with salt and extracted with CH_2Cl_2. The organic solution was washed with saturated brine, and the solvent was removed under reduced pressure: 3.4 g (37%) of crude ethyl 5,5,5-trifluoro-4-(trifluoromethyl)-2-azidovalerate was obtained. This material was dissolved in 100 ml of absolute EtOH and hydrogenated for 18 hr (1 g of 5% Pd–C). The filtered solution was saturated with HCl and evaporated to dryness. The residue was dissolved in 50 ml of concentrated HCl; the solution was refluxed for 4 hr and evaporated to dryness. The residue was dissolved in 50 ml of absolute EtOH, 10 ml of dry pyridine was added, and the mixture was kept in the refrigerator overnight. The precipitated white crystals were collected by filtration and recrystallized from H_2O–EtOH to give 0.92 g (13%) of **5**, mp 234–237° dec (sealed tube). *Anal.* ($C_6H_7F_6NO_2$) C, H, N.

Oxidation of Methionine and Trifluoromethionine.—To a mixture of 15.0 mg of methionine and 12.5 mg of trifluoromethionine were added 0.022 ml of concentrated HCl, 0.15 ml of H_2O, 0.20 ml of MeOH, and 0.10 ml of 30% H_2O_2. The mixture was left at room temperature for 3 hr, then freeze-dried. The dry residue was taken up in 25 ml of citrate buffer (pH 2.2), and appropriate aliquots were analyzed on a Phoenix Scientific Co. automatic amino acid analyzer. Under these conditions, all the methionine was converted to the corresponding sulfoxide and sulfone, whereas approximately 50% of the trifluoromethionine was converted into a derivative assumed to be the sulfoxide. All products now could be clearly distinguished from one another.

pK_a Measurements.—The pK_a measurements were carried out as described by Walborsky and Lang.[7]

Growth Experiments with Various Strains of *E. coli*.—The following strains of *E. coli* were used in our growth studies: *E. coli* B-14 Leu$^-$, *E. coli* K_{12} W-3100 (standard wild type ATCC 15153), *E. coli* K_{12} Val$^-$ (isolated at Du Pont), *E. coli* B (wild type ATCC 11303), and *E. coli* $K_{12}W_6$ 58-141 (RC^{rel} Met$^-$). Bacteria were grown in a chemically defined medium containing per liter, 7.0 g of Na_2HPO_4, 3.0 g of KH_2PO_4, 1.0 g of NH_4Cl, 0.13 g of $MgSO_4 \cdot 7H_2O$, at pH 7.0, and supplemented as required for the various auxotrophs with 20 μg/ml of the amino acids or their fluorinated analogs. Growth was followed by determinations of cells/ml of bacterial density with a Klett colorimeter using a green filter. For total amino acid analyses of bacterial proteins, the bacteria were pelletted by centrifugation, and the pellets were washed successively with 10% trichloroacetic acid and H_2O. The samples were then lyophilized, hydrolyzed with 10 N HCl, and analyzed in the usual manner.

Acknowledgments.—The authors express their thanks to Mr. D. W. Krause for the pK_a measurements.

Iodinated Phenylalanines. Tests for Selective Localization in Pancreas and Preparation of 3,4,5-Triiodophenylalanine[1a]

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Received August 17, 1967

Amino acids are among the very few classes of compounds which show any significant selective localization in pancreatic tissue.[2,3] A radioactive amino acid derivative, ^{75}Se-selenomethionine, has been used with limited success to visualize the pancreas of the human being and the dog by isotope scanning techniques.[4–6] Roentgenographic visualization of the pancreas would be a powerful diagnostic tool and in theory might be achieved with a heavily iodinated amino acid provided it retained its affinity for the pancreas after iodination. There is an excellent review of the literature by Peskin and Johnson.[3]

We have prepared two iodinated phenylalanine derivatives and have tested them for selective localization in pancreatic tissue of the rat. Initial work was done with the known[7] 4-iodophenylalanine, which we prepared from 4-iodobenzyl bromide by the diethyl acetamidomalonate method. Tests on two rats showed that the administration of 4-iodophenylalanine did cause an increase in the iodine content of the rat pancreatic tissue (Table I), although, as anticipated, this iodine level was too low to produce radiopacity.

