λ_{max}^{Nujol} 5.75 (ester C=O), 5.94 (amide C=O), 6.43 (amide NH, aryl, NH_3^+), 8.57 (ester C–O–C), 12.15 μ (*p*-disubstituted phenyl).

Anal. Calcd. for $C_{12}H_{16}N_2O_3 \cdot HCl$: C, 53.6; H, 6.37. Found: C, 53.0; H, 6.49.

(B).—Methyl 3-[*p*-(N-carbobenzoxyglycylamino)-phenyl]-propionate (X) (7.1 g., 0.019 mole) was suspended with 1.1 g. of 5% palladium-on-carbon catalyst in 50 ml. of methanol containing 0.64 ml. (0.02 mole) of concentrated hydrochloric acid. The suspension was hydrogenated at 50 p.s.i. (gauge) initial pressure for 2 hours, diluted with 50 ml. of methanol and filtered. Concentration of the filtrate *in vacuo* to appearance of crystals gave a shiny white solid which was washed with acetone and collected on a filter; yield 3.30 g. (60%), m.p. 198–208°. The infrared absorption spectrum was identical with that of preparation A.

Methyl 3-(*p*-Diazoacetamidophenyl)-propionate (XVI).—Methyl 3-(*p*-glycylaminophenyl)-propionate hydrochloride (XIV) (6.4 g., 0.022 mole) was dissolved in 25 ml. of water and cooled to 10° in an ice-bath, then 0.5 ml. of concentrated hydrochloric acid was added. A solution of 2.07 g. (0.03 mole) of sodium nitrite in 4 ml. of water was chilled and added dropwise to the acid solution. The yellow product began to precipitate when nitrite addition was nearly complete. After standing 15 minutes at 10–15°, the reaction mixture was filtered and the product washed well with water; yield 4.0 g. (74%), m.p. 110° dec.

An analytical sample was prepared by solution of the product in 95% ethanol at room temperature followed by precipitation with water. After two recrystallizations, the melting point was raised to 118° dec.; λ_{max}^{KBr} 3.10 (NH), 4.82 (diazo), 5.79 (ester C=O), 6.17 (amide C=O), 8.40, 8.58 (ester –C–O–C), 11.85 μ (*p*-disubstituted phenyl).

Anal. Calcd. for $C_{12}H_{13}N_3O_3$: C, 58.83; H, 5.29; N, 17.0. Found: C, 58.3; H, 5.26; N, 16.8.

Attempted saponification of XVI to XV under a variety of conditions was unsuccessful.

ADDED IN PROOF.—The two diazo compounds (VI and XVI) were evaluated[13] against Sarcoma 180, Carcinoma 755, Leukemia L-1210 and Ehrlich ascites. The only positive activity observed was a 37% life extension by VI on mice bearing Ehrlich ascites.

Acknowledgments.—The authors wish to thank Dr. Peter Lim for interpretation of the infrared absorption spectra and his staff for the paper chromatography and spectrophotometry. The authors are also indebted to Mr. O. P. Crews, Jr., and his staff for large-scale preparation of certain intermediates.

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MENLO PARK, CALIF.

[CONTRIBUTION FROM THE SCHOOL OF PHARMACY, UNIVERSITY OF CALIFORNIA, SAN FRANCISCO]

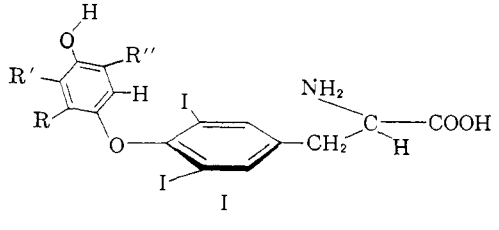
Thyroxine Analogs. I. Synthesis of 3,5-Diiodo-4-(2'-alkylphenoxy)-DL-phenylalanines[1]

BY NICOLAS ZENKER[2] AND EUGENE C. JORGENSEN

RECEIVED MARCH 10, 1959

3,5-Diiodo-4-(2'-methylphenoxy)-DL-phenylalanine and 3,5-diiodo-4-(2'-isopropylphenoxy)-DL-phenylalanine derivatives have been prepared for testing as analogs of thyroxine.

