443. *The Synthesis of Thyroxine and Related Substances. Part X.* A Synthesis of* D-*Thyroxine.*

By J. Elks and G. J. Waller.

Neither 3 : 5-dinitro- (I; R = NO_2, R′ = NH_2) nor 3 : 5-di-iodo-4-*p*-methoxyphenoxy-L-phenylalanine (I; R = I, R′ = NH_2) could be converted into the corresponding α-halogeno-acid (R′ = Cl or Br) by treatment with the nitrosyl halide. However, 3 : 5-dinitro-L-tyrosine reacted with nitrosyl bromide to give L-α-bromo-β-(4-hydroxy-3 : 5-dinitrophenyl)propionic acid (II) which underwent inversion on treatment with ammonia and yielded 3 : 5-dinitro-D-tyrosine. This was converted into D-thyroxine by the method described in Part V of this series (*J.*, 1949, 3424) for the L-isomer.

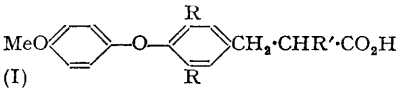
The unnatural D-isomer of thyroxine has been described twice in the literature. Harington (*Biochem. J.*, 1928, **22**, 1429) prepared it by iodination of 3 : 5-di-iodo-D-thyronine, which had itself been obtained by resolution of the synthetic racemic compound through the D-1-phenylethylamine salt of its *N*-formyl derivative. More recently Pitt-Rivers and Lerman (*J. Endocrin.*, 1948, **5**, 223) prepared the same compound in small yield by inversion of L-tyrosine, conversion of the product into 3 : 5-di-iodo-D-tyrosine, and oxidation of this compound with hydrogen peroxide under mild conditions. Neither of these processes lends itself to the preparation of D-thyroxine on any but a small scale, and so its physiological properties have not been studied in detail. Indeed, estimates of its activity by various workers using different test animals range from zero to that of L-thyroxine. The earlier work is reviewed by Pitt-Rivers and Lerman (*loc. cit.*), who found that D-thyroxine had one-eighth to one-tenth of the activity of the L-isomer when tested on myxœdematous patients. Subsequently Griesbach, Kennedy, and Purves (*Endocrinology*, 1949, **44**, 445), who used a very small number of rats, gave the activity of D-thyroxine as one-third of that of the L-isomer.

It seemed probable that D-thyroxine might be made available in reasonable amount by extension of the method already described for the synthesis of the L-isomer from L-tyrosine (Part V, *J.*, 1949, 3424), either by application of the method to D-tyrosine or, preferably, by inversion of a suitable intermediate at a later stage of the synthesis.

Owing to their very sparing solubility, neither 3 : 5-di-iodo-4-*p*-methoxyphenoxy-L-phenylalanine (I; R = I, R′ = NH_2) nor the corresponding dinitro-compound (Part V, *loc. cit.*) could be converted into (I; R = I or NO_2, R′ = Br) by means of sodium nitrite in aqueous hydrobromic acid; even when some acetic acid was added the amino-acids remained largely undissolved and were apparently unattacked. Treatment of the amino-acid (I; R = I, R′ = NH_2) with a mixture of hydrobromic and nitric acids in the presence of urea (Karrer, Reschofsky, and Kaase, *Helv. Chim. Acta*, 1947, **30**, 271) resulted in a vigorous reaction and a crude acid was isolated that gave no colour on treatment with ninhydrin but could not be purified either by crystallisation or by chromatography of its methyl ester. Similar intractable materials resulted from the addition of nitrosyl chloride to a suspension of the amino-acid in acetic acid or chloroform.

* Part IX, *J.*, 1952, 827.

The methyl ester of (I; R = I, R′ = NH_2) was similarly resistant to reaction with nitrite when suspended in ethanolic hydrochloric acid, its hydrochloride being recovered unchanged. Attention was therefore turned to the inversion of dinitro-L-tyrosine. This had the advantage over tyrosine itself that, since the positions *ortho* to the phenolic hydroxyl



(I)

group are blocked, it should not be necessary to form an ether before treatment with nitrite, and several stages might thus be eliminated.

