THE SYNTHESIS OF 7-CHLORO-1-METHYL-5-PHENYL-1,3-DIHYDRO-2H-1,4-BENZODIAZEPIN-2-ONE-5-^{14}C AND 7-NITRO-1-METHYL-5-(2'-FLUOROPHENYL)-1,3-DIHYDRO-2H-1,4-BENZODIAZEPIN-2-ONE-5-^{14}C

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SUMMARY

Two benzodiazepines, Diazepam and Flunitrazepam, labeled with carbon-14 in position 5 of the diazepine ring have been synthesized. Use of a condensation between iminochlorides and ^{14}C-benzonitriles, followed by exhaustive methylation of the resulting quinazolines and hydrolyses is described. This sequence of reactions allows the preparation of the intermediate o-methylaminobenzophenones in good yields.

Key Words: Diazepam-5-^{14}C, Flunitrazepam-5-^{14}C, Benzodiazepines-5-^{14}C

INTRODUCTION AND DISCUSSION

1,4-Benzodiazepines exhibit interesting biological effects on the central nervous system and have found use as psychotropic agents. Their pharmacology and biotransformations have been studied in depth with the aid of labeled compounds. This paper describes the synthesis of two additional carbon-14 labeled 1,4-benzodiazepines, Diazepam a widely used tranquilizer and Flunitrazepam a hypnotic agent.

The most convenient way to label 1,4-benzodiazepines consists of the introduction of a carbon-14 into position 2 of the seven-membered diazepine ring. This is accomplished by reaction of an appropriately substituted o-aminobenzo-

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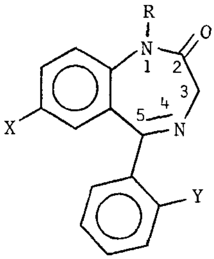
phenone with a carboxyl labeled glycine derivative(1,2) or α-haloacetic acid(3) followed by ring closure. However, incorporation of the isotope into carbon 5 of the diazepine ring would be preferred, since it appears to be the most inert portion of the molecule, both chemically and metabolically.



Syntheses of some 1,4-benzodiazepin-2-ones-5-^{14}C have appeared in the literature, i.e., Prazepam (R = cyclopropylmethyl, X = Cl, Y = H)(4), Nimetazepam (R = methyl, X = NO_2, Y = H)(5), and Fludiazepam (R = methyl, X = Cl, Y = F)(6) The key intermediates in most of these syntheses(4,5) were o-aminobenzophenone (carbonyl-^{14}C) derivatives. These intermediates are usually prepared by a condensation between a suitably p-substituted aniline and a benzoyl chloride in the presence of zinc chloride(7). This method however is not well suited for a labeled preparation. The labeled benzoyl chloride has to be used in 2.2 to 1 molar ratio to the aniline and furthermore only half (or a 0.5 molar ratio) of the tagged material gets incorporated into the o-aminobenzophenone. However, reisolation and recycling of the benzoic acid improves the yield somewhat and makes the sequence a little more attractive. Flurazepam-5-^{14}C (R = 2-diethylaminoethyl, X = Cl, Y = F), Flunitrazepam-5-^{14}C (18) (R = CH_3, X = NO_2, Y = F) have been prepared this way with overall radiochemical yields of 4.4% and 6.7% respectively(3), as was Diazepam (R = CH_3, X = Cl, Y = H) tritiated in the 5-phenyl ring.(8)

