NOTES

MINISCALE SYNTHESIS OF SPECIFICALLY TRITIUM LABELLED R28935, A NEW CENTRALLY ACTING ANTIHYPERTENSIVE AGENT.

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1. Introduction

R 28935 (fig. 1), developed in a SAR program for antihypertensive agents (1), is a potent long-acting centrally active antihypertensive drug with an unknown mechanism of action (2, 3, 4).
Its threo-isomer (R 29814) is at least ten times less active as an antihypertensive agent.
As the lipophilicity is the same for both diastereo-isomers (log P = 3.41), the differential pharmacological activity cannot be explained by a different physico-chemical distribution of the drugs in the C. N. S.
To determine if the antihypertensive effect is due to the unaltered agent or to metabolites and whether the difference in pharmacological activity is the result of differences in the pathway of metabolic degradation and/or to a selective uptake and/or binding to stereospecific "receptors", the diastereo-isomers were specifically labelled with tritium of a high specific activity.

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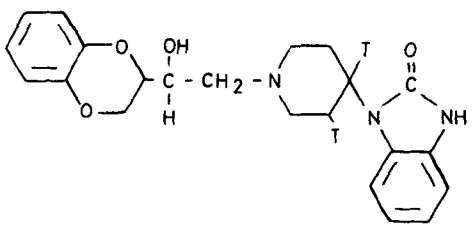

Fig. 1. Structure and position of the tritium label in 1(erythro-1-{1-[2-(2, 3 dihydro-1, 4 benzodioxin-2-yl)-2 hydroxyethyl]-4-piperidinyl-3, 4-t_2}-1, 3-dihydro-2H-benzimidazol-2 one, (R 28935 + R 29814)

2. Methods and Materials (scheme A)

1-(1, 3-dihydro-4 piperidinyl)-2H-benzimidazol-2 one was labelled with tritium at I. R. E. (Fleurus, Belgium) by catalytic tritiation of 1-(1, 2, 3, 6 tetrahydro-4-piperidinyl)-1, 3-dihydro-2H-benzimidazol-2-one (I) (Aldrich) in tetrahydrofurane and Pd/C-10 % as a catalyst.
α-(bromomethyl)-2, 3-dihydro-1, 4-benzodioxin-2-methanol (V) was obtained by a $NaBH_4$ reduction of 2-bromo-1-(2, 3 dihydro-1, 4 benzodioxin-2yl ethanone (IV), following the bromination of 1-(2, 3-dihydro-1, 4-benzodioxin-2-yl ethanone (III) as described (5) and modified by J. Vandenberk. x
As the reduction is stereoselective, resulting in 65-70 % of the erythro-bromide and 35-30 % of the threo-bromide, the erythro form was partly isolated by fractionated recrystalization.
After evaporation of the mother liquors, the ratio of the erythro : threo-bromide was about 1 : 1.

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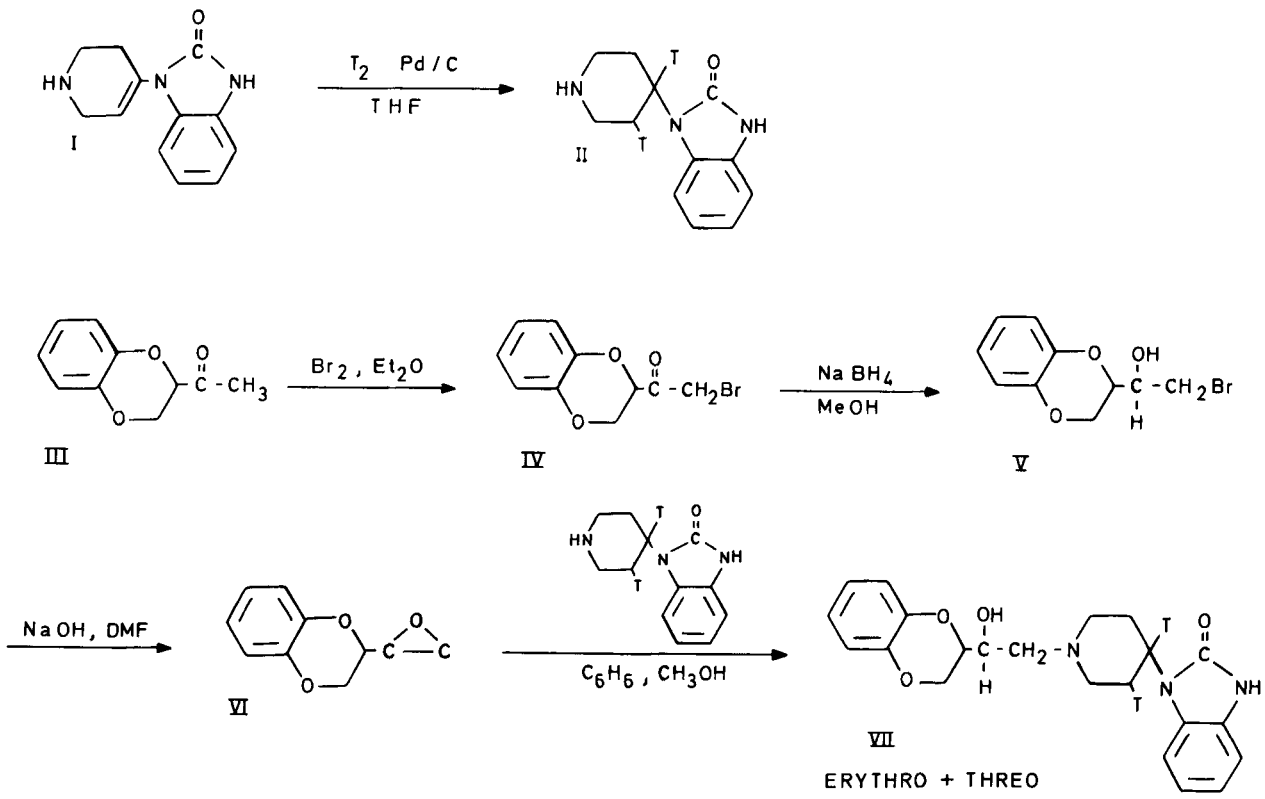

