519. *The Synthesis of Thyroxine and Related Compounds. Part XVI.*[1] *The Preparation of* 3,5-*Dichloro*-D-*thyronine: an Unexpected Walden Inversion.*

By W. K. Warburton.

When 3,5-dichloro-L-tyrosine (I) is treated with "nitrosyl bromide" the amino-group is replaced by bromine; the resulting α-bromo-acid (II) is reconverted into the L-amino-acid (I) by ammonia. Kinetic studies have shown that there is Walden inversion at each stage. With 3,5-dinitro-L-tyrosine (IV), however, inversion occurs only during ammonolysis.

In the same reactions 3-chloro-5-nitro-L-tyrosine gives a mainly racemic product, but *O*-methyl-3,5-dichloro-L-thyronine is inverted.

3,5-Dichloro-D-thyronine (IX) was required for study of its biochemical effects, but proved difficult to prepare in good yield. It can be made by methods similar to those used for 3,5-di-iodo-D-thyronine,[2] but the double Sandmeyer reaction involved proceeds poorly. 3,5-Dichloro-L-thyronine has been prepared by the iodonium-salt route,[1] and 3,5-dichloro-D-thyronine (IX) could presumably be made in the same way from *N*-acetyl-3,5-dichloro-D-tyrosine ethyl ester; but attempts to prepare 3,5-dichloro-D-tyrosine by inverting the easily obtained L-isomer unexpectedly failed.

Elks and Waller [2] converted 3,5-dinitro-L-tyrosine (IV) into the α-bromo-acid (V) (which they assumed to be the L-form) and by ammonolysis obtained 3,5-dinitro-D-tyrosine from it. When the same reactions were applied to 3,5-dichloro-L-tyrosine, the configuration was unchanged overall.

[1] Part XV, preceding paper.
[2] Elks and Waller, *J.*, 1952, 2366.

To decide whether there had been no inversion or two inversions required kinetic evidence. Neither step was suitable for direct kinetic study, but the problem was solved by preparing the azido-acid (III) under kinetically controlled conditions.[3] The azido-acid was of the L-series, for it was reduced, with slight racemization, by chromous chloride (catalytic hydrogenation was unsuccessful) to the L-amino-acid (I). It was formed from the bromo-acid (II) in an S_N2 reaction, for, when equivalent quantities of the bromo-acid and sodium azide were allowed to react in dilute (0·2M) solution, the plot of t against $1/(a - x)$, but not that of t against log $(a - x)$, was linear over the range studied (about 85% of the reaction) ($k_2 = 2{\cdot}0 \times 10^{-3}$ l. mole^{-1} sec.$^{-1}$ in water at 35°). Therefore the azidolysis had proceeded with inversion, the bromo-acid (II) had the D-configuration, and the ammonolysis had also involved inversion. No other inversion of an α-amino-acid by a " nitrosyl halide " has been reported.

A similar kinetic study of the azidolysis of the α-bromo-acid (V) derived from 3,5-dinitro-L-tyrosine (IV) showed that the reaction was bimolecular over the range studied (about 65% of the reaction) ($k_2 = 2{\cdot}3 \times 10^{-4}$ l. mole^{-1} sec.$^{-1}$ in water at 40°). This azido-acid, however, was reduced by hydrogen bromide [4] to the D-amino-acid (reduction with chromous chloride, sodium borohydride, or hydrogen was not successful). The bromo-acid was therefore of the L-series, as Elks and Waller [2] had assumed.

Molecular-rotation differences (see Table; dioxan solutions), as well as kinetic data, indicate that the bromo-acid (II) is of the D-series.

Compound	$[M]_D^{20}$	Compound	$[M]_D^{20}$
N-Acetyl-3,5-dichloro-L-tyrosine	+275°	*N*-Acetyl-2,5-dinitro-L-tyrosine	+39°
Acid (II)	−90	Acid (V)	−92

In the dinitro-series, therefore, the difference between the molecular rotations of the acetamido- and the bromo-acid is +131°. In the dichloro-series the corresponding difference is +365°, whereas the corresponding sum is +185°. The bromo-acids are therefore probably of opposite configuration.

3,5-Dichloro-D-thyronine (IX) was also made, but in poor yield, from *O*-methyl-3,5-dichloro-L-thyronine by inversion through the bromo-acid and demethylation. The bromo-acid was not obtained analytically pure. The Sandmeyer reaction still affords the best route to compound (IX).

3-Chloro-5-nitro-L-tyrosine with " nitrosyl bromide " gave an α-bromo-acid of low optical activity. This was converted into an amino-acid,* which was partly racemic but predominantly of the L-series.

