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ENZYMATIC ACYLATION USING ACID ANHYDRIDES: CRUCIAL REMOVAL OF ACID

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Abstract: An efficient enzymatic resolution of 7,7-disubstituted 1,4,5,6-tetrachlorobicyclo[2.2.1]hept-5-en-2-ols was accomplished by means of lipase AY-30 from *Candida cylindracea* in toluene. When acid anhydrides were used as acyl donors, the enantioselectivity was found to depend strongly on the reaction conditions: Whereas low selectivity (E <20) was observed without precautions taken in order to remove the co-produced acid, a more than ten fold improvement was achieved with addition of a weak base (E >200). Alternatively, adsorption of the biocatalyst onto Celite was equally effective (E >300). Complete specificity was obtained when vinyl acetate was used as acyl donor (E ~1000).

INTRODUCTION

Enzyme catalyzed acylation in organic media[1] has been shown to be advantageous over hydrolytic reactions in particular due to the following reasons:

i) Possible change of the enantioselectivity[2-6],
ii) successful transformation of lipophilic substrates being poorly soluble in aqueous systems[7],
iii) better overall yields since loss-causing extractive workup is avoided,
iv) lack of undesired side-reactions requiring water such as racemisation[9],
v) no need for immobilisation since enzymes can be recovered by simple filtration from the lipophilic media,
vi) enhanced stability of enzymes[10] and
vii) a negligible risk of microbial contamination.

In order to avoid the unfavourable equilibrium situation in trans- and interesterification reactions causing slow reaction rates[11] and low optical purity of products[4], special acyl donors making the acyl-transfer completely irreversible have recently been employed:

a) Enol esters[12],
b) oxime esters[13], and
c) acid anhydrides[3].

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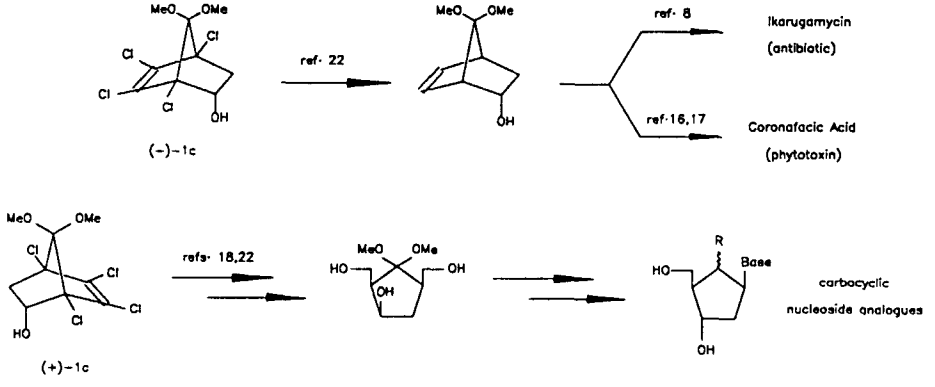
Whereas the first of these methods has already gained widespread application using vinyl acetate[14], the limited availablilty of oxime esters still represents an impedement for method b. Acid anhydrides, however, can readily be used as easily available acyl donors for enzyme catalyzed esterifications.

Aiming to compare the applicability of enol esters and acid anhydrides we investigated the enzymatic resolution of the tetrachlorobicyclo[2.2.1]-heptanols (±)-1a - (±)-1c. With respect to these particular substrates hydrolytic conversions failed due to the complete insolubility of the corresponding acetates (±)-2a - (±)-2c in water[15]. As shown in scheme 1, both enantiomers of 1c can be used as building blocks for the synthesis of antibiotics[8], phytotoxins[16,17] and functionalized carbocyclic nucleoside analogues[18].

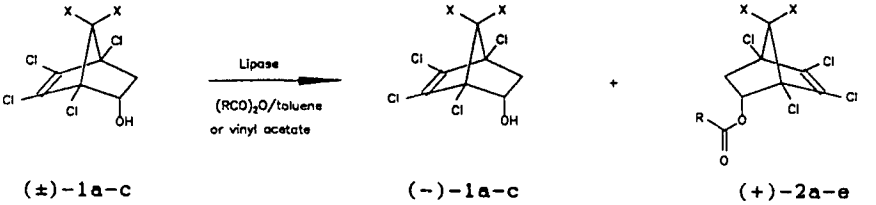
Scheme 1: Synthesis of bioactive compounds



RESULTS AND DISCUSSION

In order to test the influence of various acid anhydrides on the enantioselectivity of the enzyme, (±)-1c was subjected to enzymatic acylation in toluene.

