

EFFICIENT ENZYMATIC CYCLIZATION OF 2-(CARBAMOYLOXY)-AND 2-(SULFAMOYLOXY)-
BENZONITRILES BY ULTRASONICALLY STIMULATED BAKER'S YEAST

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Abstract - An approach to the synthesis of 4-imino substituted 1,3-benzoxazin-2-ones, 1,2,3-benzoxathiazin-2-ones and 2-quinazolinones based on the enzymatic cyclization of the labile-functionalized compounds with ultrasonically stimulated baker's yeast.

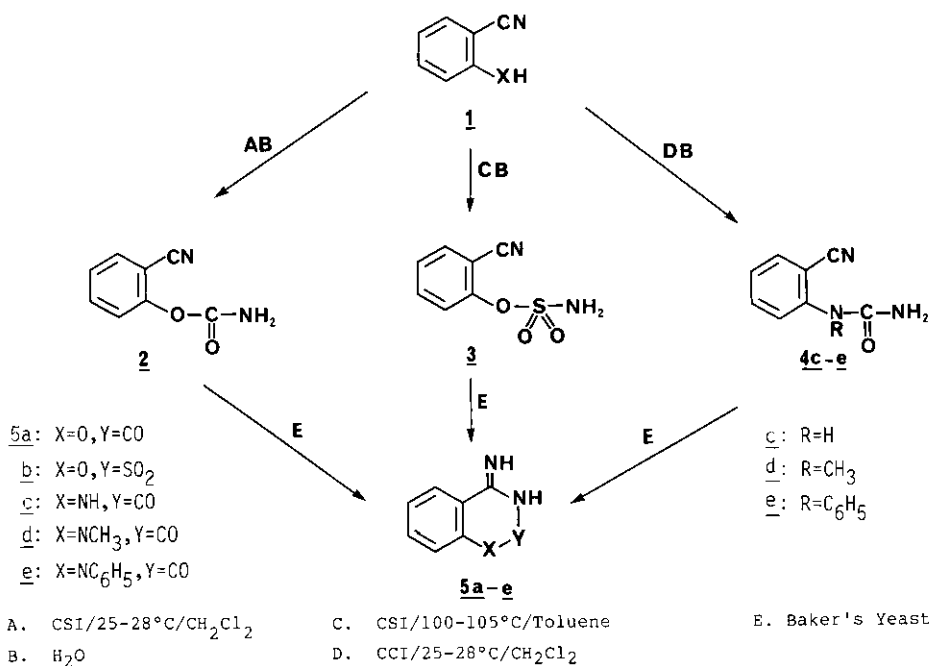
We recently demonstrated the use of catalase¹ as an effective reagent for the enzymatic cyclization of N-allylcarbamoyl anthranilonitriles and further manifested their double cyclization with baker's yeast.² However, the use of catalase has limited the enzymatic process to only small scale (100 mg or less) substrate conversions. We now wish to report that an 'ultrasonically stimulated' suspension of baker's yeast (*Saccharomyces cerevisiae*) is an efficient and inexpensive source of enzyme for cyclizations of labile-functionalized compounds such as 2-carbamoyloxy- as well as 2-sulfamoyloxybenzonitriles. Additionally, this can be employed for the gram-scale enzymatic cyclization of such substrates.

In our earlier studies³ on the synthesis of 4-imino-2(1H)-quinazolinones, it was observed that the reaction of 2-aminobenzonitriles with chlorosulfonyl isocyanate (CSI) gave the corresponding quinazolines, whereas the reaction with chlorocarbonyl isocyanate (CCI) afforded 2-benzonitrile ureas. Later attempts to prepare 4-imino-1,3-benzoxazin-2-one, in view of the biological importance of such heterocycles,³ by the reaction of CSI with 2-hydroxybenzonitrile gave the 2-carbamoyloxybenzonitrile (**2**) and not the desired cyclization in analogy with the 2-aminobenzonitrile reactions. Further, the reaction of 2-hydroxybenzonitrile with CSI at higher temperature⁴ (100-105 °C in toluene) also did not yield the cyclized compound, while 2-sulfamoyloxybenzonitrile (**3**) was obtained. Efforts to cyclize 2-carbamoyloxy- and 2-sulfamoyloxybenzonitriles non-enzymatically resulted in the cleavage of O-carbamoyl/O-sulfamoyl linkages.

In a typical reaction procedure, **2**, 1 g dissolved in ethanol (50 ml) and 0.1 M phosphate buffer

(pH 7.4) was incubated at 37 °C for 5 h with an ultrasonically preptreated⁵ suspension of baker's yeast (10 g), after filtration and extractive work up with chloroform yielded 0.86 g (86% yield, mp 165-166 °C, 98.6% purity by hplc⁶) of 4-imino-1,3-benzoxazin-2(3H)-one (5a).

A control incubation using a boiled yeast preparation afforded 97% of recovered 2. The use of a yeast suspension that was not ultrasonically stimulated prior to incubation gave approximately 30% less conversion under identical conditions.



The utility of this enzymatic cyclization method was further shown by the cyclization of O-sulfa-moylbenzonitrile (3) and 2-benzonitrile ureas (4c-e) in good yields (82-93%). The products were characterized by analytical and spectroscopic data.⁷ The possibility of the 4-amino tautomeric form has been ruled out by ir studies.³

In conclusion, this method of enzymatic cyclization by ultrasonically irradiated baker's yeast not only exhibits a facile and practical route of cyclization for substrates bearing labile functionalities but also suggests that the ultrasonic effect is associated with facilitating this type of cyclization by activating the enzyme or by increasing the substrate diffusion. This feature is being studied in greater detail.

REFERENCES AND NOTES

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5. Typical ultrasonic pretreatment⁸: 10 g of baker's yeast was suspended in 50 ml of 0.1 M phosphate buffer, pH 7.4, and irradiated ultrasonically at 0-5 °C for 1 h at about 50 W intensities employing a Branson Sonifier B-30 with titanium immersion tip.
6. Hplc was obtained by using the 6A-Shimadzu instrument with a 254 nm variable-wave length and Chromatopac C-R4A integrator. All chromatography was performed at 25 °C temperature on an UltroPac TSK Si-150, 5 μ m column (250 x 4.6 mm). The mobile phase was 95% chloroform and 5% butanol at 0.3 ml/min flow rate.
7. Selected data - 2: mp 120-122 °C (93%), ir(KBr), 3410, 3260, 2220, 1710 cm^{-1} , ^1H nmr(CDCl_3) 5.5(br s, 2H), 7.2-7.6(m, 3H), 7.8-7.9(dd, J= 7.4 and 1.5 Hz, 1H); 5a: ir(KBr), 3350, 3280, 1715, 1660 cm^{-1} , ^1H nmr(CDCl_3) 7.1-8.2(m, 5H), 9.2(br, s, 1H).
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