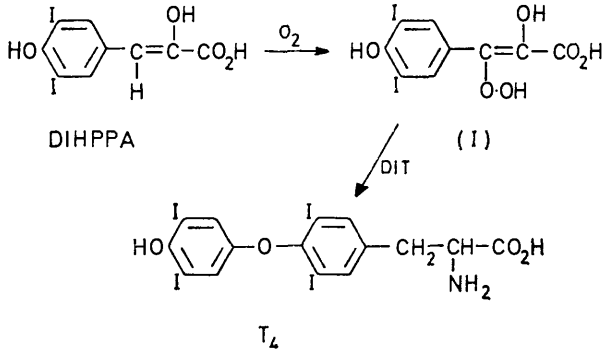
Model Reactions for the Biosynthesis of Thyroxine. Part XVII.[1] On the Mechanism of the Conversion of 4-Hydroxy-3,5-di-iodophenylpyruvic Acid into Thyroxine

By **Akira Nishinaga,*** Department of Synthetic Chemistry, Faculty of Engineering, Kyoto University Kyoto, Japan
Hans J. Cahnmann, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A.

4-Hydroxy-3,5-di-iodobenzoylglyoxylic acid is a potential intermediate in the nonenzymic formation of thyroxine from 3,5-di-iodotyrosine and 4-hydroxy-3,5-di-iodophenylpyruvic acid; its ethyl ester has been synthesised. The lack of reactivity of this ester towards 3,5-di-iodotyrosine indicates that the diketo-acid is not an intermediate in the formation of thyroxine.

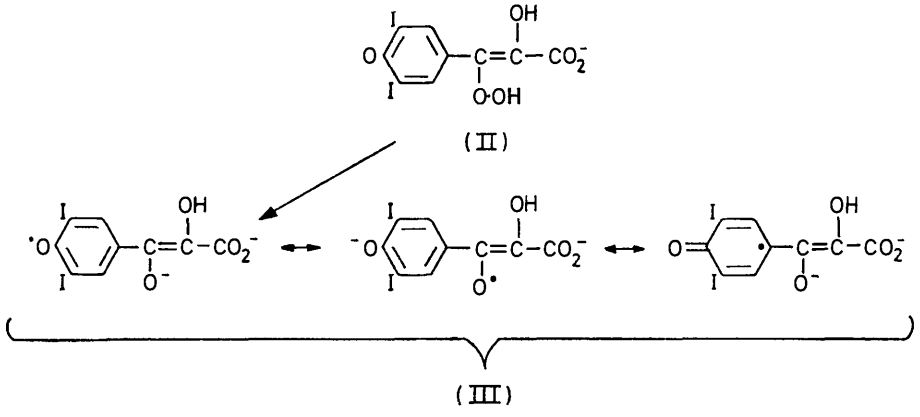
THE oxidative coupling reaction between 3,5-di-iodotyrosine (DIT) and 4-hydroxy-3,5-di-iodophenylpyruvic acid (DIHPPA) is a nonenzymic model reaction for the biosynthesis of thyroxine (T_4). The mechanism of this reaction has been investigated previously [2-6] and shown



to take place in two distinct steps, an aerobic and an anaerobic one. In the first step DIHPPA is oxidised to a hydroperoxide (I), which then reacts in the second step with DIT to form T_4 [5] in which the phenolic ring is derived from the hydroperoxide.[2]

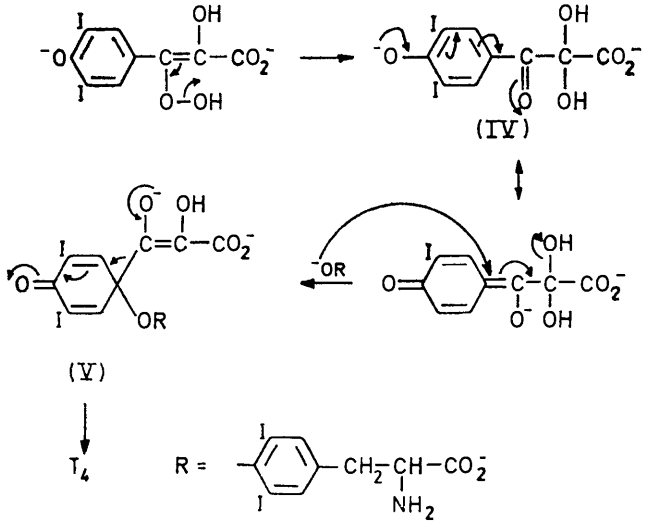
The first step apparently involves the formation of the phenoxyl radical from DIHPPA.[5,7] The mechanism of the second step is still obscure. A free-radical route analogous to that proposed [8] for the aerobic formation of T_4 from DIT is unlikely for several reasons. For example the reaction takes place in the absence of oxygen or any other one-electron transfer agent. Furthermore, homolytic fission of the hydroperoxy-group of (II), the predominant form of the hydroperoxide at pH 7·5—8·0 where the coupling reaction is carried out,[5] would lead to a semidione (III). However, there is no reason to assume that the system (III) could react with DIT to form T_4; its highest electron density should be at the α- and β-carbon atoms of the aliphatic side chain and not at the *para*-position of the phenolic ring.[9]

