Thyroxine Analogs. VIII.[1] 3-Methyl- and 3,5-Dimethyl-DL-thyronines and Iodinated Derivatives

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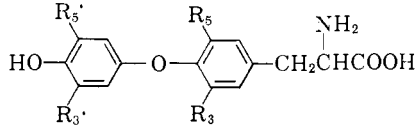
3-Methyl-, 3-methyl-5-iodo-, and 3,5-dimethyl-DL-thyronines, and their 3′-iodo derivatives have been prepared. Several showed thyroxine-like activity. 3,5,-3′,5′-Tetramethyl-DL-thyronine showed weak thyroxine-like activity, as well as antagonistic activity when administered together with thyroxine.

It has been established that methyl groups may substitute for iodine atoms in the phenolic ring of the naturally occurring thyroid hormones[1,3,4] (Ia, b) and in the phenolic ring of their propionic acid side chain analogs.[3,5,6] However, 3,5,3′,5′-tetramethyl-DL-thyro-



Ia, Thyroxine; $R_3 = R_5 = R_{3'} = R_{5'} = I$
Ib, Triiodothyronine; $R_3 = R_5 = R_{3'} = I$; $R_{5'} = H$
Ic, Tetramethylthyronine; $R_3 = R_5 = R_{3'} = R_{5'} = CH_3$

nine[7] (Ic), in which iodine atoms of both rings have been replaced by methyl groups, has been reported[3] to show no thyroxine-like activity at the levels tested. Thyroxine analogs bearing the formic acid side chain and variously substituted with methyl groups in the 3,5,3′,5′-positions have been described.[8] Within this series, iodine in the 3- and 5-positions was found to be essential for the thyroxine-like effect of lowering levels of serum and liver cholesterol.[8a] In the same test,

(1) Paper VII, E. C. Jorgensen, P. A. Lehman, C. Greenberg, and N. Zenker, *J. Biol. Chem.*, in press.

(2) In partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of California, 1962. Fellow of the American Foundation for Pharmaceutical Education, 1959–1962. Recipient of a Lunsford Richardson Pharmacy Award, 1962.

(3) C. S. Pittman, H. Shida, and S. B. Barker, *Endocrinology*, **68**, 248 (1961).

(4) T. C. Bruice, R. J. Winzler, and N. Kharasch, *J. Biol. Chem.*, **210**, 1 (1954).

(5) P. C. J. Roth, *Ann. Endocr., Paris*, **19**, 1157 (1958).

(6) N. R. Stasilli, R. L. Kroc, and R. I. Meltzer, *Endocrinology*, **64**, 62 (1959).

(7) H. J. Bielig and G. Lützel, *Ann.*, **608**, 140 (1957).

(8) E. Van Heyningen, *J. Org. Chem.*, **26**, 3850 (1961).

(8a) R. G. Hermann, C. C. Lee, and R. Parker, *Arch. Int. Pharm. Ther.*, **133**, 284 (1961).

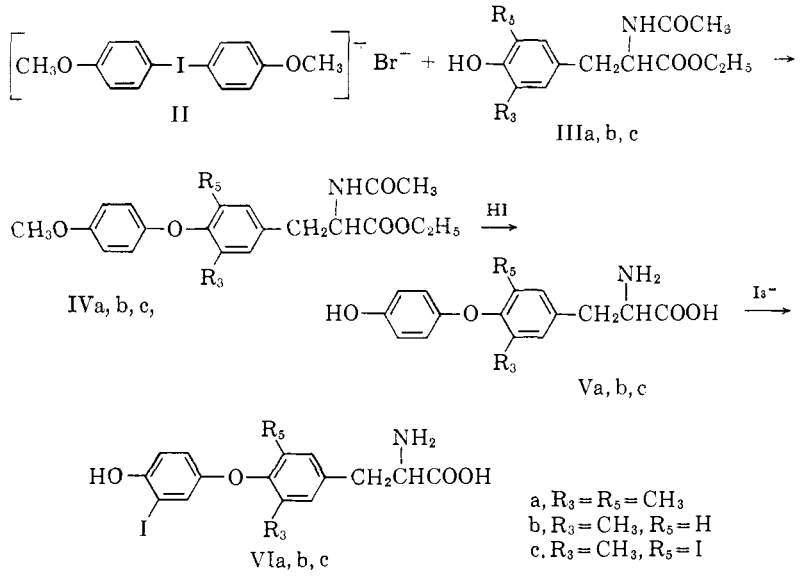
tetramethylthyronine showed no reproducible activity. Thus presently available evidence supports an activating effect for methyl groups in the phenolic ring, but no such effect in the alanine-bearing ring.

