With reference to the unprecedented color changes exhibited by $Pt(en)X_3$ when ground, the data given above rule out both isomerization and crystalline polymorphism. The color change from green to purple $Pt(en)Br_3$ is neither temperature nor pressure dependent. It therefore appears that this change is related to either particle size or a selective cleavage along a specific plane which then appears purple by reflected light. Both electron photomicrographs and the petrographic examination showed that the particle size of the green and purple forms is not significantly different but that the individual particles of the purple form consist of aggregates of very small particles. Thus a random orientation of small particles would render the purple particles opaque to polarized light in all positions. The reason for the difference in color by ordinary white light is less readily understood. If selective cleavage occurs and if the actual crystal size of the purple form is much smaller than that of the green, it is reasonable that color should be more dependent on reflected light owing to the increased surface area.

AUSTIN, TEXAS

[CONTRIBUTION FROM THE NORSK HYDRO'S INSTITUTE FOR CANCER RESEARCH, THE NORWEGIAN RADIUM HOSPITAL]

The Equilibrium Constants and Oxidation–Reduction Potentials of Some Thiol–Disulfide Systems[1]

BY LORENTZ ELDJARN AND ALEXANDER PIHL

RECEIVED APRIL 10, 1957

A new procedure for the determination of equilibrium constants of thiol–disulfide systems is described. The procedure has been used in the study of the equilibria of five X-ray protective thiols with cystine and glutathione, respectively. The approximate, practical reaction rates, and the appropriate equilibrium constants were measured at *p*H 7.4 and 37°. In all instances *K*-values were found which imply that high concentrations of mixed disulfides were present in the equilibrium mixtures. On the basis of the equilibrium constants, which were obtained at the desired *p*H and temperature under truly reversible conditions, the standard oxidation–reduction potentials relative to that of glutathione were calculated. The data have enabled us to calculate the oxidation–reduction potential of cystine/cysteine relative to that of glutathione.

In the present report a new procedure for the determination of the equilibrium constants of thiol–disulfide systems is described.

When a thiol is allowed to interact with a disulfide several reactions take place[2]

$$XSH + YSSY \rightleftarrows XSSY + YSH \quad (1)$$

$$XSSY + XSH \rightleftarrows XSSX + YSH \quad (2)$$

The equilibrium constants of such systems are defined as

$$K_2 = \frac{[XSSY][YSH]}{[XSH][YSSY]}; \quad K_3 = \frac{[XSSX][YSH]}{[XSH][XSSY]};$$

$$K_1 = K_2K_3 = \frac{[XSSX][YSH]^2}{[YSSY][XSH]^2};$$

$$K_4 = \frac{K_2}{K_3} = \frac{[XSSY]^2}{[XSSX][YSSY]}$$

Our previous determinations of *K*-values were based on measurements of the mixed disulfide content in equilibrium mixtures, in the presence and in the absence of oxygen.[2b] This method was successfully applied to the glutathione + N,N′-diacetylcystamine system. However, attempts to use this method in the study of other systems were uniformly unsuccessful.

The procedure here to be described for the determination of equilibrium constants of thiol–disulfide systems is based on the direct measurement of the equilibrium concentrations of three of the five participating molecular species. After incubation of a thiol with a disulfide, one of which is labeled with S^{35}, the three labeled molecular species (equations 1 and 2) are separated by electrophoresis at *p*H 2 in mercuric acetate treated paper. Paper electrophoresis in the absence of mercuric acetate, as previously used,[2b] permits the separation of mixed disulfides from the labeled thiol and symmetrical disulfides, which migrate together. The pretreatment of the electrophoresis paper with mercuric acetate retards the migration of the thiols so that the three labeled compounds appear as distinct peaks. When the concentrations of the three labeled components have thus been determined, the concentrations of the unlabeled constituents are obtained easily by difference.

In the present paper the procedure is used for the determination of the equilibrium constants, at *p*H 7.4 and 37°, of systems containing cystine or oxidized glutathione and various sulfur containing X-ray protective thiols. Our approach seems to offer a solution to the controversial question of the relative oxidation–reduction potentials of thiol–disulfide systems under physiological conditions.

