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# Reinvestigation of the Preparation of $^{131}$I-4-Iodoantipyrine from $^{131}$I-Iodide

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Radioiodine labeled 4-iodoantipyrine (4-IAP) is rapidly formed from radioiodide and nonradioactive 4-IAP or 4-bromoantipyrine (4-BrAP) at 100°C in acidic aqueous solution. Quantitative conversion to labeled 4-IAP is observed between pH 2 and 6 when 4-IAP is the nonradioactive starting material. This is in contrast to previous reports which found rapid incorporation only under limited conditions. When 4-BrAP is used, maximum yields of 80% are found between pH 1.5 and 2.5. Mechanistic implications of these differences are not clear. Labeled 4-IAP( $\geq$98% radiochemically pure) is separated from radioiodide by TLC or anion-exchange chromatography.

## Introduction

4-IODO-1,5-dimethyl-2-phenyl-3-pyrazolone, more commonly referred to as 4-iodoantipyrine (4-IAP), is a relatively lipophilic molecule which readily diffuses across cell membranes.[1-4] It has been shown that, immediately after injection, levels of labeled 4-IAP within brain tissue are higher than those for ionic tracers such as potassium-42.[5]

Recently, USZLER *et al.*[6,7] have studied the feasibility of directly assessing human CNS perfusion using 4-IAP and the scintillation camera. For these studies, iodine-123 was proposed as the label of choice because of the larger doses of radioactivity which can be safely administered to man.

Extension of this technique to the routine clinical setting is dependent upon a convenient, reliable source of $^{123}$I-4-IAP. In previous reports, $^{131}$I-4-IAP has been prepared by exchange between radioiodide[8,9] or elemental radioiodine[10] and unlabeled 4-IAP at elevated temperatures in acidic aqueous solution. Although the influence of pH, polarity of solvent, ionic strength, reactant concentrations, and intensity and wave length of illumination on these reactions have been studied, we chose to reinvestigate the effects of pH, time of heating at 100°C and quantity of starting material on the preparation of $^{131}$I-4-IAP from $^{131}$I-iodide because none of the methods previously reported were directly applicable to a "kit"-type labeling procedure. Also, since unlabeled 4-IAP is not a readily available starting material, interhalogen displacement labeling, using 4-bromoantipyrine (4-BrAP), was tested in addition to the direct radioiodine exchange reaction.

## Material and Methods

The effect of pH upon labeling was measured using standard reaction solutions containing 4 mg of 4-IAP† or 10 mg of 4-BrAP‡ in 3 ml of aqueous solution in a 10-ml serum vial. Acidity was adjusted by addition of 0.05 N HCl and the final pH was measured with a pH meter. 150 $\mu$Ci of $^{131}$I-iodide in 0.1 ml of aqueous solution was added and the vial was stoppered and sealed. The vial, containing the acidified reaction mixture, was then immersed in boiling water for 15 min. After the vial was removed from the boiling-water bath and allowed to cool, it was vented by insertion of a 25-gauge syringe needle and the pH of the reaction mixture was adjusted to 7.0 by addition of 1 ml of 0.1 M phosphate buffer (pH 7.0) containing a quantity of NaOH equivalent to that of the HCl initially added. The quantities of starting material used were selected for convenience and because of the limited solubility of 4-IAP ($\cong$1 mg ml$^{-1}$) compared with that of 4-BrAP ($\leq$7 mg ml$^{-1}$). To assure that, at these levels, the quantity of starting material had no significant effect upon labeling yields, 1 mg of 4-IAP and 25 mg of 4-BrAP at pH 2 were also used. Radiochemical analysis of reaction products was by thin-layer chromatography (TLC) on silica gel using 1:1 (v/v) toluene:ethyl acetate as solvent. With this analytical system, iodide remains near the origin while 4-IAP migrates with an $R_f$ value of 0.55, as illustrated in Fig. 1.

