The Biological Action of Substances Related to Thyroxine

6. THE EFFECT OF COMPOUNDS CONTAINING FRAGMENTS OF THE THYROXINE MOLECULE ON THE OXYGEN CONSUMPTION OF MICE

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All the thyroxine-inhibitory compounds described by Woolley (1946), Frieden & Winzler (1949), Sheahan, Wilkinson & MacLagan (1951), Wilkinson, Sheahan & MacLagan (1951*a, b*) and Wilkinson, Sprott & MacLagan (1953) contain the group

in their molecules. This may be considered as a fragment of the thyroxine molecule of type I, as shown in the accompanying scheme. A number of other fragments can be visualized such as those of types II and III:

Compounds of type II are represented by the iodinated phenoxyacetic acids of Barker *et al.* (1950). Apart from 2':6'-diiodothyronine, reported to have antithyroxine activity by Cortell (1949), no compounds of type III appear to have been investigated. The present paper deals mainly with some examples of types II and III and with some compounds having a nitrogen atom meta to the iodine atoms (type IV).

2:6-Diiodophenol (Woollett, Davis, Jones & Neill, 1937) and 2:6-diiodoanisole were prepared as examples of type II substances by deamination of the corresponding 4-amino compounds. 2:6-Diiodo-*p*-cresol (2:6-diiodo-4-methylphenol) and its methyl ether, which can be grouped either in type I or II, were also tested. The quinol derivative, 3:5-diiodo-4-methoxyphenol, obtained by hydrolysis of 3:5-diiodo-4-methoxybenzenediazonium sulphate, was tested as an example of type III.

EXPERIMENTAL

Chemical methods

Melting points are uncorrected. The microanalyses were by Weiler and Strauss, Oxford.

2:6-Diiodo-4-aminophenol, m.p. 173–174°, was prepared by reduction of 2:6-diiodo-4-nitrophenol as described by Woollett *et al.* (1937), who reported its m.p. as 170°. The ON-diacetyl derivative crystallized from ethanol in colourless plates, m.p. 215–216°. (Found: C, 27.0; H, 1.7; I, 56.6. $C_{10}H_8O_5NI_2$ requires C, 27.0; H, 2.0; I, 57.1%.) 2:6-Diiodophenol was prepared by the method of Woollett *et al.* (1937) by deamination of the 4-aminophenol. Our product had m.p. 63–65° (lit., 67°). 2:6-Diiodo-4-nitrophenol was methylated with methyl sulphate by the method of Kalb, Schweizer, Zellner & Barthold (1926) and the resulting nitroanisole reduced to 2:6-diiodo-4-aminoanisole, m.p. 100°.

Kalb *et al.* (1926) report m.p. 100°. The acetyl derivative melted at 200° (lit., 200°). (Found: C, 26.1; H, 2.0; I, 60.7. Calc. for $C_9H_7O_2NI_2$: C, 25.9; H, 2.2; I, 61.0%.) The propionyl derivative crystallized from 70% ethanol in colourless needles, m.p. 152°. (Found: C, 27.6; H, 2.8; I, 58.7. $C_{10}H_{11}O_2NI_2$ requires C, 27.9; H, 2.6; I, 59.0%.)

2:6-Diiodoanisole. 2:6-Diiodo-4-aminoanisole (5 g.) was dissolved in a mixture of ethanol (125 ml.) and H_2SO_4 (4 ml.), and cooled to about 10°. The resulting suspension was diazotized in the usual manner with a solution of sodium nitrite (0.92 g.) in water (10 ml.). After 1 hr. at 10°, the mixture was gradually heated to boiling over a period of 30 min., and heated under reflux for a further hour. Most of the ethanol was removed by distillation and the residue made alkaline with NaOH, and distilled at 10⁻⁵ mm. 2:6-Diiodoanisole (yield 1.87 g., 39%) was obtained as a pale yellow oil, b.p. 63–66°. (Found: C, 23.5; H, 2.0; I, 70.7. $C_7H_5OI_2$ requires C, 23.3; H, 1.7; I, 70.6%.)

3:5-Diiodo-4-methoxyphenol. 2:6-Diiodo-4-aminoanisole (5 g.) was converted to the sulphate by boiling for 30 min. with H_2SO_4 (2.5 ml.) and water (30 ml.). After cooling to

Table 1. *Antithyroxine activities in mice of simple compounds containing fragments of the thyroxine molecule*

Compound	Type* of fragment	Total dose at which active (mg./kg.)	Inhibition of thyroxine effect at dose stated (%)	Significance (Student's <i>t</i> test)	Max. dose which failed to produce a response (mg./kg.)
2:6-Diiodo- <i>p</i> -cresol	I	400	49	$P < 0.01$	—
3:5-Diiodo-4-methoxytoluene	I	50	62	$P < 0.01$	25
2:6-Diiodophenol	II	—	—	—	400
2:6-Diiodoanisole	II	200	25	$P < 0.01$	100
3:5-Diiodo-4-methoxyphenol	III	200	47	$P < 0.01$	100
2:6-Diiodo-4-nitroanisole	IV	—	—	—	400
2:6-Diiodo-4-aminophenol	IV	—	—	—	400
3:5-Diiodo-4-acetoxyacetanilide	IV	—	—	—	400
3:5-Diiodo-4-methoxyacetanilide	IV	400	80	$P < 0.001$	—
3:5-Diiodo-4-methoxypropionanilide	IV	400	45	$P < 0.05$	—

* See text, p. 491.

