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No-carrier-added radiobromination via the Gattermann reaction. — Synthesis of 75Br- and 77Br-bromperidol —

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SUMMARY

The Gattermann reaction was applied to the no-carrier-added radiobromination of bromperidol. The synthesis with diazotized precursor, no-carrier-added 75Br- or 77Br-, and copper powder proved to give the radiobrominated neuroleptic drug in high radiochemical yield of 60 - 70 %, and in high specific activity (~5 Ci/μmole). The radiobromination also proved to be regioselective. In the reaction, the copper catalyst was observed to play an important role.

Formation of unlabeled by-products due to stable chlorine or bromine contamination in the reaction mixture was also studied by neutron activation analysis. Removal of such undesirable halogen nuclides and separation of the unavoidably-formed chlorinated compound from the radiobrominated bromperidol is discussed.

Key words: No-carrier-added radiobromination, Gattermann reaction, 75Br-, 77Br-bromperidol

INTRODUCTION

In nuclear medicine, neuroreceptor ligands labeled with positron or single photon emitting nuclides have been utilized for imaging various brain neuroreceptors by means of positron or single photon emission tomographic technique. Benzodiazepine receptors have been visualized with 11C-labeled flunitrazepam (1) or Ro 15-1788 (2,3), and dopamine receptors with 11C-, 18F-, or 76Br-labeled spiperone or its alkylated derivatives (4-7) or a 11C-labeled sulpiride analogue (8). Opiate receptor mapping was also performed (9).

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The labeled receptor ligands for such purposes are required to be synthesized via simple methods giving high radiochemical yields within a limited time, and high specific activity. Sometimes it is also required that the labeling is regio-selective.

We report here a labeling technique with no-carrier-added radiobromine, which meets these requirements.

Among radiobromine nuclides, the positron emitting nuclides 75Br and 76Br, and the gamma ray emitter 77Br which are produced by a medium size cyclotron are suitable for imaging. In spite of some shortcomings in use of those nuclides for imaging, such as high energy of the positrons lowering the spacial resolution of the images or co-emitting gamma rays giving interfering signals etc, they are still useful. First, the half lives of those radiobromine nuclides (75Br:98min., 76Br:16hrs., 77Br:57hrs.) are long enough for time-coursed observation of the images, which usually takes 2 or 3 hours, second, no-carrier-added radiobromine nuclides are obtainable in high specific activity in comparison with such nuclides as 11C, and third, chemistry for no-carrier-added radiobromination is easier than that of radiofluorination with 18F, often giving higher radiochemical yields.

Radiobrominated spiperone exploited by Dejesus or Huang et al. (10,11) has recently been used for the human brain receptor imaging by Mazière et al.(7) and their images appeared to be clear enough for diagnosis.

Like spiperone, bromperidol is one of the butyrophenone neuroleptic drugs, which has proved to be effective for the treatment of schizophrenia (for example:12,13), and has been reported to have high affinity to dopamine receptors (14). Vincent et al. described the synthesis of 82Br-labeled-brom-peridol via the Sandmeyer reaction (15). However, unfortunately,

their method with the low specific acvtivity radiobromine nuclide 82Br is not directly applicable to the no-carrier-added radiobromination. The alternative synthetic method we are presenting is the Gattermann reaction. The Gattermann reaction is a method modified and simplified from the Sandmeyer reaction, in which copper powder in place of copper bromide is used (16).

In this article, details of the radiobromination technique and quality of the radiobrominated product will be described. This labeling method can be used not only for the radiobromination of bromperidol but also for other receptor ligands and aromatic compounds.

75Br- and 77Br-bromperidol were synthesized also by Moerlein et al. via an electrophilic method using a trimethylstannylated precursor (17). They reported a regiospecific radiobromination giving the labeled bromperidol in a radiochemical yield of 35 % and in high specific activity(>10Ci/ umole).

