change in absorbance at 340 nm was measured as before and initial rates were calculated.

Preparation of 3-Methylpentane-1,5-diol (1). 3-Methylglutaric anhydride (16 g, 125 mmol) from Aldrich and $LiAlH_4$ (7.2 g, 190 mmol) were refluxed in dry THF for 4 h. The solution was cooled, quenched with 70 mL of saturated aqueous NH_4Cl, and decanted from the residual sluge. The sluge was washed with methylene chloride. The organic phases were combined, dried with $MgSO_4$, and evaporated. The residual was extracted into acetone and the acetone evaporated. Distillation of the resulting oil under reduced pressure yielded 1 (10.1 g, 68%): bp 83–85 °C (0.1 torr) [lit.[3] bp 139–146 °C (17 torr)]; NMR ($CDCl_3$) δ 3.6–4.0 (6 H, s overlapping t, J = 6.6 Hz), 1.3–1.9 (5 H, m), 0.9 (3 H, d, J = 5.9 Hz).

HLADH-Catalyzed Oxidation of 3-Methylpentane-1,5-diol (1). Soluble Enzyme System. The diol 1 (1.5 g, 12.7 mmol), NAD (11.2 mg, 0.02 mmol), and FMN (0.5 g, 1 mmol) were dissolved in 0.05 M glycine–NaOH buffer (200 mL, pH 9). The pH of the resulting solution was adjusted to 9 with 4 M NaOH. HLADH (25 mg) and FMN reductase (10 units) were added and the solution was kept at room temperature. The pH was readjusted to 9 periodically with 4 M aqueous NaOH. The progress of the reaction was monitored by removing a 2-μL sample from the reactor and adding to 1 mL of glycine buffer, pH 9, containing 1.4 M NAD in a cuvette. The enzyme HLADH (1 unit) was added to the cuvette and the subsequent increase in absorbance at 340 nm was measured. From this the concentration of substrate remaining in the reactor was calculated. Upon completion of the reaction in 1 day the pH was raised to 12 with 4 M aqueous NaOH, and the solution was continuously extracted with chloroform for 2 days. The aqueous solution was acidified to pH 3 with 12 M HCl and continuously extracted with chloroform for 1 day. Evaporation of the chloroform yielded 2 in 68% yield (see the following for characterization).

Large-Scale HLADH-Catalyzed Oxidation of 3-Methylpentane-1,5-diol (1). Immobilized-Enzyme System. The diol 1 (8.9 g, 75 mmol), NAD (0.114 g, 0.15 mmol), and FMN (0.755 g, 1.5 mmol) were dissolved in 0.05 M glycine–NaOH buffer (1.5 L, pH 9). The immobilized enzymes HLADH (16 units), FMN reductase (9 units), and catalase (100 units) were added. The pH of the solution was readjusted to 9 with 4 M NaOH. The solution was agitated with a magnetic stirrer at room temperature. The pH was readjusted to 9 occasionally by adding 4 M NaOH. The progress of the reaction was monitored by assaying the remaining substrate using HLADH as described for the soluble enzyme reaction. After 14 days the assay procedure showed little substrate remaining. The contents of the reactor were centrifuged and the liquid was decanted from the enzyme containing gel. The gel was washed with 200 mL of distilled water and the suspension was again separated by centrifugation. The washing procedure was repeated with a second 200-mL portion of water and the washes were combined with the reactor solution. The solution was evaporated to 400 mL under reduced pressure. The pH of the solution was raised to 12 with 10 M NaOH. The solution was continuously extracted with $CHCl_3$ for 2 days. The aqueous solution was then acidified to pH 3 with 12 M HCl and continuously extracted with $CHCl_3$ for 1 day. Evaporation and distillation of the latter extract gave (–)-(3*S*)-3-methylvalerolactone (2) (5.85 g, 68% yield): bp 121–123 (0.2 torr) [lit.[3] bp 93–94 (0.02 torr)]; $[\alpha]^{27}$ –24.8° (c 5.6, $CHCl_3$) (90% optical purity); NMR ($CDCl_3$) δ 4.3 (2 H, m), 1.1–2.8 (5 H, m), 1.05 (3 H, d).

