and could be used as such in the next step. ^{1}H NMR (Me_2SO-d_6): δ 4.29 (2 H, s, benzylic), 7.95-8.35 (9 H, m, aromatic), 12.49 (1 H, br s, CO_2H).

When the above reaction was performed on a 1-g scale, isolation of the methyl ester was not required and the acid was obtained in quantitative yield.

Acid-Catalyzed Decomposition of 4(5)-Nitroso-5(4)-phenylimidazole in Methanol and Water

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Metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole] and other 5-nitroimidazoles are used extensively for the treatment of infections with protozoa and anaerobic bacteria.[1,2] Although exact details are unknown, these drugs are believed to be activated by reduction of the nitro group,[3-5] with an implication that one (or more) of the reduction products is the cause of biological activity. The amine derivative from metronidazole[6,7] is observed in biological systems, but it lacks antimicrobial activity.[7] This focuses attention on the nitroso and hydroxylamine derivatives, but little is known of their chemistry. Various reductions of metronidazole give ring-fragmented products,[8-10] some of which have also been observed as metabolites in biological systems.[8,11,12] The fragmentation indicates that a product (or products) of intermediate oxidation states is not stable. An examination of the literature reveals no examples of simple 5-hydroxylamino or 5-nitrosoimidazoles. There is one 4-hydroxylamine[13] and two reports of 4(5)-nitroso-5(4)-phenylimidazoles.[14,15] Frustrated in our attempts to prepare derivatives of metronidazole, we have reexamined the latter system. We find that the nitroso compound can be prepared, but it is unstable in aqueous solution, undergoing ring opening via a nucleophilic addition reaction that may model some of the fragmentations associated with metronidazole reduction.

Table I. NMR Spectral Characteristics

	nitroso[a] compd	methanol[b] adduct	intermed[c] in water	major product[a] in water
H_2'	8.53	8.28	8.43	8.26
H_3'	7.85-7.90	7.49	7.85	7.73
H_4'		7.58	7.75	7.90
H_2	8.22	6.39	6.67	9.62
		(CH_3) 3.38[f]		
C_1'	132.1	132.03		134.8
C_2'[d]	131.1	131.2		130.3
C_3'[d]	130.9	130.1		129.0
C_4'	132.3	133.7		135.0
C_2	140.3	103.7		168.0
C_4[e]	147.3	154.3		164.3
C_5[e]	159.6	167.6		192.9
		(CH_3) 51.0[f]		

[a] ^{1}H NMR, 1:1 Me_2SO-d_6/D_2O; ^{13}C NMR, Me_2SO-d_6. [b] CD_3OD. [c] 1:1 Me_2SO-d_6/D_2O. [d] C_2', C_3' cannot be distinguished. [e] C_4, C_5 cannot be distinguished. [f] Signal does not correspond to CH_3OH.

Results and Discussion

The literature route[14] to 4(5)-nitroso-5(4)-phenylimidazole involves the reaction of 4(5)-phenylimidazole with isoamyl nitrite in ethanol containing sodium ethoxide, followed by acidification. With this procedure we obtained the green solid previously reported in good yield, although there was substantial loss of material on recrystallization. Our experiments with 4(5)-methylimidazole and with 2-phenylimidazole failed to give characterizable products. The procedure however did work with 2,4(2,5)-diphenylimidazole.[14] The indication therefore is that a 4(5)-phenyl is required.

The 4(5)-nitroso-5(4)-phenylimidazole had ^{1}H and ^{13}C NMR spectra (Table I), mass spectrum, and analysis consistent with this structure. The absorption spectrum in water had a strong band at 365 nm with a weak band at 725 nm. It is well established that a *C*-nitroso compound can exist as the simple monomer or as an azodioxy dimer.[16] A weak visible band near 700 nm is characteristic of the monomer, the dimer usually not absorbing above 400 nm.[16] Thus, the presence of the 725-nm band indicates that 4(5)-nitroso-5(4)-phenylimidazole is monomeric. Moreover, in methanol, the visible absorbance followed Beer's law in solutions as concentrated as 20 mM, implying that this compound remains monomeric at least up to this point. The brilliant green indicates that the solid form is also monomeric.

