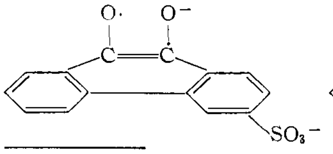
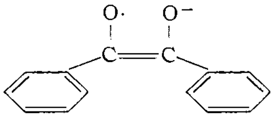
actions. Of course, these cannot be detected magnetically and so recourse was had to the indirect chemical tests described previously. These tests proved positive for either hydroxyl or perhydroxyl radicals or both, but it was not felt possible to decide between them.[15] In any case, there is strong evidence for the presence of labile radical intermediates in addition to the relatively stable semiquinone.

Conclusions

The magnetic and chemical evidence point quite definitely to a free radical mechanism operative in this oxidation. Thus the view of Michaelis and Fetcher that a stable aliphatic semiquinone is formed appears to be confirmed. It is interesting to compare the maximum radical concentration of 3% found here with that of the semiquinone of phenanthrenequinone-3-sulfonate (9,10-dihydro-9,-10-dioxo-3-phenanthrenesulfonate)[16] which has an equilibrium radical concentration of 31–44%. Structurally the two semiquinones are not unlike as shown below in their enediolate form





It can be seen that radical stabilization due to resonance structures with the odd electron or the charge, or both, located in the benzene rings must be much greater in the case of the phenanthraquinone semiquinone than with its benzil counterpart. For such structures to play an important role it is necessary that the benzene rings be coplanar with the enediolate portion.[17] This coplanarity is of course, fixed at all times in the phenanthrene semiquinone thus making a much greater stability possible.

Although a complete formulation of the reaction mechanism is probably premature at this stage, one final point may be of interest in this connection.

The data so far obtained in the study of the benzoin oxidation by Weissberger and by us have given no indication that this oxidation is a chain reaction. This may be offered as further support, at least, for the differentiation theory of Merz and Waters.[18] These authors have concluded that a substance having a free radical intermediate in which the electron density at the point of free valency is high will display a chain oxidation. The unpaired electron of the benzil semiquinone is considerably delocalized and thus should not be oxidized by a chain mechanism.

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(15) Stein and Weiss have stated that the phenolic tests are specifically diagnostic for hydroxyl radicals. However, we feel that there is no unequivocal evidence to dispute the possibility of benzene dehydrogenation by perhydroxyl radicals as well.

(16) L. Michaelis, G. F. Boeker and R. K. Reber, THIS JOURNAL, **60**, 202 (1938).

(17) G. W. Wheland, "The Theory of Resonance," John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 92–94.

(18) J. H. Merz and W. A. Waters, *J. Chem. Soc.*, 2427 (1949).

HONOLULU 14, HAWAII

[A JOINT CONTRIBUTION FROM OAK RIDGE NATIONAL LABORATORY AND EMORY UNIVERSITY]

The Application of Isotope Dilution Technique in Studies of Reaction Rates and Equilibria: The Effect of Diene Structure on the Rate of the Diels–Alder Reaction

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By applying a combination of techniques, it has been found possible to make for the first time a rigorous study of the effect of the electronic character of dienes on the rates of Diels–Alder reaction with maleic anhydride. Pairs of *para*-substituted 1-phenyl-1,3-butadienes were permitted to compete for maleic anhydride at low temperatures. The product mixtures were analyzed by making application of isotope dilution technique, and the effects of the *para* substituents on the reaction rate constants were quantitatively evaluated at several temperatures. Results were correlated using the Hammett equation, and the effect of the electronic character of the series of substituents was found to be in accord with accepted ideas of the Diels–Alder reaction mechanism.

I. Method

The use of competitive rate studies is a well-established method of investigating a chemical reaction.[3] If proper precautions regarding changes in concentration and reversibility are taken, it is possible to equate product ratios with reaction rate ratios. Many reaction rates can be studied only in this way.

The practical utility of competitive rate studies has been greatly enhanced in recent years by the development of the isotope dilution technique.[4] By the use of C-14 labeled compounds, followed by radioassay[5] of the carbon dioxide produced by the oxidation of a quantitatively diluted sample one can determine the yield of a particular compound without the tedium of quantitative isolation.

The application of these techniques has been made to the Diels–Alder reaction. This is a reaction for which precise rate studies have been reported in only a few cases. For a number of other

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(3) For a concise discussion of competing reactions, see the section by T. S. Lee in "Techniques of Organic Chemistry, Volume VIII, Rates and Mechanisms of Reactions," A. Weissberger, Interscience Publishers, Inc., New York, N. Y., 1953, p. 100.

