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PII: S1381-1177(15)30096-5
DOI: http://dx.doi.org/doi:10.1016/j.molcatb.2015.11.003
Reference: MOLCAB 3267

To appear in: *Journal of Molecular Catalysis B: Enzymatic*

Received date: 4-7-2015
Revised date: 24-10-2015
Accepted date: 1-11-2015

Please cite this article as: Kilwoong Mo, Jin Hyeong Park, Soon Bang Kang, Youseung Kim, Yong Sup Lee, Jae Wook Lee, Gyochang Keum, Synthesis of Pyrazine via Chemoselective Reduction of *rmbeta*-Keto-*rmalpha*-Oximino Ester using Baker's Yeast, Journal of Molecular Catalysis B: Enzymatic http://dx.doi.org/10.1016/j.molcatb.2015.11.003

Synthesis of Pyrazine via Chemoselective Reduction of β-Keto-α-Oximino Ester using Baker's Yeast

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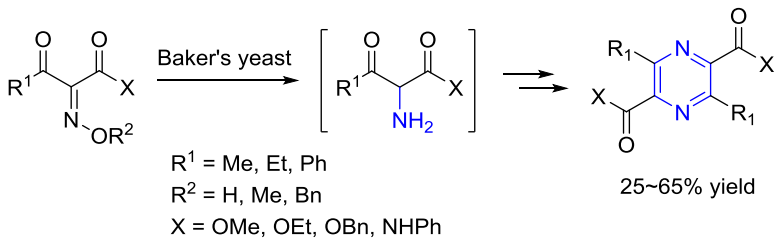
Graphical abstract:



Highlights:

- Pyrazines were prepared from β-keto-α-oximino esters using baker's yeast.
- Baker's yeast reduced oxime selectively over ketone.
- β-Keto-α-amino ester might be a key intermediate toward pyrazines.
- β-Keto-α-oximino amide reacted with baker's yeast to give pyrazines too.
- Pyrazines were prepared efficiently and eco-friendly by biocatalyzed reaction

Abstract: The synthesis of pyrazines by the baker's yeast-mediated reaction of β-keto-α-oximino esters and amides is described. Baker's yeast reduced oximes selectively over ketones of β-keto-α-oximino esters to give the corresponding β-keto-α-amino ester intermediates, which underwent spontaneous dimerization followed by air-induced aromatization to yield pyrazines. The chemoselective reduction of β-keto-α-oximino amides using baker's yeast also afforded the corresponding pyrazines. Interestingly, both hydroximes and alkoximes gave the pyrazines by the baker's yeast-mediated reduction via the corresponding amino ketones, the known precursors of pyrazines. The reaction was strongly dependent upon pH of reaction medium, and gave optimum yields at pH 5. These results demonstrate that pyrazines were synthesized efficiently and eco-friendly using a whole-cell biocatalytic system as an alternative to chemical reduction.

Keywords: Baker's yeast • Reduction • Chemoselectivity • Pyrazine • β-Keto-α-oximino esters

1. Introduction.

Baker's yeast (*Saccharomyces cerevisiae*) is a convenient whole-cell biocatalytic system that has been used extensively for the asymmetric reduction of a variety of ketone and carbonyl functionalities [1-5]. Many researchers have shown that baker's yeast is an efficient bio-catalyst for the reduction of C-C double bonds,[6] hydrolysis,[7] oxidation,[8] C-C bond formation,[9] and nitro group reduction.[10] In addition, baker's yeast has been shown to reduce oximes to amines [11]. Hydroxylamines have also been formed from oximes through the reduction of C-N double bonds using an immobilized baker's yeast in a hexane-water (1:9) solvent system [12]. The baker's yeast-mediated reduction of oximes into alcohols or amines depends on the relative rates of reaction between the hydrolysis of an oxime to its corresponding carbonyl group and the reduction of the oxime to an amine [13]. Interestingly, Kreutz *et al*.[14,15] reported that α-oximino ketones, which have both oxime and ketone groups, can be reduced into optically active α-hydroxyoximes by baker's yeast (Figure 1). This stereoselective reduction of ketone by baker's yeast is influenced by the size of the R group on the various substrates.

