Synthesis of Derivatives of Isophthalamic Acid as X-Ray Contrast Agents

G. BROOKE HOEY, ROBERT D. RANDS, GEORGE DELAMATER, DOUGLAS W. CHAPMAN AND PHILIP E. WIEGERT

Chemical Research Department, Medicinal Division, Mallinckrodt Chemical Works, St. Louis, Mo.

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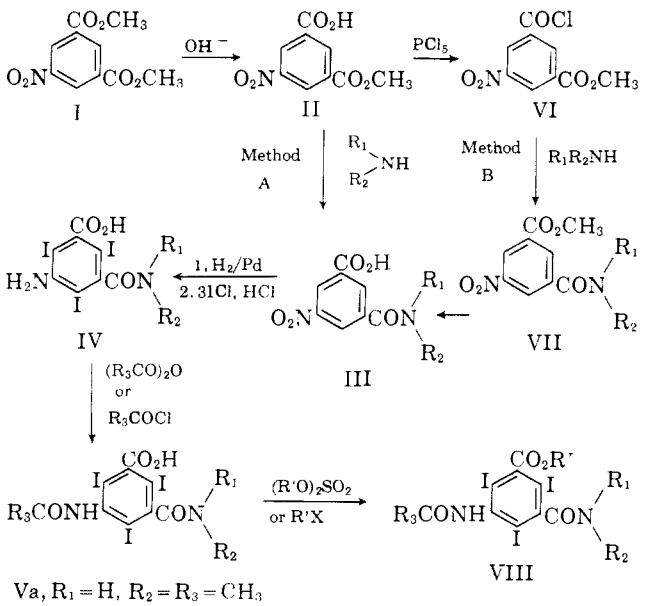
A number of derivatives of 5-amino-2,5,6-triiodoisophthalamic acid have been synthesized. Preliminary pharmacological evaluation indicates that the salts of the lower members of this series are among the least toxic iodinated organic compounds known.

For the past several years we have engaged in a research project directed toward the development of less toxic iodinated organic compounds to be screened as X-ray contrast agents in intravascular radiography. Our approach has been to vary substituents in the 3- and 5-positions of the 2,4,6-triiodobenzoic acid nucleus in a search for functional groups which would impart both higher water solubility and lower toxicity. As a part of this program a number of derivatives of 5-amino-2,4,6-triiodoisophthalamic acid have been synthesized.

The syntheses were carried out as outlined in the flow diagram. With ammonia and the lower primary alkylamines, ammonolysis of methyl hydrogen 5-nitroisophthalate (Method A) gave the desired N-alkyl 5-nitroisophthalamic acid (III) in good yield. With the higher primary alkylamines and with dimethylamine, the simple ammonolysis procedure failed and it was necessary to synthesize the desired monoamides (III) using the acid chloride (VI–Method B).



Va, $R_1 = H$, $R_2 = R_3 = CH_3$

Certain members of this series of compounds (as their sodium or N-methylglucamine salts) have been found to be less toxic and more water soluble than previously known radiopaque media.[1] The solubilities and acute toxicities obtained for these compounds are listed in Table III.

As a result of additional pharmacological testing,[2a] 5-acetamido-2,4,6-triiodo-N-methylisophthalamic acid (isothalamic acid[3]) has shown to be the most promising compound in the series and, therefore, has been selected for clinical evaluation.[2b]

Experimental[4]

Dimethyl 5-nitroisophthalate (I) was prepared from 5-nitroisophthalic acid by the method of Beyer[4a] or by the reaction of 5-nitroisophthaloyl chloride with excess methanol; m.p. 120.3–122.3° (lit., 121.5°,[5a] 121°,[5b] 123°[5c]).

