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STUDIES ON THE METABOLISM OF THE NEUROTOXIC TRI-*o*-CRESYL PHOSPHATE. SYNTHESIS AND IDENTIFICATION BY INFRARED, PROTON NUCLEAR MAGNETIC RESONANCE AND MASS SPECTROMETRY OF FIVE OF ITS METABOLITES*

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SUMMARY

Five metabolites of the industrial neurotoxic chemical tri-*o*-cresyl phosphate (TOCP) were synthesized and their structures were verified by infrared, IR; proton nuclear magnetic resonance, ^{1}H-NMR; and mass spectrometry. The 2 acids, *o*-cresyl dihydrogen phosphate and di-*o*-cresyl hydrogen phosphate were prepared in 2 steps. Step 1, $POCl_3$ was reacted with *o*-cresol, using 1:1 and 1:2 molar ratios, in the presence of anhydrous $AlCl_3$ as a catalyst, to form the 2 intermediates *o*-cresyl phosphorodichloridate and di-*o*-cresyl phosphorochloridate, respectively. Step 2, the chloridate intermediates were hydrolyzed under the appropriate condition to the corresponding acids. These acids were further derivatized to the corresponding methyl ester and the products were analyzed by the spectroscopic techniques. Saligenin cyclic-*o*-tolyl phosphate[2-(*o*-cresyl)-4*H*-1:3:2-benzodioxaphosphoran-2-one] was synthesized by reacting the potassium salt of *o*-hydroxybenzyl alcohol with *o*-cresyl phosphorodichloridate. Hydroxymethyl TOCP [di-*o*-cresyl *o*-hydroxymethylphenyl phosphate] and dihydroxymethyl TOCP [*o*-cresyl di-*o*-hydroxymethylphenyl phosphate] were synthesized by

*A preliminary account of this work has been presented [23].

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Abbreviations: HPLC, high performance liquid chromatography; IR, infrared; NMR, nuclear magnetic resonance; OPIDN, organophosphorus-induced delayed neurotoxicity; TLC, thin-layer chromatography; TOCP, tri-*o*-cresyl phosphate.

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reacting di-*o*-cresyl phosphorochloridate with the potassium salt of *o*-hydroxybenzyl alcohol. The products were separated and purified by repeated preparative thin-layer chromatography (TLC) using 3 different solvent systems. The purity of the 5 metabolites, which was determined by high performance liquid chromatography (HPLC), ranged from 92% to 99%.

Keywords: Tri-*o*-cresyl phosphate; Saligenin cyclic-*o*-tolyl phosphate; Di-*o*-cresyl *o*-hydroxymethylphenyl phosphate; *o*-Cresyl di-*o*-hydroxymethylphenyl phosphate.

INTRODUCTION

Tri-*o*-cresyl phosphate (TOCP) is an industrial chemical used as a plasticizer in lacquers and varnishes [1]. TOCP is among the group of organophosphorus compounds known to cause a condition known as organophosphorus-induced delayed neurotoxicity (OPIDN) [2]. OPIDN was demonstrated in humans exposed to TOCP-adulterated food and experimentally in some animal species [2—5]. After a delay period of 6—14 days, clinical signs are observed; there is an initial ataxia followed by paralysis. Neuronal lesions are characterized by degeneration of axons. There is also a Wallerian-type degeneration of myelin in the central and peripheral nervous systems.

Although an estimated 40 000 cases of OPIDN in humans have been attributed to TOCP [2], its metabolism has received meager attention. *o*-Cresol was identified in the urine of cats and dogs treated with TOCP [6]. Saligenin cyclic-*o*-tolyl phosphate was isolated and identified as a metabolic product of TOCP in several species [7—10]. Also indirect evidences were obtained for the presence of hydroxymethyl TOCP and dihydroxymethyl TOCP as metabolites of TOCP [8].

