N, 5.90; S, 13.51. Found: C, 45.57; H, 8.11; N, 5.67; S, 13.50.

N-2-Cyanopropyl-ε-aminocaproic Acid (XI).—To a solution of 2 g (0.0153 mole) of ε-aminocaproic acid in 17 ml of water and 2.3 ml of triethylamine (0.0165 mole) was added 1.3 ml (0.0160 mole) of *trans*-crotononitrile. The solution was stirred magnetically for 18 days, evaporated until nearly dry, washed with water, and evaporated. The residue consisted of a moist mass. It was spread out on filter paper and air dried for 20 hr. The powdery white solid was collected and recrystallized from ethanol–water as white needles. The yield of the first crop was around 1 g (28%), mp 125°. The infrared spectrum gave a signal at 2247 (CN).

Anal. Calcd for $C_{10}H_{18}N_2O_2$ (198.27): C, 60.57; H, 9.15; N, 14.13. Found: C, 60.51; H, 9.19; N, 14.10.

Determination of pK_2 Values of Amino Groups.—Automatic titrations were carried out in a TTT1C titrator with Titrigraph (Radiometer-Copenhagen) standardized with NBS as previously described.[6,7] The pK_2 values were determined graphically and the accuracy is estimated to be ±0.05 pK_2 units.

Registry No.—II, 15095-71-3; III, 15095-72-4; IV, 15095-73-5; V, 15095-74-6; VI, 15095-75-7; VII, 15095-76-8; IX, 15095-77-9; X, 15206-35-6; XI, 15095-78-0.

Model Reactions for the Biosynthesis of Thyroxine. XI. The Nature of a Free Radical Formed in the Autoxidation of 4-Hydroxy-3,5-diiodophenylpyruvic Acid[1]

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The structures of various free radicals formed by oxidation of 4-hydroxy-3,5-diiodophenylpyruvic acid (DIHPPA) have been determined by means of electron spin resonance. Two short-lived radicals were identified as the enol and keto tautomers, respectively, of the phenoxyl radical of DIHPPA. The previously reported long-lived radical, formed in the autoxidation of DIHPPA, which had been suspected to be an intermediate in the synthesis of thyroxine from DIHPPA and 3,5-diiodotyrosine, has now been identified as 2,6-diiodobenzosemiquinone (DISQ). DISQ does not react with 3,5-diiodotyrosine to form thyroxine. ^{17}O labeling was used in the structure determination of the long-lived radical by means of electron spin resonance.

In the von Mutzenbecher reaction,[4] in which a slightly alkaline solution of 3,5-diiodotyrosine is exposed to air for several days, a slow self-coupling process takes place which leads to the formation of thyroxine. Hillmann[5] suggested that in the biosynthesis of thyroxine DIHPPA[6], the keto acid analog of diiodotyrosine, might be an intermediate. Meltzer and Stanaback[7] found that in the presence of oxygen DIHPPA reacts indeed with diiodotyrosine to form thyroxine in far better yield than that obtained by von Mutzenbecher. Subsequently this model reaction was investigated in greater detail.[8]

Johnson and Tewkesbury[9] offered a hypothesis for the mechanism by which diiodotyrosine is converted to thyroxine in the von Mutzenbecher reaction. According to this hypothesis diiodotyrosine is first oxidized to its phenoxyl radical. Then two molecules of the phenoxyl radical combine to form thyroxine with the concomitant loss of one aliphatic side chain. If one applies this kind of mechanism to the model reaction in which thyroxine is formed from DIHPPA and diiodotyrosine, one will have to assume that both DIHPPA and diiodotyrosine are oxidized to their radicals which then couple.

A few years ago, we reported the formation of a relatively stable free radical when oxygen is bubbled through a buffered solution (pH 7.8) of DIHPPA.[10] This radical gives rise to an electron spin resonance (esr) signal consisting of three bands with an intensity ratio of 1:2:1. We described some of the properties of the radical and suggested that it might be DIHPPA·.[6] Since, however, various characteristics of the observed radical cannot easily be reconciled with the structure of DIHPPA·, a new effort was made to identify the radical. This reinvestigation showed that the radical is 2,6-diiodobenzosemiquinone (DISQ)[6] and that it is not an intermediate in the formation of thyroxine from DIHPPA and diiodotyrosine. Oxidation of DIHPPA with permanganate or ceric sulfate led to the formation of other radicals which have all the characteristics predicted for the enol and keto tautomer respectively of DIHPPA·.

Results and Discussion

Formation of the Stable Radical.—The extent to which the previously described stable radical[10] is formed in the autoxidation of DIHPPA depends very much on the pH, the temperature, the nature of the buffer salt used, the salt concentration, and the presence or absence of certain additives. When oxygen is bubbled at room temperature through a 10^{-3} *M* solution of DIHPPA in 0.2 *M* borate buffer, pH 7.5, for 10 min, and this is followed by nitrogen bubbling, an esr signal can be observed. The size of the signal increases considerably when the pH is raised to 8.5. Upon raising the pH further, the signal still increases, but undergoes rapid deformation. Deformation is slower

(1) Paper X: K. Toi, G. Salvatore, and H. J. Cahnmann, *Biochim. Biophys. Acta*, **97**, 523 (1965).

