ored solute formed in the sulfuric acid reacted with water subsequently to regenerate 4,4′-dichlorobenzilic acid. The color was of an intensity characteristic of stable aryl-substituted carbonium ions.

The Synthesis of Bis-(*p*-chlorophenyl)-α-chloroacetyl Chloride.—A 5.00-g. quantity (0.0178 mole) of bis-(*p*-chlorophenyl)-acetic acid obtained as described earlier was treated with 10 ml. of thionyl chloride. The mixture was kept at reflux temperature for 90 minutes. Most of the thionyl chloride was then removed by evaporation on a steam-plate. The crude acid chloride was dissolved in 20 ml. of dry carbon tetrachloride to which was added 1 ml. of phosphorus trichloride. The solution was transferred to an 8-inch Pyrex test-tube. The original container was washed with 10 ml. of carbon tetrachloride which was then added to that in the test-tube. The test-tube was fitted with a reflux condenser and a fritted glass bubbler tube, these being held in place by a two-hole rubber stopper. A 300-watt bulb was mounted next to the test-tube, which was warmed by a water-bath. As soon as reflux temperature was reached, chlorine was bubbled vigorously through the irradiated solution.

After four to five hours the chlorination was stopped. The solvent was evaporated by being warmed under suction. Then 10 ml. of low boiling petroleum ether was added. The solution was decanted from a small amount of solid impurities. Evaporation of the solvent then gave a crude product which crystallized on being chilled and stirred. The material was recrystallized three times from 6–8 ml. portions of low boiling petroleum ether, the process being facilitated by seeding. Additional solid was obtained by concentrating the mother liquors and recrystallizing the recovered material. The product melted at 60.4–61.4°. The yield was 3.91 g. (66%). After an additional recrystallization it melted at 60.7–61.7°.

Anal.[21] Calcd. for $C_{14}H_8OCl_4$: C, 50.34; H, 2.41. Found: C, 50.38; H, 2.54.

4,4′-Dichlorobenzilic Acid.—A 1.00-g. quantity (0.00299 mole) of the bis-(*p*-chlorophenyl)-α-chloroacetyl chloride was added to a solution of 2.0 g. of sodium carbonate in 25 ml. of water. The mixture was heated to reflux temperature for one hour, during which most of the organic material went into solution. After the mixture had been allowed to cool, it was poured into 200 ml. of water. The solution was filtered and the filtrate was acidified by adding a mixture of 2 ml. of concentrated sulfuric acid in 15 ml. of water. The mixture was shaken with 100 ml. of ether The ether layer was separated, and was evaporated to dryness by gentle warming. The residue was a viscous sirup which solidified after standing for three days with occasional stirring.

It was dissolved in 150 ml. of hot 60–90° ligroin and the solution was decanted from a small quantity of insoluble material. The solution was evaporated to a volume of 50 ml., seeded and allowed to cool slowly with stirring. Crystallization occurred readily. The cooled mixture was filtered under suction. The solid was dried at 45° under suction. There was obtained 0.65 g. of a product melting at 91.9–92.7°. The yield was 73% of the theoretical value based on the α-chloroacetyl chloride. The product gave a neutral equivalent of 296.9. The theoretical value for 4,4′-dichlorobenzilic acid is 297.1.

The acid was at first prepared by the method of Montagne.[12] It was obtained in a low melting form (72–76°) which was difficult to purify. On standing overnight in ligroin solution, it deposited crystals melting at 88–90°. The low melting form did not depress the melting point of these crystals. The latter did not depress the melting point of the product obtained by hydrolyzing bis-(*p*-chlorophenyl)-α-chloroacetyl chloride. Montagne reported a melting point of 101.75° for his product, which he may have obtained in still a third crystalline modification.

1,1-Dichloro-2,2-bis-(*p*-chlorophenyl)-ethylene.—Incidental to the above research, the preparation of this olefin was carried out as described by Grummitt, Buck and Egan.[22] The product was at first obtained in a form not previously reported. It melted at 74.5–75.3°. Oxidation with boiling 70% nitric acid for 40 minutes converted it to *p,p*′-dichlorobenzophenenone, m.p. 147.0–148.0.[23]

After one month a sample of the olefin was recrystallized, yielding the stable form, m.p. 88.4–89.1°. A saturated solution of the low melting form seeded with the high melting form and cooled gave only the latter form. A mixture of the two forms melted at 88.4–89.1°.

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[21] Galbraith Microanalytical Laboratories, Box 32, Knoxville, Tennessee.

[22] O. Grummitt, A. Buck and R. Egan, *Org. Syntheses*, **26**, 22 (1946).

[23] *Cf.* J. F. Norris and W. C. Twieg, *Am. Chem. J.*, **26**, 497 (1901).