Hydrogen Methyl 5-Nitroisophthalate (II).—Normal aqueous sodium hydroxide (0.02 eq.) was added at room temperature with rapid swirling to a solution of dimethyl 5-nitroisophthalate (4.8 g., 0.02 mole) in acetone–methanol (100 ml. each). After standing overnight, the solvents were evaporated at steam bath temperature and the residue extracted with warm water (50 ml.). The unsaponified diester (0.23 g., 4.2%, m.p. 115–117°) was filtered and the filtrate acidified with dilute hydrochloric acid to precipitate crude II; yield, 3.4 g. (75%), m.p. 170.5–175.5°.

The preparation was also carried out on a larger scale essentially as described above by adding 42.6 g. (0.76 mole) of potassium hydroxide in 500 ml. of methanol to 182 g. (0.76 mole) of the diester dissolved in 1900 ml. of acetone; yield, 78%, m.p. 175–179°; m.p. pure II, 179.5–181.0°.

Anal. Calcd. for $C_9H_7NO_6$: neut. equiv., 225. Found: neut. equiv., 222.

N-Methyl-5-nitroisophthalamic Acid (III, $R_1 = H$, $R_2 = CH_3$).—Crude methyl hydrogen 5-nitroisophthalate (46.3 g., 0.21 mole) was dissolved in 35% aqueous methylamine solution (500 ml.). The reaction mixture was evaporated overnight on a steam bath, the cooled residue treated with 50 ml. of water and the solution acidified with hydrochloric acid. The crude material was purified by reprecipitation from dilute ammonia solution (after charcoal treatment at pH 5.2) followed by recrystallization from 50% aqueous ethanol.

5-Amino-N-methylisophthalamic Acid.—Crude N-methyl-5-nitroisophthalamic acid (neut. equiv. 216, 11.2 g., 0.05 mole) in 250 ml. of methanol was shaken with hydrogen and 5% Pd/C in a Parr low pressure hydrogenator. After the theoretical quantity of hydrogen for reduction of the nitro group had been absorbed, the solution was filtered to remove the catalyst and the solvent evaporated under reduced pressure to give a white residue of crude 5-amino-N-methylisophthalamic acid, m.p. 227–230°. (For the synthesis of all other compounds in this series, the amino intermediate was used in the iodination step immediately after evaporation of the solvent and without further purification.)

(1) J. O. Hoppe, *Ann. N. Y. Acad. Sci.*, **78**, 727 (1959), and other references therein.

(2) (a) J. K. Kodama, W. M. Butler, T. W. Tusing and F. P. Hallett, to be published; (b) C. T. Dotter, K. R. Straube, M. K. Bilbao, and V. C. Hinck, *Northwest Medicine*, **61**, 41 (1962).

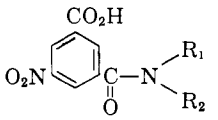
(3) Official generic name. Early clinical testing was carried out under the generic name "methalamic acid."

(4) All melting points are corrected. Neutral equivalents were determined by potentiometric titration of a weighed sample of acid with standard sodium hydroxide solution using a Beckman Model H2 pH meter. The solvent system used for these titrations was a mixture of dimethylformamide (2 vol., added first), absolute ethanol (8 vol.) and distilled water (10 vol.). Elemental analyses were made by Dr. W. J. Larson and his staff of the Department of Chemical Control, Mallinckrodt Chemical Works, or by Mrs. J. A. Macksey of this department. The infrared absorption spectra of the majority of the compounds reported here were taken using the Perkin-Elmer Spectrophotometer Model 21 (potassium bromide disc). All spectra were consistent with the postulated structures. The assistance of Mr. B. D. Field and staff of the Department of Chemical Control in determining and interpreting these spectra is acknowledged.

(5) (a) B. Beyer, *J. prakt. Chem.*, (2) **25**, 490 (1882); (b) J. B. Cohen and D. McCandlish, *J. Chem. Soc.*, **87**, 1269 (1905); (c) E. Müller, *Ber.*, **42**, 433 (1909), footnote.

