ACTA CHEMICA SCANDINAVICA 9 (1955) 203—209

Studies on Antimetabolites

VI. Synthesis of D,L-"Neothyroxine", the β,β-Dimethyl Analogue of D,L-Thyroxine *

E. DUINTJER and Å. JÖNSSON

Organisk-kemiska Institutionen, Kungl. Tekniska Högskolan, Stockholm, Sweden

The β,β-dimethyl analogue of D,L-thyroxine has been prepared by the reaction sequence shown in Fig. 1. A second series of reactions with the same goal was also carried out (Fig. 2) but had to be abandoned, as the hydantoins VII and VIII could not be hydrolysed to the corresponding 3,5-diiodothyroxine derivative.

The search for substances possessing antithyroid activity has lead to the preparation of a number of analogues of thyroxine. Among the compounds tested as thyroxine antagonists are aliphatic ethers of 3,5-diiodotyrosine, the sulphide analogue of thyroxine, 4-benzyloxy-3,5-diiodobenzoic acid and similar compounds, halogeno and nitro derivatives of diphenyl ether, thyronine and 4-(*p*-hydroxyphenoxy)benzoic acid [1], α-thyroxine and some derivatives of thyroxine in which the side chain had been modified by conversion of the carboxyl group into the methyl ketone [2]. Many of these compounds possessed some anti-thyroxine activity, but none of them appears to have been used clinically.

In continuation of our researches on the physiological effect of the introduction of a *gem*dimethyl group in various amino acids, hormones and other compounds of biochemical interest [3] we have now prepared the α,α-dimethyl analogue (XVI) of D,L-thyroxine, and it is the purpose of the present paper to describe the synthetic work. The results of the physiological testing of the compound will be reported elsewhere. For convenience the compound is henceforth called D,L-"*neo*thyroxine", thus indicating the analogy with *neo*pentyl derivatives.

The synthesis was started from D,L-"*neo*tyrosine" (I), the preparation of which was described in Part I of this series [3], and follows the same route, which was worked out by Burrows *et al.*[4] and by Chalmers *et al.*[5] for the preparation of thyroxine from tyrosine, with some alterations in experimental details.

* Part V. *Acta Chem. Scand.* 8 (1954) 1492.

R = CH3 C CH · COOC2H5 CH2 NHCOCH3
I
HONO2
II
(CH3CO)2O NaOH
IX
HONO2
X
C2H5OH SOCl2
XI
p-CH3 · C6H4 · SO2Cl + pyridine then p-CH3O · C6H4 · OH
XII
H2/Pd
XIII
HONO then KI+I2
XIV
HI
XV
I2 C2H5NH2
"Neothyroxine"
XVI

Fig. 1.

