The Hydroxylation of Aromatic Compounds by Hydrogen Peroxide in the Presence of Catalytic Amounts of Ferric Ion and Catechol. Product Studies, Mechanism, and Relation to Some Enzymic Reaction[1,2]

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Abstract: A number of aromatic compounds are hydroxylated by hydrogen peroxide in an aqueous system in the presence of catalytic amounts of ferric ion and an enediol such as catechol. The isomer distribution of the phenols which are formed and the reactivity of a number of aromatic compounds to the oxidizing agent have been determined. The results indicate that the oxidizing agent is very nonselective and that the hydroxyl radical is not the species which reacts with the aromatic compounds in this system. It is proposed that the oxidizing agent is a complexed iron oxide formed, by the elimination of a molecule of water, from an intermediate containing ferric ion, hydrogen peroxide, and the enediol catalyst. The possible relation of the proposed mechanism to the mechanisms of several enzymic reactions, especially those catalyzed by peroxidase, catalase, and tyrosinase, is discussed.

In the previous paper[4] the kinetics of the disappearance of H_2O_2 in a system composed of ferric ion, catechol (or hydroquinone), and anisole was reported. The main products obtained from this reaction are monohydroxyanisoles.[2] If anisole is replaced by other aromatic compounds it was found that hydrogen peroxide would also hydroxylate these compounds.[5] It was thus of interest to determine the isomer distribution of the phenols which are formed from aromatic compounds with differing electronic properties because such information would aid in the characterization of the oxidizing agent. To elucidate the nature of the oxidizing species further, a series of competition experiments was also carried out to determine the relative reactivity of the aromatic compounds. The results of these experiments are reported in this paper.[6]

From the product and competition experiments it is possible to conclude that the oxidizing agent is a very nonselective reagent and that it is not the hydroxyl radical. Other mechanisms for the oxidation are considered, and it appears that the most likely mechanism is one involving a complexed iron oxide as the oxidizing agent. Since the proposed intermediate bears some resemblance to intermediates which have been suggested for catalase- and peroxidase-catalyzed reactions, the possible relevance of this reaction to the mechanism of some enzymic reactions is discussed.

Experimental Section

Materials. Unless described otherwise all compounds used in this research were the same as those described in the previous paper[4] or were commercially available materials and were used as received. Anisole, chlorobenzene, and nitrobenzene were redistilled and were essentially pure as shown by gas chromatography. The phenols and phenyl methyl ethers were redistilled or recrystallized by standard procedures. In most cases these compounds were shown to be homogeneous by gas chromatography. Hexadeuteriobenzene (98% deuterium) was obtained from Calbiochem and hexadeuteriophenol (98% deuterium) was obtained from Nuclear Equipment Corp.

Product Studies. The products obtained from the monohydroxylation of benzene, anisole, and chlorobenzene were determined quantitatively by gas chromatography after conversion of the phenols to phenyl methyl ethers by treatment with dimethyl sulfate in basic solution. The ethers were separated quantitatively at 100° by a Perkin-Elmer Golay "R" column.

For a typical product experiment with benzene, anisole, or chlorobenzene as substrate, 50 ml of the reaction solution was prepared in the same way as for the kinetic experiments.[4] The solutions were allowed to stand at room temperature for 2 to 2.5 hr (over 10 half-times) after initiation with H_2O_2. An accurately weighed amount (approximately 0.01 g) of some phenol not formed in the reaction (usually *p*-cresol) was added to the solution to serve as an internal standard. Approximately 0.7 g of sodium hydroxide was dissolved in the solution (under N_2); a 0.5- to 1.0-ml aliquot of dimethyl sulfate was added, and the mixture was heated until the dimethyl sulfate went into solution (reacted with phenol or water). The solution was cooled immediately and extracted with 5 ml of benzene, and the extract was injected directly into the gas chromatograph. From control experiments with known amounts of phenols it was shown that the above conditions caused essentially quantitative conversion of the phenols to the methyl ethers. Extended heating of the basic solution of the ethers must be avoided, however, since this caused some decomposition of the ethers, possibly by hydrolysis back to the phenols.

