Table IV but were not considered in the determination of the average rate constant. The extreme deviation of this calculated rate constant from the average is undoubtedly due to the unavoidable errors in observing the optical rotations. These errors would be appreciable relative to the expected decrease in optical rotation for this substrate of low initial optical activity.

That the thermally produced decrease in optical rotation of the unreacted α-pinene is not a function of its initial optical activity is shown by the racemization rate constants obtained from the prepared sample containing α-pinene of different optical rotations. These constants with corresponding data are contained in Table VI. The data are presented as the average values of duplicate determinations. The per cent. average deviation of all the constants was 5.6% and the average deviation of the individually calculated results was 0.24 × 10^5. The average rate constant was almost identical with that determined from the α-pinene samples recovered from the previously studied reaction mixtures which had been heated at the same temperature.

TABLE VI

RACEMIZATION OF α-PINENE OF DIFFERENT INITIAL OPTICAL ROTATIONS

Temperature, 204.5°; time heated, 1620 min.

Tube nos.	α_i (2 dm.), 25°	α_t (2 dm.), 25°	$k_t \times 10^5$, min.$^{-1}$
101–102	−80.75	−70.54	4.2
103–104	−60.75	−53.02	4.2
105–106	−45.54	−39.49	4.4
107–108	−31.40	−27.25	4.4
109–110	+10.80	+ 9.50	4.0
111	+21.95	+18.92	4.6
113–114	+38.70	+33.80	4.2
115–116	+58.05	+50.03	4.6
		Av. k	4.3×10^{-5}
		Av. dev.	0.24×10^{-5}
		% Av. dev.	5.6

The energies of activation for these reactions were calculated from the standard Arrhenius equation which relates the rate constants observed at two different temperatures. These are listed in Table VII. The values of log s, in equation (4), calculated at 189.5° are also included in the table.

$$\log s = \log k + E/2.303RT \qquad (4)$$

TABLE VII

SUMMARY OF RESULTS

Reaction	$k \times 10^5$ min.$^{-1}$ (189.5°)	$k \times 10^5$ min.$^{-1}$ (204.5°)	$E_{\text{act.}}$ cal./mole	log s (time in min.)
Racemization of α-pinene	0.93	4.2	44,150	15.8
α-Pinene ⟶ dipentene	5.96	22.9	37,000	13.3
α-Pinene ⟶ *allo*-ocimene	3.03	13.0	42,650	15.6

These values are of the order of magnitude which might be expected for such first order thermal reactions.

Summary

1. The reaction velocities at 189.5 and 204.5° and the energies of activation have been determined for the thermal racemization of α-pinene and the thermal isomerization of α-pinene to dipentene and *allo*-ocimene. These isomerization reactions were of the expected first order, indicating that there were no obscure complicating effects.
2. The observed rate of racemization evidences no dependence upon the initial optical rotation of the α-pinene.
3. The dipentene and *allo*-ocimene are produced from the α-pinene by simultaneous first order side reactions.
4. The racemization of α-pinene requires the greatest activation energy and the thermal isomerization of α-pinene to dipentene the least.

GAINESVILLE, FLORIDA RECEIVED JULY 15, 1946

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE]

Oxidation–Reduction Potentials of Thiol–Dithio Systems: Thiourea–Formamidine Disulfide

BY PAUL W. PREISLER AND LOUIS BERGER

Many attempts have been made to estimate the oxidation–reduction potentials of theoretically reversible systems of the type, 2 Thiol − 2H ⇄ Dithio (2RSH − 2H ⇄ RSSR). The methods used were[1] direct measurement of electrode potentials and[2] the action of oxidation–reduction potential indicators or other reactants of known potential upon the reductant or upon the oxidant.[3] Except possibly for the recent work by Ryklan and Schmidt[4] on such systems, the data of these investigations do not comply with criteria for freely reversible systems; nor do they approach the degree of accuracy readily

