monium oxide), each of which melts congruently—although with considerable dissociation—at −79°. The corresponding compounds in the acetic acid–ammonia system, $NH_3 \cdot HC_2H_3O_2$ (ammonium acetate) and $2NH_3 \cdot HC_2H_3O_2$, have melting points, respectively, 196° and 95° higher than their ammonia–water analogs. The other three acetic acid–ammonia compounds have no analogs in the water–ammonia system. The light thrown by this contrast on the question of the weakness of aqueous "ammonium hydroxide" as a base at ordinary temperatures, has been discussed previously.[2]

Summary

1. The temperature–concentration curve for the system acetic acid–ammonia has been completed throughout the entire concentration range.
2. The melting point of pure anhydrous ammonium acetate has been found to be 117°.
3. The existence of the new solid compounds $5NH_3 \cdot 4HC_2H_3O_2$ and $9NH_3 \cdot HC_2H_3O_2$, and of a third having the probable composition $2NH_3 \cdot HC_2H_3O_2$, has been demonstrated.
4. Attention has been directed to the contrast between this system and that of water–ammonia.

LAWRENCE, KANSAS RECEIVED FEBRUARY 14, 1944

[CONTRIBUTION FROM THE AMMONIA DEPARTMENT OF E. I. DU PONT DE NEMOURS & COMPANY, INC.]

Analytical Procedures Employing Karl Fischer Reagent. XI. The Determination of Primary Plus Secondary Amines[1]

BY J. MITCHELL, JR., WALTER HAWKINS AND DONALD MILTON SMITH

The identification of amines by acylation techniques has been used to some extent,[2,3] but little has been done on their quantitative acetylation. Olson and Feldman[4] modified the acidimetric acetyl chloride procedure of Smith and Bryant[5] so that most amines reacted 90% or better. However, some amides also reacted so that this technique did not differentiate between these classes of compounds. Although acetic anhydride has been used in the determination of tertiary amines,[6,7] it apparently has not been used in the quantitative estimation of primary and secondary amines.

In the present research an acetylation method has been developed which is based on the selective reaction of acetic anhydride with the primary and secondary amines, according to the equations

$$RNH_2 + (CH_3CO)_2O = CH_3CONHR + CH_3COOH$$
$$RNHR' + (CH_3CO)_2O = CH_3CONRR' + CH_3COOH$$
$$(CH_3CO)_2O + H_2O = 2CH_3COOH$$

The new technique, independent of acid–base titrimetry, determines the excess acetic anhydride, after acetylation, by hydrolysis followed by titration of excess water with Karl Fischer reagent under conditions reported in an earlier publication for the determination of anhydrides.[8]

The new procedure is applicable to primary and secondary amines generally including aliphatic, alicyclic, heterocyclic and aromatic types. However, diaryl secondary amines and pyrrole fail to react. Since tertiary amines do not interfere, the method can be used indirectly for the determination of tertiary amine by correcting the total base titer for primary plus secondary amine.

In the presence of primary alcoholic hydroxyl the procedure is modified to effect quantitative acetylation of both the amine and alcohol.

Experimental

Reagents.—The acetylating reagent is prepared by mixing 1.5 moles (142 ml.) of pure acetic anhydride in sufficient dry pyridine to make one liter of solution.[9] The hydrolysis reagent consists of 100 g. of J. T. Baker C. P. or Merck A. R. grade dry sodium iodide and 22 ml. of water in 1 liter of pyridine solution. The preparation of the Karl Fischer reagent has been reported in a previous publication.[10]

Analytical Procedure.—In the absence of hydroxyl the sample, containing up to 10 milliequivalents of primary plus secondary amine, is transferred to a 250-ml. glass-stoppered volumetric flask.[11] To this is added exactly 20 ml. of the acetylating reagent. The flask together with a blank is then stoppered, shaken and allowed to stand for thirty minutes at room temperature.[12] At the end of this time 25 ml. (calibrated pipet) of the hydrolysis reagent is added. The flask is placed in a water-bath at 60 ± 1°, and after momentarily raising the stopper to allow for expansion of the included air, is firmly stoppered and maintained at that temperature for thirty minutes. After cooling spontaneously to room temperature, the contents are titrated directly for water with Karl Fischer reagent.

