

Essentially the same sequence of rates and spectral changes can be observed in the reaction of I with the bacterial protease subtilisin, a protein which differs from chymotrypsin in secondary and tertiary structure and in sequence about the reactive serine.⁵ The pH dependence of the rates in the subtilisin reaction are as in Figure 1, although the pK_A' is somewhat higher.

Since at low pH the deacylation rate of indoleacryloyl-chymotrypsin is significantly slower than the preceding acylation reaction, it is possible to measure the optical density of the acyl intermediate at any pH by the rapid addition of concentrated buffer to a low pH solution of this intermediate. The optical density at 380 $m\mu$ is pH dependent and is consistent, over the range pH 4.0–9.8, with that predicted for a weak acid acyl-enzyme intermediate with a single pK_A' of 7.68. This pK_A' is the same as the apparent pK_A' calculated for activation of the "deacylation" process (Figure 1).

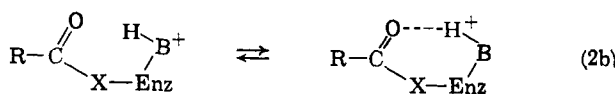
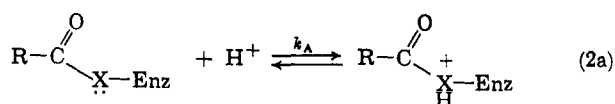
The existence of spectrophotometrically distinct acyl-enzyme acid–base conjugates (Table I) has been demonstrated. These conjugates might arise either

Table I. Spectral Properties of Indoleacryloyl Chymotrypsin

λ , $m\mu$	$\epsilon \times 10^4$, $cm^{-1} M^{-1}$	
	pH 4.0	pH 9.5
400	0.30	0.18
390	0.69	0.38
380	1.20	0.72
370	1.65	1.17
360	1.84 ^a	1.56
353	1.78	1.63 ^a
340	1.40	1.38
330	1.01	1.04
320	0.68	0.76

^a λ_{max} .

from chemical coupling of the indoleacryloyl resonance chain with a proton (eq. 2) where pK_A or $pK_{BH^+} = 7.7$, or from a perturbation of the (solvent) environment of the chromophore upon protonation of a basic substituent of the protein. Equation 2a is incompatible



with the (presumed) acyl–serine ester linkage. In this regard, the dissimilarity of the spectrum of either of the acyl-enzyme conjugates with that of model acyl–serine peptide esters⁶ (λ_{max} 330–335 $m\mu$) should be noted.

N-(Indole-3-acryloyl)imidazole was prepared from the recrystallized carboxylic acid and imidazole *via* the mixed anhydride method. It was recrystallized three times from benzene, m.p. 190°. *Anal.* Calcd. for $C_{14}H_{10}ON_3$: C, 71.0; H, 6.8; O, 4.2; N, 17.9. Found: C, 70.7; H, 7.0; O, 4.4; N, 18.0.

A prior preparation from the carboxylic acid, imidazole and dicyclohexylcarbodiimide yielded a

(5) F. Sanger and D. C. Shaw, *Nature*, **197**, 872 (1960).

(6) S. A. Bernhard, S. J. Lau, and H. Noller, *Biochemistry*, in press.

product (II, m.p. 182°) with identical elemental analysis and saponification equivalent. With this latter product, three steps in the reaction pathway could be detected kinetically, although the acyl-enzyme intermediate (following the first two steps) was kinetically and spectrophotometrically identical with that described herein. The extra step in the reaction sequence with II could be abolished, by incubation of the substrate in buffer, at a rate equal to its own rate of spectrophotometric change in the presence of buffered enzyme, as was pointed out to us by Professor M. Bender. Surprisingly, only a single hydrolytic step is observable in the "uncatalyzed" reaction of II in buffer, and this rate is identical with that obtained with the presumably clean product (I). We are indebted to Professor Bender for informing us of his observations prior to publication.

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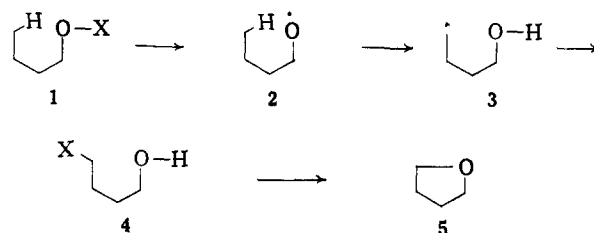
Received August 5, 1964

Silver-Catalyzed Decomposition of Hypobromites

Sir:

Recently a number of closely related methods for the *in situ* generation of hypohalites have been described and their reactions extensively studied.¹ Hypohalites can decompose in either an ionic or a radical manner, depending on experimental conditions.^{1b} It has generally been recognized that the transformation of hypohalites (of type I) to oxides (of type 5) takes place through free-radical intermediates.¹ This is represented in Scheme I.

