Tetrahedron: Asymmetry Vol. 3, No. 1, pp. 65-72, 1992
Printed in Great Britain

0957-4166/92 $3.00+.00
Pergamon Press plc

ENZYMATIC RESOLUTION OF 2,2-DISUBSTITUTED-1,3-DIOXOLANE-4-METHANOL CARBOXYLIC ESTERS

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(*Received* 19 *November* 1991)

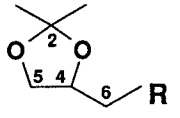
Abstract: The enantioselectivity of enzymatic hydrolysis of carboxylic esters of various 1,2-ketals of glycerol has been investigated. The influence of the ketal group has been studied. A number of lipases and proteinases have been tested and the best enantioselectivity was obtained with proteinase from *Aspergillus oryzae* which gave an *E*-value of 9 with 2,2-dimethyl-1,3-dioxolane-4-methanol butanoate. Variations in the acyl part revealed that butanoyl was optimal. All hydrolysis products have been synthesised in homochiral forms from homochiral starting materials.

INTRODUCTION

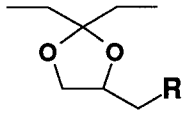
Apart from taking advantage of the socalled chiral pool, there are in principal two different ways to obtain homochiral (optically pure) compounds. That is by asymmetric synthesis from prochiral starting materials, or by resolution of racemic mixtures. Asymmetric synthesis will ideally yield 100% of one enantiomer while racemate resolution will at best give 50% of each enantiomer. In the present paper we report our results with the aim of obtaining homochiral C-3 compounds by racemate resolution.

Homochiral C-3 compounds are important starting materials for the preparation of chemicals for use in agriculture[1] and valuable intermediates for synthesis of various biologically active compounds such as β-blockers.[2] Also since they generally have great potential as synthons there has been a considerable interest in the synthesis of homochiral C-3 compounds. In particular due to their versatility, homochiral epoxyalcohols like glycidol (oxiranemethanol) has been the target for many workers in the field. An extensive review covering in particular chemical

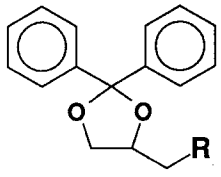
methods has recently been published.[3] Enzymatic methods utilizing lipase catalysed hydrolysis have been patented.[4] An extensive screening has been undertaken in order to find enzymes that stereoselectively hydrolyse 2-benzylglyceroldiacetate. For the synthesis of *S*-monoacetate hydrolytic enzymes from *Aspergillus fumigatus* and *Mucor javanicus* were successful.[5] Similar glycerol derivatives were obtained in excellent optical purity by lipase catalysed acetylation with



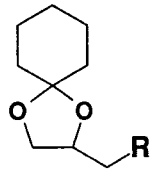
1 R = OH
2 R = $OCOC_3H_7$
3 R = Cl



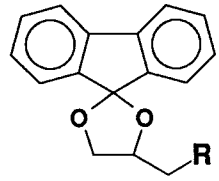
4 R = OH
5 R = $OCOCH_3$
6 R = $OCOC_3H_7$
7 R = $OCOC_7H_{15}$
8 R = $OCOC_{15}H_{31}$
9 R = Cl



10 R = OH
11 R = $OCOC_3H_7$
12 R = Cl



13 R = OH
14 R = $OCOC_3H_7$
15 R = Cl



16 R = $OCOC_3H_7$
17 R = Cl

vinylacetate.[6] Derivatives of *(R)*-2,2-disubstituted-1,3-dioxolane-4-methanol (isopropylidene glycerol) have been prepared from the racemate by microbial consumption of one enantiomer.[7] Lipase catalysed esterification of various 1,2-ketals of glycerol with succinic anhydride gave products in good chemical yield and enantiomeric excess.[8] A bioreactor system for optical resolution of racemic 2,2-dimethyl-1,3-dioxolane-4-methanol butanoate **(2)** has also been suggested.[9]

RESULTS AND DISCUSSION

We report here the enzymatic resolution of 2,2-dimethyl-1,3-dioxolane-4-methanol carboxylic esters by enzyme catalysed partial hydrolysis with the lipases Amano P and Finnsugar K 30, and the proteinases Subtilisin A and proteinase from *Aspergillus oryzae* in water which was kept at

constant pH by titration with N NaOH by means of a pH-stat apparatus. In all cases when hydrolysis took place, the *(S)*-alcohols were formed in excess. The ketal-ester substrates were synthesized from 3-chloro-1,2-propanediol by use of the appropriate ketone followed by esterification. In order to identify the enantiomers formed in the enzyme catalysed reactions, the *(S)*-enantiomers of **4, 10** and **13** were prepared starting with *(R)*-3-chloro-1,2-propanediol.

