ISOLATION AND CHARACTERIZATION OF NOCARDICIN B

MASARU KURITA, KAZUYOSHI JOMON, TADAAKI KOMORI, NORIMASA MIYAIRI, HATSUO AOKI, SAKAE KUGE, TAKASHI KAMIYA and HIROSHI IMANAKA

Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan

(Received for publication August 26, 1976)

Previously, H. AOKI *et al.*[1] reported the isolation of nocardicin A from the fermentation broth of *Nocardia uniformis* subsp. *tsuyamaensis* ATCC 21806. During further investigations to detect minor metabolites in the broth, it has been found that the microorganism produces a new antibiotic named nocardicin B, together with nocardicin A. As presented in Chart 1, nocardicin B was newly isolated from the fermentation broth. The broth was filtered at pH 4.0. The filtrate was passed through a column of Diaion HP 20. After rinsing with water, elution was performed with 30% aqueous methanol. The eluate was concentrated *in vacuo*, followed by acidification (pH 2.5) to give crude crystals containing nocardicins A and B.

Complete separation of nocardicin B from nocardicin A was achieved by using Diaion HP 20* in the presence of sodium chloride. The crude crystals were dissolved in water at pH 7.0 with 6 N sodium hydroxide solution, to which an equal volume of 6% sodium chloride solution was added, then the solution was applied to a column of Diaion HP 20. Nocardicin A was completely washed out with 3% sodium chloride solution, before the elution of nocardicin B was carried out with 30% aqueous methanol. The fraction containing nocardicin B was concentrated *in vacuo*. The concentrate was acidified to pH 2.3 with 6 N hydrochloric acid solution to afford nocardicin B as colorless needles (110 mg). Another preparation of nocardicin B was successfully carried out by using preparative liquid chromatography under the conditions described in Table 1. The thin-layer chromatogram (Fig. 1) of an aliquot of the broth on a cellulose plate revealed two active metabolites corresponding to nocardicins A and B. Therefore, nocardicin B is likely not an artifact but a natural antibiotic.

The purified nocardicin B decomposed at

* A nonionic macroporous copolymer of stylene cross linked with divinylbenzene. Mitsubishi Chemical Industry Ltd.

Chart 1. Isolation of nocardicins A and B

Fermentation broth
↓ pH 4.0 (N HCl)
Filtrate
↓
Diaion HP 20
↓ eluted with 30% MeOH
Eluate
↓ conc. *in vacuo* pH 2.5 (N HCl)
Crude crystals
↓ dissolved in dil. NaOH (pH 7.0)
↓ diluted with 6% NaCl soln.
Diaion HP 20
↓ stepwise elution
1) 3% NaCl soln. 2) 30% MeOH

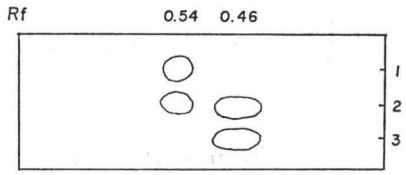
Eluate (3% NaCl soln.)	Eluate (30% MeOH)
↓	↓
Colorless needles	Colorless needles
(Nocardicin A)	(Nocardicin B)

Table 1. Retention times of nocardicins A and B on preparative liquid chromatography

Compound	Retention time in minutes
Nocardicin A	10.8
Nocardicin B	17.6

The chromatography was performed by Waters Liquid Chromatography by using a Bondapak C 18/Porasil B column (3/8 inch × 2 feet) × 2
Mobile phase; 0.01M Na_2HPO_4 - 0.01M KH_2PO_4 - MeOH (95:95:10, by volume)
Flow rate; 5.5 ml/min
Pressure; 1,000 psi
Instrument; Model No. M-6000A

Fig. 1. Thin-layer chromatogram of metabolites in the fermentation broth



Thin-layer chromatography on cellulose plate (Eastman chromatogram sheet No. 6065) was performed in *n*-BuOH-AcOH-H_2O (4:1:2). Metabolites were detected bioautographically by using *Pseudomonas aeruginosa* NCTC 10490 as the test organism.
Sample (1) Authentic nocardicin B
(2) Fermentation broth
(3) Authentic nocardicin A

