Desamino Analogs of Thyroxine[1]

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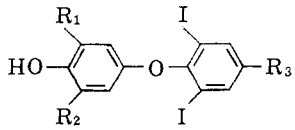
Received May 23, 1963

A synthesis of analogs of thyroxine and of 3,5,3′-triiodothyronine in which the amino group is replaced with a carboxy group (Ia) or a carbethoxy group (Ib) is presented. Preliminary biological tests show that the dicarboxylic acids (Ia) are weak thyromimetic agents.

In the course of a study on the conversion of various desamino analogs of diiodotyrosine to the corresponding analogs of thyroxine[3] it became necessary to synthesize analogs of 3,5,3′-triiodothyronine and of thyroxine in which the amino group is replaced with a carboxy group (Ia) or with a carbethoxy group (Ib).

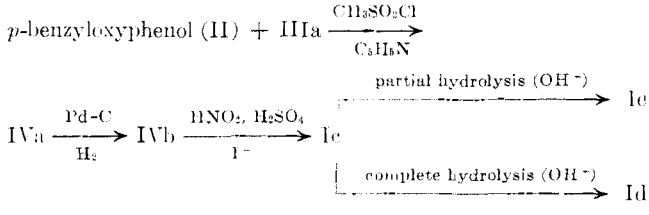


Ia, R_1 = I; R_2 = H or I; R_3 = $CH_2CH(COOH)_2$
Ib, R_1 = I; R_2 = H or I; R_3 = $CH_2CH(CO_2C_2H_5)COOH$
Ic, R_1 = R_2 = H; R_3 = $CH_2CH(CO_2C_2H_5)_2$
Id, R_1 and R_2 = H; R_3 = $CH_2CH(COOH)_2$
Ie, R_1 and R_2 = H; R_3 = $CH_2CH(CO_2C_2H_5)COOH$

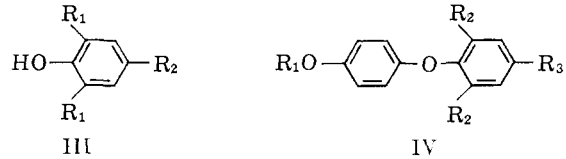
The present paper describes the synthesis of these compounds.

The classical synthesis of thyroxine by Chalmers, *et al.*,[4] involves the protection of the phenolic hydroxyl by formation of its methyl ether; toward the end of the synthesis this protective group is removed by hydrolysis with hydrogen iodide or hydrogen bromide. This principle of temporary protection is not applicable to the synthesis of the analogs of thyroxine described in this paper, since treatment of the methyl ether with acid would cause the loss of carbon dioxide and lead to the formation of a propioinc acid side chain. In the present investigation, decarboxylation was avoided by protecting the phenol through its benzyl ether and by removing this blocking group by hydrogenolysis prior to the introduction of iodine into the molecule.

The dicarboxylic acid Id and its monoester Ie were synthesized using the following sequence of reactions.



Iodination of Id and of Ie gave Ia and Ib, respectively.



III IV

IIIa, R_1 = NO_2; R_2 = $CH{=}C(C_2H_5)_2$
IIIb, R_1 = I; R_2 = $CH_2CH(CO)OH)_2$
IVa, R_1 = $C_6H_5CH_2$; R_2 = NO_2; R_3 = $CH{=}C(C_2H_5)_2$
IVb, R_1 = H; R_2 = NH_2; R_3 = $CH_2CH(C_2H_5)_2$

On hydrogenation of IVa three reactions, removal of the benzyl group, reduction of the nitro groups, and hydrogenation of the double bond in the side chain, were carried out in a single operation. Protection of the phenolic hydroxyl through the benzyl rather than the methyl ether may be advantageous in the synthesis of analogs of thyroxine other than the ones described in this paper, since a separate step for the removal of the protective group is avoided.

When the dicarboxylic acids Ia, Id, and IIIb were dried *in vacuo* at room temperature, they retained tenaciously the solvents used for their crystallization or precipitation. On heating they lose carbon dioxide. Their isolation and purification had therefore to be carried out in the cold.

The tetraiodinated acid Ia (R_2 = I) was isolated as its crystalline monopyridinium salt. Purification of the corresponding triiodinated acid Ia (R_2 = H) was carried out by partition chromatography.

