recrystallization during 2 days in a cold room gives the diacid (**4**, 87.0 g) as colorless crystals in a 7% yield starting from **1**.

5,7-Dioxobicyclo[2.2.2]oct-2-ene (5). The diacid (**4**, 27.0 g, 0.12 mol) and lead tetraacetate (102.0 g, 0.23 mol) in dioxane (260 mL) are purged with nitrogen for 15 min and then placed in a water bath at 12–15 °C. The mixture is vigorously stirred while nitrogen continues to be passed through it. Pyridine (250 mL) is next admixed. The mixture is kept in a bath of water at 60 °C for 10 min, when the carbon dioxide should all be released. The mixture is then rapidly cooled and poured into nitric acid (2 N, 1350 mL). The acid solution is extracted with chloroform (8 × 100 mL). The organic phase is washed with water (1 × 100 mL), with saturated aqueous sodium bicarbonate (2 × 100 mL), and with saturated sodium chloride solution (1 × 100 mL). Drying over Na_2SO_4 and evaporation gives practically pure (as judged by NMR) diketone (**5**, 7.2 g, 53 mmol) in 42% yield.

The Bistosylhydrazone (6) of 5. The diketone (**5**, 3.8 g, 28 mmol) in ethanol (10 mL) is added dropwise to tosylhydrazine (10.4 g, 56 mmol) in ethanol (64 mL) over 10 min. The solution is then heated under reflux for 4 h; the resulting precipitate is filtered warm, washed with ethanol, and dried in vacuo giving the bistosylhydrazone (13.0 g, 27.5 mmol) as pure product in 98% yield.

Bicyclo[2.2.2]octa-2,5,7-triene (Barrelene, 7). To a solution of bistosylhydrazone (**7**, 5.7 g, 12 mmol) in 1,2-bis(dimethylamino) ethane (70 mL) cooled to −23 °C is added dropwise during 1 h a solution of methyllithium (2 M) in ether (48 mL).[9,10] The mixture is stirred under nitrogen for 4 h at −23 °C and then overnight at 20 °C. Excess reagent is decomposed carefully with water (ca. 150 mL). Ether extraction (5 × 30 mL) followed by washing with water (1 × 50 mL), hydrochloric acid (2 N, 2 × 50 mL), water (1 × 50 mL), and saturated aqueous sodium chloride (1 × 50 mL) and drying (over $NaSO_4$) affords a solution which must be carefully evaporated. Most solvent can be removed by distilling at atmospheric pressure using a Vigreux column 20 cm long. In the residue, barrelene (**7**) is present (190–200 mg, 15% yield). Separation of pure **7** can be effected by GLC using a column of 20% SE-30 or 10% OV-17 on Chromosorb W at 150 and 100 °C, respectively. On average, 120 mg (10% yield) of pure barrelene (**7**) is isolated.[11]

Registry No.—**1**, 123-31-9; **2**, 108-31-6; **3**, 61586-14-9; **4**, 61543-84-8; **5**, 17660-74-1; **6**, 61543-85-9; **7**, 500-24-3; tosylhydrazine, 1576-35-8.

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(5) Fifty milliliters of cyclooctatetraene sells for 230 Swiss francs (Fluka AG, Chemische Fabrik, CH-9470 Buchs SG, Switzerland).
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(7) Originally we followed the method of C. M. Cimarusti and J. Wolinsky [*J. Am. Chem. Soc.*, **90**, 113 (1968)], but we found that yields of **5** were only some 10–17% using pyridine as solvent under oxygen.
(8) An earlier preparation of **5** reports a yield of 7–10%: C. A. Grob and A. Weiss, *Helv. Chim. Acta*, **53**, 1390 (1960).
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(10) J. E. Stemke and F. T. Bond, *Tetrahedron Lett.*, 1815 (1975).
(11) A similar short route, without experimental details, has been recently realized: C. Weitemeyer and A. de Meijere, *Angew. Chem., Int. Ed. Engl.*, **15**, 686 (1976).

Preparation and Reactivity of a New Spin Label Reagent

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In the course of developing a reagent which could be used to selectively attach a nitroxide spin label to tyrosine residues in proteins, we have prepared *N*-(2,2,5,5-tetramethyl-3-carbonylpyrrolidine-1-oxyl)imidazole (I), a stable, crystalline solid. Preliminary studies indicate that this reagent may be generally useful for attaching the nitroxide spin label to molecules of biological interest. In this communication, we report the preparation of I, its hydrolytic reactivity, and its utility as a reagent for synthesis of spin-labeled molecules.