Ten ml. (calibrated pipet) of the hydrolysis reagent is titrated for water. Free water in the sample is determined by titration of a portion of the sample acidified with glacial acetic acid.

(1) Presented in part before the Division of Analytical and Micro Chemistry at the Pittsburgh meeting of the American Chemical Society, Sept. 9, 1943.

(2) Alexander and McElvain, THIS JOURNAL, **60**, 2285 (1938).

(3) Billman and O'Mahony, *ibid.*, **61**, 2340 (1939).

(4) Olson and Feldman, *ibid.*, **59**, 2003 (1937).

(5) Smith and Bryant, *ibid.*, **57**, 61 (1935).

(6) Blumrich, *Angew. Chem.*, 374 (1941), used excess acetic anhydride to acetylate primary and secondary amines in order to titrate tertiary amine in glacial acetic acid solution.

(7) Haslam and Guthrie, *Analyst*, **68**, 328 (1943); acetylated ethylaniline quantitatively to determine diethylaniline.

(8) Smith, Bryant and Mitchell, THIS JOURNAL, **63**, 1700 (1941).

(9) Eastman Kodak Co. No. 4 acetic anhydride and J. T. Baker C. P. or Eastman Kodak Co. No. 214-H pyridine meet these requirements.

(10) Smith, Bryant and Mitchell, THIS JOURNAL, **61**, 2407 (1939).

(11) The sample may be weighed directly or added volumetrically in pyridine solution.

(12) In the presence of primary hydroxyl an additional thirty minutes at 60 ± 1° is required to effect complete esterification. In this case sample size is limited to 10 milliequivalents of amine plus hydroxyl.

Total anhydride added is equivalent to the net water used up during hydrolysis of the blank. This is found by subtraction of the water found from total water added before hydrolysis. Excess anhydride after acetylation is determined by subtracting water found from total water added to the sample, including free water originally present. The net anhydride is equivalent to the primary plus secondary amine in the sample.

Analytical Results

Thirteen primary amines were analyzed by this method; some were checked acidimetrically by titration to brom phenol blue. The trade products with the exception of hexamethylenediamine, which was sublimed, and *p*-bromoaniline, which was recrystallized from chloroform, were used without further purification. Experimental results are given in Table I. The precision and accuracy are usually within ±0.2%.

TABLE I

ANALYTICAL DATA FOR PRIMARY AMINES

Amine		Found, wt. % Acidimetric	Acetylation	H_2O	Total
n-Butylamine	(10)[a]	97.7	98.0 ± 0.5	1.7	99.7
Propylenediamine[b]	(2)		84.1 ± .2	13.9	98.0
Hexamethylenediamine[c]	(4)		99.9 ± .1	0.1	100.0
Cyclohexylamine	(2)		100.2 ± .2	.0	100.2
o-Aminodicyclohexyl[d]	(2)	96.2[h]	96.7 ± .3	.0	96.7
Benzylamine	(2)	92.8	92.7 ± .2	.2	92.9
Aniline	(4)		100.8 ± .1	.0	100.8
p-Toluidine[e]	(2)		99.2 ± .1	.0	99.2
p-Phenylenediamine	(4)		99.6 ± .1	.0	99.6
p-Bromoaniline	(4)		99.4 ± .1	.0	99.4
β-Naphthylamine[f]		97.5	97.1 ± .2	.0	97.1
o-Aminodiphenyl[d]	(2)		99.0 ± .2	.0	99.0
2-Aminopyridine[g]	(2)		100.0 ± .3	.0	100.0

[a] Figures in parentheses represent number of indivdual determinations. [b] Carbide & Carbon Chemical, 85%. [c] du Pont. [d] Monsanto. [e] Arthur H. Thomas Co. [f] J. T. Baker. [g] Reilly Tar and Chemical Co.; all others Eastman Kodak Chemicals. [h] Calculated from Kjeldahl nitrogen value.

