[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF RADCLIFFE COLLEGE]

The Reaction of Mustard Gas with Proteins

BY SELBY B. DAVIS[1] AND WILLIAM F. ROSS[2]

The inhibition of enzyme systems by mustard gas (bis-(2-chloroethyl)-sulfide) has been attributed[3] to a reaction of the mustard gas with the proteins involved; specifically to attachment of mustard gas residues to the free sulfhydryl groups. It has further been observed that horse serum exposed to the action of mustard gas develops new antigenic properties.[4] The latter effect was attributed to a chemical reaction between mustard gas and the free amino groups of the proteins.

TABLE I

PREPARATION OF MUSTARD GAS-TREATED PROTEINS

Product[a]	Molar concn.[b] of protein	Moles mustard per mole protein	*p*H during reaction
T-HbO_2-1	0.00127	2.4	7.5
T-HbO_2-2	.00157	9.6	7.5
T-HbO_2-3	.00157	38.5	7.5
T-HbO_2-4	.00295	20.5	7.5
T-HbO_2-5	.00149	40.6	5.5
T-SA	.00158	38.3	7.5

[a] T-HbO_2-1 = mustard gas-treated horse oxyhemoglobin, preparation #1, etc. T-SA = mustard gas-treated horse serum albumin. [b] Calculated from Kjeldahl nitrogen determinations and the nitrogen content (*cf.* Table II, footnotes *d* and *g*) and molecular weights (Table II, footnote *b*) of the proteins.

More direct evidence of the chemical combination of mustard gas with proteins has recently been obtained by Kistiakowsky, Henriques and Schneider.[5] These investigators found that when mustard gas made from radioactive sulfur was applied to the skin of pigs, a portion of the radioactive sulfur could not be removed by solvents capable of extracting the unreacted substance or its hydrolysis product, thiodiglycol. E. G. Ball and collaborators[6] fractionated such skin preparations and found that most of the "fixed" sulfur was attached to the insoluble skin proteins.

In the present study the reaction of mustard gas with two well-characterized, water-soluble proteins, horse oxyhemoglobin and horse serum albumin, has been investigated, especially in regard to the nature of the functional groups involved.

Aqueous solutions of the proteins were stirred with varying amounts of mustard gas (Table I) at a *p*H level maintained constant by the addition of alkali. When the release of hydrogen ion had ceased, the resulting products were dialyzed against water. Portions of the solutions thus prepared were exhaustively electrodialyzed in order to determine the approximate isoelectric points of the products.

Inspection of the data of Tables I and II shows that the number of sulfur atoms introduced per molecule is roughly proportional to the amount of mustard gas used, and furthermore that with increasing sulfur content there is an increase in

TABLE II

PROPERTIES OF PROTEIN PREPARATIONS

Prepn. (*cf.* Table I)	Titratable groups per molecule: *p*H 2 to 5.5	Change	*p*H 5.5 to 8.5	Change	*p*H 8.5 to 11	Change	Total groups *p*H 2 to 11	Total change	*p*I (±0.05 units)	Analytical data: % Nitrogen[a]	% Sulfur[a]	Total S[b] atoms per molecule	S Atoms added per molecule
HbO_2	94		32		36		162		7.10[c]	16.86[d]	0.39[d]	8.1	0.0
T-HbO_2-1									7.15	15.18	.43	10.0	1.9
T-HbO_2-2	89	− 5	30	−2	36	±0	155	− 7	7.25	14.79	.60	14.3	6.2
T-HbO_2-3	83	−11	25	−7	33	−3	141	−21	8.10	14.04	1.62	40.6	32.5
T-HbO_2-4										14.18	1.12	27.8	19.7
T-HbO_2-5	80	−14	30	−2	38	+2	148	−14	7.80	15.10	0.80	18.6	10.5[e]
SA	97		22		46		165		4.85[f]	16.1[g]	1.93[h]	42.1	0.0
T-SA	82	−15	18	−4	44	−2	144	−21	6.70	13.66	2.32	59.8	17.7