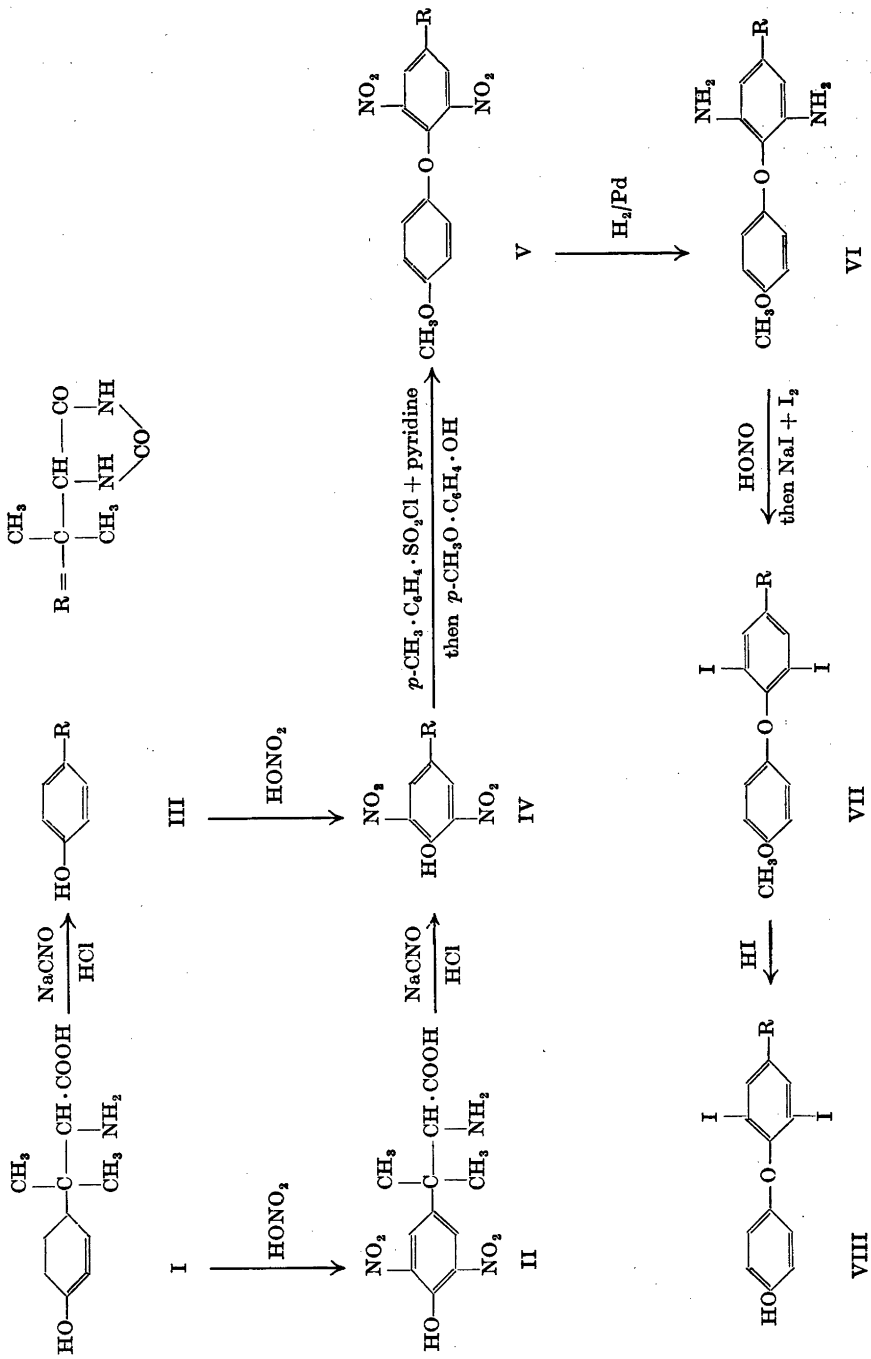

Fig. 2.

The steps involved are shown in Fig. 1. The side chain of the aminoacid was protected during these reactions by esterification of the carboxyl group and acetylation of the amino nitrogen. A series of reactions were also carried out during which the side chain was protected by conversion into a hydantoin ring. This route had, however to be abandoned as all attempts to hydrolyse 5-(α,α-dimethyl-3,5-diiodo-4-*p*-hydroxyphenoxybenzyl)hydantoin (VIII) and its methyl ether (VII) to the free aminoacid under a variety of acid and alkaline conditions failed. This was probably due to steric hindrance by the bulky substituent in position 5. (It is known that hydantoins doubly substituted in position 5 are much more resistant to hydrolysis than the monosubstituted ones. Probably a single *tertiary* alkyl group has a similar effect.) The reaction sequence is shown in Fig. 2.

The intermediate N-acetyl-3,5-dinitro-"*neo*tyrosine" (IX) was prepared by two ways, either by nitration of "*neo*tyrosine" followed by acetylation, or by carrying out these steps in the opposite order. Though it normally gave somewhat lower yield the latter method is to be preferred because the nitration of "*neo*tyrosine" was a little erratic. In most cases high yields were obtained but in a few experiments were almost zero. The reason for this could not be found.

