ester are shorter by 0.2 Å. than can be calculated from bond radii. Since then the P–O bonds are essentially double, the force constants for bending the O–P–O angle, and possibly the P–O–C angles, must be large. This double bond character might directly account for the strain, since even small angular distortions will require much energy. Alternatively, it might provide the stabilizing influence on geometry to tend to force the two P–O–C bonds at a sharp dihedral angle to one another, and thus[4] provide the strain. These hypotheses are under investigation.

Appendix

Equation 3 may be derived as follows

$$D \xrightarrow{k_h} \text{monoester} + \text{alcohol} \quad (7)$$

$$(D - D') \xrightarrow{k_e} D' \quad (8)$$

$$D' \xrightarrow{k_e/2} D'' \quad (9)$$

where D and D' have already been defined, and D'' is double labeled diester. Since in these experiments, D'' is very small, almost all the enrichment is in D', and

$$dD'/dt = k_e(D - D') + k_eD'/2 - k_hD', \text{ where } D = D_0e^{-k_ht} \quad (10)$$

Substitution and integration yields eq. 3.

When the extent of the reaction is small

$$k_h/k_e = (D/D') \ln (D_0/D) \quad (11)$$

If M is the total concentration of monoester, and M' is the concentration of enriched monoester, then

$$M = D_0(1 - e^{-k_ht}) \quad (12)$$

$$(D - D') \xrightarrow{k_h} M' \quad (13)$$

$$D' \xrightarrow{k_h} 2M' \quad (14)$$

$$dM'/dt = k_h(D + D') = k_hD_0e^{-k_ht}(3 - 2e^{-k_et/2}) \quad (15)$$

After integration

$$M'/M = 3 - 4k_h/(2k_h + k_e) - 2k_hD'/(2k_h + k_e)(D_0 - D) \quad (4)$$

At complete reaction

$$M'/M = (2k_h + 3k_e)/(2k_h + k_e) \quad (16)$$

Acknowledgments.—The authors express their gratitude to the National Science Foundation for the support of this work.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NOTRE DAME, NOTRE DAME, IND.]

The Alkaline Decomposition of Organic Disulfides. I. Some Dithiodicarboxylic Acids[1]

BY JAMES P. DANEHY AND JOHN A. KREUZ[2]

RECEIVED SEPTEMBER 9, 1960

The decompositions of three dithiodicarboxylic acids, in aqueous alkaline solution at 35.2° in an atmosphere of nitrogen, were followed as a function of time at several constant *p*H values by quantitative measurement of residual disulfide, mercaptan and hydrogen sulfide, the sum of which accounted for 93 to 100% of the original sulfur. The rate of decomposition of dithiodiacetic acid (DTDA) increases rapidly over the *p*H range of 11.9 to 12.4. A detailed mechanism is proposed which accounts for the quantitative data. The decomposition of dithiodipropionic acid ($HOOCCH_2CH_2SSCH_2CH_2COOH$, DTDP) as anticipated, is much slower than that of DTDA and may proceed by a different mechanism since no hydrogen sulfide is produced. The decomposition of 2,2′-dimethyldithiodiacetic acid ($HOOCCH(CH_3)SSCH(CH_3)COOH$, DMDT) proceeds at rates intermediate between those found for DTDA and for DTDP, by a mechanism analogous to that for DTDA.

Introduction

More than a century ago Mulder[3] observed that relatively mild alkaline treatment sufficed to remove sulfur (though not all of it, as he reports) from several proteins. Eventually it was established that the sulfur was lost at the expense of the disulfide linkage of the cystinyl residues of the protein. Many studies on the alkaline decomposition of disulfide linkages in proteins, of cystinyl peptides, and of simpler aliphatic disulfides have been reviewed in some detail by Schöberl and Wagner[4] and more recently Parker and Kharasch[5] have set the problem in the larger context.

Briefly, four different mechanistic interpretations have been offered to account for the alkaline cleavage of aliphatic disulfides, the fourth of which is a more modern and more inclusive version of the first.

