

## A Spectrophotometric Study in Infra-Red of the Hydrolysis of Dimethylamido-ethoxy-phosphoryl Cyanide (Tabun)

LENNART LARSSON

*Organic Division, Research Institute of National Defence, Dept. 1, Sundbyberg 4, and  
Institute of Organic Chemistry and Biochemistry, University of Stockholm, Sweden*

Dimethylamido-ethoxy-phosphoryl cyanide, generally known as Tabun belongs to a class of organic phosphorus compounds which are biochemically characterized by their ability to inhibit the vital enzyme cholinesterase. Tabun is one of the most potent of these compounds.

Information concerning the hydrolysis of Tabun and similar derivatives is of great toxicological interest. In the first place every physiological action must occur in an aqueous phase, in which there exists the possibility of hydrolysis of the inhibitor. In the second place, according to a theory proposed by Burgen<sup>1</sup>, it is probable that the enzyme inhibition is caused by a phosphorylation of some active group in the enzyme. If this is the case it is possible that the rate of hydrolysis runs parallel with the power to phosphorylate enzyme protein, *i.e.* that the rate of hydrolysis may be the measure of the inhibitory capacity of the compound as pointed out by Topley<sup>2</sup>. Several exceptions of this rule, however, will be found. The polarity of the molecule, for instance, will certainly influence the inhibition mechanism, and for reasons easily understood a very active phosphorylating compound, which reacts with water very quickly, will act as a weak inhibitor, as it is destroyed before meeting the enzyme.

The hydrolysis of the cyanide group of Tabun has previously been investigated by Holmstedt<sup>3</sup>. He compared these results with toxicity tests made concurrently in white mice. However, he found that the rapidly decreasing toxicity during the hydrolysis was not proportional to the splitting off of the cyanide group. This discrepancy may be explained by the dimethylamino group also being easily split off as pointed out by the present writer in a work recently carried out by Homstedt and Larsson<sup>4</sup>.

The present investigation was undertaken to give a more clear view of the course of the hydrolysis of Tabun. By following the alterations of the absorption bands in the infra-red spectrum, the author has succeeded in elucidating the reaction in a simple and perspicuous manner. As far as the author knows, no examinations of this kind have been carried out before. In the work by Holmstedt and Larsson, quoted above, the absorption at  $13.70\text{--}14.25\ \mu$  ( $730\text{--}702\ \text{cm}^{-1}$ ) was assigned to the vibrations of the P—N linkage in the

combination  $\text{P—N} \begin{matrix} \diagup \text{C} \\ \diagdown \text{C} \end{matrix}$  analogously to Meyrick and Thompson's<sup>5</sup> correlation

of the absorption at  $12.20\text{--}12.70\ \mu$  ( $820\text{--}788\ \text{cm}^{-1}$ ) with the vibrations of the P—O bond in the skeleton P—O—C. The latter correlation, however, has been met with some criticism by Daasch and Smith<sup>6</sup>. To prove that the absorption at  $13.70\ \mu$  ( $730\ \text{cm}^{-1}$ ) in the spectrum of Tabun really originates from the P—N linkage, the author has followed the decreasing absorption at this wave-length during the hydrolysis. The amount of the dimethylamine formed has simultaneously been determined by a colorimetric method according to Dowden<sup>7</sup>. Furthermore the infra-red spectra of two bis-(dimethylamido)-phosphoryl derivatives have been recorded and the absorption band of their dimethylamino groups has been discussed.

#### EXPERIMENTAL

As the measuring of the infra-red spectra was performed in a rock salt cell, it was necessary to transfer Tabun and its hydrolysis products to a suitable solvent, from which the water could be removed. The best result was obtained with dioxane.

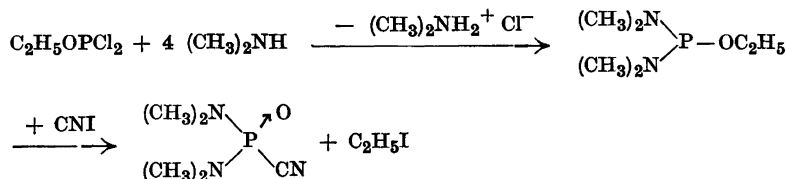
A series of samples consisting of 0.300 ml of Tabun in 0.150 ml of distilled water, the amounts measured by means of an AGLA micrometer syringe, were vigorously shaken and kept in a water bath at  $25.0 \pm 0.2^\circ\text{C}$ . At intervals a sample was mixed with 4.00 ml of dioxane. The solution was dried with 1.0 g of anhydrous sodium sulphate for 30 minutes with shaking, after which the solution was sucked off through a glass filter. The infra-red spectrum of the filtrate was then recorded between  $3.8$  to  $4.9\ \mu$  and between  $12.0$  to  $14.0\ \mu$  with a Beckman IR 2 Infrared Spectrophotometer. A rock salt cell was used and the thickness of the sample was 0.10 mm.

