Anal. Calcd. for $C_8H_{15}NO_2$: C, 61.12; H, 9.62; N, 8.91. Found: C, 60.98; H, 9.61; N, 8.73.

***cis*-3-Amino-2,2,4,4-tetramethyl-1-cyclobutanol (XVI).**—A mixture of 10 g. (0.064 mole) of the oxime XV and 2 teaspoonfuls of nickel catalyst[11] in 100 ml. of absolute ethanol was hydrogenated on a Parr shaker. The reduction was rapid and within 20 min. the theoretical amount of hydrogen was taken up. The mixture was filtered and the solvent was distilled under diminished pressure to obtain 10 g. (theoretical yield) of crude XVI; $\lambda_{max}^{CHCl_3}$ 3.07, 6.24, 6.86, 8.93, and 9.5 μ.

Five grams (0.036 mole) of the free base XVI in anhydrous ether was converted to the corresponding amine hydrochloride by treating the solution with hydrogen chloride. After recrystallization from ethanol–ether, 4.5 g. (over-all 80%) of *cis*-amino alcohol XVI hydrochloride, m.p. 243–245° dec., was obtained; λ_{max}^{KBr} 3.03, 4.77, 6.14, 6.57, 7.45, 8.95, 9.58, and 9.95 μ.

Anal. Calcd. for $C_8H_{17}NO \cdot HCl$: C, 53.50; H, 10.11; N, 7.80. Found: C, 53.70; H, 10.25; N, 7.55.

***cis*-3-Dimethylamino-2,2,4,4-tetramethyl-1-cyclobutanol (XVII).**—Thirty-seven grams (0.7 mole) of cold 88% formic acid was added to 20 g. (0.14 mole) of the *cis*-aminocyclobutanol XVI, and to the resulting clear solution was added 34 g. (0.42 mole) of 37% formaldehyde solution. The mixture was placed in an oil bath which had been heated to 100°. A vigorous evolution of carbon dioxide began after 3–5 min., at which time the reaction mixture was removed from the bath until the gas evolution notably subsided; then it was returned to the bath and heated at 100° for 18 hr. After the mixture was cooled, hydrochloric acid (30 ml. of concentrated hydrochloric acid in 70 ml. of distilled water) was added and the acidic solution was distilled under diminished pressure to remove the solvent. The resulting sirupy residue was dissolved in 80 ml. of water, made basic by the addition of 100 ml. of 30% sodium hydroxide, and extracted with three 150-ml. portions of ether and three 150-ml. portions of methylene chloride.

The combined solution was dried over anhydrous magnesium sulfate and the solvent was distilled under diminished pressure, giving 18 g. (75%) of *cis*-3-dimethylamino-2,2,4,4-tetramethyl-1-cyclobutanol (XVII), m.p. 115–118°. Recrystallization from hexane gave a sample melting at 129° (lit.[9] m.p. 129–130°); λ_{max}^{KBr} 3.16, 3.58, and 3.65 μ.

A 6.6-g. sample (0.038 mole) of the free base in anhydrous ether was converted to the corresponding amine hydrochloride by treating the solution with hydrogen chloride. After one recrystallization from ethanol–ether, 6.3 g. (over-all 64%) of *cis*-3-dimethylamino-2,2,4,4-tetramethyl-1-cyclobutanol hydrochloride, m.p. 292–293° dec., was obtained; λ_{max}^{KBr} 3.12, 3.50, 3.84, 6.76, 6.81, 7.43, 7.60, 8.15, 8.34, 8.75, and 8.94 μ.

Anal. Calcd. for $C_{10}H_{21}NO \cdot HCl$: C, 57.56; H, 10.20; N, 6.74. Found: C, 57.37; H, 10.30; N, 6.66.

***cis*-3-Hydroxy-2,2,4,4-tetramethyl-1-cyclobutylcarbamonitrile (XVIII).**—To a solution of 5 g. (0.035 mole) of the 3-aminocyclobutanol XVI and 3.1 g. (0.037 mole) of sodium acetate in 50 ml. of 95% methanol was added, with cooling a solution of 3.9 g. (0.037 mole) of cyanogen bromide in 25 ml. of methanol. The resulting solution was allowed to stand at room temperature for 90 min. and the solvent was distilled under diminished pressure. A 75-ml. portion of water was added to the residue. The resulting white solid was filtered and recrystallized from benzene to give 3 g. (51%) of the hydroxycyclobutylcarbamonitrile XVIII, m.p. 164–166°; λ_{max}^{KBr} 2.96, 4.48, 8.45, and 9.07 μ.

Anal. Calcd. for $C_9H_{16}N_2O$: C, 64.25, H, 9.59; N, 16.65. Found: C, 64.46; H, 9.75; N, 16.42.

Chloro-N-(*cis*-3-hydroxy-2,2,4,4-tetramethyl-1-cyclobutyl)-formamidine Hydrochloride (XIX).—A solution of 7.8 g. (0.046 mole) of *cis*-3-hydroxy-2,2,4,4 tetramethyl-1-cyclobutylcarbamonitrile (XVIII) in 80 ml. of anhydrous tetrahydrofuran was cooled in an ice bath. An excess of hydrogen chloride was dissolved in the solution and the resulting reaction mixture was allowed to stand at room temperature. The precipitated product was filtered and dried to give 7.5 g. (67%) of chloroformamidine hydrochloride (XIX), m.p. 162–164°; λ_{max}^{KBr} 2.93, 6.01, 6.19, 9.07, and 9.53 μ.

Anal. Calcd. for $C_9H_{17}ClN_2O \cdot HCl$: N, 11.61. Found: N, 11.58.

(*cis*-3-Hydroxy-2,2,4,4-tetramethyl-1-cyclobutyl)urea (XX).—A solution of 1 g. (0.006 mole) of *cis*-3-hydroxy-2,2,4,4-tetramethylcyclobutylcarbamonitrile (XVIII) in 80 ml. of 60% sodium hydroxide solution was allowed to stand at room temperature for 15 hr. The resulting white solid product was filtered and recrystallized from benzene–ethanol to give 0.7 g. (63%) of the hydroxycyclobutylurea XX, m.p. 183–184°; λ_{max}^{KBr} 2.95, 3.07, 6.02, 6.18, and 9.25 μ.