The tri- and tetraiodinated acids Ia were also formed, although in very low yield, when an aqueous solution (pH 7.5) was incubated in the presence of oxygen at 37° for about 1 week. Similar incubations of other desamino analogs of diiodotyrosine have been reported previously.[3] Together with the coupling reaction, partial deiodination, and decarboxylation of the starting material took place during the incubation. The starting material (IIIb) was prepared by catalytic hydrogenation of diethyl *p*-hydroxybenzalmalonate,[5] followed by hydrolysis and iodination.

The purity of the various compounds mentioned in this paper was checked routinely by paper chromatography and by high voltage electrophoresis. Table I gives the R_f values in 1-butanol–dioxane–2 *N* ammonia (4:1:5), the most frequently used solvent system.

In preliminary biological assays the tri- and tetraiodinated acids Ia exhibited a weak thyromimetic activity. In the rat goiter prevention test[6] carried out together with Dr. J. Wolff of this Institute, the triiodinated acid was as active as thyroxine when administered at a molar ratio of 240:1. With the tetraiodinated acid, the molar ratio required for equal activity lay between 240 and 480:1. Tadpole meta-

(1) A preliminary report of this work has been presented at the 144th National Meeting of the American Chemical Society, Los Angeles, Calif., March, 1963.

(2) Visiting scientist from the University of Frankfurt am Main, Germany.

(3) T. Matsuura and H. J. Cahnmann, *J. Am. Chem. Soc.*, **81**, 871 (1959).

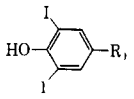
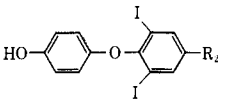
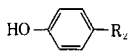
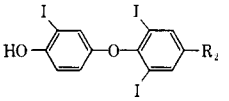
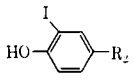
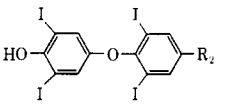
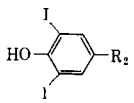
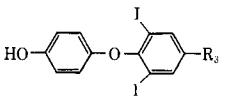
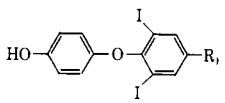
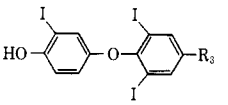
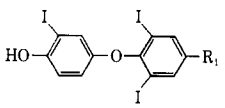
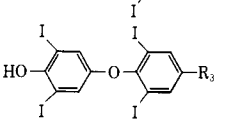
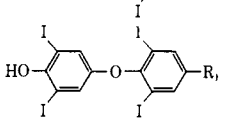
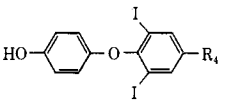
(4) J. R. Chalmers, G. T. Dickson, J. Elks, and B. A. Heus, *J. Chem. Soc.*, 3424 (1949).

(5) R. I. Meltzer, D. M. Lustgarten, and A. Fischman, *J. Org. Chem.*, **22**, 1577 (1957).

(6) E. B. Astwood and E. W. Dempsey, *Endocrinol.*, **4**, 312 (1952).

TABLE I

R_f VALUES IN 1-BUTANOL–DIOXANE–2 N AMMONIA (4:1:5), UPPER PHASE

Compound[a]	R_f[b]	Compound[a]	R_f[b]
	0.23		0.32
	0.10		0.33
	0.12		0.27
	0.06		0.73
	0.69		0.72
	0.64		0.50
	0.47		~0.93

[a] R_1 = $(CH_2)_2COOH$; R_2 = $CH_2CH(COOH)_2$; R_3 = $CH_2CH(CO_2C_2H_5)COOH$; R_4 = $CH_2CH(CO_2C_2H_5)_2$. [b] Mean values of several runs.

morphosis tests were carried out in two laboratories.[7] When the compounds were administered by injection,[8] the tetraiodinated acid had roughly 20% of the activity of thyroxine and the triiodinated acid roughly 1% of the activity of 3,3,5′-triiodothyronine. In the immersion test, the activities of both the tri- and the tetraiodinated acid were of the same order of magnitude as that of thyroxine.