The configuration of the bromo-acid (II) is proved by the kinetic studies reported, but the molecularity of the reaction in which it is formed is not so certain. It is nevertheless highly probable that this reaction is of the S_N2 type, for complete inversion has never been observed to accompany an S_N1 reaction, and on theoretical grounds [5] is not to be expected. On the other hand, complete retention is consistent only with an S_Ni or S_Ni' reaction,† and the conversion of 3,5-dinitro-L-tyrosine into its corresponding α-bromo-acid must be of one of these types. Both ammonolyses are almost certainly S_N2 reactions.

It is not easy to understand why 3,5-dichloro- and 3,5-dinitro-L-tyrosine should differ in their reaction with " nitrosyl bromide." Related amino-acids that have been inverted are: L-phenylalanine,[6] *O*-methyl-L-tyrosine,[7] *O*-methyl-3,5-dichloro-L-thyronine, and

* The rotations of substituted tyrosines are small, and conclusions in this paper are based on the much larger rotations of *N*-acetyl- or *ON*-diacetyl-tyrosines.

† I thank a Referee for suggesting the S_Ni' mechanism.

[3] Cf. Brewster, Hiron, Hughes, Ingold, and Rao, *Nature*, 1950, **166**, 178; Hiron and Hughes, *J.*, 1960, 795.

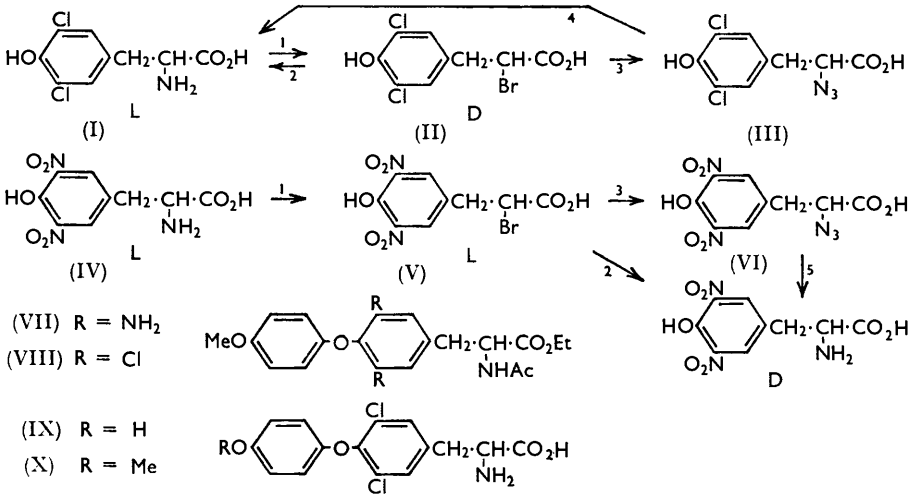
[4] Cf. Smith and Brown, *J. Amer. Chem. Soc.*, 1951, **73**, 2438.

[5] Ingold, " Structure and Mechanism in Organic Chemistry," G. Bell and Sons Ltd., London, 1953, pp. 381—382.

[6] Fischer and Carl, *Ber.*, 1906, **39**, 3996.

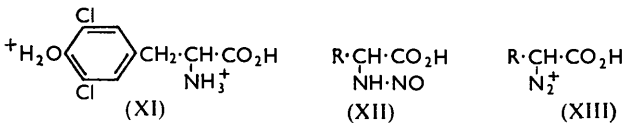
[7] Pitt-Rivers and Lermann, *J. Endocrinol.*, 1948, **5**. 223.

3,5-dinitro-L-tyrosine. 3-Chloro-5-nitro-L-tyrosine probably reacts by both the S_N1 and the S_N2 mechanism simultaneously, and the one reaction that is entirely exceptional in its steric results is that of 3,5-dichloro-L-tyrosine. This is also the only compound of those named with a relatively free *p*-hydroxyl group, a group that is absent from L-phenylalanine, bound in an ether linkage in *O*-methyl-L-tyrosine and *O*-methyl-3,5-dichloro-L-thyronine, and strongly hydrogen-bonded in 3,5-dinitro-L-tyrosine.* Although the conjugate acid (XI) could have been present only in small concentration in the 3N-sulphuric acid in which the nitrosyl bromide was generated, it could nevertheless have been the species through which the S_N2 reaction occurred. Deamination, an S_N1 reaction,[9] proceeds through the



Reagents: 1, "NOBr." 2, NH_3. 3, NaN_3 (S_N2). 4, $CrCl_2$. 5, HBr.

nitrosamine (XII), which is attacked by a proton to give the diazonium ion (XIII). The latter decomposes to a carbonium ion, which then combines with, *e.g.*, bromide ion. However, the inductive effect of a positively charged group in the *para*-position would probably make the nitrosamino-group less liable to attack by a proton. The S_N1 path would then be closed, and the reaction would be able to proceed only by the S_N2 route, with simultaneous entry of a bromide ion and expulsion of a nitrosamino-ion; this would then combine with a proton to give nitrogen and water.



