SYNTHESIS OF [^{3}H]WIN 35,065-2; A NEW RADIOLIGAND FOR COCAINE RECEPTORS

T. M. Naseree, P. Abraham, J. A. Kepler, F. I. Carroll, A. H. Lewin and M. J. Kuhar*

Research Triangle Institute, P.O. Box 12194, Research Triangle Park, NC 27709, and Neuroscience Branch*, Addiction Research Center, Baltimore MD 21224

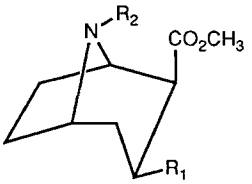
SUMMARY

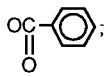
Treatment of methyl (-)-3β-phenylnortropane-2β-carboxylate with [^{3}H]CH_3I afforded [^{3}H]WIN 35,065-2 with specific activity of 25 Ci/mmol, a new ligand for the cocaine recognition site.

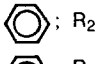
Key Words: cocaine analog, radiolabeled, tritiated

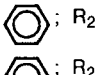
INTRODUCTION

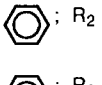
Cocaine (I), initially recognized for its stimulant and local anesthetic pharmacological actions, has become a major problem due to its dependence liability and toxicity. Recent investigations have demonstrated the existence of

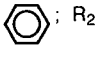


I. R_1 =  ; $R_2 = CH_3$

II. R_1 =  ; $R_2 = CH_3$

III. R_1 =  ; $R_2 = H$

IV. R_1 =  ; $R_2 = C^3H_3$

V. R_1 =  ; $R_2 = COCH_2CCl_3$

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specific binding sites for cocaine in brain tissues of mammals, including humans (1). One of these sites is at the dopamine transporter, and this site appears to be related to the abuse liability of cocaine (2). Binding studies of the dopamine transporter have been carried out using ligands other than [^{3}H]cocaine. Thus, [^{3}H]mazindol (2,3) and [^{3}H]GBR 12935 (4) have been widely used to compensate for the relative low affinity (1a) and high dissociation rate (1d) of [^{3}H]-cocaine. While these ligands have provided invaluable information, their binding sites may not be identical to those labeled by cocaine, and they may not be totally satisfactory substitutes for [^{3}H]cocaine. These limitations point to the need for molecular probes possessing high affinity and selectivity for the cocaine recognition site(s) coupled with lower dissociation rates of the bound state.

A number of years ago a series of 3β-phenyltropane-2-carboxylates (e.g., II) was identified as possessing strongly enhanced stimulant activity, relative to cocaine (I) (6). The local anaesthetic activity of this series of compounds was lower than that of cocaine while the inhibitory effect of some of these compounds on norepinephrine uptake in adregenically enervated tissue in mouse heart and rat brain was 15-20 times greater than that of cocaine. These compounds have also been shown to have binding characteristics similar to cocaine while displaying considerably higher affinity for the cocaine recognition site (1,2). Taken together, these observations suggested to us that radiolabeled methyl (-)-3β-phenyltropane-2β-carboxylate (II) may be a useful probe for elucidating the mode of action of cocaine. In order to maximize the chemical stability of the radioligand and to minimize handling of radioactive materials, it was decided to label the N-methyl group. Thus the specific target compound was methyl (-)-[N-C^{3}H$_3$]-3β-phenyltropane-2β-carboxylate (IV).

RESULTS AND DISCUSSION

In order to prepare the target compound IV, it was planned to methylate the known (6) N-nor analog, methyl (-)-3β-phenylnortropane-2β-carboxylate (III), with commercially available, high specific activity (85 Ci/mmol), iodomethane. This

presented two problems. First, such reactions are typically carried out in somewhat polar media (e.g., ether/methanol), but the high specific activity iodomethane is supplied in toluene to optimize its shelf-life. Second, such alkylations are usually performed with a large (e.g., 200-fold) excess of iodomethane. Pilot experiments with unlabeled iodomethane demonstrated that it would be feasible to carry out the methylation in toluene using excess amine. Since we were interested in preparing the product with specific activity approximately 30 Ci/mmol, the labeled iodomethane was diluted to a calculated specific activity of 28 Ci/mmol by vacuum transferring two equivalents of unlabeled iodomethane into a vessel containing the commercial labeled material. The resultant solution was vacuum transferred into a flask containing excess methyl (-)-3β-phenylnortropane-2β-carboxylate (III). The product, methyl (-)-[N-C^{3}H$_3$]-3β-phenyltropane-2β-carboxylate IV), was obtained in ca. 10% yield after purification; the specific activity was only 11 Ci/mmol (i.e., approximately one-third of the expected value of 28 Ci/mmol). This unexpectedly low specific activity could, in principle, be due to (a) contamination of the N-nor starting material III with ca. 7% of the N-methyl compound II; (b) selective reactivity with unlabeled iodomethane (kinetic isotope effect) in the methylation reaction; and (c) isotopic partitioning in the chromatographic purification of the product. Of these possibilities the first is ruled out by the workup method of the intermediate V and by the purity (>98%) of III. In particular, washing of the product V with hydrochloric acid should remove any unreacted II; in addition, the absence of II in the starting material III is readily shown by TLC and by ^{1}H NMR (no N-CH$_3$ signal at δ 3.0 ppm in the spectrum of III•HCl). A secondary kinetic isotope effect would be unprecedented and is therefore unlikely, but isotopic partitioning under chromatographic conditions has been documented (7). To address this problem it was decided to perform the reaction with undiluted radiolabeled iodomethane and dilute the final product to obtain the desired specific activity. The reaction was repeated using a second portion of the same batch of commercial iodomethane. The product, again isolated in ca 10% yield after purification, had specific activity 25 Ci/mmol, approximately one-third of the expected value of 85 Ci/mmol. In this case there was no possibility of isotopic dilution due to a kinetic isotope effect, isotopic