Many analogs of thyroxine have been synthesized in attempts to define structural requirements for thyroid hormonal activity, to elicit selective physiological responses, and to prepare substances capable of antagonizing thyroid hormones.[3] One factor which appears to have received no consideration is the potential difference in orientation for groups occupying the 3'- and 5'-positions of 3,5-diiodothyronine and related diphenyl ethers (I). Molecular models of thyroxine (I, R = H, R' = B'' = I) indicate a favored perpendicular orientation for the planes of the two phenyl rings, thus



providing a minimal interaction between the bulky 3,5-iodines and the 2',6'-hydrogens. This preferred orientation may be fixed by placing a bulky group in the 2'-position. This 2'-substituent would be forced to orient away from (distally to) the alanine-bearing ring, but would not be likely to distort the normal diphenyl ether bond angle. A series of such 3,5-diiodo-4-(2'-alkylphenoxy)-DL-phenylalanines was prepared, carrying additional substituents *ortho*, *meta* and *para* to the orienting 2'-group. In this way, the position in space relative to the diiodophenylalanine ring was known for each substituent.

Compounds such as 3,5-diiodo-3',5'-dimethyl-L-thyronine[4,5] and its thyropropionic acid analog[6,7] have demonstrated the ability of alkyl groups to replace the 3',5'-iodines of thyroxine analogs with retention of thyroxine-like activity. Thus, oriented alkylthyronines were themselves of biological interest, in addition to their anticipated use in directing iodine substitution into known spatial positions of the phenolic ring. Analogs containing oriented alkyl groups but lacking the 4'-hydroxyl group were prepared to provide further informa-

(1) Reported in part before the Division of Medicinal Chemistry at the 134th Meeting of the American Chemical Society, Chicago, Ill., September, 1958.

(2) In partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California, September, 1958.

(3) H. A. Selenkow and S. P. Asper, Jr., *Physiol. Rev.*, **35**, 426 (1955).

(4) T. C. Bruice, N. Kharasch and R. J. Winzler, *J. Org. Chem.*, **18**, 83 (1953).

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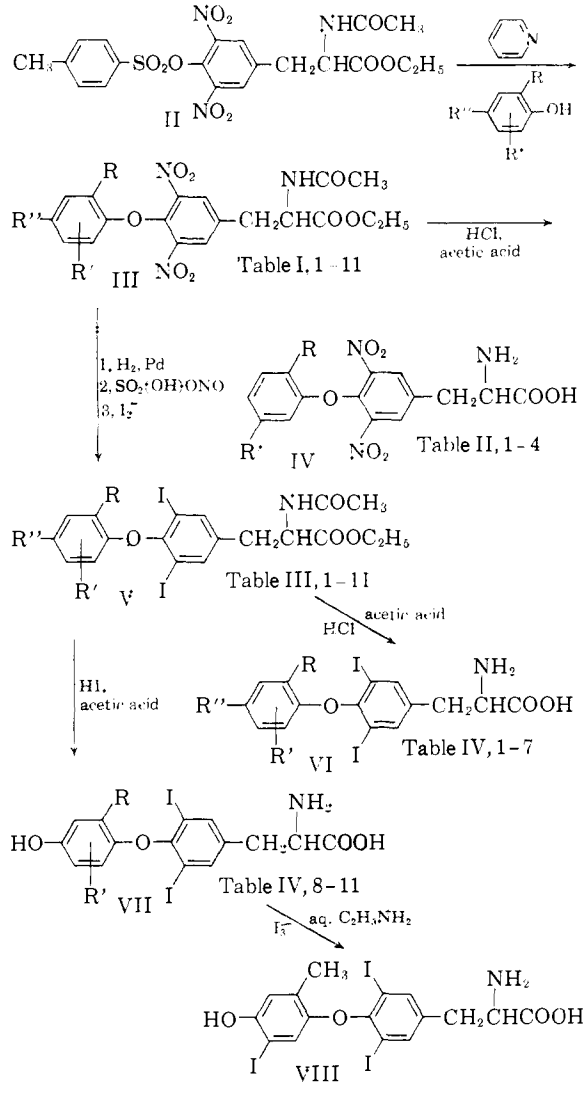
tion on the structure–activity relationships of compounds such as 3,5-diiodo-4-(3′-methyl-5′-ethylphenoxy)-DL-phenylalanine and 3,5-diiodo-4-(3′,4′-dimethylphenoxy)-DL-phenylalanine, which have been shown[8] to act as thyroxine antagonists.

