SYNTHESIS OF CARBON-14 LABELED 1,4-BENZODIAZEPINES. II.[1] DIAZEPAM-^{14}C*, DEMETHYLDIAZEPAM-^{14}C, AND KETAZOLAM-^{14}C**.

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Received on March 6, 1974.

SUMMARY

Diazepam-^{14}C and demethyldiazepam-^{14}C are synthesized from glycine-1-^{14}C. Addition of diketene to diazepam-^{14}C leads to ketazolam-^{14}C. The reaction conditions for the formation of ketazolam from diazepam is studied using high-pressure liquid chromatography in conjunction with radioactivity determinations.

Current interest in 1,4-benzodiazepines[2] and their biological effects on the central nervous system prompts us to report the synthesis of carbon-14 labeled ketazolam,[3] an antianxiety agent which is undergoing clinical investigation. The compound is labeled with carbon-14 to facilitate absorption, distribution, excretion and metabolism studies with ketazolam in test animals and man.

* Diazepam (Hoffmann-LaRoche, Inc.) is generic USAN name for 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepine-2-one.

** Ketazolam (The Upjohn Company) is generic USAN name for 11-chloro-8-12b-dihydro-2,8-dimethyl-12b-phenyl-4H[1,3]-oxazino[3,2-d][1,4]benzodiazepine-4,7(6H)-dione, also referred to as U-28,774 in the literature.

DISCUSSION AND RESULTS

The synthetic route for preparing ketazolam-^{14}C (IV) is shown in Scheme 1. Diazepam-^{14}C (IIIb) and demethyldiazepam-^{14}C (IIIa) were synthesized from glycine-1-^{14}C by modification of known procedures.[4,5] Treatment of glycine-1-^{14}C with N-carbethoxyphthalimide[6] gave phthaloylglycine-1-^{14}C (I). The acid chloride of I was used to acylate 2-amino-5-chlorobenzophenone and 5-chloro-2-methylaminobenzophenone[7] to give respectively 5-chloro-2-(2-phthalimidoacet-1-^{14}C-amido)benzophenone (IIa) and 5-chloro-2-(N-methyl-2-phthalimidoacet-1-^{14}C-amido)benzophenone (IIb). Removal of the phthaloyl group in IIb by treatment with hydrazine, followed by cyclization, led to diazepam-^{14}C (IIIb) with the label in the 2- position. We found that, contrary to the reported[4] requirement of excess hydrazine in this reaction, the best yield and a cleaner product were obtained by allowing only molar equivalent amounts of IIb and hydrazine to react in refluxing ethanol. That the reaction could be carried out also at room temperature was demonstrated by the production of demethyldiazepam-^{14}C (IIIa) from IIa, though only in low yield (36%). In refluxing ethanol the same reaction gave IIIa in 90% yield.

The addition of diketene to diazepam proceeded extremely slowly at room temperature. At 65°C the reaction occurred more readily but was accompanied by polymerization of diketene and on prolonged heating the product reverted partially to the starting material. No other diazepam related products were found in the reaction mixture. To determine the optimum reaction time, a rate study was attempted using the high pressure liquid chromatography (hplc) analysis procedure reported by Weber.[8] Diazepam-^{14}C (IIIb) was allowed to react with an excess of diketene at 65°C and aliquots of the reaction mixture were subjected to hplc to separate IIIb and ketazolam-^{14}C (IV) which were quantified by radioactivity. A linear relationship (Figure 1) was found

