CLXXIX. SOME DERIVATIVES OF THYROXINE.

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SINCE thyroxine has been available in the pure condition its therapeutic use has been limited by the fact that it is only possible to elicit with certainty the full physiological activity of the substance when it is administered intravenously. When thyroxine or its sodium salt is administered by the mouth absorption appears to be irregular, and scarcely ever is the full effect of a given dose obtained. Except, therefore, in hospital practice where the intravenous injection offers no difficulties, thyroxine compares unfavourably with crude desiccated thyroid gland as a therapeutic agent. In our opinion thyroxine fails to be absorbed after oral administration owing to its extreme insolubility when in the free condition. The necessity of a drastic hydrolysis of the thyroid gland before thyroxine can be isolated points to the probability that the compound exists in the gland as a constituent of a peptide or protein, which one would expect to be readily soluble in water. If this hypothesis be correct one may assume that after ingestion of the whole gland substance, the active principle is either absorbed as a peptide or is liberated by enzymic action, but liberated in a physical condition favourable to absorption. It was, therefore, thought worth while to attempt the preparation of some simple peptides containing thyroxine, in order to see whether a relatively soluble compound could be obtained, and, if so, whether such a compound, when administered by mouth, would exercise the physiological effect of thyroxine with a certainty comparable to that attainable by the intravenous injection of the latter. The preparation of glycyl- and *dl*-alanyl-thyroxine was undertaken and is described in detail below. Unfortunately, however, the introduction of a single amino-acid residue is insufficient to affect the solubility of thyroxine to an appreciable extent; these two peptides are very nearly as insoluble in water as is thyroxine itself. On administration to rats by mouth they give similar effects to those obtained by the oral administration of equivalent amounts of the sodium salt of thyroxine, over which therefore they offer no therapeutic advantage. This result is however of some interest, in that it indicates either that these peptides must undergo hydrolysis in the body, in spite of their insolubility, or that the blocking of the amino-group of

thyroxine in this way offers no obstacle to the exercise of the typical physiological effect. As might be expected, the preparation of even the simple peptides containing thyroxine offers considerable technical difficulties owing to the extreme insolubility of all thyroxine derivatives; it was not therefore considered practicable to pursue this line of investigation further by attempting the preparation of more complex thyroxine-containing peptides.

It was thought that the desired increase in solubility might be attained by the acylation of thyroxine with a hydroxy-acid, and with this object *N*-lactylthyroxine has been synthesised. Here again, however, disappointment was encountered, in that the *N*-lactyl derivative, whilst being extremely soluble in alcohol, was almost insoluble in water. It must, therefore, be confessed that, up to the present, no practicable method of increasing the solubility of thyroxine has been devised.

It is convenient also to include in this paper an account of the amines corresponding to thyroxine and to 3 : 5-diiodothyronine; the latter amino-acid was readily decarboxylated by a modification of the method recently described by Johnson and Daschavsky [1925] and Abderhalden and Gebelein [1926] and the resulting amine could be iodinated to give thyroxamine. But little physiological interest appears to attach to these substances; when tested on tadpoles [Gaddum, 1927] thyroxamine shows similar effects to those obtained with thyroxine; neither of the amines, however, shows any marked pressor effect such as might have been anticipated from their constitution. Their preparation and properties are only recorded as a matter of chemical interest.

EXPERIMENTAL.

A. *Glycyl- and dl-alanyl-thyroxine.*

The preparation of these peptides was accomplished in two ways: (a) by coupling chloroacetyl chloride and α -bromopropionyl chloride respectively with the methyl ester of 3 : 5-diiodothyronine, followed by hydrolysis of the ester group, treatment with ammonia, and finally introduction of the remaining two iodine atoms; (b) by coupling the acid chlorides with thyroxine methyl ester, followed by hydrolysis of the ester group and treatment with ammonia.

(a) *3 : 5-Diiodothyronine methyl ester hydrochloride.* The amino-acid was suspended in five parts of methyl alcohol and the solution saturated with a vigorous stream of dry hydrogen chloride; after cooling, the saturation was repeated. The solution was concentrated to a small volume by distillation under diminished pressure whereupon the ester hydrochloride crystallised out; it was filtered off and a small further crop was obtained by cautious addition of ether to the mother liquor. The whole was recrystallised from dilute alcohol containing some hydrochloric acid, and formed colourless needles, m.p. 230° (decomp.). The yield was 87 % of the theoretical. The compound was but little soluble in water or absolute alcohol, but fairly readily soluble in mixtures of the two solvents.

