Thermal Rearrangement of Substituted Acetophenone *O*-(Diethylphosphoryl)oximes and Synthesis and Biological Activity Studies of Series of Related Phosphoramidates

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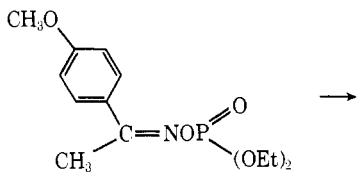
The rearrangement of substituted acetophenone *O*-(diethylphosphoryl)oximes to imidoyl phosphates was investigated. The synthesis of *p*-methoxyacetophenone *O*-(diethylphosphoryl)oxime was accomplished at reduced temperatures. This compound was observed to undergo rearrangement to its imidoyl phosphate upon introduction of heat at any point in the synthesis or work-up. All other substituted acetophenone *O*-(diethylphosphoryl)-oximes examined were found to undergo the same rearrangement when heated. An activation energy (E_a) of 8.2 kcal was obtained for the rearrangement of *p*-methoxyacetophenone *O*-(diethylphosphoryl)-oxime to *p*-methoxyphenylacetimidoyl diethyl phosphate. The synthesis and biological activity of a series of *O*,*O*-diethyl *N*-acetyl-*N*-phenylphosphoramidates were also examined. All the amidates were found to be moderately good inhibitors of housefly cholinesterase, having bimolecular inhibition constants on the order of 10^4 M^{-1} min^{-1}. Several of the phosphoramidates were toxic to the housefly and toxicity was enhanced by the use of the synergist piperonyl butoxide.

In a previous report (Fukuto *et al.*, 1969) from this laboratory concerning the relation between structure of a series of *O*-(diethylphosphoryl)oximes of ring-substituted acetophenones and biological activity, preliminary evidence was presented which indicated that oxime phosphate esters containing electron-donating substituents on the benzene ring were unstable and rearranged to the imidoyl phosphate as exemplified below with the *p*-methoxy derivative.



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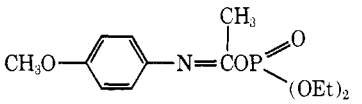

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Further, since both rates of alkaline hydrolysis and anticholinesterase activity of the various oxime phosphate esters were linearly correlated with the electron-donating tendency of the substituent, the suggestion was made that it was the imidoyl phosphate (**2**) formed by a Beckmann-type rearrangement of the oxime phosphate (**1**) that was involved in hydrolysis and cholinesterase inhibition. The possible formation of imidoyl phosphates through a Beckmann-type rearrangement was examined by Atherton *et al.* (1955). It was not clear, however, whether **2** was formed during the hydrolysis and inhibition process or whether it was formed during the preparation of **1**, prior to measurement of hydrolysis or anticholinesterase rates. Owing to the possible implication of the imidoyl phosphate in the mode of action of oxime phosphate esters, further study of the rearrangement of **1** to **2** was undertaken.

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MATERIALS AND METHODS

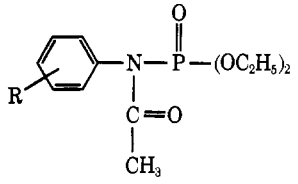
O-(Diethylphosphoryl)oximes. The substituted acetophenone *O*-(diethylphosphoryl)oximes were prepared as previously described (Fukuto *et al.*, 1969) except for minor modifications to avoid rearrangement to the imidoyl phosphate. The following procedure for the synthesis of *p*-methoxyacetophenone *O*-(diethylphosphoryl)oxime is typical. To a mixture of 7.5 g of *p*-methoxyacetophenone oxime sodium salt and 150 ml of anhydrous toluene, chilled in an ice bath at 0–5°, was added dropwise 6.9 g of diethylphosphorochloridate. After stirring for 2 hr at 5° the mixture was washed three times with chilled 5% aqueous sodium hydroxide and twice with chilled water and dried over anhydrous sodium sulfate at 0–5°. Removal of the solvent under vacuum (below 10°) gave a viscous oil, $n^{25}D$ 1.5359, which was immediately stored at 0°. Pmr spectrum (*d*-chloroform TMS) showed a single 3 H peak for the α-methyl protons at δ 2.29 and a single 3 H peak for methoxy protons at δ 3.75. Relevant absorptions for ring and ethoxy protons also were present.

Anal. Calcd for $C_{13}H_{20}NO_5P$: C, 51.82; H, 6.69. Found: C, 51.25; H, 6.69.

