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Investigations on enzyme catalytic promiscuity: The first attempts at a hydrolytic enzyme-promoted conjugate addition of nucleophiles to α,β-unsaturated sulfinyl acceptors

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ABSTRACT

Looking for new examples of enzyme catalytic promiscuity, attempts were made to use hydrolytic enzymes as catalysts for a conjugate addition of nucleophiles toα,β-unsaturated sulfinyl derivatives. The addition of piperidine to phenyl vinyl sulfoxide in chloroform proceeded both in the enzyme-catalyzed and non-catalyzed process, while in the former case the reaction was 2.5-fold faster. On the contrary, the conjugate addition of benzenethiol to phenyl vinyl sulfoxide proceeded only in the presence of enzymes and when ethanol was used as solvent. In no case were the products enantiomerically enriched. However, the addition of benzenethiol to a better Michael acceptor, namely a cyclic α-sulfinylalkenylphosphonate, performed in the presence of various lipases under kinetic resolution conditions gave in certain instances both the product and the recovered substrate with up to 25% optical purity. Although the stereoselctivity and the rates of these reactions were quite low, this are the first examples of the lipase-catalyzed Michael addition of heteroatom nucleophiles to α,β-unsaturated heteroorganic acceptors. Some mechanistic considerations are presented.

1. Introduction

Enzyme catalytic promiscuity, i.e. the ability of a single active site of the enzyme to catalyze more than one reaction, has been a subject of increasing research interest in the recent few years (for recent overviews see [1–6]). Enzyme promiscuity is clearly advantageous to chemists since it broadens the applicability of enzymes in chemical synthesis. So far, several interesting examples of this phenomenon have been reported.

Thus, selected aminopeptidases, besides their natural hydrolytic activity toward peptides, act also as phosphodiesterases [7,8] and phosphotriesterases [9–12]. The latter is of particular importance, since these enzymes can hydrolyze unnatural substrates – triesters of phosphoric acid and diesters of phosphonic acids – such as organophosphorus pesticides or organophosphorus warfare agents [13]. This means that they are capable of catalyzing the reaction that does not exist in nature. It should be noted that all the enzymes presented above belong to the class of dimetalloenzymes, which would to some extent explain their similar hydrolytic activity toward entirely different substrates. The mechanism of their action always rests upon the activation of a water molecule by the metal cations present in the active sites.

An intriguing behavior of other types of proteases, belonging to the family of serine hydrolases, is their ability to hydrolyze sulfur—nitrogen bond in N-acyl sulfinamides. When some N-acyl arenesulfinamides are treated with a buffer in the presence of subtilisin Carlsberg, the S—N bond hydrolysis unexpectedly becomes favored over the expected C—N bond hydrolysis to give, under kinetic resolution conditions, the corresponding sulfinic acids and carboxamides, together with the enantiomerically enriched recovered substrates. The proof for the direct involvement of the enzyme is the formation of an intermediate – O-sulfinyl enzyme, sulfinylated most probably on the active site serine [14]. Thus, also in this case, the hydrolysis proceeds according to a similar mechanism for both types of substrates, i.e. carboxylic and sulfinic esters, and involves attack of the catalytically active serine on the electrophilic center of the substrate.

A much more interesting phenomenon seems the ability of serine hydrolases to catalyze a carbon-carbon and carbon-heteroatom bond formation, i.e. to exhibit a lyase activity. Lipases are the enzymes for which a number of examples of such a promiscuous activity have been reported. In addition to their original activity comprising hydrolysis of lipids and, generally, catalysis of the hydrolysis or formation of carboxylic esters [15], lipases have been found to catalyze also the carbon–carbon and carbon-heteroatom bond forming reactions. The first example of a lipase-catalyzed Michael addition of a number of nucleophiles to 2-(trifluoromethyl)propenoic acid was described as early as in 1986

