# Photooxygenation and Other Oxidation Reactions of 2,3-Dehydro-1-nitrosopiperidine

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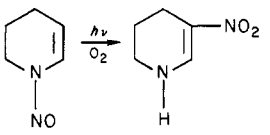
The *N*-nitroso enamine, 2,3-dehydro-1-nitrosopiperidine (1), reacted with oxygen in a light-catalyzed reaction to form a novel oxidation product, 2,3-dehydro-3-nitropiperidine (2). The nitro enamine 2 was also formed when 1 was oxidized with *m*-chloroperbenzoic acid or with iodosobenzene. The reaction involves the photodissociation of the N–N bond of 1, followed by the oxidation of the nitric oxide so produced to nitrogen dioxide. The latter combines with the enaminyl radical to form 2, after a prototropic hydrogen shift. It was shown that the photooxygenation does not involve singlet oxygen, although it is catalyzed by polymer-bound Rose Bengal. It was also shown that 1-nitro-2,3-dehydropiperidine (4) and 3-(hydroxyimino)-1,2-dehydropiperidine (3) were not intermediates in the photooxygenation. Microsome-catalyzed oxidation of 1 did not lead to 2, but a new metabolite of 1, 1-nitroso-4-hydroxy-2,3-dehydropiperidine, (6), was detected.

We have recently reported several procedures for the preparation of $\alpha,\beta$-unsaturated nitrosamines,[2] and some of their reactions.[3] We also investigated[4] the carcinogenic properties of one member of this class of compound, 2,3-dehydro-1-nitrosopiperidine (1). In an effort to understand the chemistry of 1, which, in turn, may be helpful to the understanding of its metabolic reactions, we investigated several oxidation reactions of the compound. This paper concerns itself with some of these reactions, particularly the photooxygenation reaction.

## Results

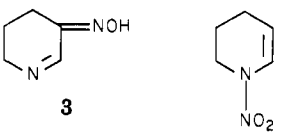
**Photooxygenation Reactions.** Compound 1 is photolabile. Thus, storage of solutions of 1 in dichloromethane, under normal laboratory light conditions, resulted in a gradual disappearance of the compound and the formation of new, crystalline material. It was determined that the reaction required light and the presence of oxygen. The structure of the product was shown to be 2 by NMR ($^1$H and $^{13}$C) and high-resolution mass spectrometry and IR and UV spectroscopy (see Experimental Section).




1 2

The ambient light reaction was slow (13% conversion after 7 days); consequently most of the experiments reported herein were carried out with a high-intensity incandescent lamp.

The photoreaction in the absence of oxygen did not give 2, although photolysis did occur to form the oxime 3 as the only identifiable product. Compound 3 could not be photooxygenated to 2 under our conditions, although it could be converted in low yield to 2 with *m*-chloroperbenzoic acid. Moreover, the *N*-nitramine 4, which was detected as a very minor product (<1%) of the photooxygenation of 1, could not be converted to 2 under our conditions. Thus, neither 3 nor 4 are intermediates in the formation of 2 from 1.

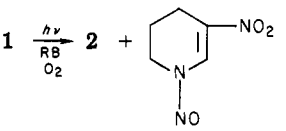



3 4

The conversion of 1 to 2 was completely inhibited by inclusion of BHT (2,6-di-*tert*-butyl-4-methylphenol) in the reaction mixture, although 1 disappeared at approximately the same rate as in the absence of BHT. Dabco (1,4-diazabicyclo[2.2.2]octane) also slightly inhibited the conversion.

Since Dabco is a singlet oxygen inhibitor,[5] it was possible that the conversion of 1 to 2 was the result of a singlet oxygen reaction. Consequently, the photooxygenation was carried out in acetonitrile and acetonitrile-$d_3$. The formation of the photoproduct, however, occurred slightly more rapidly (×1.6) in the undeuterated solvent. Since the lifetime of singlet oxygen in acetonitrile-$d_3$ is longer than in the undeuterated solvent,[6] these data are inconsistent with a singlet oxygen mechanism.

In an additional effort to probe the possibility of singlet oxygen involvement in the photooxygenation, we studied the photosensitized reaction using polymer-bound Rose Bengal (RB) as the sensitizer.[7] The reaction was more rapid in the presence of RB. Thus, photolysis of 1 in the presence of RB and oxygen resulted in the formation of 2 and a new product, 3-nitro-2,3-dehydro-1-nitrosopiperidine (5).

