4-Hydroxy-3,5-diiodophenylacetaldehyde (X).—To a solution of 1.18 g. (3.1 mmoles) of the semicarbazone of 4-hydroxy-3,5-diiodophenylacetaldehyde in 30 ml. of acetic acid and 15 ml. of water, was added 3 ml. (43 mmoles) of pyruvic acid. The solution was kept for 20 hr. at 40° in an atmosphere of nitrogen. Water was then added until a slight turbidity persisted. On standing overnight at 4°, 0.81 g. of crystals were obtained. The mother liquors yielded another crop of 0.11 g. The crude product was recrystallized from aqueous acetic acid yielding 0.29 g. (28%) of needles, m.p. 111–113°.

Anal. Calcd. for $C_8H_6I_2O_2$: C, 24.75; H, 1.55. Found: C, 24.92; H, 1.76.

Coupling Reactions of Analogs of DIHPPA with DIT.—These reactions were carried out essentially as described previously[6] with several minor modifications imposed by the nature of the analog used. The amount of analog used in a run ranged from 0.5 to 6 mmoles. The analog VIII was insoluble in butanol and was therefore dissolved in 2 *N* NaOH and the pH was kept at 7.6 by the slow addition of 4 *N* HCl. In several cases, the addition of *t*-butylhydroperoxide was omitted since preliminary tests indicated that it had little if any influence on the yield of the analog of thyroxine. The method of working up the reaction mixture also depended on the nature of the starting material and on the yield of coupling product. In the case of very small yields the thyroxine formed could only be detected by paper chromatography in the usual solvent systems.[18]

3,5,3′-Triiodo-5′-methoxythyronine.—A coupling reaction was carried out with 2.02 g. (6 mmoles) of the keto acid IV and 2.01 g. (4.6 mmoles) of DIT. The crude product was extracted with acetone in order to remove side products of the reaction, mainly 5-iodovanillin. The undissolved material, m.p. 218–219° dec., weighed 0.13 g. (4%). It was chromatographically pure. Crystallization from a 5% sodium carbonate solution gave the sodium salt in the form of fine crystals.

3,5,3′-Triiodo-5′-methoxythyropropionic Acid.—A coupling reaction of 2.02 g. (6 mmoles) of the keto acid IV and 1.94 g. (4.6 mmoles) of 3,5-diiodophloretic acid[18] gave a crude product which was crystallized from benzene to give 17 mg. (1%) of prisms, m.p. 196–198°.

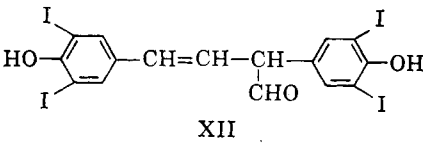
Anal. Calcd. for $C_{16}H_{13}I_3O_5$: C, 28.79; H, 1.97. Found: C, 28.26; H, 2.08.

3′,5′-Dibromo-3,5-diiodothyronine.—A coupling reaction was carried out with 2.02 g. (6 mmoles) of the keto acid V and 2.17 g. (4.6 mmoles) of DIT dihydrate. The crude product was extracted with acetone in order to remove side products of the reaction, mainly 3,5-dibromo-4-hydroxybenzaldehyde. The undissolved material, m.p. 243° dec., weighed 0.28 g. (9%). It was chromatographically pure. Treatment of a solution in methanol–ammonia (95:1) with dilute acetic acid gave a colorless precipitate, m.p. 244° dec. (lit. m.p. 245–246° dec.[19] and 244.5° dec.[20]).

3′,5′-Dibromo-3,5-diiodothyropropionic Acid.—The crude product obtained in a coupling reaction between 2.02 g. (6 mmoles) of keto acid V and 1.94 g. (4.6 mmoles) of 3,5-diiodophloretic acid[18] was crystallized from benzene yielding 0.1 g. (3%) of needles, m.p. 203–205°. The infrared spectrum was identical with that of a sample prepared by a different synthetic route.[21]

3,5,3′,5′-Tetrabromothyropropionic Acid.—A coupling reaction between 1.27 g. (3.5 mmoles) of the keto acid V and 0.59 g. (2.9 mmoles) of 3,5-dibromophloretic acid[22] gave a crude product which, after crystallization from benzene, yielded 18 mg. (1%) of needles, m.p. 184–186°. The infrared spectrum was identical with that of a sample prepared by a different synthetic route.[21]

