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A SIMPLE PROCEDURE FOR THE PREPARATION OF TRITIATED HOMOVANILLIC ACID

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SUMMARY

Homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid) was labeled with tritium by exchange reaction in tritiated water catalyzed by sulfuric acid. The product was obtained in good yield and specific activities sufficient be utilized in metabolic experiments. Reactions carried out under identical conditions, but in the presence of deuterated water gave a product in which all the aromatic hydrogens and those α - to the carboxyl group were replaced with deuterium indicating that the tritium-labeled homovanillic acid was labeled in these positions.

Key Words: Homovanillic acid, HVA, tritium labeling

INTRODUCTION

Homovanillic acid (HVA) is the major endproduct of dopamine metabolism in primates and for this reason its levels in plasma and urine provide an indication of the rate of dopamine production and turnover in normal and disease states. Homovanillic acid turnover was determined in man and monkeys using deuterated HVA that was measured by gas-liquid chromatography-mass spectrometry (1,2,3). The deuterated compound used in these studies was prepared by exchange reactions in deuterium oxide (4). The results of these investigations in which deuterated HVA was employed were that only approximately half of the administered HVA could be recovered in the urine. This led the authors to speculate that a significant portion of the metabolite may be eliminated by an as yet undescribed mechanism (1,2). These observations prompted a reinvestigation of this problem to determine if these results could be duplicated and to explore possible alternate routes of elimination of HVA. To

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increase the sensitivity of the assays it was decided to utilize tritiated HVA. Although the approach to the preparation of the radiolabeled compound is based on earlier work in which the deuterated compound was prepared (4), the procedure had to be modified to be applied for tritiation of HVA, and a simple approach to the isolation of the labeled material was developed. Using this procedure, the labeled compound, that is not commercially available can be prepared at relatively little cost in good yield and purity.

EXPERIMENTAL AND RESULTS

Materials

Tritiated water, 1 Ci/gm was obtained from NEN Research Products, deuterium oxide 100% atom excess from Aldrich Chemical Company, and homovanillic acid from Sigma Chemical Company. Preparative silica gel thin-layer chromotography plates 2000 microns thick with fluorescent indicator were purchased from Analtech Inc. The HPLC grade solvents employed in the HPLC assays were obtained from Fisher Chemical Company, while all other solvents and reagents were reagent grade.

High Performance Liquid Chromatography

The purity of the tritiated HVA was determined by high-performance liquid chromatography using a system composed of a Laboratory Data Control Constametric Model III pump and a Waters Radial-Pac C 18, 10 μ reversed phase column in a Model RCM-100 Radial Compression Module. The ultraviolet absorbance was monitored with a Gilson Model HM Holochrome detector set at 282 nM. The effluent from the detector was passed through a Flo-One-β Model 1C radioactivity detector manufactured by Radiomatic Instruments Inc. for the determination of radioactivity. The mobile phase that was run at a rate of 1 ml/min was - 75mM sodium citrate adjusted to pH 3.5 with phosphoric acid: acetontrile: methanol: 17.3:1.35:1.0 (v/v).

^{1}H NMR Spectroscopy

^{1}H NMR spectra were taken on a JOEL FX 90-Q spectrometer at room temperature using tetramethylsilane as an internal standard. ^{1}H HVA (CD_3OD) δ 3.49 (s, 2H-CH_2-).3.83 (s, H_3,-OCH_3), 6.72 (s, 2H, H-Ar), 6.85 (s, 1H, H-Ar).

Preparation of Isotopically Labeled HVA

Ten mg (0.054 mmol) of HVA was added to a 1 ml capacity conical glass vial together with 0.05 ml of tritiated water. The mixture was frozen by placing the tube in an acetone-Co_2 bath and 0.0085 ml of conc H_2SO_4 was added. The vial was sealed with a teflon lined cap and kept in a heating block at 105°C for 22 hrs. The reaction mixture was cooled, diluted to 0.4 ml with water and extracted 3 times with approximately equal volumes of ethyl acetate. The combined ethyl acetate extract was washed with water and dried over anhydrous sodium sulfate. Aliquots of this light-brown extract equivalent to about one half the total extract were applied to one preparative thin-layer-chromatograpy plate that was developed with chloroform: methanol: ammonium hydroxide 65:35:5 (v/v). HVA, which was the major uv absorbing material moved as a discrete band with an Rf of about 0.3. The silica gel from the ultraviolet absorbing area of the plate corresponding to standard HVA was transferred to a small chromatography column and the HVA was eluted with approximately 10 ml of methanol. Deuterated HVA was prepared by the same procedure, except that deuterated water was substituted for the tritiated water. The yield of isolated material was approximately 50% based on HVA. The tritiated HVA had a specific activity of 25.2 mC/m Mol.

