

# Stability of Anionic Complexes of Some Barbituric Acid Derivatives and Silver

By JAMES P. LEYDA† and LOYD E. HARRIS

Stability constants for the anionic silver complexes for a series of barbituric acid derivatives were determined potentiometrically. The titration efficiency or per cent recovery was shown to be acceptable for reproducible results and accurate calculation of the stability constants. The effect of silver ion on the stability of barbital was determined. The presence of silver ion was shown not to interfere with the assay of barbital in alkaline solution. A structure for the anionic silver barbiturate complex was proposed.

NUMEROUS procedures for the qualitative and quantitative analysis of barbituric acid derivatives are found in the literature which employ metal ion interactions. These methods include complexation by silver, cobaltous, cupric, and mercuric ions.

In 1934, Budde (1) first employed silver nitrate as a titrant for barbiturates in alkaline solution. The appearance of a permanent turbidity was used as the end point. This turbidity is caused by the formation of the insoluble disilver barbiturate.

Danielsson (2) modified the original Budde method by replacing the sodium carbonate solution with a solution of potassium metaborate. Potassium chromate was used as the indicator. At the end point in this method one mole of silver ion is equivalent to two moles of 5,5-disubstituted barbituric acid.

Potentiometric titration procedures have recently been reported employing silver complexation in barbiturate analysis. Gautier, *et al.* (3), titrated the protonated pyridine which forms after the interaction of silver ion with barbiturate in anhydrous pyridine. Perelman (4, 5) and Poethke and Furst (6) have potentiometrically titrated barbiturates with silver nitrate in a sodium carbonate solution.

The aims of this investigation were to determine the stability of the monosilver barbiturate anionic complexes of a series of nine barbiturates and the effect of the silver complex on the stability of barbital.

## EXPERIMENTAL

**Reagents.**—The barbiturates used in this investigation were recrystallized from ethanol-water mixtures with the exception of butethal which was recrystallized from an ether-petroleum ether (b.p. 30–60°) mixture. Sodium carbonate monohydrate

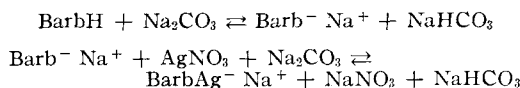
and potassium nitrate were of analytical reagent grade and were not further purified.

The silver nitrate solution was obtained from the Ohio State University reagent laboratory and was standardized against ammonium thiocyanate using acidified ferric alum as the indicator.

**Apparatus.**—A 5-ml. buret graduated in 0.01-ml. increments; magnetic stirrer; Rubicon model B and a Leeds-Northrup potentiometer with a General Electric galvanometer as the null instrument; silver electrode, prepared from 24-gauge silver wire; calomel electrode, containing a saturated potassium chloride solution; Beckman DU spectrophotometer, and Beckman pH meter, model GS.

**Potentiometric Procedure.**—An accurately weighed sample (between 15 and 80 mg./100 ml.) was dissolved in a sufficient amount of an alkaline carbonate solution (pH 11.0, 0.16 *M* sodium carbonate and 0.1 *M* potassium nitrate) to make 100 ml. A 25-ml. aliquot of the sample solution was placed in a 180-ml. titration vessel and titrated with the standardized 0.1 *N* silver nitrate solution. After each addition of titrant the E.M.F. was measured using a silver electrode as indicator electrode and a calomel reference electrode. The titration was continued until the permanent turbidity appeared, which was the visual end point described by Budde. A plot of  $\Delta$  mv./ $\Delta$  ml. vs. ml. of titrant determined the end point used in the calculation of titration efficiency and stability constants. At the end point, the average  $\Delta$  mv./ $\Delta$  ml. was about 300. A typical titration curve for barbital appears in Fig. 1.

**Calculation of Stability Constants.**—The reactions which lead to the formation of the monosilver barbiturate anionic complex in alkaline solution is shown by the following sequence



The equilibrium constant (instability constant) which defines the reaction is

$$K_{\text{ins}} = \frac{[\text{Barb}^-][\text{Ag}^+]}{[\text{BarbAg}^-]}$$

The stability constant of this anionic complex is the reciprocal of the  $K_{\text{ins}}$

$$K_s = \frac{[\text{BarbAg}^-]}{[\text{Barb}^-][\text{Ag}^+]}$$

Kolthoff (7) has given an equation for the calcula-

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TABLE I.—POTENTIOMETRIC TITRATION DATA

