SYNTHESIS OF CARBON-14 LABELED 1,4-BENZODIAZEPINES. IV. CARBON-13 AND CARBON-14 LABELED 8-CHLORO-1-(2-DIMETHYLAMINO)ETHYL-6-PHENYL-4H-*s*-TRIAZOLO[4,3-a][1,4]-BENZODIAZEPINE TOSYLATE

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SUMMARY

Carbon-13 and carbon-14 labeled forms of the title compound have been prepared from the correspondingly labeled forms of 8-chloro-1-methyl-6-phenyl-4H-*s*-triazolo[4,3-a][1,4]benzodiazepine (*3*)*. A modified and more convenient version of the previously reported procedure for preparing *3* is described.

Key Words: Synthesis, Carbon-13, Carbon-14, Triazolobenzodiazepine

INTRODUCTION

We previously described the syntheses of a group of carbon-14 labeled 1,4-benzodiazepines (1-3). The 1-methyl-6-aryl-4H-*s*-triazolo[4,3-a][1,4]benzodiazepines (*viz.* compound *3*) possess anxiolytic activity in both experimental animals (4) and man (5). The 1-(aminoalkyl)-6-aryl-4H-*s*-triazolo[4,3-a][1,4]-benzodiazepine series, on the other hand, exhibits both antidepressant and antianxiety activities (6). The apparent optimization of the antidepressant properties of the latter series with a two-carbon side chain (7) led to the development of an efficient synthesis of these compounds (8) and the selection of the title compound *4* as an agent of possible clinical interest. This report

*Alprazolam is the generic USAN name for this compound.

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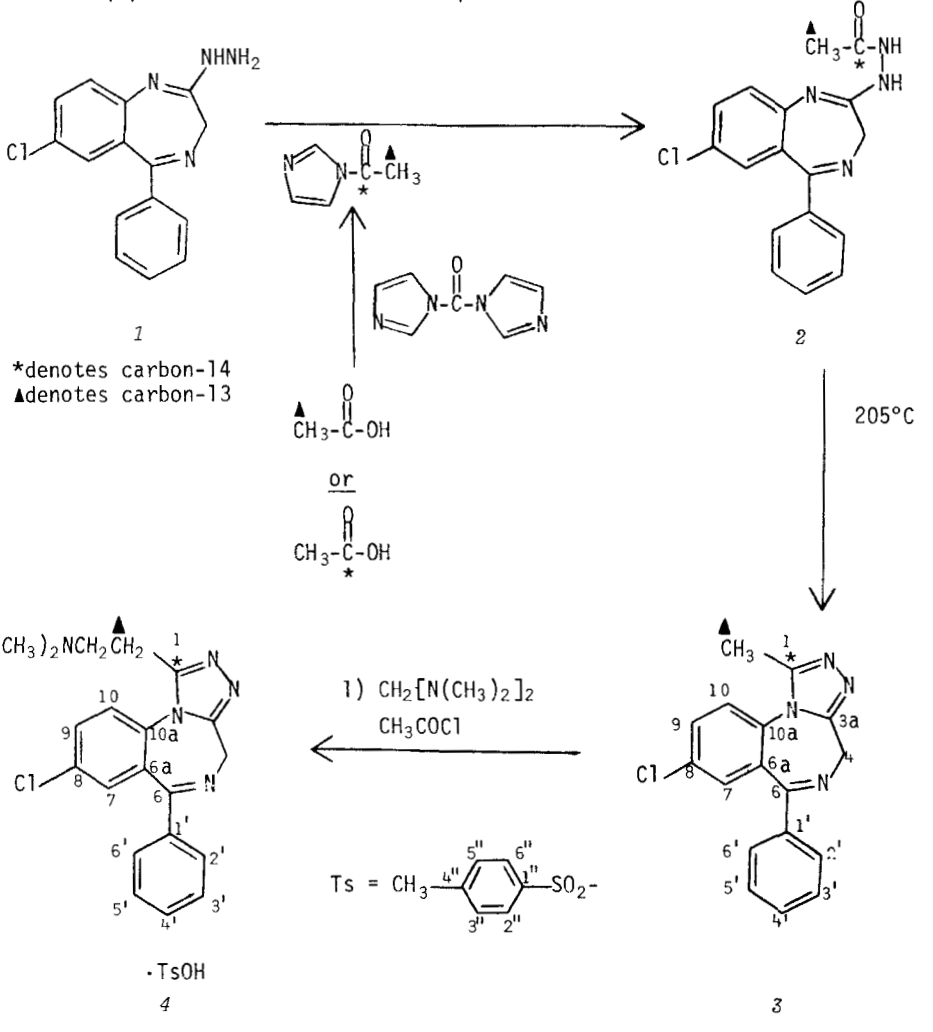
describes the preparation of carbon-14 and carbon-13 labeled compound *4* for studying its biotransformations in test animals and man.