Whatever the explanation of the difference in behaviour between 3,5-dichloro- and 3,5-dinitro-L-tyrosine in their reactions with " nitrosyl bromide " may be, it is likely that an S_N2 deamination for which there is no precedent has been observed. The alternative is an S_N1 (or S_Ni) reaction with complete inversion.

When 3,5-dichloro-L-tyrosine was converted into the bromo-acid (II) in more dilute (0·5N) sulphuric acid, the product was mainly the D-form, but was partly racemic.

* Infrared measurements in bromoform solution show that intramolecular hydrogen bonding is very strong in *N*-acetyl-3,5-dinitro-L-tyrosine ethyl ester [8] ($\nu_{max.}$ 3200—3100 cm.$^{-1}$), but negligible in the corresponding dichloro-compound ($\nu_{max.}$ 3580 cm.$^{-1}$). In *N*-acetyl-3-chloro-5-nitro-L-tyrosine ethyl ester ($\nu_{max.}$ 3220 cm.$^{-1}$) it is weaker than in the dinitro- but stronger than in the dichloro-compound. The same is almost certainly true of the hydrogen bonding in the amino-acids.

[8] Chalmers, Dickson, Elks, and Hems, *J.*, 1949, 3424.
[9] Ref. 5, p. 397; see also Bunton, *Ann. Reports*, 1958, **55**, 193.

3-Chloro-5-nitro-L-tyrosine under the same conditions gave a bromo-acid not differing significantly in rotation from one prepared in more concentrated (3N) acid.

Valine and isoleucine cannot be inverted as the free amino-acids by the usual method, but, as Neuberger has explained,[10] this is probably because there is retention at each stage.

An attempt to prepare 3,5-dichloro-D-tyrosine by three successive inversions failed. It was not possible to obtain the pure L-α-bromo-acid stereoisomeric with (II) by preparing the hydroxy-acid and treating this with phosphorus tribromide in pyridine (each of which reactions should have caused inversion), probably because there was some replacement of the phenolic hydroxyl group by bromine.

The bromo-acid (II) reacted with potassium phthalimide in dimethylformamide to give an optically inactive phthalimido-acid, which was decomposed to give 3,5-dichloro-DL-tyrosine.

Experimental

M. p.s are corrected. Rotations were measured in dioxan (*c* 1) unless otherwise stated.

D-α-*Bromo*-β-(3,5-*dichloro*-4-*hydroxyphenyl*)*propionic Acid* (II).—3,5-Dichloro-L-tyrosine [1] (20·0 g.) was dissolved in 3N-sulphuric acid and cooled, with stirring, to −12°. Potassium bromide (37·5 g.) was added, then a solution of pure sodium nitrite (12·5 g.) in water (20 ml.) during 2½ hr. at −12°. The mixture was stirred for ½ hr. more without cooling. The cream solid was washed with a little ice-cold water and dried in a neutral desiccator, leaving a poorly crystalline α-*bromo-acid* (20·0 g., 80%), m. p. 70—80°. Recrystallized from benzene–light petroleum (b. p. 100—120°) it had m. p. 85·5—86·5°, $[\alpha]_D^{20}$ −28·3° (*c* 3) (Found: C, 34·5; H, 1·7; Hal., 49·0. $C_9H_7BrCl_2O_3$ requires C, 34·5; H, 2·2; Hal., 48·1%), but was only slightly purer (infrared) than the solid of m. p. 70—80°. Preparation in 0·5N-sulphuric acid gave a bromo-acid of $[\alpha]_D^{20}$ −20° in 65% yield.