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[1] J. C. Ghosh and S. C. Ganguli, *Biochem. J.*, **28**, 381 (1934); D. E. Green, *ibid.*, **27**, 678 (1933); J. C. Ghosh, S. N. Raychandhuri and S. C. Ganguli, *J. Ind. Chem. Soc.*, **9**, 43 (1932); J. W. Williams and E. M. Drissen, *J. Biol. Chem.*, **87**, 441 (1930); E. K. Fischer, *ibid.*, **89**, 753 (1930); L. Michaelis and L. B. Flexner, *ibid.*, **79**, 689 (1928); E. C. Kendall and D. F. Loween, *Biochem. J.*, **22**, 669 (1928); D. C. Harrison and J. H. Quastel, *ibid.*, **22**, 683 (1928); M. Dixon and H. E. Tunnicliffe, *ibid.*, **21**, 844 (1927); E. C. Kendall and F. F. Nord, *J. Biol. Chem.*, **69**, 295 (1926); M. Dixon and J. H. Quastel, *J. Chem. Soc.*, **123**, 2943 (1923).

[2] H. Borsook, E. L. Ellis and H. M. Huffman, *J. Biol. Chem.*, **117**, 281 (1937); J. S. Fruton and H. T. Clarke, *ibid.*, **106**, 667 (1934); E. C. Kendall and F. F. Nord, *ibid.*, **69**, 295 (1926).

[3] P. W. Preisler, *ibid.*, **87**, 767 (1930).

[4] L. R. Ryklan and C. L. A. Schmidt, *Univ. Calif. Pub. Physiol.*, **8**, 257 (1944).

attained with many organic and inorganic systems.

The potentials reported by Ryklan and Schmidt,[4] for several examples of the thiol-dithio type system, do satisfy the appropriate potential equations. Their results were obtained by titration of the reductants (RSH) with suitable oxidizing agents; and in the case of cysteine–cystine were obtained also with known mixtures of these two components in molar hydriodic acid, which acid was believed necessary for the establishment of the equilibria at the electrode. This work[4] was brought to our attention by Professor Philip A. Shaffer, who suggested a similar potentiometric study of thiourea which has been shown by Werner[5] to form a reversible system with iodide–iodine.

Our experimental results with this system, thiourea–formamidine disulfide, fulfil the requirements for a truly reversible system in satisfying the equations applicable to this type. In acid solutions the equilibrium potentials are rapidly attained and agree with those predicted, generally to within 0.001 volt. This holds for oxidative and for reductive titrations, for known mixtures of oxidant and reductant, for changes in total concentration by dilution at a fixed ratio of oxidant and reductant, as well as for changes in acidity of the solutions below about *p*H 3.

The oxidation–reduction potential apparatus and procedure for conducting titrations in the absence of oxygen, previously described[6] were employed. The potentials were measured at 30° with bright platinum wire electrodes against a saturated potassium chloride–calomel half-cell joined by a saturated potassium chloride–agar bridge. The junction potentials are ignored. The solutions were stirred by purified nitrogen gas. Dilute sulfuric or hydrochloric acids of known concentrations were used as the solvent. The recorded potentials are converted to E_h values (normal hydrogen electrode as zero).

The materials and solutions were prepared from commercial analytical grade reagents. The thiourea was recrystallized from hot aqueous solution.

Several salts of formamidine disulfide were prepared by oxidizing thiourea in a solution of the corresponding acid by the slow addition of the theoretical amount of 30% hydrogen peroxide. The di-hydrochloride and di-trichloroacetate were well-crystallized products giving satisfactory analyses for nitrogen content and were relatively stable when kept dry. The preparations from sulfuric acid solutions were apparently mixtures of the sulfate and acid sulfate; on standing, these slowly decomposed forming some sulfur.

Preparation of the di-trichloroacetate of formamidine disulfide: 30 g. of thiourea was dissolved in 300 cc. of water containing 80 g. of trichloroacetic acid and 22 cc. of 30% hydrogen peroxide was added slowly while cooling. The precipitate which formed was filtered off by suction and washed seven times with 100-cc. portions of 0.1 *M* trichloroacetic acid and dried in a high vacuum maintained by an electric pump and then over sulfuric acid; yield, 44 g., 47% of theoretical.

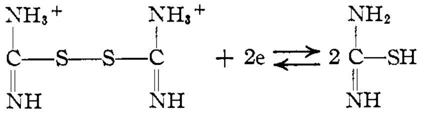
Preparation of di-hydrochloride of formamidine disulfide: 7.6 g. of thiourea was dissolved in 75 cc. of 3 *N* hydrochloric acid, and 7.5 cc. of 30% hydrogen peroxide was added slowly while cooling. Four volumes of ethanol was then added followed by 83 cc. of concentrated hydrochloric acid; the precipitate which formed was filtered off by suction, washed several times with ethanol, and dried *in vacuo* over sulfuric acid; yield 5 g., 67% of theoretical.

