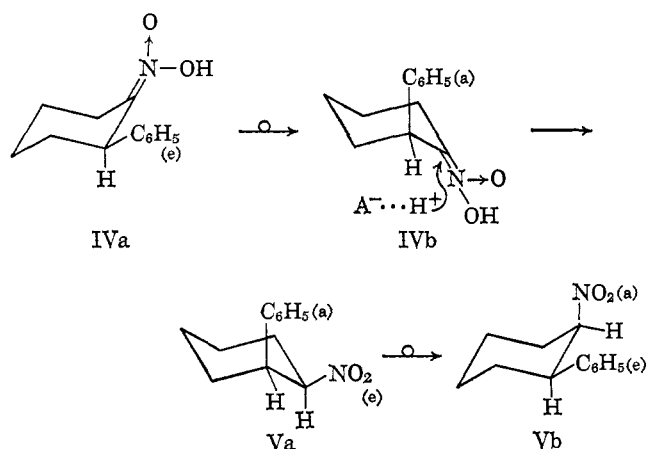


Another case discussed by Zimmerman, and of great similarity to the protonation of I above, is the C-protonation of 1-*aci*-nitro-2-phenylcyclohexane (IV).^{2d} Here it was thought that C-protonation occurred equatorially (*i.e.*, from the less hindered side)⁷ on conformation IVa. However, by $A^{(1,3)}$ strain theory the more stable ground state of IV should be conformer IVb. Protonation of this conformer axially (*i.e.*, from the less hindered side) would also give the known product *cis*-1-phenyl-2-nitrocyclohexane (Vb) *via* the less stable conformer Va.



We find that in the n.m.r. spectrum of IV the benzylic proton appears as a narrow triplet ($J = 4.0$ c.p.s.; $W_H = 9.0$ c.p.s.) at 4.6 p.p.m. Again *this is highly characteristic of a benzylic equatorial proton on a cyclohexyl ring split by adjacent CH_2 hydrogen atoms.*⁴

The cases discussed above demonstrate unequivocally that $A^{(1,3)}$ strain in these exocyclic double bond systems is real and that the much ignored conformers with axial substituents at the 2 position are in fact the stable ground states. In addition we arrive at the conclusion that Zimmerman's views of the ketonization of the exocyclic enolates under discussion must be abandoned in favor of the alternate interpretation presented above.

This general principle of $A^{(1,3)}$ strain surely opens up new vistas in conformational analysis, for now it becomes possible, as will be shown in future articles, to relate quite simply the reactivity of nonrigid molecules to that of their rigid homologs, a relationship lacking in the past.

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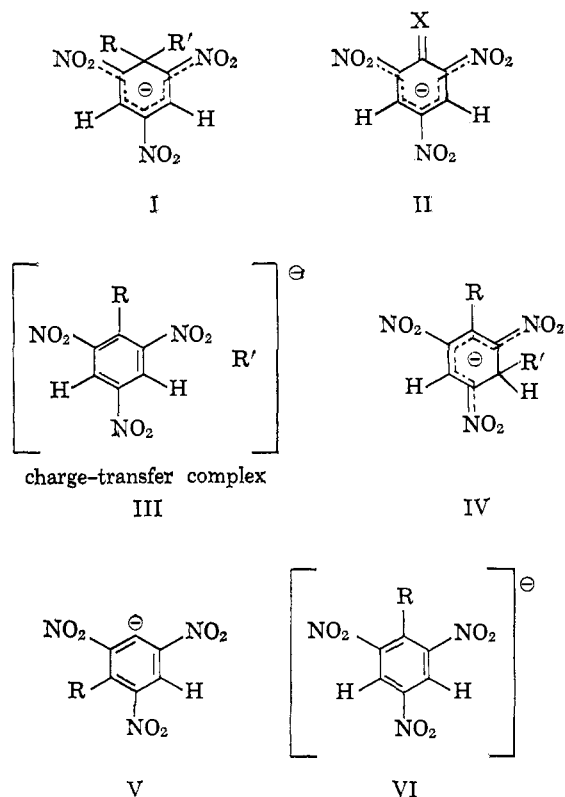
Nuclear Magnetic Resonance Studies of Meisenheimer Complexes

Sir:

In 1902 Meisenheimer¹ succeeded in the isolation and structure determination of a compound, I ($R = OCH_3$, $R' = OCH_2CH_3$), which is today commonly taken as the prototype of the activated complex in nucleophilic aromatic substitution.² Since that time,

(1) J. Meisenheimer, *Ann.*, **323**, 205 (1902).

the structure of the anions formed from other trinitroaromatic compounds has been the subject of numerous investigations and much speculation.³ Proposed structures include the anions I-VI.^{3a-d,4} We wish to report on the nuclear magnetic resonance spectra of the anions obtained on treating a series of 1-substituted 2,4,6-trinitrobenzenes with sodium methoxide in dimethyl sulfoxide (DMSO). These results clearly establish the structures of the resultant anions. Crampton and Gold⁵ have recently reported their conclusions from a similar study. Our conclusions differ from theirs in several important aspects; since they report no data it is difficult to learn the origin of the differences. Their apparent failure to observe some of the species may be due to the rapid equilibration of these anions at the higher methanol concentrations used in their study.



A solution of sodium methoxide in methanol (7 M) was added to a 1 M solution of the aromatic compound in DMSO; the n.m.r. spectra were recorded immediately after mixing using a Varian A-60 n.m.r. spectrometer. The results are summarized in Table I. Due to overlap with the solvent peaks areas for the NH_3 and OCH_3 resonances could not be measured but were visually estimated and were consistent with the assignments in Table I. In all cases, the ring

(2) For recent reviews see: (a) R. Huisgen and J. Sauer, *Angew. Chem.*, **72**, 91, 294 (1960); (b) S. D. Ross, *Progr. Phys. Org. Chem.*, **1**, 3 (1963).

(3) (a) J. B. Ainscough and E. F. Caldin, *J. Chem. Soc.*, 2528, 2540, 2546 (1956); (b) L. K. Dyllal, *ibid.*, 5160 (1960); (c) R. C. Farmer, *ibid.*, 3425, 3430, 3433 (1959); (d) V. Gold and C. H. Rochester, *ibid.*, 1687, 1692, 1697 (1964); (e) R. Foster, *ibid.*, 3508 (1959); (f) R. Destro, C. M. Gramaccioli, A. Mugnoli, and M. Simonetta, *Tetrahedron Letters*, 2611 (1965); (g) W. P. Norris and J. Osmudsen, *J. Org. Chem.*, **30**, 2407 (1965).

(4) (a) R. Foster and R. K. Mackie, *J. Chem. Soc.*, 3843 (1962); (b) R. Foster and R. K. Mackie, *Tetrahedron*, **18**, 161 (1962); (c) R. E. Miller and W. F. K. Wynne-Jones, *J. Chem. Soc.*, 2375 (1959); (d) R. J. Pollitt and B. C. Saunders, *Proc. Chem. Soc.*, 176 (1962); (e) E. F. Caldin and G. Long, *Proc. Roy. Soc. (London)*, **228A**, 263 (1955).

(5) M. R. Crampton and V. Gold, *Chem. Commun.*, 256 (1965).

Table I. Chemical Shift of Anions from 1-Substituted 2,4,6-Trinitrobenzenes and Methoxide in Dimethyl Sulfoxide

		R ₁	R ₂	R ₃	Chemical shifts, p.p.m. ^a			
					R ₁	R ₂	R ₃	OCH ₃
	IVa (Ia)	H	H	H	8.42	6.12	8.42	3.22
	IVb	OCH ₃	H	H	3.85	6.17	8.42	3.20
	IVc	NH ₂	H	H	<i>b</i>	6.09	8.48	<i>b</i>
	IVd	NHCH ₃	H	H	<i>b</i>	6.18	8.50	<i>b</i>
	IVf	N(CH ₃) ₂	H	H	2.85	6.18	8.49	3.19
	Ib	H	OCH ₃	H	8.67	3.06	8.67	3.06
		X	R ₁	R ₂	X	R ₁	R ₂	
	IIc	NH	H	H	<i>b</i>	8.37	8.37	
	IId	NCH ₃	H	H	2.85	8.54	8.20	
	IIe	NC ₆ H ₅	H	H	<i>c</i>	8.37	8.37	

^a Relative to tetramethylsilane internal standard. ^b Not observed. ^c The resonance extends from 6.57 to 7.40 p.p.m.; for N-phenyl-2,4,6-trinitroaniline the resonance extends from 7.09 to 7.34 p.p.m.

protons had resonance areas consistent with the structures assigned and were found to be coupled with $J = 1-2$ c.p.s.