The synthetic route used was based on the sequence of reactions developed by Hems and coworkers.[9] The general synthetic intermediate, 3,5-dinitro-4-(*p*-toluenesulfonyloxy)-DL-phenylalanine-N-acetyl ethyl ester (II) was prepared by the method of Barnes, *et al.*[8] Condensation of this sulfonyl ester with the appropriate phenol[10] in the presence of pyridine yielded 3,5-dinitro-4-(substituted phenoxy)-DL-phenylalanine-N-acetyl-ethyl esters (III). Some members of this series were hydrolyzed to the 3,5-dinitro-4-(alkylphenoxy)-DL-phenylalanines (IV) by hydrochloric and acetic acids. The dinitro-N-acetyl ethyl esters (III) were hydrogenated to the diamines with Pd-on-charcoal catalyst. Without isolation, these unstable intermediates were tetrazotized with nitrosylsulfuric acid and converted to the 3,5-diiodo-4-(substituted phenoxy)-DL-phenylalanine-N-acetyl ethyl esters (V) with iodine and potassium iodide. Those diiodo-N-acetyl ethyl esters without a 4′-methoxy substituent were refluxed with hydrochloric and acetic acids to form the 3,5-diiodo-4-(substituted phenoxy)-DL-phenylalanines (VI). Those possessing the 4′-methoxy group were treated with hydriodic and acetic acids to yield the 3,5-diiodo-4-(4′-hydroxyalkylphenoxy)-DL-phenyl-alanines (VII). Iodination of 3,5-diiodo-2′-methyl-DL-thyronine (VII) with iodine and potassium iodide in aqueous ethylamine produced a triiodo derivative which was assigned the 3,5,5′-triiodo-2′-methyl-DL-thyronine structure (VIII) on the basis of the analogous synthesis of 3,5,5′-triiodo-4-(2′-methyl-4′-hydroxyphenoxy)-hydrocinnamic acid.[6] Attempts to iodinate 3,5-diiodo-2′-isopropyl-DL-thyronine and 3,5-diiodo-2′,5′-dimethyl-DL-thyronine were unsuccessful under similar conditions. Reports of a successful iodination of 2,4-dichloro-3,5-dimethylphenol with iodine monochloride[11] indicated the desirability of applying this agent in the iodination of hindered phenols. However, attempts with iodine monochloride in acetic acid[12] on 3,5-diiodo-2′,5′-dimethyl-DL-thyronine resulted in incomplete iodination under mild conditions and iodine loss when the reaction temperature was raised.

Thyroxine-like activity was demonstrated in the rat for 3,5,5′-triiodo-2′-methyl-DL-thyronine (VIII) and the 3,5-diiodo-4-(4′-hydroxyalkylphenoxy)-DL-phenylalanines (VII, Table IV, no. 8–11) by the antigoiter assay of Cortell[13] and oxygen consumption method of Holtkamp and Heming.[14] The 3,5-diiodo-4-(substituted phenoxy)-DL-phenylalanines (VI, Table IV, no. 1–7) were inactive as thyroxine-like analogs in the oxygen consumption assay, and in the antigoiter assay were inactive (Table IV, no. 1, 4, 5) or thyroxine antagonists (Table IV, no. 2, 3, 6, 7). A detailed discussion of these findings, relating stereochemical orientation of substituents to biological response, will be submitted elsewhere.



Experimental[15]

3,5-Dinitro-4-(*p*-toluenesulfonyloxy)-DL-phenylalanine-N-acetyl ethyl ester (II), crystallized from aqueous acetone, m.p. 159–160.5°; prepared in 68% yield by the method of Barnes, *et al.*,[8] who reported m.p. 157–158°.

Anal. Calcd. for $C_{20}H_{21}O_{10}N_3S$: N, 8.48; S, 6.48. Found: N, 8.41; S, 6.38.

3,5-Dinitro-4-(substituted phenoxy)-DL-phenylalanine-N-acetyl Ethyl Esters (III). Table I.—3,5-Dinitro-4-(*p*-toluenesulfonyloxy)-DL-phenylalanine-N-acetyl ethyl ester (29.7 g., 0.06 mole) was heated under reflux with dry pyridine (16 ml., 0.2 mole) and dry chloroform (80 ml.) for 30 minutes. The *o*-alkylphenol[10] (0.09 to 0.42 mole) was added and the mixture refluxed for an additional 2 to 6 hours. After cooling, the chloroform solution was washed successively with 2 *N* hydrochloric acid, water, 2 *N* sodium hydroxide and water, dried over calcium chloride, filtered,

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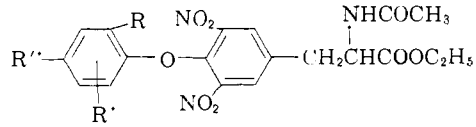
(15) Melting points obtained on Fisher–Johns melting point apparatus and are uncorrected. Microanalysis by the Microanalytical Laboratory, Department of Chemistry, University of California.

