SYNTHESIS OF 2-^{14}C-LABELLED 3H-1,4-BENZODIAZEPINES

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SUMMARY

2-^{14}C-oxazepam (7) 2-^{14}C-(+)- and -(-)-7-chloro-1-methyl-5-phenyl-1,3,4,5-tetrahydro-3H-1,4-benzodiazepine-2-one (8a and 8b) as well as 2-^{14}C-7-chloro-4-carbamoyl-1-methyl-5-phenyl-1,3,4,5-tetrahydro-3H-1,4-benzodiazepine-2-one (9) were synthesised from carbobenzoxy-glycine-1-^{14}C. The overall radiochemical yields from carbobenzoxy-glycine-1-^{14}C were 28.9 % (7), 9.5 % (8a and 8b) and 25.3 % (9), respectively.

Key Words: 1,4-benzodiazepines, synthesis, carbon-14

INTRODUCTION

Our interest in the biological fate of benzodiazepine derivatives prompted us to prepare a series of ^{14}C-labelled compounds. Using carbobenzoxy-glycine-1-^{14}C (Cbz) as the starting material we adopted procedures known from the literature for non-radioactive 1,4-benzodiazepines and worked out modifications of some of these methods to preserve the isotopic material.

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DISCUSSION

Laevorotary 7-chloro-1-methyl-5-phenyl-1,3,4,5-tetrahydro-3H-1,4-benzodiazepine-2-one (8a) (1) and its racemic N^4-carbamoyl derivative (9) (2) showed interesting biological properties determined by known methods. They were needed in labelled form for pharmacokinetic, including metabolic, studies. In order to study the *in vivo* hydrolysis of esters of oxazepam (7) (3), 7 was also synthesized in its labelled form.

Though there exists an available method (4) for preparing 2-^{14}C labelled 3 and 4, we could obtain higher yields using Cbz-glycylamidobenzophenones (1 and 2) as intermediates. Reaction pathways used for preparing 7, 8a, 8b and 9 are shown in Figure 1.

Glycine-1-^{14}C was prepared from a mixture of K^{14}CN and NaCN by a standard method (5) given for glycine-1-^{13}C and its Cbz derivative (6) prepared by carbobenzoxy-chloride. Cbz-glycylamidobenzophenones (1 and 2) were obtained from 2-amino- and 2-methylamino-5-chloro-benzophenones, by acylation with the chloride of Cbz-glycine-1-^{14}C by a modified method of Röhricht at al.(7). Removal of the protective group by treatment with 30 % hydrobromic acid in glacial acetic acid (8) was followed by cyclization without isolation of the glycylamidobenzophenones.

3 was oxidised with 40 % peracetic acid (9) but this reaction gave a comparatively low yield. We successfully used 3-chloro perbenzoic acid as oxidative agent. 6 and 7 were prepared by the method of Bell and Childress (10).

Reduction of 4 with zinc in acetic led to 8 in good yield. The resolution of 8 was performed with D-camphorsulfonic acid (11). HCl of 8 was reacted with KOCN to obtain its N^4-carbamoyl-derivative (9) (12).

