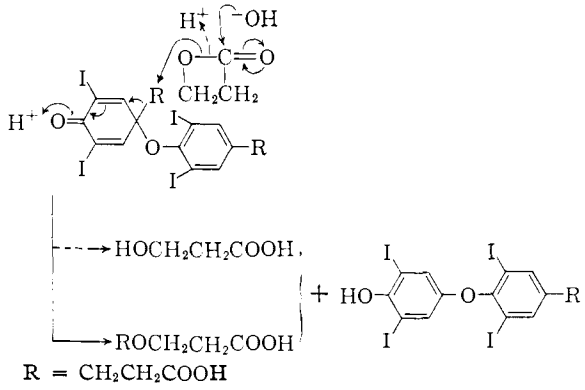


adjacent to the aromatic ring to $-CH(OH)-$. They based this suggestion on a model reaction in which tetraiodothyroacetic acid is formed when 3,5-diiodo-4-hydroxyphenylacetic acid is incubated. The reaction mixture contained glyoxylic acid, the formation of which is easily explained by an oxidation of the starting material to 3,5-diiodo-4-hydroxyphenylglycolic acid, followed by a hydroxylation of the aliphatic side chain according to Johnson and Tewkesbury's mechanism. However, the choice of the acetic acid analog of diiodotyrosine as a model for the study of the fate of the "lost side chain" in the synthesis of thyroxine is not a fortunate one. In contrast to diiodotyrosine and to its propionic acid analog which we chose as a model, the acetic acid analog contains an active methylene group that is very susceptible to oxidation. While 3-(3,5-diiodo-4-hydroxyphenyl)-hydracrylic acid could not be detected with certainty in the reaction mixture from the incubation of diiodophloretic acid,[37] the homologous 3,5-diiodo-4-hydroxyphenylglycolic acid is present in fairly large amounts in the reaction mixture from the incubation of 3,5-diiodo-4-hydroxyphenylacetic acid.[1] Furthermore, in order to explain the formation of hydracrylic acid from 3-(3,5-diiodo-4-hydroxyphenyl)-hydracrylic acid one would have to assume that the aliphatic side chain is eliminated as a carbanion $^{-}CH(OH)CH_2COOH$, a reaction mechanism that is difficult to conceive. The much more plausible mechanism of Johnson and Tewkesbury would lead not to hydracrylic acid but to the semi-aldehyde of malonic acid. Decarboxylation of this unstable compound followed by oxidation would yield acetic acid.[38] The small amount of acetic acid found in fraction C-2 may have been formed in this manner since hydracrylic acid does not give rise to even traces of acetic acid under the conditions of the incubation experiment. Only a small fraction, if any, of the incubated diiodophloretic acid follows this pathway. Reduction of an aliquot of fraction C-2 by diphosphopyridine nucleotide in the presence of alcohol dehydrogenase from horse liver showed that only a minute amount (about 0.01 mmole in the total fraction) of a carbonyl compound reducible by this system, presumably acetaldehyde, was present.

It can be concluded from the present model experiment that in the synthesis of thyroxine from diiodotyrosine the alanine side chain which is eliminated is probably converted to a hydroxylated compound (either serine or hydroxypyruvic acid), not to alanine or dehydroalanine (pyruvic acid and ammonia). It is not likely that the elimination is preceeded by a hydroxylation of the side chain.

(37) A small amount of an unknown substance was found which may be this acid. It had R_f 0.05 in 1-butanol–2 *N* ammonia and 0.11 in 1-butanol–dioxane–ammonia.[1] It does not seem to be 3,5-diiodo-4-hydroxybenzoic acid since it has a somewhat different R_f-value in 1-butanol–pyridine–water.[1] It gives a brownish-yellow spot on paper chromatograms when sprayed with reagent 2, which indicates that an oxygen function is probably present on the carbon atom adjacent to the aromatic ring.[1]

(38) *Cf.* H. D. Dakin, *J. Biol. Chem.*, **5**, 409 (1909).

BETHESDA 14, MD.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH]

Model Reactions for the Biosynthesis of Thyroxine. III. The Synthesis of Hindered Quinol Ethers and their Conversion to Hindered Analogs of Thyroxine[1,2]

BY TERUO MATSUURA[3] AND H. J. CAHNMANN

RECEIVED AUGUST 27, 1959

Model reactions are presented for the biosynthetic scheme (equations 1–3) proposed by Johnson and Tewkesbury for the formation of thyroxine from diiodotyrosine. Sterically hindered analogs of the quinol ether III have been synthesized in a sequence of free radical reactions and then converted to the corresponding analogs of thyroxine. Some of the properties of the quinol ethers and of the analogs of thyroxine are described.

The mechanism of the reaction in which thyroxine is formed from diiodotyrosine *in vivo* or *in vitro*[4] is still obscure. Various hypotheses concerning this mechanism have been proposed.[5] The present report deals with the mechanism that was first suggested by Johnson and Tewkesbury[6] and elaborated by Harington.[7] This mechanism is based on the extensive studies of Pummerer and his co-workers[8a–k] who have found that many oxidations of

(1) Paper II, H. J. Cahnmann and T. Matsuura, THIS JOURNAL, **82**, 2050 (1960).

(2) A preliminary report of this work has been presented at the 134th Meeting of the American Chemical Society, September, 1958, Chicago, Ill.

(3) Visiting Scientist from Osaka City University, Japan.

(4) P. von Mutzenbecher, *Z. physiol. Chem.*, **261**, 253 (1939).

(5) For references *cf.* paper I of this series.[1a]

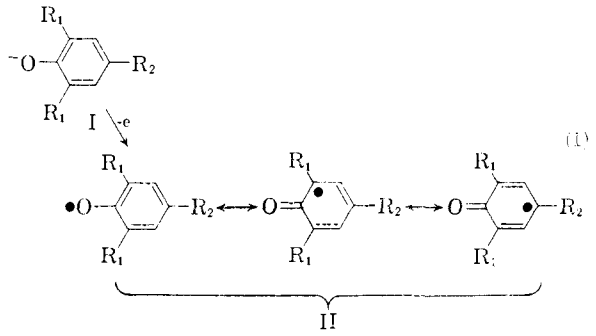
(6) T. B. Johnson and L. B. Tewkesbury, Jr., *Proc. Natl. Acad. Sci. U. S.*, **28**, 73 (1942).

(7) C. K. Harington, *J. Chem. Soc.*, 193 (1944).

(8) (a) R. Pummerer, H. Puttfarcken and P. Schopflocher, *Ber.*, **58**, 1808 (1925); see also (b) R. Pummerer and F. Frankfurter, *ibid.*, **47**, 1472 (1914); (c) **52**, 1416 (1919); (d) R. Pummerer and E. Cherbuliez, *ibid.*, **47**, 2957 (1914); (e) **52**, 1392 (1919); (f) R. Pummerer, *ibid.*, **52**, 1403 (1919); (g) R. Pummerer, D. Melamid and H. Puttfarcken, *ibid.*, **55**, 3116 (1922); (h) R. Pummerer and A. Rieche, *ibid.*, **59**, 2161 (1926); (i) R. Pummerer and F. Luther, *ibid.*, **61**, 1102 (1928); (j) R. Pummerer, G. Schmidutz and H. Seifert, *Chem. Ber.*, **85**, 535 (1952); (k) R. Pummerer and I. Veit, *ibid.*, **86**, 412 (1953).

phenols take place *via* a free radical mechanism. That the first step of most, if not all, oxidations of phenols is the formation of a free radical was borne out by the classical work of Michaelis on the mechanism of the oxidation of hydroquinones to quinones[9a,b] and by many subsequent investigations.[10]

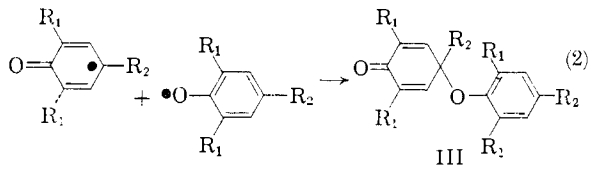
According to the hypothesis of Johnson and Tewkesbury, the first step in the synthesis of thyroxine from diiodotyrosine (I) is an oxidation of the latter to a free radical II.



(1)

R_1 = I; R_2 = $CH_2CH(NH_2)COOH$

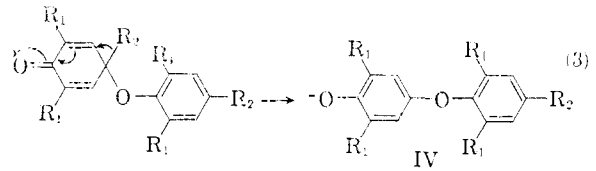
The second step is a dimerization of two molecules of the free radical to form a quinol ether III.