Except as otherwise noted, these are average values obtained from samples equilibrated in an air conditioned room. 'Calculated from the number of nitrogen atoms per molecule (804 in the case of hemoglobin, mol. wt. 66,800 and 804 for serum albumin, mol. wt. 70,000) and the atomic ratio of sulfur to nitrogen. [c] This value is appreciably higher than that generally accepted for oxyhemoglobin, *ca.* 6.7 [Hastings, *et al.*, *J. Biol. Chem.*, **60**, 89 (1924)] but does not differ greatly from the value of 6.92 obtained by Pedersen, as quoted by Theorell [*Biochem. Z.*, **268**, 46 (1934)]. Considerable denaturation and precipitation took place during the electrodialysis of oxyhemoglobin samples which did not occur in the case of the mustard gas-treated proteins prepared at *p*H 7.5. [d] Based on dry weight [Cohn, Green and Blanchard, THIS JOURNAL, **59**, 509 (1937)]. [e] During electrodialysis of this preparation a considerable amount of material precipitated and was not included in the analytical sample. Subsequent analysis of the precipitate showed it to contain 20 atoms of added sulfur per molecule. [f] McMeekin [THIS JOURNAL, **61**, 2884 (1939)] reports 4.8. [g] Based on dry weight, McMeekin [ref. *f*]. [h] Based on dry weight. Calculated from our analytical values of 14.08% N, 1.69% S.

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(3) (a) Bacq, *Enzymologia*, **10**, 48 (1941); (b) Fischer, *Bull. soc. roy. sci. Liége*, **12**, 235 (1943); *C. A.*, **38**, 5850 (1944).

(4) Berenblum and Wormall, *Biochem. J.*, **33**, 75 (1939).

(5) Personal communication.

(6) To be published.

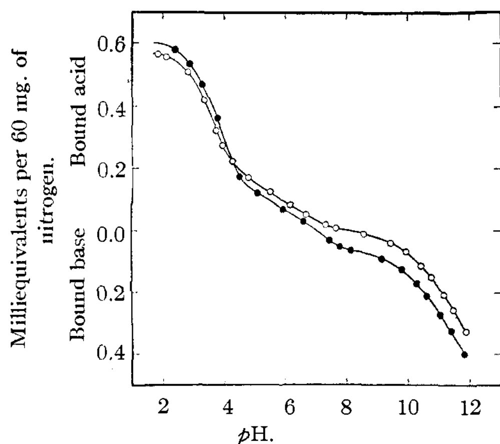


Fig. 1.—Titration curves: ●, oxyhemoglobin (HbO_2); ○, mustard-treated oxyhemoglobin (T-HbO_2-3).

the basicity of the derived proteins, as reflected by their isoelectric points (*p*I).

Potentiometric titration of certain of the preparations (Table II and Fig. 1) showed a consistent decrease in the number of titratable groups with increasing sulfur content. The greatest decrease occurred in the range *p*H 2 to 5.5, where the free carboxyl groups of proteins are normally titrated. In view of the recent observation that mustard gas reacts readily with carboxylate ions in aqueous media yielding esters of thiodiglycol,[7] this decrease is attributed to esterification of the protein carboxyl groups.

In the case of hemoglobin, an appreciable decrease in titratable groups was also observed in the range from *p*H 5.5 to 8.5. Since hemoglobin is known to contain a large portion of histidine, the imidazole groups of which are titrated in this region,[8] this decrease was attributed tentatively to reaction of the mustard gas with the imidazole residues of the protein, yielding quaternary ammonium groups.[9] The mustard gas-treated serum albumin (T-SA) showed a smaller decrease in titratable groups in the region *p*H 5.5 to 8.5 than did hemoglobin treated with a similar portion of mustard gas (T-HbO_2-3). Serum albumin is known to have a smaller histidine content than hemoglobin.[10]

In order to support the above interpretation, the reaction of mustard gas with an equimolar quantity of imidazole in dilute aqueous solution at *p*H 8.5 was investigated. Analysis of the reaction mixture by potentiometric titration and colorimetric estimation of imidazole showed the nitrogen present to be distributed thus: 49% quaternary amine, 27% tertiary amine, 24% imidazole.

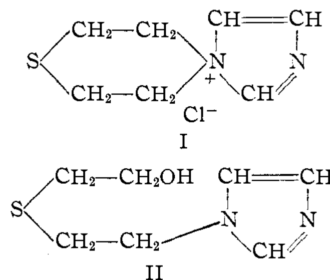
(7) R. P. Linstead and W. E. Doering, personal communication.

(8) Cohn, Green and Blanchard, THIS JOURNAL, **59**, 509 (1937).

(9) Reaction to give the corresponding tertiary amino groups would not be expected to modify the titration curve since the *pK* values of N-alkyl imidazoles differ but little from that of imidazole itself [Dedichen, *Ber.*, **39**, 1831 (1906)].

