except for the repetition of adjacent alanine residues. This randomness makes itself evident in the greater array of peptides from TSF as compared to BSF. Thus, in BSF the sequence ser-gly seems to exist to the exclusion of other sequences such as ser-X where X is any amino acid; in TSF, a variety of sequences is to be found in ser-ala, ser-asp, ser-ser and ser-tyr in addition to ser-gly. Likewise, gly-gly has been isolated from the hydrolysate of TSF but no evidence of its presence in BSF hydrolysates has ever been found. Differences in the type of tyrosine-containing peptides have been noted above.

The chemical data on the sequence of amino acids in the two fibroins and the interpretation of the X-ray diffraction patterns are in accord. Thus, the X-ray pattern of BSF can best be explained[3] in terms of two spacings between adjacent pleated sheets and these spacings can be achieved only if glycine residues occupy alternate positions in the chains: the chemical data show this alternation. The X-ray pattern of TSF indicates a single spacing between pleated sheets[4] but no conclusions about regularity of sequence can be drawn; the chemical data show little evidence of regularity.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, SCHOOL OF MEDICINE, AND THE COBB CHEMICAL LABORATORY, UNIVERSITY OF VIRGINIA]

3-Iodo-, 3,3′-Diiodo- and 3,3′-Diiodo-5-bromothyronine[1]

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Syntheses of DL-3-iodothyronine and D,L-3,3′-diiodo-5-bromothyronine have been performed, and these compounds as well as 3,3′-diiodothyronine have been described accurately. 3,3′-Diiodothyronine has weak or no thyromimetic activity while 3,3′-diiodo-5-bromothyronine is markedly active.

It is generally accepted[2] that in diphenyl ether derivatives structurally related to thyroxine, iodine substitution in positions 3 and 5 is necessary for minimal thyroxine-like activity, and that thyronine derivatives with halogens in 3′,5′ only are devoid of thyromimetic action. In view of the marked metabolic activity of (−)3,3′,5-triiodothyronine, introduction of iodine into position 3′ raises minimal activity to a high level. This fact reopens the question as to the significance of the 5-iodine atom. We began to synthesize 3,3′-diiodothyronine according to the general pattern set for 3,5-diiodothyronine by Harington and Barger,[3] starting with 3,4-diiodonitrobenzene and 3-iodo-4-methoxyphenol. While this work was in progress, a communication by Roche, Michel and Wolf[4] described the preparation of 3,3′-diiodothyronine by monodeiodination of 3,5-diiodothyronine, and subsequent monoreiodination of the resulting 3-iodothyronine. They reported[5] that DL-3,3′-diiodothyronine had about 82% of the antigoitrogenic activity of thyroxine in the rat; DL-3,3′,5′-triiodothyronine had little or no activity.

The last step of our synthetic approach to 3,3′-diiodothyronine was the reduction and hydrolysis of α-benzamido-3-iodo-4-(3′-iodo-4′-methoxyphenoxy)-cinnamic acid. Under a variety of conditions, using hydriodic acid, or combinations of hydriodic and hydrobromic acid with different amounts of phosphorus, one atom of iodine was lost, and a monoiodothyronine of melting point 235–237° was obtained in yields up to 64%. It was chromatographically homogeneous, and did not consist of a chance mixture of the 3- and 3′-iodo isomers. It differed in melting point from the 3-iodothyronine (m.p. 206°) and from 3′-iodothyronine (m.p. 207°) as reported by Roche, *et al.*[4] Confirmation of the structure of our monoiodo derivative as 3-iodothyronine was secured by unequivocal synthesis. Condensation of 3,4-diiodonitrobenzene with *p*-methoxyphenol to 2-iodo-4-nitro-4′-methoxydiphenyl ether was followed by reduction of the nitro group of this compound with iron and aqueous ethanol. A Sandmeyer reaction with the resulting amine gave 2-iodo-4-cyano-4′-methoxydiphenyl ether which was purified by chromatography and converted to the corresponding aldehyde by the Stephen method. The aldehyde was subjected to an azlactone synthesis to yield, in two steps, α-benzamido-3-iodo-4-(4′-methoxyphenoxy)-cinnamic acid which was reduced and cleaved to 3-iodothyronine. The product thus obtained was identical with the deiodination product of melting point 235–237° above, and vindicated our datum as contrasted with that in the literature.[4]

Since the direct synthesis of 3,3′-diiodothyronine had failed, we iodinated 3-iodothyronine and obtained a product which, as described,[4] melted at 198–199°. However, even after drying over phosphorus pentoxide at 115° (0.2 mm.) for eight hours it retained two molecules of water of crystallization. The iodine analysis reported[4] for the water-washed and dried product points to anhydrous material; this divergence cannot be explained at this time.