TABLE I

3,5-DINITRO-4-(SUBSTITUTED PHENOXY)-DL-PHENYLALANINE-N-ACETYL ETHYL ESTERS

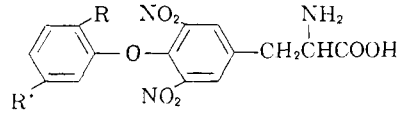


No.	Substitution R	R′	R″	Reaction conditions[a] Mole phenol/ Mole sulfonyl ester	Reflux, hr.	Yield,[b] %	M.p., °C.	Formula[c]	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
1	CH_3	H	H	0.18/0.06	2.5	58	127–128	$C_{20}H_{21}N_3O_8$	55.68	55.51	4.91	4.82
2	CH_3	H	CH_3	0.09/0.03	2	52	120–122	$C_{21}H_{23}N_3O_8$	56.62	56.77	5.21	5.43
3	CH_3	5′-CH_3	H	0.18/0.06	3.5	51	112–113	$C_{21}H_{23}N_3O_8$	56.62	56.90	5.21	5.10
4	CH_3	3′-CH_3	Cl	0.11/0.04	7	55	139–140	$C_{21}H_{22}ClN_3O_8$	52.56	52.36	4.62	4.39
5	CH_3	5′-CH_3	Cl	0.11/0.04	7	53	168–169	$C_{21}H_{22}ClN_3O_8$	52.56	52.81	4.62	4.69
6	$CH(CH_3)_2$	H	H	0.20/0.03	3.5	46	107–108	$C_{22}H_{25}N_3O_8$	57.51	57.32	5.48	5.54
7	$CH(CH_3)_2$	5′-CH_3	H	0.42/0.06	6	43	142–144	$C_{23}H_{27}N_3O_8$	58.34	58.56	5.75	5.76
8	CH_3	H	OCH_3	0.28/0.10	4	63	124–125	$C_{21}H_{23}N_3O_9$	54.66	54.90	5.02	4.94
9	CH_3	5′-CH_3	OCH_3	0.13/0.05	4	51	152–153	$C_{22}H_{25}N_3O_9$	55.57	55.34	5.30	5.40
10	$CH(CH_3)_2$	H	OCH_3	0.15/0.05	8	51	105–106	$C_{23}H_{27}N_3O_9$	56.43	56.31	5.56	5.74
11	$CH(CH_3)_2$	5′-CH_3	OCH_3	0.14/0.05	8	56	160–162	$C_{24}H_{29}N_3O_9$	57.25	57.57	5.81	5.93

[a] The general procedure is given in the Experimental section. [b] Yields are based on purified material. [c] Dried at 65° (2 mm.) in 24 hours.

TABLE II

3,5-DINITRO-4-(ALKYLPHENOXY)-DL-PHENYLALANINES

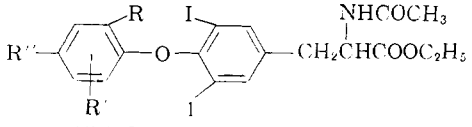


No.	Substitution R	R′	Yield,[a] %	M.p., °C.	Formula[b]	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
1	CH_3	H	23	192–193	$C_{16}H_{15}N_3O_7$	53.19	53.0[c]	4.18	4.3[c]
2	CH_3	CH_3	13	211–214	$C_{17}H_{17}N_3O_7$	54.40	54.24	4.57	4.82
3	$CH(CH_3)_2$	H	8	187–190	$C_{18}H_{19}N_3O_7$	55.52	55.37	4.92	4.82
4	$CH(CH_3)_2$	CH_3	60	196–199	$C_{19}H_{21}N_3O_7$	56.58	56.84	5.25	5.51

[a] Yields are based on purified material; considerable losses resulted during purification. [b] Dried at 100° (2 mm.) over P_2O_5 for 24 hours. [c] Hygroscopic.

