[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND CHEMISTRY, UNIVERSITY OF SOUTHERN CALIFORNIA]

A NEW "*META*" ANALOG OF THYROXINE

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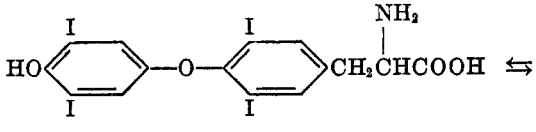
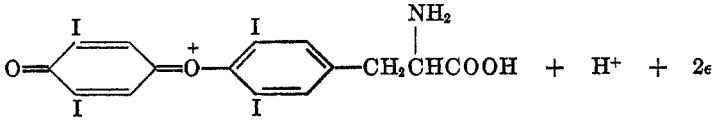
The contention that the diversified physiological responses brought about by thyroxine (I) are associated with its participation in an oxidative reaction has been prevalent since the early considerations of Plummer and Kendall (1). Niemann and co-workers (2a, b, c) suggested the interesting working hypothesis that thyroxine and its analogs may be involved in the following redox equilibrium.

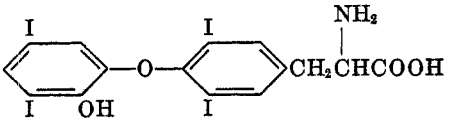


I

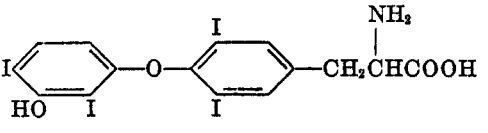


II

The basis for the latter suggestion was the observation that structure III showed low thyroxine activity in the rat, whereas a compound to which structure IV was originally assigned was found to be inactive (2). The lack of activity of IV was explained on the basis that this compound—having the phenolic hydroxyl



III



IV

group in position *meta* to the oxygen ether linkage—should be incapable of a quinoid structure (as required by the hypothesis), whereas either I or III may assume the quinoid structures.

Bruice, Winzler, and Kharasch (3) have recently found that if the alanyl side chain of I is replaced by a propionic acid side chain, the resulting compound (V) shows a remarkably high activity (*ca.* 130 times the activity of D,L-thyroxine) for tadpole metamorphosis. Since this activity is the highest yet found for tadpole metamorphosis, and in view of the Niemann-Mead hypothesis, it seemed of

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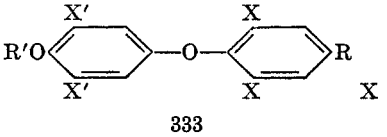
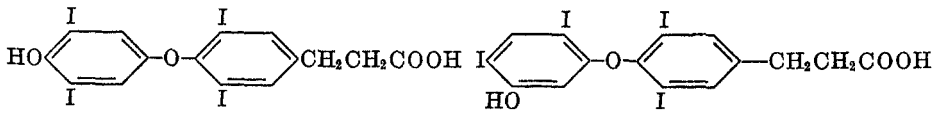
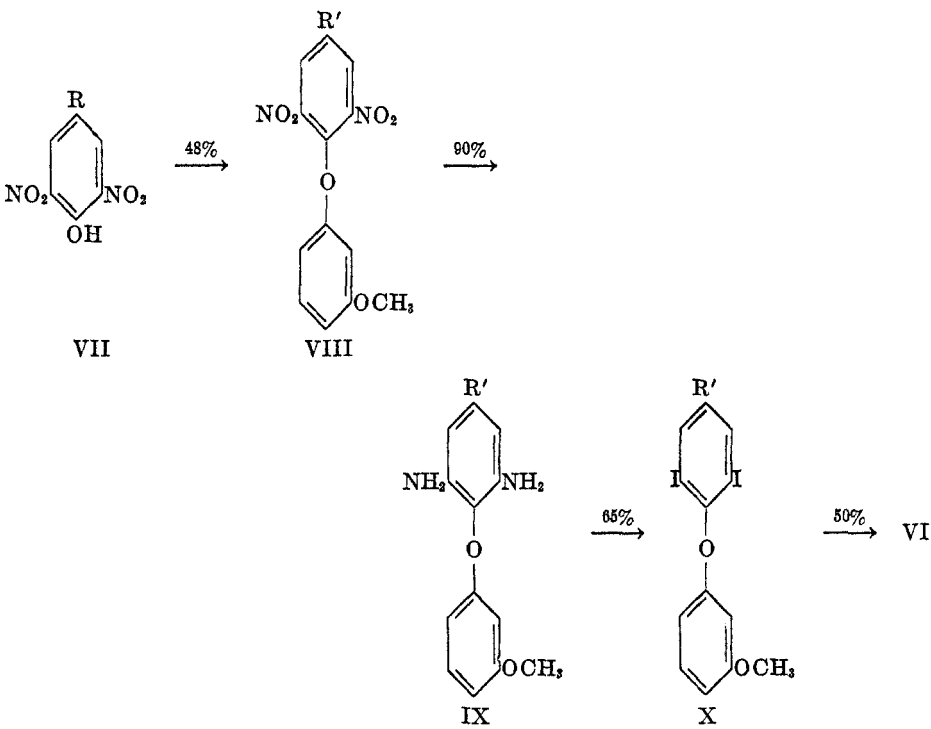