Analysis. 1.33 mg. required 5.5 cc. *N*/200 thiosulphate [Kendall, 1914].
31.1 mg. gave 0.79 mg. N (micro-Kjeldahl).

	I	N
Found	43.8	2.5
Calculated for $C_{16}H_{16}O_4NClH_2$	44.1	2.4

3 : 5-*Diiodothyronine methyl ester*. The ester hydrochloride was dissolved in 50 % alcohol and the cold solution treated with one equivalent of 0.1 *N* sodium hydroxide; crystallisation rapidly set in and was completed by dilution with water. The yield was quantitative. On recrystallisation from dilute alcohol the ester formed clusters of small needles melting at 174–175°. It was insoluble in water, fairly soluble in alcohol, but very sparingly so in all other ordinary organic solvents.

Analysis. 2.07 mg. required 9.25 cc. *N*/200 thiosulphate [Kendall, 1914].
30.1 mg. gave 0.76 mg. N (micro-Kjeldahl).

	I	N
Found	47.4	2.5
Calculated for $C_{16}H_{16}O_4NI_2$...	47.1	2.6

Chloroacetyl-3 : 5-diiodothyronine methyl ester. Owing to the great insolubility of the amino-acid ester in benzene and chloroform, the coupling with the acid chloride could not be conveniently brought about in these solvents; for the purposes of this reaction it was found better to employ anisole as a solvent; 0.54 g. of the ester was dissolved in 12 cc. of redistilled anisole and the solution cooled in ice; with continual cooling and good shaking 0.125 g. of chloroacetyl chloride was added; the solution was then shaken with 0.4 g. of sodium bicarbonate dissolved in a little water and a further addition of 0.125 g. of chloroacetyl chloride was made. The solution was filtered from a trace of undissolved matter and the anisole layer separated and dried over calcium chloride; the anisole was removed by distillation at a pressure of 1–2 mm., the bath temperature not being raised above 60°. The oily residue was dissolved in a little warm benzene; on spontaneous evaporation of the solvent the ester crystallised out and was filtered off and washed with ether. The yield was 75–80 % of the theoretical. Recrystallised from dilute alcohol it formed fine hair-like needles, M.P. 160°. It was readily soluble in alcohol and benzene.

Analysis. 1.72 mg. required 6.65 cc. of *N*/200 thiosulphate [Kendall, 1914].
33.3 mg. gave 0.72 mg. N (micro-Kjeldahl).

	I	N
Found	41.2	2.3
Calculated for $C_{18}H_{16}ONClH_2$	41.0	2.2

Chloroacetyl-3 : 5-diiodothyronine. The above ester was dissolved in *N* sodium hydroxide (two equivalents) and the solution was allowed to stand for 15 minutes at room temperature; two equivalents of *N* hydrochloric acid were then added, whereupon the acid separated as an oil which, on rubbing, solidified; it was purified by dissolving in hot 30 % alcohol, from which, on slow cooling,

it separated as a sphaero-crystalline powder; on heating it sintered at 156° and melted at 166–168°.

Analysis. 1.66 mg. required 6.55 cc. *N*/200 thiosulphate [Kendall, 1914].
35.7 mg. gave 0.851 mg. N (micro-Kjeldahl).

	I	N
Found	41.8	2.4
Calculated for $C_{17}H_{14}O_5NCH_2$	42.2	2.3

This acid was easily soluble in alcohol and glacial acetic acid, but sparingly so in other organic solvents and in water.

Glycyl-3 : 5-diiodothyronine. Chloroacetyl-3 : 5-diiodothyronine was covered with a large excess of 25 % aqueous ammonia and allowed to stand in a sealed tube at the ordinary temperature for 5 days. The contents of the tube were then mixed with an equal volume of alcohol, and evaporated under diminished pressure. The residue was extracted with hot absolute alcohol, and the insoluble portion crystallised from 30 % alcohol from which it separated in colourless needles. On heating, it darkened above 150°, but did not melt definitely below 290°.

Analysis. 1.59 mg. required 6.5 cc. *N*/200 thiosulphate [Kendall, 1914].
15.5 mg. gave 0.716 mg. N (micro-Kjeldahl).
26.5 mg. gave 1.24 cc. moist N at 758 mm. and 21° (Van Slyke).