TABLE III

3,5-DIIODO-4-(SUBSTITUTED PHENOXY)-DL-PHENYLALANINE-N-ACETYL ETHYL ESTERS



No.	Substitution R	R′	R″	Yield,[a] %	M.p., °C.	Formula[b]	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
1	CH_3	H	H	61	111–112[c]	$C_{20}H_{21}I_2NO_4$	40.49	40.69	3.57	3.46
2	CH_3	H	CH_3	55	139–140[c]	$C_{21}H_{23}I_2NO_4$	41.53	41.71	3.82	3.89
3	CH_3	5′-CH_3	H	33	147–148[d]	$C_{21}H_{23}I_2NO_4$	41.53	41.69	3.82	3.73
4	CH_3	3′-CH_3	Cl	57	175–176[c]	$C_{21}H_{22}ClI_2NO_4$[e]	39.31	39.58	3.46	3.71
5	CH_3	5′-CH_3	Cl	61	153–154[c]	$C_{21}H_{22}ClI_2NO_4$[f]	39.31	39.57	3.46	3.62
6	$CH(CH_3)_2$	H	H	57	142–143[d]	$C_{22}H_{25}I_2NO_4$	42.53	42.75	4.06	4.19
7	$CH(CH_3)_2$	5′-CH_3	H	65	178–179[c]	$C_{23}H_{27}I_2NO_4$[g]	43.48	43.56	4.28	4.27
8	CH_3	H	OCH_3	57	150–151[c]	$C_{21}H_{23}I_2NO_5$	40.47	40.66	3.72	3.96
9	CH_3	5′-CH_3	OCH_3	52	163–164[c]	$C_{22}H_{25}I_2NO_5$	41.46	41.18	3.95	3.63
10	$CH(CH_3)_2$	H	OCH_3	80	122–123[c]	$C_{23}H_{27}I_2NO_5$	42.41	42.59	4.18	4.13
11	$CH(CH_3)_2$	5′-CH_3	OCH_3	60	136–137[c]	$C_{24}H_{29}I_2NO_5$	43.32	43.12	4.39	4.23

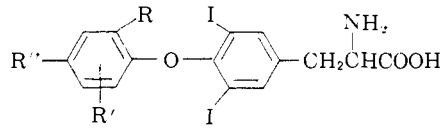
[a] Yields are based on purified material. [b] Dried at 65° (2 mm.) for 24 hours. [c] From aqueous ethanol. [d] From aqueous acetone. [e] Calcd.: I, 39.56. Found: I, 39.80. [f] Calcd.: I, 39.56. Found: I, 39.32. [g] Calcd.: I, 39.96. Found: I, 39.78.

and the chloroform removed under reduced pressure. The residue of crude *o*-methyl compound was recrystallized from ethanol and then aqueous ethanol. The residue of *o*-isopropyl compound was dissolved in a small volume of chloroform, adsorbed on a column of activated alumina and developed with chloroform. The eluate of the first band was evaporated to dryness and recrystallized from aqueous ethanol.

3,5-Dinitro-4-(alkylphenoxy)-DL-phenylalanines (IV). Table II.—3,5-Dinitro-4-(alkylphenoxy)-DL-phenylalanine-N-acetyl ethyl ester (4.9 mmoles) was heated under reflux for 2 hours with glacial acetic acid (23 ml.) and concentrated

TABLE IV

3,5-DIIODO-4-(SUBSTITUTED PHENOXY)-DL-PHENYLALANINES



No.	Substitution R	R'	R''	Yield,[a] %	M.p., °C. (d.)	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Iodine, % Calcd.	Iodine, % Found
1	CH_3	H	H	87	234–237	$C_{16}H_{13}I_2NO_3$[b]	36.73	36.42	2.89	2.89		
2	CH_3	H	CH_3	88	190–192	$C_{17}H_{17}I_2NO_3$[b]	38.01	37.99	3.19	3.48		
3	CH_3	5'-CH_3	H	56	196–197	$C_{17}H_{17}I_2NO_3$[b]	38.01	38.30	3.19	3.41		
4	CH_3	3'-CH_3	Cl	71	213–214	$C_{17}H_{16}ClI_2NO_3\cdot H_2O$[b]	34.63	34.92	3.08	2.89	43.05	43.00
5	CH_3	5'-CH_3	Cl	71	205–207	$C_{17}H_{16}ClI_2NO_3\cdot H_2O$[b]	34.63	34.92	3.08	2.99	43.05	42.94
6	$CH(CH_3)_2$	H	H	50	202–205	$C_{18}H_{19}I_2NO_3$[b]	39.22	39.44	3.48	3.69		
7	$CH(CH_3)_2$	5'-CH_3	H	78	183–185	$C_{19}H_{21}I_2NO_3$[b]	40.37	40.19	3.75	3.67	44.91	44.58
8	CH_3	H	OH	67	227–229	$C_{16}H_{13}I_2NO_4$[c]	35.64	35.44	2.81	2.82	47.08	46.85
9	CH_3	5'-CH_3	OH	87	199–201	$C_{17}H_{17}I_2NO_4$[c]	36.91	37.16	3.10	3.34	45.89	45.40
10	$CH(CH_3)_2$	H	OH	87	184–186	$C_{18}H_{19}I_2NO_4$[c]	38.12	37.9	3.38	3.3	44.76	44.88
11	$CH(CH_3)_2$	5'-CH_3	OH	67	190–191	$C_{19}H_{21}I_2NO_4$[c]	39.26	39.4	3.64	3.8	43.68	43.72

[a] Yields are based on purified material. [b] Dried at 60° (50 mm.) over $CaCl_2$ for 24 hours. [c] Hygroscopic; dried at 100° (1 mm.) over P_2O_5 for 24 hours.

hydrochloric acid (23 ml.). The cooled mixture was diluted with water (100 ml.) and brought to *p*H 5.0 with 2 *N* sodium hydroxide. The precipitate was filtered, redissolved in an acetic acid–hydrochloric acid mixture, reprecipitated at *p*H 5.0 and crystallized from aqueous pyridine.

