Tumor Localizing Agents VIII

Radioiodinated Phenylalanine Analogs

By R. E. COUNSELL, T. D. SMITH, W. DIGUILIO, and W. H. BEIERWALTES

Use of the currently available *l*-selenomethionine-^{75}Se for external pancreatic photoscanning is limited by the long biological half-life of the amino acid and the dense radiations arising from high liver concentrations of the compound. The present study was initiated in an effort to find superior pancreas-specific photoscanning agents. The approach presented here is based upon the previously established specificity of natural amino acids for pancreatic tissue. In the anticipation that this specificity might extend to other radiolabeled synthetic amino acids, *ortho-*, *meta-*, and *para*-iodophenylalanine-^{125}I and the corresponding acetylated derivatives were synthesized for tissue distribution analysis. All three iodophenylalanine-^{126}I isomers showed a specificity for pancreatic tissue in mice but not in dogs. Acetylation of the radioiodinated amino acids caused no qualitative changes in the tissue distribution results in either species.

FOR SEVERAL YEARS a research program has been directed toward the investigation of new radioscanning agents for selected tumors (1–5). A recognition of the current problems associated with presently available pancreatic radioscanning agents prompted the authors to synthesize and evaluate a series of radioiodinated phenylalanine derivatives for this purpose.

The lack of suitable diagnostic agents for pancreatic carcinoma was emphasized in a recent statistical, clinical, and pathological survey of the disease (6). It is the most difficult abdominal tumor to diagnose, and clinically correct diagnoses occur in only about 30% of autopsied cases. The prognosis of pancreatic carcinoma is poor mainly because existing diagnostic procedures confirm the pathological condition too late for proper treatment.

Radiolabeled zinc (^{65}Zn) compounds were used in previous attempts to design pancreas-specific radioscanning agents (7). Zinc-65 was expected to exhibit a predilection for pancreatic tissue because zinc is a common constituent of insulin. Pancreatic concentrations of the radionuclide, however, were insufficient to be of clinical value.

The demonstration that radiolabeled, natural amino acids concentrate in the pancreas shortly after intravenous administration (8, 9) initiated efforts to utilize this selectivity for the radiodiagnosis of pancreatic disorders. The elements (C, H, N, O, and S) present in natural amino acids, however, do not possess gamma-emitting radionuclides suitable for external *in vivo* scanning purposes. Consequently, it was necessary to examine synthetic amino acids for pancreatic selectivity. Reports have indicated that externally labeled synthetic amino acids such as 3-iodotyrosine-^{131}I (10, 11) are not selectively taken up by the pancreas, presumably because these agents take no part in protein synthesis. On the other hand, Blau and Manske (10) demonstrated that *l*-selenomethionine-^{75}Se possessed biological properties very close to those of methionine and that it did, indeed, show a predilection for the pancreas. Selenium-75 also possessed the necessary gamma emissions suitable for external photoscanning.

Subsequent clinical evaluations (11–26) of *l*-selenomethionine-^{75}Se resulted in mixed conclusions about its value as a pancreatic scanning agent. Certain problems such as high concentrations of radioactivity in the liver, patient variations in pancreas size and configuration, the distribution of radioactivity, and the long biological half-life of *l*-selenomethionine-^{75}Se were uniformly encountered.

Published data indicating the pancreatic specificity of other synthetic amino acids, such as 1-aminocyclopentane-^{14}C-carboxylic acid (27–29) and *p*-fluorophenylalanine-^{14}C (30, 31), suggested the feasibility of utilizing other radionuclides incorporated into synthetic amino acids. The suitability of iodine-125 and iodine-131 for external photoscanning and the reported pancreatic selectivity of phenylalanine-^{14}C (9) suggested the synthesis of a series of radioiodinated phenylalanines for tissue distribution studies.

The *ortho-*, *meta-*, and *para*-iodophenylalanines were prepared by the alkylation of diethyl acetamidomalonate and subsequent acid hydrolysis and decarboxylation according to the method of Redemann and Dunn (32) (Scheme I). All

Received May 22, 1968, from the Laboratory of Medicinal Chemistry, College of Pharmacy and the Department of Internal Medicine (Nuclear Medicine), University of Michigan, Ann Arbor, MI 48104

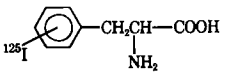
Accepted for publication August 1, 1968.

Presented to the Medicinal Chemistry Section, APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, May 1968.