Schöberl[6] proposed the direct hydrolytic cleavage of the disulfide bond to furnish mercaptide ion and sulfenic acid, with the further decomposition of the latter providing the products actually obtained. He has consistently interpreted the results of his numerous studies on model compounds[7] on the basis of this assumption, though direct evidence for aliphatic sulfenic acids is lacking.

$$RS\text{–}SR + OH^- \longrightarrow RS^- + RSOH \quad (1)$$

Tarbell and Harnish[8] suggested an alternative elimination mechanism, initiated by the ionization of a hydrogen from a carbon β to a sulfur atom.

(1) Presented at the 136th Meeting of the American Chemical Society, Atlantic City, N. J., September 15, 1959.

(2) Toni-Gillette Fellow, 1956–1959.

(3) G. J. Mulder, *Ann.*, **28**, 73 (1838).

(4) A. Schöberl and A. Wagner, Houben-Weyl's "Methoden der organischen Chemie," 4th edition, Thieme, Stuttgart; Vol. 9, 1955, p. 75; Vol. 11 (2), 1958, p. 436.

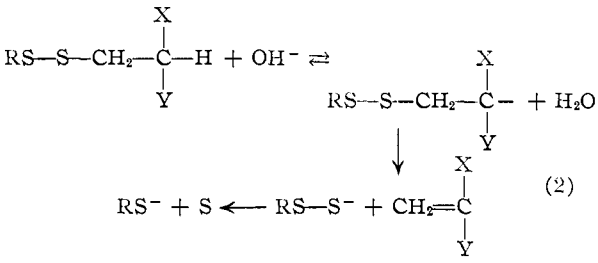
(5) A. J. Parker and N. Kharasch, *Chem. Revs.*, **59**, 583 (1959).

(6) A. Schöberl, *Ann.*, **507**, 111 (1933); A. Schöberl, E. Berninger and F. Harren, *Ber.*, **67B**, 1545 (1934).

(7) A. Schöberl and H. Eck, *Naturwissenschaften*, **23**, 391 (1935); A. Schöberl, *Ber.*, **69B**, 1955 (1936); A. Schöberl and H. Eck, *Ann.*, **522**, 97 (1936); A. Schöberl, *Ber.*, **70B**, 1186 (1937); A. Schöberl and T. Hornung, *Ann.*, **534**, 210 (1938); A. Schöberl and P. Rambacher, *ibid.*, **538**, 84 (1939); A. Schöberl and P. Rambacher, *Biochem. Z.*, **306**, 269 (1940); A. Schöberl, P. Rambacher and A. Wagner, *ibid.*, **317**, 171 (1944).

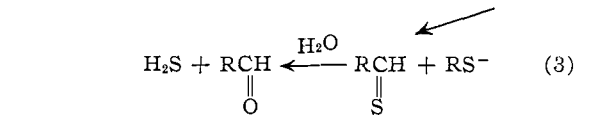
(8) D. S. Tarbell and D. P. Harnish, *Chem. Revs.*, **49**, 11 (1951).

Both Swan[9] and Dann, Oliver and Gates[10] consider their experimental results to be in accord with this hypothesis.



(2)

Rosenthal and Oster[11] propose that the initial step is the dissociation of a hydrogen from a carbon α to a sulfur atom.



(3)

Finally, Parker and Kharasch[12] prefer to represent the nucleophilic cleavage of the sulfur-sulfur bond by a base Y^- with the equation

$$RS{-}SR' + Y' \longrightarrow SRY + R'S^- \qquad (4)$$

This view agrees very well with the unequivocally established products of reaction when the nucleophile is HSO_3^-, CN^-, $R'S^-$ or HS^-, but when the base is OH^- then RSY is a sulfenic acid and scheme 4 reverts to scheme 1.