Experimental[9]

Diethyl 4-(*p*-Benzyloxyphenoxy)-3,5-dinitrobenzalmalonate (IVa).—A solution of 57 g. (0.16 mole) of diethyl 4-hydroxy-3,5-dinitrobenzalmalonate[5] in 150 ml. of dry pyridine was heated to 80°. Then 22 g. (0.19 mole) of methanesulfonyl chloride was added dropwise with stirring and gentle cooling to keep the temperature at 80°. This was followed by the addition of 96 g. (0.48 mole) of *p*-benzyloxyphenol.[10] The solution was stirred for another 10 min. and then poured into 2.4 l. of cold water. A brown oil precipitated which crystallized when the reaction mixture was stirred for a short time. The solid material was collected by filtration, washed with water, and dissolved in benzene. The benzene solution was washed with 4 *N* HCl, then with water. Some benzyloxyphenol precipitated during the washing and also when the washed solution was permitted to stand overnight at 4°. It was removed by filtration. Upon evaporation of the benzene under reduced pressure, a yellow residue was obtained which was recrystallized from benzene; yield 75.5 g. (87%). The crude material could also be purified by recrystallization from 95% ethanol or by chromatography on a short column of alumina (Woelm neutral) partially deactivated with 6% water.[11] Dichloromethane was used as the eluent. All eluate fractions except the first one gave pure material upon evaporation of the solvent.

The diphenyl ether IVa exists in two crystal forms, short thick needles, m.p. 127° and long thin needles, m.p. 115–116°. The former are less soluble in ethanol than the latter. When a mixture of the two types of crystals was partially dissolved in warm ethanol, the undissolved fraction, after washing with ethanol, consisted only of crystals melting at 127°. The elemental analyses and the infrared solution spectra of the two forms were identical.

Anal. Calcd. for $C_{27}H_{24}N_2O_{16}$: C, 60.44; H, 4.51; N, 5.22. Found: C, 60.46; H, 4.50; N, 5.39.

Diethyl 4-(*p*-Hydroxyphenoxy)-3,5-diiodobenzylmalonate (Ic).—A solution of 16.1 g. (30 mmoles) of diethyl 4-(*p*-benzyloxyphenoxy)-3,5-dinitrobenzalmalonate (IVa) in 325 ml. of ethyl acetate was hydrogenated in a Parr shaker in the presence of 2.4 g. of 10% palladium-on-charcoal. When the theoretical amount of hydrogen (240 mmoles) was consumed, the uptake of hydrogen stopped. Two hydrogenation batches (60 mmoles) were combined and, after removal of the catalyst, evaporated under reduced pressure at room temperature. Both the filtration and the evaporation were carried out in an atmosphere of nitrogen in order to avoid autoxidation. The diamine IVb was obtained as a light brown oily residue which was dissolved in 50 ml. of glacial acetic acid. This solution was cooled in an ice bath and 37.5 ml. of concentrated sulfuric acid was added dropwise. The solution thus obtained was immediately added dropwise with stirring and cooling (−5°) to a mixture of 11 g. (160 mmoles) of sodium nitrate, 80 ml. of concentrated sulfuric acid, and 160 ml. of glacial acetic acid. The reaction flask was placed in an ice bath, and stirring was continued for 1 hr. The reaction mixture was then added rapidly to a stirred mixture of 64 g. (425 mmoles) of sodium iodide, 44 g. (175 mmoles) of iodine, 7 g. (117 mmoles) of urea, 860 ml. of water, and 500 ml. of chloroform. After stirring at room temperature for 1 hr. the temperature was raised to 40°, the chloroform layer separated, and the aqueous layer extracted 5 times with chloroform. The combined chloroform layers were washed successively with water, an aqueous solution of sodium metabisulfite, and again with water. After drying and concentration to a small volume, the dark brown solution was chroma-

(7) The authors wish to thank Dr. E. Frieden of the Florida State University, Tallahassee, Fla., and Dr. W. L. Money of the Memorial Center for Cancer and Allied Diseases, New York, N. Y., for these tests.

(8) *Cf.* E. Frieden and G. W. Westmark, *Science*, **133**, 1487 (1961).

(9) The microanalyses were made by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y., and by Mr. H. McCann and his associates of this Institute.

