Journal of Chromatography, 316 (1984) 487–494
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 508

SCREENING FOR NEW MICROBIAL PRODUCTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING A PHOTODIODE ARRAY DETECTOR

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SUMMARY

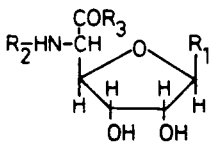
The screening for new microbial metabolites in culture filtrates from *Streptomyces tendae* TÜ 901 by high-performance liquid chromatography using a photodiode array detector resulted in detection of new compounds related to the parent compounds. Six peaks in the elution profiles of different strains could be identified as potential new nikkomycins, a group of nucleoside-peptide antibiotics. Structure elucidation confirmed the assumption for new nikkomycin structures.

INTRODUCTION

High-performance liquid chromatography (HPLC) is an excellent method for the quantitation and characterization of secondary metabolites in biological fluids, such as culture filtrates of organic extracts. Nevertheless, the search for new substances resulting from chemically derivatization programs, genetic or mutasynthetic programs, emphasises the difficulty of the classification of peaks in an HPLC elution profile, *i.e.* the classification of compounds that are related to the parent compound. One method is the isolation and characterization of all detected peaks, but this is very time-consuming.

The photodiode array technique is a very high efficient tool for comparing known and unknown compounds. The storage of UV spectra of all peaks during the HPLC run, without stopped flow, allows the classification of the parent compound and the derivatives in a single chromatographic run[1]. Culture filtrates from different strains of *Streptomyces tendae* TÜ 901, the producer of nikkomycins, which belongs to the group of nucleoside-peptide antibiotics[2–4], were screened with a diode array detector for new nikkomycin compounds. These antibiotics can be classified into two groups: one contains a pyrimidine ring system, *e.g.* nikkomycins B_z, C_z, J, N and Z, the other contains an imidazolone ring system, *e.g.* nikkomycins B_x, C_x, I, M and X (Fig. 1). Both groups show characteristic, but different, UV spectra. Of particular interest are new nikkomycins with a wide-spread activity spectrum and better stability in at alkaline pH.

0021-9673/84/$03.00



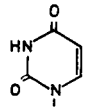
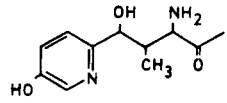
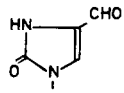
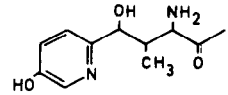
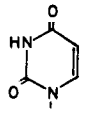
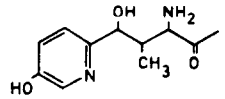
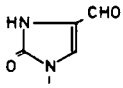
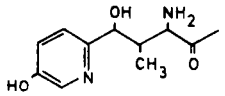
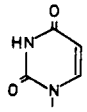
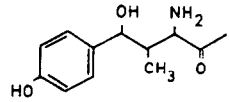
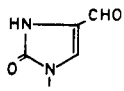
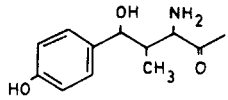
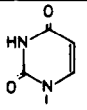
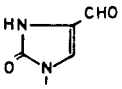
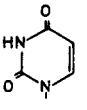
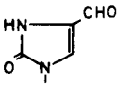
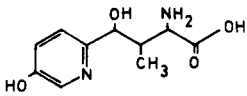
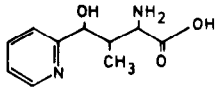
NIKKOMYCIN	R_1	R_2	R_3
Z			OH
X			OH
J			Glu
I			Glu
B_Z			OH
B_X			OH
N		H	Glu
M		H	Glu
C_Z		H	OH
C_X		H	OH
D			
E			

Fig. 1. Structures of nikkomycins.

EXPERIMENTAL

Chemicals

Acetonitrile was HPLC grade and orthophosphoric acid was analytical grade, obtained from Merck (Darmstadt, F.R.G.). Hexanesulphonic acid sodium salt monohydrate was from Fluka (Buchs, Switzerland). Water was purified by means of a Milli-Q-system (Millipore, Eschborn, F.R.G.).

