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Autoxidation Processes. X.[1] The Oxidation of Desylamine and of Benzoin Methyl Ether

BY T. H. JAMES AND A. WEISSBERGER

It has been shown in previous papers of this series that α-hydroxy ketones, RCHOHCOR, in alkaline solution racemize or are oxidized—by molecular oxygen or by Fehling's solution—at identical rates. These rates are proportional to the hydroxyl-ion concentration, the speed-limiting reaction being the enolization RCHOHCOR + OH^- = $RCOHCOR^-$ + H_2O. A characteristic group similar to that of α-hydroxy ketones exists in the α-amino ketones $RCHNH_2COR$. These substances are known to be unstable in the presence of oxygen.[2] The following investigation indicates a close parallel between their autoxidation and that of the α-ketols.

Fehling's solution oxidizes *α-aminodesoxybenzoin (desylamine)*, $C_6H_5CHNH_2COC_6H_5$, to benzil—identified by melting point, mixed melting point, and the reaction of Liebermann and Homeyer[3]—with splitting off of ammonia, which forms the characteristic copper complex.

Molecular oxygen oxidizes desylamine in alkaline solution to give ammonia and benzoic acid. The latter is the result of the interaction between benzil and hydrogen peroxide which—as in the case of the α-ketols—are initial products of the autoxidation. They can be identified if barium hydroxide is used as the alkali. The procedure is the same as that employed in the study of the autoxidation of benzoin.[4] A kinetic study showed that the rate of oxidation is proportional to the hydroxyl-ion concentration and to the desylamine concentration and is independent of the oxygen concentration. It appears, therefore, that here, as in the oxidation of the α-ketols, the speed-limiting process is the enolization

$$RCHNH_2COR + OH^- = RCNH_2COR^- + H_2O$$

A comparison of the rates of autoxidation of desylamine and benzoin shows that the aminoketone autoxidizes 1.7_5 times as fast as the hydroxy ketone. It would appear that the rate of enolization is higher if an amino group is attached to the carbon next to the carbonyl group than if an hydroxyl group is attached to this atom. This result is interesting from the point of view of the electronic theory of organic reactions. Further experiments must be carried out, however, before a reliable interpretation can be given.

Another aspect of our results arises from the similarity of the characteristic groups of α-amino ketones $—CHNH_2COR$ and of the α-amino acids $—CHNH_2COOH$. It suggests that the oxidative deamination of α-amino acids[5] might also proceed over an enolization, and that hydrogen peroxide be formed in the process.

The analogy in the reactions of α-amino ketones and of α-hydroxy ketones is also interesting in view of the process which follows the speed-limiting enolization. With the α-hydroxy ketones, this might be a second dissociation

$$RCHOCOR^- + OH^- = RCOCOR^= + H_2O$$

The oxidation proper would then virtually consist in the removal of two electrons, and the process would be analogous to the oxidation of hydroquinone in alkaline solution. This substance is active as a photographic developer after formation of the divalent anion,[6] and its autoxidation also runs through this stage.[7] Another possibility would be the direct interaction of the monovalent ion with oxygen with loss of one electron and one hydrogen atom, or with elimination of a hydroxyl ion and replacement by an oxygen atom. This mechanism deserves more attention in view of the reaction of desylamine where the amino group is eliminated, very probably from a monovalent ion.

That such an ion can autoxidize is shown conclusively by the reaction of *benzoin methyl ether*, $C_6H_5CH(OCH_3)COC_6H_5$. This substance is oxidized by molecular oxygen in alkaline solution at a rate which is about 0.005 of that of benzoin under the same conditions, and proportional to the hydroxyl-ion concentration. It takes up about one mole of oxygen per mole and as a reac-

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[1] IX. *J. Chem. Soc.*, 226 (1935).

[2] Vahlen, *Arch. Exptl. Path. Pharmakol.*, **47**, 368 (1902).

[3] C. Liebermann and J. Homeyer, *Ber.*, **12**, 1975 (1879).

[4] A. Weissberger, H. Mainz and E. Strasser, *ibid.*, **62**, 1942 (1929).

[5] *Cf.* H. A. Krebs, *Am. Rev. Biochem.*, **5**, 247 (1936); *Biochem. J.*, **29**, 1620 (1935).

[6] S. E. Sheppard and C. E. K. Mees, "Investigations on the Theory of the Photographic Process," Longmans, Green and Co., London, 1907.

[7] H. von Euler and E. Brunius, *Z. physik. Chem.*, **139**, 615 (1928); P. Dingemans, Dissertation, Delft, 1928.

tion product benzoic acid was identified by melting point and mixed melting point. Thus, it appears that, in this process, the methoxy group is eliminated in a manner similar to the elimination of the amino group in the deamination of the amino ketone and there is a possibility that the oxidation of α-hydroxy ketones proceeds correspondingly. This would be in analogy to catechol which, presumably, autoxidizes as the monovalent ion.[8]

An answer to the question whether in the autoxidation of the α-hydroxy ketones the molecular oxygen acts merely as an electron acceptor, or whether it actually enters the molecule might be obtained by running the reaction RCHOHCOR + O_2 = RCOCOR + H_2O_2 in barium hydroxide solution with "labelled" (heavy or radioactive) oxygen and analyzing the precipitated barium peroxide.