TABLE I

5-NITROISOPHTHALAMIC ACIDS

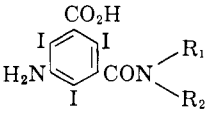


R_1	R_2	Method	Yield, %	M.p., °C.	Neutral equivalent Calcd.	Neutral equivalent Found
H	H	A	78	238.6–239.6	210	208
H	CH_3	A	89	251.0–252.5	224	222[a]
CH_3	CH_3	B	72[b]	211.3–213.3	238	238
H	C_2H_5	A	80	206.0–208.0	238	238

[a] *Anal.* Calcd. for $C_9H_8N_2O_5$: N, 12.5. Found: N, 12.3. [b] Yield by hydrolysis of corresponding methyl ester.

TABLE II

5-AMINO-2,4,6-TRIIODO-N-ALKYLISOPHTHALAMIC ACIDS

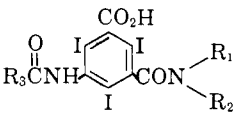


R_1	R_2	Yield, %	M.p., °C.	Neutral equivalent Calcd.	Neutral equivalent Found
H	H	74.6[a]	284.1–285.1 dec.	558	555
H	CH_3	72[a], 54[b]	266–268 dec.	572	568
H	C_2H_5	61[b]	242.5–244.5	586	578
CH_3	CH_3	74[a]	274.9–275.9 dec.	586	573

[a] Prepared using ICl–HCl. [b] Prepared using $KICl_2$.

TABLE III

5-ACYLAMINO-2,4,6-TRIIODO-N-ALKYLISOPHTHALAMIC ACIDS



R_1	R_2	R_3	Yield, %	M.p., °C.	Neutral equivalent Calcd.	Neutral equivalent Found	Iodine, % Calcd.	Iodine, % Found	Solubility,[a] g./100 ml. soln.	IVLD$_{50}$[b] mice (mg./kg.)
H	H	CH_3	64[c]	293[d]	600	602	63.5	63.4	84	21,200[e]
H	CH_3	CH_3	76[c]	285 dec.	614	619	62.0	61.8	85	19,200[e]
CH_3	CH_3	CH_3	60[c]	296.1–297.1[d]	628	630	60.6	59.9	103	20,500[e]
H	CH_3	C_2H_5	79[c]	300.1–301.1[d]	628	627	60.6	60.6	43	18,600[f]
H	CH_3	n-C_3H_7	32[c]	292.6–293.6[d]	642	646	59.3	59.1	54	17,800[f]
H	CH_3	n-C_5H_{11}	87[c]	275[d]	670	668	56.9	56.6	34	6,160[f]
H	n-C_5H_{11}[g]	CH_3	[h]	291.8–292.8[d]	670	672	56.9	56.0	...	...
H	C_2H_5	CH_3	74[c]	294[d]	628	623	60.6	60.5	80	19,800[e]
H	n-C_5H_{11}[g]	n-C_3H_7	34[c]	301–303	698	695	54.6	54.1	40	3,710[e]
H	H	C_2H_5	53[c]	281.0–282.0[d]	614	609	62.0	61.6	50	...
H	H	n-C_5H_{11}	33[c]	271.7–272.7[d]	656	653	58.0	57.9	...	...

[a] Determined by equilibrating at 25°, a saturated solution of the sodium salt with sodium salt crystals and then determining the sodium salt concentration in the supernatant liquid. [b] Administered to mice as the sodium or N-methylglucamine salt in aqueous solution, wherein the salt concentration was adjusted so that each solution contained 300 mg. I/ml. Ref. 2a. [c] Using R_3CO Cl as the acylating agent. [d] Decomposition. [e] Sodium salt. [f] N-Methylglucamine salt. [g] Prepared by Method B. [h] Using $(R_3CO)_2O$ as the acylating agent.

5-Amino-2,4,6-triiodo-N-methylisophthalamic Acid (IV, R_1 = H, R_2 = CH_3).—The following two experiments are illustrative of the iodination procedures used.