KNOXVILLE, TENNESSEE

[CONTRIBUTION FROM THE CHEMISTRY LABORATORIES, TEMPLE UNIVERSITY, AND THE PITMAN–DUNN LABORATORIES, FRANKFORD ARSENAL]

Autoxidation of the Saturated Aliphatic Diesters[1]

BY M. WESLEY RIGG AND HENRY GISSER

RECEIVED SEPTEMBER 24, 1952

This report describes an investigation of the thermal oxidation of the saturated esters of adipic acid. The preparation and physical properties of esters from alcohols having 5 and 6 carbon atoms are given. The esters were oxidized both by continuous passage of air through the esters and by oxidation in an apparatus which recorded rate of oxygen consumption. It was found that the distance of a tertiary carbon atom from the ester group exerts a considerable effect on oxidation rate, the rate decreasing as the distance is decreased. This effect is attributed to resonance of the ester group which results in an inductive effect along the carbon chain lowering the availability of electrons at the carbon in which oxidation is taking place. In general, esters from the *n*-alcohols oxidize at a lower rate than esters from the branched-chain alcohols. Activation energies for autoxidation of several esters were determined. The values reflect the effect of alcohol structure with respect to distance of the tertiary carbon from the ester group.

Kinetics of di-(3-methylbutyl) adipate autoxidation was studied in diethyl adipate solutions. In the early stages of oxidation (before appreciable hydroperoxide decomposition takes place) the rate of oxygen consumption, calculated per mole of ester, is proportional to the square of the ester concentration. As oxidation proceeds the rate of oxidation increases and becomes constant. At this stage, the rate of oxygen consumption is proportional to the square root of the ester concentration (when extent of oxidation is still small so that the concentration of ester has changed very little from the original). It is proposed that the oxidation mechanism involves chain branching due to hydroperoxide decomposition and this mechanism is found to explain the observed kinetics. Data are presented to show that the concentration of peroxide is independent of ester concentration.

In the autoxidation of saturated hydrocarbons the preponderance of evidence indicates that oxidation takes place preferentially at a tertiary C–H bond.[2] Secondary C–H bonds are attacked less readily and primary C–H bonds are still more stable

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[1] From the Ph.D. Thesis of H. Gisser.

[2] (a) A. D. Walsh, *Trans. Faraday Soc.*, **42**, 269 (1946); (b) A. D. Walsh, *ibid.*, **43**, 297 (1947); (c) A. W. Burwell, *Ind. Eng. Chem.*, **26**, 204 (1934).

TABLE I

PREPARATION OF THE ESTERS

Ester	Adipic acid, g.	Adipic acid, Moles	Reflux time, hr.	Yield, g.	Yield, %	Sapn. equiv. Found	Sapn. equiv. Calcd.
Diethyl adipate	1460	10	30	1652	81.8	101.4	101.1
Di-*n*-pentyl adipate	876	6	6	1270	74.2	143.8	143.2
Di-(1-methylbutyl) adipate	731	5	24	1216	84.9	143.6	143.2
Di-(1-ethylpropyl) adipate	876	6	24	1444	84.1	142.6	143.2
Di-(2-methylbutyl) adipate	584	4	6	898	78.7	142.8	143.2
Di-(3-methylbutyl) adipate	876	6	8	1458	85.0	143.7	143.2
Di-*n*-hexyl adipate	584	4	16	908	72.1	156.9	157.3
Di-(2-ethylbutyl) adipate	876	6	6	1520	80.5	156.5	157.3
Di-(1,3-dimethylbutyl) adipate	584	4	6	1025	81.4	157.5	157.3
Di-(2-methylpentyl) adipate	730	5	6	1286	81.7	157.7	157.3
Diethyl azelate	600[a]	3.2	30	560	72.4	120.7	121.2

[a] Azelaic acid.

TABLE II

PHYSICAL CONSTANTS OF THE ESTERS

Ester	B.p. °C.	B.p. Mm.	F.p., °C.	n^{20}_D	d^{25}_4
Diethyl adipate	99	1.4	−18.0	1.42776	1.0016
Di-*n*-butyl adipate	149.5	2.2	−23.2	1.43520	0.9563
Di-*n*-pentyl adipate	155	3	−14.0	1.43892	.9433
Di-(1-methylbutyl) adipate	125	1	−45.0	1.43350	.9335
Di-(1-ethylpropyl) adipate	145.2	2.5	−23.1	1.43463	.9371
Di-(2-methylbutyl) adipate	143	0.7	−21.1	1.43797	.9420
Di-(3-methylbutyl) adipate	122	0.2	−45.0	1.43730	.9394
Di-*n*-hexyl adipate	164.5	3	−13.8	1.44059	.9323
Di-(1,3-dimethylbutyl) adipate	155	1.3	− 7.1	1.43457	.9184
Di-(2-ethylbutyl) adipate	166.5	1	−25.3	1.44378	.9400
Di-(2-methylpentyl) adipate	161	2.0	−27.0	1.44092	.9321
Diethyl azelate	134.5	1.0	−15.8	1.43474	.9673

