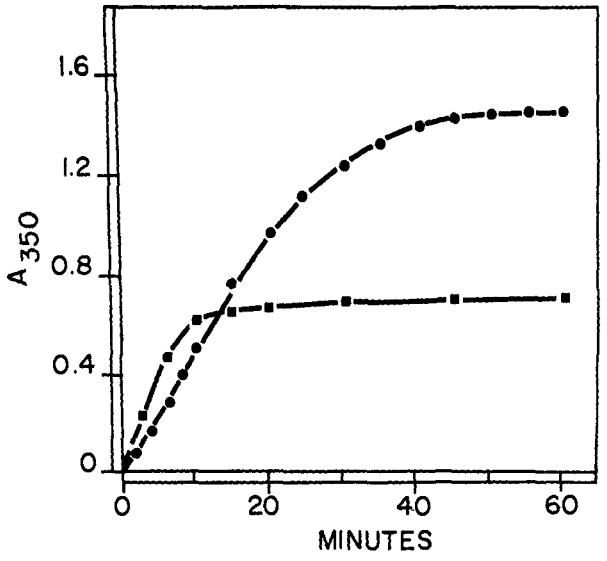


Figure 1. Increase in absorbance at 350 mμ on nitration of N-acetyltyrosine (●) and glutathione (■), both $10^{-4}$ *M*; TNM, 5 μl (42 μmoles)/3 ml; 0.05 *M* Tris-Cl, pH 8.0, 20°. The data for N-acetyltyrosine are corrected for the absorbance due to N-acetyl-3-nitrotyrosine.

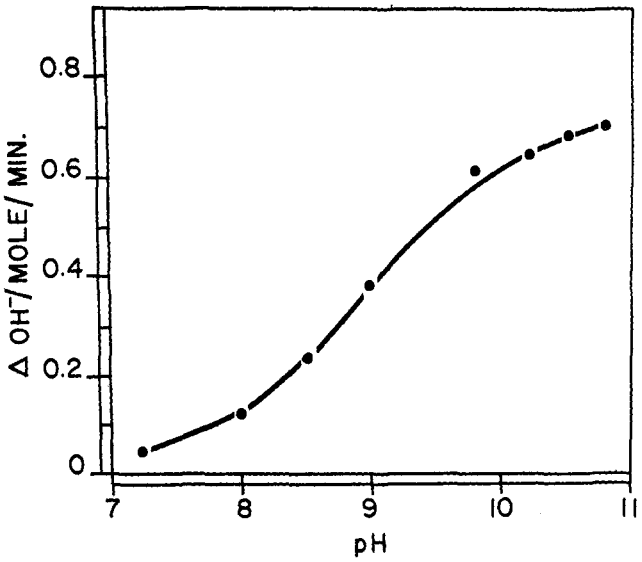


Figure 2. pH dependence of the rate of nitration of N-acetyltyrosine. Rates were calculated from the initial linear slope of the titration curves obtained when nitrations were performed on the pH-Stat.

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.

**Acknowledgment.** The authors wish to thank Mary Buchakjian and Suzanne Juhola for excellent technical assistance.

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## 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).

palladium chloride complexes with a variety of nucleophiles have been reported.[2] However, the cyanation of olefins has been unsuccessful as yet.

We wish to make a preliminary report on the first synthesis of olefinic cyanides from olefins by means of some metal cyanides.

$$CH_2{=}CHR \xrightarrow[\text{solvent}]{M(CN)_2} CH_2{=}CR{-}CN$$

$$R = H, CH_3$$

In the present study, palladium(II) cyanide, nickel(II) cyanide, and copper(I) cyanide were selected as metal cyanides because of the ability of the metal to $\pi$ complex with the olefin.

The reaction of ethylene was carried out in an autoclave under 55 kg/cm$^2$ pressure of ethylene in the presence of the metal cyanide (0.03 mole) and solvent (30 ml) at 150° for 5 hr. The results of cyanation with palladium(II) cyanide in several polar solvents are listed in Table I.

**Table I.** Cyanation of Ethylene with $Pd(CN)_2$

| Solvent | Yield,[a] % Acrylonitrile | Propionitrile |
|---|---|---|
| $C_6H_5CN$ | 50.8 | 6.9 |
| $CH_3CN$ | 17.2 | 2.7 |
| DMF | 7.7 | 15.6 |
| DMSO | 12.6 | 2.4 |

[a] Based on $Pd(CN)_2$ used.

In all cases with the exception of the reaction of copper(I) cyanide, small amounts of polyethylene ranging from gaseous oligomers to crystalline polymers of high molecular weight were always found in addition to the above cyanation products.