Fig. 2. UV spectrum of nocardicin B in phosphate buffer at pH 8.0

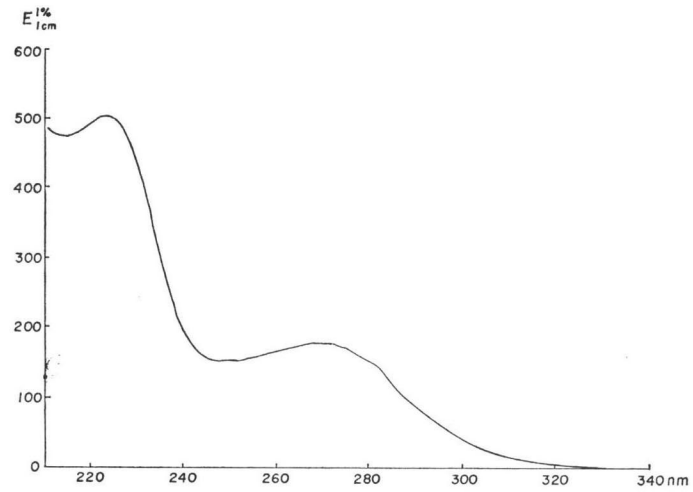
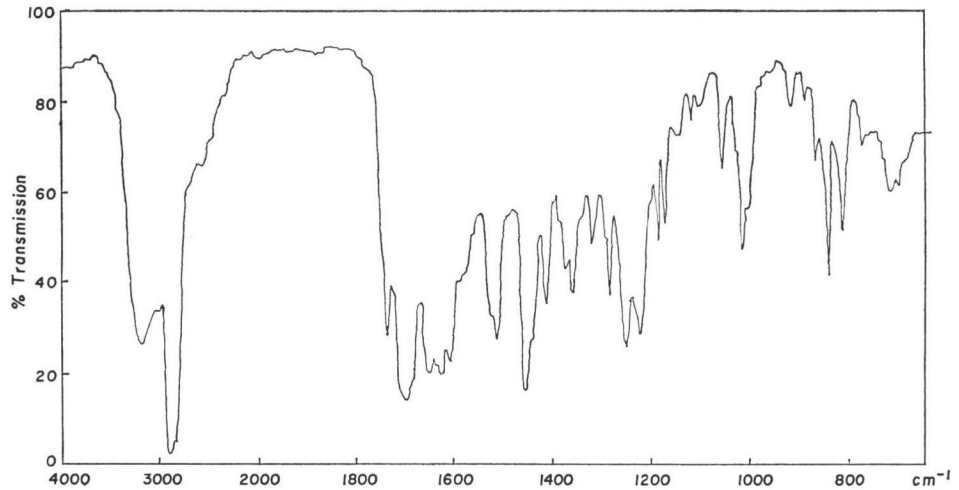


Fig. 3. IR spectrum of nocardicin B (Nujol)



262~264°C, and was found to have the empirical formula $C_{23}H_{24}N_4O_9 \cdot 1/3\ H_2O$ by elemental analysis and water content determination by the KARL FISCHER method. Anal. Calcd. for $C_{23}H_{24}N_4O_9 \cdot 1/3\ H_2O$; C 54.55 H 4.88 N 11.07 H_2O 1.18. Found; C 54.71 H 4.79 N 10.78 H_2O 1.10. It is soluble in alkaline solutions such as aqueous sodium hydroxide, aqueous ammonia, and aqueous pyridine and in dimethyl sulfoxide, but insoluble in other organic solvents. Its monosodium salt exhibits $[\alpha]_D^{20}$ —162° (*c* 1, water), and gave positive reaction with ninhydrin and $FeCl_3$-$K_3Fe(CN)_6$ reagents. The UV absorption spectrum of nocardicin B (Fig. 2) in phosphate buffer at pH 8.0 showed two absorption maxima at 223 nm ($E^{1\%}_{1cm}$ 507) and at 271 ($E^{1\%}_{1cm}$ 181). The IR spectrum and the NMR spectrum of the antibiotic are presented in Figs. 3 and 4. As reported in detail[2], the chemical structure of nocardicin B has been shown to be stereoisomeric to nocardicin A at the oxime group (Fig. 5).

