# PREPARATION OF A RADIOBROMINATED FLUORESCEIN FOR LIVER SCANNING

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## SUMMARY

A radiobrominated compound, 4,5,6,7-tetrachloro-2',4',5',7'-tetrabromofluorescein-$^{77}Br_4$ was prepared from resorcinol, tetrachlorophthallic anhydride and $Na^{77}Br$ by a two-step synthesis. The precursor was identified by spectrometry. Final purification utilized column chromatography. The product was identified by thin layer chromatography and spectrometry. The yield was 60% of theoretical.

Key Words: Bromine-77, bromination, radiobrominated eosin, radiobrominated Phloxine-B.

## INTRODUCTION

A number of radiopharmaceuticals have been developed for use in studying liver function. Of these $^{131}$I-Rose Bengal assumed a role in differentiating hepatocellular disease from partial common bile duct obstruction in patients presenting with symptoms attributable to both hepatitis and biliary atresia.(1) It is apparent that $^{131}$I-Rose Bengal is not an optimum agent. A small but variable fraction (±5%) is removed from the body through the renal system.(2) Also, partly because of the presence of endogenous deiodinase enzymes, the C-I bond is not adequately stable *in vivo*.(3)

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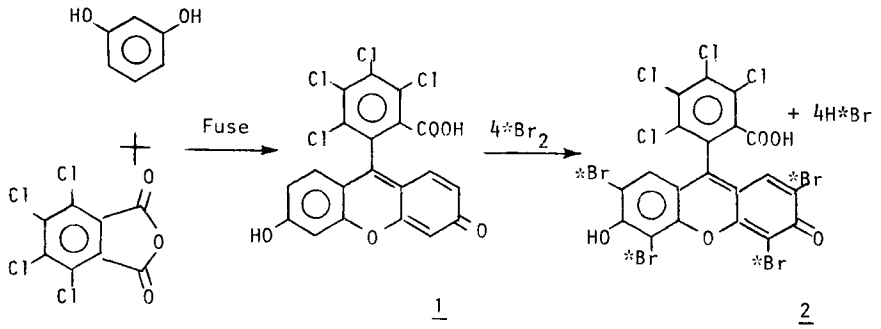
More recently developed $^{99m}$Tc-hepatobiliary agents may[4] or may not[5] prove suitable for this use in humans. We here present data on the synthesis of a radiobrominated Rose Bengal analogue, wherein the more stable C-Br bond[6] has been utilized in an attempt to overcome the instability of the C-I compounds.

The radionuclide was obtained as $Na^{77}Br$ in slightly basic solution. A typical shipment contained less than 1% $Na^{77}BrO_3$ and no detectible $NaO^{77}Br$. Bromine-77 has a physical half-life of 57 hours[7] and decays by electron capture (99%) and positron emission (1%). Principle gamma energies are 240 KeV (29%), 300 KeV (6%) and 520 KeV (17%).

## RESULTS

Synthesis of 4,5,6,7-tetrachloro-2',4',5',7'-tetrabromofluorescein-$^{77}Br_4$ (2) was achieved by bubbling gaseous $^{77}Br_2$ into a sodium bicarbonate solution of 4,5,6,7-tetrachlorofluorescein (1). Using a modification of the method of Slaunwhite and Neely[8], the $^{77}Br_2$ was generated by boiling a mixture of $Na^{77}Br$, manganese dioxide and sulfuric acid in a pear-shaped double-neck flask, the bromine being channeled to the bromination tube through a glass side arm.

Synthesis of 1 consisted of fusion of a mixture of resorcinol and tetrachlorophthallic anhydride. The purified product was obtained by precipitation from a sodium bicarbonate solution using hydrochloric acid.




REACTION SEQUENCE

The identity of 1 was confirmed by spectrometry and melting point determination. Column chromatography was performed to isolate the metabolically active product (2) from any radiochemical contamination.

Column chromatography with Sephadex G-25 was utilized to isolate 2. The elution diagram (Figure 1) was obtained from 2 washed twice with hydrochloric acid and sodium bicarbonate. The diagram shows that the $Na^{77}Br$ activity was eluted within the first 10 $cm^3$, and the radioactivity peak at 105-115 $cm^3$ corresponded with the elution volume of an authentic sample of Phloxine-B. The structure of a substance giving rise to a third radioactivity peak at 25-30 $cm^3$ remains unknown, and its biological activity has not been determined.