Further confirmation of the structure was obtained by hydrolyzing 1 g of the *O*-(diethylphosphoryl)oxime in aqueous ethanolic sodium hydroxide at room temperature for 24 hr. *p*-Methoxyacetophenone oxime, mp 85–86°, was isolated as the only organosoluble material.

Diethyl *N*-Phenylacetimidoylphosphates. Dialkyl *N*-phenylacetimidoylphosphates evidently are quite unstable, and although some imidoyl phosphates have been prepared (Atherton *et al.*, 1955) they have not been isolated in the pure state. For the preparation of *N*-*p*-methoxyphenylacetimidoyl diethylphosphate, the procedure described by Atherton *et al.* (1955) was used by reacting equimolar amounts of silver diethyl phosphate, prepared by the reaction of silver nitrate with barium diethyl phosphate (Kumamoto, 1965), and *p*-methoxyphenylacetimidoyl chloride (Abe *et al.*, 1965) in anhydrous toluene at 0–5°. Filtration and removal of the solvent under vacuum gave a crude product which could not be purified because of its instability. The following pmr data provided support for the presence of *N*-*p*-methoxy-

Table I. Physical Properties of *O,O*-Diethyl *N*-Acetyl-*N*-phenylphosphoramidates



Compound R	Yield, %	bp, °C	$n^{25}D$	Analysis	
				Theory	Found
H	76	102–104 (0.05 mm)	1.4989	C, 53.13 H, 6.68	C, 52.75 H, 6.90
p-CH_3S	69	124–126 (0.05 mm)	1.5354	C, 49.19 H, 6.35	C, 49.12 H, 6.16
p-CH_3	61	106–108 (0.05 mm)	1.4990	C, 54.73 H, 7.06	C, 54.46 H, 6.81
p-CH_3O	73	107–108 (0.1 mm)	1.5036	C, 51.82 H, 6.69	C, 51.35 H, 6.10
o-CH_3O	70	109–111 (0.5 mm)	1.5048	C, 51.82 H, 6.69	C, 52.30 H, 6.79
p-Cl	78	118–120 (0.05 mm)	1.5063	C, 47.15 H, 5.60	C, 46.98 H, 5.66
o-Cl	69	94–96 (0.05 mm)	1.4955	C, 47.15 H, 5.60	C, 47.15 H, 5.04
m-Cl	63	114–116 (0.05 mm)	1.5100	C, 47.15 H, 5.60	C, 47.67 H, 5.69
p-Br	70	124–126 (0.05 mm)	1.5219	C, 41.10 H, 4.89	C, 41.17 H, 4.34
p-F	72	126–128 (0.05 mm)	1.4848	C, 49.83 H, 5.92	C, 49.52 H, 6.31
o-*i*-Pr	63	96–98 (0.10 mm)	1.4959	C, 57.49 H, 7.72	C, 58.01 H, 7.59

phenylacetimidoyl diethylphosphate: a singlet at δ 2.06 for the C-methyl proton, a singlet at δ 3.73 for the methoxy protons, a triplet and multiplet centered at δ 1.15 and 4.05 for ethoxy protons, and a quartet centered at δ 7.02 for aromatic protons. A smaller singlet at δ 2.11 also was present and this was attributed to the C-methyl protons of *p*-methoxyacetanilide, which undoubtedly was present as an impurity. Pmr spectrum was obtained immediately after removal of the solvent, since the product decomposed upon standing with the separation of *p*-methoxyacetanilide, mp 127–128°.

Ethyl *N*-*p*-Methoxyphenylacetimidate. This compound was prepared according to the method of DeWolfe (1961) by reacting 24.6 g (0.2 mol) of *p*-anisidine and 48.6 g (0.3 mol) of triethyl orthoacetate to afford 33.6 g of (88.5%) product, bp 101–2° (1.2 mm), $n^{25}D$ 1.5241. Pmr spectrum revealed a methyl singlet at δ 1.75, a methoxy singlet at δ 3.66, an A–B quartet for aromatic protons centered at δ 7.3, and a triplet and a quartet for ethoxy protons centered at δ 1.30 and 4.20, respectively.

Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.32; H, 7.79. Found: C, 68.54; H, 7.81.