No comprehensive metabolism study of TOCP in animals is available, probably because of the lack of availability of the possible metabolites as well as a specific analytical method for isolation and quantification of TOCP and its metabolites. In this study, simple methods for the synthesis and purification of 5 TOCP metabolites are reported. A comprehensive discussion of their IR, ^{1}H-NMR, and mass spectrometry is presented.

METHODS

Chemicals

Phosphorus oxychloride was purchased from Mallinckrodt, Inc., St. Louis, MO, *o*-cresol (99 + %), 2-hydroxybenzyl alcohol (salicyl alcohol, 97%), and ethylene glycol dimethyl ether (1,2-dimethyoxyethane, 99 + %) were obtained from Aldrich Chemical Company, Inc., Milwaukee, WI. Analytical plates (250 μm precoated silica gel G.F. 10 X 20 cm) and preparative plates

(1000 μm precoated silica gel G.F. 20 X 20 cm) Uniplate, Analtech, Inc., were obtained from Fisher Scientific, Raleigh, NC. All other chemicals used were of the highest purity available.

Synthesis of TOCP metabolites

1. o-Cresyl dihydrogen phosphate

This metabolite was synthesized in 2 steps. The precursor, *o*-cresyl phosphorodichloridate, was prepared followed by the hydrolysis of the chloridate to the acid.

a. o-Cresyl phosphorodichloridate. This intermediate was prepared according to a method described previously [11] with minor modifications. To a 500-ml round bottom three-necked flask fitted with a dropping funnel, thermometer, and a reflux condenser, 45 g (0.3 mol) of $POCl_3$ were added and the flask was immersed in an ice bath. The contents of the flask were stirred by magnetic stirrer, and 1 g of anhydrous $AlCl_3$ was added. Melted *o*-cresol, 32.4 g (0.3 mol), was added gradually to the cooled stirred mixture. The reaction mixture was slowly heated to 85°C and kept at this temperature until HCl gas was completely evolved (checked by pH indicator paper). The reaction mixture was heated to 120—140°C and kept at this temperature for 2 h. The product was found to be phenol free by TLC using hexane/acetone (9:1) as the solvent system. The chloridate was purified by distillation under vacuum; and the yield was 35 g (52%).

b. o-Cresyl dihydrogen phosphate. Two grams of *o*-cresyl phosphorodichloridate were hydrolyzed in a 100 ml of 5% aqueous sodium bicarbonate solution. The reaction mixture was stirred occasionally at room temperature until the cloudy oily droplets disappeared (approx. 2 h). The mixture was extracted with ether, and the ether layer was discarded. The aqueous phase was acidified to pH 1 using 9 N H_2SO_4, saturated with sodium sulfate, and extracted with 4 X 100 ml ether. The ether layer was dried over anhydrous $MgSO_4$, filtered and evaporated under vacuum. The yield was 1.5 g (90%). The purity of the acid was determined by HPLC [12] to be 96% based on peak area. To verify the chemical structure of the product, a portion of the acid was converted to its dimethyl ester by reacting with diazomethane in ether [13], and the ester was analyzed by the spectroscopic methods described in the method section.

2. Di-o-cresyl hydrogen phosphate

The synthesis of this compound was carried out in a similar manner to that described for the previous compound with the following modifications.

a. The intermediate, di-*o*-cresyl phosphorochloridate, was synthesized using a 2:1 molar ratio of *o*-cresol/$POCl_3$. The yield was 60% based on the theoretical product.
b. The hydrolysis of di-*o*-cresyl phosphorochloridate was carried out using 4 g of the chloridate in a 100 ml of 10% NaOH. The reaction was

stirred at room temperature for 5 h then acidified to pH 5 with 9 N H_2SO_4 and extracted with ether. Di-*o*-cresyl hydrogen phosphate was isolated as described above for *o*-cresyl dihydrogen phosphate. The product was 3.4 g (90%). To verify the structure of the product, a portion of the acid was reacted with diazomethane in ether, and the product was examined by spectroscopy. The purity of the acid was 99% by HPLC.