Scheme I


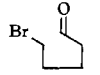



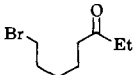
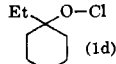
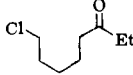


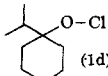

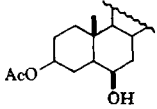
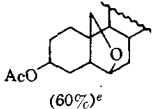
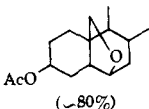


In a recent communication, Sneen and Matheny² have claimed that, in the presence of silver acetate (or silver oxide) and bromine, alcohols are transformed into oxides by an ionic mechanism. This transformation has been represented as

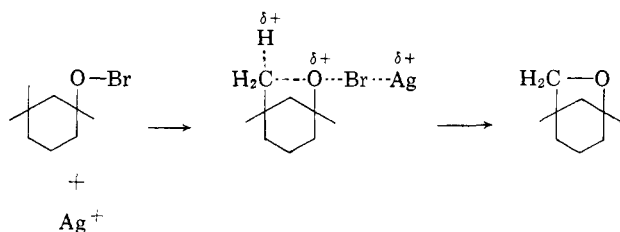
(1) (a) M. Akhtar and D. H. R. Barton, *J. Am. Chem. Soc.*, **83**, 2213 (1961); (b) M. Akhtar and D. H. R. Barton, *ibid.*, **86**, 1528 (1964); (c) C. Walling and A. Padwa, *ibid.*, **83**, 2207 (1961); (d) F. D. Greene, M. L. Savitz, H. H. Lau, D. Osterholz, and W. N. Smith, *ibid.*, **83**, 2196 (1961); F. D. Greene, M. L. Savitz, H. H. Lau, D. Osterholz, W. N. Smith, and P. M. Zanet, *J. Org. Chem.*, **28**, 55, (1963); (e) Ch. Meystre, K. Heusler, J. Kalvoda, and P. Wieland, *Experientia*, **17**, 475 (1961); *Helv. Chim. Acta*, **45**, 1317 (1962), and later papers. The work of the Ciba group is adequately reviewed: K. Heusler and J. Kalvoda, *Angew. Chem., Intern. Ed. Engl.*, **3**, 525 (1964), and M. Akhtar, *Advan. Photochem.*, **2**, 263 (1964).

(2) R. A. Sneen and N. P. Matheny, *J. Am. Chem. Soc.*, **86**, 3905 (1964); **86**, 5503 (1964).

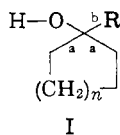
Table I

ROH	Main product ^a	Ratio, AgOAc:Br ₂	Analogous reaction	Main product
	 (14%) ^{b-d,f}	1.25:1.1	(i) Photolytic cleavage with mercuric oxide and iodine ^{1b} (ii) Photolytic decomposition of cyclopentyl nitrite in CBrCl ₃ ⁶	 
	 (24%) ^{b-d}	4:3.75	Photolytic decomposition of  (1d)	 (68%)
	 (38%) ^{c,d,g}	4:3.75	Photolytic decomposition of  (1d)	 (66%)
	 (60%) ^c	12.5:4	Photolysis with mercuric oxide and iodine ^{1b}	 (~80%)

^a From reaction of alcohol with AgOAc and Br₂. ^b Isolated as 2,4-dinitrophenylhydrazone; yield based on starting material. (Note: under identical conditions the 2,4-DNP of *n*-butyaldehyde was obtained in 38% yield.) ^c Reaction in carbon tetrachloride at room temperature. ^d Only a slight variation in percentage conversion over the range 5–40°. ^e Reaction in petroleum ether at 40°. ^f Formation of cyclopentanone less than 5% of δ -bromovaleraldehyde. ^g Yield estimated by g.l.c. and based on alcohol consumed.

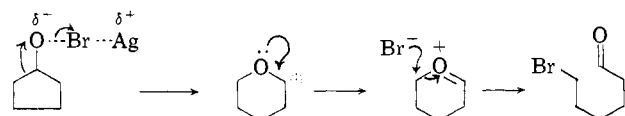


In this communication we wish to report the course of reactions of four alcohols (I: $n = 1$, R = H; $n = 2$, R = ethyl, isopropyl; and 3 β -acetoxycholestan-6 β -ol) with silver acetate and bromine in the dark.



