Tetraphenylborate Spot Test for Detection of Amines and Their Salts

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A spot test has been developed for detecting micro quantities of all types of basic organic nitrogen compounds as well as their salts. This test, which is based upon the precipitation of amine cations by aqueous sodium tetraphenylborate solution at pH 3, is sensitive and selective, yet simple. The sensitivities of various amine cations to 1 drop of 6% tetraphenylborate reagent were determined, and correlated with the solubilities, structures, and dissociation constants of their parent bases. The identification limits varied from approximately 2.5 γ for primary amines to 0.4 γ for quaternary salts. Amines, which are either extremely weak bases or contain several solubilizing groups, are insensitive to the tetraphenylborate reagent. Certain inorganic and organic onium cations interfere.

NUMEROUS methods for identifying and determining amines and their salts, as well as for detecting specific structural types of amines, have been described. However, detection procedures which are applicable to all amine cations (primary, secondary, tertiary, or quaternary) are rare, and are somewhat lacking in selectivity, sensitivity, or simplicity.

The precipitation of the cations from organic bases by large anions is well known. Because many of the resulting salts have been used for identification and determination of amines, it is surprising how few of these precipitates are insoluble enough to serve as detection media for amine ions at low concentrations. Krumholz and Watzek (16) found that organic bases gave (with tetraiodobismuthic acid) yellow precipitates, which could be used to detect the base in question. However, the sensitivity of the test was reported only for heterocyclic amines. Stein (20) reported that white phospho-12-tungstate precipitates were obtained with creatinine at a dilution of 1 to 7000, with choline at 1 to 10,000, and with acetylcholine at 1 to 100,000. Koszegi and Salgo (15) suggested dipotassium mercuric tetrathiocyanate as a reagent for the detection of amines and alkaloids. Unfortunately, some of the precipitates are too soluble in water, thus decreasing sensitivity, and the test must be carried out in alkaline solution.

Three colorimetric methods have been described for the detection of amines. Blair and Pantony (2) state that a majority of amines give a golden yellow color with vanadium-8-quinolinolate. Unfortunately, the reaction is not completely limited to amines. An acidified, alcoholic quinone reagent has been reported (12) to give a red color with amine solutions. However, too few amines were tested and no mention is made of the limits of detection. In the third method (18) dichlorourea gives intense colors with dilute aqueous or ethanolic solutions of amines, suitable for sensitive qualitative tests, but results are good only for aromatic amines. Feigl (11) recommends an ingenious, indirect micro test for the detection of primary, secondary, and tertiary amines. Hydrogen sulfide is liberated and detected when alkali thiocyanate reacts with amine or ammonium salts. However, only very faint tests were given by 2000 γ of the heterocyclic nitrogen bases. Two paper chromatographic techniques have appeared for the detection of amines. The first (21) is a complicated and special technique for isolating amines in plants; it requires many reagents and a long time to perform. The second (7) is a test for amine hydrochlorides, whereby the amine cation is detected by precipitation of the accompanying chloride ion with silver nitrate. Even though the test is sensitive to as little as 3 γ of chloride, and does not depend on the structure of the original amine, it suffers from the fact that it is indirect.

The test for amines by precipitation as their tetraphenylborate salts is very sensitive as well as selective for amine cations (4). The purpose of this investigation was to develop a rapid spot test procedure, based on the tetraphenylborate reaction, for detecting submicro quantities of all four types of amines and their salts. This test combines good sensitivity, selectivity, and remarkable simplicity. The sensitivities (expressed as identification and dilution limits) of the amine cations tested to the sodium tetraphenylborate reagent are reported.

MATERIALS

A 6.0% solution of sodium tetraphenylmetaborate (J. T. Baker's reagent grade) was prepared by dissolving 2.0 grams of solid in 33.3 ml. of distilled water. The solution was clarified with aluminum oxide and filtered according to the recommendations of Gloss and Olson (14). Because cloudiness appears after several weeks' standing even in the refrigerator, it was occasionally necessary to refilter the reagent before using.

The inorganic chemicals used—hydrochloric acid, sodium hydroxide, potassium chloride, silver nitrate, and various ammonium salts—were all of analytical reagent grade.