Experimental

Chemicals.[3]—**Cystamine-S^{35}** and **N,N′-diacetylcystamine-S^{35}** were synthesized as previously described.[4,2b] The corresponding thiols were prepared by electrolytic reduction of the disulfides.[5] **Cystine-S^{35}** and **glutathione-S^{35}** were purchased from The Radiochemical Centre, Amersham, England, and from Schwarz Laboratories, Inc., Mount Vernon, N. Y., U. S. A.

Tetramethylcystamine, tetraethylcystamine and N-pi-

(1) Supported by grants from The Norwegian Cancer Society and from The Norwegian Research Council for Science and the Humanities.

(2) (a) T. Bersin and J. Steudel, *Ber.*, **71B**, 1015 (1938); (b) L. Eldjarn and A. Pihl, *J. Biol. Chem.*, **225**, 499 (1957).

(3) The following abbreviations are used in this paper: CSH = cysteine, GSH = reduced glutathione, AcRSH = acetylcysteamine, RSH = cysteamine, Me_2RSH = N-dimethylcysteamine, Et_2RSH = N-diethylcysteamine, and PRSH = N-piperidylcysteamine. E_0' = standard oxidation–reduction potential, at *p*H 7.4 and 37°, relative to that of GSSG/GSH, with sign opposite to that used by Latimer (W. M. Latimer, "Oxidation Potentials," Prentice-Hall, Inc., New York, N. Y., 1952).

(4) L. Eldjarn, *Scand. J. Clin. & Labor. Invest.*, suppl. 13 (1954).

(5) L. Eldjarn and A. Pihl, *J. Biol. Chem.*, **223**, 341 (1956).

peridylcysteamine were generously supplied by Deutsche Gold und Silber Scheideanstalt, vormals Roessler, Frankfurt am Main, Germany.

Incubation Procedure.—The reactants (0.1 to 1 mg. amounts) were incubated at 37° for the desired length of time in oxygen-free phosphate buffer (0.067 *M*). The solution was made 0.02 *M* with respect to Versene to eliminate further the possibility of spontaneous oxidation.[2b] The final *p*H of the reaction mixture was determined at the end of the experiment.

Analytical Procedure.—After the incubation, the reaction mixture was acidified to *p*H 2 and subjected to paper electrophoresis on paper strips (Whatman No. 1, 1.8 cm. width) at 1 ma. per strip. Prior to the equilibration the paper strips were immersed in 0.3 *M* phthalate buffer, *p*H 2, containing 0.01 *M* mercuric acetate. The excess liquid was removed by touching the strips with a sheet of filter paper. The anode and cathode chambers contained the phthalate buffer without mercuric acetate. After the electrophoresis the radioactivity on the paper strips was measured by means of a Geiger–Müller strip counter, the radioactivity pattern being obtained graphically.

In Fig. 1 a typical radioactivity pattern obtained is shown. Clearly, the three labeled components (RS*S*R, RS*H, CSS*R) of the equilibrium mixture become completely separated by the described method of electrophoresis. From the areas of their peaks, the concentrations can be calculated when the initial radioactivity concentration is known. Similarly, complete separation of the three radioactive components was obtained in all systems here studied.