Behavior of 4-Hydroxy-3,5-diiodophenylacetaldehyde (X) in the Coupling Reaction with DIT.—The reaction was carried out in the usual manner with 0.25 g. (0.64 mmole) of the aldehyde X and 0.25 g. (0.53 mmole) of DIT. When the residue obtained after evaporation of the butanol extract was acidified, 0.1 g. of a precipitate was obtained which, after crystallization from ethyl acetate, gave fine needles, m.p. 248–249° dec. No thyroxine was detected by paper chromatography of the mother liquor. Further investigation showed that the presence of DIT was not required for the formation of this product which was tentatively identified as the aldehyde XII, formed by aldol condensation of 2 molecules of X. The aldehyde X was fairly stable when it was treated with oxygen at pH 7.6 However, when alkali was added the condensation product XII formed. Structure XII is compatible with the elemental analysis and the infrared spectrum. A carbonyl band at 1730 cm.$^{-1}$ indicated that the carbonyl is not in conjugation with a double bond.[23] The ultraviolet spectrum showed bands at λ_{max}^{EtOH} 220 mμ (ε 37,000), 245 (26,000), 260 (24,000), 348 (28,400), and shoulder at 415 (6300).



XII

Anal. Calcd. for $C_{16}H_{10}I_4O_3$: C, 25.33; H, 1.32. Found: C, 25.21; H, 1.55.

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

(19) K. Schuegraf, *Helv. Chim. Acta*, **12**, 405 (1929).

(20) C. R. Harington and W. McCartney, *J. Chem. Soc.*, **892** (1929).

(21) T. Matsuura, unpublished work.

(22) T. Matsuura and H. J. Cahnmann, *J. Am. Chem. Soc.*, **82**, 2055 (1962).

(23) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, p. 133.

Model Reactions for the Biosynthesis of Thyroxine. IX. Synthesis of Peptides of L-Thyroxine[1,2]

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Only a few peptides of thyroxine[4,5] have been synthesized.[6] The method used, heating of halogenoacylthyroxine with ammonia, permits the preparation of only a limited number of peptides, leads to racemization, and cannot be applied to the synthesis of those peptides in which the carboxyl group of thyroxine is peptide linked. The numerous methods for the synthesis of peptides published during the last few decades are not easily applicable to the synthesis of peptides of thyroxine since most of them involve the removal of a blocking group by either strong acid or hydrogenolysis, which leads to partial or total deiodination. It is also not possible to synthesize peptides of thyroxine by iodinating peptides of thyronine since this would lead to peptides of 3′,5′-diiodothyronine.

In the present investigation a new principle, etherification of peptides of diiodotyrosine with 4-hydroxy-3,5-diiodophenylpyruvic acid, was applied to the synthesis of peptides of thyroxine. This reaction is analogous to that in which the same keto acid converts diiodotyrosine to thyroxine[7] and various analogs of diiodotyrosine to the corresponding analogs of thyroxine.[8,9] The

(1) Paper VIII: T. Shiba, H. J. Cahnmann, T. Matsuura, A. Nishinaga, and H. Sakamoto, *J. Org. Chem.*, **29**, 3061 (1964).

(2) A preliminary report of this work was presented at the 141st National Meeting of the American Chemical Society, Washington, D. C., March, 1962.

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(4) J. N. Ashley and C. R. Harington, *Biochem. J.*, **22**, 1436 (1928).

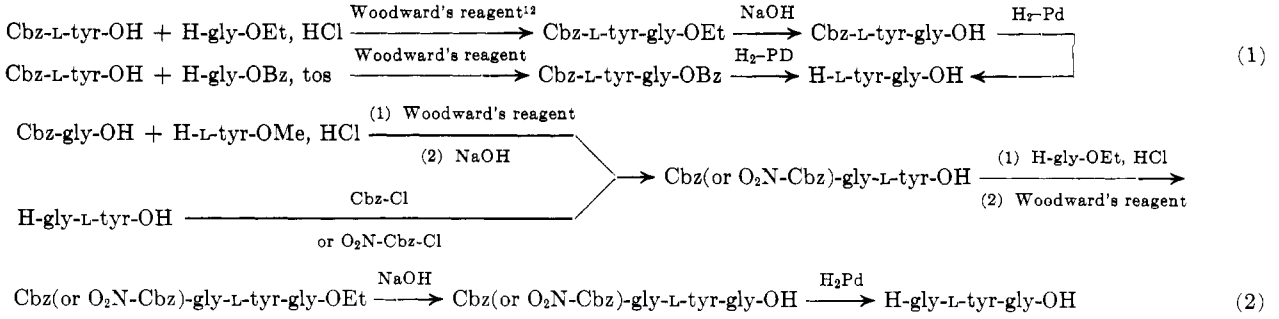
(5) E. Abderhalden and E. Schwab, *Fermentforschung.*, **11**, 164 (1930).

