Note

Synthesis of [*N*-methyl-³H]-7-Chloro-1,3-dihydro-5-(2-fluorophenyl)-1-methyl-2 *H*-1,4-benzodiazepin-2-one (³H-Fludiazepam) by Phase Transfer Catalysis†

Futoshi UOZUMI, Minoru MAEDA and Masaharu KOJIMA

Faculty of Pharmaceutical Sciences, Kyushu University
1-1, Maidashi 3-chome, Higashi-ku, Fukuoka 812, Japan

Received September 10, 1983

[*N*-methyl-³H]-7-Chloro-1,3-dihydro-5-(2-fluorophenyl)-1-methyl-2*H*-1,4-benzodiazepin-2-one (³H)fludiazepam) was prepared by phase transfer catalytic *N*-methylation of the corresponding nor-derivative with [³H]methyl iodide [407 GBq/mmol (11 Ci/mmol)] in a radiochemical yield of 34%. The specific activity was 271.6 GBq/mmol (7.34 Ci/mmol).

Key Words: tritium methyl iodide, *N*-methylation, nor-fludiazepam, fludiazepam, phase transfer catalysis

1. Introduction

Fludiazepam, 7-chloro-1,3-dihydro-5-(2-fluorophenyl)-1-methyl-2*H*-1,4-benzodiazepin-2-one, is one of the most potent anti-anxiety agents^{1,2)} and has a high binding affinity for brain benzodiazepine receptors³⁾. Fludiazepam labelled with ¹⁴C at the C-5 position⁴⁾ or with ¹¹C in the *N*-1 methyl group⁵⁾ has been prepared for its metabolic or *in vivo* receptor studies. For brain autoradiographic studies of fludiazepam in animals, the tritium labelled compound with specific activity in the range of 37~370 GBq/mmol (1~10 Ci/mmol) was required. Therefore, we planned to prepare [³H]fludiazepam *via N*-methylation of the nor-derivative with commercial available [³H]methyl iodide. This paper describes the preparation of [*N*-methyl-³H]fludiazepam by phase transfer catalysis, including the comparative evaluation of several methods for the non-radioactive methylation at a micro level.

2. Results and Discussion

Initial experiment with [³H]methyl iodide [185 MBq (5 mCi), 481 GBq/mmol (13 Ci/mmol)] was performed by adding a solution of 7-chloro-1,3-dihydro-5-(2-fluorophenyl)-2*H*-1,4-benzodiazepin-2-one (nor-fludiazepam) (111 μg) in 650 μl of dry acetone containing 2 μl of 5*N* sodium hydroxide and heating at 90°C for 25 min in a sealed tube, in analogous reaction conditions to those already reported for the ¹¹C analogue⁵⁾. However, the yield of [³H]fludiazepam was only less than 1%. Radioactivity other than unreacted [³H]methyl iodide and the [³H]fludiazepam was detected in the first eluted fraction together with acetone used as the solvent by high pressure liquid chromatographic (HPLC) analysis.

This unsuccessful result led us to investigate alternative approaches using non-radioactive methyl iodide in order to obtain sufficient amounts of [³H]fludiazepam. Only a few methods for *N*-1 methylation of 2*H*-1,4-benzodiazepin-2-one ring system have appeared in the literature, although there are several techniques available for *N*-methylation. In order to prevent the reduction of specific activity, the use of small masses of methyl iodide and the minimum volume of solvent added for labelling were required.

† 相間移動触媒を用いた [*N*-メチル-³H]-7-クロロ-1,3-ジヒドロ-5-(2-フルオロフェニル)-1-メチル-2*H*-1,4-ベンゾジアゼピン-2-オン (³H-フルジアゼパム) の合成。魚住太志, 前田 稔, 小嶋正治: 九州大学薬学部放射性薬品化学教室, 812福岡市東区馬出 3-1-1。

Furthermore, a method of preventing hydrolysis of the amide and the azomethine groups of the ring system in basic media was also necessary for achieving a satisfactory radiochemical yield. It was difficult, however, with larger excess of nor-fludiazepam to devise satisfactory system to separate the desired compound from the reaction mixture. Accordingly, small masses (μg amounts) of nor-fludiazepam must be used.