to oxidation. George and Robertson[3] showed that the thermal oxidation of tetralin proceeded *via* an energy chain, while the peroxide-catalyzed autoxidation of tetralin proceeded *via* a radical chain.[4] In the former case the autoxidation rate was proportional to the square of the tetralin concentration. Walsh[2a] proposed that hydroperoxides may decompose by thermal scission yielding RO· and HO· radicals. With tertiary hydroperoxides, the RO· radical decomposes further by breaking the weakest α C–C bond with the formation of a ketone and R′·. The latter may then serve to initiate new oxidation changes.

These observations and ideas have been applied to the study of the autoxidation of saturated aliphatic diesters, which are currently of interest as lubricants.

Experimental

Materials.—The alcohols were prepared by fractionation of commercial materials, except for ethyl alcohol where the USP grade was used without further treatment.

Adipic acid was obtained from Distillation Products Company (Eastman grade) and used without further treatment. The azelaic acid was a commercial grade obtained from Emery Industries and was recrystallized once from thiophene-free benzene and twice from distilled water before use. The melting point of the purified material was 105°.

The esters were prepared by mixing the alcohol and adipic acid, in the ratio of 2.5 moles of alcohol per mole of adipic acid, with 1 g. of *p*-toluenesulfonic acid monohydrate per mole of alcohol and refluxing in an apparatus fitted with a continuous water take-off adapter. For alcohols having more than five carbon atoms, 100 ml. of xylene were added per mole of acid. When no more water collected, the excess alcohol and xylene (if present) were removed under reduced pressure using a water-pump. Three grams of finely powdered anhydrous potassium carbonate per mole of adipic acid were then added and the mixture distilled with an oil-pump (1 to 5 mm.). The distillate was mixed with 1 g. of finely powdered anhydrous potassium carbonate per mole of adipic acid and permitted to remain overnight. The mixture was filtered and the filtrate distilled, the ester being collected over a maximum boiling range of 0.3° (except for di-*n*-pentyl adipate which was collected over a 0.5° range). All of the distillations were conducted in a current of dry nitrogen.

Esters of ethyl alcohol were made in a similar manner except that 50 ml. of thiophene-free benzene were added per mole of adipic acid and a fractionating column was interposed between the reaction flask and the reflux condenser to separate the alcohol from the water–benzene azeotrope. Considerable benzene and alcohol remained dissolved in the collected water and to replace these, 30 ml. each of ethyl alcohol and thiophene-free benzene were added to the reaction mixture for each mole of adipic acid initially used when the reaction was approximately half completed.

The freshly distilled esters were colorless and had acid numbers less than 0.01 mg. of KOH per gram of ester. The ethyl esters had faint fruity odors, while the other esters were odorless. These properties were retained for at least six months. Details of the preparations of the esters and physical properties of esters are given in Tables I and II, respectively.

Oxidation Techniques.—The esters were autoxidized by two methods, in one of which oxygen consumption rate was recorded and in the other the rate of formation of peroxide and acid during passage of a stream of air through the ester were determined.

The oxygen absorption apparatus was that described by Von Fuchs and Diamond[5] using the gas pump of Larsen and his co-workers.[6] Most of the oxygen absorption measurements were made at 110°. Tenth-mole quantities of the esters were oxidized and oxidation was continued until 200 ml. of oxygen had been consumed.

Air oxidation was accomplished by passing dry air (previously passed through towers of soda lime and cotton wool) at a rate of 8.0 ± 0.5 liters per hour through 150 g. of ester in a tube fitted with a reflux condenser. The effluent gases were passed through traps to collect peroxide or acid escaping from the system (in different runs). At intervals, samples of the esters were removed and peroxide[7] and acid were determined. (Peroxide and acid data were corrected for "volatile" material collected in the traps.) The air oxidations were run at 100°.

Results and Discussion

Effect of Structure.—The oxygen absorption data are shown in Figs. 1 and 2. The course of

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(3) P. George and A. Robertson, *Proc. Roy. Soc.* (*London*), **185A**, 309 (1946).

(4) P. George, *ibid.*, **185A**, 337 (1946).

(5) G. H. Von Fuchs and H. Diamond, *Ind. Eng. Chem.*, **34**, 927 (1942).

(6) R. G. Larsen, R. E. Thorpe and F. A. Armfield, *ibid.*, **34**, 183 (1942).