The nitrophenols formed by the hydroxylation of nitrobenzene were analyzed by a method similar to that used by Buhler and Mason.[7] The reaction mixture (500 ml) was left to stand for 2.5 hr, after which time it was made more acidic by the addition of approximately 25 ml of 10% HCl, and then extracted three times with 150 ml ether. After the ether solution was washed with water it was extracted with 0.084 *N* NaOH and immediately acidified. The *o*-nitrophenol was steam distilled and trapped in 0.084 *N* NaOH

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(2) Presented in part at the 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1964, Abstracts, p 94S.

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(4) G. A. Hamilton, J. P. Friedman, and P. M. Campbell, *J. Am. Chem. Soc.*, **88**, 5266 (1966).

(5) In the preliminary communication (G. A. Hamilton and J. P. Friedman, *ibid.*, **85**, 1008 (1963)) it was suggested that chlorobenzene was not hydroxylated by the catalytic system. This result was implied from the observation that not all of the hydrogen peroxide reacted when anisole was replaced by chlorobenzene, and in the absence of any aromatic compound some (about 20 to 30%) of the hydrogen peroxide reacts. However, when the analysis for the hydroxychlorobenzenes was carried out it was found that about a 20% yield of hydroxychlorobenzenes was obtained at pH 4.3.

(6) Some of these results have been obtained independently by R. O. C. Norman and J. R. L. Smith in "Oxidases and Related Redox Systems," Vol. 1, T. S. King, H. S. Mason, and M. Morrison, Ed., John Wiley and Sons, Inc., 1965, p 131.

(7) D. R. Buhler and H. S. Mason, *Arch. Biochem. Biophys.*, **92**, 424 (1961).

for spectrophotometric determination at 415 mμ. The residue was extracted three times with 25 ml of ethyl acetate and the extracts were concentrated under vacuum to a total volume of 10 ml. Suitable aliquots were removed and chromatographed on paper using a solvent system of isopropyl alcohol–aqueous ammonia–water (8:1:1, v/v). The *meta* and *para* isomers did not separate cleanly with this system but they were separated from other light-absorbing species which were present. Consequently, the spot containing the *m*- and *p*-nitrophenols was cut out and the phenols were eluted with 0.084 *N* NaOH. The concentrations of *m*- and *p*-nitrophenols were determined by measurement of the absorbance of the solution at two different wavelengths (400 and 252 mμ) and solution of two simultaneous equations. In order to verify the accuracy of these techniques, known amounts of the three nitrophenols were added to the reaction mixture (minus H_2O_2) and carried through the extraction, steam distillation, chromatography, and assay procedures. Over 90% of each of the nitrophenols was recovered.

Competition Experiments. The competition reactions were carried out between each pair of aromatic compounds. The solutions contained: acetate buffer, 0.005 *M* (pH 4.2); $Fe(ClO_4)_3$, 8×10^{-5} *M*; catechol, 8×10^{-5} *M*; H_2O_2, 2×10^{-3} *M*; the two aromatic compounds, 0.004 to 0.006 *M* (in any one experiment the aromatic compounds were present in equimolar quantities). The solutions were all homogeneous and were left to stand at room temperature for 2.5 hr. Each competition experiment was run at least twice and the reproducibility was better than 5%. The products were analyzed in the same way as described above. For the competition experiment between anisole and benzene the procedure was modified in order to remove the phenols from the unreacted anisole. Before methylation the unreacted anisole was separated from the phenols by benzene extraction of a basic solution of the products. During the analysis of the products formed in the competition experiment with hexadeuteriobenzene it was found that the peak for a given number of moles of pentadeuteriophenyl methyl ether was 11% larger than the peak for the same number of moles of phenyl methyl ether. This was taken into account when calculating the amount of pentadeuteriophenol produced in the competition experiment.

Results

Product Studies. The yield and isomer distribution of phenols formed by the reaction of the catalytic system with a number of aromatic compounds are shown in Table I. The isomer distribution of products formed in the Fenton reaction[8,9] is included in Table I for comparison. The data for the catalytic reaction indicate clearly that ferric ion and catechol (or other enediol) act as true catalysts in the hydroxylation by H_2O_2; up to 20 molecules of the product phenol are produced for each initial molecule of ferric ion or the enediol. It is also clear (as implied by the kinetic experiments[4]) that an enediol is absolutely necessary for the hydroxylation to proceed and the reduced form of the catalyst is the form which is catalytically active. It is not obvious why the yield should be greater at lower pH unless the destruction of the catalyst occurs at a greater rate at the higher pH's. It is significant, however, that the isomer distribution remains essentially the same for all conditions. The reactions reported in Table I were done with oxygen present (air atmosphere). However, if the hydroxylation is carried out under nitrogen essentially the same yield and isomer distribution of products are obtained. The presence of 0.15 *M* $NaClO_4$ has no effect on the yield or isomer distribution of the products.