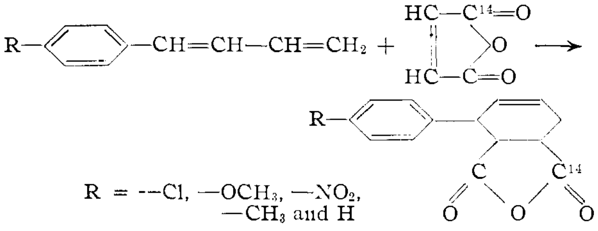
(4) For a discussion of such applications to organic chemistry, see G. A. Ropp and O. K. Neville, *Nucleonics*, **9**, 22 (1951).

(5) (a) O. K. Neville, THIS JOURNAL, **70**, 3501 (1948); (b) V. F. Raaen and G. A. Ropp, *Anal. Chem.*, **25**, 174 (1953); (c) C. J. Collins and G. A. Ropp, THIS JOURNAL, **77**, 4160 (1955).

cases, approximate relative rates have been studied by comparing yields of different reactions run for similar lengths of time under similar conditions. Some of these studies have attempted to correlate reaction rates with the steric and electronic effects of substituents in the dienes or dienophiles, but prior to the present publication no really rigorous study of this type has been made for the Diels–Alder reaction.

Hammett[6] has pointed out the value of studies of the electronic effects on rates and equilibria of *para* and *meta* substituents which are far enough removed from the reaction centers to eliminate steric effects and entropy considerations. Quantitative correlation by the Hammett equation[6] of a series of reaction rates of compounds differing only in the identity of a *meta* or *para* substituent is usually considered to be of great significance in mechanism studies.

In the present case this correlation was sought in a series of studies of the effect on the relative reaction rate of *para* substituents[7] in the diene, 1-phenyl-1,3-butadiene. Maleic anhydride labeled with carbon-14 in the carbonyl group was placed in a solution containing equimolar amounts of two competing dienes, each of which was present in about tenfold molar excess of the dienophile. Analyses of the resulting product mixture by dilution technique gave the ratio of the specific rates for the two dienes. After running a number of pairs of dienes in a series of such competitive experiments, the relative values of the rate constants for the reaction of maleic anhydride with *p*-chloro, *p*-methoxy, *p*-nitro, *p*-tolyl and unsubstituted 1-phenyl-1,3-butadiene were determined at several temperatures. The reactions, all of which were found to proceed quantitatively when carried out separately in benzene or dioxane at temperatures near 25°, may be represented by the equation



R = —Cl, —OCH$_3$, —NO$_2$, —CH$_3$ and H

II. Experimental Procedures

A. **Preparation of Starting Materials.**—Dienes used were prepared by previously described procedures.[7,8] The carbonyl-C^{14}-maleic anhydride was synthesized[9] at an activity level of approximately 100 μc. per mmole by way of succinic-1-C^{14} acid and maleic-1-C^{14} acid which was dehydrated with phosphorus pentoxide.

B. **Competitive Studies.**—All of the competitive rate studies were carried out in the same manner at three different temperatures, 25, 35 and 45°. The method is best described by an example.

Into a ground-glass stoppered test-tube was placed 0.5 mmole (49 mg.) of radioactive maleic anhydride dissolved in 6 ml. of purified and dried dioxane. A mixture of 5 mmoles (651 mg.) of 1-phenylbutadiene-1,3 and 5 mmoles (721 mg.) of 1-*p*-tolylbutadiene-1,3 was added to this solution and the total volume was brought to about 10 ml. with dry dioxane. This solution was allowed to react for about one week at a constant temperature. At the end of this time the dilution technique was applied after first diluting the reaction solution to exactly 100 ml. with benzene. From this solution 50 ml. was pipetted into a beaker containing 2.5 mmoles of inactive 3-phenyltetrahydrophthalic anhydride (adduct I). In the remaining 50-ml. portion was dissolved 2.5 mmoles of inactive 3-*p*-tolyltetrahydrophthalic anhydride (adduct II). The solutions were boiled down to about 10 ml., petroleum ether (b.p. 90–120°) was added and the rest of the benzene was boiled off. The adducts were allowed to crystallize and were collected on sintered glass filters. After two more recrystallizations by first dissolving the adduct in a little benzene, adding petroleum ether and boiling until most of the benzene was removed, about 50 mg. of the opposite dead adduct was added to each one, *i.e.*, 50 mg. of I was added to II and *vice versa* (see Section IIc on hold-back carrier technique). Each product was recrystallized twice, sublimed at 110–115° (0.02 mm.), recrystallized to purity and assayed for activity. The nitro and methoxy derivatives were not sublimed when they were used in competitive reactions.