0–10°, the suspension was treated with sodium nitrite (0.92 g.) in water (10 ml.). After 1 hr. at 10°, the mixture was gently boiled for a further hour, cooled, and extracted with ether. The ether layer was extracted with 0.5N-NaOH. The alkaline solution was washed with ether and acidified with HCl, and again extracted with ether. After removal of the solvent, the residue was distilled under 10⁻⁵ mm. pressure at 131–138°. Some difficulty was experienced during distillation and the yield was markedly reduced at this stage, but we were unable to obtain analytically pure material when this step was omitted. The distillate crystallized from benzene in buff needles, m.p. 126–127°. Yield, 0.57 g. (Found: C, 22.3; H, 1.8; I, 67.4. C₇H₆O₂I₂ requires C, 22.3; H, 1.6; I, 67.6%.)

3:5-Diiodo-4-methoxytoluene was obtained from 2:6-diiodo-*p*-cresol (Datta & Prosad, 1917) by the method of Wilkinson (1951).

Measurement of O₂ consumption

The compounds were tested for antithyroxine activity in mice by the O₂ consumption method of MacLagan & Sheahan (1950) as modified by Sheahan *et al.* (1951). Two groups of eight mice were used for each dose level.

RESULTS AND DISCUSSION

The results of the tests of antithyroxine activity are shown in Table 1. Of the ten simple compounds studied, six exhibited some thyroxine-inhibitory action, but it is clear that the type IV substances having nitrogen meta to the iodine atoms were only feebly active.

It is interesting to note that 3:5-diiodo-4-methoxytoluene displayed high activity; this is the simplest compound to do so. This result suggests that competition with thyroxine requires only the part of the molecule represented in compounds of types I and II. 2:6-Diiodoanisole and 3:5-diiodo-4-

methoxyphenol were moderately active, but 2:6-diiodophenol was without action. This suggests that some distribution effect governing rates of absorption and cell penetration, such as those discussed by Wilkinson (1953), is responsible for this difference in activity.

The variation in the reports of the activities of various substituted diiodophenoxyacetic acids could probably be explained in similar terms. Thus 2:4-diiodophenoxyacetic acid, found by Barker *et al.* (1950) to be highly active in a number of tests in rats, failed to produce any response in mice in our experiments. Several other diiodophenoxyacetic acids (Barnes, Elks, Stephens & Waller, 1953), however, did show antithyroxine activity by the present method of testing.

SUMMARY

1. The preparation of new simple thyroxine analogues is described.

2. 2:6-Diiodoanisole, 3:5-diiodo-4-methoxyphenol and 3:5-diiodo-4-methoxytoluene exhibited thyroxine-inhibitory activity at total dosages of 200, 200 and 50 mg./kg., respectively. These are the simplest compounds yet tested to show such an effect.

3. The evidence suggests that the diiodophenoxy group is responsible for the antithyroxine effect and that differences in the activity of analogous compounds are due mainly to variations in rates of absorption and cell penetration.

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The Biosynthesis of Kojic Acid

1. PRODUCTION FROM [1-¹⁴C] AND [3:4-¹⁴C₂] GLUCOSE AND [2-¹⁴C]-1:3-DIHYDROXYACETONE

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Kojic acid was first isolated by Saito (1907) from cultures of *Aspergillus oryzae* on steamed rice. It was shown to be 5-hydroxy-2-hydroxymethyl- γ -pyrone by Yabuta (1924). During the last 30 years kojic acid has been obtained as a metabolic product of many other fungi (mainly *Aspergilli*) and certain bacteria (for reviews of early work see Barham & Smits, 1934; Foster, 1949). As early as 1934 it was reported that kojic acid inhibited the growth of Gram-negative more strongly than that of Gram-positive bacteria (Reed & Bushnell, 1934). This property was rediscovered much later and the antibiotic action of culture filtrates of several fungi was shown to be due to the presence of kojic acid (Foster & Karow, 1945).

A striking feature of kojic acid biosynthesis is the wide variety of substrates which can be utilized for its production. Thus kojic acid is formed from C₂ compounds (e.g. ethanol, glycollic acid), C₃ compounds (e.g. glycerol, dihydroxyacetone), pentose and hexose sugars, C₇ compounds (quinic and shikimic acids), disaccharides and more complex polysaccharides (e.g. starch, dextrin).

Although the way in which kojic acid is formed from so large a number of substrates has been widely discussed, the precise nature of the biosynthetic process has remained virtually unknown. Several

authors (Yabuta, 1924; Kinoshita, 1927; Haworth, 1929) have pointed out the close structural similarity of kojic acid and the pyranose structure of glucose; since both compounds have the same number of carbon atoms and the hydroxymethyl groups at position 2 of the pyrone ring, it is formally possible that kojic acid may be produced from glucose by dehydration and oxidation. A chemical synthesis of kojic acid from acetobromoglucose has been described (Maurer, 1930; Stacey & Turton, 1946) but the theory that the biosynthesis proceeds directly from glucose without rupture of the carbon chain has in general found little support.

The main objection to this theory has been the fact that kojic acid can be formed from compounds having fewer carbon atoms than glucose. Thus, arabinose is converted to kojic acid (Challenger, Klein & Walker, 1929) and not, as might be expected, to 2-hydroxy- γ -pyrone, or alternatively, 3-hydroxy- γ -pyrone (pyromeconic acid). A possible explanation, however, was provided by Birkinshaw, Charles, Lilly & Raistrick (1931) who suggested that all carbon sources were first converted to a 'reserve carbohydrate' which after hydrolysis to a monosaccharide would yield kojic acid.

The following theory has received wider support: kojic acid is formed from these widely different compounds by primary splitting to a common, small, reactive molecule (e.g. C₂ or C₃ fragments) followed by condensation directly to kojic acid

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