Ammonolysis of α-*Bromo*-β-(3,5-*dichloro*-4-*hydroxyphenyl*)*propionic Acid*.—The crude α-bromo-acid (4·0 g.) was dissolved in concentrated ammonia solution (80 ml.) and left in a stoppered flask for 5 days at room temperature. Most of the ammonia was then removed under reduced pressure, 2N-sodium hydroxide (12·5 ml.) was added, and the rest of the ammonia was removed at <40°. Sodium hydrogen carbonate (1·7 g.) was added, then, with stirring, acetic anhydride (1·7 ml.), and the solution was stirred for 1 hr. longer. Addition of 2N-hydrochloric acid precipitated a grey solid, which recrystallized from water (charcoal), giving *N*-acetyl-3,5-dichloro-L-tyrosine (1·5 g., 40%) as needles, m. p. and mixed m. p. 133·5—134·5°, $[\alpha]_D^{20}$ +82·0° (*c* 6) (Found: C, 43·7; H, 4·1; N, 4·85. Calc. for $C_{11}H_{11}Cl_2NO_4,\frac{1}{2}H_2O$: C, 44·0; H, 4·0; N, 4·7%) (lit.,[1] m. p. 136—139°, $[\alpha]_D^{20}$ +83·0°).

Acetylation in the presence of a large excess of sodium hydrogen carbonate (not of sodium hydroxide) with an excess of acetic anhydride gave a 40% yield of *ON*-diacetyl-3,5-dichloro-L-tyrosine, m. p. 169—172°, mixed m. p. 170—172°, $[\alpha]_D^{20}$ +83·5° (Found: C, 46·7; H, 4·0; N, 4·3. Calc. for $C_{13}H_{13}Cl_2NO_5$: C, 46·8; H, 3·9; N, 4·2%).

The *N*-acetyl derivative, and the *ON*-diacetyl derivative (preparation described below), were identical (infrared spectra) with the respective authentic derivatives.

DL-β-(3,5-*Dichloro*-4-*hydroxyphenyl*)-α-*phthalimidopropionic Acid*.—The α-bromo-acid (II) (6·24 g.) was heated at 90—95° with potassium phthalimide (12·2 g.) in pure dimethylformamide (45 ml.) for 50 min. The mixture was filtered to remove unchanged potassium phthalimide, and the pale brown filtrate poured into water (200 ml.). Phthalimide was removed by filtration, the mixture extracted with chloroform, and the chloroform extracted with 2N-sodium hydroxide. Neutralization of the sodium hydroxide solution at 0° gave a colourless solid (1·25 g.), m. p. 120—122°, which was recrystallized from ethanol to give the impure DL-α-phthalimido-acid (0·77 g., 11%), needles, m. p. 154—154·5° (Found: C, 52·8; H, 3·8. Calc. for $C_{17}H_{11}Cl_2NO_5$: C, 53·8; H, 2·9%). The phthalimido-acid was decomposed with hydrazine to give 3,5-dichloro-DL-tyrosine (m. p. 215°) in 30% yield.

L-α-*Azido*-β-(3,5-*dichloro*-4-*hydroxyphenyl*)*propionic Acid* (III).—The α-bromo-acid (II) (2·00 g.) was dissolved in water (25 ml.) containing sodium hydrogen carbonate (1·07 g.), and a concentrated solution of sodium azide (1·0 g.) in water was cautiously added. The mixture was kept at 45—50° for 2 hr. Sodium acetate (5 g.) was added and the mixture cooled to 0°.

[10] Neuberger, *Adv. Protein Chem.*, 1948, **4**, 332.

2N-Hydrochloric acid was added until the solution was acid to Congo Red. The off-white solid was collected after refrigeration and recrystallized from water (40 ml.) (charcoal), giving the *azido-acid* as needles (0·92 g., 53%), m. p. 130—135°, $[\alpha]_D^{20}$ −70° (Found: C, 39·6; H, 2·5; N, 15·3; Cl, 25·5. $C_9H_7N_3Cl_2O_3$ requires C, 39·2; H, 2·5; N, 15·2; Cl, 25·7%).

Reduction of L-α-*Azido*-β-(3,5-*dichloro*-4-*hydroxyphenyl*)*propionic Acid.*—The azido-acid (2·76 g.) was dissolved in acetone (40 ml.), and an acid 2·3N-solution (10 ml.) of chromous chloride was cautiously added, with shaking, in an atmosphere of nitrogen. After 10 min., 2N-ammonia (90 ml.) was added and the mixture filtered. The green residue was extracted with 2N-ammonia (3 × 20 ml.), and the combined filtrates were shaken with kieselguhr and filtered again. 2N-Sodium hydroxide (10 ml.) was added and the filtrate concentrated under reduced pressure to 90 ml. Sodium hydrogen carbonate (5·0 g.) was added, then acetic anhydride (5·0 ml.) was added dropwise, with stirring, at room temperature, during 1 hr. The solution was stirred for 1 hr. more, filtered, and acidified with 2N-hydrochloric acid. *ON*-Diacetyl-3,5-dichloro-L-tyrosine (1·03 g., 31%) slowly separated, having m. p. and mixed m. p. 173—174°, $[\alpha]_D^{20}$ +67° (Found: C, 46·85; H, 3·9; N, 3·9%). This compound was identical (infrared spectrum) with authentic *ON*-diacetyl-3,5-dichloro-L-tyrosine.

