CHROM. 3819

## A note on the synthesis of N-methyl-labeled quaternary amines and betaines

During a study on the formation and degradation of carnitine (3-hydroxy-4-trimethylaminobutyric acid)[1,2] we needed several methyl-labeled quaternary amines related to carnitine. In order to obtain high specific radioactivity of the quaternary amines, the corresponding tertiary amines were treated with $^{14}C$- or $^{3}H$-labeled methyl iodide of high specific activity. The tertiary amines were added in large excess to ensure a high isotopic yield in the syntheses. When dimethylamino acids were used, a suspension of barium hydroxide was also added. Ion-exchange column chromatography on polystyrene-sulfonic acid resins[3] was used for the purification of the reaction products, using dilute solutions of hydrochloric acid for the elution. However, as a large excess of the tertiary amines were used, pure preparations of the radioactive quaternary amines were obtained only after repeated chromatographies when the carbon chain in the amine contained more than three carbon atoms. We now report simple procedures which we have used in the purification of N-trimethyl derivatives of amines, amino alcohols and amino acids with 2–6 carbon atoms in the chain.

The quaternary amines and amino alcohols were purified by ion-exclusion chromatography on columns of the anion exchanger Dowex 1-X2 (200–400 mesh, $CO_3^{2-}$-form) with water as the eluting agent. The quaternary amines were excluded from the resin phase and were obtained as the carbonates. The chlorides were obtained after the addition of dilute hydrochloric acid or after filtering the aqueous solutions of the carbonates through columns of Dowex 1-X2 (200–400 mesh, $Cl^-$-form). The dimethylamines, being in base form at the high pH value in the column, were adsorbed

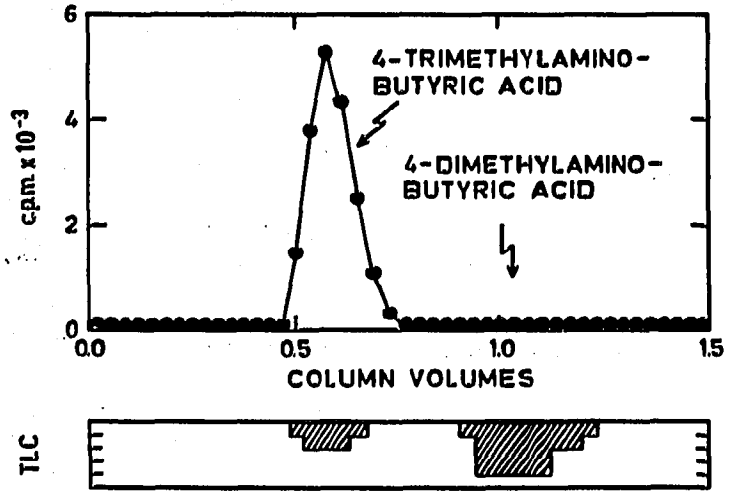


Fig. 1. Purification of [methyl-$^{14}C$]-4-trimethylaminobutyric acid. 4-Dimethylaminobutyric acid (300 $\mu$moles) and 200 $\mu$moles of barium hydroxide had been added to 33 $\mu$moles of $^{14}C$-methyl iodide (0.5 mC) in 5 ml of aqueous methanol[1]. After 3 days at room temperature the reaction mixture had been taken to dryness, redissolved in a minimum amount of dilute hydrochloric acid and applied to a column of Retardion AG 11A8 (2.4 × 40 cm, 50–100 mesh, "self-adsorbed" form; BioRad Laboratories, Inc., Richmond, Calif., U.S.A.) which was eluted with water. The radioactivity in the eluate was determined in a methane-flow proportional counter, and a semi-quantitative estimate of the relative amounts of methylated ammonium derivatives was obtained by TLC[4].

onto the resin matrix and were not eluted until several column volumes of water had passed.

The same procedure may be used for the separation of betaines from dimethylamino acids, but barium ions are then eluted together with the betaines. In this case, the purification was carried out by means of ion-retardation chromatography on Retardion AG 11A8 (50–100 mesh, "self-adsorbed" form) with water as the eluent. Betaines, being zwitterions, are not retarded during filtration through the column. Dimethylamino acids are weak bases[4], and as the pH value in the resin phase is slightly above 8, they behave as negatively charged ions and are retarded in their passage through the column. The barium ions are eluted very slowly. Quaternary amines and amino alcohols are not separated from the tertiary amines on this resin. It may be noted that amino acids are eluted together with the betaines (*cf.* ref. 5).

Fig. 1 gives the results obtained in the synthesis of [methyl-$^{14}$C]-4-trimethylaminobutyric acid. The betaine is eluted before the dimethylamino acid and there is a complete separation between the two compounds.

This work was supported by a grant from the Swedish Medical Research Council (13X-585).

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Received September 30th, 1968

*J. Chromatog.*, 39 (1969) 78–79

CHROM. 3824

## An improved method for preparing buffers that have low baselines in automated amino acid analysis

An improved method for preparing citrate buffers for automated amino acid analysis is to use anhydrous citric acid and NaOH rather than sodium citrate and HCl. Baseline rise, usually due to ammonia, is a common problem with amino acid analyzers. Elaborate precautions are often necessary to reduce ammonia (or other ninhydrin-positive contaminants) in preparing the buffers used for amino acid elution on ion-exchange columns.

A Technicon Amino Acid Analyzer with a single 125 cm column is in use in this laboratory. The common gradient of citrate buffers, pH 2.87 to pH 5.00 and 0.2 *M* $Na^+$ to 0.8 *M* $Na^+$, is routinely used. The buffers which are used to produce the gradient have been made up from sodium citrate, 2 *N* NaOH, Brij 35, thiodiglycol