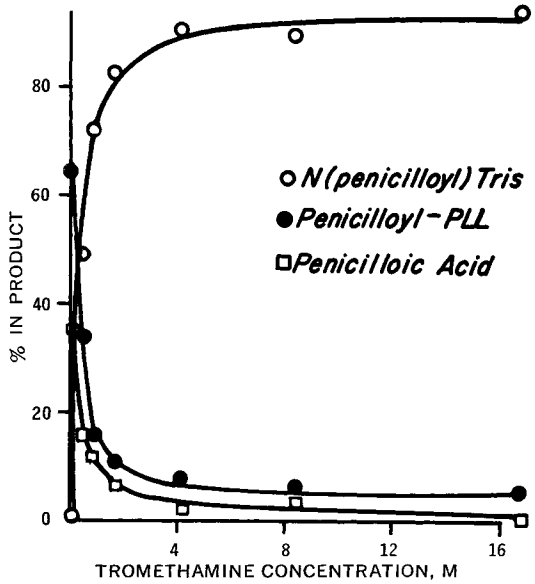

Figure 4—*Results of assays of products of reaction of penicillin with Tris and PLL at pH 8.8.*

zero time providing more accurate determination of penicilloic acid The extinction values of the amides are lower than previously observed by Levine (3), who found values about 23,000. The decrease may be a result of the difference in pH since Levine's work was done at pH 9.2, and the present study at pH 7.0.

The effect of $HgCl_2$ concentration on absorbance of penamaldate from the penicilloic acids is shown in Fig. 3. It can be seen that, not only are there differences in the maximal absorbance reached with each penicillin but the amount of $HgCl_2$ required to produce this maximum varies. Thus, assay conditions must be established for each penicillin individually.

The results of assay of products of reaction at pH 8.8 of benzylpenicillin with tromethamine and PLL at 35° are shown in Fig. 4. These confirm the previous hypothesis (5) that the penicillin reacts principally with tromethamine and the polymer serves as a catalyst. The curve for *N*-(penicilloyl)tromethamine markedly resembles the dependence of rate constant upon tromethamine concentration. One new aspect of the reaction revealed here is that both penicilloic acid and penicilloyl-PLL are formed in the absence of tromethamine, where about 38% of the original penicillin goes to penicilloic acid. The first-order rate constant for loss of penicillin under these conditions was 0.006 min.$^{-1}$. Multiplying by 0.38 gives 0.00228 min.$^{-1}$ as the rate constant for hydrolysis of benzylpenicillin at pH 8.8 in presence of PLL. An approximate value, calculated from data obtained at 31.5° (8), for hydrolysis of benzylpenicillin in absence of PLL is 2×10^{-4} min.$^{-1}$. It thus appears, that PLL is catalyzing the hydrolysis of benzylpenicillin. While the mechanism of this catalysis is unknown, it may be a clue as to one means by which the enzyme penicillinase may exert its catalytic effect.

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Thyroxine Analogs XVI: Synthesis and Activity of 3,5-Dibromo-3′-isopropyl-L-thyronine

EUGENE C. JORGENSEN and J. RAO NULU

Abstract □ 3,5-Dibromo-3′-isopropyl-L-thyronine (V) has been synthesized by two independent routes. Its biological potency, greater than that of L-thyroxine in a number of test systems, demonstrates that iodine is not a required substituent for thyroxine-like effects.

Keyphrases □ Thyroxine analogs—synthesis □ 3,5-Dibromo-3′-isopropyl-L-thyronine—synthesis □ Pharmacological screening—thyroxine analog □ Polarimetry—identity □ NMR spectroscopy—structure