The antimicrobial activities of nocardicin B, determined by the serial agar dilution streak method, are shown in Table 2. Up to 750 mg of nocardicin B per kg body weight were injected intravenously, intraperitoneally or subcutaneously to mice, or 2,000 mg administered orally without inducing any obvious toxic effects. The above dose for injection was due to the solubility.

Fig. 4. NMR spectrum of nocardicin B in D_6-dimethylsulfoxide
(100 MHz, internal standard - tetramethyl silane)

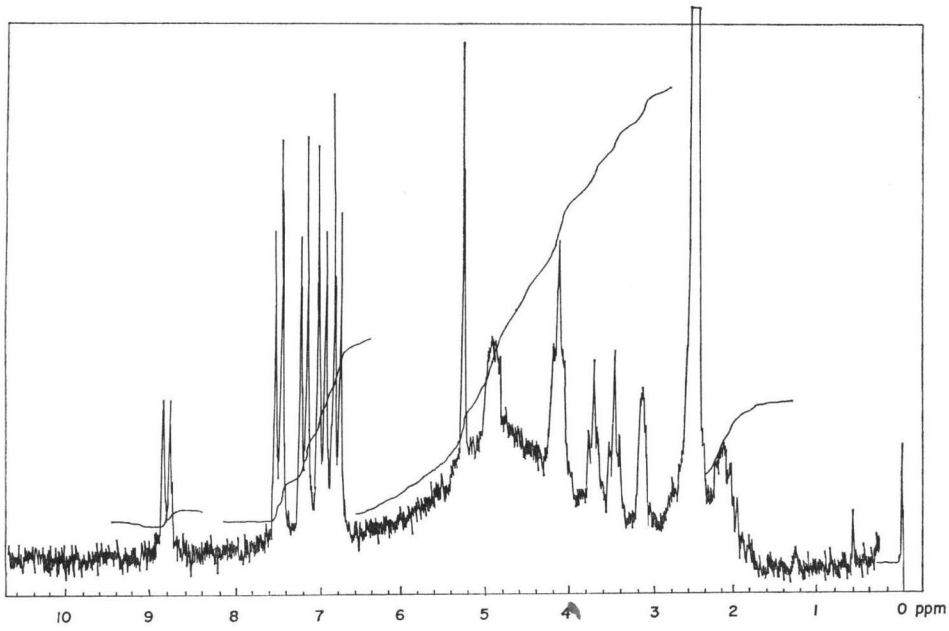


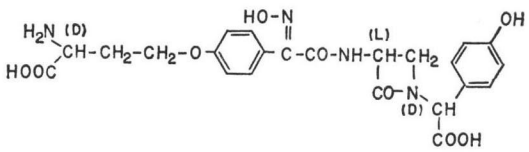
Table 2. Antimicrobial activities of nocardicin B

Test organism	MIC (mcg/ml)	
	Nocardicin B	Nocardicin A
Staphylococcus aureus FDA 209P	>800	>800
Bacillus subtilis W 23	>800	50
Escherichia coli NIHJ JC-2	>800	100
Proteus vulgaris IAM-1025	400	3.13
Pseudomonas aeruginosa NCTC 10490	200	3.13

Acknowledgements

We are grateful to members of Nagoya Pilot Plant of our company for carrying out the fermentation.

Fig. 5. Chemical structure of nocardicin B



References

1) Aoki, H.; H. Sakai, M. Kohsaka, T. Konomi, J. Hosoda, Y. Kubochi, E. Iguchi & H. Imanaka: Nocardicin A, a new monocyclic β-lactam antibiotic. I. Discovery, isolation and characterization. J. Antibiotics 29: 492~500, 1976

2) Hashimoto, M.; T. Komori & T. Kamiya: Nocardicins A and B, novel monocyclic β-lactam antibiotics from a *Nocardia* species. J. Am. Chem. Soc. 98: 3023~3025, 1976