^{14}C-Labeled pyrrolidonecarboxylic acid as a contaminant in chromatography*

Chromatographic techniques are employed frequently to separate amino acids, keto acids and related compounds.

In recent separations of ^{14}C-labeled amino acids from brain tissue extracts, we used both chromatographic and ionophoretic techniques. After two-dimensional paper chromatography with water-saturated phenol and water-saturated lutidine[1] as the first and second solvent systems, respectively, we found that the "alanine spot" on the chromatogram was quite highly labeled. Ionophoretic separation[2], on the other hand, yielded an "alanine spot" with no appreciable radioactivity.

When the spot of "radioactive alanine" on the paper chromatogram was cut out, eluted with water, concentrated and rerun ionophoretically (pH 1.9), a radioactive, ninhydrin negative spot remained close to the origin whereas the ninhydrin positive unlabeled alanine migrated a long distance.

The radioactive spot was again eluted, hydrolyzed[3] and rechromatographed. It yielded a ninhydrin-positive radioactive compound which migrated like glutamic acid. The ninhydrin-negative radioactive component had the same R_F as authentic pyrrolidonecarboxylic acid in four different chromatographic solvent systems[4] and in electrophoretic separations under two different pH conditions. It appears probable, therefore, that the contaminant is pyrrolidonecarboxylic acid.

We have detected this cyclized ninhydrin-negative derivative of glutamic acid and glutamine in many brain tissue extracts, particularly when ethyl alcohol was used as a deproteinizing agent and extracts were concentrated by evaporation. Pyrrolidonecarboxylic acid may have been formed as an artifact during sample preparation or represents a normal constituent of the tissues[5].

Investigators working with tissue extracts containing radioactive glutamate-^{14}C, glutamine-^{14}C, glutathione-^{14}C or other γ-glutamyl amino acids would be well advised to check separately the migration of pyrrolidonecarboxylic acid-^{14}C in their chromatographic, electrophoretic or column systems so as to avoid erroneous results due to the unknown coincidence of pyrrolidonecarboxylic acid-^{14}C with other compounds.

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