Diethyl *N*-Acetyl-*N*-substituted Phenylphosphoramidate. The various anilines used in the preparation of the substituted phenyl azides were obtained from commercial sources. All substituted phenyl azides were synthesized in the same manner, according to Lindsay and Allen (1955). The following triethyl *N*-substituted phenylphosphorimidates were prepared according to Kabachnik and Gilgarov (1956) from triethyl phosphite and the appropriate substituted phenyl azide (substituent, bp $n^{25}D$, respectively): H, 106.8° (0.6 mm), 1.5005; *p*-CH_3S, 165–166° (0.15 mm), 1.5352; *p*-CH_3, 127–128° (0.15 mm), 1.4985; *p*-CH_3O, 133–134° (0.15 mm), 1.5012; *o*-OCH_3, 134–135° (0.15 mm), 1.5044; *p*-Cl, 141–142 (0.5 mm), 1.5091; *o*-Cl, 142–144° (0.1 mm), 1.5094; *m*-Cl, 138–140° (0.1 mm), 1.5093; *p*-Br, 142–144° (0.3 mm), 1.5243; *p*-F, 114–115° (0.4 mm), 1.4840; *o*-*i*-C_3H_7, 108–110 (0.25 mm), 1.4962. *O,O*-Diethyl *N*-acetyl-*N*-substituted phenylphosphoramidates were prepared according to Kabachnik and Gilgarov (1956) by reacting the triethyl *N*-phenylphosphorimidate with acetyl chloride in ligroin. Physical properties and elemental analyses are presented in Table I.

For the identification of the alkaline hydrolysis products of *O,O*-diethyl *N*-acetyl-*N*-phenylphosphoramidate, a 2-g sample was stirred overnight in a mixture of ethanol and 10% aqueous sodium hydroxide. After removal of the solvent in a rotary evaporator, the single organosoluble product isolated was identified as *O,O*-diethyl *N*-phenylphosphoramidate, mp 93–95°. The structure also was supported by infrared and pmr analyses. Similar experiments were carried out with the corresponding *p*-chloro and *p*-methoxy derivatives. Hydrolysis of these compounds at pH 7.0 was examined in phosphate buffer, heated at reflux for 24 hr. The respective acetanilide was isolated as the only organosoluble product and was identified by mp, pmr, and infrared analysis.

Rate of Thermal Rearrangement. The rates of thermal rearrangement of *p*-methoxyacetophenone *O*-(diethylphosphoryl)oxime was determined by means of a Varian T-60 nmr spectrometer. Samples of freshly prepared (diethylphosphoryl)oxime were placed in pmr tubes, capped, and placed in a constant temperature bath. At different time intervals the tubes were removed, *d*-chloroform was added, the pmr spectra were taken. The rate of rearrangement was monitored by estimating the appearance of the C-methyl-protons of the imidoyl phosphate at δ 2.06 relative to the disappearance of the acetophenone C-methyl protons at δ 2.30. The relative amount of rearranged product was determined from the ratio of the peak integral at δ 2.06 to the total integral for the two C-methyl absorptions. First-order plots

of log $a/a - x$ against time, where a is the initial amount of *p*-methoxyacetophenone *O*-(diethylphosphoryl)oxime and x is the amount of rearranged product, gave straight lines from which the rate constant, k, for thermal rearrangement was calculated. Activation energy, E_a, was obtained from an Arrhenius plot of $1/T$, °K, against log k.

The solvolysis of *p*-methoxyacetophenone *O*-(diethylphosphoryl)oxime in absolute ethyl alcohol was examined at 70° by means of a Unicam SP-800 uv visible spectrophotometer.

Insecticidal activity was determined against a susceptible strain of houseflies (*Musca domestica* L., S_{NAIDM} strain) according to March and Metcalf (1949) and mosquito larvae (*Culex pipiens quinquefasciatus* Say.) according to Mulla *et al.* (1966). Anticholinesterase activity, *i.e.*, I_{50} (molar concentration for 50% inhibition) and k_i (bimolecular inhibition constant), was determined against housefly-head cholinesterase according to previous methods (Fukuto and Metcalf, 1956; Aldridge, 1950; Ellman *et al.*, 1961).

RESULTS

Pmr analysis was the principal method used for the study of the rearrangement of *p*-methoxyacetophenone *O*-(diethylphosphoryl)oxime (**1**). Synthesis of this compound without contamination by the rearranged product was accomplished by keeping the temperature of the reaction mixture below 5° at all times. The pmr spectrum of this material gave a singlet for the acetophenone methyl at δ 2.29 and a singlet for ring methoxy at δ 3.75. Hydrolysis of **1** in aqueous ethanolic sodium hydroxide gave *p*-methoxyacetophenoneoxime as the sole organosoluble product. Synthesis of **1** by the method previously used (Fukuto *et al.*, 1969) afforded a product whose pmr spectrum (carbon tetrachloride) showed two different singlets for C-methyl protons at δ 2.03 and 2.30, and two singlets for methoxy protons at δ 3.66 and 3.75. Alkaline hydrolysis of this material gave a mixture of *p*-methoxyacetophenone and *p*-methoxyacetanilide as the organosoluble products, indicating that the compound previously examined as **1** for toxicity and anticholinesterase activity (Fukuto *et al.*, 1969) was actually a mixture of **1** and the rearranged product.

