CXCVI. NOTE ON THE KETONIC ACID ANALOGOUS WITH THYROXINE.

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THE facts which are at present known regarding the relationship of chemical structure to physiological action in the thyroxine series indicate that the exhibition of typical thyroid-like activity by a compound depends on the presence in its molecule of the thyronine nucleus halogenated at least in the 3:5-positions.

Thus if we take thyroxine as our starting point we find that complete loss of physiological activity follows elimination of one of the benzene rings (3:5-diiodotyrosine), abbreviation of the side chain (thyroxamine) and total elimination of halogen (thyronine); on the other hand elimination of the 3':5'-iodine

Fig. 1. Comparison of effects of thyroxine and its ketonic acid analogue on the metabolism of a dog. The total excess Cals. are 160 or 11 Cals./mg./kg. for the thyroxine and 246 or 3 Cals./mg./kg. for the keto-acid.

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atoms only and substitution of iodine by bromine do not involve total loss of activity since 3:5-diiodothyronine and 3:5:3':5'-tetrabromothyronine both possess physiological properties which are qualitatively similar to those of thyroxine.

Since many amino-acids which perform essential metabolic functions can be effectively replaced in the diet by the corresponding keto-acids it appeared of interest to see whether the keto-acid corresponding to thyroxine would exhibit the typical physiological action of the latter.

The synthesis of this keto-acid was effected by well-known reactions and calls for no detailed comment. Its physiological action was examined by determination of its effect on the metabolic rate of a dog under standardised conditions, the methods employed being those described by Canzanelli and Rapport [1933]. The results are shown in Fig. 1 in which the effects of two separate doses of 10 mg./kg. of the keto-acid are compared with the effect of 2 mg./kg. of thyroxine. It will be seen that there is good agreement between the two experiments with the keto-acid and that the total excess metabolism produced amounts to 11 Cals./mg./kg. for thyroxine and to 3 Cals./mg./kg. for the keto-acid.

It is clear therefore that the keto-acid does exhibit the characteristic physiological activity of thyroxine, although in lower degree, and that here again we have an example of the ready interchangeability of amino- and keto-acids in the animal body.

EXPERIMENTAL.

Synthesis of 3:5-diiodo-4-(3':5'-diiodo-4'-hydroxyphenoxy)phenylpyruvic acid.

Azlactone from acetylglucine and 3:5-diiodo-4-(4'-methoxyphenoxy)benzaldehyde. An intimate mixture of the aldehyde [Harington and Barger, 1927] (5 g.), acetylglucine (1.22 g.) and freshly fused sodium acetate (5 g.) was treated with freshly distilled acetic anhydride (15 ml.) and the whole was heated for 3 hours on the steam-bath. After cooling the mixture was triturated with water and the yellow solid collected, washed with water and dried. Yield 6.0 g. Recrystallised from glacial acetic acid it formed yellow needles, m.p. 227° (decomp.). (Found: I, 46.2 %; $C_{18}H_{13}O_4NI_2$ requires I, 45.3 %.)

3:5-Diiodo-4-(4'-methoxyphenoxy)phenylpyruvic acid. The above azlactone (9.5 g.) was boiled under reflux for an hour with 30 % aqueous potassium hydroxide (50 ml.). After keeping overnight the somewhat sticky potassium salt was collected and dissolved in water; this solution was saturated with sulphur dioxide as was also the alkaline mother liquor. The two solids thus obtained were collected together, thoroughly washed with ether and dissolved in boiling water (300–400 ml.) with the aid of sodium carbonate; the solution whilst still hot was acidified to Congo red with hydrochloric acid; after cooling the acid was extracted with ether and the ethereal extract dried over sodium sulphate and evaporated. The residue was crystallised from glacial acetic acid from which it separated in needles, m.p. 205° (decomp.). (Found: I, 46.9 %; $C_{16}H_{12}O_5I_2$ requires I, 47.2 %.)

0.0877 g. required 1.65 ml. 0.1 N NaOH for neutralisation to phenolphthalein; whence mol. wt. = 531.5; calc. mol. wt. = 536.

The acid gave a dark green colour with ferric chloride in alcoholic solution.

3:5-Diiodo-4-(4'-hydroxyphenoxy)phenylpyruvic acid. The demethylation of the acid just described offered some difficulty owing to the destructive effect of the demethylating reagent on the keto-acid grouping; success was finally attained by reducing the duration of the reaction to a minimum as follows. The methoxyketo-acid (2.2 g.) was dissolved in 24 ml. of a mixture of equal parts of hydriodic acid (sp. gr. 1.7) and glacial acetic acid; the solution was boiled for

6 min., cooled and diluted to about 100 ml. with water. Free iodine was removed by addition of a little sodium thiosulphate and the solution was extracted five times with ether; the combined ethereal extracts were evaporated without drying and the residue treated with water and a little thiosulphate. The sticky precipitate which separated became partly crystalline on keeping overnight; it was collected, washed with a very little 30 % acetic acid and dried. Yield 1.32 g.

After several recrystallisations from 30 % acetic acid, in which it is easily soluble in the heat, the acid formed colourless plates, M.P. 156° after sintering. (Found: I, 48.3 %; $C_{15}H_{10}O_5I_2$ requires I, 48.5 %.)

3:5-Diiodo-4-(3':5'-diiodo-4'-hydroxyphenoxy)phenylpyruvic acid. The hydroxyphenoxyketo-acid (0.105 g.) was dissolved in a mixture of purified methyl alcohol (1 ml.) and concentrated aqueous ammonia (sp. gr. 0.880; 1 ml.). The solution was cooled in ice and treated, drop by drop with shaking, with the theoretical amount of concentrated solution of iodine in potassium iodide (0.286 ml. of 2.8*N*); the uptake of iodine was rapid at first but became sluggish after about half had been added. After all the iodine had been introduced the solution was kept for some time in ice until the colour had faded to yellowish-brown; it was then diluted with water, treated with a drop of bisulphite solution and acidified. The precipitate was collected, washed and dissolved in boiling water with the aid of the minimum of sodium carbonate; the sodium salt which separated on cooling was collected and decomposed by solution in hot water followed by acidification; the precipitated acid was finally collected and purified by two crystallisations from acetic acid slightly diluted with water. It formed colourless prisms, M.P. 173°, and gave an intense reaction with nitrous acid and ammonia. (Found: I, 65.0 %, $C_{15}H_8O_5I_4$ requires I, 65.3 %). The yield was poor.

Metabolic effect of the keto-acid.

The keto-acid was tested for its effect on the metabolism of an adult female dog living on a constant maintenance diet, the conditions of the experiments being similar to those described by Canzanelli and Rapport [1933], except that the material was administered subcutaneously. The results of two such experiments are shown graphically in Fig. 1 in comparison with the effect on the same animal of a dose of thyroxine. The relative magnitudes of the effects are calculated from the areas enclosed between the respective curves and the line representing the basal metabolic rate of the dog, and it will be seen that the activities of thyroxine and the keto-acid stand in the ratio of 11 : 3.

SUMMARY.

The ketonic acid analogous with thyroxine has been synthesised and shown to possess the characteristic physiological activity of the latter compound in lower degree; the ratio of the activities of thyroxine and of the keto-acid is about 11 : 3.

REFERENCES.

- Canzanelli and Rapport (1933). *Amer. J. Physiol.* **103**, 279.
 Harington and Barger (1927). *Biochem. J.* **21**, 169.