Characterization of the Labeled HVA

The ultraviolet spectrum of the isolated labeled compound was identical with that of the HVA standard, and the radioactivity was found to co-chromatograph with HVA in several thin-layer systems. The figure below shows that the radioactivity is co-eluted with HVA on high performance chromatography and no other labeled material was present.

The NMR spectra of the deuterated compound showed all of the protons except those on the methoxy-methyl group of HVA exchanged with the deuterium in the deuterated water. These findings are in agreement with the mass spectral data reported for deuterated HVA (4).

DISCUSSION

The procedure described in this communication provides a simple and inexpensive means for the preparation of tritiated HVA. The specific activity

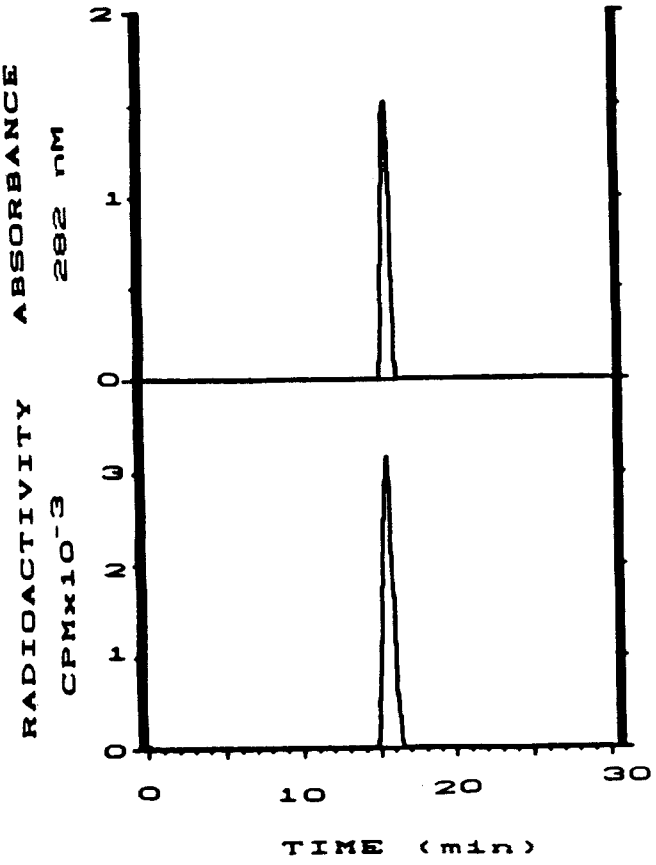

Fig. 1 The High Performance Liquid Chromatography of HVA

(^{3}H)-HVA (ca 1 nMol) was mixed with unlabeled HVA and chromatographed on a reversed phase column. The chromatographic conditions and assay for radioactivity are as described above.

of the material that is obtained is only limited by the specific activity of the tritiated water that is utilized for the exchange reaction. It was shown that all of the protons in the molecule other than those on the methoxy carbon undergo exchange with deuterium under these conditions, however, in the tritium exchange reaction, the specific activity of the isolated compound was only 2.9 times greater than that of the exchange medium rather than the theoretical 5 times. Thus, complete equilibrium was not achieved under these conditions and the exact degree of labeling of each position could not be determined. This is not a disadvantage in most of the studies for which this compound might be used.

The stability of the radioactive label in the HVA was checked by incubating the compound in 0.1 N HCl or 0.1 N NaOH at room temperature. There was no indication that any radioactivity was lost under acidic conditions in two weeks, while the base treated sample lost approximately 4% tritium after 48 hours and 17% after 14 days. The exchange in basic solution is almost certainly due to the exchange of the tritons on the α-carbon, but the rate of exchange is slow enough that under physiological conditions it would not be significant.

The availability of tritium labeled HVA of relatively high specific activity and radiopurity will permit experiments to be conducted that have greater sensitivity than those reported and may provide answers to the question of whether there is an alternate route of elimination for this compound.

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