D-α-*Azido*-β-(4-*hydroxy*-3,5-*dinitrophenyl*)*propionic Acid* (VI).—The α-bromo-acid (V) (5·0 g.) was dissolved in water (50 ml.) containing sodium hydrogen carbonate (1·5 g.), and sodium azide (5·0 g.) in water (15 ml.) was cautiously added. The mixture was kept at 45° overnight, then ice was added and the mixture made strongly acid with 2N-hydrochloric acid. A yellow oil separated and quickly solidified. The solid (3·6 g., 81%), m. p. 130—133°, $[\alpha]_D^{20}$ −35°, was extracted with a little warm benzene, leaving a solid, m. p. 139—141°, $[\alpha]_D^{20}$ −30°. This recrystallized from benzene containing a little ethanol (charcoal), giving lemon-yellow needles of the *azido-acid*, m. p. 141—143·5° (2·3 g., 50%), $[\alpha]_D^{20}$ −30° (Found: C, 36·2; H, 2·5; N, 23·1. $C_9H_7N_5O_7$ requires C, 36·4; H, 2·4; N, 23·5%).

Reduction of D-α-*Azido*-β-(4-*hydroxy*-3,5-*dinitrophenyl*)*propionic Acid.*—The azido-acid (1·02 g.) was dissolved in glacial acetic acid (15 ml.). Phenol (530 mg.) was added, then a 30% w/v solution of hydrogen bromide in acetic acid (15 ml.). Nitrogen was evolved at once. The mixture was left at room temperature overnight, then the precipitate of 3,5-dinitro-D-tyrosine hydrobromide (0·60 g., 50%) was washed with a little cold acetic acid and dried. The hydrobromide was dissolved in 2N-sodium hydroxide (4·2 ml.), then treated with acetic anhydride (0·24 ml.). *N*-Acetyl-3,5-dinitro-D-tyrosine separated on acidification, and recrystallized from 50% ethanol (7 ml.), giving pale yellow needles (230 mg., 43%), m. p. and mixed m. p. 216—217°, $[\alpha]_D^{20}$ −13·3° (Found: C, 42·4; H, 3·6; N, 13·5. Calc. for $C_{11}H_{11}N_3O_8$: C, 42·2; H, 3·5; N, 13·4%). This compound, which is dimorphic, was identical (infrared) with *N*-acetyl-3,5-dinitro-D-tyrosine, for which Elks and Waller [2] reported m. p. 192—194° (uncorr.), $[\alpha]_D^{20}$ −12·7°.

N-*Acetyl*-3-*chloro*-5-*nitro*-L-*tyrosine.*—3-Chloro-5-nitro-L-tyrosine [11] was acetylated as described for 3,5-dichloro-L-tyrosine, giving yellow needles (from water) of an *acid*, m. p. 184·5—185·5°, $[\alpha]_D^{20}$ +81° (Found: C, 44·0; H, 3·8. $C_{11}H_{11}ClN_2O_6$ requires C, 43·7; H, 3·6%).

The *ethyl ester* formed lemon-yellow needles (from ethanol), m. p. 129—131° (Found: C, 47·0; H, 4·55. $C_{13}H_{15}ClN_2O_6$ requires C, 47·2; H, 4·5%).

α-*Bromo*-β-(3-*chloro*-4-*hydroxy*-5-*nitrophenyl*)*propionic Acid.*—3-Chloro-5-nitro-L-tyrosine (4·0 g.) was dissolved in 3N-sulphuric acid (50 ml.) and treated with stirring, at −10° to −12°, with potassium bromide (7·5 g.), then with sodium nitrite (2·5 g.) in water (4 ml.) during 1½ hr. The pale brown oil which separated was washed with cold water and crystallized from benzene–light petroleum (b. p. 100—120°), giving the α-*bromo-acid* as lemon-yellow needles, m. p. 95—97°, $[\alpha]_D^{20}$ −17·0° (Found: C, 33·5; H, 2·2; N, 4·0; Hal, 34·5. $C_9H_7BrClNO_5$ requires C, 33·3; H, 2·15; N, 4·3; Hal, 35·5%).