An alternative mechanism involves heterolytic fission of the O–O bond of the hydroperoxy-group of (II). Such a fission could conceivably lead to a hydrated diketo-acid (IV), which in turn could form T_4 *via* the quinol ether (V). We report an investigation of the validity of such a mechanism. There are no literature reports of the synthesis or the reactions of phenolic diketo-acids of type (IV). Our attempts to synthesise



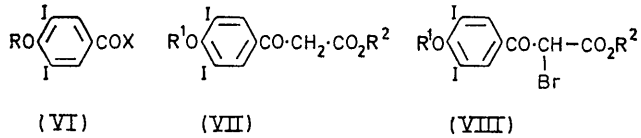
[1] Part XVI, H. Ogawara, J. M. Bilstad, and H. J. Cahnmann, *Biochem. Biophys. Acta*, 1972, **257**, 339.
[2] T. Shiba and H. J. Cahnmann, *J. Org. Chem.*, 1962, **27**, 1773.
[3] T. Shiba, H. J. Cahnmann, T. Matsuura, A. Nishinaga, and H. Sakamoto, *J. Org. Chem.*, 1964, **29**, 3061.
[4] T. Matsuura and A. Nishinaga, *Nippon Kagaku Zasshi*, 1965, **86**, 282.
[5] A. Nishinaga, H. J. Cahnmann, H. Kon, and T. Matsuura, *Biochemistry*, 1968, **7**, 388.
[6] F. Blasi, F. Fragomele, and I. Covelli, *European J. Biochem.*, 1968, **5**, 215.
[7] H. J. Cahnmann and K. Funakoshi, *Biochemistry*, 1969, **9**, 90.
[8] T. B. Johnson and L. B. Tewkesbury, jun., *Proc. Nat. Acad. Sci., U.S.A.*, 1942, **28**, 73.
[9] A. Nishinaga, H. Kon, H. J. Cahnmann, and T. Matsuura, *J. Org. Chem.*, 1968, **33**, 157.

the acid (IV) were not successful, but the ethyl ester (IXb) was prepared. The ester was found to be unreactive towards DIT under experimental conditions



similar to those used for the conversion of the hydroperoxide (I) into T_4.[5] Since a conversion of the hydroperoxide (I) into T_4 requires an attack at the *para*-position of the phenolic ring by DIT, for which it is immaterial whether the carboxy-group of (I) is free or esterified, we conclude that the acid (IV) is not an intermediate in this conversion.

The ester (IXb) was synthesised by the reaction of 4-acetoxy-3,5-di-iodobenzoyl chloride (VIa) with ethyl acetoacetate, followed by deacetylation with sodium ethoxide [to form (VIIb)], bromination [to form (VIIIb)], and oxidation with dimethyl sulphoxide. The structure of the ester (IXb) was confirmed by i.r. and n.m.r.

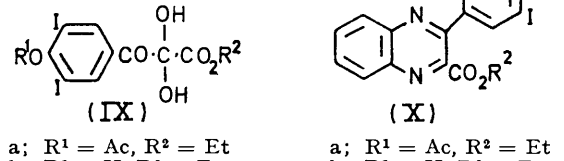


(VI) (VII) (VIII)

a; R = Ac, X = Cl
b; R = H, X = OH

a; R^1 = Ac, R^2 = Et
b; R^1 = H, R^2 = Et
c; R^1 = R^2 = H

a; R^1 = Ac, R^2 = Et
b; R^1 = H, R^2 = Et



(IX) (X)