The treatment of 3 : 5-dinitro-L-tyrosine in sulphuric acid solution with potassium bromide and sodium nitrite (cf. Pitt-Rivers and Lerman, *loc. cit.*) gave a satisfactory yield of L-α-bromo-β-(4-hydroxy-3 : 5-dinitrophenyl)propionic acid (II). In one large-scale experiment a very small quantity of a second product was obtained, which had the same analysis as (II) but melted at 158—162° instead of 130—132°. It was optically inactive and was shown to be the racemic form of (II) by comparison with a specimen prepared from 3 : 5-dinitro-DL-tyrosine.

The L-isomer of (II) was treated with aqueous ammonia at room temperature and the crude product was converted into D-5-(4-hydroxy-3 : 5-dinitrobenzyl)hydantoin (V) by means of sodium cyanate. This compound had $[\alpha]_D$ + 63·0°, which agrees well with the specific rotation (−61·6°) quoted for the L-isomer (Part V, *loc. cit.*). Since the inversion had, then, been successful, the ammonolysis was repeated and the crude 3 : 5-dinitro-D-tyrosine (IV) was acetylated to give, in rather poor yield, *N*-acetyl-3 : 5-dinitro-D-tyrosine (VI). The yield was not increased when a solution of ammonia saturated at 0° was used for the ammonolysis. Further investigation showed that the low yield of the *N*-acetyl compound was due in part to dehydrobromination during the ammonolysis, 4-hydroxy-3 : 5-dinitrocinnamic acid (III) being isolated as a by-product. More important, however, was the fact that under the conditions used (excess of acetic anhydride in the presence of sodium hydrogen carbonate) acetylation was incomplete and some unchanged amino-acid could be isolated. By acetylation of this material the yield of (VI) was increased to 51%. The melting point and rotation of (VI), as well as those of the other intermediates, are shown in the Table alongside those of the corresponding L-compounds.

	D-Form		L-Form *	
Compound	M. p.	$[\alpha]_D$	M. p.	$[\alpha]_D$
V	255—256° †	+63·0° (acetone)	249—250° †	−61·6° (acetone)
VI	192—194	−12·7 (dioxan)	189—190	+12·2 (dioxan)
VII	119—121	+ 6·7 (dioxan)	120—121	− 6·75 (dioxan)
VIII	109—110	+ 8·2 (dioxan)	109—110	− 8·2 (dioxan)
IX	132—136	−43·4 (dioxan)	135—136	+42·4 (dioxan)
X	143—145	−30·5 ± 0·15 (dioxan)	143—144	+30·5 ± 0·15 (dioxan)
XI	265 †	−27·1 (N-HCl & EtOH)	255 †	+26 (N-HCl & EtOH)
XII	229—230 †	+ 5·1 (N-NaOH & EtOH) −17·5 (N-HCl & EtOH)	233—235 †	− 5·7 (N-NaOH & EtOH) +17·8 (N-HCl & EtOH)

* The specific rotations for the L-compounds are taken from Part V (*loc. cit.*) with the exception of those for compound (X), which was determined on a specially purified specimen, and for (XII) in acid solution, which has not previously been reported.

