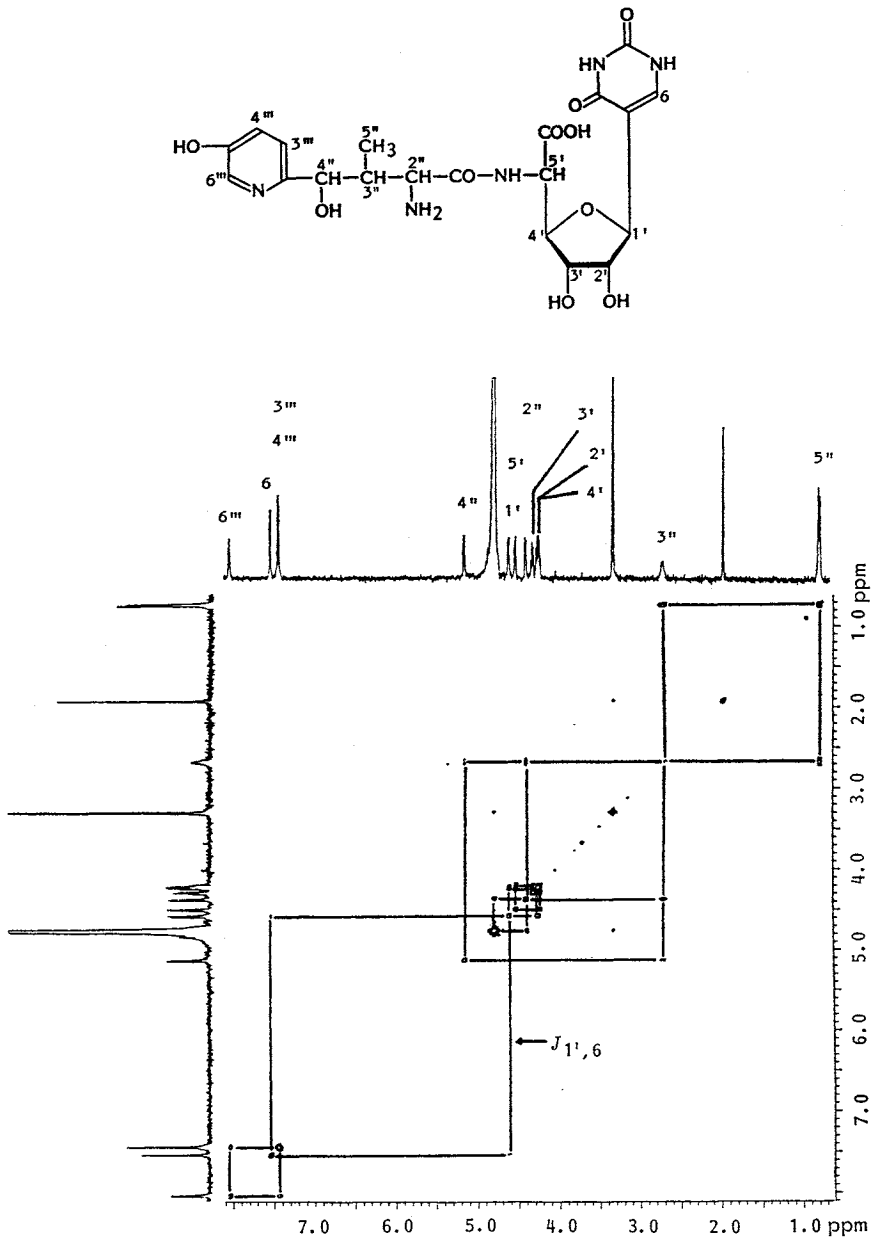
Fig. 1. ^{1}H-^{1}H COSY NMR spectrum (400 MHz, D_2O - CD_3OD) of nikkomycin Z.



7.63 appear as doublets. The spectrum of nikkomycin ϕ-Z shows a singlet of the C-6 proton at δ 7.55. The signal of the C-5 proton has disappeared and the doublet of the C-1′ proton is shifted high field from δ 5.80 (nikkomycin Z) to δ 4.58 (nikkomycin ϕ-Z). These findings are in accordance with the assumption of a *C*-glycosidic bond from C-5 of uracil to C-1′ of the sugar residue. This is further confirmed by a cross peak between the allylic protons at C-6 and C-1′ (Fig. 2).

The assumed structure of nikkomycin ϕ-Z is also corroborated by a ^{1}H-^{13}C COSY spectrum (Fig. 3). The asymmetric shape of the cross-peak between the ^{13}C signal at δ 80.18 and the 1′-H and 5′-H protons below the DOH signal indicates that both the C-1′ and C-5′ appear at the same chemical shift.