For the oxidizing solutions, ceric or thallic sulfates in sulfuric acid were prepared from the corresponding oxides and standardized. For the reducing solutions, stannous sulfate was dissolved in sulfuric acid and standardized.

The mathematical equation applicable to reactions of the type

$$\mathrm{RSSR} + 2\mathrm{e} + 2\mathrm{H}^+ \rightleftarrows 2\mathrm{RSH}$$

may be simplfied for use with the data here reported. Thiourea does not ionize appreciably within the range of acidity of our measurements; whereas formamidine disulfide exists in crystal form and in acid solution as the acid salt of a divalent base. The reaction is therefore



$$\mathrm{NH_3^+C(=NH)}\!-\!\mathrm{S}\!-\!\mathrm{S}\!-\!\mathrm{C(=NH)NH_3^+} + 2\mathrm{e} \rightleftarrows 2\ \mathrm{NH_2C(=NH)}\!-\!\mathrm{SH} \quad (1)$$

Since H^+ is not involved in the process (within the *p*H range studied), the potential should be unaffected by change of *p*H. This proves to be the case for our data. The electrode equation[4] may then be written

$$E_h = E_0' + \frac{RT}{2F} \ln \frac{(\mathrm{RSSR})}{(\mathrm{RSH})^2} \qquad (2)$$

or for 30°

$$E_h = E_0' + 0.030 \log \frac{(\mathrm{RSSR})}{(\mathrm{RSH})^2} \qquad (3)$$

where E_h is the electrode potential referred to the normal hydrogen electrode, E_0' is a constant characteristic of the specific system, and (RSSR) and (RSH) are the concentrations in *moles* per liter of the oxidant and reductant, respectively.

The potentials of other reversible organic systems follow equations involving only the first power of the concentration of the oxidant and reductant. Systems similar to thio-dithio in having di-equivalent oxidant and mono-equivalent reductant are the halogens, for example bromine[7] where $Br_2 + 2e \rightleftarrows 2\ Br^-$. A mono-

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[5] E. A. Werner, *J. Chem. Soc.*, **101**, 2166 (1912).

[6] P. W. Preisler and L. H. Hemplemann, THIS JOURNAL, **58**, 2305 (1936).

[7] G. N. Lewis and H. Storch, *ibid.*, **39**, 2544 (1917).

equivalent oxidant with diequivalent reductant is represented by the mercuric–mercurous system[8] where $2\,Hg^{++} + 2e \rightleftarrows (Hg\text{-}Hg)^{++}$.

The potentials of mixtures of oxidant and reductant prepared from weighed amounts and dissolved in 1 *N* sulfuric acid are plotted in Fig. 1 (curve for 0.01 *N* total concentration). The solid line curves are drawn from points calculated from equation (3), using stoichiometric *molar* concentrations. The points fit the theoretical curves as drawn with a degree of accuracy that can be judged by the size of the points which are 0.003 volt in diameter. Stable potentials were attained within a minute and remained within 0.0002 volt usually for over thirty minutes, two electrodes agreeing to within 0.0002 volt.

In Fig. 1, the total concentration is expressed in *equivalents* per liter, or normality, and is, therefore, the sum of the equivalent concentration of thiourea plus that of formamidine disulfide (the equivalent concentration of the disulfide being twice its molar concentration). The solid lines are theoretical curves for values of E_h plotted against per cent. of total equivalents in the form of oxidant, for total concentrations from 1 to 0.0001 normal. At the mid-point (50%) the *equivalents* of oxidant and reductant are equal or the *ratio* in moles is 2 RSH to 1 RSSR.