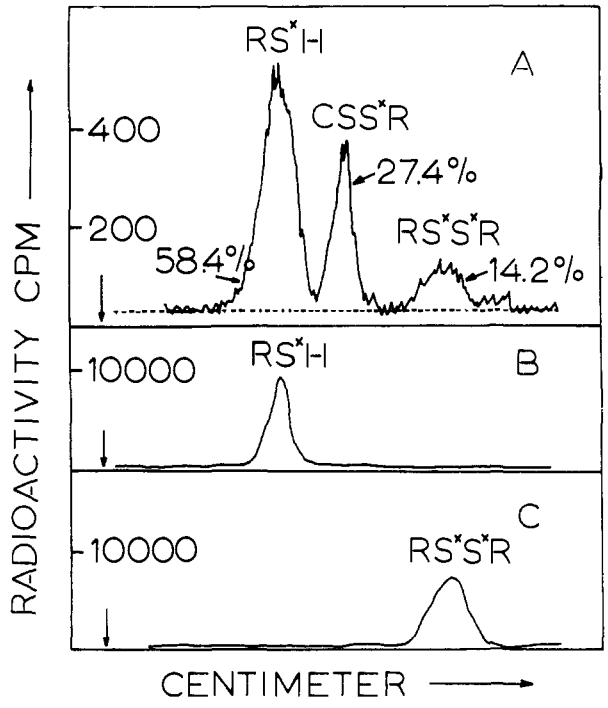


Fig. 1.—The paper electrophoretic separation of the three labeled components of the CSH + RS*S*R system: A, 2.50 × 10^{-3} *M* CSH was incubated for 15 minutes with 0.54 × 10^{-3} *M* RS*S*R at *p*H 7.4 and 37°. The mixture was acidified to *p*H 2 and subjected to electrophoresis in mercuric acetate treated paper. For further details see the text. B and C, paper electrophoresis of RS*H and RS*S*R, respectively.

In each equilibrium experiment, 8 to 14 separate samples were analyzed. The average percentage of radioactivity within each peak was used for the calculation of the equilibrium concentration of the corresponding compound. The equilibrium concentrations of the two remaining unlabeled constituents were obtained by difference, and the equilibrium constants were subsequently calculated. Although we have previously shown that the thiols enter such reactions in the ionized form,[2b] the dissociation of the thiols has not been taken into consideration in the present calculation of the *K*-values.

The practical, approximate reaction rates were determined by stopping the reactions by acidification after varying lengths of time and by analyzing the reaction mixture as described.

Control Experiments.—In strongly acid solution mercuric salts are known to dismutate disulfides to the corresponding thiols and sulfinic acids.[6,7] It was therefore necessary to test the stability of the labeled symmetrical and unsymmetrical disulfides under our conditions of analysis. Mixtures of symmetrical and unsymmetrical disulfides from the systems to be studied were prepared by bubbling oxygen through solutions of the labeled thiols and disulfides at *p*H 7.4[2b] till no thiols could be detected. Upon paper electrophoresis of the acidified mixtures, no thiols reappeared, demonstrating that the labeled disulfides of our systems are stable under our conditions of separation.

Results

Equilibrium Constants.—In the present report the equilibrium constants K_2 and K_3 refer to the situation as written in equations 1 and 2, when the "biological component" (CSSC or GSSG) in the disulfide form interacts with the X-ray protective compound in the thiol form. In some cases the actual experiments were, for practical reasons, performed in the reversed order. The equilibrium constants obtained under the latter conditions (K_2' and K_3') are related to the above constants by the equations: $K_2 = 1/K_3'$ and $K_3 = 1/K_2'$.

The results for the ten thiol–disulfide systems here studied, are shown in Table I, in which details of the experimental conditions also are given.

The commercially obtained labeled glutathione was found to contain 20.35% of the radioactivity in the form of GS*S*G. However, this could be corrected for easily in the calculation of the equilibrium constants.

The Standard Oxidation–Reduction Potentials.—The reversible nucleophilic exchange reactions 1 and 2 also can be treated as oxidation–reduction reactions. The standard oxidation–reduction potentials, at *p*H 7.4 and 37°, of the three couples involved ($E_0'_{XSSX/XSH}$, $E_0'_{YSSY/YSH}$, $E_0'_{XSSY/XSH,YSH}$), are related by the equations

$$E_0'_{XSSX/XSH} = E_0'_{YSSY/YSH} - 0.0307 \log (K_2K_3) \quad (3)$$

$$E_0'_{XSSY/XSH,\,YSH} = E_0'_{YSSY/YSH} - 0.0307 \log K_2 \quad (4)$$

Clearly, when the equilibrium constants are known, the relative standard oxidation–reduction potentials can be calculated.