Fig. 2. ^{1}H-^{1}H COSY NMR spectrum (400 MHz, D_2O - CD_3OD) of nikkomycin ψ-Z.



The C-5 of the uracil residue absorbs at δ 111.36.

In the ^{13}C NMR spectrum of nikkomycin Z the corresponding signals appear at δ 90.09 for C-1′ and 103.50 for C-5 of uracil. Again the high-field shift of the C-1′ signal and the low-field shift of the C-5 signal indicate the *C*-glycosidic bond.

In Table 1 these data are compared with the chemical shifts of pseudouridine (**5**)[7,8]. The structural relationship is clearly expressed in almost identical chemical shifts of the compounds with a *C*-glycosidic bond.

Fig. 3. ^{1}H-^{13}C COSY NMR spectrum (400 MHz/100 MHz, D_2O - CD_3OD, 40°C) of nikkomycin ϕ-Z.

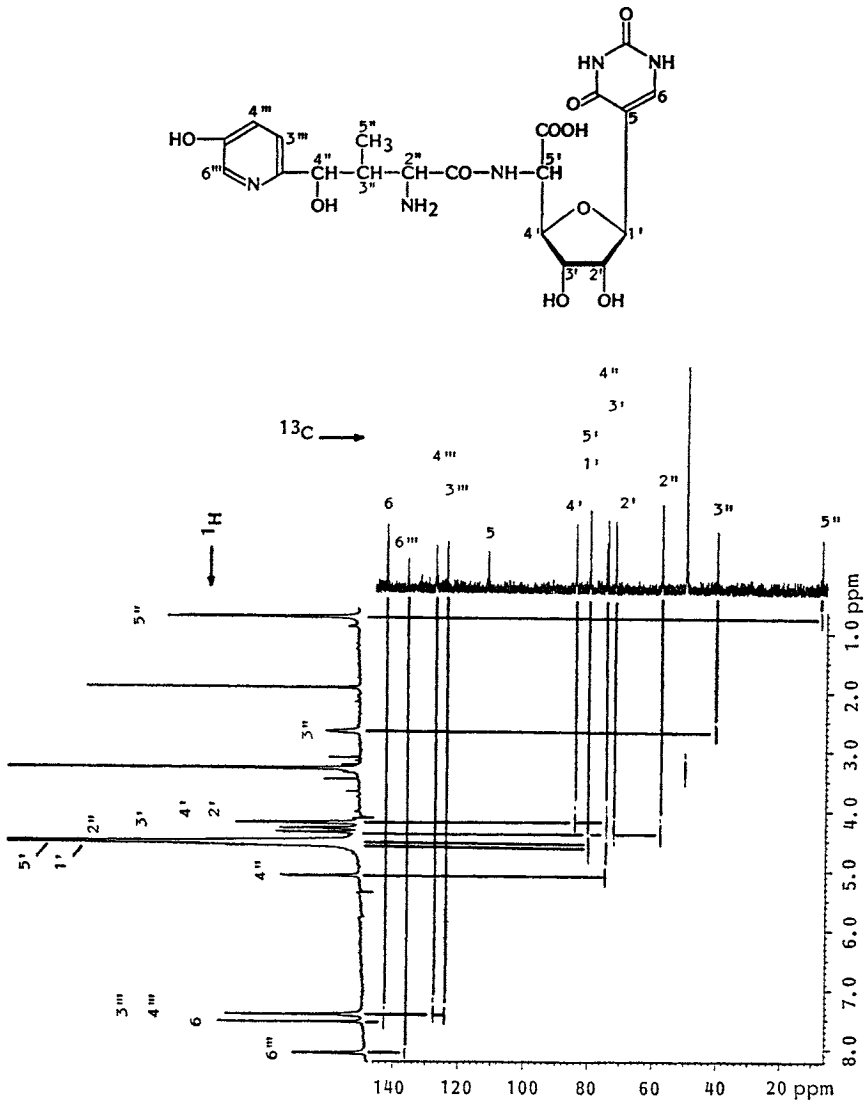
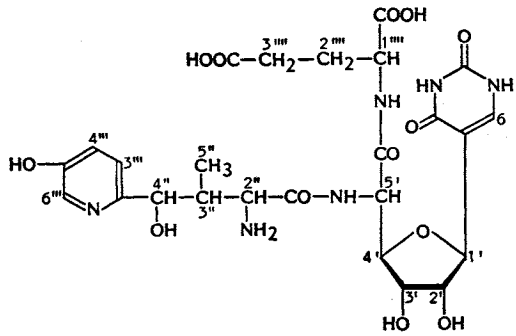
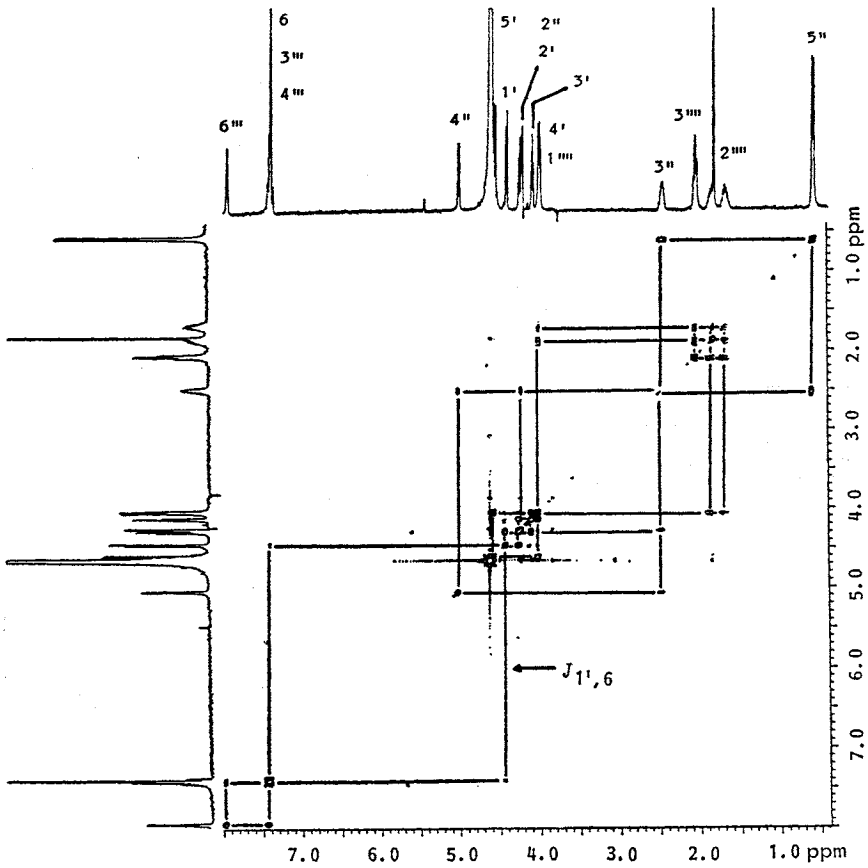