3. Saligenin cyclic o-tolyl phosphate

This compound was prepared and purified by a simpler method than that reported earlier [8]. To a solution of 2.82 g of 88% KOH (44 mmol) in 20 ml of deionized water, 5.69 g (44 mmol) of *o*-hydroxybenzyl alcohol (97%) were added and stirred until it completely dissolved. The solution was lyophilized to remove water, and the residue was suspended in 100 ml 1,2-dimethoxyethane (freshly opened). The suspension was added dropwise to a stirred solution of 5 g (22 mmol) *o*-cresyl phosphorodichloridate in 1,2-dimethoxyethane. The reaction mixture was stirred for 2 days at room temperature during which time a white salt was precipitated. The salt was removed by filtration, and the solvent was evaporated under vacuum. An attempt to purify the product by vacuum distillation [8] resulted in the decomposition of the compound similar to the results reported earlier [10]. The residue was dissolved in chloroform, washed with ice cold 2% aqueous NaOH, followed by a water wash to remove NaOH. The chloroform solution was dried over anhydrous $MgSO_4$ and Na_2SO_4, filtered and the chloroform was evaporated under vacuum. The product was found to be 99% pure by the HPLC. The yield was 3.5 g 65% of the theoretical yield.

4. Hydroxymethyl TOCP

The potassium salt of *o*-hydroxybenzyl alcohol (2.37 g, 18.5 mmol) was prepared as described above and suspended in 50 ml of 1,2-dimethoxyethane. To the stirred suspension, a solution of 5.5 g (18.5 mmol) di-*o*-cresyl phosphorochloridate in 50 ml dimethoxyethane was added dropwise, and the reaction mixture was stirred at room temperature for 2 days. The mixture was filtered, the solvent was evaporated under vacuum, and the residue dissolved in chloroform. The chloroform solution was washed with 2% NaOH followed by water wash, dried over anhydrous $MgSO_4$, and evaporated under vacuum. The residue was separated on preparative TLC using benzene/methanol (9:1) as solvent system (R_f = 0.45). The compound which could be observed under ultraviolet light, was extracted from the silica gel by acetone. Acetone was evaporated, and the residue was further purified by successive separations using preparative TLC and the same solvent system. The product was then purified on preparative TLC using hexane/acetone (5:1) as the developing system (2 times separated), (R_f = 0.18) followed by additional purification using hexane/ethyl acetate (5:1) as the developing solvent (R_f = 0.14). The purity of the product was determined to be 94% using HPLC. No further improvement in the purity

was observed by additional purification on preparative TLC. Only 50 mg of this compound was so purified.

5. Dihydroxymethyl TOCP

This compound was isolated during the synthesis of the previous compound. It was separated by preparative TLC using benzene/methanol (9:1) as the developing system (R_f = 0.65). The compound was extracted from silica gel by acetone and further purified by 3 successive preparative TLC separations using the same solvent system. It was then purified twice by preparative TLC using hexane/acetone (5:1) as a solvent system (R_f = 0.12) followed by hexane/ethyl acetate (5:1) (R_f = 0.13). The compound was found to be 92% pure by HPLC. Only 150 mg of this compound was purified.

Infrared spectroscopy (IR)

IR spectra for TOCP and its metabolites were determined using Perkin Elmer 727 Infrared Spectrophotometer. The spectrum for each compound was determined as a neat film between 2 sodium chloride windows.

Proton nuclear magnetic resonance spectroscopy

Fourier transform ^{1}H-NMR spectra for TOCP and its metabolites were recorded using an NR/80 Spectrometer (IBM Instruments., Inc.) equipped with a Silent 700 Electronic Data Terminal from Texas Instruments. The compounds were dissolved in either deuterated acetone or deuterated chloroform. Tetramethyl silane, TMS, was used as the internal standard.