When a sample of pure **1** was heated in a sealed tube under nitrogen at 90° for 15 hr, the singlets (*d*-chloroform) at δ 2.30 and 3.75 disappeared and were replaced by singlets at δ 2.06 and 2.11 in the C-methyl proton region and a single discernible peak for methoxy protons at δ 3.68. Further examination of this isomerization by placing a sample of pure **1** in the pmr probe and slowly increasing the temperature of the probe from 5° to 65° showed that the singlet at δ 2.30 gradually disappeared with the simultaneous appearance of the singlet at δ 2.06. At high temperatures and after 4 hr, another singlet appeared at δ 2.11, attributable to the C-methyl in *p*-methoxyacetanilide. *p*-Methoxyacetanilide examined separately revealed a C-methyl singlet at δ 2.11 (2.08 in carbon tetrachloride). The singlet at δ 2.06 (*d*-chloroform, 2.03 in carbon tetrachloride) therefore was assigned to the C-methyl protons of **2**. Long-range coupling between the C-methyl protons and phosphorus in **2** was not observed. Alkaline hydrolysis of the heated material produced only *p*-methoxyacetanilide. Similar results were obtained when pure samples of the *O*-(diethylphosphoryl)oximes of acetophenone, *p*-methyl-, *p*-fluoro-, *p*-bromo-, and *p*-chloroacetophenone were treated under the same conditions. In all cases the substituted acetanilide was the only product isolated after hydrolysis with sodium hydroxide.

Further, passage of pure **1** through the falling-film molecular distillation apparatus at a temperature range of 82–105° (0.08 mm) gave successive fractions whose pmr spectra (carbon tetrachloride) were virtually identical with methyl absorptions at δ 2.03, 2.08, and 2.30 and methoxy absorptions at δ 3.70 and 3.80. Alkaline hydrolysis of each fraction produced a mixture of *p*-methoxyacetophenoneoxime and *p*-methoxyacetanilide.

Table II. Data for Rates of Rearrangement of *p*-Methoxyacetophenone *O*-(Diethylphosphoryl)oxime to Diethyl *N*-*p*-Methoxyphenylacetimidoylphosphate

Temp, °C	Time, min	2.303 log $a/a - x$	k (min^{-1})
82	0	0.113	0.054
	8	0.559	
	15	0.865	
	20	1.156	
92	0	0.223	0.090
	5	0.541	
	8	0.875	
	12	1.204	
	15	1.391	
105	0	0.113	0.297
	3	0.978	
	5	1.619	
	13	4.043	

Although purification of diethyl *N*-*p*-methoxyphenylacetimidoyl phosphate (**2**) prepared from silver diethyl phosphate and *p*-methoxyphenylacetimidoyl chloride was not possible owing to the instability of this compound, pmr spectra of the impure material gave methyl absorptions at δ 2.06 and 2.11 and methoxy absorption at δ 3.73 (in *d*-chloroform). This material decomposed upon standing to precipitate *p*-methoxyacetanilide. The presence of C-methyl proton absorption at δ 2.06 provides evidence that the product obtained from the thermal rearrangement of **1** is **2**.

Data for the rate of thermal rearrangement of **1** to **2** at 82°, 92°, and 105° are given in Table II. From the first-order rate constants (k) at the three temperatures, the activation energy, E_a, was calculated to be 8.2 kcal/mol, a value which reflects the thermal instability of **1**. The Arrhenius plot is given in Figure 1.

The thermal rearrangement of **1** was examined also in the presence of a nucleophilic solvent, ethyl alcohol, at 70°. The reaction was examined spectrophotometrically every 10 min for 150 min and the spectral changes are shown in Figure 2. **1** in ethyl alcohol shows an absorption maximum at 265 nm which, upon standing at 70°, gradually disappeared with the simultaneous appearance of a new maximum at 237 nm. The reaction was essentially complete after 150 min, since no further change in the spectrum occurred after another 2.5 hr. The spectrum at this time was identical to the spectrum obtained from equimolar amounts of ethyl *N*-*p*-methoxyphenylacetimidate and diethyl phosphoric acid in ethyl alcohol (Figure 3).

