

SYNTHESIS OF CARBON-14 LABELED 1,4-BENZODIAZEPINES

1. 8-Chloro-1-methyl-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine-1-¹⁴C and 8-Chloro-6-(2-chlorophenyl)-1-methyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine-1-¹⁴C.

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SUMMARY

Reaction of 7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepine-2-thione (Ia) and 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-2H,1,4-benzodiazepine-2-thione (Ib) with hydrazine produces, respectively, 7-chloro-5-phenyl-3H-1,4-benzodiazepin-2-ylhydrazine (IIa) and 7-chloro-5-(2-chlorophenyl)-3H-1,4-benzodiazepin-2-ylhydrazine (IIb). Acetylation of IIa and IIb with N-acetyl-1-¹⁴C-imidazole affords acetic acid-1-¹⁴C 2-(7-chloro-5-phenyl-3H-1,4-benzodiazepin-2-yl)hydrazide (IIIa) and acetic acid-1-¹⁴C 2-[7-chloro-5-(2-chlorophenyl)-3H-1,4-benzodiazepin-2-yl]hydrazide (IIIb). Compounds IIIa and IIIb are thermally cyclised to give 8-chloro-1-methyl-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine-1-¹⁴C (IVa) and 8-chloro-6-(2-chlorophenyl)-1-methyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine-1-¹⁴C (IVb).

INTRODUCTION

The biological effects exerted on the central nervous system by compounds which possess the 1,4-benzodiazepine ring system have been the subject of decade-

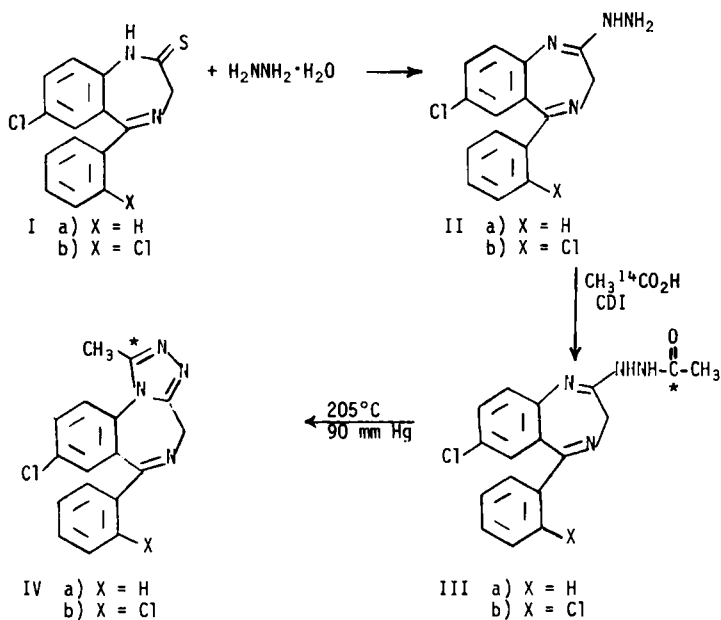
long and continuing intensive investigation by various research groups.^{1,2} 8-Chloro-1-methyl-6-phenyl-4H-*s*-triazolo[4,3-*a*][1,4]benzodiazepine (IVa) and 8-chloro-6-(2-chlorophenyl)-1-methyl-4H-*s*-triazolo[4,3-*a*][1,4]benzodiazepine (IVb)^{3,4} are members of a large group of 6-phenyl-4H-*s*-triazolo[4,3-*a*][1,4]-benzodiazepines⁵ which have central nervous system depressant activity. Radioactive forms of these two compounds were needed for metabolism studies in animals and man. The high potency of IVa and IVb indicated that these drugs would have to be administered in very low doses. Therefore, the labeled materials must be of high specific activity. This report describes the synthesis of IVa and IVb labeled with carbon-14 in the C-1 position of the 4H-*s*-triazolo[4,3-*a*][1,4]benzodiazepine ring system.

DISCUSSION AND RESULTS

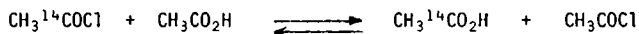
In the previously reported procedure⁴ for preparing compounds IVa and IVb, the thiones (Ia) and (Ib)⁶ were refluxed with acethydrazide in *n*-butanol to give the desired products. Because of the expected inconvenience in preparing and purifying small quantities of the low-melting and hygroscopic acethydrazide-1-¹⁴C, it was decided to follow an alternate synthetic route as shown in Scheme 1.