In the chemical reduction, the selectivity towards oxime and carbonyl groups can be controlled by selecting the appropriate reducing agent. The ketones in β-keto-α-alkyloximino esters can be selectively reduced to alcohols by sodium borohydride ($NaBH_4$) [16]. The oxime groups in β-keto-α-hydroximino esters can be selectively reduced to amines by aluminum amalgam, Zn, Pd/C, $SnCl_2$, or indium [17–23]. The selective reduction of oximes in β-keto–α-hydroximino esters is useful for stereoselective syntheses of threonine analogues and natural products [19, 24–27]. Furthermore, pyrazine can be synthesized via the oxime-selective reduction of α-hydroximino-β-keto esters with aluminum amalgam,[18] zinc,[20] Raney nickel,[28] Pd/C,[29] or excess aqueous $TiCl_3$,[30] followed by condensation and aromatization. However, to the best of our knowledge, alkyloxime selective reduction over ketone of β-keto-α-alkyloximino ester was not reported previously even by chemical reducing agents.

Pyrazines are nitrogen-containing, six-membered heterocyclic compounds with a long history in organic chemistry[31] and have been utilized in a broad range of academic and industrial fields including materials science,[32] food science,[33] and pharmaceutical research.[34] Pyrazines are found in fermented and heated foods and are widely used in the food industry as flavor ingredients [33, 35]. They are also found in many vegetables, insects, and microorganisms [36–39]. Many pyrazine derivatives boast various pharmacological

activities including antimicrobial,[40–43] herbicidal,[44] cardiovascular,[45–46] anti-inflammatory,[47] antidepressant,[48] analgesic,[49–51] and anticancer activities [52–54].

Biosynthesis[55–56] and other synthetic approaches[31,57-59] for pyrazines have been widely studied because of the broad application base of pyrazine scaffolds. We recently reported the baker's yeast-mediated, ketone-selective reduction of β-keto-α-oximino nitriles to give β-hydroxy-α-oximino nitriles with high stereoselectivity [60]. However, the oxime-selective reduction of α-oximinoketone provides the corresponding α-aminoketone, which is a known precursor in the chemical preparation of pyrazine. Baker's yeast, which has the ability to reduce both ketones and oximes, can be used to synthesize pyrazines from α-oximinoketones. These reactions can be also applied to the enantioselective synthesis of β-hydroxy-α-oximino esters and threonine-class amino acid derivatives, as shown in Figure 1. Therefore, this study explores the baker's yeast-mediated selective reduction of β-keto-α-oximino esters.

2. Materials and methods

2.1. Yeast

Baker's yeast (BY) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and Oriental (Tokyo, Japan), and stored in a refriderator at 2–8 ºC.

2.2. Chemicals

All chemicals and solvents were obtained from commercial suppliers (Fisher Scientific Co. and Sigma-Aldrich Co.) and used without further purification. Unless otherwise indicated, biocatalyzed reactions were performed under shaking incubator with temperature control.

*2.3. Synthesis of β-keto-α-alkyloximino esters (**1b–f**) and amide (**4b**)*

Ethyl 2-methoxyimino-3-oxobutanote (1b): To a stirred solution of **1a** (2.0 g, 12.6 mmol) and dimethyl sulfate (1.43 mL, 15.1 mmol) in acetone (20 mL), K_2CO_3 (1.04 g, 7.56 mmol) was added slowly at 0 ºC. The reaction mixture was stirred at room temperature for 5 h. After completion of the reaction, monitored by TLC, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc = 20:1) to afford the desired product **1b** (2.18 g, 98% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.35 (q, *J* = 7.14 Hz, 2H), 4.10 (s, 3H), 2.40 (s,

3H), 1.33 (t, *J* = 7.14 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 193.2, 161.5, 150.4, 64.7, 62.5, 25.5, 14.4 ppm. MS (EI): *m/z* 173 [M^+]. TLC: R_f 0.31 (*n*-hexane/EtOAc 20:1).

Ethyl 2-(benzyloxyimino)-3-oxobutanoate (1c): The title compound was obtained from **1a** and benzyl bromide according to the similar procedure by the *O*-alkylation described above. Yield: 87.4%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40–7.25 (m, 5H), 5.31 (s, 2H), 4.34 (q, *J* = 7.14 Hz, 2H), 2.36 (s, 1H), 1.30 (q, *J* = 7.15 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 193.3, 161.6, 150.9, 136.3, 129.0, 128.9, 128.6, 79.0, 62.5, 25.6, 14.4 ppm. MS (EI): *m/z* 249 [M^+]. TLC: R_f 0.28 (*n*-hexane/EtOAc 20:1).