These alcohols were specially chosen because the alkoxy radical derived from each of them is known to show a characteristic mode of decomposition. Thus cyclopentanol ($n = 1$, R = H)³ and 1-ethylcyclohexanol ($n = 2$, R = ethyl) with silver acetate and bromine, in the dark, gave δ -bromovaleraldehyde³⁻⁵ and 8-bromo-3-octanone,⁴ respectively, as the main products. A similar preference for the cleavage of bond "a" of cyclopentoxyl^{1b} and ethylcyclohexoxyl radicals^{1d} has

(3) The possibility that δ -bromovaleraldehyde might have originated through an ionic mechanism as follows

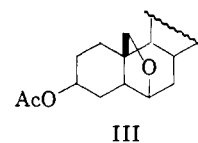
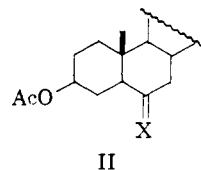


has been eliminated. The action of hydrogen bromide on dihydropyran gave no detectable amount of carbonyl compound. Cf. R. Paul, *Bull. soc. chim. France*, [5] 1, 1397 (1934).

(4) Satisfactory analyses of these compounds have been obtained.

(5) M. Akhtar, D. H. R. Barton, and P. G. Sammes, *J. Am. Chem. Soc.*, 86, 3394 (1964).

been observed before. Isopropylcyclohexanol ($n = 2$, R = isopropyl) with silver acetate and bromine gave cyclohexanone as the main product. This behavior parallels the decomposition of the isopropylcyclohexoxyl radical,^{1d} i.e., the cleavage of bond "b" is favored.



The hypohalites corresponding to alcohol II (X = —OH, —H) are known to undergo both ionic and free-radical decomposition.^{1b,e} The product from the former type of decomposition is the 6-ketone (X = O) and from the latter the 6 β -oxide (III). On treatment with silver acetate and bromine in the dark at 19° the alcohol (II) gave a mixture of the 6-ketone (X = O) and the oxide (III), favoring the oxide. However, at 40° the only product obtained (60% yield) from this reaction was the oxide (III).

These observations lead us to conclude that the reaction of silver acetate and bromine on alcohols results in the formation of hypobromites⁶ which then yield products normally expected from free-radical decomposition.⁷

From this work it is not possible to ascribe th favorable catalytic role to a particular silver species.⁸

Acknowledgments. We thank Professor K. A. Munday for his kind interest and encouragement.

(6) The formation of hypobromites with these reagents is implied by previous workers (see ref. 1e, and C. Walling and A. Padwa, *J. Org. Chem.*, 27, 2976 (1962)).

(7) We may, however, point out that thermal and photolytic decomposition of hypohalites results in higher yields of products; see ref. 1.

(8) It is interesting to note that in the case of alcohol II (X = —OH, —H) where, at room temperature, ionic decomposition of the intermediate hypobromite competes with the formation of the oxide (III), pretreatment of silver acetate with ultraviolet light depresses the ketone formation and improves the oxide formation.

We gratefully acknowledge a research grant from the Department of Scientific and Industrial Research for the purchase of an infrared spectrophotometer. P. B. D. thanks the Medical Research Council for a research studentship.

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 Received January 11, 1965

Photoregeneration of Faded Alkali Metal Solutions¹

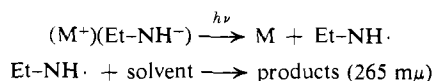
Sir:

Solutions of alkali metals in ammonia or amines decompose to form the corresponding amides. We have found that illumination of such faded solutions regenerates the metal or solvated electron components characteristic of the original metal-solvent combination. In addition to its general interest in connection with the fundamental properties of these systems, the regeneration reaction affords a convenient method for the preparation of metal solutions of controlled concentration, particularly in amine solvents.

In ethylamine solution, the absorption spectra of potassium or rubidium exhibit two bands near 650 and 850 m μ ,^{2,3} which we have previously shown to be due, respectively, to monomer and dimer species.² The 650-m μ band of potassium is associated with an e.s.r. spectrum having a characteristic hyperfine pattern.² Illumination of faded potassium or rubidium ethylamine solutions in the amide absorption region (~ 315 m μ) regenerates the original optical bands, as shown in Figure 1a,b. The e.s.r. patterns of the regenerated potassium solutions are likewise identical with the original spectra, and the fading rates are also similar. Repeating the cycle of fading and photorecovery gradually develops a strong absorption at 265 m μ , presumably due to some irreversible reaction associated with the process. This absorption does not correspond to *sym*-diethylhydrazine.

