appeared to be more than 95% complete after 16 hr. at reflux temperature. Without recourse to alkaline conditions, most of the *m*-nitroaniline hydrochloride could be extracted from most of the mustard acid hydrochloride with acetonitrile followed by extraction with ether. Although paper chromatography indicated the presence of the DL-alanine α-mustard in the residue, it was not possible to obtain the crystalline mustard or the crystalline picrylsulfonate reported by Izumi.[3]

2-Benzyloxycarbonylamino-*m'*-nitro-3-phenylpropionanilide (XIII).—To a stirred and cooled (0°) suspension of 2.99 g. (10 mmoles) of N-benzyloxycarbonyl-DL-phenylalanine in 20 ml. of freshly distilled tetrahydrofuran was added 0.95 ml. (10 mmoles) of ethyl chloroformate followed by 1.39 ml. (10 mmoles) of triethylamine and finally after 15 min. by 1.38 g. (10 mmoles) of *m*-nitroaniline. After being stirred for 15 min., the cooling bath was removed and the reaction mixture stirred for 2.5 hr. at room temperature. The precipitate of 1.25 g. (91%) of triethylamine hydrochloride was collected and the filtrate was evaporated *in vacuo* to leave 4.31 g. (103%) of crude product. Extraction with two 50-ml. portions of hot ethyl acetate gave, on chilling, 2.28 g. (55%) of product, m.p. 179–182°. Recrystallization from benzene–cyclohexane gave an analytical sample, m.p. 183–183.5°; $\lambda_{max(\mu)}^{Nujol}$ 3.05 (N—H), 5.85, 5.95 (C=O), 6.44, 6.50 (amide II, NO_2), 7.37 (NO_2).

Anal. Calcd. for $C_{23}H_{21}N_3O_5$: C, 65.9; H, 5.05; N, 10.0. Found: C, 66.2, 66.3, H, 5.33, 5.35; N, 9.47, 9.54.

2-Amino-*m'*-nitro-3-phenylpropionanilide (XIV) Hydrobromide.—A solution of 0.50 g. (1.20 mmole) of the carbobenzyloxy anilide XIII in 25 ml. of saturated hydrogen bromide in glacial acetic acid was allowed to stand at room temperature for 2 hr., then was poured into ether, and the precipitate collected to give 0.41 g. (93%) of the product as the hydrobromide, m.p. 270–273° dec., $\lambda_{max(\mu)}^{Nujol}$ 3.11 (N—H), 3.3–4.0 ($-NH_3^+$), 5.88 (C=O), 6.52 ($-NH_3^+$, NO_2), 7.40 (NO_2).

Anal. Calcd. for $C_{15}H_{15}N_3O_3 \cdot HBr$: C, 49.2; H, 4.40; Br, 21.8; N, 11.5. Found: C, 49.0; H, 4.63; Br, 22.0; N, 10.9.

To a solution of 0.80 g. (20 mmoles) of sodium hydroxide in 50 ml. of methanol was added 7.33 g. (20 mmoles) of the above hydrobromide. The mixture was stirred at room temperature for 15 min., filtered, and the filtrate evaporated *in vacuo* to give 5.08 g. (89%) of product as an oil. Digestion of this oil with ether and evaporation *in vacuo* of the ether solution left 3.34 g. of product as an oil; $\lambda_{max(\mu)}^{Nujol}$ 2.94, 3.03 (NH_2), 5.90 (amide C=O), 6.52 (amide II, NO_2), 7.40 (NO_2); R_f 0.34 in solvent B.

Anal. Calcd. for $C_{15}H_{15}N_3O_3$: C, 63.2; H, 5.30; N, 14.7. Found: C, 63.3; H, 5.49; N, 13.6.

Acknowledgment.—The authors wish to thank Dr. Peter Lim for interpretation of the infrared spectra and his staff for paper chromatography. They also wish to thank Mr. O. P. Crews and his staff for the large-scale preparation of certain intermediates.