Experimental

Desylamine hydrochloride was prepared according to the method of Pschorr and Brüggemann[9] from benzil-α-monoxime.[10]

Benzoin methyl ether was prepared according to Fischer[11] and Staudinger and Kon,[12] recrystallized from petroleum ether and from methyl alcohol, with m. p. at 50.5–51.5°.

For the qualitative *peroxide test*, 0.05 g. of desylamine hydrochloride was dissolved in 5 cc. of 95% ethyl alcohol, 4 cc. of about 0.5 saturated aqueous barium hydroxide solution was added, and the mixture shaken for five minutes. The precipitate was collected at the pump, extracted with 4 cc. of 1 *M* sulfuric acid, and the filtered solution tested for hydrogen peroxide

For the quantitative *peroxide test*, 0.4 *M* mole of desylamine was dissolved in 60 cc. of 0.5 saturated barium hydroxide solution, and shaken with oxygen at 20° in the thermostat. In ten minutes the solution had absorbed 8.9 cc. (N. T. P.) of oxygen and the reaction was complete. The resulting mixture, upon the addition of 20 cc. of 0.1 *M* potassium permanganate solution and 5 cc. of 2.5 *M* sulfuric acid evolved 7.1 cc. oxygen in twenty seconds which corresponds to a formation of 80% peroxide.

C. P. 95% ethanol was used without further purification in the preparation of the solutions for oxidation rate comparisons.

The potassium hydroxide employed was de Haen's "Fixanal."

The apparatus employed in making the autoxidation velocity measurements was essentially that described by Weissberger, Mainz and Strasser.[4] The reaction vessel was of Pyrex glass and possessed a volume of approximately 150 cc. This was attached to a gas buret and the whole thermostated. The thermostat temperature was maintained at 20.00 ± 0.02° throughout the experiments. The reaction chamber was suspended in the thermostat in such a way that it could be moved rapidly back and forth sidewise by means of a motor-driven shaker.

The compound whose oxidation rate was to be determined was dissolved in 75% ethanol contained in the reaction vessel, and the alkali, likewise in 75% ethanol, was placed in the dropping funnel attached to the reaction vessel. The total volume of solution was in every case 60.0 cc. The air in the reaction vessel and buret was replaced by oxygen and the whole allowed to attain the temperature of the thermostat. Then, at time zero, the alkali was precipitated into the reaction chamber and the shaker started. The mixing time was about ten seconds. Preliminary experiments were made to determine the shaking rate required to maintain saturation of the solution with oxygen,[4] and in all oxidation velocity determinations the shaker rate was maintained considerably above the critical value.

Time readings were made for each 0.2 cc. of oxygen absorbed by the reaction mixture. The measured absorption of oxygen was then plotted against the time, and smooth curves drawn through the points so obtained. From the resulting curves, the initial rates of oxidation were determined.

The rates for benzoin and desylamine at various degrees of alkalinity are listed in the following table. The "initial velocities" are expressed in cubic centimeters of oxygen (N. T. P.) per minute.

TABLE I

Excess KOH, *M* moles	Amount of compound, *M* moles	Partial pressure of oxygen, cm.	V_1 (benzoin)	V_1/KOH	V_2 (desylamine)	V_2/KOH	Ratio V_2/V_1
0.40	0.400	73	0.215	0.54	0.375	0.94	1.75
.70	.400	73	.37	.525	.645	.92	1.76
1.00	.400	73	.53	.53	.915	.915	1.73
2.00	.400	73	—	—	1.83	.915	——
0.40	.400	15	—	—	0.37	.925	
2.00	.200	73	—	—	.92	.46	

The initial velocity in the experiment in which 0.2 *M* moles of desylamine was oxidized in the presence of 2.00 *M* moles of excess potassium hydroxide was 0.92, almost exactly half of that obtained with 0.4 *M* moles of material (1.83). Moreover, since in this experiment the alkali was present in tenfold excess, it was possible to test the reaction by means of the simple first-order reaction equation.[13] Table II shows that a satisfactory constant was obtained.

TABLE II

0.2 *M* MOLE OF DESYLAMINE + 2.00 *M* MOLES OF KOH

Time	*V*	*K*
0.5	0.55	—
1.5	1.50	0.25
2.5	2.20	.23
4	3.00	.235
7	3.95	.24
12	4.60	.23
∞	4.90	

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[8] M. A. Joslyn and G. E. K. Branch, THIS JOURNAL, **57**, 1779 (1935).

[9] R. Pschorr and F. Brüggemann, *Ber.*, **35**, 2740 (1902).

[10] T. W. J. Taylor and M. S. Marks, *J. Chem. Soc.*, 2302 (1930).

