Enzymatic Cyclizations Mediated by Ultrasonically Stimulated Baker's Yeast: Synthesis of Imidazo-fused Heterocycles

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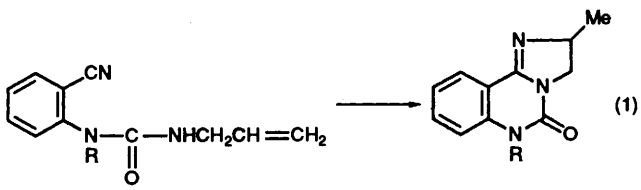
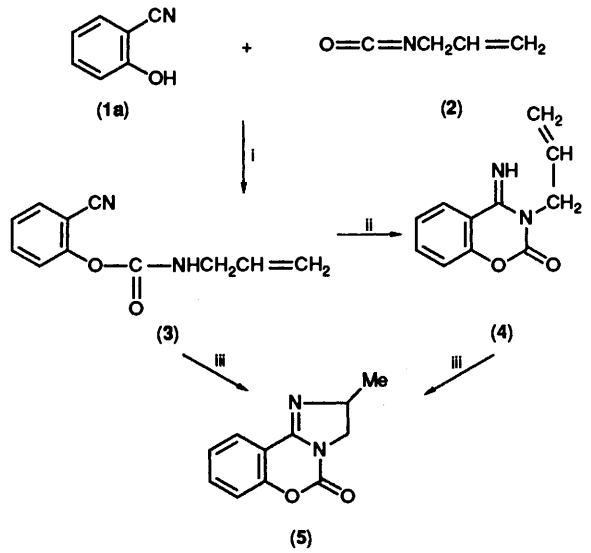
o-Cyanophenyl allyl carbamate (**3**), 3-allyl-3,4-dihydro-4-imino-2*H*-1,3-benzoxazine-2-thione (**8a**), and -quinazoline-2(1*H*)-thiones (**8b–d**) have been cyclized to 2,3-dihydro-2-methyl-5*H*-imidazo[1,2-*c*][1,3]benzoxazin-5-one (**5**), the corresponding 5-thione (**9a**), and 2,3-dihydro-2-methyl-5*H*-imidazo[1,2-*c*]quinazoline-5(6*H*)-thiones (**9b–d**), respectively, by ultrasonically stimulated baker's yeast. The effect of ultrasound on the yeast cells employed for the enzymatic reactions has been investigated. It is observed that ultrasonic pretreatment of yeast cells not only accelerates the cyclization process but improves the yields significantly.

Recent investigations[1,2] in our laboratories have focussed attention on the cyclization of compounds, particularly of those with labile functionalities, to afford biologically important heterocycles by employing enzymes in order to establish their potential as useful biocatalysts. In this research, we have recently demonstrated a novel enzymatic double cyclization[3] of 1-allyl-3-(*o*-cyanophenyl)urea by baker's yeast (*Saccharomyces cerevisiae*) as shown in equation (1). This successful cyclization of the functionalized substrate led us to consider the application of this strategy to the cyclization of other types of heteroatom-modified substrates, *e.g.* *o*-cyanophenyl allylcarbamate. Such an approach would be potentially useful for cyclization of labile-group-functionalized substrates that would be difficult to cyclize by conventional synthetic methods.[4] Furthermore, we decided to investigate the effect of ultrasonication of baker's yeast employed in enzymatic cyclizations.



(1)



Scheme 1. *Reagents and conditions:* i, CH_2Cl_2; ii, catalase; iii, baker's yeast.