(2) Visiting Scientist from Kyoto University, Japan.

(3) Recipient of U. S. Public Health Service Grant AM 07955 from the National Institute of Arthritis and Metabolic Diseases.

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

(5) G. Hillmann, *Z. Naturforsch.*, **11b**, 424 (1956).

(6) Abbreviations: DIHPPA, 4-hydroxy-3,5-diiodophenylpyruvic acid; DIHPPA·, the phenoxyl radical of DIHPPA; DISQ, 2,6-diiodobenzosemiquinone.

(7) R. I. Meltzer and R. J. Stanaback, *J. Org. Chem.*, **26**, 1977 (1961).

(8) *Cf.* papers V through X of this series and H. J. Cohnmann and T. Shiba, *Biochem. Prep.*, **10**, 171, 176 (1963).

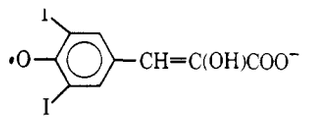
(9) T. B. Johnson and L. B. Tewkesbury, Jr., *Proc. Natl. Acad. Sci. U. S.*, **28**, 73 (1942).

(10) T. Matsuura, H. Kon, and H. J. Cahnmann, *J. Org. Chem.*, **29**, 3058 (1964).

in nitrogen than in oxygen. About 10% of 1-butanol was usually added to the reaction mixture as an antifoaming agent. The conditions just outlined will be called standard conditions (see Experimental Section). Deviations from these standard conditions led to either an increase or decrease of the size of the esr signal.

A DIHPPA concentration of about 10^{-3} moles/l. gives a strong signal under standard conditions. Weaker signals are obtained not only with lower but also with much higher concentrations, and no signal could be observed above 10^{-2} moles/l., unless the oxygen bubbling time was extended. The inhibition of the radical formation at high concentrations will be discussed below. The fact that the radical is formed by raising the pH even after oxygen has been replaced with nitrogen shows that autoxidation of DIHPPA leads to the formation of a radical precursor. Below pH 5.6 no precursor is formed since an increase of the pH to 8.5 does not give rise to any signal. Between pH 5.6 and 7.5 increasing amounts of precursor are formed. As the pK_a of DIHPPA should be about 6–6.5[11] it appears therefore that DIHPPA can undergo autoxidation only if it is present as the phenolate anion. The precursor is also formed at 0°, but in this case raising the pH to 8.5 does not cause the appearance of the esr signal unless the temperature is also raised. The length of time of oxygen bubbling may be varied within moderate limits (5 to 30 min) without greatly affecting the signal size. Under standard conditions, the addition of 10% 1-butanol (which may be replaced by other organic solvents such as ethanol, methanol, isopropanol, or acetone) has no influence on the size or shape of the signal. In phosphate buffer, however, only a very weak and deformed signal is obtained unless an organic solvent is added to the aqueous buffer. In the presence of organic solvents the size of the signal is of the same order of magnitude as that obtained in borate buffer. It is possible, but not proven, that this is at least in part due to the fact that DIHPPA in organic solvents as well as in borate buffer is present in its enol form and that oxygen is much more efficiently taken up by the enol than by the keto tautomer of DIHPPA.[12] Also the addition of catalase or horseradish peroxidase to a solution of DIHPPA in phosphate buffer leads to an increase of the signal size, although to a lesser extent than organic solvents. This indicates that hydrogen peroxide is formed in the course of the autoxidation of DIHPPA. The amount of hydrogen peroxide must, however, be small since the addition to the reaction mixture of 1 mole of hydrogen peroxide per mole of DIHPPA prevents the formation of the radical and decreases considerably the signal of the radical formed under standard conditions.

Nonidentity of the Stable Radical with DIHPPA·.— A few years ago, when the formation of a free radical in the autoxidation of DIHPPA was discovered,[10] it was thought that this radical might be DIHPPA· (I)[13] whose formation should be expected on the basis of Johnson and Tewkesbury's hypothesis. There was some circumstantial evidence for such a formulation, *viz.* the disappearance of the radical signal upon addition of an excess of diiodotyrosine and the concomitant formation of thyroxine, but proof for it was lacking. Several characteristics of the radical are difficult to explain, however, if one assumes that it is DIHPPA·. Thus the observed radical has a long life time while one would expect DIHPPA· to be short-lived, since one of the apparent requirements for a phenoxyl radical to be stable is not fulfilled. According to Cook, *et al.*,[14] stable phenoxyl radicals must have bulky *ortho* and *para* substituents which have no hydrogen attached to the carbon atom adjacent to the aromatic ring (α carbon). Furthermore, we found that the esr signal of the stable radical disappears upon addition of a small amount of DIHPPA. If the radical were DIHPPA·, the addition of DIHPPA should not decrease the concentration of its own phenoxyl radical. It should be mentioned in this connection that the reaction of the radical with DIHPPA explains the fact that no signal is observed in the autoxidation of concentrated solutions of DIHPPA (see above), where at the end of the oxygenation period sufficient DIHPPA remains in the reaction mixture to destroy the radical formed. Finally, since the α-carbon atom of DIHPPA· carries one or two protons (enol or keto form), one would expect to see esr hyperfine splitting (hfs) in addition to the splitting caused by the aromatic protons; no such hfs was ever observed.