(6) The formation of N-acylated peptides of thyroxine in the aerobic incubation of N-acylated peptides of diiodotyrosine was described by R. Pitt-Rivers [*Biochem. J.*, **43**, 223 (1948)] and by R. Pitt-Rivers and A. T. James [*ibid.*, **70**, 173 (1958)]. Attempts to convert these acyl derivatives to the free thyroxine peptides failed, because removal of the acyl group was accompanied by fission of the peptide bond (R. Pitt-Rivers, personal communication).

(7) R. I. Meltzer and R. J. Stanaback, *J. Org. Chem.*, **26**, 1977 (1961).

(8) A. Nishinaga and T. Matsuura, *ibid.*, **29**, 1812 (1964).

(9) T. Shiba and H. J. Cahnmann, *ibid.*, **29**, 1652 (1964).



reaction takes place under mild conditions (room temperature, neutral pH), does not lead to racemization,[10] and permits the preparation of peptides in which either the amino group or the carboxyl group of thyroxine, or both, are peptide linked. Glycyl-L-thyroxine, L-thyroxylglycine, and glycyl-L-thyroxylglycine were prepared in this manner in 7–11% yield from the corresponding peptides of diiodotyrosine. The same synthetic principle is presumably applicable to the synthesis of any other peptide of thyroxine.

The required peptides of diiodotyrosine were prepared by iodination of the corresponding peptides of tyrosine. Tyrosylglycine was refractory to iodination with iodine monochloride or potassium triiodide. When, however, the free amino group was blocked by adsorption of this peptide on a strong cation exchanger (Dowex 50), iodination proceeded smoothly.

Glycyl-L-tyrosine was a commercial product. L-Tyrosylglycine and glycyl-L-tyrosylglycine were prepared by reactions 1 and 2.[11]

Since most of the compounds shown in this reaction scheme had been prepared previously, although by other methods, only new intermediates are described in the Experimental part. The method of Woodward, *et al.*,[12] for the synthesis of the tyrosine peptides always gave solid reaction products, while the mixed anhydride and dicyclohexylcarbodiimid methods often yielded oily products. The only shortcoming of the use of Woodward's reagent is that the reaction product sometimes contains traces of sulfur that poison the catalyst in the subsequent hydrogenolysis. Therefore, when the Cbz-peptide, obtained after treatment of the reaction product with sodium hydroxide, showed a positive sodium nitroprusside reaction, it was dissolved in ethyl acetate and extracted several times with a solution of sodium carbonate. The precipitate obtained after acidification of the combined extracts was then free of sulfur.

Paper chromatography was used routinely to follow the purification of synthetic intermediates and end products (see Table I).

The fact that peptides of diiodotyrosine can replace free diiodotyrosine in the reaction with 4-hydroxy-3,5-diiodophenylpyruvic acid again shows that in this coupling reaction, the structural requirements are less stringent for the amino acid component than for the keto acid component.[1,8,9] The conversion under mild conditions of diiodotyrosine to thyroxine within a peptide is of particular interest because it is generally assumed that the biosynthesis of thyroxine from diiodotyrosine takes place within a protein, thyroglobulin.

TABLE I

R_f VALUES OF PEPTIDES OF TYROSINE, DIIODOTYROSINE, AND THYROXINE[a]

Peptide	Solvent 1[b]	Solvent 2[c]
gly-L-tyr	0.12	0.11
gly-L-DIT	0.13	0.28[d]
gly-L-T_4	0.27	0.57[d]
L-tyr-gly	0.14	0.12
L-DIT-gly	0.09	0.32[d]
L-T_4-gly	0.37	0.58[d]
gly-L-tyr-gly	0.09	0.12
gly-L-DIT-gly	0.05	0.27[d]
gly-L-T_4-gly	0.33	0.51[d]

[a] Abbreviations: gly, glycine; tyr, tyrosine; DIT, 3,5-diiodotyrosine; T_4, thyroxine. [b] 1-Butanol–dioxane–2 *N* ammonia (4:1:5). [c] 1-Butanol–acetic acid–water (78:10:12). [d] Elongated spot.