Mass spectrometry

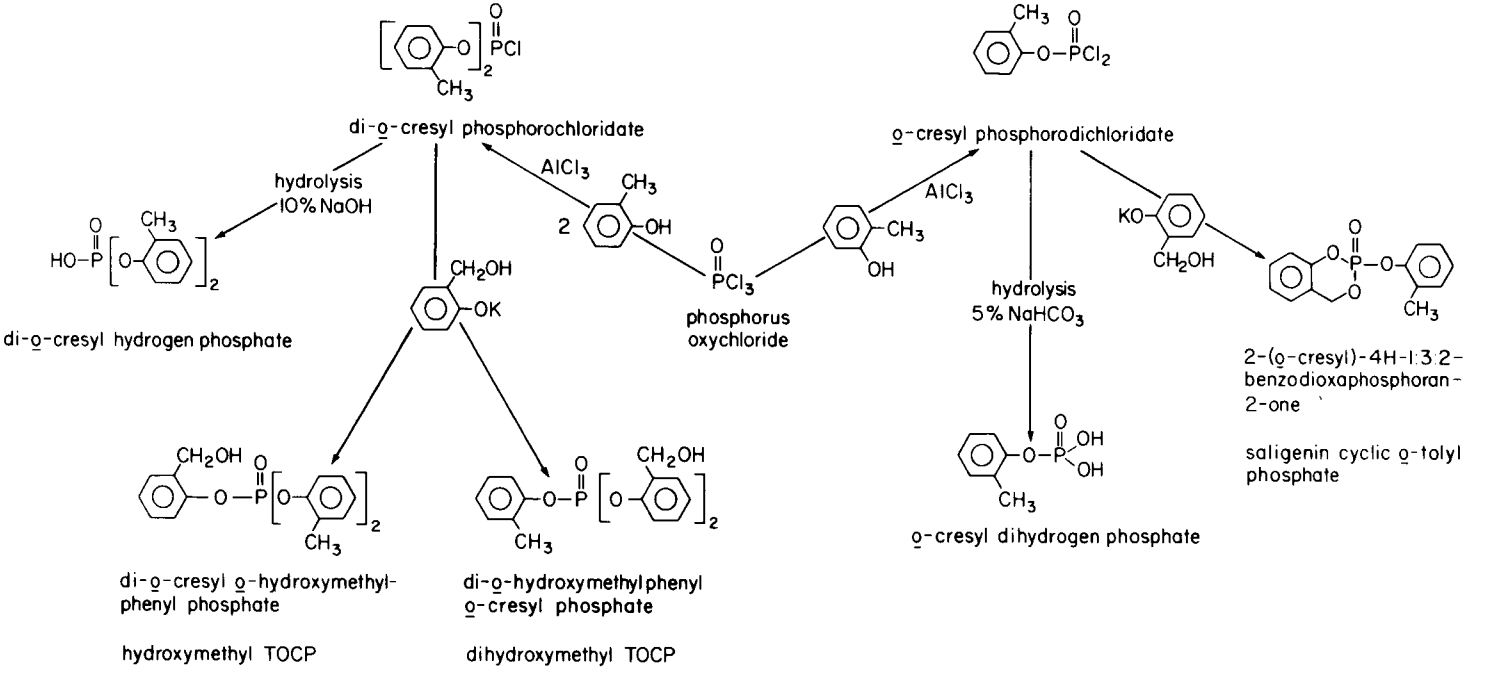
Electron impact mass spectra for various compounds were determined at 70 eV, the source temperature ranging from 120°C to 170°C depending on the compound. The instrument used was an AEI/MS 902 Mass Spectrometer (AEI Scientific Instrument, San Diego, CA). High resolution mass spectra were obtained for the molecular ions.

RESULTS AND DISCUSSION

This report describes the synthesis, purification, and identification of 5 metabolites of the industrial neurotoxic chemical TOCP. Scheme 1 summarizes the synthetic pathways of these compounds starting from $POCl_3$.

Spectroscopy

The IR, ^{1}H-NMR, and mass spectra for these metabolites and some of their derivatives are discussed in relation to the parent compound TOCP.



Scheme 1. Synthetic pathways of TOCP metabolites starting from phosphorus oxychloride.