In enzyme catalysed resolutions of racemates, the most crucial factor for success is the enantioselectivity factor *E* which is the ratio k_{cat}/K_m of the two enantiomers. A high *E* for a given substrate/enzyme combination ensures a high *ee* combined with a high chemical yield. In kinetic resolutions, the *E*-value may be calculated when the degree of conversion is known and the enantiomeric excess (*%ee*) of the substrate remaining fraction or the product fraction is measured. If one is comparing pure enantiomers, k_{cat}/K_m for each enantiomer must be determined in order to allow a valid calculation of *E*.[10] In the present work *E*-values were

Enzyme	Conversion (%)	Reaction time (h)	Yield[a]	$[\alpha]_D$ hexane	*ee*(%)[b]	*E*
Amano P lipase	40 60	0.4 0.6	8	+3.1	46 68[c]	3.5 5.1
Proteinase from *Aspergillus oryzae*	40 60	1 1.5	10	+4.0	65 84[c]	7.2 9.0
Subtilisin	40 72	2 3.6	12	+3.4	51 76[c]	4.1 3.8

Table 1. Enzymatic hydrolysis of 2,2-dimethyl-1,3-dioxolane-4-methanol butanoate **(2)**. a) Measured on isolated alcohol, b) GLC on chiral column and optical rotation of produced alcohol, c) as for b), but on unreacted ester.

calculated on the basis of the degree of conversion and *%ee* of the produced alcohol, assuming irreversible conditions. In one case, with **2** , the *ee* of the remaining substrate ester was also analysed.

We have varied the i) enzymes for one single substrate, ii) the acyl part and iii) the ketal group, and the three sets of data for enzymatic hydrolyses are reported in Tables 1, 2 and 3 respectively. In Table 1 are presented the results of the hydrolysis of 2,2-dimethyl-1,3-dioxolane-4 methanol butanoate **(2)** by three different enzymes, one lipase and two proteinases. The best results were obtained with the proteinase from *Aspergillus oryzae* which gave a maximum *E*-value of 9.

In another set of experiments we have varied the acyl part of the ester moiety for a series of diethyl ketals. Four different esters: acetyl, butanoyl, octanoyl and hexadecanoyl, were tested and the results are given in Table 2. Neither of the enzymes were able to hydrolyse the C-16 ester. This observation, which accords with previous results,[4] is probably due to the longest ester being

Sub-strate	Enzyme	Conver-sion,%	Reaction time (h)	Yield[a]	$[\alpha]_D$[b]	*ee*(%)[c]	*E*
5	Amano P lipase	42	26			10	1.3
	Aspergillus oryzae	35	46			10	1.3
	Subtilisin	31	50			10	1.1
6	Amano P lipase	40	0.5	88	+2.5	19	1.7
	Aspergillus oryzae	40	2	50	+5.6(H)	58	5.1
		30	1.5	45	+8.6	65	6.0
		20	1	31	+9.2	67	6.0
	Finnsugar K30	45	1	63	+2.3(H)	24	1.9
	Subtilisin	31	3	25	+4.2(H)	44	3.1
7	*Aspergillus oryzae*	20	68	25	+4.4	35	2.3
8	Amano P lipase	-					
	Aspergillus oryzae	-					
	Subtilisin	-					

Table 2. Enzymatic hydrolysis of 2,2-diethyl-1,3-dioxolane-4-methanol acetate **(5)**, butanoate **(6)**, octanoate **(7)** and hexadecanoate **(8)**. a) Measured on isolated alcohol, b) In MeOH, concentration 0.01, (H) in hexane, c) GLC on chiral column and optical rotation of produced alcohol.

unacceptable as a substrate by these enzymes. It is however, remarkable that a lipase behaves in this way and it can therefore not be excluded that the negative results are due to lack of contact between the enzyme and the hydrophobic substrate.