(2)

R_1 = I; R_2 = $CH_2CH(NH_2)COOH$

Finally, the loss of the side chain R_2 attached to the dienone ring leads to thyroxine (IV).



(3)

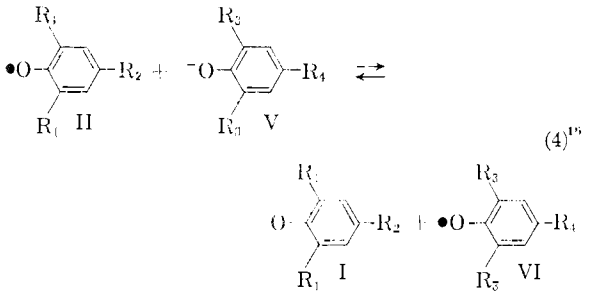
R_1 = I; R_2 = $CH_2CH(NH_2)COOH$

Although this hypothetical mechanism appears plausible, it has not previously been substantiated experimentally. No attempts to demonstrate the formation of a quinol ether intermediate in the synthesis of thyroxine or its analogs[5] have been reported. Furthermore, no aromatic quinol ethers of the type III carrying an aliphatic acid side chain in *p*-position to the ether bridge are known. Only a few other aromatic *p*-quinol ethers have been synthesized.[8j,11,12] The possibility of their conversion to diphenyl ethers, a reaction that is analogous to the last step in Johnson and Tewkesbury's reaction scheme, has not been investigated.

In the present paper we report the synthesis of several analogs of the quinol ether III in a sequence of free radical reactions closely resembling the first two steps of Johnson and Tewkesbury's mechanism. We further report the conversion of some of the quinol ethers thus synthesized to the corresponding analogs of thyroxine.

From what is known about the relationship between the stability of free radicals and their chemical structure[13a,b] the free radical II, if formed at all in the course of the synthesis of thyroxine, must be expected to be unstable. In contrast, the analogous free radical II (R_1 and R_2 = $C(CH_3)_3$) is known to be quite stable in the absence of oxygen[14a,b,15a,b] due to the resonance stabilization and steric hindrance provided by the three *t*-butyl groups. Therefore, the corresponding phenol 2,4,6-tri-*t*-butylphenol (I, R_1 and R_2 = $C(CH_3)_3$) has been selected as a starting material for the synthesis of several analogs of the quinol ether III.

The first step in this synthesis is the removal of an electron from this phenol leading to the corresponding free radical (reaction 1; R_1 and R_2 = $C(CH_3)_3$). This reaction was carried out with potassium ferricyanide as the electron-removing agent.[8a-i,14,15] A solution of an analog of tyrosine was then added to the blue solution of the stable free radical. The analogs of tyrosine used for this purpose were of the general type V characterized by a free or esterified aliphatic acid side chain in the *p*-position to the phenolic hydroxyl and by *o*-positions that are either free or substituted with bromine or iodine. An electron transfer takes place resulting in the oxidation of the added analog of tyrosine to a free radical VI and the concomitant reduction of the blue free radical to tri-*t*-butylphenol.



(4)[16]

R_1 and R_2 = $C(CH_3)_3$; R_3 = H or Br or I; R_4 = $CH_2COOC_2H_5$ or $(CH_2)_2COOC_2H_5$ or $(CH_2)_2COOH$

In the course of the addition of the analog of tyrosine the blue solution becomes colorless or almost

(9) (a) L. Michaelis, *Cold Spring Harbor Symposia Quant. Biol.*, **7**, 33 (1939); (b) *Ann. N. Y. Acad. Sci.*, **40**, 39 (1940).

(10) For leading references concerning the mechanism of biological oxidations of phenols see D. H. R. Barton and T. Cohen, "Festschrift A. Stoll, Birkhäuser, Basel, 1957, p. 117, and H. Erdtman and C. A. Wachtmeister, *ibid.*, p. 144. Extensive studies on the mechanism of the *in vitro* oxidation of phenols have been made by Pummerer and co-workers[8a-k] and by Goldschmidt and co-workers [first contribution: St. Goldschmidt, *Ber.*, **55**, 3194 (1922); last contribution: St. Goldschmidt, E. Schulz and H. Bernard, *Ann.*, **478**, 1 (1930)].

(11) E. Müller, K. Ley and G. Schlechte, *Chem. Ber.*, **90**, 2660 (1957).

(12) The transient formation of an aromatic *p*-quinol ether in the course of the oxidation of *p*-cresol to "Pummerer's ketone" reported by R. Pummerer, H. Puttfarcken and P. Schopflocher[8a] was subsequently shown not to occur [D. H. R. Barton, A. M. Deflorin and O. E. Edwards, *J. Chem. Soc.*, 530 (1956); V. Arkley, F. M. Dean, A. Robertson and P. Sidisunthorn, *ibid.*, 2322 (1956)].

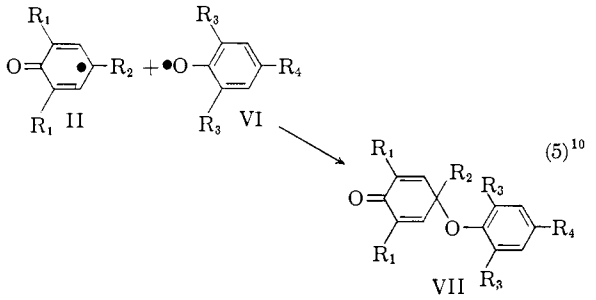
(13) (a) C. D. Cook, N. G. Nash and H. R. Flanagau, THIS JOURNAL, **77**, 1783 (1955); (b) C. D. Cook, D. A. Kuhn and P. Fianu, *ibid.*, **78**, 2002 (1956).

(14) (a) C. D. Cook, *J. Org. Chem.*, **18**, 261 (1953); (b) C. D. Cook and R. C. Woodworth, THIS JOURNAL, **75**, 6242 (1953).

(15) (a) E. Müller and K. Ley, *Chem. Ber.*, **87**, 922 (1954); (b) E. Müller, K. Ley and W. Kiedaisch, *ibid.*, **87**, 1605 (1954).

(16) For the sake of simplicity only one of the canonical structures of the free radicals is shown.

colorless since the free radical VI reacts, as soon as it is formed, with unreduced free radical II to form the quinol ether VII which is an analog of Johnson and Tewkesbury's hypothetical quinol ether III.



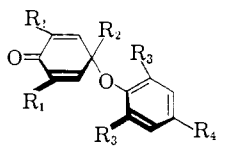
(5)[10]

R_1 and R_2 = $C(CH_3)_3$; R_3 = H or Br or I; R_4 = $CH_2COOC_2H_5$ or $(CH_2)_2COOC_2H_5$ or $(CH_2)_2COOH$

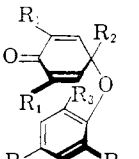
Thus two molecules of the blue free radical II react with one molecule of the analog of tyrosine (V) to form one molecule of quinol ether VII and one molecule of tri-*t*-butylphenol (I). By subjecting the reaction mixture to a second treatment with potassium ferricyanide the latter can again be oxidized to the stable blue radical which, with more of the analog of tyrosine, forms an additional amount of quinol ether. If the process of oxidation, followed by the addition of the analog of tyrosine, is repeated a few times practically all of the tri-*t*-butyl originally present is converted to the quinol ether.[11] Only a single oxidation could be carried out when a phenol (V) was used in which R_3 was iodine or R_4 a side chain with a free carboxyl group. The crude quinol ethers were contaminated with the starting phenols and with bis-(4-oxo-1,3,5-tri-*t*-butyl-2,5-cyclohexadien-1-yl) peroxide,[14b] formed from two molecules of the free radical II (R_1, R_2 and R_3 = *t*-butyl) and one molecule of oxygen. These impurities can be removed by recrystallization or by washing with methanol and *n*-pentane.

One of the quinol ethers (VII, R_1 and R_2 = $C(CH_3)_3$, R_3 = H, R_4 = $CH_2COOC_2H_5$) was also prepared by a different route described earlier for the synthesis of other quinol ethers.[11] This procedure in which the sodium salt of ethyl *p*-hydroxyphenylacetate was condensed with 2,4,6-tri-*t*-butyl-4-bromo-2,5-cyclohexadienone gave, however, a much lower yield than the oxido-reduction procedure described above.

