Biomedical Mass Spectrometry 1976, 3, 316 to 328

Synthesis and Mass Spectrometry of Crufomate Metabolites and Related Compounds

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(*Received* 11 *May* 1976)

Abstract—Phosphates and phosphoramidates related to 2-chloro-4-*t*-butylphenyl methyl methylphosphoramidate (*crufomate*) were synthesized to aid in the identification of crufomate metabolites. Deuterium labeling, metastable determinations and precise mass measurements were used to establish fragmentation pathways. Evidence was obtained for the rearrangement of an *N*-formyl phosphoramidate (the expected result of oxidative metabolism of *N*-methyl phosphoramidates) to an iminomethyl phosphate.

Introduction

METABOLISM studies on crufomate (4-*t*-butyl-2-chlorophenyl methyl methylphosphoramidate, **1**) required extensive use of mass spectrometry for metabolite identification.[1] Mass spectra of a considerable number of phosphorus compounds have been reported,[2–6] but a systematic investigation of the fragmentation of phosphorus compounds has not been carried out. Some established fragmentation routes, especially those involving cleavage reactions, were not observed in the mass spectrometry of the metabolites because substituents present provided more favorable routes.

The ideal structure proof, unambiguous synthesis and spectral comparison, was accomplished for some of the metabolites, but for others the effort required for synthesis appeared to be too great to make this method of identification practical. The identification of these metabolites was based on deductive reasoning through the establishment of fragmentation routes with model compounds. This paper reports the synthesis and mass spectra of these compounds. The designation used for coding the metabolites by a letter-number sequence is as described in Ref. 1.

Experimental

Melting points were taken with a Thomas-Hoover capillary melting point apparatus. Infrared spectra were taken with a Perkin-Elmer 337 infrared spectrometer. Gas chromatography separations were done with a Barber-Colman Series 5000 gas chromatograph equipped with an effluent splitter for sample trapping; 6 ft, 4 mm i.d. glass columns were used. Mass spectra were obtained using the solid inlet probes of either a Varian M-66 mass spectrometer equipped with a V-5500 control console or a Varian MAT CH-5 DF mass spectrometer capable of peak matching and determining decoupled metastable transitions. Nuclear magnetic resonance spectra were taken with a Varian A-60A spectrometer in conjunction with a Digilab FTS/NMR-3 Fourier transform system. 4-*t*-Butyl-3-chlorophenyl dihydrogen phosphate (**2**) was obtained from Dow Chemical Co.

4-*t*-BUTYL-2-CHLOROPHENYL METHYL METHYL-d_3-PHOSPHORAMIDATE (3)

A solution of 1.5 g (0.005 mol) of 4-*t*-butyl-2-chlorophenyl phosphorodichloridate, 0.18 g (0.0056 mol) of methanol and 2 ml of triethylamine was stirred for 20 min. Then a solution of 0.78 g (0.011 mol) of [$^2H_3$]methylamine hydrochloride in 3 ml of triethylamine was added and the reaction stirred for 4 h. The solid was filtered off and the solvent removed at reduced pressure. The residue was chromatographed on alumina; methylene chloride eluted some 4-*t*-butyl-2-chlorophenyl dimethyl phosphate, while ethyl acetate eluted the desired product.

METHYL 2-(4-METHOXYPHENYL)-2-METHYL-PROPIONATE (4)

A mixture of 93.2 g (0.52 mol) of methyl 4-methoxyphenylacetate (prepared from 4-methoxyphenylacetic acid and boron trifluoride in methanol), 23.3 g (0.44 mol) of 57% sodium hydride dispersion, 400 ml of dimethylformamide and 400 ml of tetrahydrofuran was refluxed until the evolution of hydrogen ceased. Then a 100 g (0.7 mol) of methyl iodide was added dropwise at reflux. After the addition was completed the mixture was refluxed for 1 h. Without purification, the products of this reaction were reacted again with 13.9 g (0.33 mol) of 57% sodium hydride dispersion and 50 g (0.35 mol) of methyl iodide. Ether was added to decrease the viscosity of the reaction and the sodium iodide was removed by filtration. The sodium iodide was washed with ether and the ether removed by distillation from the combined filtrates. The products were reacted twice more with sodium hydride (13.9 g) and methyl iodide (50 g) to

ensure complete methylation. Ether was again added and the sodium iodide removed by filtration. The solvents were removed at reduced pressure and the residue was distilled; the material boiling from 98–108 °C at 0.1 Torr was of sufficient purity for subsequent reactions: i.r. (neat, KBr plates) 1740 cm^{-1} (C=O); n.m.r. (CCl_4) δ 1.49 (s, CH_3CCH_3), 3.56 (s, $COOCH_3$), 3.72 (s, $ArOCH_3$), 6.74 (d, ArH, $J = 9$ Hz), 7.16 (d, ArH, $J = 9$ Hz).

METHYL 2-(3-CHLORO-4-METHOXYPHENYL)-2-METHYLPROPIONATE (**5**)

Sulfuryl chloride (32.4 g, 0.24 mol) was added with cooling to 47.7 g (0.23 mol) of methyl 2-(4-methoxyphenyl)-2-methylpropionate. After the addition was completed, the reaction was stirred at room temperature for 15 min, then heated on a steam bath for 90 min. The product distilled at 115–118 °C and 0.1 Torr: i.r. (neat, KBr plates) 1740 cm^{-1} (C=O); n.m.r. (CCl_4) δ 1.54 (s, CH_3CCH_3), 3.63 (s, $COOCH_3$), 3.88 (s, $ArOCH_3$), 6.84 (d, 6-ArH, $J = 8.5$ Hz), 7.14 (dd, 5-ArH, $J = 8.5$ and 2.3 Hz), 7.30 (d, 3-ArH, $J = 2.3$ Hz); m.s. Fig. 1(b).

2-(3-CHLORO-4-HYDROXYPHENYL)-2-METHYL-PROPIONIC ACID (**6**) (C-9)

A solution of 40 g of methyl 2-(3-chloro-4-methoxyphenyl)-2-methylpropionate, 200 ml of glacial acetic acid and 25 ml of 48% hydrobromic acid was refluxed for 4 h. Water was added and the mixture extracted with ether. The solvent and glacial acid were removed at reduced pressure. An n.m.r. spectrum on the residue indicated that the ester had been hydrolyzed but with very little cleavage of the ether. This material was heated with pyridine hydrochloride at 210 °C for 10 min, poured into ice water and extracted with ether. The solvent was removed and the residue recrystallized from ether+hexane to yield 21.5 g of product, m.p. 99–102 °C: i.r. (KBr) 1715 cm^{-1} (C=O); n.m.r. ($CDCl_3$) δ 1.58 (s, CH_3CCH_3), 7.03 (d, 5-ArH, $J = 8.5$ Hz), 7.27 (dd, 6-ArH, $J = 8.5$ and 2.3 Hz), 7.43 (d, 2-ArH, $J = 2.3$ Hz); m.s. of bis-TMS derivative Fig. 1(c).

2-(3-CHLORO-4-METHOXYPHENYL)-2-METHYL-1-PROPANOL (**7**)

An excess of lithium aluminum hydride was added to an ether solution of methyl 2-(3-chloro-4- methoxyphenyl)-2-methylpropionate at −70 °C and the mixture stirred for 15 min. Dilute sulfuric acid was added and the mixture allowed to warm to room temperature. The organic layer was dried over magnesium sulfate and the ether removed. The product was used without purification (g.c. and n.m.r. indicated >90% purity): n.m.r. (CCl_4) δ 1.21 (s, CH_3CCH_3), 2.33 (s, OH), 3.37 (s, CH_2), 3.82 (s, OCH_3), 6.79 (d, 5-ArH, $J = 8.6$ Hz), 7.12 (dd, 6-ArH, $J = 8.6$ and 2.3 Hz), 7.31 (d, 2-ArH, $J = 2.3$ Hz).

2-(3-CHLORO-4-HYDROXYPHENYL)-2-METHYL-1-PROPANOL (**8**) (B-9)

An ether solution containing 21.5 g (0.1 mol) of 2-(3-chloro-4-hydroxyphenyl)-2-methylpropionic acid was added to a suspension of 9.0 g (0.24 mol) of lithium aluminum hydride in ether. After the addition was completed the reaction was refluxed for 90 min. The excess hydride was decomposed by the addition of ethyl acetate. Dilute hydrochloric acid was added and the mixture extracted with ether. The ether extracts were dried over magnesium sulfate, the solvent removed and the residue triturated with hexane. Recrystallization from ether+hexane yielded 10.8 g, m.p. 97–100 °C: n.m.r. (acetone-d_6) δ 1.28 (s, CH_3CCH_3), 3.6 (broad s, CH_2 and OH), 6.96 (d, 5-ArH, $J = 8.2$ Hz), 7.23 (dd, 6-ArH, $J = 8.2$ and 2.0 Hz), 7.39 (d, 2-ArH, $J = 2.0$ Hz); m.s. of bis-TMS derivative Fig. 1(d).