In general, when the equilibrium constants of the systems XSH + GSSG and XSH + CSSC are known, $E_0'_{CSSC/CSH}$ and $E_0'_{GSSG/GSH}$ are related by the equation

$$E_0'_{CSSC/CSH} = E_0'_{GSSG/GSH} + 0.0307 \log \frac{(K_2K_3)_{XSH+CSSC}}{(K_2 \cdot K_3)_{XSH+GSSG}} \quad (5)$$

By the use of eq. 5 it is possible to calculate, from our five independent sets of equilibrium constants, the standard oxidation–reduction potential of cystine/cysteine, relative to that of glutathione. The data are given in Table II.

The standard potentials of our 15 oxidation–reduction couples, relative to that of glutathione, are presented in Table III. The data have been

(6) T. F. Lavine, *J. Biol. Chem.*, **117**, 309 (1937).

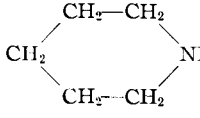
(7) L. Eldjarn, A. Pihl and A. Sverdrup, *ibid.*, **223**, 353 (1956).

TABLE I

THE EQUILIBRIUM CONSTANTS AT 37° OF VARIOUS THIOL–DISULFIDE SYSTEMS

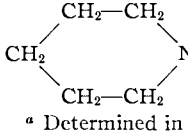
$$XSH + CSSC \underset{}{\overset{K_2}{\rightleftharpoons}} XSSC + CSH$$

$$XSSC + XSH \overset{K_3}{\rightleftharpoons} XSSX + CSH$$

System	Compd. incubated	No. of expt.	Time needed to reach equilibrium,[a] min.	Incubation time, min.	Final *p*H	Electrophoresis time, hr.	Migration distance, cm. XS*H	XS*SC	XS*S*X	K_2 Mean	K_2 Av. dev.[b]	K_3 Mean	K_3 Av. dev.[b]
$CH_3CONHCH_2CH_2SH$ + CSSC	$(AcRS^*)_2$ + CSH	4	10	45–60	7.32–7.34	6.5	1	6.5	5	5.0	1.25	0.62	0.05
$ClH_3NCH_2CH_2SH$ + CSSC	RS*S*R + CSH	6	1	15–30	7.34–7.40	3.5	7	9.5	13.5	4.76	0.68	.75	.05
							CS*H	CS*SX	CS*S*C				
$(CH_3)_2NH(Cl)CH_2CH_2SH$ + CSSC	Me_2RSH + CS*S*C	2	2	20	7.44	3.5	6	9.5	8	2.29	.11	.30	.01
$(CH_3)_2NH(Cl)CH_2CH_2SH$ + CSSC	$(Me_2RS)_2$ + CS*H	2	2	40	7.32	3.5	6	9.5	8	2.31	.26	.42	.02
$(C_2H_5)_2NH(Cl)CH_2CH_2SH$ + CSSC	Et_2RSH + CS*S*C	3	3	20	7.41–7.44	3.5	6	9	8	2.82	.15	.36	.02
$(C_2H_5)_2NH(Cl)CH_2CH_2SH$ + CSSC	$(Et_2RS)_2$ + CS*H	2	3	30	7.50	3.5	6	9	8	2.76	.18	.37	.03
 + CSSC	PRSH + CS*S*C	4	3	20–30	7.40–7.41	3	4	8	6	2.81	.40	.31	.06

$$XSH + GSSG \underset{}{\overset{K_2}{\rightleftharpoons}} XSSG + GSH$$

$$XSSG + XSH \overset{K_3}{\rightleftharpoons} XSSX + GSH$$

							Migration distance, cm. XS*H	XS*SG	XS*S*X				
$CH_3CONHCH_2CH_2SH$ + GSSG	$(AcRS^*)_2$ + GSH	5	12	25–45	7.20–7.36	6	1.5	6	4.5	2.86	0.56	0.28	0.02
$ClH_3NCH_2CH_2SH$ + GSSG	RS*S*R + GSH	4	2	15–30	7.40	3	9.5	7	11.5	5.0	1.0	.34	.02
							GS*H	GS*SX	GS*S*G				
$(CH_3)_2NH(Cl)CH_2CH_2SH$ + GSSG	$(Me_2RS)_2$ + GS*H	5	2	45–60	7.30–7.36	4	6	10	8	1.56	0.15	0.32	0.03
$(C_2H_5)_2NH(Cl)CH_2CH_2SH$ + GSSG	$(Et_2RS)_2$ + GS*H	3	3	45–60	7.30–7.34	4.5	6	8.5	7	1.92	.20	.29	.01
 + GSSG	$(PRS)_2$ + GS*H	4	2	45–80	7.31–7.34	6	6	9.5	8	1.89	.11	.25	.01

[a] Determined in separate experiments. [b] Average deviations from the mean.

