LETTERS TO THE EDITORS

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Formation of Derivatives of Thyroxine from Derivatives of Diiodotyrosine

THE observation of von Mutzenbecher[1] that traces of thyroxine are formed on incubation of an alkaline solution of diiodotyrosine was developed by Harington and Pitt Rivers[2]. These authors showed that the course of the reaction was greatly influenced by *p*H; the largest yield of thyroxine, amounting to 2·7 per cent calculated on the diiodotyrosine not recoverable at the end of the oxidation, was obtained at *p*H 10; at this *p*H the acidic groups of diiodotyrosine are 95 per cent neutralized. In highly alkaline solution, there was little destruction of diiodotyrosine and no thyroxine formation: at lower alkalinity (*p*H 8–9) the destruction of diiodotyrosine was very extensive and the formation of thyroxine was minute. In all experiments in which diiodotyrosine was oxidatively destroyed, tarry by-products appeared and the liberation of ammonia was evident.

It seemed reasonable to suppose that protection of the amino-group of diiodotyrosine by acylation would diminish the tendency towards oxidative destruction of the amino-acid, and might then favour the oxidative intramolecular coupling which is the essential step in thyroxine formation. As a preliminary test of this hypothesis, solutions of *N*-acetyl-*l*-diiodotyrosine were incubated at 37° for fourteen days, the *p*H varying from 6·5 (60 per cent neutralization) to 8·5 (90 per cent neutralization). At the end of the incubation, the solutions were made strongly alkaline and boiled under reflux to hydrolyse the acetamino-group; butanol separation then led to the isolation of thyroxine. The amounts of thyroxine formed were small, the maximum formation occurring at *p*H 7·5.

It had been noticed that during these incubations a white crystalline salt slowly separated. In a further experiment at *p*H 7·5, this salt was collected after fourteen days. After decomposition with acid and purification of the product by the method of Ashley and Harington[3], there was obtained *N-acetyl-l-thyroxine*, m.p. 227–228° (decomp.), $[\alpha]_D^{25}$ 25·7° (c = 4 per cent in a mixture of equal volumes of alcohol and *N* sodium hydroxide). Hydrolysis of this compound by heating with a mixture of 15 parts concentrated hydrochloric acid and 25 parts of acetic acid yielded *l*-thyroxine, $[\alpha]_D^{21}$ −5·42° (c = 3·3 per cent in a mixture of 2 parts alcohol and 1 part *N* sodium hydroxide); found: I, 62·0 per cent; $C_{17}H_{13}O_5NI_4$ requires I, 62·0 per cent. The yield of acetylthyroxine calculated on the acetyldiiodotyrosine destroyed was 15–20 per cent.

These experiments showed clearly that the reaction of oxidative coupling of two molecules of diiodotyrosine to form a diphenyl ether is not confined to the free amino-acid, but on the contrary is favoured by acylation of the amino-group; moreover, with the acylamino-derivative the coupling occurs most readily at the *p*H of body tissues. In view of the obvious bearing of this result on the question of the formation of thyroxine in natural proteins, it was desirable to extend the experiments to a derivative of diiodotyrosine in which both amino- and carboxyl-groups were blocked. The derivative selected was *N*-acetyl-*dl*-diiodotyrosylglutamic acid.

A preliminary incubation of this compound at *p*H 7·2, followed by hydrolysis and butanol fractionation, did indeed lead to thyroxine formation; but in this case no insoluble sodium salt separated and the ultimate yield was low. The experiment was therefore repeated at the same *p*H in baryta solution; under these conditions a barium salt soon began to separate and increased in amount until a month had elapsed. At this time it was collected and decomposed with acid, and the product crystallized from aqueous methanol. The *N-acetyl*-dl-*thyroxylglutamic acid* thus obtained formed fine needles, m.p. 195–200° (decomp.); found: C, 27·8; H, 2·77; I, 52·5 per cent; $C_{17}H_{17}O_5NI_4$, H_2O requires C, 27·3; H, 2·3; I, 52·6 per cent. Hydrolysis of this compound with a mixture of acetic and hydrochloric acids yielded pure *dl*-thyroxine and glutamic acid. The yield of *N*-acetyl-*dl*-thyroxylglutamic acid was 36 per cent after allowing for recovered starting material. It is clear, therefore, that combination in peptide linkage of the carboxyl group of diiodotyrosine favours still more its conversion at biological *p*H into thyroxine.

In the experiments just described, the final reaction mixture is relatively free from tarry by-products; it is therefore hoped that it may be possible to isolate an acylamino-compound corresponding to the side-chain lost from one of the molecules of diiodotyrosine participating in thyroxine formation; attempts in this direction are now in progress.

A full account of the work described above will be published elsewhere.

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[1] von Mutzenbecher, P., *Z. Physiol. Chem.*, **261**, 253 (1939).
[2] Harington, C. R., and Pitt Rivers, R. V., *Biochem. J*, **39**, 157 (1945).
[3] Ashley, J. N., and Harington, C. R., *Biochem. J.*, **23**, 1178 (1929).

Condensation of 2 : 4 : 5-Triamino-6-hydroxypyrimidine with Glucose and Fructose

WE have been engaged for some time on a study of the condensation of 2 : 4 : 5-triamino-6-hydroxypyrimidine (I) with glucose and with fructose. As Ohle and Hielscher[1] have carefully established the optimal conditions for the condensation of *o*-phenylenediamine with glucose and with fructose in the preparation of tetrahydroxybutylquinoxaline, we have, from the outset, applied these conditions to (I). Glucose afforded a product (II) having $[\alpha]_D^{20°}$ approximately − 81·5° in *N* hydrochloric acid or in *N*/10 sodium hydroxide solution, while fructose afforded a substance (III) with a slightly higher specific rotation, $[\alpha]_D^{20°}$ approximately − 86·6°, under the same conditions. At this point, a paper by Karrer, Schwyzer, Erden and Siegwart[2] appeared describing the condensation of (I) with a variety of simple sugars using less elaborate conditions than ours. Their product from glucose had $[\alpha]_D^{20°}$ − 68·93°, and their product from fructose had $[\alpha]_D^{20°}$ − 46·0°, respectively, in *N*/10 sodium hydroxide solution; these figures are significantly different from ours, particularly in the case of the product from fructose. Although final conclusions were not reached, Karrer and his collaborators considered their products from glucose and fructose to be respectively (IV, or V ?) and (V, or IV ?), the constitution of their product from glucose being indicated as (IV) with some degree of certainty.