Tetrahedron Letters,Vol.30,No.49,pp 6917-6918,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

STEREOSELECTIVE PAPAIN-CATALYZED SYNTHESIS OF ALAFOSFALIN

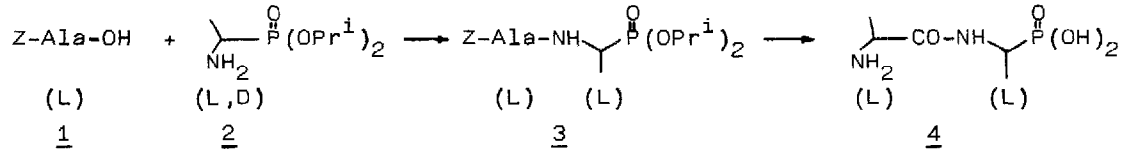
Vladimir A. Solodenko, Valeri P. Kukhar

Institute of Bioorganic Chemistry of the Ukrainian Academy of Sciences, Murmanskaya Str., 5, Kiev 252660, USSR

Summary: Papain in powdered form efficiently catalyzes alafosfalin synthesis in miscible aqueous-organic solvent involving L-aminophosphonate in peptide bond formation.

Bioactivity of phosphonopeptides depends essentially on the stereochemistry of incorporated aminophosphonic acids, which, as previously reported, must correspond to L-configuration of natural amino acids[1,2]. Several methods were developed for the preparation of such compounds[3,4], but the main problem is the separation of fragments with L-aminophosphonic acids since racemic aminophosphonates were used as starting reagents. Recently we have found that phosphonopeptides can be obtained by the enzymatic route using papain as catalyst in medium with high content of organic solvent[5], but the stereoselectivity of the enzyme under these specific conditions has not been estimated.

We demonstrate now that this stereoselectivity can be high and describe successful application of this approach for stereoselective synthesis of antimicrobial agent alafosfalin[1]. The reaction was carried out in a miscible aqueous-organic solvent with low water content, all components being in solution except the enzyme, which was in powdered form. Under these conditions, starting with N-benzyloxycarbonyl-L-alanine (1) and racemic diisopropyl ester of 1-aminoethylphosphonic acid (2), alafosfalin (4) was obtained as shown in the following scheme:



Thus, papain reveals high stereoselectivity in regard to racemic amino component: only L-aminophosphonate is involved in the peptide bond formation. It allows the racemic aminophosphonates to be used for the synthesis of phosphonopeptides with moieties of optically active aminophosphonic acids at P-terminus.

Alafosfalin: Papain (50 mg; 2.2 U/mg) was added to the solution of 0.5 mmol

Z-L-Ala (1), 1.15 mmol racemic diisopropyl ester of 1-aminoethylphosphonic acid (2) and 50 μl 2-mercaptoethanol in the mixture of 2.3 ml acetonitrile and 0.2 ml water. The suspension was shaken for about 2 days until all the Z-L-Ala was consumed (TLC-control). The enzyme was filtered off and washed on the filter with 25 ml of ethyl acetate. The filtrate was washed successively with 10% $KHSO_4$, water, saturated $NaHCO_3$, water, dried with anhydrous Na_2SO_4 and evaporated under reduced pressure. The resulting phosphonopeptide (3)[6] was dissolved in 2 ml of 40% HBr in glacial acetic acid and left overnight. Anhydrous ether (10 ml) was added and the mixture was stirred for 10 min and the upper phase decanted. The residue was evaporated, the remaining gum was dissolved in 2 ml of methanol and treated with excess of propylene oxide. The precipitated material was filtered off and crystallized from water-ethanol to give pure alafosfalin (4), yield 60%, mp 273-276°C dec, $[\alpha]_D^{20}$ -45° (C 0.2, H_2O). Spectroscopic data[7] were in agreement with those reported in the literature[3].

References and Notes

1. J.G.Allen, F.R.Atherton, M.J.Hall, C.H.Hassall, S.W.Holmes, R.W.Lambert, L.J.Nisbet, P.S.Ringrose. Nature, 272, 56 (1978).
2. P.Mastalerz, L.Kupczyk-Subotkowska, Z.S.Herman, G.Laskawiec. Naturwissenschaften, 69, 46 (1982).
3. USA Patent 4016148 (1977); F.R.Atherton, C.H.Hassall, R.W.Lambert. J. Med. Chem., 29, 29 (1986).
4. L.Kupczyk-Subotkowska, P.Mastalerz. Int.J.Peptide Protein Res., 21, 485 (1983).
5. V.A.Solodenko, Yu.V.Mitin, V.P.Kukhar. J.Gen.Chem.(Russ),58, 700 (1988).
6. NMR-^{1}H ($CDCl_3$, 200 MHz), δ, ppm: 1.30 (m, 18H), 4.35 (m, 2H), 4.68 (dq, 2H), 5.09 (s, 2H), 5.72 (d, 1H), 7.01 (d, 1H), 7.33 (s, 5H). NMR-^{31}P - $\{^1H\}$ ($CHCl_3$, 81 MHz): δ_P +24.91 ppm.
7. NMR-^{1}H (D_2O, 300 MHz), δ, ppm: 1.04 (dd, J_{HP} 15 Hz, J_{HH} 6.8 Hz, 3H), 1.28 (d, J_{HH} 6.8 Hz, 3H), 3.78 (m, 2H). NMR-^{31}P-$\{^1H\}$ (D_2O, 81 MHz): δ_P +18.92 ppm. As expected, chemical synthesis starting with compounds (1) and (2) leads to the nearly equimolar mixture of two L-L and L-D diastereomeric phosphonopeptides, which are distinctly distinguishable: two signals with δ_P 18.92 and 19.04 ppm are observed in NMR-^{31}P-$\{^1H\}$ spectrum; in NMR-^{1}H spectrum the alanyl methyl resonance appears as a pair of overlapping doublets with $\Delta\delta$ 0.03 ppm.

(Received in UK 27 September 1989)