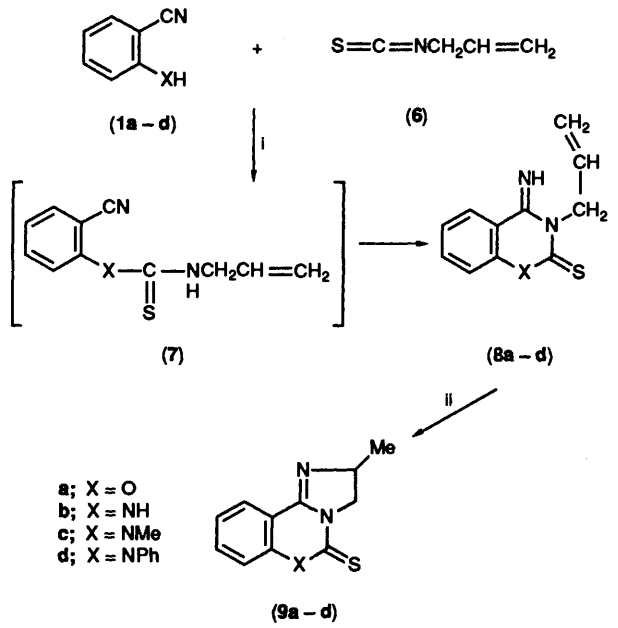
Results and Discussion

The required precursor, 2-(allylcarbamoyloxy)benzonitrile (*o*-cyanophenyl allylcarbamate) (**3**), was prepared by the reaction of allyl isocyanate (**2**) with 2-hydroxybenzonitrile (**1a**). However, when attempts were made to prepare the anthranilonitrile and salicylonitrile adducts by reaction with allyl isothiocyanate (**6**), the reactions did not proceed. However, the use of a base such as triethylamine in this reaction afforded 3-allyl-3,4-dihydro-4-imino-2*H*-1,3-benzoxazine-2-thione (**8a**) and - quinazoline-2(1*H*)-thiones (**8b–d**), while the uncyclized benzonitrile adducts (**7**) could not be isolated in these reactions.

Incubation of compound (**3**) with baker's yeast gave 2,3-dihydro-2-methyl-5*H*-imidazo[1,2-*c*][1,3]benzoxazin-5-one (**5**) *via* the 3-allyl-4-imino intermediate (**4**) (Scheme 1). Imidazobenzoxazinone (**5**) was also obtained by the direct cyclization of compound (**4**) with baker's yeast. Compound (**4**) was obtained by the cyclization of adduct (**3**) with catalase. Various 3-allyl-4-imino-substituted substrates (**8a–d**) were also cyclized to the corresponding imidazo-fused 1,3-benzoxazine-5-thione and quinazoline-5-thiones by baker's yeast (Scheme 2). These reactions were monitored by HPLC. It was observed that the cyclization took 6–7 days and gave *ca.* 50% conversion in the cases of compounds (**3**) and (**4**), and *ca.* 30% conversion in the case of substrates (**8**), these values remained constant even after 10 days of incubation. The products were characterized by analytical and spectroscopic data.

In an attempt to improve the efficiency of this enzymatic double cyclization, ultrasonically pretreated baker's yeast was employed in these reactions. This study was undertaken in view of the fact that in recent years the use of ultrasound has gained much importance as a method for improving the efficiency and spontaneity of organic synthetic reactions.[5,6] Furthermore, its application to the activation of microbial cells employed for biotransformations[7] is generating interest not only for accelerating and improving yields in enzyme-mediated reactions but also in our understanding of enzymatic reactions.

To determine the effect of ultrasound on the baker's yeast employed in the enzymatic reaction, the time course of product formation as a function of ultrasonic irradiation (see Figure) was examined. Experiments were carried out on sonicated baker's yeast with whole cells as well as cell-free homogenates. It was observed that in the incubations performed with whole cells, there occurred a significant increase in the cyclization



Scheme 2. *Reagents and conditions:* i, Et_2O, Et_3N; ii, baker's yeast.

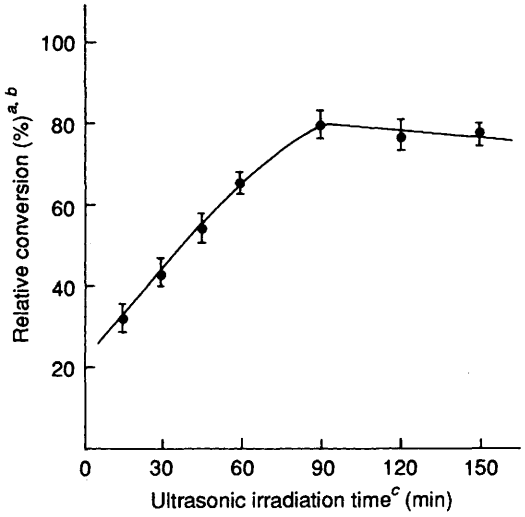


Figure. (*a*) The given conversions are relative to the maximum conversion at 1.5 h and each point is an average of four separate runs for the case (**8a**) ⟶ (**9a**) as an example. (*b*) Incubations were performed aerobically for 2 days at 37 °C. (*c*) Baker's yeast was sonicated prior to incubation, while the incubations were terminated by heating of the mixture to 80 °C for 10 min; mixtures were then shaken with chloroform, centrifuged, and analysed by HPLC.

products when cells were sonicated. It was also noticed that the increase in yield of cyclic product was appreciable when cells were sonicated for 1 h, while the maximum conversion efficiency was reached at 1.5 h sonification. On the other hand, the cyclization process employing a cell-free system was insensitive to ultrasonic irradiation and, in addition, was inefficient. The results are illustrated in the Table.