Data from competitive runs are reported in tables below, the runs being numbered from 1 to 12. In runs 1 through 3 and 5 through 8 the maleic anhydride used had a specific activity of 70.2 μc. per mmole; in all other runs the specific activity of maleic anhydride used was 90.4 μc. per mmole. In each of runs 4, 10 and 12, 3.5 mmoles of inactive adduct was used as inactive diluent; in each of the other runs 2.5 mmoles was used.

C. **Application of Hold-back Carrier Technique.**—Frequently analysis of a mixture of several isotopically-labeled compounds by dilution technique is experimentally difficult. When a component A, for example, is diluted with unlabeled A while components B, C, etc., are not diluted, the isolated sample of A may be seriously contaminated with a very small amount of B or C, etc. Since, of all the components of the original mixture, only A has been isotopically diluted, the contaminant will have a much higher specific activity than A and can, therefore, cause an error in the measured activity of A far out of proportion to its weight. Sometimes, in spite of careful purification, A may remain contaminated with sufficient undiluted B or C to invalidate the results of the analysis. In such cases the practice is to employ "hold-back carrier" technique. Essentially this involves diluting the impurity, which cannot be removed from A, with the corresponding unlabeled compound. Then A is repurified and although it may again retain the contaminant, the latter will now have been isotopically diluted and will, therefore, exert little effect on the radiochemical analysis of A. This technique was applied to the purification of diluted adducts from the competitive kinetic runs. From each of the competitive runs two adducts were formed. When a large excess of inactive adduct, identical to one, was added for dilution, the other was the contaminating adduct and was in several cases difficult to remove completely. Therefore, hold-back technique was carried out in the following manner. After two or three recrystallizations of the diluted adduct most of the impurities were removed except for a small amount of highly active contaminating adduct. About 50 mg. of inactive adduct identical to the contaminating one was added and the mixture was subjected to repeated recrystallizations. In this manner, coupled with a sublimation (except in the cases of the nitro and methoxy adducts which did not require sublimation) radiochemical purity was achieved.

D. **Proof of Irreversibility.**—Two reactions were run. In each reaction 0.5 millimole of the phenyl adduct, specific activity 90.4 μc. per mmole (made by reaction of phenylbutadiene with undiluted maleic anhydride), was dissolved in 6 ml. of dioxane. In one reaction 0.5 mmole of *p*-chlorophenylbutadiene was added to the dioxane solution. In the other 0.5 mmole of *p*-tolylbutadiene was added. After 72 hours at 35° each reaction mixture was diluted with inactive phenyl adduct. The assay in each case showed that 100% of the activity was still in the phenyl adduct, establishing the absence of any exchange of diene under the conditions prevailing in the reaction rate determinations.

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(6) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, Chapter VII.

(7) Related studies of a more qualitative nature were reported earlier with other dienophiles: G. A. Ropp and E. C. Coyner, THIS JOURNAL, **71**, 1832 (1949); **72**, 3960 (1950).

(8) E. A. Braude, E. R. H. Jones and E. S. Stern, *J. Chem. Soc.*, 1087 (1947).

(9) R. F. Nystrom, Y. H. Loo and J. C. Leak, THIS JOURNAL, **74**, 3434 (1952); M. Kushner and S. Weinhouse, *ibid.*, **71**, 3558 (1949).

III. Results and Conclusions

Details and results of the twelve competitive runs are presented in Tables I and II. Table II also presents the derived values of $\log_{10} k/k_0$ for the runs at three temperatures. Plotted against published values of σ, the substituent constant, as described by Hammett,[6] these data give three curves, one for each temperature. From the slopes of these lines, the three reaction constants were found to be approximately

$$\rho_{25^\circ} = -0.685$$
$$\rho_{35^\circ} = -0.621$$
$$\rho_{45^\circ} = -0.606$$

The fact that these values of ρ are negative means simply that electron releasing groups accelerate the reaction. The sign and magnitude of ρ is like that of the reaction of substituted anilines with formic acid in aqueous pyridine at 100° to give formanilides.[10] Thus the rates of this reaction and the Diels–Alder reaction are quantitatively alike in response to nuclear substituents. The structure of the dienophile with similar variations in substituents has already been correlated with the Hammett equation and found to give $\rho = +0.27$.[11]