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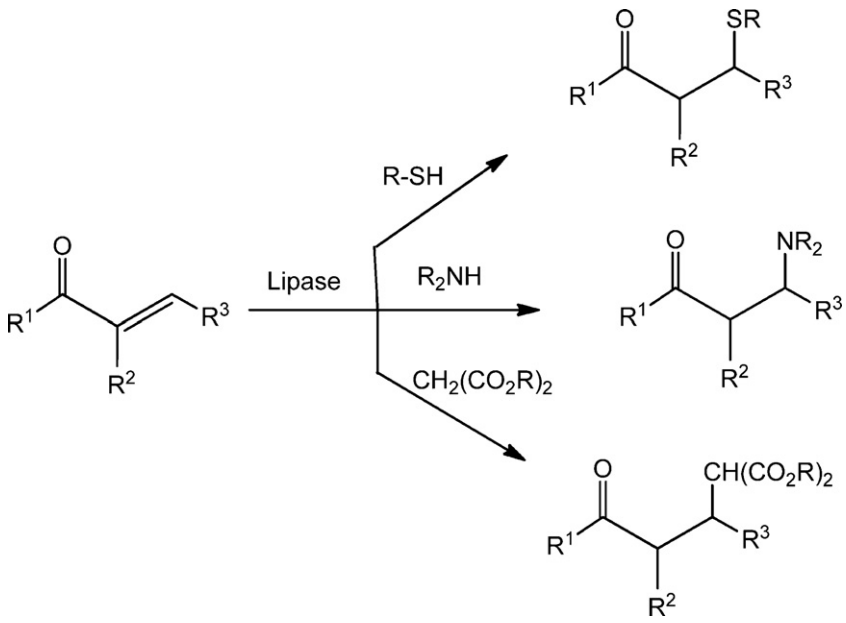


Scheme 1. Lipase-catalyzed Michael additions.

[16]. Michael addition of secondary amines to acrylonitrile is up to 100-fold faster in the presence of various preparations of lipase from *Candida antarctica* (CAL-B) than in the absence of a biocatalyst [17]. In a similar way, lipases catalyze Michael addition of amines, thiols [18], 1,3-dicarbonyl derivatives [19,20] and other C-acids [21] to α,β-unsaturated carbonyl compounds (Scheme 1). Interestingly, all such reactions proceed without enantioselectivity, the exceptions being a CAL-B-promoted Michael addition of benzylamine to methyl crotonate, which gives the adduct with enantiomeric excess of 60% [22] and Michael additions of C-acids to nitroalkenes, giving products with ee's from 7 to 86% [23]. Similarly, the lipase-catalyzed aldol condensation proceeds with low enantioselectivity, giving the products with enantiomeric excess of 9 to 43% [24]. Other types of hydrolytic enzymes, namely some serine proteases, also catalyze Michael addition of substituted pyrimidines [25], imidazoles [26] and purines [27] to acrylates.

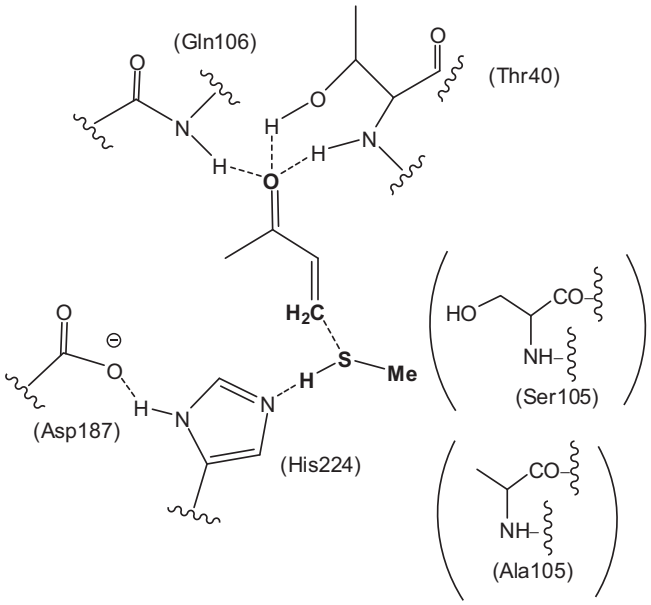
On the basis of the quantum-chemical studies, it has been concluded that it is the so called "oxyanion hole" of the enzyme that binds the carbonyl oxygen or nitrile nitrogen, increasing electrophilicity of the corresponding carbon atom and enhancing the attack of a nucleophile, which is, in turn, activated by histidine (Scheme 2) [18]. This model clearly shows that the catalytic machinery involves a dyad of histidine and aspartate together with the oxyanion hole. Hence, it does not involve serine, which is the key amino acid in the hydrolytic activity of lipases, and, together with aspartate and histidine, constitutes the active site catalytic triad. This has been confirmed by constructing a mutant in which serine was replaced with alanine (Ser105Ala), and finding that it catalyzes the Michael additions even more efficiently than the wild-type enzyme [28]. In a similar way, the catalytic activity of CAL-B and its Ser105Ala mutant in an aldol addition, thus another C—C bond forming reaction, has been explained [29].