(7) C. D. Wagner, R. H. Smith and R. D. Peters, *Anal. Chem.*, **19**, 976 (1947).

oxidation is similar to that found in the autoxidation of tetralin.[8] The oxygen consumption curve is linear for a shorter or longer period of time depending on the material under study. After this period the oxidation rate progressively increases and eventually becomes constant. This rate is referred to in the following as the "steady" rate. The steady rate was obtained in most instances when approximately 0.04 mole of oxygen had been consumed per mole of ester. The initial and steady rates (calculated from the slopes of the linear portions of the curves in Figs. 1 and 2) are given in Tables III and IV.

The oxygen absorption data indicate that as the distance of the side chain from the ester group increases, the rate of oxidation also increases. The greater susceptibility to oxidation of the tertiary C–H bond than the secondary C–H bond is also evident from Tables III and IV, when the tertiary C–H bond is sufficiently far removed from the ester group. An increase in the molecular weight of the ester is expected to increase the oxidation rate but it is improper to compare rates of oxidation per gram of ester because no relative values are available for tertiary and secondary C–H bonds. In addition, the secondary C–H bonds are also affected by their distance from the ester group. The oxidation rates of the *n*-alkyl adipates per CH_2 group are given in Table V assuming that no oxidation takes place in the end methyl group. Evidently the CH_2 groups of ethyl azelate are more susceptible to oxidation than those of the adipate.

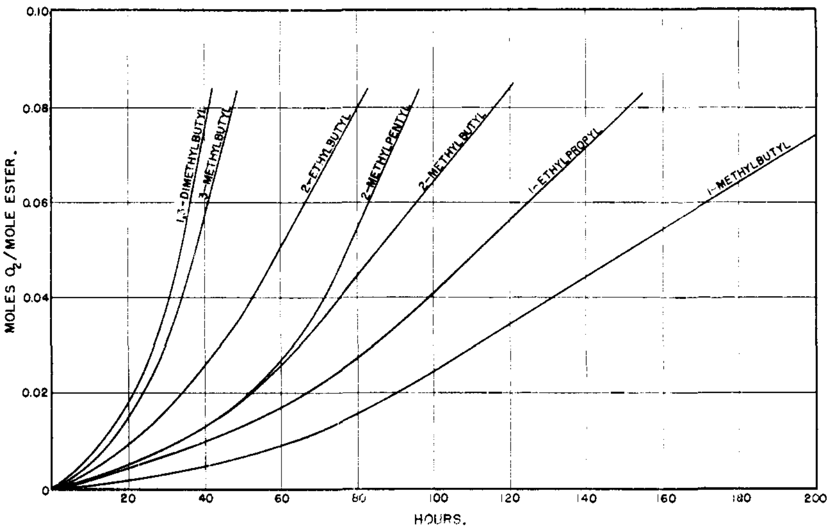

Fig. 1.—Oxidation of branched-chain dialkyl adipates at 110°.

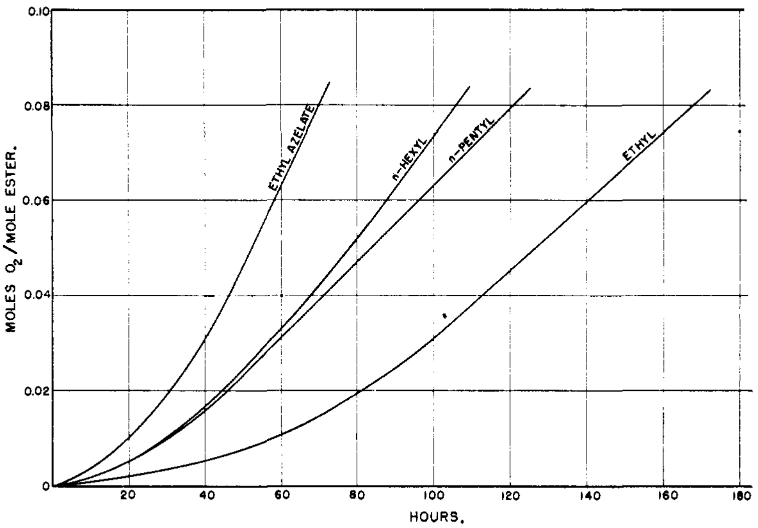

Fig. 2.—Oxidation of di-*n*-alkyl adipates at 110°.

