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New Compounds: Synthesis of Aliphatic Seleno Amino Acids as Potential Pancreatic Imaging Agents

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Abstract □ External imaging of the pancreas is made possible by taking advantage of that organ's large requirement for exogenous amino acids. The only successful approach thus far has been the substitution of selenium for the sulfur atom in amino acids naturally containing sulfur, specifically selenomethionine labeled with selenium-75. The synthesis of a class of selenium-containing amino acids has been undertaken where the selenium atom replaces a methylene group of common amino acids that do not contain a sulfur atom. Reported here are the synthesis and toxicological evaluation of one such analog, 3-[(2-aminoethyl)selenyl]alanine (L-4-selenalysine).

Keyphrases □ Seleno amino acids, aliphatic—synthesis of 3-[(2-aminoethyl)selenyl]alanine, potential pancreatic imaging agent, toxicological evaluation □ Amino acids, aliphatic—selenium substituted, synthesis of 3-[(2-aminoethyl)selenyl]alanine, potential pancreatic imaging agent, toxicological evaluation □ Pancreatic imaging agents, potential—3-[(2-aminoethyl)selenyl]alanine synthesized, toxicological evaluation

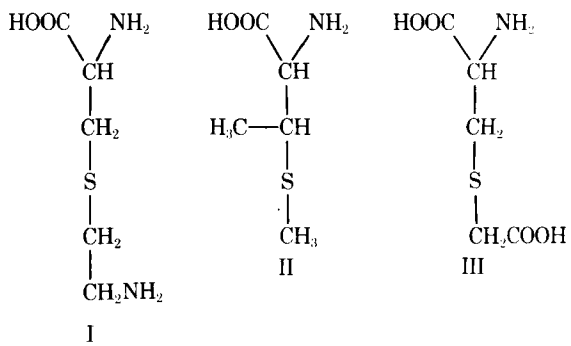
It is well known that the pancreas has a large requirement for exogenous amino acids. This specific characteristic was the basis for choosing radiolabeled amino acids to detect pancreatic tumors. Hansson (1) demonstrated this feature by using ^{14}C- and ^{35}S-labeled amino acids and proved that these compounds localized in the exocrine portion of the organ.

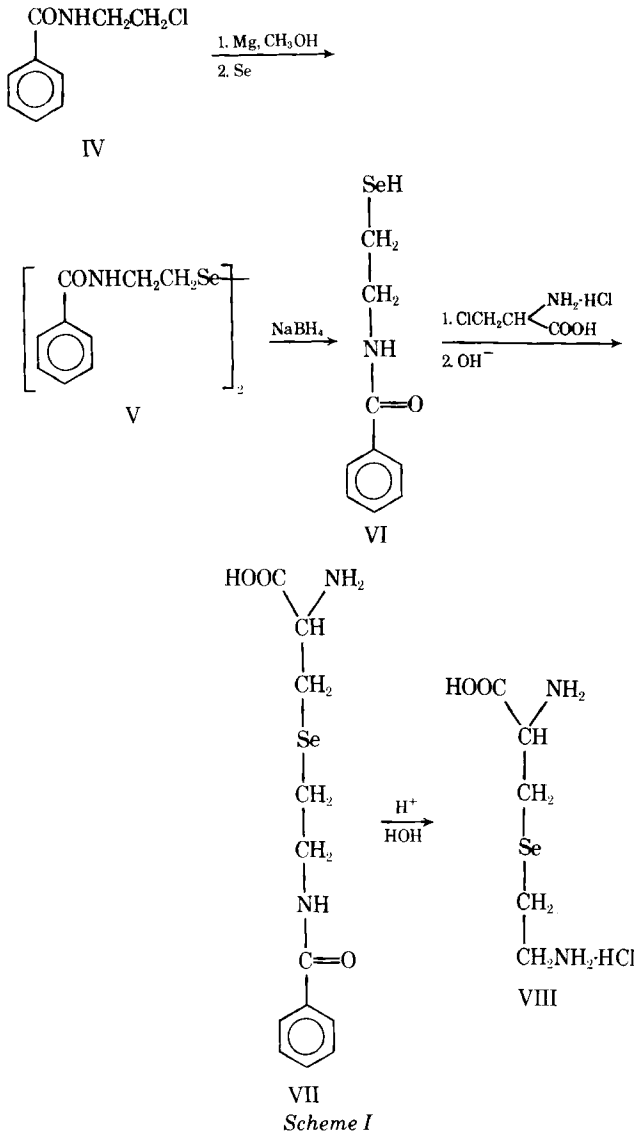
To obtain gamma-emitting amino acids for external detection and visualization of the pancreas, it was proposed to replace the sulfur atom in sulfur-containing amino acids such as methionine and cysteine. ^{75}Se-Selenomethionine was subsequently synthesized (2) but has achieved only limited clinical utility as a pancreatic imaging agent due to the unfavorable pancreas to liver concentration ratio. Several studies comparing ^{14}C-labeled amino acids showed that methionine has one of the poorest pancreas to liver ratios of all naturally occurring amino acids (3).