Scheme 2: Enzymatic acylation



1	a	b	c
X	H	Cl	OCH_3

2	a	b	c	d	e
X	H	Cl	OCH_3	OCH_3	OCH_3
R	CH_3	CH_3	CH_3	n-C_3H_7	i-C_3H_7

Table 1: Enzymatic acylation of (±)-1c using acid anhydrides

Enzyme[19]	$(RCO)_2O$ R =	Base	Conversion [%]	Alcohol[a] e.e.[%]	Ester[a] e.e.[%]	E[20]
GC-4	CH_3	none	42	56	(+)-2c 77	13
	n-C_3H_7		55	(-)-1c 52	(+)-2d 41	4
	i-C_3H_7		55	74	(+)-2e 60	9
AY-30	CH_3	none	54	87	74	19
		2,6-lutidine	47	86	97	180
		$KHCO_3$	45	(-)-1c 80	(+)-2c 98	240
		$KHCO_3$/18-cr-6	42	47	66	8
AY-30 on celite		none	45	80	99	490

[a] For absolute configuration see scheme 2.

As shown in table 1, *Geotrichum candidum* lipase (GC-4) exhibited a relatively low enantioselectivity on (±)-1c using different acid anhydrides, acetic anhydride being the best. With *Candida cylindracea* lipase (AY-30) the enantiomeric ratio[20] (E) remained moderate as well. Addition of dissolved organic or suspended inorganic base, however, resulted in a more than ten-fold improvement (E ~200). An attempt to increase the moderate reaction rate of the highly selective heterogeneous $KHCO_3$-system by adding 18-crown-6 led to a substantial drop in selectivity (E = 8), caused by concomitant chemical - and hence nonselective - acylation catalyzed by solubilized bicarbonate. This assumption was proven *via* an independent experiment in the absence of enzyme. In all of the other acylating systems, no chemical acylation - a prerequisite for a high optical purity of products - could be observed. Even better results were obtained when lipase AY-30 was adsorbed onto Celite 145[3] (E ~500).

From these results we conclude that removal of the carboxylic acid formed as co-product when acid anhydrides are used as acyl donors seems to be essential in order to avoid a substantial drop in enzyme selectivity. For this purpose, addition of base can be almost equally effective as an adsorption of the enzyme onto diatomaceous earth. In the latter system one can assume that the acid is bound by metal oxides present in the carrier.

For comparison of different techniques, alcohol (±)-1c was acylated using vinyl acetate both as solvent and as acyl donor[12]. As shown in table 2, (±)-1c could completely be resolved with both lipases GC-4 and AY-30, the latter leading to an enantiomeric ratio (E) of about 1000.

When this process was repeated several times with 200g-batches of (±)-1c using recovered lipase AY-30, a substantial loss in enzyme activity was observed. A detailed study on this phenomenon is in progress.

Table 2: Enzymatic acylation using vinyl acetate

Substrate	Enzyme[19]	Conversion [%]	Alcohol[a] e.e. [%]		Ester[a] e.e. [%]		E[20]
(±)-1a	AY-30	51	(-)-1a	99	(+)-2a	97	350
(±)-1b	AY-30	43	(-)-1b	70	(+)-2b	95	80
(±)-1c	GC-4	49	(-)-1c	94	(+)-2c	99	710
(±)-1c	AY-30	50	(-)-1c	98	(+)-2c	>99	~1000

[a] For absolute configuration see scheme 2.

A change of the substitutional pattern in the 7-position leading to the 7,7-dichloro derivative (±)-1b gave acceptable selectivities, and the 7,7-unsubstituted alcohol (±)-1a was well resolved again with E >300. Regardless of the acyl donor used both lipases from *Candida cylindracea* (AY-30) and *Geotrichum candidum* (GC-4) exhibited the same enantiospecificity by preferring the substrates which possess an *R*-configurated alcoholic center, a tendency which was expected from our previous experience[21].