Also the short acetate ester and the medium sized C-8 ester were not good substrates. By far the best results, both with respect to reactivity and enantioselectivity were obtained with the butanoate. An enantioselectivity $E = 6$ was obtained after only 1 - 1.5 h reaction time.

Finally we have investigated the influence of the nature (size) of the ketal group. The butanoates of the glycerol ketals of benzophenone (diphenyl) **(11)**, cyclohexanone (a spiro compound) **(14)** and fluorenone **(16)** were hydrolysed. The results are presented in Table 3. The bulkyest of them

16, was not hydrolysed at all. The spiro ketal was hydrolysed by all the enzymes, but the enantioselectivity was poor to medium. The diphenyl ketal however, gave good results with the Amano lipase, but was not accepted by any of the other three enzymes. The results from Table 3 may also be compared with the results with **2** and **6** (Tables 1 and 2 respectively) which also are butanoates, but with even less bulky ketal groups. It seems that smaller ketal groups give faster reactions. The dimethyl ketal **2** was hydrolysed to 60% after 1.5 h with the proteinase from *Aspergillus oryzae* to give 84 % *ee* (unreacted ester) and as previously mentioned an enantioselectivity of 9. In order to improve the *ee* of the produced alcohol it is possible to reesterify the product and hydrolyse it over again.[10]

Sub-strate	Enzyme	Conver-sion,%	Reaction time (h)	Yield[a]	$[\alpha]_D$[b]	*ee*(%)[c]	*E*
11	Amano P lipase	40	5	80	+15.3	68	8.1
		20	2.5	46	+15.5	69	6.4
	Aspergillus oryzae	-					
	Finnsugar K30	-					
	Subtilisin	-					
14	Amano P lipase	40		47	+2.7	40	2.9
	Aspergillus oryzae	40	3	77	+3.6	53	4.5
		20	1	30	+4.6	68	6.2
	Subtilisin	40	5	70	+2.8	41	3.1
		20	2.5	35	+3.1	46	3.0
16	Amano P lipase	-					
	Aspergillus oryzae	-					

Table 3 Enzymatic hydrolysis of the butanoates **11, 14** and **16**. a) Measured on isolated alcohol, b) In MeOH, concentration 0.01, c) GLC on chiral column and optical rotation of produced alcohol.

ACKNOWLEDGEMENTS

We are grateful to Novo-Nordisk, Bagsværd, Denmark for a gift of pure subtilisin, and The Royal Norwegian Council for Scientific and Industrial Research, NTNF and to Borregaard Fine Chemicals, Sarpsborg, Norway for financial support.

EXPERIMENTAL

Enzymes. The enzymes subtilisin, Amano P lipase and Finnsugar K30 were gifts from Novo-Nordisk, Amano Pharmaceutical Co and Finnsugar respectively. The proteinase from *Aspergillus oryzae* was purchased from Sigma Chemical Co.

Analytical Methods. NMR spectroscopy was performed on a Bruker WM-400 and Jeol EX 400 at 400 MHz for ^1H using $CDCl_3$ as solvent. Chemical shifts are given in ppm rel. TMS and coupling constants in Hz. The enantiomeric excess *(ee)* of the alcohol was determined by GLC using a Varian 3400 instrument equipped with a chiral column, CP cyclodex β 236-M-19, and by optical rotation using a Optical Activity Ltd. AA-10 Automatic polarimeter. Concentrations (c) are given in g/ml. Homochiral enantiomers of 1,2-isopropylidene glycerol [CAS. Reg. No. 100-79-8] were purchased from Sigma, $[\alpha]_D^{20}$ = +6.7 (c = 0.0225 in hexane) for the *(S)*-form.