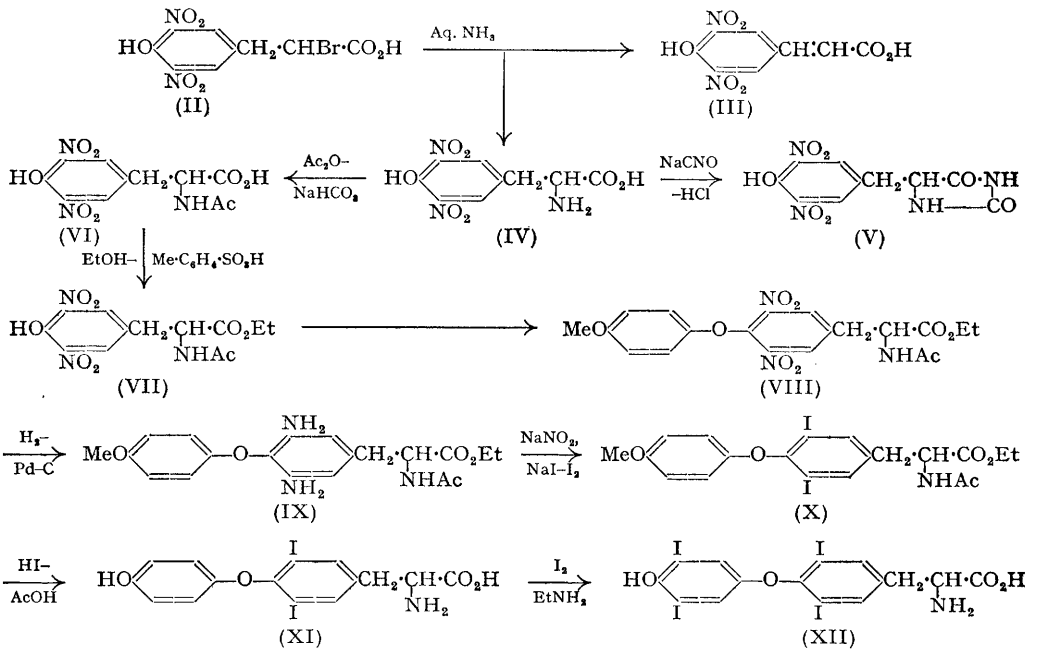
† With decomposition.

The subsequent reactions were the same as those used for the L-series (Part V, *loc. cit.*). The acetamido-acid (VI) was esterified by an azeotropic method and the ester (VII) was converted into *N*-acetyl-4-*p*-methoxyphenoxy-3 : 5-dinitro-D-phenylalanine ethyl ester (VIII) by successive treatment with toluene-*p*-sulphonyl chloride and *p*-methoxyphenol in pyridine.

The dinitro-compound (VIII) was converted *via* the diamine (IX) into *N*-acetyl-3 : 5-di-iodo-4-*p*-methoxyphenoxy-D-phenylalanine ethyl ester (X). This compound appeared to be particularly suitable for an accurate assessment of optical purity. It can be obtained very pure without difficulty and it has a moderately high specific rotation in dioxan, in which solvent colourless solutions can be obtained of quite high concentration. In addition,

our experience in the L-series would indicate that no racemisation is to be expected in the conversion of (X) into D-thyroxine (XII). Samples of both the L- and the D-isomer of (X) were crystallised to constant rotation and, as shown in the Table, the magnitudes of the specific rotations were identical.

Demethylation and hydrolysis of (X) to 3 : 5-di-iodo-D-thyronine (XI) was accomplished by means of a boiling mixture of hydriodic and acetic acids, and D-thyroxine (XII) was prepared by iodination of (XI) in ethylamine solution. The rotation of thyroxine has hitherto been measured in mixtures of aqueous sodium hydroxide and ethanol, in which the specific rotation (*ca.* −5° for L-thyroxine) is too low to permit accurate measurement. It has been found that in a mixture of aqueous hydrochloric acid and ethanol the rotation is



reversed in sign and increased in magnitude (+18° for L-thyroxine). A comparison of the rotation of our D-thyroxine with that of the L-isomer in such a solvent shows satisfactory agreement (see Table).

Experimental

3 : 5-*Di-iodo*-4-p-*methoxyphenoxy*-L-*phenylalanine Methyl Ester*.—3 : 5-Di-iodo-4-*p*-methoxyphenoxy-L-phenylalanine (Part V, *loc. cit.*) (10 g.) was boiled under reflux for 4 hours with methyl-alcoholic hydrogen chloride (12%; 100 c.c.). The solid did not dissolve but became noticeably more crystalline. After filtration and drying it melted at 238° (9·7 g., 89%). The *hydrochloride* separated from ethanol–ether as fine, white needles, m. p. 238° (decomp.) (Found: N, 2·3; OMe, 11·1. $C_{11}H_{17}O_4NClI_2$ requires N, 2·4; OMe, 10·5%).

