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Synthesis of [^{203}Hg]methyl mercuric nitrate

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INTRODUCTION.

Organic mercurials, particularly the alkyl mercurials, have been extensively used in the study and characterization of the sulfur containing groups of proteins because of their high degree of specificity for the sulfhydryl group. Electrometric and spectrophotometric methods employing some of these compounds have been developed for the quantitative assay of sulfhydryl groups of a number of soluble proteins [1–5]. Radiochemical techniques using carbon-14 labeled methyl mercuric iodide and mercury-203 labeled phenyl mercuric acetate have also recently been reported for some insoluble proteins [6]. Applications of these techniques and the syntheses of several alkyl mercurials have been discussed [7–9]. However, the relative insolubility in aqueous systems of the organic mercurials of the type RHgX where X is acetate, bromide, chloride, cyanide or iodide and the relative unavailability of the salts, $RHg^+ X^-$, where X^- is fluoride, nitrate, phosphate or sulfate, have limited the wider use of these reagents in radiochemical techniques in the assay of -SH and -S-S- groups of soluble proteins.

Hamilton [7] (p. 77) has pointed out some of the advantages of using methyl mercuric nitrate in the titrimetric assay of sulfhydryl groups. This mercurial is water soluble, stable, monofunctional and the small methyl group is more easily able to penetrate to a reactive site than larger alkyl or aryl substituted mercurials. While $[^{14}C]CH_3HgNO_3$ can be prepared from $[^{14}C]CH_3I$ this reagent is expensive to use on a routine basis. In contrast, mercury-203 is inexpensive, readily available, and, since it is both a beta and gamma emitter, can be read out in a liquid scintillation or gamma-ray spectrometer. In the course of our investigations of some soluble proteins we have prepared [^{203}Hg]methylmercuri-labeled proteins using [^{203}Hg]methyl mercuric nitrate. The radioactivity in these proteins is routinely monitored at 60 % efficiency in an aqueous system using an anthracene-packed flow cell mounted

in a liquid scintillation spectrometer [10]. The potential utility of the methyl mercury ion is not limited to structure studies of the sulfur proteins. The extensive occurrence of alkyl mercury poisoning in Sweden and Japan has been traced to bacterial alkylation in anaerobic systems [11]. Westoo [12] has suggested that methylmercury in animal foodstuffs is probably attached to thiol groups. Recently, Millar has utilized the monovalent mercurial methyl mercury hydroxide to study the destruction of the helical form of polyuridylic acid [13].

In this communication, we report the synthesis of [^{203}Hg]CH_3HgNO_3. While the procedure is similar to that of Hamilton [7], Strecker [14] and Johns *et al.* [15] for the preparation of the unlabeled mercury salt, all operations subsequent to the reduction of the mercuric ion are carried out in a "Dry Box". In addition, the hot methanol extraction of the methyl mercuric iodide is carried out in a "Glove Bag" placed inside the "Dry Box". In view of the inherent sensitivity of the radiochemical method of assay and the desirable properties of this reagent, mercury-203 labeled methyl mercuric nitrate should prove to be a valuable tool in environmental pollution studies involving methyl mercury poisoning. The reagent should also prove useful as a probe in structure studies of polynucleotides.

EXPERIMENTAL.

[^{203}Hg]$HgNO_3$ was obtained from Union Carbide Corp., Tuxedo Park, New York. All other reagents were analytical grade. The solvents were routinely redistilled before using. All operations following the reduction of the mercury are carried out behind lead brick shielding under nitrogen purge in a "Dry Box" since mercurials are volatile and toxic [16]. The exhaust from the "Dry Box" is passed to an aspirator through a trap containing a solution of silver nitrate in a 1 : 1 mixture of methanol : water.

Preparation of [^{203}*Hg*]CH_3HgI.

Two grams (5.85×10^{-3} mole) of mercuric nitrate monohydrate, placed in a 50 ml glass stoppered round bottom flask, is dissolved in 5.0 ml water acidified by the addition of two drops of concentrated nitric acid. Ten millicuries of [^{203}Hg]$HgNO_3$ is added and the solution carefully heated on a steam bath to 70°C. One gram of ascorbic acid is added and the flask is shaken for one minute. Reduction of the mercury is immediate and complete. No detectable radioactivity remains in the supernatant. Five grams (0.025 mole) of elemental mercury is added to the reaction flask and the mixture is vigorously swirled. When the mercury droplets have settled, the supernatant is carefully removed by suction and the mercury is washed twice with cold water, once with ethanol, and allowed to dry. No radioactivity was detected in any of the washings. Ten grams (0.070 mole) of methyl iodide is added to the dried

mercury in the reaction vessel together with a stirring bar and the vessel is stoppered. The reaction mixture is agitated for twenty-four hours with a magnetic stirrer under ultraviolet light (Bali Sun Lamp, Hanovia Model 30410-1) placed 0.3 meter distant from the reaction vessel. The methyl mercuric iodide which appears as a solid yellow mass on the walls of the flask is ground up and repeatedly extracted with 150 ml of boiling methanol (Glove Bag). The extracts are filtered and collected in a beaker chilled in an ice bath. White crystals of methyl mercuric iodide appear immediately. The solid is recrystallized once from methanol and allowed to dry by evaporation. Yield 8.8 g (0.026 mole). A check of the radioactivity of the methanol wash revealed that a small amount of product remained in the methanol. No attempt was made to recover this fraction.

Preparation of $[^{203}Hg]CH_3HgNO_3$.

Three grams (0.009 mole) of the $[^{203}Hg]CH_3HgI$ is dissolved at room temperature in 25 ml of methanol in a 50 ml round bottom flask. One and one half grams (0.009 mole) of silver nitrate dissolved in 2.0 ml of water is added to the reaction vessel and the mixture is stirred for two hours. The supernatant is decanted, filtered and collected in a beaker. The precipitate is extracted twice with 10 ml portions of methanol and the combined filtrates allowed to evaporate. The white crystals of $[^{203}Hg]CH_3HgNO_3$ are recrystallized from methanol until the filtrate gives no test for silver ion, then dried. M. P. 100° C [17]; yield, 2.1 g (84 % based on CH_3HgI); specific activity 180 μC/mmole. The material, assayed with glutathione, showed a single peak with an elution to void volume ratio of 3.85 when eluted from G-10 Sephadex with 1 % cationic surfactant 0.1 M in citrate. Since organic mercurials have been implicated in alkyl mercury poisoning [11, 12, 18] we recommend that none of these compounds be discharged into sewage lines but that excess or spent reagent be destroyed with ascorbic acid or other appropriate reducing agent.

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