TRITIUM LABELLING OF BENZAMIDE DRUGS

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Key words: sultopride, sulpiride, metoclopramide, tritiated benzamides.

SUMMARY

Methods for tritiation of sulpiride, sultopride and metoclopramide were developed and optimized. ^{3}H-sulpiride (7.1 Ci/mmol), ^{3}H-sultopride (2.1 Ci/mmol) and ^{3}H-metoclopramide (18.0 Ci/mmol) were obtained by hydrodebromination of the related bromo derivatives. The bromo derivatives were prepared, in turn, by appropriate procedures specified for each case.

INTRODUCTION

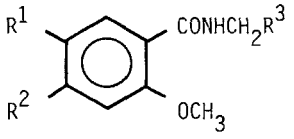
Substituted benzamides have been introduced into clinical use[1] as antipsychotic drugs. Compounds such as sulpiride, sultopride and metoclopramide (table 1) are related to phenomena which are associated with dopaminergic mechanisms. However, their mechanism of action is practically unknown.

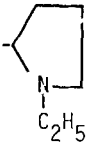
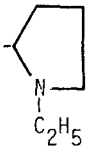
In order to study the role of these derivatives as neuroleptic drugs and their ability to be bound to specific receptor sites, the need for high specific activity tritium-labelled benzamides arose. As no detailed publication of attempts to radiolabel this family of compounds is found in the literature[2], we wish to report in this work procedures for the preparation of tritium labelled benzamides at high specific activity[3].

While attempting to prepare ^{3}H-labelled benzamides, we tried to avoid long and tedious synthetic pathways which are usually of low yields and involve a waste of expensive, some time unavailable, starting materials[4]. Instead, we preferred to start with the drug itself, as it is usually the most common precursor available and to find an efficient approach to tritium-label it, according to its chemical structure.

0362-4803/83/101153-06$01.00 Received March 24, 1983

Table 1: Chemical structure of benzamide derivatives.



Compound	R^1	R^2	R^3
Metoclopramide	-Cl	$-NH_2$	$-CH_2N(C_2H_5)_2$
Sulpiride	$-SO_2NH_2$	-H	
Sultopride	$-SO_2C_2H_5$	-H	

RESULTS AND DISCUSSION

In the preparation of labelled sulpiride and sultopride, the compounds were brominated in the aromatic ring by various bromination procedures in order to obtain high yields of the bromo derivative.

Sulpiride was brominated with an aqueous solution of hydrogen bromide and hydrogen peroxide[5] to give a crude bromo derivative which was purified by t.l.c. Catalytic hydrodebromination of this purified product gave ^{3}H-sulpiride with a specific activity of 7.1 Ci/mmol. A few attempts to brominate sulpiride with bromine in acetic acid solution failed and most of the unreacted starting compound was recovered.

Sultopride was found to be too sensitive to survive under the same experimental conditions, probably as a result of destruction of the ethylsulfonyl moiety under the acidic conditions involved. Instead, a relatively mild bromination procedure was adopted using $FeCl_3$ as catalyst. In this case, attempts to perform the hydrodebromination of the crude bromo derivative resulted in tritium labelled sultopride, where the highest specific activity reached was 2.1 Ci/mmol.

When attempting to prepare ^{3}H-metoclopramide, the possibility of competitive dehalogenation of the chlorine atom in position R^1 (table 1) arose. Indeed, after a short contact of metoclopramide with tritium gas in the presence of palladium as catalyst, no starting material was recovered; the main tritiated product obtained was a result of hydrodechlorination. Such dehalogenation was found to be complete after 15 min. under 300 mm hydrogen pressure at ambient temperature.

Attempts to overcome this undesired reaction consisted of the pre-reduction of palladium oxide with tritium[6], the removal of the residual gas followed by the addition of metoclopramide to perform a catalyzed hydrogen-tritium exchange with the T_2O formed. The best results obtained were tritiated metoclopramide at specific activity of 1.2 Ci/mmol.

Direct bromination of metoclopramide was also performed and the bromo derivative obtained was used in further experiments. It was found that the relative rates of hydrodechlorination and hydrodebromination are in favor of the latter process. Thus, when the tritiation of the brominated metoclopramide was stopped after 20 min., hydrodebromination was completed and less than 10% of hydrodechlorinated product was detected.

Addition of a catalyst poison, such as pyridine, caused a decrease in both reaction rates of one order of magnitude, as measured by tritium consumption rate. After 15 min. of reaction, no trace of dechlorination was detected while the hydrodebromination was almost completed, giving ^{3}H-metoclopramide with a specific activity of 18.0 Ci/mmol.

EXPERIMENTAL

1. General

Reagents of CP grade were used without further purification. Dioxane was dried on sodium and distilled prior to use. Tritium gas of 99% radiochemical purity was introduced into an evacuated capillary vacuum line to which the reaction vessel was attached. After the completion of the reaction, the solvent was removed by lyophilization and two successive portions of 3 ml methanol were introduced and lyophilized in order to eliminate any labile tritium left. Silica gel t.l.c. plates of 0.25 mm width were used for preparative purification of products.

Radiochemical purity was determined by radioscanning of analytical t.l.c. plates developed with two different solvent systems. The amount of labelled compound for specific activity calculations was determined by UV absorption as referred to a known standard.

