ACTA CHEMICA SCANDINAVICA 17 (1963) 2688–2690

Synthesis of ^{32}P-labelled Dimethylamido Ethoxyphosphoryl Cyanide (Tabun)

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^{32}P-labelled Tabun has been synthesized from phosphoryl trichloride and carrier-free ^{32}P-labelled orthophosphoric acid. A chromatographic method for the purification of dimethylamido phosphoryl dichloride and of Tabun has been worked out.

Anticholinesterases of the organophosphorus type are extremely toxic substances,[1] which inhibit cholinesterases at very low concentrations. Biochemical studies with these compounds often require labelling, as analytical reactions of more conventional type are not sensitive enough. In the course of a study on the reactions between Tabun, cholinesterases and reactivators (oximes) it was of interest to follow the distribution of Tabun in the animal body and to identify the products obtained from Tabun after its reaction *in vitro* with cholinesterases and subsequent reactivation. These studies required a labelled compound. Whereas the synthesis of unlabelled Tabun has been described by Holmstedt,[2] the present paper describes the small scale synthesis of ^{32}P-labelled Tabun.

SYNTHESIS

Preparation of labelled phosphoryl trichloride

$$H_3{}^{32}PO_4 + POCl_3 \rightleftharpoons H_3PO_4 + {}^{32}POCl_3$$

The method described by Vigne *et al.*[3] was used with smaller modifications. 300 mC of ^{32}P-labelled carrier-free orthophosphoric acid, dissolved in 4 ml hydrochloric acid (pH 2, Amersham, Buckingham, England), were added to a thickwalled test tube. With the aid of a melting point capillary nitrogen was bubbled through the solution over night to remove the hydrochloric acid and water. Preliminary experiments showed that the loss of radioactivity during this step could be neglected. After removing the hydrochloric acid 2.4 mmoles of redistilled phosphoryl trichloride were added by means of an Agla syringe and the test tube was sealed. The tube was kept at 150°C during 8 h. After cooling to about −50°C, the test tube was opened in dry argon atmosphere. The obtained labelled compound was used without purification.

Preparation of dimethylamido phosphoryl dichloride

$$^{32}POCl_3 + 2(CH_3)_2NH \rightarrow (CH_3)_2N^{32}POCl_2 + (CH_3)_2NH \cdot HCl$$

The test tube containing the labelled phosphoryl trichloride in 1 ml of ether was provided with a rubber stopper carrying a drying tube and an injection needle and immersed into a cooling bath (−40°C). With a previously chilled syringe 2.5 ml of dry ether containing 8 % of dimethylamine (−15°C) were added slowly under magnetic stirring. Then the temperature was allowed to rise to room temperature and finally the solution was refluxed for 30 min. After cooling, the formed salt was filtered off and washed with dry ether. Filtrate and washings were combined and the ether was distilled off until about 1 ml remained. The compound was then purified by column chromatography. After experimenting with several sorption media and eluents it was decided that silicagel with acetone or ether gave the best results. The highest yield was obtained after ether elution. Thus a column (12 × 200 mm) provided with a cooling jacket connected with tap water was prepared from silicagel (100−200 mesh) in dry ether. The reaction mixture was chromatographed and eluted with dry ether. Dimethylamido phosphoryl dichloride was located with the aid of diisonitrosoacetone [4] (an 0.4 % solution of the monobutylaminosalt in acetone). The positive fractions were collected, pooled and concentrated to about 2 ml.

Preparation of dimethylamido ethoxyphosphoryl cyanide

$$(CH_3)_2N^{32}POCl_2 + C_2H_5OH + 2NaCN \rightarrow C_2H_5O-{}^{32}P(N(CH_3)_2)(CN)=O + 2NaCl + HCN$$

