trum between 220 and 600 mμ was read in a DU Beckman spectrophotometer.

DEPARTMENT OF RADIATION BIOLOGY AND
   DEPARTMENT OF BIOCHEMISTRY
SCHOOL OF MEDICINE AND DENTISTRY
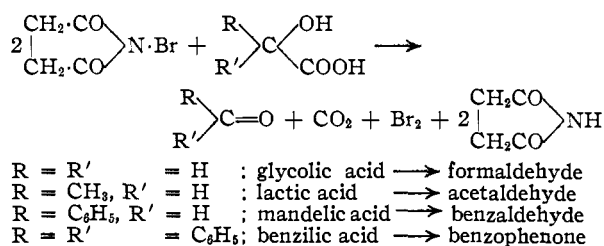UNIVERSITY OF ROCHESTER
ROCHESTER, NEW YORK

Action of N-Bromosuccinimide on Aliphatic α-Hydroxy Acids

BY MOHAMED ZAKI BARAKAT AND MOHAMED FATHY ABD EL-WAHAB

It has already been shown that N-bromosuccinimide functions as an oxidizing agent, e.g., it converts primary and secondary alcohols into the corresponding aldehydes and ketones, respectively,[1,2] and in many cases the action is highly selective. Fieser and Rajagopalan have reported high selectivity in the oxidation of the 7α-hydroxyl group of cholic acid and the 6β-hydroxyl group of cholestane-3β,5α,6β-triol by use of N-bromosuccinimide. Whereas 3-hydroxyl groups usually resist attack by N-bromosuccinimide in aqueous acetone, methyl 3α-hydroxy-9α,11α-oxidocholanate[3] is oxidized to the 3-ketone. Selective oxidation of a 3-acyl derivative of methyl cholate[4] to the 7-ketone can be accomplished in high yield with N-bromosuccinimide.

There appears to have been no report on the action of this reagent with α-hydroxy acids. We have shown that N-bromosuccinimide reacts readily on heating in an aqueous solution with aliphatic α-hydroxy acids, e.g., glycolic, lactic, mandelic and benzilic acids, yielding aldehydes or ketones containing one carbon atom less, e.g., formaldehyde, acetaldehyde, benzaldehyde and benzophenone, respectively. Evolution of carbon dioxide and bromine was demonstrated in all cases. Succinimide has been isolated in the reaction with lactic and benzilic acids.

$$2\ \substack{CH_2 \cdot CO \\ | \\ CH_2 \cdot CO} \Big\rangle N \cdot Br + \substack{R \\ R'} \Big\rangle C \Big\langle \substack{OH \\ COOH} \longrightarrow$$

$$\substack{R \\ R'} \Big\rangle C = O + CO_2 + Br_2 + 2\ \substack{CH_2CO \\ | \\ CH_2CO} \Big\rangle NH$$

R = R'   = H   : glycolic acid ⟶ formaldehyde
R = CH₃, R' = H   : lactic acid   ⟶ acetaldehyde
R = C₆H₅, R' = H   : mandelic acid ⟶ benzaldehyde
R = R'   = C₆H₅; benzilic acid ⟶ benzophenone

Compared with the fatty acids the corresponding α-hydroxy acids possess higher dissociation constants, and this may explain why such a reaction takes place; an analogous case may be the degradation of aliphatic dicarboxylic acids, e.g., oxalic acid,[5] by N-bromosuccinimide in aqueous medium at room temperature.

The conversion of benzilic acid to benzophenone

(1) L. F. Fieser and S. Rajagopalan. THIS JOURNAL. 71, 3935 (1949); ibid., 71, 3938 (1949).

(2) M. Z. Barakat and G. M. Mousa, J. Pharm. and Pharmacol., 4, 115 (1952).

(3) L. F. Fieser, H. Heymann and S. Rajagopalan, THIS JOURNAL, 72, 2306 (1950).

(4) L. F. Fieser and S. Rajagopalan, ibid., 72, 5530 (1950).

(5) M. Z. Barakat, J. Pharm. and Pharmacol., 4, 889 (1952).

when treated with N-bromosuccinimide provides a new route to pass from α-diketones, e.g., benzil, to aromatic ketones, e.g., benzophenone.