The esterification of (X) met with some difficulties, as it was found that esterification by azeotropic distillation with chloroform, found so useful for N-acetyl-3,5-dinitrotyrosine by Chalmers *et al.*[5], was not successful with the α,α-dimethyl analogue. Esterification in a large excess of ethanol with *p*-toluenesulphonic acid as catalyst afforded a low yield of the ester; the main product was a neutral solid, which was not further investigated. (The authors cited report, that a similar result was obtained, when they tried to esterify N-acetyl-3,5-dinitrotyrosine under Fischer-Speier conditions.) It was ultimately found that esterification with a mixture of ethanol and thionyl chloride afforded the desired product in excellent yield. As the diamine (XIII) was sensitive to air at least in an impure state it was converted directly into the diiodo compound (XIV). This was obtained as a glass which could not be induced to crystallise. Simultaneous demethylation, deacetylation and hydrolysis of the ester group by hydriodic acid, however, converted the crude (XIV) into the crystalline 3,5-diiodo-D,L-"*neo*thyronine" (XV). Iodination of (XV) with iodine in aqueous ethylamine produced D,L-"neothyroxine" in a good yield.

EXPERIMENTAL *

3,5-Dinitro-D,L-"*neotyrosine*", D,L-α-*amino*-β-*(3,5-dinitro-4-hydroxyphenyl)isovaleric acid* (II). "*Neo*tyrosine" (25 g) was added in portions to concentrated sulphuric acid (90 ml) at 5 to 10°. The solution was cooled to −5° and stirred while nitric acid (17.5 ml, *d* 1.42) was added drop by drop. After the addition was complete the mixture was stirred at 0° for 15 minutes, poured onto crushed ice (400 g) and the pH brought to 4.0−4.5 by careful addition of 30 % aqueous sodium hydroxide solution, the temperature being kept below 20°. After some hours the precipitate was collected, washed with water and dried. Yellow needles. Yield 32 g (89 %). For analysis a sample was crystallised from aqueous ethanol and then melted at 222° (decomp.). (Found: C 44.2; H 4.63; N 13.8. $C_{11}H_{13}N_3O_7$ requires: C 44.2; H 4.38; N 14.0.)

* All melting points uncorrected. Petrol refers to the fraction b.p. 40—60°.

*N-Acetyl-*D,L-*"neotyrosine"* (IX). D,L-*"Neo*tyrosine (20.9 g) was dissolved in 2 *N* sodium hydroxide (225 ml) and acetylated below 20° by dropwise addition of acetic anhydride (20 ml) with constant stirring which was continued for a further hour after the addition. Excess anhydride was destroyed by stirring at 40° for ½ hour, and the reaction product was precipitated by addition of 6 *N* hydrochloric acid to the cooled solution. After storing overnight in the refrigerator the solid was filtered off. An additional amount could be obtained on concentration of the mother liquor. Total yield of product melting at 178–180° (lit.[3] 181–181.5°) 20.0 g (83 %).

*N-Acetyl-3,5-dinitro-*D,L-*"neotyrosine"* (X). *a) From N-acetyl-*D,L-*"neotyrosine"*. The foregoing N-acetylderivative (18.0 g) was added to conc. sulphuric acid (75 ml) with stirring below 10°. Nitric acid (*d* 1.42; 10 ml) was added drop by drop below −5°. Stirring was continued for one further hour after complete addition, and the reaction mixture was then poured onto ice. After some hours the solid was filtered off, carefully washed with water and dried. Yield 20.8 g (80 %). For analysis the compound was crystallised from aqueous ethanol, yellow needles, m. p. 222–225°. (Found: C 45.6; H 4.68; N 12.2. $C_{13}H_{15}N_3O_8$ requires: C 45.7; H 4.43; N 12.3.)

b) *From 3,5-dinitro-*D,L-*"neotyrosine"*. The nitrocompound was acetylated as described above for *"neo*tyrosine". Yield 90 %. M. p. 222–225° alone and in admixture with the compound just described.