DISCUSSION

The sulfur analogs of numerous natural and synthetic amino acids have been synthesized but no attempts have been made to substitute selenium for the hetero sulfur atom. Compounds I–III are prime examples of reported sulfur analogs of common amino acids.

Compound I is a derivative of alanine, 3-[(2-aminoethyl)thio]alanine, but can also be considered as a hetero analog of lysine (4-thialysine). This compound has been shown to be a potent inhibitor of lysine utilization (4). Compound II, 3-methyl-3-(methylthio)alanine, is an isostere of isoleucine (4-thiaisoleucine and 4-thiaalloisoleucine) (5). α-Aminoadipic acid is a lysine precursor, and Compound III, 3-[(carboxymethyl)thio]alanine, can be viewed as a sulfur-containing α-aminoadipic acid (4-thia-α-aminoadipic acid) (6).





Scheme I

Interest in obtaining an improved agent for external visualization of the pancreas has led to an extensive synthetic program involving selenium-containing amino acids. This first report describes the successful synthesis and toxicological evaluation of 3-[(2-aminoethyl)selenyl]alanine (L-4-selenalysine), the selenium analog of I.

Chemistry—Reaction of benzoyl chloride with ethyleneimine gave *N*-(2-chloroethyl)benzamide (IV) in 75% yield (7). Refluxing IV with a solution of dimethoxydimagnesium diselenide (8) afforded bis[(2-aminoethyl)-*N*-benzoyl] diselenide (V) in 88% yield (9). Reduction of V with sodium borohydride or sodium amalgam gave (2-aminoethyl)-*N*-benzoylselenol (VI) which, upon subsequent treatment with L-3-chloroalanine (10), formed the sparingly soluble 3-[(2-aminoethyl)selenyl]-*N*-benzoylalanine (VII), also called ϵ-*N*-benzoyl-4-selenalysine.

Hydrolysis of VII with dilute hydrochloric acid led to the formation of the desired 3-[(2-aminoethyl)selenyl]alanine (L-4-selenalysine) (VIII), which was isolated as the monohydrochloride (overall yield 53%) (Scheme I). The analogous sulfur-containing compound (I) is well known (11).

Toxicology—Acute toxicity studies were carried out in Ness Ziona strain albino mice (20 ± 2 g). The LD_{50} for three different routes of administration, subcutaneous, intraperitoneal, and intravenous, was determined to be 130, 110, and 95 mg/kg, respectively. No physiological or behavioral changes were noted when sublethal doses were given.

Following the administration of lethal quantities, increased body activity accompanied by tremors, twitching, and labored breathing was readily apparent. Lethality was not immediate, however, but took place from 2 to 24 hr postadministration. When a dose four times the LD_{50} was given, death occurred within minutes. The relative lack of toxicity of selenalysine was an interesting finding since it had been shown that selenomethionine has an LD_{50} (intraperitoneal administration) of 4.25 mg/kg (12).