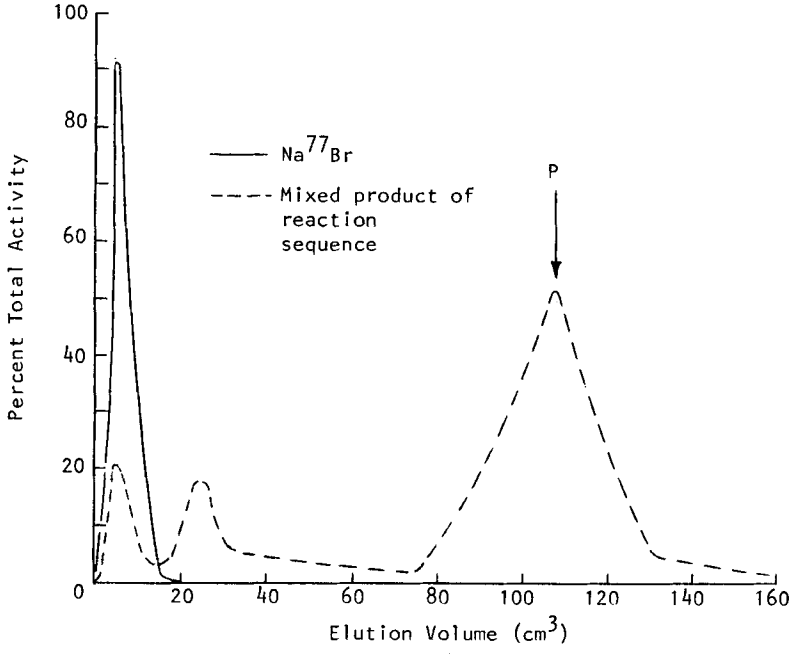



FIGURE 1. Composite elution diagram showing the location of the radioactivity peaks for $Na^{77}Br$ and 2 washed twice with HCl and $NaHCO_3$. The arrow indicates the elution volume of authentic Phloxine-B. See text for details.

Thin layer chromatography of the substance eluted in the radioactivity peak at 105-115 $cm^3$ indicated the presence of two minor radiochemical contaminants (Figure 2). Sodium bromide-$^{77}Br$ migrated with an $R_f$=0.05. An intermediate radioactive mixture at $R_f$=0.62-0.67 was also noted. The radioactivity peak at $R_f$=0.94 corresponded with the $R_f$ of pure Phloxine-B (P in the chromatogram insert of Figure 2). The identity and biologic activity of the radioactive mixture at $R_f$=0.62-0.67 remains unknown.

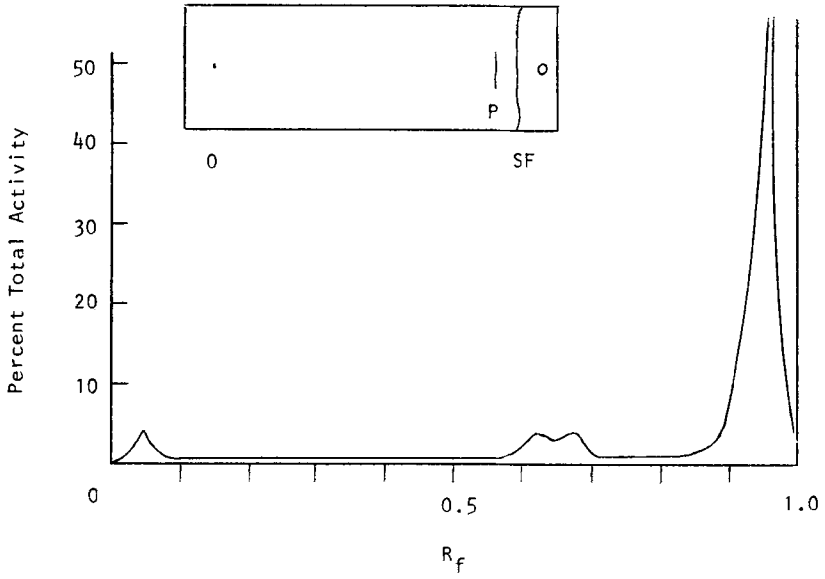



Figure 2. The thin layer radiochromatogram of purified 2 showing $Na^{77}Br$ at $R_f$=0.05. Insert shows a chromatogram of Phloxine-B (dark streak at P). O= Origin, P= Phloxine-B, SF= solvent front. See text.