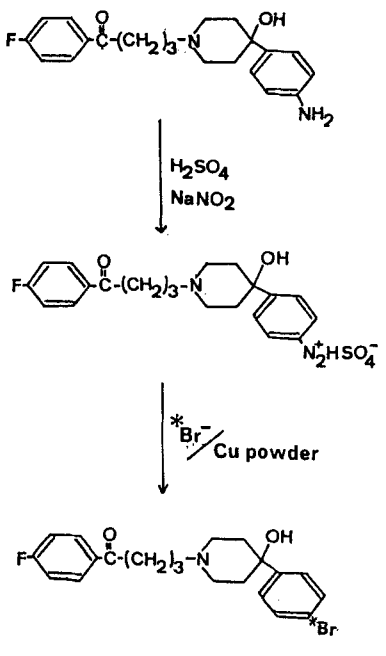
MATERIALS AND METHOD

Production of 75Br and 77Br

75Br and 77Br were produced by a 160 cm cyclotron installed in the Institute of Chemical & Physial Researches (Wakoshi, Saitama), via the reactions of 75As(3He,3n)75Br and 75As(α , 2n) 77Br (18). In both cases, GaAs was used as the target. Irradiation with 6 μA of 3He particles of 40 MeV for 100 minutes gave 15-20 mCi of 75Br and with alpha particles of 35 MeV for 5-6 hours, 10-15 mCi of 77Br(EOB). The target material was put on a small quartz boat with copper grains, placed in a quartz fusion vessel, and 75Br (or 77Br) was separated as sublimate by the radiofrequency heating. 75Br (or 77Br) on the inside wall of the fusion vessel was washed out with distilled water.

Synthesis of 75Br- and 77Br-bromperidol via the Gattermann reaction

The synthetic procedure is schematically outlined in Scheme 1. 1mg (3 μmoles) of 4-[4-hydroxy-4-(4-amino-phenyl)-piperidynyl]-1-(4-fluoro-phenyl)butanone, i.e. "aminoperidol" (Yoshitomi Pharmaceutical Co.) was dissolved in 0.5 ml of 2N sulfuric acid and diazotized in an ice-water bath by adding slightly excess amount of sodium nitrite dissolved in 10 μl of distilled water. 75Br (or 77Br) solution, which had previously been concentrated to a small volume, ~0.2 ml, was added. The catalyst, copper grains, was prepared by adding 5 - 7 mg of zinc powder and 10 - 50 μl of 2N sulfuric acid to 100 - 200 μl of 0.5 M copper sulfate solution. The copper grains formed were washed twice with distilled water and immediately added to the reaction mixture. Then, the copper grains were sonicated into powder from outside of the reaction vessel with an ultra-sonicator for a few minutes. And the reaction mixture was heated in a water bath to 80 °C. Ten minutes later, the vessel was taken out of the hot water and cooled. Then, the mixture was diluted with distilled water, alkalized with 8N ammonium hydroxide, and the products were extracted with chloroform. The extract was washed with water, and the solvent was evaporated for purification or for analysis.



Scheme 1

All the reagents and the solvents used were of guaranteed purity.

Optimization of the synthetic reaction conditions

The following factors were varied in the synthetic reaction described above to obtain the optimal conditions. Those factors were, the amount of the substrate aminoperidol, which was varied from 1 to 7 mg, the amount of sodium nitrite, from 1 to 5 equivalents of the amount of the substrate, the concentration of sulfuric acid in which diazotation and radiobromination took place, from 0.5 to 18 N, the amount of 77Br, from 10 μCi to 1 mCi, the amount of copper catalyst, from 0 to 17 mg, and the temperature for the bromination, from 40 to 80 °C.

Purification of 75Br(or 77Br)-bromperidol

Similar procedures as Vincent et al. reported (15) were used: After extraction with chloroform following radiobromination, alumina column chromatography was performed using a glass column of 10 mm inner diameter and 70 - 80 mm length packed with aluminum oxide (activation stage II - III). Chloroform was used as eluant. The eluate containing radioactivity was collected and evaporated to dryness for further purification by high performance liquid chromatography (HPLC). HPLC was carried out using Tri Rotor III (JASCO, Tokyo) with a reverse phase preparative column (ODS; 20 mmID x 250 mmL) and with a solvent system of ethanol-acetic acid-0.2M ammonium acetate (80:4:143) or methanol-0.8N ammonium hydroxide (7:3). The eluate was continuously monitored with UV spectrometer (UVIDEC 100 ;JASCO) at 245 nm and with NaI scintillation detector (OKen ;'Tokyo). The fractions containing radioactivity were collected and evaporated to dryness for analysis. From the UV absorption and the radioactivity, specific activity of the radiobrominated bromperidol was also estimated.

