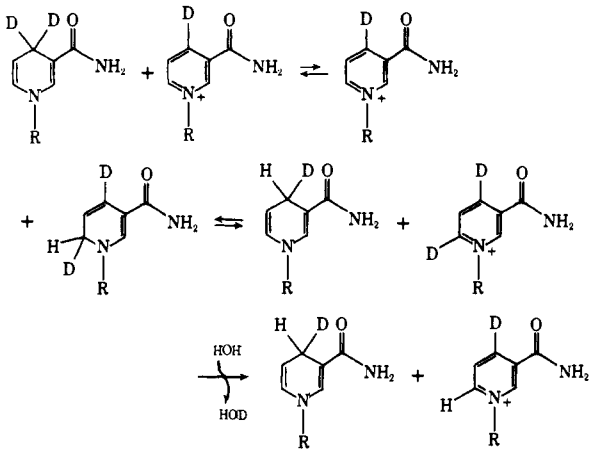

Experiments are in progress to test this hypothesis.

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(11) This, of course, excludes those reactions where the product undergoes facile proton exchange with the solvent.

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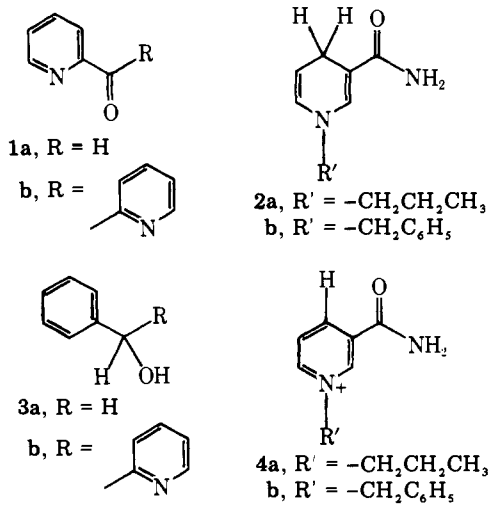
Models for NADH Coenzymes. Reactions of 2-Carboxyl-Substituted Pyridines with *N*-Alkyl-1,4-dihydronicotinamides

Sir:

The reductions of carbonyls by *N*-alkyl-1,4-dihydronicotinamides have been extensively studied[1-3] as model reactions for the enzyme-catalyzed reduction of a carbonyl by NADH. Since we previously observed that the rate of reduction of trifluoroacetophenone by *N*-propyl-1,4-dihydronicotinamide[4] was markedly accelerated by the inclusion of water in the reaction mixture,[5] we decided to study the reactions of 2-carboxyl-substituted pyridines (**1**) with *N*-alkyldihydronicotinamides (**2**) in aqueous solution as model reactions for NADH



dependent redox processes. In this communication, we report that the reaction of 2-carboxyl-substituted pyridines with *N*-alkyldihydronicotinamides leads to the formation of three products: the expected redox products, the primary hydration product of the dihydronicotinamides, and a 1:1 adduct of the carbonyl and dihydronicotinamide to which one molecule of water has been added. The course of the reaction is dependent on the steric hinderance between reactants and the reaction conditions. The implications of the results on the reported disparity between the measured kinetic and partitioning isotope effect in several model systems are discussed.

Pyridine-2-carboxaldehyde (**1a**) reacts rapidly with *N*-propyldihydronicotinamide (**2a**) in aqueous solution. The kinetics of the reaction were studied spectrophotometrically in 0.1 M aqueous (pH 9.9) carbonate buffered solvent or solvent mixtures maintained at constant temperature in a thermostated cell holder. In the presence of an excess of pyridine-2-carboxaldehyde (**1a**), the disappearance of *N*-propyldihydronicotinamide (**2a**) monitored at 360 nm, follows first-order kinetics for over 4 half-lives. The variation in the pseudo-first-order rate constant, k_{obsd}, with increasing concentrations of pyridine-2-carboxaldehyde is linear and provides the self-decomposition rate, k_{dec}, of **2a** and the second-order rate constant, k, for the reaction of **1a** and **2a** through the following equation:

$$k_{obsd} = k_{dec} + k[\mathbf{1a}]$$

Rate constants were evaluated by computer via the method of least squares.