interest to prepare a corresponding "*meta*" analog with the propionic acid side chain (VI), and to test its activity in tadpole metamorphosis. The work toward this end is reported in the present paper.



V VI

The synthetic sequence leading to the compound to which structure VI is assigned is shown below (R = $-CH_2CH_2COOH$; R′ = $-CH_2CH_2COOC_2H_5$). The details of the synthesis are given in the experimental part.



VII VIII IX X VI

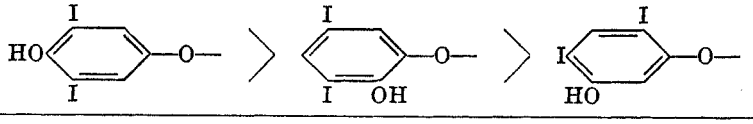
The disposition of the iodine atoms in the phenolic nucleus of the final product (designated as VI) is not completely certain. It seems most reasonable to assume that VI is the correct structure (that is, that iodination occurred in positions 2 and 4 relative to both the ether linkage and the phenolic hydroxyl group), since it is known that the mono-methyl ether of resorcinol and similar compounds yield 2,4- rather than 2,6-disubstituted products when treated with electrophilic reagents (4, 5), and the product did not give a Kendall test (6, 7) when treated with nitrous acid and ammonia. In view of the lack of data regarding the Kendall test, as applied to 3-substituted 2,6-diiodophenols, the negative test above is only suggestive of the alternate structure (VI). Niemann (2c) has re-

cently pointed out that the structure originally assigned to his "*meta*" thyroxine analog (IV) is subject to doubt as regards the location of the iodine atoms. Experimental support for the structure first assigned (IV) was not given in the original paper (2a); and it would seem more reasonable that the disposition of the iodine atoms in Niemann's product is as proposed above for the analogous compound (VI).

Compound VI was assayed on the larvae of *Rana catesbeiana* by a method recently developed in this laboratory (3). Ten gamma *per* ml. produced a response analogous to that obtained by the use of 0.1 gamma *per* ml. of *l*-thyroxine. Because of its toxicity the compound could not be given *in toto* in higher doses. However, when the animals were successively subjected to 10, 5, 5, and 5 gamma *per* ml. of VI for 48 hour periods, their response increased to that obtained by the single exposure to 0.3 gamma *per* ml. of *l*-thyroxine for a 48 hour period. The effects upon the larvae were entirely similar to those of thyroxine (a short period of no visible response, followed by a prolonged period of response—continuing a week after withdrawal of the drug—and marked by resorption of the tail, appearance of left fore-limb, a respiratory rate approximately twice that of the controls, and typical changes in the eye and mouth structures). On this basis, it is concluded that compound VI possesses approximately 2% thyroxine-like activity based on D,L-thyroxine as 100% in the larvae of *Rana catesbeiana*. An assay was also carried out on *Xenopus laevis* larvae by the method of Deansly and Parkes (8). Compound VI proved to be lethal to these larvae at a concentration of 3.2 gamma *per* ml. At 2 gamma *per* ml., no response was obtained unless the animals were repeatedly subjected to the compound at 2 gamma *per* ml. for eight days—at which time all the test animals exhibited premature formation of fore-limb buds which did not erupt by the time of expiration.