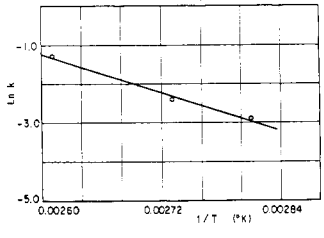


Figure 1. Arrhenius plot for the isomerization of *p*-methoxyacetophenone *O*-(diethylphosphoryl)oxime to *N*-*p*-methoxyphenylacetimidoyl diethylphosphate

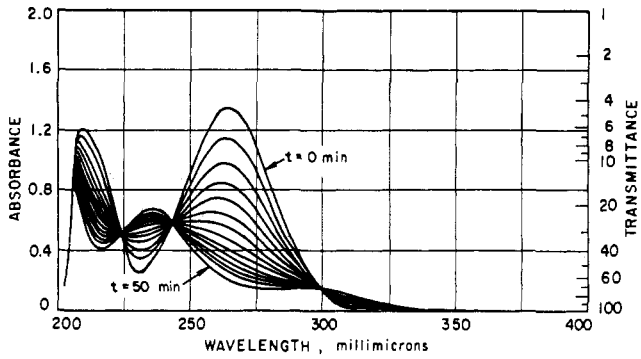


Figure 2. Absorption spectra showing the rearrangement and solvolysis of *p*-methoxyacetophenone *O*-(diethylphosphoryl)oxime in absolute ethanol at 70°

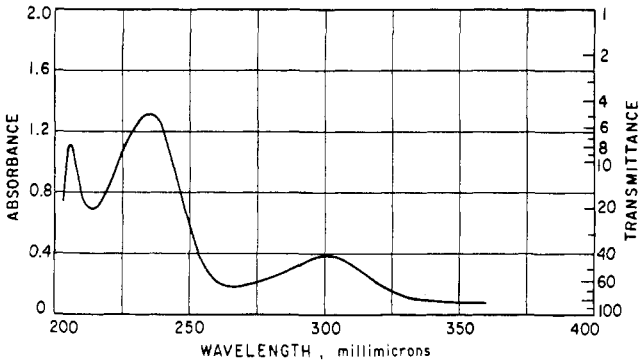
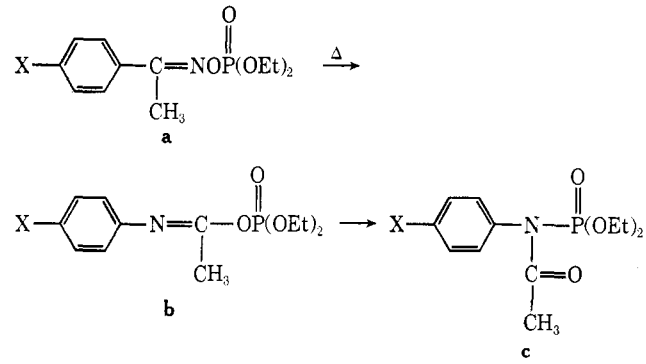


Figure 3. Absorption spectrum of equimolar amounts of diethyl phosphoric acid and ethyl *N*-*p*-methoxyphenylacetimidate in absolute ethanol

Diethyl *N*-Acetyl-*N*-substituted Phenylphosphoramidates. During the course of this study of the thermal rearrangement of substituted acetophenone *O*-(diethylphosphoryl)oximes to the substituted *N*-phenylacetimidoyl diethyl phosphate, it occurred to us that further rearrangement of the latter to diethyl *N*-acetyl-*N*-phenylphosphoramidates was possible according to the equation below. The phosphoramidate **c** having the same empirical formula as **a** or **b** would give the same elemental analysis, and alkaline hydrolysis conceivably

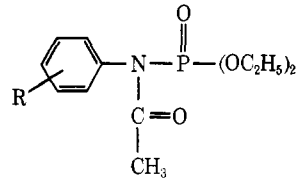


would give acetanilide by cleavage of the P–N bond. In order to check out this possibility, several key diethyl *N*-acetyl-*N*-phenylphosphoramidates were prepared and compared by nmr and infrared analysis with the products obtained from the thermal rearrangement of the oxime phosphate. The methyl protons in all cases appeared as singlets at δ 1.98–2.00 and this, coupled with infrared spectral data, indicated that **c** was not obtained upon heating **a**.

Examination of diethyl *N*-acetyl-*N*-phenylphosphoramidate and *N*-acetyl-*N*-*p*-methoxyphenylphosphoramidate, however, showed that each was moderately toxic to houseflies and effective as an anticholinesterase. The unexpected biological activity, therefore, stimulated further examination of these compounds and a series of diethyl *N*-acetyl-*N*-phenylphosphoramidates were synthesized and assessed for insecticidal and anticholinesterase activity. Biological data for the various compounds are given in Table III.