In a similar series the splitting off of the dimethylamine was determined colorimetrically according to the method reported by Dowden<sup>7</sup>. The principle of this method is to convert the dimethylamine into dimethyl-dithiocarbamic acid with carbon disulphide in a benzene solution. The cupric salt of this acid, which is insoluble in water but rather soluble in benzene, has an amber tint. The color-intensity of the samples was measured in 10 mm cells with a Beckman DU Spectrophotometer at  $4360\ \text{\AA}$  (absorption maximum). The amount of dimethylamine was calculated from the expression:

$$E = \epsilon \cdot c \cdot d$$

where  $E$  = optical density,  $\epsilon$  = extinction coefficient,  $c$  = concentration expressed in micromole dimethylamine per ml and  $d$  = cell thickness in cm. From a standard curve which obeys Lambert-Beer's law within the actual concentration range, the value of  $\epsilon$  was calculated to  $5.14 \pm 0.07$ .

The infra-red absorption spectra of bis-(dimethylamido)-phosphoryl cyanide and the corresponding fluoride were recorded between 2.5 and 15.0  $\mu$  exactly in the same manner as reported in the work quoted above <sup>4</sup>. The former was prepared in the following way:



The dimethylamine, dissolved in ether, was slowly added to a solution of ethoxyphosphine dichloride in ether with strong cooling and stirring and in an atmosphere of nitrogen. When the reaction was complete, the mixture was filtered and the ether was distilled off. The residue was fractionated three times *in vacuo*. To the bis-(dimethylamido)-ethyl phosphite obtained, dissolved in ether, a solution of cyanogen iodide in ether was added. The reaction mixture was kept between +10° to +20° C. After addition of CNI the ether was evaporated and the residue fractionated twice *in vacuo*. B.p. 91–94°/0.9 mm Hg. The latter reaction is a variation of the isomerization of Arbusow <sup>8</sup>. (Found: P 19.4; CN 15.4.  $\text{C}_5\text{H}_{12}\text{N}_3\text{OP}$  (161.15) requires P 19.22; CN 16.15.  $n_D^{20} = 1.4518$ ).

The syntheses of Tabun and of bis-(dimethylamido)-phosphoryl fluoride were performed by Holmstedt <sup>3</sup>, the latter in the way described by Schrader <sup>9</sup>.

## RESULTS AND DISCUSSION

As shown from Fig. 1 the decrease in per cent of the absorption at 13.70  $\mu$  agrees well with the amount of dimethylamine liberated during the hydrolysis. This proves the proposition made before that the stated absorption band can

be correlated with the vibrations in the  $\text{P}-\text{N} \begin{array}{c} \diagup \text{C} \\ \diagdown \text{C} \end{array}$  group. The infra-red spectro-

photometric method, however, is not quite suitable to quantitative determinations, because it is affected with some sources of error which cause too large scattering of the values. The sigmoid nature of the curve is due to the successively increasing acidity in the solution which catalyses the splitting off of the dimethylamino group <sup>10</sup>.

The absorption spectra of the two bis-(dimethylamido)-phosphoryl derivatives are illustrated in Fig. 2. and their absorption bands given in Table 1. In the spectra of these two compounds the absorption band characteristic of the P–N linkage is displaced to about 13.20  $\mu$  ( $758 \text{ cm}^{-1}$ ). The displacement

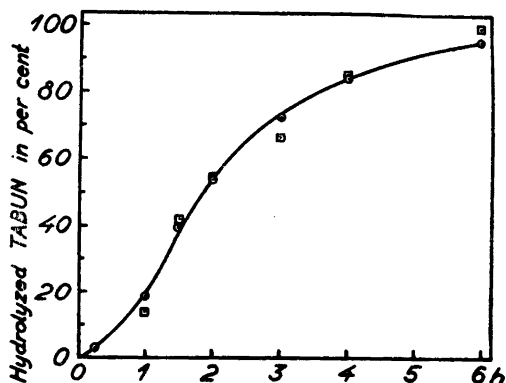


Fig. 1. Hydrolysis of Tabun in regard to the dimethylamino group.  
 ○ Colorimetric determination.  
 □ Infra-red spectrophotometric determination.