Tetra-*n*-butylammonium bromide, 1,2-di-4-pyridylethane, and its dimethiodide salt were prepared as directed by Bergmann, Crane, and Fuoss (1).

The methyl-2-picolinium and methyl-4-picolinium iodides were synthesized according to the method of Crane and Fuoss (6).

The synthesis of tetramethylammonium, methylpyridinium, and methylquinolinium iodide salts has been described $(4, \bar{o})$.

Methyllepidinium iodide was prepared by addition of methyl iodide to redistilled lepidine. The resulting yellow crystals melted at $174-175^{\circ}$ and were found by potentiometric titration to contain 44.27% iodide (44.51% theory). In water solution the crystals exhibited two ultraviolet absorption maxima at 317 and 225 m μ .

Trimethyl - 2 - phenylethylammonium iodide (I) was prepared by the addition of trimethylamine (T) to 2-phenyl-ethyl iodide (P). 2-Phenylethyl iodide was synthesized first by the action of red phosphorus (0.20 mole) and C.P. Baker's iodine (0.68 mole) on 0.56 mole of redistilled 2-phenylethanol (boiling point 58-60° at 3 mm.). By following a procedure similar to that of Lee and Spinks (17) a 70% yield of the desired was obtained. Then, a portion (0.188 mole) of this iodide was dissolved in 49 ml. of absolute methanol and to it was added 36 ml. (0.21 mole) of 25% tri-methylamine in methanol. During the dropwise addition of the amine to the well-swirled iodide solution at room temperature, no heat was evolved and no color change occurred. After standing for 22 hours at 27°, the solution was scratched with a stirring rod, whereupon it set to a white, crystalline solid.

After filtration and washing, first with cold methanol and secondly with ethyl

ether, the yield was 21 grams of white solid (melting point 206–14° with de-composition). This was found to be a mixture of the desired iodide salt (I)and unwanted trimethylamine hydroiodide. The hydroiodide was decom-posed and removed by heating with alkali. The crude white material, melt-ing point 206–214°, was dissolved in 50 ml. of water and a drop of 2% phenol-phthalein was added. A 4% aqueous sodium hydroxide solution was added dropwise to this boiling mixture until a permanent red color resulted. The odor of trimethylamine was very distinct up to the neutral point. The solution was filtered while at 90°, and, upon cooling, 16 grams of snow white needles (melting point 237.5–239.5°) de-posited from it. This corresponds to a 29% yield of I. Further purification of the needles, by recrystallization from 112 grams of 97% ethyl alcohol, yielded 11 grams of white crystals (melting point 239.5°). Iodide content found by potentiometric titration with silver nitrate for these crystals was 43.55%, compared with 43.59%, the theoretical value for $C_{11}H_{18}NI$.

The remaining organic compounds, which were tested, were commercially available. Whenever necessary, their purity was improved by recrystallization or simple distillation through a Vigreux column.

SPOT TEST PROCEDURE

If not already present in aqueous solution form, a small sample of the material is dissolved in dilute acetic acid or mineral acids, and the pH is adjusted to ca. 3 with dilute sodium hydroxide. One drop of the resulting solution is then placed on a black spot plate, and one drop of a 3 to 7% aqueous sodium tetraphenylborate (NaTPB) solution is added to it. The presence of an amine cation is indicated by the immediate appearance of a precipitate (usually white) or turbidity, the intensity of which depends upon the concentration of the reacting ions. Although the turbidity appears first at the junction of the two drops, it soon spreads throughout the entire liquid. This test could also be performed on black waterrepellent paper, or in a micro test tube against a black background.

DETERMINATION OF SENSITIVITIES

The sensitivities of various amine cations to the tetraphenylborate (TPB) ion were determined as follows:

A suitable sample (10 to 100 mg.) of the compound to be measured was dissolved in water or dilute hydrochloric acid. The solution was poured quantitatively into a volumetric flask (25- or 100-ml.). Just before it was diluted with distilled water to the mark, the solution was adjusted to pH 3 with 0.06N sodium hydroxide solution. Then one drop of the resulting solution, whose concentration can be calculated, was tested with 6.0% sodium tetraphenylborate (TPB) solution according to the spot test procedure. This original solution was diluted repeatedly (using a pipet and volumetric flask) until the test became negative. The sensitivity was recorded as the lowest quantity and concentration of cation in the test solution which still gave a detectable turbidity with one drop of a 6.0% sodium tetraphenylborate solution. To obtain the weight of amine cation used in the test as well as the dilution limit, the medicine droppers were calibrated. The average weight of one drop of the amine solutions was 0.042 gram.

