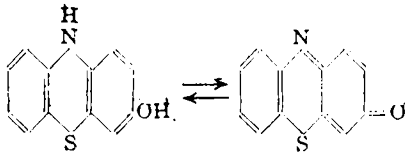
[FOOD RESEARCH DIVISION CONTRIBUTION NO. 369 FROM THE BUREAU OF CHEMISTRY AND SOILS, UNITED STATES DEPARTMENT OF AGRICULTURE, AT THE DEPARTMENT OF PHARMACOLOGY, STANFORD UNIVERSITY SCHOOL OF MEDICINE]

Studies of Phenothiazine. III. The Conversion of Phenothiazine to Thionol

BY FLOYD DEEDS AND C. W. EDDY

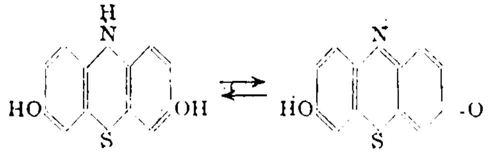
The growing interest in the subject of biological oxidations and reductions together with the desirability of increasing the number of oxidation-reduction indicators is sufficient justification for the description of a simple method for the conversion of phenothiazine to hydroxyphenthiazone, more commonly known as thionol.

Phenthiazone and hydroxyphenthiazone (see formulas I and II) are the simplest thiazines which in the presence of their respective leuco bases show the properties of reversible oxidation-reduction systems.



I

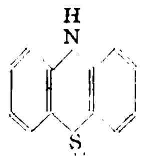
Leuco-phenthiazone ⇄ Phenthiazone



II

Leucohydroxyphenthiazone ⇄ Hydroxyphenthiazone

Two other thiazine dyes, Lauth's violet and methylene blue, are used as oxidation-reduction indicators, and have been characterized potentiometrically by Clark and co-workers.[1] The parent substance of these thiazine dyes is phenothiazine (see formula III).



III

Phenothiazine

The relationship of these four thiazines to phenothiazine was first described adequately by Bernthsen.[2] According to the nomenclature employed by Bernthsen, phenthiazone was called oxythiodiphenylimid and its leuco base (leuco phenthiazone) designated as oxythiodiphenylamine. Similarly, hydroxyphenthiazone and its leuco base, leuco hydroxyphenthiazone, were known as dioxythiodiphenylimide and dioxythiodiphenylamine, respectively.

Bernthsen[2] described a method for the preparation of thionol from phenothiazine, which depends upon oxidation in a strongly acid medium. For each 50 g. of phenothiazine 1 kg. of concentrated sulfuric acid diluted with 200 cc. of water is added to the phenothiazine and the mixture heated at 150 to 160° for twenty-five hours. The isolation of the resulting thionol is rather laborious and the yield about 10%.

The procedure adopted by us is essentially the same in principle, but is much more rapid, less troublesome, and gives material freer from products of lower oxidation and in a yield of at least 80%. The materials required for the conversion of 2 g. of phenothiazine are 200 cc. of ethyl alcohol, 80 cc. of concentrated hydrochloric acid, 1000 cc. of distilled water and 4 cc. of 30% hydrogen peroxide.

The conversion is best carried out in the following manner to obtain a product of highest purity. The phenothiazine is dissolved in the alcohol. The hydrochloric acid is added to the water, brought to a temperature of 80° and constantly stirred with a mechanical stirrer. The hydrogen peroxide is then added. While the temperature is maintained at 80° and the mixture vigorously stirred; the alcoholic solution of phenothiazine is added slowly at a constant rate of one or two drops per second, the addition being completed in forty-five minutes, and the total heating time being fifty-five to sixty minutes from the first addition of phenothiazine solution. The solution develops an intense red color. At the end of the specified heating time the solution is cooled under tap water and extracted with chloroform. The chloroform solution is concentrated to about 100 cc. by distillation, evaporated to dryness in a current of air, and the residue is transferred to an Abderhalden dryer. When pulverized, a dark red, almost black powder results. The product has all the characteristics described by Bernthsen for dioxythiodiphenylimide.

When 0.5 g. of the thionol prepared in this manner is dissolved in 50 cc. of acetic anhydride and boiled under a reflux condenser, reduction to the leuco base takes place according to Bernthsen,[2] followed by acetylation. Bernthsen[2] describes the triacetyl derivative melting at 155 to 156°. We have prepared the triacetyl derivative with a melting point of 156.5° by the above method but more frequently have obtained an acetylated product melting sharply at 212° which we believe to be the diacetyl derivative as judged by nitrogen determinations. The theoretical nitrogen content for diacetyl leuco thionol is 4.44%. Analysis gave values of 4.26, 4.30, 4.28, and 4.26, or an average of 4.275% nitrogen. *Further evidence* of an acetyl derivative of thionol melting at 212° is found in the

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(1) W. Mansfield Clark, Barnett Cohen and H. D. Gibbs, U. S. Public Health Reports, Vol. XL, No. 23, June, 1925.

