Model Reactions for the Metabolism of Thyroxine. I. Nonenzymic Cleavage of the Diphenyl Ether Linkage of 3′-Hydroxythyropropionic Acid

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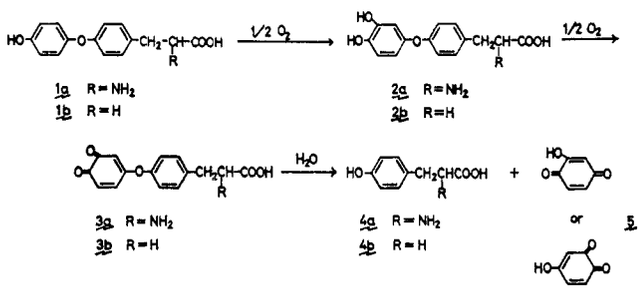
In connection with the hypothetical catabolic pathway by which thyroxine is converted into 3′-hydroxy-3,5,5′-triiodothyronine which in turn cleaves at the diphenyl ether linkage to form 3,5-diiodotyrosine, nonenzymic autoxidations of 3′-hydroxythyropropionic acid and of related compounds were carried out under various conditions. A facile cleavage of the diphenyl ether linkage of 3′-hydroxythyropropionic acid occurred at pH 7.6 and above and phloretic acid was formed in nearly quantitative yield. Chemical and electron spin resonance spectroscopic evidence indicates that in this reaction an initially formed semiquinone radical is converted, probably *via* an *o*-quinone, into the semiquinone radical of 1,2,4-trihydroxybenzene and phloretic acid.

Rupture of the diphenyl ether linkage of thyroxine (T_4)[2] is one of the possible pathways in the metabolism of T_4. Various investigators have found that the formation of 3,5-diiodotyrosine (DIT)[2] and other reaction products in the enzymic degradation of T_4 *in vitro* is always accompanied by deiodination. (For a review of earlier work see Rall, *et al.*,[3] for a more recent report see Björkstén.[4]) Lissitzky, *et al.*,[5,6] postulated a mechanism for the oxidation of thyronine by polyphenol oxidase as shown in Scheme I. They

SCHEME I



reported that aerobic incubation of thyronine (**1a**) with a polyphenol oxidase resulted in the formation of 3′-hydroxythyronine (**2a**) and tyrosine (**4a**) in addition to hydroxybenzoquinone (**5**) which was detected spectroscopically as its phenazine derivative. It is reasonable to assume an analogous pathway in the enzymic degradation of T_4 to DIT. In such a hypothetical pathway the first step would be an oxidative deiodination at the 3′ position. The 3′-hydroxy-3,5,5′-triiodothyronine thus formed would be oxidized to an *o*-quinone which then undergoes hydrolytic splitting at the diphenyl ether linkage to give diiodotyrosine and hydroxybenzoquinone (**5**). However, 3′-hydroxy-3,5,5′-triiodothyronine, an intermediate in this scheme, has so far neither been detected nor synthesized.

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(2) Abbreviations used: T_4, thyroxine; DIT, 3,5-diiodotyrosine; HTP, 3′-hydroxythyropropionic acid; THB, 1,2,4-trihydroxybenzene; hfsc, hyperfine splitting constant(s).

(3) J. E. Rall, J. Robbins, and C. G. Lewallen in "The Hormones," Vol. V, G. Pincus, K. V. Thimann, and E. B. Astwood, Ed., Academic Press, New York, N. Y., 1964, p 249.

(4) F. Björkstén, *Acta Chem. Scand.*, **20**, 1438 (1966).

(5) S. Lissitzky and S. Bouchilloux, *Bull. Soc. Chim. Biol.*, **39**, 133, 1215 (1957).

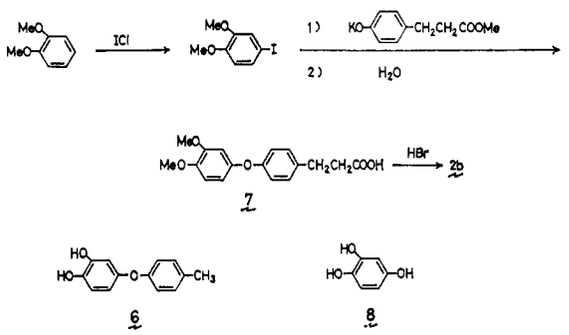
(6) S. Lissitzky, M-T. Bénévent, J. Nunez, C. Jacquemin, and J. Roche, *C. R. Soc. Biol.*, **154**, 267 (1960).