	I	N	NH ₂ -N
Found	43.3	4.6	2.6
Calculated for $C_{17}H_{16}O_5N_2I_2$	43.6	4.8	2.4

The compound was almost insoluble in water and alcohol, and only very sparingly soluble in mixtures of these two solvents.

Glycylthyroxine. Glycyl-3 : 5-diiodothyronine was dissolved in concentrated ammonia and iodinated in the manner employed for the preparation of thyroxine; towards the end of the addition of the iodine, the product began to separate as a micro-crystalline powder. After standing in the cold for some time this material was separated on the centrifuge; it was purified by dissolving in strong ammonia, from which, on spontaneous evaporation of the latter, it separated as a micro-crystalline powder. For analysis it was dried *in vacuo* at 110° over phosphorus pentoxide.

Analysis. 1.13 mg. required 6.43 cc. *N*/200 thiosulphate [Kendall, 1914].
17.6 mg. gave 0.595 mg. N (micro-Kjeldahl).

	I	N
Found	60.3	3.4
Calculated for $C_{17}H_{14}O_5N_2I_4$	60.9	3.4

The preparation of *dl*-alanyl-3 : 5-diiodothyronine was carried out by a precisely similar series of reactions which do not call for any detailed comment.

α-Bromopropionyl-3 : 5-diiodothyronine methyl ester. This was prepared similarly to the corresponding chloroacetyl compound. On recrystallisation

from dilute alcohol, it formed clusters of colourless needles, melting at 161–162°.

Analysis. 1.78 mg. required 6.3 cc. *N*/200 thiosulphate [Kendall, 1914].
38.1 mg. gave 0.751 mg. N (micro-Kjeldahl).

	I	N
Found	37.5	1.9
Calculated for $C_{19}H_{18}O_5NBrI_2$	37.7	2.1

α-Bromopropionyl-3 : 5-diiodothyronine. The above ester was hydrolysed by treatment with *N* sodium hydroxide (two equivalents) at the ordinary temperature for 15 minutes. On acidification, the crude acid separated as an oil which slowly solidified; this was dissolved in dilute sodium carbonate, and the sodium salt of the acid precipitated by addition of saturated sodium chloride; the sodium salt was redissolved and again precipitated, and was then converted into the free acid, which was crystallised first from dilute acetic acid and then from dilute alcohol. It formed a sphaero-crystalline powder, melting at 194–195°. It was readily soluble in alcohol and glacial acetic acid, sparingly so in other organic solvents and in water.

Analysis. 2.05 mg. required 7.4 cc. *N*/200 thiosulphate [Kendall, 1914].
42.3 mg. gave 0.876 mg. N (micro-Kjeldahl).

	I	N
Found	38.3	2.1
Calculated for $C_{18}H_{16}O_5NBrI_2$	38.5	2.1

dl-Alanyl-3 : 5-diiodothyronine. This was prepared by the action of 25 % aqueous ammonia solution on the above acid for 5 days at the ordinary temperature. On slow separation from its solution in hot 30 % alcohol, it formed a sphaero-crystalline powder, M.P. 207°.

Analysis. 1.83 mg. required 7.29 cc. *N*/200 thiosulphate [Kendall, 1914].
20.1 mg. gave 0.905 mg. N (micro-Kjeldahl).
30.4 mg. gave 1.2 cc. moist N at 763 mm. and 21° (Van Slyke).

	I	N	NH ₃ -N
Found	42.2	4.5	2.2
Calculated for $C_{18}H_{14}O_5N_2I_2$	42.6	4.7	2.3

The solubilities of this dipeptide were similar to those of the corresponding glycyl derivative, but its conversion into alanylthyroxine by iodination did not proceed satisfactorily, the product being extremely difficult to purify. The preparation of alanylthyroxine, as also a second synthesis of glycylthyroxine, was therefore carried out, using thyroxine itself as a starting point, by the following series of reactions.

(b) *Thyroxine methyl ester hydrochloride.* Thyroxine (5.8 g.) was covered with methyl alcohol (60 cc.) and the solution saturated with a vigorous stream of dry hydrogen chloride; after cooling, the saturation was repeated, and the solution was then concentrated by distillation under diminished pressure. The crystalline precipitate was filtered off, washed with alcohol and ether, and

dried. The yield was 90 % of the theoretical. For analysis it was recrystallised from dilute alcohol containing a little hydrochloric acid, when it formed colourless needles melting at 221.5° with decomposition. The ester hydrochloride was sparingly soluble in water or alcohol, fairly readily so in mixtures of the two solvents.