Competition Experiments. From the competition experiments with the catalytic system it is possible to calculate the reactivity series shown in Table II. Included in Table II is the reactivity of the same compounds toward the hydroxyl radical[8] (Fenton reaction). In the competition experiments with the catalytic system the yield of phenols is 20 to 30%, and the isomer distribution is the same as given in Table I; it is not affected by the presence of the other aromatic compound. If the experiments are run at a lower pH the total yield of phenols is increased but the relative reactivity and isomer distribution remain constant.

(8) R. O. C. Norman and G. K. Radda, *Proc. Chem. Soc.*, 138 (1962).
(9) For a recent review of the Fenton reaction with references, see G. H. Williams, "Homolytic Aromatic Substitution," Pergamon Press, New York, N. Y., 1960, p 110 ff.

Table I. Products from the Hydroxylation of Aromatic Compounds by the Catalytic System

Aromatic compound	Conditions,[a] pH	Yield of phenols,[b] %	Isomer distribution—Catalytic system *o*	*m*	*p*	Isomer distribution—Fenton reaction[c] *o*	*m*	*p*
Benzene	4.2	26						
	2.9	52						
	2.9[d]	<1						
	4.2[e]	28						
	4.2[f]	<3						
Nitrobenzene	4.2	27	48	26	26	24	30	46
	2.9	42	47	26	27			
	2.9[d]	<1						
Chlorobenzene	4.2	24	45	15	40	42	29	29
Anisole	4.3[g]	55	64	3	33	84	0	16
	4.3[h]	58	65	<5	35			
	4.3[i]	37	74	...	26			

[a] Unless described otherwise the reaction solutions contained: acetate buffer, 0.005 *M*; $Fe(ClO_4)_3$, 8×10^{-5} *M*; catechol, 8×10^{-5} *M*; initial H_2O_2, 2×10^{-3} *M*; and were saturated with the aromatic compound. The reactions were left to stand for 2 to 2.5 hr at room temperature and the pH was measured after standing. [b] Yield based on the initial amount of H_2O_2. [c] Data taken from Norman and Radda.[8] [d] No catechol present. [e] Hydroquinone (8×10^{-5} *M*) present instead of catechol. [f] Benzoquinone (8×10^{-5} *M*) present instead of catechol. [g] Catechol concentration, 15×10^{-5} *M*. [h] Hydroquinone (15×10^{-5} *M*) present instead of catechol. [i] Chloranilic acid (7.7×10^{-5} M) present instead of catechol.

Table II. Reactivity of Various Aromatic Compounds to the Catalytic Hydroxylation and the Hydroxyl Radical Hydroxylation

Compound	Reactivity to the catalytic hydroxylation (rel to benzene) At all positions	At each position[b] *o*	*m*	*p*	Reactivity to the hydroxyl radical[a] (rel to benzene) At all positions	At each position[b] *o*	*m*	*p*
Benzene	(1.0)	...	...	...	(1.0)	...	...	...
Benzene-d_6	0.75[c]	...	...	...	1.0[d]	...	...	...
Anisole	1.4	2.8	0.0	2.8	6.35	16	0	6
Chlorobenzene	0.6	0.7	0.3	1.5	0.55	0.7	0.5	1.0
Nitrobenzene	0.6	0.9	0.5	1.1	0.14	0.1	0.13	0.4

[a] Taken or calculated from the data of Norman and Radda.[8] [b] Reactivity of one of a given position relative to one position on benzene. The numbers are the partial rate factors for the reactions at the various positions. [c] The error in this value is probably less than 0.07. [d] This value was reported by J. R. L. Smith and R. O. C. Norman, *J. Chem. Soc.*, 2897 (1963). These workers obtained different values for the isotope effect of hexadeuteriobenzene depending on whether it was obtained in a competition experiment with chlorobenzene or by direct measurement. The value, 1.0, is believed to be the isotope effect for the attack of the hydroxyl radical on the aromatic ring.

