Penicillinacylase and α-Chymotrypsin Catalysed Hydrolysis of Phenylacetate and Phenylpropionate Esters of 2,2-Dimethyl-1,3-dioxolane-4-methanols

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Penicillinacylase catalysed hydrolysis of phenylacetate esters affords the methanol derivatives (**1**)—(**9**), of moderate to high optical purity, whereas α-chymotrypsin acting on the phenylpropionate esters provides the alcohols of opposite configuration and low optical purity.

The enzyme penicillinacylase (E.C. 3.5.1.11) selectively transfers the phenylacetyl moiety of benzylpenicillin to water[1] and is used in the production of 6-aminopenicillanic acid.[2] Despite the wide industrial application of this hydrolytic enzyme, little is known about its behaviour towards unnatural substrates. In view of the current interest[3] in new synthetic applications of commercially available enzymes, we recently reported on substrate specificity and reaction stereospecificity of immobilized penicillinacylase in transformations involving the selective hydrolysis of *N*-phenylacetyl protected dipeptide esters[4] and the enantioselective cleavage of the *N*-phenylacetyl moiety present in racemic α-hydroxy primary amides.[5] We report now on the penicillinacylase catalysed enantioselective hydrolysis of the *O*-phenylacetyl moiety in the esters of a set of substituted 2,2-dimethyl-1,3-dioxolane-4-methanols, and on the comparison of these results with those obtained when α-chymotrypsin acts on the phenylpropionate esters of materials of the same type.

The choice of the optically active forms of 4- and/or 5-substituted 2,2-dimethyl-1,3-dioxolane-4-methanols as target molecules is due to the relevance these synthons and the derived aldehydes hold as starting materials in the synthesis of chiral natural products and drugs belonging to quite different structural classes. Furthermore, the above materials are chemically related to epoxyalcohols, whose preparation in optically active form through enzymatic hydrolysis has been recently reported.[6] Thus, the hydrolysis of the phenylacetyl esters of racemic (**1**)—(**9**) proceeds at pH 7.5 and 23 °C in water, containing 10% MeCN, at 50 mmol/l, at a rate analogous to that of benzylpenicillin in the presence of a commercial preparation of immobilized penicillinacylase,† which was eventually recovered without noticeable loss of activity.

The assignment of the absolute stereochemistry depicted in structures (**1**)—(**9**) (Table 1) to the products of hydrolysis is based on optical rotation measurements and comparison with authentic samples. However, the (2*S*,3*R*) structure (**5**) was assigned to the product of hydrolysis because the material obtained at *ca.* 30% conversion, $[\alpha]_D^{20}$ −19.5° (*c* 2, $CHCl_3$), on oxidation (oxalyl chloride, Me_2SO, Et_3N, −78 °C) and condensation with $Ph_3P{=}CHCO_2Et$ afforded the (4*S*,5*R*) ester (**10**), $[\alpha]_D^{20}$ +4.8° (*c* 4, $CHCl_3$), in 65% overall yield. Its (4*R*,5*S*) enantiomer, prepared from L-rhamnose in the synthesis of (+)-citreodiol, showed $[\alpha]_D^{20}$ −4.6°.[7] Similarly, the alcohol (**8**), $[\alpha]_D^{20}$ +31.2° (*c* 2, $CHCl_3$), at 30% conversion, was assigned the (2*R*,3*S*) stereochemistry because, on acid hydrolysis it afforded 1,2-dideoxy-L-*erythro*-pentitol,

Table 1. Absolute configuration and enantiomeric excess values for the products of hydrolysis at 50% conversion with penicillinacylase and α-chymotrypsin of phenylacetate and phenylpropionate esters.

Penicillinacylase		α-Chymotrypsin	
Product	E.e.	Product	E.e.
(2*S*)-(**1**)[a]	0.6[f]	(2*R*)-(**1**)	0.15
(2*S*)-(**2**)[b]	0.9[f,g,i]	(2*R*)-(**2**)	0.1
(2*S*,3*S*)-(**3**)[c]	0.5[f,h]	(**3**)	0[j]
(2*S*,3*S*)-(**4**)[d]	0.52[f,h]	—	—
(2*S*,3*R*)-(**5**)	0.65[i]	(2*S*,3*R*)-(**5**)	0.2
(2*R*)-(**6**)[e]	0.33[f,h]	—	
(2*R*,3*S*)-(**7**)	0.46[f,h]	(2*S*,3*R*)-(**7**)	0.33
(2*R*,3*S*)-(**8**)	0.8[h]	(2*S*,3*R*)-(**8**)	0.45
(2*R*,3*S*)-(**9**)	0.9[h]	(2*S*,3*R*)-(**9**)	0.32