Methyl 2-(methoxyimino)-3-oxobutanoate (1d): The title compound was obtained from methyl 2-(hydroxyimino)-3-oxobutanoate according to the same procedure by the *O*-methylation described above. Yield: 92%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.09 (s, 3H), 3.85 (s, 3H), 2.38 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 192.9, 161.7, 150.1, 64.6, 52.8, 25.3 ppm. TLC: R_f 0.28 (*n*-hexane/EtOAc 20:1).

Methyl 2-(methoxyimino)-3-oxopentanoate (1e): The title compound was obtained from methyl 2-(hydroxyimino)-3-oxopentanoate according to the same procedure by the *O*-methylation described above. Yield: 94%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.08 (s, 3H), 3.86 (s, 3H), 2.81 (q, *J* = 7.8 Hz, 2H), 1.12 (t, *J* = 7.8, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 195.9, 161.9, 149.5, 64.5, 52.8, 31.1, 7.7 ppm. TLC: R_f 0.26 (*n*-hexane/EtOAc 20:1).

Benzyl 2-(methoxyimino)-3-oxobutanoate (1f): The title compound was obtained from benzyl 2-(hydroxyimino)-3-oxobutanoate according to the same procedure by the *O*-methylation described above. Yield: 94%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40–7.37 (m, 5H), 5.33 (s, 2H), 4.10 (s, 3H), 2.41 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 192.9, 161.3, 150.0, 134.9, 128.8, 128.7, 128.4, 67.7, 64.6, 25.4 ppm. MS (EI): *m/z* 235 [M^+]. TLC: R_f 0.31 (*n*-hexane/EtOAc 15:1).

2-(Methoxyimino)-3-oxo-*N*-phenylbutamide (4b): The title compound was obtained from **4a** according to the same procedure by the *O*-methylation described above. Yield: 97%. ^{1}H NMR (300MHz, $CDCl_3$) δ: 7.50–6.90 (m, 5H), 4.07 (s, 3H), 2.38 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 194.5, 155.5, 150.9, 137.1, 129.5, 129.4, 125.5, 121.2, 120.6, 65.1, 26.4 ppm. MS (EI): *m/z* 220 [M^+]. TLC: R_f 0.38 (*n*-hexane/EtOAc 10:1).

*2.4. Synthesis of pyrazines (**3**, **3a–c** and **6**) using Baker's yeast*

Diethyl 3,6-dimethylpyrazine-2,5-dicarboxylate (3): Ethyl-2-methoxyimino-3-oxobutanote **1b** (69.2 mg, 0.4 mmol) dissolved in ethanol (1.5 mL) was added to a suspension of baker's yeast (2.0 g) and saccharose (3.0 g) in tap water (60 mL) with shaking at 30 ^{o}C. The mixture was shaken for the specified time by monitoring the complete consumption of the starting material with TLC, and then saturated with sodium chloride. The resulting mixture was filtered through a celite pad and washed with chloroform. The filtrate was extracted with chloroform three times, and the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (*n*-hexane/EtOAc = 5:1) to afford the desired product **3** (26.4 mg, 53% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.02 (s, 6H), 3.12 (q, J = 7.47 Hz, 4H), 1.32 (t, *J* = 7.47 Hz, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.5, 151.2, 144.5, 62.8, 22.7, 14.6 ppm. HRMS (EI): *m/z* calcd for $C_{12}H_{16}N_2O_4$: 252.1110 [M^+]; found: 252.1151; elemental analysis calcd (%) for $C_{12}H_{16}N_2O_4$: C 57.13, H 6.39, N 11.10, O 25.37; found: C 57.95, H 6.46, N 10.58, O 25.01. TLC: R_f 0.44 (*n*-hexane/EtOAc 5:1).

Dimethyl 3,6-dimethylpyrazine-2,5-dicarboxylate (3a): The title compound was obtained from **1d** according to the similar procedure using the baker's yeast described above. Yield: 65%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.03 (s, 6H), 2.84 (s, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.6, 151.6, 143.9, 53.6, 22.7 ppm. MS (FAB): *m/z* 225.22 [M+H]$^+$; elemental analysis calcd (%) for $C_{10}H_{12}N_2O_4$: C 53.57, H 5.39, N 12.49, O 28.54; found: C 53.94, H 5.54, N 11.95, O 27.82. TLC: R_f 0.32 (*n*-hexane/EtOAc 10:1).