Experimental[13]

***p*-Nitrocarbobenzyloxyglycyl-L-tyrosine.**—To a stirred and ice-cooled solution of 1.90 g. (8.0 mmoles) of glycyl-L-tyrosine in 8 ml. of 1 *N* NaOH were added simultaneously over a period of 30 min. 10 ml. of 1 *N* NaOH and a solution of 2.16 g. (10.0 mmoles) of *p*-nitrocarbobenzyloxychloride in 10 ml. of dioxane. Stirring was continued for 30 min. with cooling and for another 30 min. at room temperature. A small amount of insoluble material was removed by filtration and the filtrate was acidified (congo red) and extracted three times with ethyl acetate. The combined extracts were washed once with 1 *N* HCl, then three times with a 5% solution of sodium carbonate. After acidification of the combined alkaline washings with HCl, the mixture was permitted to stand overnight at 4°. The crystals formed were washed with water and dried yielding 1.68 g. (51%), m.p. 168–170°. An analytical sample was recrystallized from water; m.p. 172–174°.

Anal. Calcd. for $C_{19}H_{19}N_3O_8$: C, 54.67; H, 4.59; N, 10.07. Found: C, 54.68; H, 4.76; N, 10.23.

***p*-Nitrocarbobenzyloxyglycyl-L-tyrosylglycine Ethyl ester.**—A suspension of 1.27 g. (5.0 mmoles) of N-ethyl-5-phenylisoxazolium-3′-sulfonate[14] in 30 ml. of nitromethane was added to a solution of 2.09 g. (5.0 mmoles) of *p*-nitrocarbobenzyloxyglycyl-L-tyrosine in 0.51 g. (5.0 mmoles) of triethylamine and 20 ml. of nitromethane. After stirring for 30 min. the reagent was completely dissolved. A solution of 0.70 g. (5.0 mmoles) of glycine ethyl ester hydrochloride in 0.51 g. (5.0 mmoles) of triethylamine and 50 ml. of nitromethane was then added and stirring was continued overnight. The residue obtained after evaporation of the solvent was dissolved in ethyl acetate. The solution was washed with 1 *N* Na_2CO_3, 1 *N* HCl, and water. The solution was evaporated and the residue was crystallized from ethanol–water yielding 1.96 g. (78%), m.p. 95–97° dec.

(10) T. Shiba and H. J. Cahnmann, *J. Org. Chem.* **27**, 1773 (1962).

(11) Abbreviations: Cbz, carbobenzyloxy; O_2N-Cbz, *p*-nitrocarbobenzyloxy; tyr, tyrosyl; gly, glycyl; Me, methyl; Et, ethyl; Bz, benzyl; tos, *p*-toluenesulfonate.

(12) R. B. Woodward, R. A. Olofson, and H. Mayer, *J. Am. Chem. Soc.*, **83**, 1010 (1961).

(13) The microanalyses were made by Mr. McCann and his associates of this Institute and by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y. Melting points were taken in capillary tubes and are uncorrected.

(14) Woodward's reagent K, Pilot Chemicals, Inc., Watertown 72, Mass. (*cf.* ref. 12).

Anal. Calcd. for $C_{28}H_{26}N_4O_9$: C, 54.97; H, 5.22; N, 11.15. Found: C, 54.94; H, 5.23; N, 11.29.

p-Nitrocarbobenzyloxyglycyl-L-tyrosylglycine.—To 1.86 g. (3.7 mmoles) of p-nitrocarbobenzyloxyglycyl-L-tyrosylglycine ethyl ester was added 1 *N* NaOH (9.5 ml.) with stirring at such a rate that a pH of about 12 was maintained. After 1.5 hr. the solution was clarified by filtration, and the filtrate was acidified (congo red) with 4 *N* HCl, then left at 4° for a few hours. The crystals formed were washed with water and dried yielding 1.19 g. (68%), m.p. 218–220° dec.

Anal. Calcd. for $C_{21}H_{22}N_4O_9$: C, 53.16; H, 4.67; N, 11.81. Found: C, 53.07; H, 4.93; N, 11.81.

