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NICOTINE SALICYLATE AS A PRIMARY STANDARD FOR NICOTINE

OBJECT:

The object of this study was to investigate the use of nicotine salicylate as a nicotine standard.

SUMMARY:

Nicotine salicylate has been studied as a substitute for nicotine in the standardization of the automated determination of nicotine alkaloids using cyanogen bromide. It has been shown that nicotine salicylate is chemically equivalent to nicotine and can be used instead of the highly toxic nicotine.

Nicotine salicylate has been synthesized and has been shown to be comparable in quality to the commercially available nicotine salicylate.

STATUS:

This study has been completed.

ABSTRACT:

Nicotine salicylate has been studied for use as a primary standard for nicotine. It has been shown that standard solutions of nicotine salicylate are chemically equivalent to standard solutions of freshly vacuum-distilled nicotine.

The stability of nicotine standard solutions has been shown to be excellent for at least 21 months. Stabilities of at least two years for the solid nicotine salicylate were found.

Nicotine salicylate has been synthesized. Studies using ultraviolet, infrared, and nuclear magnetic resonance spectroscopy, in addition to differential thermal analysis, indicate that the synthesized salt is comparable in quality to the commercially available salt.

Nicotine salicylate can be used as a primary standard for nicotine in the cyanogen bromide method for the nicotine alkaloids. It can also be used as a standard for the ultraviolet determination of nicotine if the nicotine standard solution is prepared by steam distillation from nicotine salicylate.

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I. INTRODUCTION

There has long existed a need for a convenient solid nicotine standard. There are numerous disadvantages to using nicotine itself. Although it can be purchased in a reasonably pure state, nicotine very rapidly undergoes chemical change, most probably oxidation. Thus to be absolutely certain of its purity, freshly vacuum-distilled nicotine is required. Vacuum-distilled nicotine is water-white; however, even freezer storage fails to prevent eventual development of a yellow coloration.

In addition to the chemical instability of nicotine and the obvious inconvenience involved in its purification, an important consideration in its handling and use is its toxicity. Nicotine is one of the most violent poisons, being comparable to hydrocyanic acid in toxicity and rapidity of action. It is probably lethal to all classes of animals. The absorption of nicotine takes place in a few seconds from the tongue and eyes, and somewhat more slowly from the stomach; it is readily absorbed from the skin. Due to the speed of absorption, nicotine exhibits its toxicity very rapidly, in a period of minutes exerting a central paralyzing effect upon the brain and spinal cord. In acute nicotine poisoning, death ensues from paralysis of the respiratory muscles, acute cardiac standstill, or fixation of the respiratory muscles during a convulsion. The oral LD_{50} for mammals is given as 55 mg nicotine per kilogram body-weight. Solid compounds of nicotine are also toxic but to a much lesser extent than nicotine itself.

There are only a limited number of solid nicotine compounds (the dipicrate, dihydrochloride, hydrochloride, salicylate, sulfate, and tartrate salts) commercially available. Preliminary experiments showed that for use as a primary standard the majority of these salts are not pure enough to be used as purchased. From this group only nicotine salicylate seemed to offer real hope as a primary standard for nicotine. One obvious disadvantage to the use of nicotine salicylate is that it cannot be used directly as a standard in the ultraviolet method for nicotine, due to the UV absorption of the salicylic acid moiety. It was decided therefore to study the suitability of nicotine salicylate as a standard for the cyanogen bromide (BrCN) procedure for the nicotine alkaloids and to attempt the preparation of a suitable single standard for nicotine later.

It would seem that the preparation of nicotine salts with organic acids should take place quite readily. In practice this does not appear to be true. Attempts have been made by workers in the Research Department to prepare nicotine salts in the past. Efforts were made to prepare the oxalate, citrate, and tartrate salts with only limited success. Nicotine oxalate was prepared in small quantity with difficulty. The most notable success was with some salts prepared with substituted tartaric acids.

The problems associated with the synthesis of nicotine salts were discussed with several members of the Research Department experienced in this area. Several points presented themselves during these discussions: (i) many nicotine salts are liquids or oils, (ii) the hydroscopic nature of nicotine was believed to be detrimental to the formation of crystalline salts, (iii) a salt formed from an acid with a high molecular weight should precipitate more easily, due to the weighting effect, (iv) drying of all solvents used during the preparation of the salt should aid crystal formation, and (v) it seemed to be the general consensus that, given the proper solvent system, an aromatic acid of suitable pK value should form a crystalline salt.