The only known thyroxine analog containing

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(2) E. Frieden and R. J. Winzler, *J. Biol. Chem.*, **176**, 155 (1948); C. Niemann, in "Fortschritte der Chemie der organischen Naturstoffe," VIII, L. Zechmeister, ed., Springer, Vienna, 1950, p. 167; H. A. Selenkow and S. P. Asper, Jr., *Physiol. Revs.*, **35**, 426 (1955), *cf.* p. 443.

(3) C. R. Harington and G. Barger, *Biochem. J.*, **21**, 169 (1927).

(4) J. Roche, R. Michel and W. Wolf, *Compt. rend.*, **239**, 597 (1954).

(5) J. Roche, R. Michel, W. Wolf and N. Etling, *Compt. rend. soc. biol.*, **148**, 1738 (1954).

mixed halogens in one aromatic ring is DL-3,3',5-triiodo-5'-fluorothyronine which shows about one-third of the effect of thyroxine by the goiter prevention method.[6] No derivative containing mixed halogens in the 3,5-positions has been recorded. Replacement of iodine by the relatively large bromine atom in position 5 of the highly active 3,3',5-triiodothyronine could be expected to give further information about the role of halogen substitution in that position. Consequently, DL-3,3'-diiodo-5-bromothyronine has been synthesized for comparison with 3,3',5-triiodothyronine. The synthetic sequence started with 3-bromo-4,5-diiodonitrobenzene and *p*-methoxyphenol, and proceeded in the traditional manner to 3-iodo-5-bromothyronine which was then iodinated in ammoniacal medium.

3,3'-Diiodothyronine has been found to have little or no effect on oxygen consumption of thyroidectomized rats and weak activity in the antigoiter tests. By contrast, 3,3'-diiodo-5-bromothyronine is markedly active both in increasing the oxygen consumption of thyroidectomized rats and in reducing the size of the thyroid gland in the antigoiter tests. Details of these experiments will be reported elsewhere.

Experimental[7]

3-Iodo-4-methoxyphenol.[8]—A suspension of 44.0 g. (1.77 moles) of finely ground 2-iodo-4-aminoanisole[9] in 710 ml. of glacial acetic acid and 9.5 ml. of concentrated sulfuric acid was diazotized with 22 g. of butyl nitrite at 15–18°, and after standing for 30 minutes was added to a solution of 385 ml. of sulfuric acid and 710 ml. of water at 90–100°. The dark orange solution was heated for another hour, cooled, and diluted with 765 ml. of water containing a little sodium bisulfite. A dark brown precipitate separated on standing. It was extracted with 0.5 *N* sodium hydroxide solution, the alkaline extract was cleared with Norite, and the phenol precipitated with hydrochloric acid. Repetition of this treatment gave 45–50% of yellow platelets which were recrystallized from dilute ethanol; m.p. 106–107°.

Anal. Calcd. for $C_7H_7IO_2$: C, 33.62; H, 2.82. Found: C, 33.5; H, 2.84.

3-Bromo-4,5-diiodonitrobenzene.—The preparation of this compound, attributed to Körner and Contardi,[10] has not been described. A solution of 20 g. of 2-bromo-4-nitro-6-iodoaniline in 75 ml. of concentrated sulfuric acid was diazotized with 22.5 g. of sodium nitrite below 10°. After two hours it was poured slowly into 500 g. of ice, and treated with stirring with a solution of 104 g. of potassium iodide in 75 ml. of water. The mixture was stirred for 15 minutes, heated on a steam-bath for 15 minutes, cooled, cleared with a little sodium bisulfite and filtered. Recrystallization from chloroform gave 43 g. (90%) of product melting at 144–146°. The reported melting point[10] is 146.5°.