The free base was prepared by dissolving the hydrochloride (2·0 g.) in a warm mixture of ethanol and water and adding the solution to 2N-sodium carbonate solution (1·8 c.c.) diluted with water and ice. After a short time the solid was filtered off and crystallised from aqueous alcohol. 3 : 5-*Di-iodo*-4-p-*methoxyphenoxy*-L-*phenylalanine methyl ester* (1·54 g., 82%) had m. p. 128—129°, unchanged after further crystallisation (Found: N, 2·55; I, 46·2. $C_{17}H_{17}O_4NI_2$ requires N, 2·5; I, 45·9%).

L-α-*Bromo*-β-(4-*hydroxy*-3 : 5-*dinitrophenyl*)*propionic Acid* (II).—A solution of 3 : 5-dinitro-L-tyrosine sodium salt (trihydrate; 34·7 g.) in warm 3N-sulphuric acid (400 c.c.) was cooled rapidly with shaking to precipitate the sulphate in a finely divided state. Potassium bromide (60 g.) was added and the mixture was cooled to −15° and stirred while a concentrated solution of sodium nitrite (20 g.) was added during 2½ hours, the original solid being replaced by a powdery yellow solid. The mixture was left overnight at room temperature, and the solid

(28·8 g., 86%) was filtered off, washed with a little water, and dried in a desiccator; it melted at 126—131°. L-α-*Bromo*-β-(4-*hydroxy*-3 : 5-*dinitrophenyl*)*propionic acid* separated from chloroform as yellow cubes, m. p. 134—136°, $[\alpha]_D^{20}$ −15·6° (*c*, 1·0 in ethanol) (Found: C, 32·15; H, 2·0; N, 8·4; Br, 24·4. $C_9H_7O_7N_2Br$ requires C, 32·3; H, 2·1; N, 8·4; Br, 23·85%). It crystallised less satisfactorily from water to give material of m. p. 66—67°. When this was dried in a vacuum desiccator the m. p. rose to that given above.

In one experiment on a large scale the chloroform mother-liquors from crystallisation of the bromo-acid gave, on concentration, a very small quantity of powdery yellow solid which, on crystallisation from aqueous methanol, melted at 158—162° (Found: N, 8·3; Br, 23·0%). It was optically inactive and was found by comparison with an authentic specimen, prepared as described below, to be the DL-*acid*.

DL-α-*Bromo*-β-(4-*hydroxy*-3 : 5-*dinitrophenyl*)*propionic Acid*.—This was prepared from 3 : 5-dinitro-DL-tyrosine sodium salt (2·2 g.) as described above for the L-isomer. The product (1·2 g., 56%) after crystallisation from aqueous methanol had m. p. 158—162° not depressed on admixture with a specimen of the material isolated as described above.

D-5-(4-*Hydroxy*-3 : 5-*dinitrobenzyl*)*hydantoin* (V).—L-α-Bromo-β-(4-hydroxy-3 : 5-dinitrophenyl)propionic acid (1 g.) was dissolved in ammonia (*d* 0·88; 20 c.c.) and the solution was left at room temperature for 4 days in a securely stoppered flask, then evaporated to dryness, and the residual red solid was dissolved in warm water (5 c.c.). Sodium cyanate (0·75 g.) was added, and the mixture boiled under reflux for 15 minutes; more sodium cyanate (0·4 g.) was added, and the mixture boiled for a further 30 minutes. The deep red solution was cooled and carefully acidified to Congo-red with concentrated hydrochloric acid. The solid was filtered off and heated under reflux for 30 minutes with 5N-hydrochloric acid (5 c.c.). After cooling, the yellow-brown solid was filtered off, washed with water, and dried. Crystallisation from alcohol with charcoal gave the *hydantoin* as bright yellow needles (0·24 g.), m. p. 255—256° (decomp.) (Found: C, 40·5; H, 2·8; N, 18·7. $C_{10}H_8O_7N_4$ requires C, 40·5; H, 2·7; N, 18·9%); $[\alpha]_D$ +63·0° (*c*, 0·84 in acetone).

