

## Pyrolysis Gas Chromatographic Studies on Pyridinium Compounds

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The well known antidotes in the therapy of organophosphorus intoxication, the mono- and bis-pyridinium oximes and their synthetic intermediates have been analyzed by pyrolysis gas chromatography (PGC). The results obtained on PGC at 500°C and 800°C indicate that the technique is simple but of restricted applicability in the characterization of the antidotes.

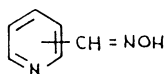
### INTRODUCTION

Pyrolysis gas chromatography (PGC) is applicable to any material that is capable of being thermally decomposed to yield pyrolysate which can be separated by gas chromatography (GC)<sup>1</sup>. While it is probably true that most PGC applications have been in investigations of macromolecules such as synthetic polymers and biomolecules<sup>2,3</sup> useful information can also be gained on materials of relatively low molecular weights such as surfactants, soaps, fatty acids and drugs<sup>3,4</sup>. The quaternary pyridinium salts due to their non-volatile nature are not easily amenable to GC investigations, hence their analysis by PGC has been considered. For this purpose PGC of well known antidotes in the therapy of organophosphorus intoxication, the mono- and bis-pyridinium oximes and their synthetic intermediates has been studied.

### EXPERIMENTAL

*Chemical:* The 2-pyridine aldoxime (2-PA, 1), 4-pyridine aldoxime (4-PA, 2), 2-pyridinium aldoxime methochloride (2-PAM, 3) and 1,3-dibromopropane (4) were obtained from Aldrich/Sigma Chem. Co. (U.S.A.) and were used without further purification. The bis-pyridinium oxime derivatives 1,1'-oxydimethylene-bis-[4-(hydroxyiminomethyl)-pyridinium]-dichloride (Toxogonin, 5), 1-[2-(hydroxyiminomethyl)-pyridinium]-1'-[4-(carboxamido)-pyridinium]-2-oxopropane dichloride (HI-6), 1,3-bis[4-(hydroxyiminomethyl)-pyridinium]-propanedibromide (TMB-4, 7) and the synthetic intermediates 1-bromo-1'-[4-(hydroxyiminomethyl)-pyridinium]-propane bromide (8) and 1-chloro-1'-[4-(hydroxyiminomethyl) pyridinium]-2-oxopropane chloride (9) were synthesized according to the described procedures<sup>5,6</sup> and were characterized by analysis, IR and <sup>1</sup>H-NMR spectroscopy.

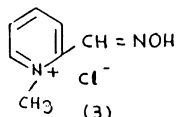
### Chemical Structures of Compounds



(1-2)

1, 2-CH=NOH

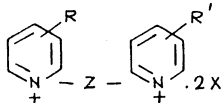
2, 4-CH=NOH



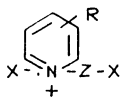
(3)

Br CH<sub>2</sub> CH<sub>2</sub> CH<sub>2</sub> Br

(4)



(5-7)

5, R = R' = 4-CH=NOH; Z = -CH<sub>2</sub>-O-CH<sub>2</sub>- X = Cl<sup>-</sup>6, R = -2CH=NOH; R' = 4-CONH<sub>2</sub>, Z = -CH<sub>2</sub>OCH<sub>2</sub>-, X = Cl<sup>-</sup>7, R = R' = 4-CH=NOH, Z = -(CH<sub>2</sub>)<sub>3</sub>-, X = Br

(8-9)

8, R = 4-CH=NOH; Z = -(CH<sub>2</sub>)<sub>3</sub>- X = Br9, R = -4CH=NOH, Z = -CH<sub>2</sub>OCH<sub>2</sub>-, X = Cl<sup>-</sup>

**Pyrolysis Gas Chromatography:** A Shimadzu pyrolyser PYR-2A attached to gas chromatograph GC-9A equipped with a flame ionisation detector and a steel column packed with FFAP on poropak was employed. The carrier gas was nitrogen at 40 mL/min, column and injection part were at 170°C and 220°C respectively; the chromatography was carried out in isothermal mode.

A weighed amount of the sample 0.2–0.3 mg in case of monopyridinium compounds and 0.7–0.8 mg for bis-pyridinium derivatives was taken in a platinum boat which was introduced and retention time (Rt) was measured by a time controller on the instrument which was reproducible within +3 seconds.