General Synthetic Directions. (a) Diphenyl Ether Condensations.—The synthesis of 3-iodothyronine started with 3,4-diiodonitrobenzene and *p*-methoxyphenol, that of 3-iodo-5-bromothyronine with 3-bromo-4,5-diiodonitrobenzene and *p*-methoxyphenol. In the 3,3'-diiodothyronine series, 3-iodo-4-methoxyphenol and 3,4-diiodonitrobenzene served as starting materials.

The *p*-iodonitro derivative (0.1 mole) and the required phenol (0.2 mole) were dissolved in about 250 ml. of dry boiling butanone, 0.21 mole of powdered anhydrous potassium carbonate was added, and the mixture refluxed for 18 hours. After treatment with 6% sodium hydroxide solution (0.11 mole), the solvent was steam distilled, and the solidified product recrystallized. Some unreacted phenolic starting material could usually be recovered from the cleared alkaline filtrate upon acidification.

4-Aminodiphenyl Ether Derivatives.—A mixture of 0.01 mole of the nitrodiphenyl ether derivative, 50 ml. of ethanol, 45 ml. of water, 5 ml. of acetic acid, 4 g. of iron powder and 4 g. of iron filings was refluxed for 3.5–6 hours, and worked up as described for analogous cases.[11] The hydrochloride was precipitated from the benzene solution of the base with hydrogen chloride, and the amine was liberated from the salt with alkali.

4-Cyanodiphenyl Ether Derivatives.—A suspension of 0.06 mole of the powdered amine hydrochloride in 300 ml. of 90% acetic acid was diazotized slowly with 10 ml. of butyl nitrite at 15–18°. The salt went slowly into solution with a reddish color. After standing for 30 minutes the mixture was added slowly, below 10°, to 1,000 ml. of a cuprous cyanide solution prepared from 170 g. of potassium cyanide and 150 g. of cupric sulfate. The diazo mixture was stirred at 25° for one hour, heated to 80°, allowed to stand overnight, and filtered. The dry precipitate was extracted with two 500-ml. portions of benzene, and the combined dark extracts were chromatographed through activated alumina. The pale-colored eluate was concentrated under reduced pressure, diluted with ether to 30 ml., cooled to −17°, and the yellow solid precipitate was collected.

Stephen Reduction.—A solution of 0.0168 mole of the nitrile derivative in 35 ml. of dry chloroform was added to a solution of 20 g. of anhydrous stannous chloride in 110 ml. of dry ether saturated with hydrogen chloride at 0°, and the mixture was allowed to stand overnight. The precipitated yellow complex salt was filtered, washed with ether, and hydrolyzed with 30 ml. of 17% hydrochloric acid. The resulting colorless solid was filtered and washed with water.

Preparation of Azlactones.—A mixture of 5.2 mmoles of the 4-formyldiphenyl ether derivative, 5.3 mmoles of hippuric acid, 11 ml. of acetic anhydride and 2.5 g. of anhydrous sodium acetate was heated at 95° for one hour. The azlactone separated soon. Excess acetic anhydride was hydrolyzed with 200 ml. of ice-water, and the product collected.

Hydrolysis of Azlactones.—The azlactone was boiled with 50 volumes of a 2% sodium hydroxide solution in 50% aqueous ethanol for 6 minutes, the mixture was filtered, and the filtrate acidified with 17% hydrochloric acid. The precipitated solid was filtered.

DL-3-Iodothyronine.—A mixture of 1.5 g. of α-benzamido-3-iodo-4-(4'-methoxyphenoxy)-cinnamic acid, 1.5 g. of red phosphorus, 22.5 ml. of acetic acid and 0.6 ml. of constant boiling hydriodic acid was refluxed for 1.5 hours, 3.75 ml. of 48% hydrobromic acid was added, and refluxing continued for 3.5 hours. After filtration the solution was evaporated to near-dryness in a vacuum, the residue was dissolved in 40 ml. of water, treated with Norite, and the clear filtrate was adjusted to *p*H 5–6 with sodium acetate. The amino acid precipitated immediately as colorless crystals. It was washed with water and recrystallized. The material was chromatographed on filter paper using butanol–6 *N* ammonium hydroxide as a solvent, and produced only one spot, R_f 0.65.