Taking into account the above mechanism and, particularly, the fact that the carbonyl oxygen atom (or the nitrogen atom of the nitrile) is H-bound with the amino acids of the oxyanion hole, we have decided to check whether the same interaction will take place with the oxygen connected with a heteroatom, e.g. with sulfur in sulfinyl derivatives. Such an approach seems quite justifiable since the sulfinyl oxygen forms strong hydrogen bonds with H-donors. Moreover, sulfinyl derivatives are tetrahedral which, in contrast to the planar carbonyl or cyano groups, raises a possibility of their stereoselective recognition by a chiral enzyme leading to enantiomerically enriched products. The present investigations are a continuation of our previous studies which have revealed that heteroatom stereogenic and prostereogenic centers are stereoselectively recognized by common hydrolytic enzymes allowing for the synthesis of enantiomerically enriched heteroorganic compounds [30–36].

2. Experimental

2.1. General

The synthesized products were purified by column chromatography on silica gel. Solvents were dried using general procedures



Scheme 2. Hypothetical mechanism of the lipase-catalyzed Michael addition of methanethiol to acrolein [18,29]. In parentheses aminoacids: serine present in the active site of a native lipase and alanine replacing serine in the Ser105Ala mutant.

Table 1
Conjugate addition of benzenethiol to phenyl vinyl sulfoxide **rac-1**.

Entry	Enzyme	Solvent	Reaction time [days]	Unreacted substrate **1** yield [%]	Product **3** yield [%]
1	MJL	EtOH	7	43	35
2	PPL	EtOH	7	53	29
3	CRL	EtOH	8	43	21
4	CAL B	EtOH	8	42	22
5	PS	EtOH	5	36	35
6	BSP	EtOH	5	40	28
7	AK	EtOH	6	46	24
8	CCL	EtOH	6	44	25
9	MJL	CH_2Cl_2	8	No reaction	
10	PPL	CH_2Cl_2	8	No reaction	

Enzyme: see Section 2.1.

and distilled prior to use. The NMR spectra were recorded in $CDCl_3$ with a 'Bruker AC 200′ spectrometer. The chemical shifts (δ) are expressed in ppm, the coupling constants (J) are given in Hz. Optical rotation values were measured on a Perkin–Elmer-241 photopolarimeter for the sodium D line at 20 °C. Mass spectra were recorded with a Finnigan MAT 95 Voyager Elite spectrometer.

Enzymes: MJL: lipase from *Mucor javanicus* SIGMA, 300 U/mg solid; PPL: lipase from porcine pancreas, SIGMA, 30–90 U/mg protein; CRL: lipase from *Candida rugosa*, SIGMA, 700 U/mg solid; CAL-B (Novozym 435): lipase acrylic resin form *Candida Antarctica*, SIGMA, 10 U/mg; PS: lipase form *Pseudomonas cepacia*, AMANO, >30 U/mg; BSP: proteinase from *Bacillus subtilis* (Subtilisin Carlsberg), SIGMA, 20 U/mg; AK: lipase from *Pseudomonas fluorescens*, AMANO, >20 U/mg; CCL: lipase from *Candida cylindracea* (*rugosa*), SIGMA, 25 U/mg lyophilized powder.

2.2. Enzyme-catalyzed conjugate addition of piperidine to phenyl vinyl sulfoxide

Racemic sulfoxide **1** (100 mg, 0.66 mmol) was dissolved in dry chloroform (5 mL). To this solution, protease from *Bacillus subtilis* (100 mg) and piperidine (0.5 equiv., 0.33 mmol) were added. The reaction was monitored by TLC (CH_2Cl_2/MeOH 40:1). The enzyme was filtered off and the solvent was evaporated. The residue was separated by column chromatography CH_2Cl_2/MeOH in gradient as eluent. The yields of the product and recovered substrate are shown in Scheme 3.

2-(N-piperidyl)ethyl phenyl sulfoxide **2**: ^{1}H NMR ($CDCl_3$) δ = 1.41–1.62 (m, 6H), 2.30–2.58 (m, 5H), 2.72–3.02 (m, 3H), 7.48–7.67 (m, 5H). MS (CI) m/z 237 (M + H).