2-(3-CHLORO-4-METHOXYPHENYL)-2-METHYL-PROPIONALDEHYDE (**9**)

2-(3-Chloro-4-methoxyphenyl)-2-methyl-1-propanol was oxidized with chromium trioxide and pyridine in methylene chloride.[7] The crude aldehyde could be used in subsequent reactions without purification or could be purified by chromatography on alumina with carbon tetrachloride, albeit with considerable loss due to conversion to 2-(3-chloro-4-methoxyphenyl)-2-methyl-1-propanol: n.m.r. (CCl_4) δ 1.37 (s, CH_3CCH_3), 3.80 (s, OCH_3), 6.81 (d, 6-ArH, $J = 8.2$ Hz), 6.97 (dd, 5-ArH, $J = 8.2$ and 2.0 Hz), 7.18 (d, 2-ArH, $J = 2.0$ Hz), 9.30 (s, CHO).

2-(3-CHLORO-4-HYDROXYPHENYL)-2-METHYL-PROPIONALDEHYDE (**10**)

An excess of boron tribromide was added to a hexane solution of 2-(3-chloro-4-methoxyphenyl)-2-methylpropionaldehyde at 0 °C with stirring. After the addition was completed, the mixture was allowed to warm to room temperature and stirred for 30 min, then poured into ice water and extracted with ether. Nonphenolic material was removed by a sodium hydroxide-acidification-ether extraction sequence. The ether extracts were dried over magnesium sulfate. The solvent was removed and the product used without purification.

2-[3-CHLORO-4-[(DICHLOROPHOSPHINYL)OXY]-PHENYL]-2-METHYLPROPIONYL CHLORIDE (**11**)

A mixture of 0.2 g 2-(3-chloro-4-hydroxyphenyl)-2-methylpropionic acid, 25 ml phosphorus oxychloride and 2 ml pyridine was stirred at room temperature for 1 h. Anhydrous ether was added and the mixture filtered. The solvent and excess phosphorus oxychloride were removed at reduced pressure (20 Torr). The crude material was used without purification.

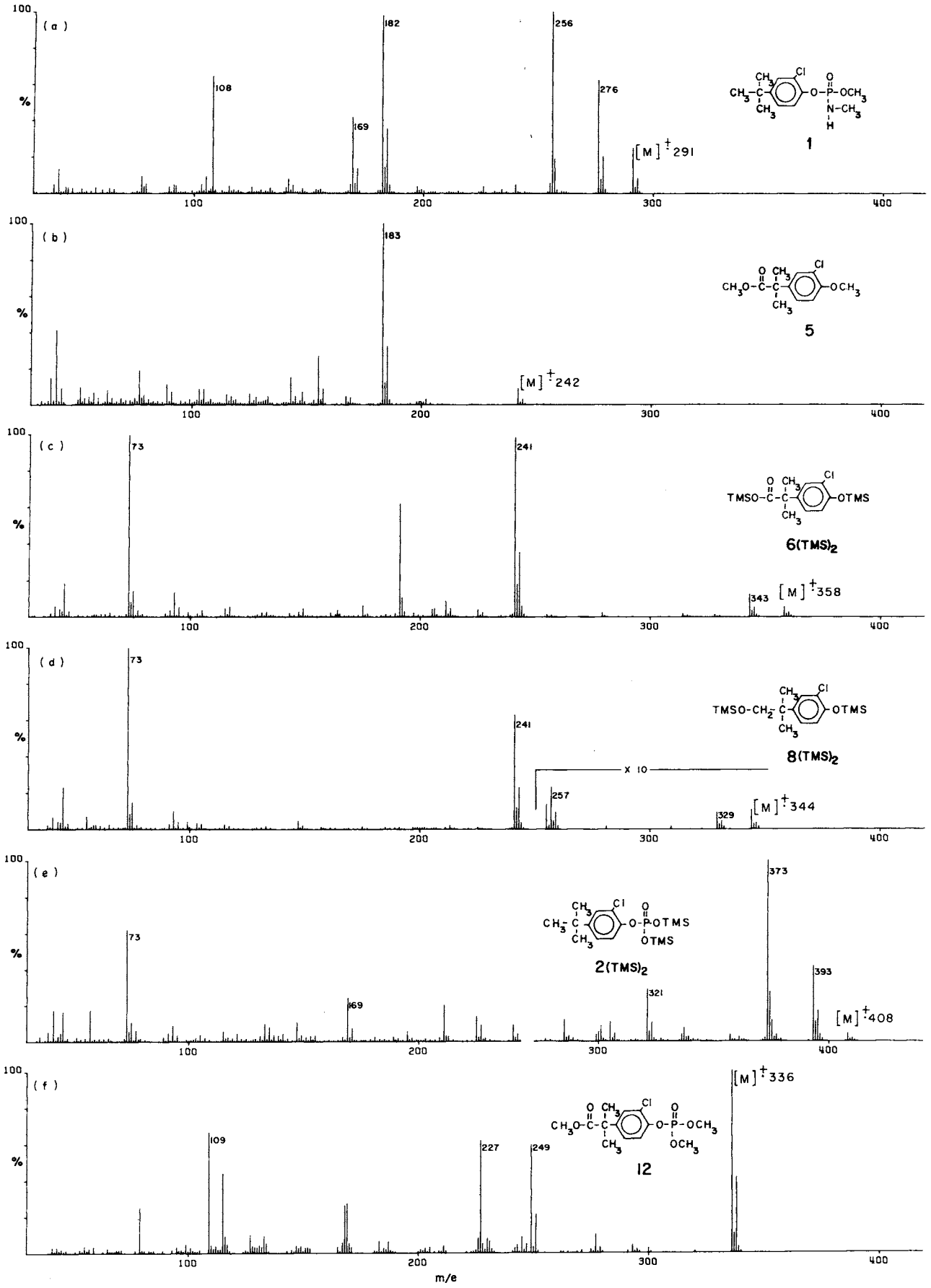


FIG. 1. 70 eV spectra of compounds used in the identification of crufomate metabolites.