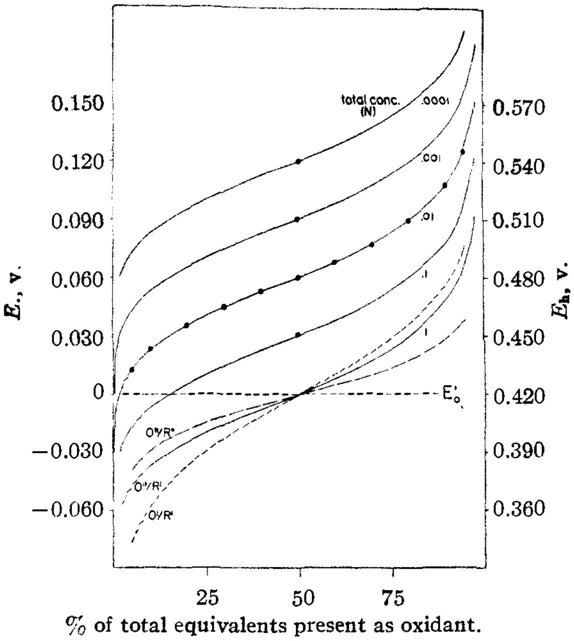

Fig. 1.

E'_0 is defined for this, as for other systems, as the E_h value when the logarithmic term becomes zero; that is, when log (oxidant)/(reductant)$^2 = 0$ or when (oxidant)/(reductant)$^2 = 1$, the concentration being expressed in *moles* per liter. Because of the square relationship, E'_0 has a different position on the various concentration curves (see dotted line on Fig. 1) and cannot alone be used to characterize the oxidation–reduction intensity, since the total concentration is also a factor. For example, only when the *total* concentration is 1 *N* (0.5 *M* RSH + 0.25 *M* RSSR) is the E'_0 at the midpoint of the curve; at 0.1 *N* it is at the point where 14.6% of the total concentration is as oxidant (0.0854 *M* RSH + 0.0073 *M* RSSR).

The inflection point of the curve may be calculated by setting the second derivative of the equation to equal zero. It occurs when the fraction of total equivalents as reductant is $2 - \sqrt{2}$, that is, when the total equivalents present as oxidant is 41.4%.

A comparison of the shape of the curve of this type system, di-equivalent oxidant and mono-equivalent reductant (O″/R′ in Fig. 1), to the more common mono-mono-equivalent (O′/R′) or di-di-equivalent (O″/R″) shows that it is asymmetrical about its inflection point and approaches closer to the O″/R″ curve when the reductant is of relatively higher effective concentration, and to the O′/R′ when the oxidant predominates. The slope is steeper on the side of the inflection point where the relative effective concentration of oxidant is higher.

The effect of total concentration of the oxidate and reductant, as shown by the experimental points on the several theoretical curves at 50% in Fig. 1, is to cause a 0.03 volt rise in potential for each ten-fold decrease in the total concentration of any fixed ratio of oxidant to reductant. The system would thus became more oxidizing by diluting any mixture.

The effect of *p*H or acidity upon the potential of a fixed mixture of oxidant to reductant (0.0025 *M* RSSR and 0.005 *M* RSH) is shown in Fig. 2. The solid line in the graph is E_h plotted against −log(HCl) at constant ionic strength (with potassium chloride) and has a slope of 0.00 which is to be expected since the oxidant carries a double positive charge and the reductant is un-ionized, H^+ from the solution not being involved in the transfer of the electrons (see equation 1).

The slope of 0.00 volt per *p*H continues at least through *p*H 4.3, since the initial potential of the above mixture in an acetate buffer at this *p*H was the same as for the hydrochloric acid solutions. At *p*H 4.3 the potential fell at the rate of 0.001 volt per minute and at *p*H 5.5 even more rapidly, so that significant initial potentials could not be obtained at the higher *p*H either by direct measurement or by extrapolation to zero time.

The fall in potential is due to the decomposition of the formamidine disulfide which is accompanied by the liberation of sulfur. The ionization constants, therefore, could not be determined by the method involving the change in slope of the E'_0 − *p*H curve.

The effect of ionic strength upon the potential of a fixed mixture is shown by comparing the re-

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(8) S. Popoff, J. A. Riddick, V. I. Wirth and L. D. Ough, *ibid.*, **53**, 1195 (1931).

sults at changing ionic strength, represented by the experimental points on the dotted line in Fig. 2, with the results at constant ionic strength represented by the points on the solid line. The difference between these curves on the graph is consistent with that calculated by standard equations for ionic strength effects.[8]

The potentials of the quinhydrone electrode measured in the same buffers or acid solutions when plotted gave the proper 0.06 slope expected.