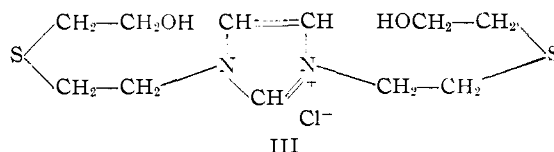
(10) 3.40% [Hartley, *Biochem. J.*, **8**, 541 (1914)], compared to 7.64% in hemoglobin [Vickery and Leavenworth, *J. Biol. Chem.*, **79**, 377 (1928)].

The reaction of one mole of mustard gas to give the quaternary compound (I) involves the liberation of only one equivalent of hydrogen ion, whereas the formation of the tertiary amine (II)



and the reaction with water to give thiodiglycol both involve the liberation of two equivalents of hydrogen ion per mole of mustard gas. The theoretical hydrogen ion release calculated for 49% quaternization is thus $100 - 49/2 = 76\%$, in agreement with the experimental value of 79%.

Formation of a quaternary compound of type (III) appeared improbable since 98% of the total available mustard gas would have been required for the formation of the observed amount of quaternary salt.



Ether extraction of a similar reaction mixture yielded both imidazole and the expected tertiary amine, 2-(1-imidazolyl)-2′-hydroxydiethyl sulfide (II).

Evaporation of the reaction mixture, from which the amines had been extracted, yielded the quaternary ammonium compound (I), spiro[imidazole-1,4′-thiamorpholinium] chloride.

In an experiment carried out at *p*H 5.5, potentiometric titration and colorimetry indicated a complete recovery of imidazole, *i. e.*, that no reaction had taken place. Similarly, when a hemoglobin solution was allowed to react with mustard gas at *p*H 5.5 (T-HbO_2-5) very little reduction in groups titratable in the region *p*H 5.5 to 8.5 was observed.

In the most heavily treated hemoglobin (T-HbO_2-3) and in the treated serum albumin (T-SA) a few groups titrating in the region *p*H 8.5 to 11 were lost. Reaction with the sulfhydryl group of cysteine (*pK* 10.28)[11] or with the phenolic hydroxyl of tyrosine (*pK* 10.07)[12] may be responsible for this observation.

In view of the involvement of imidazole moieties in the union of the hemes with the protein portion of the hemoglobin molecule,[13] the evidence that

(11) Cohn and Edsall, "Proteins, Amino Acids, and Peptides," Reinhold Publishing Corporation, New York, N. Y., 1943, p. 85.

(12) Winnek and Schmidt, *J. Gen. Physiol.*, **18**, 889 (1935).

(13) For a recent review of this subject, see ref. 11, pp. 482 to 486.

reaction with these groups had taken place suggested that there might be a concomitant effect on the oxygen affinity of the treated protein.

The oxygen dissociation curves of the hemoglobin preparations (Fig. 2) show a progression to the left, indicating an increase in oxygen affinity parallel to the increase in the number of sulfur atoms introduced into the molecule. The oxygen capacities of the equilibrated solutions, however, showed the presence of a considerable portion of inactive hemoglobin (Table III). The possible presence of methemoglobin assumed particular significance in view of the work of Darling and Roughton[14] who have shown that the presence of methemoglobin appreciably increases the oxygen affinity of hemoglobin.

Freshly prepared solutions of the stock oxyhemoglobin, analyzed spectrophotometrically,[15] were found to contain 8 to 10% of methemoglobin. Solutions of mustard gas-treated hemoglobins which had been stored for periods of three to eight weeks in the cold contained from 50 to 90% methemoglobin.

TABLE III

Preparation	N Content of soln., mg./ml.	Oxygen capacity, mM/liter Calcd.[a]	Found	Inactive Hb, %
HbO_2	16.50	5.86	4.68	20.1
T-HbO_2-1	22.00	7.81	5.00	36.0
T-HbO_2-2	27.75	9.86	5.18	47.5
T-HbO_2-3	16.03	5.69	2.40	57.8
T-HbO_2-4	21.85	7.76	4.77	38.5

[a] The nitrogen content and molecular weight of hemoglobin are taken as 16.86% and 66,800, respectively, as before (*cf.* Table II).