Similar treatment of α-benzamido-3-iodo-4-(3'-iodo-4'-methoxyphenoxy)-cinnamic acid resulted in loss of one atom of iodine. The monoiodothyronine from this reaction was obtained in a yield of 64%, and was identical with 3-iodothyronine as shown by elementary analysis, melting and mixture melting points as well as by chromatographic behavior.

DL-3-Bromo-5-iodothyronine was obtained from α-benzamido-3-bromo-5-iodo-4-(4'-methoxyphenoxy)-cinnamic acid by an analogous procedure.

DL-3,3'-Diiodothyronine.—Iodination of 3-iodothyronine in ammoniacal solution was carried out according to Roche, *et al.*,[4] but chromatographically pure material was obtained only when 1.2 molar proportions of iodine were used. The colorless product, obtained in 84% yield, was recrystallized repeatedly from dilute methanol, m.p. 198–199° dec. It was dried over phosphorus pentoxide at 115° (0.2 mm.) for 8 hours.

(6) R. E. Cortell, *J. Clin. Endocrinol.*, **9**, 955 (1949).

(7) All melting points are corrected. Microanalyses by Miss May Lai, and Weiler and Strauss Laboratories, Oxford.

(8) This compound has been mentioned, without amplification, by W. Schoeller and K. Schmidt, U. S. Patent 1,693,055 (1929); *C. A.*, **23**, 1216 (1929).

(9) F. Reverdin, *Ber.*, **29**, 997 (1896) gave m.p. 74–75° but did not report an analysis for this amine. We found m.p. 76–77°. *Anal.* Calcd. for C_7H_8INO: C, 33.75; H, 3.24. Found: C, 33.84; H, 3.26.

(10) E. Repossi, *Z. Kryst. Min.*, **55**, 287 (1912–1913).

(11) P. Block and G. Powell, THIS JOURNAL, **64**, 1070 (1942).

TABLE I

DERIVATIVES OF DIPHENYL ETHER

Derivative[a]	Yield, %	M.p., °C. (cor.)	Solvent of crystallization	Composition	Analyses, % Calcd. C	Calcd. H	Found C	Found H
2-Iodo-4-nitro-4'-methoxy-[b]	90	70–71	AcOH or EtOH	$C_{13}H_{10}INO_4$	42.07	2.71	42.05	2.66
2,3'-Diiodo-4-nitro-4'-methoxy-[b]	80	139–140	AcOH or MeCOEt	$C_{13}H_9I_2NO_4$	31.41	1.83	31.65	1.94
2-Iodo-4-nitro-6-bromo-4'-methoxy-[c]	84	141.5–142	MeCOEt	$C_{13}H_9BrINO_4$	34.69	2.02	34.97	2.07
2-Iodo-4-amino-4'-methoxy-Hydrochloride	80	(sint., 194) 202–210 dec.	EtOH–H_2O(HCl)	$C_{13}H_{13}ClINO_2$	41.35	3.47	41.25	3.50
2,3'-Diiodo-4-amino-4'-methoxy-		121–123	EtOH	$C_{13}H_{11}I_2NO_2$	33.43	2.37	33.38	2.61
Hydrochloride	90	233–236 dec.	EtOH	$C_{13}H_{12}ClINO_2$	31.01	2.40	31.15	2.62
2-Iodo-4-amino-6-bromo-4'-methoxy-	90[d]	113.5–114	EtOH	$C_{13}H_{11}BrINO_2$	37.17	2.64	37.39	2.64
2-Iodo-4-cyano-4'-methoxy-[b]	36	115–116	Et_2O	$C_{14}H_{10}INO_2$	47.88	2.87	47.79	2.94
2,3'-Diiodo-4-cyano-4'-methoxy-	60	161–162	MeCOEt	$C_{14}H_9I_2NO_2$	35.25	1.90	35.09	1.95
2-Iodo-4-cyano-6-bromo-4'-methoxy-	51	142–143	MeCOEt	$C_{14}H_9BrINO_2$	39.10	2.11	39.15	2.11
2-Iodo-4-formyl-4'-methoxy-	68	81–83	EtOH–H_2O	$C_{14}H_{11}IO_3$	47.48	3.13	47.44	3.10
2,3'-Diiodo-4-formyl-4'-methoxy-	70	147–149	AcOH–H_2O	$C_{14}H_{10}I_2O_3$	35.02	2.10	35.09	2.03
2-Iodo-4-formyl-6-bromo-4'-methoxy-	70	98–100	EtOH, AcOH, or petroleum ether	$C_{14}H_{10}BrIO_3$	38.82	2.33	39.10	2.11
4-[3-Iodo-4-(4'-methoxyphenoxy)-benzal]-2-phenyl-5-oxazolone[e]	100	157–159	AcOH	$C_{23}H_{16}INO_4$	55.55	3.24	55.45	3.25
4-[3-Iodo-4-(3'-iodo-4'-methoxy-phenoxy)-benzal]-2-phenyl-5-oxazolone[f]	70	203–203.5	AcOH	$C_{23}H_{15}I_2NO_4$	44.32	2.43	44.15	2.48
4-[3-Bromo-5-iodo-4-(4'-methoxy-phenoxy)benzal]-2-phenyl-5-oxazolone[e]	99	199–200	AcOH	$C_{23}H_{15}BrINO_4$	47.94	2.62	48.06	2.65
α-Benzamido-3-iodo-4-(4'-methoxyphenoxy)-cinnamic acid	65	216–218	AcOH	$C_{23}H_{18}INO_5$	53.61	3.52	53.56	3.62
α-Benzamido-3-iodo-4-(3'-iodo-4'-methoxyphenoxy)-cinnamic acid	90	223–224	AcOH, MeCOEt	$C_{23}H_{17}I_2NO_5$	43.08	2.67	42.79	2.93
α-Benzamido-3-bromo-5-iodo-4-(4'-methoxyphenoxy)-cinnamic acid	98	246–248	AcOH, MeCOEt	$C_{23}H_{17}BrINO_5$	46.49	2.88	46.69	2.92
DL-3-Iodothyronine[g]	45	236–238	Me_2CO–H_2O	$C_{15}H_{14}INO_4$	45.12	3.53	44.94	3.63
DL-3-Iodothyronine[h]	64	236–238	Me_2CO–H_2O	$C_{15}H_{14}INO_4$	45.12	3.53	44.85	3.57
DL-3-Bromo-5-iodothyronine	83	248–250	[i]	$C_{15}H_{13}BrINO_4$	37.68	2.74	37.25	2.80