Infrared

The IR spectra of TOCP and its metabolites are summarized in Table I. These compounds can be classified according to their IR spectra into 3 groups: non-polar, moderately polar, and highly polar. The first includes TOCP and saligenin cyclic-*o*-tolyl phosphate. The second group consists of hydroxymethyl TOCP and dihydroxymethyl TOCP, while the third includes *o*-cresyl dihydrogen phosphate and di-*o*-cresyl hydrogen phosphate. The IR spectra of all of these compounds showed common features due to the similarity in their structures as well as differences that characterize each group or individual compounds. Typical C—H aromatic and aliphatic stretching vibrations in the regions of 3125—3030 cm^{-1} and 2980—2910 cm^{-1} [14], respectively, are present in the spectra of these compounds. Benzene skeletal stretching vibration modes in the region of 1605—1465 cm^{-1} [15] are highly visible in all compounds. TOCP and its possible metabolites are characterized by the presence of 1,2-disubstituted benzene in their structures, i.e. they have 4 adjacent C—H bonds. This is manifested by the presence of C—H in-plane and out-of-plane deformation modes for 4 adjacent C—H bonds in the regions of 1180—1020 cm^{-1} and 770—760 cm^{-1}, respectively [15]. All chemicals showed a distinct P=O stretching vibration in the region of 1325—1240 cm^{-1} [15,16]. Each group of compounds, however, showed a slight difference in the position of the P=O stretching as indicated in Table I. The first group, TOCP and saligenin cyclic-*o*-tolyl phosphate showed a strong P=O stretching at 1320 and 1325 cm^{-1}, respectively. The second group, hydroxymethyl TOCP and dihydroxymethyl TOCP, showed the same bond stretching at identical 1310 cm^{-1}, and the third group, *o*-cresyl dihydrogen phosphate and di-*o*-cresyl hydrogen phosphate, showed the above stretching at 1245 and 1240 cm^{-1}, respectively. The other organophosphorus bond stretching vibrations of PO—C and P—OC (aromatic) are present at the regions of 1240—1185 and 990—965 cm^{-1}, respectively [15,17]. Saligenin cyclic-*o*-tolyl phosphate showed an extra peak at 1035 cm^{-1} which is probably due to the PO—C aliphatic stretching [15]. The IR spectrum of saligenin cyclic-*o*-tolyl phosphate was found to be very similar to that reported earlier for this compound [8].

The 2 highly polar acids, *o*-cresyl dihydrogen phosphate and di-*o*-cresyl hydrogen phosphate, showed lower intensity peaks than that of the other compounds. This is attributed to a strong hydrogen bonding that gave rise to a very broad absorption starting at 3800 cm^{-1} which masked the other peaks in the spectra of these 2 acids [17]. This is more evident in the spectrum of *o*-cresyl dihydrogen phosphate, which may be related to the presence of 2 hydroxyl groups allowing more hydrogen bonding.

The IR spectra of the moderately polar hydroxymenthyl TOCP and dihydroxymethyl TOCP were very similar. Strong broad peaks at 3525 and 3540 cm^{-1} were present in the spectra of the hydroxymethyl TOCP and dihydroxymethyl TOCP, respectively, indicating the presence of hydroxyl groups in these 2 compounds [15]. This is coupled with the presence of a