This work was supported by training grant GM 1367-03 and grants CA-08349-02 and CA 08429-02 from the National Institutes of Health, Bethesda, Md. and award PRA-18 from the American Cancer Society, New York, N. Y.

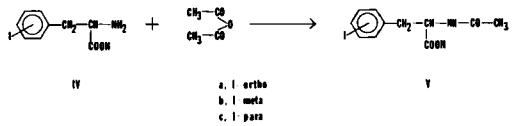
Part VII of this series, R. E. Counsell, P. Pocha, V. V. Ranade, J. Sterngold, and W. H. Beierwaltes, in preparation.

TABLE I—TISSUE DISTRIBUTION IN MICE 4 hr. AFTER INJECTION[a] (c.p.m./mg. TISSUE)



^{125}I	Pancreas	Liver	Kidney	Melanoma	Eyes
ortho	3823	411	622	807	646
meta	1192	196	396	244	501
para	496	91	205	132	114

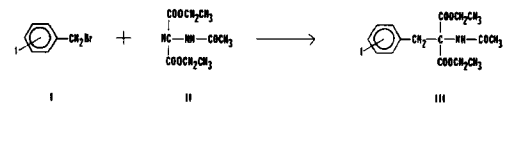
[a] Dose, 10 μc./mouse.



Scheme I—Syntheses of iodophenylalanines.

three iodophenylalanine isomers (33–36) have been reported previously but were inadequately characterized.

Radioiodinated derivatives were prepared by halogen exchange of the appropriate iodophenylalanine with $Na^{125}I$ in aqueous acetic acid. Initial attempts to carry out the exchange reaction in this solvent resulted in partial *N*-acetylation of the iodophenylalanines. Similar reactions of amino acids with acetic acid have been reported (37). The acetylated derivatives were isolated by ion-exchange chromatography during model exchange reactions and their structures elucidated. In addition, the *N*-acetyl iodophenylalanines were synthesized according to a U. S. patent (38) (Scheme II) and found to agree in all respects with the products isolated above.



Scheme II—Syntheses of N-acetyl iodophenylalanines

Tissue Distribution Studies—The preliminary studies to determine the biological distribution of *ortho-*, *meta-*, and *para*-iodophenylalanine-^{125}I in mice (Table I) indicated that all three isomers displayed a predilection for pancreatic tissue. Moreover, *para*-iodophenylalanine exhibited pancreatic specificity in mice when administered either intraperitoneally or intracardially. In contrast to these results, one previous study (39) reported an insufficient concentration of *para*-iodophenylalanine in the rat pancreas to be useful as a radiopaque media. In that report the presence of *para*-iodophenylalanine in pancreatic tissue was based upon a chemical analysis for protein-bound iodine. At the time of this writing, however, Ullberg and Blomquist (40) showed by autoradiography that mouse pancreas selectively concentrated *para*-iodophenylalanine-^{125}I in good agreement with the authors' own data. The radioiodinated *N*-acetyl amino acid analogs showed a similar predilection for pancreatic tissue at early time periods following administration.

In contrast to the pancreatic selectivity of the radioiodinated amino acids in mice, none of the three iodophenylalanine-^{125}I isomers showed any selective localization in the pancreas of dogs (Table II). Studies utilizing the *N*-acetyl derivative of *meta*-iodophenylalanine-^{125}I in dogs indicated that acetylation caused no significant change in tissue distribution.

Similar species differences between mice and dogs in the pancreatic localization of synthetic amino acids have been reported previously. For example *dl*-1-aminocyclopentane-^{14}C-carboxylic acid has been shown to exhibit a pancreatic selectivity in mice but not in dogs (29). Studies are now in progress to resolve the *dl*-amino acids prepared in this investigation to see if the *l*-isomers show a similar species variation in distribution.

EXPERIMENTAL[1]

Syntheses of Isomeric Iodophenylalanines—Sodium metal (*n* g. atoms) was dissolved in absolute ethanol[2] (8 *n* l.). Dry solid diethyl acetamidomalonate (*n* mole) was added and the stirred reaction mixture refluxed for 0.5 hr. The formation of the sodiomalonate was evidenced by turbidity. Addition of the appropriate iodobenzyl bromide[3] (*n* mole)

[1] Melting points were taken on a Fisher-Johns melting point apparatus and are corrected. NMR spectra were obtained with a Varian A-60A spectrometer using 10% solutions and tetramethylsilane as internal reference. Elemental analyses were performed by Spang Microanalytical Laboratories, Ann Arbor, Mich. Thin-layer chromatograms were run using 1-in. Eastman Chromatogram strips, type K301R with fluorescence indicator, and spots detected with UV light. Chromatograms of radioiodinated compounds were scanned with an Atomic Associates RCS-363 radiochromatogram scanner.