The quinol ethers VII are yellow crystalline substances. Those in which R_3 is hydrogen are quite stable in the solid state. Replacement of these hydrogen atoms with bromine, and much more so with iodine, renders the quinol ethers less stable. Those in which R_3 is iodine decomposed on long standing at room temperature. In solution, all the quinol ethers dissociate to a greater or lesser degree at the ether linkage into the corresponding free radicals II and VI, particularly at elevated temperatures. The yellow or greenish-yellow solutions of the quinol ethers turn blue on heating and assume again their original color on cooling. The bulkier the substituents R_3 are the lower is the transition temperature from yellow to blue. The blue color is evidently caused by the free radical II. The free radicals formed by dissociation of the quinol ethers, particularly the iodinated ones, tend to react further with the formation of various decomposition products (the parent phenols, polymers, etc.). The dissociation of the quinol ethers is therefore only partially reversible. The quinol ethers in which R_3 is iodine are so unstable in solution that they can be isolated only under constant cooling in an ice- or Dry Ice–acetone-bath. The instability of the quinol ethers in which R_3 is iodine or bromine is at least in part due to the steric hindrance caused by the close proximity of several bulky substituents (*t*-butyl and halogen). An inspection of atom models (Stuart–Briegleb) suggests that there exists a strong barrier to free rotation about the ether oxygen as a result of *t*-butyl–halogen (R_2–R_3) interaction. An inspection of atom models shows further that as a result of this interaction, two geometrical forms are theoretically possible, one an extended form (VIIa) with an extremely rigid ether bridge, the other less rigid one, a dihydral structure approaching a sandwich (VIIb).



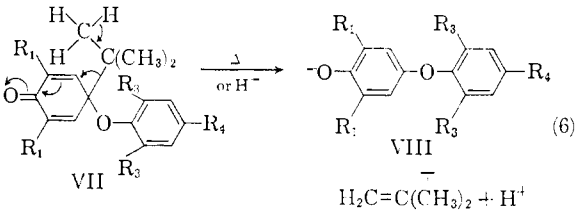
VIIa



VIIb

The infrared spectra of the quinol ethers VII, determined in the solid state, show a twin band at about 6 μ characteristic for the dienone structure in quinols and bands at about 8 and 10 μ characteristic for semi-aromatic ethers.[11,14b]

When the quinol ethers VII in which R_3 is hydrogen are heated above their melting points to about 150–180°, isobutene is evolved (bubbling). The residual melt consists largely of the corresponding analog of thyroxine (VIII). The same conversion can be carried out in boiling ethyl acetate in the presence of an acidic catalyst.



(6)

R_1 = $C(CH_3)_3$; R_3 = H; R_4 = $CH_2COOC_2H_5$ or $(CH_2)_2COOH$

If R_3 is not hydrogen but bromine the yield of the analog of thyroxine (VIII) is considerably lower since in this case the competitive reaction, *viz.*, the earlier described dissociation of the quinol ether into two free radicals, followed by further decomposition, takes place to a much larger extent. If R_3 is iodine, this competitive reaction is the predominant one. When the quinol ether VII (R_1 and R_2 = $C(CH_3)_3$, R_3 = I, R_4 = $(CH_2)_2COOH$) was heated above its melting point until bubbling ceased, the weight loss was 27% of the theoretical amount corresponding to the loss of one *t*-butyl group.[17]

(17) Part of this weight loss may be due to the sublimation of some iodine liberated in the course of the pyrolysis.

However, no analog of thyroxine could be isolated from the complex dark brown reaction mixture in which various other reaction products, *e.g.*, phloretic acid and iodinated phloretic acids, iodine and unidentified polymerized material, were present. Evidently, milder methods not requiring elevated temperatures are required for the conversion of the iodinated quinol ethers to the corresponding analogs of thyroxine.

Those hindered analogs of thyroxine (VIII) in which the carboxyl group of the side chain R_4 is esterified can be converted to the free acids by alkaline hydrolysis. The analogs of thyroxine in which R_4 is a propionic acid side chain and R_3 hydrogen could not be obtained in crystalline form and was therefore isolated as its crystalline monosodium salt.

The infrared spectra of these analogs of thyroxine show a band at about 2.8 μ characteristic for a phenolic hydroxyl and a series of bands at about 7.7, 8.0, 8.2, 8.4 and 10.4 μ, most of which must be attributed to the diaromatic ether linkage.[18] The twin band at about 6 μ that characterizes the quinol ethers from which the analogs of thyroxine are prepared is absent.

The hindered analogs of thyroxine do not couple with diazonium compounds. They cannot therefore be detected on paper chromatograms by means of diazotized sulfanilic acid (Pauly reagent) or of diazotized N^1,N^1-diethylsulfanilamide.[19] They react, however, with ferric chloride–potassium ferricyanide[20] to form ferrous ferricyanide. They appear as blue spots on paper chromatograms after spraying with this reagent.

It has been predicted that analogs of thyroxine in which the iodine atoms in *o*-position to the phenolic hydroxyl are replaced with electron-releasing groups should exhibit thyromimetic activity.[21] It has also been pointed out, however, that these analogs might act as inhibitors of thyroxine if the *o*-substituents are bulky, *e.g.*, *t*-butyl groups.[22] The reasoning behind this prediction was that bulky groups might inhibit the dehydrogenation of the analog to a quinoid form, a reaction which, according to a hypothesis of Niemann,[23] is a prerequisite for thyromimetic activity.

Two of the hindered analogs of thyroxine synthesized in this Laboratory (VIII, R_3 = H or Br, R_4 = $(CH_2)_2COOH$) were tested for their thyromimetic activity in the tadpole metamorphosis and in the rat oxygen consumption tests[24] and for both their thyromimetic and thyroxine-antagonistic activities in the rat goiter prevention test.[25] Both substances were devoid of any activity in the doses used.[26] It may be assumed that the steric hindrance caused by the *t*-butyl groups prevents the fixation of the hindered analogs to the natural receptor sites for thyroxine.

The synthesis of analogs of thyroxine from the corresponding analogs of diiodotyrosine, involving first the formation of a quinol ether *via* a free radical mechanism and then the conversion of this quinol ether to a diphenyl ether, represents a complete model for the mechanism by which, according to Johnson and Tewkesbury, thyroxine is synthesized *in vivo*. It offers the first experimental proof that such a mechanism is indeed possible.

Experimental[27]

4-[*p*-(Carbethoxymethyl)-phenoxy]-2,4,6-tri-*t*-butyl-2,5-cyclohexadien-1-one (VII, R_3 = H, R_4 = $CH_2COOC_2H_5$). —A solution of 3.93 g. (15 mmoles) of 2,4,6-tri-*t*-butylphenol[28] in 200 ml. of benzene was stirred for 2 hours with a solution of 15 g. (46 mmoles) of potassium ferricyanide and 8.4 g. (0.15 mole) of KOH in 75 ml. of water. The aqueous layer was removed and the blue benzene layer washed with water. To the washed benzene layer a 0.3 *M* solution of ethyl *p*-hydroxyphenylacetate (V, R_3 = H, R_4 = $CH_2COOC_2H_5$)[29] in benzene was added until the reaction mixture became pale green (24.7 ml., 7.4 mmoles). A solution of 7.5 g. (23 mmoles) of $K_3[Fe(CN)_6]$ and 4 g. (71 mmoles) of KOH in 50 ml. of water was then added and the reaction mixture stirred for 2 hours. The aqueous layer was removed and the benzene layer washed with water and titrated with a 0.3 *M* solution of ethyl *p*-hydroxyphenylacetate as before (8.9 ml., 2.7 mmoles). The treatment with $K_3[Fe(CN)_6]$, followed by washing and titration with ethyl *p*-hydroxyphenylacetate (8.2 ml., 2.5 mmoles), was repeated. This time the stirring time was extended to 5 hours. A total amount of 12.5 mmoles of ester was used in the three additions.

(18) *Cf.* S. Kimoto, *J. Pharm. Soc., Japan*, **75**, 763 (1955).

(19) T. Matsuura and H. J. Cahnmann, THIS JOURNAL, **81**, 871 (1959).

(20) G. M. Barton, R. S. Evans and J. A. F. Gardner, *Nature*, **170**, 249 (1952).

(21) T. C. Bruice, N. Kharasch and R. J. Winzler, *Arch. Biochem. Biophys.*, **62**, 305 (1956).