As part of a program to elucidate the nature of the reaction which involves cleavage of the diphenyl ether linkage of T_4, we investigated the nonenzymic autoxidation of 3′-hydroxythyropropionic acid (HTP)[2] (**2b**) and related catechols as simple models for the hypothetical intermediate, 3′-hydroxy-3,5,5′-triiodothyronine. Exposure of nearly neutral or alkaline aqueous solutions of HTP to air at room temperature resulted in the splitting of the molecule at the diphenyl ether bridge and the formation of phloretic acid (**4b**) in nearly quantitative yield. The nature of various free-radical intermediates formed in the course of this autoxidation was established by esr spectroscopy in conjunction with oxygen uptake experiments. The characterization of these intermediates made it possible to derive a mechanism for the autoxidative degradation of HTP.

Results

Synthesis of HTP.—HTP (**2b**) was synthesized from veratrole in 30% over-all yield as summarized in Scheme II. Iodination of veratrole with ICl gave 4-iodoveratrole in 67% yield. The acid **7** was obtained in 51.5% yield by condensation of 4-iodoveratrole with the potassium salt of methyl 3-(4-hydroxyphenyl)propionate, followed by alkaline hydrolysis. Demethylation of **7** with hydrobromic acid gave HTP in 58% yield. The catechol **6** was synthesized in a similar manner (see Experimental Section).

SCHEME II



Autoxidation of Catechols.—When a solution of HTP (**2b**) in sodium phosphate buffer (pH 7.6) was allowed to stand in an open container at room temperature, the reaction mixture turned orange-brown and after 3 days

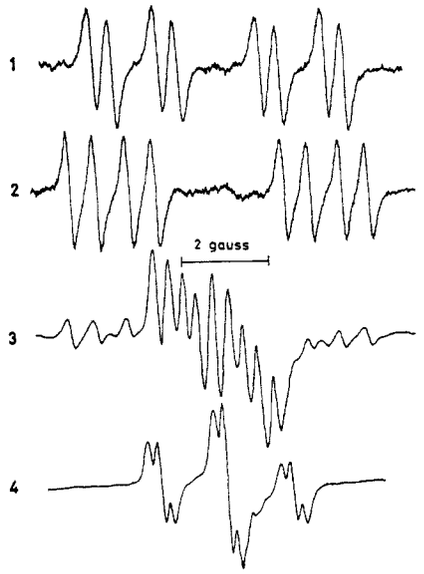


Figure 1.—Esr signals: signal 1, signal of the semiquinone radical **10** (see Scheme III), first signal observed at pH 9.6 in the autoxidation of HTP, g = 2.0039, a_3 = 1.53, a_5 = 3.96, a_6 = 0.43 G (numbering of the semiquinone ring protons according to Stone and Waters[7]); signal 2, signal of the semiquinone radical **13** of THB (see Scheme III) observed at pH >9.5 in the autoxidation of THB and also at pH 12 in the autoxidation of HTP, a_3 = 1.34, a_5 = 4.85, a_6 = 0.61; signals 3 and 4; unidentified signals observed at pH 12 in the autoxidation of HTB and/or THB.

no starting material could be detected by tlc. The reaction mixture, after acidification with HCl, was extracted with ether. The ether extract was chromatographed on a column of silica gel. Phloretic acid (**4b**) was isolated in 96% yield by eluting the column with $CHCl_3$–actone (9:1). The yield of **4b** under various experimental conditions is shown in Table I.

TABLE I

AUTOXIDATION OF HTP AT VARIOUS pH VALUES

pH	Autoxidation period, hr[a]	Temp, °C	Yield of phloretic acid (**4c**), %[b]
7.6	68	25	96
10.0	19	25	100
12.0	1	25	94

[a] Time required for complete disappearance of HTP. [b] The yield was determined by vpc of the trimethylsilyl derivative.

Extraction of the aqueous layer which remained after the above-mentioned ether extraction with 1-butanol gave substances which were identical with the products of the autoxidation of THB[2] (**8**) as shown by tlc and paper electrophoretic analysis. The autoxidation of THB was carried out in the same manner as that of HTP. These results suggest that **8** is formed when the diphenyl ether linkage is cleaved in the course of the autoxidation of HTP. The intermediary formation of **8** or of an oxidation product of **8** such as the semiquinone **13** is also supported by the esr studies reported below.

