Analytical Determination of Purity of 1-Piperidinocyclohexanecarbonitrile and Its Hydrobromide Salt

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Abstract □ A common contaminant of illicit phencyclidine, 1-piperidinocyclohexanecarbonitrile (I), is a very weak base compared to tertiary amines such as phencyclidine, 1-(cyclohexenyl)piperidine, and piperidine. Compound I and its hydrobromide salt can be selectively detected and quantitated in the presence of synthetic contaminants by nonaqueous titration with perchloric acid in acetonitrile. This method allows direct assay of I under conditions where decomposition is not observed.

Keyphrases □ Phencyclidine—analysis of 1-piperidinocyclohexanecarbonitrile and its hydrobromide salt as synthetic contaminants □ 1-Piperidinocyclohexanecarbonitrile—nonaqueous titrimetric analysis as synthetic contaminant of phencyclidine □ Potentiometric titration—analysis of 1-piperidinocyclohexanecarbonitrile and its hydrobromide salt as synthetic contaminants of phencyclidine

Phencyclidine, a widely abused illicit drug (1), is manufactured primarily in clandestine laboratories and is reported to be contaminated with the synthetic intermediate 1-piperidinocyclohexanecarbonitrile (I) (2–7). The concern over the presence of I in illicit phencyclidine prompted the National Institute of Drug Abuse to make it available for pharmacological evaluation.

Due to the reported instability of I (3, 6–9), a convenient method for determining its purity is needed before carrying out pharmacological studies. This method also must be applicable to the assay of I·HBr, which is the preferred form for use in pharmacological studies. Compound I is unstable during TLC (8–10), GLC (3, 8–10), and mass spectrometry using electron-impact or chemical-ionization procedures (9). In addition, it does not possess a distinctive UV spectrum (10). Therefore, a volumetric method using nonaqueous titration was developed to determine the purity of I and its hydrobromide salt.

EXPERIMENTAL

Apparatus and Materials—A suitable recording pH meter[1] equipped with a combination electrode[2] and coupled with a constant-volume syringe pump[3] (50-ml capacity) was used. All solvents and reagents were analytical reagent grade. Compound I was synthesized by the method of Kalir *et al.* (11) and converted to the hydrobromide salt by dissolving it in acetone followed by the slow addition of 30% HBr in acetic acid until a pH of ~2 was reached. The salt was recrystallized in chloroform–anhydrous ethanol (3:1) brought to the cloud point with anhydrous ether, mp 260–270° dec.

Solutions—The following reagents were used: 0.1 *N* perchloric acid in acetic acid[4], 0.1 *N* perchloric acid in dioxane, 6% (w/v) mercuric acetate in acetic acid, and 1% (w/v) methyl crystal violet in chlorobenzene.

The perchloric acid in acetic acid solution was restandardized by potentiometric titration against potassium biphthalate in acetic acid to which 2.0 ml of 6% mercuric acetate solution was added.

The perchloric acid in dioxane solution was standardized against potassium biphthalate in acetic acid.

Determination of I or I·HBr by Direct Titration with 0.1 *N* Perchloric Acid in Acetic Acid—An accurately weighed sample (90.0–120.0 mg) was placed in a 50-ml beaker with 20 ml of acetic acid and 2 ml of 6% mercuric acetate solution. Dissolution of I and its hydrobromide salt was effected by continuous magnetic stirring for ~10 min; occasionally, heat was applied to aid in solution. The solution, magnetically stirred, was titrated with standard 0.1 *N* perchloric acid in acetic acid. The end-point was determined from the inflection in the titration curve. When the methyl crystal violet test solution was included in the titration, appearance of the emerald-green color was found to coincide with the maximum change in the millivolt reading.

Determination of I by Direct Titration with 0.1 *N* Perchloric Acid in Acetonitrile—An accurately weighed sample (90.0–120.0 mg) was placed in a 50-ml beaker with 20 ml of acetonitrile. The solution, magnetically stirred, was titrated with the standard 0.1 *N* perchloric acid in dioxane. The end-point was determined from the inflection in the titration curve.

Determination of I·HBr by Direct Titration with 0.1 *N* Perchloric Acid in Acetonitrile—An accurately weighed sample (90.0–120.0 mg) was placed in a 50-ml beaker with 20 ml of acetonitrile and 75 μl (0.54 mmole) of triethylamine. The solution, magnetically stirred, was titrated with standard 0.1 *N* perchloric acid in dioxane. The end-point was determined from the inflection in the titration curve; I exhibited a 335-mv difference from the synthetic impurities, 1-(cyclohexenyl)piperidine (II) and piperidine (III), and triethylamine at half-neutralization.