TABLE II

THE STANDARD OXIDATION-REDUCTION POTENTIAL OF CYSTINE/CYSTEINE, RELATIVE TO THAT OF GLUTATHIONE[a]

	CSSC		GSSG		$E_0'_{CSSC/CSH}$,[b]
	K_2	K_3	K_2	K_3	v.
AcRSH	5.00	0.62	2.86	0.28	0.0180
RSH	4.76	.75	5.00	.34	.0099
Me_2RSH	2.30	.36	1.56	.32	.0068
Et_2RSH	2.79	.36	1.92	.29	.0079
PRSH	2.81	.31	1.89	.25	.0081
				Mean	0.0101

[a] The values have been calculated by means of eq. 5, on the basis of the five independent sets of equilibrium constants, summarized in the table. [b] E_0' = standard oxidation–reduction potential, at *p*H 7.4 and 37°, relative to that of glutathione ($E_0'_{GSSG/GSH}$ = 0 v.).

calculated by the use of eq. 3 and 4. In the calculations the average value of $E_0'_{CSSC/CSH^-}$ + 0.0101 volt (Table II), has been used.

Discussion

In the present paper the equilibria involving five "foreign" thiols + cystine and glutathione, respectively, have been studied. The particular thiols here used were chosen for two reasons. Firstly, they all possess protective activity against ionizing radiation. Secondly, they belong to a homologous series, the members of which differ only in the nature of the substituents on the nitrogen atom.

None of the equilibrium constants here measured depart very much from unity. Readily detectable amounts of all constituents were therefore present at equilibrium. In all systems measurements were carried out at different concentrations of reactants. The over-all precision of the measurements is indicated by the average deviations from the mean values. The fact that the values for the relative oxidation–reduction potential of cystine/cysteine, calculated on the basis of independent sets of *K*-values, show satisfactory agreement, testifies to the reliability and accuracy of the present method for the determination of equilibrium constants. The values for the N-acetylcysteamine + oxidized glutathione system found with the present procedure, are in satisfactory agreement with those found with our previous method.[2b]

The equilibrium constants here measured refer to 37° and to the physiological *p*H range. For technical reasons we were unable to achieve *p*H 7.40 in all instances. It is clear that, unless the *pK*-values of the thiols involved are identical, variations in the *p*H of the reaction mixture will affect the equilibrium constants.[2b] The effects of the small variations of *p*H in our experiments cannot be evaluated from the present data.

In Table II the equilibrium constants of our systems have been listed according to the anticipated basic strength of the nitrogen function of the protective compounds. It appears that, in the systems involving cystine, an increase in the nitrogen basicity is associated with a decrease in the K_2 and K_3 values. More data are needed to warrant generalizations as to the effects of inductive, electromeric and steric factors on the equilibrium constants.

During the past decades considerable efforts have been devoted to measurements of the oxidation–reduction potentials of thiol–disulfide systems; however, it is generally agreed that, due to the well known difficulties encountered in potentiometric studies of such systems, the available data are fraught with numerous inconsistencies and uncertainties.[8] The most reliable measurements seem to be those carried out under strongly acid conditions,[9] but it is doubtful whether the extrapolations to the physiological *p*H range are valid.[8] The oxidation–reduction potentials presented in this paper are derived from equilibrium constants, which were measured at the desired *p*H and temperature, under truly reversible conditions. Thus, in several instances equilibrium was obtained by bringing "products" together initially, as well as reactants. The oxidation–reduction potential of cystine/cysteine was calculated to be 0.01 volt higher than that of GSSG/GSH, a value which differs significantly from those reported in the literature.[8] However, for reasons given above, the present measurements would seem to merit confidence.