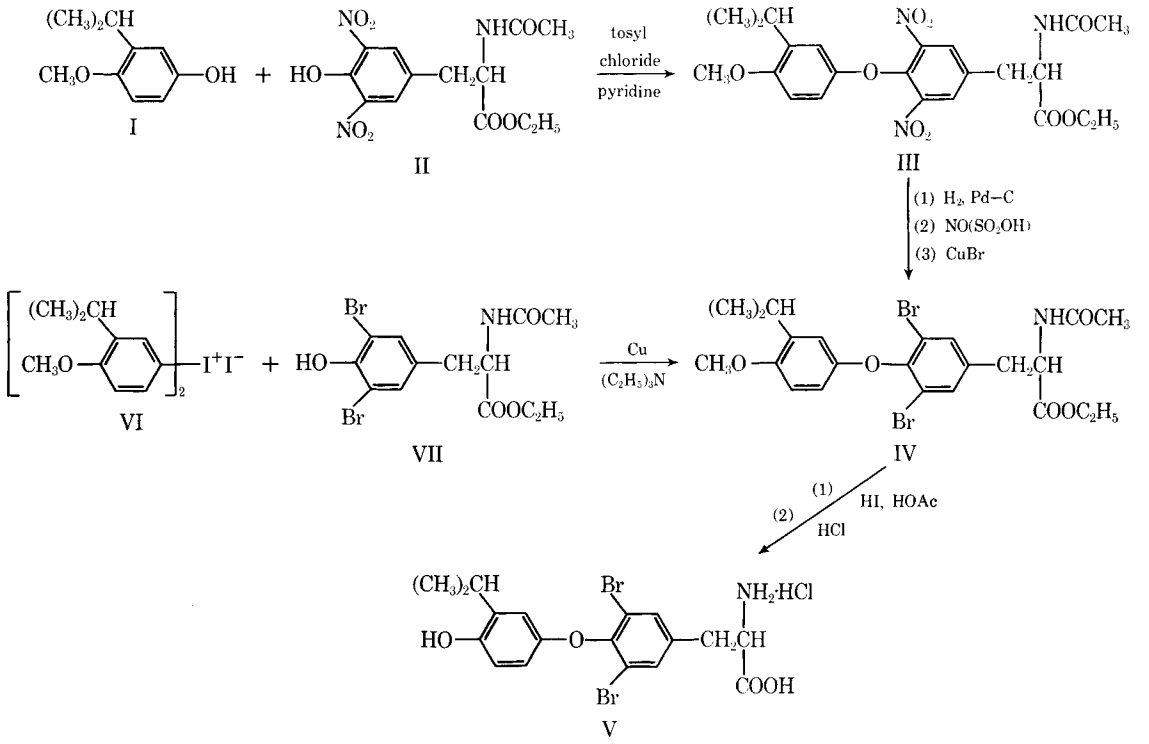
The importance of iodine in thyroid function has been recognized since the early establishment of its therapeutic value in certain types of goiter. Characterization of the thyroid hormones as iodinated thyronines provided a rationale for the biological importance of the element, but no insight into its functional role or relative importance in the molecule for hormonal actions. Some theories have emphasized a direct participation of iodine, with the rest of the molecule relegated to a lesser role as carrier (1–3).

Iodine on the phenolic ring of the thyroid hormones or their analogs has been replaced by aliphatic, alicyclic, and aromatic residues with retention and even enhancement of hormonal activity (4–10).[1] Methyl groups have

[1] *Reference* 7 is paper XV in this series.

replaced one or both iodine atoms in the 3- and 5-positions of the alanine-bearing ring of 3,5,3′-triiodothyronine with retention of hormonal activity (11). However, in no case has a significantly active iodine-free molecule been prepared. Brominated thyronines have shown moderate activities, but these were too low for general acceptance that iodine was not an essential component of the hormones (12). The excellent activating effects of the 3′-isopropyl group [*e.g.*, 3,5-diiodo-3′-isopropyl-L-thyronine, 7 to 12 times as active as L-thyroxine (13)] and of 3,5-dibromosubstitution [*e.g.*, 3,5-dibromo-3′-iodo-DL-thyronine, 1.3 times as active as DL-thyroxine (12)] encouraged synthesis in this laboratory of 3,5-dibromo-3′-isopropyl-L-thyronine (V) as an iodine-free thyroxine analog with the potential for significant hormonal activity.