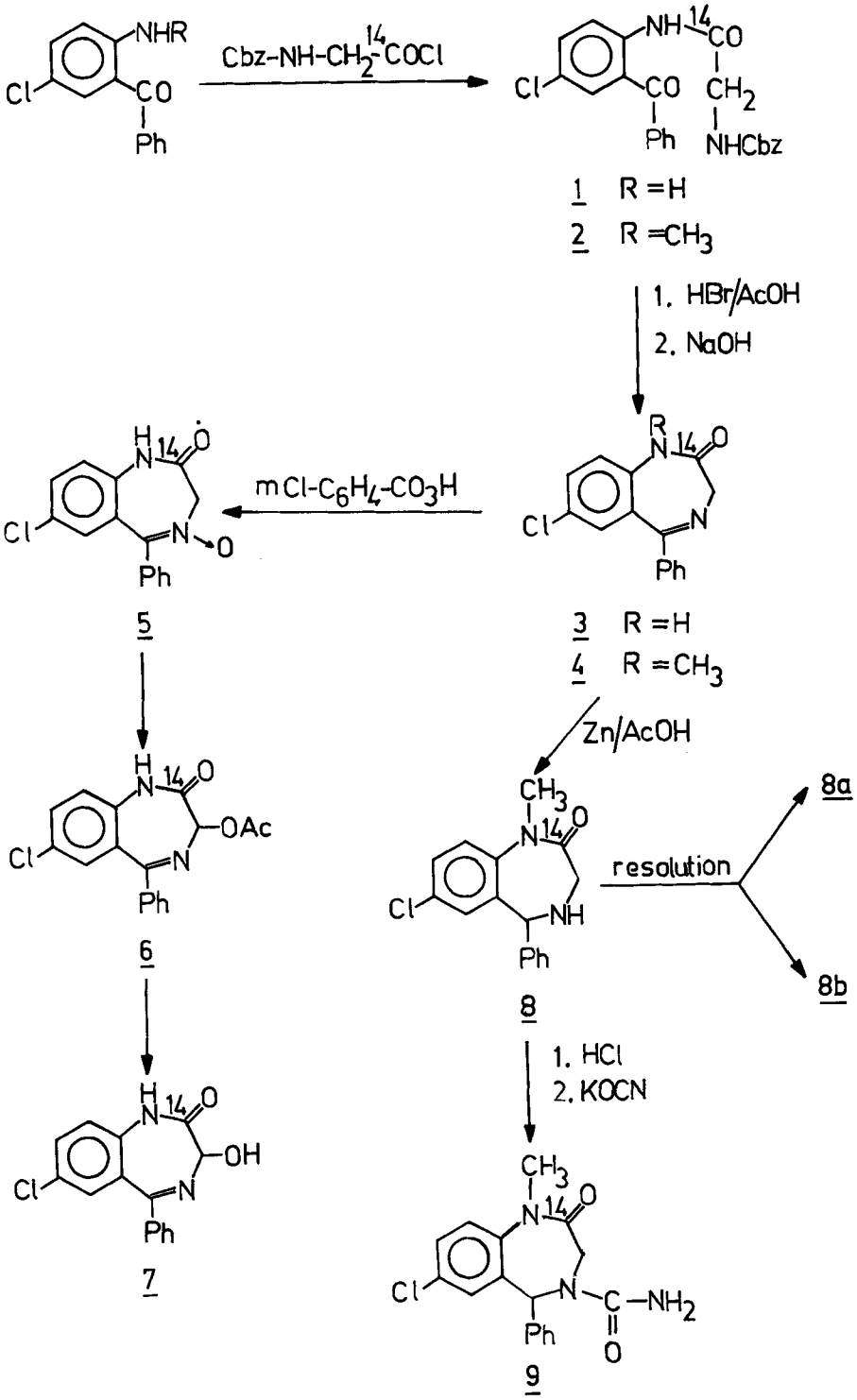
NHR
Cl
CO
Ph
Cbz-NH-CH2-14COCl
NH-14CO
CH2
NHCbz
1 R=H
2 R=CH3
1. HBr/AcOH
2. NaOH
mCl-C6H4-CO3H
3 R=H
4 R=CH3
5
Zn/AcOH
OAc
6
CH3
resolution
8a
8b
8
1. HCl
2. KOCN
OH
7
NH2
9

FIGURE 1

EXPERIMENTAL

Radioactivity determinations were carried out with a liquid scintillation spectrometer (Packard 3003) using an internal standard method. Thin layer chromatography (TLC) analyses were carried out on silica gel coated glass plates, in a solvent heptane:chloroform:acetic acid:ethanol=5:5:1:0.3 visualization by UV (254 and 366 nm) illumination. Materials were identical in every respect with authentic samples.

