peratures as high as 54°. An increase in the vapor pressure of the sample prevented nmr measurements above this temperature. A characteristic AB pattern is also observed for α-chloropinacolone and $BBr_3$. Analysis of recovered ketone showed it to be a mixture of α-chloropinacolone (56%) and α-bromopinacolone (13%), which results from slow exchange of the α-chloro substituent. Only α-bromopinacolone (89%) is recovered from the α-bromopinacolone–boron trichloride complex. This is probably due to the fact that chloride is a better leaving group than bromide and $BBr_3$ is a stronger acceptor than $BCl_3$. The chemical shifts of the nonequivalent protons of the α-chloropinacolone–boron tribromide complex parallel those of the α-bromopinacolone–boron tribromide complex, signifying a dominating influence of the boron halide moiety. Chemical shifts of the α-bromopinacolone– and α-chloropinacolone–boron trichloride complexes are consistent with this postulate.

The spectra of α-bromopinacolone plus $BCl_3$ are unique in that they contain an additional peak in the vicinity of the *t*-butyl (τ 8.45) and methylene (τ 5.13) regions at temperatures below 10°. The additional peaks merge with the methylene quartet and *t*-butyl resonance as the temperature is raised. This occurs as the quartet is coalescing. This is characteristic of three species in equilibrium. A 2:1 complex has been postulated[9] to account for additional peaks found in the nmr spectrum of $BCl_3$ complexes of N,N-dimethylformamide.

Nonequivalence of methylene protons was not observed at temperatures as low as −30° when $BF_3$ was used as the complexing agent. The absence of a magnetically asymmetric center in the $BF_3$ complexes or rapid interconversion of equilibrating species at −30° can account for the results (eq 2). This is consistent

$$(CH_3)_3CC(=O)CH_2Y + BF_3 \rightleftharpoons (CH_3)_3CC(=O{\rightarrow}BF_3)-CH_2Y \qquad (2)$$

with the relative acceptor ability of boron halides,[10,11] $BBr_3 > BCl_3 > BF_3$, and results obtained by Coyle and Stone[12] on $BH_3$ and $BF_3$ complexes of diethyl sulfide. One would expect the tendency for $BF_3$ to ionize to be smaller than $BCl_3$ or $BBr_3$ because of the stronger boron–fluorine bond and smaller steric requirements of $BF_3$.

Addition of Lewis acids to α-halo ketones is expected to be more facile than to simple ketones. The lower basicity of the former makes coordination with boron halides less favorable. Unfortunately, attempts to extend this study to complexes of 3-pentanone with $BBr_3$ were unsuccessful because of rapid polymerization of the solution. A detailed discussion of the energies of activation of several processes described herein will be the subject of a separate publication.

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(9) E. Gore, D. Blears and S. Danyluk, *Can. J. Chem.*, 43, 2135 (1965).
(10) H. Brown and R. Holmes, *J. Am. Chem. Soc.*, 78, 2173 (1956).
(11) C. Bax, A. Katritzky, and L. Sutton, *J. Chem. Soc.*, 1254 (1958).
(12) T. Coyle and F. Stone, *J. Am. Chem. Soc.*, 83, 4138 (1961).

**Fred Kaplan, David Weisleder**[13]
*Department of Chemistry, University of Cincinnati*
*Cincinnati, Ohio 45221*
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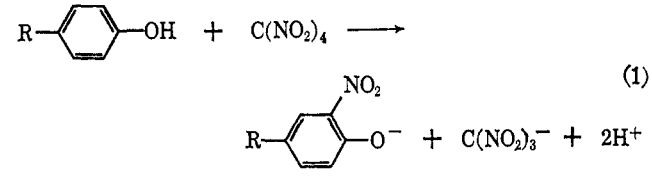
## Tetranitromethane. A Reagent for the Nitration of Tyrosine and Tyrosyl Residues of Proteins[1]

*Sir:*

The suitability of reagents for chemical modification of enzymes depends not only on their specificity toward a particular functional group but also on the reaction conditions. Available procedures for nitration,[2] while effective with stable aromatic compounds, employ conditions too severe to be applicable to biological macromolecules.

Attention was directed to the potential usefulness of tetranitromethane (TNM).[3] While this reagent had been employed to modify the biological function of proteins,[4] its chemical specificity was not examined. Since earlier studies had indicated that TNM could serve to nitrate phenols,[5] our initial efforts were aimed at the reaction with tyrosine and its derivatives as here reported.

