atom) of sodium in 40 ml. of allyl alcohol, and the mixture was heated for 1 hr. at 50°. Excess allyl alcohol was removed at reduced pressure (50° at 0.5 mm.) and the residue was extracted with chloroform. Removal of the solvent under reduced pressure gave a crude yield of 2.8 g. (46%) of I as a light tan oil.

The product contained approximately 5% 1-alloxy-2-pyridone (III) as determined by infrared analysis. Further purification by alumina chromatography using chloroform as the eluent gave 2.3 g. (38%) of pure I.

Anal. Calcd. for $C_8H_9NO_2$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.57; H, 6.10; N, 9.40.

1-Alloxy-2-pyridone (III).—2-Alloxypyridine 1-oxide, 1.00 g. was heated at 100° for 3.5 hr. After cooling, the resulting dark brown oil was purified by chromatography on alumina. Gradient elution with benzene, chloroform, and ethyl acetate gave 0.83 g. (83%) of III as a light tan oil. This material was further purified by preparative scale gas chromatography to obtain an analytical sample. A 2-ft. 20% General Electric XF-1150 polymer on Chromosorb-W was used. The column temperature was maintained at 190° with a helium flow of 60 ml./min. The ultraviolet spectrum showed λ_{max}^{water} 296 mμ (ε 5400) and 226 mμ (ε 5500); and the infrared spectrum showed carbonyl absorption at 6.01 μ.

Anal. Calcd. for $C_8H_9NO_2$: C, 63.56; H, 6.00; N, 9.27. Found: C, 61.98; H, 6.25; N, 9.47.

1-Benzyloxy-2-pyridone (VII).—2-Benzyloxypyridine 1-oxide (VIII),[2] 1.00 g., was heated for 2.5 hr. at 100°. Upon cooling, the reaction mixture solidified. Recrystallization from ethyl acetate–ligroin gave 0.92 g. (92%) of VIII, m.p. 78–79°, lit.[3] m.p. 76–78°. A mixture melting point of VIII prepared by the literature procedure and as above was not depressed. The infrared spectra of the two products are identical.

1-Methoxy-2-pyridone (VI).—2-Methoxypyridine 1-oxide (IV), 1.00 g., was heated for 1.5 hr. at 140°. The resulting dark brown oil was purified by chromatography on alumina. Gradient elution with benzene, chloroform, and ethyl acetate gave 0.89 g. (89%) of VI as a tan oil which could not be crystallized.[11] An aqueous solution of the product gave the same ultraviolet spectrum reported for authentic VI.[3] The infrared spectrum showed carbonyl absorption at 6.02 μ.

This procedure is also typical for the preparation of the following compound.

1-Ethoxy-2-pyridone (IX).—2-Ethoxypyridine 1-oxide (V) was heated for 3 hr. at 140° to give crude IX. Gradient elution chromatography as above gave 85% of IX as a light tan oil. The ultraviolet spectrum showed λ_{max}^{water} 295 mμ (ε 5900) and 225 mμ (ε 6100); the infrared spectrum showed carbonyl absorption at 6.02 μ.

Anal. Calcd. for $C_7H_9NO_2$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.62; H, 6.90; N, 9.89.

Effect of *p*-Benzoquinone on Rate of Rearrangement.—*p*-Benzoquinone, 3% by weight, was added to the 2-alkoxypyridine 1-oxide to be investigated. Portions of this sample together with pure samples of the same 2-alkoxypyridine 1-oxide were heated together in sealed tubes in a stirred oil bath set at the desired temperature so that variations in the bath temperature could not change the rate of one rearrangement with respect to the other. Tubes containing the experimental and control samples were simultaneously withdrawn from the bath at 15-min. intervals and the infrared spectra were determined. The rate of rearrangement of the 2-alkoxypyridine 1-oxide was determined by the disappearance of the 1-oxide bands in the 7.8–8.4-μ region. Formation of product was indicated by the appearance of a carbonyl absorption band at approximately 6.0 μ.

In this manner, the rearrangements of 2-alloxypyridine 1-oxide, 2-benzyloxypyridine 1-oxide, and 2-methoxypyridine 1-oxide to the corresponding 1-alkoxy-2-pyridones were shown to proceed at the same rate in the presence and absence of added *p*-benzoquinone.