to higher frequencies indicates that this linkage in the bis-(dimethylamido) derivatives is stronger than in the monoderivatives which is also manifested by the much slower hydrolytic rate of the former. The bis-(dimethylamido) compounds are very weak inhibitors of cholinesterase *in vitro* which agrees with the hydrolytic stability. *In vivo* or in presence of liver slices, however, they become strong inhibitors which depends upon these compounds being somehow activated in the body<sup>11</sup>. In the spectrum of the bis-(dimethylamido)-phosphoryl fluoride an absorption band appears at 11.80  $\mu$  (847  $\text{cm}^{-1}$ ) which arises from the P—F bond. This band has been pointed out by Daasch and

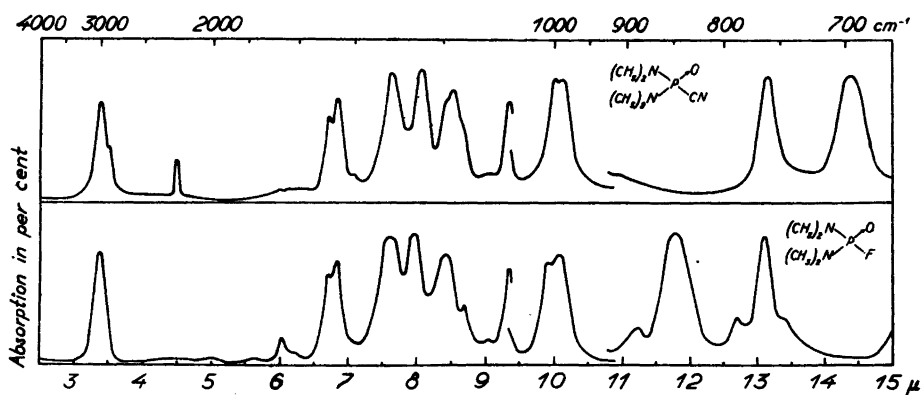


Fig. 2. Absorption curves of bis-(dimethylamido)-phosphoryl cyanide and the corresponding fluoride.

Smith<sup>6</sup> also. The absorption bands of the remaining groups agree well with these obtained in an earlier study of the spectra of phosphoryl derivatives<sup>4</sup>.

In Fig. 3 is shown the absorption spectrum of Tabun hydrolyzed for four hours in comparison with the spectrum of unhydrolyzed Tabun. The hydrolysis was followed using the method mentioned above.

The very strong absorption band which appears at  $3.97 \mu$  ( $2519 \text{ cm}^{-1}$ ) can probably be assigned to the vibrations of the bonded hydroxyl group according to Daasch and Smith<sup>6</sup>.

It appears from the curves that the absorption at  $4.48 \mu$  ( $2232 \text{ cm}^{-1}$ ) caused by the vibrations of the cyanide group decreases during the hydrolysis, but not so fast as the absorption of the dimethylamino group. The hydrocyanic acid simultaneously formed is expected to give an absorption band at  $4.73 \mu$  ( $2114 \text{ cm}^{-1}$ ). This absorption is not arisen in the spectrum, because some of the hydrocyanic acid evaporates from the very concentrated reaction mixture. This has been established by analysis of the gasphase. The residue is probably adsorbed by the sodium sulphate. Furthermore this absorption band has been recorded in preliminary experiments using diluted aqueous solutions which have been measured in calcium fluoride cells directly. In the figure this band is marked with a dotted line. A quantitative determination of the splitting off of the cyanide group by following the decreasing absorption at  $4.48 \mu$  is even more difficult than that of the dimethylamino group. An

Table 1. Absorption bands of bis-(dimethylamido)-phosphoryl cyanide and the corresponding fluoride.

$\lambda$  = Wave-length in  $\mu$ .

$\nu$  = Frequency in  $\text{cm}^{-1}$ .

s = strong, m = medium, w = weak.

Bis-(dimethylamido)-phosphoryl cyanide				Bis-(dimethylamido)-phosphoryl fluoride			
$\lambda$	$\nu$	$\lambda$	$\nu$	$\lambda$	$\nu$	$\lambda$	$\nu$
3.38	2 958 s	8.43	1 186 s	3.36	2 976 s	9.36	1 068 s
3.51	2 849 m	8.54	1 171 s	6.03	1 658 w	9.94	1 006 s
4.48	2 232 m	9.05	1 105 w	6.70	1 493 m	10.09	991 s
6.72	1 488 m	9.36	1 068 s	6.83	1 464 s	11.26	888 w
6.85	1 460 s	10.03	997 s	7.62	1 312 s	11.80	847 s
7.08	1 412 w	10.14	986 s	7.97	1 255 s	12.71	787 m
7.64	1 309 s	13.18	759 s	8.43	1 186 s	13.13	762 s
8.07	1 239 s	14.41	694 s	8.70	1 149 m	13.40	746 m
				9.05	1 105 w		