I

A review of known examples of *para*-substituted phenoxyl radicals[15] indicates that the splitting due to the α proton(s) seems to depend on the substituent. Thus no hfs due to the α proton could be observed with the phenoxyl radical of 3,5-di-*t*-butyl-4-hydroxybenzaldehyde,[15a] whereas the phenoxyl radicals of various 3,5-di-*t*-butyl-4-hydroxycinnamic acid derivatives[15b] gave signals with an α proton splitting of $\sim$1 gauss. As it is conceivable that the absence of hfs in certain cases is due to a low spin density at the α carbon, the spin density distribution of DIHPPA· was investigated by means of molecular orbital calculations according to McLachlan's method.[16] In spite of the crude nature of such calculations when applied to a radical with several heteroatoms such as DIHPPA·, it is known that in the case of structurally related radicals such calculations give results which are at least qualitatively in agreement with the observed data if proper parameters are chosen.[17] The correction for the oxygen coulomb integral (ζ) and that for the C–O resonance integral (η) were chosen as shown in Table I so as to give the best agreement with the experimental results for the unsubstituted phenoxyl radical.

Table I shows that the agreement between the calculated and the experimental spin densities is reasonably good. The calculations indicate that the phenoxyl

(11) *Cf.* J. R. Tata, *Biochem. J.*, **72**, 214 (1959).

(12) A. Nishinaga, H. J. Cahnmann, H. Kon, and T. Matsuura, *Biochem.*, in press.

(13) Only one of several possible canonical resonance forms and tautomeric structures is shown.

(14) C. D. Cook, N. G. Nash, and H. R. Flanagan, *J. Am. Chem. Soc.*, **77**, 1783 (1955).

(15) (a) A. L. Buchachenko, "Stable Radicals," Consultation Bureau, New York, N. Y., 1965, Chapter III, Table 9; (b) Chapter III, Table 13.

(16) A. D. McLachlan, *Mol. Phys.*, **2**, 233 (1960).

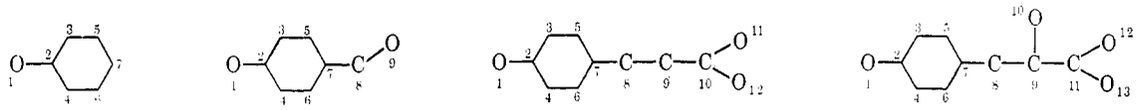
(17) For examples see P. H. Rieger and G. Fraenkel, *J. Chem. Phys.*, **37**, 2811 (1962), and R. L. Walter, *J. Am. Chem. Soc.*, **88**, 1930 (1966).

TABLE I

CALCULATED AND EXPERIMENTAL SPIN DENSITIES OF VARIOUS PHENOXYL RADICALS[a]

Parent phenol	Spin density (ρ) at position[b] 1	2	3,4	5,6	7	8	9	10	11	12	13
Phenol	0.37	−0.03	0.18	−0.07	0.43						
			0.29[c]	0.08[c]	0.46[c]						
p-Hydroxybenzaldehyde	0.33	−0.04	0.17	−0.07	0.36	−0.03	0.16				
			0.30[c]	0.10[c]							
3,5-Di-*t*-butyl-4-hydroxy-cinnamic esters	0.26	−0.01	0.13	−0.05	0.29	−0.10	0.33	−0.01	0.03	0.03	
						0.05[d]	0.38[d]				
DIHPPA (enol)	0.16	0.04	0.05	0.03	0.07	0.29	0.04	0.21	0.003	0.007	0.007
					0.10[e]	0.34[e]					

[a] For each column the upper row gives the calculated and the lower row the experimental values (for which no sign is given). The molecular orbital calculation was done with $\xi = 0.8$, $\eta = 1.4$, and McLachlan's parameter $\lambda = 1.2$; the resonance integral of the *ortho–meta* bonds in the aromatic ring was also corrected with $\eta = 1.4$. No correction was made for the iodine in DIHPPA. The experimental values were obtained by $a = 22.5\rho$, where a is the observed hfs constant [H. M. McConnell, *J. Chem. Phys.*, **24**, 632 (1956)]. [b] The positions are numbered as follows:



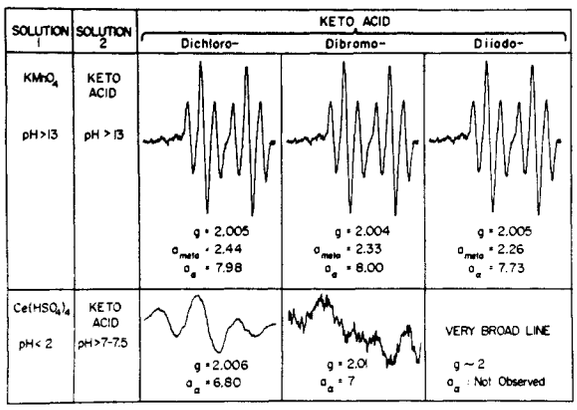
[c] From T. J. Stone and W. A. Waters, *J. Chem. Soc.*, 213 (1964). [d] From ref 15a. [e] Present work (see Chart I).

radical of *p*-hydroxybenzaldehyde has its lowest spin density at the α carbon (carbon 8). This means that the splitting due to the α proton should indeed be extremely small. It is noteworthy that the calculated spin density at carbon 2 has a negative sign in agreement with the more elaborate calculations on *para*-substituted phenoxyl radicals by Atherton, *et al.*[18] The most striking fact that can be derived from the calculations shown in Table I is that in DIHPPA· (enol form) the spin density is almost completely drained out of the aromatic ring and is localized mainly at oxygens 1 and 10 and at the α carbon (carbon 8). In fact, the latter, the point of our immediate interest, carries the highest spin density according to these calculations. One would therefore certainly expect a sizable hfs due to the α proton in contradiction to the observation with the stable radical. The assumption that DIHPPA· might be the keto rather than the enol tautomer does not eliminate the discrepancy between calculation and observation since in the keto form the spin density distribution in the aromatic ring should be nearly the same as in the simple phenoxyl radical. The high spin density at the *para* carbon should therefore cause a hfs of at least several gauss.[19,20]

Thus it appears that the stable radical is not DIHPPA·. In order to confirm this nonidentity, an attempt was made to produce the "true" DIHPPA·. When DIHPPA or its chloro or bromo analog was oxidized with potassium permanganate or ceric sulfate under the conditions shown in Chart I, short-lived radicals were obtained which could be detected only by using the rapid mixing flow technique.[21] An accurate evaluation of the hfs constants and an unambiguous interpretation of the esr spectra are possible in most cases.

In strongly alkaline media, in which DIHPPA is present as the enolate, oxidation of the 3,5-dihalogeno keto acids produces signals consisting of a pair of triplets. Both the coupling constant and the g value

CHART I



are virtually unaffected by varying the halogen. This type of spectrum can be interpreted as the keto acid radical in the enol form as indicated in Chart I. The molecular orbital calculation shows that the unpaired electron is largely withdrawn from the aromatic ring and that the α carbon has a high spin density in agreement with the observation (Table I).

In neutral or acid media, in which DIHPPA is mainly present in the keto form, oxidation of the 3,5-dihalogeno keto acids gives rise to different signals, *viz.* triplets whose line width increases considerably with the atomic weight of the halogen. These signals can be interpreted as the keto form of the phenoxyl radicals for which one can predict high spin densities at the *ortho* (and *para*) carbons since there is no conjugated unsaturation in the side chain. This should cause a greater spin-orbit interaction at the halogen (increasing with an increase in the atomic number of the halogen) and consequently a broadening of the signal and a larger g value. A quite analogous case is that of the phenoxyl radical of diiodotyrosine which also has no double bond in conjugation with the aromatic ring and which gives a very broad, unresolved spectrum. This has equally been interpreted as being due to a strong spin-orbit coupling at the iodine.[22]

This interpretation of the triplet spectra is further supported by the agreement between the observed

(18) N. M. Atherton, E. J. Land, and G. Porter, *Trans. Faraday Soc.*, **59**, 818 (1963).

(19) L. H. Piette, R. N. Adams, and P. Ludwig, *J. Am. Chem. Soc.*, **84**, 4212 (1962).

(20) E. W. Stone and A. H. Maki, *J. Chem. Phys.*, **37**, 1326 (1962).

(21) W. T. Dixon and R. O. C. Norman, *J. Chem. Soc.*, 572 (1963).

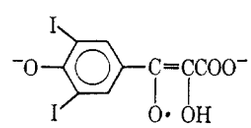
(22) D. C. Borg, *Proc. Natl. Acad. Sci. U. S.*, **53**, 829 (1965).

and the theoretical CH_2 coupling constants in the case of the dichloro keto acid. The hfs (a_H) of the methylene protons due to the unpaired π electron at the adjacent *para* carbon depends in general on the dihedral angle (θ) between the axis of the p_z orbital and the C–H bond. However, if the system is assumed to carry out hindered rotation around the equilibrium configuration ($\theta \simeq 60°$) as in the case of nitroalkane anion radicals,[20] the quantum mechanical calculation gives $a_H = 0.34B\rho_\pi$ where ρ_π is the spin density at the *para* carbon and B a constant. Using $\rho_\pi = 0.46$, the experimental spin density at the *para* carbon of the simple phenoxyl radical, and B = 45 gauss,[20] the calculated value for a_H is 7.1 gauss which is in good agreement with the observed value of 6.8 gauss (Chart I).