The rate of the reaction of **1a** and **2a** is very sensitive to the nature of the solvent or solvent mixture. The reaction occurs in methanol at 35.0 ± 0.1 °C with a second-order rate constant of (6.0 ± 0.8) × 10^{-3} M^{-1} min^{-1}. Changing the solvent to water increases the rate of the reaction by a factor of over 7000 as shown by a second-order rate constant of 43.4 ± 1.2 M^{-1} min^{-1} at the same temperature. The measured value of k_{dec} in aqueous solution was 0 in accord with the known stability of dihydronicotinamides at high pH.[6] To determine if the C–H bond at the 4 position of the dihydronicotinamide was broken in the rate-determining step, the primary isotope effect for the reaction was measured. A comparison of the second-order rate constants at 30.7 ± 0.1 °C in 0.1 M (pH 9.9) carbonate buffer for the reaction of **1a** with *N*-propyldihydronicotinamide (k_{HH} = 32.5 ± 0.9 M^{-1} min^{-1}) and [4-^{2}H]-*N*-propyldihydronicotinamide (k_{HD} = 31.4 ± 1.2 M^{-1} min^{-1}) indicates no significant primary isotope effect.

The course of the reaction of pyridine-2-carboxaldehyde (**1a**) and *N*-alkyldihydronicotinamides (**2**) in aqueous solution was also monitored by high pressure liquid chromatography (HPLC). In a typical experiment, preequilibrated buffered solutions of pyridine-2-carboxaldehyde (**1a**) and [7-^{14}C]-*N*-benzyldihydronicotinamide (**2b**) are mixed and maintained at 40.0 ± 0.1 °C. Aliquots are removed at various times and the reaction components separated by a high pressure liquid chromatograph coupled to a UV detector. Peaks corresponding to the reactants are quantified by their peak heights and the use of standard curves. All peaks are individually collected and assayed for radioactivity by liquid scintillation counting. Observed counts per minute are corrected for quenching by the use of an external standard.

Time dependent HPLC studies of the above reaction demonstrated the complete consumption of pyridine-2-carboxaldehyde (**1a**, by UV detection) and *N*-alkyldihydronicotinamide (**2**, by UV and radiochemical detection) during the course of the reaction. A typical run is shown in Figure 1. HPLC analysis, however, failed to demonstrate the production of the expected reduction product, the carbinol (**3a**). Instead, HPLC analysis (UV and radiochemical detection) demonstrated the appearance of three other products during the course of the reaction. Radiochemical measurements demonstrated that these three products account for >90% of the *N*-alkyldihydronicotinamide consumed in the course of the reaction. All these products showed a UV maximum at 290 nm. The peaks

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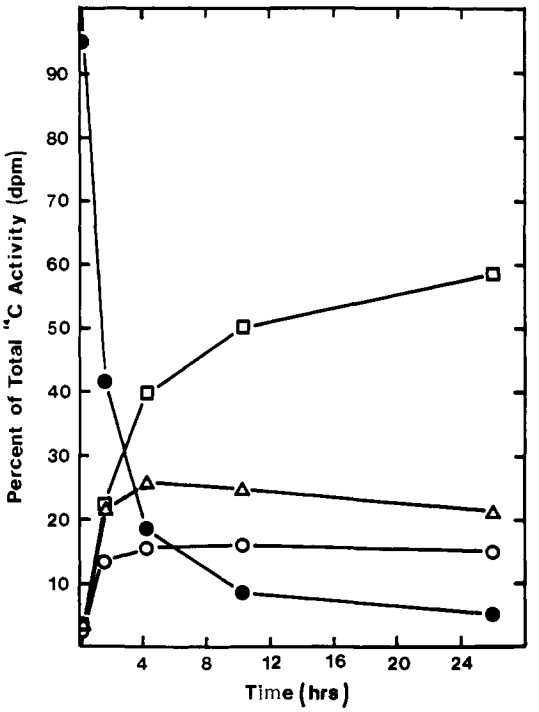
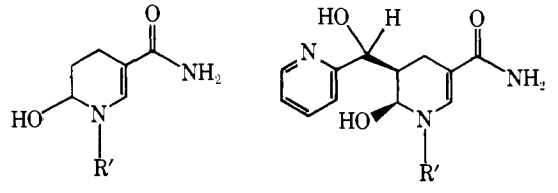


Figure 1. Time course of the reaction [7-^{14}C]-*N*-benzyldihydronicotinamide (●) and pyridine-2-carboxaldehyde in 0.1 M (pH 9.9) carbonate buffer maintained at 40.0 ± 0.1 °C. The products correspond to the primary hydration product of the dihydronicotinamide (**5b**, ○), the cis adduct (**6b**, △), and the trans adduct (**7b**, □).