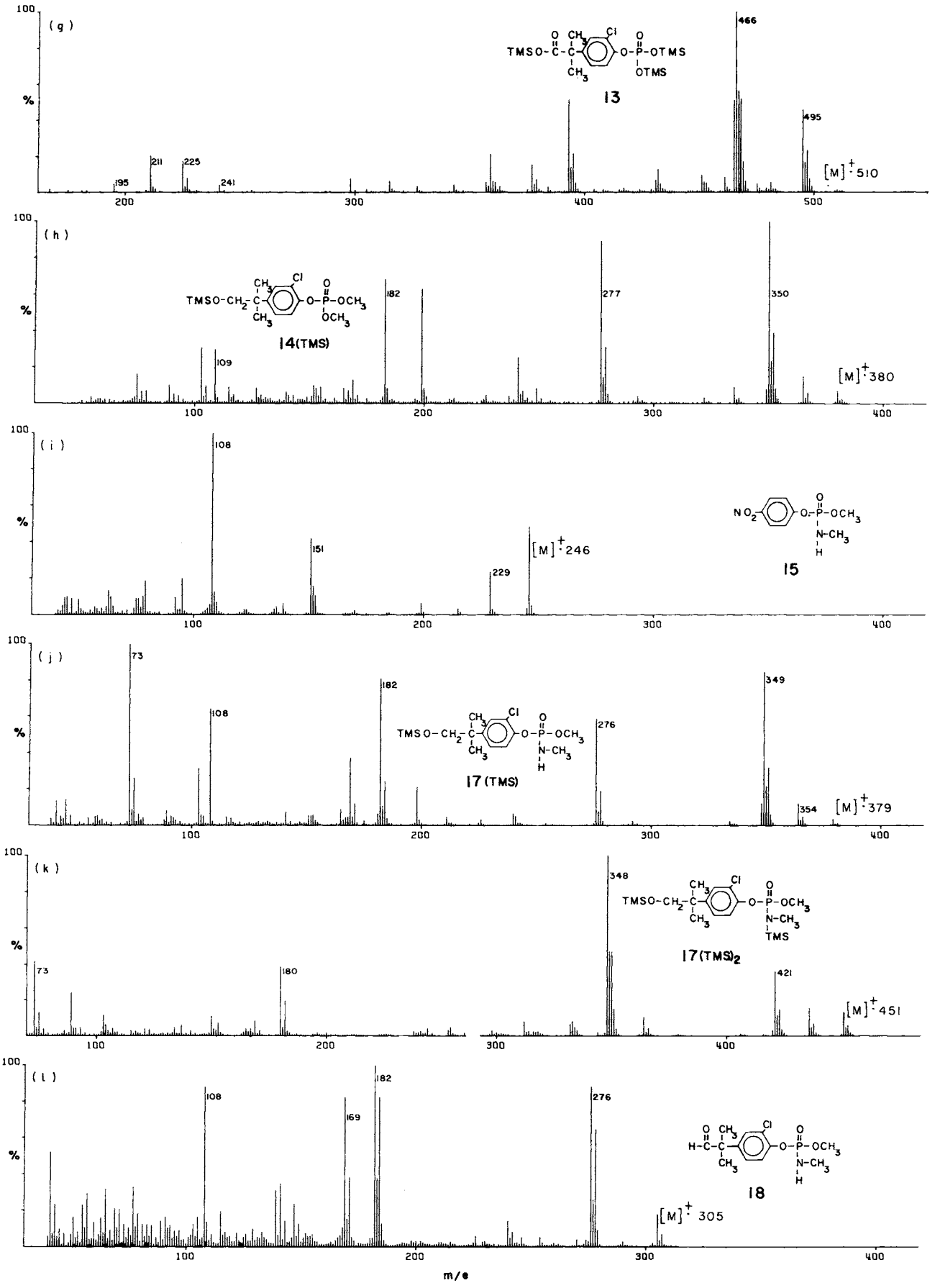


FIG. 1 (*continued*) (g–l)

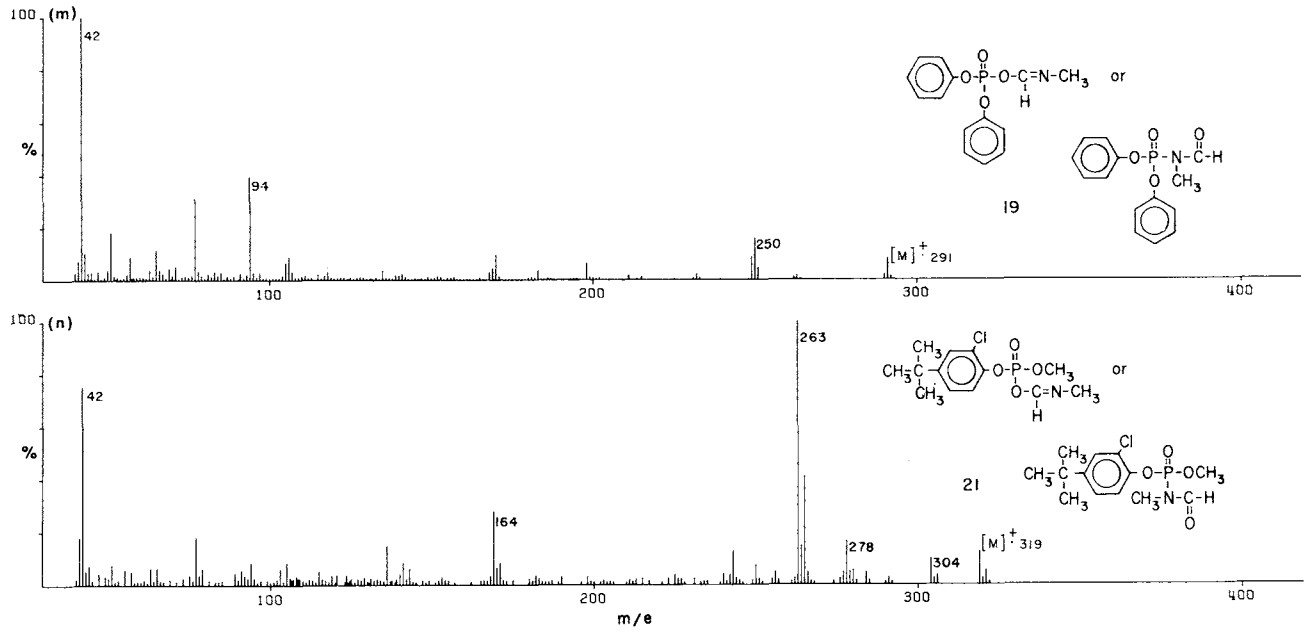


FIG. 1 (*continued*) (m and n)

METHYL 2-[3-CHLORO-4-[(DIMETHOXYPHOSPHINYL)-OXY]PENYL]-2-METHYLPROPIONATE [C(CH$_3$-4(CH$_3$)$_2$] (**12**)

A solution of 100 ml methanol and 5 ml pyridine was added to the crude 2-[3-chloro-4-[(dichlorophosphinyl)oxy]phenyl]2-methylpropionyl chloride obtained above and the reaction was stirred for 1 h. The solvent was removed at reduced pressure, water was added and the mixture extracted with ether. The ether extracts were dried over magnesium sulfate and the solvent was removed. Gas chromatography (10% OV-1, Gas-Chrom Q, 150–250 °C at 10 ° min^{-1}) indicated that the residue contained approximately 90% of the desired compound; m.s. Fig. 1(f).

TRIMETHYLSILYL 2-[[[4-BIS(TRIMETHYLSILYLOXY)-PHOSPHINYL]OXY]-3-CHLOROPHENYL]-2-METHYLPROPIONATE (**13**) [C(TMS)-4(TMS)$_2$]

A solution of 100 ml tetrahydrofuran, 5 ml pyridine, 25 ml water, and the crude 2-[3-chloro-4-[dichlorophosphinyl)oxy]phenyl]-2-methylpropionyl chloride obtained above was stirred at room temperature for 2 h. The solvents were removed at reduced pressure and the residue was evacuated at 0.05 Torr for 3 h. The residue, after reacting with bis-trimethylsilyltrifluoroacetamide containing 1% trimethylchlorosilane for several days, yielded primarily one peak on g.c. (2% OV-1, Gas-Chrom Q, 150–250 °C at 5 ° min^{-1}); m.s. Fig. 1(g).

2-CHLORO-4-(2-HYDROXY-1,1-DIMETHYLETHYL)-PHENYL DIMETHYL PHOSPHATE (**14**)

An ether solution containing 0.64 g (0.02 mol) of methanol and 2.02 g (0.02 mol) of triethylamine was added to an ether solution containing 1.53 g (0.01 mol) of phosphorus oxychloride at 0 °C. The reaction was allowed to warm to room temperature and was stirred for 30 min. The triethylamine hydrochloride was removed by filtration and $\frac{1}{4}$ of the crude product was added to an ether solution of sodium 2-chloro-4-(2-hydroxy-1,1-dimethylethyl)phenolate prepared from 0.5 g (0.0025 mol) of 2-(3-chloro-4-hydroxyphenyl)-2-methyl-1-propanol and 0.0025 mol of sodium hydride dispersion. The reaction was stirred for 15 h, treated with charcoal and the solvent removed. Column chromatography yielded, in addition to 2-(3-chloro-4-hydroxyphenyl)-2-methyl-1-propanol, a mixture of 2-chloro-4-(2-hydroxy(1,1-dimethylethyl)-phenyl dimethyl phosphate and 2-(3-chloro-4-hydroxyphenyl)-2-methylpropyl dimethyl phosphate. Characterization of these compounds was based on mass spectral interpretation of samples trapped from the g.c. after derivation with either diazomethane or bis-trimethylsilyltrifluoroacetamide containing 1% trimethylchlorosilane; m.s. of TMS derivative Fig. 1(h).

4-NITROPHENYL PHOSPHORODICHLORIDATE (**15**)

4-Nitrophenol (41.7 g, 0.3 mol) was added with external cooling to a solution of 150 ml of phosphorus oxychloride and 48 ml of pyridine. The reaction was stirred at room temperature for 4 h, ether was added and the pyridine hydrochloride removed by filtration. The solvent was removed and the residue distilled at reduced pressure; material boiling 130–135 °C at 0.05 Torr was of adequate purity for subsequent reactions.