TABLE I

THE CHARACTERISTIC INFRARED ABSORPTION FREQUENCIES (cm^{-1}) OF TOCP AND ITS POSSIBLE METABOLITES

Compound	OH stretching	C—H aromatic	C—H aliphatic	Benzene[a] skel. st. modes	P—O stretching	P—O and C—O aromatic	C—O aliphatic	C—H[b] inplane deformation modes	C—H[c] out of deformation modes	OH out of plane deformation modes
TOCP		3125(w)[d] 3090(w) 3030(m)	2980(m) 2910(w)	1605(s) 1510(vs) 1480(vs)	1320(vs)	1240(vs) 980(vs)		1180(vs) 1125(vs) 1060(vs)	770(vs)	
Saligenin cylic-*o*-tolyl Phosphate		3125(w) 3070(w) 3050(vw)	2975(w) 2940(w)	1635(w) 1600(m) 1505(s) 1475(s)	1325(vs)	1235(m) 965(s)	1035(m)	1205(m) 1185(s) 1120(s)	760(s)	
Hydroxy-methyl TOCP	3525(s)	3120(vw) 3060(w)	2975(vw) 2920(vw)	1615(w) 1600(m) 1505(vs) 1465(s)	1310(s)	1235(vs) 980(vs)	1050(m)	1175(vs) 1120(vs) 1020(w)	760(s)	710(w) 665(w)
Dihydroxy-methyl TOCP	3540(s)	3125(vw) 3070(w)	2975(vw) 2925(vw)	1620(m) 1600(m) 1505(vs) 1465(s)	1310(s)	1235(vs) 980(vs)	1050(m)	1175(vs) 1115(vs) 1020(w)	760(s)	710(w) 665(w)
o-Cresyl dihydrogen phosphate	Broad unlocated starts at 3800	3040(vw) 3070(vw)	2980(vw)	1600(w) 1510(m) 1475(vw)	1245(m)	1190(m) 990(m)		1125(m)	765(m)	
Di-*o*-cresyl hydrogen phosphate	Broad unlocated starts at 3800	3125(vw) 3090(vw) 3030(w)	2980(w) 2920(w)	1600(w) 1510(m) 1475(w)	1240(s)	1185(s) 970(s)		1120(s) 1030(s)	765(m)	

[a] Benzene skeletal stretching modes.
[b] C—H in plane deformation modes for 1,2-disubstituted benzene.
[c] Out of plane deformation modes for 4 adjacent C—H's (aromatics).
[d] *Abbreviations:* (vw) very weak; (w) weak; (m) medium; (s) strong, (vs) very strong.

peak at 1050 cm^{-1} in both compounds which probably resulted from the aliphatic C—O stretching vibration [15]. Two additional peaks at 710 and 665 cm^{-1} were also present and are probably attributed to O—H out-of-plane deformation frequencies [15]. These 2 compounds were found to be negative in the 4-aminoantipyrene phenol test [18], suggesting that the OH is not phenolic but, rather, alcoholic.

Proton nuclear magnetic resonance

The ^{1}H-NMR spectra of TOCP and its related compounds are summarized in Table II. The aromatic hydrogen atoms showed a typical multiplet signals in the range of 7.4 and 6.6 ppm [14]. A singlet signal in the range of 2.3 and

TABLE II

PROTON NUCLEAR MAGNETIC RESONANCE (^{1}H-NMR) CHEMICAL SHIFT (PPM) FOR TOCP AND ITS POSSIBLE METABOLITES

Compound	PPM					
	Ar—H's	P—O—CH_2—Ar	Ar—CH_2—OH	OH	P—O—CH_3	Ar—CH_3
TOCP	7.40—7.04 (m)[a]					2.20 (s)
Saligenin cylic-*o*-tolyl phosphate	7.25—7.02 (m)	5.51 5.39 (d)				2.20 (s)
o-Cresyl dihydrogen phosphate	7.21—7.07 (m)					2.27 (s)
Di-*o*-cresyl hydrogen phosphate	7.38—7.05 (m)					2.21 (s)
o-Cresyl *O,O*-dimethyl phosphate	7.26—7.09 (m)				3.90 3.79 (d)	2.32 (s)
Di-*o*-cresyl *O*-methyl phosphate	7.33—7.09 (m)				4.02 3.91 (d)	2.25 (s)
Hydroxymethyl TOCP	7.42—6.67 (m)		5.01 (s)	4.68 (s)		2.19 (s)
Dihydroxymethyl TOCP	7.40—6.97 (m)		5.16 (s) 5.05 (s)	4.70 (s) 4.75 (s)		2.18 (s)

[a] *Abbreviations:* (m) Multiplet, (d) doublet, (s) singlet.