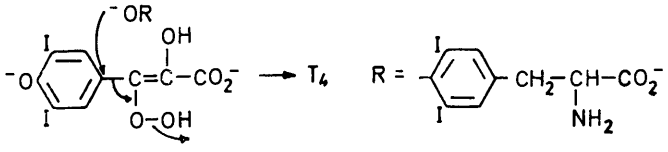
a; R^1 = Ac, R^2 = Et
b; R^1 = H, R^2 = Et

a; R^1 = Ac, R^2 = Et
b; R^1 = H, R^2 = Et
c; R^1 = R^2 = H

spectroscopy, elemental analysis, and conversion into the quinoxaline (Xb) by treatment with *o*-phenylenediamine. The diketo-esters (IXa and b) were obtained as hydrates and are represented as such. The tendency of αβ-diketo-esters to form hydrates is well known.[10]

Attempts to convert the ester (IXb) into the free acid (IV) failed: treatment with alkali (pH > 12) resulted in side-chain fission with formation of 4-hydroxy-3,5-di-iodobenzoic acid (VIb). Under mild conditions (pH <10) no reaction took place.

Since the diketo-acid hydrate (IV) does not seem to be an intermediate in the formation of thyroxine from the hydroperoxide (I), other pathways must be considered. An initiation or promotion of the reaction by fission of the hydroperoxy-group appears likely in view of the low bond energy of the O–O bond (*ca.* 35 kcal). Heterolytic fission with elimination of HO^- would result in the formation of an electron-deficient oxygen system. This, in concert with an attack by the phenolate anion of DIT would facilitate the splitting of the C–C bond between the phenolic ring and the adjacent carbon atom of the side chain.



(II)

Competition by HO^- from the aqueous medium with RO^- would lead to the formation of 2,6-di-iodohydroquinone. This hydroquinone is indeed always found as a minor side product in the conversion of the hydroperoxide (I) into T_4,[9] and its amount is greater the higher the pH. The preferential attack by the phenolate anion of DIT can perhaps be explained in terms of initial formation of an electron donor–acceptor complex between it and the hydroperoxide (I). The anions of DIT and of related di-iodophenols are known to have a tendency to form such charge-transfer complexes.[11]

Although the formation of a charge-transfer complex in the conversion of the hydroperoxide (I) into T_4 has not been proven, a mechanism involving such a complex and the subsequent elimination of HO^- appears reasonable since other mechanisms considered have been eliminated.

EXPERIMENTAL

Spectra.—N.m.r. spectra were determined with a JEOL JMN-3H-60 recording spectrometer (tetramethylsilane as internal standard); i.r. spectra (Nujol mulls) were recorded with a Nihon Bunko IR-S spectrometer.

4-Acetoxy-3,5-di-iodobenzoyl Chloride (VIa).—A suspension of 4-acetoxy-3,5-di-iodobenzoic acid (43 g) in thionyl chloride (50 g) and dry benzene (100 ml) was refluxed. After a clear solution had been obtained (8 h), refluxing was continued for 1 h. The solution was concentrated under reduced pressure to *ca.* 20 ml. Addition of light petroleum (100 ml) gave crystals which were rapidly collected and used without further purification.

Ethyl 4-Acetoxy-3,5-di-iodobenzoylacetate (VIIa).—The procedure is similar to that used by Straley and Adams [12]

[10] M. Doll, *Rev. Gen. Chim.*, 1917, **20**, 33.
[11] J. Mauchamp and M. Shinitzky, *Biochemistry*, 1969, **8**, 1554.
[12] J. M. Straley and A. C. Adams, *Org. Synth.*, 1963, Coll. Vol. IV, p. 415.

for the synthesis of ethyl benzoylacetate. A mixture of water (100 ml), light petroleum (100 ml), and freshly distilled ethyl acetate (16 g) was cooled (5°), and aqueous sodium hydroxide (33%; 5 ml) was added. To this solution was added the acid chloride (VIa) (43 g) during 30 min, while the solution was stirred, cooled to below 10°, and kept at about pH 11 by the controlled addition of aqueous 33% sodium hydroxide. Stirring and temperature and pH control were maintained for an additional 1 h. Then ammonium chloride (8 g) was added and stirring was continued overnight at room temperature. The solid product was collected and dissolved in chloroform. The solution was washed with water, dried (Na_2SO_4), and evaporated. The residue gave the *ester* (VIIa) (20 g, 40%) as prisms, m.p. 118—120° (from ethanol) (Found: C, 31·05; H, 2·6; I, 50·35. $C_{13}H_{12}I_2O_5$ requires C, 31·1; H, 2·4; I, 50·55%); $\nu_{max.}$ 1750 (AcO), 1720 (ester), and 1680 cm^{-1} (ArCO); δ 8·38 (2H, s), 4·24 (2H, q, *J* 7·2 Hz), 3·97 (2H, s), 2·46 (3H, s), and 1·29 (3H, t, *J* 7·2 Hz) (keto-form), and 8·10 (2H, s), 5·55 (1H, s), 4·25 (2H, q, *J* 7·2 Hz), 2·46 (3H, s), and 1·32 p.p.m. (3H, t, *J* 7·2 Hz) (enol form) (keto : enol, 5 : 1).