2-(Carbobenzoxyglycyl-1-^{14}C)-amido-5-chloro-benzophenone (1). 821 mg of phosphorus-pentachloride were added in three portions to a cooled and stirred suspension of 731.8 mg (3,5 mmoles; 18.65 mCi; 5.32 mCi/mmole) Cbz-glycine-1-^{14}C in 4.5 ml of dry ether. After being stirred for 30 minutes, Cbz-glycine-chloride is obtainded and to this solution 895.8 mg (3.85 mmoles) 2-amino--5-chlorobenzophenone was added dropwise in 20 ml of dry ether within 40 minutes. Stirring was continued for 2 hrs and the product began to crystallize. 15 ml of 2 N NaOH was added dropwise to pH=11 and stirred for 2 hrs. The organic layer was separated, washed with water to neutral. The water phase was diluted with water and extracted with chloroform. The combined ether and chloroform solution was dried over $MgSO_4$, solvents were removed in vacuo and the residue was purified on a silica gel column in a solvent mixture: hexane:chloroform=1:1. The product was recrystallized with 100 mg of unlabelled 1 from ethanol to produce 928.1 mg 1. Radiochemical yield: 66.9 %.

2-Methyl-(carbobenzoxyglycyl-1-^{14}C)-amido-5-chloro-benzophenone (2).
Cbz-glycine-1-^{14}C-chloride was prepared from 313.6 mg (1.5 mmoles; 12.77 mCi; 8.51 mCi/mmole) Cbz-glycine-1-^{14}C as described above. 370 mg (1.5 mmoles) of 2-methylamino-5-chlorobenzophenone in 7.5 ml of dry ether was added dropwise during 1 hr. Stirring was continued for 3 hrs then 8 ml of 2 N NaOH was added dropwise in

1 hr. Cooling and stirring was continued for 4 hrs and the organic layer was separated, washed with water to neutral, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified on a silica gel column prepared in hexane and eluted with hexane--ether mixtures by raising the ether concentration. The oily product (506.7 mg) was chemically and radiochemically pure by TLC, and used to prepare 4. Radiochemical yield: 79.4 %.

7-Chloro-5-phenyl-1,3-dihydro-3H-1,4-benzodiazepine-2-one-2-^{14}C (3).
A solution of 928.1 mg (2.19 mmoles) of 1 in 5 ml of 30 % hydrobromic acid in glacial acetic acid was stirred for 1 hr at room temperature. On addition of 100 ml of dry ether, a gummy solid separated. After several minutes, the ether solution was decanted and the solid was washed with 50 ml of dry ether. The suspension of the residue in 25 ml of ether was chilled on an ice bath and 5 ml of 10 % sodium hydroxide was added. The reaction mixture was stirred and the solid went into solution after several minutes. The ethereal layer was separated, washed with water, dried over $MgSO_4$ and concentrated to dryness in vacuo. The residue was crystallized from 8 ml of benzene to yield 483.8 mg of 3.
Radiochemical yield: 82.8 %.

7-Chloro-1-methyl-5-phenyl-1,3-dihydro-3H-4-benzodiazepine-2--one-2-^{14}C (4) was prepared from 502.4 mg (1.15 mmoles) of 2 as described above. The crude product was purified on a silica gel column in a hexane:chloroform=1:1 solution to yield 330.2 mg of 4 after recristallization from isopropyl alcohol together with 100 mg of radioinactive 4.
Radiochemical yield: 77.4 %.

7-Chloro-5-phenyl-1,3-dihydro-3H-1,4-benzodiazepine-2-one-2-^{14}C-4-oxide (5).
A solution of 483.8 mg (1.78 mmoles) of 3 in 65 ml of methylene chloride was added dropwise to a stirred solution of 460.7 mg (2.67 mmoles) of 3-chloroperbenzoic acid in 5 ml of methylene chloride at 18-20 °C in 90 minutes. Stirring was continued for 10 hrs at room temperature. The reaction mixture was brought to pH=8 with 25 % ammonium hydroxide, washed with water to neutral and dried over $MgSO_4$. The solvent was removed in vacuo, and the residue was crystallized from 7 ml of ethanol to yield 354.9 mg of 5. 70 mg of non-radioactive 5 was added to the mother liquor and the product weighed 127.7 mg. Radiochemical yield: 76 %.

3-Acetoxy-7-chloro-5-phenyl-1,3-dihydro-3H-1,4-benzodiazepine-2-one-2-^{14}C (6) was prepared by the procedure established by Bell and Childress (10). The product was obtained from 476.6 mg (1.67 mmoles) of 5 to yield 424.1 mg of 6. Radiochemical yield: 77.8 %.

