The Synthesis and Purification of Pentylenetetrazole-10-^{14}C

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Received September 15, 1969.

SUMMARY

Pentylenetrazole-10-^{14}C was synthesized by reacting hydrazoic acid with cyclohexanone-1-^{14}C. Purification was carried out using sublimation and thick layer chromatography. Proof of radiochemical purity was determined using thin layer chromatography and autoradiography. The specific activity was found using liquid scintillation counting.

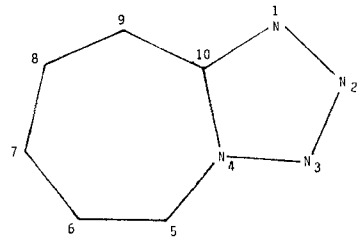
INTRODUCTION.

Pentylenetetrazole (PTZ) is a rapidly acting analeptic agent which has been used for barbiturate and alcoholic poisoning [1]. Today it is used most often in combination with nicotinic acid to alleviate senile confusion and depression in geriatric patients. Improvement is noted in alertness, memory, emotional stability and cooperation [2, 3].

A radioactive tracer technique has been used to follow the absorption and excretion of nicotinic-7-^{14}C acid in combination with PTZ in a sustained release dosage form (Geroniazol TT, Philips Roxane Laboratories) and non-sustained release dosage form in humans [4]. Esplin and Woodbury have studied the absorption and excretion of PTZ in rats but were unable to identify metabolites [5]. Pentylenetetrazole-10-^{14}C was synthesized in the work reported in this paper and a portion of it was used in another study in combination with nicotinic acid to follow the release pattern from non-sustained and sustained release dosage forms. The latter work was done in humans counting blood and urine levels at various time periods [6]. Tissue distribution and metabolite studies are currently in progress using the labeled compound.

Chapman, McCombie and Saunders worked out a one step synthesis for PTZ (I) by reacting hydrazoic acid with cyclohexanone [7]. Murray and

Ronzio [8] synthesized PTZ-^{14}C utilizing cyclohexanone-2-^{14}C using the method described by Chapman. The reaction gave labeled PTZ in the 5 and 9 positions and a purified yield of 22 %.



I

In the work reported in this paper the method of Chapman [7] was utilized by reacting hydrazoic acid with cyclohexanone-1-^{14}C to give pentylenetetrazole-10-^{14}C. The C-14 atom is exclusively in the 10 position. The yield was 47 % after four sublimations and 36 % after thick layer chromatography. The melting point was 58-59° C. The proof of radiochemical purity was shown using thin layer chromatography and autoradiography.

Experimental.

Hydrazoic Acid [9]. — To 1.183 grams (0.0182 mole) of sodium azide mixed with an equal weight of water in a 25 ml 3-necked flask fitted with a stirrer and thermometer, there was added 10 ml of a ligroin benzene mixture (15 : 85) followed by the dropwise addition of 0.93 grams (0.0091 mole) of 96 % sulfuric acid to the stirred mixture which was kept cold (0° C) using an acetone-ice bath. When all of the acid was added, the mixture was stirred for an additional three hours, and then most of the organic layer was decanted into a flask. The remainder of the organic layer was separated on a Buchner funnel from the inorganic salts. The combined organic layer was dried over calcium chloride for two hours.

Pentylenetetrazole-10-^{14}C [7]. — The dry hydrazoic acid solution obtained above was placed in a 25 ml 3-necked flask fitted with a stirrer and condenser. After the hydrazoic acid solution was cooled to 0° C using an acetone-ice bath, anhydrous ferric chloride (0.355 gm) was added to the solution followed immediately by the dropwise addition of 5 drops of diluted cyclohexanone-1-^{14}C [a] [10 mCi (312 mg) of the isotope diluted to 5 ml in benzene]. Thereafter, fifteen drops of solution were added at a time at five minute intervals. After the last addition of the radioactive isotope, 43 mg of cold cyclohexanone was added to equal 0.355 gm (0.00364 mole) of ketone. The mixture was

[a] New England Nuclear Corp., Boston, Mass.

allowed to stir for 48 hours during which time the temperature was allowed to rise to room temperature.

The resulting solution was made alkaline with 30 % sodium hydroxide and filtered using a Buchner funnel to remove the ferric hydroxide. The two layers were separated and the aqueous portion was saturated with ammonium sulfate and then extracted twelve times with 15 ml. portions of benzene. The combined organic layer was dried over sodium sulfate for 36 hours, the benzene solvent was removed under reduced pressure, and the impure solid product remained.