Synthesis of β-Phenylserines

S. WAWZONEK AND W. G. GAFFIELD

Department of Chemistry, State University of Iowa, Iowa City, Iowa

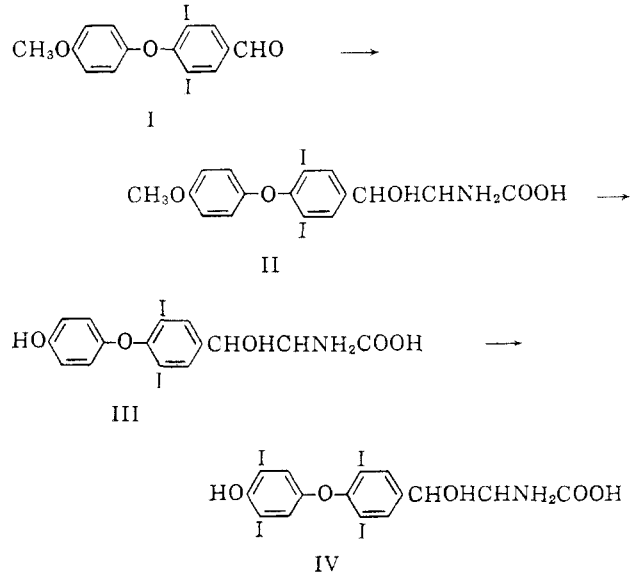
Received January 28, 1963

β-[3,5-Diiodo-4-(3,5-diiodo-4-hydroxyphenoxy)phenyl]serine, β-[3,5-diiodo-4-(4-hydroxyphenoxy)phenyl]-serine, β-[3,5-diiodo-4-(4-methoxyphenoxy)phenyl]serine, the isomeric β-(4-hydroxy-3,5-diiodophenyl)serines, and the hydrochloride of β-(4-benzoyloxyphenyl)serine ethyl ester have been prepared for testing as possible antithyroids. None of the compounds was able to inhibit the activity of L-triiodothyronine on a thiouracil-induced goiter. The first compound, however, did show some thyromimetic activity.

The report that β-phenylserine is an antagonist for the utilization of phenylalanine in microorganisms[2] suggested the synthesis of the serine analog (IV) of thyroxine and simpler β-(4-hydroxyphenyl)serine derivatives for testing as possible antithyroids.

The analog of thyroxine was prepared from 3,5-diiodo-4-(4-methoxyphenoxy)benzaldehyde (I) by the following sequence of reactions.

The condensation of 3,5-diiodo-4-(4-methoxyphenoxy)benzaldehyde (I) with glycine was carried out by an adaptation of the method of Erhart and Ott[3] for various β-(hydroxyphenyl)serines and involved the reaction of two moles of the aldehyde (I) with one mole of glycine in the presence of sodium hydroxide. The precipitate which appeared upon standing was presumably the Schiff's base and was not characterized further but was hydrolyzed to the serine (II) with dilute hydrochloric acid. The product differed in melting point from the serine (II) obtained by Friedenberg and Nobles[4] by the condensation of equimolar amounts of the organic reactants with dilute alkali. The latter reaction, which may produce a different diastereoisomer, could not be repeated in this Laboratory; only starting materials were obtained.



The structure of the serine (II) obtained in this study was in agreement with its infrared spectrum.

(1) Abstracted in part from the Ph.D. thesis, February, 1963, of W. G. Gaffield.

(2) E. Beerstecher, Jr., and W. Shive, *J. Biol. Chem.*, **164**, 53 (1946).

(3) G. Ehrhart and H. Ott, U. S. Patent 2,737,526 (1956); *Chem. Abstr.*, **50**, 15587c (1956).

(4) R. Friedenberg and W. L. Nobles, *J. Am. Pharm. Assoc. (Sci. Ed.)*, **46**, 387 (1957).