(10) The Eastman Kodak product was recrystallized from aqueous ethanol after the addition of some Norit.

(11) P. B. Müller, *Helv. Chim. Acta*, **26**, 1945 (1943).

tographed on 550 g. of alumina (Woelm acid) partially deactivated with 6% water.[11] The chromatogram was developed with chloroform. Upon evaporation, all eluate fractions (except the first and last) gave crystalline residues. Two recrystallizations from carbon tetrachloride gave 11.9 g. of crystals melting at 128–129°. The combined mother liquors and noncrystalline chromatographic fractions gave upon rechromatography and recrystallization another 3.4 g. of crystals melting at 129–130°; total yield, 15.3 g. (42%). An analytical sample which was recrystallized once more melted at 130–130.5°.

Anal. Calcd. for $C_{20}H_{20}I_2O_6$: C, 39.37; H, 3.30; I, 41.66. Found: C, 39.36; H, 3.39; I, 41.44.

4-(*p*-Hydroxyphenoxy)-3,5-diiodobenzylmalonic Acid (Id).—A solution of 3.4 g. (5.6 mmoles) of diethyl 4-(*p*-hydroxyphenoxy)-3,5-diiodobenzylmalonate (Ic) in about 35 ml. of ethanol was added to a solution of 1.4 g. (29 mmoles) of potassium hydroxide (87%) in 1.25 ml. of water. The mixture was heated in an open flask to 80°. After most of the ethanol had evaporated, the temperature was raised to 90–95° and kept there for 3–5 hr. The completeness of the hydrolysis was checked by paper chromatography (Table I).[12] Some solid material, which formed during the hydrolysis, was redissolved by the addition of a small amount of water. The reaction mixture was cooled to 0–5°, then acidified (congo red) by the slow addition of ice-cold 20% hydrochloric acid. The cold mixture was extracted 3 times with ether. The combined ether extracts were washed with water, dried, and evaporated under reduced pressure at room temperature. The residue was dissolved at room temperature in as little ethanol as possible. Crystallization was induced by the addition of about 2 volumes of isooctane and completed by slow cooling to −10°. A second crop was obtained from the mother liquors; total yield 2.5 g., m.p. 165–169° dec., with evolution of gas (carbon dioxide). The melt resolidified and melted again at about 245°. Paper chromatograms in 1-butanol–dioxane–2 *N* ammonia (4:1:5) gave an R_f value of 0.70 for the final melt, of 0.32 for the crystals before heating, and of 0.69 for authentic 3,5-diiodothyropropionic acid, m.p. 246°.[13] When chromatographically pure crystals were dried at room temperature, the solvent from which the material was crystallized was retained tenaciously. A solvent-free material was obtained when an ethanolic solution of the crystals was evaporated to dryness, the amorphous residue finely powdered, and then dried under high vacuum at room temperature.

Anal. Calcd. for $C_{16}H_{12}I_2O_6$: C, 34.68; H, 2.18; I, 45.81. Found: C, 34.90; H, 2.52; I, 45.78.

4-(4-Hydroxy-3-iodophenoxy)-3,5-diiodobenzylmalonic Acid (Ia; R_2 = H).—A solution of 1.1 g. (2 mmoles) of 4-(*p*-hydroxyphenoxy)-3,5-diiodobenzylmalonic acid (Id) in 10 ml. of a 20% aqueous solution of methylamine was cooled in an ice bath. A solution of 508 mg. (2 mmoles) of iodine and of 1 g. of potassium iodide in 3.3 ml. of water was then added slowly (2 hr.) by means of a fine polyethylene tubing immersed in the stirred solution. After completion of the addition, stirring in the cold was continued for another 30 min. The solution was acidified (congo red) by the slow addition of ice-cold 20% hydrochloric acid. The precipitate formed was washed with ice-cold water by centrifugation until the supernatant liquid gave a negative silver nitrate test, then dried *in vacuo* at room temperature; yield, 1.32 g. This material was contaminated with Ia (R_2 = I) and with Id. Contamination with the former was shown by paper chromatography (Table I) and contamination with the latter by high voltage paper electrophoresis at pH 6 in acetic acid–pyridine–water (2:20:80) in which system Id migrates faster and Ia (R_2 = I) slower than the desired product.