By following the change in absorbance at 365 nm, a pK_a of 7.1 was obtained for deprotonation. Thus, the nitrosoimidazole is relatively acidic, probably more acidic than the corresponding nitroimidazole. This acidity constant, for 4(5)-nitro-5(4)-phenylnitroimidazole, is not known. However, 4(5)-nitroimidazole has a pK_a of 9.3,[17] and the additional phenyl is unlikely to have a large effect.

Under acid conditions, the nitrosoimidazole is not stable. In the absorption spectrum, the 365-nm band and the visible band were observed to disappear, with a new band eventually (see later text) appearing at 260 nm. The absorbance decreases obeyed excellent first-order kinetics at constant pH, with the first-order rate constants k_{obsd} being proportional to H^+ concentration over the range of pH studied (pH 1-6). A second-order rate constant at 25 °C was obtained by plotting k_{obsd} vs. H^+ concentration; the value is 1.3×10^2 M^{-1} s^{-1}. The half-life at pH 6 is 1.5 h; in 0.1 M HCl it is only 50 ms.

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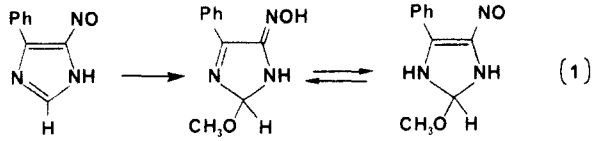
The decomposition was probed by ^1H NMR spectroscopy, in a 6:1 CD_3COOD/CD_3COONa buffer in 50:50 D_2O/Me_2SO-d_6. The starting nitrosoimidazole has a singlet at 8.22 ppm due to the imidazole C2 hydrogen. This disappeared with a new singlet appearing upfield at 6.67 ppm. There were accompanying albeit less pronounced changes in the phenyl resonances. The signals due to the starting material had almost completely disappeared after 1 h in this solution, but before they were completely gone, a further change started occurring with the initial product. This was characterized by a disappearance in the 6.67 ppm singlet and the appearance of a new singlet considerably downfield at 9.62 ppm. This further process was slow, requiring about 1 week to go to completion. The spectrum at this time had the downfield singlet and peaks associated with two phenyl rings, in a ratio of about 4:1. Less detailed experiments in solutions with different pH showed the same general features and also indicated that both the initial change and the subsequent change are H^+ dependent. In 0.01 M DCl in 50:50 D_2O/Me_2SO-d_6 the spectrum recorded after the 1–2 min required to set up the instrument corresponded to the final spectrum. The occurrence of consecutive processes could also be seen in the UV spectra, in that there were quite complex changes in the 230–270-nm region, with no isosbestic behaviour.

The nitrosoimidazole also reacted in methanol in an H^+-dependent process, but in this solvent the product with the upfield singlet was stable and could be isolated. A mass spectrum and elemental analysis provide a formula of $C_{10}H_{11}N_3O_2$, corresponding to a product containing 1 equiv each of the starting material and methanol. NMR parameters are shown in Table I; an extra signal corresponding to an OCH_3 group has appeared.

Structure 1 arising from 1,6 nucleophilic addition of methanol to the nitrosoimidazole satisfies the various observations (eq 1). The tautomer 2 can be excluded since



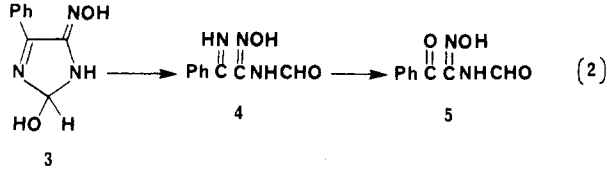
(1)

1 2

this contains a nitrosoalkene group that would have a visible spectrum (green).[18] There are several mechanistic possibilities to account for the reaction. One would involve nucleophilic C2 addition on the 5-nitroso tautomer of the starting material, with oxygen protonation of the nitroso group to explain the H^+ dependency. A second possibility has ring protonation[19] and addition to give 2, followed by tautomerization.

The saturation of the C2 carbon of the imidazole ring explains the upfield NMR shifts in the ^{13}C signal for this carbon and in the signal for the attached proton. The ^{13}C signals associated with C4 and C5 remain downfield in positions characteristic of C=N groupings.[20] A proton-coupled ^{13}C NMR spectrum provided further confirmation. This had the signal for the C2 carbon at 103.7 ppm split into two because of the directly attached hydrogen. In addition, because of long-range coupling to the methyl, each of these was split further into a quartet (with a much smaller coupling constant). A corresponding change was seen in the resonance for the methyl that appeared as a quartet with each peak further split in two.