The pyrolysis temperatures used for PGC studies of pyridinium compounds were 500°C and 800°C. These were selected from the initial studies with TMB-4 carried out over a temperature range of 400–850°C; the thermal decomposition was not significant at 400°C, PGC at 500°C and 600°C were qualitatively similar; also similar PGC were observed at 700°C, 800°C and 850°C.

### RESULTS AND DISCUSSION

The quaternary pyridinium oximes reactivate the organophosphorus inhibited acetylcholinesterase (EC 3.1.1.7) by displacing the phosphoryl residue from the active site thereby restoring enzymatic activity; thus these compounds act as antidote in the therapy of organophosphorus intoxication. Some of the effective antidotes are monopyridinium oxime such as 2-PAM and bis-pyridinium oximes

such as TMB-4, toxogonin and HI-6<sup>7</sup>. The PGC analysis of these compounds and their synthetic intermediates have been carried out at two selected temperatures of 500°C and 800°C. The retention time of the major peaks obtained on PGC at 800°C are listed in Table-1 and the pyrograms of the selected compounds are shown in Fig. 1.

TABLE-1  
RETENTION TIME IN SECS. OF PYROLYSED PRODUCTS FROM  
PYRIDINIUM COMPOUNDS AND THEIR INTERMEDIATES

Compounds	Retention time in secs.					
2-PAM (Cl)	31	40	—	75	86	—
2-Pyridine aldoxime (2-PA, 1)	31	40	—	—	87	—
4-Pyridine aldoxime (4-PA, 2)	31	41	—	—	87	—
2-Pa: 4-PA (1 : 1)	31	39	—	—	87	—
TMB-4 (7)	31	41	61	75	89	560
8	31	41	61	—	87*	560
1,3-dibromopropane (4)	31	38	58	—	—	560
Toxogonin (5)	31	41	—	77*	89	—
9	30	40	61*	—	86	—
HI-6	34	45	66*	74*	86	—

\*Indicate minor peaks at retention time.

The PGC of monopyridinium oxime 2-PAM at 500°C exhibited two major peaks at retention time 72 and 87 seconds; the latter was also observed as the only major peak with 2-PA. At 800°C both 2-PAM and 2-PA showed two additional peaks at retention time 31 and 40 seconds. It can thus be inferred that 2-PAM exhibits all peaks corresponding to that observed with the 2-PA pyrolysate with only one extra peak at retention time characteristic stability is lost when quaternisation takes place<sup>8</sup>. Some of the ring fission, ring contraction, ring expansion as well as recyclisation reactions described in thermal reaction as pyridinium compound<sup>9,10</sup> can also occur during the pyrolysis and may account for the various major and minor peaks observed in the PGC of pyridine oximes.

Toxogonin (5) and its mono-pyridinium intermediates (9) at 500°C show two peaks at retention time 75 and 87 seconds was also observed with 4-PA similar to that of 2-isomer. In fact, the 2- and 4-PA pyrolysate were not resolvable on FFAP column as a 1 : 1 mixture of the two isomers showed a pyrogram similar to each other. Another column OV-17 which even though separated 2- and 4-PA was not found to be useful as other characteristic peaks in the pyrogram of bis-pyridinium compounds observed on FFAP column were not well resolved on OV-17 column. Therefore, most work was carried out using FFAP column. At 800°C both HI-6 and its monopyridinium intermediate (9) showed qualitatively similar pyrogram to that of 4-PA.

The bis-pyridinium and mono-pyridinium compounds containing trimethylene bridge chain such as compounds 7 and 8, in their PGC at 500°C exhibited