Acknowledgment. This research was supported by the National Science Foundation, Grant CHE-8318217, and the Robert A. Welch Foundation, Grant A-1004. D.G.D. thanks NSF for a graduate fellowship. We thank Professor T. O. Baldwin of the Biochemistry and Biophysics Department for the supply of the enzyme FMN reductase and for the use of their facilities for the preparation of the enzyme.

Registry No. 1, 4457-71-0; 2, 61898-56-4; NAD, 53-84-9; NADP, 53-59-8; NADH, 58-68-4; NADPH, 53-57-6; FMN, 146-17-8; FMN-reductase, 39346-42-4; catalase, 9001-05-2; alcohol dehydrogenase, 9031-72-5; 3-methylglutaric anhydride, 4166-53-4.

New Method for Preparation of Superoxide Ion by Use of Amino Oxide

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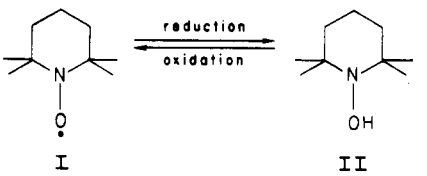
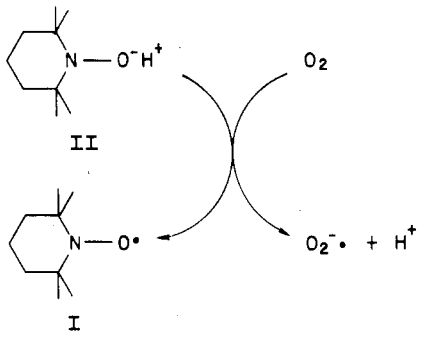
2,2,6,6-Tetramethylpiperidine-1-oxyl (I) is known as a stable radical[1] and is used as a spin trap[2] or spin label[3] reagent. As shown in eq 1, nitroxyl radical I is reduced



I II (1)

to the corresponding hydroxylamine II with ascorbic acid,[4] phenylhydrazine,[5] or hydrazine.[6] The hydroxylamine II in turn can be oxidized with Ag_2O,[7] PbO_2,[8] $NaIO_4$,[8] or $Pb(OAc)_4$[7] to the corresponding nitroxyl radical, demonstrating a reversible redox reaction between I and II.

Hydroxylamine II may be obtained as a colorless crystals which are very sensitive to air. Solutions of II in solvents containing small amounts of dissolved oxygen are oxidized especially easily. This fact suggests that II is oxidized by oxygen to give I and oxygen itself may be converted to superoxide ion by a one-electron reduction as shown in eq 2.



(2)

We now report a new method for the preparation of superoxide ion by use of an analogue of II, 4-(benzyloxy)-1-hydroxy-2,2,6,6-tetramethylpiperidine (V),[9] as a reducing agent of oxygen. Compound V was prepared from 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (III) in two steps (eq 3).

(1) Golubev, V. A.; Rozantsev, E. G.; Neiman, M. B.; *Bull. Acad. Sci. USSR, Div. Chem. Sci. (Engl. Transl.)* **1965**, 1898.
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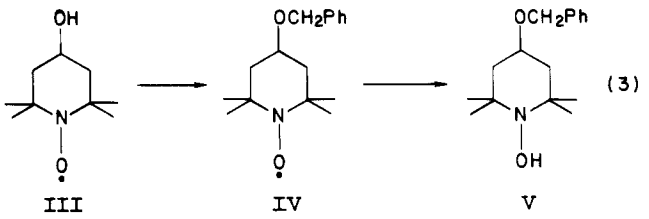
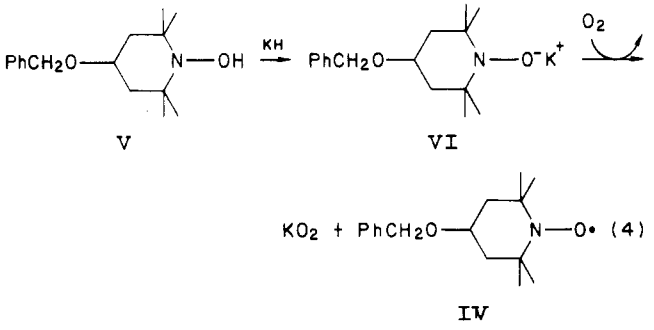


In preliminary experiments, when V was dissolved in dimethylformamide (DMF), tetrahydrofuran (THF), dimethylsulfoxide (Me_2SO), benzene, CCl_4, or EtOH and oxygen was bubbled into these solutions, the production of the red colored IV (λ_{max} 470 nm) by one-electron oxidation was observed (>NOH + O_2 → >NO· + HO_2·).

