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IMMOBILIZED PENICILLINACYLASE: APPLICATION TO THE SYNTHESIS OF THE DIPEPTIDE ASPARTAME

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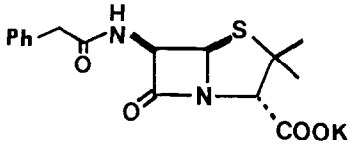
Immobilized penicillinacylase efficently catalyzes the conversion at pH 7.5 of N-phenacetyl aspartame (4) into aspartame (2) and phenylacetic acid.

There is a growing interest in new synthetic applications of enzymes in preparative organic chemistry due to the chemo- and stereospecificity and rate enhancement they can achieve in comparison with non-biologically based systems.[1] Furthermore, an increasing number of enzymes is becoming available commercially, some of which in stable immobilized form for continuous or batchwise operations, thus allowing consideration of these unique catalysts as convenient synthetic reagents. In this context, we refer to the use of a commercial preparation of penicillinacylase (EC 3.5.1.11), immobilized on Eupergit C beads,[2] in the synthesis of the dipeptide sweetener aspartame (2).[3]

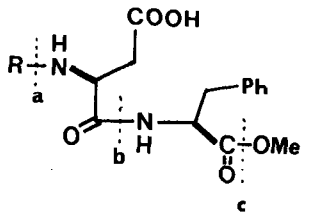
A practical synthesis of aspartame (2) involved[4] opening of N-formyl L-aspartic anhydride with L-phenylalanine methyl ester to form, as key intermediate, N-formyl α-L-asparty1-L-phenylalanine methyl ester (3), which was deformylated upon treatment with methanolic hydrochloric acid to (2), and isolated after removal of methanol and neutralization. The acid catalyzed deformylation of (3) is a delicate step, since it is difficult to devise experimental conditions assuring a precise chemoselectivity of the hydrolysis. In practice, aspartame (2) formed from (3) is accompanied by variable amounts of the bond b and/or c cleavage products, which have to be separated by fractional crystallization.

Thus, it was of interest to replace the formyl group with a nitrogen protecting group which could be selectively removed, possibly by means of an enzyme available at low cost in a reusable, stable form: we thought that penicillinacylase on Eupergit C could be suitable.

The enzyme penicillinacylase selectively transfers[5] the phenacetyl moiety of benzylpenicillin (1) to water and is used[6] in the production of 6-aminopenicillanic acid. Benzylpenicillin (1) can be formally viewed as a N-phenacetyl L-D dipeptide, and we thought that also N-phenacetyl α-L-asparty1-L-phenylalanine methyl ester (4) might be accepted as substrate by



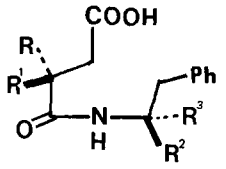
(1)



(2) R= H

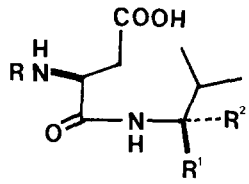
(3) R= CHO

(4) R= $COCH_2C_6H_5$



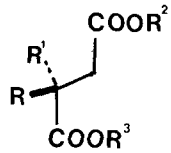
(5) R= R^3= H; R^1= $NHCOCH_2C_6H_5$; R^2= CO_2Me

(6) R= $NHCOCH_2C_6H_5$; R^1= R^2= H; R^3= CO_2Me



(7) R= $COCH_2C_6H_5$; R^1= CO_2Me; R^2= H

(8) R= $COCH_2C_6H_5$; R^1= H; R^2= CO_2Me



(9) R= $NHCOCH_2C_6H_5$; R^1= R^2= R^3= H

(10) R= R^2= R^3= H; R^1= $NHCOCH_2C_6H_5$

(11) R= $NHCOCH_2C_6H_5$; R^1= R^3= H; R^2= $CH_2C_6H_5$

(12) R= $NHCOCH_2C_6H_5$; R^1= R^2= H; R^3= Me

the above enzyme. Indeed, product (4), mp 125 °C (from methanol), $[\alpha]_D^{20}$ -3.7° (c 1, CH_3COOH) (obtained in 75% yield from N-phenacetyl L-aspartic anhydride and L-phenylalanine methyl ester) was hydrolyzed to aspartame (2) and phenylacetic acid at pH 7.5 by penicillinacylase on Eupergit C, at a rate (Table) analogous to that of the natural substrate (1). Once the calculated amount of NaOH solution was taken up, the beads were removed by filtration, the solution was first extracted at pH 3 with Et_2O/THF to remove phenylacetic acid and any unreacted (4), then concentrated at pH 5.4. Aspartame (2) separated on cooling, $[\alpha]_D^{20}$ +32° (c 1, CH_3COOH), in 87% isolated yields. The recovered beads were used for 40 runs without significan loss of activity.

TABLE: Rates of hydrolysis to the corresponding amino derivatives of N-phenacetyl derivatives. 5 mM of substrate in 300 mL of water, pH 7.5, 2 g of Eupergit C beads, carrying penicillinacylase (274 I.U./g), at 28 °C, with mechanical stirring.

entry	substrate	rate
1	benzylpenicillin (1)	1
2	N-phenacetyl L-L aspartame (4)	1
3	N-phenacetyl D-L aspartame (5)	0.38
4	N-phenacetyl L-D aspartame (6)	1.25
5	N-phenacetyl L-L α-aspartyl-valine Me ester (7)	0.2
6	N-phenacetyl L-D α-aspartyl-valine Me ester (8)	0.05
7	N-phenacetyl L-aspartic acid (9)	not hydrolysed
8	N-phenacetyl D-aspartic acid (10)	not hydrolysed
9	N-phenacetyl L-aspartic acid β-benzyl ester (11)	0.25
10	N-phenacetyl L-aspartic acid α-methyl ester (12)	1.1

In order to further define the substrate specificity and reaction stereospecificity, the N-phenacetyl α-aspartyl dipeptides (5)-(8) and the derivatives of L and D-aspartic acid (9)-(12) were prepared and submitted to the action of the immobilized enzyme. Reaction products and rates of hydrolysis of the above set of N-phenacetyl derivatives (Table) indicate the precise reaction specificity (only the phenacetyl group was claeved) and the dependence of the rate of hydrolysis on the nature of the substituent at the α-carboxyl group of aspartic acid and on the absolute configuration of aspartic acid itself (compare entries 4 and 6 — L-D dipeptides — entries 2 and 3 — L-L and D-L dipeptides, respectively — and entries 7 and 10, relative to L-aspartic acid and its α-methyl ester).

Recently, an enzyme-catalysed[7] synthesis of the peptide bond of aspartame (2) from suitably protected amino acids has been reported: if that synthesis proves compatible with

the N-phenacetyl protecting group, a combination of the above procedure with that presently described will allow a synthetic access to aspartame (2) by totally enzymatic methods.

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