

Synthesis of Labeled *o*-Iodohippuric Acid

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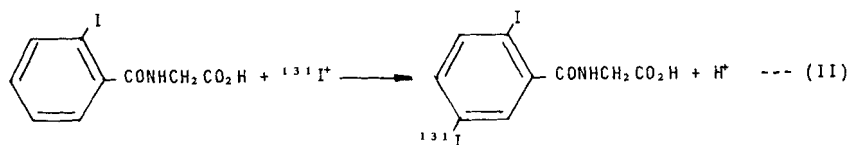
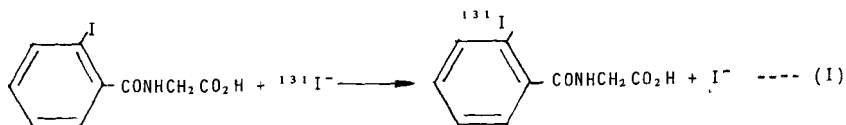
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SUMMARY

The reaction of *o*-iodohippuric acid with labeled iodide has been shown to afford exclusively labeled *o*-iodohippuric acid. The same compound has been synthesized from anthranilic acid. The rate of exchange was sufficiently rapid to permit the preparation of ^{132}I and ^{128}I labeled *o*-iodohippuric acid. The mechanism of the exchange is discussed.

INTRODUCTION

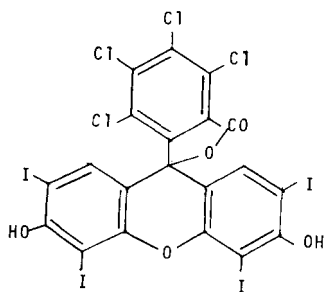
Labeled *o*-iodohippuric acid (hippuran) is usually prepared by exchange of iodohippuric acid with ^{131}I -labeled iodide ^(1, 2, 3, 4). The rate of exchange is relatively rapid (eqn. 1) ⁽⁵⁾, although nucleophilic displacement is not easy in aromatic compounds, and the *o*-iodo group is sterically hindered. The presence of free iodine is apparently necessary ⁽⁶⁾. This suggests an electrophilic substitution reaction, which would produce 2,5-diiodohippuric acid.



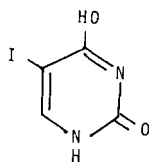
The exchange of the related iodobenzoic acids have been studied. The exchange of *o*-iodo-benzoic acid was also rapid and required the presence of sodium nitrite (7), which could oxidize iodide to iodine. The reaction has also been carried out under pressure at high temperatures (8, 9). The radiochemical purity of labeled *p*-iodo-benzoic acid formed by exchange has been confirmed (7). The specific activities of the *m*- and *p*-iodobenzoic acids were lower than that of the *o*-isomer due to the difficulty of exchange for the first two compounds. However, these facts are also consistent with electrophilic substitution, since the iodo group in *o*-iodobenzoic acid will direct the attack of an iodonium ion in the unhindered 5 position, whereas the corresponding 6 position in *m*-iodobenzoic acid is sterically hindered and deactivated by resonance from the carbonyl group, while substitution in the *p*-isomer can only occur in the hindered 3 and 5 positions.

The rate of exchange of *o*-iodohippuric acid was a maximum at pH 6 (6). This can be explained by the fact that at high pH iodine reacts with base, while at low pH the molecule is protonated thus reducing the rate of electrophilic attack. However, the effect of pH does not seem to be consistent with an exchange mechanism.

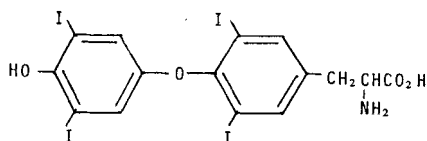
The purpose of this study was to determine the radiochemical purity of the product formed by the reaction of *o*-iodohippuric acid with radioiodine. Since nucleophilic displacement and electrophilic substitution can also occur with *o*-iodobenzoic acid, the reaction of this compound was first



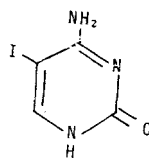
II



IV



III



V

studied. Labeled *o*-iodohippuric acid was also synthesized by an unequivocal route from anthranilic acid, and the rate of labeling of *o*-iodohippuric acid in water was determined.

EXPERIMENTAL

¹³¹I-o-Iodobenzoic Acid : by exchange.

