

ANTIBIOTIC ACTIVITY OF PURE
PENICILLIN N
AND ISOPENICILLIN N

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(Received for publication January 11, 1982)

The antibacterial spectrum of crude penicillin N solutions has been used earlier^{1,2)} to determine chirality of the α -aminoadipyl side chain in an unknown sample of this antibiotic or its enantiomer, isopenicillin N. Ready availability of pure penicillin N and isopenicillin N³⁾ prompted us to compare their antibacterial activity against a few selected organisms.

Overnight broth cultures of *Staphylococcus aureus*, strain ATCC 25923; *Micrococcus luteus*, strain ATCC 9341 (formerly *Sarcina lutea*); *Salmonella typhimurium*, strain ATCC 13311, and a *Pseudomonas* sp. (supersensitive), strain X621 were adjusted to a 0.5 McFarland standard in broth. Penassay seed agar at pH 7.2 was inoculated at an 1 percent concentration with the *Staph.*, *Salmonella*, and *Pseudomonas* cultures. *Micrococcus luteus* plates were prepared in Difco penassay base agar adjusted to pH 6.0.

Freshly prepared pure penicillin N and isopenicillin N were dissolved in water and diluted to contain 1000, 500, 200, 100, 50, 20, 10 and 5 $\mu\text{g}/\text{ml}$. Disks (6.35 mm) were placed on the

seeded plates. When 20 μl of the above antibiotic solutions were added to the disk with an Eppendorf pipette, the concentrations on the disks were 20, 10, 4, 2, 1, 0.4, 0.2, and 0.1 μg . Assays were done in triplicate. After overnight incubation at 37°C, zone size was determined with a Fisher-Lilly zone reader. An average of the 3 zone sizes for each dilution was determined.

Results of the experiment are presented in the Table 1. Detectable zones with 0.1 μg of penicillin N were demonstrated against the *Salmonella* and *Micrococcus* strains. Twice as much isopenicillin N was needed to give a detectable zone against the *Micrococcus* and 40 times as much against the *Salmonella*. Isopenicillin N was less active against all 4 organisms than penicillin N. There was a moderate difference in activity between the two enantiomers against the *Staph.*, *Micrococcus* and *Pseudomonas* sp. but penicillin N was considerably more active than isopenicillin N against *Salmonella typhimurium*. These data differ somewhat from results reported earlier²⁾ where only a crude sample of penicillin N was used as a standard.

Acknowledgements

We greatly acknowledge S. A. STROY and H. M. SULLIVAN for antibiotic assays. Freshly prepared samples of penicillin N and isopenicillin N were provided by L. L. HUCKSTEP.

References

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Table 1. Comparison of antibacterial activity of penicillin N and isopenicillin N.

Concentration in μg per disk	<i>Salmonella</i> *		<i>Pseudomonas</i> *		<i>Staph. aureus</i> *		<i>Micrococcus luteus</i> *	
	Pen N	Isopen N	Pen N	Isopen N	Pen N	Isopen N	Pen N	Isopen N
20	39.3	19.7	23.3	21.5	25.9	23.9	32.3	31.3
10	37.6	16.1	19.0	16.2	21.8	19.8	30.7	29.3
4	33.0	9.6	9.3	7.7	17.4	15.4	28.3	26.4
2	30.3	0	0	0	14.3	13.0	25.3	22.5
1	27.7	0	0	0	12.3	9.6	21.3	19.0
0.4	20.5	0	0	0	9.1	0	17.1	14.8
0.2	13.7	0	0	0	0	0	13.9	8.8
0.1	9.5	0	0	0	0	0	9.0	0

* Strains described in Text. Pen N: Penicillin N, Isopen N: Isopenicillin N.
Zone size in mm.

- ^3H from δ -(L- α -amino[4,5- ^3H]adipyl)-L-cysteinyl-D-[4,4- ^3H]valine into isopenicillin N. *Biochem. J.* 184: 421~426, 1979
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