HIGH SPECIFIC ACTIVITY LABELLING OF SOME TRICYCLIC ANTIDEPRESSANTS

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SUMMARY

Activated palladium is used as catalyst for the hydrogen-tritium exchange in tricyclic antidepressants. The specific activities obtained are: desipramine: 44.9 Ci/mmol; imipramine: 14.9 Ci/mmol; notriptyline: 2.0 Ci/mmol and amitriptyline: 1.7 Ci/mmol.

KEY WORDS: TRITIATION, DESIPRAMINE, IMIPRAMINE, NORTRIPTYLINE, AMITRIPTYLINE.

INTRODUCTION

Imipramine, a dibenzazepine derivative, amitriptyline, a dibenzocycloheptadiene derivative and other closely related compounds are the most widely used drugs for the treatment of depression. Their therapeutic efficacy has been established[1], although little is known regarding their mechanism of action.

In the last years, investigations of the metabolism and the biological action of these drugs have been undertaken[2]. The introduction of radioactive labelled antidepressants as a research tool has been proved

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useful[3]. As a result of the development of the radioimmunoassay procedure, the need has arisen for compounds of high specific activity[4].

The molecular structure of these drugs, described as tricyclic antidepressants, is constituted principally by two aromatic rings linked by an ethylene group (bibenzyl-like structure). The hydrogen atoms of the ethylene group in bibenzyl have been found very active in catalyzed isotopic exchange with molecular tritium[5]. The application of the catalytic exchange procedure, which we have developed, enable the labelling of these tricyclic compounds, producing labelled derivatives of high specific activity.

RESULTS

Using activated palladium, prepared by reduction of palladium oxide, imipramine and desipramine (desmethylimipramine) were labelled with a specific activity of 14.9 Ci/mmol and 19.7 Ci/mmol, respectively. These results were above our expectations when compared to the available literature (1.5 mCi/mmol)[6] or to our previous results (460 mCi/mmol)[7], when activated platinum was used as catalyst.

The positions undergoing exchange were confirmed by N.M.R. spectra of the compounds which were deuterated by the same procedure performed for the tritiations. The only observed effect was the decrease of about 50% of the intergated peaks ratio of the ethylenic hydrogen atoms, showing the specificity of the active sites.

In the cases of amitriptyline and nortriptyline, according to the same tritiation procedure, most of the tritium reduced the olefinic bond between the position 5 in the cycloheptene ring and the aliphatic moiety. Only a small fraction of the tritium reacted in the desired exchange reaction.

The reduced products obtained by the above tritiations included about 80 - 90% of the total radioactivity. Reference compounds of the reduced form of nortriptyline and amitriptyline were prepared by catalytic hydrogenation on palladium black.

Alternatively, we tried other catalysts usually employed for the general labelling with tritium, e.g. PtO_2[8] or $PdO/BaSO_4$[9]. In these cases the tritiated products obtained were found to be more than 90% in the reduced form. To minimize the undesired reduction, we separated the step of catalyst activation (reduction of PdO) from the exchange step of hydrogen in the organic molecule. The first step was performed without solvent in order to keep at a minimum the volume of liquid in the reaction system. It has been shown[5] that a smaller volume of solvent favors the obtention of higher specific activity of the substrate.

When the unsaturated compound was added after the complete reduction of PdO, but in the presence[10] of the residual gaseous tritium, only 20 - 30% of the olefinic bond underwent reduction. The specific activities as measured for amitriptyline and nortriptyline were relatively low: 1.7 Ci/mmol and 2.0 Ci/mmol, respectively, after purification. However, this level of labelling is high enough to perform radioimmunoassay tests and biological research.

EXPERIMENTAL

Typical tritiation of desipramine HCl; 10,11-dihydro-5-(3-methylaminopropyl)-5H-dibenz[b,f]azepine hydrochloride.

12.7 mg (0.1 mmol) PdO (Pfaltz & Bauer) are added to the reaction vessel containing 30.3mg(0.1 mmol)desipramine dissolved in 0.3 ml methanol. The

solution is frozen, the reaction system is washed twice with nitrogen gas and evacuated to a residual pressure of 10^{-2} mm Hg. Then, 7 Ci of tritium gas (C.E.A.) are transferred into the reaction vessel, developing an initial pressure of about 100 mm Hg. The solution is allowed to come back to ambient temperature and the suspension is vigorously stirred. Decrease of the pressure gives a measure of progress in reduction of the metal oxide with simultaneous formation of the activated Pd catalyst. The reduction is stopped by freezing after 3 Ci (0.05 mmol) of tritium are consumed (approx. 45 min). The residual tritium is evacuated and the solvent is separated by cryo-sublimation. The catalyst-substrate mixture is washed twice with 2 ml methanol aliquots which are then removed by cryo-sublimation. The substrate is then dissolved in 5 ml methanol and filtered from the catalyst through a Millipore pre-filter giving, without further purification, tritiated desipramine chemically and radiochemically pure (> 98%), as determined by analytical thin-layer chromatography (T.L.C.) on silica-gel precoated plates (0.25 mm, Merck). The following solvent systems were used: [A]: hexane, diethylamine (9:1), R_f: 0.20; [B]: cyclohexane, acetone, diethylamine (6:3:1), R_f: 0.50. The concentration of the solutions is determined by U.V. spectrum in methanol (max. 255 mμ). Specific activity obtained: 19.7 Ci/mmol.

