ENZYMATIC CYCLIZATION OF 2-(CARBAMOYLOXY)BENZOATES, 2-(SULFAMOYLOXY)-BENZOATES AND 2-(CARBAMOYLOXY)BENZOPHENONES WITH YEAST AND LIPASE

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Abstract - The cyclization of labile functionalized 2-(carbamoyloxy)-benzoates, 2-(sulfamoyloxy)benzoates and 2-(carbamoyloxy)benzophenones to 1,3-benzoxazine-2,4-diones, 4-oxo-3,4-dihydro-1,2,3-benzoxathiazine 2,2-dioxides and 4-phenyl-1,3-benzoxazin-2-ones with yeast as well as lipase is described. This approach establishes the application of enzymes for gram scale cyclization of such substrates under mild conditions. Further, it is revealed that baker's yeast is most suitable for this process.

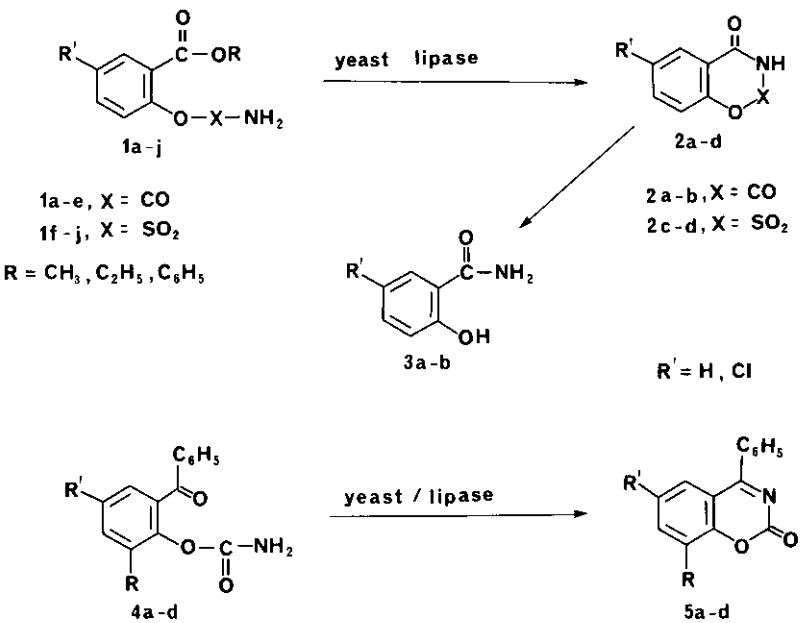
Our earlier studies[1,2] on the enzymatic formation of 1,3-benzoxazine-2,4-diones, 4-phenyl-1,3-benzoxazin-2-ones and 4-oxo-3,4-dihydro-1,2,3-benzoxathiazine 2,2-dioxides from the 2-carbamoyloxy and 2-sulfamoyloxy substrates have utilized the sedimented microsomes from mammalian (eg. rat and rabbit) liver cells as the typical source for cyclization. However, the use of the microsomal preparations has limited the enzymatic process to only small scale (about 100 mg) substrate conversions. As a result, this enzymatic process is not that useful for large scale purposes. Consequently, we decided to investigate the enzyme-catalyzed cyclizations employing enzymes from different sources which can be applied for the gram scale enzymatic cyclization.

We now report our findings for the unprecedented enzymatic cyclization of 2-(carbamoyloxy)- and 2-(sulfamoyloxy)benzoates as well as 2-(carbamoyloxy)benzophenones by employing yeast and lipase. It is well established that yeasts and lipases are valuable biocatalysts[3,4] for the asymmetric hydrolysis of racemic acid esters. Further, baker's yeast has been employed for various asymmetric reductions[5,6], though recently it has been found useful as an effective reagent for the preparative scale enantioselective enzymatic cyclization[7] of hydroxylated surrogate squalenoids. Lipase has also been reported for the enzymatic lactonization[8,9] of γ-hydroxy esters. In this paper, here the cyclization by yeasts and lipases proceeds to the desired products, whereas their efficiency and effect of incubation conditions are discussed.