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This product was prepared conveniently in 77% yield by the nitrosation of 2 in dichloromethane solution with dinitrogen tetraoxide. The formation of 2 and 5 was inhibited by addition of BHT to the reaction mixture. Photolysis of 2 in the presence of RB and oxygen proceded rapidly to give several products, including 5. Exclusion of oxygen from that reaction stopped the conversion of 2

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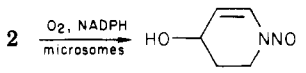
(2) Kupper, R.; Michejda, C. J. *J. Org. Chem.* **1979**, 44, 2326.
(3) Kupper, R.; Michejda, C. J. *J. Org. Chem.* **1980**, 45, 2919.
(4) Kupper, R.; Reuber, M. D.; Blackwell, B. N.; Lijinsky, W.; Koepke, S. R.; Michejda, C. J. *Carcinogenesis* **1980**, 1, 753.
(5) Ovannes, C.; Wilson, T. *J. Am. Chem. Soc.* **1968**, 90, 6527.
(6) Ogilby, P. R.; Foote, C. S. J. *Am. Chem. Soc.* **1981**, 103, 1219.
(7) Schaap, A. P.; Thayer, A. L.; Blossey, E. C.; Neckers, D. C. *J. Am. Chem. Soc.* **1975**, 97, 3741.

to 5. Thus, it appears that the RB-catalyzed reaction involves the conversion of 1 to 2, followed by the conversion of 2 to 5. It is noteworthy that the conversion of 1 to 2 is quenched by addition of BHT to the reaction mixture even though the photodecomposition of 1 does not appear to be inhibited. These results suggest that RB does not catalyze the photooxygenation of 1 by catalyzing the formation of singlet oxygen. It is, however, possible that the conversion of 2 to 5 is a singlet oxygen reaction.

**Reactions of 1 with Epoxidizing Agents.** The reaction of 1 with *m*-chloroperbenzoic acid (MCPBA) or with iodosobenzene led to the formation of 2 in 24% and 16% yields, respectively. MCPBA is a well-known epoxidizing agent, while iodosobenzene was shown to form epoxides of double bonds which are subject to nucleophilic attack, such as ketenes and tetracyanoethylene.[8] We have shown that *N*-nitroso enamines react with nucleophilic as well as electrophilic reagents.[3] The formation of an epoxide of 1 under these conditions, however, could not be demonstrated.

**Oxidation of 1 Catalyzed by Rat Liver Microsomes.** In view of the apparent ease of conversion of 1 to 2, it was interesting to examine the possibility that this transformation could occur enzymatically. Accordingly, we studied the oxidation of 1 by rat liver microsomes. The microsomal reaction was carried out in the dark in order to minimize the light-catalyzed reaction. There was no evidence that 2 was formed in the reaction. Interestingly, the microsomal reaction did yield a new metabolite, 4-hydroxy-2,3-dehydro-1-nitrosopiperidine (6), identical with an authentic sample prepared by the method of Saavedra.[9]

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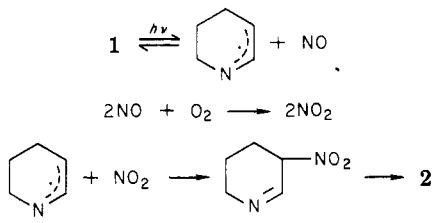
## Discussion

The foregoing data on the photooxidation of 1 to 2 allow the formulation of a mechanism which seems to satisfy all of the data. The reaction is a free-radical process in which one of the intermediates is oxidized by oxygen. Thus, the




photolysis of 1 at visible wavelength causes the reversible dissociation to the enaminyl radical and nitric oxide. The reaction is reversible because the photolysis of 1 under nitrogen to give the oxime 3 is slower than the photooxygenation to 2. Curiously, recombination of nitric oxide with the enaminyl radical appears to be favored at nitrogen. The well-known oxidation of nitric oxide to nitrogen dioxide creates another radical which appears to favor recombination at the carbon of the enaminyl radical. Only a trace of the *N*-nitro enamine 4 was detected in the photooxygenation. The formation of 2 is inhibited by BHT because that hydrogen atom donor intercepts one or both of the intermediate radicals. The slight inhibition of the reaction by Dabco was probably due to a similar effect.