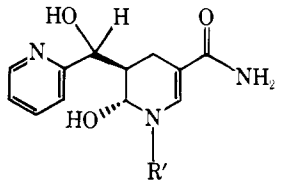
determined by UV detection at 290 nm were coincident with the peaks determined by radiochemical measurements. One of the products was identified as the primary hydration product of the starting *N*-alkyldihydronicotinamide, **5**, based on identical UV spectra and chromatographic properties with those of an authentic sample prepared by the acid-catalyzed hydration of the corresponding *N*-alkyldihydronicotinamide.[6] The structures of the other two products were shown to be the two isomers of 1:1 adducts (**6**, **7**) of pyridine-2-carboxaldehyde



5

6

a, R' = $-CH_2CH_2CH_3$
b, R' = $-CH_2-C_6H_5$

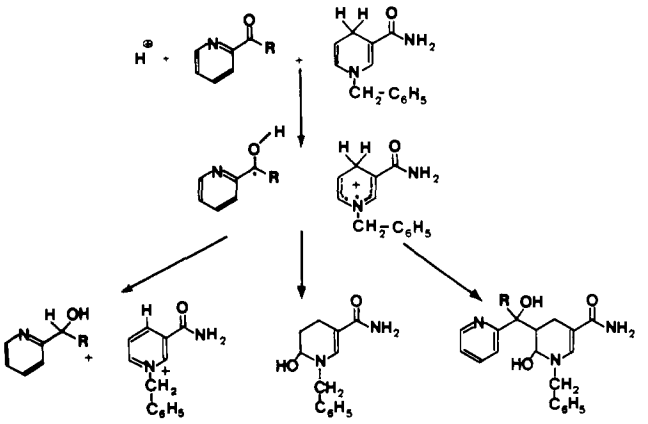


7

(**1a**) and *N*-alkyldihydronicotinamide (**2**) to which one molecule of water had been added. One of the isomeric adducts (**7**) could be isolated as a crystalline product (mp 124–126 °C) in a preparative reaction in 68% yield.[7] The assigned structure is consistent with the sample's elemental analysis and UV, NMR and mass spectra. The other isomeric adduct (**6**) was isolated by HPLC and characterized by NMR.

The formation of the adduct, **7**, is a reversible equilibrium

Scheme I



process. HPLC examination of a solution of the adduct, **7**, in anhydrous acetonitrile showed the formation of pyridine-2-carboxaldehyde (**1a**) and *N*-alkyldihydronicotinamide (**2**). This equilibrium was very sensitive to the amount of water present. Solutions of **7** in acetonitrile containing water (99:1, v/v) failed to show the formation of **1a** and **2**. Furthermore, the formation of adducts is not limited to the reaction of **1a** and **2**. We have isolated the adducts in the reaction of *N*-propyldihydronicotinamide with salicylaldehyde, benzaldehyde, glyoxylic acid, pentafluorobenzaldehyde, 5-nitrosalicylaldehyde, and trifluoroacetophenone.

The formation of the primary hydration product, **5**, of the *N*-alkyldihydronicotinamide during the course of the reaction of **1a** and **2** provides an important clue to the mechanism of the process. Formation of **5**, under conditions where the acid-catalyzed mechanism is negligible, has also been observed during the *N*-benzyldihydronicotinamide–*N*-benzylnicotinamide salt transhydrogenation reaction and has been interpreted by a mechanism involving electron transfer and resultant radical-cation formation.[8] By analogy, we propose the mechanism shown in Scheme I to explain the formation of **5**, **6**, and **7**. This mechanism is consistent with the observed bimolecular kinetics of the disappearance of the dihydronicotinamide. Furthermore, the observed rate acceleration in the presence of water is consistent with the notion that the carbonyl needs to be hydrogen bonded prior to electron transfer to avoid the formation of an unstable radical anion.[5] The scheme predicts that the resulting radical–radical-cation pair can undergo one of three possible transformations: (a) reversible collapse of the radical–radical cation followed by addition of water to give **6** and **7** with the equilibrium determined by the amount of water present, (b) addition of water to the radical cation of **2** to yield **3** in a process analogous to the one observed in the transhydrogenation reaction, and (c) hydrogen transfer to give the redox products, the carbinol (**3a**) and the *N*-alkylnicotinamide salt (**4**).