EXPERIMENTAL

Melting points[1] were determined for all of the solid compounds isolated, and elemental analyses[2] were performed for VII and VIII. Precoated silica gel plates[3] were used for TLC with the following solvent systems: A, 1-butanol–acetic acid–water (4:1:1); B, 96% ethanol–34% ammonium hydroxide (7:3); and C, methanol–isopropanol–34% ammonium hydroxide (5:3:2).

Plastic-backed TLC plates were found to have superior flow characteristics and higher resolving power compared to aluminum-backed plates, and all R_f values reported are for the plastic-backed plates. All synthetic reactions with selenium-containing compounds were performed in a hood because there is the possibility of hydrogen selenide release in several reactions.

Bis[(2-aminoethyl)-*N*-benzoyl]diselenide (V)—Compound IV (19.5 g), dissolved in methanol, was added to a methanolic solution of dimethoxydimagnesium diselenide (8 g of selenium) under gentle reflux. Refluxing was continued until the development of the typical golden-yellow color of organic diselenides was complete. The solution was then cooled, poured into cold water, and filtered. The precipitate was washed repeatedly with 10% HCl followed by a final rinse with cold water. This solid was crystallized from hot 96% ethanol, giving 20 g (88%), mp 144–145° [lit. (9) mp 144–145°].

(2-Aminoethyl)-*N*-benzoylselenol (VI, Not Isolated)—Compound V (22 g) was dissolved in 200 ml of ethanol and reduced with 5% sodium amalgam (100 g) or sodium borohydride (1.5 g) until the indicative diselenide color was discharged. Compound VI was not isolated but rather reacted immediately with chloroalanine to produce VII.

3-[(2-Aminoethyl)selenyl]-*N*-benzoylalanine (VII)—L-3-Chloroalanine hydrochloride (16 g) in aqueous solution was carefully neutralized with 10% NaOH and added to the solution of VI, which was kept at 35°. The stirred mixture was kept at pH 10 by the addition of 10% NaOH as required. A few crystals of sodium borohydride were added from time to time to prevent selenol oxidation.

After stirring at 35° for 3 hr, the solution was filtered, extracted with chloroform, brought to pH 6.5 with dilute acid, and left to crystallize. The material was purified by dissolution in the minimal volume of 10% HCl followed by filtration and reprecipitation by pH adjustment to 6.5 with ammonium hydroxide. The compound was recrystallized from boiling water, yielding 24.6 g (80%), mp 184°.

Anal.—Calc. for $C_{12}H_{16}N_2O_3Se$: C, 45.8; H, 5.1; N, 8.9; Se, 25.1. Found: C, 45.7; H, 5.2; N, 9.3; Se, 24.8.

3-[(2-Aminoethyl)selenyl]alanine (L-4-Selenalysine) (VIII)—Hydrolysis of VII was accomplished by refluxing in a 20-fold excess of 6 *N* HCl until a clear solution resulted (~3 hr). Upon cooling, benzoic acid crystallized out and was removed by filtration followed by an ether extraction of the filtrate. The aqueous phase was brought to pH 7 with ammonium hydroxide, whereupon any nonhydrolyzed starting material (VII) precipitated and was filtered off and recycled.

The filtrate was taken to dryness under vacuum, dissolved in a minimum volume of water, and refiltered. Absolute ethanol was added dropwise to the filtrate until the first sign of incipient turbidity appeared. The solution was then placed in the cold and allowed to crystallize upon standing (quantitative yield). Crystals of L-4-selenalysine monohydrochloride were obtained (small, colorless needles), mp 206°; TLC: one spot, R_f 0.78 (Solvent System B), R_f 0.76 (Solvent System C).

Anal.—Calc. for $C_5H_{13}ClN_2O_2Se$: C, 24.3; H, 5.3; Cl, 14.4; N, 11.3; Se, 31.9. Found: C, 24.4; H, 5.5; Cl, 14.4; N, 11.5; Se, 31.8.