The catalytic effect of polymer-bound Rose Bengal in the photooxygenation of 1 does not appear to be the result of the RB-sensitized singlet oxygen formation,[7] since the reaction is inhibited by the free radical trap BHT. A likely explanation for the catalysis is that RB acts as a sensitizer for the photodissociation of 1. Dye-sensitized photochemical reactions have been noted by Foote and coworkers.[10] It is possible, however, that the conversion of 2 to 5 may involve singlet oxygen, since that reaction does not occur in the absence of RB and it requires the presence of oxygen. The details of that reaction, however, are not known.

The reactions of 1 with *m*-chloroperbenzoic acid and with iodosobenzene give 2, along with other unidentified products. The intent of these reactions was to prepare the epoxide of 1. There was, however, no evidence of epoxide formation and the mechanism of 1 to 2 conversion in those reactions remains obscure.

In view of the ease of formation of 2 from 1, particularly since reagents such as MCPBA and iodosobenzene are effective in carrying it out, it was interesting to consider the possibility of the enzymatic formation of 2. Oxidation of 1, catalyzed by rat liver microsomes, failed to produce any 2. This oxidation, however, did give rise to the 4-hydroxy derivative 6. This substance is interesting because it is the vinylog of the well-known $\alpha$-hydroxylation products of many nitrosamines, including *N*-nitrosopiperidine.[11]

## Experimental Section

**General Procedures.** Unless otherwise specified, all materials were reagent grade and were used without further purification. Iodosobenzene was prepared according to a published procedure.[12] Mass spectra were obtained on a Finnigan 3300 spectrometer, equipped with a Finnigan 6000 data system (low resolution) or a VG Micromass ZAB-2F spectrometer equipped with a VG-2035 data system (high resolution). NMR spectra were obtained on Varian XL-100 spectrometer equipped with a Nicolet TT-100 Fourier-transform accessory, at an radiofrequency of 100 MHz for $^1H$ and 25.2 MHz for $^{13}C$. HPLC separations were carried out on a Laboratory Data Control Constametric II chromatograph equipped with a fixed-wavelength UV detector and a 25-cm LDC column packed with 10 $\mu$m Spherisorb $SiO_2$. The chromatograph was interfaced, via a Hewlett-Packard 18652A A/D converter, to a Hewlett-Packard 3354 computer.

**Photooxygenation of 1. Ambient Light Conditions.** A typical reaction consisted of a 30 mM solution of 1 in $CH_2Cl_2$, contained in 20 × 150 mm Pyrex test tubes, for analytical runs, or round-bottomed flasks, for preparative runs. The containers were exposed to normal, fluorescent laboratory light at room temperature, and oxygen was slowly bubbled through the solutions for the duration of the run. In some runs the solutions contained 1 equiv of BHT or Dabco. Reaction times varied from 4 to 7 days. Analysis of the reaction mixtures was accomplished by HPLC, using a 25-cm column packed with 10 $\mu$m Spherisorb $SiO_2$ and a $CH_2Cl_2/CH_3CN$ solvent gradient system. Prior to analysis, the reaction mixture was treated with a known amount of internal standard (*p*-nitroanisole). The analysis cleanly separated the standard and compounds 1, 2, 4, and 5. The reported yields are expressed as a percentage of 1 which had reacted. Product identification was accomplished by comparison with authentic samples.

**High-Intensity Incandescent Lamp Experiments.** A typical experiment involved the photolysis of a 30 mM $CH_2Cl_2$ solution of 1, contained in a 20 × 150 mm Pyrex test tube. The light source was a high-intensity microscope lamp (Scientific Instruments, Inc., Model 1200). Oxygen or nitrogen was slowly bubbled through the solution for the duration of the photolysis. Reaction times

(8) Moriarty, R. M.; Gupta, S. C.; Hu, H.; Berenschot, D. R.; White, K. B. *J. Am. Chem. Soc.* **1981**, 103, 686.
(9) Saavedra, J. *J. Org. Chem.* **1979**, 44, 4516.
(10) Mauring, L. E.; Eriksen, J.; Foote, C. S. *J. Am. Chem. Soc.* **1980**, 102, 4275.
(11) Leung, K. H.; Park, K. K.; Archer, M. C. *Res. Commun. Pathol. Pharmaçol.* **1978**, 19, 201.
(12) Saltzman, H.; Shanefkin, J. G. *Org. Syn.* **1963**, 43, 60.