To test the above scheme, we studied the reaction of dipyridyl ketone (**1b**) with *N*-propyldihydronicotinamide (**2a**) in aqueous solution. We expected that the use of a more sterically hindered carbonyl would reduce the contribution of radical pair collapse on steric grounds and thus increase the amount of the redox products. This prediction was borne out by experiment. A reaction mixture consisting of 1 mmol of **1b**, 1.2 mmol of **2a**, and an NMR internal standard was incubated in 10 mL of 0.1 M aqueous (pH 9.9) carbonate buffer at 50 °C. After 3 days the reaction mixture was extracted with chloroform, the organic layer dried over anhydrous magnesium sulfate, and the solvent evaporated in vacuo. The oily residue was examined by NMR to give a spectrum identical with that of an authentic sample of dipyridylcarbinol, **3b**. Integration of the methylene proton relative to the internal standard indicated that 82 ± 3% of the starting ketone has been reduced to the carbinol **3b**. The

Table I. Analysis of the Kinetic and Partitioning Isotope Effects by Scheme II

reactants[a]	conditions	kinetic ratio[b]	kinetic isotope[c] effect	partitioning[d] isotope effect	ref	χ[e]
N-Pr/TFA	aqueous buffer	1.16 ± 0.04	1.38 ± 0.11	3.8 ± 0.3	4	0.62 ± 0.10
N-Bz/TFA	aqueous buffer	1.47 ± 0.12	2.77 ± 0.85	3.8 ± 0.3	4	0.13 ± 0.19
N-2,6-diCl-Bz/TFA	aqueous buffer	1.68 ± 0.08	5.25 ± 0.56		4	
N-Pr/TFA	$CH_3CN/Mg(ClO_4)_2$		3.62 ± 0.15	3.44 ± 0.2	13	0
N-Pr/*N*-MAI	aqueous buffer	1.26 ± 0.07	1.70 ± 0.18	5.4 ± 1.0	14	0.49 ± 0.13
N-Bz/*N*-MAI	$CH_3CN/Mg(ClO_4)_2$	1.55 ± 0.04	3.44 ± 0.44	5.4 ± 1.0	17	0.13 ± 0.07

[a] Dihydronicotinamide/oxidant; TFA (trifluoroacetophenone), *N*-MAI (*N*-methylacridium ion). [b] Ratio of the second-order rate constants of disappearance of *N*-alkyldihydronicotinamide (k_{HH}) to [4-^{2}H]-*N*-alkyldihydronicotinamide (k_{HD}). [c] Calculated by equation $k_H/k_D = (k_{HH}/k_{HD})/[2 - (k_{HH}/k_{HD})]$. [d] Ratio of protio to deuterio product. [e] Calculated by eq 6.

kinetics of the reduction of dipyridyl ketone (**1b**) by *N*-propyldihydronicotinamide (**2a**) were followed spectrophotometrically in a manner analogous to the reaction of **1a** and **2**. In the presence of an excess of **1b** the disappearance of **2a** follows first-order kinetics. Measurements of k_{obsd} with increasing concentration of **1b** (0–0.1 M) provided the second-order rate constant of reduction, 0.093 M^{-1} min^{-1} at 30.0 ± 0.1 °C.

Scheme I was further tested by examining the reaction of pyridine-2-carboxaldehyde (**1a**) and *N*-propyldihydronicotinamide (**2a**) under anhydrous conditions where the contribution toward hydration and adduct formation should be minimized. When the reaction is carried out in methanol using $ZnCl_2$ as a catalyst, a 20% yield of the carbinol **3** was detected by HPLC. Several workers have reported similar results.[3,9]

The results reported in this communication have important implications with regard to the interpretation of the data of several NADH model systems reported in the literature. Several groups of workers[3,4,10] have observed a disparity between the kinetic isotope effect (measured by the relative rates of the disappearance of protio- vs. deuteriodihydronicotinamide) and the partitioning isotope effect (measured by the relative amount of protio vs. deuterio product). They have interpreted these observations as indicating the existence of an intermediate along the reaction pathway where the disparity resulted because the rate-determining and product-determining steps were different steps. In light of the results reported in this communication, we believe that the observed disparity may be explained through the analysis of a reaction scheme involving the reversible formation of a 1:1 adduct between the dihydronicotinamide and the oxidant as a competing reaction.