*N-Acetyl-3,5-dinitro-*D,L-*"neotyrosine" ethyl ester* (XI). Thionyl chloride (7.0 ml) was added to absolute ethanol (60 ml) in dioxan (100 ml). After 15 minutes the foregoing acetylamino acid (32 g) was added and the mixture heated for 3 hours on a steam bath. Dioxan and excess ethanol were removed under reduced pressure, and the residual solid crystallised from aqueous ethanol. Yield 27.2 g (79 %) of yellow needles, m. p. 145–145.5°. (Found: N 11.2; C_2H_5O 12.0. $C_{15}H_{19}N_3O_8$ requires: N 11.4; C_2H_5O 12.2.)

*N-Acetyl-3,5-dinitro-4-p-methoxyphenoxy-*D,L-*"neophenylalanine" ethyl ester* (XII). The foregoing ester (36 g) and *p*-toluenesulphonyl chloride (19.5 g) in dry pyridine (125 ml) were heated on the water bath for ½ hour. *p*-Methoxyphenol (36 g) was added and the mixture was refluxed in an oil bath for 1 ½ hours. The solvent was removed *in vacuo* on the water bath and the residual dark oil dissolved in chloroform and washed in turn with *N* sodium hydroxide, 2 *N* hydrochloric acid and water. After drying (Na_2SO_4) the solution was filtered through a short column of aluminium oxide. Evaporation of the solvent yielded a solid, which was crystallised from aqueous ethanol and then formed yellow needles, m. p. 124–126°. Yield 33.0 g (74 %). For analysis a sample was repeatedly recrystallised and then melted at 127–128°. (Found: C 55.7; H 5.45; N 8.65. $C_{22}H_{25}N_3O_9$ requires: C 55.6; H 5.30; N 8.84.)

*3,5-Diiodo-*D,L-*"neothyronine"* (XV). The foregoing diphenyl derivative (19.5 g) in glacial acetic acid (200 ml) was hydrogenated at room temperature and pressure using a 10 % palladium on charcoal catalyst (2 g). The filtered solution was evaporated to approximately 50 ml under reduced pressure in a nitrogen atmosphere and added with stirring to conc. sulphuric acid (25 ml) below 25°. This solution was added drop by drop below 0° with constant stirring to a solution of nitrosylsulphuric acid prepared by dissolving sodium nitrite (9 g) in conc. sulphuric acid (60 ml) and dilution of this mixture with glacial acetic acid (125 ml) below 20°. Stirring was continued for a further hour after complete addition and then urea (5 g) was added. The solution was stirred for 15 minutes and then slowly poured into a stirred solution of iodine (35 g) and potassium iodide (45 g) in water (600 ml) covering a layer of chloroform (150 ml). The addition required 15 minutes, stirring being continued for one hour after complete addition. The chloroform was separated, and the aqueous layer extracted twice with chloroform. The combined chloroform solutions were washed with water, saturated with sulphur dioxide and again washed with water. The solvent was removed on the water bath, and the residual dark oil dissolved in a little benzene and filtered through a short column of aluminium oxide. The column was washed with much benzene, and the combined filtrates freed from solvent under reduced pressure. A yellow glass (15.1 g) was obtained which could not be induced to crystallise. It was refluxed for 8 hours with 57 % hydriodic acid (50 ml) and acetic acid (50 ml). The solution was evaporated *in vacuo* on the water bath and the residual syrup was dissolved in hot 50 % ethanol (75 ml) and treated with sulphur dioxide. The amino acid was precipitated from this solution by the addition of a saturated solution of sodium acetate and filtered off after keeping for some hours in the refrigerator. The crude material was purified by dissolving in boiling 50 % ethanol containing a little conc.

hydrochloric acid and decolourising with animal charcoal and then reprecipitated by the addition of a boiling solution of sodium acetate containing a little sodium sulphite to give a faintly greyish crystalline powder which melted with decomposition at 218—220°. Yield 12.2 g (53 % calculated on the nitroderivative). (Found: C 38.2; H 3.52; I 44.3. $C_{17}H_{17}I_2NO_4$ requires: C 36.9; H 3.10; I 45.9.)