In order to demonstrate the generation of superoxide ion, it was trapped as the potassium salt (KO_2). As shown in eq 4, V was treated with KH in dry benzene or THF for 3 h under an argon atmosphere to give VI as a gelatinous precipitate with the evolution of hydrogen gas.



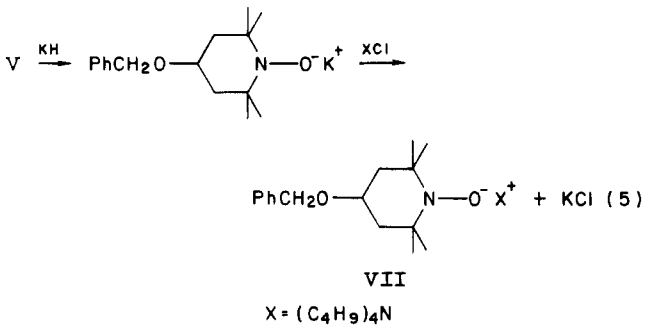
Subsequently, dry oxygen was bubbled into the solution and the gelatinous precipitate was converted to a yellow precipitate. Oxygen was smoothly reduced by the potassium salt of amino oxide VI to give a yellow precipitate of KO_2 and a solution of IV in quantitative yields.

KO_2 obtained by our method was characterized by ESR spectroscopy and showed the same ESR spectrum as that of authentic potassium superoxide.[10] In addition, it reduced nitrotetrazolium blue to diformazan (λ_{max} 560 nm)[11] and when assayed by iodometric and gasometric methods[12] was estimated to have a purity of 88%. These data corresponded completely to authentic KO_2.

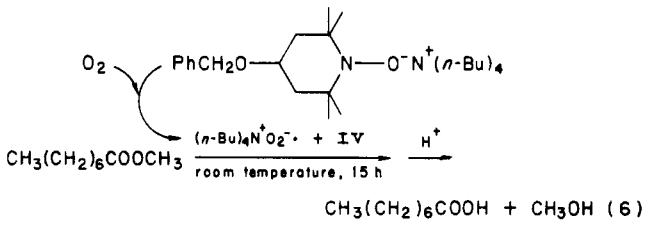
The production of superoxide ion from amino oxide VI may ascribed to the oxidative driving force converting V back to the stable nitroxyl radical. Nitroxyl radical III recovered from the mother liquor can be used repeatedly.

When oxygen was bubbled into a suspension of KH as a blank experiment, KO_2 was not obtained at all.

An example of a reaction of the superoxide ion obtained by our method was examined. Fillipo et al.[13] have reported that KO_2 can be used as a reagent in the cleavage of esters such as methyl *n*-octanoate to octanoic acid and methanol. In order to prepare a soluble superoxide salt, the tetrabutylammonium salt VIII first was prepared by an exchange reaction between VI and tetrabutylammonium chloride (eq 5). Then 4 equiv of VII and 1 equiv of methyl *n*-octanoate were dissolved in DMF and dry oxygen bubbled through the solution for several hours. After the reaction mixture was stirred at room temperature for 15 h and was concentrated in vacuo, water and benzene were



added, and the aqueous layer was acidified with 6 N HCl. Octanoic acid was isolated from the benzene solution quantitatively. (eq 6)



From the preceding results, we conclude that superoxide is produced by reduction of molecular oxygen with hydroxylamines such as V. These results represent a new method for the preparation of the chemically and biologically important superoxide anion.

Experimental Section

Synthesis of KO_2. To a stirred solution of 300 mg (1.14 mmol) of V in 6 mL of dry benzene or THF was added 46 mg (1.14 mmol) of KH at room temperature, under argon atmosphere. After the reaction mixture was stirred for 3 h at room temperature, dry oxygen was bubbled into the solution for 10 min. The produced yellow precipitate was filtered under dry nitrogen atmosphere and wased with dry benzene or THF to give 80 mg (1.13 mmol) of KO_2. The mother liquor was concentrated in vacuo to give IV (298 mg, 1.14 mmol).