2.3. Enzyme-catalyzed addition of benzenethiol to phenyl vinyl sulfoxide. General procedure

To a solution of sulfoxide **1** (152 mg, 1 mmol) in 96% EtOH (5 mL) an enzyme (10–100 mg) and benzenethiol (55 mg, 0.5 mmol) were added. The mixture was stirred at room temperature for several days (TLC control CH_2Cl_2: MeOH 20:1). Then the enzyme was filtered off and washed with CH_2Cl_2. The solvents were evaporated to give a crude mixture of the product **5** and the substrate **1**, which were separated using preparative TLC (CH_2Cl_2: MeOH 20:1). The results are summarized in Table 1.

Phenyl 2-phenylthioetyl sulfoxide **3**: ^{1}H NMR ($CDCl_3$): δ = 2.88–3.34 (m, 4H, CH_2CH_2), 7.16–7.63 (m, 10H, aromat.). MS (CI): m/z 263 (M + H). HRMS: calcd. for $C_{14}H_{15}OS_2$ 263.056435 found 263.05639.

Table 2
Conjugate addition of benzenethiol to 4.

Entry	Lipase	Solvent	Product	Yield (NMR) [%]	$[\alpha]_D$	o.p. [%]
1	CAL-B	$CHCl_3$	**4**	61	N.i.	N.i.
			6	12	–	–
			7	20	–	–
			5	7	–4.2	22
2	CRL	$CHCl_3$	**4**	46	+5.5	6.4
			6	9	–	–
			7	14	–	–
			5	23	–5.0	26
3	AK	i-Pr_2O	**4**	4	N.i.	N.i.
			6	20	–	–
			7	25	–	–
			5	48	–1.5	8
4	CRL	CH_2Cl_2	**4**	22	+1	1.2
			6	–	–	–
			7	11	–	–
			5	42	–2.5	15
5	AK	Me_2CO/Et_2O 1:1	**4**	22	N.i.	N.i.
			6	–	–	–
			7	12	–	–
			5	42	–2.8	16

Lipase: see Section 2.1; $[\alpha]_D$ in acetone, c = 1; N.i. – not isolated; o.p. – optical purity calculated by comparison of the optical rotations of the reaction products with those of the authentic samples; the measurements were performed using the same solvent and concentration [37,39].

2.4. Enzyme-catalyzed addition of benzenethiol to 2-phosphono-2,3-didehydrothiolane S-oxide **4**

2.4.1. 2-(5′,5′-Dimethyl-1′,3′,2′-dioxaphosphorinanyl)-2,3-didehydrothiolane1-oxide **4**

2-(5′,5′-Dimethyl-1′,3′,2′-dioxaphosphorinanyl)-2,3-didehydrothiolane 1-oxide **4** was prepared according to a known method [37].

2.4.2. General procedure

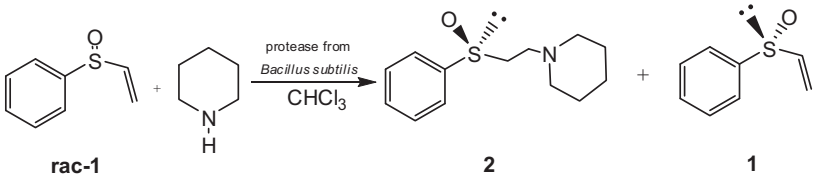
The 2-phosphono-2,3-didehydrothiolane S-oxide **4** (100 mg, 0.40 mmol) was dissolved in a dry solvent (5 mL). To this solution, an enzyme (100 mg) was added and after 15 min benzenethiol (0.5 eq., 0.20 mmol). The reaction was monitored by ^{31}P NMR spectroscopy and by TLC (CH_2Cl_2/MeOH 20:1) and stopped after 2 days. The enzyme was filtered off and the solvent was evaporated. The residue was separated by column chromatography using CH_2Cl_2/MeOH in gradient (100:1–20:1) as eluent. The products were identified on the basis of their NMR and MS spectra, as **4** (recovered substrate) and **5**. The results are shown in Scheme 5 and collected in Table 2.

2-[2′-(5,5-dimethyl-1,3,2-dioxaphosphorinanyl)]-2,3-didehydrothiolane 1-oxide (+)-(*S*)-**4**.