Analysis. 1.03 mg. required 5.93 cc. *N*/200 thiosulphate [Kendall, 1914].
33.6 mg. gave 0.536 mg. N (micro-Kjeldahl).

	I	N
Found	61.0	1.6
Calculated for $C_{16}H_{14}O_4NCII_4$	61.4	1.7

Thyroxine methyl ester. The ester hydrochloride was dissolved in 50 % alcohol and the solution treated with 2*N* sodium hydroxide (one equivalent). Crystallisation of the ester commenced immediately, and was completed by addition of water and standing in the cold for some time. Recrystallised from dilute alcohol, it formed small prismatic needles melting at 156°. The ester was readily soluble in alcohol, but sparingly so in all the usual organic solvents and in water.

Analysis. 1.04 mg. required 6.18 cc. *N*/200 thiosulphate [Kendall, 1914].
41.8 mg. gave 0.697 mg. N (micro-Kjeldahl).

	I	N
Found	63.0	1.7
Calculated for $C_{16}H_{13}O_4NI_4$	63.4	1.7

Chloroacetylthyroxine methyl ester. Thyroxine methyl ester (0.8 g.) was dissolved in redistilled anisole (16 cc.); the solution was cooled in ice and treated gradually, with continued cooling and good shaking, with 0.12 g. of chloroacetyl chloride dissolved in 4 cc. of anisole; 0.4 g. of sodium bicarbonate in a little water was added, and a further addition of 0.12 g. of chloroacetyl chloride was made. The anisole layer was separated and dried over calcium chloride, and the anisole removed by distillation under diminished pressure; the oily residue was rubbed up with light petroleum, whereupon it solidified. It was recrystallised from benzene, from which it separated in colourless prisms, melting at 159–160°; the yield was 80 % of the theoretical. The compound was easily soluble in alcohol and benzene, sparingly so in light petroleum and insoluble in water.

Analysis. 1.54 mg. required 8.4 cc. *N*/200 thiosulphate [Kendall, 1914].
31.5 mg. gave 0.478 mg. N (micro-Kjeldahl).

	I	N
Found	58.9	1.5
Calculated for $C_{18}H_{14}O_5NCII_4$	58.6	1.6

Chloroacetylthyroxine. The above ester was dissolved in 50 % alcoholic *N* sodium hydroxide (two equivalents) and the solution was allowed to stand at the ordinary temperature for 20 minutes; at the end of this period hydrochloric acid (2 equivalents) was added; the precipitated acid was filtered off

and dried, the crude yield being practically quantitative. On slow separation from its solution in dilute acetic acid, the compound formed a colourless sphaero-crystalline powder, which melted with decomposition at 201–202°. It was readily soluble in alcohol and glacial acetic acid, but practically insoluble in other organic solvents and in water.

Analysis. 1.58 mg. required 8.65 cc. *N*/196 thiosulphate [Kendall, 1914].
30.1 mg. gave 0.468 mg. N (micro-Kjeldahl).

	I	N
Found	59.1	1.55
Calculated for $C_{17}H_{12}O_5NClI_4$	59.5	1.6

Glycylthyroxine. Chloroacetylthyroxine (0.3 g.) was heated with 25 % aqueous ammonia (10 cc.) in a sealed tube at 100° for 1 hour; after cooling, the solution was filtered from a trace of insoluble matter and allowed to evaporate spontaneously, whereupon the peptide separated as a micro-crystalline powder, contaminated with ammonium chloride. When the solution was reduced to a small bulk the solid was filtered off and dried; it was then extracted with boiling absolute alcohol, and the insoluble residue was dissolved in alcohol with the addition of a little concentrated aqueous ammonia; on long standing the peptide separated as a crystalline powder, which was filtered off, washed with alcohol and ether, and dried at 110° *in vacuo* over phosphorus pentoxide. It melted with decomposition at 188–190°, and was identical with the product described above as resulting from the iodination of glycyl-3 : 5-diiodothyronine.