Analysis of the product and by-products

Mass spectrometry

The product and by-products formed via the Gattermann reaction were analyzed by mass spectrometry using a sector mass spectrometer (Shimazu-LKB 9000B). Ionization was performed by direct electron bombardment (70eV) on the sample mounted on a probe, and m/e ranging from 0 to 500 was scanned at various temperatures, 40 to 260 °C.

HPLC analysis

HPLC analysis was carried out using a reverse phase (ODS) analytical column (4.6 mmID x 250 mmL). The solvent system was the same as described above. As the references for assignment of the UV absorption peaks, authentic haloperidol, bromperidol and 4-[4-hydroxy-phenyl-piperidinyl]- 1-(4-fluorophenyl)butanone, i.e. "hydroperidol" or "peridol (17)" were used. Authentic haloperidol and bromperidol were obtained from Yoshitomi Pharmaceutical Co.. Hydroperidol was synthesized via the similar procedures as bromperidol synthesis described above, but by decomposing the diazotized aminoperidol in 50% hypophosphorous acid: MS m/e 341(M+); 1H NMR 8.00-8.04 (2H,F-Ar), 7.25-7.49 (5H,Ar), 7.12-7.16 (2H, F-Ar),1.69-3.00 (15H); Anal. (C21H24FNO2), C,H,N C:74.3, cal. 73.9

Proton NMR spectrometry

The regiospecificity of the bromination by the Gattermann reaction was examined by proton NMR spectrometry. The sample bromperidol for the NMR analysis was synthesized via the Gattermann reaction similar as described above but with unlabeled sodium bromide in place of $^{75}Br^-$ or $^{77}Br^-$, and in ten times larger scale. Purification was carried out similarly to that of radiobrominated bromperidol as described above. The sample was dissolved in $CDCl_3$ and proton NMR spectra were obtained with a 400 MHz high resolution NMR spectrometer (Jeol GX400). TMS was used as the standard. The spectrum of the

synthesized bromperidol was compared with that of the authentic bromperidol.

Assessment of stable chlorine and bromine contained in the materials used for the Gattermann reaction

Neutron activation analysis was performed. Using Rikkyo TRIGA II reactor, the materials, namely, aminoperidol, sulfuric acid, zinc powder, copper sulfate, distilled water and 77Br solution, were irradiated with a neutron flux density of 5.5 x 10E11·n·cm-2 sec-1 for 1 - 10 minutes for chlorine and 1 - 6 hours for bromine. After cooling time, radiometric analysis was carried out with a Ge(Li) detector. For all the samples except the zinc powder, 38Cl from 37Cl(n,γ)38Cl and 82Br from 81Br(n,γ)82Br were measured. For the zinc powder, 80Br from 79Br(n,γ)80Br was used to avoid the influence of radioactive zinc such as 65Zn or 69mZn.

RESULTS AND DISCUSSION

Radiochemical yield of 75Br- and 77Br-bromperidol

Among the various factors which were assumed to affect the radiochemical yield, the most important role was observed to be played by the copper grains, the catalyst for the radiobromination. Without them, no radiobrominated bromperidol was formed, although radiofrequency heating of the GaAs target with copper grains, which was carried out after cyclotron production of 75Br (or 77Br), could presumably give copper-75Br(77Br)bromide which would possibly be ready for radiobromination via the Sandmeyer reaction. With the copper grains without sonication, radiochemical yields varied from 8 to 38 %, and were independent of other factors. And the reproducibility was poor. Almost all (>97 %) the unreacted radiobromine was found to be adsorbed on the surface of the copper. By sonicating the copper grains into fine powder, radiochemical yields increased to 60 - 68 %, and were highly reproducible.

