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DETECTION OF N-ACETYLGLYCINE, N-ACETYLGLYCYLGLYCINE, AND N-ACETYLGLYCYLGLYCYLGLYCINE BY PAPER CHROMATOGRAPHY

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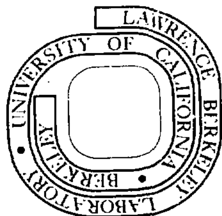

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then concentrate the ether extract, which could result in a loss of acetic acid. In addition, the acetic acid peak "rides" on the shoulder of the very large ether peak. This might require the use of lower sensitivity attenuation in order to keep the solvent peak and the acetic acid peak on scale. In addition, gas chromatography of acetic acid is often plagued by the "ghosting" phenomenon (2,3,4). Of course, in these methods for determining total acetic acid content, there is no indication of the acetyl compound from which the acid is derived.

The detection of some acyl amino acids by paper chromatography was done by Kirchenmayer and Kuffner (5). They acetylated amino acids on paper with acetic anhydride and developed the chromatogram with normal butanol-acetic acid-water (8:1:10, v/v). Reio (6) chromatographed N-acetylglycine, developed the chromatograms with three different solvent systems, and reported R_f values of 0.54, 0.08, and 0.14. Detection was made with ninhydrin reagent and bromophenol blue indicator. Whitehead (7) separated N-acetyl amino acids (including N-acetylglycine) chromatographically in various solvent systems followed by ionophoresis in ethylamine acetate. The separated N-acetyl amino acids showed up as yellow spots after they were dipped in bromocresol green indicator in acetone. Umebayashi (8) chromatographed N-acetylglycine in normal butanol-acetic acid-water (4:1:50, v/v), and then hydrolyzed the spots on the TLC plates with 6 N HCl. Later, the spots were detected with ninhydrin reagent.

Bergmann (9) determined N-acetylglycine as the hydroxamic acid derivative and measured the absorption of the ferric-hydroxamic acid complex.

Various authors formed the volatile ester (10, 11) and silyl derivatives (12, 13) of N-acetylglycine and measured this by gas chromatography.

Although these investigators studied the paper and thin-layer chromatographic and gas chromatographic behavior of N-acetylglycine (and its volatile

ester and silyl derivatives). N-acetylglycylglycine and N-acetylglycylglycylglycine were not studied. Gordon et al. (14) did study the band rates of these two compounds in partition chromatography, however, their data indicated that the two could not be satisfactorily separated using their solvent system.

In the present work we used the developing solvent of Kirchenmayer and Kuffner (5), normal butanol-acetic acid-water (8:1:10, v/v), and found that N-acetylglycine, N-acetylglycylglycine, and N-acetylglycylglycylglycine could be satisfactorily separated and detected.

PROCEDURE

All chemicals were reagent grade and used as received from the vendor. Normal butanol, glacial acetic acid, and bromocresol green indicator were obtained from J. T. Baker Chemical Co. Methanol and acetone were from Mallinckrodt Chemical Works. Eastman Organic Chemical Company supplied the morpholine. N-acetylglycine (NBC) and N-acetylglycylglycine (Cyclo Chemical Corporation) were also used directly as received. N-acetylglycylglycylglycine was prepared in this laboratory by the method of Fischer (15).

After acetyl compounds of interest were separated from peptides by ion-exchange chromatography, the effluent fraction was dried and redissolved in absolute methanol. Enough methanol was used to give a spotting solution of 0.001 to 0.005 M for each compound of interest. A 50 microliter aliquot of this methanolic solution was applied with a micropipet to the base line of Whatman #1 paper (35 to 40 cm long), and the spots were dried in a gentle stream of nitrogen. The spotted paper was equilibrated in a covered chromatographic jar for 1 hour and then dipped into the upper phase of the developing solvent. The chromatogram was developed for 18 hours at room temperature, withdrawn from the jar, and hung to dry in a hood overnight. The developed

chromatogram was next hung in a 100° C oven for 6 hours. This heating time was essential in order to see the spots under ultraviolet light without the use of an indicator. At this point the spots could be located, cut out, and processed further as desired.

To see the spots more clearly the paper was dipped into a solution of 0.01% bromocresol green indicator in acetone to which 1 drop of morpholine had been added. After drying the dipped paper for 5 min at room temperature, the spots readily showed up--yellow on a blue background. The R_f values were 0.59 (N-acetylglycine), 0.43 (N-acetylglycylglycine), and 0.33 (N-acetylglycylglycylglycine).

Although St. Onge *et al.* (10) found N-acetylglycine as a contaminant of analytical grade acetic acid, we found no paper chromatographic evidence for this in our work. Consequently, no effort was made to further purify glacial acetic acid before using it as part of the developing solvent.

This general procedure was used satisfactorily for the detection of these three compounds in aqueous solutions of diglycine, triglycine, and tetraglycine, which were used as gamma-radiolysis targets.

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