TRITIUM LABELLED COMPOUNDS OF HIGH SPECIFIC ACTIVITY III.[x] TRIFLUOPERAZINE

Gábor Zólyomi
Institute for Drug Research
H-1325 Budapest, P.O.B. 82, Hungary

SUMMARY

Trifluoperazine labelled with tritium at a specific activity of 3.15 TBq/mmol was prepared by methylation of N-desmethyltrifluoperazine with no-carrier added [^{3}H]methyl iodide. An improved method for preparation of the precursor is also described.

Key Words: tritium labelling, trifluoperazine, high specific activity

INTRODUCTION

A preceding paper (1) from these laboratories reported the synthesis of ^{14}C-labelled long-acting fluphenazine esters for pharmacokinetic studies. Animal experiments were performed; but these radioanalogues were unsuitable for human pharmacokinetic studies because of the low therapeutic plasma levels and the limited radioactivity allowed to be administered to volunteers.

Midha and coworkers (2) published a radioimmunoassay (RIA) procedure for trifluoperazine (1b), which can quantitate 0.25 ng/ml with a 200 µl plasma sample. The synthesis and properties of various haptens have been described (3) as well. This method is based on an antiserum developed to a protein conjugate of 1d,

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[x] Part II: Zólyomi G., Sirokmán F., Tóth G. and Elekes I. - J. Labelled Comp. Radiopharm. 19: 247 (1982).

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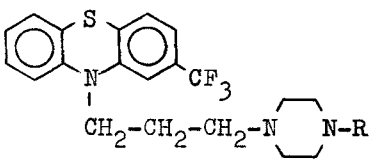
therefore, according to the authors, it should be suitable for developing an RIA for fluphenazine (1c), too.



1

	R
a:	H
b:	CH_3
c:	CH_2-CH_2-OH
d:	CH_2-CH_2-COOH
e:	C^3H_3

In order to obtain sufficient sensitivity to monitor plasma levels of patients under chronic treatment with normal therapeutic doses of depot formulations of various fluphenazine esters, a radiolabelled analogue of 1b or 1c as tracer is also required. Hawes and coworkers (3) checked the titer values of the antisera by evaluating the binding characteristics of trifluoperazine generally labelled with tritium; however, no details on the labelling procedure have been available and the specific activity was rather low, 12.8 Ci/mmol (0.47 TBq/mmol).

DISCUSSION

This paper describes a microscale synthesis of trifluoperazine labelled with tritium at a specific activity of 3.15 TBq/mmol (85 Ci/mmol). Our approach to obtain such a high specific activity tritium label was introducing the tritium by methylation; i.e. to prepare N-desmethyltrifluoperazine (1a), which can readily be methylated with no-carrier added [^{3}H]methyl iodide. For this work 1b was prepared essentially as described by Yale and Sowinski (4), while we failed in synthesizing the precursor 1a in the same manner, therefore this latter product was obtained by a modified and improved method. Each product was purified by chromatography and the identity of the compounds prepared was confirmed by microanalyses, infrared and ^{1}H-NMR spectra.

The methylation of 1a and purification of the product at nanomol level were modelled using ^{14}C-labelled methyl iodide. The yield was estimated by radioactivity measurement and found to be 73%. The reaction with [^{3}H]methyl iodide was run under experimental conditions designed to mimic those used for the model experiment with ^{14}C-labelled compound and a yield of 61% was achieved.

For preparation of the hapten 1d and drug-protein conjugate, as well as for production of the antibodies to this compound in rabbits, the method described by Hawes et al. (3) was precisely followed with complete success.

EXPERIMENTAL

Melting points were determined on a Boëtius hot stage and are uncorrected. IR spectra were recorded with a Bruker Model IFS 85 spectrometer. ^{1}H-NMR spectra were determined on a Bruker Model EM 390 instrument in dimethyl-d_6 sulfoxide with tetramethylsilane as internal reference. Radioactivity was measured by liquid scintillation technique using a Packard TRI-CARB Model 2660 spectrometer. TLC was carried out on silica gel HF_{254} (Merck) and a Berthold TLC scanner Model LB-2723 was used for evaluation [^{3}H]Methyl iodide (3.15 TBq/mmol) was purchased from Amersham International. All evaporations were carried out under reduced pressure.