O-Iodobenzoic acid (0.125 g; 0.5 mmoles) was dissolved in 1N sodium hydroxide (1 ml), 1M sodium nitrite solution (0.5 ml) added and the pH adjusted to 6.3 with 0.1N hydrochloric acid. Sodium iodide (106 mg; 0.7 mmole) containing ¹³¹I (20 μc) was added in water (2 ml). The mixture was heated to 100° for 2 hr. Chromatography on Whatman No. 4 paper using isopropanol-ammonium hydroxide-water (9 : 1 : 2) ⁽¹⁰⁾ showed the presence of labeled *o*-iodobenzoic acid (*R_f* 0.65) and iodide ion (*R_f* 0.60). No labeled 2,5-diiodobenzoic acid was detected (*R_f* 0.86). *o*-Iodobenzoic acid and 2,5-diiodobenzoic acid were not separated with *n*-butanol-acetic acid-water (4 : 1 : 1). The presence of the acids was detected by their fluorescence with ultraviolet light.

When *o*-iodobenzoic acid (0.125 g) was treated as above with sodium iodide (0.106 g) no 2,5-diiodobenzoic acid was detected in the paper chromatogram of the products. Heating at 100° for 24 hr with the same amount of sodium iodide, with an excess (0.157 g), or with iodine (0.127 g) also afforded only unchanged *o*-iodobenzoic acid as determined by m.p. and mixed m.p. with authentic *o*-iodobenzoic acid.

¹³¹I-o-Iodohippuric Acid from Anthranilic Acid.

Anthranilic acid (4 g, 29 mmoles) was dissolved in 10 % sulfuric acid (20 ml) and cooled to below 10°. Sodium nitrite (2.3 g, 33 mmoles) in water (2 ml) was added slowly and the resulting mixture added to potassium iodide (7.5 g, 45 mmole) containing labeled sodium iodide (10 μCi). The solution was then heated to boiling, cooled and filtered. The solid residue was washed with 5 % sodium thiosulfate. The residue was then extracted with sodium hydroxide and the labeled *o*-iodobenzoic acid precipitated by acidifying with dilute hydrochloric acid. The crude acid was further purified by chromatography on fluorosil and was eluted with acetone and methanol.

Labeled *o*-iodobenzoic acid (0.2 g) was heated with thionyl chloride (0.75 ml) and benzene (15 ml) for 1 hr. The reaction mixture was evaporated to dryness at reduced pressure, and the residue shaken with a solution of glycine (0.06 g) in 5 % sodium hydroxide (5 ml). The solution was then acidified with dilute hydrochloric acid. The precipitate formed was washed with water, dried and extracted with ether. The ether was evaporated to afford ¹³¹I-*o*-iodohippuric acid which was shown to be radiochemically pure by paper

chromatography. The same R_f value (of 0.74) was also given by 2,5-diiodohippuric acid ⁽¹¹⁾ in isopropanol-ammonium hydroxide-water.

¹³¹I-o-Iodohippuric Acid : by exchange.

O-iodohippuric acid (0.153 g) was dissolved in 1 N sodium hydroxide (1 ml), 1 M sodium nitrite solution (0.5 ml) added and the pH adjusted to 6.3 with 0.1 N hydrochloric acid. Sodium iodide (106 mg) and iodine-131 solution (20 μ Ci) were added in water (2 ml). The mixture was heated in a boiling water bath for 2 hr. The labeled o-iodohippuric acid was precipitated by acidifying the solution, and purified by redissolving in base and reprecipitating with acid, followed by recrystallization from acetone. The labeled compound gave an R_f value of 0.74, revealed by exposing the dried paper to chlorine gas and then spraying with 1 % potassium iodide and starch solution. The labeled compound was hydrolyzed by refluxing with concentrated hydrochloric acid for 24 hr. A precipitate formed on cooling, and had m.p. (162°) and R_f value (0.65) corresponding to o-iodobenzoic acid.

The purity of the labeled o-iodohippuric acid was also checked by reverse isotope dilution, by adding unlabeled compound and recrystallizing the mixture from acetone to constant specific activity.

Labeled o-iodohippuric acid was also obtained by carrying out the exchange without the addition of sodium iodide or sodium nitrite. Labeled o-iodohippuric acid was reacted under the same reaction conditions with unlabeled sodium iodide solution, when the radioactivity of the recovered compound was reduced.

RATE OF EXCHANGE

The exchange reaction was carried out as above and aliquots were taken at intervals of 10 minutes up to 1 hr. The incorporation of radioiodine was essentially complete in 10 min.

¹²⁸I- and ¹³²I-o-Iodohippuric Acid.

Iodine-128 was prepared by activating ethyl iodide in the reactor, diluting with chloroform and washing out the inorganic ¹²⁸I with 5 % sodium sulfite solution. Iodine-132 was prepared by washing a tellurium-132 generator with dilute aqueous ammonia ⁽¹²⁾. o-Iodohippuric acid was then labeled with ¹²⁸I and ¹³²I as above, with heating for 30 mins. The compounds were purified by precipitating with acid, washing, redissolving in base and reprecipitating and washing. The radiochemical purity of these compounds was confirmed by paper chromatography.