In the purification procedure it was found that each successive washing of 2 with hydrochloric acid and sodium bicarbonate resulted in a reduction (50%) of the radioactivity in the first 10 $cm^3$ of eluant. Also, the amount of radioactivity in the first 10 $cm^3$ was minimized when the volume of $Na^{77}Br$ used in the synthesis of 2 was less than 1 $cm^3$. Presumably large volumes of the slightly basic $Na^{77}Br$ dilute the sulfuric acid and thereby interefere with production of $^{77}Br_2$.

It may appear unusual that the lower molecular weight species were eluted from the column ahead of the high molecular weight product. However, Determann and Walter[9] have shown that there is a strong association between the less coarse Sephadex Dextran gels and aromatic heterocyclic substances. They have demonstrated that solutes with π-electrons are strongly adsorbed to the ether groups of these gels, and are thus retarded in their elution.

Figure 3 shows the absorption spectrum of non-radioactive 2 (Phloxine-B), prepared by bromination of 1. Comparative spectrophotometry, using a modification of the method of Conn[10], confirmed the identity of 2.

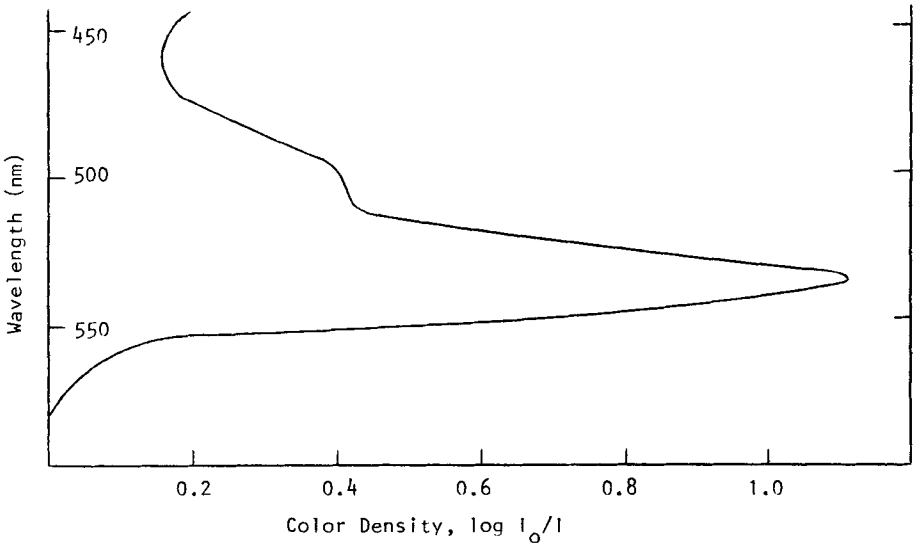



Figure 3. The spectrum of non-radioactive 2.

## EXPERIMENTAL

To prepare 4,5,6,7-Tetrachlorofluorescein (1), an intimate mixture of resorcinol (1.0g) and 1.3g tetrachlorophthallic anhydride (Aldrich Chemical Company, Milwaukee, Wisconsin 53233) was placed in a Kontes Microflex tube. The reactants were heated at 350°C for 30 min and gave a viscous maroon slurry which hardened at room temperature. The crude product was transferred to a 50 ml beaker, dissolved in 20 ml sodium bicarbonate solution, 0.08 molar, pH 8.3, and hydrochloric acid (1.0 normal) added dropwise to a pH of 1.5. The product was allowed to precipitate at 4°C overnight. Vacuum filtration of the crude product was followed by washing with 1.0 normal HCl to remove any adhering impurities. The product was dried on unglazed porcelain chips, giving a yield of 9% of theoretical. Spectroscopic analysis showed an absorption at 515 nm, which agreed well with the data from Orndorff and Hitch[11]

To prepare 4,5,6,7-Tetrachloro-2',4',5',7'-tetrabromofluorescein-$^{77}Br_4$(2), 5 mg anhydrous manganese dioxide and 5 ml of sulfuric acid were placed in a double-neck, pear-shaped flask. One neck was fitted with a Dewar condenser. To the other neck was attached a glass tube to provide for the passage of an entraining air flow through the system. A glass side arm attached to the condenser directed the air-bromine mixture to the bottom of the bromination tube.