Only palladium(II) cyanide among the three metal cyanides was an efficient reagent for the cyanation. Probably this is due to the greater ease with which it forms $\pi$ complexes with olefins. In the reaction, palladium(II) cyanide is reduced to metallic palladium, and the formation of hydrogen cyanide is observed.

When nonpolar solvents such as cyclohexane and benzene were used, the cyanation was completely repressed, while the polymerization of ethylene took place predominantly to give a large quantity of high polymers.[3] The solvent plays a key role in the cyanation; a more polar solvent accelerates the cyanation. Moreover, in the case of a polar solvent the addition of a highly polarizable nucleophile, such as triphenylphosphine, resulted in a considerable suppression of both cyanation and polymerization of ethylene because such a nucleophile, being a good $\pi$-accepting ligand, may block coordination by the ethylene. In addition, with some cyanide complexes such as potassium tetracyanopalladate(II) and potassium tetracyanonickelate(II) in place of metal cyanide, the oligomerization of ethylene occurred to some extent, but the cyanation did not take place at all, no matter what solvent was used.

(2) (a) I. I. Moiseev, M. N. Vargaftik, and Ya. K. Syrkin, *Dokl. Akad. Nauk SSSR*, **133**, 377 (1960); (b) E. W. Stern, *Proc. Chem. Soc.*, 111 (1963); (c) E. W. Stern and M. L. Spector, *ibid.*, 370 (1961); (d) J. Tsuji, M. Morikawa, and J. Kiji, *J. Am. Chem. Soc.*, **86**, 4851 (1964).

(3) A. U. Blackham, U. S. Patent 3,194,800 (1965).

A brief survey of the applicability of palladium(II) cyanide to other olefins was made. In the reaction of propylene (0.5 mole) in the presence of palladium(II) cyanide (0.02 mole) and a polar solvent (30 ml) for 5 hr, methacrylonitrile(I), 3-butenenitrile (II), crotononitrile (III), isobutyronitrile (IV), and butyronitrile (V) were obtained, along with a trace of oligomers, consisting mainly of hexene isomers. The results of the cyanation of propylene are listed in Table II.

**Table II.** Cyanation of Propylene with $Pd(CN)_2$

| Solvent | Temp, °C | Yield,[a] % I | II | III | IV | V |
|---|---|---|---|---|---|---|
| $C_6H_5CN$ | 150 | 3.6 | 0.1 | 2.5 | 0.7 | 0.5 |
| $C_6H_5CN$ | 210 | 20.5 | 4.5 | Trace | 6.4 | None |
| DMF | 210 | 20.0 | 12.0 | None | 2.5 | None |
| $C_2H_5CN$ | 210 | 22.2 | 4.5 | 7.7 | None | 2.3 |

[a] Based on $Pd(CN)_2$ used.

As in the case of ethylene, the reaction of propylene in nonpolar solvents such as cyclohexane and benzene led almost exclusively to the formation of high polymers (mp 108–110°). In addition, in the reaction of cyclohexene (0.1 mole) in the presence of palladium(II) cyanide (0.03 mole) and acetonitrile (30 ml) at 130° for 5 hr, 2-cyclohexene-1-carbonitrile (9.43%) and cyclohexane carbonitrile (5.59%) were obtained, along with cyclohexane (7.13%) and benzene (5.71%). In the absence of solvent in the above reaction, the cyanation was somewhat suppressed, while the yields of cyclohexane and benzene were increased extraordinarily to 478 and 212%, respectively. In view of these facts, it may be considered that the cyanation of cyclohexene proceeds through a $\pi$-allyl complex.

The mechanism of this unique cyanation is under investigation and detailed description of this and further work will be reported shortly.

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## The Stereochemistry of the Carbonyl Insertion Reaction

*Sir:*

Acetylmanganese pentacarbonyl is formed when carbon monoxide reacts with methylmanganese pentacarbonyl. This reaction is a carbonyl insertion reaction and is a special case of the general reaction: $CH_3Mn(CO)_5 + L \rightarrow CH_3COMn(CO)_4L$, where L is a neutral or charged nucleophile. This generalization is supported by reports that $CH_3Mn(CO)_5$ reacts with amines[1,2] and triphenylphosphine,[2,3] -arsine,[3] or -stibine[3] to form complexes of the type $CH_3COMn(CO)_4L$, and with lithium iodide[4] to give $Li^+[CH_3COMn(CO)_4I]^-$.

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(2) R. J. Mawby, F. Basolo, and R. G. Pearson, *ibid.*, **86**, 3994 (1964).

(3) W. D. Bannister, M. Green, and R. N. Haszeldine, *Chem. Commun.*, 55 (1965).

(4) F. Calderazzo and K. Noak, *J. Organometal. Chem.* (Amsterdam), **4**, 250 (1965).