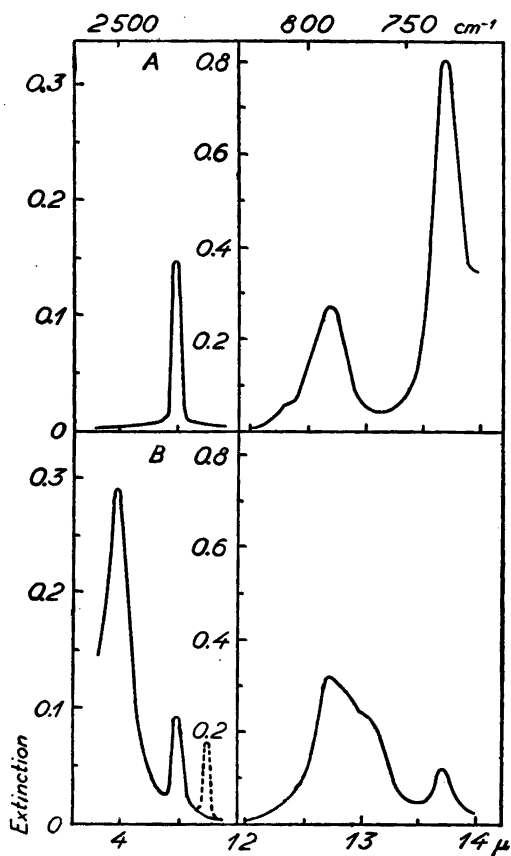


Fig. 3. Absorption curves of unhydrolyzed (A) and hydrolyzed (B) Tabun.


exact determination of the intensity of the cyanide absorption is complicated because of the overlapping absorption of the hydroxyl group.

As shown from the figure the intensity of the absorption band at  $12.70 \mu$  ( $788 \text{ cm}^{-1}$ ) is unchanged, but a little broadening of the band can be observed. This indicates that no splitting off of the ethoxy group will occur, on the assumption that this absorption corresponds to the P—O—C group as Meyrick and Thompson<sup>5</sup> have proposed. The alteration of the absorption band at  $13.70 \mu$  ( $730 \text{ cm}^{-1}$ ) has in detail been discussed above.

The spectrophotometric method is only a qualitative test of the hydrolysis, but it rapidly gives a perspicuous view of the hydrolytic course of a compound. A quantitative examination of the hydrolysis of Tabun to investigate its reaction mechanism in connection with its detoxification is now in progress.

## SUMMARY

It has been shown that the decreasing absorption at  $13.70 \mu$  ( $730 \text{ cm}^{-1}$ ) in the spectrum of Tabun is proportional to the amount of dimethylamine liberated during the hydrolysis. This argument proves the hypothesis that

the stated absorption band is caused by vibrations in the P—N  skeleton.

The infra-red absorption spectra of bis-(dimethylamido)-phosphoryl cyanide and the corresponding fluoride are recorded and the reasons of the displacement of the absorption of the dimethylamino group to shorter wave-lengths are discussed. The synthesis of the former compound is described.

A qualitative infra-red spectrophotometric method for following the course of the hydrolysis of Tabun is described. In the acid environment the dimethylamino group is split off more rapidly than the cyanide group, but the P—O—C linkage seems to be stable.

The author is indebted to the Chief of the Research Institute of National Defence, Dept. 1, Professor Gustaf Ljunggren, for the kind interest he has shown in this work.

## REFERENCES

1. Burgen, A. S. V. *Brit. J. Pharmacol.* **4** (1949) 219.
2. Topley, B. *Chemistry & Industry* **1950** 859.
3. Holmstedt, B. *Acta Physiol. Scand.* **25** (1951) Suppl. 90.
4. Holmstedt, B., and Larsson, L. *Acta Chem. Scand.* **5** (1951) 1179.
5. Meyrick, C. I., and Thompson, H. W. *J. Chem. Soc.* **1950** 225.
6. Daasch, L. W., and Smith, D. C. *Anal. Chem.* **23** (1951) 853.
7. Dowden, H. C. *Biochem. J.* **32** (1938) 455.
8. Arbusow, A. *Chem. Zentr.* **77** : 2 (1906) 1639.
9. Schrader, G. *Die Entwicklung neuer Insektizide auf Grundlage organischer Fluor- und Phosphor-Verbindungen.* Monographie 62 zu *Angew. Chem.* **63** (1951).
10. Heath, D. F., and Casapieri, P. *Trans. Faraday Soc.* **47** (1951) 1093.
11. DuBois, K. P., and Cochran, K. W. *Federation Proc.* **10** (1951) 292.

Received January 17, 1952.