Demethylation of the β-[3,5-diiodo-4-(4-methoxyphenoxy)phenyl] serine was effected with hydriodic acid in acetic acid. Neutralization of the resulting solution with sodium acetate was necessary to precipitate the demethylated product (III). The resulting serine (III) gave an infrared spectrum which was similar to the methyl ether (II) with two exceptions; the methoxyl frequency at 1030–1035 cm.$^{-1}$, was absent and the hydroxyl band was broader.

The properties of this compound (III) differed from those reported by Friedenberg and Nobles.[4] The product reported by the latter investigators precipitated directly in the acid medium and did not require neutralization.

Iodination was accomplished with potassium triiodide in 33% ethylamine[5] and gave a mixture of β-[3,5-diiodo-4-(3,5-diiodo-4-hydroxyphenoxy)phenyl]-serine (IV) together with possibly the diiodo and triiodo compounds. Purification by recrystallization was not successful but was accomplished by leaching the product with a 4:1:1 mixture of 95% ethanol, acetic acid and ethyl acetate; the desired product (IV) was insoluble in this mixture of solvents.

The structure of IV was indicated by the infrared spectrum. Bands were obtained at 3000–3500 cm.$^{-1}$ for the OH and NH stretching, 1580–1650 cm.$^{-1}$ for carboxylate absorption, 1220–1420 cm.$^{-1}$ for aryl–aryl ether absorption, and 1180 cm.$^{-1}$ for phenolic C–O stretching. The carbon–carbon stretching vibration present at 1500 cm.$^{-1}$ in the precursors (II, III) was absent in the spectrum of the tetraiodo compound since vicinal trisubstitution causes a shift of this absorption towards lower frequencies and masking by the Nujol absorption.[6] The strong out of plane C–H deformation which appeared at 820–830 cm.$^{-1}$ in the spectra of the precursors (II, III) and is characteristic of two adjacent aromatic hydrogen atoms, was absent in the spectrum of the tetraiodo compound (IV). The three serines gave no peak at 836–840 cm.$^{-1}$ in the infrared, which is reported to be characteristic of *erythro*-phenylserines,[7] and are therefore assumed to be the *threo* isomers.

The other β-phenylserines synthesized in this study were the isomeric β-(4-hydroxy-3,5-diiodophenyl)serines and β-(*p*-benzoyloxyphenyl)serine ethyl ester hydrochloride.

The various phenylserines were tested for antithyroid activity by measuring their ability to inhibit the activity of L-triiodothyronine on a thiouracil-induced goiter. None of the submitted compounds demonstrated any antithyroid activity at doses of 245.0 γ/kg./day. β-[3,5-Diiodo-4-(3,5-diiodo-4-hydroxyphenoxy)-phenyl] serine (IV), however, did show some thyromimetic activity although the effect is small considering the dose used.

Experimental[8]

β-[3,5-Diiodo-4-(4-methoxyphenoxy)phenyl]serine (II).—A solution of 3,5-diiodo-4-(4-methoxyphenoxy)benzaldehyde[9] (2.4 g.) in 95% ethanol (60 ml) was added to a solution of glycine (0.20 g.) and sodium hydroxide (0.27 g.) in water (10 ml.). The mixture was heated to 70° with stirring and then allowed to stand at room temperature for 2–3 days. The yellow precipitate, which formed, was not separated but was treated with 2 *N* hydrochloric acid (14 ml.) and the mixture was heated to 50°. Cooling the solution gave the aldehyde (I) which was removed by filtration. The filtrate was treated with anhydrous sodium acetate until it was no longer acid to congo red paper. The resulting mixture was treated with water (250 ml.), cooled overnight, and filtered. The resulting serine was recrystallized from 50% ethanol–water and leached twice with cold dry benzene; yield, 0.518 g. This compound softened at 169° and melted at 176–179° dec. Friedenberg and Nobles[4] report m.p. 165–166° for their compound.