As a part of a study of substituent requirements in the alanine-bearing ring of thyroxine analogs, we have prepared a series of thyronine derivatives with various combinations of methyl groups and iodine atoms in the 3-, 5-, and 3′-positions. These, together with 3,5,3′,5′-tetramethyl-DL-thyronine, have been tested for thyroxine-like activity in the rat antigoiter assay.

Thyronine derivatives with a single iodine substituent on the alanine-bearing ring (3-iodo-, 3,3′-diiodo-, 3,3′,5′-triiodo-DL-thyronine) have shown thyroxine-antagonistic properties in a variety of biological tests.[9,10] It was, therefore, of interest to test those analogs possessing only the 3-methyl group on the alanine-bearing ring for antagonistic effects. Because of the low thyroxine-like activity found in tests on 3,5,3′,5′-tetramethyl-DL-thyronine, this was also tested as an antagonist.

Synthesis.—Hillman[11,12] has described a synthesis of 3,5-diiodo-L-thyronine by the reaction of 4,4′-dimethoxydiphenyliodonium bromide (II) with N-acetyl-3,5-diiodo-L-tyrosine ethyl ester (III, $R_3 = R_5 = I$) in methanol at reflux temperature and in the presence of magnesium methoxide. Bevilacqua, *et al.*[13] have carried out the same reaction at room temperature in the presence of triethylamine and copper. Several attempts to condense the iodonium bromide (II) with N-acetyl-3,5-dimethyl-DL-tyrosine ethyl ester (IIIa) under the conditions described by Bevilacqua were unsuccessful, although it was possible to repeat the reaction with the 3,5-diiodotyrosine derivative in over 60% yield. Model reactions with 2,6-dimethylphenol and 4,4′-dimethoxydiphenyliodonium bromide were successful using potassium *tert*-butoxide in *tert*-butanol at reflux temperature. These conditions were used in condensations with N-acetyl-3,5-dimethyl-DL-tyrosine ethyl ester (IIIa), and the corresponding 3-methyl (IIIb) and 3-methyl-5-iodo (IIIc) derivatives. The resulting N-acetyl-O-methyl-DL-thyronine ethyl esters (IVa–c) could not be obtained in the crystalline state, and were hydrolyzed to the amino acids (Va–c).

(9) C. S. Pittman and S. B. Barker, *Am. J. Physiol.*, **197**, 1271 (1959).
(10) S. B. Barker, C. S. Pittman, J. A. Pittman, Jr., and S. R. Hill, Jr., *Ann. N. Y. Acad. Sci.*, **86**, 545 (1960).
(11) G. Hillmann, *Z. Naturforsch.*, **11b**, 419 (1956).
(12) G. Hillmann, U. S. Patent 2,886,592 (May 12, 1959).
(13) P. F. Bevilacqua, J. T. Plati, and W. Wenner, U. S. Patent 2,895,927 (July 21, 1959).



Following completion of this work, Dibbo, *et al.*[14] reported the synthesis of 3,5-dimethyl-DL-thyronine (Va) by a similar method from N-acetyl-3,5-dimethyl-DL-tyrosine.

Iodination of the thyronines (Va–c) using 90–100% of the calculated amount of iodine for monoiodination resulted in mixtures containing some diiodinated thyronine as shown by paper chromatography. Repeated isoelectric precipitations were required to produce pure samples of the desired 3′-iodothyronines (VIa–c).