RESULTS AND DISCUSSION

Due to the increased interest in I because of its presence in samples of illicit phencyclidine (2–7), it seemed important to study the metabolism of I in mammals. It was necessary to convert I to a salt form so that it would exhibit good solubility in blood and serum for the development of analytical procedures. In initial experiments, significant difficulties were encountered in extracting intact I from blood. When analyzing the stock solution of I·HBr (~10 mg/ml in normal saline), piperidine was detected by GLC. The instability of I has been documented extensively (3, 6–9). Therefore, before continuing the study, it was necessary to develop a method to determine the purity of I and its hydrobromide salt.

Potential contaminants expected to be present in the hydrobromide salt of I are the hydrobromide salts of II and III. TLC was not suitable for detecting or quantitating these contaminants. Decomposition was minimized but not completely avoided using unactivated silica plates (8). During both GLC and mass spectrometry, the hydrobromide salt of I undergoes thermal decomposition to form II (2, 8, 9), a potential synthetic contaminant. A method was needed that was simple and could be carried out easily in the laboratory.

Titrimetry was the first choice since it is used frequently in the analysis of nitrogenous bases. Initially, aqueous titration of I·HBr using 0.1 *N* NaOH was attempted. Erratic results were obtained, depending on the quantity of I·HBr analyzed (80–120 mg); however, with 100.0 mg (98.0–103.0 mg), the results were reproducible and indicated the purity to be 95.3 ± 0.6% (n = 3). A slight break in the titration curve at pH 8.5 was observed routinely. Since a precipitate was formed during the titration, it was possible that it was affecting the final results. Therefore, methanol–water (1:1) was used as the solvent. Titration in this solvent mixture indicated that the same sample of I·HBr was 73.7 ± 1.2% pure (n = 3) (Fig. 1). These observations indicated that I decomposed during titration to liberate another base that was capable of neutralizing the hydrobromide salt.

A plausible mechanism for the decomposition of I by base is shown in Scheme I, in which 2 moles of base (cyanide and piperidine) are liberated by the decomposition of 1 mole of I. This scheme explains why aqueous

[1] Model EU-301V, Heath/Schlumberger Instruments, Benton Harbor, MI 49022.
[2] Sensorex, Westminster, CA 92683.
[3] Model 341, Orion Research, Cambridge, MA 02139.
[4] N/10, Fisher Scientific Co., Fair Lawn, NJ 07410.

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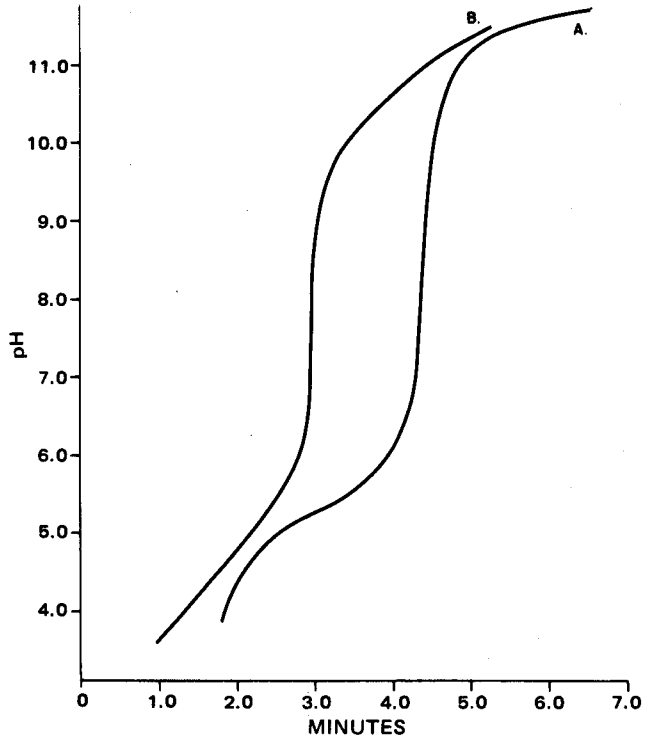


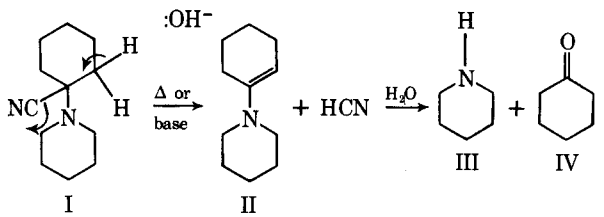
Figure 1—*Titration of the hydrobromide of I with 0.1* N *NaOH in water (A) and in methanol–water (1:1) (B).*

titration of I·HBr gave unrealistically low purity. In addition, since the contaminants of I·HBr are of a lower molecular weight, a sample analyzed by aqueous titration possibly could appear to be of high purity but be badly contaminated due to a balance of decomposition products and intermediates II and III.