Discussion

The low selectivity shown by the catalytic hydroxylation system (Table II) indicates that the aromatic compounds are attacked by a radical-like reagent.[10]

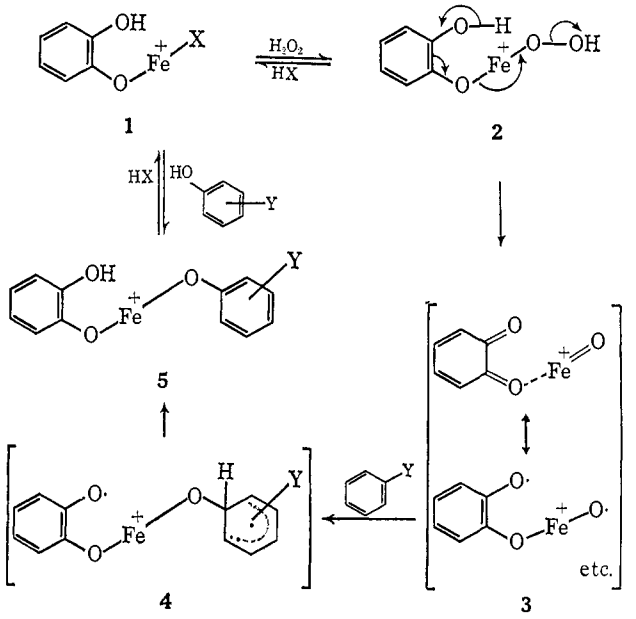
(10) In the preliminary communication[5] it was suggested that the oxidizing species was an electrophilic reagent, but the more extensive results reported here strongly indicate a radical reagent.

However, it seems clear from the comparative isomer distributions (Table I) and reactivities (Table II) that the free hydroxyl radical (HO·) is not the hydroxylating agent. The hydroxyl radical has been generated in several different ways, including the reactions of Fe(II), Cu(I), or Ti(III) with H_2O_2, the decomposition of H_2O_2 by ultraviolet light, and the reaction of X-rays with water, and in general the isomer distribution of phenols formed from aromatic compounds is similar in each case.[8,11-13] An exception is the hydroxylation of phenol. The Fenton system reacts differently with phenol than hydroxyl radicals generated by the interaction of X-rays with water.[14] This apparent anomaly can now be easily understood. In the Fenton reaction ferric ions are formed, and catechol and hydroquinone are the initial products of the reaction of hydroxyl radicals with phenol.[14] Thus, after the initial stages most of the products would probably be due to the catalytic reaction and not the Fenton reaction. Since phenols are more reactive toward the hydroxyl radical than other aromatic compounds, this same complication may have occurred in other studies of the Fenton reaction unless only the initial products were investigated.

The perhydroxyl radical (HOO·) is presumably not the hydroxylating agent either because it reacts to only a slight extent with benzene.[11] Peralkoxyl radicals[15] (ROO·) which should have a reactivity similar to HOO· also do not react appreciably with aromatic compounds to give phenols.

A mechanism which is consistent with the kinetic[4] and product results is summarized in Chart I. Catechol is depicted as the catalyst but 1,4-dihydroxybenzene

Chart I



compounds are vinylogous catechols and could react in the same way. Norman and Smith[6] have suggested that a complex like **2** transfers a hydroxyl radical to the aromatic compound. This seems unlikely because: (1) complexes like **2** should be in equilibrium with the starting materials, and thus a reaction of such a complex with the aromatic compound would require a first-order dependence, which is not observed,[4] on the concentration of the aromatic compound; (2) an oxidizing agent which *transfers* a hydroxyl radical to the aromatic compound would not be expected to be less selective (see Table II) than the hydroxyl radical itself; (3) biphenyls which are obtained in reactions involving the hydroxyl radical[11] are not observed in the catalytic reaction under study.[6]

In the mechanism shown in Chart I, the formation of **3** from **2** by the elimination of a molecule of water is considered to be the rate-determining step of the sequence.[4] Since iron should be able to transfer electrons by the overlap of its d orbitals with the p orbitals of the ligands, many resonance forms of **3** are possible[5] and these presumably add stability to the complex. If, as in complex **3**, the ligands and metal have stability in various oxidized and reduced forms, one cannot say in which oxidized state the metal or ligands are present because the complex will be a resonance hybrid of all possible states.[16] It is suggested that **3** reacts as a radical reagent with the aromatic compound to give **4** which by any of a number of steps could give **5** and ultimately regenerate **1**. With compounds which are not catalysts, presumably **3** is not stabilized sufficiently by resonance to form, or, once formed, water adds across the formal iron–oxygen double bond of **3**, thus leading to oxidation of the catalyst.[5] The inhibition by organic solvents[4] is probably due to abstraction of hydrogen atoms by **3**. The mechanism shown in Chart I and that proposed by Norman and Smith[6] both predict that if H_2O_2 is replaced by alkyl hydroperoxides the aromatic compounds should still be oxidized; phenols should be the products according to the first mechanism and either phenols or ethers would be the products according to the latter mechanism. However, under the reaction conditions ethyl hydroperoxide decomposes too rapidly to give any appreciable oxidation of the aromatic compound,[17] and perhaps for steric reasons *t*-butyl hydroperoxide is almost completely unreactive.