In a later experiment (T-HbO_2-4) the equilibration and determination of oxygen capacities and contents were carried out as soon as possible after the reaction with mustard gas was completed. Prior to equilibration the solution was found to contain 20% methemoglobin; after equilibration, 48%.[16]

Since none of the present preparations contained more than 58% methemoglobin, comparison with the results of Darling and Roughton (Fig. 2) indicates that the treatment of hemoglobin with mustard gas has increased the oxygen affinity of the protein *per se*.[17] In its high oxygen affinity, mustard gas-treated hemoglobin resembles fetal hemoglobin[18] and the muscle pigment, myoglobin.[19]

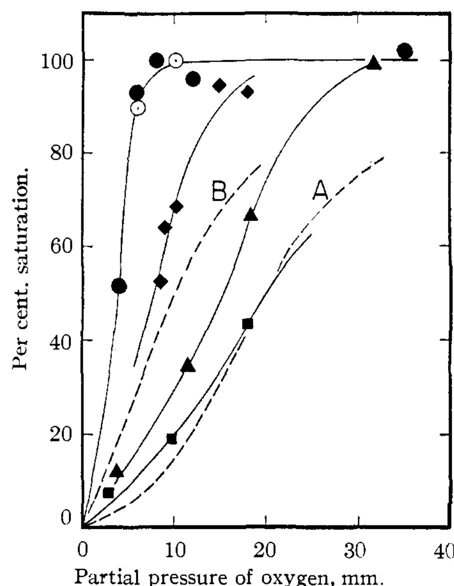


Fig. 2.—Oxygen dissociation curves: ■, HbO_2; ▲, T-HbO_2-1; ◆, T-HbO_2-2; ●, T-HbO_2-3; ⊙, T-HbO_2-4: dashed curves (Darling and Roughton[14])—A, human HbO_2; B, human HbO_2 46%, metHb 54%.

Experimental[20]

Preparation of Oxyhemoglobin.—Horse red blood cells[21] were suspended in 2 volumes of 0.9% sodium chloride containing about 0.5% of sodium citrate, and passed through a Sharples supercentrifuge. The thick semicrystalline paste from the centrifuge was cooled to 10° and stirred while the solution was gradually acidified to *p*H 6.7 by the addition of 0.1 *N* hydrochloric acid. After twenty-four hours of stirring the crystals were collected by centrifuging and washed with ice-cold, toluene-saturated water. For recrystallization, the cooled paste was mixed with one-half its volume of cold water and 1 *N* potassium hydroxide was added intermittently, with stirring, to maintain the *p*H at 7.6 until the hemoglobin had dissolved. The solution was centrifuged for one hour, and the supernate was stirred at 5° during the addition of 0.1 *N* hydrochloric acid to *p*H 6.7. After being stirred for twenty-four hours the crystals were centrifuged and washed twice with toluene-saturated, ice-cold water.

Preparation of Serum Albumin.—Crystalline, carbohydrate-free horse serum albumin was prepared according to the procedure of McMeekin.[22] The precipitate obtained in the second recrystallization was dialyzed against distilled water to remove the ammonium sulfate prior to use.

Mustard Gas.—The mustard gas used in the protein experiments was obtained by distilling the crude Levenstein product and fractionating the distillate boiling at 104–106° (14 mm.) in a seven-plate, helix-packed column yielding 33% of pure bis-(2-chloroethyl) sulfide, b. p. 67° (3 mm.).[23]

(14) Darling and Roughton, *Am. J. Physiol.*, **137**, 56 (1942).

(15) Austin and Drabkin, *J. Biol. Chem.*, **112**, 67 (1935–36).

(16) That the latter value was high was indicated by the amount of inactive hemoglobin present as estimated from the nitrogen content and oxygen capacity of the solution, 39%. The discrepancy may be attributed to partial invalidation of the Austin and Drabkin analysis due to differences between the absorption spectra of hemoglobin and mustard gas-treated hemoglobin.

(17) It is possible that treatment of hemoglobin with mustard gas successively immobilizes active centers of oxygenation and thereby, as argued by Darling and Roughton,[14] in the case of methemoglobin formation, brings about an increase in the oxygen affinity of the remaining centers. If such were the case there should be present a considerable amount of inactive protein other than methemoglobin. The uncertainty in the spectrophotometric methemoglobin analysis does not allow this point to be decided.

(18) McCarthy, *J. Physiol.*, **80**, 206 (1933).

(19) Theorell, *Biochem. Z.*, **268**, 73 (1934).

