PREPARATION OF MORPHINE-^{3}H BY MICROWAVE DISCHARGE ACTIVATION OF TRITIUM GAS.

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Radiolabelled morphine is required for the study of the biochemistry and disposition of this narcotic alkaloid. Presently available radioactive substrates suffer from certain deficiencies. In the case of the widely available morphine-N-$^{14}CH_3$ [1] the isotope is located in a metabolically active position from where it is lost by biological N-demethylation [2]. A similar problem is associated with the recently prepared morphine-N-CT_3 [3]. Morphine randomly labelled with tritium has been obtained by the Wiltzbach procedure or by acid catalyzed exchange [4]. The products obtained while apparantly biologically stable have specific activities not exceeding 10 mCi/mM and hence are of limited use in many biochemical studies and as tracers for radioimmunoassay procedures. The preparation of morphine specifically labelled with tritium at carbon 2 has also been reported but again the specific activity achieved was only 12 mCi/mM [5]. Labeling characteristics of a recently described method [6], which utilizes microwave discharge activation of tritium gas in a closed recycling system, suggested that this relatively mild procedure could be used as a superior method for morphine-^{3}H preparation.

One milligram of morphine was labelled in the presence of approximately 85 mCi of tritium at a pressure of 4 mm Hg. The reaction time was 20 minutes and the microwave discharge was maintained by 20 watts of forward power of 2450 mHz. The substrate was cooled by liquid nitrogen during the reaction. The

product was evaporated twice from ethanol-water and the residue containing 4.5 mCi, was dissolved in 5 ml of 5% hydrochloric acid and extracted three times with 5 ml chloroform. The organic extract was discarded and the aqueous layer was adjusted to pH 9 with dilute ammonium hydroxide and extracted three times with 8 ml ethyl acetate. The solvent was evaporated and the residue which contained 0.42 mCi was taken up in ethanol. An aliquot of this solution was diluted with carrier morphine which was acetylated and the morphine diacetate was recrystallized from acetone-petroleum ether to constant specific activity. Calculations showed that 3% of the radioactivity in the ethyl acetate extract was associated with morphine. Another aliquot of this extract was diluted with carrier dihydromorphine, which was then crystallized to constant specific activity. Only .05% of the ethyl acetate radioactivity was associated with dihydromorphine.

The remainder of the ethyl acetate extract was purified by preparative thin layer chromatography on silica gel in the system ethanol:acetic acid:water 60:30:10. The zone corresponding to morphine was eluted with ethanol and was shown to contain 5.8 μCi. Reverse isotope dilution of this material with carrier morphine and recrystallization to constant specific activity as the diacetate indicated that 96% of the radioactivity was morphine. To determine accurately the specific activity of this material the double isotope derivative procedure was used. A portion of the morphine-^{3}H was dissolved in 0.1 ml of dry pyridine and was acetylated with acetic anhydride-^{14}C with a specific activity of 0.38 mCi/mM. The acetylated material was first purified on silica thin layer in the above system and the heroin region was diluted with carrier heroin and recrystallized to constant isotope ratio. The final carbon 14 to tritium ratio was 0.28 which corresponds to a specific activity of 2.7 mCi/mM for the morphine-^{3}H.

The morphine-^{3}H obtained by this method was of a lower specific activity

than that obtained by other procedures. It must be emphasized, however, that this represented a single experiment and that possible and obvious variations in the conditions employed may yield material with considerably higher specific activity. This method offers two important advantages over other tritium exchange procedures. First the mild conditions required produce few organic radioactive byproducts and permit an easy purification of the desired material. Second, the reaction results in only minor reduction of the double bond, confirming similar observations with unsaturated fatty acids and aromatic amino acids [7]. This suggests that satisfactory tritium labeling can be obtained in compounds with this structural feature.

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