Compound	Samples	% Recovery	$E_{1/2}$ mv. Av.	$K_s \times 10^{-6}$	pKa (9)
Barbital	18	101.1 ± 1.7	170.7	3.46 ± 0.48	8.06
Phenobarbital	18	98.5 ± 1.5	163.4	5.03 ± 1.03	7.54
Diallylbarbituric acid	18	98.9 ± 1.3	166.6	4.60 ± 0.55	7.88
Aprobarbital	12	100.8 ± 1.9	169.0	3.81 ± 0.48	8.09
Vinbarbital	18	100.7 ± 1.7	165.0	3.85 ± 0.74	7.72
Probarbital	12	100.3 ± 2.4	170.7	3.39 ± 0.75	8.17
Butethal	12	98.2 ± 3.4	164.8	4.09 ± 1.04	8.10
Butabarbital	12	100.5 ± 2.3	171.5	3.46 ± 0.59	8.16
Amobarbital	6	98.6 ± 3.1	165.6	3.96 ± 0.20	8.02

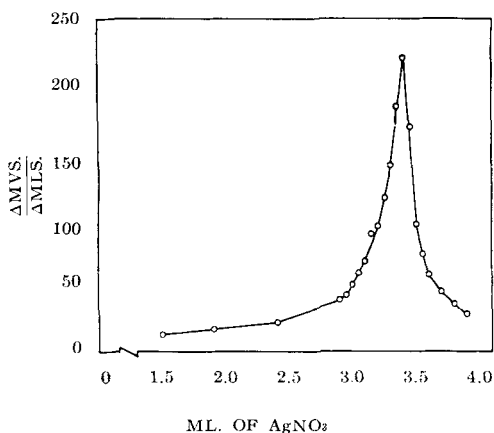


Fig. 1.—A typical first derivative titration curve for barbital titrated with silver nitrate.

tion of free silver ion concentration by measuring the potential using a silver electrode and calomel electrode system

$$pAg = \frac{0.7991 - E_s - E_c}{0.0591}$$

Where  $E_c$  is the E.M.F. of the calomel electrode which is 0.2444 v. when the electrode contains a saturated solution of potassium chloride at 25° (8).  $E_s$  is the measured E.M.F. of the system.

The concentration of anionic complex and free barbiturate was determined by the procedure described by Perelman (4, 5). Molar concentration of the free barbiturate was calculated by the equation

$$C_{\text{molar}} = \frac{(c - a) \cdot N}{a + b}$$

The molar concentration of the anionic complex was calculated in a similar manner, by the equation

$$C_{\text{molar}} = \frac{a \cdot N}{a + b}$$

Where  $a$  is the number of milliliters of titrant added;  $N$  is the normality of titrant (silver nitrate);  $b$  designates the number of milliliters in the original sample solution; and  $c$  is the number of milliliters of titrant at the end point, determined by the maximum  $\Delta \text{mv.}/\Delta \text{ml}$ . In all calculations, molar concentrations were used, since the silver ion concentration was so low it was assumed that the activity coefficients were unity.

**Results.**—The data presented in Table I were obtained from the potentiometric titration procedure.

The per cent recovery (titration efficiency) and stability constant data are the average values from a series of determinations plus the standard deviations from the mean.

Perelman (4, 5) calculated the instability constants for diallylbarbituric acid,  $K_{\text{ins}} = 1.1 \times 10^{-7}$  ( $K_s = 9.1 \times 10^6$ ) and barbital,  $K_{\text{ins}} = 4.7 \times 10^{-8}$  ( $K_s = 2.1 \times 10^7$ ). Poethke and Furst (6) have reported the instability constants for barbital and phenobarbital respectively,  $K_{\text{ins}} = 4.0 \times 10^{-7}$  ( $K_s = 2.6 \times 10^6$ ) and  $2.7 \times 10^{-7}$  ( $K_s = 3.7 \times 10^6$ ). The data presented in Table I agree with the limited number of values given by Poethke and Furst.

A general trend is noted between the stability constants of these anionic complexes and the pKa values of the parent acids. Due to the relatively narrow range which the pKa values span for the barbituric acids, it would be expected that the stability constants would be of similar magnitude. The stability of the complexes are of the same magnitude and slightly increase with the decrease in the pKa of the parent acid.

**Effect of Silver Ion on the Molar Absorptivity of Barbital.**—Danielsson (2) reported that the molar absorptivity of the silver-barbital anion at the pH of 10.9 appears to be 18,820 when determined at 238  $m\mu$ . Previously, the molar absorptivity of barbital, at 239  $m\mu$  and the pH 10.9, has been shown to be  $10.2 \times 10^3$  (9). In order to assay barbital in the presence of silver ion and silver-barbital complex, the absorbances of these various species were determined.

The absorbances of  $3.89 \times 10^{-6} M$  solutions of barbital (total) were measured by a Beckman DU spectrophotometer at 239  $m\mu$ . The solutions contained varying amounts of silver ions and were buffered at pH 10.9. The data are presented in Table II.