The present data on oxidation–reduction potentials have been expressed relative to the potential of GSSG/GSH. In so far as the dissociation of the thiols has not been taken into consideration, they are apparent values. Work is now in progress to determine the oxidation–reduction potentials, relative to the normal hydrogen electrode.

From the point of view of chemical protection it is of interest to observe that the practical, approximate reaction rates were fairly high in all systems, except those involving N,N'-diacetylcystamine.

TABLE III

THE STANDARD OXIDATION–REDUCTION POTENTIALS OF VARIOUS DISULFIDE/THIOL COUPLES[a]

Oxidation–reduction couple	E_0', v.[b]	Oxidation–reduction couple	E_0',[b] v.	Oxidation–reduction couple	E_0',[b] v.
$(AcRS)_2$/AcRSH	+0.0030	AcRSSG/ArRSH,GSH	−0.0140	AcRSSC/AcRSH,CSH	−0.0114
RSSR/RSH	− .0071	RSSG/RSH,GSH	− .0215	RSSC/RSH,CSH	− .0107
$(Me_2RS)_2$/Me_2RSH	+ .0093	Me_2RSSG/Me_2RSH,GSH	− .0059	Me_2RSSC/Me_2RSH,CSH	− .0010
$(Et_2RS)_2$/Et_2RSH	+ .0078	Et_2RSSG/Et_2RSH,GSH	− .0087	Et_2RSSC/Et_2RSH,CSH	− .0036
$(PRS)_2$/PRSH	+ .0100	PRSSG/PRSH,GSH	− .0085	PRSSC/PRSH,CSH	− .0037

[a] The potentials in columns 1 and 2 have been calculated by the use of the equilibrium constants for the systems involving glutathione. The data in column 3 have been obtained by the use of the corresponding constants for the systems involving cystine. These latter potentials have been expressed relative to that of glutathione by adding the relative oxidation–reduction potential of cystine (0.010 v.). [b] E_0' = standard oxidation–reduction potential, at *p*H 7.4 and 37°, relative to that of glutathione ($E_0'_{GSSG/GSH}$ = 0 v.).

(8) M. Calvin, in S. Colowick, *et al.*, "Glutathione," Academic Press, Inc., New York, N. Y., 1954, p. 3.

(9) L. R. Rykland and C. L. A. Schmidt, *Univ. Calif. Publ. Physiol.*, **8**, No. 17, 257 (1944).

The equilibrium constants of the first reaction (the K_2-values) all exceed unity, whereas all the K_3-values are less than one. It can be shown that, under such conditions, high concentrations of mixed disulfide are present in equilibrium mixtures.[2b] The findings emphasize the significance in general of mixed disulfides in thiol–disulfide equilibria,[2b,10] and they support the mechanism previously suggested for the mode of action of these X-ray protective agents.[11,12] A discussion of the relationship between the equilibrium constants and the X-ray protective ability of sulfur containing compounds will be the subject of a forthcoming paper.

(10) L. Eldjarn and A. Pihl, *Acta Chem. Scand.*, **10**, 1054 (1956).

(11) L. Eldjarn, A. Pihl and B. Shapiro, "Proceedings of the International Conference on the Peaceful Uses of Atomic Energy," Geneva, Vol. 11, 1956, p. 335; L. Eldjarn and A. Pihl, in J. S. Mitchell, *et al.*, "Proc. Fourth Internat. Conf. on Radiobiol.," Oliver and Boyd, London (1956), p. 249.

(12) A. Pihl and L. Eljdarn, in Proc. Fifth Internat. Conf. on Radiobiol., Oliver and Boyd, London, in press.