The data show that all of the *N*-acetylphosphoramidates listed in the table are moderately good inhibitors of housefly-head cholinesterase with bimolecular inhibition constants in the region of 10^4 M^{-1} min^{-1}. The most potent inhibitor in the series was the unsubstituted amidate (**3**), which had a k_i value of 5.1×10^4 M^{-1} min^{-1}. Examination of the inhibition data showed no obvious correlation with any of the reactivity parameters. Compounds containing electron-withdrawing substituents such as the halogen in **8**, **9**, **10**, **11**, and **12** were all less effective anticholinesterases than **3**. Compound **4**, which contains the *p*-thiomethyl moiety (σ constant of zero), was almost equal to **3** in activity.

Table III. Toxicological Properties of *O*,*O*-Diethyl *N*-Acetyl-*N*-phenylphosphoramidates



Compound R	I_{50} M	k_i M^{-1} min^{-1}	*Musca domestica* LD_{50} μg/g	*Musca domestica* + P.B. (1:5) LD_{50} μg/g	*Culex pipiens* LC_{50} ppm
3 H	2.40×10^{-7}	5.1×10^4	60.5	35	4.7
4 *p*-CH_3S	3.40×10^{-7}	4.8×10^4	425	56.5	>10
5 *p*-CH_3	1.40×10^{-6}	3.2×10^4	100	55	>10
6 *p*-CH_3O	9.40×10^{-7}	2.5×10^4	140	59	>10
7 *o*-CH_3O	1.20×10^{-6}	3.8×10^4	420	180	>10
8 *p*-Cl	1.32×10^{-6}	2.9×10^4	180	60	>10
9 *o*-Cl	7.20×10^{-5}	3.3×10^3	>500	245	>10
10 *m*-Cl	1.15×10^{-6}	1.9×10^4	180	47	>10
11 *p*-Br	2.04×10^{-6}	1.5×10^4	200	75	>10
12 *p*-F	1.60×10^{-6}	1.4×10^4	145	60	>10
13 *o*-*i*-Pr	4.40×10^{-5}	8.3×10^3	>500	170	>10

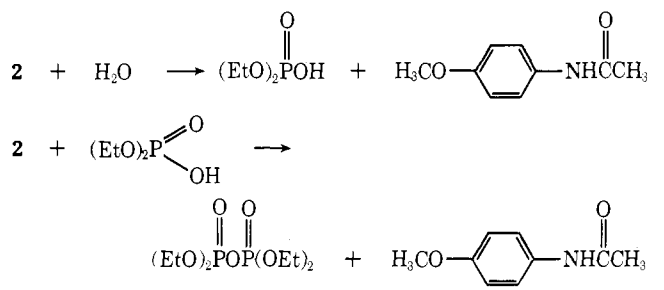
The toxicity data show that several of the diethyl *N*-acetyl-*N*-phenylphosphoramidates were toxic to the housefly and and toxicity was enhanced by the use of piperonyl butoxide. As in the case of anticholinesterase activity, **3** was most effective with an LD_{50} value of 60.5 μg/g. Again, no obvious correlation between toxicity and reactivity parameters was evident, although it is noteworthy that the two poorest anticholinesterases (**9** and **12**) were least toxic to houseflies.

DISCUSSION

The results show that *O*-(diethylphosphoryl)oxime of ring-substituted acetophenones, particularly those with electron-donating substituents, are thermally unstable and readily undergo rearrangement to give the substituted *N*-phenylacetimidoyl diethylphosphate. The rearrangement product is extremely unstable to traces of moisture and rapidly decomposes to the substituted acetanilide and diethylphosphoric acid. *p*-Methoxyacetophenone *O*-(diethylphosphoryl)oxime (**1**) may be prepared free from any rearranged material by carefully avoiding heat during any part of the reaction or work-up. Heat also must be avoided in preparing the corresponding *p*-methyl and unsubstituted analogs. In comparison, the *p*-chloro and *p*-bromo analogs are less sensitive to heat and may be distilled without rearrangement through a falling-film molecular distillation apparatus at temperatures near 100°. However, continued heating, *e.g.*, 15 hr at 90°, resulted in almost complete rearrangement.