DISCUSSION AND RESULTS

The conversion of compound *3* to compound *4*, accomplished by the treatment of *3* with *bis*-dimethylaminomethane in the presence of acetyl chloride, was reported by Hester *et al.* (8). The synthesis of carbon-14 labeled *2* from [1-^{14}C]acetic acid and 7-chloro-2-hydrazino-5-phenyl-3H-1,4-benzodiazepine (*1*) was described earlier (1). Since each of the isotopes carbon-14 and carbon-13 had its own



unique attributes as an analytical tool, radioactivity in the case of carbon-14, and nuclear magnetic resonance (nmr) and mass spectral properties in the case of carbon-13, we labeled compound *4* with both carbon-13 and carbon-14 for use in metabolism studies. The two-carbon unit of acetic acid was to be incorporated into *4* so that the carboxyl carbon would be at the C-1 position of the ring system, and the methyl carbon would become the methylene carbon adjacent to C-1. Tanayama and Kanai (9,10) have studied the metabolism of a compound similar to *3*, but devoid of methyl at C-1; namely, 8-chloro-6-phenyl-4H-*s*-triazolo[4,3-a]-[1,4]benzodiazepine. Of its metabolites which have been identified, all retain the intact triazolo ring. Hence, a label with high detection sensitivity, such as carbon-14, at the C-1 ring position would provide the means for quantification as well as detection of metabolic transformation products derived from the parent compound *4* but still containing the triazolo ring moiety. To achieve this, [1-^{14}C]acetic acid was used as the starting material for preparing ^{14}C-labeled compound *4*.

The methyl group of compound *3* undergoes hydroxylation metabolically (11). The diaminoalkyl side chain methylene carbons of compound *4* may also undergo analogous metabolic transformations. During these transformations, the two side chain methylene carbons may assume various states of oxidation (*e.g.*, primary or secondary alcohol, aldehyde, ketone, or carboxylic acid), each distinguishable from the others by means of carbon-13 nmr by virtue of their different chemical shifts (12). Labeling of one of the two side chain carbons with carbon-13 enrichment would provide the means of distinguishing one carbon from the other and thus facilitate the investigation of the metabolic fate of the side chain. We chose to incorporate ^{13}C enrichment in the carbon adjacent to the triazolo ring because we would then be able to use the readily available [2-^{13}C]acetic acid as the starting material to obtain ^{13}C-labeled *4* by employing the preparative procedures identical to those used for synthesizing ^{14}C-labeled compound *4*.

In the previously described procedure (1) for the acetylation of *1* to give the acetylhydrazine *2*, the equimolar amounts of acetic acid and N,N'-carbonyldiimidazole (CDI) were used to react with less than one molar equivalent of *1*, in

order to obtain the cyclized product *3* of high purity. This was somewhat wasteful of labeled material and required careful analysis of both the radioactive acetic acid and CDI, especially the latter because of its susceptibility to hydrolysis during storage. In the current preparation, the acylation reaction was carried out with equimolar amounts of labeled acetic acid and *1* in the presence of an excess of CDI. This led to product *3* contaminated with a by-product, but the latter was readily removable by washing with water or by chromatography on silica gel. This procedure proved more convenient to use, afforded a comparable yield of *3*, and allowed full utilization of labeled materials.

