these studies. The column and fraction collector were covered to exclude light; other operations were shielded insofar as possible. The optical density of samples at 300 mμ was determined in a Beckman DU spectrophotometer. Samples to be used later were neutralized to prevent decomposition. After each run the column was washed with about 200 ml. of 2 *N* hydrochloric acid, followed by water. The capacity of these columns for pterins has not been accurately determined, but it is much lower than their capacity for purines and pyrimidines.

Paper chromatography was carried out on strips of Whatman no. 1 paper in 1% aqueous dipotassium phosphate.[8]

To determine whether or not PGA was produced by deamination of Aminopterin during the ion-exchange procedure, combined fractions from the Aminopterin peak (285 ml.) were made alkaline with ammonium hydroxide and readsorbed by running through the column by gravity, in the dark. This required 28 hr. at room temperature. Elution in the same manner used previously gave only an Aminopterin peak, with a recovery of about 95% and no evidence of PGA.

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The Oxidation of Dialuric Acid by *o*-Iodosobenzoic Acid[1]

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The kinetics of oxidation of dialuric acid by oxygen has been studied by Hill[3] and by Richardson.[4] The dissociation constants and oxidation–reduction potentials of dialuric acid and ascorbic acid are of similar magnitude. Tautomeric formation of an enediol configuration is conceivable. In addition, the half-life of oxidized dialuric acid (alloxan)[5] is practically the same as the half-life of dehydroascorbic acid.[6] These similarities in properties suggested a study of the rate of oxidation of dialuric acid by *o*-iodosobenzoic acid to supplement work previously reported for ascorbic acid.[7]

Experimental

Dialuric acid was prepared by the method of Biltz and Damm.[8] General procedures for the preparation of reaction mixtures were the same as those previously described by us for the studies of oxidation of ascorbic acid. The rate of the reaction was followed by the spectrophotometric method using spirocyclohexylporphyrexide.[7,9] Solutions of dialuric acid were found to be too susceptible to oxidation by oxygen to permit use of the titrimetric procedure that utilizes porphyrindine.

Results and Discussion

Dialuric acid (DA) was found to be oxidized by *o*-iodosobenzoic acid (RIO) in a second-order process expressed by the differential equation

$$-\mathrm{d(DA)/dt} = k''\mathrm{(DA)(RIO)} \qquad (1)$$

The integrated form of equation 1 was used to evaluate k'' from the experimental data. Typical results are shown in Table I.

TABLE I

OXIDATION OF DIALURIC ACID BY *o*-IODOSOBENZOIC ACID

Temperature 15.0°

No.	Buffer	*p*H	(DA) × 10⁴, moles per l.	(RIO) × 10⁴, moles per l.	k'', l. mole^{-1} min.$^{-1}$
1	Phosphate, 0.10 *M*	7.05	6.91	9.43	47
2	Phosphate, .10 *M*	7.05	6.99	5.66	49
3	Phosphate, .10 *M*	7.05	13.69	9.43	46
4	Phosphate, .10 *M*	7.05	7.03	9.43	48
5[a]	Phosphate, .60 *M*	6.97	6.97	9.43	104
6[b]	Phosphate, .10 *M*	6.69	6.84	9.43	53
7	Phosphate, .10 *M*	6.04	6.91	9.43	47
8	Phosphate, .10 *M*	7.68	6.83	9.43	24
9[c]	Phosphate, .10 *M*	7.05	6.91	9.43	96
10[d]	Phosphate, .10 *M*	7.05	6.96	9.43	61
11	Veronal, 0.05 *M*	6.96	7.02	9.43	19
12[e]	Phosphate, 0.10 *M*	7.05	6.99	13.58	116

[a] Ionic strength, 1.44. [b] Ionic strength, 1.42 by addition of KCl. [c] $FeSO_4$, 1×10^{-6} *M*. [d] $CuSO_4$, 1×10^{-6} *M*. [e] Temperature, 25.0°.