[2] Dry ethanol was prepared by the magnesium ethoxide method reported in Vogel, A. I., "Elementary Practical Organic Chemistry Part I: Small Scale Preparations," 2nd ed., Wiley, New York, N. Y., 1966, p. 158.

[3] Prepared according to Weizmann, M., and Patai, S., *J. Am. Chem. Soc.*, **68**, 150(1946).

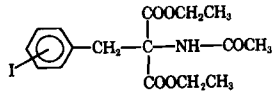
TABLE II—TISSUE DISTRIBUTION OF IODOPHENYLALANINES IN DOGS 1.5 hr. AFTER INTRAVENOUS ADMINISTRATION[a] (c.p.m./mg. TISSUE)

^{125}I-Compd.	Pancreas	Liver	Blood	Parathyroid	Thyroid	Adrenal	Kidney
o-Iodophenylalanine	1.5	1.5	7.2	0.8	1.0	1.5	2.7
m-Iodophenylalanine	1.9	3.1	10.7	1.7	3.4	1.9	4.3
p-Iodophenylalanine	3.4	4.6	11.1	1.6	2.9	3.5	11.1
N-Acetyl-*m*-iodophenylalanine	1.0	2.9	6.8	1.5	1.8	1.4	5.3

[a] Dose, 4 μc./kg.

was followed by a 4-hr. reflux period. Most of the alcohol was removed under reduced pressure and the reaction mixture diluted with water. Ether extraction afforded the crude alkylated diethyl-acetamidomalonate. A small fraction of the ether extract was dried ($MgSO_4$, charcoal) and the crude residue remaining after removal of the ether was recrystallized from ethanol–water[4] and characterized (see Table III).

TABLE III—INTERMEDIATE ACETAMIDOMALONATES



I	M.p., °C.	Found, %[a] C	H
ortho	93.8–94.5	44.40	4.57
meta	83–84.5	44.53	4.59
para	132–135	44.46	4.74

[a] *Anal.*—Calcd. for $C_{16}H_{20}INO_5$: C, 44.36; H, 4.65.

The NMR spectra were consistent in all cases with the assigned structures. The spectra of all three isomers were identical except for the benzylic protons and the aromatic region. The benzylic protons of the *ortho* isomer (3.82 δ) were shifted downfield by 14 c.p.s. with respect to the corresponding protons of the *meta* and *para* isomers (3.58 δ). Such downfield shifts are typical of *ortho*-substituted derivatives (3, 41). The aromatic resonance patterns were in good agreement with those reported for the iodotoluene isomers (41).

The remaining ether extract was evaporated to dryness yielding a crude oily residue. This residue, without further purification was refluxed with 48% HBr (3 *n* l.) for 8 hr. or until complete solution was effected. The solution was evaporated to dryness, diluted with a small amount of water, and brought to the isoelectric point[5] with 28% ammonium hydroxide. The flask was allowed to stand overnight; the solid iodophenylalanine was collected by filtration and recrystallized from ethanol–water (see Table IV). The NMR spectra of the pure iodophenylalanines taken as 10% solutions in trifluoroacetic acid were in good agreement with the reported spectra of phenylalanine taken in the same solvent (42).

Syntheses of Isomeric *N*-Acetyl Iodophenylalanines—The appropriate iodophenylalanine (200 mg.) was suspended in 25% acetic acid (2 ml.) and three 0.1-ml. portions of acetic anhydride added to the vigorously stirred suspension at 10-min. intervals. The reaction temperature was maintained at 40°. The subsequent addition of 10% HCl (2 ml.) was followed by continued stirring for 30 min. The white solid product which formed was collected by filtration and recrystallized from ethanol–water (see Table V).

^{125}I Exchange of Iodophenylalanines—The iodophenylalanine (100 mg.) was dissolved in acetic acid (0.5 ml.) and water (3 ml.) containing $Na^{125}I$ (~2 mc.). The mixture was magnetically stirred at 110° (external temperature) in a nitrogen atmosphere for 24 hr. The solvent was removed on a rotary evaporator and water (~2 ml.) was added to suspend the solid residue and wash out residual $Na^{125}I$. The solid was collected by filtration, washed with water (~2 ml.) and recrystallized from ethanol–water. Chemical purity of the exchanged product was verified by TLC (*n*-BuOH–H_2O–HOAc, 60:20:20) and IR spectra (KBr) comparisons with authentic material. Radiochemical purity was confirmed by scanning thin-layer radiochromatograms. The product was taken up in 0.1 *N* HCl for specific activity determinations[6] and animal administration.