Thus the keto–enol tautomerism, known to exist in DIHPPA, seems to be carried over to its phenoxyl radical as well as to the radicals of the chloro and bromo analogs.

Identification of the Stable Radical.—Since it has been established without doubt that the stable radical is not DIHPPA·, the question arises what the true nature of the stable radical is and whether or not it is an intermediate in the formation of thyroxine from DIHPPA and diiodotyrosine.

There are only two structural possibilities which are consistent with the absence of hfs due to the α proton. Either the proton at the α carbon of DIHPPA has been replaced by a heteroatom which has no nuclear moment and which in our case can only be oxygen, or the entire side chain of DIHPPA has been removed by oxidative splitting, in which case the radical would be DISQ. In the first case, the radical would have structure II[23] which is a semidione radical, many of



II

which are known to be generated in alkaline media and to have considerable stability.[24] Such a formulation would be in agreement with the hypothesis that an essential feature during the formation of tetraiododiphenyl ethers from diiodotyrosine or its analogs is an oxidation at the α carbon.[25] On the other hand, DISQ or the corresponding quinone or hydroquinone have been mentioned as possible intermediates in the formation of thyroxine from diiodotyrosine.[26,27] Both the *g* value and the hfs constant of the stable radical are virtually identical with those of DISQ, but the reactivities of the two radicals towards diiodotyrosine and their decay rates are different.[10] These differences, however, do not necessarily prove nonidentity since they may derive from the fact that the two radicals are generated in different surroundings. Whereas authentic DISQ is formed by mixing aqueous solutions of the corresponding quinone and hydroquinone, the unknown radical is generated in a solution which contains other oxidation products of DIHPPA. The radical may be formed from one of these products. The existence of an equilibrium between the radical and such a precursor is supported by an experiment in which a solution of the radical was acidified to pH 5.5 and then extracted five times with ether. Each ether extract was poured into borate buffer, pH 8.5, and the resulting esr signal observed. A signal of equal strength was seen in all five extracts while a mixture of diiodobenzoquinone and diiodohydroquinone should be transferred almost quantitatively into ether with only two extractions. It was possible to isolate from an oxygenated solution of DIHPPA, after reduction with sodium borohydride, a small amount of 2,6-diiodohydroquinone which is in favor of the assumption that the radical is DISQ.

In order to arrive at an unambiguous conclusion as to whether and where oxygen is incorporated in the radical, a labeling experiment with ^{17}O was carried out. The ^{17}O nucleus has a spin number of 5/2 and a nuclear moment of −1.8930 nuclear magnetons. If a labeled radical can be prepared it should therefore be possible to determine the unpaired electron density at ^{17}O, from which in turn a conclusion can be drawn as to the identity of the radical.

The labeling experiment was carried out in a specially designed reaction vessel (see Experimental Section). A solution of DIHPPA in ^{17}O-enriched water was oxidized with ^{17}O-enriched O_2 gas. An esr signal with ^{17}O hfs was obtained (Figure 1) which shows that

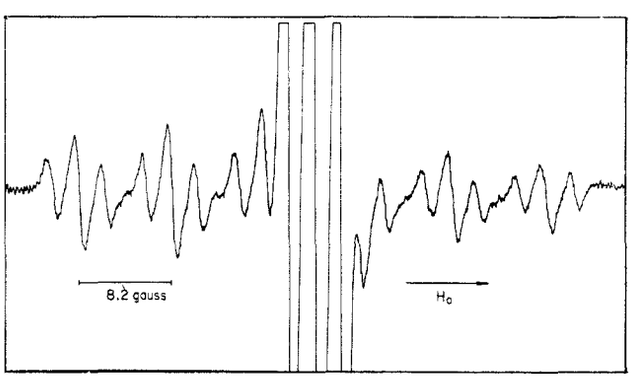


Figure 1.—Esr Spectrum of the ^{17}O-enriched stable radical.

oxygen has been incorporated. No such incorporation took place, when only ^{17}O-enriched O_2 gas, but ordinary water was used. The esr spectrum shows six sets of triplets with a coupling constant of 8.45 ± 0.03 gauss. The peaks are symmetrical and represent therefore the hyperfine structure due to *one* ^{17}O atom. This rules out the formulation of the radical as a peroxy radical. A peroxy radical has two oxygen atoms. The chance of being labeled is equal for both. The chance of double labeling is considerably less. Each of the singly labeled peroxy radicals should give rise to a different spectrum. The superposition of both spectra should therefore cause a dissymetry in the shape of the peaks. The possibility of the coupling constant of one of the two oxygen atoms being so low that it cannot be detected is very unlikely.[28] The coupling constant is very close to that of benzosemi-

(23) A peroxy radical structure (–O–O· in place of –O·) is unlikely on account of the known instability of peroxy radicals. It will be ruled out (see col. 2, this page).

(24) G. A. Russell, E. T. Strom, E. R. Talaty, K. Y. Chang, R. D. Stephens, and M. C. Young, *Record Chem. Progr.* (Kresge-Hooker Sci. Lib.), **27**, 3 (1966).