^{31}P NMR ($CDCl_3$) δ = 4.2; ^{1}H NMR ($CDCl_3$) δ = 1.05 (s, 3H, CH3), 1.24 (s, 3H, CH_3), 3.00–3.65 (m, 4H, SCH_2, CH_2), 3.98–4.40 (m, 4H, 2 × OCH_2), 7.55 (dt, J = 11.5, 2.3, 1H, C=CH). ^{13}C NMR ($CDCl_3$) δ = 20.7 (s, CH_3), 21.7 (s, CH_3), 32.3 (d, J = 6.8, CCH_3), 34.9 (d, J = 18.2, CH_2), 52.3 (d, J = 7.0, CH_2), 76.5 (d, J = 6.5, OCH_2), 77.4 (d, J = 6.4, OCH_2), 140.1 (d, J = 187.4, PC), 157.4 (d, J = 12.7, PC = CH). HRMS (CI): calcd. for $C_9H_{16}PSO_4$ (M + H): m/z 251.0506, found: m/z 251.0509.

The spectroscopic data were identical with those described previously [37].

3-Phenylsulfanyl-2-[2′-(5,5-dimethyl-1,3,2-dioxaphosphorinanyl)]thiolane 1-oxide (−)-(*1R,2S,3S*)-**5**: ^{31}P NMR ($CDCl_3$) δ 13.2; ^{1}H NMR ($CDCl_3$) δ 0.99 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 2.77–2.81 (m, 2H, CH_2), 3.00 (m, 1H, CHHSO), 3.20 (m, 1H, CHHSO), 3.31 (ddd, $^4J_{HH}$ = 1.1, $^3J_{HH}$ = 7.2, $^2J_{HP}$ = 14.6, 1H, PCH), 3.92–4.01 (m, 3H), 4.11–4.16 (m, 2H), 7.35–7.37 (m, 3H, H_{arom}), 7.54–7.57 (m, 2H, H_{arom}); ^{13}C NMR ($CDCl_3$) δ 20.98 (CH_3), 21.69 (CH_3), 32.43 (d,



Entry	Reaction time [days]	Enzyme	Recovered substrate **1** (isolated yield [%])	Product of addition **2** (isolated yield [%])
1	5	None	40	42
2	2	Protease from *Bacillus subtilis*	42	43

Scheme 3. Conjugate addition of piperidine to phenyl vinyl sulfoxide **rac-1** (expected absolute configurations are shown arbitrarily).

J = 6.8, $(CH_3)_2C$), 34.77 (d, J = 7.8, CH_2), 48.26 (CHS), 53.29 (d, J = 3.4, CH_2SO), 68.52 (d, J = 130.8, PCH), 77.05 (d, J = 6.6, CH2O), 77.33 (d, J = 6.5, CH_2O), 128.57 (CH_{arom}), 129.37 (2× CH_{arom}), 133.50 (SC_{arom}), 133.55 (2× CH_{arom}); MS (CI) m/z 361 (M + H), 161 (100); HRMS (CI) calcd. for $C_{15}H_{21}PO_4S_2$ (M + H): m/z 361.0697, found: m/z 361.0706.

The spectroscopic data were identical with those described previously [38,39].

3-Phenylsulphanyl-2-(5′,5′-dimethyl-1′,3′,2′-dioxaphosphorinanyl)-2,3-didehydrothiolane **6**: ^{31}P NMR ($CDCl_3$) δ = 4.65. ^{1}H NMR ($CDCl_3$) δ = 1.06 (s, 3H, CH_3), 1.20 (s, 3H, CH_3), 2.79–2.89 (m, 2H, $PhSCCH_2$), 3.20 (t, 2H, J = 8.60 Hz, SCH_2), 4.05–4.21 (m, 4H, 2× OCH_2), 7.31–7.34 (m, 3H, Ph), 7.45–7.50 (m, 2H, Ph). ^{13}C NMR ($CDCl_3$) δ = 21.23 (s, CH_3), 21.74 (s, CH_3), 31.51 (d, J = 6.70 Hz, CH_2), 32.50 (d, J = 6.35 Hz, $C(CH_3)CH_3$), 42.78 (d, J = 6.90 Hz, CH_2S), 76.90 (d, J = 29.35 Hz, 2× CH_2O), 127.99, 128.49, 129.20, 132.12, 132.58, 133.03 (Ph), 143.03 (d, J = 10. 14 Hz, PhSC=). MS (CI) m/z 343 (M + H), 685 (2M + H); HRMS (EI): calcd. for $C_{15}H_{19}PS_2O_3$: m/z 342.051329, found: m/z 342.05178.