Preparation of the precursor and standard material 3-[2-(Trifluoromethyl) phenothiazin-10-yl]-propyl chloride

20.0 g (75.0 mmol) of 2-trifluoromethylphenothiazine was reacted with 1-bromo-3-chloropropane according to Yale and Sowinski (4). After removing the greater part of the unreacted starting material by crystallization in ligroin (b.p. 115-125 °C), the residue was chromatographed on silica gel using a mixture of cyclohexane/ chloroform 4:1 as eluent. Yield: 12.3 g (35.8 mmol), 47.7%. M.p. 69-71 °C.

1-{3-[2-(Trifluoromethyl)phenothiazin-10-yl]-propyl}-piperazine (1a)

A mixture of 2.0 g (5.8 mmol) of the above product, 2.9 g (15 mmol) of piperazine hexahydrate and 2.6 g (15 mmol) of piperazine dihydrochloride monohydrate in 25 ml of ethanol was stirred at 70 °C for 60 hours. After cooling to -8 °C the precipitated crystals were filtered off, the filtrate was evaporated and subsequently the residue was made alkaline to pH 12 with 5 N NaOH whereupon an oil separated; it was extracted with 3 x 20 ml of chloroform, the combined extracts were washed with water, dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by chromatography on silica gel using benzene/ethanol/conc. NH_4OH 86:30:4 solvent system as eluent to give 0.8 g of a thick, yellow oil. Yield: 35.0%. Dimaleate of 1a was prepared and crystallized from a mixture of benzene and 2-propanol in a yield of 87.8%. M.p. 158-160 °C.

1-{3-[2-(Trifluoromethyl)phenothiazin-10-yl]-propyl}-4-methyl-piperazine (1b)

This compound was prepared as described by Yale and Sowinki (4), but the crude base was purified by chromatography on silica gel, using the same eluent as for the desmethyl derivative, instead of distillation. Dimaleate of 1b was obtained as pale yellow crystals from a mixture of benzene, 2-propanol and light petroleum. M.p. 189-191 °C. (Lit. 194-95 °C).

Model experiment with [^{14}C]methyl iodide

A portion of ^{14}C methyl iodide (prepared at our laboratory; specific activity: 540.2 MBq/mmol) was dissolved in toluene to obtaine a stock solution containing 1.19 x 10^5 dpm of radioactive substance, equal to 3.67 nmol of methyl iodide per ml. To 1.36 ml of this stock solution (5 nmol of methyl iodide) 25 µl of a

0.001 M solution of 1a was added and allowed to stand in a tightly closed reaction vessel at 25 °C for 96 hours. The mixture was then reduced in volume by evaporation and applied to a TLC plate in lineform by means of a Desage microdoser. The chromatogram was developed with a benzene/ethanol/conc. NH_4OH 86:30:4 solvent system, then the separated trifluoperazine was located under UV-light, removed and eluted with toluene to give a total radioactivity of 1.18×10^5 dpm (calculated: 1.62×10^5). The radiochemical yield expressed relative to methyl iodide was 72.8%.

Preparation of tritium labelled trifluoperazine (1e)

A break-seal ampoule, containing 370 MBq (approximately 0.12 μmol) of [^{3}H]methyl iodide in 1 ml of toluene, was opened and to its content transferred to a reaction vessel 0.6 ml of a 0.001 M solution of 1a was added. The reaction vessel was closed tightly and the mixture was allowed to stand at 25 °C for 96 hours resulting in trifluoperazine, which was separated as described for the model experiment. After eluting the tritiated compound (1e) with toluene, it was diluted to 10 ml and aliquots of this solution were counted for radioactivity as well as checked for purity. The radioactive concentration was 1.35×10^9 dpm/ml, accounting for 60.8% of the radioactivity, while the impurities detected by TLC amounted to less than 1%.

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