In an initial screening, the following commercially available yeasts and lipases were tested for

their cyclization ability on our substrates (most of the enzymes[10] were obtained in a crude form and all were used without purification):

Yeast from Saccharomyces cerevisiae[11] (baker's yeast), Candida utilis[12] (torula yeast), Candida pseudotropicalis[12] (ATCC No. 2512), whereas lipase from porcine pancreas[13], Rhizopus arrhizus[14] and Pseudomonas sp.[15] were employed. The results of a detailed screening on a gram-scale for a representative compound 2-carbamoyloxyethylbenzoate 1b are depicted in Table 1. All substrate-enzyme combinations led to the cyclization but their rate of cyclization varied. Increase in the duration of incubation afforded further conversion of the cyclized product to 2-hydroxybenzamide 3a by intramolecular aminotransfer as described in our earlier work on liver microsomes[2]. Therefore, this cyclization process on preparative scale by substrate-enzyme combinations required a detailed investigation. The course of conversion versus time was examined for the enzymatic cyclization of substrate 1b.



Enzymatic cyclization using baker's yeast:

The cyclization process for 1b by baker's yeast took about 20 h for 85% conversion. The increase in incubation time though increased the cyclization process and simultaneously the cyclized form was transformed to 2-hydroxybenzamide 3a. After 48 h incubation period, the cyclized product 2a was reduced on account of its further conversion to 3a. Around 7th day of the incubation period

both the cyclized and its further transformed forms were in nearly equal amounts. After 10 days of the incubation, the cyclized compound 2a was just reduced to 12%. This is clearly observed in Fig. 1.

Enzymatic cyclization using porcine pancreas lipase:

In this case, the cyclization of 1b by lipase was a slower process and the conversion to cyclized form 2a was only 45% after 24 h of incubation. Even after 5 days of incubation the conversion was 68% to the cyclized form, but on the other hand the transformation of the cyclized form to 2-hydroxybenzamide 3a was 10% at this stage. At the end of the 10-day period, the cyclized form 2a was 25% and its transformed form 3a was 49% which is depicted in Fig. 2.

Although the cyclization process with these above enzymes is a much slower process compared to the earlier study by liver microsomes[2], in view of the above results (Fig. 1), baker's yeast appears to be a suitable enzyme for cyclization of such substrates particularly on the preparative scale. Therefore, various substituted 2-carbamoyloxy and 2-sulfamoyloxy substrates were subjected to enzymatic cyclization by baker's yeast to examine the generality of this process, the results of which are illustrated in Table 2. In all the above reactions, a control experiment was carried out under similar reaction conditions employing yeast and lipase preheated at 80°C for 10 min, which did not afford the products.

Table 1. Enzyme-catalyzed conversions[a] of 2-carbamoyloxyethylbenzoate 1b to 1,3-benzoxazine-2,4-diones 2b and 2-hydroxybenzamide 3a with yeasts and lipases from different sources at 35-37°C.

Enzyme source	After 1 day of incubation %		After 5 days of incubation %		After 10 days of incubation %	
	2a	3a	2a	3a	2a	3a
1. Yeast:						
(a) Saccharomyces cerevisiae	90	0	59	35	12	68
(b) Candida utilis	96	2	55	43	14	72
(c) Candida pseudotropicalis	68	6	44	40	8	75
2. Lipase:						
(a) Porcine pancreas	45	0	68	10	25	49
(b) Rhizopus arrhizus	56	0	74	20	28	53
(c) Pseudomonas sp.	51	0	67	14	25	51

[a]By HPLC employing Ultropac TSK Si-150 column, 5 μm (250x4.6 mm), mobile phase, chloroform/10% butanol at 0.7 ml/min flow rate, 2a and 3a were calibrated with the standard samples.

By these results, it is observed that although yeasts from different sources present valuable and practical biocatalysts for the cyclization processes, the baker's yeast offers an inexpensive and convenient source of enzyme employed for the gram-scale enzymatic cyclization of substrates possessing the labile O-carbamoyloxy and O-sulfamoyloxy functionalities. Studies on the synthetic