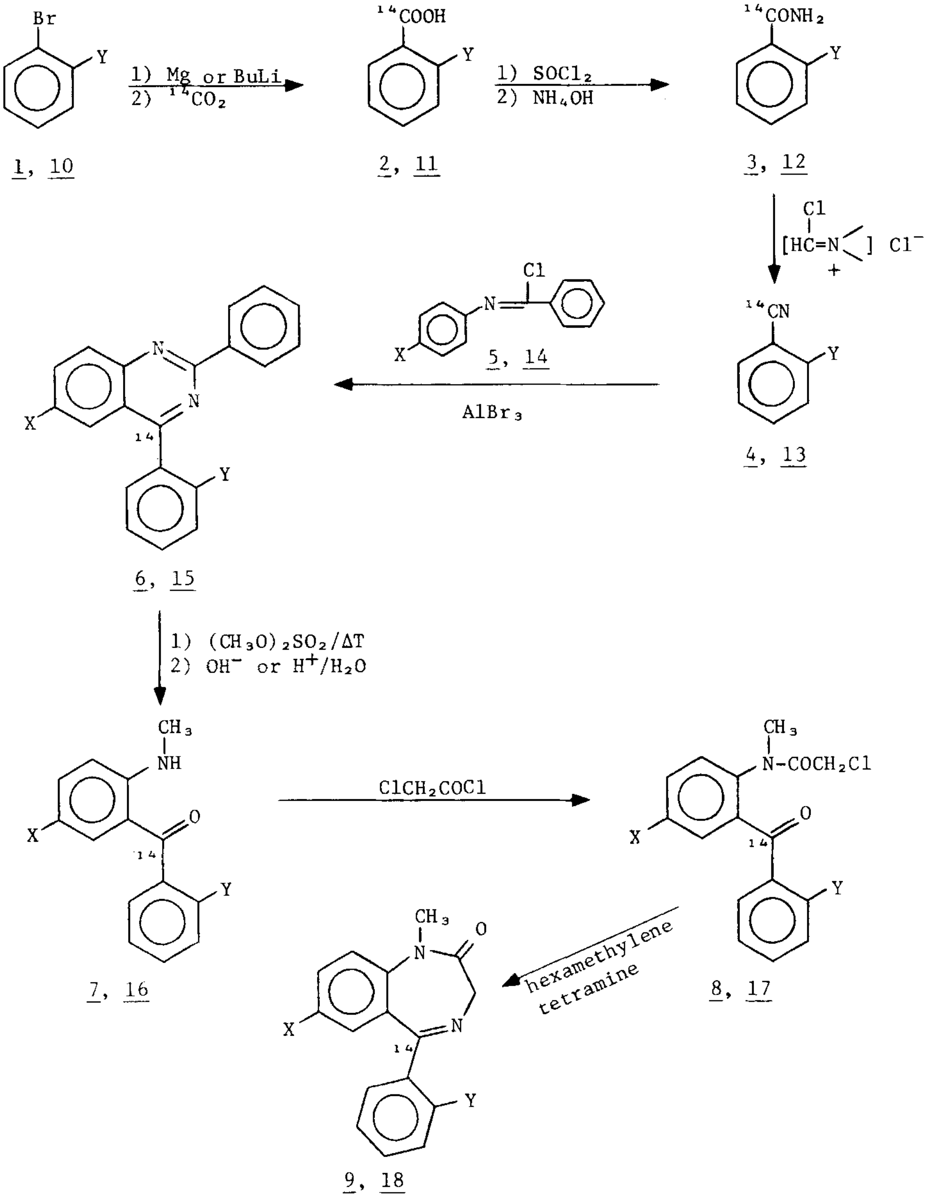
The following new method was therefore developed in order to improve the overall radiochemical yield for labeling of benzodiazepines with carbon-14 in position 5.

The synthesis of Diazepam-5-^{14}C (9) as shown in the figure below uses benzonitrile-cyano-^{14}C (4) for the introduction of the label. According to H. Meerwein et al.$^{(9)}$ quinazolines can be prepared easily and in good yield by the condensation of iminochlorides with compounds containing a nitrile function in the presence of equivalent amounts of a reagent such as aluminum chloride or other electrophilic metal halogenides. Unfortunately, these quinazolines do not easily hydrolyze to the corresponding 2-aminobenzophenones. By exhaustive methylation of 6-chloro-2,4-diphenylquinazoline (6) with dimethyl sulfate followed by hydrolysis, the quinazoline 6 was successfully degraded to 5-chloro-2-methylaminobenzophenone (7) in good yield. The two steps indeed proved to be adaptable to a small scale radioactive preparation. The same synthetic scheme was successfully adapted to a preparation of Flunitrazepam-5-^{14}C (18).