Scheme 1

Synthesis of Ketazolam-^{14}C from Glycine-1-^{14}C

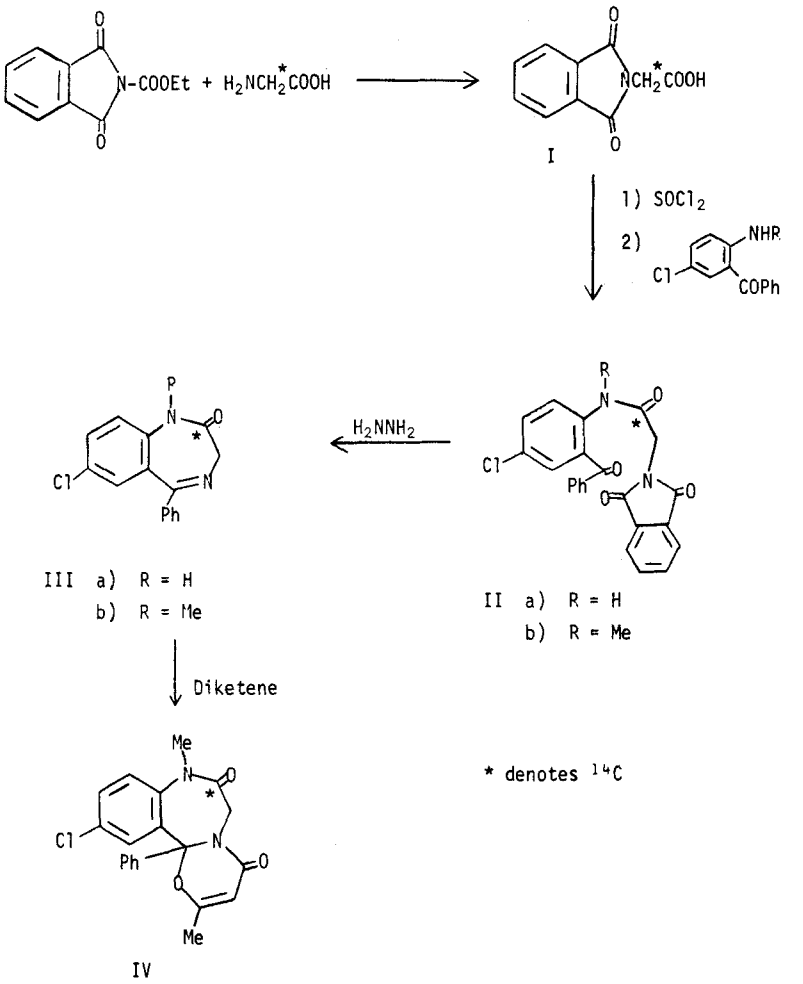
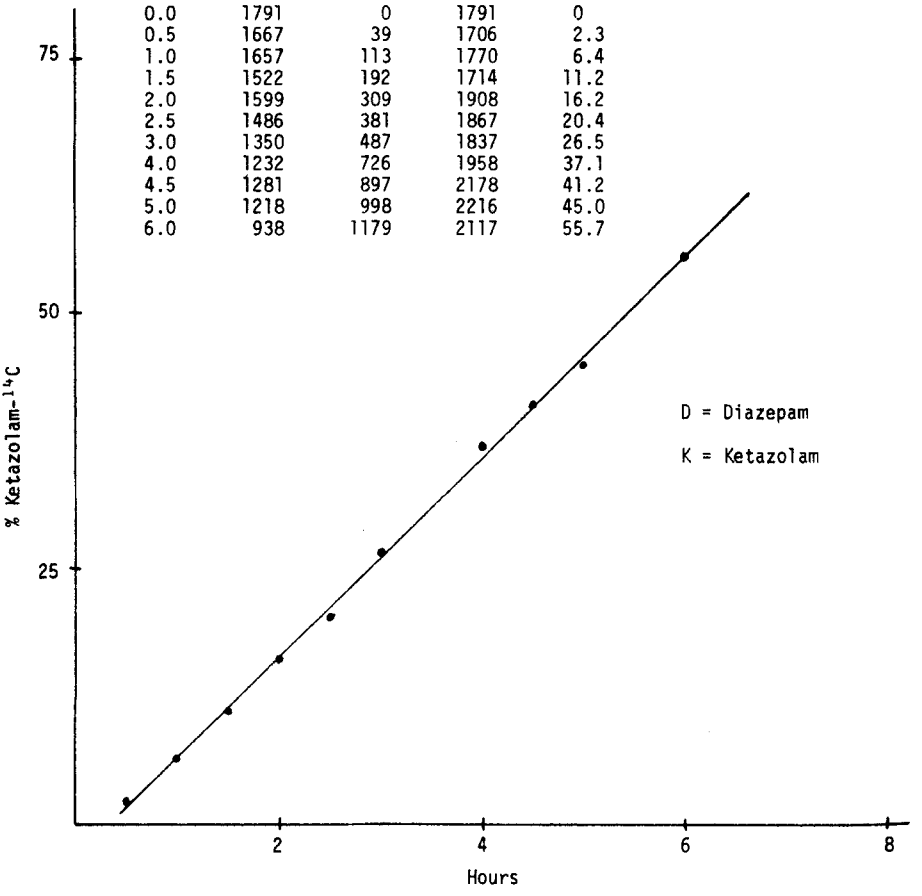


Figure 1: Formation of Ketazolam-^{14}C from Diazepam-^{14}C.

Hrs	$(CPM)_D$	$(CPM)_K$	$(CPM)_{Tot.}$	%K
0.0	1791	0	1791	0
0.5	1667	39	1706	2.3
1.0	1657	113	1770	6.4
1.5	1522	192	1714	11.2
2.0	1599	309	1908	16.2
2.5	1486	381	1867	20.4
3.0	1350	487	1837	26.5
4.0	1232	726	1958	37.1
4.5	1281	897	2178	41.2
5.0	1218	998	2216	45.0
6.0	938	1179	2117	55.7



between the formation of IIIb and reaction time, indicating that the reaction was pseudo zero order with respect to IIIb. The study was terminated at 7 hours because of precipitation of IV. Extrapolation of the data suggested that the addition should be near completion in 12 hours. This was confirmed in subsequent preparative experiments. When the reaction was allowed to proceed for 7 hours, IV was obtained in 65% yield. After 14 hours the yield was increased to 85-90%. A further increase in reaction time to 21 hours not only failed to improve the yield, but resulted in a less pure product presumably contaminated with decomposition products from diketene.