The ¹H n.m.r. spectrum obtained immediately after treating 2,4,6-trinitroanisole with sodium methoxide is shown in Figure 1A.⁶ The initially formed anion is

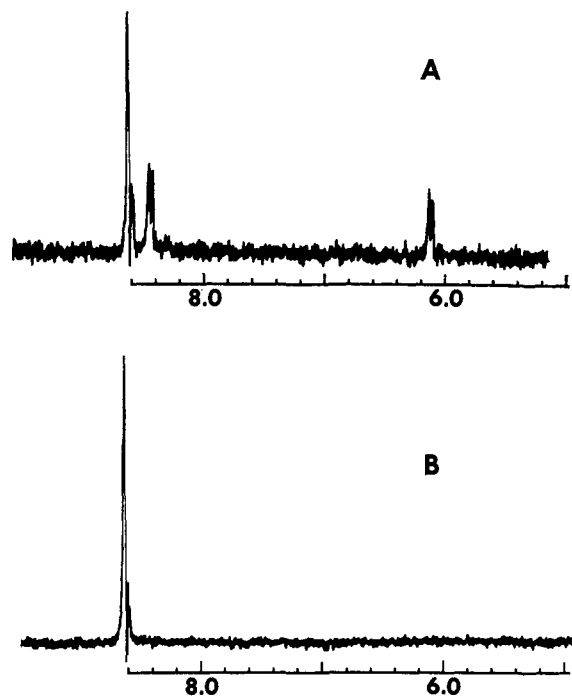


Figure 1. Proton nuclear magnetic resonance spectrum at 60.0 Mc.p.s. of trinitroanisole plus 1 equiv. of sodium methoxide in dimethyl sulfoxide: (A) immediately after mixing; (B) 15 min. after mixing. (Internal tetramethylsilane = 0.0 p.p.m.)

not the well-known Meisenheimer type complex, Ib ($R = R' = \text{OCH}_3$), but has structure IVb ($R = R' = \text{OCH}_3$). This anion is, however, unstable and is rapidly transformed into the complex Ib, whose ¹H n.m.r. spectrum is shown in Figure 1B.⁶ The apparent first-order rate constant for the rearrangement of IVb to Ib is approximately $4 \times 10^{-3} \text{ sec.}^{-1}$ in a solution prepared by the addition of 50 $\mu\text{l.}$ of 7 *M* sodium methoxide in methanol to 500 $\mu\text{l.}$ of a 1 *M* solution of

(6) See Table I for the detailed assignments.

2,4,6-trinitroanisole in DMSO at 30°. Preliminary results indicate that the reaction is catalyzed by methanol. The anion IVb is undoubtedly the type of transient intermediate to which Ainscough and Caldin^{8a}

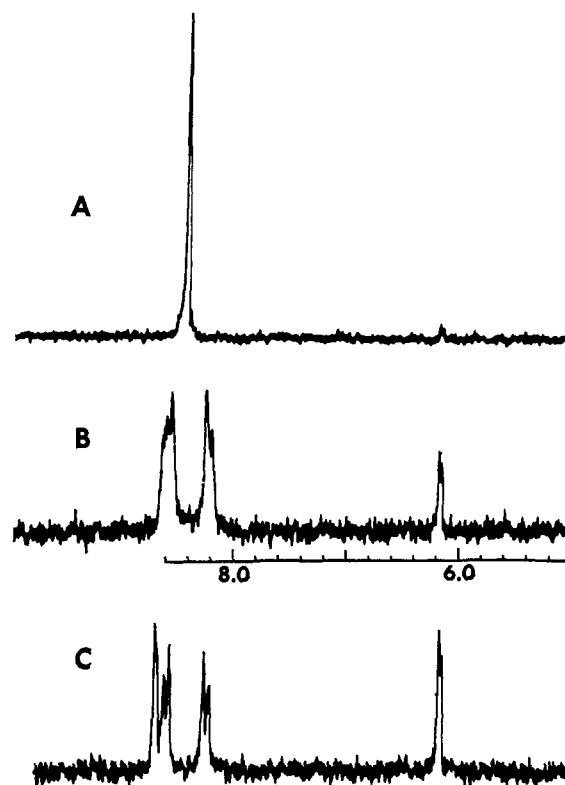


Figure 2. Proton nuclear magnetic resonance spectrum at 60.0 Mc.p.s. of N-methyl-2,4,6-trinitroaniline in dimethyl sulfoxide: (A) 0.80 equiv. of sodium methoxide added, (B) 1.1 equiv. of sodium methoxide added, (C) 1.2 equiv. of sodium methoxide added. (Internal tetramethylsilane = 0.0 p.p.m.)