We suggest that the reaction pathway depicted in Scheme II accounts for the disparity between the measured kinetic and partitioning isotope effects. In this scheme, the disappearance of the dihydronicotinamide reflects the formation of three different products: the redox products; the *reversible* adduct, and the hydration product. Consequently, the measured second-order rate constant[11] for the disappearance of the protio dihydronicotinamide represents the sum of the rate constants of three processes, i.e.,

$$k_{HH} = 2k_H + (k_{hyd} + k_A) \quad (1)$$

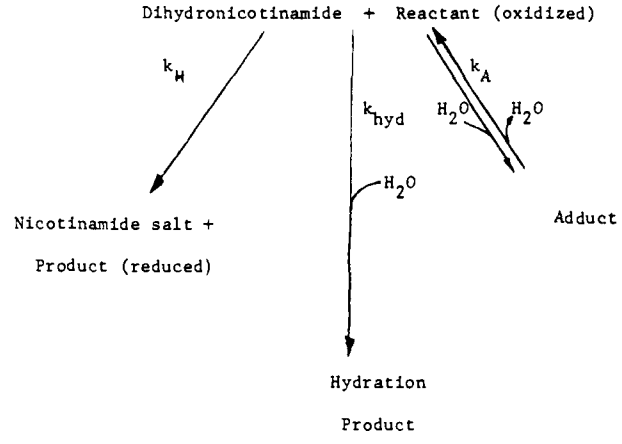
The measured second-order rate constant for the disappearance of the [4-^{2}H]dihydronicotinamide, on the other hand, is given by

$$k_{HD} = (k_H + k_D) + (k_{hyd} + k_A) \quad (2)$$

since only the process leading to the oxidation of the dihydronicotinamide is associated with a primary isotope effect.[12] Hence, the measured kinetic ratio is given by

$$\text{kinetic ratio} = \frac{k_{HH}}{k_{HD}} = \frac{2k_H + (k_{hyd} + k_A)}{(k_H + k_D) + (k_{hyd} + k_A)} \quad (3)$$

Scheme II



The consequence of this scheme is to lower the kinetic ratio toward unity as the magnitude of $(k_{hyd} + k_A)$ relative to k_{HH} increases. In contrast, the measured partitioning isotope effect is given by

$$\text{partitioning isotope effect} = k_H/k_D \quad (4)$$

and is independent of the magnitude of $(k_{hyd} + k_A)$ relative to k_{HH}. This scheme may be placed in a quantitative context by calculating the fraction of the measured second-order rate constant for the disappearance of the dihydronicotinamide, χ, which reflects the formation of adduct and hydration product. By combining

$$\chi = (k_{hyd} + k_A)/k_{HH} \quad (5)$$

with eq 3 to eliminate the $(k_{hyd} + k_A)$ term, one obtains an expression for χ

$$\chi = \frac{1 - [2/(1 + k_D/k_H)](k_{HD}/k_{HH})}{1 - [2/(1 + k_D/k_H)]} \quad (6)$$

in terms of the kinetics ratio and partitioning isotope effect. The results are summarized in Table I. The reduction of trifluoroacetophenone by *N*-propyldihydronicotinamide[4] is associated by a large disparity of the measured kinetic and partitioning isotope effect which may be explained by a large contribution of hydration and adduct formation to the measured second-order rate constant. As predicted by Scheme II, the increasingly more sterically hindered *N*-benzyl- and *N*-2,6-dichlorobenzyldihydronicotinamides have a smaller contribution to adduct formation with a resultant smaller disparity between the measured kinetic and partitioning isotope effects. Further support for Scheme II may be found in the studies of the reduction of trifluoroacetophenone by *N*-propyldihydronicotinamide under strictly anhydrous conditions (CH_3CN). Added anhydrous magnesium perchlorate serves to catalyze the reaction in addition to serving as an excellent drying agent

of the solvent. Under such conditions the reactions leading to hydration and adduct formation are not possible; Scheme II predicts the absence of a disparity between the measured kinetic and partitioning isotope effects, and no disparity is observed.[13] Similar arguments can be proposed for explaining the observed disparity in the reduction of *N*-methylacridium ion by *N*-alkyldihydronicotinamides.[14] The key to the argument is the extreme sensitivity of the equilibrium of adduct formation to the amount of water present and the well-known difficulty of preparing and maintaining totally anhydrous polar protic solvents like acetonitrile. Further support for Scheme II may be found in the reported reaction of *N*-alkylpyridines with sterically hindered oxidants carried out under conditions where the formation of hydration product and adduct would be unfavorable. For example, the reactions of thiopivalophenone,[1] benzoyl formate,[1] and hexachloroacetone[2] with *N*-benzyldihydronicotinamide, the reactions of 4-X-2,6-dinitrobenzenesulfonate with NADH,[15] and the reactions of π acceptors with *N*-methylacridan[16] show no disparity between the measured kinetic and partitioning isotope effects.

