SYNTHESIS OF TRITIUM LABELLED ADINAZOLAM WITH HIGH SPECIFIC ACTIVITY

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SUMMARY

Tritium labelled 8-chloro-1-[(dimethylamino)methyl]-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine was prepared from [^{3}H]bromomethane and 8-chloro-1-[(N-methylamino)methyl]-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine. The latter compound was efficiently obtained from 8-chloro-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine by hydroxymethylation, mesylation and mesylate displacement with aqueous methylamine. The titled product had a specific activity of 80 Ci/mmole.

Key Words: Synthesis, tritium labelled, s-triazolo[4,3-a][1,4]-benzodiazepines, antidepressant, anxiolytic

INTRODUCTION

Adinazolam (1, 8-chloro-1-[(dimethylamino)methyl]-6-phenyl-4H-s-triazolo-[4,3-a][1,4]benzodiazepine methanesulfonate) is currently undergoing clinical studies for treating depressive disorders in the CNS. This atypical antidepressant[1] is significant[1]y effective in treating depressive symptoms.[2] A sample of tritiated adinazolam (1) was required for biochemical receptor site and autoradiography binding studies for our research efforts in this program. These studies demanded that the radiolabelled product should be chemically stable and that the tritiated adinazolam should have a high final chemical and radiochemical purity. The minimum acceptable specific activity was at least 60 Ci/mmole.

Hester *et al*[3] reported the synthesis of 1 from demethyldiazepam. Using this method, Hsi and Johnson [4] reported the preparation of C-2 carbon-14 labelled 1 for

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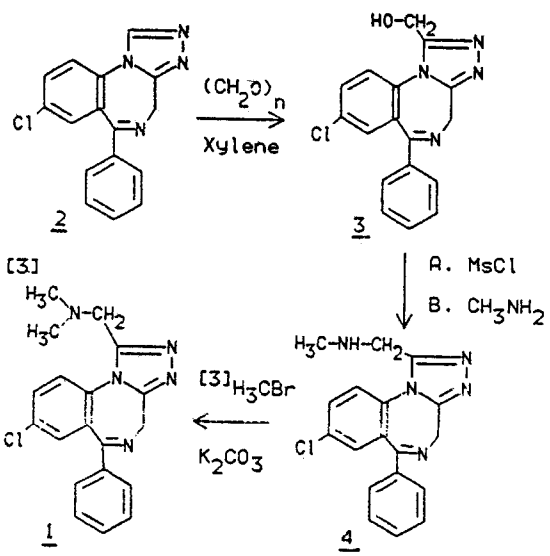
metabolism studies. We report the introduction of tritium in the final synthetic step of 1. The product has the high specific activity needed for biochemical studies.

RESULTS AND DISCUSSION

Gall *et al*[5] converted a variety of imidazo[1,5-a] -and s-triazolo[4,3a]-[1,4]benzodiazepines to their corresponding (dimethylamino) methylated products at the C-1 position. We believed that the C-1 position of the readily available estazolam 2 would be reactive with other electrophilic reagents which could easily be converted to 1.

When 2 in hot xylene was treated with portions of paraformaldehyde, the hydroxymethylated product 3 was obtained in excellent yield. Under these



conditions neither the ring cleavage of the 1,4-benzodiazepine ring nor the hydroxymethylation at the C-4 position occurred.[3,5]

The conversion of 3 to the penultimate amine 4 was accomplished in one flask. Slow addition of methanesulfonyl chloride to an ice-cold solution of 3 in dichloromethane containing an acid scavenger afforded the intermediate mesylate

which immediately was treated with an aqueous solution of methylamine. Compound 4 was the isolated product.

The alkylation conditions of 4 were developed with iodomethane rather than bromomethane. Iodomethane was added to a mixture of 4 and anhydrous potassium carbonate in acetonitrile at room temperature to afford 1. TLC indicated that the crude product was contaminated with a very polar component which disappeared after an aqueous extraction from dichloromethane. The alkylation of 2 with [^{3}H]-bromomethane using these conditions afforded the tritiated product 1. The chemical and radiochemical purity of 1 was 99% by TLC and reverse-phase HPLC. The specific activity of 1 was found to be 80 Ci/mmole.

EXPERIMENTAL

Melting points which were taken in capilliary tubes are uncorrected. The structures of all compounds were supported by IR, UV, MS and NMR. Spectra were compared with authentic spectra. The ^{1}H-NMR spectra were taken in $CDCl_3$ and are reported in ppm downfield relative to internal tetramethylsilane. IR spectra were determined in Nujol with a Digilab Model FTS 15E spectrophotometer and UV spectra were recorded on a Cary Model 15 spectrophotometer. Mass spectra were recorded using a CEC-21-110B high resolution mass spectrometer. Thin layer chromatography was done on 2.5 x 10 cm glass plates coated with a 250 μm thick layer of silica gel GF (Analtech). Solvents were purchased from Burdick and Jackson. Estazolam (2) was purchased from Takeda Chemical Ind. LTD., Japan.