Apparatus

The analytical system consisted of a Spectra-Physics SP-8000 liquid chromatograph and a Hewlett-Packard HP 1040 A diode array detector.

Columns

The column (125 × 4.6 mm I.D.) was fitted with a pre-column (20 × 4.6 mm I.D.) and filled with 5-μm Shandon ODS Hypersil; it was obtained from Bischoff (Leonberg, F.R.G.).

Mobile phases

The biological samples were analysed with gradient elution. Solvent A was water, containing 10 m*M* heptanesulphonic acid and 2 ml of acetic acid per litre; solvent B was water–acetonitrile (6:4), also containing 10 m*M* hexanesulphonic acid and 2 ml of acetic acid per litre. The linear gradient was from 13% solvent B to 45% solvent B in 9 min, and the flow-rate was 2 ml/min.

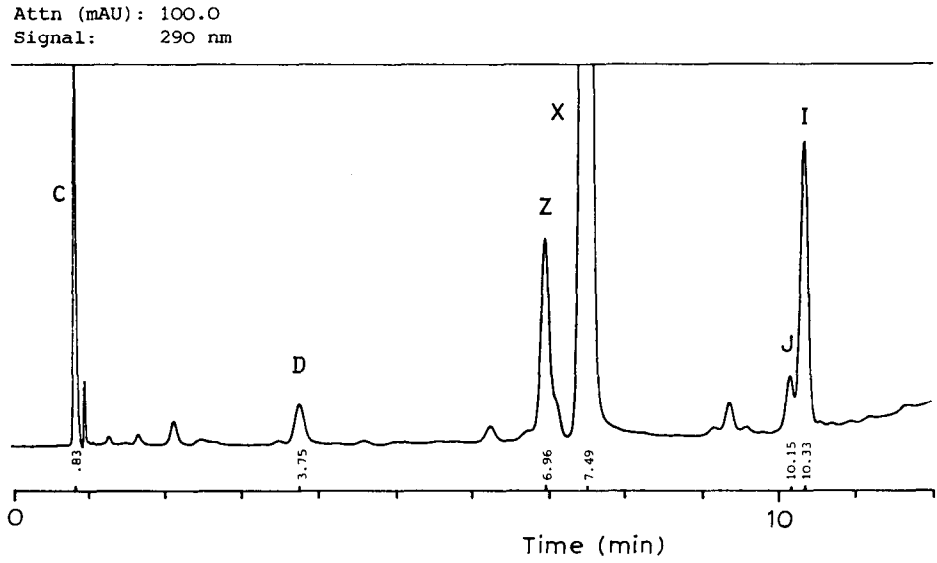
Sample preparation

The fermentation broth was centrifuged and the supernatant was filtered with a membrane filter (pore diameter 0.6 μm). A 10-μl sample was injected onto the column.

RESULTS

Fig. 2 shows the HPLC elution profile of a standard solution, which contained the main compounds of the nikkomycin group. The standard solution was prepared from a lyophilysed ion-exchange eluate with a specific nikkomycin content. In addition, Fig. 2 shows the apex UV spectrum of nikkomycin Z, a representive of the pyrimidine group with a UV maximumm at 260 nm, which can be well distinguished by the UV spectrum from nikkomycin X, a representive of the imidazolone group, which shows a maximum at 290 nm. Nikkomycins J and I have the same UV spectra as nikkomycins Z and X, respectively. The characteristic UV spectra of both groups should help in the detection of new nikkomycins related to the parent compounds in culture filtrates of mutants from the nikkomycin-producing strain *S. tendae* TÜ 901.

