and AG (free antigen). These species have the sedimentation constants $s_b > s_a > s_A$, and upon sedimentation they form the sharp boundaries **b**, **a**, and **A**, respectively. That part of the system behind **b** no longer has the original composition, and tends toward a new state of equilibrium. Behind **a** no reaction occurs, only AG being present, but between **a** and **b**, the original $(AG)_3(AB)_2$ having sedimented away, disproportionation of $(AG)_2(AB)$ takes place

$$2(AG)_2(AB) \xrightarrow{R} (AG)_3(AB)_2 + AG$$

R is the over-all rate constant of this reaction. The additional AG so produced sediments along with the AG originally present. Consider the excess AG produced at time t_1 at the position P a distance x ahead of **A** (Fig. 5a). At some later time t_2 that AG produced at P will have been overtaken by **a** and will be found in the interzone **a** − **A** (Fig. 5b).

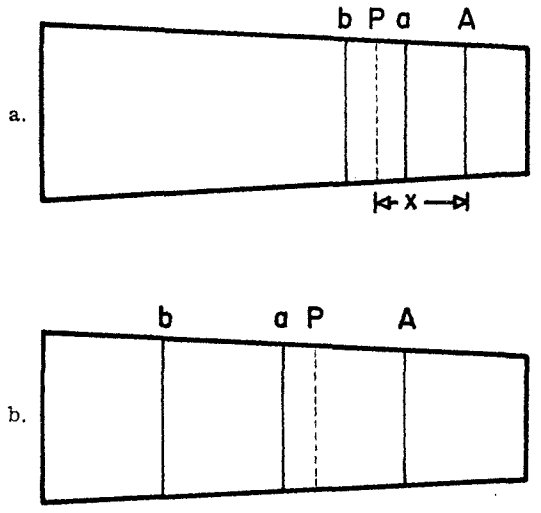

Fig. 5.—Boundary positions for the idealized three-component system in the ultracentrifuge cell. See appendix for details.

The total excess of AG found at position P, ΔAG, is simply the amount produced at P in the time interval between the arrivals of **b** and **a** at P, namely

$$\Delta AG = \int_{t(x,b)}^{t(x,a)} R(x)dt = \overline{R(x)}\left\{\frac{x}{s_a - s_A} - \frac{x}{s_b - s_A}\right\} = \frac{\overline{R(x)}\, x(s_b - s_a)}{(s_a - s_A)(s_b - s_A)}$$

Here $\overline{R(x)}$ is the effective average rate of reaction. It follows that $d(\Delta AG)/dx$ is positive (and equal to the constant $\bar{R}(s_b - s_a)/(s_a - s_A)(s_b - s_A)$ if $\bar{R}$ is constant) and that the schlieren curve in the region **a** − **A** will be elevated from the baseline correspondingly. If, on the other hand, the schlieren curve in this region does return to the baseline, then the effects of re-equilibration are negligible, and unambiguously, *the area under the free antigen peak corresponds to the concentration of free antigen present in the original solution.* With more than three species the situation is more complicated, but the same conclusion holds.

For the system BSA-5I–anti-BSA at *p*H 8.5, the electrophoretic mobilities of the various species have the order $-\mu_A > -\mu_a > -\mu_b > 0$. As a result, the ascending limb in electrophoresis exhibits a distribution of species which is similar to that produced in the ultracentrifuge, and the considerations discussed above apply, with only minor changes. The effects of re-equilibration are probably even less significant in the ascending limb in electrophoresis than in the ultracentrifuge. The electrophoresis experiment is performed near 0°, whereas sedimentation is carried out at about 20°. The rates of the re-equilibration reactions should therefore be slower in electrophoresis. Furthermore μ_a and μ_b are not very different, and the term $(\mu_a - \mu_b)/[(\mu_A - \mu_a)(\mu_A - \mu_b)]$ should be less important than the corresponding sedimentation term. For these two reasons, the ascending electrophoresis pattern should be more reliable than the sedimentation pattern, other things being equal.

In the descending limb of the electrophoresis cell, however, a markedly different distribution of components exists for this system. Between the free antigen boundary and the **a** complex boundary, *all* of the complexes are present in a region deprived of the free antigen originally in the solution. These complexes will then undergo reactions to produce free antigen. An analysis of the results by methods similar to those applied to the ascending limb has been made, but since the descending patterns have not been used quantitatively in this paper, and the effects of re-equilibration are involved, the analysis will not be detailed here. It is possible by this means to explain the experimental observation that in the descending limb, but not in the ascending, the schlieren curve between the free antigen peak and the **a** complex peak is somewhat elevated from the baseline.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND THE DEPARTMENT OF PHYSICS, KANSAS STATE COLLEGE]

Autoxidation of Ketones. II. Di-*n*-propyl Ketone[1]

BY DEXTER B. SHARP, STUART E. WHITCOMB, LEO W. PATTON AND A. DWIGHT MOORHEAD

Liquid di-*n*-propyl ketone has been oxidized by molecular oxygen to propionic and *n*-butyric acids, in the absence of inorganic catalysts. A small amount of water and propionaldehyde also was obtained, but no *n*-butyraldehyde was isolated. The oxidations were followed by chemical analyses. The peroxide content increased very slowly, but the acid content increased rapidly after the induction period. Infrared spectra were obtained periodically during the runs, and changes in spectra with oxidation time are reported. The results provided further evidence of the α-position activation by the carbonyl group during autoxidations of ketones.