The absolute configuration of alcohols 1a-1c was determined as follows: (-)-1c was dehalogenated[22] to give (-)-(1*S*,2*S*,4*S*)-7,7-dimethoxybicyclo-[2.2.1]hept-5-en-2-ol with known configuration[23]. Since the analogous reduction of 1a and 1b leading to *endo*-norborn-5-en-2-ol proceeds sluggishly[22], their absolute configuration was elucidated by CD-measurements of the corresponding hemiphthalates. The characteristic Cotton effect for hemiphthalates at about 244 nm was negative for the derivative of (+)-1a and positive for both the derivatives of (-)-1b and (-)-1c which correlates well with the absolute configuration of (-)-1c proven independently.

CONCLUSION

Vinyl acetate and acid anhydrides both proved to be useful acyl donors for enantioselective enzymatic acylation of substrates which could not be transformed in hydrolytic reactions due to their strong lipophilic character. To preserve a high selectivity of the enzyme, removal of the co-produced acid was essential when acid anhydrides were used. This could be achieved almost equally well with either addition of base or by adsorption of the biocatalyst onto celite.

EXPERIMENTAL

General

Preparative column chromatography was performed on silica gel 60 (230-400 mesh, Merck). For TLC Merck silica gel 60 F_{254} plates were used. Compounds were visualized by spraying with vanilline/conc. H_2SO_4 and heat treatment. GLC analyses were performed on a Dani 8500 chromatograph (J&W capillary column DB 1701, 30m x 0.25mm, 0.25µm film, N_2) equipped with

FID. ^{1}H-NMR spectra were recorded on a Bruker MSL 300 (300 MHz) in $CDCl_3$. Chemical shifts are reported from TMS as internal standard in ppm (δ-scale) and coupling constants (J) in Hz; s=Singlet, d=doublet. Elemental analyses (C, H, Cl) of all novel compounds were within 0.5% of calculated values. All commercially obtained compounds were used as received and enzyme preparations were employed without further purification. The following abbreviations for enzymes were used: *Candida cylindracea* lipase Amano AY-30 (AY-30) and *Geotrichum candidum* lipase Amano GC-4 (GC-4).

Synthesis of Substrates

Acetates (±)-2a-c were obtained by Diels Alder addition of the corresponding cyclopentadiene derivative to excess vinyl acetate (reflux, 4d) following literature procedures[24,25]. *Endo*-acetate (±)-2a was purified from minor accompanying *exo*-isomer[25] (~25%) by column chromatography (petroleum ether/ethyl acetate 20:1). (±)-2a: mp 52-3°C; (±)-2b: mp 42-3°C, bp 98-107°C/0.09mbar; (±)-2c: mp 75-7°C, bp 140-50°C/1mbar.

Acid catalyzed hydrolysis ($MeOH/H_2SO_4$ cat/reflux, 4h)[24] of acetates (±)-2a-c gave alcohols (±)-1a-c in >90% yield:

(1*RS*,2*RS*,4*RS*)-1,4,5,6-tetrachlorobicyclo[2.2.1]hept-5-en-2-ol[25] ((±)-1a): mp 70-2°C; ^{1}H-NMR: 1.88 (dd, J=3 and 11, 1H, *endo*-H on C-3), 2.24 (broad s, 1H, OH), 2.43 (d, J=3, 2H, H on C-7), 2.57 (dd, J=3 and 11, 1H, *exo*-H on C-3), 4.68 (dd, J=2 and 7, 1H, H on C-2).

(1*RS*,2*RS*,4*SR*)-1,4,5,6,7,7-Hexachlorobicyclo[2.2.1]hept-5-en-2-ol[25] ((±)-1b): mp 120-2°C; ^{1}H-NMR: 2.05 (dd, 1H, J=2 and 11, *endo*-H on C-3), 2.30 (broad s, 1H, OH), 2.90 (dd, J=2 and 11, 1H, *exo*-H on C-3), 4.85 (dd, J=2 and 7, 1H, H on C-2).