TABLE III

OXIDATION RATES OF BRANCHED-CHAIN DIALKYL ADIPATES

Adipate	Initial rate × 10^7 (moles O_2/mole ester/sec.)	"Steady" rate × 10^7 (moles O_2/mole ester/sec.)
1-Methylbutyl	0.143	1.38
2-Methylbutyl	0.491	2.70
3-Methylbutyl	1.15	8.18
1-Ethylpropyl	0.161	2.15
2-Methylpentyl	.562	4.95
2-Ethylbutyl	.839	3.97
1,3-Dimethylbutyl	1.65	11.3

TABLE IV

OXIDATION RATES OF *n*-ALKYL ESTERS

Ester	Initial rate × 10^7 (moles O_2/mole ester/sec.)	"Steady" rate × 10^7 (moles O_2/mole ester/sec.)
Diethyl adipate	0.178	1.98
Di-*n*-pentyl adipate	.384	2.20
Di-*n*-hexyl adipate	.348	2.97
Diethyl azelate	.798	4.62

TABLE V

OXIDATION RATES OF *n*-ALKYL ESTERS

Ester	Initial rate per CH_2 × 10^8	"Steady" rate per CH_2 × 10^8
Diethyl adipate	0.297	3.30
Di-*n*-pentyl adipate	.320	1.83
Di-*n*-hexyl adipate	.248	2.12
Diethyl azelate	.887	5.13

In the air oxidations, it was found that the concentration of peroxide built up slowly, reached a maximum and then slowly decreased probably approaching a steady or near steady state. The observed concentration of peroxide is the difference between the amount formed and the amount decomposed and is therefore an indication of both oxidation rate and hydroperoxide stability at the temperature of oxidation. As a result, the concentration of hydroperoxide is useful for rate comparison at best during the initial stages of oxidation when relatively little hydroperoxide decomposition takes place. The concentration of acid built up

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[8] P. George and A. Robertson, *Trans. Faraday Soc.*, **42**, 210 (1946).

slowly at first, and then increased quite rapidly. Except for esters in which the side chain was on the γ-carbon from the ester group, the increase in rate of acid formation took place when the rate of peroxide formation began to decrease. With esters whose tertiary carbons were in the γ-position to the ester group, acid formation approximately followed peroxide formation for approximately 200 hr., following which, the rate of peroxide formation began to decrease. The initial rates of peroxide formation and the rates of acid formation confirmed the oxygen absorption data in the effect of structure on rate.

The effect of structure on autoxidation rate may be attributed to the availability of electrons at the carbon atom in question due to the ester group.

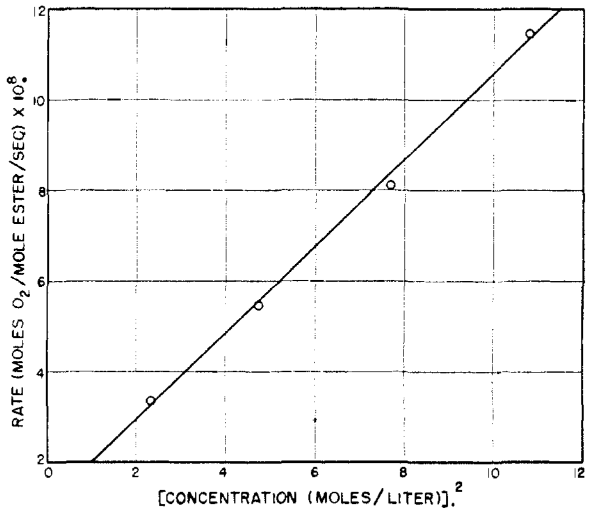


Fig. 3.—Initial oxidation rate of di-(3-methylbutyl) adipate.

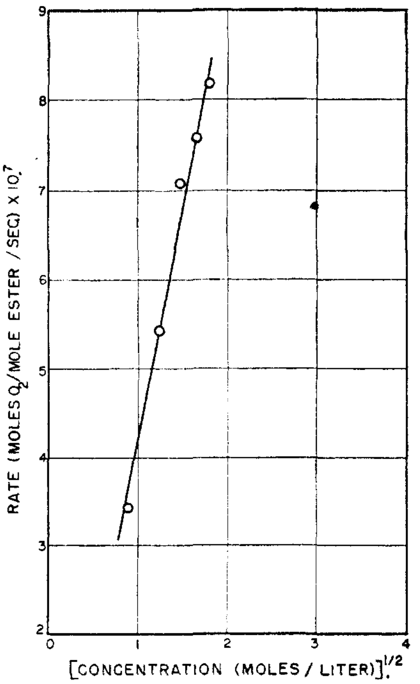
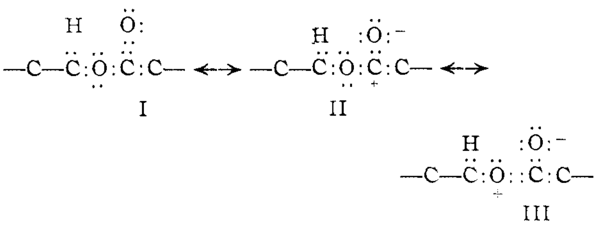


Fig. 4.—"Steady" oxidation rate of di-(3-methylbutyl) adipate.