(a) **Using Iodine Monochloride.**[6]—Crude 5-amino-N-methylisophthalamic acid (9.7 g., 0.05 mole) was dissolved in 18.5% hydrochloric acid (200 ml.) and this solution was diluted to 1 l. with water. Iodine monochloride (27.4 g. of 95% ICl, 0.16 mole) in 30 ml. of concd. hydrochloric acid was added in one portion to the stirred solution at 54°. After 2 hr. of heating on a steam bath (90°), the solution was diluted to 1.5 l. and after 3 hr. of heating, titration of an aliquot sample indicated that 50% of the ICl had been consumed. Precipitation of a solid began after 3.75 hr. Intermittent heating and stirring was continued for 4 days, 10 g. of 95% ICl being added during the third day. After 4 days, titration of an aliquot sample indicated that 96% of the theoretical quantity of iodine monochloride had been consumed. The precipitated solid was filtered, washed with water and dried at 75° under reduced pressure yielding 20.6 g. (72%) of product.

(b) **Using Potassium Iododichloride.**[7]—Potassium iododichloride solution (1144 ml., 2.22 moles) was added during 0.5 hr., to a stirred suspension of 5-amino-N-methylisophthalamic acid (196 g., 1.01 mole) in 2.5 l. of water. After 3 hr. of additional stirring, a solution of sodium hydroxide (88 g., 2.2 moles in 200 ml. of water) was added. Then, additional 1.95 *M* $KICl_2$ solution (522 ml., 1.01 mole) was added during 0.5 hr. The reaction mixture was stirred overnight after which the crude product was collected and purified by crystallization of the ammonium salt and conversion to the free acid by reaction with hydrochloric acid, yielding 310 g. (54%) of product.

5-Acetamido-2,4,6-triiodo-N-methylisophthalamic Acid (**Va,** R_1 = H, R_2 = R_3 = CH_3).—Acetyl chloride (17 ml., 0.24 mole) was added in portions to a stirred slurry (room temperature) of 5-amino-2,4,6-triiodo-N-methylisophthalamic acid (57.2 g., 0.1 mole) in dimethylacetamide (120 ml.). Solution occurred in 0.5–1 hr. and after a total of 1.5 hr., 20 ml. of water was added and the reaction mixture evaporated to a thick slurry. The product was purified by twice dissolving it as its sodium salt and precipitating the free acid by the addition of mineral acid. The nearly colorless Va decomposed at about 285° but did not melt below 300°, yield, 47 g. Acetylation could also be carried out using acetic anhydride in dimethylacetamide with 1–2 drops of concd. sulfuric acid as catalyst.

The **ethyl ester** (VIII, R′ = C_2H_5, R_1 = H, R_2 = R_3 = CH_3) was prepared by adding a solution of potassium hydroxide (22 g., 0.33 mole) in absolute ethanol (500 ml.) to a stirred slurry of 5-acetamido-2,4,6-triiodo-N-methylisophthalamic acid (205 g., 0.33 mole) in 500 ml. of absolute ethanol. Diethyl sulfate (45 ml., 0.33 mole) was added to this solution and after 2 hr. of stirring, the solution had become almost solid with crystals. After standing overnight, water (500 ml.) was added and the crystals were collected. The mother liquor was evaporated to dryness and the residue combined with the previously collected crystals. The combined solids were stirred into 800 ml. of water containing a slight excess of sodium hydroxide. The solution was acidified slightly (pH 5) and the undissolved ester collected. The crude ester was recrystallized by dissolving in 400 ml. of hot dimethylformamide, filtering, and diluting the filtrate with 400 ml. of water; yield, 102 g. (48%), m.p. 297.5–298.0° dec.

(6) V. H. Wallingford, H. Decker and M. Kruty, *J. Am. Chem. Soc.*, **74**, 4365 (1952).

(7) A. A. Larsen, C. Moore, J. Sprague, B. Cloke, J. Moss, and J. O. Hoppe, *ibid.*, **78**, 3210 (1956).

Anal. Calcd. for $C_{13}H_{13}I_3N_2O_4$: I, 59.3; N, 4.36. Found: I, 59.3; N, 4.30.