The similarity in the ^1H NMR spectra (Table I) suggests that an analogous adduct 3 forms initially in water. This is an interesting example of a heterocyclic N,N tetrahedral intermediate[21,22] and is particularly noteworthy for being so stable. The presence of the OH group however means that, in contrast to the methanol adduct, 3 can undergo further reactions involving ring opening and the formation of formyl derivatives[21a,e] (eq 2). This is consistent with the downfield shift observed in the signal for the hydrogen at C2 as 3 disappeared.



(2)

3 4 5

Unfortunately we have been unable to conclusively establish the structure of the final products. Three species were separated by preparative reversed-phase HPLC. The first two were obtained in very small quantities, and each had ^1H NMR spectra different from anything observed in situ. We suspect that these are the result of further decomposition, possibly of the minor product since this had disappeared. The third product of this separation had a ^1H NMR spectrum corresponding to the major project observed in situ (Table I) including the downfield hydrogen at 9.62 ppm. Although weak, the ion of highest mass in the mass spectrum of this material appeared at 192 mass units. This could correspond to either 5[22] or 4 H^+ of eq 2, products derived by ring opening of 3 away from the C=N bond.

Whatever the identity of the final products, it is clear from this study that the ring of a nitrosoimidazole is susceptible to nucleophilic addition and that ring opening and presumably further fragmentation reactions can occur.

Experimental Section

^1H and ^{13}C NMR spectra were recorded on 200- and 400-MHz Varian spectrophotometers and a 360-MHz Nicolet spectrophotometer. Absorption spectra were recorded with a Varian 2390 spectrophotometer. The faster kinetic runs were conducted on a Durrum-Gibson stopped-flow spectrophotometer.

4(5)-Nitroso-5(4)-phenylimidazole.[14] 4-Phenylimidazole (36 mmol) was added to 20 mL of anhydrous ethanol containing sodium ethoxide (39 mmol), and under nitrogen isoamyl nitrite (36 mmol) was added over 20 min. The solution was allowed to stand for 5–6 days and then was diluted with 250 mL of water and extracted with 3 × 100 mL of diethyl ether to remove isoamyl alcohol. Carbon dioxide gas was passed through the aqueous layer, lowering the pH to 7. A metallic green solid was filtered and washed with cold ethanol. The crude sample weighed 4.3 g (71% yield). This was recrystallized from ethanol; mp 189 °C (lit.[14] mp 193 °C). Anal. Calcd for $C_9H_7N_3O$: C, 62.42; H, 4.04; N, 24.27. Found: C, 62.73; H, 4.13; N, 24.06.

Attempted Synthesis with 4(5)-Methylimidazole and 2-Phenylimidazole. A similar procedure was employed. No precipitate was obtained upon acidification. Repeated extractions

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of the neutral solution with methylene chloride yielded small amounts of dark purple oils whose ^1H NMR spectra indicated extensive decomposition.

Methanol Adduct 1. 4(5)-Nitroso-5(4)-phenylimidazole (100 mg) dissolved in 50 mL of anhydrous methanol was allowed to stand for 1 week. Removal of the solvent by rotary evaporator and overnight drying in a vacuum desiccator over P_2O_5 left a tan solid, mp 80–100 °C dec. Anal. Calcd for $C_{10}H_{11}N_3O_2$: C, 58.53; H, 5.40; N, 20.47. Found: C, 56.60; H, 5.66; N, 20.21.

Isolation of Products from Aqueous Decomposition. 4-(5)-Nitroso-5(4)-phenylimidazole (500 mg) was vigorously stirred in 250 mL of 0.01 M HCl for 1 h, followed by removal of the solvent by lyophilization. A portion of this was subjected to HPLC separation using a Waters C-13 μBondapak reversed-phase preparative column with detection at 254 nm and 40% acetonitrile/60% water as eluting solvent. Solvents were removed from separated samples by rotary evaporation and lyophilization.