A literature survey has been made and a report written (4) on the preparation and properties of the coordination compounds and salts of nicotine.

II. EXPERIMENTAL

A. Scope

An analytical primary standard must fulfill certain requirements. Any listing of these requisites would have to include the following:

1. The compound must be pure or of known purity.
2. It must have a definite, known chemical structure.
3. It must be chemically stable; it should not be hydroscopic, nor should it be efflorescent.
4. It should be readily available commercially or easily prepared and purified in the laboratory.
5. Its cost should not be prohibitive.
6. It should have a reasonably high equivalent weight.

With nicotine salts the questions of chemical structure, purity, and stability are of major concern.

It would be advantageous to have a single standard that could be used for all of the common methods of determining nicotine; that is, the cyanogen bromide, ultraviolet, nonaqueous titration, and gas chromatographic methods. For use as a standard for spectrophotometric methods, it is essential that the salt itself not be highly colored.

This criterion would eliminate not only the use of nicotine picrate as a nicotine standard but also many of the coordination compounds that nicotine forms with the transition metal salts, since they are also deeply colored. The use of a nicotine compound as a "universal" standard for all nicotine methods would impose other practical requirements upon its selection.

All of the commercially available nicotine salts that seemed to offer possibilities as nicotine standards were purchased. Nicotinic acid and nicotinamide were considered to be excellent prospects for nicotine standards and were also purchased. However, subsequent work showed that these two compounds did not produce absorbances equal to that from an equivalent quantity of nicotine and hopes for their use were abandoned.

Using melting points or differential thermal analysis (DTA), the purity of the commerical nicotine salts was determined. Only the nicotine salicylate was found to be of sufficient purity to warrant further study. DTA gives a corrected melting point of 116-7° C for nicotine salicylate which agrees with the literature value of 116-7° C (1). In addition, the chemical composition of nicotine salicylate is well-defined, having the empirical formula $C_{10}H_{14}N_2 \cdot C_7H_6O_3$. The salt contains no water of crystallization, nor does it appear to be hydroscopic. It is freely soluble in water or alcohol.

This study is concerned with the use of nicotine salicylate as a standard for nicotine in the cyanogen bromide procedure. To investigate this possibility, standard solutions were prepared from nicotine salicylate and their absorbances compared with equivalent nicotine solutions which were prepared from freshly vacuum-distilled nicotine. Studies to determine the stability of nicotine salicylate as the salt and in dilute solution were then made. Finally, nicotine salicylate was synthesized and comparisons were made with the commercially available salt using ultraviolet, infrared, and nuclear magnetic resonance spectra, and differential thermal analysis thermograms.

B. Comparison of Nicotine Salicylate with Nicotine

Solutions of freshly vacuum-distilled nicotine and nicotine salicylate were compared using the cyanogen bromide procedure. A stock solution of nicotine was prepared by weighing 1 g of nicotine and adding extraction solution (2) to a volume of exactly 1 liter. This solution contains 1 mg nicotine per ml. A stock solution of nicotine salicylate was prepared by weighing 0.9257 g of nicotine salicylate and dissolving in extraction solution in a 500-ml total volume. This solution contains 1 mg nicotine per ml. Nicotine salicylate is easily soluble in extraction solution giving a clear solution.

The stock solutions of both the nicotine and the nicotine salicylate were diluted with extraction solution to prepare solutions containing 0.020, 0.040, 0.060, and 0.080 mg nicotine per ml. Calibration curves were prepared from these dilute standard solutions using the BrCN-nicotine procedure. These absorbances are recorded in Table I.

