phage RNA synthesis in the absence of protein synthesis, suggesting that the factor is depletable during phage protein synthesis. Such depletion may in turn limit further synthesis of phage proteins. Although the present results are consistent with this notion, other possible modes of rifampin action can not be eliminated at the present time.

Acknowledgments

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Reactions of Tetranitromethane. Mechanism of the Reaction of Tetranitromethane with Pseudo Acids†

Sandra Walters Jewett‡ and Thomas C. Bruice*

ABSTRACT: The reactions of tetranitromethane with five pseudo acids (CH) have been investigated [CH_3CN, $CH_3SO_2CH_3$, CH_3COCH_3, $CH_2(CN)_2$, and $CH_3CH_2NO_2$]. Kinetic studies, quantitative product analyses, and limited electron spin resonance (esr) studies $C(NO_2)_4$ with CH_3CN, CH_3COCH_3, and $CH_3CH_2NO_2$ were carried out. The reactions of $C(NO_2)_4$ with the pseudo acids CH_3CN, $CH_3SO_2CH_3$, and CH_3COCH_3 were found to give pseudo-first-order kinetics with respect to trinitromethane anion formation and were found to obey the following rate expression: $k_{\text{obsd}} = k_{\text{HO}^-}[\text{HO}^-] + k_1[\text{C}^-] + k_2[\text{C}^-][\text{CH}]$, where $[\text{CH}]_{\text{T}} = [\text{CH}] + [\text{C}^-]$. For the reaction of $CH_2(CN)_2$ with $C(NO_2)_4$, no hydroxide rate was detected at the pH values employed and the rate expression was simply first order in C^- at the lower carbon acid concentrations used. Due to the fact that $\text{p}K_{\text{a}}'$ of nitroethane is located in the accessible pH range, greater kinetic detail could be obtained with this pseudo acid. The pH dependence of the reaction of $CH_3CH_2NO_2$ with $C(NO_2)_4$ in deoxygenated solutions was found to be quite complex, the pseudo-first-order rate constant being provided by an expression describing a bell-shaped pH–log k_{obsd} profile with a discontinuity from slope +1 at low pH. The complicated bell-shaped pH–rate profile for the reaction of $CH_3CHNO_2^-$ with $C(NO_2)_4$ is suggested to arise not only from the reaction of the free carbanion with $C(NO_2)_4$ (giving rise to a shoulder in the pH–rate profile at the pH 6–8) but also from the reaction of the intermediate, $CH_3CH(OH)\dot{N}(O^-)_2$, with $C(NO_2)_4$. This intermediate has been suggested to occur in the basic hydrolysis of nitroethane, and its conjugate acid [$(CH_3CH(OH)\dot{N}(OH)_2$] is thought to be an intermedite in the hydrolysis of *aci*-nitroalkanes (Nef reaction). In order to account for the formation of nitrite ion as well as the esr results indicating considerable radical formation, the mechanism of the reactions of $C(NO_2)_4$ with pseudo acids is interpreted to be a nucleophilic attack of $C(NO_2)_4$ followed by subsequent formation of radical intermediates. The reaction of $CH_3CHNO_2^-$ at high pH values in oxygenated solutions was found to be zero order in the formation of $C(NO_2)_3^-$. The reaction of $CH_3CHNO_2^-$ with $C(NO_2)_4$ in the presence of oxygen is suggested to occur *via* superoxide anion $O_2^{\cdot -}$, with $C(NO_2)_4$ reacting with the $O_2^{\cdot -}$ formed from the oxidation of carbanion by O_2.

Since the introduction of tetranitromethane, $C(NO_2)_4$, as a nitrating agent for tyrosyl residues in proteins (Riordan *et al.*, 1966), studies on model systems have been undertaken to determine the specificity of the reagent (Sokolovsky *et al.*, 1966). It is now apparent that $C(NO_2)_4$ reacts with thiol (Riordan and Christen, 1968; Sokolovsky *et al.*, 1969), indolyl (Spande *et al.*, 1969), and imidazole (Ivanetich, 1971)[1] groups.

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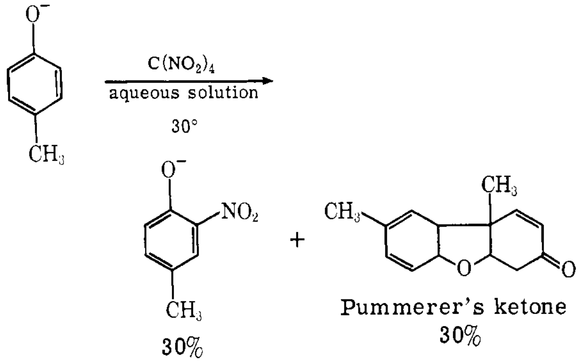
[1] Private communication.

Detailed kinetic investigations as well as quantitative identification of products have been carried out in this laboratory in order to delineate the nature of the mechanisms of the reactions of $C(NO_2)_4$ with compounds chosen as models for protein side chains. The first paper of this series (Bruice *et al.*, 1968) dealt with the mechanism of the reaction of $C(NO_2)_4$ with a series of phenols as reasonable models for protein tyrosyl residues. It was found that the reactive species was phenoxide ion and that the reaction mechanism was of a free-radical nature leading mainly to non-nitrogenous cross-linked phenol derivatives. Nitration of the phenoxide ions occurred only to the extent of 20–30%. As an example, the results for *p*-cresol (eq 1) establish that the formation of free-radical coupling products can compete with the nitration reaction. This finding was not in accord with the mechanism originally suggested by Sokolovsky in which the main reaction involved nitronium ion transfer to the phenoxide ion. Therefore, we cautioned that the reactions of $C(NO_2)_4$ with proteins could lead to radical coupling of proteins as a serious side reaction. Since then there has been numerous reports of the formation of cross-linked monomers, dimers, and higher polymers of proteins caused by treatment with $C(NO_2)_4$ (Doyle *et al.*, 1968; Bello, 1969; Boesel and Carpenter, 1970; Vincent *et al.*, 1970; Ma *et al.*, 1970; Williams and Lowe, 1971; Boyd and Smith, 1971).



(1)

In the second paper of this series, results of a study of the nitration of a series of aliphatic alkoxide ions, including *N*-acetylserinamide, by $C(NO_2)_4$ were reported. The main interest here was the possibility that $C(NO_2)_4$ reacts with protein hydroxyl groups (Walters and Bruice, 1971). While the reactions of $C(NO_2)_4$ with the simpler aliphatic alcohols leads to the nitrate ester of the alcohol, the reaction with *N*-acetylserinamide appeared to involve a complex radical mechanism possibly involving the α-CH bond rather than the alcohol moiety. Nuclear magnetic resonance investigations (Fridkin *et al.*, 1970) have indicated the α-CH protons of *N*-acetyl dipeptides such as Ac-*Ser*-Gly, Ac-*Ala*-Gly, and Ac-*Gly*-Gly exchange only at the group in italic type and, furthermore, that the α-CH protons of Ac-*Ser*-Gly exchange much faster than those of the other two dipeptides studied. These results, as well as the results presented in this paper, suggest that $C(NO_2)_4$ may be reacting with a carbanion at the α position of the *N*-acetylserinamide in a reaction more facile than a reaction at the alcohol moiety.

Most of the previous studies involving protein tyrosyl groups modified by the $C(NO_2)_4$ have been undertaken in order to study the effects of the modified side chains on the catalytic, regulatory, and physical properties of the enzymes. A more novel extension of the use of the reagent has been to modify a portion of the enzyme–substrate complex that becomes reactive with or accessible to the reagent only during the catalytic step of the enzymatic reaction. The nature of the species undergoing attack during the catalytic step has been shown to be a tyrosyl residue in the case of aspartate aminotransferase (Christen and Riordan, 1970a,b) and a substrate species in the case of an aldolase (Riordan and Christen, 1969). The species undergoing attack in the enzyme–substrate complex of aldolase was thought to be a "carbanion" intermediate on the substrate portion of the complex. This finding lead to investigations of the reactions of $C(NO_2)_4$ with pseudo acids (Christen and Riordan, 1970a,b). The present study is a mechanistic investigation of the reaction of $C(NO_2)_4$ with five pseudo acids chosen as suitable models for carbanion-forming species. We have again found a complex radical mechanism to be operative. It seems apparent that, since $C(NO_2)_4$ has the property to react by complicated radical mechanisms, the use of $C(NO_2)_4$ to modify proteins in any manner should be approached with caution. The modified proteins should be more closely analyzed in order to ascertain if more complex changes, aside from tyrosine nitration and cross-linking, have occurred.

Experimental Section

Materials. Acetone and sulfanilic acid (Mallinckrodt), acetonitrile and chloroamine T (Baker), dimethyl sulfone (Aldrich), bis(1-phenyl-1-methylpyrazoline) (Pfaltz and Bauer), trifluoroethanol (Aldrich), and all reagent quality inorganic salts and common solvents were used without further purification. 1-Phenyl-2-methylpyrazolone (Baker) was recrystallized from ethanol, mp 127–128°. 2,4-Dinitrophenylhydrazine (Aldrich) was recrystallized from absolute methanol, mp 191–193°. Tetranitromethane was purified as described in a previous paper (Walters and Bruice, 1971). α-Naphthylamine (Baker) was recrystallized from water. Malononitrile (Eastman) was doubly distilled: bp 100–103° (18), $\eta^{34.4}_{\mathrm{D}}$ 1.41370 (lit. $\eta^{34.2}_{\mathrm{D}}$ 1.41463). Nitroethane (Matheson) was purified by preparative vapor-phase chromatography on a column of 20% XF 1150 silicon on Chromosorb W 60–80. Potassium dinitroethane was prepared by adding 3 ml of a 30% solution of the acid in methylene chloride (Matheson) to 10 ml of 1.0 M KOH at 0°. The precipitate was recrystallized from warmed water.