(22) N. Kharasch and N. N. Saha, *Science*, **127**, 756 (1958).

(23) C. Niemann, *Fortschr. Chem. org. Naturstoffe*, **7**, 173 (1950).

(24) We wish to thank Dr. William Money of the Sloan–Kettering Institute, New York, for the performance of the tadpole metamorphosis test and Mr. Neil Stasilli and Dr. Robert Kroc of the Warner–Lambert Research Institute, Morris Plains, N. J., for the performance of the rat oxygen consumption test.

(25) E. B. Astwood and E. W. Dempsey, *Endocrinol.*, **4**, 312 (1952).

(26) In the tadpole metamorphosis test [C. J. Shellabarger and J. T. Godwin, *Endocrinol.*, **54**, 230 (1954); W. L. Money, R. I. Meltzer, J. Young and R. W. Rawson, *ibid.*, **63**, 20 (1958)] one group of animals received up to 180 μg. of the halogen-free compound and another group up to 100 μg. of the bromo compound. Higher doses were toxic. In the oxygen consumption test [N. R. Stasilli, R. L. Kroc and R. I. Meltzer, *Endocrinol.*, **64**, 62 (1959); administration by stomach tube] the rats were given 0.5 mg. of substance/100 g. of body weight/day for 4 days. In the goiter prevention test one group received 429 μg. of the halogen-free compound and another group 612 μg. of the bromo compound/animal (100–115 g.)/day for 10 days. In order to test the thyroxine antagonistic activities, two other groups of rats received the above-mentioned amounts of analog of thyroxine together with 3.4 μg. of sodium-L-thyroxine pentahydrate (corresponding to 3 μg. of thyroxine). This is a ratio of 300 moles of analog to 1 mole of thyroxine.

(27) Melting points were taken in capillary tubes and are uncorrected. The infrared spectra were determined by Mr. H. K. Miller of this Institute in a Perkin-Elmer recording spectrophotometer, model 21, equipped with sodium chloride optics. The microanalyses were made by Dr. W. C. Alford and his associates, also of this Institute. Paper chromatograms were made using both the ascending and the descending method (Whatman paper 3 MM). Chromatographic solvents were the upper phases of (1) 1-butanol–2 *N* ammonia; (2) 1-butanol–dioxane–2 *N* ammonia (4:1:5); (3) *t*-amyl alcohol–2 *N* ammonia. The syntheses of the quinol ethers were carried out in an atmosphere of oxygen-free nitrogen. Seaford nitrogen of the Air Reduction Corp., Baltimore, Md., containing not more than 0.002% oxygen was further purified according to L. Meites and T. Meites, *Anal. Chem.*, **20**, 984 (1948). For these syntheses a flat-bottom reaction flask was used that was equipped with a nitrogen inlet tube, an opening for the escape of nitrogen and the addition of reactants, and a stopcock arranged at angles of about 6, 30 and 280°, respectively, from the vertical center line. The nitrogen escape opening was made of a tapered joint so that it could be closed with a glass stopper. The reaction mixtures were stirred magnetically.

(28) Kindly supplied by the Gulf Research and Development Co., and by the Koppers Co., Inc., m.p. 130–131°.

(29) Ethyl *p*-hydroxyphenylacetate was synthesized according to H. Salkowski, *Ber.*, **22**, 2140 (1889); b.p. 139–140° (1 mm.), yield 78%.

The pale green solution, after evaporation *in vacuo*, gave a greenish-yellow sirup which became a yellow crystalline mass after several days at 2°. Rapid recrystallization from methanol gave yellow prisms, m.p. 64–66°; yield 3.71 g. (67%, based on ethyl *p*-hydroxyphenylacetate); after a second recrystallization, m.p. 65–67°; yield 3.06 g. (55%). When the green melt is further heated, a gas which decolorizes a bromine solution begins to evolve at about 155° (probably isobutene).

Anal. Calcd. for $C_{28}H_{40}O_4$: C, 76.32; H, 9.15. Found: C, 76.18; H, 9.27.

The ultraviolet absorption spectrum (cyclohexane) shows a main peak at 229 mμ (log ε 4.13) and minor peaks at longer wave lengths.[30] The infrared spectrum (KBr) shows bands at 5.77 (carbonyl of $COOC_2H_5$), 6.0 and 6.03 (carbonyl and double bonds of the quinol ring), 8.10 and 10.20 μ (aromatic quinol ether).[30]

The same quinol ether was also prepared from 3.41 g. (10 mmoles) of 4-bromo-2,4,6-tri-*t*-butyl-2,5-cyclohexadiene-1-one and 1.80 g. (10 mmoles) of the sodium salt of ethyl *p*-hydroxyphenylacetate according to the method described by Müller, *et al.*,[11] for other quinol ethers. The yield was 0.75 g. (17%) of yellow crystals, m.p. 61–63°.

4-[*p*-(2-Carbethoxyethyl)-phenoxy]-2,4,6-tri-*t*-butyl-2,5-cyclohexadien-1-one (VII, R_3 = H, R_4 = $(CH_2)_2COOC_2H_5$).—The synthesis of this quinol ether was similar to the one of the quinol ether VII (R_3 = H, R_4 = $CH_2COOC_2H_5$) described above. Three successive oxidations of 2,4,6-tri-*t*-butylphenol (5.25 g., 20 mmoles) dissolved in 200 ml. of benzene were made. The period of stirring during these oxidations was 2 hours each time. An aqueous solution containing 20% $K_3[Fe(CN)_6]$ and 11% KOH was used (100 ml. in the first oxidation and 50 ml. in the second and in the third oxidation). A 0.4 *M* solution of ethyl 3-(*p*-hydroxyphenyl)-propionate (ethyl ester of phloretic acid)[31] was added. After the first oxidation 24.8 ml. (9.9 mmoles) was used, after the second oxidation 10.4 ml. (4.2 mmoles), and after the third oxidation 6.6 ml. (2.6 mmoles), which is a total of 16.7 mmoles.

The residue obtained after evaporation of the pale green solution was permitted to stand at 2° for several days. Then the crystalline mass was digested with a small amount of ice-cold[32] methanol. After removal of the methanol and recrystallization from *n*-pentane, 5.0 g. (65% yield based on the ester) of yellow prisms, m.p. 32.5–33.5°, was obtained. At the melting point the fusion is greenish-yellow. It begins to bubble at about 170°.

Anal. Calcd. for $C_{29}H_{42}O_4$: C, 76.61; H, 9.31. Found: C, 76.65; H, 9.32.

The infrared spectrum (KBr) shows bands at 5.77 (carbonyl of $COOC_2H_5$), 6.00 and 6.08 (carbonyl and double bonds of the quinol ring), 8.10 and 10.20 μ (aromatic quinol ether).[30]

4-[*p*-(2-Carboxyethyl)-phenoxy]-2,4,6-tri-*t*-butyl-2,5-cyclohexadien-1-one (VII, R_3 = H, R_4 = $(CH_2)_2COOH$).—A blue free radical solution was prepared from 5.25 g. (20 mmoles) of 2,4,6-tri-*t*-butylphenol as described in the synthesis of the quinol ether VII (R_3 = H, R_4 = $(CH_2)_2COOC_2H_5$). A solution of 1.66 g. (10 mmoles) of phloretic acid[1] in a mixture of 20 ml. of benzene and 30 ml. of ethyl acetate was then added. The resulting greenish-yellow solution was evaporated *in vacuo*. The crystalline residue was well mixed with 38 ml. of 0.5 *N* NaOH. The mixture was extracted with *n*-pentane to remove 2,4,6-tri-*t*-butylphenol and the aqueous layer which contained the sparingly soluble sodium salt of the quinol ether, partially in solution and partially in suspension, was acidified with 1 *N* HCl. The yellow precipitate, m.p. 108–111° (3.94 g., 92%), gave, upon recrystallization from dilute methanol, 2.65 g. (62%) of yellow prisms, melting at 111–113°. The fusion was light green at the melting point. Bubbling started at about 130°.

Anal. Calcd. for $C_{27}H_{38}O_4$: C, 76.02; H, 8.98. Found: C, 76.67; H, 9.03.