Rates of Rearrangement of 2-Alkoxypyridine 1-Oxides.—The 2-alkoxypyridine 1-oxides were heated in a stirred oil bath set at the desired temperature. Samples were withdrawn at 15-min. intervals and their ultraviolet spectra were determined on a Beckman DK-2 spectrophotometer.

(11) Gardner and Katritzky reported that this material partially solidified on standing. This has not been observed in the present case.

Rearrangements were judged complete when the maximum at approximately 250 mμ, which characterizes the spectra of 2-alkoxypyridine 1-oxides and is missing in 1-alkoxy-2-pyridones, had disappeared. At this time, the spectra corresponded to those of the corresponding 1-alkoxy-2-pyridones.

The rearrangements of 2-alloxypyridine 1-oxide (I) and 2-benzyloxypyridine 1-oxide (VIII) were found to be complete after 3.5 and 2.5 hr. at 100°, respectively. 2-Methoxypyridine 1-oxide (IV) and 2-ethoxypyridine 1-oxide (V) were rearranged at 140° and found to be complete after 1.5 and 3 hr., respectively.

Model Reactions for the Biosynthesis of Thyroxine. V. Reaction of 4-Hydroxy-3-iodophenylpyruvic Acid and of 4-Hydroxy-3,5-diiodophenylpyruvic Acid with L-Tyrosine or Its Iodinated Congeners. A Novel Synthesis of 3,3′,5′-Triiodo-L-thyronine[1,2]

TETSUO SHIBA[3] AND H. J. CAHNMANN

The National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

Received April 22, 1963

DIHPPA[4] reacts with DIT[4] in the presence of oxygen to form thyroxine in over 20% yield.[5] The reaction takes place rapidly at room temperature and at or near neutrality. No racemization takes place when L-DIT is used.[6] It has been shown through experiments with labeled starting materials that the keto acid furnishes the phenolic ring of thyroxine, and the amino acid—the nonphenolic ring and the aliphatic side chain.[6] The side chain of the keto acid is eliminated in the course of the reaction.

The present investigation was undertaken in order to determine whether in this coupling reaction DIHPPA can be replaced with MIHPPA,[4] and DIT with MIT[4] or with tyrosine and to what extent the corresponding iodinated thyronines are formed in each case.

The coupling reaction was carried out essentially as described previously for the synthesis of thyroxine.[5,6] The amount of iodinated thyronine formed in each reaction was determined by isolation and weighing. A modification of the procedure of Nakano and Danowski[7] was used for the preparation of MIHPPA.

When in the coupling reaction described by Meltzer and Stanaback[5] L-DIT was replaced with L-MIT, the yield of the coupling product dropped from over 20% to 17%. When L-DIT was replaced with L-tyrosine, the yield of 3′,5′-diiodo-L-thyronine was about 0.2%. Reaction of MIHPPA with L-DIT gave 3,5,3′-triiodo-L-thyronine in about 2% yield. In view of this low

(1) For a preliminary report of this work see Abstracts, 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., Sept., 1962, p. 9C.

(2) Paper IV: T. Matsuura and A. Nishinaga, *J. Org. Chem.*, **27**, 3072 (1962).

(3) Visiting Scientist from the Department of Chemistry, Faculty of Science, Osaka University, Osaka, Japan.

(4) Abbreviations: HPPA, *p*-hydroxyphenylpyruvic acid; MIHPPA, 4-hydroxy-3-iodophenylpyruvic acid; DIHPPA, 4-hydroxy-3,5-diiodophenylpyruvic acid; MIT, 3-iodotyrosine; DIT, 3,5-diiodotyrosine.

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

(6) T. Shiba and H. J. Cahnmann, *ibid.*, **27**, 1773 (1962).

(7) N. Nakano and T. S. Danowski, *Endocrinology*, **65**, 889 (1959).

yield the reaction of MIHPPA with L-tyrosine was not investigated. The formation of only a trace of 3′-iodo-L-thyronine can be expected in that reaction. The reaction of HPPA[4] with L-MIT was not investigated because Meltzer and Stanaback[5] found that HPPA does not react with L-DIT.