3-Phenylsulphanyl-2-(5′,5′-dimethyl-1′,3′,2′-dioxaphosphorinanyl)-3,4-didehydrothiolane **7**: ^{31}P NMR ($CDCl_3$) δ = 10.86. ^{1}H NMR ($CDCl_3$) δ = 0.91 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 2.88–2.95 (m, 1H), 3.96–4.10 (m, 4H, 2× CH_2O), 4.34–4.46 (m, 2H), 7.28–7.52 (m, 6H, Ph, SCH_2CH); MS (CI) m/z 343 (M + H); HRMS (EI): calcd. for $C_{15}H_{19}PS_2O_3$: m/z 342.051329, found: m/z 342.05124.

3. Results and discussion

3.1. Michael additions to α,β-unsaturated sulfoxides

Commercially available racemic phenyl vinyl sulfoxide **rac-1** was chosen as the first and simplest substrate. It was subjected to the reaction with piperidine (half molar amount to ensure kinetic resolution) in the presence of protease from *Bacillus subtilis.* After completion of the reaction the mixture was separated by column chromatography to give both the unreacted substrate **1** and the addition product **2** (Scheme 3). Surprisingly, neither one was optically active. Moreover, the reaction performed under identical conditions without enzyme, gave almost the same result, although the identical conversion required a 2.5-fold longer reaction time.

In similar reactions, in which a half-molar amount of benzenethiol was used as a nucleophile and the reaction was performed in chloroform or dichloromethane in the presence of various hydrolytic enzymes, no expected product of the conjugate addition could be detected. However, when chloroform was replaced with ethanol, the desired 1,4 addition product **3** was formed. Although the time which was needed to complete the conversion was, in comparison with the analogous Michael additions to α,β-unsaturated carbonyl compounds [16,17], quite long, both the unreacted substrate **1** and the product **3** were isolated in relatively good yields (Scheme 4, Table 1). Unfortunately, again they were racemic (chiral HPLC: ee < 1%). Noteworthy, no reaction was observed under these conditions in the absence of enzyme.

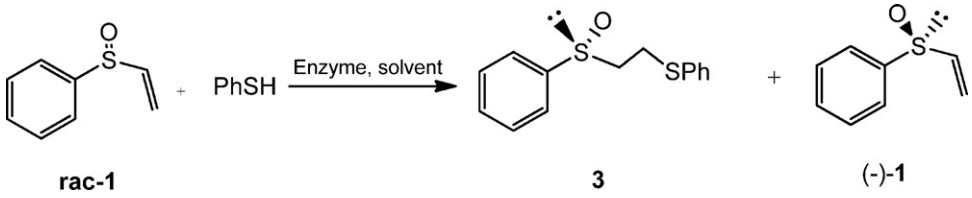
Although the lack of stereoselectivity in the above reactions was somewhat disappointing, the results undoubtedly proved that the additions were catalyzed by enzymes. A surprising effect of the application of ethanol as solvent seems at first sight difficult to explain. However, there are precedents described in the literature, which show that alcohols may enhance the activity of lipases, particularly in their promiscuous catalytic action in a C-heteroatom [22] and C—C bond formation [40].