Iodometric Method. The decomposition of KO_2 by water with respect to the following reaction is used to measure the amount of KO_2 contained in a weighed sample. In fact KO_2 is decomposed by means of an excess of sodium arsenite in water. The reactions are the following: The amount of KO_2 was estimated from the

$$2KO_2 + 2H_2O \rightarrow 2KOH + H_2O_2 + O_2$$

$$AsO_2Na + H_2O_2 \rightarrow AsO_4H_2Na$$

$$AsO_2Na \text{ (excess)} + I_2 + 2H_2O \rightarrow AsO_4H_4Na + 2HI$$

consumed I_2 as determined by iodometric method.

Tetrabutylammonium Salt of Amino Oxide VII. To a stirred solution containing 960 mg (3.6 mmol) of V in 18 mL of dry methylene chloride was added 145 mg (3.6 mmol) of KH at room temperature under argon atmosphere. After the mixture was stirred for 2 h at room temperature, 1.18 g (3.6 mmol) of tetrabutylammonium chloride (85% purity), which was dissolved in 5 mL of dry methylene chloride, was added to the reaction mixture and was stirred for 4 h at room temperature under an argon atmosphere. The KCl produced was filtered off from the reaction mixture and the mother liquor was concentrated in vacuo to give VII. VII is so sensitive to oxygen that it should be treated under an atmosphere of nitrogen or argon.

Cleavage of an Ester by Superoxide Generated from VII. To a stirred solution of 142 mg (0.8 mmol) of methyl *n*-octanoate in 10 mL of dry DMF was added 1.62 g (3.2 mmol) of VII, which was dissolved in 1 mL of DMF, at room temperature under argon atmosphere. Then, dry oxygen was bubbled into the solution for 2 h, and the reaction mixture was left standing at room temperature for 15 h. After removal of the solvent in vacuo, 15 mL of water and 50 mL of benzene were added to the residue, and the aqueous layer was acidified with 6 N HCl and the organic layer

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separated. The aqueous layer was extracted with an additional 10 mL of benzene and the combined extracts were dried (Na_2SO_4). *n*-Octanoic acid (114 mg, 0.79 mmol) was obtained (yield, 99%). The NMR, IR, and mass spectral data of *n*-octanoic acid obtained were identified with those of an authentic sample.

Registry No. IV, 31645-22-4; V, 97625-48-4; VII, 99232-63-0; KO_2, 12030-88-5; $Bu_4N^+ \cdot Cl^-$, 1112-67-0; $CH_3(CH_2)_6CO_2Me$, 111-11-5; $CH_3(CH_2)_6CO_2H$, 124-07-2; O_2, 7782-44-7; superoxide ion, 11062-77-4.

Methods for the Synthesis of Chiral Hindered Amines

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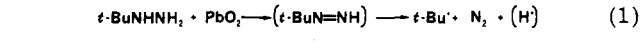
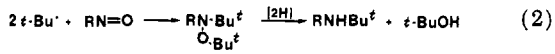
Hindered chiral amines are of interest in connection with the development of new methodology for catalytic enantioselective transformations, for example, a reaction such as cyclohexene oxide → 2-cyclohexen-1-ol. This paper describes an effective method for the synthesis of such amines which is based on a free-radical approach. We recently reported[1] that *tert*-butyl radicals can be generated on a preparative scale by reaction of commercially available *tert*-butylhydrazine with lead dioxide and that efficient trapping occurs with *tert*-alkylnitroso compounds to afford substituted N,N,O-trisubstituted hydroxylamines which can be reduced directly to di-*tert*-alkylamines as shown by the sequence in eq 1 and 2.