N-*Acetyl*-3 : 5-*dinitro*-D-*tyrosine* (VI).—L-α-Bromo-β-(4-hydroxy-3 : 5-dinitrophenyl)propionic acid (11·5 g.) was dissolved in ammonia (*d* 0·88; 2300 c.c.) and left at room temperature in a securely stoppered flask for 5 days. Sodium hydroxide (10N; 69 c.c.) was added and the solution was concentrated under reduced pressure to half of its original volume, the temperature being kept below 40°; a red solid separated during the concentration. The last traces of ammonia were removed by bubbling air through the solution. Sodium hydrogen carbonate (46 g.) was added, followed by acetic anhydride (46 c.c.) added dropwise during 90 minutes to the stirred mixture which was kept below 25°. The stirring was continued for a further hour and the undissolved solid (A) was filtered off. The filtrate was acidified to Congo-red with 15% hydrochloric acid, giving an oil which solidified on standing in the refrigerator. It was filtered off, washed with water, and crystallised from water to give 32·5 g. of material, m. p. 188—190°; a second crop (6·3 g.) was obtained from the mother-liquors. After further crystallisation from water or ethyl acetate, N-*acetyl*-3 : 5-*dinitro*-D-*tyrosine* melted at 192—194°, $[\alpha]_D^{20}$ −12·7° (*c*, 1·0 in dioxan) (Found: C, 42·4; H, 3·5; N, 13·1. $C_{11}H_{11}O_8N_3$ requires C, 42·2; H, 3·5; N, 13·4%).

Solid A (31·5 g.) was dissolved in hot water and acidified to Congo-red with concentrated hydrochloric acid. On cooling, a yellow solid (4·5 g.) separated, having m. p. 203—205° (decomp.). After crystallisation from water, this 4-*hydroxy*-3 : 5-*dinitrocinnamic acid* melted at 204—207° (decomp.) (Found: C, 42·5; H, 2·5; N, 11·0. $C_9H_6O_7N_2$ requires C, 42·5; H, 2·4; N, 11·0%).

By concentration of the filtrate from the cinnamic acid to small bulk, a yellow solid (22·8 g.) was obtained which melted at 228° (decomp.) and is believed to have been 3 : 5-dinitro-D-tyrosine hydrochloride. On acetylation by addition of acetic anhydride (11·7 c.c.) to a stirred solution of the material in 2N-sodium hydroxide (183 c.c.), kept below 25° and stirred for an additional hour, a further quantity of the acetyl compound (15·7 g.) was obtained, identical with that described above; the total yield was 54·5 g. (51%).

An attempt, in another run, to carry the acetylation to completion in the first place by treatment with further quantities of sodium hydrogen carbonate and acetic anhydride was not successful.

N-*Acetyl*-3 : 5-*dinitro*-D-*tyrosine Ethyl Ester* (VII).—*N*-Acetyl-3 : 5-dinitro-D-tyrosine (33·3 g.) was esterified by the method described in Part V (*loc. cit.*) for the L-compound. The *ester* (35·4 g., 98%) melted at 119—121° and had $[\alpha]_D^{20}$ +6·7° (*c*, 6·0 in dioxan) (Found: C, 45·8; H, 4·55; N, 12·4. $C_{13}H_{15}O_8N_3$ requires C, 45·8; H, 4·4; N, 12·3%).

N-*Acetyl*-4-p-*methoxyphenoxy*-3 : 5-*dinitro*-D-*phenylalanine Ethyl Ester* (VIII).—*N*-Acetyl-3 : 5-dinitro-D-tyrosine ethyl ester (9·6 g.) was treated as described under method (*b*) (Part V, *loc. cit.*) for the L-compound. It should be noted that the volume of pyridine is given incorrectly in that description as 80 c.c. instead of 480 c.c. The resulting *ester* (8·4 g., 67%) had m. p. 109—110° and $[\alpha]_D^{23}$ +8·2° (*c*, 1·0 in dioxan) (Found : C, 53·9; H, 4·8; N, 9·5. $C_{20}H_{21}O_9N_3$ requires C, 53·7; H, 4·7; N, 9·4%).