In 1941, a fact of signal importance, whose possibility was never considered by any of the earlier workers, was established: Horn, Jones and Ringel[13] isolated from the acid hydrolysate of alkali-treated wool a new amino acid thioether: lanthionine. Subsequent studies, especially the one by Blackburn and Lee,[14] have shown that under suitably mild conditions of alkalinity the transformation of disulfide into thioether linkages *in proteins* is remarkably efficient. All the more remarkable, then, that to date no one has demonstrated, preparatively, the formation of a thioether from any aliphatic disulfide other than a protein.[15] However, Swan[17] has presented qualitative evidence from paper chromatography of cystine and six derivatives of cystine that some thioether formation has taken place on heating in 0.1 *M* carbonate.

It appears that no one mechanistic scheme governs the alkaline cleavage of all aliphatic disulfides, even in the initial stages. There is no satisfactory explanation for the ease with which cystinyl residues in proteins are transformed into lanthionyl residues. What is needed at present is quantitative data, obtained under relatively mild alkaline conditions, on the initial changes which take place during cleavage of various disulfides, all more or less related to cystine. The present report is the first of a series on this subject.

(9) J. M. Swan, *Nature*, **179**, 965 (1957).

(10) J. R. Dann, G. L. Oliver and J. W. Gates, Jr., *J. Am. Chem. Soc.*, **79**, 1644 (1957).

(11) N. A. Rosenthal and G. Oster, *J. Soc. Cosmetic Chemists*, **5**, 286 (1954).

(12) A. J. Parker and N. Kharasch, *Chem. Revs.*, **59**, 596 (1959).

(13) M. J. Horn, D. B. Jones and S. J. Ringel, *J. Biol. Chem.*, **138**, 141 (1941).

(14) S. Blackburn and C. R. Lee, *Biochem. Biophys. Acta*, **19**, 505 (1956).

(15) In a single drastic experiment (No. 14) in which L-cystine was treated with Zn dust in 1–3 *N* NaOH on a water-bath for several days Schöberl and Wagner[16] isolated lanthionine amounting to less than 10% of the cystine (exact amount not specified).

(16) A. Schöberl and A. Wagner, *Hoppe-Seyler's Z. physiol. Chem.*, **304**, 97 (1956); Proc. Internl. Wool Textile Research Conf., Melbourne, 1955, p. C, 11.

(17) J. M. Swan, Proc. Internl. Wool Textile Research Conf., Melbourne, 1955, p. C, 25.

Experimental

Materials.—Mercaptoacetic acid, 2-mercaptopropionic acid, 3-mercaptopropionic acid, dithiodiacetic acid (DTDA) and 2,2-dimethyldithiodiacetic acid (DMDT) were obtained as gifts from the research laboratories of the Toni Co., Chicago 54, Ill. Dithiodipropionic acid (DTDP) was prepared by adding the calculated amount of 30% hydrogen peroxide to aqueous 3-mercaptopropionic acid. Within a few minutes an almost quantitative yield of colorless crystals separated from the solution; m.p. 155–156°, 98.8% pure by titration with 0.1 *N* NaOH. Mercaptan stock solutions were standardized by iodometric titration. Disulfide stock solutions were standardized by reducing aliquots with amalgamated zinc in a Jones reductor or by shaking them with mossy cadmium, and titrating the reduced solutions iodometrically.

Procedure for Following Decomposition of Dithiodicarboxylic Acids.—A 200-ml. aliquot of standard, carbonate-free aqueous sodium hydroxide was transferred to a 300-ml. round-bottom flask, the solution was aspirated with nitrogen[18] for 15 minutes, and the flask was then equilibrated in a constant temperature bath at 35.2°. In the cases of DTDA and DMDT a 5-ml. aliquot of a standard aqueous solution (about 0.04 *M*) was added while a stream of nitrogen was passed through the neck of the flask. With the much less soluble DTDP an equivalent amount of the crystalline solid was added directly. In each case, as soon as homogeneous solution was attained, an initial 2 ml. aliquot was taken for disulfide analysis, a 10-ml. aliquot was taken for *p*H determination, the nitrogen stream was discontinued and the flask quickly closed. At appropriate time intervals the flask was opened, the nitrogen stream resumed, and the aliquots taken for the determination of residual disulfide, mercaptan and hydrogen sulfide. Terminally, the *p*H value of the solution was again determined.