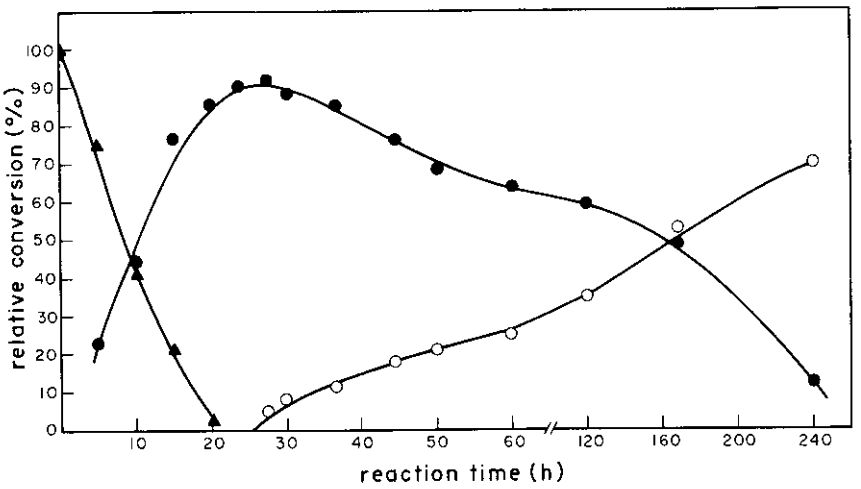


Fig. 1. Time course of the baker's yeast-mediated incubation mixture of 1b at 35-37°C at the indicated times. Percent relative concentration of 2-(carbamoyloxy)ethylbenzoate (▲), 1,3-benzoxazine-2,4-dione 2a (●) and 2-hydroxybenzamide 3a (○).

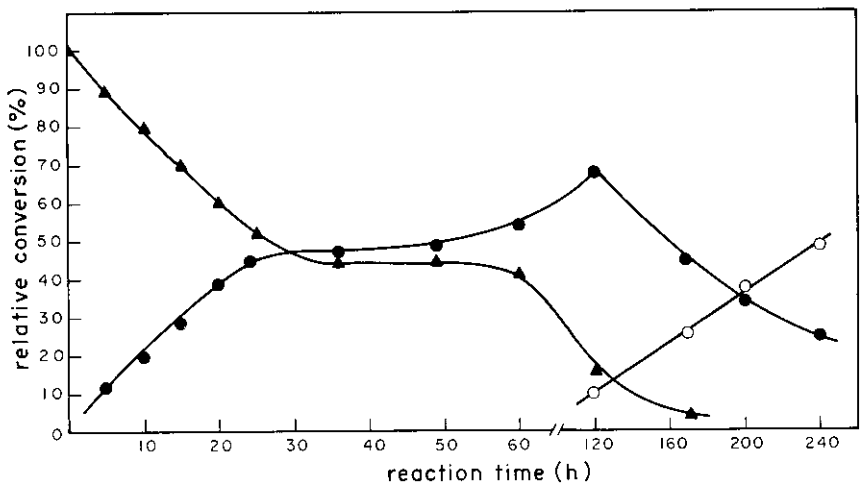


Fig. 2. Time course of the porcine pancreas lipase-mediated incubation mixture of 1b at 35-37°C at the indicated times. Percent relative concentration of 1b (▲), 2a (●) and 3a (○).

scope of such enzymatic reactions and on its extended use in bioorganic transformation are currently under investigation in this laboratory.

Table 2. Cyclization of 2-(carbamoyloxy)benzoates, 2-(sulfamoyloxy)benzoates and 2-(carbamoyloxy)-benzophenones (1 and 4) by baker's yeast after 24 h of incubation at 35-37°C.

Substrate	R	R'	X	Product	Yield of 2a-d and 5a-d, %
1a	CH_3	H	CO	2a	83
1b	C_2H_5	H	CO	2a	89
1c	C_6H_5	H	CO	2a	78
1d	CH_3	Cl	CO	2b	86
1e	C_2H_5	Cl	CO	2b	92
1f	CH_3	H	SO_2	2c	88
1g	C_2H_5	H	SO_2	2c	70
1h	C_6H_5	H	SO_2	2c	73
1i	C_2H_5	Cl	SO_2	2d	79
4a	H	H	-	5a	83
4b	H	Cl	-	5b	86
4c	H	CH_3	-	5c	91
4d	Cl	H	-	5d	88

EXPERIMENTAL

Melting points were determined on Buchi melting point apparatus and are uncorrected. Incubations were carried out on New Brunswick G-86 Aquatherm shaker. ^{1}H-Nmr spectra were recorded in $CDCl_3$ on a JEOL FX90Q FT spectrometer employing TMS as an internal standard at 90 MHz. Infrared spectra were recorded on a Perkin-Elmer 283B spectrophotometer. Mass spectra were recorded on a VG 7070H mass spectrometer. HPLC analyses were performed on a 6A-Schimadzu instrument with 254-nm variable wavelength and Chromatopac C-R4A integrator.