Introduction

In a previous paper,[2] the autoxidation of diisopropyl ketone to an α-hydroperoxide, acetone and isobutyric acid was reported. Similar studies have been made of the autoxidation of di-*n*-propyl ketone. Paquot[3] reported that di-*n*-propyl ketone was oxidized by oxygen to a mixture of *n*-butyric acid, propionic acid and smaller amounts of heptanedione-3,4 and *n*-butyraldehyde. However, he used nickel phthalocyanine, which is known to accelerate autoxidation processes. It seemed logical that the exclusion of such catalysts might provide more reliable information about the course of oxidation.

A multiple investigation of the autoxidation was carried out. Inorganic catalysts were excluded

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(2) D. B. Sharp, L. W. Patton and S. E. Whitcomb, THIS JOURNAL, **73**, 5600 (1951).

(3) C. Paquot, *Bull. soc. chim.*, **12**, 450 (1945).

and the course of oxidation was followed by chemical analyses of samples withdrawn periodically during the runs. The final mixtures were separated into all isolable components. Changes in the infrared spectra with oxidation time were studied.

Experimental

Apparatus.—The oxidations were conducted in the all-glass apparatus described previously.[2] The infrared absorption spectra were obtained with a Perkin-Elmer Model 12C Recording Spectrometer equipped with a sodium chloride or a lithium fluoride prism, as desired.

Materials.—Di-*n*-propyl ketone was prepared by passing *n*-butyric acid vapors over a heated thorium oxide catalyst, an adaptation of the method described in reference 4. The ketone was fractionated through a 48-inch, 20-mm. i.d., adiabatic column packed with 3/32-inch glass helices and fitted with a total condensation, variable take-off still-head. The di-*n*-propyl ketone used for the oxidations had the following physical constants: b.p. 142–143° (736 mm.); d^{20}_4 0.822; $n^{20}D$ 1.4064. Reported for di-*n*-propyl ketone: b.p. 144.2° (760 mm.)[5]; d^{20}_4 0.8217[6]; $n^{20}D$ 1.4073.[6] Reagent grade chemicals were used for analyses and other operations.

Oxidation Procedure.—Before an oxidation, the oxidation chamber was cleaned as described previously.[2] The chamber was filled with di-*n*-propyl ketone (425–450 g.) and Dry Ice was placed about the trap. The ketone was heated to the desired run temperature, and oxygen gas was dispersed into the liquid ketone so that bubbles of the gas always reached the top of the liquid column. Water was circulated in the reflux condenser to minimize loss of volatile products. Samples of the reaction mixture were removed at intervals. Each sample was analyzed for peroxide content by the method of Wagner, Smith and Peters,[7] and the acid content was determined by titration with standard alkali. The infrared absorption spectrum of each sample was obtained also. At the end of the oxidation the liquid was drained from the oxidation chamber, and chemical and infrared analyses were obtained for samples of this final mixture.

Separation of Products.—The same general procedure was employed for product separation as was used in the case of diisopropyl ketone.[2] Upon completion of an oxidation at 110° the small amount of liquid in the cold trap was converted to the 2,4-dinitrophenylhydrazone derivative, m.p. 151–152° alone or mixed with authentic propionaldehyde 2,4-dinitrophenylhydrazone (reported[8] 154°). In a subsequent run steam was passed through the reflux condenser to facilitate passage of volatile compounds into the cold trap. The trap-liquid (19 ml.) was fractionated through a 10-inch Vigreux column. The first fraction (13 ml.) had a b.p. 49–53°, d^{20}_4 0.805 and $n^{20}D$ 1.3594. Reported for propionaldehyde: b.p. 49.5° (740 mm.)[9]; d^{20}_4 0.8066[10]; $n^{20}D$ 1.3635.[10] The boiling point then increased from 53 to 140° while a two-ml. portion distilled. This indicated that no *n*-butyraldehyde was present (b.p. 75°) contrary to the report of Paquot.[3]

