NOTES

SYNTHESIS AND PURIFICATION OF 2-BROMO-α-ERGOCRYPTINE-^{82}Br

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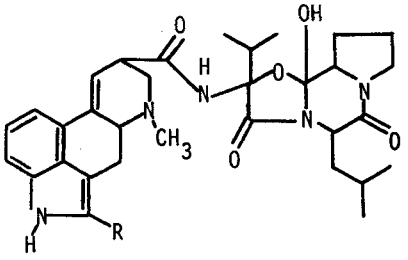
Bromocryptine (2-bromo-α-ergocryptine, I) has been found to be a dopamine agonist used for treatment of endocrine disorders and more recently for the treatment of Parkinsonism (1,2). In order to perform metabolism and pharmacokinetic studies, a radiolabelled drug was desired. Recent studies have demonstrated the utility of ^{82}Br labelled drugs prepared by synthesis (3) or direct neutron irradiation (4). We have chosen synthesis to avoid decomposition anticipated from recoil labelling. A dilute solution of α-ergocryptine (II) was found to react specifically in the 2-position with Br_2 in dichloromethane upon standing at room temperature.



R = Br = I
= H = II

Use of appropriate molar ratios of $^{82}Br_2$ (generated from K^{82}Br) and α-ergocryptine leads to a product substantially free from decomposition products and readily separable from an excess of II by liquid partition techniques or high pressure liquid chromatography. Synthesis and purification require approximately 6 hours, permitting adequate time for metabolism and pharmacokinetic studies using this isotope which has a relatively short half-life.

Experimental

Synthesis of 2-bromo-α-ergocryptine-^{82}Br (I). $K^{82}Br$ was obtained (ICN, Isotope and Nuclear Division, Cleveland, Ohio) at a concentration of 0.91 mCi Br/ml H_2O with a specific activity of 2.18 mCi Br/mg Br. Conversion to Br_2 was achieved by adding 5 ml CCl_4, 3 drops concentrated H_2SO_4 and 3 drops 1 N KMnO4 to the shipping vial which contained 5.84 ml KBr solution. The screw cap vial was resealed and shaken for 2 minutes. Approximately 4 ml of the CCl_4 extract was pipetted into 5 ml CH_2Cl_2 containing 6 mg II (generously supplied by Sandoz Pharmaceuticals, Basel, Switzerland). After standing at room temperature in a screw cap test tube for 20 minutes, the reaction mixture was washed with 3 ml 0.1 N NH_4OH to remove generated bromide ion. The organic solution was transferred to a 50 ml round bottom flask and evaporated to dryness with a mechanical vacuum pump protected by NaOH foreline and Dry Ice traps. The product was transferred to a screw-capped test tube with 5 ml ethyl-acetate and washed three times with 5 ml of 10mM HCl. The yield of I based upon specific activity or chromatography was 1.15 mg and contained 320.8 μCi ^{82}Br. The separation of I and II by partitioning was assayed by high pressure liquid chromatography on a Model 8500 instrument (Varian Inst. Div., Palo Alto, Calif.) using a Micropak-NH_2 column (25 cm x 2 mm) with a flow rate of 60 ml/hr of 3% 2-propanol-methylene chloride - 1mM triethylamine. Collection and counting of fractions eluting from the liquid chromatograph confirmed the radiochemical purity of the product.

Discussion

Several pitfalls were encountered in determining appropriate conditions for the synthesis and purification of I. An excess of bromine in the reaction led to the decomposition of both I and II. Bromination in solvents other than dichloromethane produced alternative products.

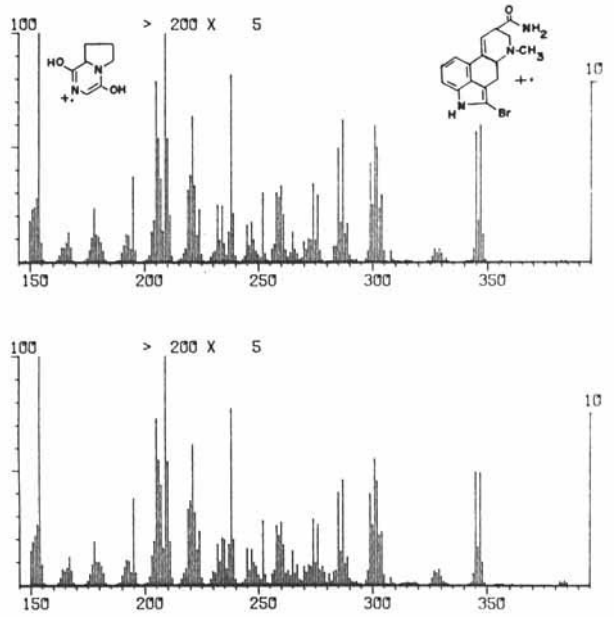


Figure 1: Partial electron ionization mass spectra of authentic (upper) and synthetic (lower) bromocryptine, scale expanded by a factor of 5 above m/e 200. A bromine doublet at m/e 345, 347 identifies the bromoindole nucleus. Ions at m/e 237, 209, and 154 derive from the tripeptide portion.

Separation of I and II on silica or neutral alumina columns with chloroform-2-propanol 95:5 is possible, but was not as rapid or effective as liquid partitioning.

Identity of I, synthesized and separated as described above, with an authentic standard (generously supplied by Sandoz Pharmaceuticals, Basel, Switzerland) was shown by co-chromatography on Micropak-NH_2, silica gel, and Micropak CH-10 (52% acetonitrile, 38% 1mM tris buffer), as well as by TLC (silica gel, benzene-chloroform-ethanol 2:4:1. Low resolution electron and chemical ionization (methane) mass spectra were also identical. Unfortunately, characteristic fragment ions containing

the bromine atom are weak, and no molecular ion was detectable (Figure 1). Selected ion recording of the bromoindole ions at m/e 345, 347 with respect to the peptide ion at m/e 209 gave identical ratios for the standard and synthesized materials. A negative reaction to Ehrlich's reagent demonstrates that the bromine is at the 2-position (5). Preliminary testing in rats indicates that synthetic bromocryptine prepared by this method is equipotent to material supplied by commercial sources.

Studies are now in progress to determine basic pharmacokinetic parameters using labelled bromocryptine in several animal species.

References

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