(25) R. Pitt-Rivers and A. T. James, *Biochem. J.*, **70**, 173 (1958).

(26) S. Lissitzky and W. Krotemberg, *Compt. Rend.*, **242**, 3002 (1956).

(27) J. G. Ljunggren, *Acta Chem. Scand.*, **15**, 1772 (1961).

(28) R. W. Fessenden and R. H. Schuler, *J. Chem. Phys.*, **44**, 434 (1966).

quinone-^{17}O in aqueous solution (8.70 gauss);[29] the small difference can be attributed to the effect of the two iodine atoms. In contrast, the predicted coupling constant[30] for the semidione radical II calculated by the molecular orbital method is about 11 gauss, which is much higher than the observed value. From this it can be concluded that the stable radical cannot be radical II but must be DISQ.

The question arises whether or not DISQ is an intermediate in the formation of thyroxine from DIHPPA and diiodotyrosine. Good yields of thyroxine are obtained in this reaction even under conditions, such as high DIHPPA concentration and low temperature, where the DISQ signal could not be observed. This makes it appear unlikely that DISQ or the corresponding quinone and hydroquinone are intermediates in the coupling reaction. That they do indeed not react with diiodotyrosine to form thyroxine was confirmed by an experiment in which an equimolar mixture of 2,6-diiodobenzoquinone and 2,6-diiodohydroquinone (giving rise to the typical esr signal of DISQ) was permitted to react with an excess of diiodotyrosine under conditions in which DIHPPA and diiodotyrosine form thyroxine in good yield. Not even a trace of thyroxine could be detected in the reaction mixture. From this it must be concluded that DISQ is a side product rather than an intermediate in the thyroxine formation. The short-lived DIHPPA· could of course still be an intermediate. This possibility will be discussed in a forthcoming paper.[12]

Experimental Section[31]

Instruments.—Esr spectra were taken as described before.[10] The 6-in. electromagnet was regulated and scanned by a Fieldial control. The magnetic field was calibrated by a proton resonance gaussmeter (Alpha Scientific, Inc.) combined with an electronic counter (Computer Measurement Co., Model 707 BN). For rapid mixing flow experiments a cell made in our laboratory according to the design of Dixon and Norman[21] or a Varian flow cell were used. The magnetic field scanning time over 50 gauss was 0.5 to 1 min. A time averaging computer (Varian C-1024) was utilized occasionally for weak signals.

Molecular orbital calculations were carried out by a Honeywell 800 or a CEIR computer with programs written in FORTRAN and BASIC, respectively.

Formation of Radicals.—Standard conditions for the formation of the stable radical were as follows. DIHPPA (32 mg) was added to 75 ml of 0.2 *M* sodium borate buffer, pH 7.5, and oxygen was bubbled through the mixture. Frequently about 10% of 1-butanol was added in order to avoid foaming. After 10 min the oxygen was replaced with nitrogen. About 3 min later circulation[10] of the slightly greenish yellow solution[32] through the esr cell was started and the signal observed. The size of the signal increases considerably when the pH is raised to 8.5.

In order to observe the esr signal of short-lived radicals the rapid mixing flow technique[21] was used. One of the feeding bottles contained a 10^{-2} *M* solution of the dihalogeno keto acid brought with NaOH to the appropriate pH; the other feeding bottle contained a 10^{-2} *M* solution of the oxidant, either $KMnO_4$ in alkali or $Ce(HSO_4)_4$ in dilute acid (see Table II). The flow rate of each solution was about 6 ml/sec. Nitrogen was bubbled through both feeding bottles.

The ^{17}O-labeled stable radical was prepared in the apparatus shown in Figure 2. The following steps were carried out. A

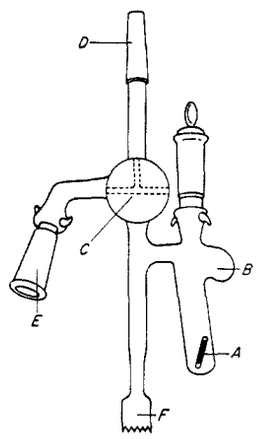


Figure 2.—Apparatus for the preparation of the ^{17}O-enriched stable radical.

1-mg portion of borax was placed in A and 10 mg in B. The system was evacuated and the borax dehydrated by gently heating both A and B for 3–5 min. The apparatus was permitted to cool, then transfered to a nitrogen-filled drybox where 5 mg of boric acid and 0.5 mg of DIHPPA were placed in A. The apparatus was then put under high vacuum for several hours in order to remove all moisture. An ampoule containing 0.5 ml of ^{17}O-enriched H_2O was opened in the drybox and placed in a cylindrical reservoir which was then connected to E. The H_2O was degassed by three cycles of freezing and thawing under reduced pressure then vacuum-distilled into A. Another cylindrical reservoir equipped with a stopcock and containing ^{17}O-enriched O_2 was connected to E. After the reagents in A had been dissolved, the O_2 was put in communication with the solution in A, which was kept at 0°. The solution was vigorously stirred for 30 min. The reaction mixture in A was then degassed by three cycles of freezing and thawing, and the borax in B dissolved in the reaction mixture by letting the solution flow back and forth between A and B. In this manner the pH is raised from 7.5 to 8.5. The solution was then permitted to flow into F and the esr signal was observed at room temperature.

