LABELING PHARMACEUTICALS
WITH
RADIOACTIVE ISOTOPES

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SUMMARY

The purpose of this research is to prepare iodo- and bromo-aliphatic amino acid analogs labeled with γ -emitting isotopes (^{131}I , ^{123}I and ^{77}Br) for possible use as pancreas localizing agents.

Studies on the halogen exchange reaction (I- for Cl-) for the synthesis of β -iodo- α -aminobutyric acid (a valine analog) have suggested that the iodo compound was formed initially. However, the desired compound cannot be isolated because of its chemical instability. Distribution studies in rats with the crude halogen exchange reaction mixture confirmed this finding. Studies on the addition of hydrogen iodide to allylglycine under various conditions for the synthesis of γ -iodo- α -aminopentanoic acid (a leucine analog) suffered the same obstacle; the chemical instability of the desired iodo compound precludes isolation and characterization.

Convinced that the iodo analogs were too unstable for use as practical localizing agents, we turned to the possible use of Br for CH_3 substituted amino acids. The ^{14}C labeled β -bromo- α -aminobutyric acid methyl ester was synthesized. This methyl ester will be hydrolyzed and the distribution of free amino acid will be studied. Labeled with ^{77}Br this compound might be useful for pancreas localization.

INTRODUCTION

Amino acid analogs, such as selenomethionine, which can participate in metabolic processes have found use as pancreas localizing agents. Replacing one of the methyl groups on L-valine or L-leucine with an iodo or bromo group (compounds I and II) results in analogs which could have similar properties. In the past year we have continued our studies on the synthesis and biological behavior of these amino acid analogs.

Valine Analogs

Leucine Analogs

I. STUDIES ON THE SYNTHESIS OF β -IODO- α -AMINO BUTYRIC ACID (Ib) - A VALINE ANALOG

A. Direct Halogen Exchange

Beta-chloro- α -aminobutyric acid (III) was prepared as described in the previous progress report (ref. 1). Studies on the direct halogen exchange reaction between compound (III) and sodium iodide under various conditions have been carried out. Refluxing in acetone or aqueous solution at different pHs or heating the reaction mixture in a steel bomb failed to yield the desired iodo compound. Under most of the conditions the starting compound was recovered.

B. Halogen Exchange with the Trimethyl-Silyl Derivative

One major problem in the synthesis of β -iodo- α -aminobutyric acid (Ib) from the corresponding β -chloro compound (III) by the direct halogen exchange reaction was that the starting compound (III) is not acetone-soluble. In order to carry out the iodo-chloro exchange reaction it is essential to solubilize the starting compound in acetone. The lipid-soluble silyl derivatives (ref. 2) of various amino acids have been used for the synthesis of amino acid derivatives and for mass spectroscopic studies. After the halogen exchange reaction, the silyl groups can be hydrolyzed by a simple treatment with a protic solvent such as methanol or ethanol.

The silylation was carried out by refluxing a suspension of compound (III) and hexamethyldisilazane in toluene. After two hours, the clear solution was condensed to syrup which was then mixed with sodium iodide in dry acetone and heated to reflux. The precipitate formed was collected by filtration and accounted for ~90% of the expected sodium chloride. The filtrate was condensed and coevaporated with absolute ethanol to hydrolyze the silyl protecting groups. Chromatography (TLC) of the resulting mixture showed a ninhydrin positive spot which had a different R_f value than the starting compound (III). This data strongly suggests the halogen exchange reaction has proceeded. However, in several attempts, only the decomposed product (shown by NMR spectrum) was recovered after the work-up. The desired product was not successfully isolated due to the instability of the C-I bond in this compound.

C. Distribution of the Trimethylsilyl Derivative of ^{125}I β -Iodo- α -Aminobutyric Acid in Rats

Beta-chloro- α -aminobutyric acid (III) was converted to the trimethylsilyl derivative of ^{125}I β -iodo- α -aminobutyric acid by the above method. The crude mixture obtained after the halogen exchange reaction was dissolved in saline and used directly without further purification. The results are shown in Table I.

Table I

Distribution of the Trimethylsilyl Derivative of ^{125}I β -Iodo- α -Aminobutyric Acid in Holtzman Rats (% Dose/organ, average value for two rats).