Anal. Calcd. for $C_{16}H_{15}I_2NO_5$: C, 34.43; H, 2.72; N, 2.52. Found: C, 34.76; H, 2.89; N, 2.36.

The infrared spectrum had bands at 3000-3500 cm.$^{-1}$ (NH, OH) 1580-1660 cm.$^{-1}$ (COO^-), 1035-1090 cm.$^{-1}$ (OCH_3), and 820-830 cm.$^{-1}$ (CH).

β-[3,5-Diiodo-4-(4-hydroxyphenoxy)phenyl]serine (III).—A suspension of β-[3,5-diiodo-4-(4-methoxyphenoxy)phenyl]serine (II) (1.0 g.) in glacial acetic acid (10 ml.) was refluxed for 2 hr. with 57% hydriodic acid (10 ml.). The resulting solution was cooled, treated with water (100 ml.), filtered 3 times, and then neutralized with sodium acetate as indicated by congo red paper. The resulting solid (0.615 g.) was filtered and melted at 203–206° dec. Friedenberg and Nobles[4] report 160° for their compound.

Anal. Calcd. for $C_{15}H_{13}I_2NO_5$: C, 33.29; H, 2.42; N, 2.58. Found: C, 33.35; H, 2.71; N, 2.36.

β-[3,5-Diiodo-4-(3,5-diiodo-4-hydroxyphenoxy)phenyl]serine (IV).—A solution of β-[3,5-diiodo-4-(4-hydroxyphenoxy)phenyl] serine (III) (0.095 g.) in 5 ml. of 33% ethylamine was treated with *N* potassium triiodide solution (2 ml.) dropwise until a permanent iodine color remained. The resulting solution was stirred for 3 hr., filtered, and then neutralized with glacial acetic acid. The crude product was filtered and purified by leaching with a 4:1:1 mixture of 95% ethanol, acetic acid, and ethyl acetate: yield, 0.021 g.; m.p. 216–219° dec. No solvent could be found for recrystallization.

Anal. Calcd. for $C_{15}H_{11}O_5NI_4$: C, 22.70; H, 1.39. Found: C, 23.09; H, 1.19.

***threo*-β-(*p*-Hydroxyphenyl)serine Monohydrate.**—This compound was obtained in 35% yield when the directions of Bolhofer[7] were used for the preparation of *threo*-β-(*p*-hydroxyphenyl)-serine. The compound began to darken at 160°, was dark brown at 170°, and melted at 193–195° dec. The infrared spectrum was similar to that reported[7] except that the OH, NH band (3000–3500 cm.$^{-1}$) was broader.

Anal. Calcd. for $C_9H_{13}NO_5$: C, 50.23; H, 6.09; N, 6.50. Found: C, 50.25; H, 6.47; N, 6.44.

***threo*-β-(4-Hydroxy-3,5-diiodophenyl)serine.**—A solution of *threo*-β-(4-hydroxyphenyl)serine monohydrate (1.0 g.), iodine (1.3 g.), and iodic acid (0.12 g.) in 50% ethanol (500 ml.) was refluxed for 2 hr. The resulting solution was concentrated under reduced pressure and the precipitated material was recrystallized from aqueous ethanol; yield, 0.5 g. The compound turns brown at 166° and decomposes at 169–170°.

Anal. Calcd. for $C_9H_9I_2NO_4$: C, 24.07; H, 2.02; N, 3.12. Found: C, 24.36; H, 2.29; N, 3.11.

***erythro*-β-(4-Hydroxy-3,5-diiodophenyl)serine.**—This compound was prepared in 65% yield from *erythro*-β-4-hydroxyphenylserine[10] using the directions given for the *threo* isomer. The compound turned brown at 150° and melted at 171–172° dec.

Anal. Calcd. for $C_9H_9I_2NO_4$: C, 24.07; H, 2.02; N, 3.12. Found: C, 24.03; H, 2.15; N, 3.35.