METHYL 4-NITROPHENYL METHYLPHOSPHORAMIDATE (**16**)

A solution of 46.2 g (0.18 mol) of 4-nitrophenyl phosphorodichloridate in 350 ml of carbon tetrachloride was reacted with 6.4 g (0.20 mol) of methanol

in 50 ml of carbon tetrachloride. The reaction was stirred for 30 min, then purged with nitrogen to remove hydrogen chloride. The product began to crystallize; it was redissolved by addition of carbon tetrachloride and methylene chloride. Methylamine was bubbled through this solution until 15 g had been absorbed; external cooling was used to keep the solution below 30 °C. The reaction was stirred at room temperature for 30 min, then ice, water and methylene chloride were added. The organic layer was removed, washed with water and dried over magnesium sulfate. The solvent was removed and the residue was recrystallized from methylene chloride + hexane or benzene + cyclohexane to yield 27.3 g of product m.p. 62–65 °C: n.m.r. (acetone-d_6) δ 2.61 and 2.70 (two d, $PNCH_3$ invertamers, J(PNCH) = 12.8 Hz), 3.82 (d, $POCH_3$, J(POCH) = 11.7 Hz), 4.4–5.0 (diffuse, NH), 7.52 (d, 2- and 6-ArH, J = 9.3 Hz), 8.29 (d, 3- and 5-ArH, J = 9.3 Hz); m.s. Fig. 1(i).

2-CHLORO-4-(2-HYDROXY-1,1-DIMETHYLETHYL)-PHENYL METHYL METHYLPHOSPHORAMIDATE (**17**) (B-1)

A hexane solution containing 0.01 mol of butyllithium was added to a solution of 2.0 g (0.01 mol) of 2-(3-chloro-4-hydroxyphenyl)-2-methyl-1-propanol in 175 ml of tetrahydrofuran. The solution was stirred at room temperature for 10 min and then added to a solution of 2.5 g (0.01 mol) of methyl 4-nitrophenyl methylphosphoramidate in 100 ml of tetrahydrofuran. The reaction was stirred for 4 h and the solvent removed at reduced pressure. Water was added and the reaction extracted with ether. The ether extracts were washed with water until the washings were no longer yellow and then with sodium bicarbonate. The extracts were dried over magnesium sulfate, the solvent removed and the residue chromatographed on alumina with ethyl acetate. The product could not be obtained in crystalline form: n.m.r. (acetone-d_6) δ 1.28 (s, CH_3CCH_3), 2.60 and 2.70 (two d, $PNCH_3$ invertamers, J(PNCH) = 12.4 Hz), 3.56 (s, CH_2), 3.79 (d, $POCH_3$, J(POCH) = 11.3 Hz), 4.1–4.7 (diffuse OH and NH), 7.2–7.6 (aryl protons inadequately resolved for assignments). Gas chromatography (3% OV-1, Gas-Chrom Q, 150–250 °C at 5 °C min^{-1}) after reaction with bis-trimethylsilyltrifluoroacetamide containing 1% trimethylchlorosilane yielded peaks for the mono- and bis-trimethylsilyl derivatives; m.s. Fig. 1(j) and 1(k).

2-CHLORO-4-(1-FORMYL-1-METHYLETHYL)PHENYL METHYL METHYLPHOSPHORAMIDATE (**18**)

Sodium hydride was added at room temperature to a tetrahydrofuran solution containing 1 g of crude 2-(3-chloro-4-hydroxyphenyl)-2-methylpropionaldehyde until the evolution of hydrogen ceased. An excess of methyl 4-nitrophenyl methylphosphoramidate was added and the mixture stirred for 2 h. Ether was added and the solid removed by filtration. The residue was chromatographed on silica gel with methylene chloride. A mixture of the desired aldehyde and methyl 4-nitrophenyl methylphosphoramidate was eluted as shown by n.m.r. and g.c. (2% OV-1, Gas-Chrom Q, 150–250 °C at 5 °C min^{-1}): n.m.r. (CCl_4) δ 1.38 (s, CH_3CCH_3), 2.54 and 2.63 (two d, $PNCH_3$ invertamers, J(PNCH) = 12.7 Hz), 3.73 (d, $POCH_3$, J(POCH) = 11.3 Hz), assignments could not be made on aryl protons, 9.34 (s, CHO); m.s. Fig. 1(l).

DIPHENYL FORMYLMETHYLPHOSPHORAMIDATE OR (METHYLIMINO)-METHYL DIPHENYL PHOSPHATE (**19**)

Method A

A solution of 0.59 g (0.01 mol) of *N*-methylformamide in 50 ml of tetrahydrofuran was added to a suspension of 0.42 g (0.01 mol) of 57% sodium hydride dispersion in 50 ml of tetrahydrofuran and the mixture was refluxed for 30 min. The mixture was cooled to room temperature and a solution of 2.68 g (0.01 mol) diphenyl phosphorochloridate in 20 ml of tetrahydrofuran was added. The mixture was stirred for 2 h, charcoal was added and the solids removed by filtration. The solvent was removed at reduced pressure and the remaining oil purified by g.c. (2% OV-1, Gas-Chrom Q, 150–250 °C at 10 °C min^{-1}): n.m.r. (CCL_4) δ 2.98 (d, NCH_3, J(PNCH) = 8.6 Hz), 7.16 (m, aromatic), 8.73 (s, CHO); m.s. Fig. 1(m).

Method B

A modification of the method of Nikonorov *et al.*[8] was used. A solution of 1 g of diphenyl methylphosphoramidate, 5 ml of chloral and 3 drops of concentrated sulfuric acid was allowed to stand at room temperature for 16 h. The excess chloral was removed by evaporation with a jet of nitrogen and the remaining residue refluxed for 4 h with an excess of triethylamine. The triethylamine was removed by evaporation with a jet of nitrogen and the residue was purified by g.c. (10% OV-1, Gas-Chrom Q 170–250 °C at 5 °C min^{-1}). The mass spectrum was identical to that of the product obtained by method A.

DIPHENYL FORMYLPHOSPHORAMIDATE OR IMINOMETHYL DIPHENYL PHOSPHATE (**20**)

When *N*-methylformamide was replaced by formamide in method A above, an unstable oil was obtained which lost hydrogen cyanide on standing and formed diphenyl hydrogen phosphate. A sample of the oil, when reacted with diazomethane immediately after the solvent had been removed, yielded a more stable methyl derivative; this compound had a mass spectrum identical to that of diphenyl formylmethylphosphoramidate.

4-*t*-BUTYL-2-CHLOROPHENYL METHYL FORMYLMETHYLPHOSPHORAMIDATE OR 4-*t*-BUTYL-2-CHLOROPHENYL IMINOMETHYL METHYL PHOSPHATE (**21**) (A-7)

