Table 1. Summary of pK values obtained from the pH dependence of V_{max} and V_{max}/K_m in water and ethanol.

V_{max}/K_m	Solvent	pK_1±SE		pK_2±SE	
		Neutral acid buffer	Cationic acid buffer	Neutral acid buffer	Cationic acid buffer
	Water	6.5±0.2	6.3±0.1	8.0±0.2	8.4±0.3
	25% Ethanol	5.7±0.2	6.2±0.2	8.2±0.1	8.4±0.2

V_{max}	Solvent	pK±SE	
		Neutral acid buffer	Cationic acid buffer
	Water	7.3±0.2	7.5±0.1
	25% Ethanol	7.2±0.3	7.5±0.4

* pK_1 indicates that the group must be protonated for enzyme activity and pK_2 indicates that the group must be deprotonated for enzyme activity.

25% ethanol (third curve), in cationic acid buffer in 25% ethanol (bottom curve). The log V_{max}/K_m plots imply the effects of these solvents on the pK values of the ionizing groups on the free enzyme. The pK values were not changed between in the neutral acid buffer and in the cationic acid buffer in water within errors in Table 1. The pK values were changed between in the neutral acid buffer and in the cationic acid buffer on the 25% ethanol. Particularly, the pK in the acidic side was shifted 0.5 pH units from 6.2 to 5.7. The pK values on the acidic side and basic side is not shifted in the presence of 25% ethanol and cationic acid buffers. However, the pK values in the presence of 25% ethanol and neutral acid buffers appears to be shifted to lower pH. The pK in the acidic side is shifted 0.8 pH units from 6.5 down to 5.7 and the pK in the basic side is shifted from 8.0 to 8.2. The pK in the acidic region is considered a real shift (0.8 pH units) within errors. But the change in the basic pK value may be only an apparent shift caused by large shift in the acidic pK value in this profile. These results suggest that the enzyme group (pK_1) for the acidic side is of the cationic type and the enzyme group (pK_2) for the basic side is of the neutral type. The most likely candidate enzyme group for acidic side is a histidine residue at which 6.3 pK value occurs. This conclusion is supported by Yoon *et al.* who examined that a general base must be deprotonated for catalysis with a pK value near 6.3 and that a general acid must be protonated for substrate binding with a pK value near 8.4[7](*vide ante*). Another additional evidence is that the chemical modification study with diethylpyrocarbonate which is specific to histidine residue also supports that histidine residue is being in active site.[10] The possible candidate enzyme group for the basic side is a cysteine residue at which 8.4 pK value occurs. Another chemical modification study using NEM and DTNB which is specific to cysteine residue has supported the requirement of cysteine residue for catalytic activity.[11] In summary, the authors strongly suggest that a general base for the catalysis is a histidine residue and if any exist, a general acid for activity is a cysteine residue.

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Cleavage of N-O Bonds by Samarium Diiodide: A New Entry to Chemoselective Methods for the Conversion of Isoxazolines to β-Hydroxy Ketones

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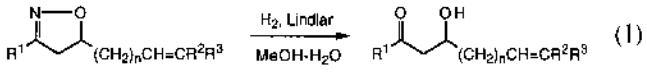
Recently we reported a practical method for the selective cleavage of isoxazoline nuclei (N-O bonds) in the presence of double bonds by the use of Lindlar catalyst Eq. (1).[1] During these studies, we also observed that the reduction of isoxazoline rings containing the C-3 phenyl group Eq. (1), (R= Ph) requires much larger amount of Lindlar catalyst and accompanies the serious hydrogenation of the double bonds, resulting in poor chemoselectivity of the

reduction.[2] An alternative method is needed in order to improve the chemoselectivity of the reductive cleavage of N-O bonds in such isoxazolines.

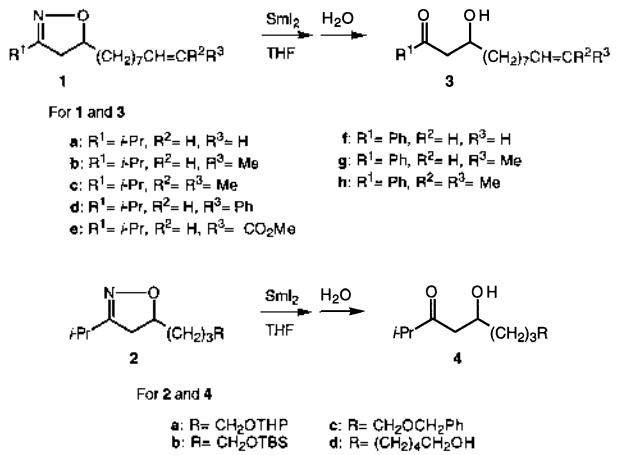


(1)

Although samarium diiodide is known to cleave N-O bonds,[3,4] to the best of our knowledge this reducing reagent has never been utilized for the reduction of isoxazoline N-O bonds.[5] We thus began to examine samarium diiodide-promoted reduction of isoxazolines (Scheme 1).