In expt. 1–3, the concentrations of dialuric acid and *o*-iodosobenzoic acid were varied independently over a limited range owing to the low solubility of *o*-iodosobenzoic acid. The agreement of the values for k'' indicates that the reaction is first order with respect to each of the reactants. The value of k'' increased from 48 to 104 as the concentration of phosphate buffer was increased from 0.10 to 0.60 *M* (expt. 4 and 5). This increase in rate is not associated primarily with an increase in ionic strength since addition of potassium chloride to produce an equivalent ionic strength had no significant effect on the rate (expt. 6). In phosphate buffer, the rates were similar at *p*H 6 and 7 but decreased at *p*H 7.7 (expt. 7 and 8). In veronal buffer at *p*H 7 (expt. 11) the rate was much less than in phosphate buffer. No experiments were conducted in nonbuffered solutions. Iron was a more effective catalyst than copper (expt. 9 and 10). In 0.10 *M* phosphate buffer at *p*H 7.05, k'' increased from 48 to 116 liters mole^{-1} min.$^{-1}$ as the temperature was increased from 15 to 25° (expt. 12).

Analyses of solutions after reactions were completed indicated that, under all conditions studied, one mole of *o*-iodosobenzoic acid had been reduced for each mole of dialuric acid oxidized. There was no evidence of formation of any products from alloxan capable of reducing either iodine or *o*-iodosobenzoic acid.

These preliminary results suggest that the oxidation of dialuric acid by *o*-iodosobenzoic acid is similar in some respects to the oxidation of ascorbic acid. The principal reactions are second order; the rate is proportional to the concentration of buffer but is independent of the ionic strength; both reactions exhibit catalysis by copper and iron.

Points of difference also may be noted. Under the same conditions, dialuric acid is oxidized at a rate fifteen times that of ascorbic acid. No reducing substance is formed from oxidized dialuric acid. The catalytic effect of copper is much greater than

(1) One of several investigations supported in part by a research grant from the National Cancer Institute, National Institutes of Health, United States Public Health Service.

(2) Predoctorate Research Fellow of the National Institutes of Health, 1949–1950.

(3) E. S. Hill, *J. Biol. Chem.*, **85**, 713 (1930); **92**, 471 (1931).

(4) G. M. Richardson, *Biochem. J.*, **26**, 1959 (1932).

(5) J. W. Patterson, A. Lazarow and S. Levy, *J. Biol. Chem.*, **177**, 187 (1949).

(6) E. G. Ball, *ibid.*, **118**, 219 (1937).

(7) W. T. Caraway and L. Hellerman, THIS JOURNAL, **75**, 5334 (1953).

(8) H. Biltz and P. Damm, *Ber.*, **46**, 3662 (1913).

(9) C. C. Porter and L. Hellerman, THIS JOURNAL, **66**, 1652 (1944).

iron on the rate of oxidation of ascorbic acid but with dialuric acid the effect is reversed. These observations are consistent with the suggestion that copper acts more effectively than iron as a catalyst for the oxidation of the enediol configuration (ascorbic acid); conversely, iron may act more effectively as a catalyst for the oxidation of the α-keto-hydroxy configuration (dialuric acid).

Levy[10] has concluded that ascorbic acid exists in its ketonic form in acid solution and Huelin and Stephens[11] have observed that the relative catalytic effects of copper and iron on the oxidation of ascorbic acid are reversed as the *p*H of the solution is decreased from 3.0 to 0.4. The specificity of copper as a catalyst for the enediol group has been discussed by Dodds.[12]

(10) L. F. Levy, *Nature*, **152**, 693 (1943).
(11) F. E. Huelin and I. M. Stephens, *ibid.*, **158**, 703 (1946).
(12) M. L. Dodds, *Arch. Biochem.*, **18**, 51 (1948).

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A New Synthesis of 4a(H)-Dodecahydrobenzo(c)-quinolizine

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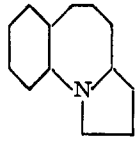
Of the four racemic modifications possible in 4a-(H)-dodecahydrobenzo(c)quinolizine, Clemo and co-workers[2] have reported the preparation of two, "A" and "B." Subsequent work by Leonard and Wildman[3] has shown that the precursor to Clemo's compound "A," 1-keto-6,7-hexahydrobenzoquinolizidine, had undergone disproportionation of two rings during a Clemmensen reduction to give a structure shown by I.



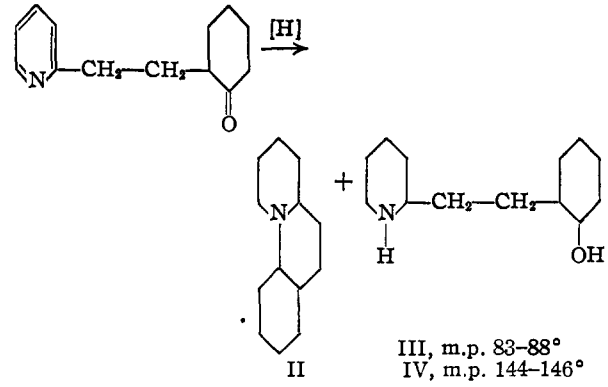
I

Our compound has physical constants differing from those of Clemo's compound "B" and is, therefore, presumed to be a heretofore unknown racemate.