[a] Colorless unless otherwise noted. [b] Pale yellow. [c] Bright yellow. [d] The hydrochloride, isolated from the reaction, melted at 186–192°, and lost hydrogen chloride on crystallization from ethanol. [e] Orange yellow. [f] Pale orange needles. [g] Obtained by direct synthesis. [h] From the deiodination in the reductive cleavage of α-benzamido-3-iodo-4-(3'-iodo-4'-methoxyphenoxy)-cinnamic acid. [i] Reprecipitated from dilute acid solution with ammonium hydroxide.

Anal. Calcd. for $C_{15}H_{13}I_2NO_4 \cdot 2H_2O$: C, 32.11; H, 3.06; I, 45.24. Found: C, 31.96; H, 3.03; I, 44.8.

In butanol–6 *N* ammonium hydroxide, R_f was 0.56; in isoamyl alcohol–6 *N* ammonium hydroxide, R_f 0.35.

DL-3,3'-Diiodo-5-bromothyronine.—A solution of 154.2 mg. of iodine in 25 ml. of ethanol was added gradually to a stirred solution of 240 mg. of 3-bromo-5-iodothyronine in 250 ml. of 28% ammonium hydroxide. After standing at 25° for two hours, the solution was concentrated to 50 ml. under reduced pressure and filtered. The amino acid was reprecipitated from acid solution with ammonium hydroxide and recrystallized from 50% aqueous methanol. The colorless product melted at 213–215° dec. and weighed 0.2 g.

Anal. Calcd. for $C_{15}H_{12}BrI_2NO_4$: C, 29.82; H, 2.00. Found: C, 30.01; H, 2.33.

The compound was indistinguishable chromatographically from 3,3',5-triiodothyronine. The R_f values for 3,3'-diiodo-5-bromothyronine were 0.87 (butanol–6 *N* ammonium hydroxide), and 0.59 (isoamyl alcohol–6 *N* ammonium hydroxide). For 3,3',5-triiodothyronine, R_f was 0.86 and 0.59, respectively.

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