Fig. 3 shows a chromatogram obtained after injection of 10 μl of culture filtrate from the selected strain 395/11. This strain produces neither nikkomycin Z or X, but appears in the chromatogram, with retention times 5.64, 5.72 and 6.24 min. The UV spectra of this peaks indicate the presence of new nikkomycins both of the pyrimidine type (5.64 min) and of the imidazolone type (5.72 and 6.24 min). When the peaks were isolated by ion-exchange chromatography on Dowex 50 WX4, Amberlite 401-



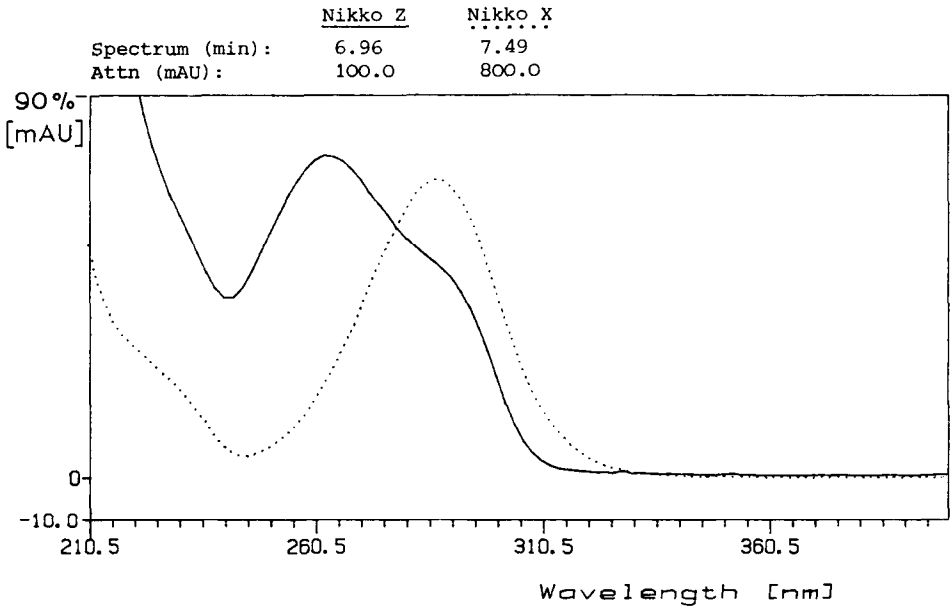
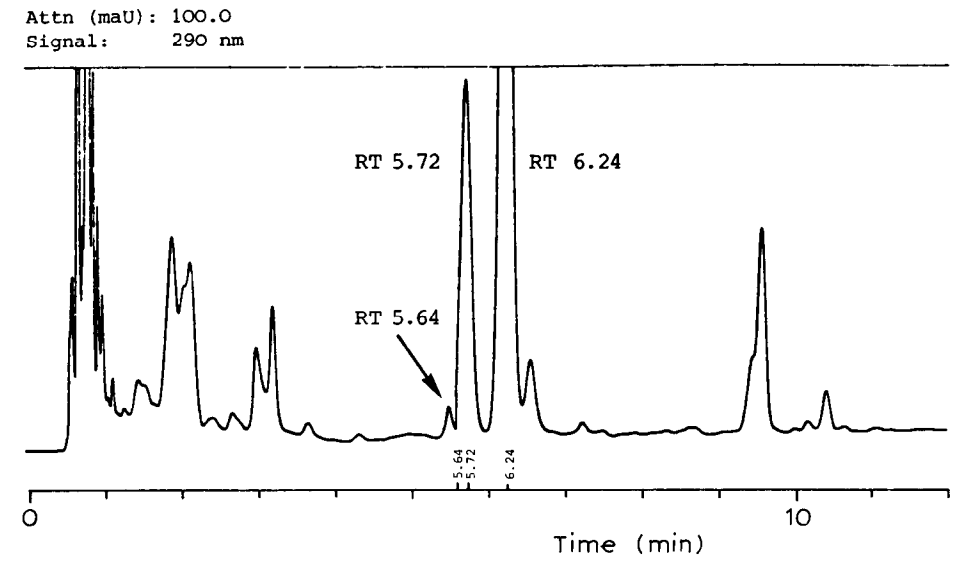


Fig. 2. HPLC chromatogram of nikkomycin standards, plotted at 290 nm, and UV spectra of nikkomycins Z and X, recorded during chromatography of the standard solution.