Synthesis of substrates

2-Carbamoyloxymethylbenzoate (1a). General procedure. Reaction of 2-hydroxymethylbenzoate (6.98 g, 0.046 mol) with chlorosulfonyl isocyanate (4 ml, 0.046 mol) at 4-6°C following a procedure previously described[16] yielded 83% of 1a, mp 149-150°C; ir (KBr): 3420, 3305, 1730, 1690 cm^{-1}; ^{1}H-nmr ($CDCl_3$): 8.2-7.1 (4H,m,Ar-H), 5.8 (2H,br s,NH_2), 3.9 (3H,s,OCH_3); CI ms (m/z) 195.

2-Sulfamoyloxymethylbenzoate (1g). General Procedure. 2-Hydroxymethylbenzoate (3.5 g, 0.023 mol) was reacted with chlorosulfonyl isocyanate (2 ml, 0.023 mol) in toluene (10 ml) at 100-105°C as described previously[2] to yield 90% of 1g, mp 87-88°C; ir (KBr): 3400, 3200, 1700, 1370, 1160 cm^{-1}; ^{1}H-nmr ($CDCl_3$): 7.8-7.9 (1H,dd, Ar-H), 7.5-7.2 (3H,m,Ar-H), 5.6 (2H,br s,NH_2), 3.8 (3H,s,OCH_3); CI ms (m/z) 231.

2-Carbamoyloxybenzophenone (4a). General procedure. Reaction of 2-hydroxybenzophenone (9.1 g, 0.046 mol) with chlorosulfonyl isocyanate (4 ml, 0.046 mol) at room temperature following a procedure previously described[17] yielded 84% of 4a, mp 130-132°C; ir (KBr): 3425, 3310, 1715, 1650 cm^{-1}; ^{1}H-nmr ($CDCl_3$): 7.8-7.1 (9H,m, Ar-H), 5.0 (2H,br s,NH_2); CI ms (m/z) 241.

General procedure for the screening of enzymes

To the substrate (1 g) dissolved in ethanol (200 ml) and phosphate buffer (0.01 M, 180 ml, pH 7.5) was added a solution of yeast (1 g) or crude lipase (1.2 g). The mixture was then agitated on a rotary shaker at about 250 rpm. Analytical samples were periodically withdrawn, extracted with chloroform and analyzed on HPLC (Table 1).

General procedure for baker's yeast-catalyzed cyclization of 2-(carbamoyloxy)benzoates 1a-f, 2-(sulfamoyloxy)benzoates 1g-j and 2-(carbamoyloxy)benzophenones 4a-d:

2H-1,3-benzoxazine-2,4(3H)-dione 2a. To 2-(carbamoyloxy)ethylbenzoate 1b (2 g) dissolved in ethanol (450 ml) and 0.01 M phosphate buffer pH 7.5 (400 ml) was added a solution of baker's yeast (2 g) in 0.01 M phosphate buffer pH 7.5 (100 ml). Incubation was performed under aerobic conditions at 35-37°C for 24 h with shaking at about 300 rpm. The reaction was stopped by the addition of chloroform (800 ml) and the reaction mixture was stirred vigorously for 10 min with a mechanical stirrer. Stirring was discontinued and the phases were separated (occasionally appearing emulsions were broken by centrifugation at 2000-4000g). After repeated extraction of the aqueous phases with chloroform (3x800 ml), the combined organic phase was dried over Na_2SO_4. The solvent was evaporated under reduced pressure to give the crude product 2a which on recrystallization with chloroform/hexane gave 89% of 2a[1], mp 228-230°C.

Other cyclization reactions were carried out similarly and the results are given in Table 2. The physical and spectroscopic data were similar to the ones described earlier[2].

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10. Except lipase from porcine pancreas, all the enzymes employed were from microbial sources.

11. Obtained from Sigma Chem. Co., type I.

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14. Lipase 14000 U/mg, obtained from Boehringer Mannheim as suspension in ammonium sulfate solution, 3.2 mol/l, potassium phosphate, 0.01 mol/l.

15. Lipase 40 U/mg lyophilisate obtained from Boehringer Mannheim.

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