Acidity Constant. A solution of 4(5)-nitroso-5(4)-phenylimidazole (200 μM) in 0.002 M NaOH was mixed with an exactly equal volume of various buffers and the absorbance at 364 nm recorded. In the case of solutions with pH <6, extrapolation to zero time was necessary because of the decomposition. The acidity constant K_a was calculated as the average of values of $(A_{acid} - A)[H^+]/(A - A_{base})$ where A_{acid} and A_{base} are absorbances at pH 4 and 11, respectively, and A is the absorbance in the intermediate range pH 6–8.

Kinetics. To 2.5 mL of an equilibrated aqueous solution at 25 °C was added 25 μL of an 0.02 M Me_2SO solution of 4(5)-nitroso-5(4)-phenylimidazole. The absorbance decrease at 365 nm was monitored. Rate constants were calculated by linear regression as the slopes of plots of ln $(A - A_\infty)$ vs. time.

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Regio- and Enantioselective Reduction of α,2-Dioxocycloalkaneacetates with Fermenting Bakers' Yeast. A New Synthesis of (*R*)-(−)-Hexahydromandelic Acid

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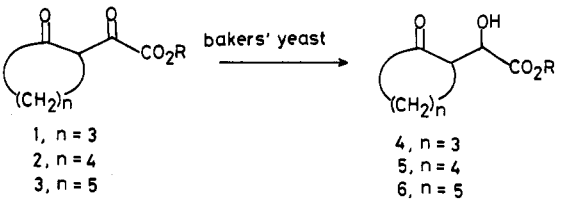
Recently, the use of actively fermenting bakers' yeast for the preparation of optically active building blocks in the synthesis of natural products has increased.[1] It is well-known that the reduction of β-keto esters by actively fermenting bakers' yeast (*Saccharomyces cerevisiae*) affords optically active β-hydroxy esters.[2-5] Although the asymmetric reductions of α-keto esters with fermenting bakers' yeast were reported,[6-8] no attempt has been made for those of α,2-dioxocycloalkaneacetic acid esters **1–3**. This prompted us to investigate the reduction of **1–3** with fermenting bakers' yeast,[9] which would be expected to give optically active α-hydroxy-2-oxocycloalkaneacetic acid esters **4–6**.



α,2-Dioxocycloalkaneacetates **1–3** are readily available from the reaction of the cycloalkanones and oxalic esters in the presence of base.[10] Treatment of **1–3** with fermenting bakers' yeast at 32 °C gave optically active α-hydroxy esters **4–6** as diastereomeric mixtures in fair yield. The chemical yields, the ratios of threo and erythro isomers, and the optical yields are tabulated in Table I.

The reaction proceeded regiospecifically to give only α-hydroxy ester, and even a trace amount of 2-hydroxy-α-oxo ester could not be detected. Diastereomers were separated by either preparative HPLC, GLC, or column chromatography. Determination of the optical purity was established by conversion of each isomer to the corresponding *N*-(3,5-dinitrophenyl)carbamate and then analysis by HPLC using an optically active column and/or by ^1H NMR in the presence of the chiral shift reagent Eu(hfc)$_3$.

Reduction of ethyl α,2-dioxocyclopentaneacetate (**1**) yielded optically active ethyl α-hydroxy-2-oxocyclopentaneacetate (**4**) in 74% yield as a diastereomeric mixture of threo (α*R*,1*S*) and erythro (α*R*,1*R*) isomers (2/3). Reduction of ethyl α,2-dioxocyclohexaneacetates (**2b**) showed better diastereoselectivity (threo/erythro = 9/1) than that in the case of methyl ester **2a**. To get the best reaction conditions, the relative amount of bakers' yeast to the substrate **2b** was varied, and the results are tabulated in Table II.

The chemical yield and threo selectivity increased as the ratio of the amount of yeast to **2b** decreased. Although immobilized bakers' yeast improved enantiomeric excess in the reduction of some α-keto esters,[11a] we found no notable improvement in chemical yield and diastereoselectivity using immobilized yeast. Reduction of ethyl α,2-dioxocycloheptaneacetate (**3**) with bakers' yeast gave the reduced product **6** in low diastereo- and enantioselectivity.

The stereochemistry of the reduced products was determined by analyzing the NMR spectra with reference to the literature.[12] In ^1H NMR spectra, the α-proton of a threo isomer generally appeared at higher field than that of the erythro one. In the ^{13}C NMR spectrum of ethyl

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