[11] E. Fischer, *Ber.*, **26**, 2413 (1893).

[12] H. Staudinger and N. Kon, *Ann.*, **384**, 113 (1911).

[13] $k = \frac{1}{\Delta T} \ln \frac{a - V_1}{a - V_2}$.

The analogy between the kinetics of the autoxidation of benzoin and desylamine thus is, within the scope of our experiment, complete.

The determination of the rate of autoxidation of benzoin monomethyl ether was made in 45.5% ethanol, employing 0.4 M mole of the ether. The initial oxidation rates at two alkali concentrations are given in Table III. The data, substituted into the first-order equation yielded a satisfactory constant.

TABLE III

0.4 M MOLE OF BENZOIN MONOMETHYL ETHER

KOH, M mole	V_i cc./mm.	V_i/KOH
3.00	0.00395	0.0013
10.00	.0140	.0014

The analysis of a control with nitrogen showed that the absorption of oxygen was not due to previous saponification of the ether under formation of benzoin which then autoxidized.

Summary

The mechanism of the oxidation of an α-amino ketone is closely analogous to that of the α-hydroxy ketones. The reaction proceeds at a rate which is proportional to the hydroxyl-ion concentration, with formation of the diketone, ammonia, and—with molecular oxygen—of hydrogen peroxide. A similar mechanism also applies to the oxidation of an α-methoxy ketone. The speed-limiting process in all these reactions is obviously the enolization, *i. e.*, the slow dissociation of the hydrogen ion from the carbon bearing the OH, NH_2, or OCH_3 group. The significance of this common behavior for the oxidation proper is discussed.

ROCHESTER, N. Y.

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[CONTRIBUTION FROM SEVERANCE CHEMICAL LABORATORY, OBERLIN COLLEGE]

The Isolation of Crystalline Vitamin A[1]

BY HARRY N. HOLMES AND RUTH E. CORBET[1a]

The existence of vitamin A was proved as early as 1913 yet up to 1937 its isolation was not accepted by workers in this field.

One of the first recorded efforts to prepare a rich concentrate was made by Takahashi and Kawakami[2] in 1923 using cod liver oil as the source. They called their product "crude biostearin" and reported a biologic activity of 0.08 mg. daily dose per rat. In 1925 Takahashi[3] and others further concentrated their crude biostearin by solvent partition and high vacuum distillation methods. In fact they claimed isolation of the pure vitamin, a claim later challenged by Drummond, Channon and Coward.[4] The latter workers distilled the nonsaponifiable portion of cod liver oil (previously freed from its cholesterol) at 2–3 mm. and found that the vitamin passed over at 180–220°. The distillate, however, contained several impurities.

British workers in their very thorough reports such as that edited by Hume and Chick[5] in 1935 set up a "provisional standard" of a concentrate with an extinction coefficient of $E_{1\,\text{cm.}}^{1\%} = 1600$. This was based upon the work of Carr and Jewell[6] who in 1933 prepared by distillation under very high vacuum a material reading $E_{1\,\text{cm.}}^{1\%} = 1600$ as measured by a quartz spectrophotometer. They also reported a feeding level of about 2,000,000 international units per gram for this preparation.

In 1933[7] Karrer and his associates, at Zürich, prepared a similar concentrate from fish liver oils but not by the vacuum distillation method. They used freezing methods and Tswett column adsorption by aluminum oxide, followed by differential adsorption on a column of calcium hydroxide. The extinction coefficient claimed was about 1700 and the rat feeding level reported by Euler, Karrer and Zubrys[8] was very low, about 0.3 gamma. This value was not reported directly in international units, as is the present custom.

Methods Used in This Research

Saponification and Extraction.—At the suggestion of Dr. C. E. Bills we used isopropanol as solvent for the saponifica-

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(1) A thesis based upon this research was submitted by Ruth Corbet in partial fulfilment of the requirements for the Doctor of Philosophy degree at The Pennsylvania State College, through a coöperative agreement between The Pennsylvania State College and Oberlin College. Paper read at the Chapel Hill meeting of the A. C. S., Division of Biochemistry, April 15, 1937.—(H. N. H.)

(1a) Research Assistant, Oberlin College, 1935–1937.

(2) Takahashi and Kawakami, *J. Chem. Soc. Japan*, **44**, 590 (1923).

(3) Takahashi, Nakamiya, Kawakami and Kitasato, *Sci. Papers Inst. Phys. Chem. Res.*, **3**, 81 (1925).

(4) Drummond, Channon and Coward, *Biochem. J.*, **19**, 1047 (1925).

(5) Hume and Chick, Medical Research Council Report IV. "The Standardisation and Estimation of Vitamin A," 1935.

(6) Carr and Jewell, *Nature*, **131**, 92 (1933).

(7) Karrer and Morf, *Helv. Chim. Acta*, **16**, 625 (1933).

(8) Euler, Karrer and Zubrys, *Helv. Chim. Acta*, **17**. 24 (1934).