2. Preparation of ^{3}H-sulpiride

a. Bromination of sulpiride

150 mg of sulpiride were dissolved in 25 ml water containing 10 ml of 40% hydrobromic acid. The solution was stirred in a 100 ml round bottom flask connected to a reflux condenser and heated to 70°C on an oil bath. 10 ml of hydrogen peroxide[5] (30%) were added slowly through the condenser and the heating was continued for 10 hours. The reaction mixture was cooled to room temperature and the solvents were evaporated under educed pressure. The crude reaction product was dissolved in dioxane and triethylamine was added until no more precipitation was formed. The precipitate was removed by filtration and washed with 5 ml dioxane. The oily brominated product obtained by evaporation of the dioxane was purified by preparative t.l.c. (t.l.c. showed that no starting sulpiride was left), using t-butanol: methylethylketone: ammonia: water (40:30:15:15) as solvent system. The spot with R_f: 0.92 was found to contain the desired bromo derivative which was extracted with methanol from the silica gel. Hydrogenation of the product extracted from the other spots resulted in compounds different from sulpiride.

b. Tritiation of bromosulpiride

The purified bromo derivative was dissolved in 0.3 ml of methanol and 0.2 ml triethylamine and the solution was transferred to a glass ampoule provided with a magnetic stirrer bar. 30 mg Pd/C (10%) and tritium gas (300 mm Hg pressure) were introduced and stirring was continued until 1 Ci of tritium was consumed (about 30 min.). The residual tritium was evacuated and after the usual work-up procedure, the tritiated product was purified by t.l.c. using cyclohexane: acetone: diethylamine (6:9:1) as solvent system (R_f: 0.18).
Tritiated sulpiride was obtained 99% radiochemically pure at a specific activity of 7.1 Ci/mmol after methanol extraction from the silica gel. The purified compound was also checked with chloroform: methanol (9:1) (R_f: 0.20).

3. Preparation of ^{3}H-sultopride

a. Bromination of sultopride

A few attempts were performed to brominate sultopride with bromine in CCl_4 or in acetic acid from which the starting material was recovered and almost no bromo derivative was formed. Better results were obtained by addition of an halogenation catalyst e.g. $FeCl_3$ or $AlCl_3$ to the reaction mixture.

150 mg of sultopride were dissolved in 15 ml glacial acetic acid and a small amount of $FeCl_3$ was added. The mixture was stirred and heated to 75°C for

18 hr, cooled to ambient temperature and the solvent was removed by distillation under reduced pressure. The brown residue was triturated with 5 ml water and a solution of 5N KOH was added until it was basic to lithmus. The aqueous solution was extracted three times with 10 ml chloroform. The organic phase was dried over $MgSO_4$ and evaporated to give the crude bromo derivative which was submitted to tritiation without any additional purification. The absence of brominated sultopride after hydrogenation was proved by t.l.c. on silica gel, using the solvent system cyclohexane: acetone: diethylamine (6:3:1).

b. Tritiation of bromosultopride

30 mg of the crude bromosultopride were dissolved in 0.5 ml of dried dioxane and 0.2 ml triethylamine. The solution was stirred in a small glass bulb with 20 mg Pd/C (10%) under 350 mm Hg pressure of tritium gas at ambient temperature until 3 Ci (0.5 mmol) of tritium were consumed. The reaction was then stopped (about 30 min.), the excess of tritium was removed, the solvent was vacuum distilled and the residue was dissolved in methanol and lyophilized in the usual work-up procedure. The product was purified by t.l.c. on silica gel using cyclohexane: acetone: diethylamine (6:3:1) (R_f: 0.25) as solvent system. ^{3}H-sultopride of 99% radiochemical purity with a specific activity of 2.1 Ci/mmol was extracted with methanol from the silica gel and checked with chloroform: methanol (9:1) (R_f: 0.14).

4. Preparation of ^{3}H-metoclopramide

a. Bromination of metoclopramide

50 mg of bromine dissolved in 5 ml glacial acetic acid were added dropwise to a solution of 100 mg metoclopramide dissolved in 10 ml acetic acid. The solution was stirred a further two hours at room temperature. The excess of bromine was removed together with the solvent by repeated lyophilizations with CCl_4, the crude bromo derivative was dissolved in dioxane, an excess of triethylamine was added and the precipitate of the salt was removed by filtration.

b. Tritiation of bromometoclopramide

50 mg of the crude bromo derivative were dissolved in 1 ml of dried dioxane containing 0.2 ml triethylamine and 0.1 ml pyridine. The solution was stirred with 30 mg of Pd/C (10%) and 300 mm Hg tritium gas in a sealed glass ampoule at ambient temperature, the reaction was stopped after 5 Ci of tritium were consumed (about 15 min.) and the excess of gas was removed. After usual work-up of lyophilization and catalyst removal, the crude ^{3}H-metoclopramide was

purified on preparative t.l.c. developed in n-butanol: acetic acid: water (2:1:1) as solvent system (R_f: 0.47). The ^{3}H-metoclopramide extracted with methanol from the silica gel was found over 98% radiochemically pure and with a specific activity of 18.0 Ci/mmol. The purity was checked with chloroform: methanol (9:1) (R_f: 0.17).

ACKNOWLEDGEMENTS

We thank SESIF Ltd (France)and Rafa Laboratories (Israel)for the generous gift of the starting substituted benzamides. We wish to thank Ms A. Cohen for helpful technical assistance.

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