The titrations of oxidant or of reductant gave potentials which agreed with the calculated potentials to within the allowable experimental error as shown in Table I.

TABLE I

COMPARISON OF OBSERVED AND CALCULATED POTENTIALS OF MIXTURES OF FORMAMIDINE DISULFIDE AND THIOUREA (AT 0.01 N TOTAL CONCENTRATION IN 1 N SULFURIC ACID) PRODUCED BY MIXTURES OF THEIR SOLUTIONS OR BY TITRATION OF ONE COMPONENT BY A SUITABLE REAGENT (POTENTIALS ARE IN VOLTS AND ARE REFERRED TO NORMAL HYDROGEN ELECTRODE)

% of total equivalents oxidized	Mixtures (at constant vol. of 50 cc.)		Titration of RSH with Ce^{++++} (Vol. change from 50 to 70 cc.)		Titration of RSSR with Sn^{++} (Vol. change from 50 to 70 cc.)	
	Obs.	Calcd.	Obs.	Calcd.	Obs.	Calcd.
5	0.4326	0.4333	0.4340	0.4335	0.4414	0.4375
10	.4433	.4437	.4443	.4443	.4486	.4477
20	.4563	.4558	.4566	.4568	.4601	.4588
30	.4644	.4646	.4658	.4661	.4681	.4678
40	.4721	.4724	.4741	.4743	.4755	.4751
50	.4800	.4800	.4822	.4824	.4824	.4824
60	.4870	.4882	.4908	.4910	.4901	.4901
70	.4968	.4977	.5007	.5009	.4993	.4992
80	.5083	.5100	.5136	.5136	.5112	.5110
90	.5285	.5296	.5342	.5336	.5295	.5301
95	.5455	.5484	.5552	.5526	.5466	.5486

Equilibrium potentials were reached for the mixtures usually within one minute. For the oxidizing titrations with ceric sulfate or thallic sulfate, the potentials became stable in about two or three minutes after each addition of reagent and for the reduction by stannous sulfate from five to ten minutes were required.

The calculated potentials in Table I were obtained by using the aforementioned equation for this system, accepting as E_0' the 0.4200 volt experimentally determined by the method of mixtures.

Because of the volume changes incident to a titration, the calculated potentials of a titration series are not identical with those calculated for the mixture series which is at constant volume. Furthermore, the calculated series of potentials resulting from an oxidative titration and of a reductive titration are not identical even though the initial concentration and final volume are the same in each case, which is to be expected, since the concentration of one of the components, RSH, occurs as the square in the electrode equation.

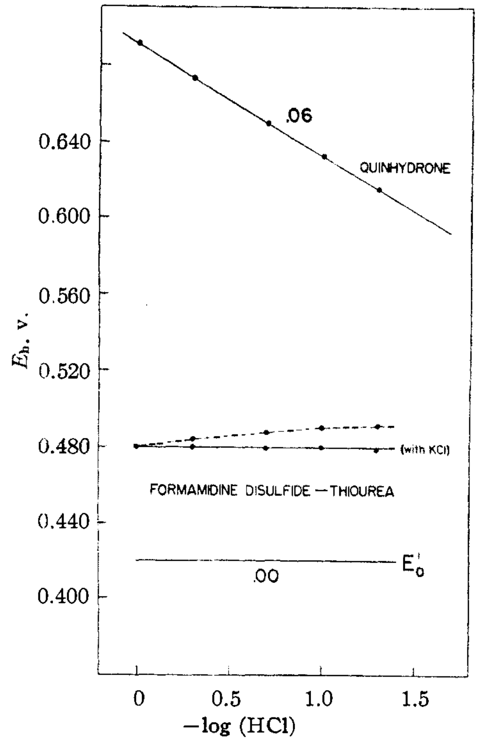

Fig. 2.

The observed potentials are in satisfactory agreement with the calculated potentials in each series.

Summary

The oxidation–reduction potentials of the thiourea–formamidine disulfide system have been measured by standard procedures and the results conclusively establish the equation

$$E_h = E_0' + \frac{RT}{2F} \ln \frac{(\mathrm{RSSR})}{(\mathrm{RSH})^2}$$

as applying to this reversible system.

The E_0' value was found to be +0.420 volt, referred to the normal hydrogen electrode, for solutions at constant ionic strength containing 0.05 to 1 N hydrochloric acid.

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