It can be inferred that the ultrasonic effect is probably associated with an enhancement of the diffusion process of the substrate on removal of the outer membrane. However, enzyme activity and membrane-associated factors cannot be ruled out. It is interesting to observe that disruption of the yeast cells by grinding did not give results much different from those obtained in the unsonicated baker's yeast-catalysed reactions. A control incubation using a boiled yeast preparation failed to produce the cyclic product, and unchanged substrate was recovered.

Table. Baker's yeast-mediated cyclization of compounds (**3**), (**4**), and (**8**) at 37 °C.

			Yield of products (**5**) and (**9a–d**) (%)		
			Unsonicated[a]	Sonicated	
Substrate	X	Product		Whole cell[b]	Cell-free[c]
(**3**)		(**5**)	51	76	22
(**4**)		(**5**)	53	82	25
(**8a**)	O	(**9a**)	32	80	12
(**8b**)	NH	(**9b**)	34	78	14
(**8c**)	NMe	(**9c**)	27	86	9
(**8d**)	NPh	(**9d**)	30	79	10

[a] Isolated yield of chromatographed product on reaction with unsonicated baker's yeast and incubation for 5 days (yields monitored by HPLC were almost identical). [b] By HPLC employing TSK ODS-120A, 5 μm column (4.6 × 250 mm), water–methanol (3:7) with 2% AcOH (v/v) at 0.5 ml min^{-1} flow rate for reactions with whole cell sonicated baker's yeast and incubation for 2 days. [c] By HPLC with cell-free sonicated baker's yeast and incubation for 4 days.

In conclusion, this investigation describes the application of ultrasonicated baker's yeast as an efficient biocatalyst for the synthesis of imidazo-fused heterocycles of biological interest, in particular for the conversion of 2-thione compounds (**8**). Furthermore, the ultrasonic effect is not only associated with improved yields but also with shortening of the incubation times required to convert the substrates into products. Hence, the present study, though lacking in stereoselectivity, provides a convenient one-pot procedure for the preparation of imidazo-fused heterocycles *via* double cyclization mediated by ultrasonically stimulated baker's yeast under mild conditions, and seems to have many potential applications in cyclizations involving labile-group-functionalized substrates.

Experimental

M.p.s were determined on a Buchi melting-point apparatus and are uncorrected. ^1H NMR spectra were taken frpm $CDCl_3$ solution with a JEOL FX90Q FT NMR spectrometer, with $SiMe_4$ as internal reference, at 90 MHz. IR spectra were recorded on a Perkin-Elmer 283B spectrophotometer. Mass spectra were recorded on a VG mass spectrometer. HPLC analyses were performed with a 6A-Shimadzu instrument with a 254 nm variable-wavelength detector and Chromatopac C-R4A integrator. Ultrasonifications were carried out employing a Branson Sonifier B-30 with a titanium immersion tip.

o-Cyanophenyl Allylcarbamate (**3**).—A solution of allyl isocyanate (**2**) (0.84 g, 10 mmol) in dry dichloromethane (2 ml) was added during 10 min to a stirred solution of 2-hydroxybenzonitrile (**1**) (1.2 g, 10 mmol) in dry dichloromethane (10 ml) at room temperature and the mixture was stirred for 6 h. The dichloromethane was then removed under reduced pressure and to the residue obtained was added cold water and the solid separated was filtered off. It was recrystallized from dichloromethane–light petroleum (40–60 °C) to give the *ester* (**3**) (1.8 g, 88%), m.p. 163–165 °C (Found: C, 65.1; H, 4.8. $C_{11}H_{10}N_2O_2$ requires C, 65.32; H, 4.97%); ν_{max} 3 325, 2 220, and 1 720 cm^{-1}; δ 3.8 (2 H, t), 5.2 (2 H, t), 5.8 (1 H, m), and 7.1–7.7 (5 H, m).