varied from 5 to 10 h. When polymer-bound Rose Bengal was used as a sensitizer, the solution was stirred with a magnetic stirrer. In some runs, the solutions also contained 2 molar equiv of BHT. The photoreactivity of **2** was also determined in this system. The analyses of the photolyzed solutions were carried out as described above. There was no qualitative difference between photolyses carried out with ambient light or the high-intensity lamp, except that the latter provided for much shorter reaction times. The structure of **2** was established from a product collected in a preparative run under ambient light conditions. A solution containing 3.0 g (24.5 mmol) of **1** in 250 mL of $CH_2Cl_2$ was exposed to ambient light for 7 days. Removal of solvent and trituration of the residue with pentane resulted in the recovery of 2.35 g of unreacted **1**. The residual brown oil was chromatographed on a Florisil column using tetrahydrofuran as the eluant. Only one product, a solid, was collected. Recrystallization from THF/hexane gave 0.33 g of a yellow, crystalline product: mp 156–157 °C; IR (KBr pellet) 3450, 1625, 1320, 1235 $cm^{-1}$; UV (THF) 233 nm ($\epsilon$ 2460), 355 ($\epsilon$ 9600); $^1H$ NMR ($CDCl_3$) $\delta$ 1.60 (m, 1 H), 1.89 (m, 2 H), 2.69 (t, 2 H), 3.25 (m, 2 H), 8.20 (d, 1 H); shaking with $D_2O$ caused the collapse of the resonance at $\delta$ 3.25 from m to t and at $\delta$ 8.20 from d to s; $^{13}C$ NMR ($CDCl_3$) $\delta$ 20.2, 21.8, 41.0, 122, 143.6; the off-resonance $^{13}C$ NMR spectrum (decoupler offset 44 707 Hz, hetero power = 80 dB) split the first three resonances (at 20.2, 21.8, 41.0) into triplets, the resonance at 143.6 into a doublet, while the weak peak at 122 remained a singlet; mass spectrum, *m/z* (relative intensity) 128 ($M^+$, 61), 111 (100), 81 (73), 80 (92); exact mass ($M^+$ peak), 128.0595 ($C_5H_8N_2O_2$).

**Solvent Deuterium Isotope Effect on Photooxygenation of 1.** A solution of 20 mg of **1** in 6.4 mL of either $CH_3CN$ or $CD_3CN$ with oxygen bubbling through the mixtures was photolyzed with the high-intensity lamp at room temperature for 4 h. The reaction mixtures were analyzed by HPLC as described above. The yield of **2** in the $CH_3CN$ reaction was 6.9 mg, while it was 4.2 mg in the $CD_3CN$ reaction. The yield ratio was 1.6 for undeuterated/deuterated solvent.

**Preparation of 1-Nitroso-2,3-dehydropiperidine (1).** The compound was prepared most conveniently by the base-catalyzed isomerization of 1-nitroso-3,4-dehydropiperidine, as described previously.[2,4] The conversion was virtually quantitative.

**Rearrangement of 1 to Oxime 3 and Oxidation of 3 to 2.** The rearrangement was carried out following the method of Seebach and Enders.[13] Hydrogen chloride gas was bubbled for 15 min through a solution of 1.0 g (9 mmol) of **1** in 100 mL of benzene. After 45 min of additional stirring, 1.28 g of powder was collected by filtration. This powder was suspended in 25 mL of ether and 5 mL of triethylamine was added dropwise. The solution was filtered and the filtrate was evaporated to give a yellow crystalline product. Recrystallization from THF/hexane afforded 0.27 g (27% yield) of product: mp 137–138 °C; IR (KBr) 3300–2000, 1616, 1495, 1445, 1320, 1020, 935 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.79 (m, 2 H), 2.62 (t, 2 H), 3.70 (br s, 2 H), 8.08 (s, 1 H); mass spectrum, *m/z* (relative intensity) 112 ($M^+$, 60), 95 (38), 68 (61), 41 (100).