8-Chloro 1-(hydroxymethyl)-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine (3). A mixture of 8-chloro-6-phenyl-4H-s-triazolo[4,3-a][1,4]benzodiazepine (2, 51.4 g, 0.17 mol) in 1.5 L of xylene was refluxed in a 3 L three-necked flask which was equipped with a mechanical stirrer and a Dean Stark trap fitted with a condenser. Water was collected. The colorless solution was cooled to 125°C and paraformaldehyde (50 g) was added over 10 min. The mixture gradually became a

colorless solution after refluxing for 15 min. A TLC developed with 10% methanol in chloroform indicated that ca. 50% of the starting material remained. After 30 min, paraformaldehyde (5 g) was added and the solution was heated for another 30 min. Additional paraformaldehyde (15 g) was added and the solution was heated for 2 h. About 500 mL of xylene was removed via the Dean Stark trap, the solution was cooled and crystallized in the reaction flask. The crystalline product was collected and washed with cold ethyl acetate to give 47.8 g (84%) of 3; mp 210-212°: (Lit.[6] mp 210-211°C); UV (MeOH) λ_{max} 222 (ε 36,650) inflections 244 (16,100), 265 (6050), 285 (3450); IR (Nujol) 3197 cm^{-1}; NMR ($CDCl_3$ + D_2O) δ 4.10, 5.45 (2H, d, d, J_{AB} = 14 Hz, CH_2OH) 4.60, 5.05 (2H, d, d, J_{AB} = 14 Hz, (C-4)H_2), 7.3-7.75 (7H, m,. aromatic), 8.10 (1H, d, J = 8Hz, ortho C-H); MS m/e 324 (M^+) 293, 289.

Anal. Calcd. for $C_{17}H_{13}ClN_4O$: C, 62.87; H, 4.03; N, 17.25; Cl, 10.92. Found: C, 62.50; H,4.03; N, 17.30; Cl, 11.08.

8-Chloro-1-[(N-methylamino)methy]-6-phenyl-4H-s-triazolo-[4,3-a][1,4]benzodiazepine (4). A solution of methanesulfonyl chloride (12.5 g, 0.11 mole) in dichloromethane (100 mL) was added dropwise to an ice-cold solution of 3 (30.9 g, 0.095 mol) and triethylamine (14 g) in dichloromethane (400 mL) under nitrogen. A solution of 40% aqueous monomethylamine (125 mL) was added rapidly, and the biphasic solution was stirred vigorously at 5°C for 15 min and at room temperature for 1 h. The two layers were separated and the aqueous layer was extracted twice with dichloromethane. The dichloromethane solutions were combined and washed once with water and then with 10% aqueous NaOH solution. They were dried over Na_2SO_4 and concentrated to give 36 g of crude foamy syrup 4 which crystallized from warm ethyl acetate-hexane to afford 15.8 g (49%) of beige needles; mp 164-166° (Lit.[3] mp 152-156°); UV (MeOH) λ_{max} 222 (ε 38,050) inflections 245 (15,600), 285 (3,250); IR (Nujol) 3295 cm^{-1}; NMR ($CDCl_3$) δ 1.69 (1H, broad, s, NH), 2.51 (3H, s, CH_3), 3.70, 4.20 (2H, d, d, J_{AB} = 12 Hz, CH_2N), 4.15, 5.55 (2H, d,d, J_{AB} = 12.8 Hz, (C-4), H_2) 7.3-7.75 (7H, m, aromatic), 8.30 (1H, d, J = 8.7 Hz, ortho C-H); MS m/e 337.339 (very small); 308, 310 (M - CH_3-NH).

Anal. Calcd. for $C_{18}H_{16}N_5Cl$: C, 64.00; H, 4.77; N, 20.73; Cl, 10.50. Found: C, 63.67; H, 4.91; N, 20.37; Cl, 10.54.

8-Chloro-1-[(dimethylamino)methyl-6-phenyl-4H-s-triazolo-[4,3-a][1,4] benzodiazepine (Adinazolam, 1). A mixture of 4, (167 mg 0.5 mmol), anhydrous potassium carbonate (220 mg) and 0.063 mL of iodomethane (1.0 mmol) in anhydrous acetonitrile (5 mL) was stirred at room temperature under nitrogen for 1.5 h. The mixture was filtered through Celite (filter aid) and concentrated to a residue. The residue was dissolved in ethyl acetate and water, and the layers separated. The ethyl acetate solution was washed with water, dried over Na_2SO_4, and concentrated to give 46 mg of a pale yellow foam. A TLC developed in 10% methanol in chloroform co-spotted with authentic adinazolam gave one homogenous spot (R_f = .6); NMR ($CDCl_3$) δ 2.35 (6H, s, CH_3), 3.6 (2H, s, CH_2N), 4.10, 5.20 (2H, d,d, J_{AB} = 13 Hz, (C-4) H_2), 7.35-7.70 (7H, m, aromatic), 8.40 (1H, d, J = 8 Hz, ortho C-H).

8-Chloro-1[(-^{3}H-dimethylamino)methyl]-6-phenyl-4H-s-triazolo-[4,3-a][1,4]benzodiazepine (1). A mixture of 4 (24 mg, 0.07 mmol), anhydrous potassium carbonate (29 mg, 0.2 mmol), and [^{3}H]-bromomethane (0.025 mmol, total activity 2000 mCi at 80.1 Ci/mmol) in dry acetonitrile (700 μL) was stirred at room temperature for 3 h. Radioactive labiles were removed under vacuum and the concentrated solution was passed through a short silica gel column. The product (R_f = .63) was separated from 4 (Rf = .34) by preparative thin layer chromatography using 250 μ silica gel plates developed with 10% methanol in chloroform. The product in absolute ethanol had a specific activity of 80.1 Ci/mmol as determined by UV absorption at 223 nm. Tritium NMR (δ) 2.51 (s). Radiochemical purity by TLC was 99% (R_f = 0.63), 10% methanol in chloroform. Radiochemical purity by HPLC was 99% (Zorbax ODS, 50% CH_3CN, 50% buffer (.01M NaH_2PO_4 solution adjusted to pH 3 with 17% H_3PO_4).

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