3,5-Diiodo-4-(substituted phenoxy)-DL-phenylalanine-N-acetyl Ethyl Esters (V). Table III.—3,5-Dinitro-4-(substituted phenoxy)-DL-phenylalanine-N-acetyl ethyl ester (0.02 mole), dissolved in acetic acid (300 ml.), was shaken for 45 minutes in the presence of Pd-on-charcoal (10%, 2.0 g.) and hydrogen (initial 35 p.s.i.). In each case the hydrogen uptake was between 89 to 98% of theoretical. Concentrated sulfuric acid (15 ml.) was added, the catalyst removed by filtration through Celite, and the resulting solution added over 2 hours to a stirred mixture of nitrosylsulfuric acid (sodium nitrite, 5.6 g., 0.081 mole, added in small portions to sulfuric acid, 120 ml., and acetic acid, 40 ml., at 60–70°) at −5°. The tetrazonium mixture was stirred for 2 more hours at −5°, then added to a well-stirred mixture at 25° of iodine (17 g., 0.07 mole) and sodium iodide (12 g., 0.08 mole) diluted with water (300 ml.) and underlayed with chloroform (300 ml.). After 2 hours the chloroform phase was separated and the aqueous layer extracted with chloroform. The combined chloroform extracts were washed with 10% aqueous sodium bisulfite, water, 1 *M* sodium bicarbonate and water, dried over calcium chloride, filtered, and the chloroform removed under reduced pressure. The residue was crystallized from ethanol. A second crop was obtained when the residue of the evaporated mother liquor was dissolved in chloroform, adsorbed on activated alumina and developed with chloroform. Final purification was carried out from aqueous alcohol or aqueous acetone.

3,5-Diiodo-4-(substituted phenoxy)-DL-phenylalanines (VI). Table IV. No. 1–7.—3,5-Diiodo-4-(substituted phenoxy)-DL-phenylalanine-N-acetyl ethyl ester (Table III, no. 1–7; 4.1 mmoles) was refluxed for 3 hours with glacial acetic acid (25 ml.) and concentrated hydrochloric acid (25 ml.). The cooled mixture was adjusted to *p*H 5.0 with 2 *N* sodium hydroxide, the precipitate filtered, dissolved in hot aqueous pyridine, and precipitated by adjustment to *p*H 5 with 2 *N* HCl. After a second precipitation from pyridine, the compound was dissolved in 1 *N* sodium hydroxide (in 50% ethanol for the 4-alkylphenoxy amino acids; Table IV, no. 1–3, 6 and 7; in 75% ethanol for the 4-*p*-chloroalkylphenoxy amino acids; Table IV, no. 4 and 5) and precipitated at *p*H 5.0 with 2 *N* hydrochloric acid in the presence of a few drops of 2 *N* sodium acetate.

3,5-Diiodo-4-(4'-hydroxyalkylphenoxy)-DL-phenylalanines (VII). Table IV, No. 8–11.—3,5-Diiodo-4-(4'-methoxyalkylphenoxy)-DL-phenylalanine-N-acetyl ethyl ester (Table III, no. 8–11; 9.5 mmoles) was refluxed for 8 hours with glacial acetic acid (40 ml.) and hydriodic acid (30 ml., 58%). The reaction mixture was distilled to near dryness from a water-bath at 50° (5 mm.). The residue was dissolved in a suspension of sodium metabisulfite in hot ethanol, additional metabisulfite added until decolorization was complete and adjusted to *p*H 5.0 with 2 *N* sodium acetate. The precipitate was centrifuged, redissolved in sodium hydroxide (1 *N*, 50% ethanolic) and reprecipitated at *p*H 5.0 with 2 *N* hydrochloric acid in the presence of a few drops of 2 *N* sodium acetate.