The oxidation of **3** was carried out by mixing 100 mg (0.9 mmol) of 3-(hydroxyimino)-1,2-dehydropiperidine with 154 mg (0.95 mmol) of *m*-chloroperbenzoic acid in 15 mL of dichloromethane, in the dark, under a nitrogen atmosphere, at room temperature. The reaction was stirred overnight. The reaction mixture was subjected to HPLC analysis, using *p*-nitroanisole as an internal standard. The analysis showed that 5.4 mg (5% yield) of **2** was formed under those conditions.

**Preparation of 1-Nitro-2,3-dehydropiperidine (4).** To a mixture of 20.0 g (0.32 mol) of fuming nitric acid and 30.6 g (0.3 mol) of acetic anhydride at 0 °C was added 13.8 g (0.14 mol) of 3-hydroxypiperidine. After being stirred for 24 h at that temperature, the reaction mixture was treated with 57 g (1 mol) of potassium hydroxide and dissolved in a mixture of 40 mL of water and 360 mL of methanol. The resulting solution was stirred for 2 h at room temperature. The solvent was pumped off in vacuo and the residue was extracted with three 150-mL portions of dichloromethane. After drying ($Na_2SO_4$) and removal of solvent, 11.9 g of a brown oil was obtained. This oil was chromatographed on silica gel, using 20% dichloromethane in ether as the eluant. The product came off in the first fraction. It was a solid, which upon recrystallization from 1:1 ether/pentane afforded 5.34 g, mp 53–54 °C. The IR spectrum suggested that this material was 1-nitropiperid-3-yl nitrate (no NH or OH stretch, characteristic asymmetric and symmetric ONO stretch at 1635 and 1240 $cm^{-1}$, respectively, and asymmetric and symmetric NNO, stretch at 1520 and 1320 $cm^{-1}$, respectively). Accordingly, 2.3 g (12 mmol) of the nitrate ester was mixed with 1.35 g (24 mmol) of potassium hydroxide in a mixture of 30 mL of water and 20 mL of methanol. The solution was heated at reflux overnight. The solvent was removed in vacuo and the residue was extracted with dichloromethane. The extracts were chromatographed on silica gel, using 20% ether in petroleum ether as the eluant. The product was an amber liquid; the yield was 0.95 g (62%). The IR spectrum in dichloromethane (3120, 2940, 1515, 1300, 980, 950 $cm^{-1}$) indicated the presence of $NNO_2$ and absence of $ONO_2$ and OH. The NMR spectrum was virtually indistinguishable from that of **1**. Mass spectrum, *m/z* (relative intensity) 128 ($M^+$, 23), 111 (8), 82 (12), 81 (10), 80 (14), 55 (100).

**Preparation of 3-Nitro-*N*-nitroso-2,3-dehydropiperidine (5).** A solution of **2** (34 mg, 27 mmol) in 10 mL of $CH_2Cl_2$ was cooled to 0 °C. A solution of $N_2O_4$ in $CH_2Cl_2$ was added dropwise until it was determined by TLC (silica gel, 1:1 benzene–ethyl acetate) that all of the starting material had been consumed. The solvent was removed in vacuo and the solid residue was recrystallized from ether/pentane. The product **5** was obtained in 77% yield, mp 56–57 °C. The spectral data were in agreement with the structure. **5**: IR ($CCl_4$) 3100, 2970, 2940, 1665, 1510, 1340, 1000, 960 $cm^{-1}$; UV ($CH_3CN$) 324 nm ($\epsilon$ 12 900); $^1H$ NMR ($CDCl_3$) $\delta$ 2.01 (m, 2 H), 2.88 (t, 2 H), 3.69 (t, 2 H), 9.23 (s, 1 H); the product was predominantly the syn rotamer, with approximately 6% of the anti, indicated by $\delta$ 4.56 (t), 8.68 (s); mass spectrum, *m/z* (relative intensity) 157 ($M^+$, 92), 127 (12), 111 (13), 81 (52), 80 (66), 30 (100). An alternative preparation of **5** involved the passage of gaseous $N_2O_4$ ($NO_2$) at room temperature over a stirred solution of **1** in $CH_2Cl_2$. Product **5** was isolated in a virtual quantitative yield.

**Reaction of 1 with Iodosobenzene.** A solution of **1** (100 mg, 0.89 mmol) in 15 mL of $CH_2Cl_2$ was treated with 216 mg (1.0 mmol) of iodosobenzene under nitrogen. The flask was shielded from light. The mixture was stirred at room temperature for 3 h. After the addition of an internal standard (*p*-nitroanisole), the composition of the reaction mixture was examined by HPLC. The yield of **2** was 16%, based on the amount of **1** (60%) which had reacted.

