SYNTHESIS OF 7β-(5-D-AMINOADIPAMIDO)-3β-HYDROXY-3α-METHYL-CEPHAM-4α-CARBOXYLIC ACID, A NEW METABOLITE FROM CEPHALOSPORIUM ACREMONIUM FERMENTATION

Sir:

A new β-lactam derivative, 7β-(5-d-amino-adipamido)-3β-hydroxy-3α-methylcepham-4α-carboxylic acid (1), has been recently isolated from the broth of Cephalosporium acremonium by Neuß and associates. The discovery of this new cephalosporin in the fermentation broth revived our interest in this type of compound. In earlier studies of the interconversion of penam and cephap systems, we proposed the hypothesis that biosynthesis of penicillin N and deacetoxycephalosporin C and closely related substances could be explained as proceeding via a common episulfonium ion E. If one assumes that the anion Y- in the formula E could be hydroxyl (or similar anion encountered in this kind of enzymatic reactions), it is conceivable that 3-hydroxy-3-methylcepham (1) and 2-hydroxymethylpenam (2) are possible products.

The idea that these compounds might be intermediates in the biosynthesis of penicillins and cephalosporins prompted one of us to develop a general method for their synthesis. Using this method we made the hydroxy-protected nucleus 5 and coupled it with the appropriately protected L- and D-α-aminoadipic acids.

Since the NMR spectrum of the fermentation product indicated only its gross structure but not the chirality, we concentrated our efforts on proving its basic structure and stereochemistry by synthesis from stereochemically defined intermediates. The starting cephap compound 3 was prepared, together with the penam chloroacetate 4, according to the procedure described by Spryr. The mixture of cephap 3 and penam 4 was separated by chromatography over a silica gel column. The phenoxacyethyl side chain was removed from 3 by treatment with phosphorous pentachloride in pyridine and the desired p-nitrobenzyl-7-amino-3β-chloroacetxy-3α-methylycepham-4α-carboxylic acid (5) isolated after chromatography over a silica gel column [NMR (CDCl₃) δ in ppm: 1.60 (s, 3H, 3α-Me), 2.0 (2H, NH₂), 3.43 (s, 2H, SCh₂), 4.10 (s, 2H, COCH₂Cl), 4.53 (d, J=4.0 Hz, 1H, β-lactam), 4.90 (s, 1H, 4β-H), 5.20 (d, J=4.0 Hz, 1H, β-lactam), and 5.33 (s, 2H, CH₂ of pNB); IR (CHCl₃) 1750 cm⁻¹]. The nucleus 5 was then coupled by the EEDQ procedure with D- and L-α-aminoadipic acid whose α-carboxy- and amino-groups had been blocked by tert-butoxycarbonyl (BOC) and benzhydryl (Bh) protecting groups. All four protecting groups in compounds 6 and 7 were clearly discernible by NMR spectra [NMR (CDCl₃) δ in ppm: 1.43 (s, tert-Bu), 1.57 (s, 3H, 3α-Me), 3.42 (s, 2H, SCh₂), 4.10 (s, 2H, COCH₂Cl), 4.90 (s, 1H, 4β-H), 5.28 (s, 2H, CH₂ of pNB), 5.5 (β-lactam H), and 7.33 (s, Bh); IR (CHCl₃) 1760 cm⁻¹]. The protected intermediates 6 and 7 were subjected to three subsequent deprotecting steps: (i) removal of the chloroacetate group with thiourea (EtOH, 60°C, 2 hours), (ii) hydrogenation of the p-nitrobenzyl (pNB) group over palladium on charcoal catalyst (MeOH, 22°C, 50 psi, 1 hour), and (iii) cleavage of...
the BOC and Bh groups (98% HCOOH, triethylsilane, 22°C, 1 hour). The NMR spectra clearly showed removal of each protecting group. After the work-up, the completely deprotected final products 1 and 8 were isolated as colorless amorphous solids by HPLC using as stationary phase microbondpak C-18 with 0.5% of formic acid, 0.5% of methanol and 99% of water as the mobile phase (preparative HPLC was carried out using Waters Associates Prep LC System 500).

The 360 MHz proton magnetic spectra of the synthetic D- and L-isomers 1 and 8 are identical and superimposable with the spectrum of the natural metabolite ([NMR spectra of 1, 8, and the natural metabolite recorded at the same pH in D$_2$O solution using a Bruker WH360 spectrometer: $\delta$ (in ppm) 1.40 (s, 3H, 3a-Me), 1.75 and 1.92 (m, 4H, $\alpha$-AAA), 2.43 (t, 2H, $\alpha$-AAA), 2.65 and 3.52 (ABq, $J=14.5$ Hz, 2H, S-CH$_2$), 3.78 [t, 1H, HC(NH$_2$)COOH], 4.21 (s, 1H, 419-H), 5.29 and 5.43 (2d, $J=4.5$ Hz, 2H, $\beta$-lactam). The mass spectrum showed M +1 at 376 (MW 375.4)]. On this basis it is apparent that the chirality at C-3, C-4, C-6, and C-7 in the cepham moiety is the same in all three compounds, but from the NMR spectra it is not possible to determine whether the $\alpha$-aminoadipyl side chain in the natural metabolite has D- or L-configuration.

Measurements of optical rotation and CD were also not conclusive because both synthetic isomers (D- and L-) have the positive sign of rotation and their values are not sufficiently different to allow a judgment about the chirality of the side chain in the natural product. Apparently the chiral contribution of the side chain is over-shadowed by that of the cepham moiety. A similar phenomenon was observed earlier in the case of D- and L-phenylglycine derivatives of 7-ACA. The chirality of the side chain in the natural metabolite was proven to be D- by an assay using a cell extract containing D-$\alpha$-amino oxidase from Trigonopsis variabilis. L-Amino oxidase from snake venom did not oxidize the acid.

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