Isomer	Recovery, mg.	Sp. Act. μc./mg.	% Exchange
ortho	36.2	16	31
meta	45.2	16.8	25
para	39.8	12.3	22

^{125}I Exchange of *N*-Acetyl Iodophenylalanines—The *N*-acetyl iodophenylalanine (100 mg.) was dissolved in acetic acid (2 ml.) and water (1 ml.) containing $Na^{125}I$ (~2 mc.). The solution was magnetically stirred and heated to 110° (external temperature) in a nitrogen atmosphere for 24 hr. At the end of the heating period, the solvent was removed *in vacuo*. The solid residue was collected by filtration, washed with water (~3 ml.), and recrystallized from ethanol–water. The final exchange product was identical with known material by melting point, IR spectra, and TLC analysis (*n*-BuOH–H_2O–HOAc, 60:20:20). Radiochromatogram scans of developed TLC strips indicated a radiochemically pure product. The final product was dissolved in ethanol (2 ml.) and the specific activity determined.[6]

Isomer[7]	Recovery, mg.	Sp. Act. μc./mg.	% Exchange
ortho	63.5	15.1	53
meta	57.5	15.4	49

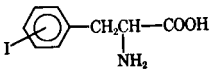
[4] The first crystallization was difficult to achieve. The products tend to form an initial oil which solidifies upon standing.

[5] The solution is titrated to the point of turbidity with NH_4OH (to about pH 7). If excess NH_4OH is accidentally added, the isoelectric point can be reached by evaporation of excess ammonia on a rotary evaporator.

[6] Samples used for specific activity determinations were counted on a Beckman ambient temperature liquid scintillation system model 200 in a dioxane cocktail composed of naphthalene, 100 g.; PPO (2,5-diphenyloxazole), 7 g.; and dioxane, a quantity sufficient to make 1,000 ml.

[7] The *para* isomer was not exchanged.

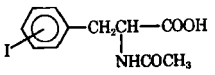
TABLE IV—IODOPHENYLALANINES



I	n	M.p.,[a] °C.	Yield, %[b]	Found,[c] % C	H
ortho	0.013	230–234	30	37.41	3.56
meta	0.009	213.5–216.5	55	37.43	3.58
para	0.017	223–226	68	37.27	3.66

[a] Melting points of amino acids are dependent upon the method of determination (43). No special precautions were used in these determinations. [b] Yields are reported as the overall yield of pure product based upon the appropriate iodobenzyl bromide. [c] *Anal.*—Calcd. for $C_9H_{10}INO_2$: C, 37.14; H, 3.46.

TABLE V—*N*-ACETYL IODOPHENYLALANINES



I	M.p., °C.	Yield, %	Found, %[a] C	H
ortho	185–187.5	31	39.97	3.71
meta	172–174.5	69	39.84	3.65
para	190–193.5	19	39.65	3.65

[a] *Anal.*—Calcd. for $C_{11}H_{12}IO_3$: C, 39.66; H, 3.63.

