Analysis of Organic Bases by Salt Partition

By JOSEPH LEVINE

Most of the pharmaceutically important organic bases will form salts with certain acids which can be extracted from aqueous solution with chloroform. The combination of partition chromatography with this property constitutes the basis of a general method for the analysis of alkoids in commercial dosage forms.

THE CLASSICAL liquid–liquid extraction procedures used in the analysis of alkaloids and of organic bases which have analogous solubility characteristics are founded on the general rule that during partition the free base will concentrate in an organic solvent phase, while the salt of the base will enter an acidic aqueous phase. This principle constitutes the basis of such analytical procedures as the proximate assay of alkaloidal drugs of the "National Formulary" (2) and the assay of salts of organic nitrogenous bases of the "United States Pharmacopeia" (3).

It has been recognized that there are deviations from this rule and that the choice of both the organic solvent and the acid used in the aqueous phase may affect the direction of the partition. Higuchi and Bodin (4) note that in the extraction of alkaloids from immiscible solvent with aqueous acid, "hydrochloric acid is avoided when chloroform is the solvent, since certain alkaloidal hydrochlorides are surprisingly soluble in chloroform."

Deviations from the general partition rule are not restricted to the hydrochloric acid: chloroform system. Methylene chloride and ethylene chloride, which like chloroform are protonated chlorinated solvents, are about equally effective in the extraction of alkoids[1] from acidic solution. Ether is effective in some cases, although to a lesser degree. For example, it will extract appreciable amounts of promethazine from hydrochloric acid solution. Nitric, sulfamic, succinic, tartaric, and sulfuric acids as well as hydrochloric acid are among those acids which form partitionable salts with certain individual alkoids.

The applications of alkoid salt partition to the analysis of drugs has been quite limited until recently. Quantitative extraction of the alkoid salts using the conventional separator technique is frequently impractical because of partition characteristics which strongly favor the aqueous phase. The effect of these unfavorable partition coefficients is to a large extent overcome by the application of partition chromatography. The use of preponderant ratios of organic solvent as the mobile phase with this technique greatly increases the efficiency of the extraction, so that quantitative recovery is achieved readily.

The variety of acids used in the applications of alkoid salt partition thus far reported illustrates the selectivity of the procedure and its broad applicability. Codeine has been determined in combination with pyrilamine and with phenindamine, antihistamines which have widely dissimilar partition characteristics (5). Codeine is separated from pyrilamine by eluting the codeine with chloroform from a chromatographic column using as stationary phase 1 *N* nitric acid, which retains the antihistamine on the column. The converse process is used to achieve separation of codeine from phenindamine. In this case, the antihistamine is eluted with chloroform from a 5% sulfamic acid column, while the codeine is retained; the narcotic then is recovered from the column by a separate procedure. Tartaric acid has been used as the stationary phase in the separation of strychnine from a degradation product of quinine which forms upon aging in elixir of iron, quinine, and strychnine (6). Miller reported (7) that this product is not separated from strychnine by the earlier procedure in which hydrochloric acid is used in the immobile phase (8).

The partitioning of alkoid salts between chloroform and aqueous acid solution is a complex phenomenon, and not all of the factors which govern it are recognized at this time. Unexpected differences occur in the behavior of various salts during partition. Codeine is eluted with chloroform from a Celite: 1 *N* nitric acid column, while chlorpheniramine is retained. If 1 *N* succinic acid is substituted for the nitric acid, however, a complete reversal results; the chlorpheniramine is eluted, while the codeine is retained. The partition of diphenhydramine between chloroform and hydrochloric acid increases progressively in favor of the chloroform phase with increasing concentration of acid. In contrast, the quantity of cyclizine hydrochloride entering the chloroform phase diminishes as the acid strength is increased (Fig. 1).

The individual acids differ markedly in their ability to form partitionable salts with individual alkoids. For instance, sulfuric acid will form such salts with a limited number of alkoids. Hydrochloric acid will do so with a greater number, including all of those which formed such salts with sulfuric acid. *p*-Toluenesulfonic acid (tosic acid) is effective with all those alkoids which form partitionable salts with any of the acids used thus far. It fails in few cases, notably with alkoids which contain a phenolic hydroxyl group in their structure. The application of alkoid partition using tosic acid in the analysis of a number of alkaloids has been described (9). In a reinvestigation of this procedure to ascertain the cause of the low recoveries reported for arecoline, apomorphine, and cocaine, it has been found that the partition of the tosic acid salts of these alkaloids between the acid solution and ether is sufficient that significant proportions of the alkaloid salts are eluted with ether during one step of the procedure.

The simplicity of the manipulations of alkoid salt partition suggests the extension of this technique to the general analysis of a broad range of alkoid salts

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[1] In a discussion of alkaloids and synthetic organic bases which present identical analytical problems, Curry (1) suggested that, "because of the absence of a single noun for the whole group, the word 'alkaloid' should include them." Since the word "alkaloid" is reserved, by long usage, for the natural products, adoption of his proposal at this time would not be feasible. In accord with the intent of his proposal, however, "alkoid" will be used herein to encompass the group.