Dimethyl 3,6-diethylpyrazine-2,5-dicarboxylate (3b): The title compound was obtained from **1e** according to the similar procedure using the baker's yeast described above. Yield: 24%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.01 (s, 6H), 3.11 (q, *J* = 7.5 Hz, 4H), 1.26 (t, *J* = 7.4 Hz, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.9, 156.2, 144.4, 51.5, 28.9, 14.5 ppm. MS (FAB): *m/z* 253.25 [M+H]$^+$. TLC: R_f 0.31 (*n*-hexane/EtOAc 10:1).

Dibenzyl 3,6-dimethylpyrazine-2,5-dicarboxylate (3c): The title compound was obtained from **1f** according to the similar procedure using the baker's yeast described above. Yield: 37%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.48–7.26 (m, 10H), 5.45 (s, 4H), 2.75 (s, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 165.2, 151.3, 144.3, 135.4, 129.1, 129.0, 128.9, 68.3, 22.7 ppm. MS (FAB): *m/z* 377.16 [M+H]$^+$. TLC: R_f 0.43 (*n*-hexane/EtOAc 10:1).

3,6-dimethyl-N^2,N^5-diphenylpyrazine-2,5-dicarboxamide (6): The title compound was obtained from **4a** according to the similar procedure using the baker's yeast described above.

Yield: 45%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.96 (s, 2H), 7.77–7.16 (m, 10H), 3.10 (s, 6H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 161.7, 151.3, 143.2, 137.8, 129.5, 125.1, 120.3, 23.1 ppm. MS (FAB): *m/z* 347.24 $[M+Na]^+$. TLC: R_f 0.32 (*n*-hexane/EtOAc 5:1).

3. Result and discussion

Ethyl-2-(hydroxyimino)-3-oxobutanoate **1a** was used as a substrate to evaluate the baker's yeast-mediated reduction of β-keto-α-oximino esters. This reaction using Sigma type II yeast under typical conditions [60] at 30 ºC for 3.5 days afforded a white solid in a 42% yield after isolation. The solid product stained lightly on a TLC plate, exhibited an unexpectedly low polarity, and was identified as pyrazine **3**. Also, GC/MS analysis confirmed the presence of pyrazine with a small amount of α-amino ketone intermediate in the reaction mixture. Interestingly, GC/MS analysis of crude product revealed none of the expected side products such as compound **2**. This result indicated that baker's yeast selectively reduced the oxime in **1a** to give the corresponding α-amino ketone intermediate followed by the spontaneous transformation to the pyrazine derivative [17–23, 28–30].

To improve the yield of pyrazine **3**, the reaction was evaluated under a variety of conditions by changing additives, temperature, and the type of baker's yeast used (table 1). The reduction with baker's yeast immobilized on montmorillorite K10[15] gave a similar yield as that obtained with entry 1. Reductions without saccharose gave lower yields with a mixture of unknown compounds (entry 3). Further modification of the reaction conditions did not improve the isolation yield of compound **3**.

In addition to hydroximes, alkyloximes were also evaluated as substrates for baker's yeast-mediated, oxime-selective reduction. A series of β-keto-α-alkyloximino esters **1b** and **1c** were prepared according to a previous method [25]. Treatment of the appropriate β-ketoesters with sodium nitrite in acetic acid gave hydroxime compounds **1a** and subsequent *O*-alkylation of the hydroximes gave the methyl- and benzyl-oximinoketones **1b** and **1c**, respectively, in high yields. The reduction of α-methyloximinoketone **1b** with a variety of baker's yeasts gave pyrazine **3** with relatively low yields. GC/MS analyses showed the presence of small amounts of alcohol **2** generated by β-ketone reduction. The results represent that alkyloxime groups in β-keto-α-alkyloximino esters can be selectively reduced to α-aminoketones by baker's yeast.

The reduction reaction in the absence of saccharose slightly improved the isolated yield of **3** (31%) following relatively long reaction times (3.5 days, entry 9). β-Keto-α-benzyloximino

ester **1c** with Sigma type II baker's yeast successfully afforded pyrazine **3** in a 38% isolated yield with a 9:91 GC ratio of **2**:**3**, which is a higher selectivity than that obtained with the immobilized yeast (entries 11 and 12). The reaction times of alkyloximes **1b** and **1c** were shorter than that of the corresponding hydroxime **1a**.