In the autoxidation of 1,2-dihydroxy-4-toluoxybenzene under similar conditions, diphenyl ether cleavage was also observed. *p*-Cresol was one of the reaction products.

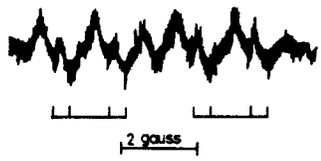


Figure 2.—Esr signal observed in the autoxidation of HTP (7×10^{-3} M) in 0.1 M phosphate buffer, pH 7.6. A time-averaging computer (404 scannings) was used to compensate for the low radical concentration at this pH.

At pH 12, autoxidation of HTP was completed after less than 1 hr, when 0.43 mol of oxygen had been taken up per mole of HTP.

Esr Studies.—Esr studies were carried out in order to obtain information on intermediates in the autoxidation of HTP. When a 1.4×10^{-3} to 1.1×10^{-2} M solution of HTP in 0.1 M phosphate buffer was circulated in the presence of air through an esr cell at pH 7.6, no signal was observed. When the pH was raised to 9.6 a pair of quartets (Figure 1, signal 1) could be observed. Signal 1 is similar to that of the semiquinone radical **13** (signal 2) obtained in the autoxidation of THB, but the hfsc[2] and the behavior of the two radicals are different. In the autoxidation of HTP at pH 7.6, the rate of reaction is very slow (see above) and the radical concentration consequently so low that no signal could be observed directly. However, when a time-averaging computer (404 scannings) was used, a pair of quartets (Figure 2) could be detected. Although the double quartet signal was somewhat deformed (additional absorption between the two quartets), its hfsc coincide well with those of signal 1. (At pH 9–10 signal 1 deformed slowly and assumed the same shape as the signal shown in Figure 2.)

When the pH of a 1.1×10^{-2} M solution of HTP was raised from 9.6 to 12.0, signal 1 changed to signal 3 which is a composite signal. One of its components (the peripheral part of the signal) is identical with signal 2, which was obtained when a solution of THB in 0.1 M phosphate buffer (pH >9.5) was circulated through an esr cell in the presence of air but without oxygen bubbling. The hfsc of signal 2 are nearly identical with those reported by Stone and Waters[7] for the semiquinone radical **13** of THB. Signal 2 was quite stable at pH 9–10, but on raising the pH to 12, it changed to a composite signal consisting of signals 2 and 4. When oxygen was then bubbled through the solution, signal 2 disappeared and only signal 4 remained. The life time of radical 4 is short and within several minutes of oxygen-bubbling signal 4 changed further to signal 3 (less the peripheral quartet).

Discussion

The radical giving rise to signal 1 (Figure 1) should be the semiquinone radical **10** of HTP, judging from its hfsc which resemble those of the semiquinone radical **13** of THB (signal 2). The facts that signal 1 could be observed in the autoxidation of HTP not only at a high pH, but also at pH 7.6, and that the autoxidation products are essentially the same at pH 7.6 and at 12.0 (Table I) indicate that the phenolic hydroxyls of HTP are partly dissociated at pH 7.6 and that the formation

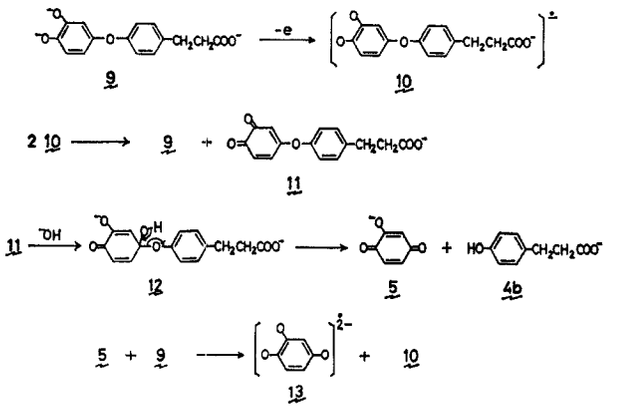
(7) T. J. Stone and W. A. Waters, *J. Chem. Soc.*, 1488 (1965).

of the semiquinone radical **10** of HTP must be the initial step in the autoxidation of HTP. This conclusion is supported by oxygen uptake experiments which show that 0.4–0.5 mol of O_2 is consumed per mole of HTP.