Partition chromatography of the crude product was carried out using a column of Celite 545 (3.5 × 80 cm.) and the two-phase solvent system obtained by mixing equal volumes of 1-butanol and chloroform with 1 *M* potassium phosphate buffer, pH 8.5.[14] The Celite was suspended in a large volume of the mobile (water-poor) phase; then 0.8 ml. of stationary (water-rich) phase was added for each gram of Celite. The mixture was shaken vigorously for a few min., then poured in small portions into the column partially filled with mobile phase. Each portion was packed down evenly by means of a perforated stainless steel disk. The packed column was completely closed and permitted to equilibrate overnight. A solution of the sample (1 g.) in a small amount of mobile phase was then chromatographed at a flow rate of about 1 ml./min. Paper chromatography of the eluate fractions showed that the early fractions contained pure triiodinated acid while the later fractions were contaminated with increasing amounts of the tetraiodinated acid. The last fractions consisted of a mixture of the tetraiodinated acid and starting material. The fractions containing both the tri- and tetraiodinated acids were further purified by descending chromatography on thick paper sheets (Whatman No. 17) in 1-butanol–dioxane–2 *N* ammonia (4:1:5). The paper was prewashed successively with 2 *N* acetic acid in 95% ethanol, 1-butanol–dioxane–2 *N* ammonia (4:1:5), and methanol containing 30% (v./v.) 2 *N* ammonia. The positions of the tri- and tetraiodinated acids on the developed chromatograms were seen clearly in short-wave ultraviolet light. The triiodinated acid was eluted with methanol containing 30% (v./v.) 2 *N* ammonia. For the purification of the later eluate fractions, two or three successive chromatographic fractionations were required. All those eluates from the column and from the paper sheets which contained only the triiodinated acid were combined and evaporated under reduced pressure at room temperature. The residue was dissolved in a small amount of water containing a few drops of ammonia. The solution was acidified at 0° and the precipitate formed washed by centrifugation as described previously. A solution of the precipitate in methanol was clarified by centrifugation, then evaporated at room temperature. The amorphous residue was powdered finely and then dried under high vacuum at room temperature (684 mg.). The substance melted at 100–105°, then resolidified, and melted again between 165 and 175°. An authentic sample of 3,5,3′-triiodothyropropionic acid melted between 178 and 185°.[13] When the melt was chromatographed on paper in 1-butanol–dioxane–2 *N* ammonia (4:1:5), its R_f value was identical with that of authentic 3,5,3′-triiodothyropropionic acid.

Anal. Calcd. for $C_{16}H_{11}I_3O_6 \cdot CH_3OH$: C, 28.68; H, 2.12; I, 53.47. Found: C, 28.64; H, 2.48; I, 54.04.

4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodobenzylmalonic Acid (Ia; R_2 = I).—A solution of 2.0 g. (3.6 mmoles) of 4-(*p*-hydroxyphenoxy)-3,5-diiodobenzylmalonic acid (Id) in 20 ml. of a 20% aqueous solution of methylamine was cooled in an ice bath. A solution of 1.83 g. (7.2 mmoles) of iodine and 1.8 g. of potassium iodide in 7.5 ml. of water was then added dropwise with stirring. Stirring was continued for 30 min. at room temperature. The solution was cooled to 0° and acidified (congo red) by the slow addition of 20% hydrochloric acid. The mixture was extracted twice with ether and the combined ether layers were washed with water and dried. Evaporation at room temperature gave 2.2 g. of crude acid which was dissolved in a small amount of pyridine–water (1:1). Dilute hydrochloric acid (20%) was added carefully until a faint cloudiness persisted. On standing overnight at 2°, crystals deposited which were collected by filtration, washed with cold water, and dried *in vacuo* at room temperature.

Anal. Calcd. for $C_{16}H_{10}I_4O_6 \cdot C_5H_5N$: C, 28.50; H, 1.71; I, 57.36; N, 1.58. Found: C, 28.35; H, 1.92; I, 57.30; N, 1.60.