TABLE I

IODINE CONTENT OF RAT TISSUE

4-Iodophenylalanine, mg iv or sc	Tissue	Total iodine found, mg	mg of iodine/g of tissue
Controls (av of 11 samples)	Pancreas	1.9 ± 0.57[a]	3.9 ± 1.1[a]
10.0	Pancreas	2.9	...
66.25	Pancreas	4.3	6.1
Controls (av of 10 samples)	Liver	4.1 ± 1.2[a]	1.7 ± 0.4[a]
10.0	Liver	10.3	...
66.25	Liver	8.0	3.2

[a] Average deviation.

In theory a triiodinated phenylalanine should offer a better chance of obtaining the required iodine concentration. However, none of the possible di- or triiodophenylalanine isomers has been reported up to the present time. We succeeded in preparing one of these, 3,4,5-triiodophenylalanine, from a condensation of diethyl acetamidomalonate with the previously unknown 3,4,5-triiodobenzyl bromide followed by work-up in acid medium.

By far the most difficult step in this synthesis was the preparation of 3,4,5-triiodobenzyl bromide (TIBB). Low yields (15%) of TIBB were finally obtained by treating 3,4,5-triiodotoluene with N-bromosuccinimide in the presence of relatively large amounts of the catalyst, dibenzoyl peroxide, added throughout the course of the reaction. The product was identified as the desired isomer by means of its chemical properties and nmr spectrum. Once sufficient quantities of TIBB were on hand, preparation of 3,4,5-triiodophenylalanine (TIPA) proceeded smoothly with good yields.

TIPA was tested for selective localization in pancreatic tissue of rats in the form of its somewhat more soluble hydrochloride salt (TIPA·HCl). Young adult female rats were starved for 24 hr, then fed and injected either intravenously or subcutaneously with 35.3–66.0 mg of TIPA·HCl in propylene glycol solution. Each animal was sacrificed 1 hr after injection, and the pancreas and liver were analyzed separately for iodine. Control animals were treated by exactly the same

(1) (a) This work was supported by a grant (C-2981) from the U. S. Public Health Service, National Institutes of Health. (b) To whom requests for reprints should be sent.

(2) V. B. Schatz, B. C. O'Brien, W. M. Chaddlerk, A. M. Kaner, A. Burger, and W. R. Sandusky, *J. Med. Pharm. Chem.*, **2**, 425 (1960).
(3) G. W. Peskin and C. Johnson, *Am. J. Med. Sci.*, **249**, 223 (1965).
(4) M. Blau and R. F. Manske, *J. Nucl. Med.*, **2**, 102 (1961).
(5) M. Blau and M. A. Bender, *Radiology*, **78**, 974 (1962).
(6) M. C. Anderson, J. M. Beal, D. N. Sim, M. K. Copass, and J. L. Quinn, Department of Surgery, Northwestern University Medical School, Chicago, Ill., personal communication.
(7) (a) H. L. Wheeler and S. H. Clapp, *Am. Chem. J.*, **40**, 458 (1908); (b) E. H. Zeller, G. Ramachander, G. A. Fleisher, T. Ishimaru, and V. Zeller, *Biochem. J.*, **95**, 262 (1965).

Table II
Iodine Content of Rat Tissue

3,4,5-Triiodophenylalanine·HCl (TIPA·HCl), mg	Tissue	Iodine added, mequiv	Total iodine found, mequiv	Iodine found less quantity added: mequiv	mequiv/g of tissue	
Blank	None	...	0.020	0.020	...	
	None	...	0.022	0.022	...	
Blank with KIO_3	None	0.316	0.336	0.020	...	
	None	0.304	0.329	0.025	...	
Blank with TIPA·HCl	None	0.201	0.208	0.007	...	
Control Tissue	Pancreas	...	0.018	0.018	0.21	
	Pancreas	...	0.018	0.018	0.20	
	Pancreas	...	0.042	0.042	0.22	av 0.17
Control tissue with KIO_3	Pancreas	0.540	0.552	0.012	0.13	
Control tissue with TIPA·HCl	Pancreas	0.079	0.104	0.025	0.10	
35.3 mg of TIPA·HCl sc	Pancreas	...	0.014	0.014	0.03	
	Pancreas	...	0.024	0.024	0.12	
	Pancreas	...	0.022	0.022	0.09	
35.3 mg of TIPA·HCl iv	Pancreas	...	0.020	0.020	0.18	av 0.13
	Pancreas	...	0.021	0.021	0.19	
	Pancreas	...	0.019	0.019	0.11	
66 mg of TIPA·HCl iv	Pancreas	...	0.034	0.034	0.19	
Control tissue	Liver	...	0.034	0.034	0.022	
	Liver	...	0.036	0.036	0.026	
35.3 mg of TIPA·HCl sc	Liver	...	0.033	0.033	0.019	
	Liver	...	0.038	0.038	0.021	

procedures except that pure propylene glycol was used for the injections.