The infrared spectrum (KBr) shows bands at 5.83 μ (carbonyl of COOH); 5.98 and 6.06 μ (carbonyl and double bonds of the quinol ring); 8.06 and 10.19 μ (aromatic quinol ether).[30]

Ethyl 3,5-Dibromo-4-hydroxyphenylacetate.—A solution of 3.09 ml. (60 milliatoms) of bromine in 20 ml. of chloroform was slowly added to a solution of 5.41 g. (30 mmoles) of ethyl *p*-hydroxyphenylacetate in chloroform. The reaction mixture was cooled in an ice-bath during this addition and then permitted to stand overnight at room temperature. The solvent was removed *in vacuo*. Recrystallization of the crystalline residue from benzene–isooctane gave colorless silky needles melting at 106–108°; yield 8.90 g. (88%).

Anal. Calcd. for $C_{10}H_{10}Br_2O_3$: C, 35.53; H, 2.98; Br, 47.29. Found: C, 35.54; H, 3.00; Br, 47.14.

4-[2,6-Dibromo-4-(carbethoxymethyl)-phenoxy]-2,4,6-tri-*t*-butyl-2,5-cyclohexadien-1-one (VII, R_3 = Br, R_4 = $CH_2COOC_2H_5$).—A free radical solution was prepared from 5.25 g. (20 mmoles) of 2,4,6-tri-*t*-butylphenol as described for the synthesis of the quinol ether VII (R_3 = H, R_4 = $(CH_2)_2COOC_2H_5$). A solution of 3.38 g. (10 mmoles) of ethyl 3,5-dibromo-4-hydroxyphenylacetate in 50 ml. of benzene was added within a few minutes. After 15 minutes standing, the solution, which had become light blue, was subjected in the usual manner to a second and then to a third oxidation, each of which was followed by the addition of ethyl 3,5-dibromo-4-hydroxyphenylacetate. After each addition of bromoester, the solution was permitted to stand for 15 minutes. After the second oxidation, 1.70 g. (5 mmoles) of bromoester was added, after the third oxidation 1.20 g. (3.6 mmoles). The total amount of ester used was 6.28 g. (18.6 mmoles).

Ice-cold methanol (25 ml.) was added to the crystalline residue obtained after evaporation of the light blue solution. The mixture was kept overnight at −25°, then filtered. Yellow crystals, m.p. 88–90° (8.50 g., 85% based on the bromoester), were obtained which, after recrystallization from *n*-pentane, gave yellow prisms melting at 89.5–90.5°; yield 6.70 g. (67%). The fusion was greenish-blue at the melting point; evolution of isobutene began at about 145°.

Anal. Calcd. for $C_{28}H_{38}Br_2O_4$: C, 56.20; H, 6.40; Br, 26.71. Found: C, 55.95; H, 6.25; Br, 26.88.

The infrared spectrum (KBr) shows bands at 5.74 (carbonyl of $COOC_2H_5$), 5.99 and 6.07 (carbonyl and double bonds of the quinol ring), 8.03 and 10.09 μ (aromatic quinol ether).[30]

Ethyl 3-(3,5-Dibromo-4-hydroxyphenyl)-propionate (Ethyl Ester of 3,5-Dibromophloretic Acid) (V, R_3 = Br, R_4 = $(CH_2)_2COOC_2H_5$).—A solution of 3.09 ml. (60 milliatoms) of bromine in 20 ml. of chloroform was added slowly with stirring and cooling (ice-bath) to a solution of 5.82 g. (30 mmoles) of the ethyl ester of phloretic acid[31] in 50 ml. of chloroform. The solution was then allowed to stand overnight at room temperature. The residue obtained after evaporation of the solvent was distilled *in vacuo*. A colorless viscous liquid, b.p. 163–164° (1 mm.), was obtained which became crystalline on standing; yield 8.22 g. (78%). This material was sufficiently pure for the preparation of the corresponding quinol ether. On recrystallization from ether–*n*-pentane it gave colorless plates, m.p. 47–48°.

Anal. Calcd. for $C_{11}H_{12}Br_2O_3$: C, 37.53; H, 3.44; Br, 45.40. Found: C, 37.27; H, 3.58; Br, 45.38.

4-[2,6-Dibromo-4-(2-Carbethoxyethyl)-phenoxy]-2,4,6-tri-*t*-butyl-2,5-cyclohexadien-1-one (VII, R_3 = Br, R_4 = $(CH_2)_2COOC_2H_5$).—This quinol ether was prepared in the same manner as the quinol ether VII (R_3 = Br, R_4 = $CH_2COOC_2H_5$) except that the ethyl ester of 3,5-dibromophloretic acid was used instead of ethyl 3,5-dibromo-4-hydroxyphenylacetate. A total amount of 6.28 g. (17.8 mmoles) of bromoester was used; 3.52 g. (10 mmoles) in 30 ml. of benzene after the first oxidation, 1.76 g. (5 mmoles) in 20 ml. of benzene after the second oxidation, and 1.00 g. (2.8 mmoles) in 20 ml. of benzene after the third oxidation.

The mixture of the crude quinol ether and methanol was permitted to stand at −25° for several hours, then filtered. Yellow crystals, m.p. 100–101°, were obtained; yield 10.0 g. (92% based on the bromoester). Recrystallization from *n*-pentane gave yellow prisms, m.p. 103–104°; yield 8.12 g. (74%).

Anal. Calcd. for $C_{29}H_{40}Br_2O_4$: C, 56.87; H, 6.58; Br, 26.10. Found: C, 57.46; H, 6.61; Br, 25.83.

The infrared spectrum (KBr) shows bands at 5.76 (carbonyl of $COOC_2H_5$), 5.97 and 6.06 (carbonyl and double

(30) *Cf.* ref. 11 and 14b.

(31) Phloretic acid was prepared as described in paper II of this series.[1] It was esterified according to the procedure used for the preparation of *p*-hydroxyphenylacetate[29]; yield 89% of a colorless, viscous liquid, b.p. 135–136° (1 mm.).

(32) The crude crystals melt slightly above room temperature.

bonds of the quinol ring), 8.01 and 10.07 μ (aromatic quinol ether).[30]

3-(3,5-Dibromo-4-hydroxyphenyl)-propionic Acid (3,5-Dibromophloretic Acid) (V, R_3 = Br, R_4 = $(CH_2)_2COOH$).—A solution of 3.09 ml. (120 milliatoms) of bromine in 40 ml. of a 20% (w./v.) aqueous solution of KBr was added dropwise with cooling (ice-bath) to a stirred solution of 4.98 g. (30 mmoles) of phloretic acid[1] in 150 ml. of water. The precipitate formed was filtered and dried, m.p. 102–103°; yield 8.16 g. (84%). Recrystallization from chloroform–benzene gave 7.33 g. (75%) of colorless prisms, m.p. 106–108°, lit. m.p. 107–108°[33] and 106–108°.[34]

4-[2,6-Dibromo-4-(2-carboxyethyl)-phenoxy]-2,4,6-tri-*t*-butyl-2,5-cyclohexadien-1-one (VII, R_3 = Br, R_4 = $(CH_2)_2COOH$).—A free radical solution was prepared from 10.5 g. (40 mmoles) of 2,4,6-tri-*t*-butylphenol as described for the synthesis of the quinol ether VII (R_3 = H, R_4 = $(CH_2)_2COOC_2H_5$). A solution of 6.48 g. (20 mmoles) of 3,5-dibromophloretic acid in 50 ml. of ether was then added within a few minutes. After standing 15 minutes the light blue solution was evaporated *in vacuo*. The crystalline residue was well mixed with a small amount of ice-cold *n*-pentane. After filtration, 9.69 g. (83%) of yellow crystals, m.p. 115° dec., were obtained. Recrystallization from 90% methanol gave yellow prisms which melted at 119° with decomposition (evolution of isobutene); yield 8.00 g. (68%).

Anal. Calcd. for $C_{27}H_{36}Br_2O_4$: C, 55.49; H, 6.21; Br, 27.35. Found: C, 55.56; H, 6.42; Br, 27.22.

The infrared spectrum (Fluorolube S from 2–7.4 μ, Nujol from 7.4–15 μ) showed bands at 5.83 (carbonyl of COOH), 5.97 and 6.06 (carbonyl and double bonds of the quinol ring), 8.00 and 10.11 μ (aromatic quinol ether).[30]

Ethyl 3,5-Diiodo-4-hydroxyphenylacetate.—A suspension of 14.70 g. (36.4 mmoles) of 3,5-diiodo-4-hydroxyphenylacetic acid[19] in 125 ml. (2.2 moles) of absolute ethanol was saturated in an ice-bath with dry HCl. First a clear solution was obtained, then a precipitate of needles formed. After standing at room temperature for one hour, the mixture was diluted with water. The crystals were collected by filtration, then recrystallized from aqueous ethanol. Colorless fine needles, m.p. 121–122°, were obtained; yield 14.76 g. (94%).

