WORKING METHODS PAPER

Fast and High-yield Synthesis of Radioactive $CH_3{}^{203}Hg(II)$

Claude Rouleau[1,*] and Mats Block[2]
[1] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Biomedicum Box 573, S-751 23 Uppsala, Sweden
[2] Department of Environmental Toxicology, Uppsala University, Norbyvägen 18A, S-752 36 Uppsala, Sweden

A simple and fast synthesis of radioactive methylmercury is presented. It is based on the methylation of inorganic $^{203}Hg(II)$ by methylcobalamin and isolation of resulting $CH_3{}^{203}Hg(II)$ in a single extraction step. Time required is less than 4 hours and the final yield is $\geqslant 90\%$. © 1997 John Wiley & Sons, Ltd.

Appl. Organometal. Chem. **11**, 751–753 (1997)
No. of Figures: 1 No. of Tables: 1 No. of Refs: 3

Keywords: methylmercury; radioactive; synthesis

Received 7 November 1996; accepted 30 January 1997

INTRODUCTION

For our studies using radioactive methylmercury as a tracer, we have previously synthesized it according to Toribara[1] or Naganuma *et al.*[2] Toribara's method is based on the methylation of inorganic $^{203}Hg(II)$ by tetramethyltin. Isolation of $CH_3{}^{203}Hg(II)$ is then performed by successive benzene–water extractions, at the end of which it is dissolved in a small volume of a dilute Na_2CO_3 solution. According to Naganuma *et al.*, methylmercury is obtained by the reaction of methylcobalamin (MeCo) with inorganic $^{203}Hg(II)$ and isolated by liquid chromatography. These multistep methods allow the preparation of a high-purity radioactive methylmercury, but they suffer from some disadvantages as both are rather time-consuming (approx. 1 day) and as the practical yield achieved (50–70%) results in the waste of one-third to one-half of the starting radioactive inorganic mercury. We have combined some steps of these two methods to develop a much simpler and faster procedure that gives a >90% yield. It is based on the methylation of inorganic $^{203}Hg(II)$ by MeCo and the isolation of $CH_3{}^{203}Hg(II)$ from the reaction mixture in a single extraction step.

MATERIALS AND METHODS

As the specific activity of inorganic $^{203}Hg(II)$ commercially available is usually $\geqslant 1.0$ mCi mg^{-1}, reaction conditions were set for the methylation of 1 mCi, representing a quantity of 1 mg Hg (5 μmol) or less. The synthesis proceeded as follows.

(1) A portion of 1 ml or less of a radioactive $^{203}HgCl_2$ solution (in 0.1–0.01 M HCl) was placed in a 100-ml separatory funnel fitted with a Teflon® stopcock. The contents of a 25-mg vial (18.6 μmol) of MeCo (Sigma), dissolved in 0.01 M HCl just before use so that the total volume was 2 ml, were added. The funnel was wrapped in aluminium foil to protect it from light and left to stand for 1 h.

(2) Ten milliliters of hexane/benzene (1:1) were added and the mixture was stirred for 10 min, as

---

† Correspondence to: Claude Rouleau, Maurice Lamontagne Institute, C.P. 1000, Mont-Joli, Québec, Canada G5H 3Z4.

CCC 0268–2605/97/090751–03 $17.50

Table 1 Yield of $CH_3{}^{203}Hg(II)$ synthesis[a]

Run no.	Starting amount of inorganic Hg (mg)	^{203}Hg remaining in the MeCo solution (%)	Final yield as $CH_3{}^{203}Hg(II)$ (%)	Other losses (%)
1	1.0	6	90	4
2	0.25	1	92	7

[a] The radioactivity of 10 μl aliquots was measured in triplicate and results are expressed as a percentage of the total $^{203}Hg(II)$ radioactivity.

described by Toribara,[1] by using a mechanical stirrer equipped with a glass stirring rod, the bottom of which was flattened and twisted so that the stirred solutions would be directed downwards. The extraction was repeated twice and the organic layers were combined in a 50-ml conical glass tube containing 2 ml of a 0.005 M Na_2CO_3 solution.