Apparatus. Quantitative absorbance measurements were made on a Gilford Model 220 recording spectrophotometer and kinetic measurements were made on a Gilford Model 2000. Kinetic solutions were maintained at 30 ± 0.1° by circulating water at this temperature through the Beckman double thermospacers. Absorbance spectra were taken on a Cary Model 15 spectrophotometer. pH measurements were made with a Radiometer Model 22 pH meter with a PHA 630 scale expander using a combined glass-calomel electrode (Radiometer GK 2021C). The electrode was thermostated at the temperature of the kinetic runs. pH-Stat measurements were carried out with the aid of a Radiometer TTT 1b autotitrator equipped with a Radiometer PHA 630 scale expander and a Radiometer SBR 2C titrigraph or with the aid of a Radiometer autoburet ABU 12 equipped with a Radiometer pH meter 26. The water-jacketed titration cell had a 25-ml volume with ports for thermometer, salt bridge for calomel electrode, a Metrohm EA 115X glass electrode, and a titration needle. Solutions were either purged with N_2 for 15 min, or made up in a degassed solvent; $C(NO_2)_4$ was added and the cell sealed for the course of the determination. Electron spin resonance spectra were taken on a Varian V-4502 X-band electron paramagnetic resonance spectrometer equipped with a Varian V-4560 100-Hz field modulation and control unit.

Kinetics. The rate of appearance of $C(NO_2)_3^-$ (*i.e.*, disappearance of $C(NO_2)_4$) in solutions of the various pseudo acids was followed spectrophotometrically by measuring the increase in absorbance at 350 mμ. For the pseudo acids acetonitrile, dimethyl sulfone and acetone, one drop of a stock solution of $C(NO_2)_4$ (1×10^{-5} to 3×10^{-5} M in doubly distilled water) was added to 3 ml of the kinetic solutions in cuvets. For nitroethane and malononitrile 0.5–1.0 ml of this same solution of $C(NO_2)_4$ was added to 25 ml of the appropriate kinetic solution in a pH-Stat cell. The rate of reaction of nitroethane with $C(NO_2)_4$ could not be followed titrimetrically; however, the increase in absorbance at 350 mμ was determined for kinetic solutions of nitroethane by withdrawing *ca.* 0.25 ml of the solution at given time intervals and reading the absorbance manually in a 0.1-cm cuvet. For malononitrile the increase in absorbance at 350 mμ was found to be identical with the rate of hydroxide uptake so that the rate of hydroxide uptake could be used as a measure of the rate of reaction. All reactions were followed to completion. Pseudo-first-order plots were obtained from slopes of plots of $-\ln\,[(OD_\infty - OD_t)/(OD_\infty - OD_0)]$ *vs.* t with the actual calculations performed on an Olivetti-Underwood Programma 101 computer. Pseudo-first-order plots were discarded unless linear from 2 to 3 half-lives. The formation of $C(NO_2)_3^-$ in the reaction of $C(NO_2)_4$ with nitroethane anion at higher pH values in deoxygenated solutions was fit to an equation of the form

$$A_t = A_\infty(1 - e^{-k_{obsd}}) + k_0t \tag{2}$$

where A_t is the total absorbance at time t; A_∞ the final absorbance due to the pseudo-first-order component; k_{obsd} the rate constant describing the pseudo-first-order reaction; and k_0t, the contribution to the absorbance at time t due to a zero-order reaction. It was possible to decrease the zero-order contribution to the overall increase in absorbance at 350 mμ by deoxygenating the $C(NO_2)_4$ stock solution by repeated freezing and degassing (five times). Presumably then, the zero-order component is due to accidental contamination by oxygen. Even though no special precautions were used to transfer the degassed $C(NO_2)_4$ to the degassed nitroethane solution in a cuvet, the pseudo-first-order rates obtained using deoxygenated $C(NO_2)_4$ were within experimental error the same as the pseudo-first-order component obtained using nondeoxygenated $C(NO_2)_4$ but fitting the rate as described in eq 2.

For the reaction of nitroethane anion in solutions which were not deoxygenated, the zero-order rate constants were obtained by converting the slope of the trace to M min^{-1} using an extinction coefficient (ϵ_{ion}) which takes into account that the product, dinitroethane anion, is also absorbing at 350 mμ (eq 3). χ_1 and χ_2 are the yields in mole fractions of $C(NO_2)_3^-$

$$\epsilon_{ion} = \chi_1\epsilon_1 + \chi_2\epsilon_2 \tag{3}$$

and $CH_3C(NO_2)_2^-$, respectively, based on $[(C(NO_2)_4]_0 = 1.0$; ϵ_1 and ϵ_2 are the respective extinction coefficients at 350 mμ. This equation is derived in the following manner. The total absorbance (A_T) at 350 mμ is given by

$$A_T = [C(NO_2)_3^-][\epsilon_1] + [CH_3C(NO_2)]_2^-][\epsilon_2] \tag{4}$$

Since$[C(NO_2)_3^-] = [C(NO_2)_4]_0\chi_1$ and $[CH_3C(NO_2)_2^-] = [C(NO_2)_4]_0\chi_2$, eq 3 becomes

$$A_T = [C(NO_2)_4]_0(\chi_1\epsilon_1 + \chi_2\epsilon_2) \tag{5}$$

It is seen that $(\chi_1\epsilon_1 + \chi_2\epsilon_2)$ is then the corrected extinction coefficient that takes into account both species absorbing at 350 mμ.

Kinetic solutions for acetonitrile, dimethyl sulfone and acetone were prepared from a 2.0–5.0 M stock solution of the reagent in water. This stock solution was diluted with 1.0 M KOH and 4.0 M KCl to give the desired hydroxide concentrations and ionic strength, 0.5. The maximum pH drift of these solutions was −0.04 pH unit. Kinetic solutions for the low-pH studies of nitroethane and malononitrile were prepared by dissolving a known amount of the carbon acid in degassed 0.5 M KCl; these solutions were used immediately. The rate of reaction of malononitrile with $C(NO_2)_4$ was not affected by additional degassing. For the low-pH studies of nitroethane, solutions were degassed an additional 10 min only before product analyses since the additional degassing procedure carried some nitroethane out of solution. For the high-pH studies of nitroethane anion, a 0.02–0.3 M stock solution of nitroethane was appropriately diluted with 0.1–1.0 M KOH and 4 M KCl to give the desired hydroxide concentrations and ionic strength. Bicarbonate buffer (0.1 M, μ = 0.5, KCl) was used for the rates at pH 10.06. No buffer catalysis by this buffer was observed. Kinetic studies were carried out with solutions of nitroethane anion that were deoxygenated for 30–60 min immediately after the addition of base as well as solutions that were saturated with oxygen at 30°. For the zero-order reactions of nitroethane at high pH in oxygenated solutions, more reproducible rates were obtained if the carbanion concentration was held constant while the $C(NO_2)_4$ concentration was varied. The reverse procedure of varying the carbanion concentration at constant $C(NO_2)_4$ was found to be less accurate due to the volatility of $C(NO_2)_4$ and hence its loss each time a vessel containing the stock solution was opened (Rabani *et al.*, 1965). While the rates of reaction of $C(NO_2)_4$ with nitroethane anion in deoxygenated solutions were found to be irreproducible if the basic solution was allowed to stand much longer than 1 hr, the oxygenated solutions gave reproducible rates for incubation of the anion in base up to 1–1.5 hr. Equilibration of the nitroethane with base was complete before the addition of $C(NO_2)_4$. The reaction of acetonitrile in the presence of trifluoroethanol was carried out using a constant concentration of acetonitrile (0.5 M) in varying trifluoroethanol (0.5–3.0 M) at pH 11.59 (*ca.* $[OH^-] = 0.0083$ M).

Product analyses were performed on solutions which had been deoxygenated by bubbling nitrogen through the solutions. While the rates were not effected by oxygen, except for the rate of nitroethane anion with $C(NO_2)_4$ the product yields showed greater variation if the solutions were not first deoxygenated. The $C(NO_2)_4$ stock solution was deoxygenated only for one product study in the reaction of nitroethane anion at pH 11.0. Product analyses were also performed on the oxygenated solutions of nitroethane anion. Product yields are calculated on the basis that the initial $C(NO_2)_4$ concentrations are 100%, therefore, making the total N or NO_2 available from the starting material, 400%.

Trinitromethane, nitrite ion, dinitroethane, and cyanide ion were analyzed spectrophotometrically. Nitrite ion concentrations were determined according to the procedure of Kolthoff *et al.* (1952); these assays were performed on diluted samples so that the carbon acid did not interfere with the assay. Nitrite ion yields for the reaction of $C(NO_2)_4$ with nitroethane anion were corrected for nitrite ion that was formed in the decomposition of nitroethane itself in these solutions. Attempts were made to estimate acetaldehyde from the nitroethane reaction

TABLE I: Reactions of $C(NO_2)_4$ with Five Carbon Acids, $\mu = 0.5$, 30°.

	pH	$[OH^-]$, M	Carbon Acid Concn Range (M)	No. of k_{obsd} Values Detd
CH_3CN		0.01–0.05	0.5–4.0	34
$CH_3SO_2CH_3$		0.01–0.10	0.2–2.0	42
CH_3COCH_3		0.0025–0.01	0.5–3.0	32
$CH_2(CN)_2$	6.0–8.0		0.001–0.10	23
$CH_3CH_2NO_2$	6.0–8.25		0.001–0.10	43
	11.0–12.4[a]	0.0025–0.05	0.0001–0.002	55
	10.06 ± 0.03[a] {(0.1 M HCO_3^-)}		0.0001–0.0012	10
		0.0025–0.005[b]	0.0001–0.001	47

[a] Nitroethane solution degassed 30–60 min before addition of $C(NO_2)_4$. [b] Saturated with O_2, 30°.

at low pH using 2,4-dinitrophenylhydrazine according to the procedure of Lappin and Clark (1951). Kinetic solutions for the reaction of $C(NO_2)_4$ with acetone were found to contain a small perturbation of the uv spectrum of $C(NO_2)_3^-$ in the region of 320 mμ, the λ_{max} associated with nitroacetone (Pearson *et al.*, 1955). The small contribution to the total absorbance of this peak was estimated from a difference spectra using a blank of $C(NO_2)_3^-$ at a concentration corresponding to 90–95%. Rigorous quantitative measurements for nitroacetone were not performed.