assigned the charge-transfer complex structure III ($R = \text{OCH}_3$, $R' = \text{OCH}_2\text{CH}_3$) and whose rate of formation and rate of rearrangement to I ($R = \text{OCH}_3$, $R' = \text{OCH}_2\text{CH}_3$) they measured.⁹

(7) The failure of previous workers to observe IVb⁸ may be due to the rapidity of this rearrangement.

(8) V. Gold and M. R. Crampton, *J. Chem. Soc.*, 4293 (1964).

(9) L. J. Andrews and R. M. Keefer, "Molecular Complexes in Organic Chemistry," Holden-Day Inc., San Francisco, Calif., 1964, p. 148.

The ^1H n.m.r. spectra of solutions of N-methyl-2,4,6-trinitroaniline (VIId) and sodium methoxide in dimethyl sulfoxide (Figure 2) result from a mixture of the anion obtained by methoxide addition at the 3 position, IVd ($\text{R} = \text{NHCH}_3$, $\text{R}' = \text{OCH}_3$), and the anion obtained by abstraction of the NH proton, IID ($\text{X} = \text{NCH}_3$).⁶ With less than 1 equiv. of methoxide proton exchange between VIId and IID is very rapid, and for these two species an averaged spectrum is observed (Figure 2A). With slightly more than 1 equiv. of methoxide a dramatic spectral change occurs (Figure 2B). This change is undoubtedly due to a sharp decrease in the rate of rotation around the nitrogen to ring carbon bond. This rotation occurs in the free amine, VIId, and when exchange between IID and VIId is rapid produces an averaged resonance for the two aromatic ring protons.¹⁰ The ratio of IID to IVd is solvent dependent, decreasing from about 9:1 in 10% methanol in DMSO to about 1:1 in 50% methanol in DMSO.

N-Phenyl-2,4,6-trinitroaniline reacts with methoxide to give a single anion whose structure appears to be IIe. That NH proton loss is occurring is verified by the large increase in chemical shift difference between the *ortho* and *meta* protons of the N-phenyl group on reaction (see footnote *c* of Table I). 2,4,6-Trinitroaniline also gives an equilibrium mixture of two anions. The assignment of structure IVc ($\text{R} = \text{NH}_2$, $\text{R}' = \text{OCH}_3$) to the major component follows from a comparison of its n.m.r. spectrum with that of the anions IVa, IVb, IVd, and IVf. Comparison of the chemical shift of the ring protons in the second anion with those of the corresponding protons in Ib, IID, and IIe argues strongly for structure IIc. The ratio of IIc to IVc is about 1:9 in 10% methanol in DMSO and decreases as the methanol concentration is increased.

Of the compounds so far examined, *only trinitroanisole gives a Meisenheimer-type complex*. If the present results may be assumed to be applicable to other solvents, the results of numerous previous studies may be readily understood without invoking a myriad of dubious hypotheses.^{3a,c,e,4c-e} A detailed discussion of the factors influencing the relative rates of formation of the isomeric ions and their thermodynamic stabilities together with the results of further studies on these systems will be presented at a later date.

(10) Rapid proton exchange is also observed between IVd and the dianion obtained by proton abstraction from IVd (see Figure 2C).¹¹ These results will be presented in a forthcoming publication.

(11) K. L. Servis, unpublished results.