OSLO, NORWAY

[CONTRIBUTION FROM THE U. S. NAVAL ORDNANCE TEST STATION]

Thermal Decomposition of Cyclopentane-d_2 and Cyclopentane–Acetone-d_6 Mixtures. Reactions of the Allyl Radical

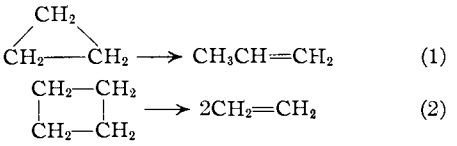
BY JAMES R. MCNESBY AND ALVIN S. GORDON

RECEIVED MARCH 15, 1957

The pyrolysis of cyclopentane is shown to be at least partly free radical. The inhibition of acetone pyrolysis by cyclopentane and the reactions of the allyl radical are discussed. It is shown that allyl radicals abstract H and D very poorly at temperatures below 400°, but that they abstract quite well at temperatures around 500°. The implications for inhibition by propylene are discussed.

Introduction

The thermal isomerization of cyclopropane[1] to propylene has been shown to be intramolecular. Also, the thermal pyrolysis of cyclobutene[2] has been shown to be intramolecular.

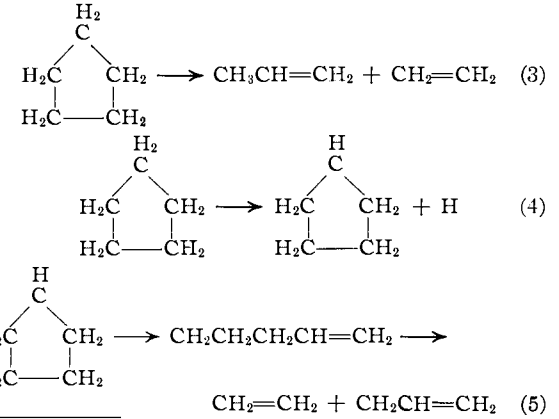


(1)

(2)

The pyrolysis of cyclopentane has been previously studied[3,4] and shown to consist of two reactions, (1) a dehydrogenation reaction to form cyclopentadiene, and (2) a ring cleavage reaction to form propylene and ethylene.

There are two possible mechanisms whereby cyclopentane can pyrolyze to ethylene and propylene: intramolecularly or *via* free radicals.



(3)

(4)

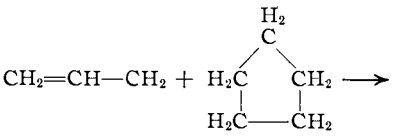
(5)

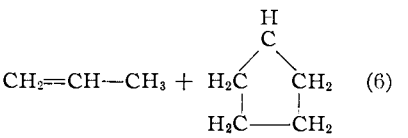
(1) J. R. McNesby and Alvin S. Gordon, *J. Chem. Phys.*, **25**, 582 (1956).

(2) C. T. Genaux, F. Kern and W. D. Walters, THIS JOURNAL, **75**, 6196 (1953).

(3) D. Vanas, N. Lodge and W. Walters, *ibid.*, **74**, 451 (1952).

(4) L. Kuchler, *Z. physik. Chem.*, **B53**, 307 (1943).





(6)

With the object of finding out which of these mechanisms is correct, the pyrolysis was carried out in the presence of D_2 and of acetone-d_6. Photolysis of the latter system also was studied over a temperature range.

Experimental

Pyrolyses were carried out by immersion of Pyrex reaction flasks in a molten salt-bath at about 500°. The pressure was 110 mm. of cyclopentane measured at room temperature. In addition, equimolar mixtures of cyclopentane and D_2 were made up to a total pressure of 110 mm. measured at room temperature. Photolyses and analyses were carried out as described previously.[5] A medium pressure mercury arc was the light source. In all cases 5% or less of the reactants were consumed.

Results and Discussion

It was found that in the presence or absence of D_2, the important pyrolysis products are ethylene and propylene in roughly equal amounts. The rough equivalence of the amounts of ethylene and propylene was demonstrated by comparing the gas chromatogram of a 1:1 mixture of ethylene and propylene with that obtained in the pyrolysis of cyclopentane. H_2 is one of the products. One of the ways to generate hydrogen is *via* hydrogen atoms. If reaction 4 is important, it could be followed by

(5) J. R. McNesby and A. S. Gordon, THIS JOURNAL, **76**, 4196 (1954).