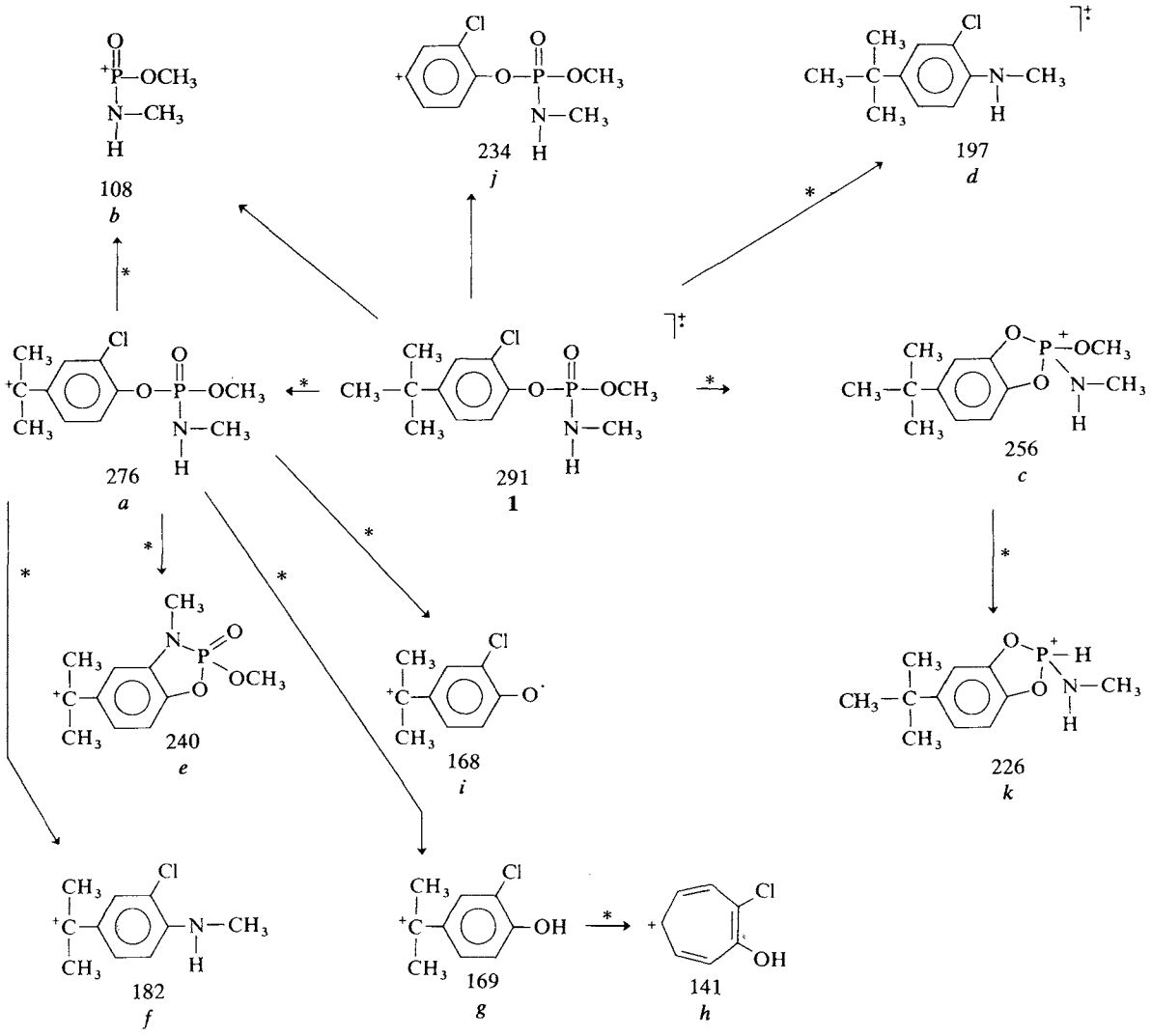
4-*t*-Butyl-2-chlorophenyl methyl phosphorochloridate was prepared by reaction of 3.0 g (0.01 mol) of 4-

t-butyl-2-chlorophenyl phosphorodichloridate with 0.35 g (0.011 mol) of methanol in carbon tetrachloride.[10] The reaction was monitored by g.c. (2% OV-17, Gas-Chrom Q, 200 °C) to determine the relative amounts of the dichloro, methoxychloro and dimethoxy compounds present.

A benzene solution containing 0.0065 mol of sodium *N*-methylformamide (13 ml of a reaction of 0.057 mol of sodium hydride and 0.05 mol of *N*-methylformamide in 100 ml of benzene) was added to a benzene solution of crude 4-*t*-butyl-2-chlorophenyl methyl phosphorochloridate calculated to contain 0.006 mol of 'chloro function' (a typical sample contained 60% methoxychloro, 20% dichloro and 20% dimethoxy). The reaction was allowed to warm to room temperature and then stirred for 1 h. Charcoal was added and the mixture filtered. The solvent was removed at reduced pressure, the residue dissolved in ether and a small amount of insoluble material was removed by filtration. The solvent was removed and the resulting oil separated into three compounds by g.c. (10% OV-1, Gas-Chrom Q, 200 °C) in a ratio of 1:1:2. Mass spectrometry provided evidence for the following compounds: 4-*t*-butyl-2-chlorophenyl dimethyl phosphate, crufomate and methyl 4-*t*-butyl-2-chlorophenyl methyl formylmethylphosphoramidate. An n.m.r. spectrum of the crude mixture also suggested that the oil contained 50% of the desired compound: i.r. (KBr) 1705 cm^{-1}; n.m.r. ($CDCl_3$) δ 1.3 [2, $C(CH_3)_3$], 3.04 (d, NCH_3, J(PNCH) = 8.6 Hz), 3.95 (d, OCH_3, J(POCH) = 11.8 Hz), 7.27 (m, aromatic), 8.85 (s, CHO); m.s. Fig. 1(n).

Results and discussion

The typical cleavage reactions of alkyl phosphates, involving the loss of an alkyl or alkoxy radical,[2–5] were either not observed or were of minor importance in the fragmentation of singly charged ions in the set of aromatic phosphoramides and phosphates examined here. A more important fragmentation mode was cleavage between the aryl oxygen and phosphorus with



SCHEME 1. Suggested fragmentation routes for crufomate.
* Observed metastables.

either portion retaining the charge. Alternatively, these ions may have resulted from fragmentation of doubly charged ions which were quite abundant in many of these spectra. The metabolites contained an aryl group with either a *t*-butyl or an oxidized *t*-butyl group. These groups had dominant effects on fragmentation.

CRUFOMATE

Scheme 1 suggests fragmentation routes for the formation of the major ions observed in the mass spectrum of crufomate. Compositions of ions *f*, *g* and *h* were established by precise mass determinations (< 3 ppm). All the proposed structures are compatible with the spectrum of N-CD_3 crufomate; all ions except *g*, *h* and *i* increased by 3 mass units.