The results (Table II) show that there is no measurable difference in the iodine concentration of pancreas or liver of animals injected with TIPA·HCl compared with control animals.

Experimental Section[8]

4-Iodophenylalanine.—4-Iodobenzyl bromide was treated with sodium ethoxide and diethyl acetamidomalonate using standard procedures,[9] and the ester thus obtained was hydrolyzed by boiling in 6 *N* HCl for 8 hr. Recrystallization from H_2O gave pure 4-iodophenylalanine.[7]

Anal. ($C_9H_{10}INO_2$) C, H, N.

3,4,5-Triiodobenzyl Bromide.—A solution of 10.89 g (0.0232 mole) of 3,4,5-triiodotoluene[10] and 4.12 g (0.0232 mole) of N-bromosuccinimide in 110 ml of CCl_4 was treated with four portions of 0.1 g each of benzoyl peroxide during 23 hr of refluxing and stirring. The solution was then filtered hot and the precipitate was washed with 40 ml of CCl_4. This solid (2.82 g) was discarded since it all dissolved readily in warm H_2O. The filtrate plus CCl_4 washing stood at 25° overnight with formation of 2.40 g of precipitate. When washed with warm H_2O, a small amount dissolved leaving 2.08 g of tan solid, mp 168–172°, which proved to be moderately pure 3,4,5-triiodobenzyl bromide. Analytically pure product (1.17 g) of white needles, mp 176–178°, was obtained after two recrystallizations from $CHCl_3$. Additional quantities of this material (total 15%) were obtained from the mother liquor after repeated recrystallizations from $CHCl_3$.

Anal. ($C_7H_4BrI_3$) C, H, Br, I.

In hot alcoholic $AgNO_3$, 0.0549 g of this material gave 0.0176 g (94%) of pure AgBr as evidence for the location of the bromo-substituent on the side chain rather than on the ring.

Confirmation of structure was obtained from the nmr spectrum (values in ppm, downfield from TMS) in $CDCl_3$, which shows two peaks of equal intensity, at 4.22 ppm (CH_2) (calculated for $C_6H_5CH_2Br$, 4.40 ppm by Shoolery's rules[11]) and 7.83 ppm (2 aromatic, *o*-H).

3,4,5-Triiodophenylalanine.—Equimolar quantities of 3,4,5-triiodobenzyl bromide (2.58 g), diethyl acetamidomalonate (1.02 g), and $NaOC_2H_5$ were dissolved in 100 ml of absolute EtOH. The clear faintly yellow solution was stirred and refluxed for 7 hr, then evaporated under N_2, with gentle heating. The residue was triturated with cold water, filtered, and dried to give 3.06 g of white sandy crystals. After three recrystallizations from absolute EtOH, 2.32 g (72%) of pure diethyl (3,4,5-triiodobenzyl)acetamidomalonate was obtained, mp 176–177.5°.

Anal. ($C_{16}H_{18}I_3NO_5$) C, H.

A mixture of 52 ml of AcOH, 82 ml of concentrated HCl, and 1.73 g of the ester was refluxed for 24 hr and the acids were distilled until 90 ml of distillate had been collected. To the cooled material in the distilling flask, 500 ml of H_2O was added, and the mixture was boiled until the solid largely dissolved. After filtering and cooling, a precipitate (1.3 g) was collected, then redissolved in 700 ml of boiling 0.14 *N* HCl. The solution was brought to pH 5 with K_2CO_3, and the white powdery precipitate was collected. After one recrystallization by the same procedure, 0.96 g (70%) of analytically pure product was obtained (mp above 250°) and dried at 80° for 24 hr *in vacuo*.

Anal. ($C_9H_8I_3NO_2$) C, H.