DISCUSSION

o-Iodobenzoic acid and 2,5-diiodobenzoic acid could be separated chromatography. However, in an exchange experiment of o-iodobenzoic acid, no labeled 2,5-diiodobenzoic acid was detected. 2,5-Diiodohippuric acid could not be separated by paper chromatography from o-iodohippuric acid. However, labeled o-iodohippuric acid prepared in the normal manner, was hydrolyzed and the only activity detected was due to o-iodobenzoic acid. The labeled o-iodohippuric acid was also shown by isotope dilution to be 100 % radiochemically pure. That the reaction was an exchange was also shown by the fact that labeled o-iodohippuric acid exchanged with unlabeled ionic iodine under the same conditions as in the exchange labeling experiments. The rate of exchange in water at 100° was also found to be rapid, being completed in about 10 min., and sufficiently fast to allow the preparation of ¹²⁸I and ¹³²I labeled o-iodohippuric acid. Thus the normal labeling procedure led to a rapid exchange producing exclusively labeled o-iodohippuric acid (hippuran). Although the exchange is normally carried out in the presence of unlabeled iodide ion and nitrite ion ^(1, 2, 3) labeling was also achieved using only carrier-free iodide. Labeled o-iodohippuric acid was also prepared from anthranilic acid via labeled o-iodobenzoic acid.

The exchange of hippuran in ethanol has been shown to be first order (in hippuran) ⁽⁴⁾. The exchange in acetonitrile of o- and p-iodonitrobenzene was second order ⁽¹³⁾, whereas m-iodonitrobenzene was first order using this solvent ⁽¹³⁾, and p-iodonitrobenzene first order using 2-octanol ⁽¹⁴⁾. The exchange in methanol of 1-iodo-2,4-dinitronaphthalene was also first order, but second order using acetone ⁽¹⁵⁾. In addition there were considerable differences in the rates of reaction (see Table I). However, a first order exchange reaction is consistent with an nucleophilic displacement mechanism in which the breaking

TABLE I. Rates of Exchange of Iodides

Iodide	Rate Constant	Ref.
o-Iodohippuric Acid (I)	~10 ⁻³ sec ⁻¹ , H ₂ O, pH 6.3, 100°	a
	2 × 10 ⁻⁶ sec ⁻¹ , EtOH, 55°	5
Rose Bengal (II)	5 × 10 ⁻³ sec ⁻¹ H ₂ O, 100°	b
Thyroxine (III)	4 × 10 ⁻³ sec ⁻¹ , H ₂ O, pH 7.5, 100°	b
5-Iodouracil (IV)	>10 ⁻² sec ⁻¹ , H ₂ O, pH 7, 50°	16
5-Iodocytosine (V)	3 × 10 ⁻⁴ sec ⁻¹ , H ₂ O, pH 7, 70°	16
o-Iodonitrobenzene	1.3 × 10 ⁻⁶ l mole ⁻¹ sec ⁻¹ , MeCN, 100°	13
1-Iodo-2,4-dinitro naphthalene	1.8 × 10 ⁻⁶ l mole ⁻¹ sec ⁻¹ , Me ₂ CO, 55°	14

^a Estimated from present work.

^b Estimated from ref. 12.

of the original carbon to iodine (^{127}I) bond is the rate determining step. Polarization of the bond will be facilitated by highly polar solvents such as methanol and 2-octanol, but was less easy in acetonitrile and acetone. The rates of exchange in aqueous solution at 100° of o-iodohippuric acid (I), Rose Bengal (II) ⁽¹³⁾, thyroxine (III) ⁽¹³⁾, 5-iodouracil (IV) ⁽¹⁶⁾ and 5-iodocytosine (V) ⁽¹⁶⁾ were all rapid. The order of ease of exchange was (see Table I), 5-iodouracil Rose Bengal \sim thyroxine $>$ 5-iodocytosine $>$ o-iodohippuric acid $>>$ o-iodo nitrobenzene. Thus an ortho hydroxy group caused greater activation than an ortho amino group, or o-carboxy and a o-nitro group had a smaller activating effect. The small steric effect of the side-chain for displacement of the ortho iodine atom in o-iodo-benzoic acid and hippuric acid must arise from the fact that in an $\text{S}_{\text{N}}2$ reaction attack of iodide ion will occur from the topside of the benzene ring, with the unlabeled iodide leaving from the underside. The side chain can then accommodate itself in the plane of the benzene ring by bending away from the ortho position, towards the 6-position of the ring.

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