

DEVELOPMENT OF TECHNIQUES FOR THE SYNTHESIS OF RADIOLABELLED
TRIMETHYLOXONIUM TETRAFLUOROBORATE

Vladimir M.Mahnir and Sergey S.Zaharov

Pacific Institute of Bioorganic Chemistry, Academy of
Sciences of the U.S.S.R., Vladivostok, 690022, U.S.S.R.

The design of a vacuum manifold for the synthesis of radiolabelled trimethyloxonium tetrafluoroborate from radiolabelled methyl iodide and triethyloxonium tetrafluoroborate is described.

Key words: Trimethyloxonium tetrafluoroborate, protein modification

Trialkyloxonium salts (Meerwin's reagent) have been applied as very reactive alkylating agents for organic syntheses [1,2] and also for modification of carboxylate groups in proteins [3-5]. Tetrafluoroborates of trimethyloxonium (TMO) and triethyloxonium (TEO) are used more frequently. At present several approaches for syntheses of radiolabelled TEO and TMO are used, all of them are based on exchange reactions. Parsons *et al.* have synthesized [^{14}C]TEO by the exchange between TEO and [^{14}C]diethyl ether [3], however, radiolabelled TEO was obtained by this way only as an oil and

The abbreviations used: TMO-Trimethyloxonium tetrafluoroborate
TEO-Triethyloxonium tetrafluoroborate

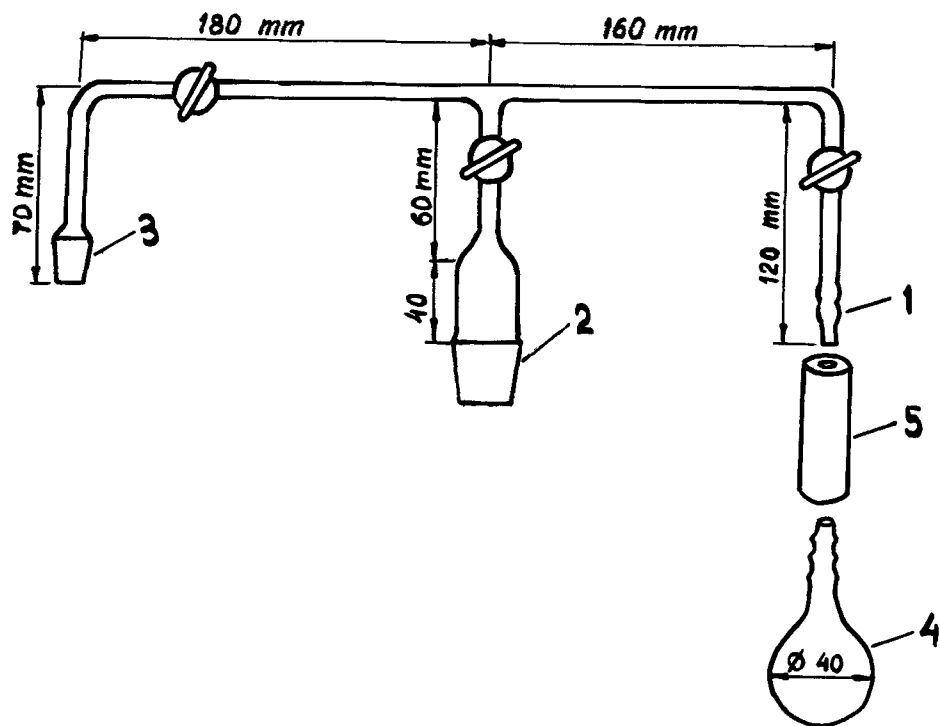


Fig. Vacuum manifold for synthesis of radiolabelled TMO

with low specific radioactivity. The method of thermal activation of gaseous tritium [6] led to TEO with high specific radioactivity but required special equipment and the maximum yield of the product was no more than 20%. Duguid *et al.* [7] have used Meerwein's method [8], according to which TMO is synthesized by exchange reaction of TEO with dimethyl ether. As a result of the reaction of TEO with [^3C]dimethyl ether Duguid *et al.* obtained a crystalline TMO with the yield, reaching 37% and a high specific radioactivity. The reaction scheme of the synthesis includes obtaining radiolabelled dimethyl ether from radiolabelled methyl iodide and sodium methylate, the purification of dimethyl ether and the exchange reaction between TEO and dimethyl ether. The advantage of this approach is that depending on the methyl iodide used the pre-