Resonance of the ester group may be illustrated as



$$-\mathrm{C}-\overset{\mathrm{H}}{\ddot{\mathrm{C}}}:\ddot{\mathrm{O}}:\overset{\ddot{\mathrm{O}}:}{\mathrm{C}}:\mathrm{C}- \longleftrightarrow -\mathrm{C}-\overset{\mathrm{H}}{\ddot{\mathrm{C}}}:\ddot{\mathrm{O}}:\overset{:\ddot{\mathrm{O}}:^{-}}{\underset{+}{\mathrm{C}}}:\mathrm{C}- \longleftrightarrow -\mathrm{C}-\overset{\mathrm{H}}{\ddot{\mathrm{C}}}:\underset{+}{\ddot{\mathrm{O}}}::\overset{:\ddot{\mathrm{O}}:^{-}}{\mathrm{C}}:\mathrm{C}-$$

I II III

In III, the positively charged oxygen results in an inductive effect along the carbon chain attracting electrons to the ester group, and this effect falls off as the distance from the ester group increases. If oxidation is initiated at the tertiary carbon by formation of a free radical (by reaction with another free radical)[9] or by the formation of an energy-rich molecule having high energy in the vibrational mode of the C–H bond,[3] it would be expected that as electron availability decreases, the ease of oxidation should decrease.

Oxidation Kinetics.—The autoxidation of di-(3-methylbutyl) adipate was studied in diethyl adipate solutions. The rates in the initial and steady parts of the oxidation, calculated from the appropriate slopes of oxygen consumption curves, (corrected for oxidation of diethyl adipate assuming no interactions) are plotted in Figs. 3 and 4, respectively. The oxidation process may be considered as taking the following course: Oxidation chains are initiated thermally with the formation of hydroperoxide. The hydroperoxide decomposes resulting in free radicals which initiate new oxidation chains. In the initial period, the amount of hydroperoxide which is decomposing is small and most of the oxidation is initiated by thermal means and the rate of oxygen consumption is proportional to the square of ester concentration. As hydroperoxide formation and decomposition continue, free radical initiation competes with thermal initiation, the reaction speeds up and, after a period of time, most of the oxidation chains are initiated by previously formed hydroperoxide. At this stage the rate is again constant and is proportional to the square root of ester concentration. It should be noted that the oxidations were run until no more than 5% of the ester had been oxidized and as a result the ester concentration had changed relatively little and its effect on rate during the run was negligible.

The dependence of the initial rate on the square of ester concentration may be explained by the high energy chain proposed by George and Robertson[3] for tetralin. Dependence of the steady rate on the square root of ester concentration may be explained by assuming a chain-branching process in which hydroperoxide decomposes by the mechanism proposed by Walsh.[2] The oxidation mechanism may be formulated as follows taking into account chain branching due to hydroperoxide decomposition

Initiation:

$$\mathrm{RH} \xrightarrow{k_1} \mathrm{X}$$

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(9) C. E. Frank, *Chem. Revs.*, **46**, 156 (1950).

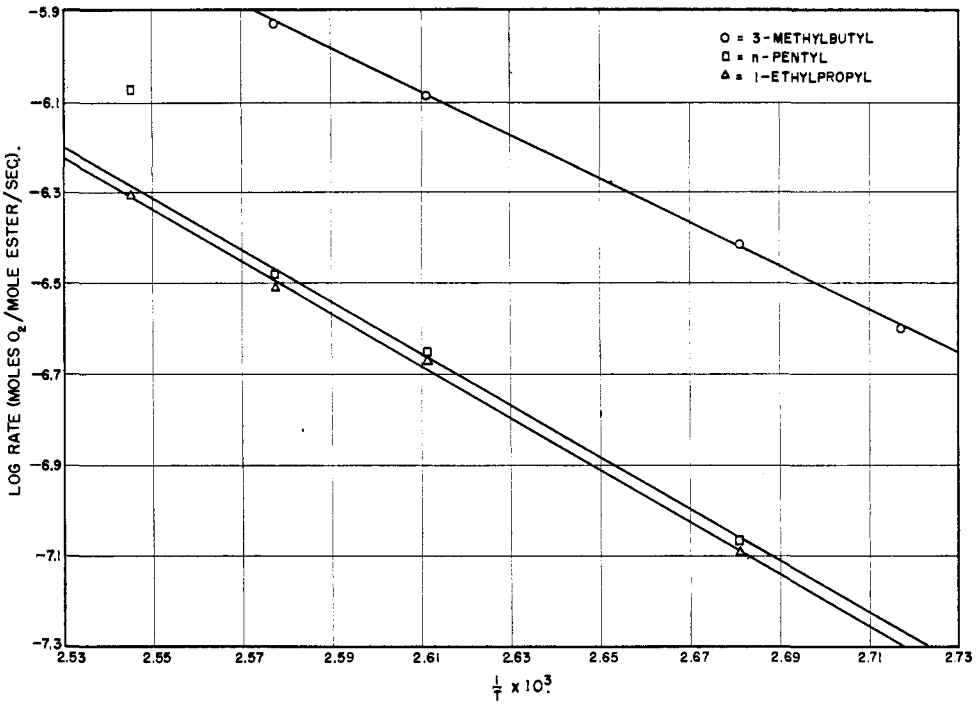

Fig. 5.—"Steady" oxidation rates of dialkyl adipates.