Analysis of Rat Tissue for Iodine.—The method of Zak, *et al.*,[12] for the determination of protein-bound iodine was modified and used. Each tissue was placed, immediately after removal from the animal, in a 300-ml tared electrolytic beaker, weighed, then dried overnight at 110° and reweighed. To each beaker were added 40 glass beads, 2 ml of 0.5% aqueous Na_2CrO_4, and 50 ml of 28% $HClO_3$ solution.[12] The solutions were boiled for about 90 min until the tissue had been digested. After cooling, 25 ml of distilled H_2O was added to each beaker to give a clear yellow solution. When rat livers were analyzed, it was necessary to triple the quantities of reagents used for digestions and titrations.

Titration.—To each beaker was added 5 ml of 4% HCl, 5 drops of 3% ammonium molybdate, and 2 g of KI. The iodine was then titrated with 0.1 *N* sodium thiosulfate. Just before the end point, when the color of the solution had become a light yellow-tan, 3 ml of starch solution was added. All samples gave clear end points.

(8) Melting points were observed in open glass capillary tubes in a stirred silicone bath, and the readings are corrected. Elemental analyses were performed at the Department of Chemistry, University of Virginia, Charlottesville, Va., except the analysis of 3,4,5-triiodobenzyl bromide, which was performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

(9) C. Kaiser and A. Burger, *J. Am. Chem. Soc.*, **79**, 4365 (1957).

(10) H. L. Wheeler and L. M. Liddle, *Am. Chem. J.*, **42**, 441 (1909).

(11) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, Inc., New York, N. Y., 1959, p 59.

(12) B. Zak, H. H. Willard, G. B. Myers, and A. J. Boyle, *Anal. Chem.*, **24**, 1345 (1952).

Acknowledgment.—The authors wish to thank Dr. Alfred Burger of the Department of Chemistry, University of Virginia, for his very helpful encouragement and advice; Dr. Bruce Chodosh for his aid with the animal studies; and Dr. Edwin D. Becker, Laboratory of Physical Biology, NIAMD, NIH, for the nmr spectra.

N^2-Hydroxyasparagine[1]

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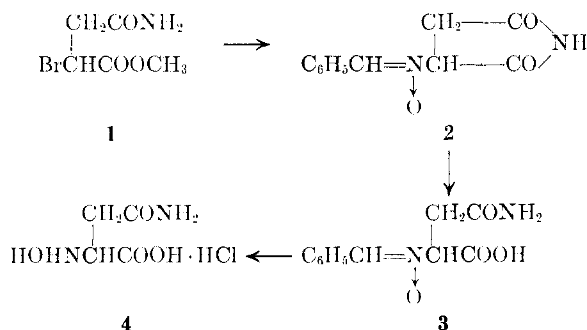
Received September 18, 1967

Because of the reported role of asparaginase in certain asparagine-requiring tumors,[2-4] the study of analogs of this amino acid seemed desirable. We have prepared the asparagine analog N^2-hydroxyasparagine through an extension of a general procedure for α-N-hydroxyamino acids published previously.[5,6]

Attempts to prepare the benzal nitrone of succinic acid or of diethyl succinate from their respective α-bromo compounds were unsatisfactory, as was an attempted condensation of β-benzaldoxime with α-bromosuccinamic acid. The reaction succeeds through the methyl ester (**1**) of the latter, in the presence of 2 equiv of sodium methoxide. Condensation with the β-benzaldoxime probably occurs after formation of α-bromosuccinimide to give the imide derivative of the nitrone (**2**).[7] Dilute hydrochloric acid led to cleavage



of the nitrone to 2-hydroxylaminosuccinimide which was identified in the reaction mixture by pmr. It failed to open without further decomposition. Treatment of **2** with only 1 equiv of base (pH 9.5) did not give complete opening of the imide, but maximum yield of open-ring material could be achieved by maintaining the pH at 11-12. This nitrone of 2-aminosuccinamic acid (**3**) proved to be homogenous by thin layer chromatography. Cleavage of the nitrone **3** was then effected by treatment with 1 *N* HCl at room temperature. The compound thus formed, N^2-hydroxyasparagine (**4**), was isolated as its hydrochloride. The product gave a positive Fehling's test and could not be crystallized either as its hydrochloride or the free base. Chromatography showed one major spot. Catalytic hydrogenolysis led to asparagine, with not more than a trace of isoasparagine, as determined by paper chromatography or by electrophoresis. As expected[7] treatment of **2** with concentrated aqueous ammonia gave the diamide, which on treatment with 1 *N* HCl gave 2-hydroxylaminosuccinamide.