Anal. Calcd. for $C_9H_{18}N_2O_2$: C, 58.03; H, 9.74; N, 15.04. Found: C, 58.31; H, 9.91; N, 14.76.

Model Reactions for the Biosynthesis of Thyroxine. VI. Structural Requirements of Analogs of Diiodotyrosine in the Reaction with 4-Hydroxy-3,5-diiodophenylpyruvic Acid to Form Analogs of Thyroxine[1,2]

AKIRA NISHINAGA[3] AND TERUO MATSUURA[3]

Faculty of Science, Osaka City University, Sumiyoshiku, Osaka, Japan

Received September 3, 1963

To contribute to the understanding of the mechanism by which thyroxine is formed in good yield from 4-hydroxy-3,5-diiodophenylpyruvic acid (I) and 3,5-diiodotyrosine in the presence of oxygen, keto acid I was permitted to react in a similar fashion with a series of analogs of diiodotyrosine. The dependence of the formation of the corresponding analogs of thyroxine on various structural features of the analogs of diiodotyrosine used has been investigated.

The biosynthetic mechanism by which thyroxine is formed from diiodotyrosine has not yet been elucidated. A few years ago, Meltzer and Stanaback[4] reported that 4-hydroxy-3,5-diiodophenylpyruvic acid (I) couples with 3,5-diiodotyrosine [IIa, X = I; R = $CH_2CH(NH_2)COOH$] in the presence of oxygen at a neutral or slightly alkaline pH to form thyroxine rapidly and in good yield. Shiba and Cahnmann[5] extended the investigation to the preparation of radioactive products. They proved that the phenolic ring of the thyroxine [IIIa, X = I; R = $CH_2CH(NH_2)COOH$] formed is derived from 4-hydroxy-3,5-diiodophenylpyruvic acid (I), and the nonphenolic ring and its alanine side chain from 3,5-diiodotyrosine (IIa). More recently the same authors found that rattlesnake venom, in the presence of oxygen and of catalase, can convert diiodotyrosine

(1) Previous paper in this series, T. Shiba and H. J. Cahnmann, *J. Org. Chem.*, **29**, 1652 (1964); for first paper in this series see ref. 8.

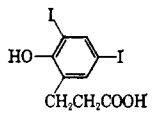
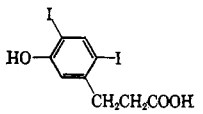
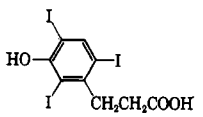
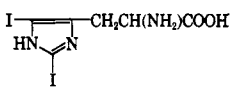
(2) This paper has been presented at the 16th Annual Meeting of the Chemical Society of Japan, April, 1963, Tokyo, Japan.

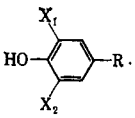
(3) Department of Synthetic Chemistry, Faculty of Engineering, Kyoto University, Kyoto, Japan.

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

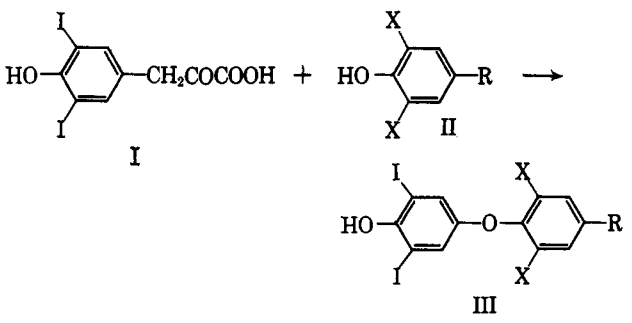
(5) T. Shiba and H. J. Cahnmann, *ibid.*, **27**, 1773 (1962).

TABLE I

	Diiodotyrosine and its analogs X_1[a]	X_2[a]	R[a]	Thyroxine and its analogs	Yield, %	Analog of thyroxine R_f[b]	Infrared bands, cm.$^{-1}$				Other products
Group 1											
IIa	I	I	$-CH_2CH(NH_2)COOH$	IIIa	18	0.36	3470	1700	1245	913	IV
IIb	I	I	$-COOH$	IIIb	5	0.50	3500	1692	1236	916	IV, V
IIc	I	I	$-CH_2COOH$	IIIc	9	0.51	3400	1700	1246	919	IV
IId	I	I	$-CH_2CH_2COOH$	IIId	10	0.50	3440	1703	1233	913	IV
IIe	I	I	$-CH_2CH_2CH_2COOH$	IIIe	4	0.48	3400	1689	1235	912	IV
IIf	I	I	$-CH_2CH(CH_3)COOH$	IIIf	4	0.59	3470	1702	1238	915	IV
IIg	I	I	$-CH_2CH(C_6H_5)COOH$	IIIg	Trace	0.60					IV
IIh	I	I	$-CH_2CH(OH)COOH$	IIIh	14	0.46	3450	1720	1234	915	IV
IIi	I	I	$-CH=CHCOOH$	IIIi	18	0.64	3450	1685	1240	918	IV
IIj	I	I	$-I$	IIIj							IV
Group 2											
IIk	Br	Br	$-CH_2CH_2COOH$	IIIk	13	0.46	3420	1700	1253	916	IV
III	Cl	Cl	$-CH_2CH_2COOH$	IIIl	16	0.45	3470	1698	1258	903	IV
IIm	H	I	$-CH_2CH_2COOH$	IIIm	Trace	0.56					IV
IIn	I	OMe	$-CH=CHCOOH$	IIIn	2	0.58	3520	1690	1280	906	IV
IIo	H	H	$-CH_2CH_2COOH$	IIIo							IV, IIIb
IIp	NO_2	NO_2	$-CH_2CH_2COOH$	IIIp							IV, IIIb
IIq	*t*-butyl	*t*-butyl	$-CH_2CH_2COOH$	IIIq							IV
Group 3											
VI				X	Trace	0.63					IV
VII				XI	1	0.42	3460	1706	1230	915	IV
VIII				XII	10[c]	0.62					IV
IX				XIV							IV, IIIb

[a] X_1, X_2, and R denote the substituents *ortho* and *para* to the phenolic group in  . [b] Paper chromatography in 1-butanol–ammonia (2 *N*) (upper phase), Toyo Roshi paper No. 51, ascending method. [c] Yield of XIII obtained by catalytic hydrogenation of the reaction products.