Cyanide ion was assayed in reaction mixtures of malononitrile according to the procedure of Kruse and Mellon (1953). Kinetic mixtures were surveyed spectrophotometrically for products other than $C(NO_2)_3^-$ using a $C(NO_2)_3^-$ blank solution containing 95–100% $C(NO_2)_3^-$ based on the initial $C(NO_2)_4$ concentration. A minor product (1,1,2,3,3-pentacyanopropenide anion, 4%) was identified by the characteristic double absorption peak λ_{max} 395, 410 mμ (Looney and Downing, 1958). The kinetic mixtures also contained ultraviolet (uv) peaks at 320 and 285 mμ. The product giving the 285-mμ peak was isolated in small quantity from a preparative mixture $[C(NO_2)_4{:}CH_2(CN)_2{:}OH^- = 1{:}2.5{:}2]$ as an amorphous impure material showing NH, C=C, and conjugated CN bands in the infrared (ir) spectrum. Further uv analyses showed that this species has $pK_a = 2.7$, λ_{max} 285 mμ (basic), λ_{max} 238 ± 2 mμ (acidic), and isosbestic point 259 mμ. Tlc revealed a total of at least six products derived from the malononitrile as well as insoluble tars and precipitates. Further products were not isolated.

For the specific base-catalyzed reaction of acetonitrile, dimethyl sulfone, acetone, and nitroethane anion with $C(NO_2)_4$, the yields of $C(NO_2)_3^-$ and nitrite ion due only to the carbon acid reaction can be obtained from the total yields after subtraction of the products due to hydroxide catalysis alone. A detailed discussion of the calculations involved is described in a previous publication (Walters and Bruice, 1971).

The generation of radicals in the base-catalyzed reaction of the carbon acids with $C(NO_2)_4$ were performed in the following manner. To 1 ml of the carbon acid was added 0.1 ml of $C(NO_2)_4$ followed by 1 ml of O_2-free 6 N KOH. The carbon acids that were studied were acetonitrile, acetone, and nitroethane. Only one phase remained with acetone while considerable mixing of the aqueous and organic phases occurred with nitroethane. Two phases separated with acetonitrile. The aqueous phase exhibited a seven-line electron spin resonance (esr) spectrum consistent with the interaction of an unpaired electron with three equivalent nitrogen nuclei. This spectrum has been assigned to $C(NO_2)_3^{\cdot\,2-}$ (Lagercrantz, 1964a,b). The spectrum of acetone and acetonitrile contained, in addition to the seven-line spectrum, a weak overlapping signal probably due to an organic derivative of the carbon acid. Acetonitrile exhibited no radicals in the light orange organic phase but the seven-line spectrum developed in the aqueous phase after several hours in the dark. No radical spectra was obtained under kinetic conditions. Spectra were scanned at room temperature ±50 G about the center of the spectrum of $C(NO_2)_3^{\cdot\,2-}$; no *g* values were measured.

Results

Reaction of $C(NO_2)_4$ with Pseudo Acids. The experimental conditions employed for the kinetic studies of the carbon acids are given in Table I. Plots of the pseudo-first-order rate constant (k_{obsd}) for the appearance of $C(NO_2)_3^-$ *vs.* total carbon acid concentration ($[CH]_T$) for acetonitrile, dimethyl sulfone, and acetone (see Figure 1) at various constant hydroxide concentrations were found to obey the following expression

$$k_{obsd} = k_{OH^-}[OH^-] + k_1'[OH^-][CH]_T + k_2'[OH^-][CH]_T^2 \quad (6)$$

$[CH]_T = [CH] + [C^-]$ but since at the pH employed $a_H \gg K_a$ it follows that $[CH]_T \approx [CH]$. Plots of $\{(k_{obsd}/[HO^-]) - k_{HO^-}\}/[CH_T]$ *vs.* $[CH_T]$ are linear (Figure 2), least-squares analyses giving the slopes and intercepts k_2' and k_1', respectively. The rate expression (eq 6) can be written in the following manner

$$k_{obsd} = k_{OH^-}[OH^-] + k_1[C^-] + k_2[CH][C^-] \quad (7a)$$

$$k_{obsd} = k_{OH^-}[OH^-] + k_1[CH]_T[K_a/(K_a + a_H)] + k_2[CH]_T^2[K_a/(K_a + a_H)] \quad (7b)$$

since $a_H \gg K_a$

$$k_{obsd} = k_{OH^-}[OH^-] + k_1[CH]_T(K_a/K_w)[OH^-] + k_2[CH]_T^2(K_a/K_w)[OH^-] \quad (7c)$$

TABLE II: Rate Constants for the Reactions of $C(NO_2)_4$ with Five Carbon Acids at 30°, $\mu = 0.5$.

	$pK_a{}^a$	k_1' (M^{-2} min^{-1})^b	k_2' (M^{-3} min^{-1})^b	k_1 (M^{-1} min^{-1})^c	k_2 (M^{-2} min^{-1})^c
CH_3CN	25^d	6.6 ± 0.06	1.89 ± 0.07	9.7×10^{11}	2.8×10^{11}
$CH_3SO_2CH_3$	23^d	9.16 ± 0.5	3.81 ± 0.31	1.35×10^{10}	5.6×10^{9}
CH_3COCH_3	20^d	144 ± 6	325 ± 9	2.1×10^{8}	4.7×10^{8}
$CH_2(CN)_2$	11.1^e	$3.83 \pm 0.23 \pm 10^7$		2.4×10^{4}	
$CH_3CH_2NO_2$	8.4^f	$1.36 \times 10^{6\,g}$		5.0	

[a] The pK_a's for acetonitrile, dimethyl sulfone, and acetone are reported at 25°. Malononitrile and nitroethane pK_a's are given at 30°. [b] These are the experimentally determined rate constants according to eq 6. [c] These are the calculated rate constants according to 8a and 8b. [d] Pearson and Dillon (1953). [e] Boyd and Wang (1965). [f] Gregory and Bruice (1967). [g] This rate constant is calculated as the ratio $k_5K_{a_1}/K_w = k_1'$ from eq 14b. It is analogous to the rate constants obtained for the other carbon acids when $a_H > K_a$. See eq 22, where $k_1' = [k_7k_8/(k_{-7} + k_8)](K_a/K_w)$ for the other carbon acids.

Hence, we can obtain the constants k_1 and k_2 by the following relationships

$$k_1'K_w/K_a = k_1 \tag{8a}$$

$$k_2'K_w/K_a = k_2 \tag{8b}$$

The rate constants for the reactions calculated from eq 6 and 8 are provided in Table II.

The rate equation for malononitrile was found to obey the following expression

$$k_{obsd} = k_1'[OH^-][CH]_T \tag{9}$$

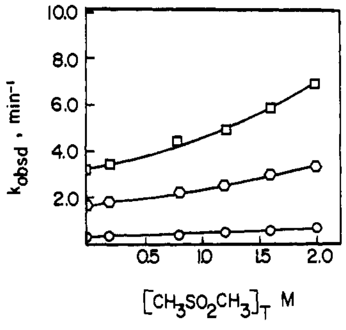


FIGURE 1: Plot of k_{obsd} *vs.* total dimethyl sulfone concentration at hydroxide concentrations of 0.01 M (○), 0.05 M (○), and 0.1 M (□), 30°, $\mu = 0.5$

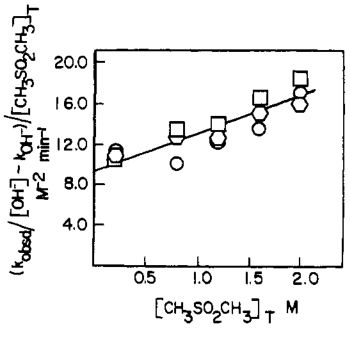


FIGURE 2: Plot of $(k_{obsd}/[OH^-] - k_{OH^-})/[CH_3SO_2CH_3]_T$ *vs.* $[CH_3SO_2CH_3]_T$ for hydroxide concentrations of 0.01 M (○), 0.05 M (○), and 0.1 M (□), 30°, $\mu = 0.5$.

Plots of k_{obsd} *vs.* $[CH]_T$ at constant pH were linear and did not exhibit a second-order term in carbon acid at the lower concentrations employed (Figure 3).

Plots of k_{obsd} *vs.* total carbon acid for nitroethane at both the high (Figure 4) and low pH values (Figure 3) were linear and also did not exhibit a second-order term in carbon acid at the lower concentrations employed. The experimental data (Table III) for the plot of log ($k_{obsd}/[CH_T]$) *vs.* pH (Figure 5) can be fit to a simple mathematical expression describing a bell-shaped curve

$$\frac{k_{obsd}}{[CH]_T} = \frac{kK_1a_H}{a_H{}^2 + K_1a_H + K_1K_2} \tag{10}$$

The two pK_{app} values obtained from this mathematical expression $pK_1 = 10.8$ and $pK_2 = 11.4$ with $k = 7.5 \times 10^3$ min^{-1}, are seen to be nearly the same and near the top of the bell. The more detailed nature of this profile is dealt with in the Discussion section. Furthermore, it is noted that between pH 6 and 8 there appears to be another pK_a perturbing the linear slope of the bell (Figure 6).

