Enzymic and Chemical Transformations of the Side Chain of Cephalosporin C

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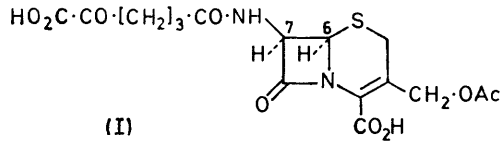
The synthesis of 7-(5-oxoadipamido)cephalosporanic acid by treating cephalosporin C with D-amino-acid oxidase is described. The same compound is obtained by a non-enzymic transamination reaction between pyridoxal 5-phosphate and cephalosporin C.

ENZYMES able to act on cephalosporin C include β-lactamases,[1] which open the β-lactam ring, and acetylesterases,[2] which hydrolyse the acetic ester function. However, enzymes with acylase activity able to detach the D-α-aminoadipoyl chain [3] are not known.

We describe here the oxidative deamination of the D-α-aminoadipoyl chain by D-amino-acid oxidase from pig kidney. This enzyme is known to transform D-α-amino-acids into α-keto-acids, but its specificity is limited and D-α-aminoadipic acid is reported not to be a suitable substrate.[4]

By use of a high enzyme–substrate ratio we have transformed cephalosporin C into an acid product identified as 7-(5-oxoadipamido)cephalosporanic acid (I) on the basis of analytical and spectroscopic data (yield 10—20%).



(I)

The same compound (I) has been prepared by a non-enzymic transamination reaction involving treatment with pyridoxal 5-phosphate; by employing a reagent–cephalosporin C ratio of 5, a 33% yield was obtained.

EXPERIMENTAL

M.p.s were taken with a Kofler hot-stage apparatus. U.v. spectra were measured for solutions in ethanol with a Cary 14 spectrometer. I.r. spectra were recorded for potassium bromide discs with a Perkin-Elmer 521 spectrometer. N.m.r. spectra were determined for solutions in hexadeuterioacetone with a Varian A-60 D instrument, with tetramethylsilane as internal standard. Mass spectroscopic analysis was performed at 70 eV with an A.E.I. MS12 spectrometer. Optical rotations were recorded at *ca.* 20° with a Schmidt–Haensch polarimeter.

Reaction of Cephalosporin C with D-*Amino-acid Oxidase.*—The potassium salt of cephalosporin C (680 mg, 1·5 mmol), D-amino-acid oxidase (50 mg) (Boehringer; crystalline suspension in 1·8M-ammonium sulphate), and catalase (2 mg) in phosphate buffer (0·1M; pH 6·2; 30 ml) under oxygen were stirred at 27° for 26 h. The mixture was then saturated at 0° with ammonium sulphate, and, after removal of precipitated enzyme by centrifugation, acidified to pH 3 with 2N-hydrochloric acid and extracted with ethyl acetate. The extract was washed with saturated ammonium sulphate solution, dried (Na_2SO_4), and evaporated to leave material (124 mg), which crystallized from ethyl acetate–hexane to give 7-(5-*oxoadipamido*)*cephalosporanic acid* (I), m.p. 163—164° (decomp.), $\lambda_{max.}$ 258 nm (ε 5600), $[\alpha]_D$ +101° (*c* 1 in EtOH), $\nu_{max.}$ 3290, 1745, 1730, 1710, 1685, 1650, 1530, and 1230 cm^{-1}, *m/e* 414 (M^+), δ 5·87 (1H, q, *J* 5 and 9 Hz, 7-H), 5·17 (1H, d, *J* 5 Hz, 6-H), 7·88 (1H, d, *J* 9 Hz, NH, exchangeable with D_2O), 3·45 and 3·80 (2H, ABq, J_{AB} 18 Hz, S·CH_2), 4·83 and 5·18 (2H, ABq, J_{AB} 13 Hz, CH_2·OAc), and 2·03 p.p.m. (3H, s, Ac) (Found: C, 46·45; H, 4·85; N, 6·95; S, 7·95. $C_{16}H_{18}N_2O_9S$ requires C, 46·35; H, 4·4; N, 6·75; S, 7·75%).

Reaction of Cephalosporin C with Pyridoxal 5-Phosphate.—A solution of the potassium salt of cephalosporin C (2·27 g, 5 mmol) and sodium pyridoxal 5-phosphate (6·75 g, 25 mmol) in phosphate buffer (0·1M; pH 6·2; 80 ml) was kept at room temperature for 16 h. The mixture was then saturated at 0° with ammonium sulphate, acidified to pH 3 with 2N-hydrochloric acid, and extracted with ethyl acetate. The extract was washed with saturated ammonium sulphate solution, dried (Na_2SO_4), and evaporated to leave a product (690 mg), which, after crystallization from ethyl acetate–hexane, was identical with the acid (I) already obtained.

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[1] L. D. Sabath, M. Jago, and E. P. Abraham, *Biochem. J.*, 1965, **96**, 739.
[2] J. d'A. Jeffery, E. P. Abraham, and G. G. F. Newton, *Biochem. J.*, 1961, **81**, 591.
[3] R. B. Morin and B. G. Jackson, *Fortschr. Chem. org. Naturstoffe*, 1970, **28**, 355.
[4] A. E. Bender and H. A. Krebs, *Biochem. J.*, 1950, **46**, 210.