3-Carbomethoxy-5-nitrobenzoyl Chloride (VI).—Hydrogen methyl 5-nitroisophthalate (II, 241 g., 1.07 mole) and phosphorus pentachloride (230 g., 1.07 mole) were mixed in toluene (100 ml.) at room temperature. The reaction proceeded slowly for 17.5 hr. after which heat (steam bath) was applied to complete solution in an additional 45 min. The toluene was evaporated under reduced pressure whereupon the crude product crystallized. Addition of carbon tetrachloride and evaporation under reduced pressure gave VI, m.p. 72.4–74°.

Anal. Calcd. for $C_9H_5ClNO_5$: neut. equiv., 244. Found: neut. equiv., 238 (determined by alcoholysis of the chloroformyl group and titration of the liberated hydrochloric acid).

Methyl N,N-Dimethyl-5-nitroisophthalamate (VII, $R_1 = R_2 = CH_3$).—One hundred grams of VI (0.41 mole) was added during 0.5 hr. to a well-stirred solution of dimethylamine (81 g. of a 25% aqueous solution, 0.45 mole) and sodium bicarbonate (69 g., 0.82 mole) in water (500 ml.), maintained at 0–5°. When the reaction appeared complete, the undissolved material was collected, slurried with sodium bicarbonate solution, and again collected, washed and air dried; yield of crude VII ($R_1 = R_2 = CH_3$), 90 g. (87%), m.p. 64.3–70.3°. A portion of the crude product was recrystallized twice from methanol, m.p. 89.3–91.5°.

N,N-Dimethyl-5-nitroisophthalamic Acid (III, $R_1 = R_2 = CH_3$).—Methanol (200 ml.) was added to 80 g. of crude methyl N,N-dimethyl-5-nitroisophthalamate and then 300 ml. water. Sodium carbonate (36.5 g.) was added in portions to the warmed mixture (pH 8–9). After filtration and acidification with hydrochloric acid, the resulting precipitate was collected and dried, yielding 54 g. of crude III ($R_1 = R_2 = CH_3$), m.p. 205.8–208.8°. This crude product was combined with 18 g. of similar material from another reaction, absolute ethanol (150 ml.) added, and the mixture digested for 10 min. Upon chilling N,N-dimethyl-5-nitroisophthalamic acid was precipitated, filtered and dried: yield, 64.3 g.

Methyl N-Amyl-5-nitroisophthalate (VII, $R_1 = H$; $R_2 = n\text{-}C_5H_{11}$).—3-Carbomethoxy-5-nitrobenzoyl chloride (55.6 g., 0.23 mole) in carbon tetrachloride (271 g.) was added slowly to a stirred mixture of *n*-amylamine (19.9 g., 0.23 mole), water (200 ml.), acetone (50 ml.), and sodium hydroxide (9.2 g.). The reaction mixture was kept alkaline at all times. The carbon tetrachloride and acetone were then removed by evaporation. The residual mixture was stirred under an air jet for 2.5 hr., cooled in an ice bath, and the aqueous layer decanted from the oily product. The crude product was utilized in the following preparation without further purification.

N-Amyl-5-nitroisophthalamic Acid (III, $R_1 = H$, $R_2 = n\text{-}C_5H_{11}$).—The crude methyl N-amyl-5-nitroisophthalamate was dissolved in the minimum amount of anhydrous alcohol and an equal volume of water added. After addition of sodium carbonate (pH 8), the mixture was heated for 0.5 hr. and allowed to stand for 2 days. Precipitation occurred during this period. The mixture was diluted with water and heated, whereupon most of the precipitate dissolved. The undissolved matter was filtered and the filtrate poured into an excess of dilute hydrochloric acid. The resulting precipitate was purified in the manner described for N-methyl-5-nitroisophthalamic acid and used directly in the synthesis of the desired 5-acylamino-2,4,6-triiodo-N-amylisophthalamic acids (Table III).