EXPERIMENTAL METHODS

Radioactivity determinations were carried out with a Packard Tri-carb Model 2425 liquid scintillation spectrometer using Diotol (Burdick & Jackson) and the external standard method. Thin layer chromatographic (tlc) analyses were done on 2.5 x 10 cm glass plates coated with a 250 μm thick layer of silica gel GF (Analtech). Developed zones were visualized under ultraviolet light (254 nm) illumination. Radioactive zones on developed plates were detected with a Vanguard Model 880 Autoscanner equipped with Model 885 glass plate scanner. Ultraviolet spectra were obtained with a Cary Model 15 spectrometer. Nuclear magnetic resonance (nmr) spectra were obtained with a Varian Model CFT-20 or A-60D spectrometer, and mass spectra with a Varian Model CH-5 spectrometer. Melting points were determined in capillary tubes and are uncorrected. Microanalyses were obtained for the listed elements where indicated, and all results were within ±0.4% of theory.

7-Chloro-2-(2-acetylhydrazino)-5-phenyl-3H-1,4-benzodizepine (*2*)

A) Carbon-13 Labeled *2*

To a stirred solution of [2-^{13}C]acetic acid* (500 mg, 8.19 mmoles) in 20 ml of anhydrous tetrahydrofuran (THF) was added a 10% excess of CDI (1.464 g, 9.03 mmoles, Aldrich Chemical Co.). After evolution of gases subsided, the mixture was stirred under N_2 at room temperature for 1 hour, cooled to 0°C, and a cold

*Supplied by Stohler Isotope Chemicals, Waltham, MA, U.S.A.; 90% enrichment.

solution of *1*** (2.335 g, 8.20 mmoles), in 45 ml of anhydrous THF was added in one portion. The mixture was stirred at 0°C for 1 hour and allowed to come to room temperature overnight. The mixture containing copious amounts of white precipitates was concentrated at reduced pressure and the residues were triturated with Et_2O-hexane, filtered, and the solids washed repeatedly with Et_2O and H_2O, and dried to give 2.309 g (86.2% yield) of carbon-13 labeled *2*, mp 205-207°C (dec), single component by tlc (silica gel; 95:6:1 V/V CH_2Cl_2:MeOH:NH_4OH, Rf 0.20, or 9:1 V/V CH_2Cl_2:MeOH, Rf 0.31) identical to a standard sample of *2* (1,5). This material was used without further purification in the cyclization step.

B) Carbon-14 Labeled *2*

Similarly, from [1-^{14}C]acetic acid*** (nominally 53.8 mg, 0.871 mmole), CDI (160 mg, 0.986 mmole), and II (248 mg, 0.871 mmole), there was obtained 295 mg (~100% yield) of crude carbon-14 labeled *2*. The tlc of this material (95:6:1 V/V CH_2Cl_2:MeOH:NH_4OH, Rf 0.20, or 9:1 V/V CH_2Cl_2:MeOH, Rf 0.31) showed presence of an impurity**** (Rf 0.16) detectable both visually and by radioactivity. However, this impurity did not interfere with the cyclization step to follow, and was readily removed in the later step. Therefore, the crude material was used in the cyclization step without purification.

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

A) Carbon-13 Labeled *3*

The carbon-13 labeled *2* (2.30 g, 7.02 mmole) described above was heated at 205°C (oil bath) and 25 mm Hg pressure for 30 minutes until all solids had turned into a melt. The melt was cooled and dissolved in 50 ml of CH_2Cl_2 and the solution was filtered. The filtrate was concentrated at reduced pressure and the residue was crystallized from benzene-hexane to give 1.934 g (89.3%) yield of carbon-13 labeled *3*, mp 227-228°C; m/z 309; λ_{Max}^{EtOH} 222 nm (ε40,550), λ_{Sh}^{EtOH} 245 nm (ε15,850), 265 (6,650), 290 (ε3,250); anal. - C,H,N; single compo-