Since aqueous titration was unsuitable, a nonaqueous titration in acetic acid was evaluated. The assay gave reproducible results and indicated that the purity was 99.9 ± 1.2% (n = 7). Due to the leveling effect of acetic acid (12), the potential contaminants cannot be differentiated by titration. To determine the sensitivity of this technique, I·HBr was contaminated with III·HBr at 4.5 and 4.6% (w/w). Titration of these samples indicated a purity of 104 and 105%, respectively (theoretical purity of 103.7%). The hydrobromide of I also was contaminated with a 65:35 mixture of the hydrobromides of II and III at 8.2, 8.6, and 10.3% (w/w). Titration of these samples indicated a purity of 103.4, 103.5, and 103.9%, respectively (theoretical purities of 103.0, 103.1 and 103.7%). Nonaqueous titration of a sample of I·HBr (mol. wt. 273.2), which was contaminated with only II·HBr (mol. wt. 246.2) could not detect a 10% (w/w) contamination (theoretical purity of 101.1%).

The nonaqueous potentiometric titration of I as the free base in acetonitrile (Fig. 2) indicated that I is a much weaker base than II and III. It exhibited a 335-mv difference from II, III, and triethylamine.

Although I decomposes rapidly in aqueous solution, a rapid potentiometric titration of I·HBr was performed in a methanol–water (1:1) solution with 0.1 N NaOH at 22°. The pKa obtained was 4.6. Under these conditions, phencyclidine hydrochloride precipitated from solution and no comparison was possible. Titration of I·HBr and phencyclidine hydrochloride was possible in ethylene glycol monomethyl ether–water (4:1), and the apparent pKa values were 3.3 and 8.3, respectively. It is apparent from these data that I is a much weaker base than most tertiary amines. This result was not unexpected since the pKa for N-cyanomethylmethylaminomethane is 4.2 in water (13).



Scheme I

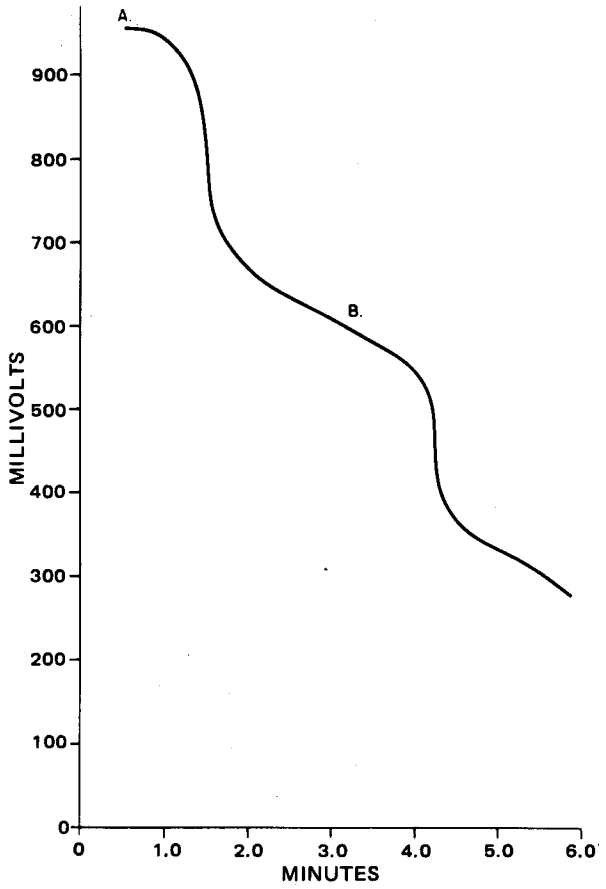


Figure 2—*Nonaqueous potentiometric titration of I in acetonitrile with 0.1* N *perchloric acid in dioxane. Key: A, II, III, or triethylamine; and B, I.*

Due to this difference in basicity and because I is unstable in aqueous solution, a specific nonaqueous potentiometric titration of the free base of I was developed. A closely related method allows analysis of I·HBr. If one assumes that the mechanism of decomposition of I is as outlined in Scheme I, the addition of a slight excess of a sterically hindered base (*i.e.*, triethylamine) effectively transfers the hydrobromide to the stronger base, enabling direct titration of I. This method was quite reproducible and indicated a purity of 98.4 ± 0.4% (n = 6). This value is lower than that observed in the nonaqueous titration in acetic acid but is more reliable since I itself is being titrated and not the hydrobromide salt or acetate anion.

The primary advantage of nonaqueous potentiometric titration is that it enables specific and quantitative analysis of I or any salt in the presence of a mixture of amines. Previous to the development of this method, direct analysis of I under conditions in which no decomposition to II–IV could take place was not possible. This method has potential application for the quantitation of I present in illicit samples of phencyclidine to verify the results obtained using GLC, in which the thermal elimination product, II, usually is analyzed. The presence of a weak acid is expected to interfere in this type of analysis. However, preliminary identification procedures (spot tests, TLC, and GLC–mass spectrometry) would indicate the presence of such a contaminant.