Purification of the Crude Product. — The crude product was sublimed four times in a microsublimator at 75° C (0.1 mm). After four sublimations a yield of 0.2331 gm (47 %) of product with a specific activity of 19.5 μCi/mg was obtained.

The product was subjected to thin layer chromatography (T.L.C.) and autoradiography analyses. The procedure was as follows :

To 1.5 mg of PTZ-10-^{14}C, 0.5 ml. of absolute ethanol was added. Three 10 λ samples of the solution along with a non-radioactive standard of the same concentration were spotted on a 20 × 20 cm thin layer chromatography plate utilizing silica gel, Adsorbosil-1[b], 250 μ thick. The plate was developed in methyl ethyl ketone, and the spots were visualized by iodine vapor. After the iodine had all desorbed, the plate was then subjected to autoradiography. The plate was sprayed with Neatan[c], a plastic preservative, to keep the silica gel intact when placing the X-ray film on the plate. After the No Screen Medical X-ray[d] film had been exposed for 2 hours, the film was developed and fixed in Eastman Kodak liquid developer and liquid fixer, respectively. Three radioactive impurities were seen on the autoradiogram along with the labeled PTZ. The R_f for impurity A was 0.73, for impurity B 0.47 and for impurity C 0.24. The R_f of the labeled PTZ was 0.58 as was the PTZ standard. The T.L.C. plate was then sectioned using the autoradiogram as a guide. Each radioactive area was scraped from the plate into a counting vial containing scintillation fluid composed of 0.4 % PPO (2,5-diphenyloxazole) in an equal volume of toluene and 2-ethoxyethanol. The present purity of PTZ-10-^{14}C was determined using the Packard Tri-Carb liquid scintillation spectrometer. For determination of absolute disintegration, toluene-^{14}C was used as the internal standard. The percent purity (determined by averaging the counts of three columns on the same T.L.C. plate) of PTZ was 94.4 %, for impurity A 0.79 %, for impurity B 0.94 %, for impurity C 3.69 % and for the rest of the column up to the solvent front 0.16 %.

Thick layer chromatography (ThLC) was used to further purify the product. Seven glass plates, 5 × 20 cm, were spread 2 mm thick with Adsorbo-

[b] Applied Science Laboratories, Ind., State College, Pa.
[c] Brinkmann Instruments Co., Westbury, L. I., N. Y.
[d] Eastman Kodak Co., Rochester, N. Y.

sil-1 which had been extracted first with spectrophotometric grade chloroform[e] and then with absolute methanol each for 24 hours using a Soxhlet extractor. The 233 mg of PTZ-10-^{14}C was dissolved in 2.30 ml of absolute ethanol. This solution was spotted equally on the seven glass plates which were then developed using methyl ethyl ketone. The plates were allowed to air dry overnight, and they were then exposed to X-ray film for 2 hours. The R_f for PTZ was 0.60, for impurity A 0.83, for impurity B 0.43, for impurity C 0.16 and for impurity D(origin) 0.00. For each plate, the section of silica which contained the PTZ was scraped into an evaporating dish and placed into a vacuum desiccator at 0.03 mm at room temperature for 16 hours to remove all methyl ethyl ketone trapped within the thick silica. The content of the evaporating dish was placed into a 1/2 inch chromatographic column which was eluted with 50 ml of spectrophotometric grade chloroform. The chloroform was then evaporated off using a steam bath. A total of 180 mg of PTZ-10-^{14}C was obtained.

A sample of the labeled PTZ was tested for radiochemical purity by thin layer chromatography. Two plates were used. The first was developed in methyl ethyl ketone and the second in methyl ethyl ketone-*n*-butanol-glacial acetic acid-water (5 : 2 : 1 : 2). The spots of unlabeled PTZ standard were detected by iodine vapor. These plates were then exposed to X-ray film which showed after developing and fixing only one spot for PTZ-10-^{14}C for each system. For the first plate the R_f of the labeled and unlabeled PTZ was 0.62, and for the second plate the R_f was 0.82.

The specific activity was found to be 17.8 μCi/mg using internal liquid scintillation counting. The compound gave a melting point of 58-59° C; reported 57-60° C [10].

ACKNOWLEDGMENTS.

This research was supported in part by a grant from Philips Roxane Laboratories, Inc., Columbus, Ohio.

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