[1] Gallenkamp capillary melting-point apparatus (uncorrected).

[2] Performed by Microanalytical Laboratory, Hebrew University, Jerusalem, Israel, and the Alfred Bernhardt Micro-Analytisches Laboratorium, Elback, German Federal Republic.

[3] C. Schleicher and Schull FR 1500 kieselgel.

Chromatography—L-3-Chloroalanine, because of its lability, must be applied to the TLC chromatogram as a fresh aqueous solution of the hydrochloride (1%) and dried immediately with a stream of warm air. The chromatogram was developed with Solvent System A, and the location of material was visualized by spraying with 1% ninhydrin in 1-butanol followed by heating at 80° until the pink spot of chloroalanine was clearly visible (about 7 min); R_f 0.31.

Comparative chromatography of L-lysine and the two isosteres, L-4-thialysine (I) and L-4-selenalysine (VIII) was carried out with their hydrochlorides. The highly polar nature of these amino acid hydrochlorides requires equally polar solvents for chromatographic separation, and Solvent Systems B and C were found to be satisfactory. The values for the hydrochloride of L-lysine, L-4-thialysine, and L-4-selenalysine were R_f 0.47, 0.75, and 0.78 in Solvent System B and R_f 0.37, 0.75, and 0.76 in Solvent System C, respectively. These results show the greater polarity of L-lysine compared to its sulfur and selenium analogs as well as the close similarity between the two hetero analogs.

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New Compounds: Synthesis of *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine Hydrochloride

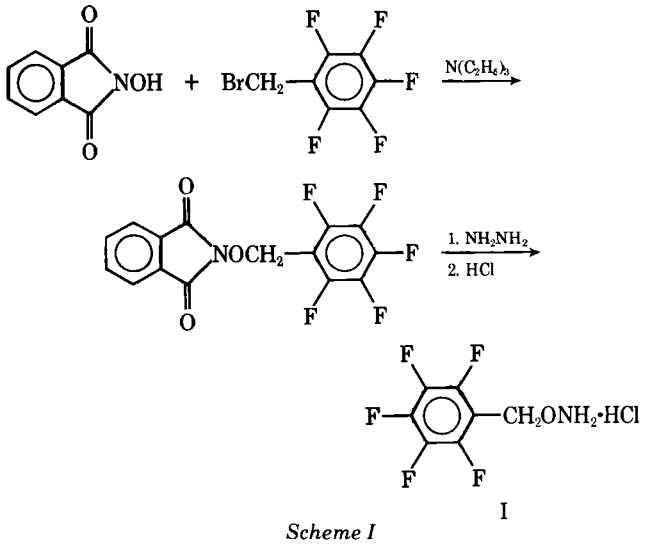
GILBERT A. YOUNGDALE

Abstract □ Reaction of 2,3,4,5,6-pentafluorobenzyl bromide with *N*-hydroxyphthalimide produced *N*-(2,3,4,5,6-pentafluorobenzyloxy)phthalimide which, after hydrazinolysis and treatment with hydrogen chloride, yielded *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride. The latter compound was used to derivatize keto steroids for their analysis by electron-capture GLC.

Keyphrases □ *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride—GLC derivatization reagent, synthesized from pentafluorobenzyl bromide □ GLC derivatization reagent—synthesis of *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride

A recent article (1) described the use of *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (I) to derivatize keto steroids for their analysis by electron-capture GLC. The synthesis of I is reported in this article.

Synthesis of I was achieved following the general method of McKay *et al.* (2). Reaction of 2,3,4,5,6-pentafluorobenzyl bromide with *N*-hydroxyphthalimide produced *N*-(2,3,4,5,6-pentafluorobenzyloxy)-phthalimide which, after hydrazinolysis and treatment with anhydrous hydrogen chloride, yielded I (Scheme I).



I

Scheme I