Ammonolysis of α-*Bromo*-β-(3-*chloro*-4-*hydroxy*-5-*nitrophenyl*)*propionic Acid.*—The bromo-acid was decomposed with ammonia and the amino-acid acetylated as described for the dichloro-compound. N-*Acetyl*-3-*chloro*-5-*nitrotyrosine* was obtained in 30% yield, having m. p. 186·5—189·5° (decomp.), $[\alpha]_D^{20}$ +10° (Found: N, 9·1; Cl, 11·4. $C_{11}H_{11}ClN_2O_6$ requires N, 9·25; Cl, 11·75%).

Ethyl α-*Acetamido*-β-(3,5-*dichloro*-4-p-*methoxyphenoxyphenyl*)*propionate* (VIII).—Ethyl α-acetamido-β-(3,5-diamino-4-*p*-methoxyphenoxyphenyl)propionate (10 g.) was tetrazotized as described by Chalmers *et al.*[8] The tetrazonium salt was poured into a stirred solution of cuprous chloride (14 g.) in 10N-hydrochloric acid (190 ml.) containing chloroform (200 ml.). The

[11] Zeynek, *Z. physiol. Chem.*, 1925, **144**, 250.

chloroform solution was worked up as for the di-iodo-compound,[8] and the residual gum passed in the minimum amount of cold chloroform through a column (8 × 2 cm.) of alumina. An orange gum which was eluted by chloroform was dissolved in propan-2-ol (4 ml.). Isopropyl ether was added and the solution was cooled to 0°; the ester crystallized. More isopropyl ether (6 ml.) was added and the mixture kept at 0° overnight. Filtration gave a slightly sticky solid, which was extracted with boiling cyclohexane (250 ml.) for 1 hr. The cyclohexane was decanted and the residual gum extracted again with boiling cyclohexane (200 ml.). The combined extracts were cooled slowly and refrigerated overnight, giving the *ester* (5·55 g., 50·5%), m. p. 81—83°, $[\alpha]_D^{20}$ +36° (Found: Cl, 16·0. $C_{20}H_{21}Cl_2NO_5$ requires Cl, 16·6%). In later preparations the yield seldom exceeded 40%.

3,5-*Dichloro*-L-*thyronine*.—The preceding ester (14·3 g.) was refluxed for 4 hr. with red phosphorus (10 g.), constant-boiling hydriodic acid (270 ml.), and acetic acid (100 ml.). The hot solution was filtered and, on cooling, the filtrate deposited crystals, which were collected after being cooled at 0° for 1 hr. The solid was dissolved in boiling ethanol (60 ml.) containing the minimum amount of 2N-sodium hydroxide, filtered, and treated with 20% acetic acid until the pH was 5·5. 3,5-*Dichloro*-L-*thyronine* separated as needles (6·35 g., 62%), m. p. 255° (decomp.), $[\alpha]_D^{20}$ −33° (*c* 1·0 in 1 : 1 EtOH–2N-HCl) (Found: C, 52·6; H, 3·8. $C_{15}H_{13}Cl_2NO_4$ requires C, 52·65; H, 3·8%).

ON-*Diacetyl*-3,5-*dichloro*-L-*tyrosine*.—3,5-Dichloro-L-tyrosine (3·0 g.) was dissolved in water (60 ml.) containing sodium hydrogen carbonate (4·0 g.) and 2N-sodium hydroxide (10 ml.). Acetic anhydride (4·0 ml.) was added dropwise with stirring at room temperature. After 30 min. the solution was filtered and the pH adjusted to 1 with 2N-hydrochloric acid. The solid which separated was washed with cold water and recrystallized from water (250 ml.). ON-*Diacetyl*-3,5-*dichloro*-L-*tyrosine* separated as needles (2·32 g., 58%), m. p. 171—172°, $[\alpha]_D^{20}$ +79·7° (Found: C, 46·5; H, 3·9; N, 4·1. $C_{13}H_{13}Cl_2NO_5$ requires C, 46·8; H, 3·9; N, 4·2%). This compound slowly racemizes in boiling water.