Table IV contains the product yields for the reactions of $C(NO_2)_4$ with the carbon acids. Product analyses for the reaction of $C(NO_2)_4$ with dimethyl sulfone and acetonitrile indicated that the yield of $C(NO_2)_3{}^-$ is not quantitative but equal to 40–50%. The nitrite yields were found to be [100% − % $C(NO_2)_3{}^-$] × 4. The reaction of $C(NO_2)_4$ with acetone gave *ca.* 100% $C(NO_2)_3{}^-$ and 93 ± 7% nitrite ion. Nitroacetone was estimated in the kinetic mixture to be 7 ± 2%. Product analyses for the reaction of malononitrile showed

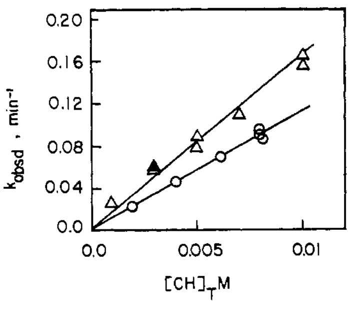


FIGURE 3: Plot of k_{obsd} *vs.* $[CH]_T$ for $CH_2(CN)_2$ at pH 7.5, titrimetric rate (△), spectrophotometric rate (▲), and for $CH_3CH_2NO_2 \times 10^{-1}$ (○) at pH 7.03, 30°, $\mu = 0.5$.

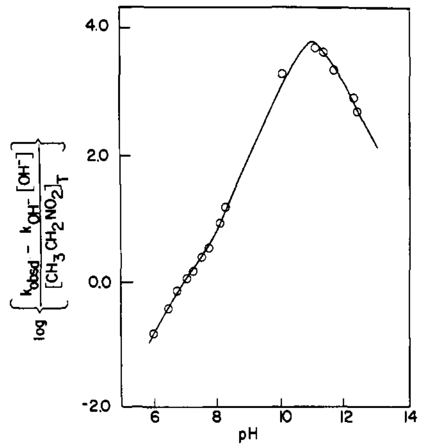


FIGURE 4: Log (k_{obsd}/[CH]$_T$) *vs.* pH for nitroethane (○) (30°, μ = 0.5). Points are experimental and the line theoretical being generated from eq 14b employing the constants of Table V.

TABLE III: Log (k_{obsd}/[CH]$_T$) Values at Various pH Values for the Reaction of $C(NO_2)_4$ with Nitroethane, 30°, μ = 0.5.

Log (k_{obsd}/[CH]$_T$)	pH
−0.815	6.0
−0.432	6.5
−0.15	6.75
0.057	7.03
0.152	7.25
0.393	7.52
0.532	7.73
0.947	8.08
1.16	8.25
3.24	10.05
3.66	11.07
3.59	11.36
3.3	11.66
2.9	12.30
2.65	12.40

that the reaction leads to complex mixtures presumably containing condensation products of malononitrile in addition to $C(NO_2)_3^-$ and nitrite ion. Details of the product distributions for malononitrile were not worked out. The product yields for the reaction of $C(NO_2)_4$ with nitroethane exhibit sensitivity to O_2 particularly as the pH is increased. Acetaldehyde (1×10^{-4} to 5×10^{-4} M), formed in the decomposition of nitroethane alone during a time comparable to the reaction with $C(NO_2)_4$, prevented accurate analyses of acetaldehyde formed in the reaction of $C(NO_2)_4$ with nitroethane $[C(NO_2)_4]_0$ $\sim 0.06 \times 10^{-4}$ to 1.0×10^{-4} M). Detection of acetaldehyde in the reaction of nitroethane with $C(NO_2)_4$ over that due to decomposition of substrate can only be taken as an indication that acetaldehyde is formed but the yield cannot be determined accurately.

The reaction of 0.5 M acetonitrile with $C(NO_2)_4$ (*ca.* 1.2×10^{-5} M) in varying amounts of trifluoroethanol (0.5–3.0 M) varied ±8% from the rate expected for 0.5 M acetonitrile at the same pH. Since the addition of increasing amounts of trifluoroethanol, shown to be inert toward $C(NO_2)_4$ (Walters and Bruice, 1971), did not produce the same apparent rate enhancements as comparable amounts of acetonitrile, it is likely that the rate enhancement over the hydroxide rate due to acetonitrile (both the first- and second-order rate terms) is probably not due to a solvent effect of the acetonitrile on the hydroxide rate.

The formation of $C(NO_2)_3^-$ in the reaction of nitroethane anion ([C⁻]) with $C(NO_2)_4$ at high pH under aerobic conditions was found to be zero order in $C(NO_2)_4$ and to obey the following rate expression

$$(k_0/[C^-]_T)^{-1} = S[1/C(NO_2)_4] + I \qquad (11a)$$

$$k_0 = \frac{[C(NO_2)_4][C^-]_T}{S + I[C(NO_2)_4]} \qquad (11b)$$

where k_0 is the observed zero-order rate constant (M min^{-1}) and S and I are determined from the slope ($S = 9.4 \times 10^{-5}$ M^{-1} min^{-1}) and intercept (I = 2.26 min^{-1}), respectively of a plot of $[C^-]_T/k_0$ *vs.* $1/[C(NO_2)_4]$ (Figure 7). Product analyses (Table IV) for these solutions indicate that the products do not vary as a function of the initial $C(NO_2)_4$ concentration.

Discussion

The log of the rate constants (k_1 and k_2) for the reactions of five pseudo acids with tetranitromethane are linearly dependent upon the pK_a of the pseudo acids (Figure 8). Inspection of Figure 8 reveals that the rate constants are as great as 10^{12} M^{-1} min^{-1} indicating that k_1 could not simply pertain to the second-order reaction of carbanion with $C(NO_2)_4$. Any mech-

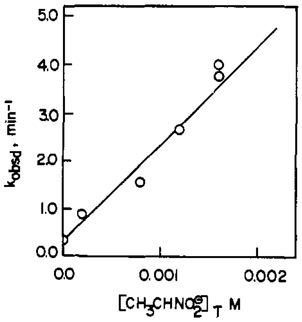


FIGURE 5: Plot of k_{obsd} *vs.* $[CH_3CHNO_2^-]_T$ at pH 11.6 (*ca.* 0.01 M OH⁻), 30°, μ = 0.5.

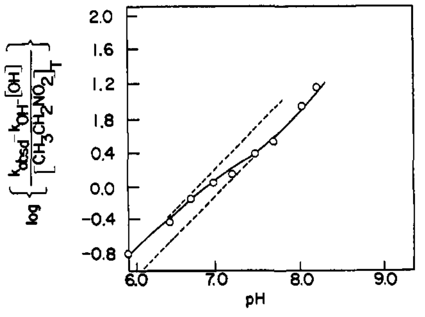


FIGURE 6: Log (k_{obsd}/$[CH_3CH_2NO_2]_T$) *vs.* pH between pH 6.0 and 8.0 showing small shoulder in pH profile. Points are experimental and the line theoretical being generated from eq 14b employing the constants of Table V.

TABLE IV: Product Analyses for the Reactions of $C(NO_2)_4$ with Carbon Acids.

	pH or [OH^-], M	[CH], M	$[C(NO_2)_4]_0$, × 10^5 M	% $C(NO_2)_3^-$	% NO_2^-	Additional Products	%	Total N[a]
CH_3CN	0.01–0.05	0.4–4.0	2–9	50 ± 4	238 ± 11			388 ± 22
$CH_3SO_2CH_3$	0.01–0.10	0.5–2.0	2–10	46 ± 5	233 ± 33			393 ± 45
CH_3COCH_3	0.0025–0.01	0.5–2.0	4–5	111 ± 9	93 ± 7	$CH_3COCHNO_2^-$	7 ± 2	433 ± 36
$CH_2(CN)_2$	pH 6.0	0.05–0.12	5–18	94 ± 5	61 ± 10			
						$(NC)_2C{=}C(CN)$-	4	
	pH 8.0	0.01	5–5	100	20	$C(CN)_2^-$	0	
						CN^-	<1	
$CH_3CH_2NO_2$	pH 7.0–8.0[b]	0.005–0.02	5–11	100 ± 3	116 ± 5	$CH_3C(NO_2)_2^-$	50 ± 5	466 ± 19
	pH 8.0[c]	0.02	0.6	98	105	$CH_3C(NO_2)_2^-$	5	399
	pH 8.0[c]	0.02	1.7	101	93	$CH_3C(NO_2)_2^-$	19	415
	pH 8.0[c]	0.02	4.9	100	92	$CH_3C(NO_2)_2^-$	35	427
	pH 8.0[c]	0.02	6.1	100	75	$CH_3C(NO_2)_2^-$	44	419
	pH 8.0[c]	0.02	10.1	100	73	$CH_3C(NO_2)_2^-$	56	449
	0.0025–0.005[d]	0.0005–0.001	0.45–0.48	83 ± 6	82 ± 11	$CH_3C(NO_2)_2^-$	26 ± 11	357 ± 40
	0.0025–0.005[d]	0.0005–0.001	0.89–0.97	96 ± 1	69 ± 7	$CH_3C(NO_2)_2^-$	31 ± 6	388 ± 16
	0.0025–0.005[d]	0.0005–0.001	1.48–1.98	98 ± 2	60 ± 7	$CH_3C(NO_2)_2^-$	43 ± 3	397 ± 16
	0.0025–0.005[d]	0.0005–0.001	2.88–2.92	99 ± 1	55 ± 1	$CH_3C(NO_2)_2^-$	49 ± 10	401 ± 14
	0.0025–0.005[d]	0.0005–0.001	3.82–4.34	101 ± 3	54 ± 10	$CH_3C(NO_2)_2^-$	53 ± 7	410 ± 26
	0.0025[e]	0.0005	1.3	85	73	$CH_3C(NO_2)_2^-$	19	347
			2.6	95	61	$CH_3C(NO_2)_2^-$	32	378
			3.8	102	58	$CH_3C(NO_2)_2^-$	42	406
			5.1	105	54	$CH_3C(NO_2)_2^-$	50	419
			6.3	97	49	$CH_3C(NO_2)_2^-$	50	390
	0.0025[f]	0.005–0.001	2	100 ± 1	63 ± 3	$CH_3C(NO_2)_2^-$	59 ± 2	422 ± 8
	0.0025[f]	0.0007	0.5–4.0	100 ± 4	62 ± 3	$CH_3C(NO_2)_2^-$	61 ± 3	423 ± 21

[a] Calculations are made on the basis that the initial $C(NO_2)_4$ concentration is equal to 100%, therefore making the total N or NO_2 available from the starting material, 400%. [b] Nitroethane solution partially deoxygenated. [c] Nitroethane solution deoxygenated an additional 10 min. [d] Nitroethane solution deoxygenated 30 min. [e] Nitroethane solutions and $C(NO_2)_4$ stock solution both deoxygenated. [f] Product yields in the presence of oxygen.

anism written for these reactions must account for reactivity of the carbon acids, the dependence of the nature of the products with change in carbon acid, the rate terms second order in carbon acid seen with acetonitrile, dimethyl sulfone, and acetone and the esr evidence indicating considerable radical formation. Additional evidence, including the formation of nitrite ion and the sensitivity to oxygen, suggests that the most probable mechanism(s) undoubtedly involve(s) the formation of radical species along the reaction pathway.