Procedure for Determination of Disulfide and Mercaptan.—An adaptation of the Folin–Looney[19] colorimetric method was employed. Aliquots for either mercaptan or disulfide (2 to 5 ml.) were immediately quenched in 2 ml. of *N* hydrochloric acid and aspirated for 10 minutes with nitrogen to remove any hydrogen sulfide. For the determination of mercaptan, the aliquot, so treated, was transferred to a 50-ml. volumetric flask containing 10 ml. of acetate buffer[20] at *p*H 5, 4 ml. of phosphotungstic acid reagent was added, a single drop of 0.002 *M* cupric sulfate was added, the blue color was allowed to develop for at least 15 minutes (but not more than thirty minutes), the solution was brought to volume with distilled water, and the optical density was determined with an Evelyn photoelectric colorimeter, using a filter with the maximum transmission at 660 $m\mu$. The concentration of mercaptan was read from a standard curve of optical density *vs.* concentration. For the determination of disulfide the quenched, aspirated aliquot was placed at the top of a Jones reductor charged with zinc amalgam, the eluate and rinsings collected directly in the 50-ml. flask containing 10 ml. of acetate buffer, and the rest of the procedure was as in the case of mercaptan. The disulfide concentration was calculated as the total sulfhydryl content minus that known to be attributable to mercaptan, divided by two.

Preparation of Phosphotungstic Acid Reagent.—To a 1-liter flask equipped with reflux condenser was added 100 g. of sodium tungstate dihydrate (Folin grade), 200 ml.

(18) Nitrogen, containing not more than 8 parts per million of oxygen, was obtained from General Electric Cleveland Wire Plant, Cleveland 17, O.

(19) O. Folin and J. M. Looney, *J. Biol. Chem.*, **51**, 421 (1922).

(20) Six hundred grams of sodium acetate trihydrate and 50 ml. of glacial acetic acid dissolved in sufficient distilled water to give 2 liters of solution.

of distilled water and 50 ml. of 85% phosphoric acid. The mixture was refluxed gently for 1 hour, 5 drops of bromine added to discharge the light blue color that developed, the solution refluxed gently for 5 minutes, and then boiled vigorously for 20 minutes to expel excess bromine. The solution was allowed to cool and was then diluted to 1 liter with distilled water.

Procedure for Determination of Hydrogen Sulfide.— The method employed was that of Budd and Bewick,[21] substantially unmodified. Aspiration of the hydrogen sulfide from the acidified solution with nitrogen was carried out for 20 minutes.

Oxalate from the Alkaline Decomposition of DTDA.— DTDA (0.500 g.) in 100 ml. of 0.100 *N* NaOH was held at 35.2° under nitrogen for 15 hours. Analyses of 1-ml. aliquots indicated that 52.6% of the disulfide had decomposed. The solution was neutralized with 10 ml. of *M* hydrochloric acid, the hydrogen sulfide was removed by aspiration with nitrogen for 30 minutes, and 5 ml. of 0.2 *M* calcium chloride solution was added. The precipitate which formed after some time was collected, washed with water, dried at 105° and weighed: 28.3 mg. Dissolved in dilute sulfuric acid, it consumed 35.3 ml. of 0.0119 *N* $KMnO_4$ (calculated for CaC_2O_4, 37.1 ml.).

Pyruvate from the Alkaline Decomposition of DMDT.— DMDT (0.526 g.) in 100 ml. of 0.100 *N* NaOH was held at 35.2° under nitrogen for 22 hours. Analyses of 1-ml. aliquots indicated that 63.5% of the disulfide had decomposed. The solution was neutralized with 10 ml. of *N* hydrochloric acid, the hydrogen sulfide was removed by aspiration with nitrogen for 30 minutes, the volume reduced to about 10 ml. by evaporation *in vacuo*, and 2,4-dinitrophenylhydrazine was added dropwise. The copious yellow precipitate was collected, washed and dried: 0.175 g.; recrystallized from glacial acetic acid, melted at 214–215°. An authentic specimen of pyruvic acid 2,4-dinitrophenylhydrazone melted at 214–215°.