N-*Acetyl*-3 : 5-*diamino*-4-p-*methoxyphenoxy*-D-*phenylalanine Ethyl Ester* (IX).—The foregoing dinitro-compound (32·2 g.) was hydrogenated as described in Part V (*loc. cit.*) for the L-compound. The *diamine* (25·2 g., 90%) melted at 72—77°, resolidified and finally melted at 132—136°, and had $[\alpha]_D^{19}$ −43·4° (*c*, 0·99 in dioxan) (Found : C, 61·5; H, 6·5; N, 10·9. $C_{20}H_{25}O_5N_3$ requires C, 62·0; H, 6·45; N, 10·85%).

N-*Acetyl*-3 : 5-*di-iodo*-4-p-*methoxyphenoxy*-D-*phenylalanine Ethyl Ester* (X).—The diamino-ester (20 g.) was tetrazotised and the tetrazonium solution decomposed with iodide as described for the L-compound in Part V (*loc. cit.*). After completion of the reaction the chloroform layer was separated and the aqueous layer extracted twice with chloroform. The combined chloroform solutions were washed with aqueous sodium thiosulphate, to remove excess of iodine, and then repeatedly with water. The solution was evaporated to dryness and the solid residue dissolved in benzene and passed through an alumina column, benzene being used for elution. Crystallisation of the eluted material from ethanol gave the *di-iodo-ester* (17·4 g., 55%), m. p. 143—145°, $[\alpha]_D^{19}$ −30·5° (*c*, 6·0 in dioxan) (Found : C, 39·65; H, 3·6; N, 2·0; I, 41·7. $C_{20}H_{21}O_5NI_2$ requires C, 39·4; H, 3·5; N, 2·3; I, 41·7%).

3 : 5-*Di-iodo*-D-*thyronine* (XI).—The foregoing di-iodo-ester (15·9 g.) was hydrolysed as described in Part V (*loc. cit.*) for the L-compound. The 3 : 5-di-iodo-D-thyronine (12·4 g., 90%) had m. p. 265° (decomp.) and $[\alpha]_D^{20}$ −27·1° (*c*, 1·0 in N-hydrochloric acid–ethanol, 1 : 2 by vol.) (Found : C, 34·6; H, 2·9; N, 2·4; I, 47·9. Calc. for $C_{15}H_{13}O_4NI_2$: C, 34·3; H, 2·5; N, 2·7; I, 48·35%). [Harington, *loc. cit.*, gives m. p. 256° (decomp.); the rotation, determined in ammonia solution, cannot usefully be compared with ours.]

D-*Thyroxine* (XII).—3 : 5-Di-iodo-D-thyronine (5·25 g.) was iodinated as described in Part V (*loc. cit.*) for the L-compound. The resulting D-thyroxine (5·9 g., 76%) had m. p. 229—230° (decomp.), $[\alpha]_D^{22}$ +5·1° [*c*, 1·98 in N-sodium hydroxide–ethanol (1 : 2 by vol.)] and −17·5° [*c*, 2·04 in N-hydrochloric acid–ethanol (1 : 4 by vol.)] (Found : C, 23·3; H, 1·7; N, 1·7; I, 65·7. Calc. for $C_{15}H_{11}O_4NI_4$: C, 23·2; H, 1·4; N, 1·8; I, 65·3%). Harington (*loc. cit.*) gives m. p. 237° (decomp.) : the rotation was determined under different conditions. Pitt-Rivers and Lerman (*loc. cit.*) do not give a m. p. but give $[\alpha]_D^{26}$ +5·15° for a 3·3% solution in N-sodium hydroxide–ethanol (1 : 2 by vol.).

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