Experimental

Action of N-Bromosuccinimide on Aliphatic α-Hydroxy Acids. (1) Isolation of Aldehydes. (a).—N-Bromosuccinimide (1.78 g., 2 moles) and glycollic acid (0.38 g., 1 mole) or lactic acid (0.43 cc., 1 mole) or mandelic acid (0.76 g., 1 mole) in distilled water (20 cc.) were refluxed in the apparatus previously described (Schonberg, Moubasher and Mostafa[6]) in a stream of carbon dioxide for 20 minutes. The receiver contained an ice-cold solution of 2,4-dinitrophenylhydrazine sulfate (0.6 g.) in alcohol (20 cc.). Yellow or orange crystals deposited and were recrystallized from the proper solvent (ligroin, alcohol and ethyl acetate) to give the 2,4-dinitrophenylhydrazone of formaldehyde, acetaldehyde and benzaldehyde, respectively, in 50% yields, identified by their m.p. and mixed m.p. with authentic samples.

(b) Formation of Bromine, Carbon Dioxide and Succinimide in the Degradation.—N-Bromosuccinimide (1.78 g.) and lactic acid (0.43 cc.) in distilled water (20 cc.) were heated for 20 minutes; the mixture was then concentrated by heat to a small volume (about 2 cc.) and allowed to cool; the colorless crystals which deposited were pressed on a porous plate and recrystallized from benzene. They were proved to be succinimide by m.p. and mixed m.p. (yield 0.5 g.).

The evolution of bromine and carbon dioxide during the degradation was demonstrated by passing the gases evolved during the reaction, first into 10% silver nitrate solution acidified with nitric acid and then into baryta water. A yellowish-white precipitate of silver bromide deposited, while the baryta water became turbid.

(2) Isolation of Ketones.—It is sufficient to describe one example in detail to illustrate the procedure.

N-Bromosuccinimide (1.78 g., 2 moles) and benzilic acid (1.14 g., 1 mole) in distilled water (100 cc.) were refluxed for 30 minutes. The reaction started after heating for 2 minutes with evolution of bromine vapor. The N-bromosuccinimide and benzilic acid gradually dissolved and an oil began to separate. At the end of the reaction, the mixture was allowed to cool and extracted with ether. The aqueous layer was concentrated to a small volume (about 5 cc.) and on standing deposited colorless crystals of succinimide, which after recrystallization from benzene were identified by m.p. and mixed m.p. (yield 0.6 g.).

The ethereal layer was dried over anhydrous sodium sulfate for 12 hours, filtered and concentrated to yield an oil which soon crystallized. The solid was recrystallized from aqueous alcohol to give benzophenone (m.p. and mixed m.p.) in 85–90% yield. The evolution of carbon dioxide during the reaction was demonstrated as above.

Acknowledgment.—The authors thank Dr. M. M. El-Sadr for his interest in this work and acknowledge their gratitude to the National Aniline Division, New York 6, New York, for supplying N-bromosuccinimide.

(6) Schönberg, Moubasher and Mostafa, J. Chem. Soc., 176 (1948).

BIOCHEMISTRY DEPARTMENT
IBRAHIM PASHA EL-KEBIR UNIVERSITY
FACULTY OF MEDICINE
ABBASSIA, CAIRO, EGYPT

The High Field Conductance of Aqueous Solutions of Ammonia at 25°[1]

BY DANIEL BERG AND ANDREW PATTERSON, JR.