Preliminary studies, using both 4-IAP and 4-BrAP, had shown yields of $^{131}$I-4-IAP were maximum in the pH range between 1 and 3. Production of labeled

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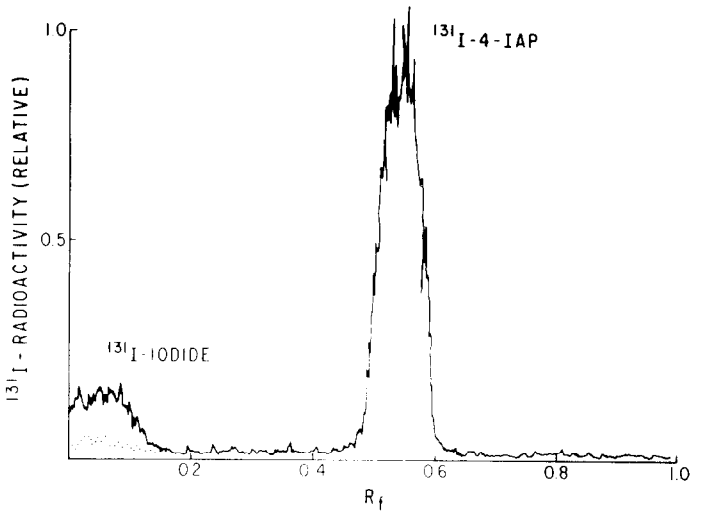


FIG. 1. Thin-layer chromatography on silica gel using 1:1 (v/v) toluene:ethyl acetate solvent shows $^{131}$I-iodide near origin and $^{131}$I-4-IAP at $R_f = 0.55$. Similar analysis shows less $^{131}$I-iodide (· · · ·) after anion exchange treatment of buffered $^{131}$I-4-IAP reaction mixture.

4-IAP at pH 2.0 was measured using reaction solutions prepared as described for time intervals from 2 to 60 min. Labeling during the first 2 min was determined by heating the acidified reaction mixture to boiling before addition of radioiodide. Addition of radioiodide to the heated solution was followed by sampling the radioactive mixture at 15, 30, 60 and 120 s. Radiochemical analysis of conversion of iodide to 4-IAP was by TLC as described previously.

## Results and Discussion

The effect of reaction pH upon radiochemical yields of $^{131}$I-4-IAP is shown in Fig. 2. When unlabeled 4-IAP served as starting material, yields of the labeled compound were greater than 80% in the pH range between 1 and 6. Over most of this range (pH 2–6) labeled 4-IAP was produced in essentially quantitative yields. A yield of 90% was obtained at pH 2 even when only 1 mg of 4-IAP was used. These findings contrast with previous reports[9, 10] in which high yields were found only under limited conditions. When 4-BrAP was used as the starting material, little labeling occurred above pH 3, and quantitative yields were never produced. Maximum labeling occurred only in the pH range between 1.5 and 2.5. Even using 25 mg of 4-BrAP at pH 2, only 93% yield was obtained: at pH 4, using this amount of 4-BrAP, the yield was less than 10%.

In Fig. 3, radiochemical yields of $^{131}$I-4-IAP are shown as a function of time of heating at pH 2.0. At this optimal pH, labeling yields of 90% or more were obtained regardless of the starting material used. Using 4-IAP, yields actually decreased from near 100% at 5 min to 94% at 60 min. Under these conditions, the reduction in radiochemical yield with increased heating time may be due to hydrolytic decomposition of the parent material. With 4-BrAP, yields in the 90% range were obtained at all heating times between 5 and 60 min. The reason for not producing higher ultimate yields when the 90% value is reached so rapidly is unknown.

Since quantitative yields of labeled 4-IAP were not achieved even under "ideal" conditions when 4-BrAP was used, the use of anion exchange for removal of radioiodide was studied. After a 4-BrAP solution containing 90% radiochemically pure $^{131}$I-4-IAP was passed through a 1-cm diameter × 2-cm column of "Dowex 1-X8" (chloride) and eluted with 1.0 ml of 0.9% NaCl, over 95% of the "free" radioiodide had been removed. TLC analyses before and after anion exchange treatment are shown in Fig. 1.

In other studies[9, 10] it has been assumed that preparation of labeled 4-IAP from its unlabeled counterpart under the conditions described here is by exchange rather than by addition of radioiodine.

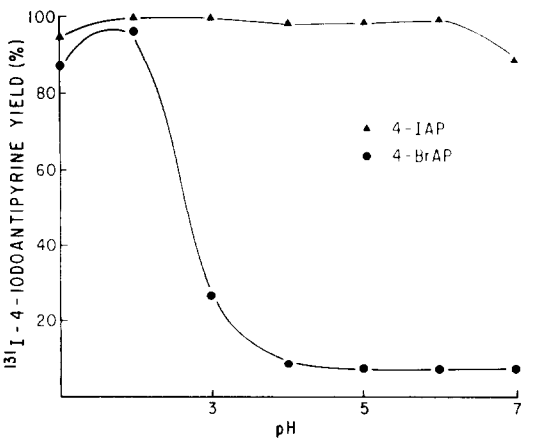


FIG. 2. Effect of reaction pH on radiochemical yield of $^{131}$I-4-IAP when 4 mg of 4-IAP (▲) or 10 mg of 4-BrAP (●) are used as unlabeled starting material.