<u>Organ/Time</u>	<u>30 min.</u>	<u>1.5 hr.</u>	<u>2.5 hr.</u>	<u>4 hr.</u>
Blood	11.62	9.04	6.93	5.44
Pancreas	0.13	0.11	0.07	0.05
Stomach	8.77	12.62	11.50	9.59
Liver	2.94	4.61	1.92	1.33
Thyroid	1.26	4.61	3.93	5.15
Kidney (2)	0.73	0.58	0.44	0.37

The distribution was very similar to that for iodide ion. Even without attempting to hydrolyze the silyl groups, it is clear that the iodo compound is too unstable to be useful.

D. Synthesis of Methyl 3-Iodo-2-Acetamidobutyrate (VI)

In order to study the synthesis of the desired iodo valine analog and investigate the stability of the C-I bond, it was of interest to "mask" the amino and carboxylic groups with protecting groups. It was thought that compound (VI) would be relatively more stable. Therefore, after the halogen exchange reaction the compound could be characterized and the chemical stability could be studied.

Methyl 3-chloro-2-acetamidobutyrate (V) was synthesized by stirring compound (IV) (synthesized by the method reported previously, ref. 1) in a mixture of pyridine and acetic anhydride at room temperature for 5 hours. After work-up, a syrup was obtained (yield 51%) and the NMR spectrum of this syrup showed a characteristic absorption peak for the acetyl group (CH_3CO , δ 2.0).

Methyl 3-chloro-2-acetamidobutyrate (V) and sodium iodide (containing 1 mCi of Na^{125}I) were refluxed in acetone. The white solid which precipitated from the solution was collected by filtration and identified as sodium chloride. The acetone filtrate was concentrated, redissolved in chloroform and again filtered.

The chloroform solution, presumably containing the desired iodo compound (VI) was then partitioned between 5 ml. of chloroform and 5 ml. of water. Unfortunately, most of the radioactivity was washed into the aqueous layer (95.2%). Only 4.8% of the radioactivity remained in the chloroform layer.

The same reaction was carried out by heating the reaction mixture in a steel bomb for 2 hours at 80-95°. From the resulting mixture sodium chloride was isolated and identified. The radioactivity in the chloroform solution was readily washed into water after shaking the chloroform solution with water in a separatory funnel.

These two experiments suggest that the halogen exchange reaction did take place. However, the product (VI) was readily decomposed by shaking with water. It is likely that the decomposition of the desired compound (VI) by water is a S_N1 reaction which reflects the weak bond energy and high polarizability of the C-I bond.

II. STUDIES ON THE SYNTHESIS OF γ -IODO- α -AMINOPENTANOIC ACID (IIb)- A LEUCINE ANALOG

A. Preparation of γ -Iodo- α -Aminopentanoic Acid (IIb)

In an attempt to add one mole of hydrogen iodide to the double bond of allylglycine (VII), compound (VII) was stirred in a mixture of potassium iodide and concentrated phosphoric acid (70%) at room temperature for 22 hours (ref. 3). Chromatography (TLC) of the resulting mixture showed a ninhydrin positive spot which has a R_f value different from that of the starting compound (VII) (solvent system, EtOH:H₂O 5:1; allylglycine R_f = 0.33, unknown R_f = 0.64). In the presence of Na¹²⁵I and under the same reaction conditions, the radioactivity was found to be associated with the ninhydrin positive spot (R_f = 0.72, 58% of the total radioactivity). However, the separation of the desired amino acid was not successful. The unsuccessful separation is most likely due to the fact that both the amino acid and inorganic side products (potassium iodide and sodium phosphate) are soluble in aqueous solution only. It was concluded that this reaction was not useful for the preparation of the desired compound (IIb).

Sodium iodide and concentrated phosphoric acid were used in order to generate hydrogen iodide in situ. Hydrogen iodide gas dissolved in alcohol would be equivalent to the reagents used for the addition reaction and the product separation would not be a problem.

Allylglycine (VII) was heated in a sealed glass bomb with 3 ml. of abs. ethanol presaturated with hydrogen iodide gas at -30° . After 5 hrs., the reaction mixture became dark brown and an oily layer was separated. These seemed to suggest that polymerization and/or decomposition had taken place. No further investigation was carried out on this particular reaction.