3,5-*Dichloro*-O-*methyl*-L-*thyronine*.—The ester (VIII) (10·5 g.) was refluxed in 10N-hydrochloric acid (50 ml.) and glacial acetic acid (50 ml.) for 2 hr., during which a solid separated. Water (100 ml.) was added, and the solid was collected and crystallized from pyridine (100 ml.) and water (50 ml.), giving 3,5-*dichloro*-O-*methyl*-L-*thyronine* as needles (6·05 g., 72·5%), m. p. 235° (decomp.), $[\alpha]_D^{20}$ +32° (*c* 1·0 in 1 : 1 : 1 dioxan–EtOH–2N-HCl) (Found: C, 49·1; H, 4·9; N, 3·8; Cl, 18·6. $C_{16}H_{15}Cl_2NO_4,2H_2O$ requires C, 49·0; H, 4·9; N, 3·6; Cl, 18·1%).

L-α-*Bromo*-β-(3,5-*dichloro*-4-p-*methoxyphenoxyphenyl*)*propionic Acid*.—3,5-Dichloro-*O*-methyl-L-thyronine (4·74 g.) was dissolved at 50° in a mixture of 36N-sulphuric acid (20 ml.), water (40 ml.), and glacial acetic acid (50 ml.). Potassium bromide (27·6 g.) in water (40 ml.) was added and the mixture cooled quickly, with shaking, to −5°. Sodium nitrite (9·2 g.) in water (20 ml.), cooled to 0°, was added in one portion, with shaking, to the suspension of the amino-acid in a strong 750-ml. flask. The flask was firmly stoppered and vigorously shaken behind a safety screen. The mixture was allowed to rise to room temperature (23°) and the pressure momentarily released from time to time. The total time of shaking was 2 hr. Gases were removed under reduced pressure. Water (50 ml.) was added to the pale orange solution which was then extracted with chloroform (3 × 100 ml.). The chloroform was washed with sodium hydrogen sulphite solution, then with water, dried ($MgSO_4$), and evaporated to dryness under reduced pressure, leaving a pale yellow oil which was washed with light petroleum. The residue (4·6 g., 80%), $[\alpha]_D^{20}$ −20°, did not crystallize.

3,5-*Dichloro*-O-*methyl*-D-*thyronine*.—The preceding α-bromo-acid (2·00 g.) was dissolved in concentrated ammonia solution (40 ml.) and left at room temperature in a tightly stoppered flask for 5 days. Most of the ammonia was removed under reduced pressure and 2N-hydrochloric acid added until the pH was 4·5. The resulting sticky, brown solid was washed with water and acetone, leaving a residue (650 mg., 37%), m. p. 220—222°. This was dissolved in alcoholic hydrochloric acid (charcoal) and reprecipitated with sodium acetate as almost colourless 3,5-*dichloro*-O-*methyl*-D-*thyronine* (279 mg.), m. p. 220°, $[\alpha]_D^{20}$ −25° (*c* 1·0 in 1 : 1 : 1 dioxan–EtOH–2N-HCl).

3,5-*Dichloro*-D-*thyronine* (IX).—(a) *After isolation of the methyl ether*. The crude methyl ether (145 mg.) was refluxed with red phosphorus (100 ml.), constant-boiling hydriodic acid (4 ml.), and glacial acetic acid (4 ml.) for 7 hr., then filtered while hot. The filtrate was taken to dryness and the almost colourless solid was converted into the *amino-acid* (70 mg.), m. p. 242° (decomp.), $[\alpha]_D^{20}$ −30·7° (*c* 1 in 1 : 1 EtOH–2N-HCl).

(b) *Without isolation of the ether.* The crude solid obtained by ammonolysis of the bromo-acid (4·5 g.) was drained and then refluxed with hydriodic and acetic acid for 17 hr. Removal of the acids and addition of a little sodium hydrogen sulphite, then of hot water (30 ml.), gave a colourless solution from which 3,5-dichloro-D-thyronine (600 mg., 13%), m. p. 250—252°, $[\alpha]_D^{20}$ −33° (*c* 1 in 1 : 1 EtOH–2N-HCl), was obtained on addition of sodium acetate.

Kinetic Studies.—Sodium azide was purified by crystallization from water and ethanol. Bromo-acids were of analytical purity. The extraction with chloroform described below was also done with pure 0·1N-potassium bromide. The normality found was then 0·0995N.

Azidolysis of α-bromo-β-(3,5-dichloro-4-hydroxyphenyl)propionic acid. A solution of sodium azide (650 mg.) in water (6 ml.), warmed to 35°, was added to a solution of the bromo-acid (3·140 g.) in water (25 ml.), also at 35°, containing sodium hydrogen carbonate (840 mg.). The solution was made up to 50 ml. and shaken, and the clock started. The flask was kept at 35° ± 0·1° and 3-ml. samples were drawn off at intervals and run into ice-cold 0·5N-nitric acid (4·5 ml.), then refrigerated for 48—72 hr.

