Preparation of L-Djenkolic acid-^{3}H*

K. R. BRODY AND R. P. SPENCER
Section of Nuclear Medicine, Department of Radiology
Yale University School of Medicine, New Haven, Connecticut, U.S.A.

Received on 28 June 1966

SUMMARY

L-djenkolic acid (a cystine analogue with an -S-CH_2-S- linkage) was tritiated by the Wilzbach technique. Tritiation was accompanied by considerable degradation as revealed by discoloration and multiple chromatographic spots. Using a modification of a literature method for djenkolic acid synthesis, the crude material was reprocessed for purification. A 60% yield of the pure tritiated amino acid was obtained (purity checked by elemental analysis, lack of -SH groups and chromatographic behavior). The levo configuration was maintained as shown by the optical rotation. The tritiated compound was unstable. Cold and an inert atmosphere did not offer significant stabilization.

Following tritiation of an organic compound by the Wilzbach technique, or an other relatively non-specific method, the problem of purification remains. An approach to solving this particular problem for the cystine analogue L-djenkolic acid is presented. The approach was to utilize the impure product in the latter steps of a chemical preparation of L-djenkolic, not for synthesis, but for purposes of purification.

METHODS

Five hundred thirteen mg of L-djenkolic acid (CalBiochem) was tritiated by exposure to three curies of tritium gas for one week at ambient temperature. Labile tritium was removed by exchange with 10 ml of 1 N HCl. The original L-djenkolic acid was white in color, while the crude tritiated product was yellow. Small samples of the radioactive material were analyzed by thin-layer chromatography (1,000 micron MN 300 cellulose on glass plate in butanol:acetic acid:water, 17:10:40). Since multiple radioactive overlapping peaks were present

* Supported by Grants CA 06519 and AM 09429 from the U.S. Public Health Service.

(which could not be separated by thin-layer chromatography), purification was begun on the remaining material.

The purification described below is a modification of the latter steps in the synthesis of djenkolic acid, as reported by Armstrong and duVigneaud [1]. Any cystine present is converted to soluble products in alkaline solution; these remain in solution while the djenkolic acid is then precipitated in an acid solution. The detailed procedure follows.

1. The tritiated material (0.5 gm) was suspended in 5 ml of water at room temperature. With agitation, concentrated NH_4OH was added dropwise to dissolve the compound.
2. The solution was filtered, and 0.075 gm NaCN was added. Mixing was performed for one-half hour.
3. Adjustment of the pH to 6.0 was accomplished by means of glacial acetic acid. Filtration was carried out and the solid was washed twice in ice cold water.
4. The solid was dispersed in 5 ml of boiling water and 6 N HCl was added dropwise until the solution was obtained.
5. Any trace of solid was filtered off and the filtrate was heated to boiling and neutralized with 6 N NaOH to pH 5.0 with agitation.
6. The mixture was cooled to room temperature and filtered and the residue was washed three times with ice cold water.
7. The solid was heated at 100 °C under 0.1 mm Hg vacuum for two hours.

RESULTS

The purified L-djenkolic acid-^{3}H was white in color. Chemical analysis was as follows: expected C 33.08%, H 5.55%, N 11.02%; found (Carol K. Fitz, Ph.D.) C 33.0%, H 5.6%, N 11.0%. Optical rotation of a 1% solution in approximately 1 N HCl at ambient temperature was determined by use of a Rudolph polarimeter. Found: tritiated material −53.9°, original L-djenkolic acid −54.1°.

Chromatography in several solvent systems revealed the tritiated material to be indistinguishable from the original L-djenkolic acid (Table 1). That is, there

TABLE 1. Thin-layer chromatography of L-djenkolic acid on MN 300 (Mackerey, Nagel & Co.) cellulose (1,000 microns).

Solvent	R_f
Water	0.94
Butanol:acetic acid:water (17:10:40)	0.72
Isopropanol:conc. NH_4OH:water (17:10:30)	0.81
Isopropanol:conc. HCl:water (17:10:40)	0.72

was only one peak of radioactivity, and that corresponded to the single ninhydrin-reacting spot. In addition, neither the original compound nor the tritiated one gave a reaction for -SH or -S-S- groups with cyanide and nitroprusside [2, 3].

Discussion

Evidence that the final tritiated compound was chemically similar to the original L-djenkolic acid was quite good: physical appearance, chemical analysis, optical rotation, and chromatographic behavior in several solvent systems. The final specific activity was about 0.3 μc/μmole. This was considerably less than the specific activity of the original crude (unpurified) product, indicating that high activity contaminants were present. Indeed, the original thin-layer chromatograms revealed large quantities of radioactivity outside of the region corresponding to djenkolic acid. Final recovery (by weight) of L-djenkolic acid-^{3}H was approximately 60% of the starting material. The purified L-djenkolic acid-^{3}H was unstable at room temperature. After three months, there was approximately 62% decomposition, as determined by thin-layer chromatograph and radiochromatogram scanning. New radioactive peaks (of about equal size) appeared at the origin and at $R_f = 0.95$ in an isopropanol:conc. HCl:water (17:10:40) system. When kept at the temperature of dry ice (with or without covering nitrogen), the preparation was only slightly more stable. Since this great instability is not observed with cystine-^{3}H, it may well be a function of the radiosensitivity of the -S-CH_2-S- group of djenkolic acid.

References

1. Armstrong, M. D. and du Vigneaud, V. — *J. Biol. Chem.*, **168** : 373 (1947).
2. Grunert, R. R. and Phillips, P. H. — *Arch. Biochem.*, **30** : 217 (1951).
3. Gawron, O. and Fernando, J. — *J. Am. Chem. Soc.*, **83** : 2906 (1961).