Ultraviolet spectra in both alkali and acid showed the expected shift of absorption maxima to longer wave lengths following introduction of the 3′-iodo substituent. Analytical data and physical constants for the compounds are presented in Table I.

Biological Results and Discussion.—The substituted thyronines (Ic, Va-c, VIa-c) were tested for thyroxine-like activity by the rat antigoiter procedure as described previously.[15] The data obtained and estimates of potency relative to L-thyroxine are presented in Table II.

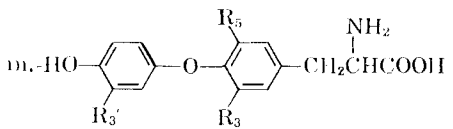
It is clear from these data that the methyl group, when present as a substituent in the alanine-bearing ring of the thyronine nucleus, effects an enhancement in thyromimetic potency with respect to the corresponding desmethyl compound. Compare, for example, 3,3′-

(14) A. Dibbo, L. Stephenson, T. Walker, and W. K. Warburton, *J. Chem. Soc.*, 2645 (1961).

(15) E. C. Jorgensen and P. Slade, *J. Med. Pharm. Chem.*, **5**, 729 (1962).

TABLE I

METHYL-SUBSTITUTED THYRONINES



Compound							Analyses,[f] %					Ultraviolet spectra[h]			
							Carbon		Hydrogen			Alkali		Acid	
No.	R_3	R_5	R_3'	M.p., °C.[a]	Yield %	Formula	Calcd.	Found	Calcd.	Found	R_f[g]	λmax., mμ	ε × 10⁻³	λmax., mμ	ε × 10⁻³
Va	CH_3	CH_3	H	217–220	8	$C_{17}H_{19}NO_4$[b]	63.93	64.24	6.63	6.69	0.79	296	5.15	280	3.79
Vb	CH_3	H	H	242–244	20	$C_{16}H_{17}NO_4$	66.88	66.44	5.97	6.02	0.76	303	2.83	275	2.54
														282(s)	2.49
Vc	CH_3	I	H	223–224	20	$C_{16}H_{16}INO_4$	46.50	46.2	3.90	4.1	0.70	304	3.38	284	3.03
VIa	CH_3	CH_3	I	212–214	70	$C_{17}H_{18}INO_4$[b,c]	45.87	45.4	4.53	4.5	0.65	317	4.81	295	4.09
VIb	CH_3	H	I	216	22	$C_{16}H_{16}INO_4$[d]	46.50	46.39	3.90	3.62	0.67	314	4.39	293	3.48
VIc	CH_3	I	I	209	66	$C_{16}H_{15}I_2NO_4$[e]	35.64	35.59	2.80	3.10	0.50	318	4.53	295	3.73

[a] Melting points were taken in capillary tubes in an oil-bath and are uncorrected. All compounds melted with decomposition. [b] Monohydrate. [c] Calcd.: I, 28.50. Found: I, 27.9. [d] Calcd.: I, 30.71. Found: I, 30.42. [e] Calcd.: I, 47.08. Found: I, 46.68. [f] Microanalyses by The Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley. [g] Chromatography on Whatman No. 1 paper, formic acid–water, 1:5. See Experimental Section. [h] Determined on a Cary Model 11 recording spectrophotometer at a concentration of 10 mg. per 100 ml. in 0.05 *N* aqueous sodium hydroxide. Acid spectra were obtained by acidifying the alkaline solutions in the spectrophotometer cells with five drops of 6 *N* hydrochloric acid. Extinction coefficients for the acid solutions are therefore approximate.