Relation of This System to Some Enzymic Reactions

The enzymes, catalase and peroxidase,[18] are ferric–porphyrin enzymes which have characteristics similar to the ferric–catechol system described here. Some time ago George[19] suggested that one of the intermediates in reactions catalyzed by these enzymes has an oxygen atom attached to the iron in much the same way as suggested for intermediate **3**, Chart I. Porphyrins have oxidation–reduction properties related to those of catechol and could presumably stabilize intermediates like **3**. The implication that **3** is involved in the model ferric–catechol system is thus additional evidence that George's suggestion for the

(11) See footnote *d*, Table II.
(12) H. Loebl, G. Stein, and J. Weiss, *J. Chem. Soc.*, 2704 (1950).
(13) G. R. A. Johnson, G. Stein, and J. Weiss, *ibid.*, 3275 (1951).
(14) G. Stein and J. Weiss, *ibid.*, 3265 (1951).
(15) For a review see C. Walling, "Free Radicals in Solution," John Wiley and Sons, Inc., New York, N. Y., 1957, p 397 ff.
(16) E. I. Stiefel, J. H. Waters, E. Billig, and H. B. Gray, *J. Am. Chem. Soc.*, **87**, 3016 (1965); S. I. Shupack, E. Billig, R. J. H. Clark, R. Williams, and H. B. Gray, *ibid.*, **86**, 4594, (1964), and references therein.
(17) H. N. Arst, Jr., Senior Thesis, Department of Chemistry, Princeton University, Princeton, N. J., 1964.
(18) For recent reviews see P. Nicholls and G. R. Schonbaum, *Enzymes*, **8**, 147 (1963); K. G. Paul, *ibid.*, **8**, 227 (1963); see also B. C. Saunders, A. G. Holmes-Siedle, and B. P. Stark, "Peroxidase," Butterworth and Co. (Publishers) Ltd., London, 1964.
(19) P. George in "Currents in Biochemical Research," D. E. Green, Ed., Interscience Publishers, Inc., New York, N. Y., 1956, p 338.

structure of the enzymic intermediates is correct. In general, catalase and peroxidase reactions can be related to similar reactions of the ferric–catechol system, but a detailed discussion of these similarities is outside the realm of this paper.

The enzyme variously known as tyrosinase, phenolase, etc.,[20] also bears some resemblance to the ferric–catechol system. This copper enzyme will catalyze the hydroxylation of phenols to catechols by O_2 but only in the presence of catalytic amounts of catechols.[21] It is not difficult to visualize mechanisms for obtaining from O_2 and the catechol a copper-containing intermediate like **3**, which possibly is the hydroxylating agent in this enzymic reaction.

(20) For a review see D. Kertesz and R. Zito in "Oxygenases," O. Hayaishi, Ed., Academic Press Inc., New York, N. Y., 1962, p 307.

(21) S. H. Pomerantz and M. C. Warner, *Biochem. Biophys. Res. Commun.*, **24**, 25 (1966), and references therein.

Formation and Thermal Decomposition of Bicyclo[1.1.0]butane-2-*exo*-d_1[1]

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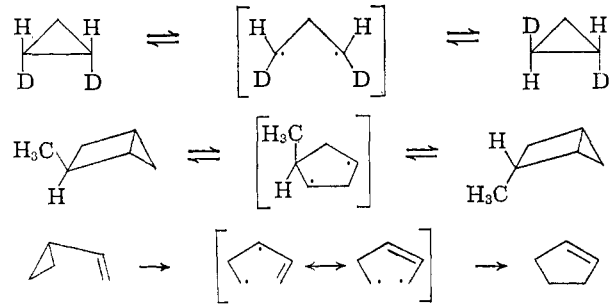
Contribution from the Department of Chemistry, Yale University, New Haven, Connecticut. *Received June 8, 1966*