Analysis. 1.16 mg. required 6.6 cc. *N*/200 thiosulphate [Kendall, 1914].
15.2 mg. gave 0.536 mg. N (micro-Kjeldahl).
16.4 mg. gave 0.51 cc. moist N at 180–752 mm. (Van Slyke).

	I	N	NH ₂ -N
Found	60.3	3.5	1.8
Calculated for $C_{17}H_{14}O_5N_2I_4$	60.9	3.4	1.7

α-Bromopropionylthyroxine methyl ester. This was prepared similarly to the chloroacetyl compound by the coupling of *α*-bromopropionyl chloride with thyroxine methyl ester in anisole solution. The yield was 70 % of the theoretical. The compound was crystallised from benzene and formed masses of prismatic needles which melted with decomposition at 199–201°.

Analysis. 1.55 mg. required 7.86 cc. *N*/196 thiosulphate [Kendall, 1914].
31.9 mg. gave 0.495 mg. N (micro-Kjeldahl).

	I	N
Found	54.7	1.6
Calculated for $C_{19}H_{16}O_5NBrI_4$	54.9	1.5

α-Bromopropionylthyroxine. The above ester was hydrolysed with cold sodium hydroxide in the usual manner, and the crude acid purified by recrystallisation from dilute acetic acid, from which it separated first as an oil which

subsequently solidified to a felted mass of fine needles, melting at 193–194° with decomposition.

Analysis. 1.11 mg. required 5.8 cc. *N*/200 thiosulphate [Kendall, 1914].

45.2 mg. gave 0.663 mg. N (micro-Kjeldahl).

	I	N
Found	55.4	1.5
Calculated for $C_{18}H_{14}O_5NBrI_4$	55.7	1.5

Alanylthyroxine. α -Bromopropionylthyroxine (0.25 g.) was heated with 25 % aqueous ammonia (9 cc.) in a sealed tube at 100° for 1 hour; the reaction mixture was worked up as described for glycylthyroxine. The separation of the alanyl compound was considerably slower, and the yield was poorer (25 % of the theoretical). The compound, which formed a colourless powder with no definite crystalline structure, melted with decomposition at 195–200° after sintering at 185°. In physical properties it was similar to glycylthyroxine. For analysis it was dried *in vacuo* at 110° over phosphorus pentoxide.

Analysis. 1.24 mg. required 6.98 cc. *N*/200 thiosulphate [Kendall, 1914].

15.3 mg. gave 0.497 mg. N (micro-Kjeldahl).

15.4 mg. gave 0.45 cc. moist N at 18°/752 mm. (Van Slyke).

	I	N	NH ₂ -N
Found	59.6	3.2	1.7
Calculated for $C_{18}H_{16}O_5N_2I_4$	59.9	3.3	1.65

B. *N*-Lactylthyroxine.

N-Acetyllactylthyroxine methyl ester. Acetyllactyl chloride was prepared by the method of Anschütz and Bertram [1904]. Thyroxine methyl ester (1.6 g.) was dissolved in redistilled anisole (32 cc.); to the solution, cooled in ice, was gradually added a solution of acetyllactyl chloride (0.5 mol.) dissolved in anisole. The precipitate of thyroxine methyl ester hydrochloride was filtered off and amounted to 0.8 g. The anisole was removed from the filtrate by distillation *in vacuo*, and the residual oil dissolved in benzene in which it was very soluble; addition of light petroleum precipitated the ester, the crude yield being quantitative. It was purified by recrystallisation from light petroleum.

Analysis. 1.43 mg. required 7.46 cc. *N*/196 thiosulphate [Kendall, 1914].

	I
Found	56.3
Calculated for $C_{21}H_{19}O_7NI_4$	56.1

N-Lactylthyroxine. The above ester was dissolved in *N* sodium hydroxide (3 mols.), and the solution was allowed to stand for 20 minutes at the ordinary temperature; a slight excess of dilute hydrochloric acid was then added; the precipitated acid was filtered off, washed with water, and recrystallised first from dilute alcohol and then from dilute acetic acid. It formed bunches of colourless needles melting at 199–200° with decomposition. It was very readily soluble in alcohol and glacial acetic acid, but almost insoluble in water.

Analysis. 1.19 mg. required 6.58 cc. *N*/196 thiosulphate [Kendall, 1914].

