CXXIX. NOTE ON THE ACETYL DERIVATIVES OF THYROXINE.

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IN the course of some experiments in another laboratory [Gaddum, 1929] regarding the physiological activity of various derivatives of thyroxine, it became desirable to include the "acetylthyroxine" of Kendall and Osterberg [1919] in the series of compounds under investigation. Further, the precise nature of the substance designated "acetylthyroxine" by Kendall is left open to doubt by the description given in his paper. It was, therefore, thought to be worth while to study the compound further from the chemical as well as from the physiological side. The physiological results are contained in a paper by Gaddum [1929]; the present note deals with the chemical investigation which has revealed an unexpected and interesting point.

The first experiment consisted in carrying through a preparation precisely as described by Kendall and Osterberg [1919]; an alcoholic solution of the sodium salt of thyroxine was treated with excess of acetic anhydride, the product was dissolved in alcohol and the solution poured into a large volume of boiling dilute sulphuric acid. The crystalline compound obtained in this way gave no nitrous acid reaction and was insoluble in boiling dilute sodium carbonate; it was evident, therefore, that both the phenolic and carboxylic groups were blocked, *i.e.* that the substance was, in reality, the ester of di-acetylthyroxine; this conclusion was confirmed by analysis, and we then proceeded to try the reaction on another amino-acid to see whether the phenomenon of simultaneous acetylation and esterification could be observed in an independent instance. On subjecting phenylalanine to similar treatment we were able in fact to demonstrate the conversion of part of the amino-acid into the ester of its acetyl derivative. It appears, therefore, that the reaction is a general one, and there can be little doubt but that it proceeds through the stage of the azlactone. Bergmann, Stern and Witte [1926] have demonstrated the readiness with which phenylalanine, on treatment with acetic anhydride, is converted into the azlactone

and compounds of this type are known to pass with ease, on treatment with

water or alcohol, to the corresponding acid or ester respectively. In the case under consideration, alcohol being present in excess from the beginning, the existence of the azlactone would naturally be only transient. In any case it is sufficiently obvious that this treatment of thyroxine does lead to simultaneous complete acetylation and esterification, and that the "acetyl sulphate" described by Kendall and Osterberg [1919] (which was, however, not analysed for sulphur) was in fact the diacetylthyroxine ester; the figures given by Kendall for the iodine content of this compound agree well with those required by the ester in question.

In his paper Kendall describes a second method of "purification" of the acetyl derivative, in which the reaction product obtained by treatment of thyroxine with acetic anhydride in alcoholic solution is dissolved in alcohol to which has been added excess of strong sodium hydroxide, the alcohol being subsequently removed by distillation under diminished pressure, and the product isolated as the sodium salt. This product is, needless to say, entirely different from that obtained by the first method of preparation outlined above. It is an acid, and gives an intense nitrous acid reaction, and we were able without difficulty to demonstrate its identity with *N*-acetylthyroxine prepared by an entirely different method.

In his book on thyroxine Kendall [1929] devotes a paragraph to the discussion of the acetyl derivative; in this he seems to have become aware of the complete acetylation accompanying treatment with acetic anhydride and alcohol and he describes yet another method of working up the reaction product, which involves the use of sodium hydroxide, and again leads to *N*-acetylthyroxine; he makes, however, no mention of the esterification which we have shown to take place.

EXPERIMENTAL.

Diacetylthyroxine ethyl ester. According to the instructions of Kendall and Osterberg [1919], 100 mg. of thyroxine were dissolved in 20 cc. of alcohol containing 100 mg. of sodium hydroxide; the solution was treated with 2 cc. of acetic anhydride and allowed to stand for 30 minutes; 5 cc. of water and 5 cc. of 50 % sulphuric acid were added, and the alcohol was removed by distillation under diminished pressure, the temperature of the bath not being allowed to rise above 40°; the white solid which separated was filtered off, washed with water and dissolved in 15 cc. of alcohol; the filtered solution was then poured into 200 cc. of boiling water containing 5 cc. of 50 % sulphuric acid. After standing overnight the product was filtered off and dried. It was recrystallised by dilution with water of its solution in a mixture of four parts alcohol and one part methyl ethyl ketone, and was thus obtained in clusters of colourless needles, which melted at 216–217° after sintering at 213°.