Upon surveying reaction conditions with isoxazoline **1b**[1] as a model substrate, it was found that the chemoselective reduction of N-O bond could be achieved by treating isoxazoline **1b** with 2.3 equivalents of samarium diiodide in tetrahydrofuran[6] containing five equivalents of triethylamine and subsequent acidic workup gave β-hydroxy ketone **3b** in high yield. The yield of β-hydroxy ketone **3b** seemed to be sensitive to reaction conditions. For example, when excess (more than 2.5 equivalents) samarium diiodide was used, reduction of the β-hydroxy group became significant. And, without the use of triethylamine, the desired conversion of **1b** to **3b** was accompanied by β-elimination of the hydroxy group, which resulted in lower yield of **3b**. The use of approximately 2.3 equivalents of samarium diiodide and five to ten equivalents of triethylamine gave the most satisfactory results.

As shown in Table 1, variously substituted olefin-containing isoxazolines could be transformed into the corresponding β-hydroxy ketones in high yields by this procedure. Most double bonds survived the reduction conditions without difficulty (entries 1-4 and 6-8). For the cleavage of most C-3 isopropyl-substituted isoxazolines (entries 1-4, except entry 5[7]), the yields are comparable to those by the Lindlar procedure.[1] However, the yields of β-hydroxy ketones from C-3 phenyl-substituted isoxazolines (entries 6-8) were far superior to those by the Lindlar process. It is also worth to note that no care is needed to prevent the reduction of monosubstituted olefins (entry 1) while the reduction with Lindlar catalyst requires careful control to prevent the saturation of the carbon-carbon double bonds. In order to test the stability



Scheme 1.

Table 1. Samarium Diiodide-Mediated Reduction of Isoxazolines.

Entry	Isoxazoline	β-Hydroxy ketone	Reaction time (min)	Yield[c] (%)
1	**1a**	**3a**	60	73
2	**1b**[a]	**3b**	60	81
3	**1c**	**3c**	60	74
4	**1d**[a]	**3d**	75	76
5	**1e**[b]	**3e**	60	43
6	**1f**	**3f**	60	78
7	**1g**[a]	**3g**	60	81
8	**1h**	**3h**	60	65
9	**2a**	**4a**	60	73
10	**2b**	**4b**	60	74
11	**2c**	**4c**	60	74
12	**2d**	**6d**	60	62[d]

[a] A mixture of (*E*)- and (*Z*)-isomers was used. [b] Only (*E*)-isomer was used. [c] Yields refer to isolated yields. [d] Yields not optimized.

of other functional groups to the reaction conditions reduction of additional isoxazolines **2a-d** were also examined. Results in Table 1 (entries 9-12) indicate that functional groups such as tetrahydropyranyl, benzyl or silyl ethers and alcohols are compatible with the reaction conditions.

The typical experimental procedure is as follows. To a solution of isoxazoline **1b** (53 mg, 0.21 mmol) in tetrahydrofuran (2 mL) at the room temperature under argon atmosphere was added triethylamine (213 mg, 2.10 mmol). Then a solution of samarium diodide (4.8 mL, 0.1 M, 0.48 mmol) in tetrahydrofuran (2 mL) at the room temperature under argon atmosphere was added triethylamine (213 mg, 2.10 mmol). Then a solution of samarium diodide (4.8 mL, 0.1 M, 0.48 mmol) in tetrahydrofuran was added dropwise over 30 min and the mixture was stirred for an additional 30 min. After addition of water and 1 N HCl, the resulting mixture was extracted with ethyl acetate (~7 mL × 3). The combined organic layers were washed with saturated sodium bicarbonate solution and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography (11% EtOAc/hexanes) to give 43 mg (81%) of **3b** as a clear oil: IR 3448 (O-H), 3012 (vinylic C-H), 1692 (C=O), 1031 (C-O) cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.30-5.52 (m, 2H), 4.02 (m, 1H), 3.14 (br d, J= 3.1 Hz, 1H), 2.46-2.72 (m, 3H), 1.90-2.10 (m, 2H), 1.56-1.72 (m, 3H), 1.32 (m, 12H), 1.12 (d, J= 6.4 Hz, 6H).