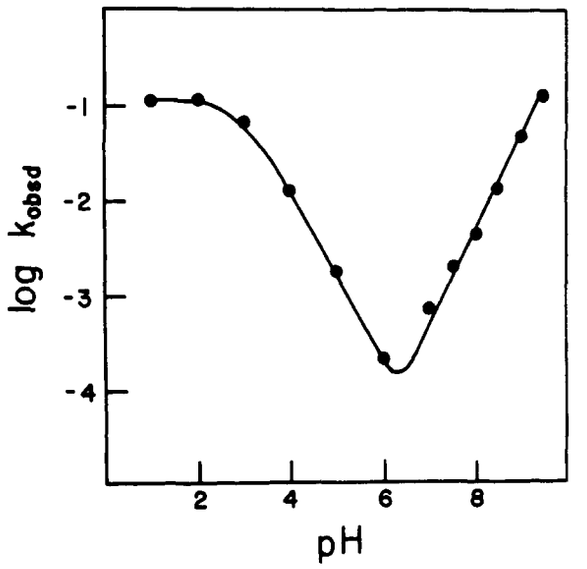
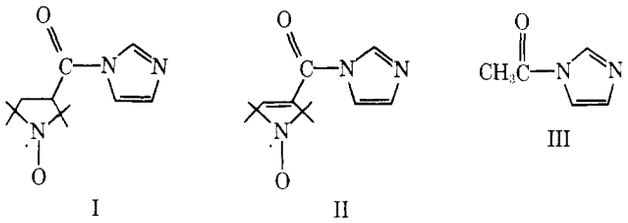


Figure 1. pH profile for hydrolysis of I.



I II

Preparation of I was achieved by allowing equimolar amounts of *N,N'*-carbonyldiimidazole and *N*-(2,2,5,5-tetramethylpyrrolidine-1-oxyl)-3-carboxylic acid[1] to react for several hours at room temperature as a suspension in dry benzene. After workup and two crystallizations from ether, a 57% yield of I was obtained. This product gave satisfactory elemental analysis, IR spectrum, and EPR spectrum. Several previous attempts to prepare I using various methods and conditions led to either destruction of the nitroxide function or to mixtures of products which could not be readily characterized. Use of solvents which afforded homogeneous solutions did not yield isolable amounts of I.

Preparation of the unsaturated analogue (II) of I has been reported.[2] This material was unstable and decomposed rapidly. In contrast, we have stored crystals of I at 4 °C for more than 1 year without noticeable decomposition.

Hydrolysis of I in aqueous solution was studied over the pH range 1.0–9.5. The results are plotted in Figure 1, where the points are experimental and the solid curve is the computer fit of the data to eq 1.

$$k_{obsd} = \frac{K_a^{SH}}{(H^+) + K_a^{SH}}\left[\frac{k_0^{SH}(H^+)}{K_a^{SH}} + \frac{k_{OH}^{S}K_w}{(H^+)}\right] \quad (1)$$

The values of the parameters of eq 1 are listed in Table I, where K_a^{SH} is the acid dissociation constant of the conjugate acid (SH) of I, k_0^{SH} is the water-catalyzed reaction of SH, and k_{OH}^{S} is the hydroxide-catalyzed reaction of S.

The rate parameters obtained for hydrolysis of I are compared to those of Jencks[3] for hydrolysis of *N*-acetylimidazole (III). Both the acid term k_0^{SH} and the base term k_{OH}^{SH} are

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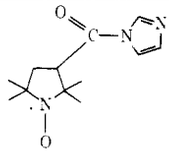
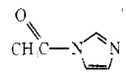
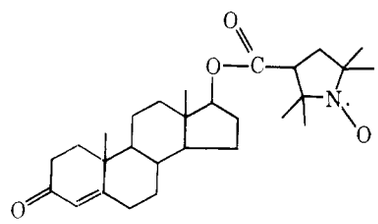
Table I. Hydrolysis Data for Acyl Imidazoles at 25 °C, μ = 0.1[a]

Substrate (S)	k_0^S, s^{-1}	k_0^{SH}, s^{-1}	k_{OH}^S, M^{-1} s^{-1}	pK_a^{SH}
 I	b	(1.22 ± 0.17) × 10^{-1}	(4.89 ± 0.4) × 10^3	3.13 ± 0.09
 III	8.3 × 10^{-5}	4.7 × 10^{-2}	3.2 × 10^2	3.6

[a] Rate constants are given with standard deviations. [b] Too small to measure. [c] Data from ref 3.

larger for I than for III. The small neutral term k_0^S observed for III was not detected for I. From these parameters it can be calculated that hydrolysis of I will be faster than hydrolysis of III by a factor of 4 at pH 7 and by a factor of 12 at pH 8.