Glycyl-3,5-diiodo-L-tyrosine.—A solution of 5.1 g. (31 mmoles) of iodine monochloride in 11.3 ml. of 20% HCl was added with stirring to a solution of 3.6 g. (15 mmoles) of glycyl-L-tyrosine[15] in 25 ml. of 1 *N* HCl. After 1.5 hr. an aqueous solution of sulfur dioxide was added until the reaction mixture became pale yellow. The precipitate formed on adjusting the pH to 4 with 6 *N* NH_4OH was washed with water, and purified by reprecipitation (pH 7) from its solution in 6 *N* NH_4OH yielding 5.1 g. (69%) of needles, m.p. 222–224° dec.; ref. 16 gives a sintering point of 122–125° and a melting point of 290–292° dec. For paper chromatography, see Table I.

Anal. Calcd. for $C_{11}H_{12}I_2N_2O_4$: C, 26.96; H, 2.47; I, 51.79; N, 5.72. Found: C, 27.08; H, 2.69; I, 51.89; N, 5.82.

3,5-Diiodo-L-tyrosylglycine.—Dowex 50 × 12, H-form (about 20 ml. of wet resin) was added to a solution of 1.42 g. (6 mmoles) of L-tyrosylglycine[17] in 60 ml. of water until the pH of the mixture was 3.2. (Addition of more resin did not cause a further change in pH). Then 3 ml. of a 4 *M* solution of iodine monochloride (12 mmoles) in 1 *N* HCl was added with stirring over a period of about 5 min. After stirring for another 30 min. the resin was collected by filtration and washed with water, then with 60 ml. of 2 *N* NH_4OH, and again with water. The combined alkaline filtrates were concentrated to about 60 ml. When the pH was adjusted to 4, a precipitate formed which was collected after cooling and washed with ice-cold water yielding 1.66 g. (57%), m.p. 195.5–196.5° dec. Dissolution in 2 *N* NH_4OH and reprecipitation with 2 *N* HCl gave 1.20 g. of small crystals (m.p. unchanged) which were dried *in vacuo* at room temperature. A second crop, m.p. 184–185° dec. (0.50 g.), was obtained from the filtrate. For paper chromatography, see Table I.

Anal. Calcd. for $C_{11}H_{12}I_2N_2O_4 \cdot 0.5H_2O$: C, 26.47; H, 2.63; I, 50.86; N, 5.61. Found: C, 26.68; H, 2.64; I, 50.83; N, 5.53.

Glycyl-3,5-diiodo-L-tyrosylglycine.—A solution of 1.7 g. (6.7 mmoles) of iodine and 1.6 g. of potassium iodide in 10 ml. of water was added slowly (1 hr.) to a stirred solution of 0.89 g. (3.0 mmoles) of glycyl-L-tyrosylglycine[18] in 10 ml. of a 2% aqueous solution of methylamine. A 20% aqueous solution of methylamine was added dropwise as needed in order to maintain the pH of the reaction mixture between 7.5 and 9. After stirring for another hour the excess of iodine was reduced with an aqueous solution of sulfur dioxide and the pH was brought to 6.5 with 4 *N* HCl. The microcrystalline precipitate was washed with a small amount of ice-cold water and dried *in vacuo* at room temperature yielding 1.21 g., m.p. 211–212° dec. A second crop was obtained from the mother liquors; total yield, 1.34 g. (80%). For paper chromatography, see Table I.

Anal. Calcd. for $C_{13}H_{15}I_2N_3O_5 \cdot 0.5H_2O$: C, 28.08; H, 2.90; I, 45.64; N, 7.56. Found: C, 28.06; H, 3.13; I, 45.49; N, 7.47.

Glycyl-L-thyroxine.—The reaction of glycyl-3,5-diiodo-L-tyrosine (2.45 g., 5.0 mmoles) with 4-hydroxy-3,5-diiodophenylpyruvic acid (2.59 g., 6.0 mmoles) was carried out essentially as described previously[10] for the reaction of 3,5-diiodo-L-tyrosine-I^{131} with the same keto acid. The solution of the keto acid[19] was freshly prepared and the extraction with 1-butanol was omitted. The reaction mixture was evaporated and the residue was washed with small amounts of a saturated solution of sodium sulfate and of ice-cold water. The crude product was purified by dissolution in 1 *N* NH_4OH and reprecipitation (pH 5) with 4 *N* HCl. The substance was dried *in vacuo* at room temperature yielding 0.46 g. (11%), m.p. 183–185° dec. For paper chromatography, see Table I.

Anal. Calcd. for $C_{17}H_{14}I_4N_2O_5 \cdot 2H_2O$: C, 23.47; H, 2.09; I, 58.35; N, 3.22. Found: C, 23.23; H, 2.14; I, 58.11; N, 3.36.