Analytical data for several secondary amines are given in Table II. Results with diphenylamine, carbazole and phenothiazine were 0.0 ± 0.0%.

TABLE II

ANALYTICAL DATA FOR SECONDARY AMINES

		Found, wt. % Acidimetric	Acetylation	H_2O	Total
Diethylamine	(2)[a]	99.7	99.7 ± 0.1	0.3	100.0
Di-*n*-butylamine	(10)	98.3	98.4 ± .2	.1	98.5
Di-*s*-butylamine	(4)	99.5	83.5 ± .2	.1	83.6
Di-isobutylamine	(2)	100.1	100.1 ± .1	.0	100.1
Methylaniline	(4)		98.8 ± .1	.0	98.8
Pyrrole	(6)		0.0 ± .1	.0	0.0
Morpholine[b]	(2)		104.1 ± .2	.0	104.1
Piperidine	(2)	99.4	99.8 ± .2	.0	99.8
Piperazine hydrate	(2)		45.8 ± .2	53.8	99.6

[a] Figures in parentheses represent number of determinations. [b] Eastman Kodak Co. practical grade chemical; all others Eastman Kodak.

The low result obtained on di-*s*-butylamine probably indicates the presence of some tertiary amine. It is quite surprising that pyrrole failed to react. Experiments conducted at 60° on the sample as received and on freshly distilled pyrrole gave values of only 0.6% after thirty minutes and 2% after three hours.

Interfering Substances.—Since the above procedure is based on acetylation, alcohols will react. By modifying the technique to include the short additional heating period primary hydroxyl will react quantitatively as shown in Table III.[13]

TABLE III

ANALYTICAL DATA FOR HYDROXYAMINES

Substance	Found, wt. % Acetylation	H_2O	Total
Monoethanolamine	99.5 ± 0.1	0.2	99.7
Diethanolamine	99.6 ± .1	.0	99.6
Hydroxyethylethylenediamine	99.1 ± .2	.0	99.1

These conditions are not sufficiently drastic to effect quantitative esterification of secondary hydroxyl; tertiary hydroxyl reacts only slightly. Under the conditions employed for the complete reaction of primary alcohols the hydroxyl of diisopropanolamine was esterified to the extent of 80%. By increasing the heating period to one hour at 60° this value was raised to 92%.

The effect of. the type of alcohol is given in Table IV. In this case 5 milliequivalents of butanol was analyzed alone and in the presence of an equivalent amount of amine.

TABLE IV

RELATIVE REACTIVITY OF ACETIC ANHYDRIDE WITH *n*-, *s*- AND *t*-BUTANOL

Substances	% OH Esterified
n-Butanol	100
n-Butanol + *n*-butylamine	100[a]
s-Butanol	71
s-Butanol + isobutylamine	72[a]
t-Butanol	6
t-Butanol + isobutylamine	13[a]

[a] Corrected for quantitative amine reaction.

In general the presence of amine had little or no effect on the acetylation of the alcohol.

No interference is encountered from amides, urethans, nitriles or tertiary amines. Acetamide, urea and acetanilide did not react. Acetonitrile and adiponitrile gave 0.7 and 0.4% reaction, respectively. Dimethylaniline reacted to the extent of 1.3 ± 0.1%. This may have been due to secondary amine impurity.

Summary

1. An accurate and precise quantitative procedure for the determination of primary plus secondary amine, based on acetylation with acetic anhydride, has been described. Analytical results for more than twenty-two primary and secondary amines are reported.

(13) When necessary the results may be corrected for primary hydroxyl by the specific esterification procedure described in the preceding paper of this series, Smith, Mitchell and Hawkins, Paper No. X.

2. The interference of alcoholic hydroxyl has been investigated, including the relative reactivity of primary, secondary and tertiary hydroxyl.