To explore the utility of I as a reagent for spin labeling other molecules, we prepared a nitroxide-labeled derivative of testosterone (IV). Heating equivalent amounts of testosterone, I, and *t*-BuOK in *t*-BuOH at 55 °C for 8 h followed by crystallization from acetone afforded a 19% yield of IV. (This yield is based on the sum of the two enantiomers which could result.) The melting point of this material was 224–225 °C, which is about 18 °C higher than that reported by Dodd[4] for the same compound. The IR spectrum of our material is similar to that reported by Dodd and we obtained a satisfactory elemental analysis and EPR spectrum. One explanation for the difference in melting points involves the possibility of isolating diastereomeric products. These would be expected to have different melting points but might have similar IR spectra.


IV

In another experiment, we reacted I with poly-L-tyrosine (mol wt 40 000–100 000) in aqueous solution at pH 7.5 according to the procedure of Barratt et al.[2] After dialysis to remove excess reagent and hydrolysis products, we obtained an EPR spectrum identical with the one reported by Barratt for the product obtained from reaction of poly-L-tyrosine with II.[2]

In summary, our results indicated that I can be readily prepared and stored but that it is somewhat more susceptible to aqueous hydrolysis than is *N*-acetylimidazole. The spin-label functionality of I can be transferred to suitable receptor molecules under aqueous or nonaqueous conditions.

We have also explored the use of I to spin label the enzyme carboxypeptidase and will report these results elsewhere.

Experimental Section

General. EPR spectra were recorded on a Varian Model E-3 spectrometer and IR spectra on a Perkin-Elmer Model 137 instrument. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. pH measurements were made with a Radiometer Model PHM62 pH meter equipped with a combination electrode.

***N*-(2,2,5,5-Tetramethyl-3-carbonylpyrrolidine-1-oxyl)imidazole (I).** To 0.5 mmol of *N*-(2,2,5,5-tetramethylpyrrolidine-1-oxyl)-3-carboxylic acid[1] suspended in 10 mL of dry benzene was added 0.5 mmol of *N,N'*-carbonyldiimidazole. The mixture was stirred under N_2 for 2 h. The solvent was removed under a stream of nitrogen and the residue crystallized twice from ether to give 68 mg (57%) of I: mp 128–129 °C; IR (KBr) 1730, 1242 cm^{-1}.

Anal. Calcd for $C_{12}H_{18}N_3O_2$: C, 61.00; H, 7.68; N, 17.78. Found: C, 60.84; H, 7.72; N, 18.06.

O^{17}-(2,2,5,5-Tetramethyl-3-carbonylpyrrolidine-1-oxyl)testosterone (IV). Testosterone (0.5 mmol) and I (0.5 mmol) were combined in 5 mL of *t*-BuOH and 500 μL of 1 M *t*-BuOK/*t*-BuOH (0.5 mmol) was added. The resulting solution was heated at 55 °C for 8 h in a sealed tube. The solvent was removed by rotary evaporation. The residue was dissolved in $CHCl_3$ and extracted with water. The $CHCl_3$ layer was dried and the solvent removed to give 213 mg of crude IV which was crystallized from acetone to give a yield of 19% based on the sum of the two enantiomeric products that could result: mp 224–225 °C (lit.[4] mp 206.5–207.5 °C); IR (KBr) 1725, 1662 cm^{-1} (lit.[4] IR 1725, 1662 cm^{-1}).

Anal. Calcd for $C_{28}H_{42}NO_4$: C, 73.64; H, 9.27; N, 3.07. Found: C, 73.71; H, 9.46; N, 3.25.

Reaction of I with Poly-L-tyrosine. According to the procedure of Barratt,[2] 8 mg of poly-L-tyrosine (Sigma, mol wt 40 000–100 000) was dissolved in basic, aqueous solution and dialyzed against 6 mM, pH 7.5 phosphate buffer at 4 °C. To the resulting solution at pH 7.5 was added 1.5 mg of I. The mixture was stirred for 4 h at 4 °C followed by dialysis against 6 mM, pH 7.5 phosphate buffer. An EPR spectrum of the resulting solution was identical with the EPR spectrum reported by Barratt for poly-L-tyrosine labeled with II.[2]

Kinetic Measurements. Pseudo-first-order rate constants were determined either spectrophotometrically at 250 nm using a Cary 14 instrument or by a pH Stat method using a Radiometer apparatus which included a TTT60 titrator, PHM62 pH meter, ABU12T buret, and a REC61 servograph recorder. Typically, 50 μL of an acetonitrile stock solution of I was used to initiate the reaction. The pseudo-first-order rate constants were fit to eq 1 using a nonlinear least-squares computer program.

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Registry No.—I, 61463-55-6; IV, 56948-71-1; *N*-2,2,5,5-tetramethylpyrrolidine-1-oxyl-3-carboxylic acid, 2154-68-9; *N,N'*-carbonyldiimidazole, 530-62-1; testosterone, 58-22-0.

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