The average molar absorptivity of the complex is  $10.10 \times 10^3$ , which correlated within 99.0% of the

TABLE II.—EFFECT OF SILVER ION ON THE ABSORBANCE OF BARBITAL

Sample	Barbital, $M \times 10^5$	Silver Complex, $M \times 10^5$	Free Barbital, $M \times 10^5$	$A_{\text{obs}}$
1	3.89	0	3.89	0.398
2	3.89	0	3.89	0.397
3	3.89	0.841	3.05	0.400
4	3.89	0.841	3.05	0.395
5	3.89	1.680	2.21	0.394
6	3.89	1.680	2.21	0.395
7	3.89	2.520	1.37	0.395
8	3.89	2.520	1.37	0.394
9	3.89	3.360	0.53	0.398
10	3.89	3.360	0.53	0.388

literature value (9) for the molar absorptivity of barbital. Thus, it appears that the spectrophotometric assay is efficient for determining the total amount of barbital in the system.

**Effect of Silver Anionic Complex on the Stability of Barbital.**—The decomposition of aliphatic substituted ureides in alkali has been shown to be retarded when intermolecular hydrogen bonding is present. Werner (10) described this as chelate formation. Since barbiturates are cyclic ureides which hydrolyze in alkaline solution, silver ions are believed to form a silver-barbiturate anionic complex which resists hydrolysis.

A 0.2 *M* solution of sodium carbonate (pH 11.0) was employed as the alkaline medium. Barbital solutions were prepared of approximately  $4.0 \times 10^{-3}$  *M* and varying amounts of silver nitrate. Samples were sealed in 2-ml. Neutraglas ampuls and placed in an oil bath at 42.5°. At various time intervals, samples were removed from the constant temperature bath and immediately frozen in a chloroform-dry ice mixture. The samples were assayed spectrophotometrically as previously described.

The degradation rate (apparent) is defined by the following equation

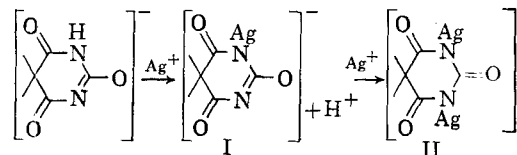
$$k_{app} = N_{barb} k_{barb} + N_{barbAg} k_{barbAg}$$

Where  $N_{barb}$  is the mole fraction of free barbital,  $N_{barbAg}$  the mole fraction of silver-barbital complex (silver mole fraction),  $k_{barb}$  the pseudo first-order degradation rate of free barbital, and  $k_{barbAg}$  the pseudo first-order degradation rate of the complex.

The results are presented in Table III, and Fig. 2 illustrates the relationship between the pseudo first-order reaction rate and the concentration of anionic silver-barbital complex. The comparison of the reaction rate constants between the control samples and the samples containing known amounts of silver ion concentration agrees within 97.7% of the calculated rate of hydrolysis (assuming that only the free barbital degrades). Calculated data (solid line) had a slope  $-5.43 \times 10^{-5}$  and the experimental data (broken line) had a slope  $-5.56 \times 10^{-5}$ , determined by the method of least squares.

## DISCUSSION AND RESULTS

Perelman (4) has described the reaction sequence for the formation of the silver complexes of barbiturates. In an alkaline solution, barbiturates react with silver ions in a step-wise fashion which is shown by the following formulas



Gautier, *et al.* (3), have followed the released protons of these reactions in pyridine. They have shown that titration of the free barbituric acid liberated two protons and that one proton is liberated when the sodium salts are employed.

Poethke and Furst (6) represent the following formulas as the anionic complex (III) and the

TABLE III.—EFFECT OF SILVER ION ON THE DEGRADATION OF BARBITAL

Sample	Mole Fraction Silver Complex	$k_{app} \times 10^3$ hr. <sup>-1</sup>	$k_{barb} \times 10^3$ hr. <sup>-1</sup> (calcd.)
1	0	5.42	5.42
2	0	5.49	5.49
3	0.268	3.78	3.97
4	0.576	1.93	2.31
5	0.836	0.82	0.89