Typical conditions involve the addition of 5 μl of TNM (42 μmoles) to 3 ml of a $10^{-4}$ *M* solution of tyrosine, buffered at pH 8.0 with 0.05 *M* tris(hydroxymethyl)aminomethane hydrochloride (Tris-Cl) at 20°. The products of the reaction are trinitromethane (nitroform), protons, and a nitrated derivative of tyrosine having an absorption maximum of 428 mμ. For kinetic studies, the high molar absorptivity of the nitroformate anion ($\epsilon_{350}$ 14,400)[6] provides a most sensitive and convenient parameter of this reaction (Figure 1). At pH 8, nitration displaces two protons and produces 1 mole of nitroformate/mole of tyrosine, suggesting the formation of 3-nitrotyrosine as in eq 1.



(1)

In support of this interpretation, chromatographic analysis of the nitration reaction mixture, using the Spinco Model 120B amino acid analyzer according to the procedure of Spackman, *et al.*,[7] revealed the presence of a substance eluting in the position corresponding to authentic 3-nitrotyrosine, *i.e.*, 228 ml relative to the elution of phenylalanine at 213 ml. Material eluting

(1) This work was supported by Grants-in-Aid HE-07297 from the National Institutes of Health of the Department of Health, Education and Welfare.
(2) J. D. Roberts and M. C. Caserio, "Basic Principles of Organic Chemistry," W. A. Benjamin, Inc., New York, N. Y., 1964, p 786.
(3) R. M. Herriott, *Advan. Protein Chem.*, 3, 170 (1947).
(4) A. Wormall, *J. Exptl. Med.*, 51, 295 (1930); L. Ehrenberg, I. Fischer, and N. Lofgren, *Svensk. Kem. Tidskr.*, 57, 303 (1945); L. Ehrenberg, I. Fischer, and N. Lofgren, *Nature*, 157, 730 (1946); T. Astrup, *Acta Chem. Scand.*, 1, 744 (1948).
(5) E. Schmidt and H. Fischer, *Ber.* 53, 1529 (1920).
(6) D. J. Glover and S. G. Landsman, *Anal. Chem.*, 36, 1690 (1964).
(7) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, 30, 1190 (1958).

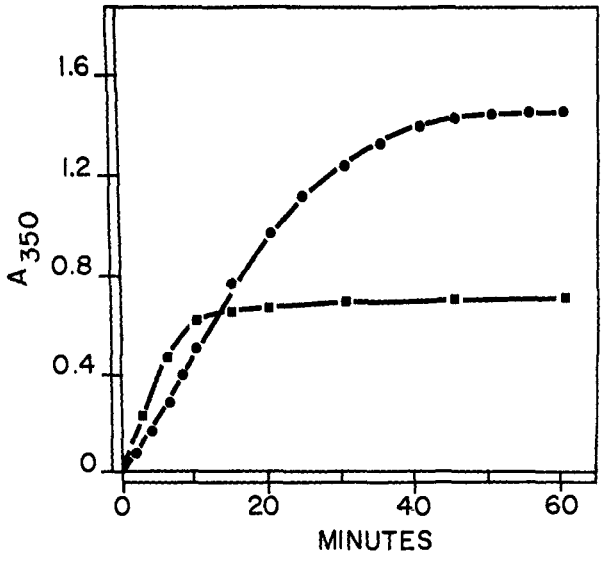



Figure 1. Increase in absorbance at 350 mμ on nitration of N-acetyltyrosine (●) and glutathione (■), both $10^{-4}$ *M*; TNM, 5 μl (42 μmoles)/3 ml; 0.05 *M* Tris-Cl, pH 8.0, 20°. The data for N-acetyltyrosine are corrected for the absorbance due to N-acetyl-3-nitrotyrosine.

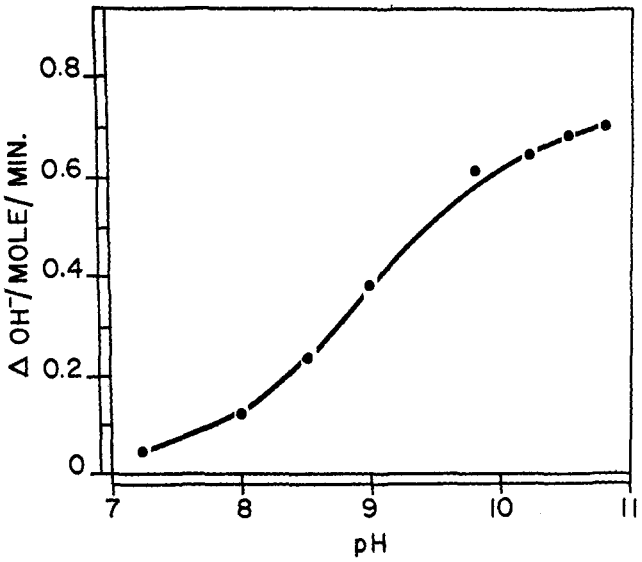



Figure 2. pH dependence of the rate of nitration of N-acetyltyrosine. Rates were calculated from the initial linear slope of the titration curves obtained when nitrations were performed on the pH-Stat.

in the position of 3,5-dinitrotyrosine, *i.e.*, at 156 ml, was not detected.