Another important observation made in the course of the esr experiments is the appearance of the signal of the semiquinone radical **13** of THB in the autoxidation of HTP. The identity of signal 2 observed in the autoxidation of HTP with signal 2 observed in the autoxidation under similar conditions of THB was proven by comparison of their shapes and hfsc and also by the identical behavior of those radicals on raising the pH to 12. This converted both signals into signal 3. The fleeting intermediary appearance of signal 4 was observed only in the case of THB and not in that of HTP. This is certainly due to the short life time of the radical 4. The nature of the free radicals giving rise to signals 3 and 4 remains to be elucidated. Furthermore, the butanol-soluble products from the autoxidation of HTP at pH 7.6 were essentially the same as those obtained from THB under similar conditions. The appearance of the semiquinone radical **13** of THB when HTP is autoxidized strongly supports Lissitzky's hypothetical mechanism for the enzymic degradation of thyronine (**1a**) (Scheme I) according to which thyronine is split at the diphenyl ether bridge to form hydroxybenzoquinone (**5**). Since the diphenyl ether linkage of T_4 can be ruptured chemically,[8] photolytically,[9] and enzymically,[4-6,10-12] it is reasonable to suspect a similar mode of breakdown of T_4 *in vivo*. In a recent investigation, however, Pittman and Chambers, Jr.,[13] found that in the rat the major excretion products arising from administered T_4 still had an intact diphenyl ether structure.

A plausible mechanism for the autoxidation of HTP, which is a model for the enzymic degradation of thyronine, can be derived from our findings. This hypothetical mechanism is shown in Scheme III.

SCHEME III



According to this scheme, the first step in the autoxidation of HTP is the formation of the semiquinone radical **10** from HTP in its dissociated form (**9**) by electron transfer involving a molecule of oxygen. Semiquinones are well known to undergo a disproportionation process. Thus **10** is converted back into **9** and into a quinone **11**. Alternatively further oxidation of **10** to **11** by oxygen is also possible, although less plausible. Nucleophilic attack of the quinone **11** by a hydroxyl anion causes cleavage of the ether linkage. Thus hydroxybenzoquinone (**5**) and phloretic acid (**4b**) are formed *via* the intermediate **12**. Electron transfer between HTP (**9**) and hydroxybenzoquinone (**5**) thus formed gives rise to the semiquinone radicals **10** and **13**, the latter of which is certainly more stable because of a more favored electron delocalization and can therefore be detected by esr as a component of signal 3.

In order to ascertain the intermediary formation of the *o*-quinone **11**, attempts were made to synthesize **11**. Treatment of thyropropionic acid with Fremy's salt yielded a red pigment which could not be isolated in pure form but which showed spectral properties which are in agreement with those expected for **11**. A solution of this pigment in phosphate buffer (pH 7.6) yielded phloretic acid (**4b**) on standing at room temperature. This finding also supports the above mechanism.

Another conceivable mechanism for the diphenyl ether cleavage in the autoxidation of HTP is "quinol ether equilibration."[14] This mechanism can, however, be ruled out by the nearly quantitative yield of phloretic acid.

Experimental Section

Spectra.—Nmr spectra were determined with a JMN-3H-60 recording spectrometer. Tetramethylsilane was used as an internal standard. Esr spectra were determined as described previously.[15,16] Ir spectra (Nujol mulls or KBr disks) were recorded with a Nihon Bunko Model IR-S spectrometer.

Chromatograms.—For vapor phase chromatography (vpc), columns (150 cm, 3.0-mm i.d.) packed with silicon DC 550 were used. The carrier gas was helium. The substances to be analyzed were injected after being converted into their trimethylsilyl derivatives by means of O,N-bis(trimethylsilyl)acetamide. For thin layer chromatography (tlc) silica gel covered glass plates containing a fluorescent indicator were used. Spots became visible in short-wave ultraviolet light or in iodine vapor.

Preparation of Starting Materials. 4-Iodoveratrole.—Iodine monochloride (16.3 g, 0.1 mol) was added dropwise during 15 min to a stirred ice-cooled solution of 13.8 g (0.1 mol) of veratrole in 10 ml of acetic acid. Stirring was continued for another 6 hr at room temperature. After the addition of 15 g of sodium carbonate, the reaction mixture was extracted with ether and the ether extract washed with water, dried (Na_2SO_4), and evaporated. Distillation of the oily residue gave 17.6 g (67%) of 4-iodoveratrole, bp 120–124° (5 mm) [lit.[17] bp 150–170° (7 mm)]. Authentic 4-iodoveratrole synthesized from 4-nitroveratrole by a known procedure[18] showed identical behavior in vpc.