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The Catalytic Versatility of Erythrocyte Carbonic Anhydrase. The Enzyme-Catalyzed Hydrolysis of *p*-Nitrophenyl Acetate

Sir:

Carbonic anhydrase (CA) is not a specific catalyst for the reversible hydration of CO_2 but powerfully catalyzes the hydration of other carbonyl systems.¹

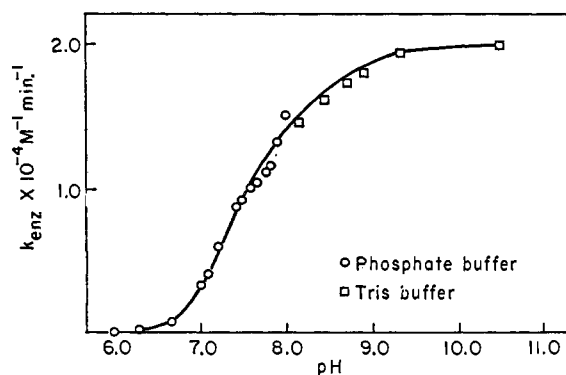


Figure 1. The hydrolysis of *p*-nitrophenyl acetate as catalyzed by bovine carbonic anhydrase.

We wish to report that CA also acts as an esterase.² Thus the enzyme efficiently catalyzes the hydrolysis of *p*-nitrophenyl acetate, a reaction which may prove to be of great value in the elucidation of the mode of action of CA because it can be studied over a very wide pH range by spectrophotometrically following the appearance of the nitrophenolate ion at 400 $\text{m}\mu$ ($\epsilon \sim 2.1 \times 10^4$), the rate of *p*-nitrophenolate ion appearance being identical with that of substrate disappearance. Excellent pseudo-first-order kinetics are obtained; the reaction proceeds to completion (>99% hydrolysis) with stable infinities being observed.

Up to pH 9, the nonenzymic components were less than 15% of the total rate, while at higher pH they became progressively significant because of hydroxide ion catalysis. The buffers, either phosphate or Tris, were approximately 0.01 *M* and the ionic strength, μ , was held at 0.09 with added NaCl. The aqueous solutions were 10% by volume in acetonitrile. (The slope of k_{obsd} vs. $[\text{E}]$ is defined as k_{enz} .) Under these conditions a pH-rate profile was obtained at 25.0° over the pH range 6.0 to 10.5³ using bovine CA (Figure 1). Both phosphate and Tris buffers gave essentially identical k_{enz} . In acidic media the enzyme is very ineffective ($k_{\text{enz}} = 82 \text{ min}^{-1} \text{ M}^{-1}$ at pH 6) while a plateau is reached in basic media where the limiting $k_{\text{enz}} = 20,000 \text{ min}^{-1} \text{ M}^{-1}$ (no drop off in rate is observed up to pH 10.5); the inflection point occurs at pH 7.5. It is instructive to compare the enzyme effectiveness with that of other catalytic species present in the buffer systems: the catalytic constants, k_c , for the proton and hydroxide ion, under our conditions, are 58 and 890 $\text{min}^{-1} \text{ M}^{-1}$, respectively, while the catalytic constants for HPO_4^{2-} , H_2PO_4^- , H_2O , and Tris are appreciably smaller.⁴ The zinc ion associated with each molecule of CA is an obligatory component for its

(1) Y. Pocker and J. Meany, Abstracts of the Sixth International Congress of Biochemistry, 1964, Vol. IV-132, New York, N. Y., p. 327; *J. Am. Chem. Soc.*, **87**, 1809 (1965).

(2) We felt that CA would possess esterase activity because of the similarity between hydration and certain bimolecular hydrolytic mechanisms [M. L. Bender, *ibid.*, **73**, 1626 (1951); **75**, 5986 (1953)]. Others have approached this activity somewhat differently [R. E. Tashian, D. P. Douglas, and Y. L. Yu, *Biochim. Biophys. Res. Commun.*, **14** (3), 256 (1964); F. Schneider and M. Lieflander, *Z. Physiol. Chem.*, **334**, 279 (1963); J. T. Edsall, private communication; S. Lindskog and P. O. Nyman, private communication.]

(3) In order to determine the catalytic efficiency of CA at high pH the inhibitory effects of basic anions had to be avoided. Pure 0.25 *M* Tris in 10% acetonitrile gives a solution whose pH as recorded by a glass electrode is 10.5 and whose "buffer" capacity is such that during initial hydrolysis the observed pH changes can be neglected.

(4) T. C. Bruce and R. Lapinski, *J. Am. Chem. Soc.*, **80**, 2265 (1958).