2.1 ppm is present in all NMR spectra, indicating the presence of $C\underline{H}_3$-Ar in these chemicals [14]. The 2 methyl esters, *o*-cresyl *O,O*-dimethyl phosphate and di-*o*-cresyl *O*-methyl phosphate, each showed an additional doublet signal at the range of 3.79—4.02 ppm resulting from the presence of O—$C\underline{H}_3$ [17,19,20]. The coupling constant of these 2 signals was calculated to be 11 Hz and is attributed to the coupling with the phosphorus [17,19]. Saligenin cyclic-*o*-tolyl phosphate showed a doublet signal for P—O—$C\underline{H}_2$—Ar at 5.51 and 5.39 ppm, with a coupling constant of 12 Hz due to coupling with the phosphorus [17,19]. The Ar—$C\underline{H}_2$—OH signals are present in both the hydroxymethyl TOCP and dihydroxymethyl TOCP. The monohydroxy derivative showed a singlet signal at 5.01 ppm which is characteristic of Ar—$C\underline{H}_2$—OH. The second compound, however, exhibited 2 signals at this region for the hydrogens of the 2 Ar—$C\underline{H}_2$—O groups. The presence of 2 signals is probably attributed to hydrogen bonding of 1 of the 2 OH groups with the oxygen of the P—O, resulting in a change of the electronic environment around one of the Ar—$C\underline{H}_2$—O groups relative to the other and thus, giving rise to the additional chemical shift. This explanation is supported by the result that hydroxymethyl TOCP gave one O$\underline{H}$ signal at 4.68 ppm while the dihydroxymethyl TOCP gave 2 signals (Table II).

Further confirmation of the structure assigned to dihydroxymethyl TOCP was obtained by running its ^{1}H-NMR in the presence of a small amount of deuterated water. D_2O exchange resulted in the disappearance of the signals at 4.70 and 4.75. Also, the integration ratio was in agreement with the structure assigned.

Mass spectra

Electron impact mass spectra of TOCP and related compounds are summerized in Table III. The 2 acids, *o*-cresyl dihydrogen phosphate and di-*o*-cresyl hydrogen phosphate, did not show the parent ion; thus, their methyl esters were used instead. Table IV present the high resolution mass (determined and calculated) for TOCP, hydroxymethyl TOCP, *o*-cresyl *O,O*-dimethyl phosphate, di-*o*-cresyl *O*-methyl phosphate and saligenin cyclic-*o*-tolyl phosphate. The calculated and determined mass of the molecular ion for each compound is almost identical, strongly confirming the structure assigned for these chemicals (Table IV).

The molecular ion of all compounds, except dihydroxymethyl TOCP, is present in each spectrum. The spectra of TOCP, di-*o*-cresyl *O*-methyl phosphate, and saligenin cyclic-*o*-tolyl phosphate showed that the base peak was the molecular ion. All compounds showed the presence of 2 ions, 1 at *m/z* 107 and the other at *m/z* 91, which are probably due to $[O\text{-}C_6H_4\text{-}o\text{-}CH_3]^+$ and $[C_6H_4—CH_3]^+$ ions, respectively. Similar fragmentation patterns of organophosphorus compounds have been reported earlier [20—22]. Also, all compounds except the dihydroxymethyl TOCP, showed the M-107 ion, from the loss of the $[O\text{-}C_6H_4\text{-}o\text{-}CH_3]$ fragment from the parent ion. The dihydroxymethyl TOCP showed an M-123 ion (*m/z* = 277) which was apparently the fragment remaining after the loss of 1 of the 2 $O\text{-}C_6H_4\text{-}o$-