1,2-Dihydroxy-4-toluoxybenzene (6).—A stirred mixture of the potassium salt of *p*-cresol prepared from 16.4 g (0.15 mol) of *p*-cresol and 4.0 g (0.15 g-atom) of potassium in dry benzene, 20 g (76 mmol) of 4-iodoveratrole, and 1 g of active copper[19] was heated at 150–180° for 4 hr. After cooling, the reaction mixture was extracted with ether and the ether extract washed with a dilute aqueous NaOH solution and with water, dried (Na_2SO_4), and evaporated *in vacuo*. Two successive distillations of the residue

(8) S. Lissitzky and M. Roques, *Bull. Soc. Chim. Biol.*, **39**, 521 (1957).
(9) S. Lissitzky, M-T. Bénévent, and M. Roques, *Biochim. Biophys. Acta*, **51**, 407 (1961).
(10) L. G. Plaskett, *Biochem. J.*, **78**, 652 (1961).
(11) J. Wynn and R. Gibbs, *J. Biol. Chem.*, **237**, 3499 (1962).
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(13) C. S. Pittman and J. B. Chambers, Jr., *Endocrinology*, **84**, 705 (1969).
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(15) T. Matsuura, H. Kon, and H. J. Cahnmann, *J. Org. Chem.*, **29**, 3058 (1964).
(16) A. Nishinaga, H. Kon, H. J. Cahnmann, and T. Matsuura, *ibid.*, **33**, 157 (1968).
(17) C. Seer and E. Karl, *Monatsh.*, **34**, 647 (1913).
(18) D. Cardwell and R. Robinson, *J. Chem. Soc.*, **107**, 256 (1915).
(19) R. Q. Brewster and R. Groening, "Organic Syntheses," Coll. Vol. II, John Wiley & Sons, Inc., New York, N. Y., 1950, p 446.

gave 11.7 g of 1,2-dimethoxy-4-toluoxybenzene, bp 178–184° (9 mm). A solution of 5 g (20.4 mmol) of this distillate in 20 ml of 48% hydrobromic acid and 60 ml of acetic acid was refluxed for 2 hr under nitrogen. Acetic and hydrobromic acid were removed by evaporation *in vacuo*. Successive distillation at 10^{-3} mm (bath temperature 150°) and crystallization from benzene–isooctane of the residue gave 2.2 g (31%) of **6**, mp 94–97°.

Anal. Calcd for $C_{13}H_{12}O_3$: C, 72.18; H, 5.83. Found: C, 72.21; H, 5.59.

3-[4-(3,4-Dimethoxyphenoxy)phenyl]propionic Acid (7).—The potassium salt of methyl 3-[4-hydroxyphenyl]propionate was prepared from 36.0 g (0.2 mol) of the free phenol[20] and 7.82 g (0.2 g-atom) of potassium in absolute methanol. After complete evaporation of the methanol, 60 g (0.22 mol) of 4-iodoveratrole and 1 g of active copper[19] was added and the mixture heated in an oil bath (150–160°) for 4 hr. After cooling, the mixture was extracted with ether and the ether extract washed with water, with a dilute NaOH solution, and again with water, dried (Na_2SO_4), and evaporated *in vacuo*. The oily residue was dissolved in 400 ml of 10% alcoholic KOH and the solution heated on a boiling water bath for 30 min. The reaction mixture was evaporated *in vacuo*, the residue dissolved in water, and the solution washed with ether, then acidified under ice cooling with concentrated hydrochloric acid. The precipitate formed was dried and crystallized from benzene–petroleum ether to give 31.3 g (51.5%) of **7** as colorless needles: mp 101–104°; nmr ($CDCl_3$) δ 2.77 (m, 4, CH_2CH_2), A_2B_2 pattern, 3.81 (s, 3, OCH_3), 3.84 (s, 3, COH_3), 6.85 (m, 7, aromatic), 10.8 (broad s, 1, COOH), disappears on addition of D_2O.

Anal. Calcd for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.29; H, 5.68.