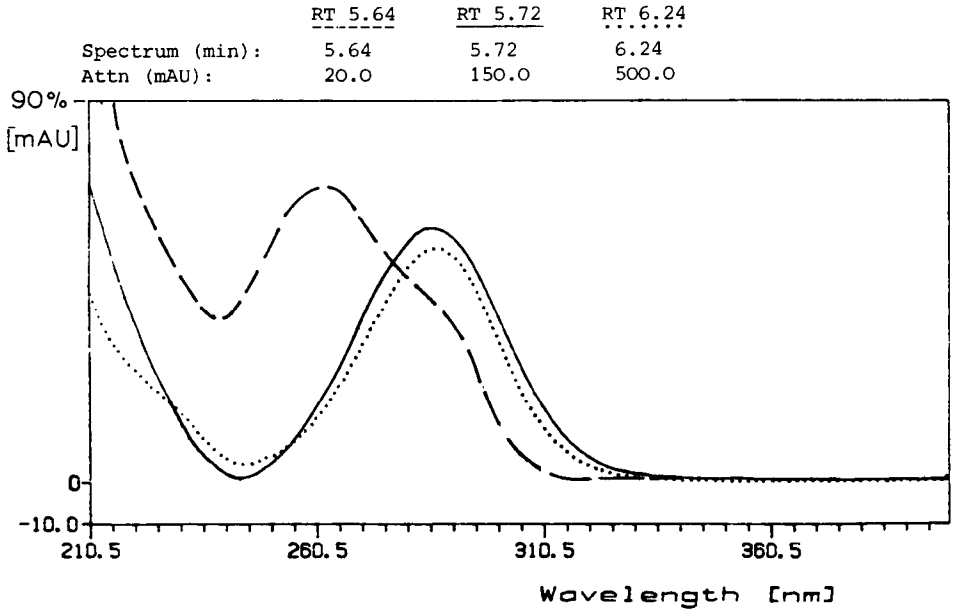


Fig. 3. HPLC chromatogram of culture filtrate from *Streptomyces tendae* strain 395/11, plotted at 290 nm, and UV spectra of compounds eluting at 5.64, 5.72 and 6.24 min, recorded during an HPLC run.