RESULTS AND DISCUSSION

The sensitivities of various cations to one drop of 6.0% sodium tetraphenylborate are recorded in Table I. The limits of identification and dilution limits of each compound in the amine test solutions were calculated according to Feigl's (9) recommendation. Previous results (4) had shown that amines whose basic ionization constants were less than 1×10^{-11} and amines that contained many water-solubilizing (polar)

Table I. Sensitivity of Test for Detecting Amines

	Compound	Ident. Limit, γ	Dilution Limit
А.	Primary amines		
	Methylamine hydrochloride Ethylamine hydrochloride <i>n</i> -Butylamine <i>tert</i> -Butylamine Glycine methyl ester Aniline <i>p</i> -Bromoaniline Benzylamine 2-Phenylethylamine <i>n</i> -Octylamine 1-Naphthylamine	$1.75 \\ 6.3 \\ 2.6 \\ 2.8 \\ 2.7 \\ 2.9 \\ 37.5 \\ 1.82 \\ 0.80 \\ 0.68 \\ 2.2$	$\begin{array}{c} 1:2.4\times 10^{4}\\ 1:6.7\times 10^{3}\\ 1:1.6\times 10^{4}\\ 1:1.5\times 10^{4}\\ 1:1.4\times 10^{4}\\ 1:1.4\times 10^{3}\\ 1:2.3\times 10^{4}\\ 1:5.3\times 10^{4}\\ 1:6.2\times 10^{4}\\ 1:1.9\times 10^{4}\\ \end{array}$
в.	Secondary amines		
	Piperidine Diethylamine Diisopropylamine N-Methylaniline Benzylmethylamine Di-n-butylamine	$\begin{array}{c} 0.30\\ 0.83\\ 0.97\\ 1.46\\ 0.45\\ 0.54 \end{array}$	$\begin{array}{c} 1:1.4 \times 10^5 \\ 1:5.1 \times 10^4 \\ 1:4.3 \times 10^4 \\ 1:2.9 \times 10^4 \\ 1:9.3 \times 10^4 \\ 1:7.8 \times 10^4 \end{array}$
C.	Tertiary amines		
	Pyridine 2-Picoline 4-Picoline Pyridine N-oxide 3-Hydroxypyridine Triethylamine Creatinine N,N-Dimethylaniline Quinoline Quinoline Guinolinol Benzyldimethylamine Triethanolamine p-Dimethylaminobenzaldehyde 1.2-Di(4-pyridyl)ethane Tri-n-butylamine Stearyldimethylamine	$\begin{array}{c} 1.3\\ 0.85\\ 0.25\\ 24.0\\ 2.6\\ 0.34\\ 11.9\\ 1.15\\ 0.97\\ 0.25\\ 0.50\\ 8.4\\ 13.0\\ 2.1\\ 0.44\\ 1.35\end{array}$	$\begin{array}{c} 1:3.2 \times 10^4 \\ 1:4.9 \times 10^4 \\ 1:1.7 \times 10^5 \\ 1:1.8 \times 10^3 \\ 1:1.6 \times 10^4 \\ 1:1.2 \times 10^5 \\ 1:3.5 \times 10^3 \\ 1:3.7 \times 10^4 \\ 1:4.3 \times 10^4 \\ 1:5.0 \times 10^3 \\ 1:3.2 \times 10^3 \\ 1:3.2 \times 10^3 \\ 1:2.0 \times 10^4 \\ 1:9.5 \times 10^4 \end{array}$
D.	Quaternary nitrogen salts		
	Tetramethylammonium iodide Methyl-2-picolinium iodide Methyl-4-picolinium iodide Methyl-4-picolinium iodide Methyllepidinium iodide Trimethyl-2-phenylethylammonium iodide 1,2-Di(4-pyridyl)ethane bismethiodide	$\begin{array}{c} 0.32 \\ 0.15 \\ 0.54 \\ 0.40 \\ 0.17 \\ 0.45 \\ 0.41 \\ 0.84 \\ 0.20 \end{array}$	$\begin{array}{c} 1:1.3 \times 10^5 \\ 1:2.8 \times 10^5 \\ 1:7.8 \times 10^4 \\ 1:1.1 \times 10^5 \\ 1:2.5 \times 10^5 \\ 1:9.3 \times 10^4 \\ 1:1.0 \times 10^5 \\ 1:5.0 \times 10^4 \\ 1:5.0 \times 10^5 \end{array}$
E.	Inorganic salts	0.25	1.1.0 \ 10
_,	Potassium chloride Ammonium chloride Silver nitrate Thallium(I) nitrate Rubidium chloride Mercuric chloride Mercurous nitrate	$\begin{array}{c} 0.13\\ 0.106\\ 0.20\\ 1.5\\ 0.38\\ 1.4\\ 1.05 \end{array}$	$\begin{array}{c} 1:3.2\times10^5\\ 1:4.0\times10^5\\ 1:2.1\times10^5\\ 1:2.8\times10^4\\ 1:1.1\times10^5\\ 1:3.0\times10^4\\ 1:4.0\times10^4\end{array}$
F.	Organic onium salts		
	S-Benzylthiuronium chloride Triphenylsulfonium chloride Tetraphenylarsonium chloride	${ \begin{smallmatrix} 0 & . \ 47 \\ 2 & 5 \\ 0 & . \ 32 \end{smallmatrix} }$	$1:8.9 \times 10^{4}$ $1:1.7 \times 10^{4}$ $1:1.3 \times 10^{5}$