When a variety of reaction conditions were evaluated, the results in table 2 show that the reaction was primarily influenced by pH. The reaction at pH 7 showed a significantly lower isolation yield after 1.5 days (entry 1, table 2) than that obtained without buffer. The product yield increased with decreasing pH until reaching an optimum at pH 5 (entry 3).

These results show that an addition of small amount of ethanol and careful control of the reaction pH (entries 1–4) range are critical to improving yields in baker's yeast-mediated reductions of **1a**. The reductions of methyl oxime **1b** also gave pyrazine **3** in high yields at pH 5 with three types of baker's yeast. The reduction of benzyl oxime **1c** showed the highest yield with Sigma type I baker's yeast at the same pH (entries 9–11). The reactions of methyl and benzyl esters **1d**–**f**, prepared using the same method, and the corresponding pentanoate **1e** also afforded the chemoselective reduction of the oxime to afford pyrazines **3a**–**c** (entries 12–14 in table 2). The reduction of methyl ester **1d** with baker's yeast purchased from Oriental showed the highest isolation yield (65%, entry 13 in table 2).

To test β-keto-α-oximino amide substrates, we prepared compounds **4a**–**b** from 2,2,6-trimethyl-4H-1,3-dioxin-4-one and aniline using a known procedure [61]. The reaction of hydroxime amide **4a** gave a 1:2 mixture of diol **5** and pyrazine **6** in GC analyses in 9% and 21% isolated yields, respectively, using Sigma type II baker's yeast without buffer (entry 1 in table 3).

The same reaction of **4a** at pH 5 gave pyrazine **6** with improved yield (45%, entry 2 in table 3). The reduction of methyloxime amide **4b** with baker's yeast also performed best at pH 5 and yielded pyrazine **6** in a low yield of 25%. With the exception of the benzyl ester **1f**, reactions with *N*-phenyl amides **4a** and **4b** required longer durations than reactions with methyl and ethyl esters **1a**–**e** (table 3).

We next explore the mechanism of formation of compound **3** (dimethyl 3,6-dimethylpyrazine-2,5-dicarboxylate) by baker's yeast mediated reduction, and the plausible mechanism was shown in Figure 2. Usually, the product which is generated by baker's yeast reduction of □-ketone was expected to be alcohol. The GC-MS analysis revealed that the molecular mass of amino ketone was found in the reaction mixture confirming the generation of amino ketone **7** as an intermediate. The baker's yeast reduced oximino ketone to amino

ketone. The amino ketone **7** were dimerized by spontaneous condensation and followed by air oxidation to give pyrazine.

4. Conclusion

In summary, the synthesis of pyrazines through the baker's yeast-mediated reaction of β-keto-α-oximino esters and amides was developed. The pyrazines were generated by the oxime-selective reduction of β-keto-α-oximino esters and amides with baker's yeast to their corresponding aminoketone intermediates, which was not isolated but underwent spontaneous dimerization followed by air-oxidized aromatization to provide pyrazines. The selective reduction with baker's yeast converted both the hydroxy and alkyl oxime moieties of β-keto-α-oximino esters and amides to the corresponding β-keto-α-amino esters and amides, the known precursors of pyrazines. The baker's yeast-mediated reduction was primarily influenced by pH and gave the optimum results at pH 5. The results demonstrate that pyrazines can be prepared in an efficient and eco-friendly green procedure using a whole-cell biocatalytic system, representing a valuable alternative to chemical reduction. Furthermore, the reactions studied herein may be representative of the processes occurring in biological systems and may account for the natural occurrence of pyrazines in foods and microorganisms.

Acknowledgements

This work was supported by the Korea Institute of Science and Technology (KIST) institutional program (2E25240 and 2Z04371).