Each sample was decanted from the oil or solid present, the residue was washed three times with water, and the aqueous solution extracted with chloroform (10 + 7 ml.; containing no chloride ion). The aqueous layer was boiled with 6N-nitric acid (1 ml.) until free from smell, then cooled to <25°. Saturated ferric ammonium alum solution (1 ml.) was added, followed by more 6N-nitric acid (2 ml.), then a measured volume of 0·02N-silver nitrate. The silver bromide was filtered off and the filtrate titrated with ammonium thiocyanate solution. One experiment is recorded in Table 1.

A control run in which the sodium azide was omitted showed that during 50—145 min.

TABLE 1.

Sample no.	Time (min.)	0·0235N-NH_4CNS (ml.)	0·02N-$AgNO_3$ added (ml.)	0·02N-$AgNO_3$ consumed (ml.)	$(a - x)$ (mole $l.^{-1}$)	$1/(a - x)$ (l. $mole^{-1}$)	$\log_{10}$ $[10^3(a - x)]$
1	2·5	15·6	20	1·7	0·189	5·29	2·277
2	5	14·25	20	3·25	0·178	5·62	2·250
3	10	12·20	20	5·7	0·162	6·18	2·210
4	15	10·25	20	8·0	0·146	6·85	2·164
5	20	8·85	20	9·6	0·136	7·36	2·134
6	30	6·45	20	12·45	0·117	8·55	2·068
7	40	4·50	20	14·7	0·102	9·81	2·009
8	50	11·45	30	16·55	0·0892	11·21	1·950
9	60	10·30	30	17·9	0·0806	12·41	1·906
10	70	9·35	30	19·0	0·0727	13·75	1·862
11	80	8·85	30	19·6	0·0692	14·45	1·840
12	100	11·80	35	21·15	0·0588	17·00	1·769
13	120	10·75	35	22·4	0·0506	19·75	1·704
14	150	9·85	35	23·45	0·0433	23·1	1·637
15	175	9·00	35	24·45	0·0367	27·3	1·565
16	207	8·25	35	25·3	0·0312	32·1	1·494

TABLE 2.

Sample no.	Time (min.)	0·02N-NH_4CNS (ml.)	0·02N-$AgNO_3$ added (ml.)	0·02N-$AgNO_3$ consumed (ml.)	$(a - x)$ (mole $l.^{-1}$)	$1/(a - x)$ (l. $mole^{-1}$)	$\log_{10}$ $[10^2(a - x)]$
1	5	19·45	20	0·55	0·196	5·10	1·292
2	10	19·05	20	0·95	0·194	5·15	1·288
3	20	8·20	10	1·80	0·188	5·32	1·274
4	30	7·50	10	2·50	0·183	5·47	1·263
5	50	6·15	10	3·85	0·174	5·75	1·241
6	70	4·90	10	5·10	0·166	6·03	1·220
7	90	3·80	10	6·20	0·159	6·30	1·201
8	120	2·20	10	7·80	0·148	6·76	1·170
9	150	1·00	10	9·00	0·140	7·15	1·146
10	180	4·75	15	10·25	0·132	7·58	1·121
11	205	3·80	15	11·20	0·125	8·00	1·097
12	235	2·80	15	12·20	0·118	8·48	1·072
13	301	1·20	15	13·80	0·108	9·27	1·033
14	360	5·10	20	14·90	0·101	9·90	1·004
15	420	3·30	20	16·70	0·088	11·40	0·945
16	480	2·30	20	17·70	0·082	12·20	0·914

the rate of bromide-ion formation due to hydrolysis was less than 4% of that formed by azidolysis.

Azidolysis of α-bromo-β-(4-hydroxy-3,5-dinitrophenyl)propionic acid. The bromo-acid (3·35 g.) was dissolved in water (35 ml.) containing sodium hydrogen carbonate (about 1 g.) and warmed to 40°. A solution of sodium azide (650 mg.) in water (5 ml.) was warmed to 40° and added. The volume was made up to 50 ml., the flask shaken, and the clock started. The flask was kept in a water-bath at 40° ± 0·1°. Samples (3 ml.) were drawn off at intervals and run into 0·5N-nitric acid (7·5 ml.). The samples were refrigerated for 24—48 hr., then filtered. The filtrate was treated with 6N-nitric acid (3 ml.), then boiled until free from hydrazoic acid. 0·02N-Silver nitrate was added and the solutions were titrated as before. One experiment is recorded in Table 2.

A control run in which the sodium azide was omitted showed that the rate of hydrolysis was negligible.

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