groups were only partially precipitated by the sodium tetraphenylborate reagent. Thus, these types of amines were not investigated here.

The choice of 6.0% for the concentration was a compromise. The stronger the reagent the smaller the quantity of cation detectable, and consequently the more sensitive the test. Unfortunately, sodium tetraphenylborate solutions that are more concentrated than 6% are unstable and must be filtered too frequently. Appearance of turbidity in the stock solution may be delayed by neutralizing it to pH 7 with sodium hydroxide (19), or storing it in a refrigerator when not in use (8). Cooper (3)has made a more comprehensive study of the stability of sodium tetraphenylborate solutions.

To convey an idea of the good sensitivity of amine cations to this tetraphenylborate reagent, the sensitivities of some inorganic cations-potassium, ammonium, silver, thallium, rubidium, mercuric, and mercurous-are listed in Table I. These inorganic cations are generally considered to be very sensitive to the reagent. Feigl and Caldas (10) report the identification limit to be 1γ of potassium, which is higher than the present value of 0.13 γ , because their sodium tetraphenylborate reagent was only 1/6 as strong. Geilmann and Gebauhr (13) found that 5 γ of potassium was detectable in 5 ml. of water by 3.4% reagent. This would calculate to be a dilution limit of 1.0×10^6 , as compared to 3.2 imes 10⁵. This discrepancy may be due to ability to detect a larger quantity of turbidity at a lower concentration level. In their study they used 5 ml.; the present authors used only 0.042 ml. With the exception of some highly water-soluble amines and certain primary amines, most of the amine cations tested possessed sensitivities of the same order of magnitude as the inorganic cations.

In addition to the potassium, ammonium, silver, thallium, rubidium, mercuric, and mercurous cations, cesium would also yield a precipitate (22) under the conditions of the test, and must be absent when testing for amine ions. Geilmann and Gebauhr (13) report the limit of detection for cesium tetraphenylborate to be 0.005 mg. per 5 ml. Certain organic onium cations (4)would also interfere. Some typical examples, whose sensitivities are recorded in Table I, are S-benzylthiuronium chloride, triphenylsulfonium chloride, and tetraphenylarsonium chloride.