diiodo-5-methyl-DL-thyronine (VIc, 20% L-thyroxine) with 3,3′-diiodo-L-thyronine (<3% L-thyroxine[6]; 0.5% L-thyroxine[16]; 4% DL-thyroxine for the DL-analog,[17,18] all in the rat antigoiter assay). Enhancement is also seen for 3′-iodo-3-methyl-DL-thyronine (VIb, 0.7% L-thyroxine) and for 3,5-dimethyl-3′-iodo-DL-thyronine (VIa, 2.7% L-thyroxine) when compared with 3′-iodo-DL-thyronine (inactive in the rat antigoiter assay).[16,19,20] When tested at higher dose levels than previously employed,[3] 3,5,3′,5′-tetramethyl-DL-thyronine showed a detectable level (0.3% L-thyroxine) of thyromimetic activity. This may be compared with the inactivity reported for the unsubstituted thyronine.[21] These methyl-substituted compounds are the first analogs of thyroxine with non-halogen substituents on the alanine-bearing ring to show significant thyroxine-like activity.

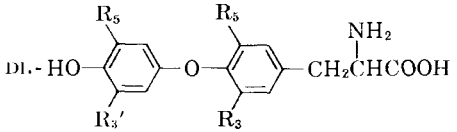
The methyl-substituted analogs are considerably less active than are the corresponding iodo- or bromo-thyronine derivatives. Thus, 3,3′-diiodo-5-methyl-DL-thyronine (VIc, 20% L-thyroxine) shows only 4% of the thyromimetic potency of 3,5,3′-triiodo-L-thyronine (Ib, 500% L-thyroxine[22]). 3,5-Dibromo-3′-iodo-DL-thyronine has been reported[23] to possess 130% of the activity of DL-thyroxine, while the corresponding 3,5-dimethyl analog (VIa) shows only 3% of L-thyroxine activity in the same assay method. Data on the corresponding chlorine analogs are not available. However, 3,5,3′,5′-tetrachloro-DL-thyronine has been reported[22,23] to show 0–0.2% the activity of L-thyroxine. The activity of about 0.3% L-thyroxine for the tetramethyl derivative (Ic) implies that the methyl group is either of the same order of effectiveness, or slightly more effective than chlorine. However, this cannot be established until compounds differing only in the alanine-bearing ring may be compared.

In providing activation of the thyromimetic effect when substituted in the 3- or 5-position of the thyronine nucleus, the order of effectiveness for substituent groups appears to be: $I > Br > Me \geq Cl > H$.

Five compounds in the series were tested for their ability to act as thyroxine antagonists by concomitant administration of thiouracil, L-thyroxine, and test compound. The weakly thyromimetic 3,5,3′,5′-tetramethyl-DL-thyronine (Ic) also proved an effective thyroxine

(16) E. G. Tonich, E. A. Wollett, and M. A. Pratt, *J. Endocrinology*, **20**, 65 (1960).
(17) C. L. Gemmill, J. J. Anderson, and A. Burger, *J. Am. Chem. Soc.*, **78**, 2434 (1956).
(18) C. L. Gemmill, *Amer. J. Physiol.*, **186**, 1 (1956).
(19) J. Roche, R. Michel, and W. Wolf, *Bull. Soc. Chim. France*, 462 (1957).
(20) E. C. Jorgensen and K. Tsutsui, *Endocrinology*, **68**, 171 (1961).
(21) H. A. Selenkow and S. P. Asper, Jr., *Physiol. Rev.*, **35**, 426 (1955).
(22) R. Pitt-Rivers and J. R. Tata, "The Thyroid Hormones," Pergamon Press, London, 1959.
(23) M. V. Mussett and R. Pitt-Rivers, *Metabolism*, **6**, 18 (1957).

TABLE II
RAT ANTIGOITER ASSAY[a]