3-[4-(3,4-Dihydroxyphenoxy)phenyl]propionic Acid (HPT) (2b).—A solution of 5 g (1.7 mmol) of **7** in 100 ml of 48% hydrobromic acid and 100 ml of acetic acid was refluxed for 2.5 hr under nitrogen. The reaction mixture was evaporated *in vacuo* and acetic acid was completely removed by repeated addition and evaporation of water. The oily residue was extracted with ether and the ether extract washed with water, dried (Na_2SO_4), and evaporated to give a syrup which solidified on standing in a refrigerator. Recrystallization from benzene containing a few drops of acetic acid gave 2.5 g (58%) of **2b** as colorless needles: mp 117–118°; nmr [$(CD_3)_2CO$] δ 2.72 (m, 4, CH_2CH_2), A_2B_2 pattern, 6.77 (m, 7, aromatic), 7.85 (broad s, 2, OH), 9.7 (very broad s, 1, COOH), signals of OH and COOH disappear on addition of D_2O.

Anal. Calcd for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15. Found: C, 65.59, H, 5.49.

3-[4-(4-Hydroxyphenoxy)phenyl]propionic Acid (1b).—The potassium salt of methyl 3-[4-hydroxyphenyl]propionate was prepared from 36.0 g (0.2 mol) of the free phenol as described above. After complete evaporation of the methanol, 52 g (0.22 mol) of 4-iodoanisole[21] and 1 g of active copper[19] was added and the mixture heated in an oil bath (150–180°) for 5 hr. After cooling, the mixture was shaken with water and ether. The ether layer was washed with ice-cooled dilute aqueous NaOH and water, then dried (Na_2SO_4) and evaporated. The residue was dissolved in 400 ml of 10% alcoholic KOH and heated at 70° for 30 min. The reaction mixture was evaporated and the residue dissolved in water. The aqueous solution was washed with ether, then acidified with dilute HCl to give 23.0 g of 3-[4-(4-methoxyphenoxy)phenyl]propionic acid as a colorless precipitate. A solution of the precipitate in a mixture of 200 ml of acetic acid and 200 ml of 48% hydrobromic acid was refluxed for 4 hr under nitrogen. The reaction mixture was evaporated and acetic and hydrobromic acid were removed by repeated addition and evaporation of water. The crystalline residue gave, after recrystallization from water, 15.2 g (30%) of **1b** as colorless crystals, mp 160–162° (lit. mp 162–163°,[22] 162°,[23] 175°[24]).

Oxygen Uptake Experiments.—Oxygen uptake was measured in a previously described apparatus.[25] A solution (1.82×10^{-3} *M*) of 200 mg of HTP in 400 ml of either 0.2 *M* sodium phosphate buffer (pH 7.6) or 0.2 *M* boric acid–NaOH (pH 7.7) or 0.3 *M* NaOH (pH ~12) was stirred under oxygen at 17°.

Autoxidation of HTP (2b) and of 1,2,4-Trihydroxybenzene (THB) (8).—A solution of 2 g (7.3 mmol) of HTP in 1 l. of 0.067 *M* sodium phosphate buffer (pH 7.6) was stirred magnetically at room temperature in an open container, until no starting material was detectable by tlc (3 days). The course of the reaction was followed by removing 1-ml aliquots from time to time and extracting them with ether after acidification (HCl, congo red). The extract was then analyzed by tlc. When starting material could no longer be detected the reaction mixture was acidified with HCl. (In some experiments the reaction mixture was treated with sodium borohydride and then worked up in the same manner. The tlc patterns obtained with or without borohydride treatment were identical.) The acidified mixture (A) was extracted with ether and the ether layer washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue (2.16 g) which showed one major and several minor tlc spots was chromatographed on a silica gel column (60 g). Elution with chloroform–acetone (9:1) gave 1.16 g (96%) of phloretic acid (**4b**) (mixture melting point and ir spectrum). Subsequent elution with chloroform–acetone (85:15) yielded a minor product (146 mg). Its methylation with diazomethane gave 120 mg of a methylated product as a syrup which was not a uniform compound, but whose nmr spectrum indicates that it consisted mainly of methylated HTP or a closely related compound. The acidified solution (A), after extraction with ether, was further extracted with 1-butanol. The butanol extract was evaporated to dryness, the residue extracted with ether, and the ether extract washed with water, dried (Na_2SO_4), and evaporated. The residue (108 mg) showed at least four tlc spots. Attempts to separate the mixture by preparative tlc were not successful. Paper electrophoresis at pH 6.15 (pyridine–acetic acid–water, 10:1:78) and tlc gave patterns which were identical with those obtained with an autoxidized solution of THB. The autoxidation of THB was carried out in the same manner as described for HTP. The autoxidation of HTP was also carried out under other conditions (higher pH and lower temperatures). In each case the tlc pattern was virtually identical with that obtained in the autoxidation of HTP at pH 7.6 and room temperature.