Benzoic-7-^{14}C acid (1) was prepared according to the standard procedure$^{(10)}$ and the preparation of 2-fluorobenzoic-7-^{14}C acid (11) was effected by carbonation of the lithio derivative of o-bromofluorobenzene, prepared according to H. Gilman et al.$^{(11)}$ The nitriles 4 and 13 were prepared by dehydration of amides 3 and 12 with dimethylchloroformiminium chloride$^{(12)}$ in benzene at room temperature. Finally, the diazepines were obtained from the o-aminobenzophenones 7 and 16 via the chloroacetamide 8 and 17 by ring closure with hexamethylenetetramine.$^{(13)}$

EXPERIMENTAL

Melting points are uncorrected. Radioactivity was measured by the liquid scintillation technique using a Packard Tricarb Model 2010 spectrometer. Radiochemical purity was determined on thin layer chromatograms with a Packard Model 7021 Radiochromatogram Scanner System. TLC analyses were conducted on 20 cm TLC plates of Merck Silica Gel GF 254. Unlabeled reference compounds and intermediates were obtained internally or synthesized and structures were confirmed by spectroscopic methods.



1 to 9, X = Cl; Y = H

10 to 18, X = NO_2; Y = F

Benzoic-carboxy-^{14}C acid (2)

Benzoic-carboxy-^{14}C acid was prepared according to standard procedures$^{(10)}$ from 103 mCi (1.7 mM) of barium carbonate-^{14}C in 96% yield.

o-Fluorobenzoic-carboxy-^{14}C acid (11)

To a degassed solution of 700 mg (4 mmole) of o-bromofluorobenzene in 15 ml of ether in a vacuum stirring flask frozen in liquid nitrogen under an inert atmosphere of nitrogen 2 mmole of n-butyllithium in 1.45 ml of hexane was added. The liquid nitrogen was replaced by a dry ice-acetone bath (-80°C). The mixture was stirred for 5 min counting from the time the mixture become liquid and stirable. The the solution was frozen in liquid nitrogen again and the flask evacuated to one micron. One mmole of carbon-^{14}C dioxide (56 mCi) was then vacuum transferred into the flask and allowed to react at -80°C for 20 min. The apparatus was filled with nitrogen and the reaction mixture quenched by addition of 10 ml of water. After warming to room temperature the phases were separated, the ether extracted twice with 10 ml of 1N sodium hydroxide and once with water. The aqueous phases were combined, made acidic by addition of conc. hydrochloric acid and extracted three times with chloroform. The organic extracts were washed once with water, dried and evaporated yielding 130 mg of colorless crystalline product (92.7% yield). The acid was found to be radiochemically pure by TLC-analyses (SiO_2; chloroform-ethylacetate-methanol-conc. ammonia 30-30-30-1).

Benzamide-carbonyl-^{14}C (3)

The benzoic acid (204 mg; 1.65 mmole) on treatment with thionyl chloride in the usual manner$^{(14)}$, gave the acid chloride. Under cooling in an ice-bath, 2 ml of conc. ammonia was added to the 231 mg of benzoyl-carbonyl-^{14}C chloride, followed by 2 ml of dioxane. The mixture was allowed to stir at room temperature for one hr, diluted with water, and the product extracted three times with chloroform. The dried organic extracts yielded 152 mg (1.24 mmole) of pure benzamide-carbonyl-^{14}C, mp. 132-33°C.

o-Fluorobenz-carbonyl-^{14}C amide (12)

o-Fluorobenzoic-carboxy-^{14}C acid (439 mg; 69.5 mCi) was refluxed for 35 hr in 7 ml of dry benzene together with one ml of thionyl chloride. The solution was allowed to cool and then added slowly at room temperature, and under stirring, to 15 ml of conc. aqueous ammonia. After adding an additional 3 ml of dry benzene the mixture was stirred at room temperature for 3 hr, then diluted with 10 ml of water and allowed to stand overnight. More water was added and the product isolated by extracting four times with chloroform. Yield: 418 mg (96%) of pure product, mp. 116-117°C.