Anal. Calcd. for $C_{10}H_{10}I_2O_3$: C, 27.80; H, 2.33; I, 58.75. Found: C, 27.50; H, 2.65; I, 59.17.

4-[2,6-Diiodo-4-(carbethoxymethyl)-phenoxy]-2,4,6-tri-*t*-butyl-2,5-cyclohexadien-1-one (VII, R_3 = I, R_4 = $CH_2COOC_2H_5$).—A free radical solution was prepared from 5.25 g. (20 mmoles) of 2,4,6-tri-*t*-butylphenol as described for the synthesis of the quinol ether VII (R_3 = H, R_4 = $(CH_2)_2COOC_2H_5$). A suspension of 4.32 g. (10 mmoles) of ethyl 3,5-diiodo-4-hydroxyphenylacetate in 50 ml. of benzene was then added within a few minutes. After 15 minutes standing the greenish-blue solution was evaporated *in vacuo* at low temperature (about 5°). The sticky residue was rapidly mixed in an ice-bath with 30 ml. of *n*-pentane. Insoluble white needles of recovered iodoester were removed by filtration (1.10 g.). The filtrate was evaporated *in vacuo* at low temperature (< 0°). The crystalline residue was rapidly digested in the cold (ice-bath) with 30 ml. of methanol to remove tri-*t*-butylphenol. Insoluble yellow crystals of the quinol ether were collected by filtration; m.p. 89–90° dec.; yield 4.31 g. (62 % based on the amount of iodoester and 83% if the recovered iodoester is taken into account).

The filtrate, upon standing at −25°, deposited yellow crystals, m.p. 144° dec., which did not depress the m.p. (146°) of bis-(4-oxo-1,3,5-tri-*t*-butyl-2,5-cyclohexadien-1-yl) peroxide.[14b]

The crystals of the quinol ether were rapidly digested in the cold (ice-bath) with 10 ml. of *n*-pentane. The residual crystals, isolated by filtration, melted at 89–90° dec., yield 3.20 g. (46%; 62% if the recovered iodoester is taken into account). The fusion begins to bubble at about 150°.

Anal. Calcd. for $C_{28}H_{38}I_2O_4$: C, 48.57; H, 5.53; I, 36.66. Found: C, 48.70; H, 5.46; I, 36.79.

The infrared spectrum (KBr) shows bands at 5.75 (carbonyl of $COOC_2H_5$), 5.97 and 6.06 (carbonyl and double bonds of the quinol ring), 8.04 and 10.10 μ (aromatic quinol ether).[30]

Ethyl 3-(3,5-Diiodo-4-hydroxyphenyl)-propionate (Ethyl Ester of 3,5-Diiodophloretic Acid) (V, R_3 = I, R_4 = $(CH_2)_2COOH$).—A suspension of 16.0 g. (38 mmoles) of diiodophloretic acid[35] in 75 ml. (1.3 moles) of absolute ethanol was saturated with dry HCl in an ice-bath. After standing for one hour at room temperature, the reaction mixture was poured into ice-water. Recrystallization of the precipitate from aqueous ethanol gave 15.25 g. (89%) of colorless prisms, m.p. 86–87°, lit.[35] m.p. 86–87°.

4-[2,6-Diiodo-4-(2-carbethoxyethyl)-phenoxy]-2,4,6-tri *t*-butyl-2,5-cyclohexadien-1-one (VII, R_3 = I, R_4 = $(CH_2)_2COOC_2H_5$).—A free radical solution was prepared from 5.25 g. (20 mmoles) of 2,4,6-tri-*t*-butylphenol as described for the synthesis of the quinol ether VII (R_3 = H, R_4 = $(CH_2)_2COOC_2H_5$). A solution of 4.6 g. (10 mmoles) of the ethyl ester of 3,5-diiodophloretic acid in 50 ml. of ethyl acetate was then added within a few minutes. After standing for 10 minutes, the slightly brownish-blue solution was evaporated *in vacuo* at low temperature (< 0°). The light brown color of the distillate indicated that some iodine had been liberated. The viscous residue was well mixed with 25 ml. of methanol in the cold (Dry Ice–acetone-bath). The mixture was then permitted to stand at −25° for 30 minutes. The yellow crystals formed were collected by filtration; m.p. 97° dec.; yield 6.39 g. (90%). Brief digestion with *n*-pentane in a Dry Ice–acetone-bath, followed by filtration, gave 5.96 g. (84 %) of yellow crystals, m.p. 97° dec. The fusion begins to bubble at about 125°.

Anal. Calcd. for $C_{29}H_{40}I_2O_4$: C, 49.30; H, 5.71; I, 35.93. Found: C, 50.60; H, 5.77; I, 34.39.

Digestion of the crystals with methanol, followed by another digestion with *n*-pentane, both with cooling (Dry Ice–acetone-bath), did not raise the melting point.

The infrared spectrum (KBr) shows bands at 5.77 (carbonyl of $COOC_2H_5$), 6.00 and 6.08 μ (carbonyl and double bonds of the quinol ring), 8.06 and 10.10 μ (aromatic quinol ether).[30]

4-[2,6-Diiodo-4-(2-carboxyethyl)-phenoxy]-2,4,6-tri-*t*-butyl-2,5-cyclohexadien-1-one (VII, R_3 = I, R_4 = $(CH_2)_2COOH$).—A free radical solution was prepared from 5.25 g. (20 mmoles) of 2,4,6-tri-*t*-butylphenol as described for the synthesis of the quinol ether (VII, R_3 = H, R_4 = $(CH_2)_2COOC_2H_5$). A solution of 4.20 g. (10 mmoles) of 3,5-diiodophloretic acid[35] in 60 ml. of ethyl acetate was then added within a few minutes. After standing for 10 minutes, the light blue solution was evaporated *in vacuo* at low temperature (0°). The light brown color of the distillate showed that some iodine had been liberated. The crystalline residue was well mixed with methanol (Dry Ice–acetone-bath). After standing overnight at −25° the yellow crystals were collected by filtration, then washed with a small amount of cold methanol; m.p. 111° dec.; yield 2.60 g. (38% based on diiodophloretic acid). Digestion with 15 ml. of *n*-pentane in the cold (Dry Ice–acetone-bath), followed by filtration, raised the melting point to 114° dec.; yield 1.80 g. (26%). Evolution of gas occurs already at the melting point.

Anal. Calcd. for $C_{27}H_{36}I_2O_4$: C, 47.80; H, 5.35; I, 37.42. Found: C, 47.84; H, 5.35; I, 37.15.

The infrared spectrum (KBr) shows bands at 5.86 (carbonyl of COOH), 6.00 and 6.09 (carbonyl and double bonds of the quinol ring), 8.06 and 10.10 μ (aromatic quinol ether).[30] The infrared spectrum in Fluorolube S (2–7.4 μ)–Nujol (7.4–15 μ) show the same bands.

Sodium 3′,5′-Di-*t*-butylthyroacetate[36] (VIII, R_3 = H, R_4 = CH_2COONa).—The quinol ether VII (R_3 = H, R_4 = $CH_2COOC_2H_5$) (1.76 g., 3 mmoles) was heated at 160–190° until bubbling ceased (about 30 minutes).

The reddish-brown reaction mixture weighed 1.55 g. (weight loss, 12%; calcd. for the elimination of one *t*-butyl group, 13%). Short path distillation *in vacuo* (about 0.1 mm.) gave two fractions. Fraction 1 (bath temperature <180°), 0.35 g. of pale yellow crystals; fraction 2 (bath temperature 180–210°), 0.92 g. (62%) of a viscous orange liquid.

Recrystallization of fraction 1 from methanol gave colorless plates of tri-*t*-butylphenol, m.p. and mixed m.p. 130–131°.

(33) C. Stöhr, *Ann.*, **225**, 57 (1884).

(34) G. Habild, *Z. physiol. Chem.*, **285**, 127 (1950).

(35) J. H. Barnes, E. T. Borrows, J. Elks, B. A. Hems and A. G. Long, *J. Chem. Soc.*, 2824 (1950).

(36) Thyroacetic acid, *p*-(*p*-hydroxyphenoxy)-phenylacetic acid; thyropropionic acid, 3-[*p*-(*p*-hydroxyphenoxy)-phenyl]-propionic acid.

Part of fraction 2 (0.66 g.) was refluxed for one hour with a mixture of 2 ml. of 2 *N* NaOH and 10 ml. of ethanol. The ethanol was evaporated and 20 ml. of water was added to the residue.