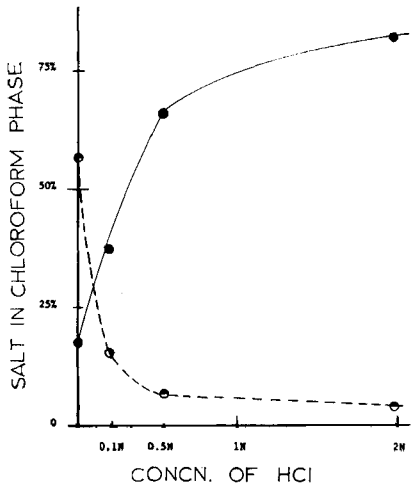

Fig. 1.—Distribution of alkoid salts between equal volumes of chloroform and HCl solution. Key: ⊖, cyclizine HCl; ●, diphenhydramine HCl.

in their various drug dosage forms. Such a general procedure would entail essentially the following steps: (*a*) preparation of a chromatographic column, using as immobile phase a solution of the sample itself and the selected acid, (*b*) removal of neutral or acidic extractives with an ether wash, (*c*) quantitative elution of the alkoid salt with chloroform, and (*d*) direct spectrophotometric quantitation of the eluate.

The acid used with the individual alkoid is selected in accord with the partition characteristics of the resultant salt. The partition coefficient must be sufficiently favorable that the salt will be eluted completely from the column with a reasonable volume of chloroform, but it must be such that none will be removed from the column during the ether wash.

For a rapid screening procedure to select the acid to be used with a given alkoid, an initial trial is made with 2 *N* hydrochloric acid under the conditions described under *Procedure*. If any of the alkoid is eluted in the ether wash, 5% sulfamic acid solution is substituted for the hydrochloric acid; if incomplete recovery of alkoid in the chloroform eluate results, 10% tosic acid solution is used.

METHOD

Chromatographic Column.—A test tube (25 × 250 mm.), to which is attached a 5-cm. length of 7-mm. tubing, is used. The tamping rod is a disk of stainless steel, aluminum, or glass with a diameter about 1 mm. less than that of the column attached to a rod 12–18 in. long. Pack a pledget of fine glass wool[2] in the base of the column as support.

Mixed Solvent.—Mix 8 ml. of methanol,[3] 1 ml. of glacial acetic acid, 4 drops of concentrated hydrochloric acid, and sufficient water-saturated chloroform[3] to make 100 ml.

Sample Preparation.—*Tablets or Capsules.*—Transfer to a 100-ml. beaker an accurately weighed portion of the sample containing the amount of substance noted in Table II. Add 2 ml. of the specified acid (use H_2O with diphenhydramine), warm on steam bath to dissolve, and cool.

Liquids.—Dilute with water so that 2 ml. contains the quantity noted in Table II. Transfer 2.0 ml. to a 100-ml. beaker, and add a quantity of solid or concentrated specified acid to reach the proper concentration.

Preparation of Column.—*A. Sodium Bicarbonate Trap.*—(Use only when *p*-toluenesulfonic acid is specified.) To 2 Gm. of Celite 545[4] add 1 ml. of 1 *M* $NaHCO_3$ solution. Mix until fluffy. Transfer to column and tamp, using gentle pressure, to compress the mixture to a uniform mass.

B. Acid Trap.—In a like manner mix 2 Gm. of Celite with 1 ml. of the specified acid, transfer to column directly above lower layer, and tamp.

C.—Add 3 Gm. of Celite to the sample preparation, mix, and transfer to column directly above acid trap. Dry wash the beaker with 1 Gm. of Celite, transfer to the column, and tamp. (The ether wash will pass through a properly prepared column in 5 to 7 min.)

Procedure.—Pass over the column 100 ml. of water-saturated ether and discard the washings. Place under the column a 100-ml. volumetric flask containing 8 ml. of methanol and 4 drops of hydrochloric acid. Pass over the column a mixture of 90 ml. of water-saturated chloroform and 1 ml. of acetic acid (2 ml. for doxylamine[5]), mix, allow to reach room temperature, and adjust to volume. Concomitantly determine the absorbance of the sample solution and of a solution of reference standard in mixed solvent at a concentration noted in Table I, using mixed solvent as the blank at the wavelength noted in Table I.

DISCUSSION

In an initial trial of this procedure as a general method, it was applied to those products for which the Procedure for Salts of Organic Nitrogenous Bases is official in U.S.P. XVI. This group encompassed nine antihistamines in several dosage forms. The behavior of each antihistamine with three acids, 10% tosic, 5% sulfamic, and 2 *N* hydrochloric, was determined. Ether was found to remove varying but appreciable quantities of phenindamine, promethazine, chlorcyclizine, cyclizine, and diphenhydramine from the tosic acid. It removed small quantities of the first two from hydrochloric acid and none from the sulfamic acid column. Chloroform readily eluted all of the nine antihistamines from the tosic acid column, all except chlorpheniramine, doxylamine, pyrilamine, and tripelennamine from the hydrochloric acid and only phenindamine and promethazine from the sulfamic acid column. Based on these results, 10% tosic acid was selected for use with chlorpheniramine, doxylamine, pyrilamine, and tripelennamine, 2 *N* hydrochloric acid with chlorcyclizine, cyclizine, and diphenhydramine, and 5% sulfamic acid with phenindamine and promethazine.