Benzonitrile-cyano-^{14}C (4)

To 146 mg (2 mmole) of N,N-dimethylformamide in 5 ml of dry benzene, 230 mg (1.8 mmole) of oxalyl chloride was added. After stirring for 30 min at room temperature, 152 mg of benzamide-carbonyl-^{14}C dissolved in 10 ml of warm benzene was added. The mixture was stirred at room temperature for one hr and then extracted three times with 15 ml of distilled water. The aqueous phases were re-extracted with benzene, all organic layers combined, dried and evaporated to yield 117 mg of benzonitrile-cyano-^{14}C.

o-Fluorobenzo-nitrile-cyano-^{14}C (13)

To 365 mg (5 mmole) of N,N-dimethylformamide in 10 ml of dry benzene 507 mg (4 mmole) of oxalyl chloride was added and the resulting mixture stirred for 30 min. To this was slowly added 418 mg (3 mmole) of 2-fluorobenz-carbonyl-^{14}C amide dissolved in 5 ml of warm benzene. The mixture was stirred at room temperature for 90 min and then extracted with water until neutral. The aqueous washes were re-extracted once with benzene. The organic phases were combined, dried and evaporated to dryness yielding 360 mg (98.7%) of product.

2,4-Diphenyl-6-chloroquinazoline-4-^{14}C (6)

To 117 mg (1.12 mmole) of benzonitrile-cyano-^{14}C dissolved in 4 ml of o-dichlorobenzene, 300 mg of aluminum bromide was added with strring followed by

285 mg (1.14 mmole) of N-(4-chlorophenyl)benzimino chloride dissolved in one ml of o-dichlorobenzene. The resulting mixture was stirred at 140-150°C for one hr. After cooling to room temperature, water was added and the mixture neutralized by the addition of sodium bicarbonate and then extracted three times with chloroform. All organic extracts were first washed once with water, then combined and after drying, evaporated. After removing the o-dichlorobenzene in vacuo, the residue, dissolved in a small amount of chloroform, was applied to a column of 20 g of silica gel. The column was developed with chloroform, taking small fractions. Those containing the quinazoline (as checked by TLC) were combined and evaporated to dryness. Yield: 245 mg (69%)

6-Nitro-2-phenyl-4-(2-fluorophenyl)quinazoline-4-^{14}C (15)

To 360 mg (2.97 mmole) of 2-fluorobenzonitrile-cyano-^{14}C and 427 mg (3.2 mmole) of aluminum chloride in 6 ml of o-dichlorobenzene a solution of 3.5 mmole of N-(p-nitrophenyl)benziminochloride in 3.5 ml of o-dichlorobenzene was added. The mixture was then stirred at 120°C for 20 hr. After removing the solvent in vacuo 20 ml of 2N sodium hydroxide was added to the residue and the mixture warmed quickly to 90°C and cooled again. The product was isolated by extraction with chloroform and the 1.29 g of crude extract purified by chromatography on 100 g of silica-gel using chloroform as solvent. Appropriate fractions were combined yielding 637 mg of the wanted quinazoline.

A chromatographic fraction of 297 mg of oil containing unreacted 2-fluorobenzonitrile-cyano-^{14}C was treated again with 2 mmole of N-(4-nitrophenyl)benziminochloride and 2 mmole of aluminum chloride to give an additional 80 mg of product for a total yield of 717 mg (70%).

5-Chloro-2-methylaminobenzophenone-carbonyl-^{14}C (7)

The 245 mg (0.77 mmole) of 2,4-diphenyl-6-chloroquinazoline-4-^{14}C was stirred in 5 ml of dimethyl sulfate at 130°C overnight for a total of 17 hr. After removing the excess dimethyl sulfate in vacuo, the residue was stirred and refluxed with 10 ml of 2N sodium hydroxide and 10 ml of ethanol for 20 hr. The mixture was then cooled, diluted with water and extracted three times with chloroform. All organic extracts were washed once with water, then combined and after drying, evaporated. The residue, dissolved in a small amount of chloroform, was applied to a column of 20 g of silica gel and the product eluted with chloroform to yield 157 mg (82%) of pure product.

2'-Fluoro-2-methylamino-5-nitrobenzophenone-carbonyl-^{14}C (16)

The 717 mg (2.08 mmole) of 6-nitro-2-phenyl-4-(2-fluorophenyl)quinazoline-4-^{14}C was stirred in 10 ml of dimethyl sulfate at 130°C for 20 hr. After removing the excess dimethyl sulfate in vacuo, the residue was stirred with 10 ml of water and 5 ml of conc. ammonia at 90°C for 5 min. After cooling the liquid was decanted from the solid. The solid was then stirred at 130°C with a mixture of 10 ml of conc. sulfuric acid and 20 ml of water for 30 min. At this time the remaining solid was filtered.* The solution was stirred at 130°C for 20 hr and the solid was kept at 130°C for seven hr with 30 ml 50% (V/V) sulfuric acid. Both mixtures were diluted with water and extracted with chloroform. The organic extracts were washed once with 1N sodium hydroxide, dried and evaporated to dryness yielding a total of 448 mg of solid material. Chromatography on 100 g of silica-gel with chloroform as solvent gave 308 mg (54%) of pure product.