to thyroxine with the intermediary formation of 4-hydroxy-3,5-diiodophenylpyruvic acid (I).[6]



I II III

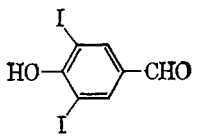
These findings suggest that the acid (I) may be an intermediate in the formation of thyroxine *in vivo*. We have, therefore, investigated the reaction of I with various analogs of diiodotyrosine to examine the structural requirements of these analogs in this reaction. The reaction was carried out according to the procedure of Shiba and Cahnmann.[5] The reaction mixture was extracted with 1-butanol after the addition of alkali. The butanol extract always contained 3,5-diiodobenzaldehyde (IV) in addition to the analog of thyroxine formed. When no analog of thyroxine was formed, a small amount of 3,5,3′,5′-tetraiodothyroformic acid (IIIb) was also present in the butanol extract. The results are shown in Table I. The analogs of thyroxine were identified by direct comparison with authentic samples[7] or by elemental analysis and through their spectroscopic and chromatographic properties.

The members of group 1 consisting of diiodotyrosine (IIa) and its side-chain analogs (IIb–IIj) were chosen to study the structural influence of the aliphatic side chain on this reaction. The formation of almost pure

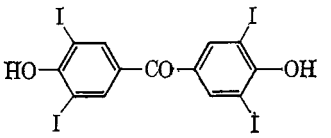
(6) T. Shiba and H. J. Cahnmann, *Biochim. Biophys. Acta*, **58**, 609 (1962).

(7) The synthesis will be published elsewhere.

thyroxine (IIIa) in 18% yield in this reaction could be confirmed. The reactions of IIb, IIc, and IId to form IIIb, IIIc, and IIId, respectively, were already mentioned, without experimental details, by Meltzer and Stanaback.[4] In the case of IIb, 4,4′-dihydroxy-3,5,-3′,5′-tetraiodobenzophenone (V) was isolated from the reaction mixture in 6% yield in addition to the analog IIIb of thyroxine. The structure of V was confirmed by conversion to 4,4′-dihydroxybenzophenone. The yields of the analogs of thyroxine obtained from IIb–IIg are almost the same as those obtained when these analogs of diiodotyrosine are incubated alone for several days.[8] In both cases the highest yield is obtained



IV



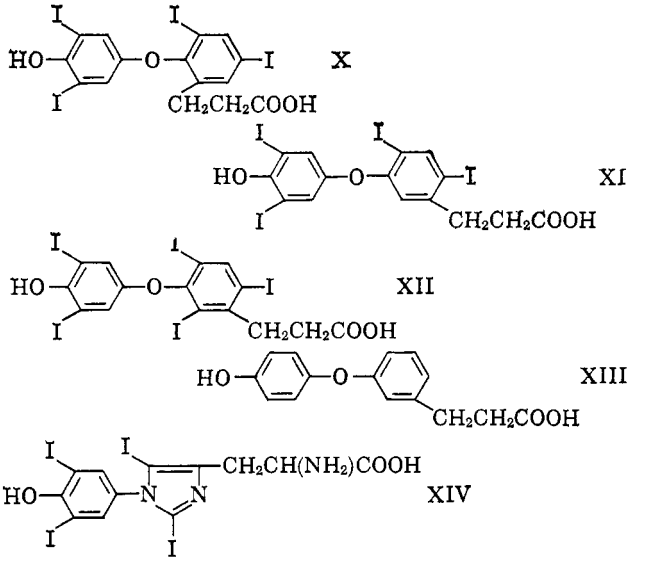
V

when the aliphatic side chain has the structure –C–C–COOH. Lengthening or shortening of this side chain as well as branching leads to poorer yields. However, in contrast to the incubation of IIa, IIh, and IIi in the absence of the keto acid I,[8] incubation of these compounds with the keto acid gave high yields of thyroxine or of the corresponding analog.

The influence of the substituents *ortho* to the phenolic hydroxyl was examined in group 2. The members of this group (IIk–IIq) are propionic or acrylic analogs of diiodotyrosine in which one or both iodine atoms are replaced with other substituents. Dibromo- and dichlorophloretic acid (IIk and IIl) gave the corresponding analogs of thyroxine in higher yield than diiodophloretic acid (IIb). The monoiodo derivatives IIm and IIn gave considerably lower yields than diiodophloretic acid, and the noniodinated derivatives IIo, IIp, and IIq yielded no analog of thyroxine at all. It seems therefore that the substitution of both *ortho* positions with halogen atoms greatly facilitates the formation of the corresponding thyronines. It should be noted, however, that Shiba and Cahnmann[1] obtained 3,3′,5′-triiodothyronine in good yield from 3-iodotyrosine and the keto acid I. The formation of IIIk, IIIl, IIIm, and IIIn provides additional evidence to support Shiba and Cahnmann's finding[5] that in this coupling reaction the phenolic ring of the iodinated thyronine formed is derived from I and the nonphenolic ring and its side chain from diiodotyrosine or its analogs.

Other structural requirements were examined with the members of group 3. The poor yields obtained in the reactions of the *ortho* and *meta* analogs (VI and VII) of diiodophloretic acid may be due to the fact that only one *ortho* position to the hydroxyl group is substituted with iodine. In contrast to triiodophenol (IIj), the triiodinated *meta* analog VIII yielded the corresponding analog (XII) of thyroxine in good yield. This analog could not be isolated in pure form and was therefore catalytically hydrogenated to form the crystalline *meta* analog (XIII) of thyropropionic acid. In a control run, thyropropionic acid was isolated in 10% yield by catalytic hydrogenation of the reaction products from IId. This shows that a shift of the propionic side chain in diiodophloretic acid from the *para* to the *meta* position does not affect the yield of the corresponding analog of thyroxine, if both *ortho* positions are substituted with iodine. Diiodohistidine (IX) which is known to be present in the thyroid gland[9] was also subjected to the reaction with the keto acid I, but the expected reaction product XIV could not be detected.

The results obtained in the reactions of the keto acid I with a series of analogs of diiodotyrosine permit the conclusion that the formation of the corresponding analog of thyroxine is favored by the following structural features of the analog of diiodotyrosine: (1) substitution of both *ortho* positions to the phenolic hydroxyl with halogen, (2) an aliphatic side chain of the type C–C–COOH in the *para* or *meta* position to the phenolic hydroxyl, (3) the presence of an amino group, a hydroxyl group, or a double bond in α-position to the carboxyl group of the side chain.