The pyridinium salt had no sharp melting point. It began to melt at about 129° dec. and was almost completely melted at about 135°. The free acid, which was obtained in poor yield by recrystallization of the crude acid from benzene containing a very small amount of methanol, melted between 172 and 176° dec. When the melt was chromatographed on paper in 1-butanol–dioxane–2 *N* ammonia (4:1:5) its R_f value was identical with that of authentic tetraiodothyropropionic acid.[13]

2-Ethoxycarbonyl-3-[4-(*p*-hydroxyphenoxy)-3,5-diiodophenyl]propionic Acid (Ie).—A solution of 4.0 g. (6.56 mmoles) of diethyl 4-(*p*-hydroxyphenoxy)-3,5-diiodobenzylmalonate (Ic) in 100 ml. of ethanol was cooled to 0°. A solution of 0.48 g. (7.4 mmoles) of potassium hydroxide (87%) in 7 ml. of ethanol was added dropwise with stirring. The solution was allowed to warm up and to stand at room temperature for 6 hr. Some anhydrous sodium sulfate was then added and the mixture evaporated at room temperature. The residue was dried *in vacuo* for 1 hr. Then the flask was cooled in an ice bath and the mixture made acid (congo red) by the addition of 50 ml. of 2% hydrochloric acid. A gummy mass precipitated which was dis-

(12) If the hydrolysis is incomplete, the final product is contaminated with monoester Ie. Such a contaminated product can be purified by dissolving it in 1.5 *M* phosphate buffer (pH 7.25) and extracting the monoester with ether.

(13) The reference sample was kindly supplied by Dr. R. J. Meltzer of the Warner Lambert Research Institute, Morris Plains, N. J.

(14) The Celite was washed with hydrochloric acid, water, and methanol, then dried at 100°. The 1-butanol was redistilled, the chloroform washed with water, then dried, and redistilled. The two phases of the solvent system have about equal densities so that the lower layer is sometimes the water-poor and sometimes the water-rich phase.

solved by the addition of about 20 ml. of ether. Then 2 *N* ammonia was added until the aqueous phase had pH 10–10.5. The mixture was shaken well and the aqueous layer extracted 5 times with ether. The combined ether extracts gave, after drying and evaporation at room temperature, 2.79 g. (70%) of starting material. The aqueous phase was cooled in an ice bath, acidified (congo red) with 2 *N* HCl, and then extracted 5 times with ether. After drying and evaporation at room temperature the combined ether extracts gave 0.96 g. (25% yield) of the half-ester; m.p. 139° (softens at 87°). The substance was recrystallized without heating from chloroform–isooctane. The melting point of the recrystallized material was unchanged.[15]

Anal. Calcd. for $C_{18}H_{16}I_2O_6$: C, 37.14; H, 2.77; I, 43.60. Found: C, 37.21; H, 2.90; I, 43.66.

Partial Iodination of 2-Ethoxycarbonyl-3-[4-(*p*-hydroxyphenoxy)-3,5-diiodophenyl]propionic Acid (Ie).—A partial iodination of Ie was carried out following the procedure described previously for the synthesis of Ia (R_2 = H). The triiodinated monoester Ib (R_2 = H) was contaminated with Ie and with Ib (R_2 = I). The substance could not be purified by crystallization. Purification was achieved on a small scale by descending chromatography in chloroform–formamide (lower phase) on paper that had been washed in formamide–acetone (1:3) and then dried. In this system Ie migrates slower and Ib (R_2 = I) faster than Ib (R_2 = H). The R_f values depend on how often the paper had been soaked in formamide–acetone.

2-Ethoxycarbonyl-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]propionic Acid (Ib, R_2 = I).—An iodination of 815 mg. (1.40 mmoles) of 2-ethoxycarbonyl-3-[4-(*p*-hydroxyphenoxy)-3,5-diiodophenyl]propionic acid (Ie) was carried out according to the procedure described for the synthesis of Ia (R_2 = I). Crystals which formed on cooling of the reaction mixture were redissolved by the addition of water. The precipitate which formed after acidification was collected by filtration, washed with water, and dried. The crude material (theoretical yield) was recrystallized several times below 35° from ethanol containing a few drops of water and from chloroform–isooctane. The substance softened at 103–105°, decomposed with the evolution of gas (carbon dioxide) at about 115°, resolidified, and melted again at 159–160°. For elemental analysis the material was finely powdered, then dried under high vacuum at room temperature.