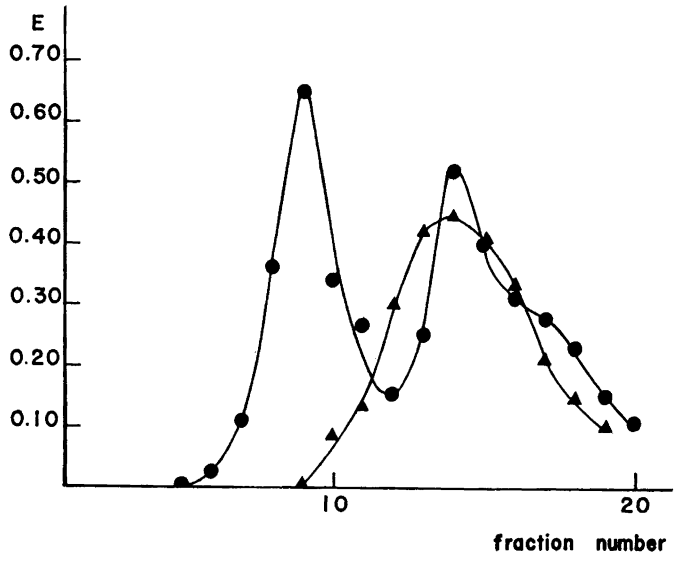

Fig. 1. Chromatogram showing the separation of Tabun from hydrocyanic acid with the aid of a silicagel column. ● = cyanide derived from hydrocyanic acid and from Tabun; ▲ = Tabun determined with diisonitrosoacetone.

400 mg of dry and finely ground sodium cyanide and 2 ml of dry ether containing 40 % of waterfree ethanol were added to the labelled dimethylamido phosphoryl dichloride in ether. The mixture was refluxed for 1.5 h. After cooling the reaction mixture was filtered through a glass filter and the salt was washed with dry ether. The combined solutions were concentrated to about 1 ml and chromatographed as described above. A chromatogram obtained from a preliminary experiment conducted with hydrocyanic acid and Tabun in alcohol is seen in Fig. 1. In the preliminary experiments cyanide derived from hydrocyanic acid and from Tabun (after hydrolysis with sodium hydroxide) was determined according to Asmus *et al.*[5] and Tabun according to Sass *et al.*[4] Between 60 and 75 % of Tabun were recovered. During the preparation of ^{32}P-labelled Tabun, when only qualitative determinations were necessary, Tabun was located with diisonitrosoacetone according to a modification of Sass' method and after addition of alkali with the Prussian Blue test for cyanides.[6] To avoid fractions containing mainly hydrocyanic acid only those positive to both methods were collected. However, to obtain a product stable in ether, the first product had to be rechromatographed. The final yield of Tabun was 8 % (see under analysis) calculated on the used amount of $POCl_3$. This low yield compared with a large scale synthesis (yield about 50 %) is probably due to the repeated concentration of small amounts of ether solutions and to the purification by column chromatography.

ANALYSIS

To be able to handle the small amount of ^{32}P-labelled Tabun the compound was kept in ether solution. The concentration of the Tabun solution was determined in three ways. After complete hydrolysis of samples in alkali the amount of cyanide was determined by the colorimetric method of Asmus and Garschagen.[5] A standard graph obtained in a similar way with unlabelled Tabun was used to read the amount of Tabun. The concentration of Tabun (cyanide) was found to be 3.5×10^{-3} M. The concentration of phosphorus, determined as described by Scheel,[7] was found to be 3.4×10^{-3} M. The concentration of Tabun was further determined by measuring the inhibitory power of the Tabun solution on cholinesterase. From numerous determinations in this laboratory of the concentration of Tabun necessary to inhibit the cholinesterase activity of a standard preparation of purified horse serum cholinesterase to 50 % (I_{50}) it was known that this concentration at pH 8.0, 25°C and after 30 min of incubation with the enzyme is $(9.3 \pm 0.7) \times 10^{-9}$ M. The Tabun-ether solution was diluted with distilled water on the basis of the cyanide determination prior to assay of anticholinesterase activity. The I_{50} of this solution was found at a concentration of 9.4×10^{-9} M.

The final product, obtained after 2 days, had a counting rate of 63×10^5 cps/mg Tabun. This value was calculated from measurements on a series of planchets. The counting rate of the purchased orthophosphoric acid solution, measured with our equipment, would have been 93×10^5 cps/mg Tabun on the same day, calculated from measurements on a series of planchets provided with varying amounts of with phosphate buffer diluted ^{32}P-labelled orthophosphoric acid.

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Received August 9, 1963.