From the evidence obtained in a related study (9),[2] it may be concluded that the activity of VI in rodents would be entirely too low to permit measurement. For this reason testing in mammalia was not attempted.

The present study permits the conclusion that if a two-electron oxidation mechanism is responsible for the varied physiological effects of thyroxine and its active analogs, then stabilization of any such oxidation product as in the case of VI, by a quinoid form, is not requisite to activity. The results suggest, moreover, that the greater is the positional deviation of a compound from thyroxine, the lower will be the biological response observed. Thus, activity in the above cases is in the order:



[2] The amphibian and mammalian responses to analogs as shown below have been found to parallel one another in their dependency upon the nature of the substituents R'O, X, and X'. Changes in R do not give parallel alterations in response between amphibia and mammalia. The mammalian responses are usually quite low, unless R is an alanyl group, whereas amphibian responses may actually be greater when R is represented by groups other than the alanyl side chain, as noted above in the case of V.

This observation accords with a correlative hypothesis of thyroxine-like activity *vs.* structure presented in another study (9).

EXPERIMENTAL[3]

Ethyl 3,5-dinitro-4-hydroxyhydrocinnamic acid. *p*-Nitrohydrocinnamic acid was prepared by the nitration of hydrocinnamic acid after the method of Konek and Pascu (10) (38% yield). The nitro-compound (0.1 mole, 18 g.) was reduced at 40 p.s.i. with hydrogen using a Raney nickel catalyst at 40° and 95% ethanol as solvent. After removal of the catalyst and solvent, the residual amine was taken up in 600 ml. of 10% sulfuric acid and diazotized (0–5°) by the addition of 100 ml. of a 5% solution of sodium nitrite (0.725 mole). After one hour of refrigeration 750 ml. of water was added, and the solution was heated to boiling, then chilled and extracted with an ether-chloroform mixture. After aspiration of the solvent and recrystallization from water, 13 g. (78% of theory) of phloretic acid, m.p. 128–129°, was obtained. Lit. m.p. 130° (11).

Phloretic acid (16.6 g.; 0.1 mole) dissolved in 100 ml. of acetic anhydride was nitrated, below 20°, by adding, cautiously, and with constant stirring, 13 ml. of concentrated nitric acid. After addition was complete the nitration mixture was warmed to room temperature then poured on 200 g. of cracked ice and water. The aqueous solution, when brought to the boil and allowed to cool slowly, with scratching, deposited yellow plates of 3,5-dinitro-4-hydroxyhydrocinnamic acid. After collection and desiccation this substance melted at 135° (18.8 g., 74%). Lit. m.p. 137.5° (12, 13).

When 18.8 g. (0.1 mole) of the above acid was esterified by the general Fisher technique (HCl), 14.3 g. (69%) of the corresponding ethyl ester (VII), m.p. 70–72° resulted. Lit. m.p. 74–75° (12).

Ethyl 3,5-dinitro-4-(3′-methoxyphenoxy) hydrocinnamate. Compound VII (5.68 g., 0.02 mole) was heated, with moisture excluded, on the steam-bath with 57 g. (0.03 mole) of tosyl chloride and 10 ml. of anhydrous pyridine, for 30 minutes. The pyridine was aspirated at 80°, and the residue was extracted with anhydrous petroleum ether (b.p. 88–99°) to remove residual tosyl chloride. To the crude pyridinium tosylate there was added 4.96 g. (0.04 mole) of pure resorcinol monomethyl ether, and the mixture was heated to 150–155° for 1.5 hours. The resultant residue, after cooling, was extracted with chloroform and the extract was washed successively with 1 *N* sodium hydroxide, 1 *N* hydrochloric acid, and water. After removal of solvent the crude product was recrystallized by the identical method as previously used for the preparation of 3,5-dinitro-4-(3′, 5′-dimethyl-4′-methoxyphenoxy)phenyl ethyl alanate (14). This gave 3.75 g. (48%) of VIII; almost colorless needles, m.p. 89–90°.