***p*-Benzoyloxybenzaldehyde.**—This compound was prepared by the directions of Kopp[11] in 55% yield. The melting point was 90–91°. The literature[11] reports 72°.

Anal. Calcd. for $C_{14}H_{10}O_3$: C, 74.34; H, 4.42. Found: 74.20; H, 4.55.

N-4-Benzoyloxybenzylidene-β-(4-benzoyloxyphenyl)serine Ethyl Ester.—A solution of *p*-benzoyloxybenzaldehyde (48 g.) and ethyl glycinate (11.3 g.) in absolute ether (1 l.) was refluxed with 10 g. of a 50% sodium mineral oil dispersion for 2 days. The ether was removed by decantation and the remaining solid

(5) R. I. Meltzer, S. Farber, E. Merrill, and A. Caro, *J. Org. Chem.*, **26**, 1413 (1961).

(6) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958.

(7) W. A. Bolhofer, *J. Am. Chem. Soc.*, **76**, 1322 (1954).

(8) Melting points are corrected. Infrared spectra were obtained using Nujol mulls.

(9) C. R. Harington and G. Bargar, *Biochem. J.*, **21**, 169 (1927).

(10) K. W. Rosenmund and H. Dornsaft, *Chem. Ber.*, **52**, 1734 (1959).

(11) K. Kopp, *Ann.*, **277**, 350 (1893).

was treated with glacial acetic acid (250 ml.). The resulting precipitate (34 g.) was washed with a 1% acetic acid solution and then recrystallized successively from methylene chloride and absolute ether; m.p. 143–145°.

Anal. Calcd. for $C_{32}H_{27}NO_7$: C, 71.49; H, 5.06; N, 2.60. Found: C, 71.44; H, 5.32; N, 2.61.

β-(4-Benzoyloxyphenyl)serine Ethyl Ester Hydrochloride Monohydrate.—N-4-Benzoyloxybenzylidene-β-(4-benzoyloxyphenyl)serine ethyl ester (5.0 g.) was treated with dry hydrogen chloride in a 1:1 mixture of methylene chloride–ethyl ether (100 ml.). The resulting hydrochloride was recrystallized 5 times from water; yield, 1.0 g.; m.p. 135–138°.

Anal. Calcd. for $C_{18}H_{22}ClNO_6$: C, 56.32; H, 5.77; N, 3.64. Found: C, 56.61; H, 5.33; N, 3.66.

Pharmacological Test.—Intact male rats were treated with a 0.1% solution of thiouracil in their drinking water for 10 days. Concommitently they were dosed with varying amounts of L-triiodothyronine to show the effect of the hormone on the development of the goiters. The test compounds were administered to groups of animals along with triiodothyronine (different sites of injection to prevent physical mixing) in a ratio of 100 parts of test compound to 1 part of triiodothyronine. After 10 days of treatment the animals were sacrificed and the thyroid glands were removed and weighed. Animals receiving saline or 0.1% solutions of thiouracil in their drinking water served as controls.

Drug	No. of rats	Thyroid gland weight (mean ± S.D.) (mg./100 g.)
None (untreated controls) (0.9% NaCl)	7	6.7 ± 1.4
None (thiouracil 0.1% treated controls) (0.9% NaCl)	8	16.0 ± 4.0
L-TIT[a] (1.25 γ/2 ml./kg.)	8	11.4 ± 2.5
L-TIT (1.75 γ/2 ml./kg.)	8	8.3 ± 2.0
L-TIT (2.45 γ/2 ml./kg.)	7	9.4 ± 2.6
L-TIT (3.45 γ/2 ml./kg.)	8	7.8 ± 2.0
L-TIT (2.45 γ/2 ml./kg.) + IV[b] (245.0 γ/2 ml./kg.)	8	5.7 ± 0.79

[a] L-Triiodothyronine. [b] β-[3,5-Diiodo-4(3,5-diiodo-4-hydroxyphenoxy)phenyl]serine.