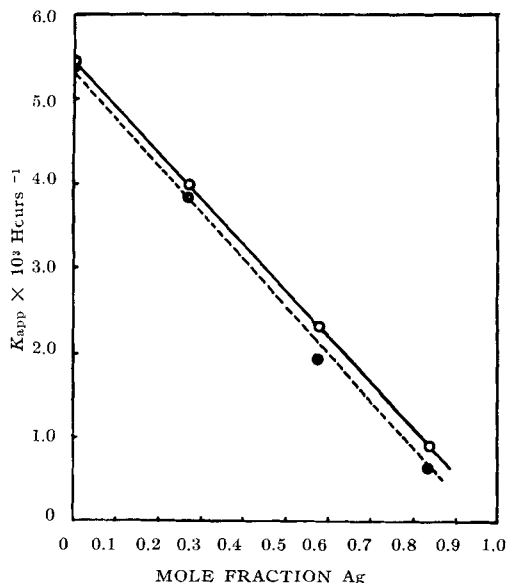
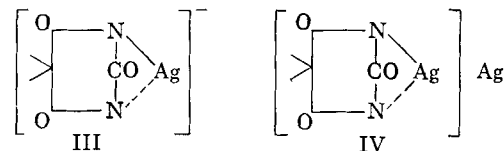
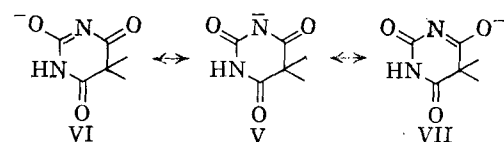


Fig. 2.—The relationship between pseudo-first order degradation rate and the mole fraction of silver. —○— represents the calculated data and —●— the experimental data.

water-insoluble disilver barbiturate (IV) as chelate compounds



Goyan, *et al.* (11), have written the resonance forms of the anionic species in the following manner

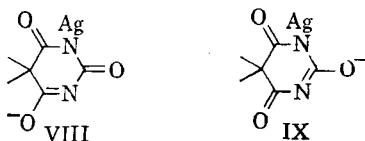


Spectral data have supported the view that species V is not the resonance form which is attacked by the silver ion. The enol forms which are present in alkaline solution are the species responsible for the increase in absorbance and are represented by structures VI and VII. We have shown that the silver-barbital anionic complex does not appear to alter the absorptivity of the barbiturate. Thus, the

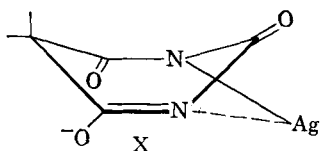
silver ion does not change the electron distribution in the enolized form.

Goyan, *et al.* (11), have suggested that barbital degradation in ammonia buffer is similar to amonolysis of an ester and that hydrolysis of the ionized species would probably be at the 4 (or 6) position. By virtue of infrared spectra of the *p*-nitrobenzyl derivatives of a series of barbiturates, Chatten and Levi (12) have proposed that the mechanism of the reaction involves enolization of the carbonyl at position 4 (or 6).

The silver-barbiturate anionic complex has been shown to be stable in alkaline solution. The silver ion, when attacking the barbiturate anion, liberates a proton, thus the nitrogen atom which is not involved in the enolization appears to be the position of the silver ion, as shown by structures VIII and IX.



These formulas do not appear to explain the stability of the complex to alkaline hydrolysis, especially if the 4 (or 6) carbonyl group is the position vulnerable to hydroxyl ion attack.



Barbiturates which have substituents on a nitrogen atom, i.e., a methyl group, are known to hydrolyze in the same way as the unsubstituted compounds. The mono silver anionic complexes are believed to be

stabilized by the formation of a chelate structure involving both the 1 and 3 nitrogen atoms. This compound was previously described by Poethke and Furst (6) and is represented by structure X.

#### SUMMARY

1. Stability constants for the anionic silver complexes for a series of nine barbituric acid derivatives were determined potentiometrically.
2. The per cent recovery or titration efficiency was determined.
3. The molar absorptivity of the silver anionic complex of barbital was shown to be the same as that of barbital in alkaline solution.
4. The effect of silver ion on the stability of barbital in a sodium carbonate solution was described.
5. A structure for the anionic silver complex was proposed.

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## Synthesis of Some Derivatives of Sorbic Acid and Their Antifungal Properties

By ROBERT W. GOETTSCH†, AUGUST G. DANTI, and DALE H. CRONK

Seven amides and three amine salts of sorbic acid were prepared and screened for their antifungal activity against four pathogenic fungi. Of the 10 compounds tested, *N*-(2-methylpiperidyl)-sorbamide, *n*-hexylsorbamide, cyclohexylamine sorbate, phenylethylamine sorbate, and *n*-hexylamine sorbate exhibited antifungal activity.

THERE ARE many types of chemical compounds that have been studied for their potential antifungal activity. Among the diverse chemical types of antifungal agents investigated are

the fatty acids, their salts, and derivatives. Hoffman, Schweitzer, and Dalby (1) have demonstrated that fatty acids and their salts are fungistatic for certain nonpathogenic fungi. Keeney and Broyles (2) reported that sodium propionate appeared effective in the treatment of certain superficial fungus infections. Keeney, Ajello, and Lankford (3) investigated the fungi-

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