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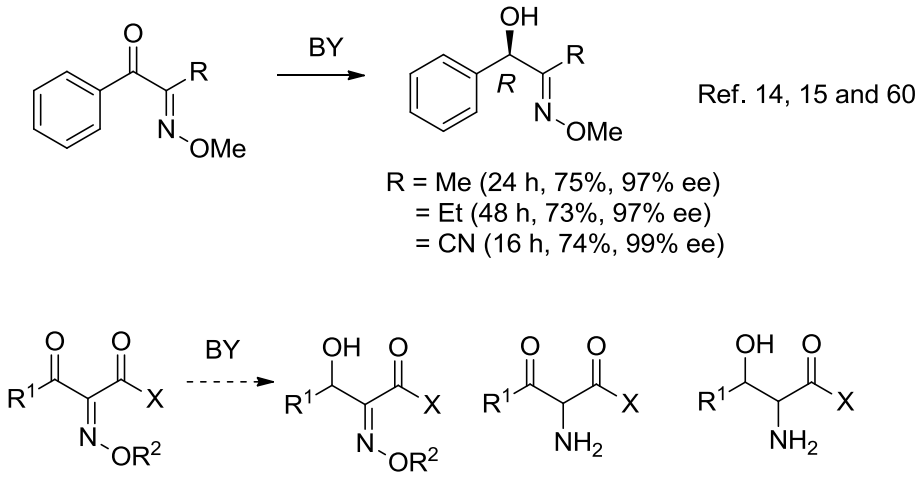


Figure 1. Baker's yeast (BY)-mediated reduction of β-keto-α-oximino carbonyl compounds.

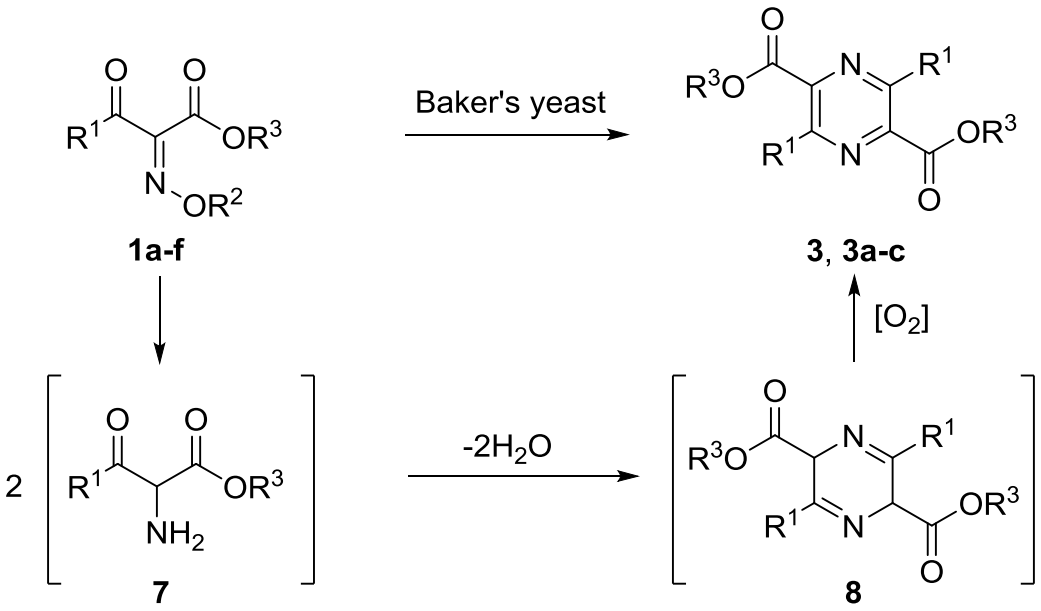
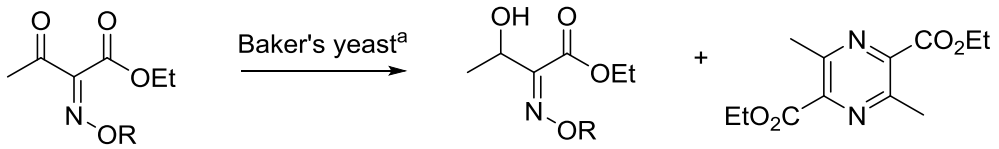


Figure 2. Proposed mechanism of the synthesis of pyrazine by baker's yeast mediated reduction.

Table 1. Reduction of β-keto-α-alkyloximino esters using baker's yeast.