The reaction mixture from a third run was extracted with portions of 5% sodium hydroxide to which phenolphthalein had been added, until the basic color of the indicator was not discharged. The aqueous extract was evaporated to dryness, 10 ml. of water was added and the mixture was acidified with 85% phosphoric acid. The mixture was extracted with five 25-ml. portions of ether, the ether extract was dried over anhydrous sodium sulfate and distilled through a 10-inch Vigreux column. After removal of the ether, a fraction (8.36 g., 0.11 mole) was collected: b.p. 120–145°; $n^{20}D$ 1.3861; *p*-bromophenacyl ester m.p. 62–63°. Reported for propionic acid: b.p. 141.3° (760 mm.)[11]; $n^{17}D$ 1.3874[12]; derivative m.p. 63°.[5] The second fraction (8.75 g., 0.10 mole) had the following constants: b.p. 145–162°; $n^{20}D$ 1.3956; *p*-bromophenacyl ester m.p. 62–63°. Reported for *n*-butyric acid: b.p. 163.2° (748.7 mm.)[13]; $n^{20}D$ 1.3983[14]; derivative m.p. 63°.[5] When each derivative was mixed with the corresponding authentic *p*-bromophenacyl ester the melting points showed no depression. A mixture of the derivatives of the acids obtained from the oxidation melted at 43–44°.

The oxidized di-*n*-propyl ketone had a yellow color which suggested the presence of an α-diketone. Attempts to separate the yellow compound from unchanged ketone by distillation through a 48-inch column were unsuccessful; the yellow substance codistilled, apparently, with di-*n*-propyl ketone. Negative results were obtained with a modification of the quinoxaline method reported by Bost and Towell[15] for the detection of α-diketones with *o*-phenylenediamine. Paquot[3] reported the isolation of heptanedione-3,4 from the oxidized di-*n*-propyl ketone.

Progress of Oxidation.—The concentrations of acid and peroxide were calculated as millimoles per gram, and these values were plotted against time of oxidation. The curves in Fig. 1 represent such plots for oxidations at 110 and 120°.

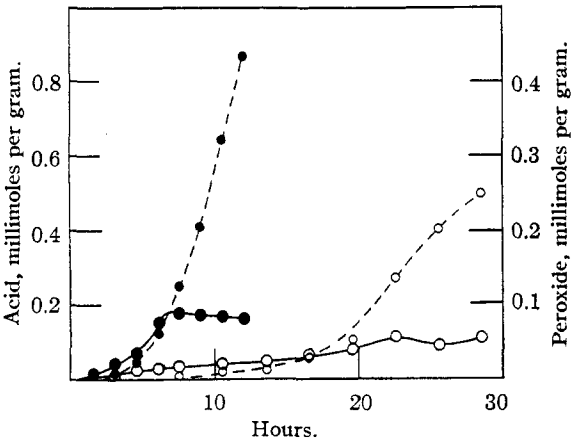

Fig. 1.—Oxidation of di-*n*-propyl ketone: ●, 120°; ○, 110°; - - -, total acid; ——, total peroxide.

Infrared Spectra.—The techniques employed in the previous study[2] were used. The absorption spectra were obtained of samples withdrawn periodically during oxidation runs. The sample cells were rock salt plates separated by

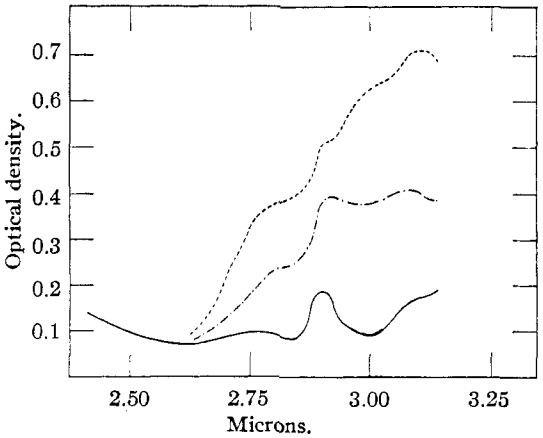

Fig. 2.—Infrared absorption spectra of oxidized di-*n*-propyl ketone (120°); sodium chloride prism, rock salt cell, thickness 0.1 mm.; ketone oxidized 1.5 hr., ——; 9.0 hr., —·—·—, 12.0 hr., - - - - -.