partitioning or isotopic dilution by tritium exchange. We therefore conclude that the specific activity of the commercial product must have been in error.

EXPERIMENTAL SECTION

Methyl (-)-3β-phenylnortropane-2β-carboxylate (III). A mixture of methyl (-)-3β-phenyltropane-2β-carboyxylate (II) (6) (259 mg, 1 mmol), 2,2,2-trichloroethylchloroformate (420 mg, 2 mmol) and K_2CO_3 (25 mg) in toluene (10 mL), was heated at 85-90°C for 28 hr. The solvent was evaporated and the residue was dissolved in $CHCl_3$. The solution was washed with dil. HCl and water, dried and evaporated. Drying under vacuum for 24 hr afforded 403 mg (89% yield) of the urethane V. This residue was dissolved in 95% HOAc (15 mL) and treated with freshly activated Zn dust (2.5 g) in portions. After stirring for 2.5 hr the solids were removed by filtration and washed with 95% HOAc. The combined filtrate and washings were evaporated to 10 mL, diluted with H_2O and extracted with $CHCl_3$ (3 x 500 mL). The combined organic extract was washed with 5% NaOH, dried over K_2CO_3 and evaporated to a cream colored solid (130 mg). This solid was dissolved in dry 3% HCl/MeOH (10 mL) and the solution was evaporated. The residue was dissolved in a minimum amount of MeOH and diluted with Et_2O to opalescence. After overnight in the freezer the HCl salt was isolated as needles (70 mg, 27% yield). TLC (SiO_2, EtOAc-MeOH-NH_4OH 50:50:1) showed a single spot (UV and I_2 visualization), R_f 0.4. No spot was observed for the starting material II (R_f 0.5).

Methyl (-)-[N-C^3H_3]-3β-phenyltropane-2β-carboxylate (IV). A commercial (Amersham Code TRK.706 Batch No 43) sample of $[^3H]CH_3I$ (10.0 mCi with claimed S.A. 85 Ci/mmol, 0.12 μmol) in toluene was vacuum transferred into a flask in which a solution of methyl (-)-3β-phenylnortropane-2β-carboxylate (III) (6) (770 μg, 2.32 μmol) in 6 mL toluene had been evaporated to dryness. After stirring for 3 1/2 h at room temperature and atmospheric pressure the solution was evaporated to dryness. The residue was dissolved in 2 mL of MeOH/CH_2Cl_2 (1:1), transferred into a conical Reacti-Vial and evaporated to dryness. The residue was purified by PTLC using 5 x 20 cm SiO_2 plates and EtOAC-NH_4OH (100:1). Since the R_f was low, the plate was developed three times. The product band, detected by

UV, was collected, the free product eluted from the scraped SiO_2 with MeOH/CH_2Cl_2 (1:1), and this solution was diluted with toluene. The volatiles were removed under a stream of N_2, and the final solution was brought to a composition of 9:1 toluene/MeOH in a 10.0 mL volumetric flask. This product (IV) was found to be pure (>98%) and to co-elute with an authentic sample of WIN 35,065-2 (II), by TLC-radioscan, using SiO_2 plates and eluting with CH_2Cl_2/MeOH/ NH_4OH (90:10:1, R_f 0.54), and by GC on a 3 ft 1.5% OV-17, 1.95% OV-210 on 80/100 Gas Chrom Q glass column at 200°C (4.84 min), with FID detection. The total amount of activity recovered was 927 μCi (9.3% radiochemical yield). The specific activity determined by GC, using methadone (8.5 min) as internal standard, was 25 Ci/mmol.

CONCLUSION

Treatment of excess methyl (-)-3β-phenylnortropane-2β-carboxylate (II) with tritiated methyl iodide in toluene affords [^{3}H]WIN 35,065-2, a new radioligand for cocaine receptors (8).

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