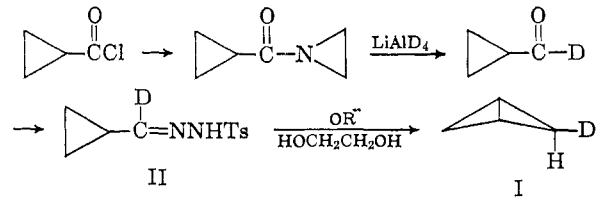
Abstract: The mechanism of the thermal decomposition of bicyclo[1.1.0]butane has been studied using a deuterium-labeled derivative. The reaction giving butadiene appears to involve partial cleavage of both carbon–carbon bonds in the activated complex, and conforms to the Woodward–Hoffmann rules for electrocyclic reactions. The formation of bicyclo[1.1.0]butane-*exo*-2-*d*, from the tosylhydrazone of cyclopropanecarboxaldehyde-*d*, is discussed. It appears that the cyclopropylcarbinyl cation, as such, is not involved in the reaction.

The thermal rearrangement of most cyclopropane derivatives may be rationalized in terms of the formation of a 1,3-diradical intermediate. Thus, the thermal reactions of cyclopropane[3] and bicyclo[2.1.0]pentane[4] both lead to *cis–trans* isomerization of the reactants. Similarly, the rearrangement of vinyl cyclopropane to cyclopentene[5] appears to be best explained as involving a 1,3-diradical intermediate.



Analogy with the reaction of bicyclo[2.1.0]pentane might lead one to expect that the initial reaction in the thermal decomposition of bicyclo[1.1.0]butane would involve the formation of the 1,3 diradical. The fact that 1,3-dimethylbicyclo[1.1.0]butane[6] and 1,3-bis(trifluoromethyl)-2,2,4,4-tetrafluorobicyclo[1.1.0]butane[7] undergo thermal rearrangement to 2,3-disubstituted butadienes indicates the central bond to be intact in the product. Thus, if the 1,3 diradical is formed, it is not involved in the formation of products.

The possibility that any diradical is formed in the reaction in equilibrium with the reactant could be tested by examining the reaction of a 2-labeled bicyclo[1.1.0]butane.[8] A deuterium label is preferred since this will not introduce extraneous steric effects. The synthesis of bicyclo[1.1.0]butane-*exo*-2-*d* (I) is



The final step in the synthesis and the evidence for the position of deuterium in the product will be discussed below. For now we shall simply state that I had at least 90% of the deuterium in the *exo* position.

The bicyclobutane, I, was heated in the gas phase at the reaction temperature of 200°, and the nmr spectrum of the reaction mixture was examined at times corresponding to 6, 21, 30, 45, 58, and 75% reaction. Because of the large chemical shift between the butadiene and bicyclobutane protons, the spectrum of the latter could

(1) This investigation was supported by the Army Research Office (Durham).

(2) National Institutes of Health Predoctoral Fellow 1963–1966; taken from the Ph.D. thesis of J. M. L., 1966.

(3) B. S. Rabinovitch, E. W. Schlag, and K. B. Wiberg, *J. Chem. Phys.*, 28, 504 (1958); E. W. Schlag and B. S. Rabinovitch, *J. Am. Chem. Soc.*, 82, 5996 (1960).

(4) J. P. Chesick, *ibid.*, 84, 3250 (1962).

(5) M. C. Flowers and H. M. Frey, *J. Chem. Soc.*, 3547 (1961); C. A. Wellington, *J. Phys. Chem.*, 66, 1671 (1962).

(6) W. von E. Doering and J. F. Coburn, Jr., *Tetrahedron Letters*, 991 (1965).

(7) W. Mahler, *J. Am. Chem. Soc.*, **84**, 4600 (1962).

(8) It should be noted that the 2-methylbicyclo[1.1.0]butanes (G. Closs, private communication) and the 2,3-dimethylbicyclo[1.1.0]-butyl 1-cyanides (A. Cairncross and E. P. Blanchard, Jr., *J. Am. Chem. Soc.*, **88**, 496 (1966)) do not undergo thermal *cis–trans* equilibration. However, steric factors could intervene in these cases since an *endo*-methyl group would probably give an unfavorable 1,3-nonbonded interaction with the cross-ring methylene group.