**Reaction of 1 with *m*-Chloroperbenzoic Acid (MCPBA).** The reaction was carried out in a manner similar to that for iodosobenzene, using 154 mg (1.0 mmol) of MCPBA. The HPLC analysis of the reaction mixture indicated that **2** was formed in 24% yield, based on the amount of **1** (75%) which had reacted.

**Microsome-Catalyzed Oxidation of 1.** Male Fischer 344 rats (10 weeks, uninduced) were used for the liver microsome preparation.[14] The microsomes were prepared at 0 °C in 0.1 M pH 7.4 potassium phosphate buffer and were stored in 4-mL aliquots at −80 °C until use. The oxidations were carried out in rubber-septum-sealed, 50-mL, foil-wrapped Erlenmeyer flasks. Care was taken to exclude light from the reaction and subsequent centrifugation. The reaction mixture consisted of 1.80 mL of 100 mM isocitrate, 0.72 mL of 10 mM $NADP^+$ solution, 0.60 mL of 50 mM $MgCl_2$ solution, 5.0 mL of pH 7.4 phosphate buffer (0.1M), 40 $\mu$L of isocitrate dehydrogenase, 4 mL of microsomal solution (15.9 mg of protein/mL), and 8.2 $\mu$L of the nitrosamine **1**. Control reactions were carried out by replacing the nitrosamine or the microsomes by buffer. The flasks were kept at 4 °C during the mixing of the reagents. Dimethyl sulfoxide (0.5 mL) was added to each flask and oxygen was bubbled through each solution for 20 s. The flasks were then sealed and placed in a 37 °C water bath shaker. After 60 min, 5 mL of methanol was added to each flask, and the contents were centrifuged for 15 min at 6000*g* to remove the precipitated protein. The volume of the supernatant was reduced in vacuo. The residue was extracted with a little dichloromethane and a known amount of an internal standard

(13) Seebach, D.; Enders, D. *Chem. Ber.* **1975**, 108, 1993.

(14) Kroeger-Koepke, M. B.; Michejda, C. J. *Cancer Res.* **1979**, 39, 1587.

(*p*-nitroanisole) was added. The samples and controls were analyzed by HPLC. Some **2** was always detected, but no more than was contained in the controls. A new product, however, was obtained. On the basis of its high-resolution mass spectrum, it was identified as 1-nitroso-4-hydroxy-2,3-dehydropiperidine (**6**): mass spectrum, *m/z* (relative intensity) 128 ($M^+$, 98), 111 (3), 98 (4), 81 (25), 80 (30), 71 (92), 63 (15), 53 (100); exact mass (*m/z* 128), 128.0566 ($C_5H_8O_2N_2$). This spectrum was virtually identical with that of authentic **6**, prepared by the method of Saavedra.

**Acknowledgment.** We thank Drs. Bruce D. Hilton and Gary McClusky for the NMR and mass spectra, respectively, and Dr. Robert Kupper for preparing authentic **6**. This work was supported by Contract No. N01-CO-75380 with the National Cancer Institute, NIH, Bethesda, MD.

**Registry No.** **1**, 70501-82-5; **2**, 81971-40-6; **3**, 74719-26-9; **4**, 81971-41-7; **5**, 81971-42-8; **6**, 71785-88-1; 3-hydroxypiperidine, 6859-99-0; 1-nitropiperid-3-yl nitrate, 81971-43-9.