III. STUDIES ON THE SYNTHESIS OF β -BROMO- α -AMINOBUTYRIC ACID (Ia)

From what we have learned in the past year, it is clear that the iodo analogs of aliphatic amino acids are very unstable molecules. In several cases the iodo compound was formed initially but decomposed during the subsequent work-up. It would not be fruitful to synthesize an amino acid analog which cannot survive even such mild conditions. We have therefore turned our attention to more stable analogs, particularly β -bromo- α -aminobutyric acid (IIa) which was mentioned in our last progress report (ref. 1).

We have chosen to synthesize ^{14}C labeled β -bromo- α -aminobutyric acid and to study its biological distribution. The feasibility of this compound as a pancreas localizing agent will be determined and then the γ -emitting ^{77}Br compound will be prepared.

At the time of writing the synthesis of ^{14}C labeled methyl β -bromo- α -aminobutyrate hydrogen bromide has been completed. The compound (VIII) was prepared by following the procedure reported previously (ref. 1). Some modifications which were necessary for small scale synthesis were adopted. The hydrolysis of compound (VIII) to its free acid on a small scale (50 mg.) is being studied. The free amino acid (IX) will be characterized and the biological distribution will be studied.

A. Preparation of ^{14}C -Labeled Methyl β -Bromo- α -Aminobutyrate Hydrogen Bromide

Uniformly labeled ^{14}C -L-threonine (500 μCi) was diluted with cold L-threonine (100 mg, 0.84 mmol). Methanol (2 ml) in a 10 ml. round bottom flask was cooled to 0°C by an ice-bath and 0.11 ml. of thionyl chloride was added. The methanol solution was then transferred by a pipet into a vial containing the L-threonine. The solid L-threonine was dissolved in the methanol solution and transferred back to the original 10 ml. round bottom flask. The mixture was heated to reflux for 3 hours. The solvent was evaporated by a stream of nitrogen, 1 ml. of methanol was added, and again evaporated. The syrup thus obtained was attached to a vacuum pump for 20 minutes and the residue was directly used for the next reaction step.

Fresh phosphopentabromide was prepared by adding 0.1 ml. of bromine in 1 ml. chloroform into a phosphotribromide solution (0.24 ml. of phosphotribromide in 1 ml. chloroform) in an ice-bath. The yellow slurry was transferred quickly into the flask containing the previous syrup residue and the flask for preparing phosphopentabromide was rinsed with 1 ml. of chloroform.

The resulting reaction mixture was stirred at room temperature for 2 hours. The solvent was evaporated by a stream of nitrogen and the residue was treated with 1 ml. of methanol and again evaporated to dryness with nitrogen. The process was repeated twice. The final residue was dissolved in 1 ml. methanol and transferred into a cone-shaped test tube. The reaction flask was rinsed with 0.5 ml. methanol and the solution was evaporated to 1 ml. by a stream of nitrogen. The methanol solution was treated with 9 ml. of anhydrous ether and mixed well by a Vortex mixer. The ether layer was decanted. The pink solid was redissolved in 1 ml. methanol and again treated with 9 ml. of anhydrous ether. After keeping in a freezer for 3 hours, the mixture was spun for 10 minutes (1,000 rpm) and the ether was decanted. The white needles obtained were again washed with 6 ml. of anhydrous ether. The resulting white crystals were first dried by a stream of nitrogen and then by a vacuum pump for 20 minutes. The white crystals gave 0.133 g. of the desired product (0.48 mmol, yield 57.2%). The IR spectrum was exactly the same as that of the authentic cold material.

In a parallel cold synthesis, the product showed exactly the same spectroscopic data (both NMR and IR) as those reported last year (ref. 2). A small sample was sent for analysis:

$C_5H_{11}NBr_2O_2$; 274.966,

Calc'd: C 21.84; H 4.03; N 5.09

Found: C 21.63; H 3.97; N 5.02.

PERSONNEL

Contribution of principal investigators will be the same as previous contract period; principal investigator, Monte Blau, Ph.D. (50% of time, 12 months) and co-investigator, Merrill A. Bender, M.D. (5% of time, 12 months).

Since January 2, 1976 the full-time chemist working on this project has been Hank F. Kung, Ph. D.

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