Table 1. ^{1}H and ^{13}C NMR chemical shifts (ppm) of nikkomycins Z and ϕ-Z, and pseudouridine.

	Nikkomycin Z	Nikkomycin ϕ-Z	Pseudouridine
^{1}H			
1′-H	5.80	4.58	4.53
5-H	5.78	—	—
6-H	7.63	7.55	7.52
^{13}C			
C-1′	90.09	80.18	80.08
C-5	103.50	111.36	111.50
C-6	143.04	142.62	142.40

Fig. 4. ^1H-^1H COSY NMR spectrum (400 MHz, D_2O) of nikkomycin ϕ-J.





Nikkomycin ϕ-J (**2**)

In Fig. 4 the ^1H-^1H COSY NMR spectrum of nikkomycin ϕ-J is shown. Again the signal of the 5-H of uracil is missing in the lower field range and the 6-H appears as a singlet as already observed in Fig. 2 for nikkomycin ϕ-Z. Moreover, a cross-peak between the 1′-H and the allylic 6-H of uracil indicates the *C*-glycosidic bond between C-1′ and C-5. The chemical shift of the 1′-H at δ 4.46 and of the 6-H at δ 7.42 (superimposed by the signals of 3-H and 4-H of the pyridine system) closely correspond to the chemical shifts of nikkomycin ϕ-Z and pseudouridine. As already found in nikko-

mycin J[4] an additional L-glutamic acid residue is present in nikkomycin ϕ-J. This is established in the ^{1}H NMR spectrum by the signals of the diastereotopic β-CH_2 group (AB system at δ 1.71 and δ 1.88), a multiplet of the γ-CH_2 at δ 2.09 and the α-CH proton at δ 4.05, which overlaps with the signal of the 4′-H of the sugar moiety. The resonances of the *N*-terminal amino acid have almost identical chemical shifts as in the spectra of nikkomycins Z and ϕ-Z.

The presence of an L-glutamic acid residue was confirmed after HCl hydrolysis of nikkomycin ϕ-J and formation of an isopropyl ester - trifluoroacetyl derivative by GC-MS and by chiral capillary GC[5].

Finally the molecular mass of nikkomycin ϕ-J could be established by a prominent $(M+H)^+$ ion at *m/z* 625 in the positive ion FAB-MS. The configuration of the 5-amino-5-deoxy-β-D-*allo*-furanuronic acid moiety of nikkomycins ϕ-Z and ϕ-J was found to be identical to nikkomycin Z by comparing ^{1}H NMR data[9].

Acknowledgments

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