The high field conductance of aqueous solutions of ammonia, between 1.3 and 1.5 × 10⁻³ M, has been measured at 25.00° relative to potassium chloride. The high field conductance data are
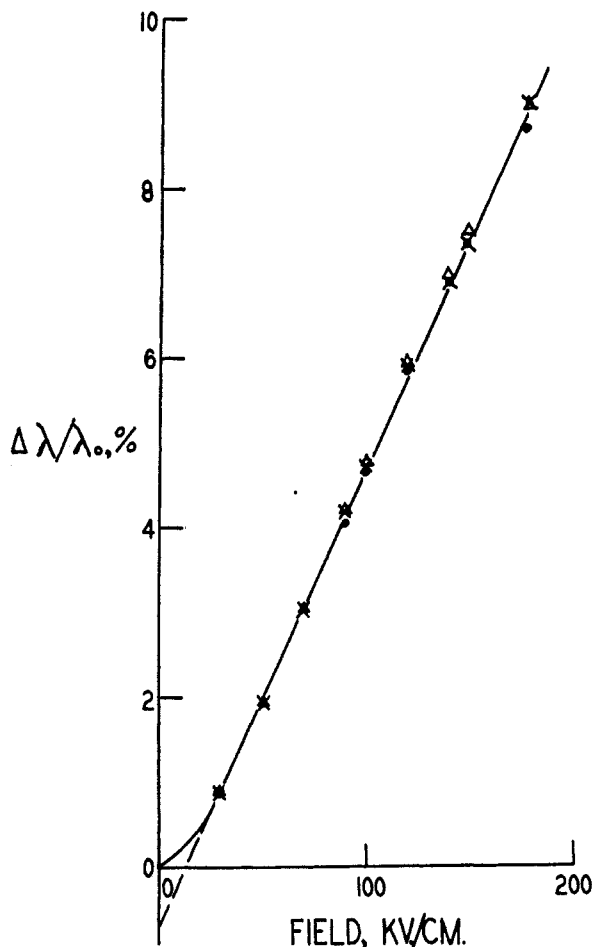
Fig. 1.—The high field conductance of ammonium hydroxide relative to potassium chloride at 25.00 ± 0.015°. The triangles refer to a 1.363 × 10⁻³ M ammonium hydroxide solution, the crosses to a 1.529 × 10⁻³ M ammonium hydroxide solution, and the dots to a 1.354 × 10⁻³ M ammonium hydroxide solution. The concentration of the potassium chloride reference solution was 3 × 10⁻⁴ M.

presented together with a calculation and discussion of the true ionization constant of the ammonium hydroxide in the solution.

Both low and high field conductance measurements were carried out according to the procedure of Gledhill and Patterson[2] using a differential pulse transformer bridge circuit. The ammonium hydroxide solutions were prepared in the conductance cell by addition of strong stock solution to degassed conductivity water in the cell until a desired resistance was reached. The concentration of the cell solution was then known from the weights of material used. The concentration of the strong stock solution was determined by titrating measured portions of the solution with standard hydrochloric acid using chlor phenol red as indicator. The concentration of the stock solution was 0.0431 M. All measurements were made with four microsecond pulse duration. The potassium chloride reference solution was prepared from a strong stock solution as described in reference 2. The concentration of the reference solution was 3 × 10⁻⁴ M. The temperature of measurement was

(2) J. A. Gledhill and A. Patterson, J. Phys. Chem., 56, 999 (1952).
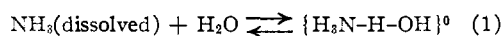
25.00 ± 0.015°, referred to a recently calibrated platinum resistance thermometer. Every precaution was taken to avoid introduction of carbonates, silicates or acidic contaminants with the stock solution or during the measurements. The measurements were made, however, in Pyrex glass cells.

The results are shown in Fig. 1. The dots refer to 1.354, the triangles to 1.363, and the crosses to 1.529 × 10⁻³ M solutions of ammonia in water. At 200 kv./cm. the fractional high field conductance quotient is 10.0%. The intercept of the extrapolated curve is −0.68%.

Ammonia is the first base for which we have reported measurements. There is no evidence of difficulty with the conductance measurements due to reaction of the ammonia with the Pyrex glass from which the conductance cells are constructed, when using solutions of 10⁻³ M concentration over a period of several days to a week. It is also notable that it was not found necessary or desirable to use a solution of a strong base as reference electrolyte; potassium chloride was entirely satisfactory. This is in contrast with our measurements on acetic acid[3] and with carbon dioxide,[4] for both of which it was necessary to use hydrochloric acid as reference electrolyte to make impedance balancing possible. In spite of the apparent cancellation of differential polarization between measured and reference electrolytes through the use of an acid for reference, with both acetic and carbonic acids at higher fields there was a downward curvature of the high field conductance quotient vs. field plot which has been explained[3] in terms of polarization due almost entirely to the hydrogen ion, and to its effect on the high field gradient to which the measured and reference electrolytes are subjected. Any such effects as these just mentioned with acids were absent with ammonium hydroxide in the range of concentrations and at the one temperature studied.