D,L-"*Neothyroxine*" (XVI). The foregoing di-iodo compound (4.2 g) was dissolved in 33 % ethylamine (50 ml) and iodinated by slow addition, with stirring, of a 1.0 *N* solution of iodine in saturated aqueous potassium iodide (30.5 ml). After a short while the solution was diluted with water (200 ml) and the "*neo*thyroxine", the ethylamine salt of which had started to separate, was precipitated by addition of 4 *N* hydrochloric acid to pH 4—5. The crude product was collected after some hours and washed well with water. It was purified by dissolving in a boiling mixture of ethanol (125 ml) and 2 *N* sodium hydroxide (50 ml), decolourising with animal charcoal, filtering and reprecipitation by the addition of boiling 2 *N* hydrochloric acid to pH 4—5. Faintly yellowish crystal powder, which decomposed without melting at 192—195°. Yield 4.9 g (80 %). (Found C 25.7; H 1.82; I 61.2. $C_{17}H_{15}I_4NO_4$ requires: C 25.4; H 1.88; I 63.1.)

D,L-*5-(α,α-Dimethyl-4-hydroxybenzyl)hydantoin* (III). D,L-"*Neo*tyrosine" (31 g), sodium cyanate (20 g), and water (150 ml) were refluxed for 15 minutes, additional sodium cyanate (10 g) was added, and the mixture refluxed for a further hour, cooled and acidified with conc. hydrochloric acid. After a few hours the colourless precipitate was collected and refluxed for one hour with 6 *N* hydrochloric acid (150 ml). After cooling the solid was filtered off and washed with water. Crystallisation from aqueous acetic acid yielded small colourless needles, (24 g, 69 %), m. p. 290—295° (decomp.). (Found: C 61.6; H 6.26; N 12.0. $C_{12}H_{14}N_2O_3$ requires: C 61.5; H 6.03; N 12.0.)

D,L-*5-(α,α-Dimethyl-3,5-dinitro-4-hydroxybenzyl)hydantoin* (IV). *a) From 3,5-dinitro-*D,L-"*neotyrosine*". The hydantoin was obtained in 75 % yield when 3,5-dinitro-D,L-"*neo*tyrosine" was treated as described above for D,L-"*neo*tyrosine". Yellow prisms from ethanol, m. p. 236—237° (decomp.). (Found: C 44.7; H 3.99; N 17.3 $C_{12}H_{12}N_4O_7$ requires: C 44.5; H 3.73; N 17.3.)

b) From D,L-*5-(α,α-dimethyl-4-hydroxybenzyl)hydantoin.* This hydantoin (20 g) was added with stirring to nitric acid (100 ml, *d* 1.42) at 30—40°. After the addition the mixture was stirred for a further 2 hours and then poured into ice and water (400 ml). The solid was filtered off, washed and dried. Yield 17.5 g (63 %). M. p. and mixed m. p. with a specimen prepared by method a) 234—236°.

D,L-*5-(α,α-Dimethyl-3,5-dinitro-4-p-methoxyphenoxybenzyl)hydantoin* (V). The foregoing dinitrohydantoin (25 g), *p*-toluenesulphonyl chloride (20 g) and dry pyridine (125 ml) were mixed and refluxed on an oil bath for 15 minutes. *p*-Methoxyphenol (36 g) was added and the solution refluxed for a further hour. Most of the pyridine was removed *in vacuo* on a water bath, and the residual oil poured into *N* hydrochloric acid (500 ml). The dark oil, which separated, crystallised on cooling and scratching. The solid was filtered off, carefully washed with *N* hydrochloric acid and water, dried, and dissolved in a little boiling acetone. Some undissolved material was filtered off, and water was added to the hot solution to turbidity. The solution was allowed to cool very slowly overnight, and the yellow needles (22 g) collected. Recrystallised from aqueous acetone the product melted at 133—134°. (Found: CH_3O 7.0; N 12.8. $C_{19}H_{18}N_4O_8$ requires: CH_3O 7.2; N 13.0.)