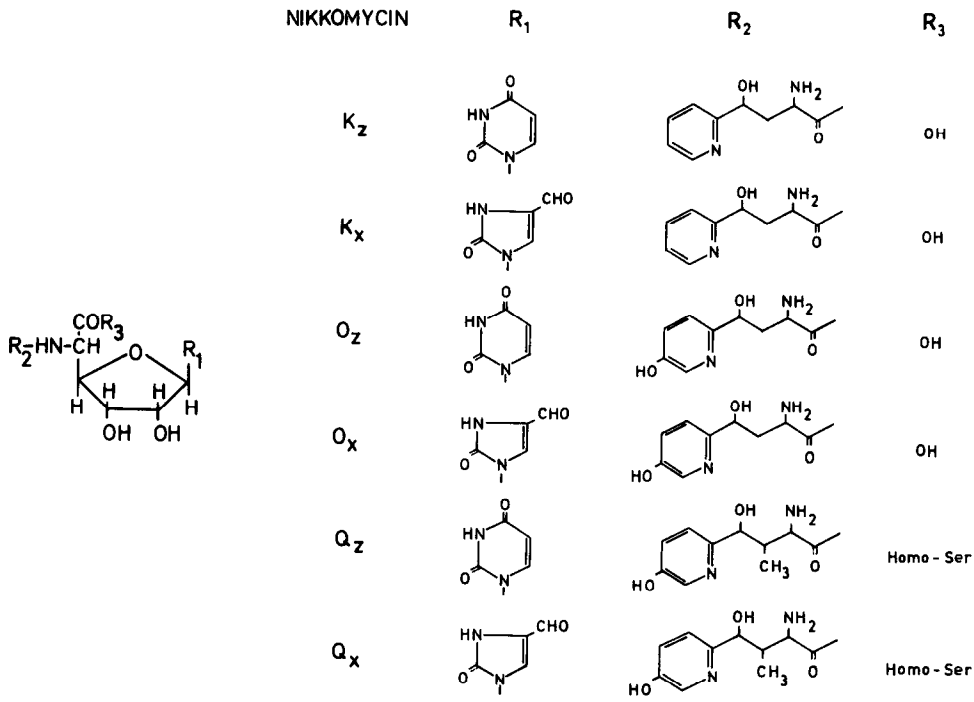
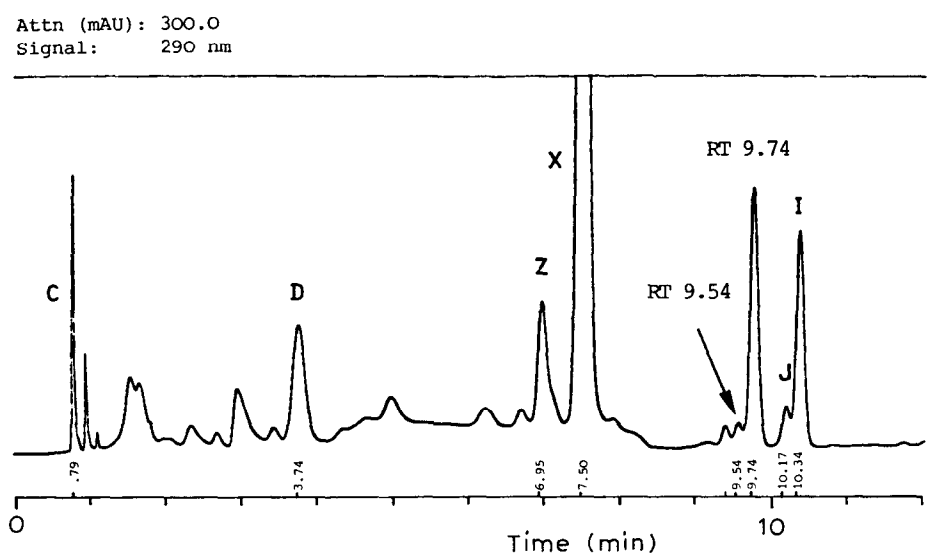


Fig. 4. Structures of new nikkomycins, isolated from strain 395/11 and strain C 37.

S, and SP-Sephadex C-25, and by size-exclusion chromatography on Bio-Gel P-2[5], and additional peak (5.55 min) was characterized as a new nikkomycin of the pyrimidine type.

Structure elucidation, as reported by Bormann *et al.*[5], confirmed the new nikkomycin structures (Fig. 4). Those at 5.55 and 5.72 min are from the pyrimidine and imidazolone group, respectively, and were named nikkomycins K_z and K_x; they differ in lacking the hydroxyl and methyl group of the amino acid part of the molecule. Those at 5.64 and 6.24 min were named nikkomycins O_z and O_x, and belong to the pyrimidine and imidazolone group, respectively; they differ in lacking the methyl group of the amino acid part of the nikkomycin molecule.