3-Allyl-3,4-dihydro-4-imino-2H-1,3-benzoxazin-2-one (**4**).—To a solution of compound (**3**) (0.3 g, 1.5 mmol) in ethanol (30

ml) and 0.1M-phosphate buffer (pH 7.4; 45 ml) was added catalase* (0.3 ml). The reaction mixture was incubated at 37 °C under aerobic conditions for 5 h while being shaken (200 rpm). The incubation mixture was extracted with chloroform (3 × 50 ml). The extract was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue obtained was recrystallized from methanol to give *compound* (**4**) (0.22 g, 73%), m.p. 89–90 °C (decomp.) (Found: C, 65.5; H, 4.8. $C_{11}H_{10}N_2O_2$ requires C, 65.32; H, 4.97%); ν_{max} 3 330, 1 715, and 1 610 cm^{-1}; δ 3.8 (2 H, t), 5.2 (2 H, t), 5.8 (1 H, m), and 6.9–8.2 (5 H, m).

3-*Allyl*-3,4-*dihydro*-4-*imino*-2H-1,3-*benzoxazine*-2-*thione* (**8a**).—To a solution of 2-hydroxybenzonitrile (**1a**) (1.2 g, 10 mmol) in diethyl ether (5 ml) was added a solution of allyl isothiocyanate (**6**) (1.0 g, 10 mmol) in dry diethyl ether (1 ml) followed by triethylamine (3 drops). The reaction mixture was stirred at room temperature for 6 h and kept overnight. The diethyl ether was then removed under reduced pressure to give the crude product, which was purified by column chromatography (silica gel; chloroform–methanol, 98:2) to furnish pale yellow crystals of compound (**8a**) (1.54 g, 71%), m.p. 178–180 °C.†

3-*Allyl*-3,4-*dihydro*-4-*iminoquinazoline*-2(1H)-*thione* (**8b**).—This compound was prepared in a similar manner as described above with 2-aminobenzonitrile (**1b**) (1.2 g, 10 mmol) and allylisothiocyanate (**6**) (1.0 g, 10 mmol) to give an oily residue. Purification by column chromatography (silica gel; chloroform–methanol, 97:3) afforded compound (**8b**) (1.73 g, 80%), m.p. 120–121 °C.

2,3-*Dihydro*-2-*methyl*-5H-*imidazo*[1,2-c][1,3]*benzoxazin*-5-*one*.—*Method A*. To a solution of *o*-cyanophenyl allylcarbamate (**3**) (0.2 g, 1.0 mmol) in ethanol (15 ml) and 0.01M-phosphate buffer (pH 7.4; 25 ml) was added baker's yeast (Sigma, Type I; 1.0 g). Incubation was performed under aerobic conditions at 37 °C for 6 days. The incubation mixture was extracted with chloroform (3 × 30 ml). The separated organic phases were combined, and dried over Na_2SO_4. The solvent was evaporated off under reduced pressure to give the crude *product* (**5**), which was purified by column chromatography (silica gel; chloroform–methanol, 96:4), m.p. 164–167 °C (0.102 g, 51%) (Found: C, 65.2; H, 4.7. $C_{11}H_{10}N_2O_2$ requires C, 65.32; H, 4.97%); ν_{max} 1 720 and 1 610 cm^{-1}; δ 1.5 (3 H, d), 5.1 (2 H, t), 6.0 (1 H, m), and 6.9–8.1 (4 H, m).

Method B. This was performed in a similar manner as described above, but used 3-allyl-3,4-dihydro-4-imino-1,3-benzoxazin-2-one (**4**) (0.2 g, 1.0 mmol) instead of ester (**3**). Incubation was carried out for 5 days to afford the product (**5**) (0.106 g, 53%).

2,3-*Dihydro*-2-*methyl*-5H-*imidazo*[1,2-c][1,3]*benzoxazine*-5-*thione* (**9a**).—This compound was prepared in a similar manner as described above, by employing baker's yeast. When whole cell, sonicated baker's yeast was employed the incubation was performed for 2 days, while for cell-free, sonicated baker's yeast the incubation was carried out for 5 days; yields obtained are given in the Table. Compound (**9a**) had m.p. 158–160 °C.

Ultrasonic Pretreatment of Baker's Yeast.—A suspension of baker's yeast (20 g) in 0.1M-phosphate buffer (pH 7.4; 100 ml) was irradiated ultrasonically (*ca.* 50 W) at 0–5 °C for 1.5 h. The resulting dispersion was employed for whole cell or cell-free sonicated baker's yeast as required.

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* Catalase from beef liver as solution in glycerol, 30% (v/v), ethanol 10% (v/v), *ca.* 260 000 units ml^{-1}; obtained from Boehringer Mannheim.

† Yields, m.p.s and ^1H NMR and analytical data for compounds (**8a–d**) and (**9a–d**) have been deposited as Supplementary Publication SUP 56789 (3 pp.), see section 4.0 *et seq.* in Instructions for Authors, in the January issue.