(20) (a) We are indebted to Miss Eleanor Werble for all analyses except the Kjeldahl nitrogen determinations on wet samples. (b) All melting points corrected.

(21) Horse red cells and serum were furnished by the Massachusetts Antitoxin Laboratory.

(22) McMeekin, THIS JOURNAL, **61**, 2884 (1939).

(23) We are indebted to Dr. W. E. Doering for this fractionation.

The experiments with imidazole were carried out with bis-(2-chloroethyl) sulfide made from thiodiglycol.[24]

Imidazole.—Commercial crude imidazole was distilled and the distillate taken up in hot benzene. The solid portion of the precipitate obtained on cooling was sublimed and the sublimate was crystallized from benzene, yielding colorless needles of pure imidazole, m. p. 89.5–90°.

Reaction of Mustard Gas with Proteins.—Freshly prepared solutions of the proteins were stirred with the desired quantities of mustard gas at 24 ± 1° in a reaction vessel equipped with a glass electrode assembly. The desired *p*H was maintained by intermittent addition of 0.5 *N* potassium hydroxide. After being stirred for five hours the mixtures were allowed to stand overnight at room temperature, and were then dialyzed against distilled water at 4° for two days.

Portions of the solutions were then electrodialyzed against distilled water at 15° until the *p*H remained constant (two to five days). The isoelectric points thus obtained were approached from both the acid and alkaline side in each case, and the two values obtained never differed by more than 0.5 *p*H unit. The resulting solutions were lyophilized and submitted to analysis for nitrogen (micro-Kjeldahl) and sulfur (Parr bomb).

For the potentiometric titrations (glass electrode) the cell was charged with 0.50 ml. of 2 *M* potassium chloride, a portion of the protein solution containing 60.0 mg. of nitrogen, and sufficient carbon dioxide-free distilled water to bring the total volume to 10.00 ml. Correction for dilution was applied by subtracting from the volume reading for each point the amount of acid or base needed to bring 10 ml. of 0.1 *M* potassium chloride to the observed *p*H.

Determination of Oxygen Dissociation Curves.—Prior to equilibration the dialyzed protein solutions were adjusted to *p*H 7.2 with solid mono- and dipotassium phosphate, 0.025 *M* in the case of oxyhemoglobin and preparations T-HbO_2-2 and -3, and 0.050 *M* in the case of T-HbO_2-1 and -4. The *p*H values remained in the range *p*H 7.10 ± 0.08 during subsequent operations.[25] The solutions were twice equilibrated with the desired air-nitrogen mixtures for three-quarter hour periods and the oxygen contents and capacities were then determined by the Van Slyke procedure.[26] To reduce the opportunity for methemoglobin formation in T-HbO_2-4, dialysis was omitted prior to equilibration.

Reaction of Mustard Gas with Imidazole.—Imidazole (513 mg.) was stirred with an equimolar quantity of mustard gas (1.20 g.) in 50 ml. of distilled water, the *p*H being maintained at 8.5 by intermittent addition of *N* sodium hydroxide solution. After seven hours the rate of liberation of hydrogen ion had become negligible and the total amount liberated was 78.8% of that theoretically obtainable from hydrolysis of the mustard gas present. The solution was then acidified to *p*H 6 and refluxed for one hour to insure completion of the reaction, destruction of sulfonium compounds, and removal of carbon dioxide.

The total nitrogen content of the resulting solution was obtained by Kjeldahl analysis. Aliquots were then titrated potentiometrically. The data showed 51.1% of the total nitrogen (2 atoms per equivalent) to be present in titratable form (*p*H 2 to 11), representing imidazole and the derived tertiary amine. Colorimetric estimation of imidazole by Macpherson's procedure[27] gave 24.2%.

When the experiment was repeated at *p*H 5.5, 99.9% of the nitrogen in the reaction mixture was titratable in the region *p*H 2 to 11, and the mixture developed 100.6% of the imidazole color.

Isolation of the Tertiary Amine (II).—Imidazole (513 mg.) and mustard gas (1.20 g.) were allowed to react in 50 ml. of distilled water. Freshly precipitated and washed silver oxide was added at intervals during the course of five hours to maintain the reaction mixture at *p*H 8.

After being stirred overnight the reaction mixture was continuously extracted with ether for twenty-eight hours. Concentrated sulfuric acid (0.5 ml.) was added to the ether reservoir to retain the amines. Distilled water (50 ml.) was added to the ether extract and the mixture was extracted continuously with ether for forty-three hours. Evaporation of the extract yielded 70 mg. of a yellow oil having the characteristic odor of thiodiglycol.