3,5,5'-Triiodo-2'-methyl-DL-thyronine (VIII).—To a well-stirred solution of 3,5-diiodo-2'-methyl-DL-thyronine (2.0 g., 3.7 mmoles) in aqueous ethylamine (33%, 40 ml.) 5.1 ml. of an aqueous solution of iodine (2.55 g., 10 mmoles) and potassium iodide (4.0 g., 24.1 mmoles) was added at room temperature over a period of 2 hours and stirring continued an additional hour. The solution was adjusted to *p*H 5.0 with acetic acid. The precipitate was collected by centrifugation, dissolved and precipitated three times from sodium hydroxide (1 *N*, 50% ethanolic) by adjustment to *p*H 5.0 with 2 *N* hydrochloric acid and a few drops of aqueous sodium acetate, washed by suspension in water and collected by centrifugation. Drying at 100° *in vacuo* (2 mm.) over P_2O_5 yielded 2.3 g. (92%) of a light tan powder, m.p. 221–224° dec.

Anal. Calcd. for $C_{16}H_{14}I_3NO_4$: C, 28.90; H, 2.12; I, 57.25. Found: C, 28.76; H, 2.34; I, 57.50.

Attempted Synthesis of 3,5,5'-Triiodo-2'-isopropyl-DL-thyronine.—To 3,5-diiodo-2'-isopropyl-DL-thyronine (1.5 g., 2.6 mmoles) in aqueous ethylamine (33%, 25 ml.) 5.3 ml. of an aqueous solution of iodine (2.7 g., 10.4 mmoles) and potassium iodide (4.1 g., 24.5 mmoles) was added at room temperature over a period of 2 hours and stirring continued an additional hour. The reaction mixture was treated as was that in the preparation of 3,5,5'-triiodo-2'-methyl-DL-thyronine, but analysis indicated incomplete iodination to the triiodo derivative.

Anal. Calcd. for $C_{18}H_{19}I_3NO_4$: C, 38.12; H, 3.38; I, 44.76. Calcd. for $C_{18}H_{18}I_3NO_4$: C, 31.19; H, 2.62; I, 54.94. Found: C, 35.73; H, 3.37; I, 48.50.

Repetition of the above experiment with addition of iodine extended over 9 hours and a molal ratio of iodine to thyronine of 2.13:1, resulted in a product which was less completely iodinated than before.

Anal. Found: C, 36.25; H, 3.72; I, 45.00.

Attempted Synthesis of 3,5,3'-Triiodo-2',5'-dimethyl-DL-thyronine. (a) Iodine–Potassium Iodide.—To 3,5-diiodo-2',5'-dimethyl-DL-thyronine (0.52 g., 0.95 mmole) in aqueous ethylamine (33%, 20 ml.) 2.0 ml. of an aqueous solution of iodine (1.0 g., 3.9 mmoles) and potassium iodide (1.3 g., 8.0 mmoles) was added at room temperature over a period of one hour and stirring continued for an additional hour. The reaction mixture was treated as was that in the preparation of 3,5,5'-triiodo-2'-methyl-DL-thyronine. Analysis indicated incomplete conversion to the triiodo derivative.

Anal. Calcd. for $C_{17}H_{17}I_2NO_4$: C, 36.91; H, 3.10; I, 45.80. Calcd. for $C_{17}H_{16}I_3NO_4$: C, 30.07; H, 2.38; I, 56.07. Found: C, 35.28; H, 2.92.

Repetition of the above experiment with addition of iodine extended over 3 hours and a molal ratio of iodine to thyronine of 34:1 resulted in apparent loss of iodine from the starting thyronine.

Anal. Found: C, 37.62; H, 3.40; I, 44.48.

(b) **Iodine Monochloride.**—To 3,5-diiodo-2',5'-dimethyl-DL-thyronine (0.75 g., 1.4 mmoles) dissolved in glacial acetic acid (5 ml.), a solution of freshly redistilled iodine monochloride (0.26 g., 1.6 mmoles) in acetic acid (2.1 ml.) was added. The reaction mixture was stirred for one hour at room temperature, then heated at 60° for 1.5 hours. The product was isolated and purified as described for 3,5,5'-triiodo-2'-methyl-DL-thyronine. Analysis showed only slight increase in iodine content.

Anal. Found: C, 35.86; H, 3.11; I, 48.60.

In a second attempt, iodine monochloride (1.3 mmoles) was added in two portions, one hour apart, to the diiodothyronine (0.56 mmole) and the reaction mixture placed in a 60° water-bath for 3 hours. Isolation and purification was carried out as before. Analysis indicated loss of iodine.

Anal. Found: C, 37.52; H, 2.94; I, 33.58.

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SAN FRANCISCO, CALIF.