In summary, we have presented evidence that the course of the reaction of a dihydronicotinamide with a carbonyl is dependent on the steric bulk of the reactant and the reaction conditions. The observed products can be rationalized by a scheme that involves initial electron transfer. The observations have important implications with regard to the interpretation of the disparity between the measured kinetic and partitioning isotope effects in several reported NADH model systems.

Acknowledgment: We acknowledge the Research Corporation and the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. Furthermore, we express our appreciation to Drs. P. C. Myhre, A. K. Colter, D. M. Chipman, and L. Kurz for encouraging and helpful discussions.

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(11) k_{HH} and k_{HD} are the second-order rate constants for the disappearance of the diprotio- and monodeuterio-*N*-alkyldihydronicotinamides, respectively, in their reactions with substrate; k_H and k_D are the second-order rate constants associated with C–H and C–D bond cleavage, respectively, during the oxidation of the *N*-alkyldihydronicotinamide; k_{hyd} and k_A are the second-order rate constants for the formation of hydration product of the dihydronicotinamide and 1:1 adduct, respectively.
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Direct Observation of the Monomeric Metaphosphate Anion

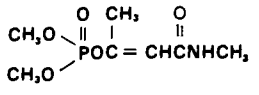
Sir:

We report herein the direct observation of the monomeric metaphosphate anion, PO_3^-.

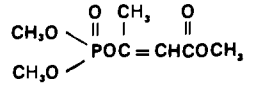
This species was first proposed in 1955[1] as an intermediate in hydrolysis of phosphomonoesters in aqueous solution. The hypothesis has since been extended to include other reactions and additional monomeric metaphosphate species as intermediates.[2] Recent work on phosphoryl transfer from aryl phosphates to alcohols and water in largely aprotic media has provided substantial evidence for the intermediacy of the monomeric metaphosphate anion.[3] Despite widespread acceptance of the concept, not until 1974 was the first unequivocal experimental evidence reported for the preparation of any monomeric metaphosphate, specifically the methyl ester in the vapor phase.[4] Subsequently, two of us and associates demonstrated the formation of a number of monomeric metaphosphate species via both thermal and electron-impact-induced reactions in the mass spectrometer, in part from our own work but largely on the basis of published data in an extensive preexisting literature on mass spectrometry of organophosphorus compounds.[5,6] Despite the passage of nearly 25 years since the original proposal, however, the monomeric metaphosphate anion has continued, understandably, to evoke references to an "evanescent" [7] or "mythical" [8] intermediate.

Some of us recently reported mass spectra, measured by a variety of experimental techniques, of several organophosphorus pesticides.[9] Negative-ion chemical-ionization[10] spectra of some of these compounds contained prominent peaks at *m/z* 79. In this context, such a peak would have to be attributed to either PO_3^- or $CH_4PO_2^-$. The precise masses of these species, 78.9585 and 78.9949, respectively, differ by 0.0364 u, enough to allow easy differentiation by high-resolution mass measurement. We have now made such measurements on the four pesticides whose structures are displayed. The results, listed in Table I, leave no doubt that we have observed the monomeric metaphosphate anion.[11]

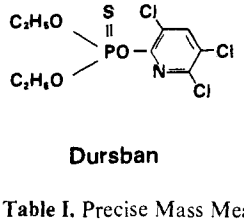
The remaining 0.1% of intensity at *m/z* 79 in the spectra of Monocrotophos and Mevinphos appears to be attributable to $CH_4PO_2^-$. The remaining ~10% in Azinphos methyl appeared



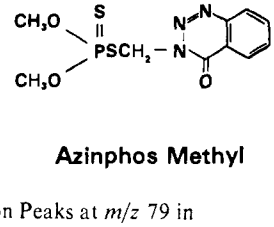
Monocrotophos



Mevinphos



Dursban



Azinphos Methyl

Table I. Precise Mass Measurements on Peaks at *m/z* 79 in Negative-Ion Chemical-Ionization Spectra

compd	measd mass	assignment ion	assignment mass	% of total ion current at *m/z* 79
Monocrotophos	78.9590	PO_3^-	78.9585	99.9
Mevinphos	78.9590	PO_3^-	78.9585	99.9
Dursban	78.9590	PO_3^-	78.9585	80
	78.9446	PSO^-	78.9407	20
Azinphos methyl	78.9409	PSO^-	78.9407	~90

0002-7863/79/1501-7409$01.00/0