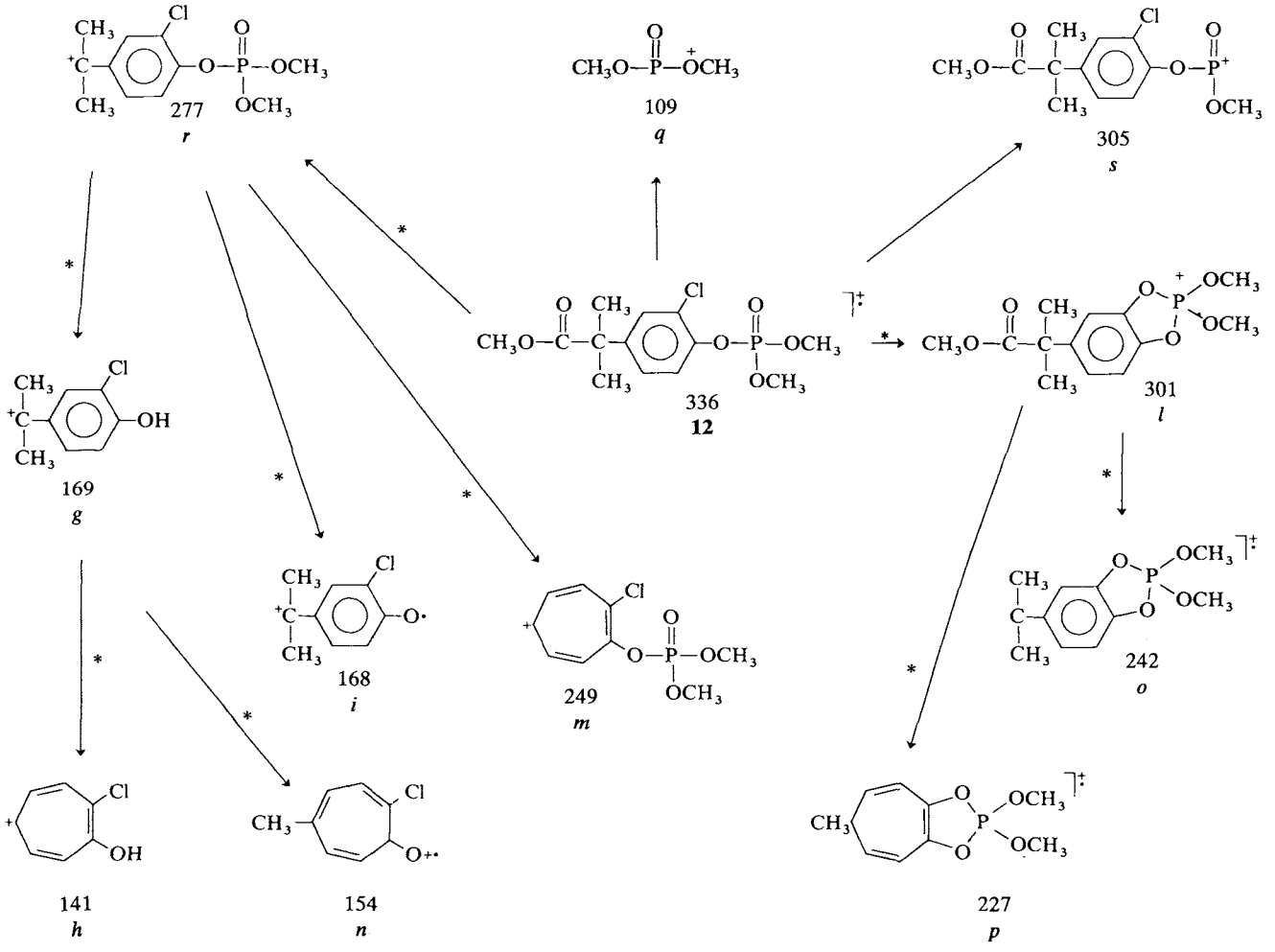
t-BUTYL OXIDATION PRODUCTS

Several isolated metabolites resulted from biological oxidation of the *t*-butyl group.[1] Representative compounds having the possible oxidation states of the *t*-butyl group were synthesized. A fragmentation scheme for the methyl ester of an acid is presented in Scheme 2. A dominant feature of the mass spectra of these compounds was the loss of the oxidized portion of the *t*-butyl group (i.e. 44, 29 and 31 for the acid, aldehyde and alcohol, respectively). The methyl esters of the acids fragmented as suggested by the example in Scheme 2; however, trimethylsilyl (TMS) derivatives of the acids and alcohols fragmented quite differently and are discussed in the next section.

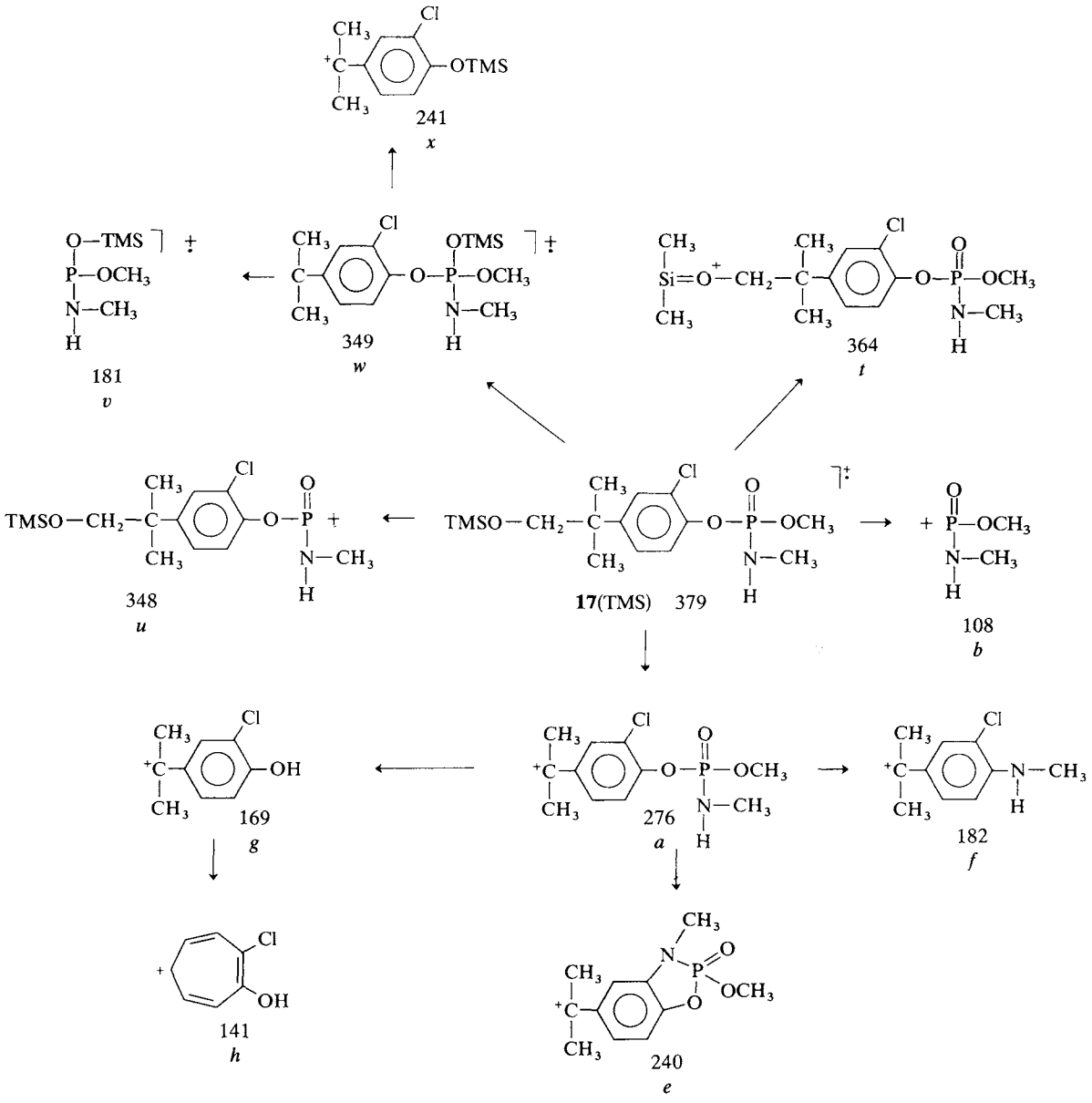
The aldehyde with crufomate substituents on phosphorus (**18**) was synthesized because a metabolite with the appropriate molecular ion was isolated (later shown to be the *N*-formyl compound) and because the aldehyde might be an expected intermediate in the oxidation sequence.[1] The instability of the aldehyde probably explained why it was not isolated as a metabolite.

REARRANGEMENT OF TRIMETHYLSILYL DERIVATIVES

Scheme 3 suggests fragmentation routes that are typical for the TMS derivatives of the phosphorus-containing *t*-butyl oxidation products. Migration of a TMS group from the oxidized *t*-butyl portion to the phosphorus portion occurred in all compounds in

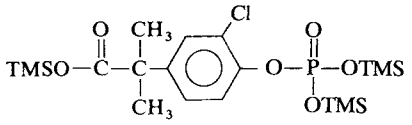


SCHEME 2. Typical fragmentation routes for crufomate metabolites that have been oxidized at the *t*-butyl groups.
*Observed metastables.



SCHEME 3. Typical fragmentation routes for TMS derivatives of phosphorus-containing *t*-butyl oxidation products.

which the *t*-butyl group contained either a TMS ester or ether. Fragment ions due to TMS migration and concurrent loss of CO_2 or CH_2O, i.e. *w*, Scheme 3, yielded large peaks (often the base peak) in the spectra of all compounds of this type. The CO_2 and CH_2O losses were established by precise mass determinations (< 3 ppm). Further evidence for the TMS rearrangement was obtained by precise mass determination (< 3 ppm) of ions with compositions of $P(OTMS)_3$ and $TMSOPOCH_3[N(CH_3)TMS]$ from **13** and **17** $(TMS)_2$, respectively.



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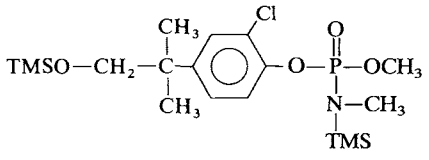
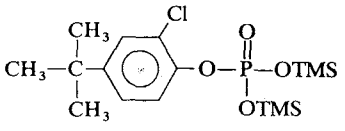