One-half to one millicurie of an aqueous solution of $Na^{77}Br$ contained in not more than 1 ml (MRC Cyclotron, Hammersmith Hospital, London, England) was introduced into the reaction flask. A slow air flow was started, the cold finger was charged with an ice-water mixture and gentle heat was applied using a Bunsen Burner. The $^{77}Br_2$ thus generated was bubbled into a test tube containing 5 mg of 1 in 4 ml of sodium bicarbonate solution (0.08 molar, pH 8.3) for 15 min or until the transfer of radioactivity was complete. To the resulting orange solution, 1.0 normal hydrochloric acid was added dropwise to pH 1.5, and the product (2) allowed to precipitate for 2 h (or overnight) at 4°C. The yield was 30% based on the starting radioactivity and corrected for

decay. The wavelength at maximum absorbance was 535 nm. (Phloxine-B, 537 nm).

Spectrophotometry (Beckman Model 25, Beckman Products, Fullerton, California 92634) was performed to confirm the identity and purity of 1 and 2. A modification of Conn's method[10] was used for these determinations in which 1 mg of the compound was dissolved in 10 ml sodium bicarbonate solution (0.08 molar) and the wavelength at maximum absorbance determined. Comparison was made with a sodium bicarbonate blank and a holmium oxide standard.

Column Chromatography was carried out using a 1 x 13 cm glass column packed with Sephadex G-25 fine (Pharmacia Fine Chemicals, Uppsala, Sweden) which had been pre-treated with 0.08 molar sodium bicarbonate solution. Hydrostatic pressure was used to maintain a flow rate of 1 ml per minute. After washing the adsorbant with bicarbonate solution, 0.5 mg of 2 in 0.08 molar bicarbonate solution was placed on the column. Using 0.08 molar sodium bicarbonate as the eluant, 5 and 10 ml fractions were collected in test tubes at the desired flow rate.

Ascending Thin Layer Chromatography of 2 was performed using the method reported by Vivian, et. al.[12] for $^{131}$I-Rose Bengal. I.T.L.C.-S.G. (Gelman Instrument Company, Ann Arbor, Michigan 48106) was used for the stationary phase. The solvent consisted of chloroform, acetic acid (9:1). Na$^{77}$Br and Phloxine-B were used as standards.

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REFERENCES

1. Poley J. and Smith W. - Clinical Nuclear Medicine 1:179 (1976)

2. Tubis M., Nordyke R., Posnick E., and Blahd W. - J. Nucl. Med. 2:282 (1961)

3. Preston D. - Personal Communication, Kansas University Medical Center, Kansas City, Kansas 66103

4. Wistow B., Subramanian G., Van Heertum R., Henderson R., Gagne G., Hall R., and McAfee J. - J. Nucl. Med. 18:455 (1977)

5. Ryan J., Cooper M., Loberg M., Harvey E., and Silorski S. - J. Nucl. Med. 18:997 (1977)

6. Weast, R. - Handbook of Chemistry and Physics, CRC Press, Inc., Cleveland, p. F-205 (1974)

7. Lederer C., Hollander J., and Pearlman I. - Table of Isotopes, John Wiley and Sons, Inc., New York, p. 213 (1967)

8. Slaunwhite W., Jr. and Neely L. - Anal. Biochem. 5:133 (1963)

9. Determann H. and Walter I. - Nature 219:604 (1968)

10. Lillie R. - H. J. Conn's Biological Stains, Williams and Wilkins Co., Baltimore p. 434 (1969)

11. Orndorff W., and Hitch F. - J. Am. Chem. Soc. 36:680 (1914)

12. Vivian A., Ice R., Shen V., and Hetzel K. - Radiochemical Purity of Radiopharmaceuticals, Procedure Manual, Gelman Instrument Co., Technical Bulletin 32, p. 32 (1977)