(1*RS*,2*SR*,4*SR*)-7,7-Dimethoxy-1,4,5,6-tetrachlorobicyclo[2.2.1]hept-5-en-2-ol[1a,24] ((±)-1c): mp 85-7°C; ^{1}H-NMR: 1.70 (dd, 1H, J=3 and 7, *endo*-H on C-3), 2.50 (s, 1H, OH), 2.64 (dd, 1H, J=7 and 11, *exo*-H on C-3), 3.59 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 4.63 (dd, 1H, J=3 and 7, H on C-2).

Determination of optical purity

The e.e. of alcohols 1a-c was determined *via* GLC-analysis of their corresponding diastereomeric mixed carbonates after derivatisation with (-)-menthyl chloroformate[26]. Esters 2a-e were hydrolyzed prior to derivatisation ($MeOH/H_2SO_4$ cat./reflux, 4h).

Determination of absolute configuration

Dehalogenation of the dimethoxy derivative (-)-(1*R*,2*S*,4*S*)-1c by dissolved metal reduction[22] gave (-)-(1*S*,2*S*,4*S*)-7,7-dimethoxybicyclo[2.2.1]hept-5-en-2-ol with known configuration[24]. Alcohols (1*R*,2*R*,4*R*)-1a, (1*S*,2*S*,4*R*)-1b and (1*R*,2*S*,4*S*)-1c were transformed into their corresponding hemiphthalates for CD-measurements: A mixture of alcohol (1mmol), phthalic anhydride (1mmol) and 4-dimethylaminopyridine (1mmol) in pyridine (5mL) was kept at r.t. until the reaction was complete as judged by TLC. The mixture was taken up in CH_2Cl_2 (25mL), washed with 1*N* HCl (2 x 10mL) and dried (Na_2SO_4). Gradual addition of petroleum ether (about 1/2 volume) precipitated the hemiphthalates in ~80 % yield:

(1*R*,2*R*,4*R*)-1a-hemiphthalate mp 165°C, (1*S*,2*S*,4*R*)-1b-hemiphthalate mp 184°C and (1*R*,2*S*,4*S*)-1c-hemiphthalate mp 142°C.

CD-Values (in acetonitrile):

Hemiphthalate of	c [mMol/L]	λ_{max} [nm]	$\Delta\varepsilon$ [L/mMol•cm]
(+)-(1*R*,2*R*,4*R*)-1a	0.44	248	-0.52
(-)-(1*S*,2*S*,4*R*)-1b	0.60	243	+3.56
(-)-(1*R*,2*S*,4*S*)-1c	0.63	245	+2.71

Optical rotation values

Compound	$[\alpha]_D^{20}$	c [g/100mL]	solvent	e.e. [%]
(1*S*,2*S*,4*S*)-1a	-60.8	1.09	$CHCl_3$	99
(1*S*,2*S*,4*R*)-1b	-14.1	3.15	$CHCl_3$	70
(1*R*,2*S*,4*S*)-1c	-34.9	2.54	MeOH	98
(1*R*,2*R*,4*R*)-2a	+0.43	3.04	$CHCl_3$	97
(1*R*,2*R*,4*S*)-2b	+1.21	4.65	$CHCl_3$	95
(1*S*,2*R*,4*R*)-2c	+47.6	2.85	MeOH	99

Enzymatic Experiments

Acylation using acid anhydrides

To a solution of substrate (±)-1a-c (10mmol) and acid anhydride (10 mmol) was added lipase (50% w/w of substrate). Bases were used either in suspension (finely powdered $KHCO_3$ anh., 10mmol) or in solution (2,6-lutidine, 10mmol). The mixture was shaken at 250 rpm on a rotary shaker at 22°C until a conversion close to 50 % was reached (monitored by

GLC). After filtration of the solids, the organic phase was washed with dil. $NaHCO_3$ solution, dried (Na_2SO_4) and evaporated. Chromatography gave esters **2a-e** and remaining alcohols **1a-c** in >90 % overall yield. Adsorption of lipase AY-30 onto Celite 145 was performed according to ref. 3.

Enzymatic acylation using vinyl acetate

Lipase (50% w/w of substrate) was added to a solution of alcohol (±)-**1a-c** (10 mmol) in vinyl acetate (10mL) and the suspension was shaken at 250 rpm (22°C). When the appropriate degree of conversion was reached, the enzyme was filtered and excess vinyl acetate was evaporated. The residue was then chromatographed as described above giving about the same overall yields.

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