17 $(TMS)_2$



2 $(TMS)_2$

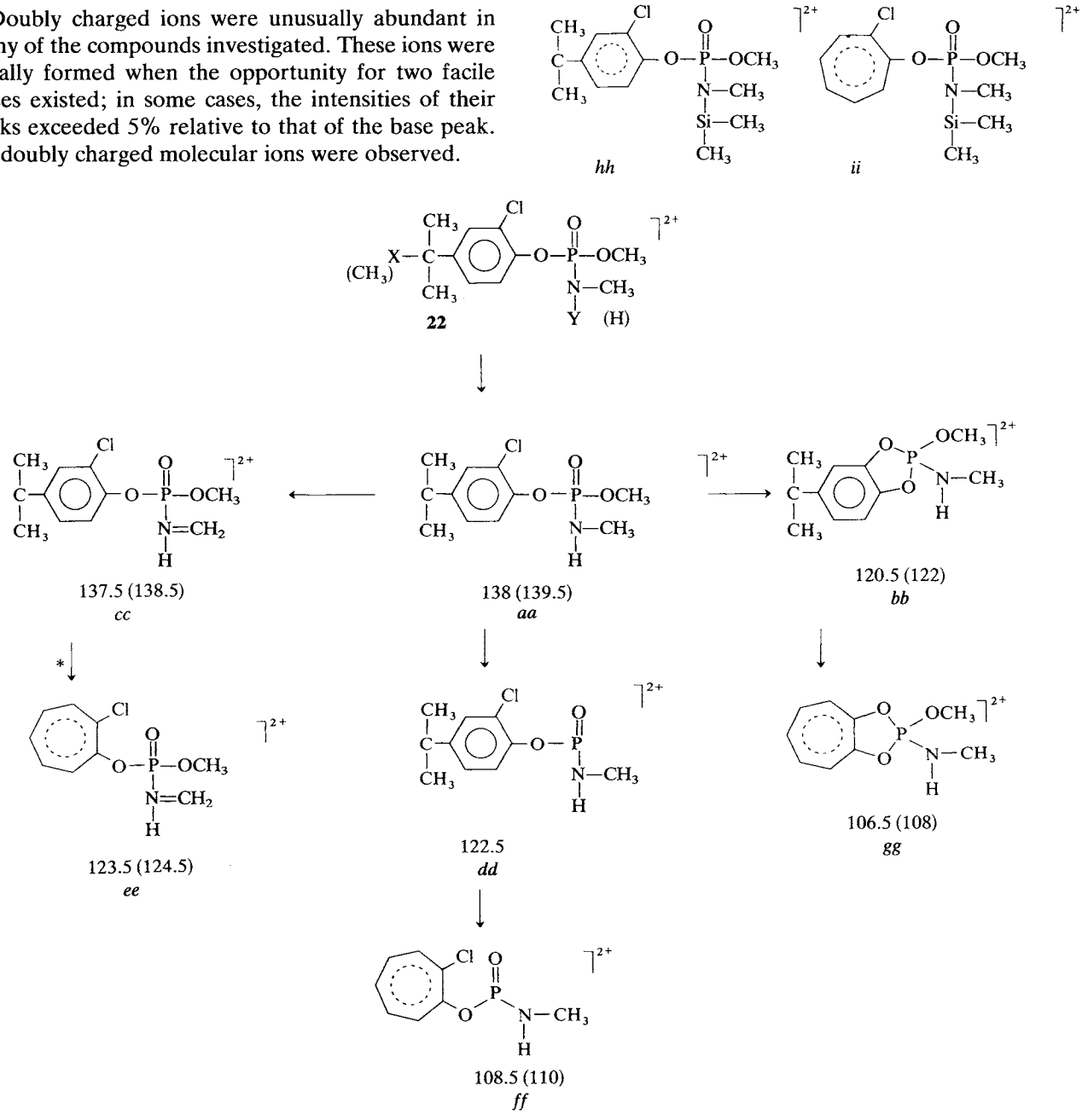
Although most of the ion current in the spectrum of **2** $(TMS)_2$ was carried by $[M-Cl]^+$ and $[M-CH_3]^+$ ions, precise mass determinations and $[^2H_9]$TMS labeling showed that **2** $(TMS)_2$ underwent rearrangements

similar to those observed by Zinbo *et al.* for $(TMSO)_3PO$.[6] [$^2H_9$]TMS labeling and peak-match studies on ions *m/e* 195, 211 and 225 provided evidence for the following fragments: $C_4H_{12}O_3PSi_2$ (8 ppm), $C_4H_{12}O_4PSi_2$ (1 ppm) and $C_6H_{18}O_3PSi_2$ (3 ppm). Evidence was also obtained for the loss of butene and a TMS methyl group to yield $C_{11}H_{19}ClPO_4Si_2$ (3 ppm), and for migration of a TMS group to the phenol oxygen and loss of methyl from the *t*-butyl group to yield *x* (Scheme 3) $C_{12}H_{18}ClOSi$ (1 ppm). The $[M-CH_3]^+$ peak was shown by [$^2H_9$]TMS labeling to involve methyl losses from the *t*-butyl and TMS groups in a 1:2 ratio.

Several doubly charged ions were diagnostic for the portion of the crufomate molecule represented by **22** (Scheme 4). Structures for some of these ions observed in the crufomate spectrum are proposed in Scheme 4; similar ions were observed when X and Y of **22** had the following compositions: CH_3, H; CH_2OH, H; CH_2OTMS, TMS; CHO, H; CH_3, CHO. Crufomate that was labeled with deuterium in the *N*-methyl group yielded ions that were compatible with the structures proposed in Scheme 4 and ions suggesting scrambling of the N-H and N-CH_3 hydrogens. Compounds with a TMS group on the phosphorus portion of the molecule, i.e. **2**, also yielded ions such as *hh* and *ii*.[1]

DOUBLY CHARGED IONS

Doubly charged ions were unusually abundant in many of the compounds investigated. These ions were usually formed when the opportunity for two facile losses existed; in some cases, the intensities of their peaks exceeded 5% relative to that of the base peak. No doubly charged molecular ions were observed.



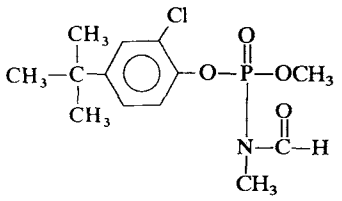
SCHEME 4. Fragmentation of doubly charged ions of crufomate. Parenthetical values were observed for the N-CD_3 derivative. The C_2H_4 loss from *cc* to *ee* was established by precise mass measurements.
*Observed metastable.

Other compounds yielding detectable doubly charged ions were: (1) 2-[3-chloro-4-[trimethylsilyl)-oxy]phenyl]-2-methyl-1-[(trimethylsilyl)oxy] propane, **8**$(TMS)_2$, $[M-2CH_3]^{2+}$, $[M-(CH_2OTMS+CH_3)]^{2+}$; (2) 2-chloro-4-[1, 1-dimethyl-2-[(trimethylsilyl)oxy]ethyl]phenyl methyl trimethylsilyl phosphate, $[M-(CH_2OTMS+CH_3)]^{2+}$, $[M-(CH_2OTMS+CH_3+C_2H_4)]^{2+}$; (3) 2-chloro-4-[1, 1-dimethyl-2-[(trimethylsilyl)oxy]ethyl]phenyl bis-(trimethylsilyl) phosphate,[1] $[M-(CH_2OTMS+CH_3)]^{2+}$; (4) trimethylsilyl derivative of crufomate, $[M-2CH_3]^{2+}$.

FORMYLPHOSPHORAMIDATES OR IMINOMETHYL PHOSPHATES

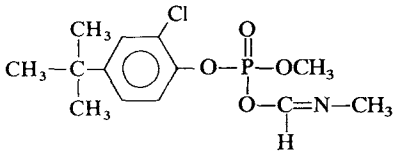
Scheme 5 summarizes the approaches to the synthesis of **24**, a model compound synthesized to aid in proving structures of metabolites B-6, B-7 and B-8.[1] The most consistently successful method of synthesis was the reaction of sodium *N*-methylformamide with a phosphorochloridate. The method of Nikonorov, synthesis via the chloral adduct,[8] failed in several attempted syntheses but yielded a small amount of **24** on one occasion. The product from the Nikonorov reaction was identical to that prepared through the sodium *N*-methylformamide method.

Compound **23** was unstable, liberating hydrogen cyanide and forming diphenyl hydrogen phosphate. Reaction of crude **23** with diazomethane immediately after its formation gave a low yield of **24**. The *N*-methyl derivative was more stable; it could be purified by g.c.