The compounds listed in Table I are arranged so that the formula weights of their resulting cations increase downward within any given group. There is some dependence of reaction sensitivity on the size or formula weight of the cation. In general, the larger (or heavier) the cation, the smaller the number

Table II. Effect of Acidity on Sensitivity of Test

	Identification Limit, γ	
a 1	HCl	No
Compound	added	HCI
Tetramethylammonium iodide	0.37	0.0015
Methyl-4-picolinium iodide	0.40	0.080
Methylquinolinium iodide	0.17	0.49
Tetra-n-butylammonium	ı	
bromide	0.29	0.68
Potassium chloride	0.21	0.42
Silver nitrate ^a	0.36	1.9
^a Nitric acid used in chloric acid.	nstead of	hydro-

Table III. Effect of Anions on Sensitivities of Ammonium and Tri-n-butyl-

Compound	Dis- solved in	$\begin{array}{c} \operatorname{Ident.} \\ \operatorname{Limit,} \\ \gamma \end{array}$		
Ammonium chloride Ammonium nitrate Ammonium sulfate Ammonium acetate	$egin{array}{c} \mathrm{HCl} \\ \mathrm{HNO}_3 \\ \mathrm{H}_2 \mathrm{SO}_4 \\ \mathrm{HAc} \end{array}$	$\begin{array}{c} 0.072 \\ 0.070 \\ 0.073 \\ 0.12 \end{array}$		
Tributylamine	HCl HNO3 H2SO4 HAc H3PO4	$\begin{array}{c} 0.44 \\ 0.46 \\ 0.39 \\ 0.47 \\ 0.46 \end{array}$		

of particles of it in solution required for detection. The relative number of particles in each case would be calculated as the minimum detection weight in micrograms divided by the formula weight. Of course, solubility, steric, and basicity factors also affect the sensitivity of the cation, and must be taken into account. Even then, several exceptions are evident in the quaternary salt group to the direct relationship between sensitivity of detection and size of cation. However, members of the quaternary salt group, as a whole, can be detected at lower levels than members of any of the other three groups.

Further examination of Table I shows that some primary amines become progressively more sensitive to the tetraphenylborate reagent, as they are transformed first into secondary, then into tertiary amines, and finally into quaternary salts. The microgram values decrease steadily as one proceeds through each of the following series (nbutylamine 2.6, di-n-butylamine 0.54, tri-n-butylamine 0.44, tetra-n-butylammonium bromide 0.29), (ethylamine 6.3, diethylamine 0.83, triethylamine 0.34), and (aniline 2.9, N-methylaniline 1.46, N,N-dimethylaniline 1.15). Actually, if the values corresponded to the number rather than the weight of the particles in solution, the amounts of decrease would be even more apparent. To rule out the possibility that the decreases are due solely to the increasing size or weight of the various cations, a series was arranged so that each cation was lighter than the previous member. The values obtained (benzylamine 1.82, diisopropylamine 0.97, triethylamine 0.34) indicate that the class to which an amine belongs has some influence in determining its sensitivity.

The data for Table II show the effect of hydrochloric acid on the sensitivity of the tetraphenylborate test. The values in the second column were obtained by the recommended procedure, in which the acidity was adjusted to pH 3 in each case. The numbers in the third column were obtained on water solutions of the various salts, to which no acid was added. In these cases the pH of the organic salt solutions was about 4.5, when the tetraphenylborate was added. The data clearly show that the identification limits depend on the pH of the solution. Experiments with uncharged amines also showed that water solutions of *n*-butylamine, diethylamine, triethylamine, pyridine, and quinoline all gave a turbidity immediately with the tetraphenylborate reagent, even though the solutions were alkaline. Apparently, sufficient cation concentrations were developed as the result of dissociation to give positive tests. However, the sensitivities of these compounds to the tetraphenylborate reagent were much better under acidic conditions. Therefore, pH was fixed at 3, which is acidic enough to convert any amine to its salt, but not acidic enough to risk precipitation of the reagent itself (22), which would be mistaken for a positive amine test.

In Table III are recorded the identification limits of various ammonium and tri-n-butylammonium salts obtained by the spot test procedures. One can conclude that the values for the two cations are not dependent on the type of anion with which each is associated. The higher value of 0.12 obtained in the case of ammonium acetate probably does not represent a real difference from the other ammonium salts, but is caused by the hygroscopic nature of the acetate. Thus, any cold mineral acid or acetic acid could be used, prior to adding the tetraphenylborate reagent, to convert a basic organic nitrogen compound into its cation.