The residual water layer was rendered alkaline with barium hydroxide. The precipitated barium sulfate was filtered and washed with hot water. Filtrate and washings were then continuously extracted with ether for forty-six hours. An oil (130 mg.) remaining after evaporation of the extract was submitted to sublimation at 1.5 mm., 65°, which removed 40 mg. of colorless, crystalline solid, m. p. 83–87°, identified as imidazole (m. p. 85–88°).

The residue was taken up in 2 ml. of water, acidified to *p*H 5 with hydrochloric acid, and 1.6 ml. of a 0.3 *N* solution of sodium picryl sulfonate[28] in 0.5 *N* hydrochloric acid was added. After standing overnight at 4° a precipitate of 110 mg. of pale yellow solid, m. p. 141–144°, was obtained. Two recrystallizations from boiling acetone by the addition of petroleum ether yielded 70 mg. of nearly colorless prisms of the picryl sulfonate of the tertiary amine, 2-(1-imidazolyl)-2′-hydroxy diethyl sulfide (II), m. p. 144–146°.

Anal. Calcd. for $C_{13}H_{15}O_{10}N_5S_2$: C, 33.55; H, 3.25; N, 15.05; S, 13.78. Found: C, 33.76; H, 3.23; N, 14.67; S, 13.74.

Color Development of the Tertiary Amine.—A sample of the free amine was prepared by repeated ether extraction of a solution of the picryl sulfonate (20 mg.) in 5 ml. of 30% sodium hydroxide. Evaporation of the dried extract left 4.9 mg. of (II), a colorless oil. An aqueous solution of this substance, at a concentration comparable to that of a standard imidazole solution, developed only 109% of the color developed by a blank prepared from the reagents alone. The color development of a standard imidazole solution was unaffected by the introduction of various proportions of (II).

Isolation of Quaternary Ammonium Compound (I).—Following the ether extraction, the original aqueous reaction mixture was rendered neutral with hydrochloric acid and the silver chloride was removed by centrifugation and filtration. Evaporation of the filtrate and washings left 1.03 g. of a colorless glass. For purification the substance was twice precipitated by dissolving it in 7 ml. of absolute ethanol and pouring the solution into 75 ml. of absolute ether. The glassy product (0.7 g.) showed no tendency to crystallize and was strongly hygroscopic.

A 250-mg. portion of the substance was dissolved in 5 ml. of water and a 2-ml. portion of the solution was acidified with hydrochloric acid and treated with 1.75 ml. of 0.3 *N* sodium picryl sulfonate in 0.5 *N* hydrochloric acid. After standing overnight at 4° the oily precipitate formed was washed with 0.5 *N* hydrochloric acid and dried. On digestion with boiling absolute ethanol the precipitate solidified and was readily pulverized. After further trituration with absolute ethanol the substance was filtered and dried, yielding 120 mg. of spiro[imidazole-1,4′-thiamorpholinium] picrylsulfonate as a bulky, pale yellow powder, m. p. 75–80°.

Anal. Calcd. for $C_{13}H_{13}O_9N_5S_2$: C, 34.90; H, 2.93; N, 15.66; S, 14.33. Found: C, 34.60; H, 3.06; N, 15.61; S, 14.16.

Acknowledgment.—This investigation was supported by a grant from the Rockefeller Foundation to Radcliffe College for which the authors wish to express their gratitude. We also wish to express our sincere thanks to Dr. E. G.

(24) Prepared at the University of Illinois under the direction of Dr. C. H. Marvel.

(25) A study of published data indicates that these variations in *p*H and salt concentration were not significant factors in the results [Ferry and Green, *J. Biol. Chem.*, **81**, 175 (1929); Sidwell, Munch, Barron and Hogness, *ibid.*, **123**, 335 (1938)].

(26) Peters and Van Slyke, "Quantitative Clinical Chemistry," The Williams and Wilkins Co., Baltimore, Md., 1932, Vol. II.

(27) Macpherson, *Biochem. J.*, **36**, 59 (1942).

(28) We are grateful to Dr. J. W. Davis for a sample of this reagent.

Ball, Official Investigator under Division 9 of the National Defense Research Committee, for facilitating the carrying out of this work, and to Professor A. B. Hastings, Dr. J. M. Buchanan, and Mr. Robert Wolf for aid in determining the oxygen dissociation curves.