TABLE I

ABSORBANCES OF NICOTINE AND NICOTINE SALICYLATE SOLUTIONS USING THE BrCN METHOD

Nicotine Concentration (mg/ml)	Absorbances	
	Nicotine	Nicotine Salicylate
0.02	0.184	0.188
0.04	0.363	0.365
0.06	0.544	0.547
0.08	0.723	0.721

In conjunction with another project (3), a study of the stability of nicotine salicylate solutions was carried out, where again it was shown that solutions of nicotine salicylate were chemically equivalent to solutions of freshly vacuum-distilled nicotine. Standard curves were prepared from dilutions of a nicotine salicylate stock solution which had been freshly prepared and from a sample of freshly vacuum-distilled nicotine using the BrCN procedure (Table II). The results indicated good stability of the solid nicotine salicylate samples. Although reported previously (3), these results are included in this report for completeness.

TABLE II

ABSORBANCES OF NICOTINE AND NICOTINE SALICYLATE SOLUTIONS USING THE BrCN METHOD*

Nicotine Concentration (mg/ml)	Absorbance	
	Nicotine	Nicotine Salicylate
0.02	0.150	0.157
0.04	0.294	0.300
0.06	0.443	0.447
0.08	0.582	0.587
0.10	0.729	0.731

* Data from reference (3)

As an additional check on the stability of nicotine solutions used as standards, BrCN calibration curves were prepared from the following primary nicotine standards: nicotine(vacuum-distilled April 1, 1970), nicotine (vacuum-distilled January 12, 1972), and nicotine salicylate. The above dates indicate the time of preparation of stock solutions; all dilute solutions were freshly prepared. The nicotine salicylate solutions were prepared January 13, 1972. It is customary to keep all nicotine standard solutions in the refrigerator when not in use. The absorbances obtained from these solutions are shown in Table III.

TABLE III

ABSORBANCES OF NICOTINE AND NICOTINE SALICYLATE SOLUTIONS

Nicotine Concentration (mg/ml)	Absorbance		
	Nicotine (Apr. 1970)	Nicotine (Jan. 1972)	Nicotine Salicylate
0.02	0.154	0.148	0.155
0.04	0.294	0.293	0.294
0.06	0.436	0.439	0.438
0.08	0.578	0.571	0.580
0.10	0.716	0.717	0.718

These results indicate that there is no difference in the nicotine standards run. Stock solutions of nicotine, if well stoppered and refrigerated, are stable for at least 21 months. Although solid samples of nicotine salicylate are stable for at least two years stored in brown bottles at room temperature, it would probably be advantageous to store the nicotine salicylate in a refrigerator.

Nicotine salicylate solutions cannot be used directly in the ultraviolet method for nicotine because of the absorbance of the salicylic acid moiety. It was believed that standard solutions of nicotine could be prepared by steam-distilling either samples of nicotine salicylate or aliquots of a nicotine salicylate solution. To investigate this possibility, three 5-ml aliquots of a nicotine salicylate solution, containing 1 mg nicotine per ml, were individually steam-distilled. All samples were predistilled with 5 ml of glacial acetic acid. After the predistillation, 10 ml of NaOH-NaCl solution were added and the nicotine distilled. Approximately 200 ml of distillate were collected in 10 ml of 2.5 M HCl and made to 250 ml with distilled water. The absorbances of the solutions were measured at 236, 259 and 282 nm. For comparison, a sample of nicotine was also steam-distilled and analyzed (Table IV).

TABLE IV

UV ABSORBANCES OF NICOTINE STEAM-DISTILLED FROM NICOTINE SALICYLATE

Sample	A_{236}	A_{259}	A_{282}
1	0.089	0.712	0.006
2	0.087	0.704	0.006
3	0.090	0.711	0.008
Nicotine (0.02 mg/ml)	0.080	0.713	0.002

A series of aliquots of a standard nicotine salicylate solution was individually steam-distilled from NaOH-NaCl solution without acid predistillation. Two-hundred milliliters of distillate were collected in 10 ml of 2.5 M HCl and diluted to 250 ml with distilled water. The absorbance was measured at 259 nm and compared with direct readings made on solutions of pure nicotine and nicotine salicylate (Table V).

TABLE V

UV ABSORBANCES OF NICOTINE AND NICOTINE SALICYLATE SOLUTIONS

Nicotine Concentration (mg/ml)	Absorbances		
	Nicotine Direct Reading	Nicotine Salicylate Direct Reading	Nicotine Salicylate Distilled
0.004	0.145	0.147	0.148
0.010	0.359	0.369	0.358
0.020	0.716	0.751	0.702
0.026	0.929	0.981	0.924

These results indicate that nicotine salicylate can be used as a UV standard, if the nicotine is separated from the salicylic acid by steam distillation. It also shows that an acid predistillation is not necessary.

