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Note

Convenient apparatus for the generation of small amounts of diazomethane

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The formation of methyl esters of organic acids by the use of ethereal diazomethane has remained an important derivatization procedure for use prior to gas chromatographic analysis for well over 25 years^{1,2}. Of the numerous ways to prepare diazomethane, the micro co-distillation procedure described by Schlenk and Gellerman² has proved most useful. This procedure results in a relatively concentrated solution while minimizing explosion and toxicity hazards. Although the product obtained from the micro co-distillation was originally used for the derivatization of fatty acids, this method has also proven to be useful with a large variety of other organic acids^{1,3,4}.

Commercially available apparatus are either for larger scale synthesis or are based on the diffusion design of Fales *et al.*⁵. In our laboratory, we have found that the smaller scale commercial devices produce only relatively dilute solutions which are insufficient to quantitatively derivatize some organic acids. A recent report by Walker *et al.*⁶ described a modification of the original Schlenk and Gellerman² apparatus. While a decided improvement over the original test tube system, the modified design was fragile and complex. It consisted of four threaded glass units each composed of seven individual pieces. These components were connected together by PTFE tubing slipped over delicate, pulled glass nipples. This present report describes a sturdy, two piece glass and PTFE apparatus incorporating "Clear-Seal" standard taper joints (Wheaton*). The device is safe and convenient to use (in fume hood) and avoids problems that result from use of rubber stoppers, sealing rings, and septa. Also, it does not have screw threads, fragile glass connectors, ground glass joints, or other fragile pieces or sharp edges. In addition, this device allows the product to be collected into small glass vials where it can be used to methylate samples directly^{2,6} or the ethereal solution can be stored for later use.

* Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

MATERIALS AND METHODS

Apparatus

The apparatus (Figs. 1 and 2) was custom made (Glassblowing by Ambrose, Takoma Park, MD, U.S.A.) using standard taper "Clear-Seal" 24/40 glass joints (Wheaton Scientific, Millville, NJ, U.S.A.: No. 757773 and No. 758973) and a glass to PTFE union (Alltech, Deerfield, IL, U.S.A.; No. 200-28). The PTFE outlet tube was 1.5 mm O.D. (0.4 mm I.D.) \times 80 mm; it was flanged using a heated device (Alltech No. 200-30) and connected to the union using a nylon end fitting (Alltech No. 20022). All other parts were fabricated from standard borosilicate glass. The diazomethane generated was collected into diethyl ether in 1/2-dram (12 \times 35 mm) vials equipped with PTFE lined screwcaps (Scientific Specialties, Randallstown, MD, U.S.A.; No. B69301).

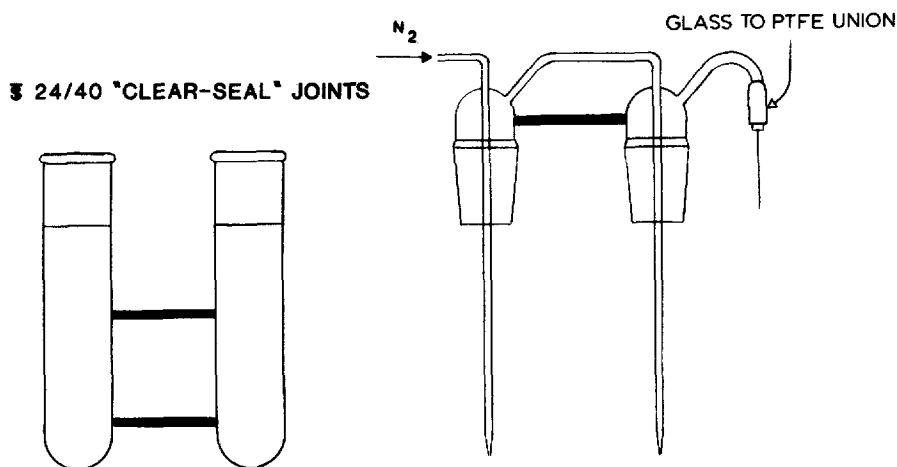


Fig. 1. Diagram of the two components of the co-distillation apparatus showing the "Clear-Seal" joints and the glass to PTFE union. Tips of the nitrogen bubbling tubes were drawn to yield an opening of 0.5 mm and were fire polished. Nitrogen flows into the first tube (left) where it is saturated with diethyl ether. The diazomethane is generated in the second tube (right) and is carried in the nitrogen stream out the PTFE tube that is connected to the union fitting.