The results show that iodinated thyronines can be obtained in good yield only with DIHPPA. The replacement of DIHPPA with MIHPPA results in poor yields. With DIHPPA, good yields of iodinated thyronines are obtained only when the other reaction partner is either DIT or MIT. DIHPPA reacts with tyrosine to give only traces of the corresponding iodinated thyronine.

It has been shown[6] that the coupling reaction of DIHPPA with L-DIT provides a simple method for the synthesis of a number of specifically labeled radioactive forms of L-thyroxine. Similarly, the ease with which 3,3′,5′-triiodo-L-thyronine is formed by coupling DIHPPA with L-MIT offers a convenient method for the synthesis of various specifically labeled radiosiomers of 3,3′,5′-triiodo-L-thyronine. This so-called "reverse" triiodothyronine occurs in the thyroid. Previously used procedures for its synthesis are tedious since they involve many steps.

It has been suggested[8,9] that DIHPPA may be an intermediate in the biosynthesis of L-thyroxine from L-DIT. In this connection it is of interest to compare the amounts of various iodinated thyronines present in the thyroid with the ease with which the same thyronines are formed in the nonenzymic coupling reaction. The amounts present in the thyroid decrease in this order: thyroxine > 3,5,3′-triiodothyronine > 3,3′,5′-triiodothyronine. 3′,5′-Diiodothyronine has not been detected in the thyroid. The yields in the nonenzymic coupling reaction decrease in this order: thyroxine > 3,3′,5′-triiodothyronine > 3,5,3′-triiodothyronine > 3′,5′-diiodothyronine.

All coupling reactions were carried out at about the same pH (6.7–7.7). A variation of pH within these limits does not greatly affect the yield of thyroxine from DIHPPA and DIT. It should be pointed out that, at this pH, the degree of dissociation of the phenolic groups of the various amino acids used as reaction partners differs greatly depending on the number of iodine atoms present in the *ortho* positions. The pK values for the phenolic groups of tyrosine, MIT, and DIT are 10.0, 8.7, and 6.5, respectively.[10] Consequently, it is possible that, at a higher pH at which the phenolic groups of MIT and of tyrosine are ionized, these two amino acids would also form the corresponding thyronines in a higher yield. It is difficult to investigate this possibility experimentally since DIHPPA is extremely unstable in alkaline medium.

Experimental[11]

4-Hydroxy-3-iodophenylpyruvic Acid.—*p*-Hydroxybenzaldehyde was iodinated with either iodine monochloride or with potassium triiodide.[12] In both cases a series of fractional crystallizations and dissolutions was required to remove starting material and the diiodinated aldehyde that was always formed together with the desired product. This purification was conveniently followed by infrared spectroscopy or by gas chromatography (1% SE 30, siliconized Gaschrom P); the pure acid had m.p. 110.5°.

A modification of the procedure of Nakano and Danowski[7] was used for the conversion of the aldehyde to 2-methyl-4-(4-acetoxy-3-iodobenzal)-5-oxazolone. The pure azlactone, m.p. 184–185°, was obtained after recrystallization from benzene–isooctane with the addition of Norit.

Anal. Calcd. for $C_{13}H_{10}INO_4$: C, 42.07; H, 2.72; I, 34.20; N, 3.78. Found: C, 42.05; H, 2.89; I, 34.31; N, 3.76.

A two-step hydrolysis[7] of the azlactone was found not to be necessary, as a single-step hydrolysis with 3 ml. of 2 N hydrochloric acid per mmole of azlactone (3 hr., 100°) gave the keto acid in the same yield. Recrystallization was done from water containing a few drops of 4 N hydrochloric acid.