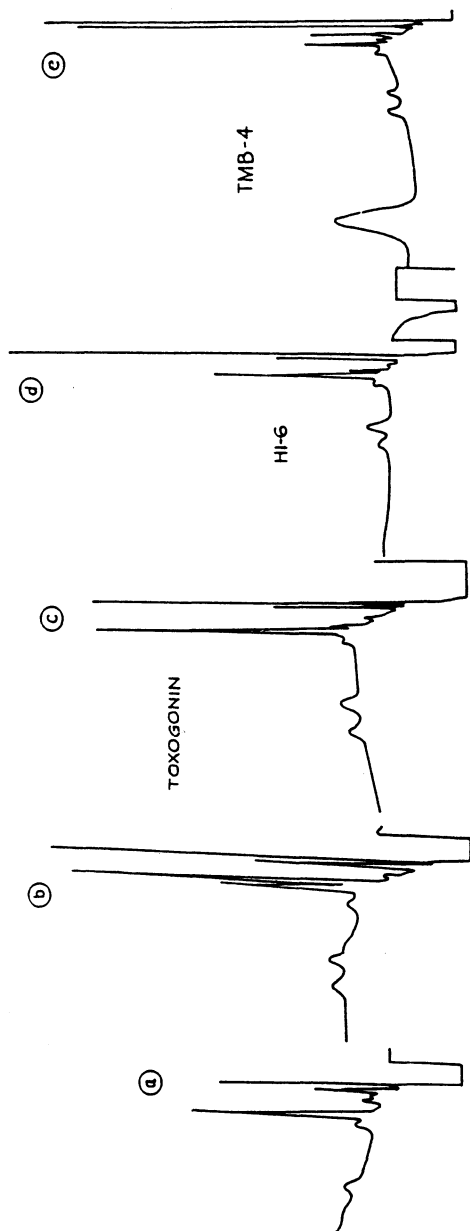
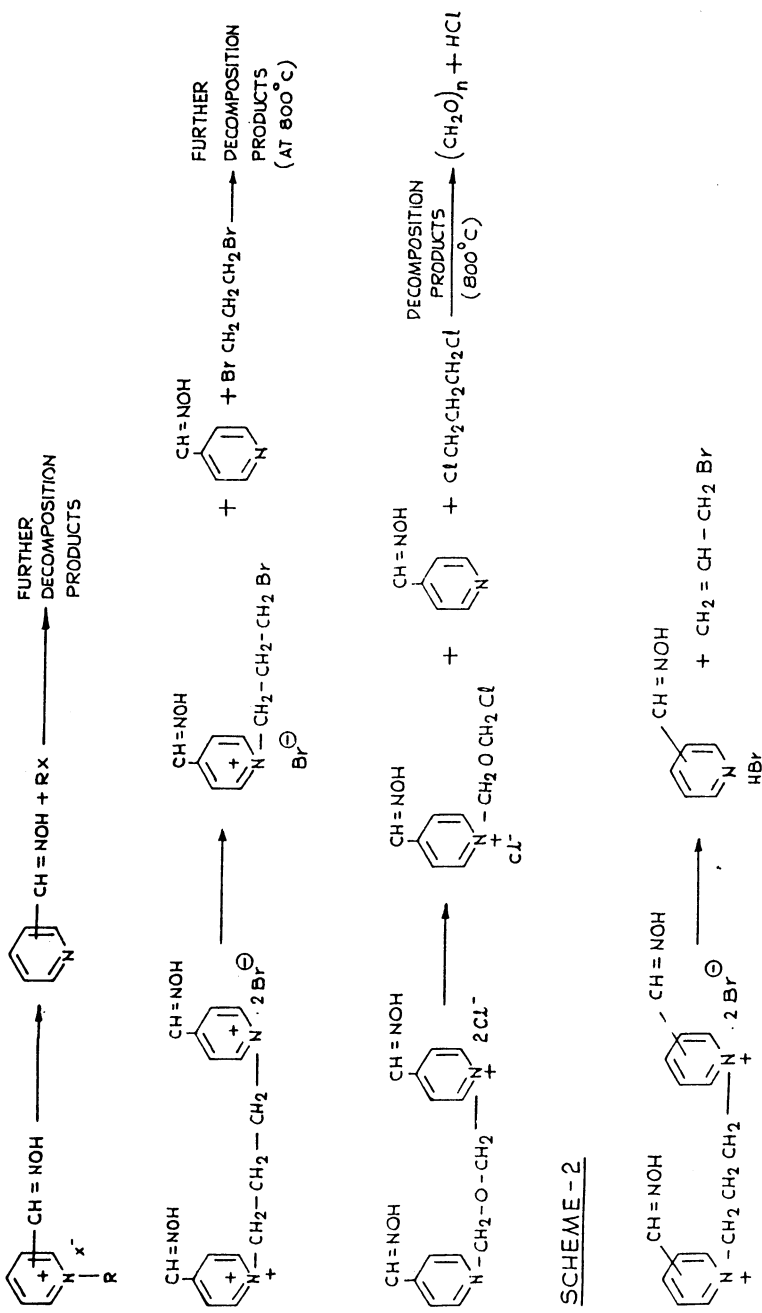
PGC OF  $F_2$  COMPOUNDS AT 800 °C