The reaction of $C(NO_2)_4$ with nitroethane was studied in greatest detail and will be discussed first. The reaction of $C(NO_2)_4$ with nitroethane even in the absence of oxygen and interfering buffers appears to be complicated. The pH-rate profile of Figure 4 can be fit to the kinetic equation which describes a bell (eq 10); however, the apparent pK_a's derived from the profile bear no obvious relationship to the pK_a of nitroethane. In going from low to high pH the profile con-

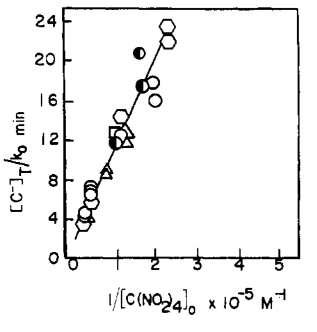


FIGURE 7: Plot of $[C^-]_T/k_0$ *vs.* $1/[C(NO_2)_4]_0$ for the reaction of $C(NO_2)_4$ with nitroethane anion in oxygen-saturated solutions, 30°, μ = 0.5 $[C^-]_T$ = 5 × 10^{-4} M (○), 7.8 × 10^{-4} M (△), 9.85 × 10^{-4} M (⬡), at 0.0025 M OH^-; $[C^-]_T$ = 5 × 10^{-4} M (◐) at 0.003 M OH^-, $[C^-]_T/k_0$ calculated from varying $[C^-]_T$ = 0.0001–0.001 M at 0.005 M OH^- with $[C(NO_2)_4]_0$ = 8.6 × 10^{-6} (□).

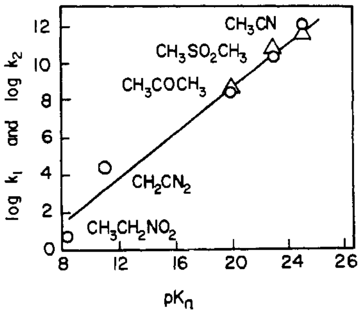


FIGURE 8: Log k_1 (○) and log k_2 (△) *vs.* pK_a for the reactions of five pseudo acids with $C(NO_2)_4$ at 30°, μ = 0.5, β = 0.6.

tinues to rise with slope +1 after the nitroethane pK_a. Therefore, the pK_{app} values do not represent acid dissociation constants. Phenomena of this nature are now common and suggest the following possibilities (see Bruice and Benkovic, 1966, for discussions of kinetically apparent pK_a values). (i) There exists a prototropic equilibrium involving $CH_3CH_2NO_2$ that occurs in the reaction sequence prior to the acid ionization thereby shifting the apparent pK_a to a higher value. The descending portion of the bell could result from a protonation step occurring after a steady-state step involving the carbanion and $C(NO_2)_4$. (ii) The ascending limb of the profile beyond the nitroethane pK_a could be described by an intermediate, reactive with $C(NO_2)_4$, which is formed by the interaction of hydroxide ion and the carbanion. Subsequent intermediates whose breakdown require acid or are accelerated by acid could form the descending portion of the bell. (iii) Another possibility is that $C(NO_2)_4$ and hydroxide ion form an intermediate which is trapped by the carbanion hence giving a rate–pH profile that continues to rise with slope +1 after the nitroethane pK_a. As in the first two possibilities, the descending portion of the bell could arise from a facile acid-catalyzed breakdown of the subsequent intermediates. We have ruled out any simpler kinetic scheme, using either a steady-state or preequilibrium treatment. For a comprehensive discussion of the kinetic deliberations leading to the conclusions that the conditions of i, ii, or iii must be met in order that the results of Figure 8 be explained (see Jewett, 1971).

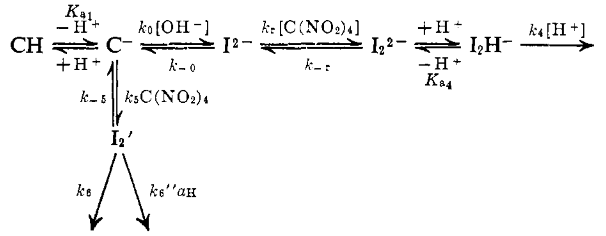
Condition i requires that a preequilibrium exists before the acid dissociation of nitroethane as in eq 12. Employing a material balance in ([CH′] + [CH] + [C^-]) and the steady-state assumption for I_1 and I_2, we arrive at equations (not shown) which can be fit to the data of Figure 8 but which require K_c to be a very small number. This small value for K_c would require nitroethane to exist almost entirely as another species (CH′) when dissolved in water. This kinetic scheme has therefore been discarded as a reasonable one since there has been nothing reported in the literature to indicate what this species could be.

$$\mathrm{CH'} \xrightleftharpoons{K_c} \mathrm{CH} \xrightleftharpoons[-\mathrm{H}^+]{K_{a_1}\ +\mathrm{H}^+} \mathrm{C}^- \xrightleftharpoons[k_{-r}]{k_r\mathrm{C(NO_2)_4}} \mathrm{I}_1 \xrightarrow{k_3[\mathrm{H}^+]} \text{product}$$

or (12)

$$\mathrm{I}_1 \xrightleftharpoons[-\mathrm{H}^+\ K_{a_2}]{+\mathrm{H}^+} \mathrm{I}_2 \xrightarrow{k_3'} \text{product}$$

Condition ii involves the formation of a hydroxide–carbanion intermediate at high pH values. Before discussing reaction schemes embodying this feature that allow a fit of the kinetic data to theroetical equations, it should be stated that there is precedence in the literature for OH^- attack upon nitroethane species (Lockhard *et al.*, 1969). The reaction sequence of eq 13 allows a kinetic derivation that can be fit to the experimental pH *vs.* log k_{obsd} profile and also a mechanism for which intermediate structures have some precedence and products may be rationalized. From eq 13 there follows eq 14a if the following assumptions are made: (a) material balance in [CH] and [C^-]; (b) steady state in the species I_2' and I_1^{2-}, I_2^{2-}; (c) $dP/dt = k_4a_H[I_2H^-] + (k_6''a_H + k_6)[I_2']$.



or

$$\mathrm{I_2H^-} \xrightleftharpoons[-\mathrm{H}^+\ \mathrm{K_d}]{+\mathrm{H}^+} \mathrm{I}_3 \xrightarrow{k_4'} \tag{13}$$

$$dP/dt = \frac{(k_5K_{a_1}a_H + k_5(k_6/k_6'')K_{a_1})[C(NO_2)_4][CH_T]}{a_H{}^2 + \left(\frac{(k_{-5} + k_6)}{k_6''} + K_{a_1}\right)a_H + \frac{K_{a_1}(k_{-5} + k_6)}{k_6''}} + \frac{(k_0/k_{-0})k_rK_{a_1}K_wa_H[C(NO_2)_4][CH_T]}{\left[a_H{}^3\left(1 + \frac{k_r[C(NO_2)_4]}{k_{-0}}\right) + K_{a_1}\left(1 + \frac{k_r[C(NO_2)_4]}{k_{-0}}\right)a_H{}^2 + \frac{k_{-r}K_{a_4}a_H}{k_4} + \frac{K_{a_1}k_{-r}K_{a_4}}{k_4}\right]} \tag{14a}$$

Since the terms containing $C(NO_2)_4$ in the denominator of the second part of eq 14a can be shown to be reasonably smaller than 1, we obtain eq 14b

$$\frac{k_{obsd}}{[CH_T]} = \frac{k_5K_{a_1}a_H + k_5(k_6/k_6'')K_{a_1}}{a_H{}^2 + \left(\frac{(k_{-5} + k_6)}{k_6''} + K_{a_1}\right)a_H + \frac{K_{a_1}(k_{-5} + k_6)}{k_6''}} + \frac{(k_0/k_{-0})k_rK_{a_1}K_wa_H}{a_H{}^3 + K_{a_1}a_H{}^2 + \left(\frac{k_{-r}K_{a_4}}{k_4}\right)a_H + \frac{K_{a_1}k_{-r}K_{a_4}}{k_4}} \tag{14b}$$

If the step $k_4'[I_3]$ replaces the kinetically equivalent $k_4a_H[I_2H^-]$, the same form of an equation is obtained with the following replacement

$$\frac{k_{-r}K_{a_4}}{k_4} \text{ is replaced by } \frac{k_{-r}K_{a_4}K_d}{k_4'} \tag{15}$$

Several features of this derivation are worth noting. The formation of I_2' from the free carbanion and $C(NO_2)_4$ and its acid catalyzed breakdown could account for the perturbation in the pH profile at pH 6–8 if $(k_{-5} + k_6)/k_6''$ is greater than K_{a_1}, such that a shift in apparent pK_a occurs to a lower value. The simpler case of a rate step $k_5[C^-][C(NO_2)_4]$ would give the same form of an equation as the $k_6''a_H[I_2'] + k_6[I_2']$ steps (titration curve) but would not account for the small shoulder in the pH–rate profile at pH 6–8. Either case requires that the profile level off at pH >14. No points were attempted in this region since the rates would not have been accessible because of the lability of $C(NO_2)_4$ to HO^--catalyzed hydrolysis at these pH values.