Chromatographic Methods.—Separations by gas–liquid partition chromatography (glpc) were done on siliconized Gaschrom P coated with 1% SE 30 (6-ft glass column, 160°, 35 cc of argon/min). For thin layer chromatography (tlc) Merck precoated fluorescent silica gel plates (Brinkman Instruments, Inc.) were used.

Materials.—Commercially available products were checked for purity and purified if necessary, DIHPPA was purchased from Osaka Laboratory of Synthetic Organic Chemicals, Nishinomiya, Japan, and recrystallized from glacial acetic acid and from aqueous alcohol. 3,5-Dibromo-4-hydroxyphenylpyruvic acid was synthesized as described previously.[33] "Ultra high purity" nitrogen containing less than 0.0015% oxygen (Southern Oxygen Co.) was used. Labeled water and O_2 gas were obtained from Yeda Research and Development Co., Rehovoth, Israel. The water contained 15.2 at.% ^{17}O and was normalized to the natural abundance of hydrogen; the O_2-gas contained 36.0 at.% ^{17}O.

3,5-Dichloro-4-hydroxybenzaldehyde.—This was prepared by a modification of the procedure of Auwers and Reis[34] in which only a 10% excess of Cl_2 was used. Colorless crystals, mp 152–154°, were obtained in 42% yield (lit. mp 156°[34] and 158–159°[35]). An additional 14% was obtained from the mother liquors. The first crop was used for the next step without recrystallization.

4-(4-Acetoxy-3,5-dichlorobenzal)-2-methyl-5-oxazolone.—A mixture of 38.2 g (0.2 mole) of 3,5-dichloro-4-hydroxybenzaldehyde, 28.1 g (0.24 moles) of acetylglycine, 19.7 g (0.24 moles)

(29) W. M. Gulick and D. H. Geske, *J. Am. Chem. Soc.*, **88**, 4119 (1966); a coupling constant of 9.02 ± 0.02 gauss was obtained in aqueous alcohol by B. L. Silver, Z. Luz, and C. Eden, *J. Chem. Phys.*, **44**, 4258 (1966).

(30) Obtained by $a = -40.41\rho_O^\pi - 16.69\rho_C^\pi$ (see ref 29) where ρ_O^π and ρ_C^π are the spin densities at the oxygen and the adjacent carbon respectively, calculated by the molecular orbital method.

(31) The elemental analyses were done by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y., and by the Analytical Service Laboratory of this institute. Melting points were determined in capillary tubes and are uncorrected.

(32) If the solution is prepared at 0°, it is practically colorless.

(33) T. Shiba, H. J. Cahnmann, T. Matsuura, A. Nishinaga, and H. Sakamoto, *J. Org. Chem.*, **29**, 3061 (1964).

(34) K. Auwers and J. Reis, *Ber.*, **29**, 2356 (1896).

(35) H. Biltz, *ibid.*, **37**, 4033 (1904).

of anhydrous sodium acetate, and 250 ml of acetic anhydride was heated on a steam bath for 3.5 hr. The reaction mixture was allowed to stand overnight at 2°. The crystals were collected by filtration, washed with some hot water, and dried: 50.3 g (84%), mp 182–183°. Recrystallization from benzene gave 40 g (67%) of pale yellow crystals, mp 184–185°.

Anal. Calcd for $C_{13}H_9Cl_2NO_4$: C, 49.70; H, 2.89; Cl, 22.57; N, 4.46. Found: C, 49.66; H, 2.97; Cl, 22.33; N, 4.43.

Colorless crystals were obtained upon two more recrystallizations with charcoal (Norit A, neutral).

3,5-Dichloro-4-hydroxyphenylpyruvic Acid.—A mixture of 29.8 g (0.1 mole) of the oxazolone, 300 ml of acetic acid, and 100 ml of 3 *N* HCl was refluxed gently for 3 hr. Upon evaporation of the reaction mixture and addition of water to the residue, 15 g of crude keto acid was obtained. Recrystallization from acetic acid gave 14 g (56%) of colorless needles, mp 208–209° dec.

Anal. Calcd for $C_9H_6Cl_2O_4$: C, 43.40; H, 2.43; Cl, 28.47. Found: C, 43.17; H, 2.40; Cl, 28.74.

Detection of 2,6-Diiodohydroquinone after Reduction of an Ether Extract of an Oxygenated Solution of DIHPPA.—Oxygen was bubbled at 0° for 20 min through a solution of 0.5 g of DIHPPA in 250 ml of 0.2 *M* borate buffer, pH 7.5. The solution was adjusted to pH 5 and extracted three times with ether. The combined extracts were washed with a small amount of water, then an excess of a freshly prepared aqueous solution of $NaBH_4$ was added. After 5 min the mixture was acidified and the ether layer dried and evaporated. Both tlc in chloroform and glpc of the residue (38 mg) showed that the major part of the residue was 2,6-diiodohydroquinone. The R_f value and the retention time of an authentic sample were identical with those of the reduction product, both in separate and mixed chromatograms.