3,3′,5′-Triiodo-L-thyronine.—A solution of 1.54 g. (5.0 mmoles) of 3-iodo-L-tyrosine[13] in a mixture of 100 ml. of 0.2 M sodium phosphate buffer (pH 6.7), 17.5 ml. of 1 N sodium hydroxide, and 17.5 ml. of a saturated aqueous solution of sodium sulfate was adjusted to pH 6.7 with 4 N hydrochloric acid. Then 50 μl. of *t*-butyl hydroperoxide[14] was added. Oxygen was bubbled through the vigorously stirred reaction mixture while 2.59 g. (6.0 mmoles) of 4-hydroxy-3,5-diiodophenylpyruvic acid[5,6,15] was added in very small portions over a period of 1.3 hr. The pH was maintained at 6.7 by adding 2 N sodium hydroxide slowly to the reaction mixture by means of an immersed thin polyethylene tubing. The rate of addition was automatically controlled with a pH-stat.[16] Bubbling of oxygen and stirring were continued for another 30 min. The reaction mixture was kept overnight at 2°, then filtered. The solid material was washed with a small amount of ice-cold water and dried, yielding 0.83 g. Paper chromatography revealed contamination with a small amount of 3-iodotyrosine. Dissolution in 4 N ammonium hydroxide and reprecipitation with 4 N hydrochloric acid at pH 5 gave 0.56 g. (17%) of practically pure 3,3′,5′-triiodo-L-thyronine, m.p. 191–193° dec.; R_f 0.34 (1-butanol–dioxane–2 N ammonium hydroxide) and 0.73 (1-butanol–acetic acid–water). For elemental analysis part of the material was reprecipitated a second time.

Anal. Calcd. for $C_{15}H_{12}I_3NO_4$: C, 27.67; H, 1.84; I, 58.48; N, 2.15. Found: C, 27.55; H, 1.79; I, 58.66; N, 2.44.

A second crop of 0.24 g. of crude 3,3′,5′-triiodo-L-thyronine was obtained from the mother liquors after concentration.

Other Coupling Reactions.—These reactions were carried out following essentially the procedure described for the synthesis of 3,3′,5′-triiodo-L-thyronine with appropriate minor modifications made necessary by the differences in the solubilities of the various starting materials and by the low coupling yields. In view of these low yields a detailed description of each coupling reaction will not be given.

The reaction of 3,5-diiodo-L-tyrosine with 4-hydroxy-3-iodophenylpyruvic acid at pH 7.0 gave chromatographically pure 3,5,3′-triiodothyronine in 2% yield. The reaction of L-tyrosine with 4-hydroxy-3,5-diiodophenylpyruvic acid at pH 7.7[17] gave 3′,5′-diiodo-L-thyronine in 0.2% yield. The identity of the coupling products was ascertained by comparison of the infrared spectra and of the chromatographic mobilities with those of authentic samples.

(8) T. Shiba and H. J. Cahnmann, *Biochim. Biophys. Acta*, **58**, 609 (1962).

(9) S. Lissitzky and C. Cheftel, *Compt. rend.*, **256**, 3898 (1963).

(10) R. Pitt-Rivers and J. R. Tata, "The Thyroid Hormones," Pergamon Press, Inc., New York, N. Y., 1959, p. 188.

(11) The microanalyses were done by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y., and by Mr. H. G. McCann and his associates of the analytical service laboratory of this institute. Melting points were determined in capillary tubes and are uncorrected. Paper chromatographic solvents were 1-butanol–dioxane–2 N ammonium hydroxide (4:1:5) and 1-butanol–acetic acid–water (78:10:12). The location of the chromatographic spots was revealed by visual inspection in short-wave ultraviolet light and by spraying with a solution of ninhydrin or of diazotized N^1,N^1-diethylsulfanilamide [T. Matsuura and H. J. Cahnmann, *J. Am. Chem. Soc.*, **81**, 871 (1959)].

(12) J. H. Barnes, E. T. Borrows, J. Elks, B. A. Hems, and A. G. Long, *J. Chem. Soc.*, 2824 (1950).

(13) A commercial sample was recrystallized from water. The recrystallized material was chromatographically pure.

(14) Lucidol Davidson, Wallace and Tiernan, Buffalo, N. Y.

(15) Now commercially available from Osaka Laboratory of Synthetic Organic Chemicals, 74 Ueda Higashimachi, Nishinomiya, Japan.

(16) Radiometer, Copenhagen, Denmark.

(17) L-Tyrosine is not completely soluble at a lower pH.