From later chromatographic fractions containing more polar material 40 mg of 2'-fluoro-2-amino-5-nitrobenzophenone-carbonyl-^{14}C could be isolated by preparative TLC.

2-Chloro-N-methyl-2'-benzoyl-carbonyl-^{14}C-4'-chloro-acetanilide (8)

The 157 mg (0.63 mmole) of aminobenzophenone 7 was refluxed in 5 ml of benzene for 15 min together with 147 mg (1.3 mmole) of chloroacetyl chloride.

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[*] During cold runs it was observed that this solid contained, besides some of the wanted aminobenzophenone, a second compound which on treatment with more concentrated sulfuric acid hydrolyzed to 2-amino-2'-fluoro-5-nitrobenzophenone. It was isolated by preparative TLC and identified as the N-benzoyl derivative of 2-amino-2'-fluoro-5-nitrobenzophenone.

After cooling to room temperature, the solution was washed with dilute sodium bicarbonate and then water until neutral. Upon evaporation to dryness in vacuo, 199 mg (97%) of crystalline product was obtained.

2-Chloro-N-methyl-2'-(2-fluorobenzoyl-carbonyl-^{14}C)-4'-nitro-acetanilide (17)

A mixture of 308 mg (1.1 mmole) of 2'-fluoro-2-methylamino-5-nitrobenzophenone-carbonyl-^{14}C and 0.5 ml (6.3 mmole) of chloroacetyl chloride in 10 ml of benzene was refluxed for six hr. The residue obtained after thorough evaporation of the solvent and excess reagent was found to be pure enough to be used in the next step without further purification.

7-Chloro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one-5-^{14}C (9)

A mixture of the 199 mg (0.62 mmole) of 8, 290 mg (2.07 mmole) of hexamethylenetetramine, 0.46 ml of 2N hydrochloric acid, 2 ml of methanol and 0.19 ml of distilled water was stirred and lightly refluxed for 16 hr. After dilution with water, the product was extracted with chloroform. The crude product obtained after drying and evaporation of the condensed organic phases was dissolved in a small amount of chloroform and applied to a column of 20 g of silica gel. For the development of the column, a mixture of chloroform-ethyl acetate was used. The small fractions taken were analyzed by TLC and those containing only Diazepam were combined. evaporated to dryness and the residue recrystallized from ethyl acetate-petroleum ether, yielding 115 mg of pure product (23.4% overall yield), mp. 125-26°C (lit. 125-126°C$^{(15)}$). The mother liquor yielded an additional 40 mg (8.1%) product of better than 98% radiochemical purity. The specific activity was 197 μCi/mg and the overall radiochemical yield was 31.5% calculated from barium carbonate-^{14}C.

1-Methyl-7-nitro-5-(2'-fluorophenyl)-1,3-dihydro-2H-1,4 benzodiazepin-2-one-5-^{14}C (18)

The residue of 17 and 505 mg of hexamethylenetetramine were refluxed for 27 hr in a mixture of 3.7 ml of methanol, 0.35 ml of water and 0.85 ml of 2N hydrochloric acid. After dilution with water the product was extracted with chloroform. The 399 mg of crude material obtained after drying and evaporation of the combined organic phases was dissolved in a small amount of chloroform and applied onto a column of 40 g of silica-gel. The column was developed first with 10x 100 ml of chloroform, followed by 2x 100 ml each of chloroform containing first 2% ether and then 5%. Appropriate fractions containing the wanted product as analyzed by TLC were combined and evaporated to dryness. The 342 mg of material so obtained was recrystallized from methylene chloride-ether yielding 252 mg of pure product, mp. 170.5-171.5°C (lit. 170-72°C(16)). The specific activity was found to be 69.4 μCi/mg and the overall radiochemical yield 25%.

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