Autoxidation of 1,2-Dihydroxy-4-toluoxybenzene (6).—A solution of 2 g (0.01 mol) of **6** in 1 l. of sodium phosphate buffer (0.067 *M*, pH 7.7) was stirred in an open container at room temperature until no starting material was detectable by tlc (3 days). The reaction mixture was acidified and extracted with ether and the ether washed with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on a column of silica gel (50 g). Elution with chloroform gave 80 mg of *p*-cresol (ir and tlc). Further elution with chloroform–acetone (9:1) gave 700 mg of a powdery product. Attempts to purify it were unsuccessful, ir (KBr) 3450 and 3350 cm^{-1} (OH), no C=O band. The aqueous layer obtained in the above-mentioned extraction with ether, was further extracted with 1-butanol. The butanol extract, upon evaporation *in vacuo*, gave a few milligrams of an oily residue, whose tlc and paper electrophoretic patterns showed similarities to those obtained with the autoxidation products of THB (**8**).

Oxidation of 3-[4-(4-Hydroxyphenoxy)phenyl]propionic Acid (1b) with Fremy's salt.—To an ice-cooled stirred solution of 774 mg (3 mmol) of **1b**, synthesized as described above, in 60 ml of acetone was added over a period of 50 min 2.1 g of Fremy's salt (potassium nitrosodisulfonate) dissolved in 145 ml of 0.06 *M* KH_2PO_4. The mixture was then diluted with 100 ml of water and stirred for 20 min under ice cooling. The reaction mixture, after addition of another 100 ml of water, was extracted with chloroform (20 ml). The residue obtained upon drying (Na_2SO_4) and evaporating the chloroform extract was dissolved in absolute ether. When the solution was cooled to −70°, a red precipitate formed: uv ($CHCl_3$) 336 mμ (log ϵ 4.04, based on quinone **11**); ir (Nujol) no OH band, 1660 and 1640 cm^{-1} (C=O).

The red precipitate was dissolved in phosphate buffer (pH 7.6) and the solution allowed to stand at room temperature for 3 hr. The reaction mixture was then acidified and repeatedly extracted with ether. Analysis of the ether extract by tlc revealed the presence of phloretic acid (**4b**) together with other unidentified products.

Formation of Radicals.—In order to observe stable radicals formed in the autoxidation of HTP, solutions of HTP (1.4×10^{-3} to 1.1×10^{-2} *M*) were prepared by adding HTP to 0.1 *M*

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(21) H. R. Frank, P. E. Fanta, and D. S. Tarbell, *J. Amer. Chem. Soc.*, **70**, 2314 (1948).

(22) A. Nishinaga and T. Matsuura, *J. Org. Chem.*, **29**, 1812 (1964).

(23) L. Petit and N. P. Buu-Hoi, *ibid.*, **26**, 3832 (1961).

(24) R. I. Meltzer, S. Farber, E. Merrill, and A. Caro, *ibid.*, **26**, 1413 (1961).

(25) A. Nishinaga, H. J. Cahnmann, H. Kon, and T. Matsuura, *Biochemistry*, **7**, 388 (1968).

sodium phosphate buffer (pH 7.6–9.6) in an open container at room temperature. Immediately after the addition, circulation (~140 ml/min) of the straw-colored solution through an esr cell was started and the signals were recorded. The pH was kept at the desired value by the occasional addition of 1 *M* NaOH. In some cases oxygen or nitrogen was bubbled through the reaction mixture. Stable radicals formed in the autoxidation of THB were observed in a similar manner.

Registry No.—Thyroxine, 51-48-9; **2b**, 20224-53-7; **6**, 20224-54-8; **7**, 20224-55-9; **10**, 12349-50-7; **13**, 12349-49-4.