(4) A. H. Blatt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 389.
(5) W. L. Judefind and E. E. Reid, THIS JOURNAL, **42**, 1043 (1920).
(6) J. W. Bruhl, *J. prakt. Chem.*, [2] **60**, 141 (1899).
(7) C. D. Wagner, R. H. Smith and E. D. Peters, *Anal. Chem.*, **19**, 976 (1947).
(8) C. F. H. Allen, THIS JOURNAL, **52**, 2955 (1930).
(9) W. Hartung and H. Adkins, *ibid.*, **49**, 2517 (1927).
(10) J. W. Bruhl, *Ann.*, **200**, 175 (1880).
(11) J. Timmermans and Hennaut-Roland, *J. chim. phys.*, **27**, 401 (1930).
(12) G. S. Whitby, *J. Chem. Soc.*, 1458 (1926).
(13) A. Lieben and A. Rossi, *Ann.*, **158**, 146 (1871).
(14) S. T. J. Tromp, *Rec. trav. chim.*, **41**, 278 (1922).
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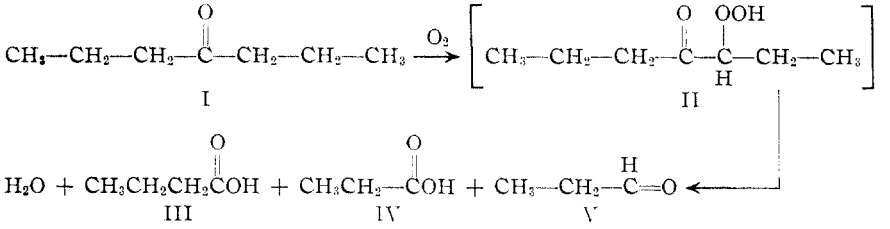
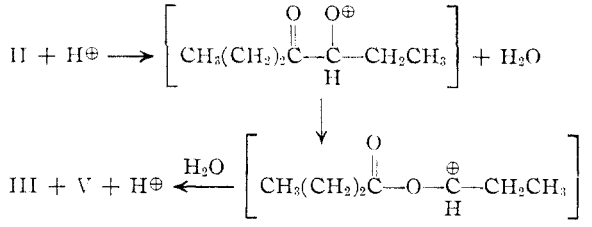
aluminum spacers which were rolled to the desired thickness. Figure 2 shows portions of the infrared spectra of di-*n*-propyl ketone which had been oxidized 1.5, 9 and 12 hours.

Discussion of Results

Approximately equimolar quantities of propionic and *n*-butyric acids were isolated from runs in which there was minimum loss of volatile components. Although the peroxide content during a run remained low, it was significantly real. It was assumed, therefore, that an unstable α-hydroperoxide was the initial oxidation product and that it in turn decomposed to give the two acids. A formulation for this oxidation is



Since appreciable quantities of propionaldehyde could be isolated by aiding passage of the volatile component to the Dry Ice-cooled trap, it seemed reasonable that it was the precursor of the propionic acid, and that the aldehyde was a primary decomposition product of the postulated α-hydroperoxide. Rapid autoxidation of the propionaldehyde may account for the formation of the propionic acid. No *n*-butyraldehyde was found in the trap contents, which is contrary to the results reported by Paquot.[3] The *n*-butyric acid may be formed from the hydroperoxide (II) by the following acid-catalyzed decomposition, analogous to the mechanisms proposed by Criegee[16] and George and Walsh[17] for hydroperoxide decompositions.



The failure to isolate a hydroperoxide from the oxidation mixture indicates that it is not as stable as the hydroperoxide of diisopropyl ketone.[2] This difference in stability is assumed to be due to the secondary and tertiary nature, respectively, of the carbon to which each hydroperoxy group is attached. The instability of the postulated α-hydroperoxide of di-*n*-propyl ketone is indicated further in Fig. 1. The maximum peroxide content was 0.1 millimole per gram at 110°. The maximum peroxide content observed during oxidation of diisopropyl ketone was almost five times this amount.[2]

Qualitatively paralleling the formation of the oxidation products is the progressive increase of optical density with oxidation time in the 3 μ region of the infrared absorption spectra (Fig. 2). This region of absorption has been attributed to O–H stretching,[18] and, in this oxidation, could be due to the combined absorption of the two carboxylic acids, the hydroperoxide and water. Absorption bands different from those of the pure ketone appeared at 3, 7.7–8.6, 9.3, 10.6, 12.1 and 12.6 microns.

The results of this study provide evidence that the carbonyl group in di-*n*-propyl ketone activates the secondary α-carbon in a manner similar to the activation of the tertiary α-carbon which was apparent in the case of diisopropyl ketone.[2] In each case, appreciable autoxidation of the ketone by molecular oxygen has been observed. It is suggested that the mechanism for the propagation step in the autoxidation of di-*n*-propyl ketone is analogous to that outlined for diisopropyl ketone.[2]

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(16) R. Criegee, *Ber.*, **77B**, 722 (1944).

(17) P. George and A. D. Walsh, *Trans. Faraday Soc.*, **42**, 94 (1946).

(18) H. M. Randall, R. C. Gore, U. Liddell and V. Z. Williams, "Infrared Spectroscopy," D. Van Nostrand Company, New York, N. Y., 1949, p. 43.