Ethyl 4-*Hydroxy*-3,5-*di-iodobenzoylacetate* (VIIb).—To a solution of sodium (2·3 g) in absolute ethanol (50 ml) was added the ester (VIIa) (20 g). After 30 min the yellow solution was cooled in an ice-bath, acidified with conc. hydrochloric acid, and then diluted with water (500 ml). Extraction with ether and evaporation of the extract gave the *hydroxy-ester* (VIIb) (17·2 g, 94%), m.p. 85—87° (from benzene–light petroleum) (Found: C, 28·7; H, 2·2; I, 55·45. $C_{11}H_{10}I_2O_4$ requires C, 28·7; H, 2·2; I, 55·2%); $\nu_{max.}$ 3273 (OH), 1724 (ester CO), and 1661 cm^{-1} (ArCO); δ 8·26 (2H, s), 5·85br (1H, s, disappeared upon addition of D_2O), 4·20 (2H, q, *J* 7·2 Hz), 3·91 (2H, s), and 1·28 (3H, t, *J* 7·2 Hz) (keto-form), and 8·06 (2H, s), 5·51 (1H, s), 4·22 (2H, q, *J* 7·2 Hz), and 1·31 p.p.m. (3H, t, *J* 7·2 Hz) (enol form) (keto : enol, 6 : 1).

Alkaline hydrolysis of the ester (VIIb) gave the keto-acid (VIIc), which underwent decarboxylation on attempted recrystallisation from aqueous methanol, even without heating.

Ethyl 4-*Acetoxy*-3,5-*di-iodobenzoyl*(*bromo*)*acetate* (VIIIa).—A solution of bromine (8 g) in chloroform (5 ml) was added during 30 min to a stirred solution of the ester (VIIa) (25·1 g) in chloroform (100 ml). Evaporation of the solvent left the *bromo-ester* (VIIIa) (23·5 g, 81%), m.p. 102—104° (from EtOH) (Found: C, 26·95; H, 1·85. $C_{13}H_{11}BrI_2O_5$ requires C, 26·85; H, 1·9%); δ 8·05 (2H, s), 5·35 (1H, s), 4·16 (2H, q, *J* 6·9 Hz), 2·35 (3H, s), and 1·23 p.p.m. (3H, t, *J* 6·9 Hz).

Ethyl Bromo-(4-*hydroxy*-3,5-*di-iodobenzoyl*)*acetate* (VIIIb).—Bromine (3·2 g) in chloroform (10 ml) was added during 15 min to a stirred and ice-cooled solution of the hydroxy-ester (VIIb) (9·2 g) in chloroform (90 ml). Evaporation left the *bromo-derivative* (VIIIb) (10·5 g, 98%), needles, m.p. 109—111° (from benzene–light petroleum) (Found: C, 24·65; H, 1·75. $C_{11}H_9BrI_2O_4$ requires C, 24·5; H, 1·7%); $\nu_{max.}$ 3393 (OH), 1753 (ester CO), and 1672 cm^{-1} (ArCO); δ 8·30 (2H, s), 5·85br (1H, s, disappeared upon addition of D_2O), 5·53 (1H, s), 4·30 (2H, q, *J* 6·9 Hz), and 1·30 p.p.m. (3H, t, *J* 6·9 Hz).