				I
Found	...	...	...	59.6
Calculated for	C ₁₈ H ₁₅ O ₆ NI ₄			59.8

C. *Amines.*

When 3 : 5-diiodothyronine was heated with diphenylamine according to the method described by Abderhalden and Gebelein [1926], decarboxylation took place, but there was considerable formation of tar and the yield of amine was poor; the operation was more successful when air was excluded from the reaction flask. Finely powdered 3 : 5-diiodothyronine accordingly was covered with diphenylamine (20 parts) and heated in a metal-bath, the air in the flask being displaced by a current of dry hydrogen; the first evolution of gas was perceptible at about 190°; from this point the temperature of the bath was slowly raised, until, after about 45 minutes a temperature of 240° had been attained; by this time the whole of the amino-acid had passed into solution and evolution of gas had ceased. The light brown solution in the flask was allowed to cool to about 70° and was then diluted with benzene and light petroleum; separation of the amine commenced at once and was complete after some hours' standing in the cold. The somewhat pigmented crystalline powder was filtered and well washed with light petroleum; it was then dissolved in dilute alcohol with the aid of a little sulphuric acid, the solution was boiled with charcoal, filtered, and treated with excess of ammonia. The amine so obtained was practically pure, though still faintly coloured; the yield was 70–80 % of the theoretical. For complete purification the amine was converted into the sparingly soluble sulphate by dissolving in much boiling dilute sulphuric acid and allowing the solution to cool. The sulphate was filtered off, redissolved in dilute alcohol, and the base precipitated from the hot solution by addition of ammonia. The pure compound formed small colourless rhomboidal crystals, melting at 243–245°. It was fairly easily soluble in alcohol, but insoluble in the other common organic solvents and in water.

Analysis. 0.98 mg. required 4.85 cc. *N*/200 thiosulphate [Kendall, 1914].
20.2 mg. gave 0.546 mg. N (micro-Kjeldahl).

				I	N
Found	...	...	...	52.5	2.7
Calculated for	C ₁₄ H ₁₃ O ₂ NI ₂			52.8	2.9

The *hydrochloride* formed glistening plates which were sparingly soluble in cold water, but more readily so in dilute alcohol; the substance melted with decomposition at 285–290°.

Analysis. 1.53 mg. required 7.05 cc. *N*/200 thiosulphate [Kendall, 1914].
29.3 mg. gave 0.790 mg. N (micro-Kjeldahl).

	I	N
Found	48.8	2.7
Calculated for $C_{14}H_{14}O_2NCl_2$	49.1	2.7

The *sulphate* formed long fine needles with similar solubilities.

Thyroxamine. This compound was first prepared by the direct decarboxylation of thyroxine according to the method described above; it was impossible, however, to avoid a good deal of decomposition of the thyroxine, with liberation of free iodine, and the yield of the amine was unsatisfactory. Better results were subsequently obtained by the iodination of 3 : 5-diiodothyronamine. The latter compound (1 part) was dissolved in pure methyl alcohol (500 parts) to which was added concentrated aqueous ammonia (10 parts); the cold solution was then treated gradually with a concentrated (2.5 *N*) solution of iodine in potassium iodide in the theoretical amount. When the addition of the iodine was complete the solution, which now had a yellow tinge, was concentrated, when the amine separated as a heavy crystalline powder. This was filtered off and purified by solution in 70 % alcohol with the addition of a little mineral acid, followed by precipitation of the base with ammonia from the hot solution. Repetition of this process once or twice gave a product which was analytically pure, and which formed small colourless needles melting at 207° with decomposition.

Analysis. 50.3 mg. gave 64.3 mg. AgI.
22.8 mg. gave 0.425 mg. N (micro-Kjeldahl).

	I	N
Found	69.1	1.9
Calculated for $C_{14}H_{11}O_2NI_4$	69.3	2.0

The *sulphate* and *hydrochloride* were both very sparingly soluble in water; they were soluble in dilute alcohol but did not crystallise well from this solvent.

The *chloroacetate* was obtained by dissolving thyroxamine in a warm aqueous solution of chloroacetic acid and allowing to cool. It formed fine hair-like needles, which were fairly readily soluble in warm water (in which, however, a clear solution could not be obtained owing to dissociation), and could be recrystallised from water containing a little chloroacetic acid. The substance darkened and melted at 152°.

Analysis. 54.3 mg. gave 0.858 mg. N (micro-Kjeldahl).

	N
Found	1.6
Calculated for $C_{16}H_{14}O_4NCl_4$	1.7

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