Anal. Calc'd for $C_{18}H_{18}N_2O_8$: C, 55.38; H, 4.65.
Found: C, 55.58; H, 5.00.

Ethyl 3,5-diamino-4-(3′-methoxyphenoxy)hydrocinnamate. The dinitro compound VIII was reduced and worked up by the procedure recorded for ethyl 3,5-diamino-4-(3′,5′-diamethyl-4′-methoxyphenoxy)phenyl alanate (14). The product was obtained in 90% yield; m.p. 89–91°. The preparation of an analytical sample by recrystallization from alcohol met with difficulty because the dissolved product was readily oxidized by air.

Anal. Calc'd for $C_{18}H_{22}N_2O_4$: C, 65.43; H, 6.73.
Found: C, 65.95; H, 6.95.

The *N,N′-diacetate*, m.p. 151–152°, was also prepared.

Anal. Calc'd for $C_{22}H_{26}N_2O_6$: C, 63.75; H, 6.32.
Found: C, 63.88; H, 6.30.

Ethyl 3,5-diiodo-4-(3′-methoxyphenoxy)hydrocinnamate. Tetrazotization of IX (0.82 g., 0.0025 mole) by the procedure reported for 3,5-diamino-4-(3′,5′-dimethyl-4′-methoxyphen-

[3] Melting points are uncorrected. The microanalyses were performed by Dr. A. Elek of the Elek Microanalytical Laboratories, Los Angeles, California.

oxy)phenyl ethyl alanate (14), followed by replacement of the tetrazo grouping with iodine, also as previously reported (14), gave 0.9 g. (65%) of X; m.p. 86–86.5°.

Anal. Calc'd for $C_{18}H_{18}I_4O_2$: C, 39.15; H, 3.29.
Found: C, 38.87; H, 3.33.

3,5-Diiodo-4-(2',4'-diiodo-5'-hydroxyphenoxy)hydrocinnamic acid. The ethyl ester (X) (0.76 g., 0.0014 mole) was refluxed with a 50% solution of acetic acid and hydriodic acid (57% aqueous) for 12 hours. Slow dilution of the chilled reaction mixture with water yielded long white needles of the intermediate, 3,5-diiodo-4-(5'-hydroxyphenoxy)hydrocinnamic acid, which were directly dissolved in 20 ml. of 33% aqueous ethylamine, then iodinated, at 5°, by adding excess iodine dissolved in the minimum quantity of concentrated, aqueous potassium iodide. After diluting with water and acidifying with hydrochloric acid, the amorphous material was collected and reprecipitated several times from sodium carbonate solution, by adding hydrochloric acid. The buff-colored amorphous material was dissolved in boiling 70% aqueous ethanol, acidified with a few drops of acetic acid, and the solution slowly was cooled to 5°, giving white needles of VI (525 mg., 50%; m.p. 203–204°). Several recrystallizations from ethanol, diluted with water, gave a product melting at 213°. This was the product used in the bio-assays, and which gave the following analysis. The analysis of other samples also gave results somewhat divergent from the calculated values.

Anal. Calc'd for $C_{15}H_{10}I_4O_4$: C, 23.65; H, 1.32; I, 66.63.
Found: C, 24.03; H, 1.44; I, 67.92.

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SUMMARY

A new "*meta*" analog of thyroxine [3,5-diiodo-4-(2',4'-diiodo-5'-hydroxyphenoxy)phenylpropionic acid] was prepared and found to be active in tadpole metamorphosis. The significance of this result is discussed in relation to the hypothesis of thyroxine activity previously proposed by Niemann and co-workers.

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