Registry No.—Enol tautomer of DIHPPA radical, 14886-10-3; keto tautomer of DIHPPA radical, 14886-11-4; DIHPPA, 780-00-7; DISQ, 14886-16-9; thyroxine, 7488-70-2; chloro analog of DIHPPA, 13990-05-1; bromo analog of DIHPPA, 13990-07-3; 4-(4-acetoxy-3,5-dichlorobenzal)-2-methyl-5-oxazolone, 14886-17-0.

The Photolysis of Some 1,6-Dienes.[1] Total Synthesis of (±)-α-Bourbonene

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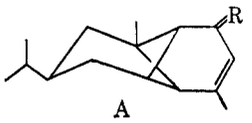
The photolysis of several 1,6-dienes has been shown to yield head-to-head cyclization products. The diketone **5c** has been converted into the naturally occurring α-bourbonene.

The sesquiterpenes are particularly interesting to the synthetic chemist because of the wide variation of structural types found in this class of compounds. Copaene (A, R = H) and mustakone (A, R = O) are

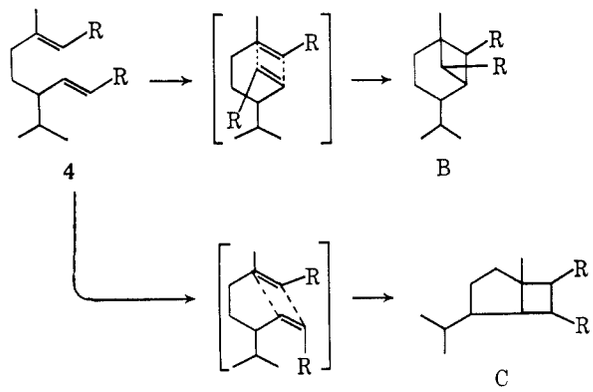


A

exceptionally interesting substances[2] which pose difficult challenges to the synthetic chemist. The presence of a four-membered ring with two fused six-membered rings is thus far unique to copaene and mustakone.

Synthesis of a complex substance such as copaene can be approached in many different ways.[3] We envisioned the synthesis of a properly substituted diene **4**, which on photolysis could yield the desired [3.1.1]bicyclohexane system B. If R were a suitably chosen group (CH_3CO–, for example) then addition of the third ring would seem to present no serious problems, and final alteration of such a tricyclic compound to the natural product should be straightforward.

However, an alternate mode of cyclization for the diene **4** is possible. This would afford the 5:4 fused-ring system C. Although there are published experimental results on the dimerization of dienes (*vide infra*), *a priori* no firm prediction could be made as to which



4 B C

product (B or C) would be produced on photolysis of diene **4**. We therefore set out to examine the synthesis and photolysis of **4**.

Production of **4** proved to be quite efficient once the proper experimental procedures were devised. The starting material was the piperidineenamine of isovaleraldehyde (**1**)[4] (see Scheme I). In the condensation of **1** with methyl vinyl ketone, the aminoenol ether **2** is first produced, as evidenced by the vinyl methyl and vinyl hydrogen singlets in the nuclear magnetic resonance (nmr) spectrum.[5] The slightly unstable ether **2** did not need to be isolated, and conversion of **1** → **3** could be accomplished in 79% yield, employing aqueous oxalic acid for the hydrolysis of **2** → **3**.[6] Ini-

(1) A preliminary communication describing some of these results has been published, M. Brown, *Chem. Commun.*, 340 (1965).

(2) (a) P. De Mayo, R. E. Williams, G. Büchi, and S. H. Feairheller, *Tetrahedron*, **21**, 619 (1965); (b) V. H. Kapadia, B. A. Nagasampagi, V. G. Naik, and S. Dev, *ibid.*, **21**, 607 (1965).

(3) For a completely different and successful approach to the problem, see C. H. Heathcock, *J. Am. Chem. Soc.*, **88**, 4110 (1966).

(4) G. Stork, A. Brizzolara, H. Landesman, J. Szmuszkovicz, and R. Terrell, *ibid.*, **85**, 207 (1963).

(5) See G. Opitz and I. Löschmann, *Angew. Chem.*, **72**, 523 (1960), for similar results.

(6) The ketoaldehyde **3** has apparently not been isolated before, although it or a closely related derivative is undoubtedly an intermediate in a synthesis of 4-isopropylcyclohexenone.[7a]

(7) (a) G. Stork, A. Brizzolara, H. Landesman, J. Szmuszkovicz, and R. Terrell, *J. Am. Chem. Soc.*, **85**, 219 (1963); (b) W. S. Wadsworth, Jr., and W. D. Emmons, *ibid.*, **83**, 1733 (1961).