When a culture filtrate of an auxotrophic mutant from *S. tendae* TÜ 901, strain C 37 met^- thr^-, was injected onto the HPLC column, two additional peaks were detected in the elution profile between nikkomycins X and J (Fig. 5). The diode array plot of these new peaks allowed the classification of the peak at 9.54 min as due to a nikkomycin of the pyrimidine group, with an UV maximum at 260 nm, and of that at 9.74 as due to a nikkomycin of the imidazolone group, with an UV maximum at 290 nm. Isolation of the peaks and structure elucidation[5] confirmed these new nikkomycin structures, which are also shown in Fig. 4. They are homologues of nikkomycins J and I, respectively, and were named nikkomycin Q_z and nikkomycin Q_z. Both compounds differ in the amino acid side-chains, since homoserine is incorporated into the molecule instead of glutamic acid.



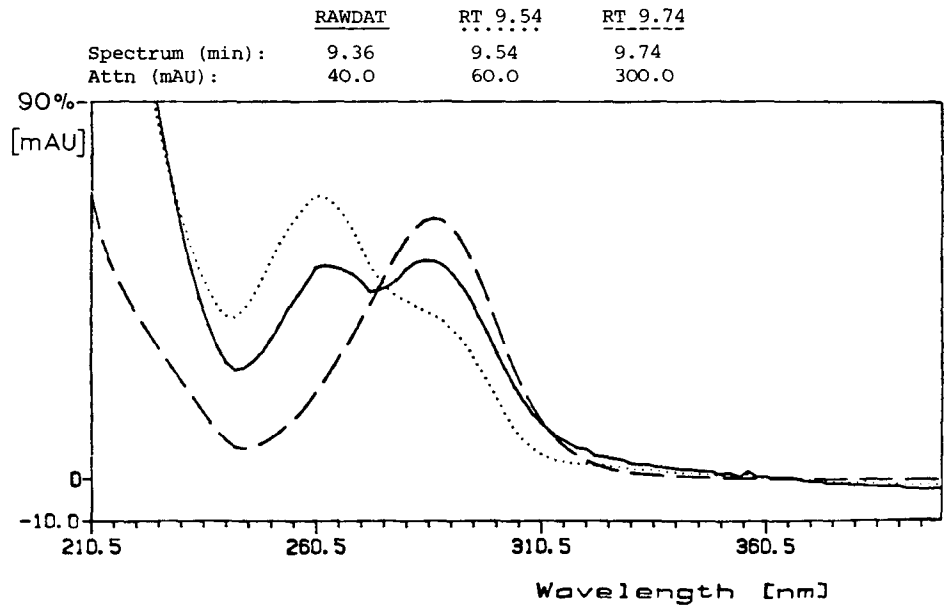


Fig. 5. HPLC chromatogram of a culture filtrate from *Streptomyces tendae* TÜ 901 strain C 37, plotted at 290 nm, and UV spectra of compounds eluting at 9.54 and 9.74 min, recorded during an HPLC run.

CONCLUSIONS

The great power of diode array detection in HPLC analysis lies in the vast amount of useful information generated in a single chromatographic run. This method simplifies the search for structure-related metabolites in biological samples by comparing the UV–visible spectra of all peaks from the HPLC elution profile with the spectrum of the parent compound, as was demonstrated in the identification of the new nikkomycins K_z, K_x, O_z, O_x, Q_z and Q_x. The method is limited only by the adsorption range, >220 nm, of the substances to be determined.

ACKNOWLEDGEMENTS

Thanks are due to Mrs. A. Döhle for her skilful technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 76).

REFERENCES

1 K. Zech, R. Huber and H. Elgass, *J. Chromatogr.*, 282 (1983) 161.
2 U. Dähn, H. Hagenmaier, H. Höhne, W. A. König, G. Wolf and H. Zähner, *Arch. Microbiol.*, 107 (1976) 143.
3 H.-P. Fiedler, *J. Chromatogr.*, 204 (1981) 313.
4 H.-P. Fiedler, R. Kurth, J. Langhärig, J. Delzer and H. Zähner, *J. Chem. Technol. Biotechnol.*, 32 (1982) 271.
5 Ch. Bormann, W. Huhn, H. Zähner, R. Rathmann, H. Hahn and W. A. König, *J. Antibiot.*, in press.