Experimental[10]

Preparation of Materials. 3-(3,5-Dichloro-4-hydroxyphenyl)-propionic Acid (IIl).—Dry chlorine gas was passed through a solution of 49.8 g. (0.3 mole) of phloretic acid[8] (IIo) in 500 ml. of acetic acid until the weight increase indicated the absorption of 1 mole equiv. of chlorine. When the reaction mixture was concentrated to 100 ml. under reduced pressure, crystals deposited which on recrystallization from chloroform gave 24.2 g. (34%) of prisms, m.p. 108–110°.

Anal. Calcd. for $C_9H_8Cl_2O_3$: C, 45.99; H, 3.43. Found: C, 45.95; H, 3.47.

3-(4-Hydroxy-3-iodophenyl)propionic Acid (IIm).—To a solution of 15 g. (0.09 mole) of phloretic acid in 80 ml. of 20% aqueous methylamine was added a solution of 22.8 g. (0.18 g.-atom) of iodine and 23 g. of potassium iodide in 80 ml. of water. The reaction mixture was acidified with dilute hydrochloric acid to separate a liquid which solidified on standing. The solid was collected by filtration, dried, dissolved in chloroform and chromatographed on a column of 300 g. of silica gel. Elution with chloroform–ether (99:1) yielded diiodophloretic acid and elution with chloroform–ether (9:1) yielded 12 g. (55%) of IIm as prisms, m.p. 114–116°, lit.[11] m.p. 112–113°.

5-Iodovanillin.—To a solution of 14.2 g. (0.1 mole) of vanillin in 400 ml. of dilute hydrochloric acid (18%) was added at 50°

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

(9) R. J. Block, R. H. Mandl, and S. Keller, *Arch. Biochem. Biophys.*, **76**, 508 (1958).

(10) Melting points were determined in capillary tubes and are uncorrected. The infrared spectra were determined in a Nippon Bunko recording spectrophotometer, Model IR-S. The ultraviolet spectra were determined in Hitachi recording spectrophotometer, Model EPS-2. The microanalyses were made by Mr. J. Goda and his associates, of this faculty.

(11) J. Runeberg, *Acta Chem. Scand.*, **12**, 188 (1958).

16.2 g. (0.1 mole) of iodine monochloride in 20 ml. of concentrated hydrochloric acid, and the mixture was allowed to stand for 2 days. The crystals which formed were collected by filtration. On standing, the filtrate deposited additional crystals and the total yield was 21.6 g. (81%).

Recrystallization from dilute ethanol gave needles, m.p. 180–181°, lit. m.p.[12] 181–182°.

3-Methoxy-4-hydroxy-5-iodocinnamic Acid (IIn).—A mixture of 15 g. (54 mmoles) of 5-iodovanillin, 13.5 g. (165 mmoles) of anhydrous sodium acetate, and 44 ml. of acetic anhydride was refluxed for 4 hr. After standing overnight at room temperature, the reaction mixture was poured into 300 ml. of water and boiled for 1 min. The precipitate formed was collected by filtration and treated with a solution of 20 g. of sodium hydroxide in 300 ml. of water. The insoluble material was removed by extraction with ether. The aqueous solution was heated on a water bath for 30 min., then cooled, and acidified with dilute hydrochloric acid (18%). The precipitate formed was collected by filtration, washed with water, dried, and dissolved in ethanol. The insoluble material was removed by filtration. Concentration of the filtrate gave crystals which were recrystallized from acetone to give 6.6 g. (38%) of IIn as yellow prisms, m.p. 250–251° dec.; λ_{max}^{EtOH} 234 mμ (log ε 4.34), 244 (4.39), and 322 (4.31).

Anal. Calcd. for $C_{10}H_9IO_4$: C, 37.52; H, 2.83. Found: C, 37.89; H, 2.99.

4-Acetoxy-3,5-di-*t*-butylcinnamic Acid.—A mixture of 17.0 g. (0.07 mole) of 3,5-di-*t*-butyl-4-hydroxybenzaldehyde,[13,14] 14.2 g. (0.17 mole) of fused sodium acetate, and 40 ml. of acetic anhydride was refluxed for 24 hr. The reaction mixture was poured into ice and, after standing overnight, extracted with ether. The ethereal layer was extracted three times with 5% aqueous sodium carbonate. Acidification of the sodium carbonate solution with dilute hydrochloric acid yielded a precipitate which was recrystallized from benzene–cyclohexane to give 4.56 g. (20%) of the acid as needles, m.p. 233–235°. Further recrystallization raised the melting point to 237–238°.

Anal. Calcd. for $C_{19}H_{26}O_4$: C, 71.67; H, 8.23. Found: C, 71.27; H, 8.02.

3-(4-Acetoxy-3,5-di-*t*-butylphenyl)propionic Acid.—To a solution of 2.0 g. of 4-acetoxy-3,5-di-*t*-butylcinnamic acid in 60 ml. of 10% sodium hydroxide aqueous solution was added at 90° 6 g. of Raney nickel alloy in portions with stirring over a period of 20 min. Stirring was continued for 1 hr., then the nickel was removed by filtration and the filtrate was poured into a mixture of concentrated hydrochloric acid and ice. The mixture was extracted with ether and the ether layer was dried and evaporated to a crystalline mass which, on recrystallization from benzene–cyclohexane, yielded 1.6 g. (80%) of crystals, m.p. 149–150°. On further recrystallization the melting point rose to 153–155°.

Anal. Calcd. for $C_{19}H_{28}O_4$: C, 71.22; H, 8.81. Found: C, 71.00; H, 8.59.

3-(3,5-Di-*t*-butyl-4-hydroxyphenyl)propionic Acid (IIq).—A solution of 1.2 g. of 3-(4-acetoxy-3,5-di-*t*-butylphenyl)propionic acid in 30 ml. of a 2 *N* solution of sodium hydroxide in 1-butanol was refluxed for 2.5 hr. The mixture was evaporated to dryness under reduced pressure and the residue was acidified with dilute hydrochloric acid to yield a precipitate which was collected by filtration, dried, and recrystallized from benzene–cyclohexane to give 0.90 g. (87%) of IIq as needles, m.p. 171–173°. On further recrystallization, the melting point rose to 173–175°, lit. m.p. 172°[15] and 172–173°.[16]

Anal. Calcd. for $C_{17}H_{26}O_3$: C, 73.34; H, 9.41. Found: C, 73.28; H, 9.65.