A study, using these sensitivity data as a guide to predict and investigate amines which would be precipitated quantitatively by the tetraphenylborate reagent, is now in progress.

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Determination of Steroid Alcohols with Acetic Anhydride-C-14

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A simple procedure for the measurement of steroid alcohols by acetylation with acetic anhydride-C-14 is described. Application to pure compounds or partially purified mixtures is intended. Preparation of the radioactive acetates permits separation, identification, and quantitation in submicrogram amounts. Excess acetic anhydride can be recovered, to decrease the cost of the analysis. Androsterone, dehydroepiandrosterone, cortisone, and corticosterone can be measured singly or in a mixture. Hydrocortisone and corticosterone can be recovered from plasma. Androsterone and dehydroepiandrosterone cannot be adequately recovered from plasma by this procedure. The procedure is capable of submicrogram determination of steroid alcohols in favorable cases, where radiopurity of the derivative can be obtained.

THIS laboratory is engaged in the separation and estimation of submicrogram quantities of steroids in crude chromatographic fractions of blood and tissue. The radioactive derivative method of Keston and colleagues (8) lends itself to this problem because of high sensitivity and manipulative ease.

In general, the method involves the coupling of the unknown with a reagent of high specific activity. After destruction of excess reagent and addition of nonradioactive product, the derivative is purified. Determination of the specific activity of the derivative readily permits calculation of the amount in the original sample.

Bojesen, Keston, and Carsiotis (3, 4)use the iodine-131 anhydride of piodobenzenesulfonic acid as a reagent for the determination of corticoids. Avivi and colleagues (1) and Berliner (2) used radioactive acetic anhydride for determination of steroid alcohols. This paper describes the preparation of acetic anhydride-C-14 of high specific activity and its use as an analytical reagent.

MATERIALS AND METHODS

Sodium acetate-1-C-14 was prepared in the usual manner from barium carbonate-C-14. No carrier barium carbonate was added, in order to achieve a specific activity of 8 to 10 mc. per mmole. The product was rendered anhydrous by solution of the sodium acetate in dry methanol, evaporation of the solvent with dry nitrogen, and continuous evacuation of the container at 10^{-3} mm. of mercury for 12 hours. During this process, the tube containing the product was maintained at 70° C. by means of an air bath.

Acetic Anhydride-C-14. Acetic anhydride-C-14 of 1 mc. per mmole specific activity or lower was prepared from sodium acetate and phthallyl chloride. A mixture of 164 mg. of sodium acetate-C-14 and 0.15 ml. of redistilled phthallyl chloride was sealed in a 10×75 mm. borosilicate test tube and heated for 2 hours in an electric oven at 70° C. The tube was cooled to room temperature and opened with care in a hood, and 0.2 ml. of dry

benzene was added by means of a syringe and fine needle. The tube was placed in a suitable ground-joint vessel for attachment to the high vacuum line and distilled at room temperature into a small U-trap containing 10 mg. of anhydrous nonradioactive sodium acetate. The sodium acetate removes traces of acetyl chloride. The product was then distilled into a second trap and diluted by the addition of nonradioactive acetic anhydride and sufficient dry benzene to make a 2 to 4%solution of acetic anhydride.

This benzene solution was stable. The yield was 90%. Nonradioactive acetic anhydride prepared by this reaction contained a trace of unidentified impurity which boiled at 130° C. No residue was observed after room temperature evaporation of 1 ml. of this material, which gave a negative or very faint test for halogen. This product was entirely satisfactory for determination and separation of microgram quantities of steroid. However, with 0.05- γ samples the acetates could not be freed of a persistent radioactive impurity which interfered with the analysis. For submicrogram analysis, acetic anhydride-C-14 prepared by exchange (1) was satisfactory.

This was prepared by heating a mixture of 164 mg. of sodium acetate-1-C-14 (8 to 10 mc. per mmole) and 0.10 ml. of nonradioactive acetic anhydride in a sealed tube at 100° C. for 12 hours. The product was then distilled on the high vacuum line and diluted with dry benzene to a concentration of