General experimental procedure for enzymatic hydrolysis. The carboxylic esters of 2,2-disubstituted-1,3-dioxolane-4-methanol (5mmol) were suspended in water (100 ml). The enzymes lipase Amano P (200 mg), proteinase from *Aspergillus oryzae* (100 mg), lipase Finnsugar K 30 (100 mg) or subtilisin (20 mg) was added and the reaction mixture was stirred at room temperature. The enzymatic hydrolysis was monitored by a pH-stat consisting of a Radiometer PHM 64 pH meter and a Metrohm Herisau Dosimat pump. Addition of N NaOH solution was controlled by a Copam PC. The hydrolysis was stopped by repeated extraction with diethyl ether and the alcohol was separated from the unreacted ester by column chromatography (silica gel, hexane : acetone = 4 : 1).

2,2-Dimethyl-1,3-dioxolane-4-methanol butanoate **(2)** was prepared by stirring at roomtemp. 1,2-isopropylidene glycerol **(1)** (75.7 mmol) with butanoic anhydride (227 mmol) in pyridine (25g) for 1 hr. Distillation afforded **2**, bp_{10}: 110-112°C, yield 63%.

(S)-*2,2-Dimethyl-1,3-dioxolane-4-methanol butanoate* **(*S*-2)** was prepared from *(R)*-1,2-isopropylidene glycerol (*R*-**1**) as for *rac.***2**, $[\alpha]_D^{20}$ = -14,4 (c = 0,0087 in hexane).

4-[Chloromethyl]-2,2-diethyl-1,3-dioxolane **(9)** was prepared by heating 3-chloro-1,2-propanediol (0,3 mol) with 3-pentanone (0,25 mol) and toluene-4-sulphonic acid monohydrate (3,7 mmol) in toluene (100 ml) in a Dean-Stark trap. The reaction mixture was extracted with Et_2O, the organic layer washed with dilute NaOH and H_2O and dried over $MgSO_4$. Distillation gave **9**, yield 66%, bp_{10}: 68-70 °C.

4-[Chloromethyl]-2,2-diphenyl-1,3-dioxolane **(12)** was prepared from 3-chloro-1,2-propanediol, benzophenone and toluene-4-sulphonic acid monohydrate as described for **9**. Crystallization from MeOH afforded **12** as a white solid, yield 64%, mp 40 °C.

2-[Chloromethyl]-1,4-dioxaspiro[4.5]decane **(15)** was prepared from 3-chloro-1,2-propanediol, cyclohexanone and toluene-4-sulphonic acid monohydrate in toluene (100 ml) as described for **9**. Distillation gave **15**, yield 62%, $bp_{0,5}$: 44-46°C.

2-[Chloromethyl]-spiro[1,3-dioxolane-5,9'-[9H]-fluorene] **(17)** was prepared from 3-chloro-1,2-propanediol, fluorenone, toluene-4-sulphonic acid monohydrate in toluene as decribed for **9** Column chromatography (Silica gel, hexane : acetone = 4 : 1) afforded **17** as a colourless viscous oil, yield 20%.

2,2-Diethyl-1,3-dioxolane-4-methanol butanoate **(6)** was prepared from **9** (22 mmol), sodium butanoate (67 mmol) and hexamethyl phosphoramide (80 ml) by heating at 140°C for 90 min. Light petroleum was added, the mixture was washed with NH_4OH soln. (0,5 mol/l) in MeOH : H_2O = 3 : 1 (v/v) and dried over $MgSO_4$. Distillation afforded **6**, bp_{10}: 130°C, yield 92%. The esters **5**, **7** and **8** were prepared as described for **6** from **9** and the sodium salts of acetic, octanoic and hexadecanoic acids respectively.

2,2-Diphenyl-1,3-dioxolane-4-methanol butanoate **(11)** was prepared from **12**, sodium butanoate, and hexamethyl phosphoramide as described for **6**. Workup as above afforded **11** as a colourless oil, yield 78%.

1,4-Dioxaspiro[4.5]decane-2-methanol butanoate **(14)** was prepared from **15**, sodium butanoate and hexamethylphosphoramide as described for **6**. Distillation afforded **14**, $bp_{0,5}$: 90-92°C, yield 88%.