EXPERIMENTAL

Radioactivity determinations were carried out with a Packard Tri-Carb Model 3375 liquid scintillation spectrometer using diotol scintillation solvent[9] and the external standard method. Thin layer chromatography (tlc) plates were analyzed by counting eluates from sections of the plates in 10 ml portions of diotol containing 3% by weight of water. The tlc plates used were 2.5 x 10 cm glass plates coated with a 250 micron thick layer of silica gel GF (Analtech, Inc.). The plates were developed with either (A) 5% v/v MeOH in CH_2Cl_2 or (B) Et_2O. Visualization was by uv (254 nm) illumination. Uv spectra were obtained with a Cary Model 15 spectrometer. Hplc analyses and separations were carried out with a DuPont Model 820 liquid chromatograph. The fractioned eluates were brought to dryness and residues dissolved in 15 ml portions of diotol for counting. Microanalyses were obtained for the listed elements and, except as noted, the results were within ±0.4% of theory.

Phthaloylglycine-1-^{14}C (I)

A solution of 339 mg of glycine-1-^{14}C (nominally 20 mCi/mM)* and 412 mg of glycine (total 751 mg, 10.0 mmoles) in 15 ml 1M Na_2CO_3 was chilled in an

* Supplied by New England Nuclear Corp., Boston, Mass.

ice bath for 15 min. The bath was removed and to the solution was added 2.411 g (11.0 mmoles) of N-carbethoxyphthalimide. The mixture was stirred at room temperature for 30 min, filtered and solids washed with 10 ml of cold H_2O. The filtrate and washings were cooled and 5.6 ml of 6N HCl was added dropwise with stirring. The resulting precipitates were filtered, washed with cold H_2O and dried to give 1.560 g (76% yield) of I.

5-Chloro-2-(2-phthalimidoacet-1-^{14}C-amido)benzophenone (IIa)

Ten ml $SOCl_2$ was cautiously added to 1.550 g of I with evolution of gases. The mixture was gently refluxed with stirring for 1 hr and 10 ml of toluene was added. The solution was distilled with simultaneous addition of 25 ml of toluene until the boiling point of the distillate reached 104°C (*ca.* 42 ml of distillate collected). The remaining solution was diluted with toluene to 10 ml. Two ml of this solution, containing 1.5 mmoles of the acid chloride of I, was added to a mixture of 1 ml of toluene and 383 mg (1.65 mmoles) of 2-amino-5-chlorobenzophenone. The mixture was stirred at room temperature overnight. The resulting paste was diluted with 2.5 ml of a 1:1 v/v mixture of benzene and Skellysolve B. The solids were thoroughly washed with benzene followed by Skellysolve B and dried to give 498 mg of IIa. From the mother liquor and washings was recovered 55 mg of the product, total yield 553 mg, 87.9%.

5-Chloro-2-(N-methyl-2-phthalimidoacet-1-^{14}C-amido)benzophenone (IIb)

Similarly from 4.0 ml (3.0 mmoles) of the above toluene solution of the acid chloride of I and 811 mg (3.3 mmoles) of 5-chloro-2-methylaminobenzophenone,[7] there was obtained 1.11 g (85.3% yield) of IIb.

Diazepam-^{14}C (IIIb)

A mixture of 1.082 g (2.5 mmoles) of IIb and 12.5 ml (2.6 mmoles) of 0.207M hydrazine hydrate in absolute EtOH was refluxed with stirring under N_2 for 21 hrs. The mixture was cooled and filtered and the solids were washed with absolute EtOH. The combined filtrate and washings were concentrated and

the residue was chromatographed on a 3 x 60 cm column of 190 g of silica gel eluted with 5% v/v MeOH in CH_2Cl_2. The crude product was recrystallized from a 1:1 v/v mixture of acetone and water to give 569 mg (79.2% yield) of IIIb, sp. act. 33.00 μCi/mg or 9.40 mCi/mM, radiochemically pure by tlc (silica gel, 5% v/v MeOH in CH_2Cl_2); *anal* ($C_{16}H_{13}ClN_2O$): C, H, Cl, N.