The thiones Ia and Ib were treated with hydrazine hydrate to obtain the corresponding hydrazines IIa and IIb. Acetylation of IIa and IIb with acetyl-1-¹⁴C chloride was considered, but it was judged unsuitable because in model experiments with non-labeled materials, the reaction required the presence of acetic acid as solvent to avoid diacetylation. In a mixture of labeled acetyl-1-¹⁴C chloride and non-labeled acetic acid, the two reagents could equilibrate according to Scheme 2, resulting in dilution of the acylating species, and making it impossible to attain the desired high specific activity. The acylation was therefore carried out with N-acetyl-1-¹⁴C-imidazole which was prepared *in situ* from acetic acid-1-¹⁴C and 1,1'-carbonyldiimidazole (CDI). The resulting acethydrazides IIIa and IIIb were of high purity and required no

Scheme 1

Synthesis of Carbon-14 Labeled
6-Phenyl-4H-*s*-triazolo[4,3-*a*][1,4]benzodiazepines

Scheme 2



purification, when the molar ratio of the acetic acid-1- ^{14}C , CDI, and the hydrazine IIa or IIb used were carefully controlled. The acetyldiazides IIIa and IIIb were cyclized thermally under vacuum, which removed water formed as the by-product, to afford IVa and IVb in 68% overall yields based on acetic acid-1- ^{14}C .

EXPERIMENTAL SECTION

Radioactivity determinations were carried out on Packard Tri-Carb Model 3375 or Model 2425 liquid scintillation spectrometers with the external standard method. Thin-layer chromatography (TLC) glass plates containing radioactive materials were scanned on a Vanguard Model 880 Autoscanner equipped with Vanguard Model 885 Glass Plate Scanner. Gas chromatographic (GC) analyses were carried out on a Hewlett-Packard Model 5754B instrument fitted with a 4' x $\frac{1}{8}$ " O.D. Teflon[®]-lined aluminum column packed with 100/120 mesh Porapak[®] Q; detection by thermal conductivity. TLC analyses were carried out on silica gel coated glass plates developed with these solvent mixtures: A) 5% v/v MeOH in CH_2Cl_2 , B) 10% v/v MeOH in CH_2Cl_2 . Microanalyses were obtained for the indicated elements, and, except as noted, all results were within $\pm 0.4\%$ of theory. Melting points were uncorrected. Infrared (IR) spectra were obtained from Nujol mulls or KBr pellets with a Perkin-Elmer Model 421 spectrometer. Ultraviolet (UV) spectra were obtained from 95% EtOH solutions with a Cary Model 14 spectrophotometer.

7-Chloro-5-(2-chlorophenyl)-3H-1,4-benzodiazepin-2-ylhydrazine (1b)

Hydrazine hydrate (4 ml, 4.12 g, 82.2 mmol) was added to a stirred suspension of 5.73 g (17.8 mmol) of 1b in 150 ml of MeOH while a stream of N_2 was bubbled through the mixture. The mildly exothermic reaction was allowed to proceed for 2.5 hrs and the resulting solution was filtered to remove trace of solids. The filtrate was concentrated at reduced pressure and 30°C. The residual oil was treated with 100 ml of H_2O and extracted with 125 ml of CHCl_3 in two portions. The combined extracts were washed with 50 ml of H_2O and dried over anhydrous K_2CO_3 . The dried extracts were concentrated at reduced pressure (19 mm Hg) and 30°C to 25 ml and mixed with 25 ml of EtOAc. The mixture was again concentrated to 20 ml and filtered. The filtration was only partially completed before precipitation caused clogging of the filter. Both the fil-

trate and the unfiltered mixture were kept at room temperature overnight. The crystals from both mixtures were collected, washed with EtOAc followed by Et₂O and dried. The filtrate yielded 3.778 g of product, mp 235-7°C (dec), while the unfiltered mixture afforded 1.339 g with mp 203-206°C (dec) (total yield was 5.117 g, 90%). Despite the widely different melting points, both crops of product appeared to be pure by TLC (solvent mixtures A and B). In subsequent trial runs of the acetylation and cyclization reactions, both crops of product led to equally pure IVb in similar yields. For preparing IIb, the higher melting product was used; IR spectrum compatible with proposed structure, UV end absorption, λ_{sh} 213 nm (ϵ 35,050), 235 (25,450), and 275 (11,900), λ_{max} 349 nm (ϵ 2,250); *anal.* (C₁₅H₁₂Cl₂N₄) C, H, Cl, N.

The above procedure is similar to the one used to prepare 7-chloro-5-phenyl-3H-1,4-benzodiazepin-2-ylhydrazine⁶ (IIa) from Ia.

GC Assay for CDI

To effectively control the molar ratio of reactants in the acylation of IIa or IIb with acetic acid-1-¹⁴C *via* N-acetyl-1-¹⁴C-imidazole, the purity of commercially supplied CDI must be known. A GC assay was devised for this purpose. To each of 5 ml aliquots of 0.35 M solution of glacial HOAc in anhydrous THF was added 284, 312, 326, 341, 355 and 369 mg of CDI (Aldrich), representing nominally 1.00, 1.10, 1.15, 1.20, 1.25 and 1.30 molar equivalents, respectively, with respect to the HOAc used in each reaction. After evolution of CO₂ had subsided, the mixtures were kept at room temperature for 1 hr. To each mixture was added 0.15 ml (2.6 mmols) of absolute EtOH. The resulting mixtures were kept at room temperature overnight and 3 μ l aliquots were subjected to GC analysis. The instrument was operated under the following conditions injection port 270°C column oven 245°C detector 275°C, He flow 50 ml/min. The detected components and their retention times were: EtOH, 1.0 min; HOAc, 1.2 min; THF and EtOAc, 1.8 min; (EtO)₂CO, 4.0 min; and imidazole, 7.6 min.