Fig. 1. (a) 2-Pyridine aldoxime (b) 2-Pyridine aldoxime methochloride (c) Toxogonin (d) HI-6- (e) TMB-4



SCHEME - 2

Scheme-1

characteristic peaks at retention times 340, 428 and 570 s, similar to that observed with 1,3-dibromopropane. At 800°C the peaks at Rt 340 and 428 seconds disappeared and an intense peak at 560 s was observed. All pyridinium compounds studied showed a peak at retention times 87 seconds. The observation suggests that pyridinium compounds probably form the corresponding pyridine aldoximes by dequaternisation during pyrolysis (Scheme-1). Krohne and Meyer-Dehing<sup>11</sup> also described the formation of pyridinium and an olefin or even acetylene on heating pyridinium salts (Scheme-2). The qualitative similarity of pyrogram of 1,3-dibromopropane for peaks at retention times 300 seconds with those from pyridinium compounds containing trimethylene bridge group suggests that these may arise by dequaternisation of the pyridinium compounds and as subsequent decomposition similar to that with 1,3-dibromopropane (Fig. 2).

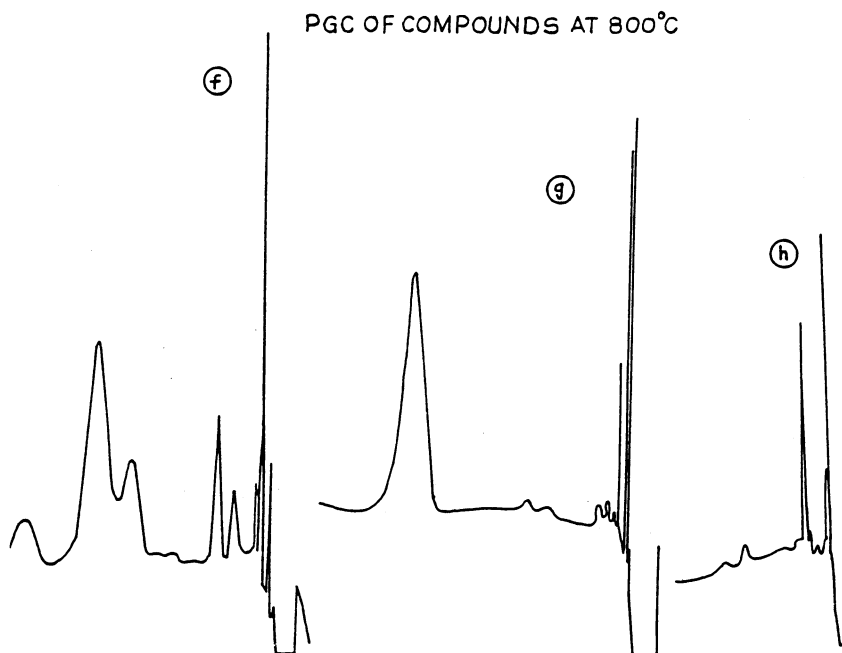


Fig. 2. (f) 1,3-Dibromo propane (g) 1-Bromo-1'-[4-(Hydroxy iminomethyl) pyridinium] propane bromide (h) 1-chloro-1'-[4-(hydroxy iminomethyl) pyridinium]-2-oxo-propane chloride

These studies suggest that PGC of pyridinium compounds is of limited application in their characterization since mono- and bis-pyridinium compounds give qualitatively similar pyrograms in most cases. However, a distinction between the pyridinium compounds which are known as potent antidotes against organophosphorus intoxication is possible based on the peak height ratio by observing the peaks at retention time 87 seconds and 75 seconds taken together with presence or absence of peak at retention time 570 seconds, but even this will

not distinguish obidoxime from HI-6. It may thus be concluded that the simple technique of PGC has a restricted applicability in characterization of antidotes against organosphosphorus intoxication.

TABLE-2  
PEAK HEIGHT MEASUREMENTS FOR SELECTED  
PYRIDINIUM COMPOUNDS IN THEIR PGC AT 800°C

Compound	Peak height ratio*	Peak 'c'†
	Peak 'a' Peak 'b'	
2-PAM (Cl)	< 1.0	Absent
Toxogonin	< 1.0	Absent
HI-6	> 1.0	Absent
TMB-4	> 1.0	Present

\*Peak 'a' Rt =  $87 \pm 2$  s; Peak 'b' Rt =  $75 \pm 2$  s

†Peak 'c' Rt = 560 s

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