Tetrahedron Letters,Vol.27,No.18,pp 2061-2062,1986 0040-4039/86 $3.00 + .00
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IMMOBILIZED BENZYLPENICILLIN ACYLASE: APPLICATION TO THE SYNTHESIS OF OPTICALLY ACTIVE FORMS OF CARNITIN AND PROPRANALOL

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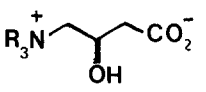
The hydrolysis at pH 7.5 in the presence of immobilized benzylpenicillin acylase of the N-phenacetyl derivatives of the primary amines (3)-(5) affords the hydroxy amines (6)-(8), key intermediates in the synthesis of optically active forms of carnitin and propranalol, of ca. 0.4, 0.3 and 0.8 ee, respectively.

A recent report[1] on the obtainment of (S) norpropranalol through kinetic enzymic resolution of an O-acetate ester by means of a lipase preparation from Pseudomonas induces us to present analogous results, arising from studies on the stereospecificity of the hydrolysis of the N-phenacetyl derivatives of racemic primary amines bearing a β hydroxyl group, catalysed by immobilized benzylpenicillin acylase (EC 3.5.1.11) from E.coli. This enzyme[2], which selectively transfers the N-phenacetyl moiety of benzylpenicillin to water yielding 6-APA, has already been shown to possess a variety of hydrolytic[3] and synthetic[4] capacities. However, in the present interest[5] for new applications in preparative organic chemistry of commercially available enzymes, we started a study on the chemo-[6] and stereospecificity of the above hydrolytic enzyme towards the transformation of substrates which might afford useful intermediates in the synthesis of substances of current interest. The work we are referring on was performed by means of benzylpenicillin acylase immobilized on Eupergit C beads[7], showing a remarkable long term stability.

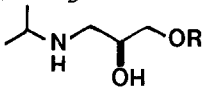
As target molecules we chosed (R) carnitin (1)[8] and propranalol (2)[9], and, accordingly, we submitted to the enzymic hydrolysis, at pH 7.5 and 28 °C, the N-phenacetyl derivatives (3)-(5). The amines and the survived N-phenacetyl derivatives, isolated at ca. 50% conversion, were shown to hold the absolute configuration depicted in formulas (6)-(8) and (9)-(11), respectively.. However, the ee values for the sets (6)-(9), (7)-(10) and (8)-(11) were ca. 0.45, 0.3 and 0.8, respectively. Indeed, the amino nitrile (6), on methylation, followed by

acid hydrolysis, afforded (S) carnitin, $[\alpha]_D^{20}$+12° (c 2, H_2O), (60% yield), whereas the survived N-phenacetyl derivative (9), $[\alpha]_D^{20}$ -4.2° (c 2, $CHCl_3$), and identical material prepared from (6), $[\alpha]_D^{20}$ +3.4° (c 2, $CHCl_3$), once converted into the (+) MTPA esters[10], resulted, on ^1H NMR studies, to be 75:25 and 30:70 mixtures, respectively, of two enantiomers. The H-3 protons in the above derivatives appear at 6 and 5.75 ppm, respectively (90 MHz, $CDCl_3$). The amine (7), obtained in the enzymic hydrolysis of (4), was N-phenacetylated to give, on CN displacement, a nitrile, $[\alpha]_D^{20}$ +2.6° (c 2, $CHCl_3$), enantiomer of (9). Similarly, survived (10), $[\alpha]_D^{20}$ -3.2° (c 1.8, $CHCl_3$), afforded (9), $[\alpha]_D^{20}$-2.8° (c 2, $CHCl_3$). These materials resulted, by the above mentioned NMR method, 65:35 and 35:65 mixtures of two enantiomers, respectively.

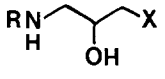
Finally, norpropranalol obtained from (5) was assigned the (R) absolute configuration (8) because it showed $[\alpha]_D^{20}$ +6.6° (c 0.5, EtOH) (lit.[1] for the (S) enantiomer (0.87 ee) -7.3°). The optical purity of (8) was supported by ^1H NMR studies in the presence of tris [3-(heptafluoropropylhydroxymethylene)-d-camphorato] europium (III) onto the N-phenacetyl-O-acetyl derivative. The latter, and identical material prepared from survived (11), resulted to be 90:10 and 10:90 mixtures, respectively, of two enantiomers. Product (11), in two steps [1) H_3O^+, 2) acetone/$NaBH_4$], afforded (S) propranalol (2), $[\alpha]_D^{20}$ -6.5° (c 1, EtOH) (lit.[1] -8.1°), in 65% overall yield.



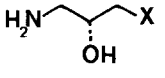
(1) R= Me



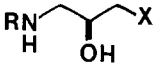
(2) R= 1-naphthyl



(3) R= $COCH_2Ph$; X= CN
(4) R= $COCH_2Ph$; X= Cl
(5) R= $COCH_2Ph$; X= $OC_{10}H_7$



(6) X= CN
(7) X= Cl
(8) X= $OC_{10}H_7$



(9) R= $COCH_2Ph$; X= CN
(10) R= $COCH_2Ph$; X= Cl
(11) R= $COCH_2Ph$; X= $OC_{10}H_7$

Considerations on the factors governing the extent of enantiomer discrimination in the enzymic hydrolysis of (3)-(5) are outside the purposes of the present short note. However, the rate of hydrolysis of (3)-(5) is comparable to that of benzylpenicillin, and much higher than that of the N-phenacetyl derivatives of racemic secondary amines bearing in α a CH_2OR group.

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(Received in UK 3 March 1986)