3. The method can be used indirectly for the determination of tertiary amine.

WILMINGTON, DELAWARE RECEIVED FEBRUARY 17, 1944

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 956]

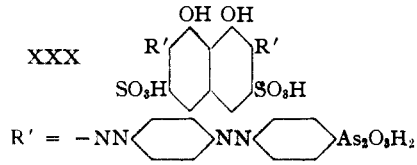
The Serological Properties of Simple Substances. VII. A Quantitative Theory of the Inhibition by Haptens of the Precipitation of Heterogeneous Antisera with Antigens, and Comparison with Experimental Results for Polyhaptenic Simple Substances and for Azoproteins

BY LINUS PAULING, DAVID PRESSMAN, AND ALLAN L. GROSSBERG

In an earlier paper in this series[1] there was developed a simple physicochemical theory of the precipitation of bivalent antibody molecules and bivalent antigen molecules and the inhibition of precipitation by haptens, and it was shown[1,2] that the results of experiments on the precipitation of specific antisera by polyhaptenic substances agree qualitatively but not quantitatively with the theory: in particular, the predicted linear decrease in amount of precipitate with increase in amount of hapten was observed only in the region of small inhibition. The deviation from linearity for larger amounts of hapten was attributed to the heterogeneity of the antisera, which were assumed, as is indicated by many experimental observations, to contain antibody molecules with greatly varying combining powers. We have now developed an extended quantitative theory of hapten inhibition on the assumption of an error-function distribution of antibody molecules with different combining powers in a heterogeneous antiserum, and have found it to be in generally satisfactory quantitative agreement with experiment. The theory permits the evaluation of two constants from each hapten-inhibition experiment, an average bond-strength constant for antibody and hapten (in competition with antigen) and an effective heterogeneity index for the antiserum; values of each of these constants can be interpreted in relation to the molecular structure of the hapten and the antigen.

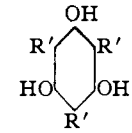
There have previously been reported[2,3] the results of quantitative investigations of the inhibition by haptens of the precipitation of polyhaptenic simple substances by antisera made by inoculating rabbits with sheep serum coupled either with diazotized *p*-arsanilic acid[2] (anti-R sera) or with diazotized *p*-(*p*-aminophenylazo)-phenylarsonic acid[3] (anti-R′ sera). It was found that the relative inhibiting powers of various haptens are essentially the same for different polyhaptenic simple substances precipitated by the same pool of antiserum, but are somewhat different for different pools of anti-R serum or of anti-R′ serum precipitated by the same polyhaptenic substance. Still greater differences are observed between anti-R sera and anti-R′ sera in general. We have now carried out a comparative study of hapten inhibition of the precipitation of a polyhaptenic simple substance (XXX) and an azo

XXX



protein (R′-ovalbumin) by anti-R serum and anti-R′ serum, and of a polyhaptenic substance (XI) by anti-R serum obtained by a single three

XI



weeks' course of inoculations, with the results described below. The data obtained in these and the earlier experiments have been analyzed by use of the new theory, and the results are discussed in relation to the molecular structure of the interacting substances.

Experimental Methods

Materials.—There have been previously described the methods of preparation of the antisera,[3,4] the haptens and antigens,[2,3,4] and the R′-ovalbumin,[3] which contained 2 per cent. arsenic.

The Reaction of Antiserum with Antigen and Hapten.—The reaction mixtures were set up in triplicate, with use in each series of experiments of the amount of antigen giving the largest amount of precipitate in the absence of hapten; borate buffer was used as diluent.[2] The tubes were allowed to stand one hour at room temperature and over two nights in the refrigerator, and the precipitates were then analyzed by our standard method.[5]

The results of the study of the inhibition by each of 24 haptens of the precipitation of antiserum with antigen

(1) L. Pauling, D. Pressman, D. H. Campbell, and C. Ikeda, THIS JOURNAL, **64**, 3003 (1942).

(2) D. Pressman, D. H. Brown, and L. Pauling, *ibid.*, **64**, 3015 (1942).

(3) D. Pressman, J. T. Maynard, A. L. Grossberg, and Linus Pauling, *ibid.*, **65**, 728 (1943).

(4) L. Pauling, D. Pressman, D. H. Campbell, C. Ikeda, and M. Ikawa, *ibid.*, **64**, 2994 (1942).

(5) D. Pressman, *Ind. Eng. Chem., Anal. Ed.*, **15**, 357 (1943).