Reaction of 4-Hydroxy-3,5-diiodophenylpyruvic Acid (I) with Analogs of 3,5-Diiodotyrosine. General Procedure.—The reactions were carried out by a slight modification of the procedure of Shiba and Cahnmann.[5] The analog of diiodotyrosine (4.63 mmoles) was dissolved in mixture of 50 ml. of 0.2 *M* borate buffer (pH 7.6), 17.5 ml. of 1 *N* sodium hydroxide and 17.5 ml. of a saturated aqueous solution of sodium sulfate. Enough 4 *N* hydrochloric acid was added to the solution to adjust the pH to 7.6. After the addition of 10 ml. of a 1% solution of *t*-butyl hydroperoxide in 1-butanol, the pH was again adjusted to 7.6. To the vigorously stirred mixture was added a solution of 2.59 g. (6.0 mmoles) of 4-hydroxy-3,5-diiodophenylpyruvic acid (I)[17] in 50-ml. of 1-butanol over a period of about 1 hr. During the addition oxygen was bubbled through the reaction mixture and the pH was kept constant at 7.6 by adding 2 *N* sodium hydroxide by means of an immersed thin polyethylene tubing. The rate of the addition of alkali was automatically controlled with a pH-Stat. Stirring and bubbling of oxygen were continued for about another hour.

After the addition of 30 ml. of 10 *N* sodium hydroxide the reaction mixture was shaken with 50 ml. of 1-butanol. The butanol layer was separated and the aqueous layer was extracted twice with 1-butanol. The combined butanol layers were washed with 150 ml. of 1 *N* sodium hydroxide, then with 100 ml. of water, and were evaporated under reduced pressure at room temperature. The residue was dissolved in 20 ml. of water and the solution was acidified with 4 *N* hydrochloric acid. The resulting precipitate (A) was analyzed by paper chromatography (Table I). Short-wave ultraviolet light was used for the detection of 4-hydroxy-3,5-diiodobenzaldehyde (IV), and diazotized N^1,N^1-diethylsulfanilamide[6] for the detection of all other compounds. Fractional recrystallization yielded the pure reaction products. The yield of the analogs of thyroxine was based on the analog of diiodotyrosine used.

Reaction with 3,5-Diiodo-L-tyrosine (IIa).—The precipitate A (0.83 g.) was treated with hot acetone. On filtration, 0.64 g. (18%) of thyroxine was obtained as an almost colorless solid, m.p. 218–220° dec., which showed a single spot on a paper chromatogram. The solid obtained by the concentration of the filtrate from A was found to consist mainly of 4-hydroxy-3,5-diiodobenzaldehyde (IV, infrared spectrum and by paper chromatogram).

Reaction with 4-Hydroxy-3,5-diiodobenzoic Acid (IIb).—The precipitate A (0.91 g.) was recrystallized from methanol to give 0.20 g. (6%) of 4,4′-dihydroxy-3,5,3′,5′-tetraiodobenzophenone (V) as colorless needles, m.p. 255° dec.; infrared spectrum (Nujol) 3600, 3400, 1622, 1572, and 1528 cm.$^{-1}$; λ_{max}^{EtOH} 209 mμ (log ε 4.53), 246.5 (4.50), and 372 (4.10).

Anal.. Calcd. for $C_{13}H_6I_4O_3$: C, 21.75; H, 0.84; I, 70.75. Found: C, 21.85; H, 1.21; I, 70.31.

A solution of 0.15 g. of this substance in 20 ml. of ethanol was hydrogenated in the presence of 0.20 g. of 10% palladium–charcoal and 0.25 g. of anhydrous sodium acetate. After the absorption of hydrogen ceased (1 hr.), the catalyst was removed by filtration and the filtrate was evaporated under reduced pressure. The residue was shaken with a mixture of ether and water. The ethereal layer was separated, dried, and evaporated. The residue was recrystallized from benzene, then from benzene–ethyl acetate to give 29 mg. of prisms, m.p. 213–214°; λ_{max}^{EtOH} 227 mμ (log ε 4.17) and 300 mμ (4.36).

Anal. Calcd. for $C_{13}H_{10}O_3$: C, 72.89; H, 4.71. Found: C, 72.91; H, 5.06.

This substance was identified as 4,4′-dihydroxybenzophenone[18] by comparison with an authentic sample (infrared spectrum and mixture melting point).

The mother liquor obtained from the recrystallization of 4,4′-dihydroxy-3,5,3′,5′-tetraiodobenzophenone (V) gave after concentration pale yellow needles, which were recrystallized from ethyl acetate to yield 0.18 g. (5.3%) of colorless needles, m.p. 269–270° dec. The infrared spectrum was identical with that of an authentic sample of tetraiodothyroformic acid (IIIb).

From the mother liquor of the recrystallization of tetraiodothyroformic acid, crystals (0.49 g.) were obtained. The infrared spectrum and paper chromatogram showed that the crystals consisted of 4-hydroxy-3,5-diiodobenzaldehyde (IV) contaminated with tetraiodothyroformic acid (IIIb).

Reaction with 4-Hydroxy-3,5-diiodophenylacetic Acid (IIc).[8]—The precipitate A (0.55 g.) was recrystallized from benzene containing a small amount of ethanol to give 0.32 g. (9.2%) of colorless needles, m.p. 229–230°. The infrared spectrum was identical with that of an authentic sample of 3,5,3′,5′-tetraiodothyroacetic acid (IIIc).[8]

(12) G. D. Thorn and C. B. Purves, *Can. J. Chem.*, **32**, 373 (1954).

(13) (a) G. M. Coppinger and T. W. Campbell, *J. Am. Chem. Soc.*, **75**, 734 (1953); (b) L. A. Cohen, *J. Org. Chem.*, **22**, 1333 (1957).

(14) The starting material, 2,6-di-*t*-butyl-4-methylphenol, for this preparation was kindly supplied by Koppers Co., Inc.

(15) S. Fujisaki, Japan Patent 11,030 (1960); *Chem. Abstr.*, **55**, 466 (1961).

(16) T. H. Coffield, H. H. Filbey, G. G. Ecke, and A. J. Kolka, *J. Am. Chem. Soc.*, **79**, 5019 (1957).