The curve of Fig. 1 is almost identical with that for the experimental results of reference 3 at 25°. The ionization constants reported for acetic acid, 1.754 × 10⁻⁵, and for ammonia, 1.774 × 10⁻⁵, are almost identical.[5,6] However, the concentration of ammonia, 1.4 × 10⁻³ M, is appreciably higher than that of acetic acid, 7 × 10⁻⁴ M, required to obtain the same conductivity; this would be expected since the limiting molecular conductance of acetic acid is appreciably higher than that of ammonia. The curve of Fig. 1 is quite different from that for carbon dioxide solutions, reference 4; the Δλ/λ₀ vs. field plot for carbon dioxide solutions has a smaller slope in keeping with the larger true ionization constant of carbonic acid.

With ammonia solutions in water it is thought that there is a set of equilibria of the type

$$NH_3(\text{dissolved}) + H_2O \rightleftharpoons \{H_3N\text{-}H\text{-}OH\}^0 \quad (1)$$

$$\{H_3N\text{-}H\text{-}OH\}^0 \rightleftharpoons NH_4^+ + OH^- \quad (2)$$

(3) F. E. Bailey and A. Patterson, THIS JOURNAL, 74, 4756 (1952).

(4) D. Berg and A. Patterson, ibid., 75, 5197 (1953).

(5) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 2nd ed., Reinhold Publishing Corp., New York, N. Y., 1950.

(6) R. G. Bates and G. D. Pinching, THIS JOURNAL, 72, 1393 (1950).

The formula $\{H_3N-H-OH\}^0$ has been written to suggest that an un-ionized molecular entity similar to an ion pair exists in solution; this entity arises from the presumed hydrogen bonding between the nitrogen and oxygen atoms from the ammonia and water molecules. While this series of equilibria is reminiscent of a similar set for carbon dioxide and water, reference 4, there is no evidence and little reason to believe that with ammonia any of these steps is slow. Indeed, reaction 1 is undoubtedly a fast one to which only a Langevin time lag could be attributed; the same is true of reaction 2. Nevertheless, and presumably this may be attributed to the enhanced stability conferred by the hydrogen bond, there is a finite concentration of dissolved but unhydrated ammonia in the solution together with an amount of undissociated ammonium hydroxide, and, finally, an equilibrium concentration of ions, as is represented by equations 1 and 2. These facts were clearly established by Moore and Winmill,[7] from whose work on ammonia and alkyl substituted amine solutions the hydrogen bond concept arose.

The ratio of the concentrations of ammonia and undissociated ammonium hydroxide, equation 1, is independent of concentration if the concentrations be small, and may be termed B.

$$B = [NH_3]/[NH_4OH] \qquad (3)$$

The value of B may be determined by measuring at at least three temperatures the ionization and the partition of ammonia between water and an immiscible solvent such as toluene. Moore and Winmill[7] thus determined how much of the undissociated ammonia in solution was present as dissolved ammonia and how much as ammonium hydroxide. Sidgwick[8] states that at 25° the value of B is 0.885. Using the relations

$$K(0)(\text{true}) = [NH_4{}^+][OH^-]/[NH_4OH] \qquad (4)$$

$$K(0)(\text{apparent}) = \frac{[NH_4{}^+][OH^-]}{[NH_4OH] + [NH_3]} = \frac{[NH_4{}^+][OH^-]}{[NH_4OH](1 + B)} \qquad (5)$$

