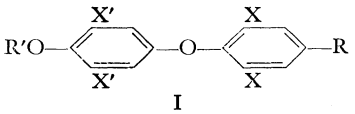
9. This analysis was carried out in the laboratory of Dr. Karl Meyer, Department of Medicine, College of Physicians and Surgeons, Columbia University. The methods have been described by K. Meyer *et al.* [*Biochem. et Biophys. Acta* 21, 506 (1956)] and by P. J. Stoffyn and R. W. Jeanloz [*Arch. Biochem. Biophys.* 52, 373 (1954)].

27 November 1957

Sterically Hindered Analogs of Thyroxine

In previous papers from this laboratory (*1–4*) the synthesis of various compounds of general structure I, related to thyroxine, was reported. In structure I, R = $-CH_2CH_2COOH$, $-CH_2CH(NH_2)COOH$, $-NH_2$, etc; X = iodine; X′ = iodine, methyl, etc.; and R′ = methyl or hydrogen.

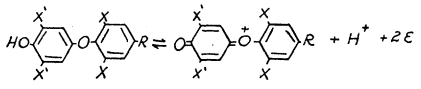


I

In an accompanying paper (*5*), an empirical correlation between structure and biological activity for 47 analogs of structure I was proposed.

The above correlation, while entirely empirical in nature, suggested significant deductions about the essential pharmacogen which is required for thyroxine-like activity. It also led to the conclusions that structural parameters, such as the electron-releasing abilities of X, X′ and OR′, the hydrogen bonding abilities of X and X′ and the *pK* values for the compounds (dependent on the nature of the ionizing side chain, R), are the probable factors which determine the comparative biological activity of these substances. Of major importance to our thinking in arriving at the correlative conclusions was the very striking fact (*5*) that the 3′,5′-dimethyl analogs of L- and D,L- thyroxine (structure I, X = iodine, X′ = methyl, R′ = H) were distinctly more active (*5*), in certain assays of thyroxine-like activity, than the corresponding stereoisomers of thyroxine. The suggestion that substitution of electron-releasing groups, such as methyl, in place of electron-attracting groups such as iodine, bromine, nitro, and so forth, can enhance thyroxine-like activity is a novel one and is in direct opposition to earlier considerations pertaining to such effects (*5, 6*).

The postulate of the Bruice-Kharasch-Winzler correlation, that electron-releasing groups in the 3′,5′-positions of structure I can enhance thyroxine-like activity, finds a possible rationale in the hypothesis of Niemann (*7*) that oxidation of thyroxine to a quinoid form, as shown below, may somehow be involved in its action. Essentially, this oxidation



appears to involve removal of the elements of a hydride group ($H^+ + 2e$) from the thyroxine analog, and this reaction should be enhanced by substituting electron-releasing groups into the back ring of thyroxine.

The above predictions of activities, and the possible relations to the Niemann hypothesis, can be tested by synthesis and biological evaluation of suitable compounds (such as L- and D,L- 3,5,3′-triiodo-5′-methylthyronines and others) in which electron-releasing groups are incorporated into the structures related to compound I. Compare, for example, reference *3*, for the synthesis of initial substances for these purposes.

We now also wish to report our studies toward the synthesis of compounds related to structure I, in which the X′ groups have favorable electron-releasing abilities, but the steric characteristics of which should be such as to cause significant steric complications toward an in vivo oxidative reaction, which may be involved in converting the analog of I to a quinoid form, as illustrated in the equation above. The first compound of this type which we wish to report is the 3′,5′-di-tertiarybutyl analog (X′ = *t*-butyl) and with R = $-CH_2CH_2COOH$. This compound was synthesized by the route shown in Fig. 1.

Compound III melted at 94° to 95°C and gave the following analysis: Calcd. for $C_{25}H_{32}N_2O_8$: C, 61.48; H, 6.56; N, 5.74; found: C, 61.68; H, 6.66; N, 5.57. The conversion of III to I (X = iodine; X′ = *t*-butyl; R′ = H; R = $-CH_2CH_2COOH$) was carried out by reducing to the diamine, diazotization and use of the Sandmeyer reaction to introduce iodine at the 3,5-positions, and hydrolysis of the intermediate ester; m.p. 120° to 121°C. Analysis (for the ester): Calcd. for $C_{25}H_{32}O_4I_2$: C, 46.16; H, 4.96; I, 39.03; found: C, 46.55; H, 4.97; I, 39.29. The final product, as the free acid, was obtained as excellent colorless needles, from aqueous ethanol, which melted at 197° to 198°C. Analysis: Calcd. for $C_{23}H_{28}O_4I_2$: C, 44.39; H, 4.53; I, 40.79; found: C, 44.53; H, 4.60; I, 40.60.

In the above compound, if the *t*-butyl groups are sufficiently large to block the in vivo oxidative reaction to the quinoid form, and if they are not removable in vivo (a question of general interest, which has yet to be resolved), then the possibility of finding a true competitive inhibitor to thyroxine is implied in this approach. The synthesis of various such molecules (the so-called "hinderins") is therefore a major objective of our studies (*8*).

A sample of "hinderin A" (structure I: X′ = *t*-butyl; X = iodine; R′ = H; R = $-CH_2CH_2COOH$) has been submitted for biological assay (effect on metabolism of glucose in *Aerobacter* aerogenes) by W. Marx and M. Gutenstein, who have reported interesting results for the initial screening.

NORMAN KHARASCH
NARENDRA N. SAHA
Chemistry Department, University of Southern California, Los Angeles

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8. This work was supported by the U.S. Public Health Service (grant No. A-703 (C3) and by the Travenol Laboratories of Morton Grove, Ill.

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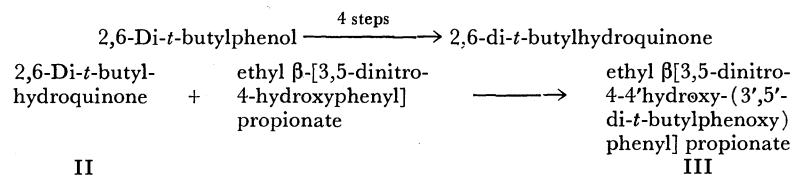

Fig. 1. Synthesis of the 3′,5′-di-tertiarybutyl analog of structure I.

On the Composition of Zymosan

Zymosan is the name given by Pillemer and Ecker (*1*) to a yeast fraction having the specific immunological property of inactivating the third component of complement, C′3. Later work indicated that zymosan adsorbs properdin, a radiation-sensitive serum protein reported to protect mammals from the spread of microbiological infection (*2*). Further interest in zymosan is derived from the finding that its injection into mice, rats, and rabbits results in an immediate decrease in the properdin titer followed by an increase to levels sometimes three times greater than the initial properdin level (*3*). Consistent with this property are the observations that zymosan decreases the lethal effect of x-radia-