(17) Chemed, Inc., Odenton, Md.

(18) K. Nakagawa, S. Matsuura, and S. Baba, *J. Pharm. Soc. Japan*, **74**, 498 (1954).

Reaction with 3-(4-Hydroxy-3,5-diiodophenyl)propionic Acid (IId).[8]—Recrystallization of the precipitate A (0.52 g.) from benzene gave 0.35 g. (10%) of colorless needles, m.p. 213–215°, whose infrared spectrum was identical with that of an authentic sample of 3,5,3′,5′-tetraiodothyropropionic acid (IIId)[8]; λ_{max}^{EtOH} 216 mμ (log ε 4.68), 225 (4.69), 238 (4.52), 295 (3.62), and 303 (3.63).

Reaction with 4-(4-Hydroxy-3,5-diiodophenyl)butyric Acid (IIe).[8]—The precipitate A (0.53 g.) was recrystallized from benzene, then from ethyl acetate to give 0.17 g. (4.4%) of 3,5,3′,5′-tetraiodothyrobutyric acid (IIIe) as colorless needles, m.p. 200–202°, lit.[19] m.p. 195–196°; λ_{max}^{EtOH} 213 mμ (log ε 4.67), 226 (4.69), 238 (4.43), 295 (3.64), and 303 (3.63).

Anal. Calcd. for $C_{16}H_{12}I_4O_4$: C, 24.77; H, 1.56. Found: C, 24.94; H, 1.85.

From the mother liquor, 50 mg. of 4-hydroxy-3,3-diiodobenzaldehyde (IV) was isolated.

Reaction with 3-(4-Hydroxy-3,5-diiodophenyl)-2-methylpropionic Acid (IIf).[8]—Recrystallization of the precipitate A (0.67 g.) from benzene gave 80 mg. of 4-hydroxy-3,5-diiodobenzaldehyde (IV). The mother liquor, on slow evaporation, deposited a mixture of two kinds of crystals. Needle-shape crystals were picked up and recrystallized twice from benzene to give 0.14 g. (3.8%) of α-methyl-3,5,3′,5′-tetraiodothyropropionic acid (IIIf) as colorless needles, m.p. 193–195°; λ_{max}^{EtOH} 213 mμ (log ε 4.70), 226 (4.69), 237 (4.45), 295 (3.65), and 303 (3.66).

Anal. Calcd. for $C_{16}H_{12}I_4O_4$: C, 24.77; H, 1.56. Found: C, 24.77; H, 2.00.

Reaction with 3-(4-Hydroxy-3,5-diiodophenyl)-2-phenylpropionic Acid (IIg) (Iodoalphionic Acid).[20]—Recrystallization of the precipitate A (0.68 g.) from benzene gave 4-hydroxy-3,5-diiodobenzaldehyde (IV). The mother liquor, on slow evaporation, deposited crystals. The paper chromatogram and the infrared spectrum (1701, 1233, and 912 cm.$^{-1}$) of the crystals indicated that they consisted of a mixture of α-phenyl-3,5,3′,5′-tetraiodothyropropionic acid (IIIg) and of IV, but IIIg could not be isolated in pure form.

Reaction with 3-(4-Hydroxy-3,5-diiodophenyl)lactic Acid (IIh).[8]—Recrystallization of the precipitate A (0.67 g.) from a mixture of ethyl acetate, benzene, and petroleum ether gave 0.52 g. (14%) of 3,5,3′,5′-tetraiodothyrolactic acid (IIIh) as colorless needles, m.p. 201–203°, lit.[8] m.p. 207–208°. The infrared spectrum was identical with that of an authentic sample of IIIh[8]; λ_{max}^{EtOH} 214 mμ (log ε 4.70), 225 (4.70), 238 (4.45), 294 (3.64), and 302 (3.65).

Reaction with 4-Hydroxy-3,5-diiodocinnamic Acid (IIi).[8]—Recrystallization of the precipitate A (1.43 g.) from ethyl acetate gave 0.64 g. (18%) of 3,5,3′,5′-tetraiodothyroacrylic acid (IIIi) as colorless needles, m.p. 277–278° dec., lit.[21,22] m.p. 270° dec.; λ_{max}^{EtOH} 213 mμ (log ε 4.58), 247 (4.61), and 279 (4.37).

Anal. Calcd. for $C_{15}H_8I_4O_4$: C, 23.71; H, 1.05. Found: C, 24.02; H, 1.23.

Catalytic Hydrogenation of Tetraiodothyroacrylic Acid (IIIi). A.—A solution of 368 mg. of IIIi and 400 mg. of anhydrous sodium acetate in 40 ml. of ethanol was hydrogenated in the presence of 200 mg. of 10% palladium–charcoal. After the absorption of hydrogen ceased, the catalyst was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in water and the solution was acidified with dilute hydrochloric acid. The resulting precipitate was collected, dried, and recrystallized from ethyl acetate–petroleum ether to give 107 mg. (85%) of colorless needles, m.p. 163–165°. This substance was identified as thyropropionic acid by mixture melting point determination and by comparison of its infrared spectrum with that of an authentic sample of thyropropionic acid, m.p. 162–163°, which was prepared by catalytic hydrogenation of 3,5,3′,5′-tetraiodothyropropionic acid,[8] lit. m.p. 161°[23] and 175°.[24]

B.—A solution of 200 mg. of IIIe and 200 mg. of anhydrous sodium acetate in 20 ml. of ethanol was hydrogenated in the presence of 100 mg. of 10% palladium–charcoal which had been stored over a year after its preparation. After the absorption of hydrogen ceased (80 min.), the mixture was treated as described in A. Recrystallization of the product from ethyl acetate gave 32 mg. of thyroacrylic acid as colorless needles, m.p. 227–228° dec.; infrared spectrum (Nujol): 3140, 1693, 1628, 1600, 1500, 980, and 837 cm.$^{-1}$; λ_{max}^{EtOH} 206.5 mμ (log ε 4.32), 224.5 (4.21), 297 (4.32), and inflexion at 308 (4.31).

Anal. Calcd. for $C_{15}H_{12}O_4$: C, 70.30; H, 4.72. Found: C, 69.97; H, 5.01.