Propagation:

$$X + O_2 \xrightarrow{k_a'} P$$

$$P + RH \xrightarrow{k_b'} RO_2H + X$$

Termination:

$$P \xrightarrow{k_c} \text{products}$$

Branching:

$$RO_2H \xrightarrow{k_3} RO\cdot + HO\cdot$$

$$RO\cdot \xrightarrow{k_4} R_1\cdot + \text{carbonyl compounds}$$

Propagation:

$$R_1\cdot + O_2 \xrightarrow{k_a} R_1O_2\cdot$$

$$R_1O_2\cdot + RH \xrightarrow{k_b} R_1O_2H + R\cdot$$

$$R\cdot + O_2 \xrightarrow{k_a} RO_2\cdot$$

$$RO_2\cdot + RH \xrightarrow{k_b} RO_2H + R\cdot \text{ etc.}$$

Termination:

$$HO\cdot \xrightarrow{k_d} ?$$

$$RO_2\cdot + R\cdot \xrightarrow{k_5} RO_2R$$

where RH is the ester, X is a high energy ester molecule, P is a high energy complex of the ester and oxygen, and the k's are rate constants.

As a result of the oxidative degradation, radicals and molecules of lower carbon content than the original ester appear, *e.g.*, R_1O_2H. It will now be assumed that all radicals and molecules of the same type undergo similar reactions with approximately equal rate constants. Subscripts will therefore be dropped, and the indicated concentration of any compound will be the sum of concentrations of all compounds of that type. Neglecting the energy chains, since their contribution to the oxidation rate is small during the steady reaction, the following steady state equations are obtained

$$d(RO\cdot)/dt = k_3(RO_2H) - K_4(RO\cdot) = 0 \quad (1)$$

$$d(R\cdot)/dt = k_4(RO\cdot) - k_a(R\cdot)(O_2\cdot) + k_b(RO_2\cdot)(RH) - K_5(R\cdot)(RO_2\cdot) = 0 \quad (2)$$

$$d(RO_2\cdot)/dt = k_a(R\cdot)(O_2) - k_b(RO_2\cdot)(RH) - k_5(R\cdot)(RO_2\cdot) = 0 \quad (3)$$

Elimination of (RO·) and ($RO_2\cdot$) yields the following equation for (R·)

$$(R\cdot)^2 - \frac{k_3(R\cdot)(RO_2)}{2k_a(O_2)} - \frac{k_bk_3(RH)(RO_2H)}{2k_ak_5(O_2)} = 0 \quad (4)$$

since (R·) << 1 and $k_b > k_5$, the term in (R·) may be neglected, and

$$(R\cdot) = \left[\frac{k_bk_3(RH)(RO_2H)}{2k_ak_5(O_2)}\right]^{1/2} \quad (5)$$

since

$$dO_2/dt = k_a(R\cdot)(O_2) \quad (6)$$

$$dO_2/dt = k(RH)^{1/2} \quad (7)$$

where

$$k = \left[\frac{k_ak_bk_3(O_2)(RO_2H)}{2k_5}\right]^{1/2} \quad (8)$$

The steady rate is therefore proportional to the square root of ester concentration provided (RO_2H) is constant. (O_2) is constant because the oxidations were conducted at constant pressure.

Solutions of di-(3-methylbutyl) adipate and diethyl adipate were oxidized by a stream of dry air at 100° and peroxide was determined at 24 hr. intervals. The results are given in Table VI (where the figures have been corrected for oxidation of diethyl adipate assuming no interactions) from which it appears that the peroxide concentration per mole of di-(3-methylbutyl) adipate is independent of concentration of di-(3-methylbutyl) adipate at mole fractions of 0.367 and higher. The rate of oxygen consumption referred to above is the rate per mole of di-(3-methylbutyl) adipate. It has already been indicated that the peroxide concentration during oxidation probably approaches a steady state. It therefore follows that concentration of hydroperoxide in the above kinetic treatment may be assumed constant.