25-A

or

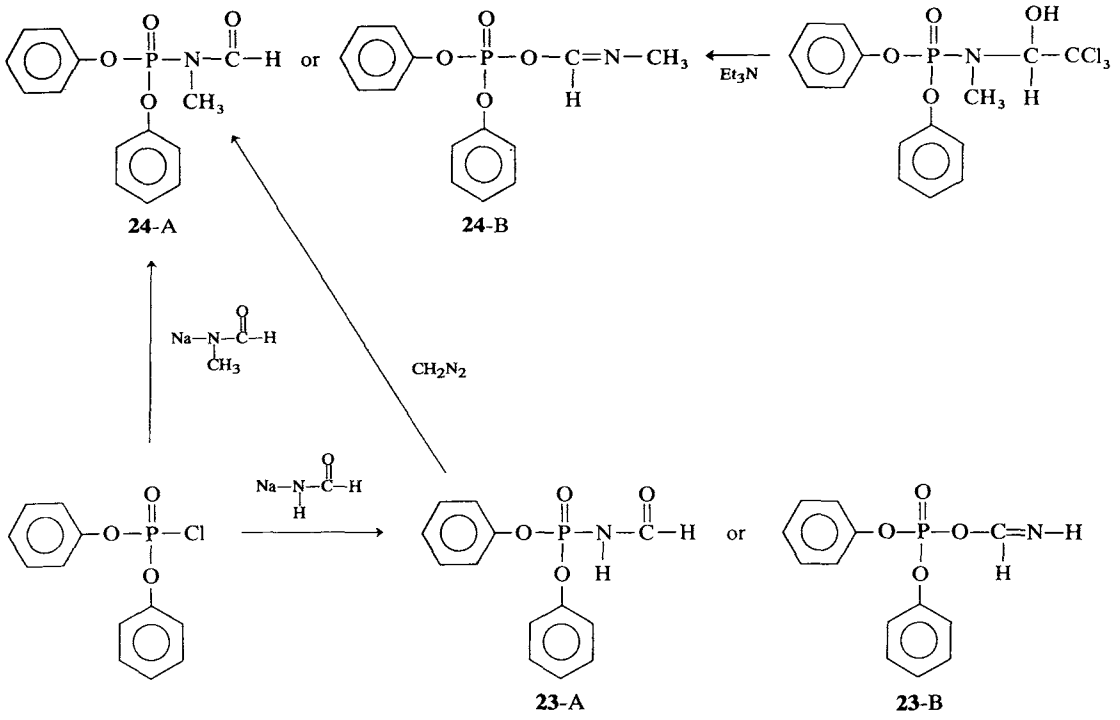


25-B

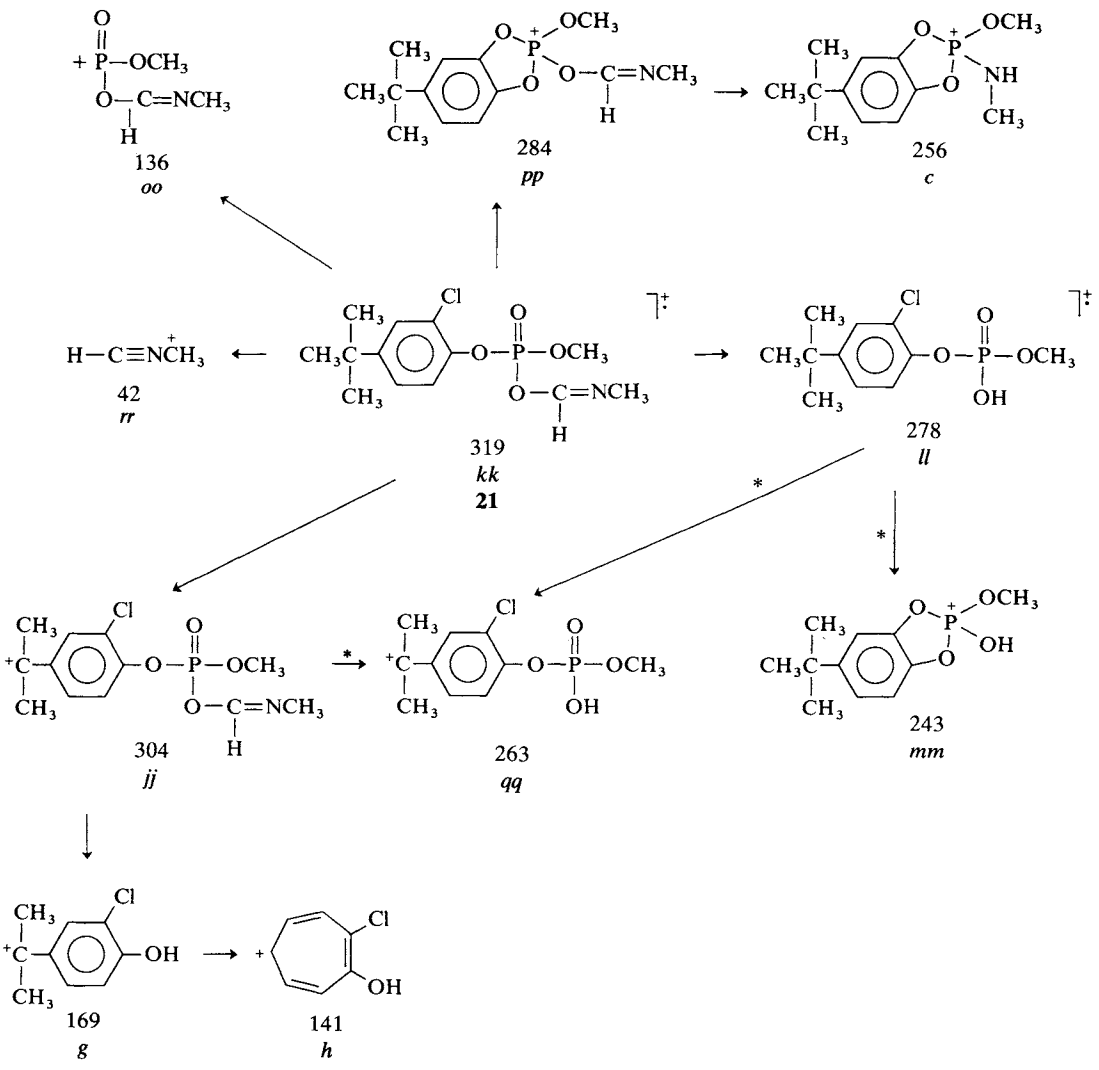
The sodium *N*-methylformamide method of Scheme 5 was used, in the synthesis of **25**.

Scheme 6 summarizes the mass spectral data obtained on **25**. Precise mass measurements established the loss of C_2H_3N as a major fragmentation route. Deuterium labeling (N-CD_3 and O-CD_3) established the source of the C_2H_3N loss and the source of the $[C_2H_4N]^+$ ion. Peaks due to ions *jj*, *kk*, *ll*, *mm*, *oo*, *pp* and *qq* increased by 3 mass units when the *O*-methyl was labeled with deuterium, whereas peaks due to ions *kk*, *oo*, *pp* and *rr* increased by 3 mass units when the *N*-methyl was labeled with deuterium. These fragmentation routes suggested the rearrangement of **26** to **27**, Scheme 7, either before or after electron impact.

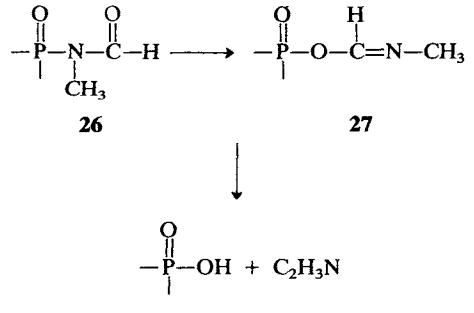
Infrared and n.m.r. spectra of **24** and **25** were similar to those reported by Terry and Borkovec[9] for the



SCHEME 5



SCHEME 6. Suggested fragmentation routes for *N*-formyl phosphoramidates or methanimidoyl phosphates. *Observed metastables.



SCHEME 7

oxidation product of hexamethylphosphoramide for which they claimed the *N*-formyl structure, *N*-bis-(dimethylamino)-phosphinyl-*N*-methylformamide. The 'carbonyl' absorption was found at 1705 cm^{-1} for **24** and **25**.The n.m.r. spectra of **24** and **25** showed slightly broadened singlets for the 'aldehydic' proton (δ 8.73 for **24** in CCl_4 and δ 8.85 for **25** in $CDCl_3$) and doublets (J(PNCH) = 8.6 Hz) for the *N*-methyl protons (δ 2.98 for **24** and δ 3.04 for **25**). *Syn-anti* type isomerization of an imino structure is an alternative explanation for the *N*-methyl doublets; however, under these circumstances a doublet would be expected for OCH=N proton. Thus the i.r. spectra are compatible with either the *N*-formyl or imino structure, while n.m.r. spectra provide limited support for the *N*-formyl structure without eliminating the imino structure.

Reaction of **24** with phenyllithium in either yielded 1,1-diphenyl-*N*-methylmethanamine but no diphenylmethanol; this result indicated that under the reaction conditions used, the imino structure, **24**-B, was far more important than the *N*-formyl structure, **24**-A. No evidence was obtained on a possible equilibrium between the two structures.

Logically, the *N*-formyl is the structure formed during metabolism with rearrangement to the imino structure taking place before or after excretion.

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