The other factors did not seem to give much determining effects except for the concentration of sulfuric acid in which diazotation and bromination took place (Fig. 1) and the reaction temperature (Table 1).

Those findings suggested that the Gattermann reaction took place on the surface of the copper and the "freshness" of the surface was important for the reaction to proceed. Presumably, on the "fresh" copper surface, CuBr*(Br*:radiobromine of tracer amount) or Cu_nBr*_m(n > m) was formed and the copper bromide was ready to react with the diazonium ion. Around the sites where the radiobromide ions were adsorbed, nucleophilic environments to which the diazonium cations could easily access were formed

Table 1 Reaction temperature and radiochemical yield (Substrate: 1mg, concentration of sulfulic acid : 2N, reaction time : 10 minutes)

Temperature(°C)	Yield(%)
40	12
60	32
80	61

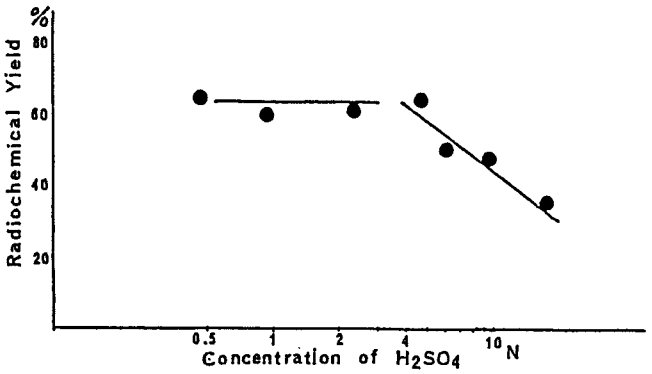


Fig. 1 Effect of sulfuric acid concentration of synthetic reaction mixture on radiochemical yield of radiobrominated bromperidol.

by the electrons that the radiobromide anions had brought in. Thus it seems more advantageous to use copper powder catalyst than copper bromide for such a purpose as no-carrier-added

radiobromination where tracer amount(<ng) of radiobromine is involved, since electron transfer from Br- to Cu and/or from CuBr(or Cu_nBr_m;n>m) to a diazonium cation would be more freely and easily performed on the surface of the catalyst. On the other hand, the reaction with copper bromide takes time (15), unless larger amount of copper bromide, for example equimolar to that of diazonium salts, is involved (19).

The surface of the copper grains prepared from zinc powder and copper sulfate was quickly oxidized turning to black. And on the oxidized surface of copper, bromination seemed to be prevented. Promotion of such oxidation might be one of the reasons why high concentration of sulfuric acid in which bromination took place lowered the radiochemical yield (Fig 1).

As the catalyst, in place of copper, iron, cobalt, or zinc might also be worth examining (20). And, since the Gattermann reaction, the Sandmeyer reaction, and other similar reactions are oxidation-reduction reactions, the electrolytic method such as described by Votocek,E., et al. (21) might also be useful.

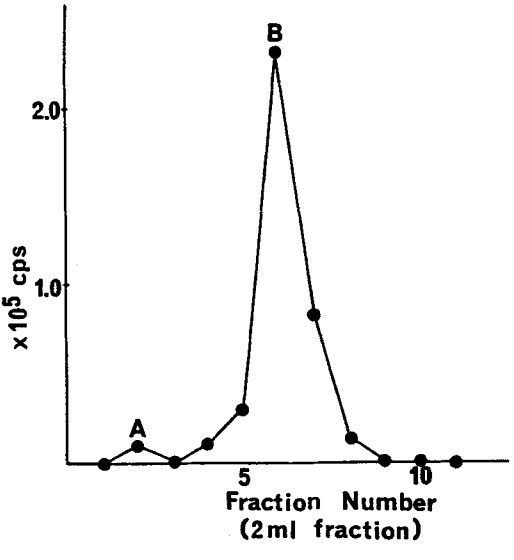


Fig.2
Elution of 77Br-bromperidol (B) from the alumina column with chloroform.
Elution conditions: see text

Labeling efficiency

Labeling efficiency was high and more than 98 % of the reacted radiobromine was recovered as radiobrominated bromperidol.(Fig 2,B) The rest , 2%, was observed to be incorporated into a lipophilic compound with low polarity, which was eluted in the first fractions from the alumina column (Fig.2,A).