Discussion

The data for the alkaline decomposition of DTDA, presented in Table I, permit the drawing of several conclusions. Over the *p*H range of 11.9 to 12.4 the speed of decomposition increases markedly. The concentration of hydrogen sulfide rather rapidly attains a value which is independent of the *p*H value of the system (over the range studied) and of the extent of decomposition of the disulfide. The system does not seem to involve an over-all equilibrium; however, it would appear that substantially complete decomposition of DTDA could be carried out in a few hours at an alkalinity still recordable on the *p*H scale. The high percentages of the total sulfur accounted for warrant the conclusion that, under the conditions employed, mercaptoacetic acid and hydrogen sulfide account for substantially all the disulfide sulfur which has disappeared.

TABLE I

ALKALINE DECOMPOSITION OF DITHIODIACETIC ACID AT 35.2°

Medium, *N* NaOH	*p*H Init.	*p*H Final	Time, hr.	DTDA, *M* × 10⁴	Mercaptoacetate, *M* × 10⁴	H_2S, *M* × 10⁴	Total sulfur accounted for, %
0.0080	11.92	...	0	10.70	..	..	..
			1.5	8.85	2.01	1.28	97.9
			3.0	7.88	2.84	1.83	95.6
			5.5	7.44	3.71	2.10	96.6
	...	11.83	9.0	6.72	4.39	2.30	93.9
0.0101	12.03	...	0	10.70	..	..	..
			1.0	8.91	2.28	1.17	99.3
			2.0	8.12	3.20	1.90	99.7
			3.0	7.67	3.73	1.98	98.3
			5.0	7.27	4.35	2.00	97.4
	...	12.03	8.0	6.83	5.08	1.98	96.7
0.0141	12.24	..	0	11.03	..	..	..
			0.5	9.15	2.20	1.00	97.2
			1.0	8.16	3.01	1.30	93.3
			2.0	7.34	4.11	1.70	92.6
			3.0	6.97	4.85	1.95	93.8
	..	12.22	5.0	6.27	5.68	2.17	92.1
0.0201	12.39	...	0	10.75	..	..	..
			0.3	9.18	2.40	1.00	100.0
			0.7	8.01	3.57	1.52	97.0
			1.0	7.40	4.38	1.87	96.8
			1.5	6.98	5.08	1.87	96.1
			2.0	6.36	5.79	1.87	93.6
	..	12.35	3.0	6.06	6.39	1.90	93.8

(21) M. S. Budd and H. A. Bewick, *Anal. Chem.*, **24**, 1536 (1952).

TABLE II

ALKALINE DECOMPOSITION OF 2,2′-DIMETHYLDITHIODIACETIC ACID AT 35.2°

Medium, *N* NaOH	*p*H Init.	*p*H Final	Time, hr.	DMDT₄, *M* × 10⁴	2-Mercaptopropionate, *M* × 10⁴	H_2S, *M* × 10⁴	Total sulfur accounted for, %
0.0100	11.99	...	0	9.88	..	..	..
			3.0	9.68	0.15	0.14	99.7
			24.0	8.95	0.70	0.66	97.4
			48.0	8.18	1.15	1.30	95.2
	...	11.96	72.0	7.52	1.96	1.97	96.0
0.0500	12.56	...	0	10.10	..	..	..
			1.0	9.83	0.30	0.23	99.8
			3.0	9.19	1.00	0.80	99.8
			6.0	8.43	1.84	1.55	100.0
			12.0	7.10	3.30	2.79	100.4
			16.0	6.23	3.95	3.62	99.1
			24.0	5.39	4.84	4.70	100.6

TABLE III

ALKALINE DECOMPOSITION OF DITHIODIPROPIONIC ACID AT 35.2°[a]