C. Synthesis of Nicotine Salicylate

Although nicotine salicylate is available commercially at the present time, a knowledge of its synthesis would be desirable. Nicotine salicylate has been prepared in this laboratory by two

procedures, both of which are rapid and simple. While different solvents were used in the two procedures, the second method uses a brief period of refluxing to promote the reaction. A third procedure, taken from the literature (1) after development of the first two procedures was complete, has been included for comparison.

Procedure 1.

Dissolve 3.0 g nicotine and 2.6 g salicylic acid in minimum amounts of diethyl ether in separate beakers. Mix these solutions and warm in a beaker of hot water (ca 50° C) for a few minutes with stirring. Add petroleum ether until precipitation occurs. Cool in an ice bath with stirring until the mass solidifies into light orange crystals.

Procedure 2.

Reflux a mixture of nicotine (6 g) and salicylic acid (5.1 g) in chloroform for about 10 minutes. Cool the mixture and add petroleum ether to cause precipitation. Recrystallize from the same solvents. The nicotine salicylate recrystallized from chloroform-petroleum ether was almost pure white. A DTA thermogram on this sample gave a melting point of 117° C.

Procedure 3.

Add 1 g nicotine to a suspension of 1.34 g of salicylic acid in absolute ethanol; heat a few minutes on a water bath; cool, shake with diethyl ether. Dissolve the precipitate in hot methanol; clear with charcoal; recrystallize from absolute ethanol and methanol. The pure colorless crystals, dried in vacuo at 60° C, are stable in air (yield 88%).

D. Spectral Comparisons of Nicotine Salicylates

To compare the quality of the nicotine salicylate synthesized with the commercial nicotine salicylate (K&K Laboratories), the ultraviolet, infrared and nuclear magnetic resonance spectra were recorded for both samples. There was excellent agreement between the spectra for each type of instrument.

Fisher-Johns melting point determinations had been attempted on nicotine salts with little success, all the salts decomposing rather than melting sharply. Using differential thermal analysis, the melting points can be easily obtained using the proper heating rate, 20° C per minute. Individual DTA thermograms were run on six commercial samples of nicotine salicylate. The melting points obtained indicate a high degree of purity (Table VI).

TABLE VI

MELTING POINTS OF NICOTINE SALICYLATE

Sample	Melting Points (°C) DTA	Fisher-Johns
1	117	All samples melted with decomposition, 113-7° C
2	117	
3	117	
4	117	
5	116	
6	117	

A thermogravimetric analysis of nicotine salicylate indicated the sample to be stable over the range 28°-110° C. A loss in weight was first observed at 112° C, with a steady decomposition following. Drying at temperatures up to 105° C would be satisfactory; however, drying in vacuo at 60° C would be preferable.

III. DISCUSSION AND CONCLUSION

These studies have shown that standard solutions of nicotine salicylate are chemically equivalent to the same concentrations of freshly vacuum-distilled nicotine in the cyanogen bromide method for nicotine alkaloids.

The stabilities of nicotine salicylate, as the salt, and standard nicotine solutions have been studied, and stabilities of at least two years for the solid nicotine salicylate and 21 months for nicotine solutions were found. Further studies in this area would most likely extend these times. Although the stability of nicotine salicylate solutions themselves was not studied, the information about the stability of nicotine solutions can be directly related to their dependability. The extraction solution used as a solvent for the standard nicotine solution contains acetic acid; the presence of acid is a dominant factor in the permanence of all nicotine solutions, whether prepared from nicotine or from nicotine salts.

Ultraviolet, infrared, and nuclear magnetic resonance spectra indicate that the synthesized nicotine salicylate is comparable in quality to the commercially available salt. Differential thermal analysis thermograms support this contention.

Nicotine salicylate can be used without reservation as a primary standard for nicotine in the cyanogen bromide method. It can also be used as a standard for the ultraviolet determination of nicotine if the nicotine standard solution is prepared by steam distillation from nicotine salicylate.

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