The rate of nitration of tyrosine increases as a function of increasing pH (Figure 2) with an inflection point near the p*K* of the phenolic hydroxyl group and suggesting an ionic[8] rather than a free radical mechanism.[9] Optimal conditions are between pH 8 and 9. At more alkaline pH, TNM breaks down spontaneously, thereby interfering with the kinetic analysis. Significant nitration of tyrosine does not occur below pH 7.0.

The specificity of TNM has been examined by nitrating a mixture of 17 amino acids[10] under the conditions described above. Amino acid analysis revealed a quantitative conversion of tyrosine to 3-nitrotyrosine. Other components of the mixture were not modified. As shown separately, tryptophan and tryptophanyl peptides are also unaffected by treatment with TNM.

In addition to tyrosine, so far only cysteinyl residues were found to react with TNM, as shown with glutathione both by spectrophotometry (Figure 1) and by titration on the pH-Stat. The product has been identified by paper chromatography as oxidized glutathione. Between pH 6 and 9 the rate of this reaction with cysteinyl residues is independent of pH, permitting its differentiation from nitration of tyrosyl residues.

Nitration with TNM offers many advantages over procedures previously reported.[11] The conditions here employed are gentle. Both the stability and specificity of the reagent allow modifications with low molar excesses of TNM, as shown by studies of several proteins and enzymes to be described elsewhere. Nitration extends the number of available procedures for the chemical modification of tyrosyl residues,[12] providing greater flexibility for the study of their role in the biological function of proteins. Further, reduction of the nitro to an amino group may lead to yet additional derivatives. Nitration should also facilitate identification of "tyrosyl" enzymes,[13] *i.e.*, those in which tyrosyl groups are involved in enzymatic activity. Here the yellow color of nitrotyrosine ($\lambda_{max}$ 428 mμ (ε 3800)) should enable the ready isolation of active center peptides. In this regard it is important that nitrotyrosine is stable under conventional conditions for acid hydrolysis of proteins and, hence, the number of nitrotyrosyl residues of proteins can be determined directly. Since nitrotyrosine is an ionizable chromophore it can be employed to probe changes in the microscopic environment of active center residues by perturbation spectra, optical rotatory dispersion, or similar methods. These and other considerations are currently under investigation in this laboratory.

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(8) J. M. Patterson, *J. Org. Chem.*, **20**, 1277 (1955).
(9) C. Lagercrantz, *Acta Chem. Scand.*, **18**, 382 (1964).
(10) Spinco amino acid calibration mixture No. 1, Beckman Instruments, Spinco Division, Palo Alto, Calif.
(11) O. Kratky, A. Sekora, H. Zahn, and E. R. Fritze, *Z. Naturforsch.*, **106**, 68 (1955).
(12) J. F. Riordan and B. L. Vallee, *Biochemistry*, **2**, 1460 (1963); H. Fraenkel-Conrat, *Enzymes*, **1**, 589 (1960).
(13) J. F. Riordan, W. E. C. Wacker, and B. L. Vallee, *Biochemistry*, **4**, 1758 (1965).
(14) On leave of absence from the Department of Biophysics, Weizmann Institute of Science, Rehovoth, Israel.

**James F. Riordan, Mordechai Sokolovsky,[14] Bert L. Vallee**
*Biophysics Research Laboratory*
*Department of Biological Chemistry*
*Harvard Medical School and Division of Medical Biology*
*Peter Bent Brigham Hospital, Boston, Massachusetts*
*Received July 18, 1966*

## The Synthesis of Olefinic Cyanides from Olefins by Means of Palladium(II) Cyanide

*Sir:*

Since the industrial production of carbonyl compounds by oxidation of olefins with palladium chloride was established,[1] a large number of studies on the syntheses of vinyl compounds by the reaction of olefin–

(1) J. Smidt, *Angew. Chem.*, 74, 93 (1962).