Scheme A :

Reaction scheme for the synthesis of 1-{1-[2-(2, 3 dihydro-1, 4 benzodioxin-2-yl)-2 hydroxyethyl] -4-piperidinyl-3, 4-t_2} -1, 3 dihydro-2H-benzimidazol-2 one.

Although the unlabelled end-products were obtained by heating the separated erythro- and threo-bromides with the amine in dimethylformamide at 90°C during 16 hours in the presence of Na_2CO_3 as an H-Hal scavenger, the labelled synthesis was econominized by converting the crude bromides into the epoxide form (NaOH, DMF (6)) which, after reacting very fast with the labelled amine resulted directly in both diastereo-isomers which could be separated in the erythro-and-threo form by preparative thin-layer chromatography and isolated by scraping off the radioactive spots identical with the reference R 28935 and R 29814 and elution with methanol.

3. Results. Scheme A

3.1 1-(1,3 dihydro-4-piperidinyl-3,4-t_2)-2H-benzimidazol - 2 one (II)

The specific activity of different batches of ^{3}H - II was 3 Ci/mM and 9 Ci/mM.

3.2 Synthesis of 1-{1 [2-(2,3 dihydro-1,4 benzodioxin-2-yl)-2-hydroxyethyl] -4-piperidinyl-3,4-t_2} -1,3 dihydro-2H-benzimidazol-2 one (VII)

In a reacti-vial of 0.6 ml (Pierce), 0.4 ml of a solution of 2.17 mg ^{3}H-II (10 µmole) in methanol was evaporated to dryness by heating in an oil-bath of 50°C in a gentle stream of dustfree N_2 gas. The residue was dissolved in 0.01 ml of methanol.
From a stock solution of 0.23 ml VI in 0.8 ml benzene, 0.01 ml (16 µmole) was introduced into the reacti-vial. 0.1 ml of benzene was added, the reacti-vial sealed with a teflon cap, the mixture vortexed and heated in an oil-bath at 100°C for 2 hours.
After cooling, the reaction mixture, consisting of a mixture of the endproducts in a yield of about 90 %, (50 % R 28935 and 40 % R 29814), was chromatographed on a preparative T. L. C. plate.

3.3 Separation of erythro-1-{1-[2-(2,3 dihydro-1,4-benzodioxin-2-yl)-2-hydroxyethyl]-4-piperidinyl-3,4-t_2}-1,3 dihydro-2H-benzimidazol-2-one and threo-1-{1-[2-(2,3 dihydro-1,4-benzodioxin-2-yl)-2-hydroxyethyl]-4-piperidinyl-3,4-t_2}-1,3 dihydro-2H-benzimidazol-2 one

The total reaction mixture (3.2) was applied to a glassplate (20 x 20 cm), covered with a 0.25 mm layer Sil 60 F 254 (Merck), in the form of a line of 17 cm length.
The plate was developed with a mixture of 90 ml $CHCl_3$ + 10 ml HCOOH, by allowing the solvent to run a distance of 17 cm.
After evaporation during 15' at room temperature, the procedure was repeated three times with freshly prepared developing solvents until a good separation of R 28935 and R 29814 was obtained (rf. respectively 0.53 and 0.41).
After location of the radioactive line and the reference compounds, using a Berthold radiochromatogram scanner LB 2723 and examination by U.V. light (254 nm) about 60 % of the pharmacologically inactive threoform (R 29814) was scrapped off (the centre of the spot), extracted with methanol and rechromatographed as described in order to avoid any contamination with the antihypertensive agent R 28935.
About 80 % of the erythro-form was isolated as described for the threo-form.
An aliquot of the methanol extracts of both diastereo-isomers were rechromatographed and appeared to be radiochemical pure and identical to the reference compounds.

The radiochemical purity was confirmed by the inverse isotope dilution method, whereas the chemical purity was tested pharmacologically using the decrease in blood pressure as a criterion (personal communication T. Loonen et al.).

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