7-Chloro-3-hydroxy-5-phenyl-1,3-dihydro-3H-1,4-benzodiazepine-2-one-2-^{14}C (oxazepam) (7) (10) was prepared by hydrolysis of 424.1 mg (1.29 mmoles) of 6 to yield 297.6 mg of 7 after recrystallization from ethanol. Radiochemical yield: 79.8 %; 4.88 mCi; 4.74 mCi/mmole. 100 mg of non-radioactive 7 was added to the mother liquor to give 118 mg of 7 (Radiochemical yield: 8.2 %; 0.5 mCi; 1.21 mCi/mmole). The products were chemically pure by TLC. Radiochemical purity: 99.6 % by TLC.

7-Chloro-1-methyl-5-phenyl-1,3,4,5-tetrahydro-3H-1,4-benzodiazepine-2-one-2-^{14}C (8).
173.6 mg (0.61 mmole) of 4 and 240 mg of zinc powder was suspended in 1.5 ml of acetic acid and stirred for 1 hr at room temperature. Zinc acetate was filtered off and washed with acetic acid. The filtrate was neutralized with 2 N NaOH, then

extracted with methylene chloride. The organic layer was dried and concentrated in vacuo to dryness, The residue was crystallized from isopropyl alcohol with 30 mg of non-radioactive 8 and yielded 160.6 mg of 8. Radiochemical yield: 88.1 %.

Resolution of 8 was carried out by the method of Kisfaludy at al. (11).
To a hot solution of 68.9 mg (0.24 mmole) of 8 in 1.5 ml of 0.1 % water in methanol, 60.2 mg of D-camphorsulfonic acid was added in 0.3 ml of 0.1 % methanol. The solution was cooled at 24 °C and stirred for 1 hr. The solid was separated by centrifugation.

The filtrate was concentrated in vacuo. The residue was heated in 1.5 ml of 1.5 % water in ethanol then crystallized at 24 °C with the salt of D-camphorsulfonic acid of 8. The crystals were centrifuged and the filtrate was concentrated, the residue was solved in 2 ml of 80 % ethanol, brought to alkaline pH with $KHCO_3$ then diluted with water followed by extraction with ether. The ethereal phase was dried ($MgSO_4$), concentrated and crystallized with non-radioactive 8a to yield 11.9 mg of 8a.
Radiochemical yield: 16.6 %; 0.25 mCi; 6.25 mCi/mmole.
Radiochemical purity: 99.7 % by TLC.
$[\alpha]_D$ = - 217.8 (c=1, $CHCl_3$)

The crystals were dissolved in 2 ml of 80 % ethanol, brought to alkaline pH with a solution of $KHCO_3$, diluted with water and extracted by ether, then dried to yield (after crystallization from isopropyl alcohol) 12.5 mg of 8b.
Radiochemical yield: 16.6 %; 0.27 mCi; 6.30 mCi/mmole.
Radiochemical purity: 99.6 by TLC.
$[\alpha]_D$ = + 216.0 (c=1, $CHCl_3$)

7-Chloro-4-carbamoyl-1-methyl-5-phenyl-1,3,4,5-tetrahydro-3H--1,4-benzodiazepine-2-one-2-^{14}C (9) (12).

93 mg (0.32 mmole) of 8 was dissolved by heating in 1.3 ml of dry acetone, and its salt was prepared with dry hydrochloric acid in methanol. Crystals separated (88.2 mg); they were suspended in 1.8 ml of acetic acid and 111.6 mg (1.37 mmoles) KOCN was given to the suspension at room temperature. The reaction mixture was stirred for 1.5 hr, neutralized with ammonium hydroxide (25 %) and extracted with methylene chloride. The organic layer was washed with water, dried ($MgSO_4$) and concentrated. The residue was crystallized with 40 mg of non--radioactive 9 from isopropyl alcohol to give 105.5 mg of 9. Radiochemical yield: 88.9 %; 1.85 mCi; 5.80 mCi/mmole. Radiochemical purity: 99.8 % by TLC.

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