3.2. Michael additions to an α-sulfinylalkenylphosphonate

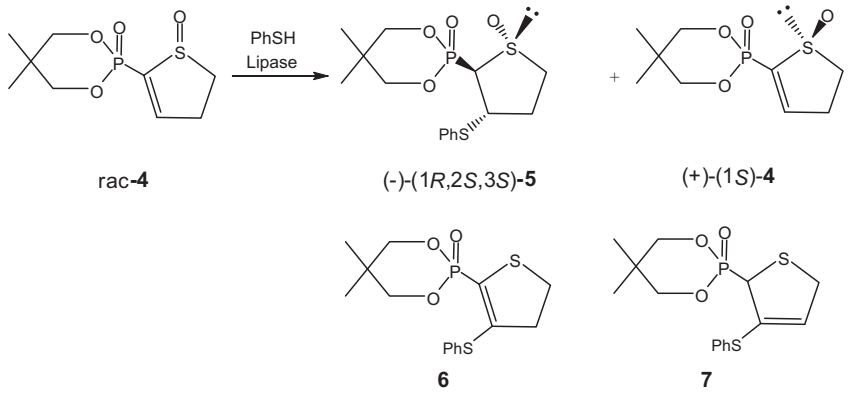
Since phenyl vinyl sulfoxide **1** proved to be a relatively weak Michael acceptor, we decided to construct an alkenyl compound bearing sulfinyl and phosphoryl moieties at the same sp^2 carbon atom. The presence of the two electron-withdrawing substituents was expected to enhance electrophilicity of the β carbon atom. We decided to use as a model compound racemic 2-(5′,5′-dimethyl-1′,3′,2′-dioxaphosphorinanyl)-2,3-didehydrothiolane 1-oxide **4**, because its triethylamine-catalyzed reaction with benzenethiol had been earlier studied by us and the relative and absolute configurations of both **4** and the conjugate addition product **5** were determined [37–39].

The enzyme-catalyzed additions to **4** were performed under kinetic resolution conditions, using half molar amount of benzenethiol and a number of commercially available lipases. The reaction was monitored by ^{31}P NMR spectroscopy of the crude samples taken out directly from the reaction solution and stopped after two days. As a result, complex mixtures of products were usually obtained from which only four compounds, among them the recovered substrate **4** and the expected adduct **5**, could be isolated and characterized (Scheme 5, Table 2). The structure and absolute configurations of **4** and **5** were ascribed by comparison with authentic samples which were obtained by us earlier and described in details in Refs. [37–39]. It should be added that compound 5 was obtained as a single diastereomer which, according to Ref. [39], is the thermodynamically controlled product. Hence, comparison of a sample of 5 with the sample of the same compound obtained earlier [39] (for which an X-ray analysis was performed and both relative and absolute configurations were determined) using TLC, HPLC, MS and ^{1}H and ^{31}P NMR, undoubtedly established its identity. Therefore, the sign and value of the $[\alpha]_D$ of compound **5** allowed to ultimately determine its absolute configuration and optical purity.

In certain cases it was impossible to obtain the pure recovered substrate **4** due to its decomposition during chromatography: the isolated substance was contaminated with unidentified products of decomposition, which could not be removed or separated. This caused severe problems and substantially lowered the isolated



Scheme 4. Conjugate addition of benzenethiol to phenyl vinyl sulfoxide **rac-1** (expected absolute configurations are shown arbitrarily).

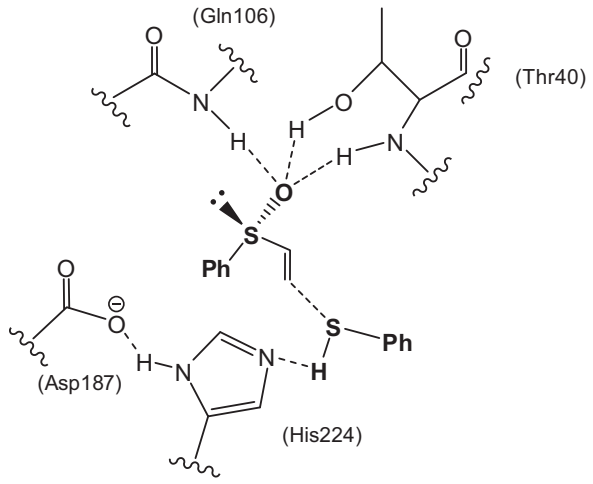


Scheme 5. Conjugate addition of benzenethiol to 2-(5′,5′-dimethyl-1′,3′,2′-dioxaphos-phorinanyl)-2,3-didehydrothiolane 1-oxide **4**.

yields of all the products. Therefore, Table 1 contains only selected examples and the yields, which were estimated on the basis of the NMR spectra of the crude reaction mixtures. It should be added that no formation of the products could be observed when the reaction was performed in the absence of enzymes or in the presence of the CAL-B which was denatured with urea [21].