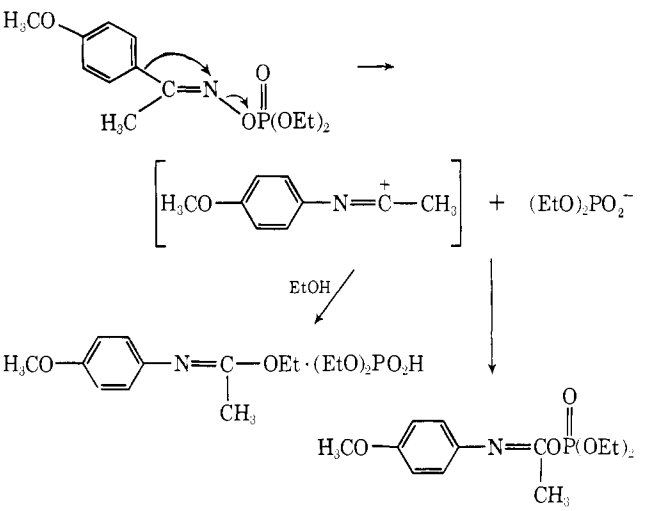
The rather low E_a value (8.2 kcal) for the thermal rearrangement of **1** provides a measure for the instability of *O*-(diethylphosphoryl)oximes of ring-substituted acetophenones containing electron-donating substituents. Overall, the results show that care must be taken in avoiding heat when compounds of this type are prepared.

The compound reported previously as **1** (Fukuto *et al.*, 1969) was unusually potent as an inhibitor of fly-head cholinesterase, the molar concentration to give 50% inhibition of enzyme (I_{50}) was 7.2×10^{-9}, and moderately toxic to houseflies, LD_{50} 27 μg/g/fly. However, **1** prepared without application of any heat was virtually nontoxic to the housefly ($LD_{50} > 500$ μg/g) and was about 500-fold less effective in inhibiting fly-head cholinesterase (I_{50} 3.45×10^{-6}). Thus, it appears that the strong anticholinesterase activity and housefly toxicity reported earlier for **1** was caused by the rearranged product **2** rather than **1**. Although **2** undoubtedly is formed when **1** is heated, it is possible that tetraethyl pyrophosphate (TEPP) also may be formed in the presence of small amounts of moisture according to the equations below.



The reaction between dialkyl imidoyl phosphate and dialkyl phosphoric acid to give the corresponding pyrophosphate has been established by Atherton *et al.* (1955). Therefore, the possibility exists that TEPP also may be contributing to the toxicological properties of impure **1**.

The spectral evidence for the formation of ethyl *p*-methoxyphenylacetimidate when **1** is heated in absolute ethanol indicates that the rearrangement process is not concerted, as suggested previously (Fukuto *et al.*, 1969), and rearrangement probably occurs through an ion intermediate according to the following equations.



The proposed mechanism is similar to that generally accepted for the Beckmann rearrangement (Donaruma and Heldt, 1960; Smith, 1963) in which migration of an anti aryl or alkyl group occurs simultaneously as the N–O bond is broken to form the intermediate iminocarbonium ion. In the absence of a nucleophilic solvent such as ethanol, rearrangement occurs to the imidoyl phosphate. In ethanol, the intermediate iminocarbonium ion reacts with the solvent to form ethyl *p*-methoxyphenylacetimidate. The imidoyl phosphate **2** evidently is not formed from **1** in ethanol, since the spectral results did not show the presence of any triethyl phosphate and *p*-methoxyacetanilide, the expected ethanolysis products of **2**.

The possibility that **1** rearranges by methyl migration rather than by phenyl migration to give diethyl *N*-methyl-*p*-methoxybenzimidoylphosphate was ruled out on the basis that no *N*-methyl-*p*-methoxybenzamide was detected after alkaline hydrolysis of thermally rearranged **1**. This observation provides evidence, although by no means conclusive, that the *p*-methoxyphenyl moiety in **1** is anti to the diethyl phosphoryloxy group.

Comparison of pmr and infrared spectra showed that diethyl *N*-acetyl-*N*-phenylphosphoramidates are not produced from the thermal rearrangement of the corresponding *O*-(diethylphosphoryl)oximes of substituted acetophenone. The *N*-acetyl-*N*-phenylphosphoramidates (**3–13**), however, were of considerable interest owing to their unexpected anticholinesterase activity and toxicity to insects. These compounds are unusual in that the most likely bond to break when cholinesterase enzyme is phosphorylated in the inhibition process is the P–N linkage and apparently the acetanilide anion is the leaving group. Compounds of this type deserve further examination as possible insecticides.