Spiro[1,3]dioxolane-5,9'-[9H]-fluorene]-2-methanol butanoate **(16)** was prepared from **17**, sodium butanoate and hexamethyl phosphoramide as for **6**. Column chromatography (as for **17**) afforded **16** as a colourless oil, yield 20%.

(S)-*2,2-Diethyl-1,3-dioxolane-4-methanol* **(S-4)**. *(R)*-**9** was prepared from *(R)*-3-chloro-1,2-propanediol as described for racemic **9**, $[\alpha]_D^{20}$ = +38.6 (c = 0.0114 in CH_2Cl_2). *(R)*-**9** was transformed into *(R)*-**6** as described for *rac.* **6**, $[\alpha]_D^{20}$ = +15.6 (c = 0,0115 in hexane). The homochiral *(R)*-**6** was finally hydrolysed by heating with 10% KOH/MeOH to yield the homochiral alcohol *(S)*-**4**, $[\alpha]_D^{20}$ = +9.6 (c = 0,0147 in hexane), $[\alpha]_D^{20}$ = +13.5 (c = 0,0140 in MeOH). ^1H NMR: 0.905 t, 0.921 t, 1.640 q and 1.678 q , J = 7.32 (2 different ethyl groups), 1.94 (1H, br. s, OH), 3.602 (1H, dd, H-6a, $J_{4,6a}$ = 5.37, $J_{6a,6b}$ = 11.72), 3.760 (1H, dd, H - 6b, $J_{4,6b}$ = 3.42), 3.741 (1H, app.t, H-5a, J = 7.6), 4.046 (1H, dd, H-5a, J = 7.8 and 6.8), 4.218 (1H, m, H - 4).

(S)-*2,2-Diphenyl-1,3-dioxolane-4-methanol* **(S-10)**. *(R)*-**12** was prepared from *(R)*-3-chloro-1,2-propanediol as described for racemic **12**, $[\alpha]_D^{20}$ = +37.1 (c = 0.0114 in CH_2Cl_2). *(R)*-**12** was further

transformed into*(R)*-**11** as described for *rac.* **11**, $[\alpha]_D^{20}$ = +24.7 (c = 0,00196 in hexane). The homochiral *(R)*-**11** was finally hydrolysed by heating with 10% KOH/MeOH to yield the homochiral alcohol *(S)*-**10**, $[\alpha]_D^{20}$ = +22.5 (c = 0.0036 in MeOH). ^{1}H NMR: 1.82 (1H, br. s, OH), 3.641 (1H, dd, H-6a, $J_{4,6a}$ = 5.4, $J_{6a,6b}$ = 11.7), 3.813 (1H, dd, H - 6b, $J_{4,6b}$ = 3.4), 3.991 (1H, app.t, H-5a, J = 7.6), 4.034 (1H, app.t, H-5a, J = 7.6), 4.332 (1H, m, H - 4), 7.23 - 7.55 (10H, Ph).

(S)-*1,4-Dioxaspiro[4.5]decane-2-methanol* (*S*-**13**). *(R)*-**15** was prepared from *(R)*-3-chloro-1,2-propanediol as described for racemic **15**, $[\alpha]_D^{20}$ = +28.0 (c = 0.0019 in CH_2Cl_2). *(R)*-**15** was further transformed into *(R)*-**14** as described for *rac* **14**, $[\alpha]_D^{20}$ = +12.5 (c = 0.00138 in hexane). The homochiral *(R)*-**14** was finally hydrolysed by heating with 10% KOH/MeOH to yield homochiral alcohol *(S)*-**13**, $[\alpha]_D^{20}$ = +6.8 (c = 0.0118 in MeOH) lit.[11]: +7.1 (c = 5, MeOH). ^{1}H NMR: 1.41 (2H, br. m), 1.61 (8H, br. m, cyclohex), 1.93 (1H, br. s, OH), 3.582 (1H, dd, H-6a, $J_{4,6a}$ = 5.37, $J_{6a,6b}$ = 11.72), 3.745 (1H, dd, H- 6b, $J_{4,6b}$ = 3.41), 3.792 (1H, app.t, H-5a, J = 7.6), 4.036 (1H, dd, H-5a, J = 7.8 and 6.8), 4.238 (1H, m, H- 4).

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