TABLE VI

AIR OXIDATION OF DI-(3-METHYLBUTYL) ADIPATE IN DIETHYL ADIPATE AT 100°. EQUIVALENTS PEROXIDE PER MOLE DI-(3-METHYLBUTYL) ADIPATE

Mole fraction di-(3-methylbutyl) adipate	24 hr.	48 hr.	72 hr.	96 hr.
0.179	2.06	3.02	6.86	..
.367	2.59	6.62	12.1	18.2
.566	..	6.32	11.7	18.1
.778	2.57	6.66	12.5	17.6
1	2.86	7.90	11.9	23.4

The kinetic analysis above does not account for the fate of HO· nor the formation of acid. While it is probable that HO· is associated with the later stages of oxidation, further work is required to clarify this point.

Energy of Activation.—Energies of activation for steady oxidations were determined on di-(3-methylbutyl) adipate, di-(1-ethylpropyl) adipate and di-*n*-pentyl adipate. The log of oxidation rate (moles oxygen consumed per mole ester per second) are plotted against reciprocal of absolute temperature in Fig. 5 and the calculated activation energies are in Table VII. These data provide further evidence for the effect of structure on oxidation rate.

TABLE VII

ENERGY OF ACTIVATION (CAL./MOLE)

Ester	Energy of activation
Di-(3-methylbutyl) adipate	21,900
Di-(1-ethylpropyl) adipate	26,300
Di-*n*-pentyl adipate	26,100

Acknowledgment.—The authors are indebted to the Chemistry Staff of the Pitman–Dunn Laboratories, Frankford Arsenal, where this investigation was conducted in part as an Army Ordnance Project.

PHILADELPHIA 37, PENNA.

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

Polarographic Analysis of the Serum Albumin–Mercury and Zinc Complexes[1a,b]

BY H. A. SAROFF AND H. J. MARK

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The polarography of protein–metal complex solutions is considered in light of the present limited theory of diffusion currents controlled by rates of reaction and diffusion. An experimental method is described for the use of the polarograph in analyzing protein–metal complexes based on a combination of amperometric titrations and dialysis experiments. Zinc in an acetate buffer at *p*H 6.1 binds bovine albumin at eight similar sites with an intrinsic constant of 1200. Mercuric chloride shows binding to at least two different sets of sites on bovine serum albumin at *p*H 4.9 in an acetate buffer, one, a single (sulfhydryl) site with a constant of 10^7, the other, five sites with a K value of 27000 to 45000.

Proteins have been reported to affect the polarographic wave by lowering the diffusion current[2] and shifting the half-wave potential.[3] Shifts in half-wave potential have been employed for the calculation of some constants of non-protein complexes.[4] Because of the size of the protein molecule (and its small diffusion coefficient) some of the shifts in half-wave potential with proteins are difficult to explain on the basis of complexation of the protein with the substance measured since marked shifts in half-wave potential have been found even under conditions where little change in diffusion current could be detected. It is probable that such shifts in potential are a function of adsorption of the protein on the surface of the mercury drop rather than complexation with the substance electrolyzed. While these shifts are important and must be explained in order to arrive at a complete description of the electrolysis process in studying protein metal complexes with the polarograph, this communication illustrates a method for calculating the binding constants of a protein–metal complex from changes in the diffusion current of metal ion solutions in the presence of proteins.

The problem involved in the electrolysis of a mixture containing free protein, free metal ion and protein–metal complex about a dropping mercury electrode has not yet been solved. The problem may be formulated by considering the diffusion and reaction rate conditions about a dropping mercury electrode in a solution at equilibrium with the reaction defined by

$$M^+ + HP \underset{k_2}{\overset{k_1}{\rightleftarrows}} MP + H^+ \qquad (1)$$

where M^+ is a reducible metal ion, HP a protein, MP the metal protein complex (reducible or non-reducible), and H^+ the hydrogen ion concentration. Solutions to an analogous problem but with the same diffusion coefficient for the reducible substance and the complex (non-reducible) have been published by Koutecky and Brdicka[5] and Delahay.[6] These solutions do not apply to the protein problem mainly because of the difference in the diffusion

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(1) (a) Parts of this paper were extracted from Report No. 1 by H. A. Saroff and H. J. Mark, Project NM 000006, Naval Medical Research Institute, Bethesda, Maryland, 1949. (b) Presented before the Division of Biological Chemistry, 116th Meeting, American Chemical Society, September, 1949.

(2) I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1941, p. 121.

(3) R. H. Coe and L. B. Rogers, THIS JOURNAL, **70**, 3276 (1948).

(4) Reference 2, p. 170.

(5) J. Koutecky and R. Brdicka, *Collection Czechoslov. Chem. Communs.*, **12**, 337 (1947).

(6) P. Delahay, THIS JOURNAL, **73**, 4944 (1951).