L-VALINE: A PRECURSOR OF CEPHALOSPORIN C

Arnold L. Demain

Merck Sharp & Dohme Research Laboratories
Division of Merck & Co., Inc.
Rahway, New Jersey

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The structure of the penicillinase-resistant antibiotic, cephalosporin C (Figure 1), has recently been elucidated (Abraham and Newton, 1961). Whereas the nucleus of the closely-related penicillin family of antibiotics consists of a fused β-lactam-thiazolidine ring system, that of cephalosporin C is composed of a fused β-lactam-dihydrothiazine ring structure to which an acetoxy group is attached. The acetoxy group has been shown to be derived from acetate (Trown et al., 1962). Since it is known that L-valine is a precursor of the thiazolidine ring in penicillin (for review, see Demain, 1959), it was of interest to determine whether this amino acid is incorporated into cephalosporin C.

A procedure employing washed resting cells (Demain, in preparation) of Cephalosporium sp. mutant 8650 was used to study the incorporation of DL- and L-valine-1-C^{14} into the antibiotic. After 60 hours of growth in chemically defined medium C (Demain, Newkirk and Hendlin, 1962), the cells were harvested by aseptic centrifugation, washed with 0.1M phosphate buffer (pH 6) and incubated in a mineral salts solution (buffered with phosphate at pH 6.0) containing DL-valine-1-C^{14}. After incubating on a rotary shaker at 28°C for 48 hours, the cells were removed by centrifugation and the supernatant solution was

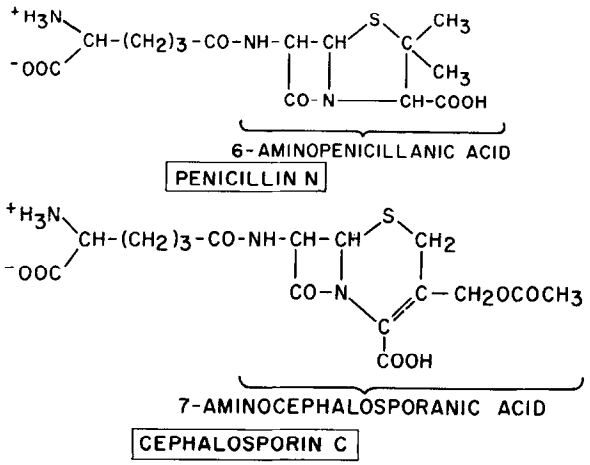

Figure 1. Structure of Penicillin N and Cephalosporin C

assayed for cephalosporin C (Demain and Newkirk, 1962). After removal of interfering materials with 70% methanol, the solution was concentrated *in vacuo* and paper chromatographed in butanol-acetic acid-water (4:1:4). The sheets were examined over an ultraviolet light source and the UV-absorbing band on each sheet corresponding to cephalosporin C was marked, cut out, eluted with water and concentrated *in vacuo*. The cephalosporin C concentration was determined to estimate the recovery of activity and the solution was rechromatographed on a single paper strip under the same conditions as described above. The strip was examined for UV-absorbing spots, for radioactive peaks in an automatic windowless paper chromatogram scanner, and for antibacterial spots by bioautography on agar seeded with *Escherichia coli* W-208. The results showed one radioactive peak, one UV-absorbing spot and one bioactive spot, all of which coincided. Mixed chromatography of the solution with authentic unlabeled cephalosporin C in the above solvent system and in two others (butanol-ethanol-water, 8:3:10 and isopropanol-pyridine-water, 65:5:30) revealed only one UV-absorbing, radioactive and bioactive spot. Mixed

chromatography with DL-valine-1-C^{14} led to two radioactive peaks; only the slower one (corresponding to cephalosporin C) was UV-absorbing and bioactive. Co-chromatography with penicillin N

TABLE I

Incorporation of valine into cephalosporin C

Expt.	Medium Supplement	Substance Measured	Amount μ moles	Total Radio-activity c.p.m.	Specific Radio-activity c.p.m./ μ mole	Incor-poration of Radio-activity %	Dilut: of Speci: Activ:
1	DL-valine-1-C^{14}	Valine added	1.8	3.5×10^6	1.9×10^6	5.1	49
		Cephalosporin C produced	4.7	1.8×10^5	3.9×10^4		
2	L-valine-1-C^{14}	Valine added	0.85	1.9×10^6	2.3×10^6	2.8	38
		Cephalosporin C produced	0.89	5.4×10^4	6.1×10^4		
	L-valine-1-C^{14}	Valine added	43.6	1.9×10^6	4.3×10^4	0.6	3.1
		Cephalosporin C produced	0.87	1.2×10^4	1.4×10^4		

The two experiments are not comparable to each other because of somewhat different conditions of incubation. In Expt. 1, volume was 41 ml. in 250 ml. Erlenmeyer flasks; incubation time was 48 hours. In Expt. 2, volume was 10.25 ml. in 50 ml. flasks; incubation time was 72 hours. Radioactivity of cephalosporin C corrected for losses during paper chromatographic procedure as judged by cephalosporin C assays. Recoveries were 49% in Expt. 1, 48% and 45% in Expt. 2 at the low and high valine concentrations respectively.

(also produced by Cephalosporium sp.) gave two bioactive spots. The faster spot, corresponding to penicillin N, carried no radio-activity or UV-absorption. Thus, it was evident that the labeled

DL-valine had been incorporated into cephalosporin C. Quantitative data obtained with the use of a liquid scintillation counter are shown in Table I. The dilution of molar specific radioactivity, presumably due to endogenous valine, was 49. Also in the table are the results of another experiment using L-valine-1-C^{14} at two concentrations. It can be seen that the L isomer was incorporated into cephalosporin C and that increasing the concentration of exogenous valine markedly decreased the dilution effect.

Thus, L-valine-1-C^{14} is efficiently incorporated into cephalosporin C. In view of previous studies on the penicillins, it would appear that the site of incorporation is the dihydrothiazine ring but further work is required to prove this point.

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