Assay no.	Food	Thyroxine,[c] dose mcg. per 100 g.	Compound No.	R_5	R_5	R_3'	R_5'	Molar ratio	Thyroid weight per 100 g. mg.	± s.d.	Thyromimetic activity, % L-thyroxine
1	Untreated								5.56	0.74	
	TU[b]								21.98	5.93	
	TU	2.0						0.67	11.16	4.12	
	TU	3.0						1.0	8.46	3.23	
	TU	4.5						1.5	4.55	0.70	
	TU		Ic	CH_3	CH_3	CH_3	CH_3	100	16.44	3.55	0.3[d]
2	Untreated								7.0	1.20	
	TU								22.5	4.67	
	TU	2.0						0.67	13.3	4.96	
	TU	3.0						1.0	11.6	6.21	
	TU	4.5						1.5	6.5	1.36	
	TU		Vc	CH_3	I	H	H	100	18.8	6.63	<1
	TU		VIc	CH_3	I	I	H	100	3.7	1.19	>1.5[e]
3	Untreated								6.57	0.75	
	TU								20.24	3.91	
	TU	2.0						0.67	14.42	3.48	
	TU	3.0						1.0	10.33	3.99	
	TU	4.5						1.5	6.09	2.95	
	TU		VIc	CH_3	I	I	H	25	3.95	0.86	>6[e]
	TU		VIa	CH_3	CH_3	I	H	100	4.35	0.65	>1.5[e]
	TU	3.0	Ic	CH_3	CH_3	CH_3	CH_3	1.0 + 100	17.32	3.45	Antag.[f]

4	Untreated								8.22	1.62	
	TU								29.8	4.45	
	TU	2.0						0.67	18.2	5.21	
	TU	3.0						1.0	10.8	3.61	
	TU	4.5						1.5	9.16	3.86	
	TU		VIc	CH_3	I	I	H	5.0	13.8	7.18	20.0[e]
	TU		VIc	CH_3	I	I	H	1.0	31.8	4.93	
	TU		VIa	CH_3	CH_3	I	H	25	17.7	6.35	2.7[e]
	TU		VIb	CH_3	H	I	H	100	23.1	6.54	0.67[d]
	TU		Vc	CH_3	I	H	H	200	21.3	4.21	0.3[e]
	TU	3.0	Ic	CH_3	CH_3	CH_3	CH_3	1.0 + 50	12.3	4.20	Antag.[g]
	TU	3.0	Vb	CH_3	H	H	H	1.0 + 200	11.4	3.52	Antag.[g]
	TU	3.0	VIb	CH_3	H	I	H	1.0 + 200	13.0	2.94	Antag.[g]
5	Untreated								7.55	0.902	
	TU								24.17	6.28	
	TU	2.0						0.67	19.53	4.83	
	TU	3.0						1.0	9.60	3.66	
	TU	4.5						1.5	6.38	2.24	
	TU	3.0	Va	CH_3	CH_3	H	H	1.0 + 200	9.05[h]	3.78	Antag.[g]
	TU	3.0	Vc	CH_3	I	H	H	1.0 + 100	8.77	1.88	Antag.[g]

[a] Analysis of variance showed control responses varied from assay to assay, making it necessary to reproduce the data in full. Six rats were used at each control and dose level. [b] Thiouracil, 0.3%. [c] Sodium L-thyroxine pentahydrate. [d] Confidence level that treated animals have significantly different thyroid gland weights than controls >90%. [e] Confidence level >99%. [f] 70.5% Reversal of thyroxine effect. [g] Reversal of thyroxine effect not significant. [h] Group of 3 rats.

antagonist, producing a 70% reversal ($P < 0.01$) of the thyroxine effect at a molar ratio of 100 to 1 (Table II, assay 3). The slight reversal produced by the tetramethyl analog at a molar ratio to L-thyroxine of 50 to 1 was not statistically significant, nor was that produced by 3′-iodo-3-methyl-DL-thyronine (VIb), 3-methyl-DL-thyronine (Vb), or 3,5-dimethyl-DL-thyronine (Va) at molar ratios to L-thyroxine of 200 to 1 (Table II, assays 4 and 5). Also inactive as a thyroxine antagonist was 5-iodo-3-methyl-DL-thyronine (Vc), tested at a molar ratio of 100:1 (Table II, assay 5).