The Kinetics of the Decarboxylative Dehydration of β-Anisyl-β-hydroxy-α-phenylpropionic Acid[1,2]

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The decarboxylative dehydrations of *erythro*- and *threo*-β-anisyl-β-hydroxy-α-phenylpropionic acids proceed at different rates in dilute aqueous sulfuric acid solution. Both stereoisomers give *trans*-4-methoxystilbene. The diastereoisomers are interconverted at a rate which is slower than decarboxylation in dilute sulfuric acid solution, but at a rate more rapid than decarboxylation in more acidic medium. These facts are interpreted in terms of generation of a dipolar ion which loses carbon dioxide more rapidly than it reacts with water.

The behavior of β-hydroxy acids under a variety of circumstances has been studied in these laboratories; in addition to studies of the mechanism of acid-catalyzed dehydration[4,5] many features of the decarboxylative dehydration have been elucidated.[6-8] The reaction shows a particularly modest increase in rate with increasing acidity of the medium;[6] a plot of log *k* *vs.* H_0 typically has a slope of 0.4–0.6. It was shown that there are circumstances in which racemization (*e.g.*, of β-hydroxy-β-arylbutyric acids) is no more rapid than decarboxylation. This situation applies at low acidities. At higher acidities racemization was much more rapid than any other reaction of the β-hydroxy acids. It was also shown[1] that both diastereoisomers of α-methyl-β-hydroxy-β-(*p*-tolyl)propionic acid gives *trans*-*p*-propenyltoluene. In order to examine the kinetic features of decarboxylative dehydration more thoroughly, particularly in relation to the stereochemistry of the process, we have sought a compound which would be more suitable than β-anisyl-β-hydroxybutyric acid. For this purpose we have chosen to examine the kinetic behavior of the two diastereoisomers of β-anisyl-β-hydroxy-α-phenylpropionic acid (**1**). Ultraviolet spectra are distinctive for the four possible products, both *cis*- and *trans*-α-phenyl-*p*-methoxycinnamic acids, which could result from simple acid-catalyzed dehydration, as well as *cis*- and *trans*-4-methoxystilbenes, which could result from decarboxylative dehydration. These differences in spectra thus make it easy to follow the course of the reaction of **1** in detail.

A mixture of the two isomers of **1** was prepared by an Ivanov reaction and separated by chromatography over alumina. The *threo* configuration is assigned to the predominant isomer (mp 151–152°) on the basis of the following arguments. Zimmerman and Traxler[9] have unambiguously determined the configuration of the two diastereoisomers of α,β-diphenyl-β-hydroxypropionic acid (**2**) by a direct chemical method. More recently Canciell, *et al.*,[10] have shown that it is generally true that *threo* isomers of compounds such as **2** show a larger coupling constant between the α and β hydrogens than do the *erythro* isomers. Coupling constants very similar to those reported for *threo* **2** and *erythro* **2** were observed for the two diastereoisomers of **1**.

In fairly dilute sulfuric acid at 65° *threo* **1** and *erythro* **1** separately showed excellent first-order kinetics as followed by the appearance of the spectrum of *trans*-4-methoxystilbene. *threo* **1** reacted more rapidly than *erythro* **1**. These observations show that there is not rapid interconversion of the two diastereoisomers.

In 0.8 *M* sulfuric acid, the exclusive product is *trans*-4-methoxystilbene from both isomers. Control experiments showed that there is essentially none of the substituted cinnamic acid formed by simple dehydration, and that less than 1% of the *cis*-4-methoxystilbene is formed. Thus, the decomposition of both stereoisomers of **1** gives the same *trans* olefin, an observation which is completely in accord with the previous stereochemical results obtained in the study of α-methyl-β-hydroxy-β-(*p*-tolyl)propionic acid.[1]

When the kinetic studies were carried out at 65° in more concentrated sulfuric acid medium (about 1 *M*) the usual first-order plot was no longer linear, but showed some curvature. For *threo* **1** a plot of (log [*threo* **1**]) *vs.* time was slightly concave upward.

The lack of simple first-order behavior shows up more clearly in our kinetic measurements at 44°. Under these conditions and working in more concentrated sulfuric acid media, neither isomer showed simple first-order behavior. For *threo* **1** the plot of (log [*threo* **1**]) *vs.* time is concave upward initially and becomes linear only after about 50% reaction. For *erythro* **1** the corresponding plot is slightly concave downward, again becoming linear after approximately 50% reaction. Moreover, the limiting slope for the later stages of reac-

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