Reaction with 2,4,6-Triiodophenol (IIj).—In this reaction an additional 30 ml. of 1-butanol was added in order to render triiodophenol soluble at pH 7.6. The precipitate A (2.31 g.) was recrystallized from methanol to give triiodophenol (1.44 g., 70% recovery), m.p. 159–160°. Paper chromatography of the mother liquor showed the presence of triiodophenol and 4-hydroxy-3,5-diiodobenzaldehyde (IV), but of no analog of thyroxine.

Reaction with 3-(3,5-Dibromo-4-hydroxyphenyl)propionic Acid (IIk).[25]—The precipitate A (0.84 g.) was recrystallized twice from benzene to give 0.22 g. of colorless needles, m.p. 172–175°. Concentration of the mother liquor yielded 0.25 g. of crystals, m.p. 160–165°, which infrared spectroscopy and paper chromatography showed to consist of a roughly equimolecular mixture of IIIk and IV. On this basis the yield of IIIk was calculated to be about 12%; λ_{max}^{EtOH} 211 mμ (log ε 4.77), 240 (3.58), 287 (3.58), and 302 (3.65).

Anal. Calcd. for $C_{15}H_{10}Br_2I_2O_4$: C, 27.01; H, 1.51. Found: C, 27.13; H, 1.71.

The infrared spectrum of this substance was identical with that of an authentic sample of 3,5-dibromo-3′,5′-diiodothyropropionic acid (IIIk).[7]

Reaction with 3-(3,5-Dichloro-4-hydroxyphenyl)propionic Acid (III).—The precipitate A (0.79 g.) was recrystallized twice from benzene to give 0.36 g. (16%) of colorless plates, m.p. 186–188°; λ_{max}^{EtOH} 213 mμ (log ε 4.65), 221 (4.63), 237 (4.24), 285 (3.59), and 303 (3.69).

Anal. Calcd. for $C_{15}H_{10}Cl_2I_2O_4$: C, 31.12; H, 1.74. Found: C, 31.12; H, 1.83.

The infrared spectrum was identical with that of an authentic sample of 3,5-dichloro-3′,5′-diiodothyropropionic acid (IIII).[7]

Reaction with 3-(4-Hydroxy-3-iodophenyl)propionic Acid (IIm).—Recrystallization of the precipitate A (0.59 g.) from ethyl acetate–petroleum ether gave 0.16 g. of 4-hydroxy-3,5-diiodobenzaldehyde (IV). Paper chromatography of the mother liquor showed the presence of IV and of 3,3′,5′-triiodothyropropionic acid (IIIm) which could not be isolated in pure form.

Reaction with 3-Methoxy-4-hydroxy-5-iodocinnamic Acid (IIn).—Recrystallization of the precipitate A (1.15 g.) from ethyl acetate gave 0.30 g. of 4-hydroxy-3,5-diiodobenzaldehyde (IV). Fractional recrystallization of crystals obtained from the mother liquor gave 76 mg. (2.2%) of 3-methoxy-5,3′,5′-triiodothyroacrylic acid (IIIn) as colorless needles, m.p. 226–228° (sintering at 220°); infrared spectrum (Nujol): 3520, 2700–2500, 1690, 1636, 1280, and 906 cm.$^{-1}$.

Anal. Calcd. for $C_{16}H_{11}I_3O_5$: C, 28.94; H, 1.67. Found: C, 29.33; H, 2.19.

Reaction with 3-(4-Hydroxyphenyl)propionic Acid (IIo).[8]—Fractional recrystallization of the precipitate A (0.29 g.) from ethyl acetate gave 45 mg. of 4-hydroxy-3,5-diiodobenzaldehyde (IV) and 9 mg. of 3,5,3′,5′-tetraiodothyroformic acid (IIIb), m.p. 250–251° dec., which was identical with an authentic sample.

Reaction with 3-(4-Hydroxy-3,5-dinitrophenyl)propionic Acid (IIp).[26]—Fractional recrystallization of the precipitate A (0.23 g.) from ethyl acetate–benzene gave 43 mg. of 4-hydroxy-3,5-diiodobenzaldehyde and 7 mg. of 3,5,3′,5′-tetraiodothyroformic acid (IIIb), both identified by comparison of infrared spectra, but no analog (IIIp) of thyroxine.

Reaction with 3-(3,5-Di-*t*-butyl-4-hydroxyphenyl)propionic Acid (IIq).—The reaction was carried out with 0.80 g. (2.9 mmoles) of IIq. Recrystallization of the precipitate A (0.85 g.) from benzene gave 0.45 g. (53% recovery) of colorless needles, m.p. 173–175°, which were identical with IIq. Paper chromatography of the mother liquor showed the presence of IV, but not of the analog IIIq of thyroxine.

Reaction with 3-(2-Hydroxy-3,5-diiodophenyl)propionic Acid (VI).[7]—Recrystallization of the precipitate A from benzene gave

(19) N. Kharasch and S. H. Kalfayan, *J. Org. Chem.*, **21**, 929 (1956).

(20) Schering Corp., Bloomfield, N. J.

(21) S. Wawzonek, S. C. Wang, and R. Lyons, *J. Org. Chem.*, **15**, 593 1950.

(22) R. C. Cookson and G. F. H. Green, *J. Chem. Soc.*, 827 (1952).

(23) J. Walker, *ibid.*, 347 (1942).

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

(25) T. Matsuura and H. J. Cahnmann, *J. Am. Chem. Soc.*, **82**, 2055 (1960).

(26) R. K. Callow, J. M. Gulland, and R. D. Haworth, *J. Chem. Soc.*, 1452 (1929).

0.38 g. of needles, m.p. 122–123°, which were identical with the starting material VI. The paper chromatogram of the mother liquor showed the presence of 4-hydroxy-3,5-diiodobenzaldehyde (IV), triiodophenol (IIj), VI, and the analog X[7] of thyroxine. R_f values of these substances were identical to those of authentic samples.

Reaction with 3-(3-Hydroxy-4,6-diiodophenyl)propionic Acid (VII).[7]—Recrystallization of the precipitate A (0.49 g.) from benzene gave 20 mg. (1%) of crystals, m.p. 219–220°; λ_{max}^{EtOH} 215 mμ (log ε 4.70), 228 (4.71), 238.5 (4.58), 293 (3.84), and inflexion at 300 (3.82).