Inspection of Table 2 reveals that the expected adduct **5** was formed in a stereoselective manner, although its optical purity was quite low. The term "optical purity" was used here, instead of the "enantiomeric excess", to evaluate the stereoselectivity of the reaction, since under no conditions could the enantiomers be separated by chiral HPLC (cf. [37,39]). Nevertheless, isolation of the optically active species undoubtedly proves that the enzymes must have been involved in the process, since they were the only source of chirality. The fact that the recovered substrate **4** was also optically active and its absolute configuration at the sulfinyl sulfur atom was opposite to the configuration of the sulfinyl moiety in **5**, clearly indicates that the result was an effect of kinetic resolution. The next two products, namely **6** and **7**, were also isolated and their structures were ascribed on the basis of ^{1}H and ^{31}P NMR and MS. The way of their formation is not known. Since each of them contained the benzenesulfenyl moiety, originating from benzenethiol, it seemed reasonable to assume that the adduct **5** was the primary intermediate. However, their formation, which undoubtedly had to be a result of a simultaneous reduction and dehydrogenation/oxidation of **5**, remains unclear. To gain a better insight into the way of their formation, some additional experiments were performed. Thus, when pure **5** was treated with lipase AK in chloroform, no changes were observed for over 15 days. However, addition of benzenethiol to this mixture caused formation of compound **7**. This ultimately proves that both **6** and **7** must be produced from **5** in a subsequent reaction, in which the presence of benzenethiol seems crucial. When the procedure was reversed, i.e. benzenethiol was first added to a solution of **5**, no changes could be observed until the lipase was added. Then, the result was as above, which may be taken as proof for the cooperative action of benzenethiol and the enzyme in the formation of by-products.

Taking into account the hypothetical mechanism shown in Scheme 2, the course of the reaction discussed above may be illustrated in the following way (Scheme 6). The sulfinyl oxygen atom is bound within the "oxyanion hole" of enzyme active site using hydrogen bonds. In turn, the nucleophilicity of the sulfur center in the benzenethiol molecule is enhanced by the histidine of the catalytic dyad. Though the latter interaction is identical as in the case of the Michael addition of thiols to enones, the H-binding of the sulfinyl oxygen atom must be different (weaker?) from that of the carbonyl oxygen atom, which results in a less efficient catalysis of the reaction by enzymes. It is known that the oxyanion hole binds transition states better than the ground state. In case of the lipase-catalyzed hydrolysis of esters, the intermediary oxy anion is tetrahedral. Although the sulfinyl group is tetrahedral as well, which would suggest that it should be bound equally well, in contrast to the oxy anion it bears no negative charge on the oxygen atom in the intermediate, which obviously decreases the H-bonding strength. Moreover, for the Michael addition of



Scheme 6. Assumed mechanism of the conjugate addition of benzenethiol to **1**.

nucleophiles to enones, the intermediary oxy anion is planar, which additionally makes it fit better in the oxyanion hole by lowering spatial requirements in comparison with the tetrahedral sulfinyl intermediate. It must be stressed that such an explanation is only a speculative one. To find a better insight into the real mechanism of the reaction further studies will be continued, which will involve attempts at the X-ray analysis of an enzyme-substrate complex as well as molecular modeling. Nevertheless, the results presented undoubtedly prove that the stereogenic sulfinyl group in **4** must be recognized and stereoselectively bound in the chiral enzyme environment which results in the stereoselective course of the addition. This result does not exclude another possible mechanism, based on a phenomenon called "alternate site promiscuity", which assumes that the reaction neither involves any of the catalytic amino acids of the natural enzymatic process, nor appears to occur in the natural binding pocket (for a recent example see Reetz and co-workers [41]).

4. Conclusions

Attempts at the use of hydrolytic enzymes as catalysts for a conjugate addition of nucleophiles to α,β-unsaturated sulfinyl derivatives have been made for the first time. The studies proved that the simple mechanism proposed for the analogous lipase-catalyzed conjugated addition of nucleophiles to enones and acrylonitrile cannot be directly applied. Although in both cases the "oxyanion hole" of the enzymes is supposed to enhance electrophilicity of the Michael acceptor by the formation of hydrogen bonds, the non-ionic tetrahedral structure of the sulfinyl group (contrary to the planar oxy anion intermediate in the case of the Michael addition to enones) is probably responsible for a less effective location of the heteroatom substrates within the space available. Nevertheless, in the case of a doubly activated acceptor, bearing both the sulfinyl and phosphoryl moieties at the same sp^2 carbon atom, the reaction takes place in a weakly stereoselective manner, which can be taken as proof for the involvement of the enzyme, being here the only source of chirality. However, an alternative mechanism, resting upon the enzyme "alternate site promiscuity", must also be taken into consideration. Further studies on this problem are under way.

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