**Prepared according to previously described procedure (1).

***Supplied by ICN, Chemical and Radioisotope Division, Irvine, CA, U.S.A.; nominally 50 mCi, 55.8 mCi/mM.

****This impurity was observed in the crude *2* as well during the carbon-13 preparation, but was removed during the repeated washing with Et_2O and H_2O. During the carbon-14 preparation, washing was kept to a minimum to avoid loss of desired *2*.

nent by tlc (9:1 V/V CH_2Cl_2:MeOH, Rf 0.50, or 95:6:1 V/V CH_2Cl_2:MeOH:NH_4OH, Rf 0.44) identical to an authentic sample of *3*; proton nmr ($CDCl_3$), δ7.3-7.8 (m, 8H, aromatic), 5.5 (A of AB, 1H, Jab = 12.7 Hz, H-C-H), 4.1 (B of AB, 1H, Jab = 12.7 Hz H-C-H), 2.6 (s and d, 3H, JH-^{13}C = 130.5 Hz, CH_3, integration ratio of unenriched methyl proton singlet to carbon-13 coupled proton doublet gave enrichment of 89.5%); ^{13}C-nmr ($CDCl_3$, ppm), 167.98 (C_6), 154.92 (C_{3a}), 150.18 (C_1, J_{C-C} = 57.3 Hz), 138.56 (C_{10a}), 133.07 (C_8), 132.21 (C_1'), 131.80 (C_9), 131.51 (C_7), 130.82 (C_4') 130.48 (C_{6a}), 129.27 (C_2',C_6'), 128.35 (C_3',C_5'), 124.87 (C_{10}), 46.30 (C_4), 12.19 (CH_3-, ^{13}C-enrichment 91%, no enrichment seen at C_1). The enrichment was determined by comparing the ^{13}C-nmr integrals at 46.30 ppm and 12.19 ppm, using a sample of known ratio of ^{13}C-enriched and unenriched *3* to obtain the spectrum with a pulse delay of 20 seconds and gated decoupling to suppress nuclear Overhauser enhancement.

B) Carbon-14 Labeled *3*

Similarly, the crude carbon-14 labeled *2* (295 mg) described above was heated at 205°C and 25 mm Hg pressure to give 200 mg of product which was shown by tlc (9:1 V/V CH_2Cl_2:MeOH) to contain a polar impurity. This material was twice chromatographed on a 35 g column of silica gel, packed in and eluted with 95:5 V/V CH_2Cl_2:MeOH) to give after crystallization from benzene-hexane 167 mg (62.0% yield based on [1-^{14}C]acetic acid) of carbon-14 labeled *3*, sp. act. 170.0 μCi/mg, λ^{EtOH}_{Max} 222 nm (ε40,600), λ^{EtOH}_{Sh} 245 (ε15,500), 265 (ε6,540) and 290 nm (ε2,950), single component radiochemically and chemically by tlc (9:1 V/V CH_2Cl_2:MeOH, Rf 0.50, or 95:6:1: V/V CH_2Cl_2:MeOH:NH_4OH, Rf 0.44), identical to a standard of *3*.

8-Chloro-1-(2-dimethylamino)ethyl-6-phenyl-4H-*s*-triazolo[4,3-a][1,4]benzodiazepine Tosylate (*4*)

A) Carbon-14 Labeled *4*

To a stirred ice cold solution of carbon-14 labeled *3* (155 mg, 0.50 mmole, 86.7 μCi/mg) in 2.4 ml of 0.4M solution of *bis*-dimethylaminomethane in dry dimethylformamide (DMF) was added dropwise 0.9 ml of 1.3M solution of acetyl chloride (Baker Chemical Co.) in dry DMF. The mixture containing precipitates