[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF THE BRITISH COLUMBIA RESEARCH COUNCIL]

Studies on Polynucleotides. V.[1] Stepwise Synthesis of Oligonucleotides. Syntheses of Thymidylyl-(5′ → 3′)-thymidylyl-(5′ → 3′)-thymidine and Deoxycytidylyl-(5′ → 3′)-deoxyadenylyl-(5′ → 3′)-thymidine[2]

BY P. T. GILHAM AND H. G. KHORANA

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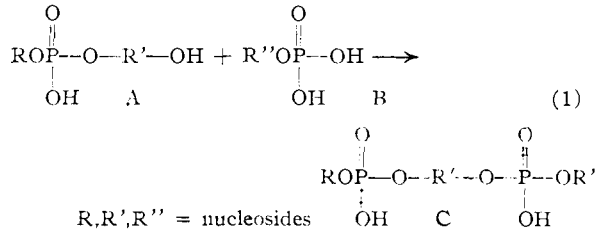
The reaction of 3′-*O*-acetylthymidylic-(5′) acid (X) with thymidylyl-(5′→3′)-5′-*O*-tritylthymidine (VIII) in the presence of dicyclohexylcarbodiimide, followed by mild alkaline and acidic treatments of the product, gave thymidylyl-(5′ → 3′)-thymidylyl-(5′ → 3′)-thymidine ("trithymidine diphosphate") (XIV) in 68% yield. In the application of this method to the stepwise synthesis of mixed oligonucleotides, *N*,*O*³′-diacetyldeoxycytidylic-(5′) acid (XI) was treated with deoxyadenylyl-(5′ → 3′)-5′-*O*-tritylthymidine (IX) and the product afforded, after successive alkaline and acidic treatments, deoxycytidylyl-(5′ → 3′)-deoxyadenylyl-(5′ → 3′)-thymidine (XV) in 31% over-all yield. The synthetic products were characterized by chemical and enzymic degradations.

In initiating a program of studies in the polynucleotide field we have recently developed a general method for synthesis of the C_5'–C_3' internucleotide linkage.[3,4] This method involves the direct activation of the *phosphomonoester* group of a mononucleotide by dicyclohexylcarbodiimide (DCC) or *p*-toluenesulfonyl chloride in the presence of a suitably protected nucleoside or nucleotide. By this method good yields of a number of dinucleoside phosphates and dinucleotides containing the typical internucleotidic linkage were obtained.[4] The synthesis of larger oligonucleotides would entail the formation of a phosphodiester linkage between a fragment bearing the phosphomonoester group and a second fragment bearing the appropriate hydroxyl group, one or both of these fragments containing preformed diester bonds. The present communication describes our initial experiments to determine whether the above method of diester synthesis can be used to form a second internucleotide bond in the presence of a preformed one. Syntheses of thymidylyl-(5′ → 3′)thymidylyl-(5′ → 3′)-thymidine[5] ("trithymidine diphosphate") (XIV) and deoxycytidylyl-(5′ → 3′)-deoxyadenylyl-(5′ → 3′)-thymidine[5] (XV) have resulted.[6]

Two modifications of the general procedure of diester synthesis were considered advisable in the present work on the formation of a second phosphodiester linkage between a preformed diester (A) and a phosphomonoester (B). (The reaction is represented simply by equation 1, the diester A



carrying the appropriate hydroxylic function on the component R′.) The first was prompted by a consideration of the possible complications due to diester linkage in (A). Although the precise mechanism of the diester synthesis remains to be elucidated, it seems likely that the phosphomonoester (B) is converted by DCC to tri- and higher meta- (I) or polyphosphates (II) and that these reactive products serve as the phosphorylating agents.[7] From previous work it is also known that diesters of phosphoric acid (A) react with DCC in pyridine to

(1) Paper IV, W. E. Razzell and H. G. Khorana, *J. Biol. Chem.*, **234**, 2114 (1959).

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(3) H. G. Khorana, G. M. Tener, J. G. Moffatt and E. H. Pol, *Chemistry & Industry*, 1523 (1956); H. G. Khorana, W. E. Razzell, P. T. Gilham, G. M. Tener and E. H. Pol, THIS JOURNAL, **79**, 1002 (1957).

(4) P. T. Gilham and H. G. Khorana, *ibid.*, **80**, 6212 (1958).

(5) The nomenclature used is that which was proposed in the first paper of this series (ref. 4).

(6) This work has been reported briefly; H. G. Khorana and P. T. Gilham, *Federation Proc.*, **18**, 259 (1959).

(7) Ref. 4 and unpublished results by H. G. Khorana and J. P. Vizsolyi.