L-Thyroxylglycine.—Solid 4-hydroxy-3,5-diiodophenylpyruvic acid (1.73 g., 4.0 mmoles) was added in small portions to a buffered solution (pH 6.7) of 3,5-diiodo-L-tyrosylglycine hemihydrate (1.47 g., 2.9 mmoles). Other reaction conditions were similar to those described previously (*cf.* glycyl-L-thyroxine). After reprecipitation at pH 7 of the crude product, the peptide was dried *in vacuo* at room temperature yielding 0.23 g. (9%), m.p. 207–210° dec. For paper chromatography, see Table I.

Anal. Calcd. for $C_{17}H_{14}I_4N_2O_5 \cdot H_2O$: C, 23.96; H, 1.89; I, 59.58; N, 3.29. Found: C, 23.77; H, 1.87; I, 59.10; N, 3.54.

Glycyl-L-thyroxylglycine.—Reaction conditions were similar to those described in the preceding paragraph. Starting materials were 1.73 g. (4.0 mmoles) of 4-hydroxy-3,5-diiodophenylpyruvic acid and 1.64 g. (2.9 mmoles) of glycyl-3,5-diiodo-L-tyrosylglycine hemihydrate. The crude reaction product was washed by centrifugation, then reprecipitated at pH 4.5, and dried *in vacuo* at room temperature yielding 0.18 g. (7%) of fine crystals, m.p. 198–201° dec.

Anal. Calcd. for $C_{19}H_{17}I_4N_3O_6 \cdot 2H_2O$: C, 24.62; H, 2.28; I, 54.76; N, 4.53. Found: C, 24.89; H, 2.29; I, 54.76; N, 4.76.

Inspection of paper chromatograms of the substance in short wave ultraviolet light revealed the presence of a faintly fluorescent spot. The fluorescent impurity was removed by extracting a solution of the tripeptide in 1 *N* NaOH several times with 1-butanol, washing the combined butanol extracts with water, then removing the butanol by evaporation under reduced pressure. Recrystallization of the residue gave a chromatographically pure product. For R_f values, see Table I.

(15) Nutritional Biochemicals Corp., Cleveland, Ohio.

(16) E. Abderhalden and M. Guggenheim, *Ber.*, **41**, 1237 (1908).

(17) M.p. 259–264° dec.; after recrystallization from acetone–water, 282–283° dec.; *cf.* E. Abderhalden, R. Abderhalden, H. Weidle, E. Baertich and W. Morneweg [*Fermentforschung.*, **16**, 98 (1938)] and H. Zahn and K. Ziegler [*Ann.*, **610**, 132 (1957)].

(18) M.p. 239–242° dec.; *cf.* ref. 17 and T. Yamashita, *J. Biochem.* (Tokyo), **48**, 651 (1960).

(19) Commercially available from Osaka Laboratory of Synthetic Organic Chemicals, Nishinomiya, Japan.

Synthesis of 2-(1-Nonenyl)-4-quinolinol (Pyo III) and Some Related 2,4-Disubstituted Quinolines

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In 1945 Hays and co-workers[2] reported the isolation of five closely related antibiotic metabolites of *Pseudomonas aeruginosa*. These substances were designated Pyo Ib, Pyo Ic, Pyo II, Pyo III, and Pyo IV. In 1952 Wells[3] reported the structural elucidation and synthesis of Pyo Ib, Pyo Ic, and Pyo III. Pyo Ib and Pyo Ic were shown to be the homologous 2-heptyl-4-quinolinol and 2-nonyl-4-quinolinol, respectively. Application of the Conrad–Limpach reaction[4] afforded synthetic Pyo Ib and Pyo Ic in yields above 25%. Structural studies featuring hydrogenation and ozonization[5] provided convincing evidence that the structure of Pyo III was 2-(1-nonenyl)-4-quinolinol. By use of chromatography an 0.8% yield of Pyo III was obtained by

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(2) E. E. Hays, *et al.*, *J. Biol. Chem.*, **159**, 725 (1945).

(3) I. C. Wells, *ibid.*, **196**, 331 (1952).

(4) N. J. Leonard, H. F. Herbrandson, and E. M. Van Heyningen, *J. Am. Chem. Soc.*, **68**, 1279 (1946).

(5) I. C. Wells, W. H. Elliott, S. A. Thayer, and E. A. Doisy, *J. Biol. Chem.*, **196**, 321 (1952).