In summary, we have developed a new method for the chemoselective cleavage of the isoxazoline nuclei, which serve a valuable and complementary means for the conversion of isoxazolines to β-hydroxy ketones.

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7. Further work is in progress to locate the origin of lower yield in this case and to improve the yield of **3e**.

Diastereoselective Routes in the Paterno-Büchi Reaction of Cyclic Enol Ortho Ester with Aldehydes†

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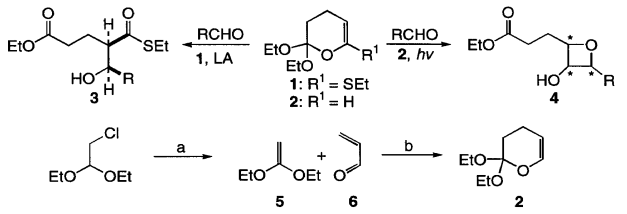
In confluence with the wide spread advances in synthetic methods, particularly diastereoselective routes, the Paterno-Büchi reaction has prospered and led to significant developments in the area of synthetic organic chemistry.[1] The Paterno-Büchi reaction represented by the [2+2] photocycloaddition of carbonyl functionality to alkene provides a unique way of route to functionalized oxetane.[2] There have been several investigations directed towards diastereoselectivity in this reaction using enol ethers mainly due to the generation of up to three new stereogenic centers during the process.[3] We recently reported the diastereoselective synthesis of highly functionalized aldol products of glutarates **3** from the reaction of dihydropyran **1** with aldehydes promoted by a Lewis acid catalyst.[4] The efficiency of this transformation concerning diastereocontrolled process has encouraged us to apply the extension of this substrate to structurally unique systems. We report herein our discovery of a broadly useful method for assembling products **4** from the photochemical reaction of 2,2-diethoxy-3,4-dihydro-2*H*-pyran (**2**) with aldehydes as depicted in Scheme 1. The method described herein is successful with several aldehydes and affords products with useful levels of diastereoselectivity.

Starting material **2** was prepared in quantity by a two step sequence, purified by distillation, and stable to storage. Treatment of chloroacetaldehyde diethyl acetal with *t*-BuOK in *t*-BuOH afforded ketene acetal **5** in 78% yield after distillation. The hetero Diels-Alder reaction of **5** with acrolein in the presence of 2,6-di-*tert*-butylphenol (0.5 mol %) at 180 °C for 2h in a sealed tube afforded adduct **7** in 74% yield.[5] The stage was thus set for the photochemical cycloaddition of **2** with aldehydes.



Scheme 1. (a) *t*-BuOK, *t*-BuOH, (b) 2,6-Di-*tert*-butylphenol (0.5 mol%), 180 °C, 2 h, sealed tube.

The initial study for orienting experiments focused on the feasibility of substrate **2** for the photochemical cyclization with achiral aldehydes. Preliminary studies on the reaction of **2** with benzaldehyde indicated that the conversion to the corresponding product could be realized by irradiation of the reaction mixture at 300 nm. After surveying numerous conditions, several key findings emerged: (i) optimal chemical yields and diastereo-selectivities were observed with the use of CH_2Cl_2 as a solvent compared to other solvents such as benzene and acetonitrile; (ii) a 2 : 1 mixture of aldehyde/dihydropyran **2** proved to be the most effective in terms of chemical yield; (iii) benzaldehyde exhibited better reactivity than aliphatic aldehydes; (iv) interesting observation was made that the formation of rearranged product **9** during the photocycloaddition was detected. The photochemical cycloaddtion was performed according to the following general procedure: A quartz tube was charged with benzaldehyde Eq. (2) and dihydropyran **2** Eq. (1) dissolved in degased CH_2Cl_2. The sample was irradiated at 300 nm at room temperature for 3 h, and then the irradiation was stopped. After cooled to 0 °C, the reaction mixture was treated with 0.5 N aqueous HCl followed by work up and silica gel chromatography to afford inseparable oxetanes **7a** and **8a** in a ratio of 92 : 8 as judged by ^{1}H NMR along with the minor product **9a** (3 : 2 diastereomeric mixture). The results obtained with other aldehydes are summarized in Scheme 2.

Although the exact mechanistic aspects of this transformation have not been rigorously elucidated, the following pathway could be a probable chemical and stereochemical routes on the basis of product population. According to the mechanistic studies for photocycloadd-itions,[6] a putative 1,4-diradical intermediate for the oxetane formation resulted from the addition of n,π^* triplet state of carbonyl compound to

†Dedicated to Prof. Sang Chul Shim on the occasion of his 60th birthday.