Tetrahedron Letters,Vol.29,No.36,pp 4623-4624,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

PHENYLACETYLOXYMETHYLENE, A CARBOXYL PROTECTING GROUP REMOVABLE WITH IMMOBILIZED PENICILLIN ACYLASE, USEFUL IN BENZYL PENICILLIN CHEMISTRY

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Abstract: The phenylacetyloxymethylene group serves to protect the carboxyl group of benzyl-penicillin sulphoxide (**2a**) during the transformation into 7-ADCA and 7β-amino-3-methoxyceph-3-em-4-carboxylic acid (**9**), being removed together with the 7-phenylacetyl side chain by means of immobilized penicillin acylase in a single step.

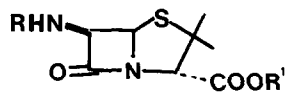
The chemical conversion of penicillin G (**1a**) into the ring expanded products (**3**)-(**5**), key intermediates in the manufacture of therapeutically important cephalosporins, starts off with the sulphoxide (**2a**).[1,2,3] A major problem faced with this synthetic context is the choice of the carboxyl protecting group, which most be resistent to the variety of conditions experienced in the sequence, but removable under conditions enabling the survival of the ceph-3-em nucleus.[4] At the light of the practical importance of the enzymic deacylation of (**1a**) and (**3**) by means of penicillin acylase (EC 3.5.1.11)[5] and of the growing interest for the substitution of chemical processes for milder, reduced-waste enzymic processes in penicillin and cephalosporin chemistry,[6] we thought to devise a protecting group of the carboxyl of (**2a**), useful during the conversion into (**8**) and (**9**) and removeable together with the phenylacetyl side chain in a single enzymic process using penicillin acylase. We now refer on the results of our studies which indicate that the phenylacetyloxymethylene group meets the above requisites.

To this end, the double esters (**1b**), $[\alpha]_D^{25}$ +123° and (**2b**), $[\alpha]_D^{25}$ +169° were obtained in 65% not optimized yield from (**1a**) and (**2a**), respectively, upon treatment with 1.2 mol eq of $C_6H_5CH_2COCCH_2Cl$[7] and Et_3N in DMF at 23 °C over 24 h. The latter material was converted[1,2] into the expanded products (**6**) and (**7**) (m.p. 151°C and 236°C, respectively). Products (**1b**), (**2b**), (**6**) and (**7**) were submitted to the action of penicillin acylase immobilized on Eupergit C beads, in water-acetonitrile (9:1) at pH 7.3 and 23 °C, by dropping an acetonitrile solution of the substrates into the reaction medium at a rate assuring a clear solution. The course of the reaction is measured from the consumption of 1N NaOH, while HPLC analysis indicated the appearance, as transformation products, of 6-APA, 6-APA sulphoxide, 7-ADCA and of (**9**) and phenylacetic acid in 1:2 molar ratio. The relative rate of hydrolysis of (**1b**), (**2b**), (**6**) and (**7**) is 1:3.3:1.5:1.3. At the end of the hydrolysis, phenylacetic acid is recoverd by extraction at pH 3, the individual amino acids being isolated in solid form in 70-90% yield concentrating the aqueous solution at the isoelectric pH. We would expect membrane bioreactors operating in organic solvent-water as most suitable for these transformations.[8] The yield of (**9**) from (**2b**) is ca. 15% (not optimized).

The ability of penicillin acylase to hydrolyze phenylacetyloxymethylene double esters is not limited to the penicillin field. Indeed, racemic products (**10**) and (**11**) afford readily, on penicillin acylase treatment, at pH 7.5, mandelic acid and phenylalanine, respectively. However, the material obtained at 30% conversion resulted devoid of optical activity. Furthermore, the quaternary salt (**12**)[9] afforded slowly the corresponding aminodiol. Again, the

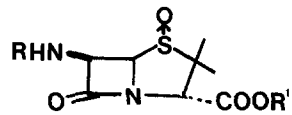
material at 30% conversion was optically inactive. The lack of discrimination in the rate of hydrolysis of the two enantiomers, at variance with other phenylacetate esters,[10] is likely to be due to the distance between the bond to be broken and the chiral center. The formaldehyde formed in the hydrolysis is not affecting the enzymic activity.

The present results thus support the general significance[11] of this industrial enzyme.



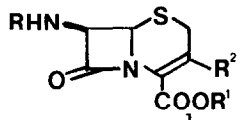
(1a) R= $COCH_2C_6H_5$; R^1= H

(1b) R= $COCH_2C_6H_5$; R^1= $CH_2OCOCH_2C_6H_5$



(2a) R= $COCH_2C_6H_5$; R^1= H

(2b) R= $COCH_2C_6H_5$; R^1= $CH_2OCOCH_2C_6H_5$



(3) R= $COCH_2C_6H_5$; R^1= H; R^2= Me

(4) R= $COCH_2C_6H_5$; R^1= H; R^2= OMe

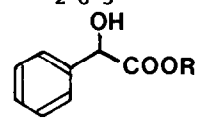
(5) R= $COCH_2C_6H_5$; R^1= H; R^2= Cl

(6) R= $COCH_2C_6H_5$; R^1= $CH_2OCOCH_2C_6H_5$; R^2= Me

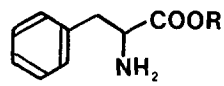
(7) R= $COCH_2C_6H_5$; R^1= $CH_2OCOCH_2C_6H_5$; R^2= OMe

(8) R= R^1= H; R^2= Me

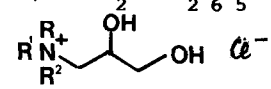
(9) R= R^1= H; R^2= OMe



(10) R= $CH_2OCOCH_2C_6H_5$



(11) R= $CH_2OCOCH_2C_6H_5$



(12) R= $CH_2OCOCH_2C_6H_5$
R^1= $CH_2C_6H_5$; R^2= Me

REFERENCES AND NOTES

$[\alpha]_D^{20}$ measured in $CHCl_3$, c= 1

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(Received in UK 20 July 1988)