Summary

Crystalline horse oxyhemoglobin and serum albumin were treated with mustard gas under controlled *p*H conditions. It was found that:

1. The number of sulfur atoms introduced per molecule of protein is a function of the amount of mustard gas used.
2. The isoelectric points of the treated proteins are shifted in the direction of increasing alkalinity with increase in the number of sulfur atoms introduced.
3. The greatest reduction in titratable groups occurs in the region *p*H 2 to 5.5 where the carboxyl groups are normally titrated. It is concluded that some of the free carboxyl groups of the proteins have been esterified.
4. Further loss in titratable groups occurs in the region *p*H 5.5 to 8.5 where imidazole groups are normally titrated. The results of a study of the reaction of mustard gas with imidazole substantiate the conclusion that at physiological *p*H values mustard gas may react with the imidazole groups of these proteins.
5. With the proportions of mustard gas employed very little reduction in titratable groups in the region *p*H 8 to 11 takes place.
6. The oxygen dissociation curves of mustard gas-treated hemoglobins are shifted in a direction indicating that the modified proteins have a greater affinity for oxygen. It is suggested that this effect is due to the reaction of mustard gas with the imidazole groups of the hemoglobin molecule.

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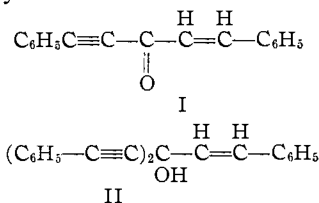
[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF MISSOURI]

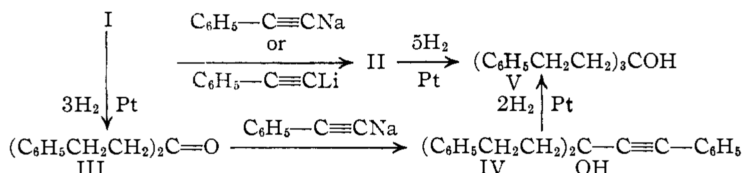
The Action of Sodium, Lithium and Potassium Phenylacetylene on Acid Derivatives

BY DOROTHY NIGHTINGALE AND FRANCIS T. WADSWORTH[1]

In a preceding paper[2] we have noted that the properties of cinnamoylphenylacetylene I prepared from cinnamic anhydride and sodium phenylacetylene differ markedly from those of the compound which Worrall[3] reported as cinnamoylphenylacetylene.



The identity of Worrall's compound has now been established as bis-phenylethynylstyrylcarbinol II by the reactions



Ketone III, obtained by the hydrogenation of I, was identified as dibenzylacetone through its oxime which did not depress the melting point of an authentic specimen. In the Grignard machine, I added one mole of methylmagnesium iodide while II and V liberated one mole of methane. Most of the properties which Worrall described for his compound are those to be expected of a tertiary carbinol.

The tertiary carbinol II was readily obtained from the reaction of either cinnamic anhydride or ethyl cinnamate with lithium phenylacetylene in yields of 54 and 90%, respectively. This fact led us to compare the action of lithium, potassium, and sodium phenylacetylene with the acid derivatives listed in Table I.

In all cases except ethyl formate, lithium phenylacetylene yielded tertiary carbinols, while sodium phenylacetylene yielded ketones with the acid chlorides, tertiary carbinols with ethyl cinnamate and ethyl phenylpropiolate, and only traces of viscous oil with the aliphatic esters. The isolation of phenylpropargyl aldehyde from ethyl formate and lithium phenylacetylene rather than bis-phenylethynylcarbinol was surprising.

Potassium phenylacetylene formed small amounts of acetylphenylacetylene with acetic anhydride and acetyl chloride and a 50% yield of cinnamic acid with ethylcinnamate, but only a trace of viscous oil with all of the other acid derivatives.

The formation of tertiary carbinols rather than ketones from lithium phenylacetylene and the acid derivatives may be due to a greater solubility of the lithium addition product in ether, which may

(1) Abstract of a portion of the dissertation to be submitted by Francis T. Wadsworth in partial fulfillment of the requirement for the degree of Doctor of Philosophy at the University of Missouri. Present address: Pan American Refining Company, Texas City, Texas.

(2) Nightingale and Wadsworth, THIS JOURNAL, **67**, 416 (1945).

(3) Worrall, *ibid.*, **60**, 1266 (1938).