Experimental

3- or 3,5-Substituted-DL-thyronines (Va–c).—Potassium (5 mmoles) was dissolved in 100 ml. of refluxing anhydrous *tert*-butyl alcohol. The 3- or 3,5-substituted N-acetyl-DL-tyrosine ethyl ester[24] (IIIa–c, 5 mmoles) was added and the solution stirred for 2 min. 4,4′-Dimethoxydiphenyliodonium bromide[25] (II, 10 mmoles) was added and the mixture heated under reflux and stirred vigorously for 18 to 24 hr. Insoluble material was removed by filtration, the filtrate was evaporated to dryness *in vacuo*, and the residue was shaken for 3 min. with 100 ml. of chloroform and 150 ml. of 3% aqueous hydrochloric acid. The chloroform layer was washed successively with water, 1 *N* sodium hydroxide, and water, dried over sodium sulfate, and the chloroform removed *in vacuo*. The residue was dissolved in a minimum volume of warm benzene and chromatographed on 50 g. of acid-washed alumina. Fractions were eluted with successive 200 ml. portions of benzene, 50% chloroform-benzene, chloroform, and methanol. The residue from the chloroform (Va) or chloroform-benzene (Vb,c) fractions was heated under reflux for 4 hr. with 10 ml. of glacial acetic acid and 15 ml. of 47% hydriodic acid. The acids were removed by distillation at reduced pressure, the residue was taken up in a small amount of hot water and the pH of the solution adjusted to 5 with 40% aqueous sodium hydroxide. The resulting precipitate was collected and purified by several isoelectric precipitations at pH 5. Analytical samples were dried over P_2O_5 at 100° (1 mm.) for at least 24 hr. In all cases, it was possible to obtain a small additional quantity of the thyronine derivative by hydrolysis of the fraction eluted from the alumina column by methanol. Analytical data and physical constants are listed in Table I.

3′-Iodo-DL-thyronines (VIa–c).—To the preceding thyronines (Va–c, 0.4–0.8 mmole) dissolved in 10 ml. of 33% aqueous ethylamine was quickly added 90–100% of the calculated quantity of iodine as a 1 *N* solution in aqueous potassium iodide. The addition was carried out at 5–10° with vigorous stirring, and stirring was continued for 10 min. after the addition was complete. The pH of the solution was then adjusted to 5 with concd. hydrochloric acid, the resulting precipitate collected and purified by several isoelectric precipitations from 50% aqueous ethanol at pH 5. Analytical samples were dried over P_2O_5 for 8 hr. at 60° (1 mm.); drying at 110° resulted in loss of iodine. Homogeneity of all materials was verified by paper chromatography. Analytical data and physical constants are listed in Table I.

(24) E. C. Jorgensen and R. A. Wiley, *J. Pharm. Sci.*, in press.
(25) J. T. Plati, U. S. Patent 2,839,583 (June 17, 1958).

Paper Chromatography.—One mg. samples were dissolved in 1 ml. of ethanol-2 *N* ammonium hydroxide (3:1 v./v.), and diluted to 10 ml. with distilled water. The spots (1–3 mcg., 10–30 μl.) were applied with a Hamilton syringe to Whatman No. 1 paper which had been previously washed with the solvent system to be used. Descending chromatograms were equilibrated for 2 hr. and run for 2.5–3 hr. in formic acid–water (1:5 v./v.).[26] The chromatograms were dried, then with all operations in the dark, sprayed with the "FFCA" reagent of Gmelin and Virtanen,[27] allowed to develop for 2.5–3 min., washed with dil. hydrochloric acid and with water, and dried. It was found that this method was easily sensitive to 5% of 3′,5′-diiodo-3-methyl-DL-thyronine (R_f 0.50) as impurity in samples of 3′-iodo-3-methyl-DL-thyronine (VIc, R_f 0.67), or to a similar quantity of 3′,5′-diiodo-3,5-dimethyl-DL-thyronine (R_f 0.39) as impurity in 3,5-dimethyl-3′-iodo-DL-thyronine (VIa, R_f 0.65).

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(26) F. Bjorksten, R. Grasbeck, and B. A. Lamberg, *Acta Chem. Scand.*, **15**, 1165 (1961).
(27) R. Gmelin and A. I. Virtanen, *Acta Chem. Scand.*, **13**, 1469 (1959).