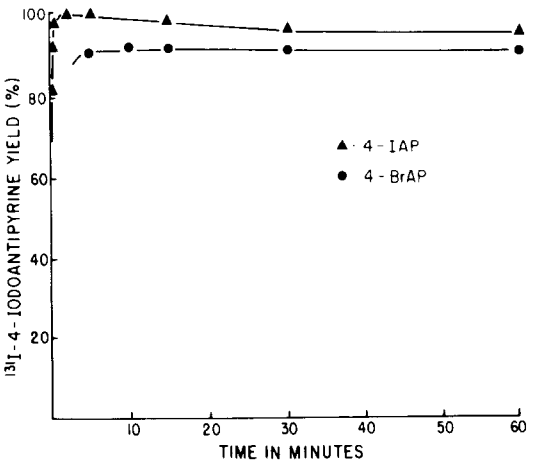


FIG. 3. Effect of time of heating (100°C) at pH 2 on radiochemical yield of $^{131}$I-4-IAP when 4 mg of 4-IAP (▲) or 10 mg of 4-BrAP (●) are used as unlabeled starting material.

Although no previous studies have been made, we assumed that formation of labeled 4-IAP from 4-BrAP and radioiodide would proceed via an analogous mechanism. The marked differences in effect of reaction pH on radiochemical yields when 4-IAP or 4-BrAP were used as the starting material were unexpected.

Inspection of the antipyrine structure, shown in Fig. 4, indicates that an electrophilic displacement mechanism is most likely, since the bond between carbon atoms in positions 4 and 5 is unsaturated. Indeed, KÖRÖS *et al.*,[10] after studies of the influence of solvent on the reaction between elemental radioiodine and 4-IAP, concluded that "exchange" between $I^+$ and 4-IAP was the dominant route and that the reaction occurs essentially instantaneously. In contrast, no reaction between $IO_3^-$, and 4-IAP was observed, with the reaction between $I^-$ and 4-IAP being extremely slow ($T_{\frac{1}{2}} \cong 2$ days). If this is the case, even transient formation of $I^+$ in an aqueous solution containing 4-IAP could lead to formation of labeled 4-IAP. Even so, if electrophilic displacement is involved the labeling reaction may not occur as readily in equivalent solution containing 4-BrAP compared with 4-IAP. This could be either because $Br^+$ (or an equivalent, more complex specie) is a poorer leaving group than $I^+$, or because the C–Br bond is stronger than the C–I bond. There is evidence that the stability of $I^+$ in aqueous solution is improved by increasing acidity.[11] The resulting increased lifetime of $I^+$ in acid solution, which allows longer interaction between a given $I^+$ and 4-BrAP, may be required for the electrophilic substitution to occur.

4-IODOANTIPYRINE

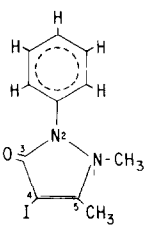


FIG. 4. Structure of 4-iodoantipyrine.

However, there seems to be no evidence to support even transient formation of $I^+$ under our experimental conditions. Indeed, other workers[9] suggest that their data are consistent with positive or negative reaction species or a neutral intermediate, but the basis for their conclusion is not stated and, from their data, it is not obvious why this should be so. Nucleophilic displacement, presumably by $I^-$, is an attractive alternative; however, such a mechanism, involving the electron-rich carbon atom in the 4 position, seems unlikely. Displacement or addition of radioiodide at an alternative position, though possible, does not seem likely because replacement of I by Br in the starting molecule should have no dramatic effect upon a reaction which occurs at a position far removed from the halogenated site. Thus, the exact mechanism by which labeling occurs remains uncertain.

Even so, as a result of our findings, and considering the commercial availability of $^{123}$I-iodide, we have recently reported development of a simple, reliable, "dual-buffer"-kit method for routine preparation of sterile, pyrogen free $^{123}$I-4-IAP.[12] The kit has been used successfully for preparation of $^{123}$I-4-IAP applied in preliminary clinical studies of direct CNS perfusion imaging.[6, 7]

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