Esterification

The first tube was filled approximately half full (25 ml) with diethyl ether (Mallinckrodt, Paris, KY, U.S.A.; No. 0804) and the second tube contained 7 ml of 2-(2-ethoxyethoxy)ethanol (Aldrich, Milwaukee, WI, U.S.A.; No. E455-0), 7 ml of diethyl ether and 5–10 ml of 7 *M* sodium hydroxide. A flow of nitrogen was initiated and adjusted to 5–10 ml/min. Approximately 1 g of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide ("Diazald", Aldrich; No. D2,8000) was added to the second tube and the apparatus sealed with springs. The PTFE outlet tube was placed into a 1/2-dram vial containing 1.5–2 ml of diethyl ether. After a few minutes the ether in the vial developed a bright yellow appearance characteristic of ethereal diazomethane and the first vial was replaced by a similar vial. This process was continued until the amount of diazomethane production had declined so that full color would not de-

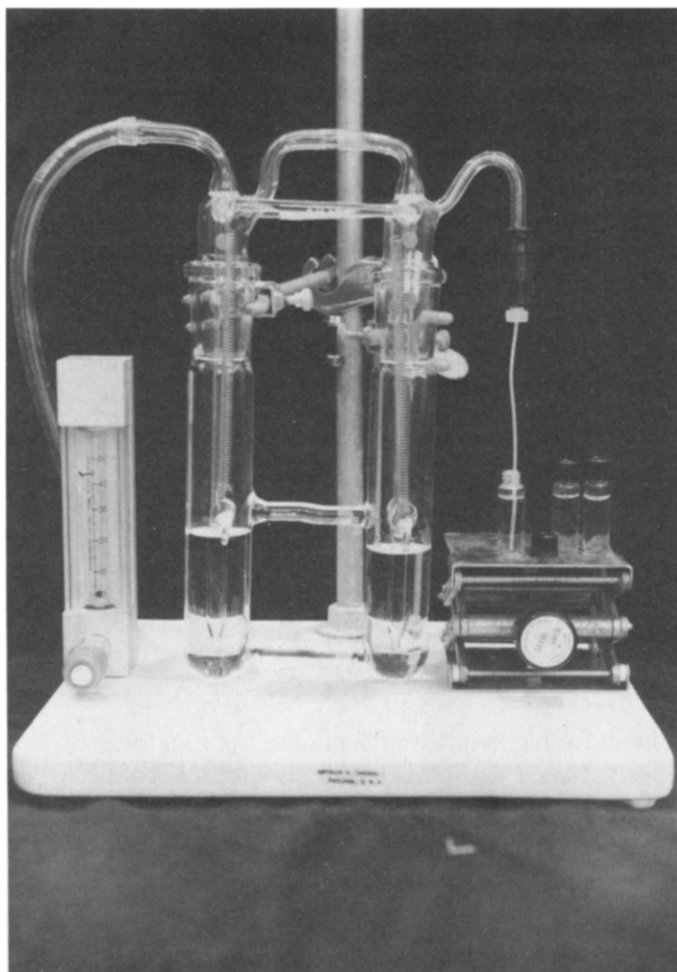


Fig. 2. Photograph of the apparatus set up for the production of ethereal diazomethane with nitrogen connected and a collection vial in place at the outlet tube.

velop in the diethyl ether in the next vial. Usually 10–12 vials can be produced from one reaction.

Numerous methods have been described for methylation with diazomethane^{1–4}. The procedure used in our laboratory for esterification of small organic acids, such as indole-3-acetic acid, is as follows: The sample is dried in a 1/2-dram vial and resuspended in 100 μ l of methanol. Approximately 1 ml of a bright yellow solution of ethereal diazomethane is added and the reaction is allowed to continue at room temperature for 5 min. The sample is dried using a nitrogen stream and hand warming (in a hood and with vinyl gloves!). This technique is useful for quantities of up to 50 nmol and for as little as a few femtomoles. With some indole derivatives, such as indole-3-acetyl-L-aspartic acid, the 5-min reaction time is eliminated and the sample is dried immediately after the diazomethane is added⁷. For critical samples we prefer to use freshly prepared diazomethane; however, we have obtained good results from

solutions of diazomethane which have been stored for as long as ten weeks provided that they still exhibited the characteristic color.

DISCUSSION

Diazomethane is a useful mild agent for methylation, but its toxicity and explosion hazards require that precautions be taken with its use. Diazomethane is toxic and will cause development of specific sensitivity after repeated exposure. In addition, it is potentially explosive and it is known that sharp edges (ground joints, scratched or broken glass, crystal formation) and strong light greatly increase the risk of detonation. All work with diazomethane should be carried out in a properly vented hood and workers should be made aware of the hazards involved. A particularly useful cautionary message is given by De Boer and Backer⁸. As an additional safety precaution it is recommended that the N-methyl-N-nitroso-*p*-toluenesulfonamide be recrystallized prior to storage⁹. The device described in this report and the small amount of compound involved, however, minimize the hazards involved. An even smaller scale device is currently being built using 14/20 "Clear-Seal" joints for use in studies involving generation of diazo[¹⁴C]methane. The availability of an apparatus of this type simplifies the process of diazomethane generation and thus increases the utility of this important derivatization reagent.

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