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Synthesis of Radiolabeled Ethyl *O*-Benzoyl-3-chloro-2,6-dimethoxybenzohydroximates: Oxime-^{14}C, Carbonyl-^{14}C, and 2-Methoxy-^{14}C

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Ethyl *O*-benzoyl-3-chloro-2,6-dimethoxybenzohydroximate, benzomate acaricide, was prepared with specific ^{14}C-labeling at the oxime, carbonyl, and 2-methoxy positions. Benzomate-*oxime*-^{14}C (0.396 mCi/mmol) was prepared with the overall radiochemical yield of 4.2% from acetic anhydride-*1*-^{14}C and 4-methoxy-7-hydroxycoumarin through nine-step reactions. Benzomate-*carbonyl*-^{14}C (1.13 mCi/mmol) was prepared with the yield of 32.2% by a one-step reaction, which gave preferentially an *N*-benzoyl isomer with rather high yield (49.8%). Benzomate-*2-methoxy*-^{14}C (0.117 mCi/mmol) was prepared in four-step reactions, starting from methylation by a substituted salicylate with diazomethane-^{14}C.

Benzomate (ethyl *O*-benzoyl-3-chloro-2,6-dimethoxybenzohydroximate) is a new acaricide developed by Nippon Soda Co., Ltd., and effective especially against *Panonychus citri* (Noguchi *et al.*, 1971). The study of the metabolic fate of benzomate is facilitated by the use of radiolabeled materials in different parts of the molecule. It was recently found by using these labeled compounds that the debenzoylation of benzomate occurred moderately on citrus fruit (Soeda *et al.*, 1971). This report deals with the synthesis of benzomate labeled with ^{14}C at different positions.

By either acid or alkaline hydrolysis, benzomate is easily debenzoylated to give ethyl 3-chloro-2,6-dimethoxybenzohydroximate. On heating in 47% hydroiodic acid, debenzoyl benzomate is demethylated exclusively at the 2-methoxy grouping and the resulting ethyl 3-chloro-2-hydroxy-6-methoxybenzohydroximate was resistant to further demethylation (Tohyama *et al.*, 1970).

Of the several groupings retained in the major skeleton of the molecule under these chemical reactions, the oxime carbon was selected for labeling, since it was supposed to survive with the benzene ring in the metabolic process. In addition, two chemically labile groupings, carbonyl and 2-methoxy, were also selected for labeling. With those differently labeled compounds available, a metabolite will be more easily identified by the presence or absence of radioactivity, depending on the kind of the starting labeled compounds. Therefore the three differently labeled benzomates were synthesized.

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The synthetic schemes are shown in Figure 1. According to the known procedures (Russell and Frye, 1955), nonlabeled resorcinol was converted to 2,6-dihydroxyacetophenone-^{14}C (**4**) *via* 4-methyl-7-acetoxycoumarin-^{14}C (**3**), the radioactive carbon of which had been introduced by acetic anhydride-*1*-^{14}C with some modification. Acetylation by the known procedure of 4-methyl-7-hydroxycoumarin (**1**) with acetic anhydride without any solvent gave 95% yield of the product in a preliminary cold run. Acetic anhydride-^{14}C, however, was only available in benzene, which was a poor solvent for the compound to be acetylated. Therefore pyridine had to be added for the reaction to take place.

In the preliminary study, methylation of 2,6-dihydroxyacetophenone-^{14}C (**4**) with dimethyl sulfate in 2 *N* sodium hydroxide gave a mixture of mono- and dimethyl derivatives. Therefore the methylation was actually accomplished in two steps. Compound **4** was treated with excess diazomethane to give the monomethyl derivative, which was further methylated with dimethyl sulfate in 4 *N* alkaline solution. In view of this result, however, it is likely that 2,6-hydroxydiacetophenone can be fully methylated in one step at both phenolic hydroxyl groupings with dimethyl sulfate, if 4 *N* sodium hydroxide is used.

The monomethyl derivative, 6-hydroxy-2-methoxyacetophenone, was soluble in benzene but sparingly soluble in alkaline water. When the silica gel tlc had been developed with benzene, the R_f value (0.37) of this compound was greater than that (R_f 0.21) of the dimethyl derivative **5**. So, this compound might have an intramolecular hydrogen bonding (>C=O · · · H–O–).

The same reagent, sodium hypochlorite, was used for the oxidation and the subsequent chlorination of 2,6-dimethoxyacetophenone (**5**), so the latter reaction could be performed either with or without isolation of 2,6-dimethoxybenzoic acid (**6**). In either case the chemical yield was found to be around 60%. 3-Chloro-2,6-dimethoxybenzoic acid (**7**) thus obtained