(3) The combined organic layers were stirred over the Na_2CO_3 solution for 10 min, then evaporated by blowing a gentle stream of clean air or nitrogen over the surface. The radioactive $CH_3{}^{203}Hg(II)$ was left dissolved in the Na_2CO_3 solution. The final solution could be transferred to a suitable vial and stored at −20 °C.

Radiochemical purity was assayed against a standard of methylmercury by TLC, using TLC 0.2-mm silica-gel plates with hexane/acetic acid (12:1) for elution, and dithizone as developer. After processing, TLC plates were applied to a X-ray film (Hyperfilm MP, Amersham) for autoradiography.

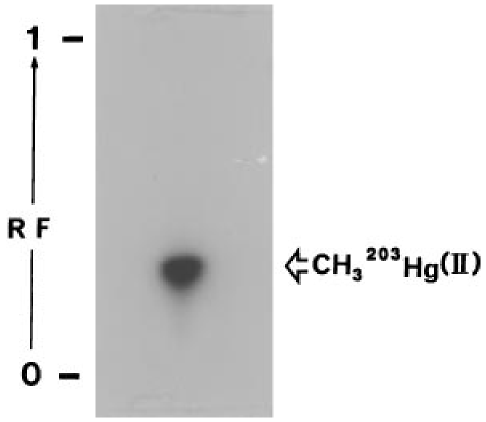

Figure 1 Autoradiogram of a thin-layer chromatogram made from a benzene extract of the final $CH_3{}^{203}Hg(II)$ solution.

RESULTS AND DISCUSSION

This procedure gives excellent results, both qualitatively and quantitatively. Table 1 shows the yield from two typical runs. The methylation reaction (step 1), the extraction from the MeCo solution of the lipophilic $CH_3{}^{203}HgCl$ produced (step 2), and the transfer to the Na_2CO_3 solution (step 3) are very effective and lead to a final yield of $\geqslant$90%. Volatilization of some methylmercury during the evaporation phase and small droplets of benzene and/or aqueous solutions sticking to glass labware are probably responsible for the other losses. An autoradiogram of a thin-layer chromatogram made from a benzene extract of the final sodium carbonate solution (Fig. 1) shows a single spot with a retention factor in the range of values (0.25–0.30) found when performing TLC with the nonradioactive standard solution of methylmercury. As no nonradioactive mercury is added, the specific activity of $CH_3{}^{203}Hg(II)$ obtained is the same as that of the starting $^{203}Hg(II)$.

In practice, this method is problem-free if the operator closely follows the steps and reaction conditions described. The use of a basic aqueous solution for the final step is essential as the methylmercury chloride produced initially is converted to methylmercury hydroxide. The latter is much more hydrophilic[3] and this favours its transfer from the organic phase toward the aqueous phase during mixing. Reducing methylmercury content of the organic phase prior to evaporation also helps to reduce the volatilization of the radioactive compound, if any. Occasionally, a lower yield was obtained: this was related to either insufficient agitation or to the use of excess acid (i.e. the HCl concentration of the starting inorganic $^{203}Hg(II)$ solution $>$0.1 M). If the solution is too acidic, it is preferable to dilute the inorganic mercury solution in order to have a HCl concentration within the working range of 0.01–0.1 M.

In summary, this simple synthesis method is advantageous in terms of time and cost. The time needed for the whole procedure, from the beginning of methylation to isolation of the final $CH_3{}^{203}Hg(II)$ solution, is less than 4 h, which is much faster than our previous experience with the other methods. The reduced number of methodological steps results in high final yield of radiochemically pure $CH_3{}^{203}Hg(II)$ and minimizes the losses of expensive radioisotope. Direct manipulations by the operator are also reduced to a minimum, decreasing the radiation dose received.

Acknowledgement Financial support was provided by the Swedish Institute (C.R., visiting fellowships).

REFERENCES

1. T. Y. Toribara, *Int. J. Appl. Radiat. Isot.* **36**, 903 (1985).
2. A. Naganuma, T. Urano and N. Imara, *J. Pharm.-Dyn.* **8**, 69 (1985).
3. M. A. Major, D. H. Rosenblat and K. A. Bostian, *Environ. Toxicol. Chem.* **10**, 5 (1991).