The compound failed to give the nitrous acid reaction, and was insoluble in sodium carbonate solution even at the boiling-point. On analysis it gave figures in agreement with those required by diacetylthyroxine ethyl ester.

Analysis. 64.0 mg. gave 0.974 mg. N (micro-Kjeldahl).
62.4 mg. gave 65.4 mg. AgI.

	N	I
Found	1.5 %	57.1 %
Calculated for $C_{21}H_{19}O_6NI_4$	1.6	56.6

Action of acetic anhydride on phenylalanine in alcoholic solution. *dl*-Phenylalanine (1.65 g.) was dissolved in alcohol (30 cc.) in which 0.23 g. of sodium had been dissolved; 5 cc. of acetic anhydride were added and the mixture was allowed to stand for 30 minutes. At the end of this time the alcohol was removed by distillation under diminished pressure. On addition of water to the residue a part passed into solution; the insoluble oil remaining was taken up in ether, and the ethereal solution was washed with dilute sodium carbonate, dried and evaporated. The residue consisted of a light brown oil which, on keeping overnight, crystallised. It was distilled and had B.P. 155–160° at 2 mm.; the distillate solidified at once, and, on recrystallisation from a mixture of ether and light petroleum, yielded fine colourless needles melting at 67°.

Cherbuliez and Plattner [1929] have recently described acetylphenylalanine ethyl ester, resulting from the action of acetic anhydride on phenylalanine ester, as having B.P. 151–157° at 2 mm. and M.P. 68°. We ourselves prepared some of the ester by esterification of acetylphenylalanine; the product had M.P. 68°, and the melting-point of a mixture of this preparation with the product of the action of acetic anhydride and alcohol on phenylalanine melted at 67–68°.

N-Acetylthyroxine methyl ester. A solution of 0.8 g. of thyroxine methyl ester [compare Ashley and Harington, 1928] in 16 cc. of redistilled anisole was cooled to 0° and treated gradually with 0.04 g. of acetyl chloride dissolved in 2 cc. of anisole; after standing for some time at 0°, the precipitate of thyroxine methyl ester hydrochloride was filtered off and the filtrate evaporated under diminished pressure, the temperature not being allowed to rise above 50°. The residue was dissolved in warm benzene; on spontaneous evaporation of the solvent, the ester crystallised out; on recrystallisation from anisole it formed colourless plates of a somewhat irregular shape, melting at 208–209° with decomposition after sintering at 205°.

Analysis. 1.02 mg. required 5.8 cc. *N*/200 thiosulphate [Kendall, 1914].

	I
Found	60.7 %
Calculated for $C_{18}H_{15}O_5NI_4$	61.0

N-Acetylthyroxine. The above ester was dissolved in 50 % alcoholic *N* sodium hydroxide (two equivalents); after keeping for 30 minutes at the ordinary temperature the solution was neutralised with the calculated amount of *N* hydrochloric acid. The precipitated acid was at first oily but rapidly hardened; for purification it was dissolved in boiling dilute sodium carbonate, the sparingly soluble sodium salt, which separated on cooling, being filtered

off and decomposed with dilute hydrochloric acid. The free acid was crystallised by pouring its concentrated solution in hot glacial acetic acid into a large volume of boiling water. The substance gave an intense nitrous acid reaction. On heating it sintered at 205° and melted with decomposition at 210–215°.

Analysis. 50.7 mg. gave 0.816 mg. N (micro-Kjeldahl).

0.74 mg. required 4.28 cc. *N*/200 thiosulphate [Kendall, 1914].

	N	I
Found	1.6 %	61.7 %
Calculated for $C_{17}H_{13}O_5NI_4$	1.7	62.0

The above compound was identical in all respects with products obtained (a) by the second "method of purification" described by Kendall and Osterberg in their paper [1919], and (b) by the method more recently described by Kendall in his book [1929].

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