The slightly colored crystals formed were collected by filtration; yield 0.55 g. (51% based on the quinol ether). Recrystallization from aqueous ethanol gave colorless plates which contained water of crystallization. This was eliminated by drying at 80° *in vacuo*. On heating, the dried crystals turned brown at about 230°, without melting.

Anal. Calcd. for $C_{22}H_{27}O_4Na$: C, 69.82; H, 7.19. Found: C, 69.57; H, 7.34.

The infrared spectrum (KBr) shows bands at 2.84 (phenolic OH); 6.29–6.34 (carbonyl of COO^-); 7.71, 8.01, 8.22, 8.35. 10.36 μ (several of these bands are caused by the diphenyl ether linkage[18]).

Acidification of the sodium salt gave the free acid in the form of a viscous oil that could not be crystallized.

The R_f-values in paper chromatograms are 0.83 (solvent 1), 0.75 (solvent 2) and 0.67 (solvent 3).

3,5-Di-*t*-butylthyropropionic Acid[36] (VIII, R_3 = H, R_4 = $(CH_2)_2COOH$).—A. By Pyrolysis.—The quinol ether (VII R_3 = H, R_4 = $(CH_2)_2COOH$) (2.13 g., 5 mmoles) was heated at 165° for 10 minutes, then at 190° until bubbling ceased (about 20 minutes). The brownish-yellow reaction mixture weighed 1.88 g. (weight loss, 12%; calcd. for the elimination of one *t*-butyl group 13%). It was dissolved in 50 ml. of 0.1 *N* NaOH and this solution was extracted with *n*-pentane. Evaporation of the pentane extract gave brownish crystals (0.30 g.) which, upon recrystallization from methanol, yielded colorless plates of 2,4,6-tri-*t*-butylphenol, m.p. and mixed m.p. 130–131°.

The aqueous layer was acidified with 5 ml. of 1 *N* HCl. The light brown precipitate formed (1.30 g., 70%) gave, upon two recrystallizations from benzene–isoöctane, 0.65 g. (35%) of colorless needles, m.p. 187–189°.

Anal. Calcd. for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.80; H, 8.08.

The infrared spectrum (KBr) showed bands at 2.84 (phenolic OH); 5.87 (carbonyl of COOH); 7.72, 7.97, 8.19, 8.38, 10.37 μ (several of these bands are caused by the diphenyl ether linkage[18]).

The R_f-values in paper chromatograms are 0.80 (solvent 1), 0.74 (solvent 2) and 0.67 (solvent 3).

Paper chromatography of the mother liquors from the recrystallization of the di-*t*-butylthyropropionic acid followed by spraying with diazotized N^1,N^1-diethylsulfanilamide[19] revealed the presence of phloretic acid.

B. **By Acid Catalysis.**—A solution of 0.21 g. (0.5 mmole) of the quinol ether VII (R_3 = H, R_4 = $(CH_2)_2COOH$) and of 0.01 g. of β-naphthalenesulfonic acid in 5 ml. of ethyl acetate was refluxed for 20 minutes. The originally yellow solution became first green, then brownish-yellow. The residue obtained after the removal of the solvent *in vacuo* was dissolved in benzene. The solution was filtered to remove insoluble β-naphthalenesulfonic acid and the filtrate was evaporated *in vacuo*. The residue was recrystallized from benzene–isoöctane. The less soluble fraction (0.02 g.), upon recrystallization from benzene–ethyl acetate, gave colorless plates, m.p. 118–122°, which were identified by paper chromatography[19] as impure phloretic acid. The more soluble fraction (0.09 g., 48%), m.p. 178–183°, upon recrystallization from benzene–ethyl acetate and then from aqueous ethanol, gave colorless needles of 3,5-di-*t*-butylthyropropionic acid, m.p. 187–189°. A mixture of this acid and the one prepared by pyrolysis (procedure A), also melts at 187–189°.

In a blank experiment in which a solution of the quinol ether in ethyl acetate was refluxed without β-naphthalenesulfonic acid, no 3,5-di-*t*-butylthyropropionic acid could be detected in the reaction mixture that remained green. Most of the starting material was recovered unchanged.

3,5-Dibromo-3′,5′-di-*t*-butylthyropropionic Acid[36] (VIII, R_3 = Br, R_4 = $(CH_2)_2COOH$). A. By Pyrolysis.—The quinol ether VII (R_3 = Br, R_4 = $(CH_2)_2COOH$) (2.92 g., 5 mmoles) was heated at 135–140° for 5 minutes, then at 165–170° for another 5 minutes. Vigorous bubbling ceased after the first 3 minutes at 135–140°; thereafter, only little evolution of gas took place. The dark brown reaction mixture weighed 2.74 g. (weight loss, 6.2%; calcd. for the elimination of one *t*-butyl group, 9.6%). After the addition of a mixture of 25 ml. of 0.2 *N* NaOH and 15 ml. of ethanol, the reaction mixture was extracted several times with ligroin (b.p. 66–75°). Evaporation of the ligroin extract gave 1.15 g. of crude 2,4,6-tri-*t*-butylphenol. The aqueous layer was acidified with 1 *N* HCl, then extracted with ether. The ether extract was evaporated and the residue dissolved in 50 ml. of 1-butanol. The butanol layer was washed 20 ml. of 2 *N* NaOH and then with 20 ml. of water.[19,37] Evaporation *in vacuo* of the butanol layer, followed by acidification of the residue with dil. HCl, gave 0.75 g. of a sticky brown material which was recrystallized from benzene; yield 0.28 g. (11%). Recrystallization from benzene gave 0.23 g. (9%) of colorless needles, m.p. 169–171°.

Anal. Calcd. for $C_{23}H_{28}Br_2O_4$: C, 52.29; H, 5.34; Br, 30.26. Found: C, 52.19; H, 5.37; Br, 30.08.

The infrared spectrum (Fluorolube S for 2–7.4 μ; Nujol for 7.4–15 μ) shows bands at 2.82 (phenolic OH); 5.82 (carbonyl of COOH); 7.72, 7.95, 8.24, 8.38, 10.41 μ (several of these bands are caused by the diphenyl ether linkage[18]).

The R_f-values in paper chromatograms are 0.85 (solvent 1), 0.82 (solvent 2) and 0.78 (solvent 3).

Acidification of the combined aqueous layers obtained in the butanol extraction with dil. HCl gave 0.91 g. of a reddish-brown solid. Recrystallization from benzene gave 0.40 g. of colorless prisms, m.p. 105–108°, which were shown by mixed m.p. and by paper chromatography[19] to be 3,5-dibromophloretic acid.

B. **By Acid Catalysis.**—A solution of 1.0 g. (1.7 mmoles) of the quinol ether VII (R_3 = Br, R_4 = $(CH_2)_2COOH$) and of 0.03 g. of β-naphthalenesulfonic acid in 17 ml. of ethyl acetate was refluxed for 20 minutes. The brownish-green solution was evaporated *in vacuo* to dryness, and the residue was taken up in benzene. Insoluble β-naphthalenesulfonic acid was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was dissolved in 15 ml. of 1-butanol. This solution was treated with 2 *N* NaOH and worked up as described in procedure A. Needles (0.06 g., 7%) were obtained which, upon recrystallization, melted at 168–170°. A mixture with 3,5-dibromo-3′,5′-di-*t*-butylthyropropionic acid prepared by pyrolysis (procedure A) had the same melting point.

Acidification of the combined aqueous layers from the butanol extraction again yielded 3,5-dibromophloretic acid (see procedure A).

Decomposition of the Quinol Ether VII (R_3 = I, R_4 = $(CH_2)_2COOH$). A. By Pyrolysis.—The quinol ether VII (R_3 = I, R_4 = $(CH_2)_2COOH$) (0.3441 g., 0.51 mmole) was heated at 125–135° for 5 minutes; weight loss 3.9 mg. (1.1%; calcd. for the elimination of one *t*-butyl group, 8.2%). The reaction mixture was then heated at 155–160° until bubbling ceased (2 minutes). The total weight loss was 7.8 mg. (2.3% of the starting material or 27% of the theory). The dark brown reaction mixture was dissolved in 20 ml. of 1-butanol. This solution was washed with 15 ml. of 2 *N* NaOH and with 10 ml. of water, then evaporated *in vacuo* to dryness. The residue was acidified with aqueous HCl and the acid mixture again evaporated. Extraction of the residue with benzene, followed by evaporation of the extract, gave 0.20 g. of a sticky dark brown mass. Attempts to obtain crystals failed. The paper chromatogram (solvent 2), after spraying with $FeCl_3$–$K_3[Fe(CN)_6]$ followed by washing with dil. HCl and water,[20] showed no distinct spots between R_f 0.8 and 0.9, where 3,5-diiodo-3′,5′-di-*t*-butylthyropropionic acid must be expected. (There was a spot with an R_f-value of 0.97 which is probably too high for the expected analog of thyroxine.)