Propyl 5-Acetamido-2,4,6-triiodoisophthalamate (VIII, $R_1 = R_2 = H$; $R_3 = CH_3$, $R' = C_3H_7$).—5-Acetamido-2,4,6-triiodoisophthalamic acid (Table III, 110 g., 0.18 mole) was added to a stirred solution of sodium ethoxide, prepared by dissolving sodium (4.23 g., 0.18 mole) in absolute ethanol (500 ml.). After a few minutes *n*-propyl iodide (34.4 g., 0.20 mole) was added and the mixture heated and stirred under gentle reflux for 3 hr. A portion of the solvent was evaporated, and water added to the remaining solution to precipitate the ester. The white precipitate was collected, slurried with sodium bicarbonate solution and filtered. Recrystallization from 50% aqueous dimethylformamide gave propyl 5-acetamido-2,4,6-triiodoisophthalamate, 40 g. (34%), m.p. 271.0–271.5°.

Anal. Calcd. for $C_{13}H_{13}I_3N_2O_4$: I, 59.3. Found: I, 58.2.

Toxicity Determinations.—The acute toxicity studies were carried out by Drs. T. W. Tusing and J. K. Kodama, Hazleton Laboratories, Falls Church, Virginia. The methods employed in these studies are described in reference 2a.

The Synthesis of Arylalkylaminopropionamide Analogs of Lysergic Acid Diethylamide and their Effect upon Isolated Cholinesterase Systems. II[1]

ANDREW LASSLO, PAULINE D. WALLER AND GLENDA J. EPPERSON

Department of Pharmacology, Division of Basic Health Sciences, Emory University, Atlanta 22, Georgia; and Department of Pharmaceutical and Medicinal Chemistry, College of Pharmacy, University of Tennessee, Memphis 3, Tennessee

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A series of α-arylalkylaminopropionic acid diethylamides, patterned after a component of the LSD molecule, was synthesized. Their effect upon isolated human plasma "pseudo"-cholinesterase systems was studied, and relationships between molecular constitution and biochemical response were explored.

In the preceding paper,[2] we suggested that the substituted (>N–C–C–CON<), β-aminopropionamide moiety present in (+)-lysergic acid diethylamide (LSD) as well as in the β-(arylalkylamino)propionamide[2] and piperidinecarboxamide[3–6] compounds derived from the corresponding components of the parent LSD molecule, might be involved in the inhibitor–enzyme complex formation in human plasma "pseudo"-cholinesterase systems. In order to complement our findings we prepared and evaluated the β-(methylamino), β-(dimethylamino) and β-(trimethylammonium) substituted diethylpropionamides (Fig. 1). Next, the pronounced difference between inductive effects elicited by some substituents on α- and β-carbons of aliphatic acids,[7] prompted us to examine the biochemical responses of the α-(arylalkylamino) substituted analogs (Fig. 2) of the previously studied β-(arylalkylamino)propionic acid diethylamides[2]; the fact that these two series of compounds also could be viewed as

(1) This investigation was supported by grants from the National Institute of Mental Health (USPHS MY-2072/MY-4379) and the Geschickter Fund for Medical Research, Inc.

(2) A. Lasslo, P. D. Waller, A. Meyer and B. V. Rama Sastry, *J. Med. Pharm. Chem.*, **2**, 617 (1960).

(3) A. Lasslo, W. M. Marine and P. D. Waller, *J. Org. Chem.*, **21**, 958 (1956).

(4) A. Lasslo and P. D. Waller, *J. Org. Chem.*, **22**, 837 (1957).

(5) S. E. Jordan, A. Lasslo, H. L. Livingston, H. Alperin and A. Gersing, *Arch. intern. pharmacodynamie*, **115**, 452 (1958).

(6) A. Lasslo and P. D. Waller, *J. Med. Pharm. Chem.*, **2**, 107 (1960).

(7) L. F. Fieser and M. Fieser, "Advanced Organic Chemistry," Reinhold Publishing Corporation, New York, N. Y., 1961, p. 362.