The equimolar reaction of nor-fludiazepam and methyl iodide on a chemical scale (mg amounts) in the presence of *n*-butyllithium or sodium hydroxide in *N,N*-dimethylformamide (DMF) or tetrahydrofuran gave insignificant yields of fludiazepam in all cases. Schwob, et al. prepared [*N*-methyl- ^3H]-7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one by *N*-methylation of its nor-derivative in DMF in the presence of thallos ethoxide⁶. We examined the response of nor-fludiazepam toward methyl iodide in DMF containing thallos ethoxide. When this reaction was carried out at a milligram level (using equimolar ratio of nor-fludiazepam and methyl iodide) for 1 h at room temperature, the desired fludiazepam was obtained in 85% yield. However, the methylation on a microgram scale which approximated the quantities of nor-fludiazepam to be used in ^3H -labelling experiment proceeded with remarkable difficulty, giving less than 20% yield of fludiazepam. This means that deprotonation of the amide-NH group by thallos ethoxide is not effective when the amounts of substrates are used at a micro level. Thus, using thallos ethoxide-DMF to prepare [^3H]fludiazepam was considered inadequate.

Phase transfer technique has been employed for extractive methylation of 1,3-dihydro-7-nitro-5-phenyl-2*H*-1,4-benzodiazepin-2-one from biological samples by Ehrsson, et al.⁷ A more preferable approach to [^3H]fludiazepam appeared to be the use of phase transfer catalyst⁸, although relatively little attention has been paid to the application of phase transfer catalyst in radio-synthetic works. Thus, milligrams amounts of nor-fludiazepam was methylated in benzene containing aqueous sodium hydroxide with tetra-*n*-butylammonium hydrogen sulphate (TBA- HSO_4). When this reaction was carried out for 24 h at room temperature, the desired fludiazepam was obtained in 76% yield. Furthermore, this method allowed the methylation of micro quantities of nor-fludiazepam and methyl iodide. Numerous trial experiments using the phase transfer technique led to the most favorable reaction conditions for performing ^3H -labelling experiment. It consisted of ① only molar equivalent amounts of nor-fludiazepam and methyl iodide, ② 10 μl of 100 mM TBA- HSO_4 in 4-5*N* sodium hydroxide, ③ 48 h at room temperature, and ④ total volume of 1000 μl benzene as solvent. Thus, we adapted the above reaction conditions to prepare [^3H]fludiazepam. Using 370 MBq (10 mCi) of [^3H]methyl iodide [407 GBq/mmol (11 Ci/mmol)] we synthesized [^3H]fludiazepam having a specific activity of 271.6 GBq/mmol (7.34 Ci/mmol). The radiochemical yield was 34%. After chromatographic separation, the labelled compound was chemically and radiochemically pure, and its identity was performed by HPLC and ultraviolet (UV) spectroscopic analyses compared with a standard sample.

Fig. 1 Synthesis of [^3H]fludiazepam.

This phase transfer technique appears to be of general application to radiosynthetic works, although in the present study a little drop of specific activity from 407 GBq/mmol (11 Ci/mmol) to 271.6 GBq/mmol (7.34 Ci/mmol) was noted (Fig. 1).

3. Experimental

Fludiazepam and nor-fludiazepam were supplied by Sumitomo Chemical Co. Ltd., Japan. [^3H]Methyl iodide was purchased from Amer-sham International plc, England, and supplied in a borosilicate glass break-seal ampoule containing a small piece of copper. Radioactivity was determined in an Aloka LSC-703 liquid scintillation spectrometer. Counting efficiency was determined by the external standard-channels ratio techniques. Analytical thin layer chromatography (TLC) was carried out on silica gel 60F₂₅₄ (0.2 mm, Merck). The UV spectra were recorded using a Hitachi 220A spectrophotometer. High pressure liquid chromatography (HPLC) (Waters) was carried out using a reverse phase column (25 cm Partisil M 9 10/25 ODS-2, Whatman) and/or a normal phase column (50 cm Partisil M 9 10/50, Whatman) equipped with a 254 nm UV detector and coupled to a LB 503 flow glass scintillation counter (Berthold Laboratories). Radiochemical purity was determined by scanning of the TLC plate using an Aloka 101 radiochromatogram scanner.

3.1 *N*-Methylation using thallos ethoxide

(1) To a solution of nor-fludiazepam (24.0 mg, 79 μmol) in dry DMF (500 μl) containing a small piece of copper was added thallos ethoxide (5 μl , 80 μmol). After the mixture was stirred for 12 min at room temperature, methyl iodide (5 μl , 80 μmol) was added to this mixture. The mixture was left standing for 1 h at room temperature with stirring in a sealed glass tube and then evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel (silica gel 60, 70~230 mesh, Merck) [benzene-chloroform (1:1 v/v)] and gave 21.5 mg (85%) of fludiazepam.