paration of both [³H]TMO and [¹⁴C]TMO may be obtained. In spite of repeated attempts we have failed to reproduce the synthesis of TMO according to Duguid *et al.* We believe that the main reason of our failure was the dissolution of vacuum grease during the synthesis under the action of fumes of methylene chloride leading to leaks in the vacuum system and contamination of the reaction mixture by the grease material. Furthermore, in our opinion, the published sources [7,3] contain insufficient experimental details which complicated the reproduction of the synthesis. We succeeded in achieving stable results due to the application of a simple vacuum manifold (Fig.) including a vacuum stopcock with a tip (1) and two vacuum stopcocks with joints (2,3). To avoid the dissolution of vacuum grease, the flask (4) where the exchange reaction took place was attached to the manifold, using a piece of vacuum rubber hose (5). The hermetic sealing of the flask was carried out by pinching the hose with a clamp. Small size of the flask (4) ensured performing the exchange reaction at risen pressure of dimethyl ether. We believe that this promotes the increase of TMO yield.

EXPERIMENTAL

TEO was synthesized by the method of Meerwein [9], kept in a desiccator under absolute diethyl ether at -20°C over phosphorous pentoxide. So stored TEO preserved its properties during several years.

Unlabelled methyl iodide was dried by magnesium sulphate and distilled. It was kept in the dark at -5°C. Methanol absolutization was performed according to [4].

Sodium methylate (approximately 2 M) was obtained by dissolution of 2,3 g of metallic sodium in 50 ml of absolute methanol and was usable for a long time when kept in germetically sealed bottle.

Synthesis of radiolabelled dimethyl ether Ten g of sodium methylate (20 mmoles) was introduced into 250 ml round-bottom flask, attached to the joint (2) of the vacuum manifold, frozen in liquid nitrogen and evacuated. Ampule containing [^3H]methyl iodide ("Isotope", U.S.S.R., 22 mCi; 357 Ci/mol) was scored, frozen in liquid nitrogen, opened and placed into the test tube with a joint, already containing 0,7 ml (11 mmoles) of unlabelled methyl iodide. The test tube was attached to the joint (3), frozen in liquid nitrogen and evacuated. Methyl iodide was allowed to distil at room temperature into the flask containing sodium methylate immersed in liquid nitrogen, then the cooling was removed and the reaction mixture was left at room temperature overnight. A vacuum pump was connected through the tip (1); joints were greased with vacuum grease. Since radiolabelled methyl iodide can not be stored, the final product specific radioactivity largely depends on the time of its manufacturing.

The purification of dimethyl ether A test tube was attached to the joint (3), evacuated, frozen in liquid nitrogen and dimethyl ether was allowed to distil into it. The flask containing the sodium methylate was replaced by a 250 ml round-bottom flask containing a small quantity of phosphorous pentoxide, cooled in liquid nitrogen and evacuated. The test tube containing dimethyl ether was immersed in a Dewar vessel with chloroform/solid chloroform cooling mixture (-68°C) which was prepared by adding portions of liquid nitrogen to chloroform with vigorous stirring. Dimethyl ether was allowed to distil into the flask with phosphorous pentoxide immersed in liquid nitrogen, incidentally, the majority of the methanol remained in the test tube. The flask was then allowed to stand for 5 hr at room temperature to remove the traces of methanol.

Exchange reaction TEO was dried in a vacuum desiccator.

One g (5.8 mole) of TEO was quickly weighed and then dissolved in 8 ml of methylene dichloride. This solution was introduced by pipette into the flask (4) the latter was attached to the tip (1) through the piece of the vacuum hose (5). The flask was frozen with liquid nitrogen, evacuated and dimethyl ether was allowed to distil into it, then the hose was pinched with a clamp and the flask was allowed to stand overnight at room temperature. A vacuum pump was connected to the joint (3) using a corresponding connecting adapter. If the reaction mixture was periodically shaken the first TMO crystals were observed after 30 min. The crystals of [³H]TMO were collected on a glass filter, quickly washed with methylene dichloride, kept under methylene dichloride in a vacuum desiccator over phosphorous pentoxide at -20°C. The total TMO yield was 239 mg (1,71 mmole) or about 30%. Specific radioactivity was determined according to method [7] using benzoic acid as a model compound. The methyl benzoate obtained was purified by thin layer chromatography on silica gel L 5/40 µm (Lachema, CSSR), eluting using benzene-petroleum ether (4:1 v/v), R_f 0,6.

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