Acknowledgment.—The authors wish to thank Smith Kline and French Laboratories for the pharmacological tests reported.

Synthetic Schistosomicides. III. 5-(4-Amino-1-naphthylazo)uracil and Related Heterocyclic Azo Compounds[1]

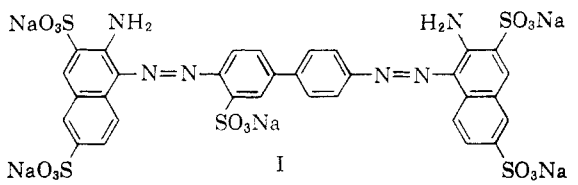
EDWARD F. ELSLAGER AND DONALD F. WORTH

Research Laboratories, Parke, Davis and Company, Ann Arbor, Michigan

Received January 11, 1963

A variety of 4-amino-1-naphthylazoheterocyclic compounds and 8-amino-5-(heterocyclicazo)quinoline derivatives have been prepared by allowing a diazotized heterocyclic amine to react with the appropriate 1-naphthylamine or 8-aminoquinoline precursor or by coupling 4-acetamido-1-naphthalenediazonium chloride with a substituted pyrimidinol followed by acid hydrolysis. 5-(4-Amino-1-naphthylazo)uracil (ANU) was highly active against experimental *Schistosoma mansoni* infections in the mouse, hamster, and monkey.

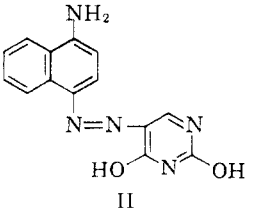
Over half a century ago, Ehrlich demonstrated that the cotton substantive azo dye Trypan Red (I) was capable of sterilizing *Trypanosoma equinum* infections



I

in the mouse when administered in a single subcutaneous dose 24 hr. before the death of untreated controls.[2] Subsequent researches with literally thousands of azo derivatives have demonstrated that certain specific structures among this large class of compounds not only possess antitrypanosomal activity but also possess antibacterial, antimalarial, antiviral, antifungal, and antihaemorrhagic properties.[3] Notable among the azo compounds used as medicinals during this era are chloroazodin, 3,5-diamino-2-[4′-(sulfamylphenyl)azo]-benzoic acid, 4-*o*-tolylazo-*o*-diacetotoluide, salicylazosulfapyridine, 4′-sulfamyl-2,4-diaminoazobenzene hydrochloride, 2,6-diamino-3-phenylazopyridine hydrochloride, and 2′,6′-diamino-2-butyloxy-5,5′-azopyridine.

During the course of a continuing program in these laboratories to develop new schistosomicidal agents, several hundred azo compounds of diverse structure have been tested against *Schistosoma mansoni* in mice. Although most of these compounds were ineffective, unexpected activity was observed with 5-(4-amino-1-naphthylazo)uracil (ANU) (II), which proved to be highly effective against *S. mansoni* in mice, hamsters, and monkeys.[4] The present communication is concerned with the synthesis of ANU and certain closely related compounds.



II

ANU (II) was prepared by allowing diazotized 5-aminouracil (IV) to couple with 1-naphthylamine in aqueous ethanol. In like manner, a variety of substituted 5-(4-amino-1-naphthylazo)uracils of formula V (where X represents a hydrogen atom or a methyl, hydroxy, or ethoxy group and NR_2 represents an amino,

(1) For previous paper in this series, see E. F. Elslager, J. F. Cavalla, W. D. Closson, and D. F. Worth, *J. Org. Chem.*, **26**, 2837 (1961).

(2) P. Ehrlich, *Berlin. klin. Wochschr.*, **44**, 233 (1907).

(3) G. N. Mahapata, *J. Proc. Inst. Chemists* (India), **29**, 33 (1957).

(4) P. E. Thompson, J. E. Meisenhelder, and H. Najarian, unpublished results, Parke, Davis and Company, Ann Arbor, Michigan.