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COMMUNICATIONS

New Direct Calculation of $K_{1:1}$ and $K_{1:2}$ Complexation Constants Using Solubility Method

Keyphrases □ Complexation—calculation of complexation constants using solubility method, derivation of new equation □ Solubility—direct calculation of complexation constants using new equation □ Models, mathematical—equation for direct calculation of complexation constants using solubility method

To the Editor:

The solubility method is used frequently to determine the extent of molecular interactions between compounds. A detailed discussion of this method is available (1). In this method, a solution is maintained saturated with one component, S, and incremental amounts of a second complexing agent, L, are added. At equilibrium, the total S in solution is determined. If the complexes are soluble, an increase in the solubility of S is observed as a function of added L.

If a 1:1 complex is formed (Scheme I), complexation is represented by:

$$S + L \rightleftharpoons SL$$

Scheme I

$$K_{1:1} = \frac{[SL]}{S_0[L]} \quad \text{(Eq. 1)}$$

$$[S_T] = \frac{K_{1:1}S_0}{1 + K_{1:1}S_0}[L_T] + S_0 \quad \text{(Eq. 2)}$$

where $[S_T]$ is the total S concentration in the solution, S_0 is the original solubility of S, $[SL]$ is the concentration of the 1:1 complex, $[L]$ is the concentration of the free complexing agent, $[L_T]$ is the total concentration of the complexing agent, and $K_{1:1}$ is the 1:1 complexation constant. The complexation constant can be determined easily and accurately from Eq. 2.

However, for a system in which both 1:1 and 1:2 complexes are formed, the 1:1 complex (Scheme I) is represented by Eqs. 1 and 2 and the 1:2 complex (Scheme II) is represented by:

$$S + 2L \rightleftharpoons SL_2$$

Scheme II

$$K_{1:2} = \frac{[SL_2]}{S_0[L]^2} \quad \text{(Eq. 3)}$$

where $K_{1:2}$ is the 1:2 complexation constant and $[SL_2]$ is the concentration of the 1:2 complex. The mass balance equation for S becomes:

$$[S_T] = S_0 + [SL] + [SL_2] \quad \text{(Eq. 4)}$$

The combination of Eqs. 1, 3, and 4 results in:

$$[S_T] - S_0 = K_{1:1}S_0[L] + K_{1:2}S_0[L]^2 \quad \text{(Eq. 5)}$$

Since the exact amount of $[L]$ in a system is not known, it has been recognized (1–5) that Eq. 5 cannot be used directly to calculate $K_{1:1}$ and $K_{1:2}$ unless a certain assumption is made. This assumption is that $[L] = [L_T]$ or that all of the complex is in the form of either SL or SL_2. Then the data are manipulated using several approximations to arrive at the values of $K_{1:1}$ and $K_{1:2}$.

These assumptions are totally invalid if the SL and SL_2 concentrations are both very large. The purpose of this article is to derive an equation for calculating the two complexation constants directly and without assumptions.

Since:

$$[L_T] = [L] + [SL] + 2[SL_2] \quad \text{(Eq. 6)}$$

the combination of Eqs. 4 and 6 results in:

$$[L_T] = [L] + [SL] + 2([S_T] - S_0 - [SL]) \quad \text{(Eq. 7)}$$

and:

$$[L_T] = 2[S_T] - 2S_0 + [L] - [SL] \quad \text{(Eq. 8)}$$

Substituting for $[SL]$ in Eq. 8 using Eq. 1 gives:

$$[L_T] = 2[S_T] - 2S_0 + [L] - K_{1:1}S_0[L] \quad \text{(Eq. 9)}$$

Rearranging Eq. 9 results in:

$$[L] = \frac{[L_T] - 2([S_T] - S_0)}{1 - K_{1:1}S_0} \quad \text{(Eq. 10)}$$

Substituting this expression for $[L]$ in Eq. 5 gives:

$$[S_T] - S_0 = \frac{K_{1:1}S_0}{1 - K_{1:1}S_0}\{[L_T] - 2([S_T] - S_0)\} + \frac{K_{1:2}S_0}{(1 - K_{1:1}S_0)^2}\{[L_T] - 2([S_T] - S_0)\}^2 \quad \text{(Eq. 11)}$$

Let:

$$\frac{K_{1:1}S_0}{1 - K_{1:1}S_0} = \alpha \quad \text{(Eq. 12)}$$

and:

$$\frac{K_{1:2}S_0}{[1 - K_{1:1}S_0]^2} = \beta \quad \text{(Eq. 13)}$$

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