D,L-*5-(α,α-Dimethyl-3,5-diamino-4-p-methoxyphenoxybenzyl)hydantoin* (VI). The foregoing diphenyl ether (10.4 g) in glacial acetic acid (250 ml) was hydrogenated at room temperature and pressure with 10 % palladium on charcoal catalyst (1 g). The hydrogen uptake was very slow but quantitative. After complete reduction the catalyst was filtered off and the filtrate evaporated *in vacuo*, these and subsequent operations being carried out in an atmosphere of nitrogen. A light brown gum was obtained, which was dissolved in anhydrous ethanolic hydrogen chloride, evaporated to dryness *in vacuo*, freed from excess hydrogen chloride by drying *in vacuo* overnight over potassium hydroxide, and dissolved in a small volume of anhydrous ethanol. Careful addition of much dry ether yielded a solid precipitate, which was filtered off, washed with ether, and dried *in vacuo*. Yield 9.8 g. As the product was very difficult to purify and sensitive to air when moist, it was used for the next step without further purification. (For this purpose the original gum after evaporation of the acetic acid could be used as well.)

D,L-*5-(α,α-Dimethyl-3,5-diiodo-4-p-methoxyphenoxybenzyl)hydantoin* (VII). The foregoing diamine hydrochloride (7.5 g) was dissolved in a mixture of glacial acetic acid (70 ml) and concentrated sulphuric acid (70 ml) and added dropwise with stirring below −2° to a solution of nitrosylsulphuric acid, prepared by dissolving sodium nitrite (3.5 g) in cold concentrated sulphuric acid (40 ml) and diluting with glacial acetic acid (40 ml). Stirring and cooling was continued for one hour after the addition was complete, and excess diazotising agent was destroyed by addition of a little urea and stirring for 15 minutes. This mixture was added slowly to a stirred solution of sodium iodide (20 g) and iodine (21 g) in water (350 ml), the temperature being kept below 40° by addition of ice. Stirring was continued for a further 4 to 6 hours, and the clear solution was decanted from the heavy, dark oil, which was ground with sodium bisulphite solution until completely solid and free from excess iodine. The solid was collected and thoroughly washed with water. Faintly brown crystal powder. Yield 9.2 g. This crude product was further purified by filtration in acetone solution through a short column of aluminium oxide. The filtrate was evaporated to dryness, and the solid residue crystallised from chloroform-petrol. Minute, colourless platelets, m. p. 258−259° (decomp.) (7.2 g). (Found: CH_3O 5.32; N 4.70. $C_{19}H_{18}I_2N_2O_4$ requires: CH_3O 5.24; N 4.73.)

D,L-*5-(α,α-Dimethyl-3,5-diiodo-4-p-hydroxyphenoxybenzyl)hydantoin* (VIII). The foregoing methoxydiiodocompound (7.0 g) was refluxed with a mixture of glacial acetic acid (20 ml) and 57 % hydriodic acid (20 ml). It dissolved rapidly, and the hydroxycompound soon started to separate. Heating was continued for one hour, water was added to turbidity and the mixture was stored overnight in the refrigerator. The crystals were collected and washed with aqueous acetic acid. Clusters of minute colourless spears. Yield 5.7 g. For analysis a sample was crystallised from aqueous acetic acid. The decomposition point of the compound varied between 280−290°. (Found: C 37.6; H 2.9; I 43.8; N 4.9. $C_{16}H_{16}I_2N_2O_4$ requires: C 37.4; H 2.8; I 43.9; N 4.8.)

The authors wish to acknowledge their indebtedness to *Statens Tekniska Forskningsråd* for a Fellowship to one of them (Å. J.) and to *Cancernämnden* for financial support.

REFERENCES

1. Clayton, J. C., Green, G. F. H. and Hems, B. A. *J. Chem. Soc.* **1951** 2467.
2. Cookson, R. C. and Green, G. F. H. *J. Chem. Soc.* **1952** 827.
3. Jönsson, Å. *Acta Chem. Scand.* **8** (1954) 1203.
4. Borrows, E. T., Clayton, J. C. and Hems, B. A. *J. Chem. Soc.* **1949** S 199.
5. Chalmers, J. R., Dickson, G. T, Elks, J. and Hems, B. A. *J. Chem. Soc.* **1949** 3424.

Received November 1, 1954.