Further kinetic analyses of eq 13 indicate that the minimum requirements for the equation to fit the experimental curve above pH 8 involves I_1^{2-} and the pathway through k_r and a_H/K_{a_4}, followed by a rate step involving acid (or its kinetic equivalent). If the pathway through I_1^{2-} is considered alone,

TABLE V: Analysis of the pH–Rate Profile for the Reactions of Nitroethane with $C(NO_2)_4$.

Kinetic Expression	Experimentally Determined Constants
12	$k_2 = 1.5 \times 10^4\ \text{M}^{-1}\ \text{min}^{-1}$ $K_{a_1} = 4 \times 10^{-9}\ \text{M}$ $K_c = 2 \times 10^{-3}$ $\frac{k_{-r}K_{a_2}}{k_3'} \equiv \frac{k_{-r}}{k_3} = 8 \times 10^{-12}\ \text{M}$
14b	$k_5 = 39\ \text{M}^{-1}\ \text{min}^{-1}$ $k_6/k_6'' = 1.98 \times 10^{-8}\ \text{M}$ $\frac{k_{-5} + k_6}{k_6''} = 8.0 \times 10^{-8}\ \text{M}$ $k_r(k_0/k_{-0})K_{a_1}K_w = 4.0 \times 10^{-16}\ \text{M min}^{-1}$ $K_{a_1} = 4 \times 10^{-9}\ \text{M}$ $K_w = 1.47 \times 10^{-14}\ \text{M}^2$ Estimated $pK_{a_4} = 12 \pm 1$ $\frac{k_{-r}K_{a_H}}{k_4} \equiv \frac{k_{-r}K_{a_4}K_d}{k_4'} = 9.23 \times 10^{-23}\ \text{M}$ If $k_r \sim 10^{11}\ \text{M}^{-1}\ \text{min}^{-1}$, $k_0/k_{-0} \sim 10^{-4}\ \text{M}^{-1}$

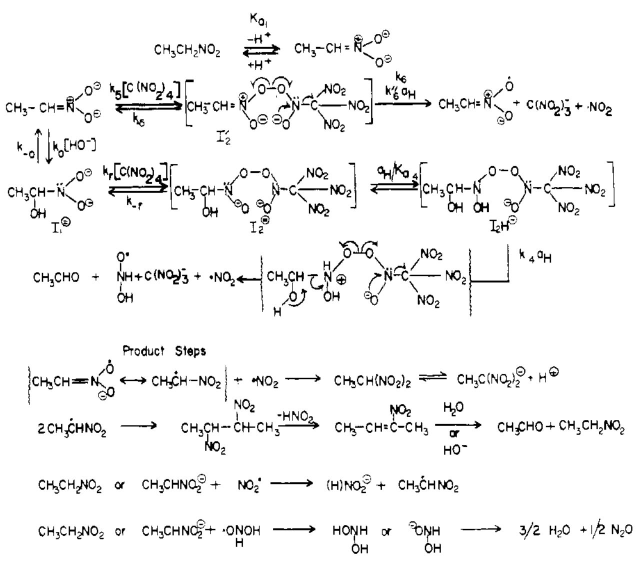
then the derivation leads to a profile of slope +2 in the low pH region changing to +1 in the region of pK_{a_1}. This pathway alone would necessitate writing a rate step from the free carbanion (as k_5) in order to assure that the profile have slope +1 at low pH. It seems most reasonable that $C(NO_2)_4$ does react with the free carbanion, perhaps in a rate step that is written in 13 so that we can also account for the perturbation of the pH–rate profile at pH 6–8. The experimentally determined constants which can be associated with parameters in this derivation are given in Table V.

A proposal for the chemical intermediates in equation 13 is presented in Scheme I. The requirement of a species as I_2^{2-}, which proceeds to $C(NO_2)_3^-$ formation *via* gain of protons is dictated by decrease in rate at pH 11–13. In order to suggest a plausible structure for this intermediate one must take into account what is known of the chemistry of nitroethane anion and the mechanisms by which $C(NO_2)_4$ has been shown to react. It is instructive to consider first the possibilities for the nature of the intermediate I_2' since discussions about this intermediate can help lead to suggestions for I_2^{2-}.

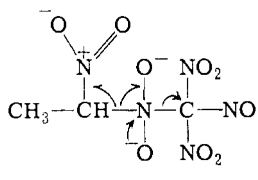
Not only are nitroalkane anions known to undergo one-electron oxidations (Russell *et al.*, 1962a,b; Russell and Janzen, 1962; Russell and Danen, 1968; Pagano and Shechter, 1970), but $C(NO_2)_4$ is also known to react as an acceptor in one-electron oxidations (Bruice *et al.*, 1968; Walters and Bruice, 1971; Henglein, 1959). These observations indicate the possibility that $C(NO_2)_4$ is oxidizing the carbanion by a one-electron transfer (eq 16) (a possibility for the pathway involving I_2' in Scheme I). This simple oxidation step can account for the observed products of this reaction but does not explain the kinetics observed at lower pH values (perturbation of the pH–rate profile at pH 6–8). However, an additional possibility for the initial step of this reaction is as follows.

$$C(NO_2)_4 + CH_3CHNO_2^- \xrightarrow{1\ e^-\ \text{transfer}} NO_2\cdot + C(NO_2)_3^- + CH_3\dot{C}HNO_2 \quad (16)$$

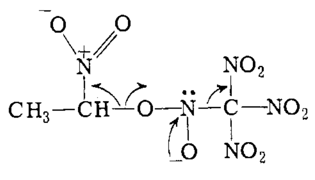
SCHEME I



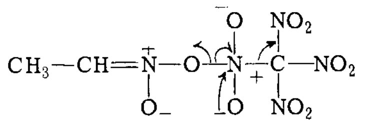
Since nitroalkane anions are known to undergo reactions at both carbon and oxygen (nitro group) centers (Kerber *et al.*, 1965), nucleophilic attack on $C(NO_2)_4$ may occur either through the oxygen or through the carbon of the nitroethane anion. Furthermore, since $C(NO_2)_4$ has been shown to undergo reactions at both the nitrogen (Walters and Bruice, 1971; Glover, 1968, 1970) and oxygen centers (Sager and Hoffsommer, 1969) of the nitro groups, there are four possible intermediates one can write involving nitroethane anion attack on $C(NO_2)_4$ (see a–d). The reactions of Scheme I have been



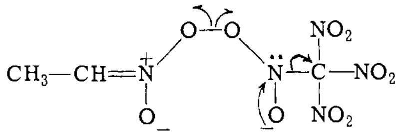
a



b



c



d

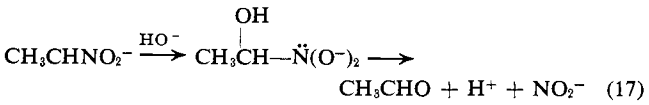
written employing step d though it is possible that the desired products may be obtained from any of the four. For step d the peroxy bond is anticipated to readily undergo homolytic cleavage and hence give the observed products. Furthermore, there should be less steric hindrance to attack of nitroalkane anion if an oxygen of $C(NO_2)_4$ is the electrophilic center. Further support for step d arises from the fact that the ambient nitroalkane anions exhibit pronounced oxygen nucleophilicity (Weisler and Helmkamp, 1945; Hass and Bender, 1949; Kornblum and Brown, 1964).

In order to account for all the electrons in the proposed intermediate I_2' in Scheme I, we have written a structure involving nucleophilic addition of the nitroethane anion to $C(NO_2)_4$. We have also indicated possible modes of breakdown of these intermediates to give radical species. However, it is possible that the attack of the anion, its oxidation by $C(NO_2)_4$, and the subsequent fragmentation of the intermediate to products occurs in the same step. This possibility is suggested from what is known about the instability of $C(NO_2)_4$ after capture of an electron (Rabani *et al.*, 1965; Bielski and Allen, 1967). The possibility that electron transfer accompanies nucleophilic attack has been proposed earlier (Bilevitch *et al.*, 1968). It is important to point out again that the acid-catalyzed breakdown of I_2' as written in Scheme I can account for the perturbation of the pH–rate profile from pH 6 to 8 if the term $(k_{-5} + k_6)/k_6''$ is greater than K_{a_1} (eq 14b). Even though the basicity of the radical species I_2' may not be expected to be great, the acid-catalyzed breakdown of this species at pH <7 may still be larger than its spontaneous breakdown. For instance the H^+ catalysis of the decomposition of nitrobenzene anion radical is found to be diffusion controlled (Metcalf and Waters, 1969).

After consideration of the possible chemical nature of I_2', let us now consider possibilities for I_2^{2-}. The kinetic derivation for eq 13 demands that a hydroxide–carbanion intermediate is being formed. No kinetically equivalent step can be fit to the pH profile above pH 8. There is ample evidence that the intermediate involving hydroxide ion and the carbanion could be the structure I_1^{2-} in Scheme I, $CH_3CH(OH)\ddot{N}(O^-)_2$. Structure I_1^{2-} has been suggested as an intermediate in the basic hydrolysis of nitroethane (Lockhard *et al.*, 1969) and its conjugate acid as an intermediate in the Nef reaction (Hawthorne, 1957; Sun and Folliard, 1971). The intermediate considered to be formed on reaction of I_2^{2-} with $C(NO_2)_4$ (I_2^{2-}) is structurally akin to that suggested for I_2' (*i.e.*, the HO^- adduct of C^- as well as C^- itself yield intermediate of similar structure in the reaction with $C(NO_2)_4$). It should be noted that in the formation of I_1^{2-}, we have employed the most expeditious path which would involve attack of HO^- on nitroethane anion. More reasonable, but kinetically equivalent steps might involve formation of the *aci*-nitroethane species, $CH_3CH{=}N^+(OH)O^-$ from the carbanion followed by HO^- attack on this species to generate $CH_3CH(OH)\ddot{N}(OH)O^-$ which could then form the dianionic species I_1^{2-} by ionization.