Anal. Calcd. for $C_{18}H_{14}I_4O_6$: C, 25.92; H, 1.69; I, 60.87. Found: C, 26.10; H, 1.86; I, 60.86.

***p*-Hydroxybenzylmalonic Acid.**—A solution of 130 g. (0.49 mole) of diethyl *p*-hydroxybenzalmalonate[5] (m.p. 90–90.5°) in 600 ml. of ethanol was hydrogenated slightly above atmospheric pressure in the presence of 10 g. of 10% palladium-on-charcoal. The catalyst was removed by filtration and the filtrate was added dropwise (1.2 hr.) to a stirred hot solution (80°) of 100 g. (1.6 moles) of potassium hydroxide (87%) in 100 ml. of water. The reaction flask was kept on a steam bath for another 2.5 hr. during which period a funnel connected to a water pump was placed over the neck of the flask in order to remove the ethanol vapors. Some precipitate which formed was redissolved by the addition of a small amount of water. The reaction mixture was cooled in an ice bath, then acidified (congo red) by the slow addition of 20% hydrochloric acid. This was followed by the addition of some water and 4 extractions with ether. The combined ether extracts were dried over calcium chloride and evaporated at room temperature. The residue weighed 100 g. (97% yield) and melted at 160–161° dec.; lit.[16] m.p 160.5° dec. The substance was recrystallized from ethyl acetate–benzene with the addition of Norit; m.p. 160.5–161.5° dec.

4-Hydroxy-3,5-diiodobenzylmalonic Acid (IIIb).—*p*-Hydroxybenzylmalonic acid (3.15 g., 15 mmoles) was iodinated according to the procedure described for the synthesis of Ia (R_2 = I). After evaporation of the ether extract 6.7 g. (97% yield) of crude product, m.p. 167.5–168.5° dec. was obtained. It was contaminated with a small amount of 3,5-diiodophloretic acid from which it was freed by fractional acidification of a solution in 0.5 *N* NaOH at 0° with small increments of dilute hydrochloric acid. The first precipitates which contained most of the contaminant were eliminated and the remaining precipitates were recrystallized from methanol below room temperature; white needles, m.p. 172–174° dec.

Anal. Calcd. for $C_{10}H_8I_2O_5 \cdot CH_3OH$: C, 26.74; H, 2.45; I, 51.38. Found: C, 26.75; H, 2.69; I, 51.82.

Incubation of 4-Hydroxy-3,5-diiodobenzylmalonic Acid.—An 0.25 *M* solution of IIIb (pH 7.5) was incubated aerobically at 37° following the procedure described previously[3] for other analogs of diiodotyrosine. After various time intervals aliquots of the incubation mixture were analyzed by paper chromatography and by high voltage electrophoresis. The solvent systems and methods used were the same as those described earlier.[3] Identification of the incubation products was made by comparison with the R_f values and mobilities of authentic samples. Starting material was present at all times. Extensive deiodination of the starting material gave rise to 4-hydroxy-3-iodobenzylmalonic acid. After 3 days 3,5-diiodophloretic acid could be detected and after 4 or 5 days also traces of 3-iodophloretic acid. After 5 days, Ia (R_2 = I) and Ia (R_2 = H) began to appear in the reaction mixture. These two acids could be detected only after extraction of the reaction mixture with 1-butanol at pH 7.5 and evaporation of the butanol extracts at room temperature.

(15) Before optimal conditions for the partial hydrolysis of diester Ic had been established, monoester Ie was frequently contaminated with dicarboxylic acid Id. These two substances were then separated as described in footnote 12.

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Hydroxylamine Chemistry. IV. O-Aralkylhydroxylamines

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Received November 21, 1963

Several synthetic procedures were used to prepare a series of O-aralkylhydroxylamines. Some of the products are structurally analogous to amines of biological interest. These compounds are in general 5-hydroxytryptophan decarboxylase inhibitors and mild depressants.

Interest in hydroxylamine derivatives designed as pharmacodynamic or chemotherapeutic agents has increased in recent years.[1–6] Our efforts in this field have been directed in part to the synthesis of O-

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