Demethyldiazepam-^{14}C (IIIa)

A mixture of 503 mg (1.20 mmoles) of IIa and 15 ml (3.0 mmoles) of 0.207M hydrazine hydrate in absolute EtOH was stirred at room temperature for 23 hrs. The resulting mixture was diluted with absolute EtOH, filtered and the solids washed with absolute EtOH. The combined filtrate and washings were concentrated and the residue was triturated with 5% v/v MeOH in CH_2Cl_2. Traces of insoluble solids were removed by filtration and the filtrate was concentrated. The resulting residue was chromatographed on a column (3 x 32 cm) of 100 g of silica gel eluted with 5% v/v MeOH in CH_2Cl_2. The crude product was recrystallized from a 1:1 v/v mixture of CH_2Cl_2 and Skellysolve B to give 104 mg of IIIa, sp. act. 34.54 μCi/mg or 9.36 mCi/mM, (a second crop of 20 mg was obtained from the mother liquor, total yield 124 mg, 36.2%), radiochemically pure by tlc (silica gel, 5% v/v MeOH in CH_2Cl_2, *anal* ($C_{15}H_{11}ClN_2O$): C, H, Cl (+0.75%), N.

Hplc Monitoring of Conversion of IIIb to Ketazolam-^{14}C (IV)

A mixture of 15 mg of IIIb (sp. act. 9.40 mCi/mM), 135 mg of diazepam (total 0.526 mmole) and 458 mg (5.45 mmoles) of diketene* was stirred at 65°C. The reaction mixture became a clear solution within one minute after heating began. Aliquots of 2 μl each were taken from the reaction mixture at 30 min

* Twice redistilled, second distillation under vacuum at room temperature; stored in freezer in crystalline form.

intervals and dissolved in 5 ml portions of a mixture of 15% v/v tetrahydrofuran in diisopropyl ether. Precipitates began to appear in the reaction mixture at between 6 and 7 hrs and homogenous samples could no longer be obtained. Aliquots of 3 µl each of the above solutions were subjected to hplc separation of diazepam and ketazolam. The eluate for each sample was collected from 2.0 to 5.5 min for diazepam and from 5.5 to 10.5 min for ketazolam in separate 20 ml counting vials. The eluates were evaporated to dryness and the residues were dissolved in 15 ml portions of diotol and counted. The liquid chromatograph was equipped with a 100 cm column of Corasil II (Waters Associates) eluted with 15% v/v tetrahydrofuran in diisopropyl ether at room temperature at a flow rate of 1 ml/min under 200 psi. Under these conditions the retention times for diazepam, ketazolam and diketene were respectively 3.5, 7.5 and 15-25 min as determined by uv (254 nm) detection. The data and results are shown in Figure 1. The reaction was terminated after 8 hrs and the mixture was diluted with Et_2O and filtered to give 134 mg (74% yield) ketazolam-^{14}C, 99.1% radiochemically pure by tlc (silica gel, Et_2O), sp. act. 0.945 mCi/mM after column chromatography according to procedure described below.

Ketazolam-^{14}C (IV)

A mixture of 285 mg (1.0 mmole) of IIIb, sp. act. 9.40 mCi/mM, and 873 mg (10.4 mmoles) of diketene was stirred at 65°C for 13 hrs and then at room temperature overnight for 11 hrs. The mixture containing precipitates was diluted with 4 ml of Et_2O and the crystals were filtered, washed with Et_2O and dried to give 315 mg (85.3% yield) of crude IV. Although this material was 99% pure radiochemically by tlc, its specific activity (6.84 mCi/mM) and uv spectrum [$\lambda_{sh}^{CH_3CN}$ 240 nm, (ε13,000)] indicated it was only 73-75% pure chemically. The crude was rapidly passed under pressure (N_2) through a short column (1.6 x 24 cm) of 20 g of silica gel eluted with acetone. The residue

from the eluate (60 ml) was passed under pressure through a second column (1.6 x 12 cm, 10 g) of silica gel eluted with 30 ml of acetone. The transit time on each column was less than 5 min. The residue for the second eluate was recrystallized from a 2:1 v/v mixture of water and acetone to give 207 mg of IV, 58.1% yield, sp. act. 25.14 μCi/mg or 9.28 mCi/mM, $\lambda_{sh}^{CH_3CN}$ 240 nm (ε17,750). The product was 99.4% radiochemically pure by tlc (silica gel, Et_2O, 5 min) and 99.44% by hplc. Its diazepam-^{14}C content was 0.33% by tlc and 0.40% by hplc, *anal* ($C_{20}H_{17}ClN_2O_3$): C, H, Cl, N.

ACKNOWLEDGEMENT

The author is indebted to Dr. D. J. Weber for his help in carrying out the high pressure liquid chromatography analyses. Microanalyses by Messrs. R. C. Anderson and P. A. Spinelli and ultraviolet spectra by Mrs. B. F. Zimmer are gratefully acknowledged.

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