The synthesis was carried out by two routes (Scheme I). 3-Isopropyl-4-methoxyphenol (I) was prepared in five steps by a method which assured the relative positions of all substituents (9). This was condensed with the *p*-toluenesulfonyl derivative of *N*-acetyl-3,5-dinitro-L-tyrosine ethyl ester (II) to form the dinitro diphenyl ether (III). Reduction to the diamine, bis-diazotization, and reaction with cuprous bromide yielded the dibromo ether (IV). Hydrolysis with hydriodic acid produced 3,5-dibromo-3′-isopropyl-L-thyronine (V), which was isolated as the hydrochloride to avoid the presence of iodine. A shorter synthesis of IV was achieved by the reaction of di(3-isopropyl-4-methoxyphenyl)iodonium iodide (VI) with *N*-acetyl-3,5-dibromo-L-tyrosine ethyl ester (VII) in the presence of copper and triethylamine (14, 15). Isomer formation has not been reported in this reaction, but the possibility was a source of concern. However, both IV and V obtained by both routes, were identical with their counterparts in all respects. In particular, NMR spectra which would have detected potential isomers of IV (16), were identical for the compounds prepared by both methods.



Scheme I

The demonstration that 3,5-dibromo-3′-isopropyl-L-thyronine (V) is more potent than L-thyroxine in a variety of biological systems, establishes the fact that iodine, as a substituent on the thyronine nucleus, plays no unique role in hormone action.

BIOLOGICAL RESULTS AND DISCUSSSION

The biological potency of V was evaluated in several systems (17). It was 7.3 times as potent as L-thyroxine in enhancing the resting heart rate of thyroidectomized rats. In two variations of the tadpole metamorphosis test, as measured by decrease in tail width or length, it was 2.0–2.1 times as active as L-thyroxine. In the rat antigoiter assay, it was 1.7 times as active as L-thyroxine. It appears that heart tissue in the rat is either more sensitive or more accessible to the analog than is the pituitary-thyroid system, or to the factors which initiate metamorphosis in the tadpole.

EXPERIMENTAL[2]

N-Acetyl-3[4-(3-*i*-propyl-4-methoxyphenoxy)-3,5-dibromophenyl]-L-alanine Ethyl Ester (IV)—*Method* A—*N*-Acetyl-3-[4-(3-*i*-propyl-4-methoxyphenoxy)-3,5-dinitrophenyl]-L-alanine ethyl ester (9) (III,

[2] Melting points were determined in a Thomas-Hoover apparatus and are uncorrected. Optical rotations were measured in a Rudolph photoelectric polarimeter. Microanalyses by the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley, Calif. NMR spectra were determined in $CDCl_3$ on a Varian A-60 spectrometer using tetramethylsilane (δ, 0) as internal standard. Chemical shifts are reported as δ values in p.p.m.; J values in c.p.s.

4.89 g., 0.01 mole) dissolved in 100 ml. of acetic acid was shaken for 1 hr. in the presence of palladium-on-charcoal (10%, 1.0 g.) and hydrogen (45 p.s.i. initial pressure). Hydrogen uptake was 98% of theoretical. Concentrated sulfuric acid (15 ml.) was added to the cooled solution, the catalyst removed by filtration through diatomaceous earth,[3] and the solution of the diamine added over 1 hr. to a stirred cooled solution (−5–0°) of nitrosylsulfuric acid (prepared by adding sodium nitrite, 4.2 g. (0.06 mole) in small portions to 90 ml. sulfuric acid at 60°, then diluted when cool with 35 ml. acetic acid). After addition was complete, the bisdiazonium solution was stirred for an additional hour at −5°, then added in a slow stream to a stirred mixture of cuprous bromide (4.8 g., 0.033 mole) in 60 ml. of 50% aqueous hydrobromic acid and 40 ml. of chloroform. The mixture was stirred for 2 hr. at room temperature, 80 ml. of water was added, the chloroform separated, and the aqueous layer extracted several times with chloroform. The combined chloroform extracts were washed in sequence with water, 10% sodium bisulfite, water, 5% sodium bicarbonate, and water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was dissolved in chloroform, adsorbed on an acid-washed alumina column, and eluted with chloroform. Evaporation and crystallization from ethanol yielded 3.3 g. (59%) of colorless crystals, m.p. 109–110°; $[\alpha]_D^{22}$ + 57.0° (c 2.0 $CHCl_3$).