# Kinetics of Epimerization of (+)-Catechin and Its Rearrangement to Catechinic Acid[1]

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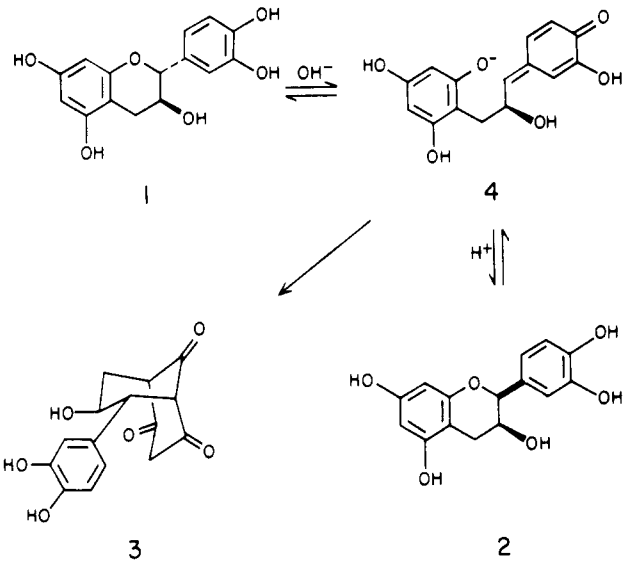
The rates of epimerization of (+)-catechin (**1**) to (+)-epicatechin (**2**) and of (−)-epicatechin to (−)-catechin in aqueous solution were measured over the pH range 5.4–11.0 and the temperature range 34–100 °C. The rate of conversion of (+)-catechin to catechinic acid (**3**) also was measured under these conditions. First-order kinetics were observed for all three processes. At low pH, $k$(epimerization) ≫ $k$(rearrangement), and epimerization approached an equilibrium in which (+)-catechin predominated over (+)-epicatechin. Near pH 11 and at elevated temperatures, $k$(epimerization) was only slightly greater than $k$(rearrangement), and the rapid, irreversible formation of catechinic acid under these conditions determined product composition. Both the epimerization of catechin and its rearrangement to catechinic acid can be rationalized in terms of a quinone methide intermediate (**4**).

Condensed tannins from conifer bark are polyflavanoids containing flavan-3-ol repeat units such as (+)-catechin [**1**; (2*R*,3*S*)-3,3′,4′,5,7-pentahydroxyflavan] and (−)-epicatechin [(2*R*,3*R*)-3,3′,4′,5,7-pentahydroxyflavan] linked between C-4 of one unit and either C-6 or C-8 of the next unit.[3] The possibility of using these subunits in polymers cross-linked with formaldehyde or methylolated phenol has been considered as a means of improving the adhesive properties of phenol–formaldehyde resins, but the results have been generally disappointing.[4]

A primary concern in the design of polymeric systems derived from catechins is the instability of these flavanoids toward epimerization and rearrangement. Epimerization of **1** in hot water or dilute caustic solution to (+)-epicatechin (**2**) is well-known,[5] and, more recently, it was shown by Sears et al.[6] that **1** undergoes rearrangement to catechinic acid (**3**) in hot alkaline solution. The quinone methide **4** suggested by Mehta and Whalley[7] is a logical intermediate in both processes (see Scheme I). Because these competitive reactions could potentially interfere with tannin extraction from plant material and with resin synthesis based on flavanoids, it was important to determine their relative rates over a range of pH and temperature. We report the results of kinetic studies (a) on the epimerization of **1** to **2** and of (−)-epicatechin to (−)-catechin and (b) on the rearrangement of **1** and **2** to **3**. These studies were conducted in aqueous solution over the pH range 5.4–11.0 and at temperatures from 34 to 100 °C.

Scheme I




## Results

Catechin, epicatechin, and catechinic acid can readily be resolved by high-pressure liquid chromatography (HPLC); this permits an accurate assay by measurement of peak area. A preliminary survey with **1** and **2** revealed

(1) (a) Taken in part from the Ph.D. thesis of P.K., Oregon State University, 1980. (b) Paper 1575, Forest Research Laboratory, School of Forestry, Oregon State University.

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(3) Roux, D. G. *Phytochemistry* **1972**, *11*, 1219.

(4) Hemingway, R. W. "Complete Tree Utilization of Southern Pine"; MacMillin, C. W., Ed.; Forest Products Research Society: Madison WI, 1978; pp 443–457.

(5) Freudenberg, K.; Böhme, O.; Purrman, L. *Ber. Dtsch. Chem. Ges. B* **1922**, *55*, 1734. Freudenberg, K.; Purrman, L. *Justus Liebigs Ann. Chem.* **1924**, *437*, 274.

(6) Sears, K. D.; Casebier, R. L.; Hergert, H. L.; Stout, G. H.; McCandlish, L. E. *J. Org. Chem.* **1974**, *39*, 3244.

(7) Mehta, P. P.; Whalley, W. E. *J. Chem. Soc.* **1963**, 5327.

0022-3263/82/1947-2910$01.25/0 