The presence or absence of HOAc and $(\text{EtO})_2\text{CO}$ in the mixture were used as end points in the GC analysis. A mixture which showed presence of HOAc but absence of $(\text{EtO})_2\text{CO}$ indicated that less than equimolar amount of CDI relative to HOAc had been used. Absence of both HOAc and $(\text{EtO})_2\text{CO}$ would indicate interaction of equimolar amounts of HOAc and CDI, while absence of HOAc and presence of $(\text{EtO})_2\text{CO}$ resulted from excess CDI with respect to HOAc. A sample of the CDI to be used in the acetylation reactions described below was found to be $89 \pm 2\%$ pure.

Acetic Acid-1- ^{14}C 2-(7-chloro-5-phenyl-3H-1,4-benzodiazepin-2-yl)hydrazide (IIa)

All items of glassware were flame dried and flushed with dry N_2 prior to use. To a mixture of 326 mg (effectively 1.79 mmole) of CDI and 5 ml of anhydrous THF in an addition funnel was added 105 mg (1.75 mmole, 48 mCi, 100 μl delivered from a 250 μl micro syringe) of anhydrous HOAc-1- ^{14}C .^{*} The mixture was briefly agitated until evolution of CO_2 subsided. The resulting solution was kept under N_2 for 1 hr and added dropwise, with stirring in 5 min, to a solution of 525 mg (1.84 mmole, 5% excess) of IIa in 6 ml of anhydrous THF at 0°C . The mixture was stirred at 0°C for 15 min and allowed to warm to room temperature. After 5-6 hrs, stirring was stopped and mixture was kept at room temperature overnight. The solids were filtered, washed with 10 ml of THF in portions followed by Et_2O , and dried to give 504 mg (88.1% yield) of IIIa, mp $198-205^\circ\text{C}$ (dec) softening at 183°C and resolidifying at 185°C (Lit.⁴ mp $199-200^\circ\text{C}$); specific activity 77.8 $\mu\text{Ci}/\text{mg}$, 25.45 mCi/mM. TLC (solvent mixture B) indicated the material was radiochemically pure. TLC of the melt showed that IIIa on heating had been converted to chemically and radiochemically pure IVa.

Acetic Acid-1- ^{14}C 2-[7-chloro-5-(2-chlorophenyl)-3H-1,4-benzodiazepin-2-yl]hydrazide (IIb)

Analogous to the reaction described above, 543 mg (86.0% yield) of IIb was

* Supplied by International Chemical and Nuclear Corp., sp. act. 27.3 mCi/ml

prepared from 588 mg (1.84 mmole) of IIb in 22 ml of anhydrous THF; mp 198-200°C (dec); sp. act. 24.85 mCi/mM; radiochemically pure by TLC (solvent mixture B). TLC of the melt showed IIIb had been quantitatively converted thermally to IVb.

8-Chloro-1-methyl-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine-1-¹⁴C (IVa)

IIIa (458 mg, 1.40 mmole) was heated in an oil bath at 295°C and reduced pressure (~90 mm Hg) for 15 min and the melt was cooled to room temperature under H₂. The resulting glassy material was dissolved in 25 ml of CH₂Cl₂ and the solution was filtered. The filtrate was evaporated on steam bath until the volume was reduced to 5 ml. The remaining CH₂Cl₂ was displaced by simultaneous distillation and addition of PhH while the total volume was maintained at ~10 ml. Addition of 20 ml of Skellysolve B caused rapid precipitation of fine crystals. The mixture was kept at room temperature overnight and the crystals were filtered, 370 mg (85.7%) of IVa, mp 225-227°C; sp. act. 89.9 μCi/mg, 27.7 mCi/mM; UV and IR spectra conformed with standards; radiochemically pure by TLC (solvent mixtures A and B); *anal.* (C₁₇H₁₃ClN₄) C, H, Cl, N.

8-Chloro-6-(2-chlorophenyl)-1-methyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine-1-¹⁴C (IVb)

Similarly as above, IVb was obtained by the thermal cyclization of 506 mg (1.40 mmole) of IIb. The product, 422 mg (88% yield), was isolated and recrystallized from mixtures of Me₂CO and CH₂Cl₂, mp 239-241°C; sp. act. 80.7 μCi/mg, 27.7 mCi/mM; radiochemically pure by TLC (solvent mixture A); UV, IR spectra conformed with standards, *anal.* (C₁₇H₁₂Cl₂N₄) C, H, Cl, N.

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