Anal.—Calcd. for $C_{23}H_{27}Br_2NO_5$: C, 49.56; H, 4.89; Br, 28.41. Found: C, 49.48; H, 5.15; Br, 28.28.

Method B—A mixture of di(3-*i*-propyl-4-methoxyphenyl)iodonium iodide (9) (VI, 5.0 g., 90 mmoles), *N*-acetyl 3,5-dibromo-L-tyrosine ethyl ester (18) (2.0 g., 50 mmoles), 0.75 ml. of triethylamine (50 mmoles), and 50 mg. of copper powder in 60 ml. of methanol was stirred at room temperature for 36 hr. The mixture was filtered, the solvent removed *in vacuo*, and the residue dissolved in 40 ml. of benzene. The benzene solution was shaken with 25 ml. of 3% aqueous hydrochloric acid for 5 min. The resulting insoluble triethylamine hydrochloride was removed by filtration and the separated benzene layer washed twice with water, twice with 10-ml. portions of 1 *N* sodium hydroxide, and with water. The benzene solution was dried briefly over sodium sulfate and distilled. The residue solidified on trituration with cold petroleum ether and was crystallized from ethanol to yield 1.4 g. (51%), m.p. 109–110°; $[\alpha]_D^{22}$ + 56.2° (c 2.0 $CHCl_3$). No depression of m.p. on mixture with IV prepared by Method A.

Anal.—Found: C, 49.68; H, 4.83; Br, 28.48.

The NMR spectra of (IV) prepared by Methods A and B were identical: 2,6-protons, singlet at 7.46 p.p.m.; 2′-proton, doublet at 6.88 p.p.m. (*J*, 3 c.p.s.); 5′-proton, doublet at 6.78 p.p.m. (*J*, 9 c.p.s.); 6′-proton, quartet at 6.56 p.p.m. (*J*, 9, 3 c.p.s.); methine proton, quartet at 4.88 p.p.m. (*J*, 7 c.p.s.); β-carbon-protons and methine proton of isopropyl, doublet at 3.13 p.p.m. (*J*, 6 c.p.s.); amido proton, broad doublet at 6.39 p.p.m.; methoxy proton, singlet at 3.81 p.p.m.; ethyl methylene protons, quartet at 4.25 p.p.m. (*J*, 7 c.p.s.); ethyl methyl protons, triplet at 1.29 p.p.m. (*J*, 7 c.p.s.); acetyl methyl protons, singlet at 2.04 p.p.m.; isopropyl methyl protons, doublet at 1.19 p.p.m. (*J*, 7 c.p.s.).

3-[4-(3-i-Propyl-4-hydroxyphenoxy)-3,5-dibromophenyl]-L-alanine Hydrochloride (V)—A solution of 500 mg. (0.9 mmole) of IV in 10 ml. of 48% hydriodic acid and 10 ml. of acetic acid was heated under reflux for 4 hr. The cooled solution was poured into 80 ml. of ice water, and the pH adjusted to 5.5 with aqueous sodium hydroxide. The precipitated solid was removed by filtration, washed with cold water, heated to boiling with 15 ml. of 0.5 *N* hydrochloric acid, and the hot solution filtered. The filtrate was cooled to yield white crystals, 300 mg. (65%), m.p. 214–218° (dec.).

From IV by Method A: Calcd. for $C_{18}H_{20}Br_2ClNO_4$: C, 42.42; H, 3.95; Br, 31.36. Found: C, 42.68; H, 3.88; Br, 30.96. $[\alpha]_D^{25}$ + 34.6 (c 1.0, ethanol : 1 *N* hydrochloric acid, 3:1).

From IV by Method B: Found: C, 42.30; H, 3.85; Br, 31.34. $[\alpha]_D^{25}$ + 35.2 (c 1.0, ethanol : 1 *N* hydrochloric acid, 3:1).

Samples of V prepared by both methods were identical on descending chromatography on Whatman No. 1 filter paper. R_f 0.88, *n*-butanol–acetic acid–water, 10:1:3; R_f 0.94, isoamyl alcohol saturated with 2 *N* ammonium hydroxide.

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