Further tritiation, performed following the above experimental details, but with 23.8 mg desipramine and 78 mg PdO which reacted with 10 Ci tritium gives a chemically and radiochemically pure product (> 98%) with a specific activity of 44.9 Ci/mmol.

Typical tritiation of imipramine HCl; 10,11-dihydro-5-(3-dimethylaminopropyl)-5H-dibenz[b,f]azepine hydrochloride.

24.8 mg (0.2 mmol) PdO are added to the reaction vessel containing

31.7 mg (0.1 mmol) imipramine* dissolved in 0.3 ml methanol. 6 Ci (0.1 mmol) tritium reacted with PdO. The experimental details are the same as for desipramine. After filtration from the catalyst and without further purification, tritiated imipramine is obtained chemically and radiochemically pure (> 98%) as determined by T.L.C. in the solvent systems [A]: R_f: 0.55; [B]: R_f: 0.80 and by U.V. spectrum in methanol (max. 255 mμ). Specific activity: 14.9 Ci/mmol.

Typical tritiation of nortriptyline HCl; 10,11-dihydro-5-(3-methylaminopropylidene)-5H-dibenzo[a,d]cyclopentene hydrochloride.

The exchange reaction is performed in a specially designed vessel (fig.1). 29.5 mg (0.1 mmol) nortriptyline** , dissolved in 0.2 ml methanol, are introduced in the side arm (a) and 28.0 mg (0.23 mmol) PdO are introduced in the main arm (b). It is necessary to introduce 20 Ci tritium in the vessel to develop an initial tritium pressure of about 100 mm Hg. After PdO reacted with 13 Ci (0.22 mmol) tritium (approx. 90 min.), the solution of substrate is added to the activated catalyst by opening the stopcocks (c), the suspension is vigorously stirred 30 min. and the reaction is stopped by freezing the solution. Further experimental details are as for desipramine. The crude product is purified by preparative T.L.C. on silica-gel precoated plates (2 mm, Merck) in the solvent system [A] and extracted from silica-gel with methanol. The purified product is checked by analytical T.L.C. in the solvent systems [A]: R_f: 0.38, (reduced form, R_f: 0.25); [C]: benzene, ethanol, ammonia (95:15:5), R_f: 0.63, (reduced form, R_f: 0.56); [D]: methanol, ammonia (100:1.5), R_f: 0.40, (reduced form, R_f: 0.25) and by U.V. spectrum in methanol (max. 240 mμ). Chemical and radiochemical purity: > 98%; specific activity obtained: 2.0 Ci/mmol.

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*gift from Plantex Ltd.,Chemical and Pharmaceutical works, Netanya, Israel.

**gift from Ikapharm, Ramat-Gan, Israel.

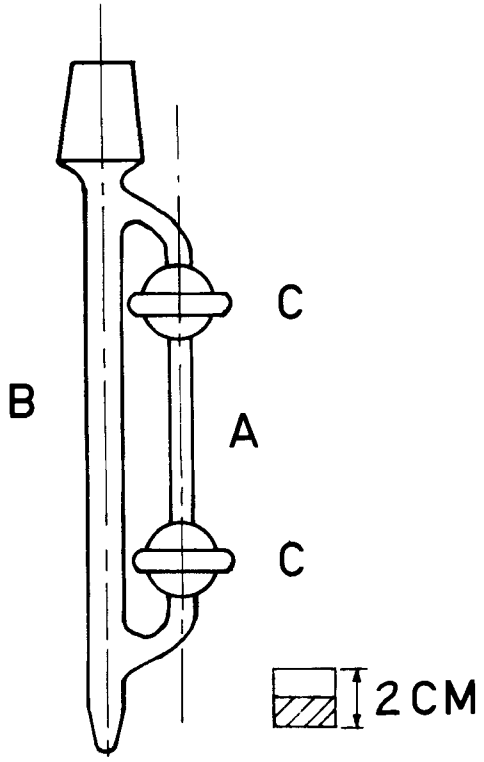

Fig. 1: Vessel for the two-step tritiation.

Typical tritiation of amitriptyline HCl; 10,11-dihydro-5-(3-dimethylamino-propylidene)-5H-dibenzo[a,d]cycloheptene hydrochloride.

The reaction is performed as for nortriptyline. After reaction of 28.0 mg (0.23 mmol) PdO with 13 Ci (0.22 mmol) tritium, 15.1 mg (0.05 mmol) amitriptyline*** dissolved in 0.2 ml methanol is added to the activated palladium. The crude product is purified by preparative T.L.C. on silica-gel in the solvent system [E]: acetone, ammonia (99:1) and extracted with methanol. The purified product is checked by analytical T.L.C. in the solvent systems [E]: R_f: 0.74, (reduced form, R_f: 0.48); [D]: R_f: 0.65, (reduced form, R_f: 0.50); [G]: acetone, methanol (88:22), R_f: 0.20, (reduced form, R_f: 0.15), and by U.V. spectrum in methanol (max. 240 mμ). Chemical and radiochemical purity: > 98%; specific activity obtained: 1.7 Ci/mmol.

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