was stirred at 0°C under N_2 for 6 hours and basified with 2.5 ml of 1N NaOH. The resulting mixture was partitioned with 20 ml of brine and 25 ml of $CHCl_3$. The aqueous phase was extracted with 5 x 15 ml of $CHCl_3$. The combined $CHCl_3$ extracts were washed with 35 ml of brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residual thick yellow syrup, dissolved in 5 ml of $CHCl_3$, was chromatographed on a column of 50 g of silica gel packed in MeOH. The column was eluted with 700 ml of MeOH which was collected in 11 ml fractions at 3 minutes per fraction. The combined fractions 25 through 55 were concentrated to give 9.21 mCi of the free base of *4* (theory 126 mg, 0.344 mmole). This material was dissolved in 1 ml of absolute EtOH and treated with 0.66 ml of 0.5M solution of *p*-toluenesulfonic acid (Eastman Organic Chemicals) in absolute EtOH, seeded, and allowed to crystallize overnight. There was obtained, after washing with 4:1 V/V Et_2O:EtOH, then Et_2O, and drying at room temperature and 25 μm Hg pressure, 157 mg (58.3% yield) of carbon-14 labeled *4*, sp. act. 49.9 μCi/mg; single component radiochemically by tlc (90:10:1 V/V CH_2Cl_2:MeOH:NH_4OH, Rf 0.33, or MeOH, Rf 0.25); λ_{Max}^{EtOH} 222 nm (ε50,750), λ_{Sh}^{EtOH} 245 (ε15,950), 268 (ε6,300), and 285 nm (ε3,800); anal. - C,H,N.

B) Carbon-13 Labeled *4*

Similarly, carbon-13 labeled *3* (1.625 g, 5.25 mmoles) in dry DMF (25 ml) was treated with *bis*-dimethylaminomethane (0.644 g, 6.30 mmoles) and acetyl chloride (0.546 g, 6.83 mmoles) at 0°C under N_2 for 3.5 hours to give, after chromatographic purification on a 150 g column of silica gel, 1.8 g of the free base of carbon-13 labeled *4* (fractions 61-106, 10 ml each of MeOH eluent). The free base on treatment with *p*-toluenesulfonic acid (821 mg, 4.31 mmoles) in absolute EtOH afforded 1.908 g (67.5% yield) of carbon-13 labeled *4*, mp 204-205°C; λ_{Max}^{EtOH} 222 nm (ε51,200), λ_{SH}^{EtOH} 245 (ε16,150), 268 (ε6,350), and 285 (ε3,700); single component by tlc (90:10:1 V/V CH_2Cl_2:MeOH:NH_4OH, Rf 0.33, or MeOH, Rf 0.25), identical to an authentic sample of *4*; m/z 321 (loss of dimethylamine from the free base); anal. - C,H,N; proton nmr (DMSO-d_6): δ9.4 (bs, 1H, N-H), 7.1-7.9 (m, 12H, aromatic) 5.25 (A of AB, 1H, H-C-H), 4.2 (B of AB, 1H, H-C-H), ca. 3.5 (m, 4H, $-CH_2-{}^{13}CH_2-$), 2.9 (s, 6H, $N(CH_3)_2$), 2.27 (s, 3H, ar-CH_3); ^{13}C-nmr (10% V/V

D_2O/DMSO-d_6, ppm); 167.96 (C_6), 154.97 (C_{3a}), 150.48 (C_1, J_{C-C} = 56.0 Hz), 144.11 (C_1"), 138.62 (C_4"), 138.42 (C_{10a}), 131.96 (C_9), 131.96, 131.49 (C_8,C_1'), 130.78, 130.62 (C_7,C_4'), 130.16 (C_{6a}), 129.17 (C_2',C_6'), 128.30 (C_2",C_6",C_3',C_5'), 125.85 (C_{10}), 125.41 (C_3",C_5"), 53.63 (N-$\underline{C}H_2$-CH_2-, J_{C-C} = 36.2 Hz), 45.33 (C_4), 42.69 (N-($\underline{C}H_3$)$_2$), 20.89 (N-CH_2-$\underline{C}H_2$-, ^{13}C-enrichment, no enrichment at C_1), 20.67* (Ar-$\underline{C}H_3$).

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*Determined from a spectrum of a standard sample of *4* of natural ^{13}C-abundance. In the spectrum of the ^{13}C-enriched sample, the aromatic CH_3 signal is masked by the nearby overwhelming -$\underline{C}H_2$-CH_2-N< signal.