In Scheme I we have indicated a probable mode of decomposition of I_2^{2-} or I_2H^- which could lead to the observed products, nitrite ion and dinitroethane. Since acetaldehyde yields could not be determined accurately, we cannot state with certainty that this is the only mode of decomposition of I_2H^-. Alternate pathways of decomposition of I_2H^- involving the steps kinetically equivalent to $k_4a_H(I_2H^-)$, that is $k_4'(I_3)$, have been considered (Jewett, 1971) but will not be discussed here.

Lockhart *et al.* (1969) proposed that alkaline hydrolysis of nitroethane yields acetaldehyde and nitrite ion by way of addition of HO^- to the nitroethane anion (eq 20). A closer look at this reaction reveals that a two-electron oxidation is



$$\mathrm{CH_3CHNO_2^-} \xrightarrow{\mathrm{HO^-}} \mathrm{CH_3CH(OH)}\text{—}\ddot{\mathrm{N}}(\mathrm{O^-})_2 \longrightarrow \mathrm{CH_3CHO} + \mathrm{H^+} + \mathrm{NO_2^-} \quad (17)$$

required before nitrite ion could be formed. In our attempts to assay for acetaldehyde in the $C(NO_2)_4$ reaction with nitroethane, it was observed that the hydrolysis of nitroethane alone under our kinetic conditions leads to acetaldehyde and nitrite ion in the ratio of *ca.* 100:1. This observation is in accord with a major pathway of alkaline hydrolysis which yields acetaldehyde but not nitrite ion. Presumably N_2O is the product instead of nitrite ion, just as it is the product in the Nef reaction.

In the reaction of $C(NO_2)_4$ with nitroethane, the total nitrogen in the products (Table IV) exceeds that of the $C(NO_2)_4$ employed. This excess of N can be accounted for by the nitrite ion formed in the dimerization of nitroethane radicals suggested in Scheme I (Weinberg and Weinberg, 1968; Levy and Rose, 1948). The recombination of the nitroethane radical with a nitro radical to form dinitroethane (Titov, 1963; Titov and Smirnov, 1952) as well as the reactions of the nitro radical to form nitrite ion as in Scheme I (Titov, 1952; Gray and Yoffe, 1955; Russell and Danen, 1968) have precedent in the literature. Furthermore, the less than quantitative nitration yields for the nitroethane reaction with $C(NO_2)_4$ are also in accord with other studies in the nitration of nitroalkanes with $C(NO_2)_4$ (Noble *et al.*, 1964). The product dependence on $C(NO_2)_4$ may be rationalized by the scavenging of radical species such as nitroethane radical by $C(NO_2)_4$ after the rate-determining steps of the reaction (Rabani *et al.*, 1965; Henglein *et al.*, 1959).

The third kinetic scheme for the reaction of $C(NO_2)_4$ with nitroethane that was found to give a derivation fitting the experimental pH–rate profile is the following

$$\begin{tikzcd}
\mathrm{C(NO_2)_4} + \mathrm{OH^-} \arrow[r, shift left, "K_0"] \arrow[d, shift left, "k_5{[\mathrm{C^-}]}"] & \mathrm{I_1} \arrow[l, shift left] \arrow[r, shift left, "k_r{[\mathrm{C^-}]}"] & \mathrm{I_2} \arrow[l, shift left, "k_{-r}"] \arrow[r, shift left, "\substack{K_d\\ +\mathrm{H^+}}"] & \mathrm{I_3} \arrow[l, shift left, "-\mathrm{H^+}"] \arrow[r, "k_3{[\mathrm{H^+}]}"] & {} \\
\mathrm{I_2}' \arrow[u, shift left, "k_{-5}"] \arrow[r, "k_6{[\mathrm{H^+}]}"] & {}
\end{tikzcd} \quad (18)$$

The possibilities for the chemical nature of the intermediates in eq 18 are again complex. We will not discuss this scheme in this paper but a discussion is presented elsewhere (Jewett, 1971).

Under conditions in which the other carbon acids (malononitrile, acetone, dimethyl sulfone, and acetonitrile) were studied ($a_H >> K_a$), no complexities were observed in the pH dependence of the reaction with $C(NO_2)_4$. We can write the following equation to explain these reactions (eq 19). Solving

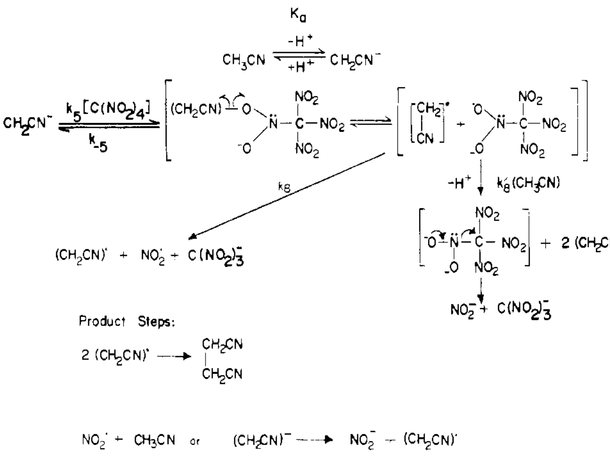
$$\begin{tikzcd}
 & & & {} \\
\mathrm{CH} \arrow[r, shift left, "\substack{K_a\\ -\mathrm{H^+}}"] & \mathrm{C^-} \arrow[l, shift left, "+\mathrm{H^+}"] \arrow[r, shift left, "K_7{[\mathrm{C(NO_2)_4}]}"] & \mathrm{I_2}'' \arrow[l, shift left] \arrow[ur, "k_8"] \arrow[dr, "k_8'{[\mathrm{CH}]}"] & \\
 & & & {}
\end{tikzcd} \quad (19)$$

the steady-state equation for I_2'' and using $[CH]_T = [CH]$, since $a_H > K_a$, we can write eq 20. This equation contains rate

$$k_{obsd} = \frac{k_7k_8[CH]_T(K_a/K_w)[HO^-] + k_7k_8'[CH]_T^2(K_a/K_w)[HO^-]}{k_{-7} + k_8 + k_8'[CH]_T + k_7[C(NO_2)_4](k_8 + k_8'[CH]_T)} \quad (20)$$

terms analogous to those proposed for the reaction of nitroethane anion with $C(NO_2)_4$ (eq 13). Not only for simplicity have we left out an acid-catalyzed step from I_2'', but also because these reactions were investigated over a limited range of pH and it, therefore, cannot be definitely stated whether or not such an acid-catalyzed step is present. In order that the equation resemble the experimentally determined rate expres-

SCHEME II



sion (eq 6) (ignoring the hydroxide rate), the following must hold

$$\{k_7[C(NO_2)_4](k_8 + k_8'[CH]_T)\} < \{k_{-7} + k_8 + k_8'[CH]_T\} \qquad (21a)$$

$$\{k_{-7} + k_8\} > \{k_8'[CH]_T\} \qquad (21b)$$

We now have the expressions in eq 22 where we have equated rate constants from 8a and 8b with the constants of eq 20.

$$\frac{k_7k_8}{k_{-7} + k_8} = k_1 \text{ and } \frac{k_7k_8'}{k_{-7} + k_8} = k_2 \qquad (22)$$

For malononitrile as well as nitroethane no $k_8'[CH]_T$ term was observed under the conditions employed; however, it is possible that these carbon acids could exhibit a second-order term at higher $[CH]_T$ concentrations. Without going into laborious detail, we can state that the ionization (K_a/a_H) of eq 19 can be treated as if the equilibration of the carbon acid is complete. Furthermore, additional analysis of eq 20 indicates that the conditions demanded in 21b are reasonable (Jewett, 1971). We suggest that the nature of the intermediate I_2'' in the reactions of the other carbon acids arises from a nucleophilic attack by the carbanion followed by an oxidation reaction similar to that proposed for the reaction of $C(NO_2)_4$ with nitroethane anion. Furthermore, a reasonable explanation for the second-order term in carbon acid is that the intermediate I_2'' in eq 19 is reacting with a neutral molecule of carbon acid in a H-abstraction step. Scheme II illustrates a plausible chemical mechanism for the reactions of $C(NO_2)_4$ with acetonitrile, dimethyl sulfone, and acetone. The reaction of $C(NO_2)_4$ with malononitrile could be written without the H-abstraction step. The lower $C(NO_2)_3^-$ yields in the reactions of acetonitrile and dimethyl sulfone might be explained by the subsequent reaction of the organic radical (ex.CH_2CN^-) with $C(NO_2)_3^-$. Based on previous studies of radical stabilities as a function of substituent groups (Russell, 1956; Timberlake and Hodges, 1970), we would expect these radicals to be more unstable and probably more reactive than the radicals generated from pseudo acids of lower pK_a's. The Brønsted plot of Figure 8 suggests that the reactivity of the carbanions toward $C(NO_2)_4$ increases with increasing pK_a. This is in accord with a nucleophilic mechanism (Leffler and Grunwald, 1963).

We have written the decomposition of the intermediate in Scheme II in two steps. In this manner we are able to account for the rate term involving a second-order dependence on the carbon acid. We should like to point out that the decomposition of the intermediates, I_2' and I_2H^- in Scheme I, could also occur in two steps, *via* the $C(NO_2)_4^{\cdot -}$ radical as indicated in Scheme II. Though this radical species, $C(NO_2)_4{\cdot}^-$, has never been observed in esr studies (Lagercrantz, 1964a,b), it is possible that it is a reactive intermediate along the reaction pathway.