Medium, *N* NaOH	*p*H Init.	*p*H Final	Time, days	DTDP *M* × 10⁴	3-Mercaptopropionate, *M* × 10⁴[b]	Total sulfur accounted for, %
0.1006	12.82	...	0	9.85	..	..
			5.9	9.60	0.20	98.3
			17.9	9.08	1.60	100.3
	...	12.82	26.9	8.76	2.24	100.3
1.006	...	...	0	9.83	..	..
			1.0	7.97	3.70	99.9
			2.0	6.63	6.20	99.0
			3.0	6.56	6.96	102.0
			5.0	5.56	7.16	93.0

[a] No H_2S detected at any time. [b] Perhaps actually a mixture of two isomeric mercaptans.

If, in each *p*H series, we calculate the ratios of moles of mercaptoacetic acid formed to moles of DTDA lost at each given time, and then calculate the arithmetic mean of the ratios, we get the following numbers for *p*H and average ratio, respectively: 11.92, 1.08; 12.03, 1.25; 12.24, 1.14; 12.39, 1.43. These averages should not be allowed to obscure the fact that there is some tendency for the ratios to increase in a given series as the amount of disulfide decomposed increases. This is most apparent for the first series where, of course, there are more data (not all included in Table I) for the initial stages of

decomposition. For any appreciable degree of decomposition the ratio is probably at least 1.20 and it seems to tend toward a maximum of 1.5.

All of the data available can be rationalized by the mechanism

$$2\text{-}^{-}OOCCH_2SSCH_2COO^{-} + 2OH^{-} \rightleftarrows 2\bar{O}OCCH_2SS\bar{C}HCO\bar{O} + 2H_2O$$

$$2\text{-}^{-}OOCCH_2S{-}S\bar{C}HCOO^{-} \longrightarrow 2\text{-}^{-}OOCCH_2S^{-} + 2S{=}CHCOO^{-}$$

$$S{=}CHCOO^{-} + OH^{-} \rightleftarrows O{=}CHCOO^{-} + HS^{-}$$

$$O{=}CHCOO^{-} + OH^{-} \longrightarrow H{-}C(OH){-}COO^{-} \quad (O^{-})$$

$$H{-}C(OH){-}COO^{-} + OH^{-} \longrightarrow H{-}C(O^{-}){-}COO^{-} + H_2O \quad (O^{-})$$

$$H{-}C(O^{-}){-}COO^{-} + S{=}CHCOO^{-} \xrightarrow{H^{-}} \bar{O}OCCOO^{-} + \bar{S}CH_2COO^{-} \quad (O^{-})$$

$$2\text{-}^{-}OOCCH_2S{-}SCH_2COO^{-} + 5OH^{-} \longrightarrow 3\bar{S}CH_2COO^{-} + HS^{-} + \bar{O}OCCO\bar{O} + 3H_2O$$

The first three steps are identical with those proposed by Rosenthal and Oster.[11] The remaining steps, however, as well as the quantitative data, are required for a rational justification of the over-all stoichiometric equation. While quantitative data on oxalate were not obtained, its presence was demonstrated qualitatively by a single preparative experiment carried out for that purpose. It is worth noting that the data on the alkaline decomposition of DTDA, published 27 years ago by Schöberl,[6] is qualitatively, and even semi-quantitatively, in agreement with ours, though the two interpretations differ considerably.

It will be noted that the stoichiometry calls for three molecules of mercaptan formed from each two molecules of disulfide. This is the ratio of 1.5 toward which the experimental ratios seem to tend. However, it is possible to formulate at least four variants of the Cannizzaro reaction, two of which yield glycolic acid rather than mercaptoacetic acid. It is possible to write a stoichiometric equation assuming the ratio to be 1.25.

$$4\text{-}^{-}OOCCH_2SSCH_2COO^{-} + 10OH^{-} \longrightarrow 5\text{-}^{-}OOCCH_2S^{-} + 2\text{-}^{-}OOCCOO^{-} + \bar{O}OCCH_2OH + 3HS^{-} + 5H_2O$$

To the extent that Cannizzaro reactions other than the one first hypothesized (involving both glyoxylate and thioglyoxylate) take place, experimental ratios less than 1.5 will be found.