REFERENCES

(1) Counsell, R. E., Smith, T. D., Doelle, J., Meier, D. and Beierwaltes, W. H., *J. Pharm. Sci.*, **56**, 1019(1967).
(2) Counsell, R. E., and Willette, R. E., *ibid.*, **55**, 1012 (1966).
(3) Counsell, R. E., Willette, R. E., and DiGuilio, W., *J. Med. Chem.*, **10**, 975(1967).
(4) Counsell, R. E., Pocha, P., Morales, J. O., and Beierwaltes, W. H., *J. Pharm. Sci.*, **56**, 1042(1967).
(5) Counsell, R. E., Ranade, V. V., Lala, L. K., and Hong, B. H., *J. Med. Chem.*, **11**, 380(1968).
(6) Klintrup, H.-E., *Acta Chir. Scand. Suppl.*, **No. 362**, 1 (1966).
(7) Meschau, I., Quinn, J. L., Witcofski, R. L., and Hosick, T. A., *Radiology*, **73**, 62(1959).
(8) Wheeler, J. E., Lubens, F. D. W., and György, P., *Proc. Soc. Exptl. Biol. Med.*, **70**, 187(1949).
(9) Hansson, E., *Acta Physiol. Scand. Suppl.*, **No. 161** (1959).
(10) Blau, M., and Manske, R. F., *J. Nucl. Med.*, **2**, 102(1961).
(11) Burke, G., and Goldstein, M. S., *Am. J. Roentgenol.*, **92**, 1156(1964).
(12) Blau, M., and Bender, M. A., *Radiology*, **78**, 974 (1962).
(13) Zuidema, G. D., Kirsh, M., Turcotte, J. G., Gaisford, W. D., Powers, W., and Kowalczyk, R. S., *Ann. Surg.*, **158**, 894(1963).
(14) Rodríguez-Antúñez, A., *Cleveland Clin. Quart.*, **31**, 213(1964).
(15) Beierwaltes, W. H., *Northwest Med.*, **63**, 771(1964).
(16) Sodee, D. B., *Radiology*, **83**, 910(1964).
(17) Haynie, T. P., Svoboda, A. C., and Zuidema, G. D., *J. Nucl. Med.*, **5**, 90(1964).
(18) Tabern, D. L., Kearney, J., and Dolbow, A., *ibid.*, **6**, 762(1965).
(19) Burdine, J. A., and Haynie, T. P., *J. Am. Med. Assoc.*, **194**, 979(1965).
(20) Aronsen, K.-F., Gynning, I., and Waldeskog, B., *Acta Chir. Scand.*, **129**, 624(1965).
(21) Sodee, D. B., *Radiology*, **87**, 641(1966).
(22) Rodríguez-Antúñez, A., Filson, E. J., Sullivan, B. H., and Brown, C. H., *Ann. Intern. Med.*, **65**, 730(1966).
(23) Hansson, E., and Jacobson, S.-O., *Biochim. Biophys. Acta*, **115**, 285(1966).
(24) Lahdevirta, J., Räsänen, T., and Haikonen, M., *Ann. Med. Intern. Fenn.*, **55**, 115(1966).
(25) Sodee, D. B., *Geriatrics*, **22**, 133(1967).
(26) Eaton, S. B., Potsaid, M. S., Lo, H. H., and Beaulieu, E., *Radiology*, **89**, 933(1967).
(27) Berlinguet, L., Bégin, N., and Babineau, L. M., *Can. J. Biochem. Physiol.*, **40**, 1111(1962).
(28) Bégin, N., and Scholefield, P. G., *Biochim. Biophys. Acta*, **90**, 82(1964).
(29) Sherman, F. D., Paredes, A. M., Chambers, D. A., and Nardi, G. L., *Can. J. Biochem.*, **43**, 1243(1965).
(30) Garzo, T., Hansson, E., and Ullberg, S., *Experientia*, **18**, 43(1962).
(31) Hansson, E., and Garzo, T., *Biochim. Biophys. Acta*, **61**, 121(1962).
(32) Redemann, C. E., and Dunn, M. S., *J. Biol. Chem.*, **130**, 341(1939).
(33) Wheeler, H. L., and Clapp, S. H., *Am. Chem. J.*, **40**, 458(1908).
(34) Abderhalden, E., and Brossa, G. A., *Ber.*, **42**, 3411 (1910).
(35) McGeer, E. G., and McGeer, P. L., *Can. J. Biochem.*, **45**, 115(1967).
(36) Zeller, E. A., Ramachander, G., Fleisher, G. A., Ishimaru, T., and Zeller, V., *Biochem. J.*, **95**, 262(1965).
(37) Bell, E. A., *J. Chem. Soc.*, **1958**, 2423.
(38) Town, B. W., U. S. pat. 2,867,654 (1959); through *Chem. Abstr.*, **53**, 9086*b*(1959).
(39) Schatz, V. B., O'Brien, B. C., and Sandusky, W. R., *J. Med. Chem.*, **11**, 140(1968).
(40) Ullberg, S., and Blomquist, L., *Acta Pharm. Suecica*, **5**, 45(1968).
(41) Stehling, F. C., *Anal. Chem.*, **35**, 773(1963).
(42) Tiers, G. V. D., and Bovey, F. A., *J. Am. Chem. Soc.*, **81**, 2870(1959).
(43) Burckhalter, J. H., and Stephens, V. C., *ibid.*, **73** 56(1951).

Keyphrases

Tumor localizing agents
Phenylalanine analogs, radioiodinated—synthesis
Radioiodinated phenylalanines—tissue distribution
NMR spectroscopy—structure
Radiochromatography—purity