Ethyl 4-*Acetoxy*-3,5-*di-iodobenzoylglyoxylate Hydrate* (IXa).—A solution of the bromo-ester (VIIIa) (20 g) in dimethyl sulphoxide (100 ml) was kept at 45° for 20 h and then poured into ice–water. The mixture was extracted with ether and the extract washed with water, aqueous sodium hydrogen carbonate, and water again, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a silica gel column (200 g), which was eluted with benzene (100 ml) and then with ether (300 ml). The latter eluate gave the *hydrate* (IXa) as a viscous yellow liquid (9·5 g, 54%), which formed prisms (8·3 g), m.p. 95—97° (from benzene saturated with water) (Found: C, 29·2; H, 2·15; I, 47·5. $C_{13}H_{10}I_2O_6,H_2O$ requires C, 29·25; H, 2·25; I, 47·55%); $\nu_{max.}$ 3400 (OH), 1783 (AcO), 1758 (ester CO), and 1693 cm^{-1} (ArCO); δ 8·47 (2H, s), 4·26 (2H, q, *J* 7·2 Hz), 2·49 (3H, s), and 1·15 p.p.m. (3H, t, *J* 7·2 Hz).

Reduction of the diketo-ester (IXa) with hydrogen sulphide gave the monoketo-ester (VIIa) (60%). Alkaline hydrolysis of the diketo-ester (IXa) in ethanolic M-sodium hydroxide (1 h) gave the acid (VIb) (95%).

Ethyl 4-*Hydroxy*-3,5-*di-iodobenzoylglyoxylate Hemihydrate* (IXb).—A solution of the bromo-ester (VIIIb) (4 g) in dimethyl sulphoxide (30 ml) was kept at 45° for 20 h and then poured into ice–water (300 ml). The mixture was extracted with ether and the extract washed with aqueous 5% solutions of sodium chloride, sodium hydrogen sulphite, and sodium chloride again, dried (Na_2SO_4), and evaporated under reduced pressure to give the *diketo-ester* (IXb) (3·4 g, 88%) as prisms, m.p. 116—118° (with foaming) (from H_2O–EtOH) (Found: C, 27·6; H, 1·9; I, 52·4. $C_{11}H_8I_2O_5,0{\cdot}5H_2O$ requires C, 27·35; H, 1·9; I, 52·55%); $\nu_{max.}$ 3200 (OH), 1723 (ester CO), and 1679 cm^{-1} (ArCO).

Alkaline hydrolysis of the diketo-ester (IXb) in M-sodium hydroxide at room temperature (1 h) gave the acid (VIb) (96%).

Both diketo-esters (IXa and b) formed quinoxaline derivatives (Xa and b) with *o*-phenylenediamine: (Xa), yellow *needles* (94%), m.p. 181—182° (Found: C, 39·1; H, 2·4; I, 43·0; N, 4·7. $C_{19}H_{14}I_2N_2O_4$ requires C, 83·8; H, 2·4; I, 43·15; N, 4·75%); $\nu_{max.}$ 1780 and 1730 cm^{-1} [hydrolysis (M-NaOH) at room temp. gave (Xc)]; (Xb), yellow *needles* (73%), m.p. 172—174° (Found: C, 37·7; H, 2·05; I, 46·9; N, 5·2. $C_{17}H_{12}I_2N_2O_3$ requires C, 37·4; H, 2·2; I, 46·5; N, 5·15%), $\nu_{max.}$ 1720 cm^{-1} [acetylation (Ac_2O–C_5H_5N) gave the quinoxaline derivative of (IXa)].

Attempts to Convert the Diketo-ester (IXb) *into* T_4.—To an ice-cooled stirred solution of DIT (3 g, 6·9 mmol) in 0·2M-borate buffer (pH 8·0; 150 ml) was added the diketo-ester (IXb) (0·5 g, 1 mmol) in ethanol (15 ml). The solution was warmed to 30° within 10 min, and kept at that temperature for 40 h, during which time the pH was maintained at 8·0. The mixture was cooled, acidified to pH 1, and extracted with ether. The extract was washed with water, dried (Na_2SO_4), and evaporated. Starting material (IXb) was recovered in 92% yield (0·47 g). The aqueous solution was made alkaline (pH 13) and extracted with butan-1-ol. No T_4 was detected (t.l.c.) in the extract. When the incubation of (IXb) with DIT was carried out with oxygen bubbling through the mixture the same results were obtained.

Compounds (VIIb) (an isomer of the ethyl ester of DIHPPA) and (VIIIb) were also unreactive towards DIT under conditions similar to those used for the reaction of DIHPPA with DIT to form T_4.

We thank Professor Teruo Matsuura, Kyoto University, for discussions.

[2/473 *Received*, 29*th February*, 1972]