(2) August Bernthsen, *Ann.*, **230**, 73-211 (1885).

work of Wheeler,[3] who obtained such an acetyl derivative from thionol prepared by (1) action of 84% sulfuric acid on phenothiazine and (2) hydrolysis of thionine by 65% sulfuric acid.

As pointed out in the introduction, thionol–leuco thionol possesses the properties of a reversible oxidation–reduction system. Since it is conceivable that a sample of thionol prepared from phenothiazine may be more or less contaminated with products representing lower states of oxidation, it appeared logical that the oxidation–reduction potential of a solution in which the ratio of oxidant to reductant is unity could be used as a criterion of the purity of the thionol produced from phenothiazine. For determination of the potential of pure thionol the diacetyl leuco thionol was used as a source of material. A sample of the pure diacetyl derivative with a melting point of 212° was dissolved in a small volume of ethyl alcohol, diluted with water, strongly acidified with hydrochloric acid, and heated to promote hydrolysis. The liberated leuco thionol was oxidized to thionol in the presence of air by adding sodium hydroxide to the cooled solution until an alkaline reaction was produced. The thionol was extracted with chloroform, and brought into solution in a phosphate buffer of *p*H 4.47 by volatilizing the chloroform from the heated buffer, the mixture being stirred constantly until all traces of chloroform were removed. The oxidation–reduction potential of the thionol solution thus prepared was measured by a standard procedure. The potential of this thionol at a temperature of 21° and *p*H 4.47 when the ratio of the concentration of oxidant to reductant was unity was found to be 301.9 mv. more positive than the standard normal hydrogen electrode. Using this value as a standard of reference, it was found that when the method of converting phenothiazine to thionol was successfully carried out there was produced a red dye having a potential of 300 ± 2 mv. at a *p*H of 4.47. The potential values at other hydrogen ion concentrations are given in Table I.

TABLE I

OXIDATION–REDUCTION POTENTIALS OF THIONOL AT DIFFERENT *p*H VALUES. AUTHORS' PREPARATION 81, TEMPERATURE 21°

*p*H	E_h, v.	*p*H	E_h, v.
1.42	+0.4717	6.57	+0.1882
1.85	+ .4576	6.84	+ .1687
2.65	+ .4105	7.14	+ .1516
3.47	+ .3917	7.44	+ .1333
4.03	+ .3450	7.78	+ .1132
4.47	+ .3022	8.70	+ .0700
4.99	+ .2710	9.90	− .0040
5.53	+ .2479		

Potentials have been determined on samples of thionol prepared by oxidation of phenothiazine orthosulfoxide, and thionol prepared by the method of Bernthsen. A summary of potential values obtained on various preparations is given in Table II.

TABLE II

OXIDATION–REDUCTION POTENTIAL OF THIONOL AT *p*H 4.47 AND TEMPERATURE 21°

Method	E_h, v.
Hydrolysis of diacetyl leuco thionol	0.3019
Oxidation of phenothiazine orthosulfoxide	.3010
Thionol by method of Bernthsen	.3040
Authors' Preparation 75	.3008
Authors' Preparation 81	.3022

Summary and Conclusions

1. A simple and rapid method for the conversion of phenothiazine to thionol (hydroxyphenthiazone) has been described.

2. The oxidation–reduction potential of this thiazine dye has been determined.

3. The oxidation–reduction potential has been used as a criterion for purity of thionol.

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(3) Donald H. Wheeler, personal communication.

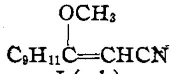
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

The Reaction of Phenylmagnesium Bromide with β-Methoxy-β-mesitylacrylonitriles

BY REYNOLD C. FUSON, G. E. ULLYOT, R. F. STEDMAN AND P. O. TAWNEY

β-Methoxy-β-mesitylacrylonitriles derived from α-cyanoacetomesitylene and α-cyanopropiomesitylene and described elsewhere[1] generally react normally, but their behavior toward the Grignard reagent has led to interesting results. This paper reports a study of their reactions with phenylmagnesium bromide.

The methylation product derived from α-cyanoacetomesitylene, β-methoxy-β-mesitylacrylonitrile (I), exists in two forms which are presumably *cis* and *trans* isomers. These melt at 66° (Ia) and 82.5–83.5 (Ib). When mixed in equal amounts they form a solid solution melting at 67–68°.



$$C_9H_{11}\overset{OCH_3}{\overset{|}{C}}{=}CHCN$$

I (a,b)

The action of phenylmagnesium bromide was essentially the same on the two forms, and most

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(1) Fuson, Ullyot and Gehrt, THIS JOURNAL, 60, 1199 (1938).