Anal. Calcd. for $C_{15}H_{10}I_4O_4$: C, 23.65; H, 1.33. Found: C, 24.94; H, 1.64.

The microanalysis shows that the product XI was partly deiodinated.

Fractional recrystallization of crystals obtained from the mother liquor gave 172 mg. of the starting material VII and 55 mg. of 4-hydroxy-3,5-diiodobenzaldehyde (IV).

Reaction with 3-(3-Hydroxy-2,4,6-triiodophenyl)propionic Acid (VIII).[7]—The paper chromatogram of the precipitate A (1.02 g.) showed the presence of a considerable amount of the starting material VIII, in addition to 4-hydroxy-3,5-diiodobenzaldehyde (IV) and the analog XII[7] of thyroxine. Attempts to isolate XII were unsuccessful. The precipitate and A 1 g. of anhydrous sodium acetate were therefore dissolved in 100 ml. of ethanol and the solution was hydrogenated in the presence of 0.5 g. of 10% palladium–charcoal. After the absorption of hydrogen ceased, the catalyst was removed by filtration, the solvent was evaporated under reduced pressure, and the residue was taken up 50 ml. of water. Acidification of the mixture with dilute hydrochloric acid yielded 0.12 g. (10%) of 3-[3-(4-hydroxyphenoxyphenyl)]-propionic acid (XIII) as colorless plates, m.p. 145–149°. Recrystallization from water raised the melting point to 149–151°. The infrared spectrum was identical with that of an authentic sample of XIII.[7]

In a control run, catalytic hydrogenation of the precipitate A obtained from 3-(4-hydroxy-3,5-diiodophenyl)propionic acid (IId) yielded thyropropionic acid in 10% yield.

Reaction with Diiodo-L-histidine (IX).[27]—The precipitate A (0.31 g.) was treated with hot benzene. The insoluble material (25 mg.) was removed by filtration. This material was dissolved in a small volume of 7 *N* aqueous ammonia and the solution was then acidified. The infrared spectrum of the precipitate formed (19 mg.) was almost identical with that of tetraiodothyroformic acid (IIIb). The filtrate from the above mentioned insoluble material was evaporated and the residue was fractionally recrystallized from benzene to yield 105 mg. of 4-hydroxy-3,5-diiodobenzaldehyde (IV) and 9 mg. of IIIb. No coupling product (XIV) was detected.

Acknowledgment.—This work was supported by Grant A-3706 from the National Institutes of Health, U. S. Public Health Service. The authors are indebted to Dr. H. J. Cahnmann of the National Institutes of Health and to Dr. T. Shiba of Osaka University for helpful discussions and also to Professor T. Kubota for his interest in this work.

(27) K. J. Brunnings, *J. Am. Chem. Soc.*, **69**, 205 (1947).

Phosphorylating Agents by the Activation of Phosphates with Ethoxyacetylene[1,2]

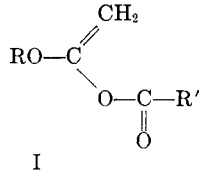
HARRY H. WASSERMAN AND DAVID COHEN

Contribution No. 1678 from the Sterling Chemistry Laboratory, Yale University, New Haven, Connecticut

Received November 19, 1963

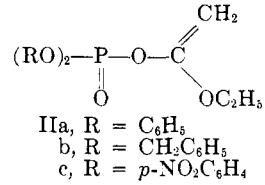
1-Alkoxy vinyl esters of phosphoric acids (IIa and b, R = C_6H_5 and $CH_2C_6H_5$) have been isolated from the reaction of the corresponding phosphoric acids with ethoxyacetylene and shown to be active phosphorylating agents for a variety of nucleophiles. Monoesters of phosphoric acid were activated and allowed to react *in situ* to form methyl adenylate, thymidine-3′ thymidine-5′ phosphate, and flavine adenine dinucleotide (FAD). Uridine-5′ diphosphate (UDP) was prepared by reaction of IIb (R = $CH_2C_6H_5$) with uridine-5′ monophosphate (UMP) followed by debenzylation.

Arens and his co-workers have, in the course of extensive studies,[3] shown that alkoxy acetylenes are effective agents for the conversion of carboxylic acids to anhydrides, and, in the case of acids containing strongly electronegative groups, intermediates of the type I have been isolated.[4]



I

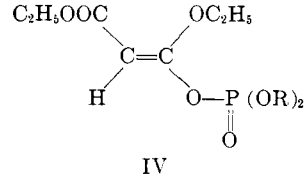
In these laboratories, we have found that such 1-alkoxy vinyl esters of carboxylic acids can *generally* be prepared at moderate temperatures either with the aid of a mercuric ion catalyst or by the use of a large excess of alkoxy acetylene.[5,6] In this report, we describe the extension of this method to the activation of



IIa, R = C_6H_5
b, R = $CH_2C_6H_5$
c, R = p-$NO_2C_6H_4$

phosphoric acid esters. The utility of these enol phosphate intermediates in synthesis has been demonstrated by the formation of internucleotidic and coenzyme linkages. As described below, we have selected a number of applications, and although effects were not made to work out optimum yields for each case, we have shown the potential and versatility of the method.

The active intermediates (II)[7] were prepared by re-



IV

sulfonic, and phosphoric acids and also adenylic acid. However, the fact that symmetrical triesters of phosphorous acid are not readily available limits the applicability of this type of intermediate.

(1) A preliminary report of these findings has already appeared: H. H. Wasserman and D. Cohen, *J. Am. Chem. Soc.*, **82**, 4435 (1960).

(2) Supported by Grant RG 7874, U. S. Public Health Service.

(3) See J. F. Arens and H. C. Volger, *Rec. trav. chim.*, **77**, 1170 (1958), and earlier papers in this series.

(4) R. Broekema, S. van der Werf, and J. F. Arens, *ibid.*, **77**, 258 (1958).

(5) H. H. Wasserman and P. S. Wharton, *Tetrahedron*, **3**, 321 (1958).

(6) H. H. Wasserman and P. S. Wharton, *J. Am. Chem. Soc.*, **82**, 661 (1960).

(7) F. Cramer [*Angew. Chem.*, **69**, 727 (1957); **72**, 246 (1960)] has reported the preparation of similar systems (IV) using the Perkow reaction between triesters of phosphorous acid and bromomalonic esters. Intermediates of this type have been shown to be useful phosphorylating agents of carboxylic,