TABLE III

MASS SPECTRAL DATA FOR TOCP AND RELATED COMPOUNDS

Compound	*m/z* and relative abundance of the molecular ion and prominent fragments	
TOCP	368 (100%)	107 (92%)
	277 (29%)	91 (16.9%)
	261 (4.2%)	
o-Cresyl *O,O*-dimethyl phosphate	216 (67%)	109 (11.1%)
	201 (9.3%)	107 (22.2%)
	185 (1.3%)	91 (18.5%)
Di-*o*-cresyl *O*-methyl phosphate	292 (100%)	185 (8.6%)
	277 (9.3%)	107 (22.9%)
	201 (40%)	91 (37.1%)
Saligenin cyclic-*o*-tolyl phosphate	276 (100%)	185 (5.4%)
	230 (70%)	169 (7.9%)
	201 (20.8%)	107 (69.3%)
		91 (19.8%)
Hydroxymethyl TOCP	384 (1%)	277 (5%)
	367 (100%)	107 (7%)
		91 (11%)
Dihydroxymethyl TOCP	383 (0.1%)	277 (2%)
	368 (43%)	107 (15%)
	367 (100%)	91 (10%)

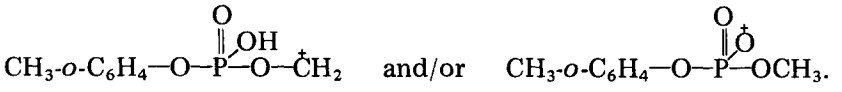
CH_2OH groups [21,22]. Three compounds, TOCP, di-*o*-cresyl *O*-methyl phosphate, and saligenin cyclic-*o*-tolyl phosphate showed the M-91 ion; probably the fragment remaining after loosing the [C_6H_4—CH_3] group from the parent ions [21,22]. The spectrum of *o*-cresyl *O,O*-dimethyl phosphate showed 2 ions one at *m/z* 201 (M-15) and the other at *m/z* 185 (M-31). These 2 ions are probably the fragment remaining after the loss of the CH_3 and OCH_3 from the parent ion, respectively. The spectrum of di-*o*-cresyl

TABLE IV

ELECTRON IMPACT HIGH RESOLUTION MASS SPECTROSCOPY OF THE MOLECULAR ION OF TOCP AND SOME OF ITS RELATED COMPOUNDS

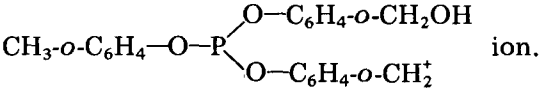
Compound	Determined mass	Calculated mass
TOCP	368.1172	368.1177
Hydroxymethyl TOCP	384.1124	384.1127
o-Cresyl *O,O*-dimethyl phosphate	216.0553	216.0551
Di-*o*-cresyl *O*-methyl phosphate	292.0867	292.0864
Saligenin cyclic *o*-tolyl phosphate	276.0555	276.0550

O-methyl phosphate also has the M-15 fragment ion (m/z = 277). Saligenin cyclic-*o*-tolyl phosphate showed the presence of a high intensity ion at m/z 230. This ion is probably due to CH_3-*o*-C_6H_4—O—P=$\overset{+}{O}$—C_6H_4 fragment. The ion at m/z 201, which is also present in the spectrum of saligenin cyclic-*o*-tolyl phosphate probably has the following structure



and/or

The spectrum of hydroxymethyl TOCP contained an M-17 ion which may have resulted from the removal of the hydroxyl group from the parent ion. The ion at m/z 367 (M-33), which is present in the spectrum of dihydroxymethyl TOCP, is probably attributed to the



ion.

In summary this report describes simplified methods for the synthesis of saligenin cyclic-*o*-tolyl phosphate and dihydroxymethyl TOCP. Reported also, are the procedures for synthesis of the di-*o*-cresyl hydrogen phosphate and *o*-cresyl dihydrogen phosphate in good yield. All compounds were purified to analytical grade purity. The infrared, proton nuclear magnetic resonance, and mass spectra presented for these metabolites of TOCP are consistent with the structure assigned to each compound. The spectroscopic techniques should provide better and unequivocal indentification of the TOCP breakdown products. These compounds have been used as reference metabolites in a metabolism study of [^{14}C]TOCP in the cat.

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