$$= K(0)(\text{true})/(1 + B) \qquad (6)$$

and the value of $K(0)$ (apparent) at 25° from reference 6, we find that the method of Moore and Winmill gives $K(0)$ (true) $= 3.34 \times 10^{-5}$, while Moore and Winmill themselves report $K(0)$ (true) at 20° from their measurements to be $5.2 \pm 1.3 \times 10^{-5}$. Bates and Pinching[6] give the values of $K(0)$ (apparent) at 20° and at 25° as 1.710 and 1.774×10^{-5}. The difference between the computed quantity 3.34×10^{-5} and Moore and Winmill's value of $5.2 \pm 1.3 \times 10^{-5}$ is thus too large to be accounted for by the limits of error specified, and is in the wrong direction as a function of temperature, unless other unaccounted potent factors affecting the true ionization constant in a different manner than the apparent constant were present.

We may employ the method described in reference 4 to obtain $K(0)$ (true) from the high field conductance data. From a series of plots of the coefficients A_2 and A_3 against b (see eq. 21, ref. 4),

the reduced slope Q and the function ρ_0 (eq. 17, ref. 4) are obtained. From the data presented in Fig. 1 ρ_0 has the average value 0.1639, and, with the value for $K(0)$ (apparent) $= 1.77 \times 10^{-5}$, $K(0)$ (true) is found to be $6.3 \pm 0.5 \times 10^{-5}$. The precision of measurement is at least ten times poorer than for carbon dioxide, as may be seen from the discussion of errors in ref. 4. This value for $K(0)$ (true) must be compared with those in the paragraph above; it is larger than any of the values of $K(0)$ (true) either quoted or computed from other data. A larger $K(0)$ bespeaks a smaller Wien effect. It is indeed possible that electrolytic impurities might cause the measurements here reported to be low, although every precaution has been taken to exclude contaminants. It seems more probable that the rapidity of reactions 1 and 2 would give a high field conductance increase larger than would truly represent the equilibrium concentrations of the several ionic species at low fields and thus a $K(0)$ (true) which is too small. This possibility hinges upon the relative speeds of reactions 1 and 2, direct information on which is not available. Experiments at a variety of pulse lengths might help elucidate this point. It is planned to extend these measurements to a range of temperatures for which values of $K(0)$ (apparent) are available in order to test the internal consistency of data derived from the high field conductance measurements.

Thiohydantoins of Amino Acids[1]

BY E. CAMPAIGNE AND WESLEY L. ARCHER[2]

Recent reports[3] have shown that some thiosemicarbazones have an inhibitory effect on the growth of vaccinia viruses. Thompson and Wilkin have shown that the phenylalanine antagonist, β-2-thienylalanine, prevented the multiplication of vaccinia virus in chick embryonic tissues.[4] 5-Substituted thiohydantoins possess certain structural similarities to both of these types of virus inhibitors, having an α-aminocarbonyl and an N-substituted thiourea portion in the same molecule. In pursuing a program of virus chemotherapy[5] we have synthesized a number of thiohydantoins in order to determine whether these compounds had antiviral activity. Tests conducted by Dr. R. L. Thompson at the Indiana University Medical

(7) T. Moore and T. Winmill, *J. Chem. Soc.*, **101**, 1635 (1912); also, **91**, 1373 (1907).

(8) N. V. Sidgwick. "The Chemical Elements and their Compounds," Oxford University Press. New York. N. Y., 1950. p. 660.

(1) Contribution No. 587 from the Chemistry Laboratory of Indiana University. This work was supported by a contract between the Office of Naval Research, Department of the Navy, and Indiana University.

(2) Abstracted from the thesis of Wesley L. Archer, to be submitted to Indiana University in partial fulfillment for the Degree of Doctor of Philosophy.

(3) *Cf.* R. L. Thompson, S. A. Minton, Jr., J. E. Officer and G. A. Hitchings, *J. Immunology*, **70**, 229 (1953); D. Hamre, J. Bernstein and R. Donovick, *Proc. Soc. Exp. Biol. Med.*, **73**, 275 (1950).

(4) R. L. Thompson and M. L. Wilkin, *ibid.*, **68**, 434 (1948).

(5) *Cf.* E. Campaigne, *et al.*, THIS JOURNAL, **75**, 988 (1953).