Regiospecificity of the bromination reaction

High resolution NMR spectrometry demonstrated the regiospecificity of the bromination via the Gattermann reaction carried out under the conditions described above. The spectrum of the synthesized bromperidol showed 4 peaks at δ 8.00 - 8.04 ppm and 3 peaks at 7.12 - 7.16 ppm which were related to 4 protons in the aromatic ring with florine, and, 2 peaks at 7.47-7.45 ppm and 2 peaks at 7.34 to 7.32 ppm which were related to 4 protons in the other aromatic ring that was involved in the bromination. This spectrum agreed with that of authentic bromperidol in which bromine is bound at the para position. No additional peaks nor chemical shifts which indicated the presence of brominated isomers were observed. Bromination or chlorination via the Sandmeyer reaction or other similar reactions with copper bromide or chloride has been considered to be regiospecific, and our result demonstrated that this is the case also in the Gattermann reaction. On the other hand, in the electrophilic radiobromination with oxidizing agents such as hydrogen peroxide, which is also a simple and useful technique for the synthesis of various radiobrominated neuroleptic drugs (10,11, 22,23), brominated position is not usually controllable (24) except for the case where specific precursor such as the one stannylated at the specific position is used (17).

Unlabeled by-products of the reaction, specific activity and effective specific activity of the radiobrominated bromperidol

The mass spectra showed major by-product molecular peaks (M+)

at m/e 339, 341, 356, and 377+375 (twin). The compounds with m/e 339 and 356 were removed by the alumina column while the others passed through it. The observation suggested that the compound corresponding to m/e 339 was 4-[(4-phenyl)-1,2,3,6-tetrahydropyridinyl]-1-(4-fluorophenyl)-butanone ($C_{21}H_{22}NO_2F$) which was apparently derived by substitution of the amino group of aminoperidol by the hydroxy group, and m/e 356 was unreacted aminoperidol. The other peaks were assigned to be hydroperidol ($C_{21}H_{24}NO_2F$), and haloperidol ($C_{21}H_{23}NO_2F^{35}Cl$+$C_{21}H_{23}NO_2F^{37}Cl$). The mass spectra of the authentic compounds supported the assignment. In comparison with hydroperidol and haloperidol mass ion currents, those of bromperidol(m/e: 419 & 421) were small, and only slightly detectable.

In no-carrier-added radiobromination, formation of chlorinated by-products deserves attention, since such chlorinated compounds are often biologically as active as the brominated one, and are usually formed in larger amount, and are hard to separate from the objective product due to their similarity in chemical structure. In this case also, a chlorinated compound, i.e. haloperidol which has an affinity to dopamine receptors as high as bromperidol (14), was demonstrated to be produced. It was obvious that the undesirable chlorinated compound was formed with stable chlorine contained in the reaction mixture as impurity. Neutron activation analysis revealed that the materials which were used for the Gattermann reaction contained 30 mg/g to 4 μg/g of chlorine (Table 2). The analysis also revealed the contamination with stable bromine as high as 0.31 mg/g, which significantly lowered the specific activity of the radiobrominated compound. The highest contamination with both chlorine and bromine was found in the substrate aminoperidol (Table 2,I), which suggests that when 1 mg of aminoperidol was used for the synthesis, 30 μg of Cl and 0.3 μg of Br were

incorporated into the reaction mixture. A simple repurification of aminoperidol by extraction with $CHCl_3$ from alkalized solution reduced the amount of chlorine by 22 times, but still remained 1.4 mg/g, whereas the amount of bromine was not much changed. (Table 2,II) Contamination in the radiobromine solution was also quite significant, which was presumably derived from the target material GaAs.

Table 2 Stable chlorine and bromine contamination in the materials used for the Gattermann reaction.