(2) A solution of nor-fludiazepam (24.0 mg, 79 μmol) in dry DMF (500 μl) was added to a vial containing thallos ethoxide (6 μl). After the mixture was stirred for 15 min at room tem-

perature, 2.5 μl of this solution was added to a solution of methyl iodide (57 μg) in dry DMF (10 μl) containing a small piece of copper. The reaction mixture was stirred for 1 h at room temperature in a sealed glass tube. HPLC of the reaction mixture on a reverse phase column which was eluted with ethanol-water (47:53 v/v) at a flow rate of 4.0 ml/min gave only 20% yield of fludiazepam. Variation in the solvent volume (12.5~415 μl) and the reaction time (up to 24 h), or elevated temperatures (up to 60°C) gave no further enhancement of reaction.

3.2 *N*-Methylation using tetra-*n*-butylammonium hydrogen sulphate (TBA-HSO₄)-sodium hydroxide

(1) A small piece of copper was added to a solution of nor-fludiazepam (11.5 mg, 40 μmol) in benzene (1.0 ml). To this mixture were added methyl iodide (2.5 μl , 40 μmol) and quickly thereafter 500 μl of 80 mM tetra-*n*-butylammonium hydrogen sulphate in 0.2*N* sodium hydroxide. The reaction mixture was stirred for 24 h at room temperature in a sealed glass tube. The organic phase was separated and evaporated to dryness under reduced pressure. The residue was chromatographed on silica gel [chloroform-acetone (95:5 v/v)] to give 9.3 mg (76%) of fludiazepam.

(2) The above reaction was carried out on a microgram scale of nor-fludiazepam under a variety of conditions by changing the ratio of methyl iodide to nor-fludiazepam, the amount of TBA-HSO₄, the concentration of sodium hydroxide, the reaction time and the total volume of benzene. The reaction progress was followed by HPLC using a reverse phase column as described above. The best yield (about 57%) and a cleaner reaction mixture were obtained by allowing only molar equivalent amounts of nor-fludiazepam and methyl iodide in the presence of 10 μl of 100 mM TBA-HSO₄ in 4~5*N* sodium hydroxide to react for 48 h at room temperature.

3.3 [*N*-methyl- ^3H]-7-Chloro-1,3-dihydro-5-(2-fluorophenyl)-1-methyl-2*H*-1, 4-benzodiazepin-2-one

To a borosilicate glass ampoule containing [^3H]methyl iodide [370 MBq (10 mCi), 407 GBq

/mmol(11Ci/mmol)] and a small piece of copper, which had been maintained at 0°C , were quickly added $10\mu\text{l}$ of 100 mM TBA- HSO_4 in 4.0N sodium hydroxide and a solution of nor-fludiazepam ($262\mu\text{g}$, $0.909\mu\text{mol}$) in 1 ml of absolute benzene. The glass ampoule was sealed and stirred for 48 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in DMF ($700\mu\text{l}$) and subjected to HPLC using a reverse phase column which was eluted with ethanol-water (47:53 v/v) at a flow rate of 4.0 ml/min. Separation was monitored both with a UV detector set at 254 nm and with a flow scintillation counter. The eluant fraction containing the [^3H] fludiazepam was separated and the eluant was evaporated under reduced pressure. This separation procedure was repeated twice. Final purification by HPLC using a normal phase column with chloroform elution (4.0 ml/min) gave 125.8 MBq (3.4 mCi) of the pure product. The radiochemical purity was also determined by TLC in chloroform-methanol (49:1 v/v) to confirm the HPLC result. The mass amount and chemical purity were determined by UV spectroscopy in ethanol solution from the ratio of absorbance at 254 and 242 nm. The radiochemical yield was 34% and the specific activity was 271.6 GBq/

mmol (7.34 Ci/mmol).

Acknowledgment

The authors wish to thank Sumitomo Chemical Co. Ltd., Japan, for providing fludiazepam and nor-fludiazepam. This research was partially supported by a grant from the Ministry of Health and Welfare, Japan.

References

- 1) Asai, Y., et al.: *Arzneimittel-Forsch.*, **24**, 1563 (1974)
- 2) Borea, P.A., et al.: *Biochem. Pharmac.*, **32**, 603 (1983)
- 3) Hasegawa, N., et al.: *Jap. J. Clin. Exp. Med.*, **54**, 2075 (1977)
- 4) Nakatsuka, I., et al.: *J. Label. Compd. Radiopharm.*, **13**, 453 (1977)
- 5) Ariyoshi, Y., et al.: *Radioisotopes*, **31**, 431 (1982)
- 6) Schwob, R., et al.: *J. Label. Compd. Radiopharm.*, **14**, 355 (1978)
- 7) Ehrsson, H., et al.: *Anal. Lett.*, **6**, 197 (1973)
- 8) Dehmlow, E.V., et al.: "Phase Transfer Catalysis, Monographs in Modern Chemistry", Vol. 11, Verlag Chemie, Weinheim (1980)