The formation of trinitromethane dianion radical [$C(NO_2)_3^{\cdot -2}$] in the reactions of $C(NO_2)_4$ with carbon acids in concentrated basic solution can be explained not only by the secondary oxidation of the carbanion-derived radical by $C(NO_2)_3^-$ but also by the oxidation of the carbanion itself by $C(NO_2)_3^-$. These suggestions are in accord with those proposed by Lagercrantz (1964a,b) for the formation of $C(NO_2)^{\cdot 2-}$ in the reactions of $C(NO_2)_4$ with 1,3-dicarbonyl compounds and unsaturated compounds in strongly alkaline media. Our findings (esr) of no detectable dianion radical formation under kinetic conditions is in accord with the work of Lagercrantz and Torssell (1968) who found that no detectable concentration of the radical was seen in systems containing an excess of the alkaline water solution.

The formation of the dianion radical from the intermediates proposed in Schemes I and II could also be possible. This apparently does not occur extensively under our kinetic conditions since the total nitrite ion yields can be accounted for by the reduction of a nitro radical, derived from $C(NO_2)_4$, that does not recombine with a nitroethane radical to form dinitroethane.

The reaction of nitroethane anion with $C(NO_2)_4$ in the presence of oxygen can be rationalized by the following Scheme III. The product steps in this scheme require a total yield of nitrite ion of 80%. The observed yield is 60%. This discrepancy can be explained by the reaction of some of the $C(NO_2)_4$ with nitroethane radical to give products which do not lead to nitrite ion. The constants in the rate equation in Scheme III can be related to the experimentally determined constants (see Results section) in the following manner

$$\frac{k_{-e}}{k_e k_f[O_2]} = S = 9.4 \times 10^{-5}\ \text{M min} \qquad (23a)$$

$$\frac{1}{k_e[O_2]} = I = 2.26\ \text{min} \qquad (23b)$$

By using the known value for $k_f = 1.1 \times 10^{11}\ \text{M}^{-1}\ \text{min}^{-1}$ (Rabani *et al.*, 1965) and assuming a value for the oxygen concentration in water at 30°, $[O_2] = 1.1 \times 10^{-3}$ M (Handbook of Chemistry and Physics, 1961), we can calculate k_e and $k_{-e}:k_e = 400\ \text{M}^{-1}\ \text{min}^{-1}$, $k_{-e} = 4.6 \times 10^6\ \text{min}^{-1}$, and $k_e/k_{-e} = 8.8 \times 10^{-5}\ \text{M}^{-1}$.

The oxidation of carbanions by molecular oxygen has been previously suggested to occurt hrough intermediate radicals such as $C\cdot$ and $O_2^{\cdot -}$ (Russell *et al.*, 1962a,b), hence our suggestion of such species as intermediates is not unique. Furthermore $C(NO_2)_4$ has been shown to react with $O_2^{\cdot -}$ (Rabani *et al.*, 1965). The zero order rate of $C(NO_2)_3^-$ formation in oxygenated solutions of nitroethane anion is derived from the reaction of $C(NO_2)_4$ with $O_2^{\cdot -}$ can compete with the reverse step of transfer of an electron from $O_2^{\cdot -}$ back to the carbanion.

The detail in which we have discussed the reactions of $C(NO_2)_4$ with pseudo acids has indicated several factors.

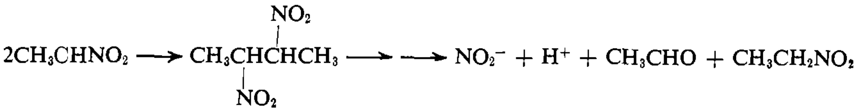
Scheme III

$$\mathrm{CH_3CHNO_2^-} + \mathrm{O_2} \underset{k_{-\mathrm{e}}}{\overset{k_\mathrm{e}}{\rightleftharpoons}} \{\mathrm{CH_3CHNO_2\cdot} + \mathrm{O_2\cdot^-}\} \xrightarrow{k_\mathrm{f}\mathrm{C(NO_2)_4}} \mathrm{CH_3CHNO_2\cdot} + \mathrm{C(NO_2)_3^-} + \mathrm{O_2} + \cdot\mathrm{NO_2}$$

Product Steps

$$\mathrm{CH_3\cdot}\ \ \mathrm{HNO_2} + \cdot\mathrm{NO_2} \longrightarrow \mathrm{CH_3CH(NO_2)_2} \rightleftharpoons \mathrm{CH_3C(NO_2)_2^-} + \mathrm{H^+}$$

$$\mathrm{NO_2\cdot} + \mathrm{CH_3CHNO_2^-} \longrightarrow \mathrm{NO_2^-} + \mathrm{CH_3CHNO_2\cdot}$$



$$2\mathrm{CH_3CHNO_2} \longrightarrow \mathrm{CH_3CH(NO_2)CH(NO_2)CH_3} \longrightarrow \longrightarrow \mathrm{NO_2^-} + \mathrm{H^+} + \mathrm{CH_3CHO} + \mathrm{CH_3CH_2NO_2}$$

$$\mathrm{d}P/\mathrm{d}t = k_0 = \frac{k_\mathrm{e}k_\mathrm{f}[\mathrm{C(NO_2)_4}][\mathrm{C^-}]_\mathrm{T}[\mathrm{O_2}]}{k_{-\mathrm{e}} + k_\mathrm{f}[\mathrm{C(NO_2)_4}]} = \frac{[\mathrm{C(NO_2)_4}][\mathrm{C^-}]}{\frac{k_{-\mathrm{e}}}{k_\mathrm{e}k_\mathrm{f}[\mathrm{O_2}]} + \frac{[\mathrm{C(NO_2)_4}]}{k_\mathrm{e}[\mathrm{O_2}]}}$$

Our results for the reaction of $C(NO_2)_4$ with nitroethane are not in accord with the initial studies of this reaction. Our studies of the reactions of $C(NO_2)_4$ have made it evident that $C(NO_2)_4$ is capable of reacting by very complex radical mechanisms. *It is perhaps appropriate to caution again concerning the use of $C(NO_2)_4$ to modify proteins, whether to modify tyrosines or to trap carbanions. The reagent has been shown to cross-link tyrosyl residues and it may well initiate radical coupling between substrate and enzyme through carbanion intermediates.*

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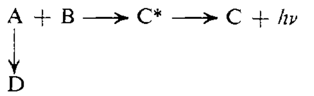
Bacterial Bioluminescence. Quantum Yields and Stoichiometry of the Reactants Reduced Flavin Mononucleotide, Dodecanal, and Oxygen, and of a Product Hydrogen Peroxide†

John Lee

ABSTRACT: Conditions are described for obtaining the maximum bioluminescence quantum yields for each component of the *in vitro* bacterial bioluminescence reaction. With optimum concentration of *Photobacterium fischeri* luciferase the quantum yields are: for reduced flavin mononucleotide 0.048, dodecanal 0.1, oxygen 0.048, and for an identified product hydrogen peroxide 0.12. Under optimum conditions the reaction of reduced flavin and luciferase is probably the sole chemical pathway and its overall stoichiometry can be inferred from the quantum yield ratios as: $2FMNH_2 + 2O_2 + RCHO \rightarrow 2FMN + H_2O + H_2O_2 +$ product. The quantum yield ratio 2:1 of dodecanal to reduced flavin mononucleotide is strong evidence for aldehyde utilization in the reaction. Present evidence is unable to distinguish whether this overall reaction represents a single pathway or is the sum of two independent enzyme reactions which proceed with equal velocities. The maximum flavin quantum yield is independent of oxygen concentration and of the purity of the luciferase preparation used (60% to crystalline). Both the flavin and dodecanal maximum quantum yields are reduced at very high luciferase concentration and both are unaffected by the presence of catalase which excludes free peroxide as a reaction intermediate.

A chemiluminescence reaction may be represented by a molecule A reacting with B to give the excited state of the product, C*:



$$\begin{array}{l} A + B \longrightarrow C^* \longrightarrow C + h\nu \\ \downarrow \\ D \end{array} \qquad (1)$$

Competing with this light path may be a dark reaction yielding a product D. The observed chemiluminescence quantum yield with respect to A, $Q_c(A)$[1] will depend on the number of A molecules which disappear by the light over the dark path.

If the concentration of B is sufficiently high the dark reaction rate will be negligible in comparison to the light reaction and $Q_c(A)$ will approach a maximum value. Under this condition it can be predicted for reaction 1 that $Q_c(A) = Q_c(B) = Q_c(C)$.

As a corollary the relative maximum Q_c of each component in a reaction will be a measure of the stoichiometry of that component. This stoichiometry is unity for each component in reaction 1 but if instead two molecules of A were consumed for each C produced then $Q_c(A) = 0.5Q_c(B)$.

Chemiluminescence and bioluminescence reactions usually take place under vigorous oxidizing conditions and the presence of dark reactions cannot be neglected. In a study of the luminol chemiluminescence, reaction conditions were found to fall into two groups according to the magnitude of Q_c (luminol) (Lee and Seliger, 1972). Aqueous hydrogen peroxide oxidation (pH > 11) or oxidation under basic aprotic conditions led to maximum values of Q_c, around 0.0125. Under these conditions various factors such as temperature, solvent polarity, or reactant concentration were without effect on Q_c, from which it was inferred that there were no competing dark chemical reactions. These factors strongly influence Q_c under other oxidizing conditions such as ferricyanide, where Q_c was much lower and competing chemical reactions were suggested. The Methylene Blue photosensitized chemi-

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[1] The quantum yield of chemiluminescence or bioluminescence with respect to a reaction component is the total number of photons emitted divided by the total change as a result of the reaction in the number of molecules of that component.