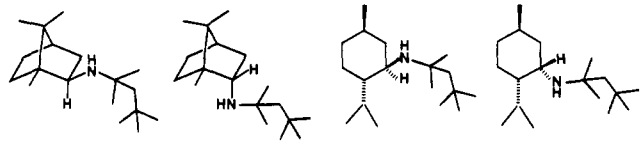


(1)

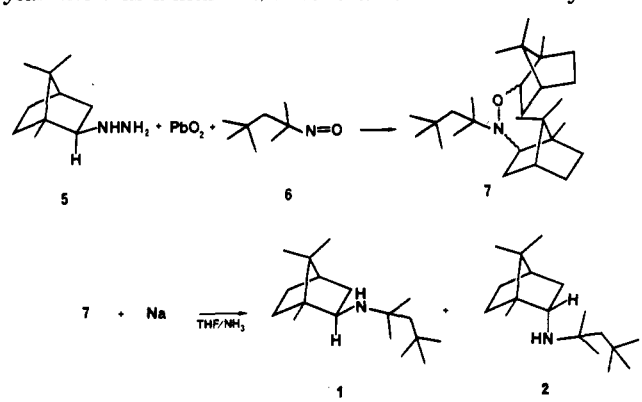


(2)

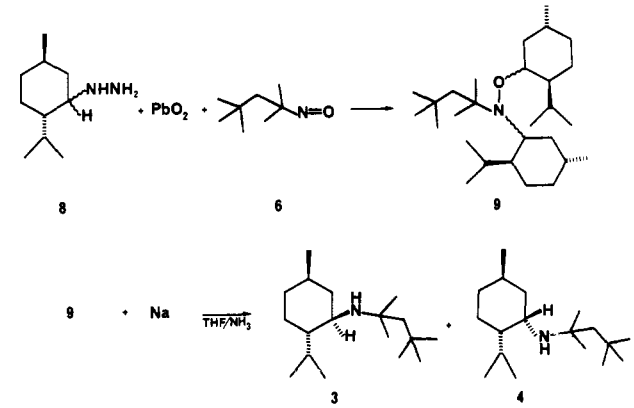
The synthesis of the chiral amines (1–4) was carried out using this new methodology starting from (+)-camphor and (−)-menthone. The key step in these syntheses was the



generation of the secondary alkyl radicals from camphor and menthone. As for the reaction of *tert*-butylhydrazine, reaction of bornylhydrazine **5** with PbO_2 in the presence of nitroso-*tert*-octane (**6**) gave the trisubstituted hydroxylamine **7** as a mixture of several isomers. The hydroxylamine **7** was unstable to acid and heat (80 °C) and therefore was reduced directly without purification. Reduction of **7** with either sodium in tetrahydrofuran (THF)–ammonia or sodium naphthalenide in THF afforded the chiral *exo*- and *endo*-bornyl-*tert*-octylamines **1** and **2** in a 1:1 ratio. The amines were easily separated by chromatography on silica gel (ethyl acetate–hexane) with the less polar amine (R_f 0.7) being assigned as the exo compound **1** and the more polar amine (R_f 0.2) as the Endo compound **2** by analogy with the observed polarities of bornyl and isobornylamines.

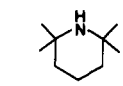
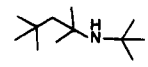
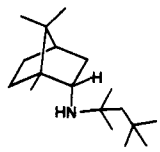
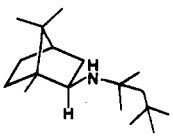
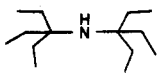
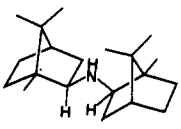


Similarly, reaction of menthylhydrazine **8** with PbO_2 in the presence of nitroso-*tert*-octane gave the hydroxylamine **9** as a mixture of isomers which upon reduction with sodium in THF–ammonia gave the equatorial amine **3** and the epimer **4** in a ratio of 20:1, respectively. Again the amines **3** and **4** were readily separable by chromatography, and their respective configurations were assigned on the basis of their polarities. The high selectivity for the equatorial amine indicates a strong steric influence in the addition of the menthone radical to the nitroso compound.



Bornylhydrazine (**5**) and menthylhydrazine (**8**) were readily prepared by condensation of (+)-camphor and (−)-menthone with ethyl carbazate[2] to yield the carbo-

Table I

	pK_a
	10.1
	9.6
	8.6
	7.9
	7.1
	4.7

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(2) (a) Ghali, N.; Venton, D.; Hung, S.; Le Baron, G. *J. Org. Chem.* **1981**, *46*, 5413. (b) Chaco, M. C.; Stapp, P. R.; Ross, J. A.; Rabjohn, N. *J. Org. Chem.* **1962**, *27*, 3371. (c) Geigy, J. R. A. G. Swiss 307 629, **1955**; *Chem. Abstr.* **1957**, *51*, 5113.

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