The combined aqueous layers from the butanol extraction were acidified with dil. HCl, then extracted with ether. Evaporation of the ether gave a sticky mass in which phloretic acid, 3-iodophloretic acid and 3,5-diiodophloretic acid were identified by paper chromatography.[19]

B. **By Acid Catalysis.**—The quinol ether VII (R_3 = I, R_4 = $(CH_2)_2COOH$) (0.20 g.) and β-naphthalenesulfonic acid (0.02 g.) were dissolved in 5 ml. of ethyl acetate. The solution which was first blue slowly became dark brown. After standing at −25° for 18 days, the reaction mixture was evaporated. The residue was extracted with methanol. Yellow crystals (0.05 g.), m.p. 144° dec., which did not dissolve in methanol, proved to be bis-(4-oxo-1,3,5-tri-*t*-butyl-2,5-cyclohexadien-1-yl) peroxide.[14b] Paper chromatography of the methanol extract carried out as described in procedure A showed the presence of the same substances that were ob-

(37) J. P. Leland and G. L. Foster, *J. Biol. Chem.*, **95**, 165 (1932).

tained during pyrolysis (procedure A) and in addition a compound with an R_f value of 0.82. As this compound reacted not only with $FeCl_3$–$K_3[Fe(CN)_6]$ but also with diazotized N^1,N^1-diethylsulfanilamide (brownish-yellow spot), it cannot be a 3′,5′-di-*t*-butyl analog of thyroxine. It is suspected to be 2,4,6-triiodophenol.[19]

Spontaneous Decomposition of the Quinol Ether VII (R_3 = I, R_4 = $CH_2COOC_2H_5$).—The following is an example for the spontaneous decomposition of solutions of the iodinated quinol ethers of the type VII, in the absence of an acidic catalyst.

The quinol ether VII (R_3 = I, R_4 = $CH_2COOC_2H_5$) (0.80 g., 1.2 mmoles) was dissolved at room temperature in 30 ml. of *n*-pentane. The resulting light-blue solution began to turn brownish-red after a few minutes. After standing 3 days at room temperature the reaction mixture was filtered to remove an amorphous precipitate that had formed (0.13 g.). The filtrate was titrated with 0.1 *N* sodium thiosulfate. This titration showed that 24% of the iodine of the quinol ether had been liberated as iodine. The pentane layer was concentrated, and the concentrate filtered to remove some more amorphous precipitate (0.18 g.). Attempts to crystallize the two batches of amorphous powder (total weight 0.31 g.), failed. The powder seems to consist of a mixture of polymerized substances. It was apparently formed by free radical-induced polymerization.

The filtrate, upon standing at −25°, deposited yellow crystals of bis-(4-oxo-1,3,5-tri-*t*-butyl-2,5-cyclohexadien-1-yl) peroxide,[14b] m.p. 144° dec.

BETHESDA, 14 MD.

[CONTRIBUTION FROM THE HORMONE RESEARCH LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY]

The Synthesis of L-Histidyl-L-phenylalanyl-L-ornithyl-L-tryptophyl-glycine and L-Histidyl-D-phenylalanyl-L-ornithyl-L-tryptophyl-glycine and their Melanocyte-stimulating Activity

BY CHOH HAO LI, EUGEN SCHNABEL AND DAVID CHUNG

RECEIVED AUGUST 7, 1959

The synthesis of peptides L-histidyl-L-phenylalanyl-L-ornithyl-L-tryptophyl-glycine and L-histidyl-D-phenylalanyl-L-ornithyl-L-tryptophyl-glycine is described. These two synthetic peptides have been shown by bioassay to possess melanotropic activities identical to their L-phenylalanyl- and L-arginyl analogs.

In the course of the purification and isolation of adrenocorticotropins (ACTH) from pituitary glands of various species, it has been observed by a number of investigators that every adrenocortically-active fraction always possesses melanocyte-stimulating activity.[1] It is now well established that the melanocyte-stimulating activity found in the adrenocorticotropins is an intrinsic biologic property of the hormone.[1,2] When the chemical structure of the melanotropins (MSH)[3,4] is compared with that of the adrenocorticotropins,[1,5] it is evident that a heptapeptide sequence, L-methionyl-glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl-glycine, occurs in all preparations of these two hormones. Indeed, synthetic peptides[6,7,8] related to this heptapeptide have recently been shown to possess melanocyte-stimulating activity. One of the outstanding features of these natural and synthetic peptides is the effect on them of alkali-heat treatment. For example, when a solution of L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl-glycine (1 mg. per ml.) in 0.1 *M* NaOH was kept in a boiling water-bath for 15 minutes, the activity which is responsible for darkening the skin of hypophysectomized frogs was greatly prolonged.[7,9] It was suspected from unpublished observations in this Laboratory that either the conversion of arginine to ornithine or the racemization of L-phenylalanine, or both, may be responsible for the "prolongation" effect of the alkali–heat treatment; consequently, the synthesis of L-histidyl-L-phenylalanyl-L-ornithyl-L-tryptophyl-glycine (I) and L-histidyl-D-phenylalanyl-L-ornithyl-L-tryptophyl-glycine (II) was undertaken.

The synthesis of I was accomplished in two different ways, which differed only in the blocking of the imidazo group of histidine. In one instance, the benzyl group was used to protect the imidazo group, whereas in the other the imidazo group remained free. Carbobenzoxy-L-histidine azide was coupled with L-phenylalanine methyl ester and converted to the carbobenzoxy-dipeptide hydrazide in the manner described by Hofmann, *et al.*[10] The azide was only slightly soluble in ethyl acetate and chloroform; therefore, after the amorphous material was filtered, it was suspended in an ethyl acetate solution of δ-carbobenzoxy-L-ornithine methyl ester[11] and kept in the refrigerator for 3 days with occasional shaking. The carbobenzoxy-L-histidyl-L-phenylalanyl-δ-carbobenzoxy-L-ornithine methyl ester was contaminated with some histidyl carbobenzoxy-L-histidyl-L-phenylalanine[10] and material formed by Curtius degradation, and could be purified only by recrystallization from a mixture of boiling water and ethyl acetate. The crystalline product was saponified with Ba(OH)$_2$, and carbobenzoxy-L-histidyl-L-phenylalanine-δ-carbobenzoxy-L-ornithine (III) was obtained in crystalline form. The condensation of III with L-tryptophyl-glycine benzyl ester (IV) was accomplished by means of the dicyclohexylcarbodiimide (DCCI) method.[12] The product V was

(1) C. H. Li, *Adv. Protein Chem.*, **11**, 101 (1956).

(2) C. H. Li, P. Fønss-Bech, I. I. Geschwind, T. Hayashida, G. Hungerford, A. J. Lostroh, W. R. Lyons, H. D. Moon, W. O. Reinhardt and M. Sideman, *J. Expt. Med.*, **105**, 335 (1957).

(3) C. H. Li, *Adv. Protein Chem.*, **12**, 269 (1957).

(4) J. I. Harris and A. B. Lerner, *Nature*, **179**, 1346 (1957).

(5) C. H. Li, J. S. Dixon and D. Chung, THIS JOURNAL, **80**, 2587 (1958).

(6) K. Hofmann, T. A. Thompson and E. T. Schwartz, *ibid.*, **79**, 6087 (1957).

(7) R. Schwyzer and C. H. Li, *Nature*, **182**, 1669 (1958).

(8) K. Hofmann, M. E. Woolner, H. Yajima, G. Spiokler, T. A. Thompson and E. T. Schwartz, THIS JOURNAL, **80**, 6458 (1958).

(9) C. H. Li, *Laboratory Investigation*, **8**, 574 (1959).

(10) K. Hofmann, H. Kappeler, A. E. Furlenmeier, M. E. Wooner, E. T. Schwartz and T. A. Thompson, THIS JOURNAL, **79**, 1641 (1957).

(11) R. L. Synge, *Biochem. J.*, **42**, 99 (1948).

(12) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955).