In ammonia, the photoregeneration of potassium solutions shows two phases, which are conveniently studied by flash technique, using apparatus previously described.⁴ Flashing potassium amide solutions in ammonia, in the region 240-390 m μ , produces an infrared transient whose absorption spectrum (at least as far as 1000 m μ) closely resembles the solvated electron band of metal-ammonia systems.⁵ Most of this transient disappears rapidly ($\tau \approx 40$ μ sec.), but a residual absorption, having the same spectrum, persists for as long as a minute (Figure 1c).

Under the conditions of these experiments, the metal amides in ethylamine solution are probably highly ion-paired, and it appears that the relevant reactions are



(1) This work was supported by a grant from the U. S. Atomic Energy Commission to Brandeis University (Grant No. AT(30-1)-2003).

(2) M. Ottolenghi, K. Bar-Eli, H. Linschitz, and T. Tuttle, *J. Chem. Phys.*, **40**, 3729 (1964).

(3) R. R. Dewald and J. L. Dye, *J. Phys. Chem.*, **68**, 121 (1964).

(4) H. Linschitz, C. Steel, and J. A. Bell, *ibid.*, **66**, 2574 (1962).

(5) "Metal-Ammonia Solutions," G. Lepoutre and M. J. Sienko, Ed., Benjamin Publishing Co., New York, N. Y., 1963.

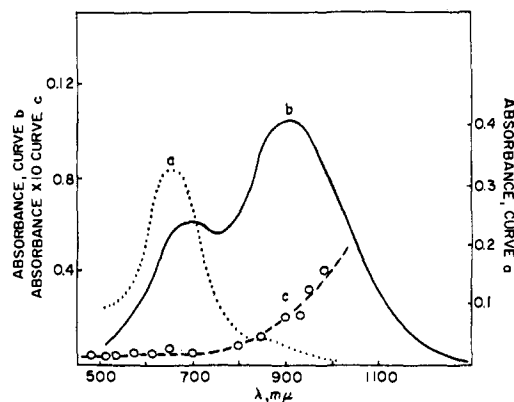


Figure 1. Photoregeneration of decomposed metal solutions (Hanovia Type HS (150 w.) quartz mercury arc, 10 cm. from sample): (a) absorption spectrum of decomposed potassium ethylamine solution (amide $\sim 10^{-3}$ M), 5-min. irradiation, 1-cm. Pyrex cell, -78° ; (b) decomposed rubidium ethylamine solution (amide $\sim 10^{-3}$ M), 7-min. irradiation, 1-cm. quartz cell, room temperature; (c) flash transient spectrum, potassium amide in ammonia ($\sim 10^{-4}$ M), time after flash, 20 μ sec., 5-cm. cell, -75° .

In ammonia, where ion-unpairing is more extensive,⁶ it is possible that charge transfer occurs from excited amide ions directly to solvent to yield solvated electrons. This is evidently closely related to the formation of hydrated electrons by photoionization of negative ions in water.⁷

Quantitative aspects of this work will be presented shortly.

(6) W. H. Hawes, *J. Am. Chem. Soc.*, **55**, 4422 (1933).

(7) M. Matheson, W. A. Mulac, and J. Rabani, *J. Phys. Chem.*, **67**, 2613 (1963).

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The Catalytic Versatility of Carbonic Anhydrase from Erythrocytes. The Enzyme-Catalyzed Hydration of Acetaldehyde

Sir:

Although several workers have studied the catalytic effect of erythrocyte carbonic anhydrase (EC4.2.1.1) upon the reversible hydration of carbon dioxide,^{1a-f} the accelerative effect of this enzyme in the hydration of other carbonyl systems does not appear to have been considered. We have recently established that the catalytic effect of carbonic anhydrase is not limited to CO₂ hydration, but that the enzyme very powerfully and reversibly catalyzes the hydration of acetaldehyde and many other related carbonyl systems. The hydration of acetaldehyde was studied in the ultraviolet by following the decrease in the carbonyl band at 278 m μ (ϵ 16.8). For the determination of the catalytic constant k_{enz} the initial concentration of acetaldehyde

(1) (a) F. J. W. Roughton and V. H. Booth, *Biochem. J.*, **40**, 319 (1946). (b) R. P. Davis, *J. Am. Chem. Soc.*, **80**, 5209 (1958); **81**, 5674 (1959). (c) H. De Voe and B. G. Kistiakowsky, *ibid.*, **83**, 274 (1961). (d) J. C. Kernohan, *Biochim. Biophys. Acta*, **81**, 346 (1964). In this paper imidazole buffers were employed but the rapid and reversible carbamate formation prior to hydration appears to have been ignored. (e) B. H. Gibbons, and J. T. Edsall, *J. Biol. Chem.*, **239**, 2539 (1964). (f) S. Lindskog and B. G. Malmstrom, *ibid.*, **237**, 1129 (1962).