TABLE I

ASSAY DATA OF PURIFIED ADDUCTS FROM COMPETITIVE RUNS

Run	Temp., °C.	Compound[a]	Assay μc./mmole	M.p., °C.	M.p.,[b] °C.
1	25	*p*-H	4.78	119–119.6	120–121
		p-Cl	2.87	105.8–106.2	106–107
2	25	*p*-H	3.68	119.5–120.5	120–121
		p-CH_3	4.045	115–116	115–116
3	25	*p*-H	5.06	117–118	120–121
		p-MeO	4.85	144–144.5	144–145
4	25	*p*-H	4.81	120.1–120.8	120–121
		p-NO_2	1.364	177–178.5	178–179
5	35	*p*-H	4.47	118.5–119.5	120–121
		p-Cl	2.89	106.4–107.4	106–107
6	35	*p*-H	3.09	118.5–119.5	120–121
		p-CH_3	3.94	115–115.5	115–116
7	35	*p*-H	2.96	116.5–118.5	120–121
		p-MeO	4.70	144–145	144–145
8	35	*p*-H	4.93	119–120	120–121
		p-NO_2	1.55	178–179	178–179
9	45	*p*-H	5.37	120–120.4	120–121
		p-Cl	3.50	105.3–106	106–107
10	45	*p*-H	2.70	119.9–120.8	120–121
		p-CH_3	3.70	115.3–116	115–116
11	45	*p*-H	3.395	116.5–118	120–121
		p-MeO	5.97	144–145	144–145
12	45	*p*-H	4.85	120–120.6	120–121
		p-NO_2	1.415	177.8–178.3	178–179

[a] Refers to the 3-*p*-substituted phenyl-1,2,3,6-tetrahydrophthalic anhydride. [b] Melting point of purified dead adduct before it was used for dilution.

The point corresponding to the σ-value of 1-(*p*-methoxyphenyl)-butadiene-1,3 in its reaction with maleic anhydride lies far above the curve signifying that the rate of reaction of this diene is much too rapid to be merely the result of the permanent polarization caused by the methoxy group. Since conjugated diene systems are known to have a high degree of polarizability, the exceptionally high rate of reaction of the 1-(*p*-methoxyphenyl)-butadiene-1,3 is to be expected on the basis of accepted principles.[12]

TABLE II

KINETIC RESULTS FROM COMPETITIVE RUNS

Run	Temp., °C.	Compound	Yield, mg.	k/k_0	$\log_{10} k/k_0$
1	25	*p*-H	0.355	0.586	−0.2321
		p-Cl	.208		
2	25	*p*-H	.269	1.11	.0453
		p-CH_3	.298		
3	25	*p*-H	.137[a]	2.65	.4216
		p-MeO	.361		
4	25	*p*-H	.393	0.275	− .5607
		p-NO_2	.108		
5	35	*p*-H	.331	.632	− .1993
		p-Cl	.209		
6	35	*p*-H	.224	1.29	.1106
		p-CH_3	.289		
7	35	*p*-H	.150	2.33	.3674
		p-MeO	.349		
8	35	*p*-H	.367	0.300	− .5229
		p-NO_2	.110		
9	45	*p*-H	.316	.636	− .1965
		p-Cl	.201		
10	45	*p*-H	.218	1.37	.137
		p-CH_3	.299		
11	45	*p*-H	.147[a]	2.40	.3802
		p-MeO	.353		
12	45	*p*-H	.397	0.280	− .5528
		p-NO_2	.110		

[a] Corrected value. Assuming the methoxy adduct to be pure.

Calculation of the σ-value for the methoxyl group from our data gives an average value of −0.60 rather than −0.268. This agrees quite well with the σ-value of −0.58 for methoxyl reported by Pearson and co-workers.[13] Hammett himself has shown how the σ-value for a highly polarizable group might need to be modified.[14]

The negative sign of ρ found here for the Diels–Alder reaction is in accord with accepted concepts of the reaction mechanism.[15]

From further studies now in progress, it should be possible to measure the absolute rates of all the reactions reported here and to calculate their activation energies and pre-exponential factors.

OAK RIDGE, TENNESSEE

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[10] O. C. M. Davis, *Z. physik. Chem.*, **78**, 353 (1912).

[11] H. D. Barnstorff and J. S. Meek, Abstracts of Papers, 125th A.C.S. Meeting, Kansas City, Mo., March 23–April 1, 1954.

[12] A. E. Remick, "Electronic Interpretations of Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1943, Chapter V and Appendix V.

[13] D. E. Pearson, J. F. Baxter and J. C. Martin, *J. Org. Chem.*, **17**, 1511 (1952).

[14] L. P. Hammett, paper presented before the Fourth Symposium on Organic Reaction Mechanisms, Bryn Mawr, Pennsylvania, September, 1952.

[15] C. C. Price, "Reactions at Carbon to Carbon Double Bonds," Interscience Publishers, Inc., New York, N. Y., 1946, p. 49.