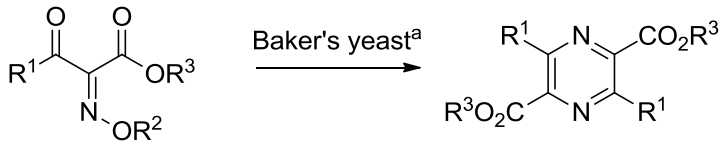


1 R = H (**a**), Me (**b**), Bn (**c**) **2** **3**

entry	Conditions			GC ratio				yield **3**
	sub.	B.Y.[b]	time	sm[c]	**2**	**3**	uk[d]	(%)[e]
1	**1a**	Sigma-II	3.5 d	0	0	100	0	42
2		ImS-II	3.5 d	0	0	100	0	42
3		ImS-II	3.5 d	0	0	51	49	-[f]
4		ImS-II	3.5 d	0	0	94	6	22[g]
5		Sigma-I	3.5 d	14	0	57	29	-
6		Oriental	3.5 d	0	0	79	21	22
7	**1b**	Sigma-II	6 h	0	14	86	-	-
8		ImS-II	14 h	0	2	98	-	29
9		ImS-II	3.5 d	0	4	96	-	31[f]
10		Oriental	14 h	0	10	49	41	-
11	**1c**	Sigma-II	18 h	0	9	91	-	38
12		ImS-II	14 h	0	16	84	-	-

[a] Typical conditions: Baker's yeast (2.0 g), substrate (0.4 mmol), saccharose (3.0 g) in water (30 mL) and ethanol (1.5 mL) at 30 °C. [b] Baker's yeast: Sigma-II (Sigma type II), Sigma-I (Sigma type I), ImS-II (Sigma type II immobilized on montmorillorite K10)[15], Oriental (Tokyo, Japan). [c] Starting material. [d] Unknown mixture. [e] Isolated yields. [f] Saccharose was not used. [g] Reaction temperature: at 37 °C.

Table 2. Reduction of various β-keto-α-oximino esters **1a-f** with baker's yeast under pH control.



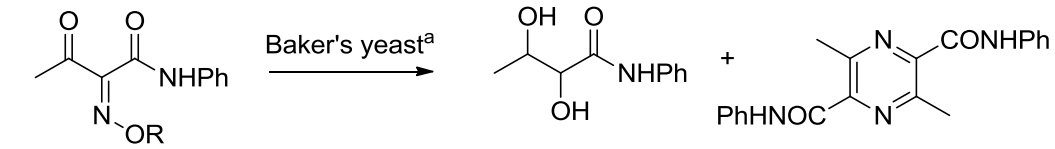
1a-f **3, 3a-c**

entry	sub.	Conditions						pdt	yield **3** (%)[b]
		R^1	R^2	R^3	B.Y.	time	pH		
1	**1a**	Me	H	Et	Sigma-II	1.5 d	7	**3**	3
2					Sigma-II	3.5 d	6	**3**	42
3					Sigma-II	1.5 d	5	**3**	47
4					Sigma-II	1.5 d	4	**3**	27
5					Oriental	2 d	5	**3**	34
6	**1b**	Me	Me	Et	Sigma-II	1.5 d	5	**3**	53
7					Sigma-I	1.5 d	5	**3**	35
8					Oriental	2 d	5	**3**	44
9	**1c**	Me	Bn	Et	Sigma-II	2 d	5	**3**	29
10					Sigma-I	1.5 d	5	**3**	40
11					Oriental	2 d	5	**3**	25
12	**1d**[c]	Me	Me	Me	Oriental	3 d	5	**3a**	65
13	**1e**[c]	Et	Me	Me	Sigma II	2 d	5	**3b**	24
14	**1f**[c]	Me	Me	Bn	Oriental	6 d	5	**3c**	38

[a] Typical conditions: Baker's yeast (2.0 g), substrate (0.4 mmol), and saccharose (3.0 g) in water (60 mL) and ethanol (1.5 mL) at 30°C. The pH was adjusted with 1 *N* HCl and NaOH.

[b] Isolated yields. [c] The best results from the three types of baker's yeast were reported.

Table 3. Reaction of β-keto-α-oximino amide compounds with baker's yeast.



R = H (**4a**), Me (**4b**) **5** **6**

entry	sub.	Conditions			yield (%) of **6**[c]
		yeast type	time (day)	pH	
1	**4a**	Sigma II	3.5	-[b]	21 (9)[d]
2		Sigma II	5	5	45
3		Oriental	5	5	37
4	**4b**	Sigma II	7	5	25
5		Oriental	3	5	25

[a] Reactions were carried out with 0.4 mmol substrate, baker's yeast (2.0 g), and saccharose (3.0 g) in water (60 mL) and ethanol (1.5 mL) at 30°C. The pH was adjusted with 1 *N* HCl and NaOH.

[b] Reaction was carried out without buffer. [c] Isolated yields. [d] The isolated yield of diol **5** is in parentheses.