Sample	Cl			Br		
Aminoperidol (I)	31	± 2	mg/g	0.31	± 0.02	mg/g
(II)	1.4	± 0.07	mg/g	0.27	± 0.01	mg/g
Distilled water	0.55	± 0.03	μg/ml	0.006	± 0.001	μg/ml
Sulfuric acid		<0.4	μg/ml		<0.01	μg/ml
Copper sulfate	0.006	± 0.004	mg/g		<0.0002	mg/g
Zinc powder	0.014	± 0.006	mg/g	0.015	± 0.001	mg/g
^{77}Br solution	0.077	± 0.004	mg/ml	0.001	± 0.0002	mg/ml

Copper sulfate or zinc, also, appeared to contain rather high amount of Cl and Br which could give 30-40 μg of Cl and Br to the reaction mixture. However, these materials were used for preparing the copper catalyst, and Cl and Br in those materials were probably removed or reduced in significant amounts by washing the catalyst with distilled water. Contamination from other sources such as sulfuric acid was relatively negligible. Adam et al. also reported on the stable bromine contamination ranging from 0.09 to 650 μg/g in the reagents, solvents and substrates for the electrophilic radiobromination (25).

HPLC separation of the ^{75}Br-bromperidol from other by-products which passed through alumina column is shown in Fig.3. The UV absorption peak a represented that of hydroperidol, b haloperidol, and c bromperidol. In this case, repurified aminoperidol was used as substrate, and elution was performed with CH_3OH - 0.8N NH_4OH (7:3). The resolution between haloperidol and bromperidol was 1.52. The alternative elution system with

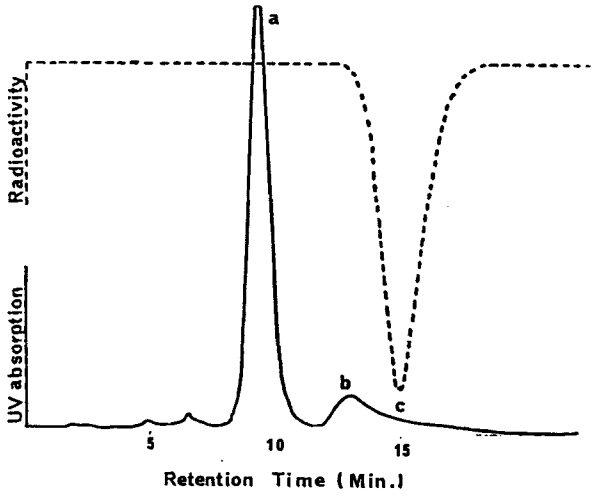


Fig.3
HPLC separation of 75Br-bromperidol from other by-products.
Column:ODS(20mmφx250mmL)
Eluant:CH3OH-0.8NNH4OH (7:3)
a : hydroperidol
b : haloperidol
c : bromperidol

ethanol-acetic acid-0.23M ammonium acetate (80:4:143) gave the resolution of 1.26. With such resolutions, 1/10 - 1/15 of haloperidol was presumed to be incorporated into the radiobrominated bromperidol due to the tailing effect.

Thus, taking all those findings described above into account, and assuming that the radiochemical yield of the Gattermann reaction was 60 %, 20 mCi of 75Br(or 77Br) was calculated to give the radiobrominated bromperidol of 5 Ci/μmole, which agreed well with the actual observation. By additional assumptions that 1/10 of the unavoidably-formed haloperidol was incorporated into the radiobrominated bromperidol due to the tailing effect of HPLC, and that the affinity of bromperidol for the dopamine receptor was 1.2 times higher than that of haloperidol (14), the effective specific activity was calculated to be 1.2 Ci/μmole.

CONCLUSION

The Gattermann reaction proved to be useful for the no-carrier-added radiobromination. 75Br- and 77Br- bromperidol, as examples, were demonstrated to be synthesized via the reaction in good chemical yield (60-70 %), in high specific activity (~5

Ci/μmole), and regiospecifically. Stable chlorine and bromine contained in the reaction mixture as impurities caused limitation of specific activity and effective specific activity of the radiobrominated bromperidol.

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