Optical activity, retained from L-asparagine to L-α-bromosuccinamic acid,[8] was lost during the formation of the nitrone. Thus, in this reaction series, L-asparagine leads to racemic hydroxylamino compound.

In preliminary assays the presence of 2 molar equiv of DL-N-hydroxyasparagine did not inhibit the hydrolysis of L-asparagine by L-asparaginase. The assay[4] includes recovery of ammonia by aeration at pH 11, and in the absence of the enzyme the analog releases slightly over 1 equiv of ammonia. In the presence of guinea pig serum L-asparaginase or *Escherichia coli* L-asparaginase (EC-2)[4] no additional ammonia was released from the analog. Tests were made at both pH 5.0 (0.1 *M* acetate) and pH 8.5 (0.1 *M* borate) at 37°.

Although it is unstable in strong alkali, we find that N-hydroxyasparagine is considerably more stable than indicated by Emery[9] for a presumed N-hydroxyaspartic acid.

We thank Dr. H. A. Campbell for the assay with asparaginase.

Experimental Section[10]

Benzalnitrone of Succinimide (2).—To a solution of 8.5 g (0.043 mole) of L-α-bromosuccinamic acid prepared from L-asparagine[8] in 20 ml of MeOH was added an excess of CH_2N_2 in 100 ml of dry ether. The reaction mixture was taken to dryness under vacuum and the crude ester was dissolved in 50 ml of EtOH and added to a solution of β-benzaldoxime[11] (5.3 g) and $NaOCH_3$ (2 g of Na) in 100 ml of MeOH. After standing at room temperature overnight, the reaction mixture was concentrated *in vacuo* and the residue was taken up in 200 ml of H_2O. Neutralization with AcOH yielded a precipitate (7 g, 75%) which was recrystallized from MeOH; mp 187-192° dec. *Anal.* ($C_{11}H_{10}N_2O_3$) C, H, N.

Benzalnitrone of 2-Aminosuccinamic Acid (3).—To 2.55 g (0.012 mole) of **2** was added 60 ml of 0.2 *N* NaOH plus enough additional alkali to bring the solution to pH 12. After 17 hr at room temperature, the solution was adjusted to pH 3 by the addition of 1 *N* HCl. On standing, glistening plates separated (1.5 g, 53%) which after recrystallization from absolute EtOH melted at 175-176°. Thin layer chromatography on silica gel with BuOH-EtOH-H_2O (3:1:1) and with uv light or Tollens reagent for visualization indicated one component. *Anal.* ($C_{11}H_{12}N_2O_4$) C, H, N.

N^2-Hydroxyasparagine Hydrochloride (4).—To 1.3 g of nitrone **3** was added 10 ml of 1 *N* HCl. The HCl was then removed under vacuum below 30°. The residue was dissolved three times in 10 ml of EtOH and taken to dryness *in vacuo*. The resulting white gum was taken up in 10 ml of EtOH, filtered, and precipitated by the addition of ether. After repeating this twice the residue

(1) This investigation was supported in part by funds from the National Cancer Institute (Grant No. CA 08748).

(2) H. A. Campbell, L. T. Mashburn, E. A. Boyse, and L. J. Old, *Biochemistry*, **6**, 721 (1967).

(3) L. J. Old, E. A. Boyse, H. A. Campbell, R. S. Brodey, J. Fidler, and J. D. Teller, *Lancet*, **1**, 447 (1967).

(4) E. A. Boyse, L. J. Old, H. A. Campbell, and L. T. Mashburn, *J. Exptl. Med.*, **125**, 17 (1967).

(5) E. Buehler, *J. Org. Chem.*, **32**, 261 (1967).

(6) E. Buehler and G. B. Brown, *ibid.*, **32**, 265 (1967).

(7) E. Sondheimer and R. W. Holley, *J. Am. Chem. Soc.*, **79**, 3767 (1957).

(8) S. Kallenberg, *Ber.*, **50**, 90 (1917).

(9) T. F. Emery, *Biochemistry*, **2**, 1041 (1963).

(10) Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements were within ±0.4% of theoretical values.

(11) E. F. Schoenewaldt, R. B. Kinnel, and P. Davis, *J. Org. Chem.*, in press (private communication). Since that procedure is superior to that of Sele and Buehler, mentioned in footnote 32 of ref 5, the latter has been withdrawn from publication.