From the data in Table II, in which the time unit is the day rather than the hour, the relatively greater stability of DMDT, as compared with that of DTDA, is seen. In DMDT the methyl groups not only decrease the original number of potentially acidic protons from four to two, but reduce the acidity of these remaining ionizable protons and hinder their attack. At *p*H 12, DMDT decomposed to the same extent in 3 days as DTDA did in 3 hours. A further difference is that in the case of DMDT the formation of mercaptan and hydrogen sulfide almost exactly parallel each other, and the average for the ratio of mercaptan formed to disulfide decomposed is close to unity: 0.85 at *p*H 11.99 and 1.08 at 12.56. The available data are completely consistent with the sequence of reactions

$$\bar{O}OC{-}C(H)(CH_3){-}S{-}S{-}C(H)(CH_3){-}COO^{-} + OH^{-} \rightleftarrows {}^{-}OC{-}C(H)(CH_3){-}S{-}S{-}\bar{C}(CH_3){-}COO^{-} + H_2O$$

$$\bar{O}OC{-}C(H)(CH_3){-}S{-}S{-}\bar{C}(CH_3){-}COO^{-} \longrightarrow \bar{O}OC{-}C(H)(CH_3){-}S^{-} + S{=}C(CH_3){-}COO^{-}$$

$$S{=}C(CH_3){-}COO^{-} + OH^{-} \rightleftarrows O{=}C(CH_3){-}COO^{-} + SH^{-}$$

$$\bar{O}OC{-}C(H)(CH_3){-}S{-}S{-}C(H)(CH_3){-}COO^{-} + 2OH^{-} \longrightarrow {}^{-}OOC{-}C(H)(CH_3){-}S^{-} + SH^{-} + CH_3COCOO^{-}$$

This scheme differs from the one previously proposed for DTDA only in that a thioketone (thiopyruvate) rather than a thioaldehyde is a postulated intermediate. But this important difference successfully accounts for the observed experimental difference: the unitary ratio of disulfide decomposed to mercaptan formed to hydrogen sulfide formed. Since no Cannizzaro reaction can take place, the metathetical reaction between thiopyruvate ion and hydroxide ion, displaced far to the right, is the final one in the decomposition studied. In a single experiment on a preparative basis pyruvic acid was isolated as its 2,4-dinitrophenylhydrazone.

Schöberl[22] has reported that DTDP is not appreciably attacked by alkali. The data in Table III show that this compound is indeed much more resistant to alkaline decomposition than either of the disulfides previously discussed: barely 10% decomposition in 22 days at *p*H 12.82. The increase in stability is consonant with the decrease in acidity of the potentially ionizable protons, comparable to that found in going from malonic acid to succinic acid. Here, too, however, though at a different level, the accelerating effect of increasing alkalinity on speed of decomposition is quite apparent. Two experimental facts require that the mechanism for this decomposition be distinctly different from that for the other two disulfides. First, not a trace of hydrogen sulfide is detectable, even when 50% of the disulfide has decomposed. Second, the disulfide is converted quantitatively into mercaptan. At present we are seeking experimental evidence in support of a mechanism involving the formation of

(22) A. Schöberl, *Ber.*, **70B**, 1186 (1937).

two mercaptans in equivalent amounts: β-mercaptopropionic acid and α-hydroxy-β-mercaptopropionic acid.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ARIZONA STATE UNIVERSITY, TEMPE, ARIZ.]

