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NOTE

TRITIUM LABELLING OF NITROARYLDIHYDROPYRIDINES

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Dedicated to Professor Dr. Karl-Heinz Büchel on his 60th birthday

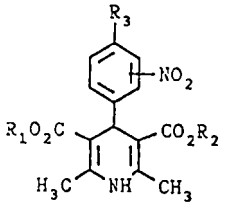
SUMMARY

The synthesis of tritium labelled nitroaryldihydropyridines was achieved by dehalogenation of a brominated precursor using the palladium hydroxide on charcoal as catalyst. The investigations described show the example [^{3}H]nimodipine which was prepared with a specific activity of 16,4 Ci/mmol (607 GBq/mmol).

Key words: Tritiated nitroaryldihydropyridines, [^{3}H]nimodipine

Introduction

Nitroaryldihydropyridines of type 1 with a nitro group in the ortho or meta position of the aromatic ring are highly effective drugs used in a variety of indications. For binding studies and for investigations of biotransformation in tissues with a low concentration these active compounds are required in a tritiated form, and the labelling position must be both chemically and metabolically stable. Only by labelling in the aromatic ring can both these conditions be fulfilled. Tritium labelling in the aromatic part of the molecule in dihydropyridines 1 has not previously been described in the literature although the synthesis of [2-C^{14}]nimodipine has been published [5].



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a) m-NO_2, R_1=$CH(CH_3)_2$, R_2=$(CH_2)_2OCH_3$, R_3=H (nimodipine)
b) R_3=Cl (as 1a)
c) R_3=Br (as 1a)
d) m-NH_2, R_3=Cl
e) m-NH_2, R_3=H

We have therefore investigated the possibilities of tritium labelling dihydropyridines 1 in the aromatic ring using the labelling of nimodipine 1a as a model compound.

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Aromatic compounds can usually be successfully labelled with tritium by catalytic dehalogenation of a precursor [1], but since under the conditions of dehalogenation nitro groups are also reduced to amino groups it was a matter of finding a selective catalyst which would catalyse only dehalogenation or at least would tend to do so preferentially.

Discussion

As in the synthesis of nimodipine 1a itself [2], the halogenated precursors 1b or 1c can be prepared for tritium labelling in one synthesis step by condensation of a chlorinated or brominated nitrobenzaldehyde with an acetoacetic acid ester and a 3-aminocrotonic acid ester. In the search for suitable reaction conditions for the dehalogenation of 1b and 1c, hydrogen or deuterium was used instead of tritium.
From the literature tetrakis(triphenylphosphine)palladium(0) is known as a catalyst [3] which dehalogenates selectively halogenated nitroaromatics. However, for reasons of safety the specified reaction conditions of tritium labelling (reaction temperature 100°C, current of hydrogen) cannot be applied. We therefore tried commercial catalysts such as palladium on carbon (10 % Pd), palladium on calcium carbonate (10 % Pd), and palladium oxide and tris(triphenylphosphine) - palladium(II) chloride which have often been used successfully in reductive dehalogenations.
Unfortunately, the reduction of the nitro group was catalysed preferentially by all these catalysts. We did, however, find one noble metal catalyst, palladium hydroxide on carbon (20 % Pd) [4] which at room temperature and a partial hydrogen pressure below 90 kPa dehalogenated 1b somewhat more rapidly than it reduced the nitro group, resulting in distinct quantities of 1d and 1e being formed after a reaction time of 30 min. However, the brominated compound 1c was dehalogenated almost completely to 1a under the same reaction conditions with discontinuation of the reaction at the right time. In this case the undesired reduction of the nitro group occurred considerably more slowly than the debromination and by keeping to the optimal reaction time, yields of over 80 % of 1a were achieved using this catalyst.
Following the use of deuterium instead of hydrogen, approximately 62 % incorporation of deuterium in position 4 of the aromatic ring was confirmed by NMR spectroscopy. Complete deuteration of the 4-position cannot in fact be expected, since the NH group in the molecule dilutes the deuterium at the catalyst surface by isotope exchange.

The optimal reaction conditions determined for the dehalogenation of 1c were applied to the tritium labelling. The course of the reaction was followed by changes in the tritium gas pressure in the labelling apparatus. Compared with deuteration the reaction took considerably longer which can be explained by an isotope effect between deuterium and tritium. To prevent overhydrogenation the reaction was in each case interrupted after 23 min, although only 80 % of the theoretical quantity of tritium gas had by then been used up. Apart from nearly 25 % of the initial product 1c the crude product was essentially tritium-labelled nimodipine 1a. The total proportion of by-products was under 5 %. After purification, determination of the specific activity yielded a value of 607 $GBq \cdot mmol^{-1}$, corresponding to 57 % labelling in

position 4 of the aromatic ring. The degree of incorporation is somewhat lower than that after deuteration, but this can be explained by an isotope effect between deuterium and tritium.

The labelling was checked by ^3H-NMR spectroscopy. The ^1H-decoupled ^3H-NMR spectrum shows a singlet at 7.96 ppm. This is in accord with the expectation that the corresponding proton signal of the aromatic position 4 would show the same chemical shift. The ^1H-coupled spectrum shows a doublet caused by coupling with the proton in position 5. Since no other signals were detected, the tritium labelling was only in position 4 which had been the objective of the present work.

Experimental

40 mg (80.4 μmol) 1c was stirred for 23 min in a tritium-labelling apparatus with 8.1 mg palladium hydroxide on active carbon [4] in a mixture of 1.5 ml tetrahydrofuran and 0.2 ml triethylamine with 314.5 GBq tritium gas at a pressure of 46.8 kPa. After readsorption of non-incorporated tritium the catalyst was filtered off and the clear solution was freeze-dried. The strongly radioactive filtrate was adsorbed in a plastic cartridge packed with active carbon which was sealed at the end of the freeze-drying. To separate off labile tritium the crude product was taken up in 2 ml of a 1:1 mixture of dioxan and water and freeze-dried. This procedure was repeated three times. All manipulations should if possible be carried with exclusion of daylight because 1a is light-sensitive.

The dry crude product was dissolved in a mixture of 1 ml acetonitrile and 0.9 ml water and redissolved into 4 equal portions under the following HPLC conditions: column Lichrosorb®RP18, 7 μm, 250 x 10 mm (Merck), eluent 1:1 acetonitrile/water, flow rate 6 ml.min^{-1}, detection in the UV at 275 nm, Uvicord® S II detector (Pharmacia LKB GmbH), k' for [^3H]nimodipine = 7.58. The eluate containing [^3H]nimodipine was fractioned in each case on the basis of the UV signal and each of the fractions were purified. The substance content of the solution was determined by comparing the UV extinction of solutions having known 1a
concentrations. According to this method the total quantity was 13.2 mg (31.4 μmol) [^3H]nimodipine = 39 % of theory. The total radioactivity, determined with a Philips PW 4700 liquid scintillation counter, was 519 mCi (19.2 GBq). The specific activity calculated was 16.4 Ci/mmol (607 GBq/mmol). The labelling position was confirmed by ^3H-NMR, using a Bruker AM 300 spectrometer, measurement frequency 320.14 MHz, [D_6]DMSO, ^1H-decoupled: δ = 7.96 ppm (S, 1T), ^1H-coupled: δ = 7.96 ppm (d, 1T, $^3J(H,T)$ = 8.4 Hz).

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