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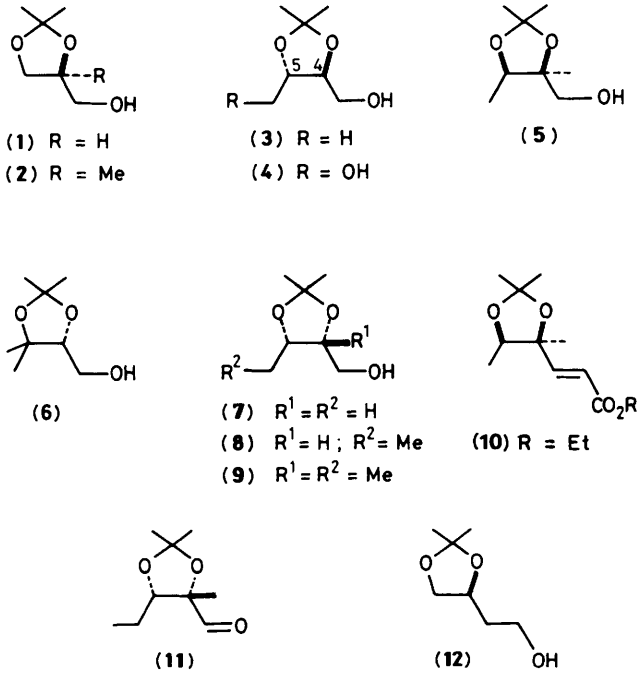

† Immobilized on Eupergit C (Röhm Pharma, D-6100 Darmstadt), oxirane activated acrylic beads.

$[\alpha]_D^{20}$ +6.35° (*c* 1, H_2O) (lit.[8] +6.2°), in 70% yield. Finally, the alcohol (**9**), obtained under the same conditions, was converted, as above, into the aldehyde (**11**), $[\alpha]_D^{20}$ +10.7° (*c* 1, EtOH) (lit.[9] +11.9°).

The enantiomeric excess (e.e) values at 50% conversion, as measured from the consumption of 1 M NaOH, of products (**1**)—(**9**) (Table 1) and of the residual esters which had not been hydrolysed were determined by optical and/or n.m.r. methods on the appropriate derivative, as indicated. There is an inversion of the absolute configuration of the products of hydrolysis on going from the alcohols (**1**)—(**5**) to (**6**)—(**9**), the higher e.e. values being observed for the 4-methyl substituted materials (**2**) and (**9**). However, the absolute stereochemistry is apparently dependent upon the nature of the 5-substituent(s) and on the relative stereochemistry at positions 4 and 5.

We then decided to compare the above results, obtained with penicillinacylase which holds a specific binding site for phenylacetate, with those accessible with α-chymotrypsin, bearing a binding site for phenylpropionate.[10] To this end, the phenylpropionate esters of racemic (**1**)—(**3**), (**5**), and (**7**)—(**9**) were submitted to the action of α-chymotrypsin, at pH 7.5, under the conditions used for penicillinacylase. Apart from optically inactive (**3**) and (2*S*,3*R*)-(**5**), the products of hydrolysis (Table 1) have an absolute stereochemistry opposite to that observed with penicillinacylase. Furthermore, the e.e. values at 50% conversion (Table 1) were rather low, the higher figures being observed with the 4,5-*erythro* products (**7**)—(**9**).

The temperature influences the e.e. values of the products obtained with penicillinacylase; for product (**7**), the e.e. changes from 0.51 to 0.38 on going from 0 to 40 °C. Similarly, for compound (**3**) at 23 °C, the e.e. value increases from 0.5 to 0.64 when MeCN in the reaction medium is replaced by 10% glycerol.[11] Furthermore, with penicillinacylase acting on the racemic phenylacetate, (2*S*)-(**12**) was obtained with 0.28 e.e., as determined from the optical rotation of the derived 3,5-dinitrobenzoate.[12] Comparison of this result with that obtained with the lower homologue (**1**) indicates a decrease of optical purity on introduction of an additional CH_2 group between the bond to be broken and the asymmetric carbon atom. Finally, the phenylacetate of glycidol (2,3-epoxypropan-1-ol) was hydrolysed rapidly by penicillinacylase, but the ester remaining at 50% hydrolysis was devoid of optical activity. It is interesting to note that lipase-catalysed hydrolysis of the butyrate of glycidol and of 2,2-dimethyl-1,3-dioxolane-4-methanol leads at 60% conversion to residual esters of *ca.* 0.9 and 0.4 e.e., respectively.[6] Apart from the mechanistic interest of the results obtained with these two hydrolytic enzymes, the present work illustrates the synthetic potential of immobilized penicillinacylase in the preparation of optically active chiral synthons. Experiments designed to increase the e.e. values according to known principles[11,13] are in progress.

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