Potential Purine Antagonists. XXVI. Preparation of Certain 8-Triazenopurine Nitrogen Mustards[1]

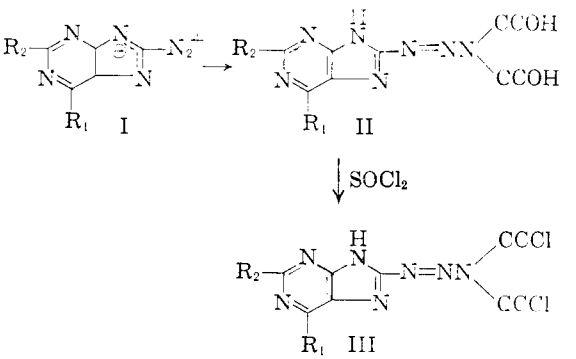
BY GERHARD A. USBECK,[2] JESSE W. JONES AND ROLAND K. ROBINS

RECEIVED SEPTEMBER 22, 1960

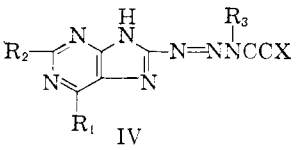
A number of 8-diazopurines has been coupled with diethanolamine and the resulting compounds treated with thionyl chloride to give the novel 8-triazenopurine nitrogen mustards (III). Several of these new derivatives have been prepared by coupling the appropriate 8-diazopurine directly with β,β-dichlorodiethylamine hydrochloride under carefully controlled conditions.

Bergel[3] first introduced the concept that several alkylating agents which are active in cancer chemotherapy can be considered as composed of an alkylating function and a "carrier" moiety. Recent synthesis of 5-bis-(2-chloroethyl)-aminouracil[4,5] and a study of the antitumor properties of this compound[4-7] have focused attention on this idea. The possibility that a certain biologically important carrier might provide nitrogen mustards of high specificity and less gross toxicity to the host provides an interesting area for synthetic study. Our current interest in purine antagonists has prompted an investigation which would utilize certain purine derivatives as carriers of the nitrogen mustard grouping because of the known biological importance of various purines in nucleic acid biosynthesis.

A literature survey revealed that DiPaco and Tauro[8] have previously reported the synthesis of three purines containing nitrogen mustard groupings, 6-bis-(β-chloroethyl)-aminopurine, 6-bis-(β-chloroethyl)-amino-2,8-dichloropurine and 7-bis-(β-chloroethyl)-amino-1,3-dimethylethylpurine-2,6-dione. However, to date no biological activity has been reported for these derivatives. In a preliminary study, position 8 was selected as the best position for substitution of the nitrogen mustard function since this would then allow the functional groups of the naturally occurring purines in positions 2 and 6 and allow position 9 to be free for possible ribosidation. It was decided that the nitrogen mustard function should be slightly removed from the 8-position to prevent possible cyclization with the imidazole nitrogens. Recent studies of various 8-diazopurines (I)[9] suggested that coupling might occur with diethanolamine. This reaction indeed proceeded readily to give the corresponding 8-[bis-(2-hydroxyethyl)-triazeno]-purine (II) which was then treated with thionyl chloride to give the desired 8-[bis-(2-chloroethyl)-triazeno]-purine (III).



Several secondary alkylamines containing a β-hydroxyethyl group were likewise coupled to the appropriate 8-diazopurine followed by treatment with thionyl chloride to give purines containing a "one-arm" mustard of the type IV, X = Cl. The mustards of this type and their hydroxy intermediates are listed in Table I. These compounds were



essentially prepared by the procedure described in the Experimental section for the appropriate corresponding 8-[bis-(2-chloroethyl)-triazeno]-purine. The 8-diazopurines chosen for study were 8-diazoadenine,[9] 8-diazoguanine,[9] 8-diazohypoxanthine,[9] 8-diazoxanthine[9] and 8-diazotheophylline.[9] It was discovered during the course of investigation that under certain conditions coupling could be effected directly with bis-(2-chloroethyl)-amine hydrochloride to give the desired 8-[bis-(2-chloroethyl) triazeno]-purine. Thus, in the case of 8-[bis-(2-chloroethyl)-triazeno]-theophylline (VI), this compound was prepared both by coupling 8-diazothe-

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