Results and Discussion

Reduction of 2-(2-ethylpyridyl)-cyclohexanone in aqueous HCl over PtO_2 gave three products, of which one IV, was formed to the extent of about one per cent. under all temperatures employed. The ratio of II and III formed was found to be a function of the temperature, with II predominating at higher temperatures at the expense of III.

The uncyclized alcohol III could not be dehydrated to give the cyclic 4a(H)-dodecahydrobenzo-(c)quinolizine (II) when subjected to the same conditions prevalent at the formation of II. It is possible that this racemic form of the alcohol III is not an intermediate in the formation of II.



II III, m.p. 83–88°
IV, m.p. 144–146°

Reaction of II with methyl iodide gave two methiodides which could not be completely separated by fractional crystallization. The partially separated fractions had melting points of 185–186° and 242–249° and both analyzed for the quaternary ammonium compound expected from 4a(H)-dodecahydrobenzo(c)quinolizine and one equivalent of methyl iodide. The fact that II forms a single, constant-melting picrate is taken as proof that it is a single racemic modification; the two methiodides represent a pair of diastereoisomers which owe their existence to the asymmetric nitrogen atom.

Experimental Part

2-(2-Ethylpyridyl)-cyclohexanone was prepared by the procedure of Levine and Wilt[4] in 38% yield, b.p. 137–143° (1 mm.), $n^{20}D$ 1.5311.

2-(2-Ethylpiperidyl)-cyclohexanol (IV).—Fifty-one grams (0.25 mole) of 2-(2-ethylpyridyl)-cyclohexanone was reduced in aqueous HCl at 55–60° over one gram of PtO_2 at 1500 p.s.i. of hydrogen. The theoretical uptake of hydrogen (4 equivalents) required 5 hours. The solution was decanted from the catalyst, made basic with aqueous alkali, and extracted with benzene. The benzene was evaporated, ether was added, and the mixture kept at 0° for 2 days. A precipitate of white crystals was filtered off (1.5 g., m.p. 107–112°) and recrystallized twice from ether, giving 0.52 g. of fine white needles, m.p. 144–146°.

Anal. Calcd. for $C_{13}H_{25}NO$: C, 73.88; H, 11.92; N, 6.63. Found: C, 73.70; H, 11.54; N, 6.82.

The *p*-nitrobenzoate (N-*p*-nitrobenzoyl), prepared in the usual manner, crystallized from ethanol in yellow prisms, m.p. 144–146°. An intimate mixture of it and the starting alcohol IV melted at 132–140°.

Anal. Calcd. for $C_{27}H_{31}N_3O_7$: C, 63.64; H, 6.13; N, 8.25. Found: C, 63.44; H, 6.03; N, 8.31.

4a(H)-Dodecahydrobenzo(c)quinolizine (II).—The ether filtrate from the filtration of IV above was evaporated and distilled, giving 38 g. (79%) of colorless liquid, b.p. 113–115° (1.5 mm.) and a small amount of higher boiling material, subsequently identified as compound III. Redistillation gave 30 g. of colorless liquid, b.p. 71–74° (0.5 mm.), $n^{24}D$ 1.5080.

Anal. Calcd. for $C_{13}H_{23}N$: C, 80.76; H, 11.99; N, 7.25. Found: C, 80.52; H, 12.25; N, 7.38.

The picrate, prepared in ether, crystallized from an ethanol–water mixture in fine yellow crystals, m.p. 178–180°.

Anal. Calcd. for $C_{19}H_{26}N_4O_7$: C, 54.02; H, 6.20; N, 13.26. Found: C, 54.45; H, 6.03; N, 13.67.

The methiodides were prepared by refluxing the base with excess methyl iodide in ethyl acetate for 0.5 hour. Repeated fractional crystallization from methanol–acetone mixtures gave two fractions which could not be purified to constant melting points.

Anal. Calcd. for $C_{14}H_{26}NI$: C, 50.15; H, 7.81; N, 4.18. Found: fraction a, m.p. 242–249°: C, 49.73; H, 7.58; N,

(1) Abstracted from a thesis submitted by Warren E. Solodar in partial fulfillment of the Master of Science degree, Stevens Institute of Technology.
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