A 1% solution of acetic acid in chloroform is used in actual practice for elution of the alkoid salt, rather than chloroform alone. Although the latter is generally suitable, tailing occurs in some cases. The addition of acetic acid effectively eliminates the tailing.

[2] Pyrex Filtering Fibre, Corning Glass, catalog No. 3950.
[3] Use solvents conforming to spectro-grade specifications
[4] Johns-Manville Corp.
[5] Esterification, with separation of solvent into two phases, will occur with this concentration of acetic acid in about 4 hr.

TABLE I

Ref. Std.	Recovery, %	Wavelength for Measurement, mμ	Specified Acid	Concn. of Standard (Approx.), mcg./ml.
Chlorpheniramine maleate	100.0 100.9	266	10% Tosic[a]	25
Chlorcyclizine HCl	99.5 99.9 100.1	263	2 *N* HCl	250
Cyclizine HCl	99.6 99.7	264	2 *N* HCl	250
Diphenhydramine HCl	100.0 100.8	257	2 *N* HCl	250
Doxylamine succinate	99.4 99.2	268	10% Tosic	25
Phenindamine tartrate	99.6	261	5% Sulfamic	25
Promethazine HCl	100.0 101.0	304	5% Sulfamic	50
Pyrilamine maleate	100.4 100.2	318	10% Tosic	25
Tripelennamine HCl	99.5 100.3	318	10% Tosic	25

[a] Tosic = *p*-toluenesulfonic acid.

TABLE II

Product	Amt. Used in Anal., mg.	Sample	Found, mg. Proposed Procedure	Found, mg. U.S.P. XVI Procedure
Chlorpheniramine maleate	2.5	Tablets, 4 mg.	4.19 4.19	4.06 4.08
		Injection, 10 mg./ml.	10.5 10.4	... ...
Chlorcyclizine HCl	25	Tablets, 25 mg.	24.9 24.6	24.4 24.1
		Tablets, 50 mg.	47.1 47.7	48.4 47.6
Cyclizine HCl	25	Tablets, 50 mg.	47.4 47.8	46.1 45.3
Diphenhydramine HCl	25	Capsules, 25 mg.	25.0 25.0	24.8 25.1
Doxylamine succinate	2.5	Tablets, 12.5 mg.	12.5 12.4	12.2 12.1
Phenindamine tartrate	2.5	Tablets, 10 mg.	10.2 10.2	9.2 9.7
Promethazine HCl	5	Tablets, 12.5 mg.	12.2 12.1	12.0 12.0
		Injection, 25 mg./ml.	24.7 24.5	... ...
Pyrilamine maleate	2.5	Tablets, 25 mg.	24.7 24.5	23.7 24.0
Tripelennamine HCl	2.5	Tablets, 25 mg.	24.5	24.7
Tripelennamine citrate		Elixir, 30 mg./4 ml.	30.5	30.8

In measuring the ultraviolet absorbance of the eluate, it is essential, in the case of those alkoids in which the basic nitrogen atom constitutes a portion of the chromophoric group, that protonation be complete, since the absorbance and wavelength of the maxima vary with the degree of ionization. To accomplish this, concentrated hydrochloric acid, together with methanol to achieve miscibility, is added to the chloroform eluate.

For those analyses using tosic acid, the eluate is passed through a column segment containing sodium bicarbonate solution to remove the anion, since this acid absorbs in the ultraviolet region.

In applying the procedure to commercial products, two minor modifications of the general procedure were found to be necessary for individual products. (*a*) Unlike the other antihistamines, diphenhydramine cannot be heated with acid to facilitate solution, since it is cleaved readily to benzhydrol. Therefore, water is used for solution of samples containing this product. The specified acid still is used in the trap layer. (*b*) A small amount of tailing occurred occasionally with doxylamine. This is remedied by increasing the acetic acid content of the chloroform eluant to 2%.

Samples of U.S.P. reference standard material and of commercial dosage forms of the nine antihistamines were analyzed by associates who had varying amounts of experience with partition chromatographic techniques. The commercial products were also analyzed by the U.S.P. XVI procedure. Results of the analyses are presented in Tables I and II.

In the investigation of the extraction patterns of the salts of the nine antihistamines, the number of acids used in the study arbitrarily was limited to three to establish the minimal requirements for a general procedure. These are not necessarily the

optimum acids in all cases. It is obvious from Fig. 1, for instance, that in the analysis of cyclizine it would be logical to substitute 0.5 *N* HCl for the 2 *N* acid specified. The latter acid was entirely suitable, however, for effecting quantitative recoveries. A simple screening, as described above, with the three acids was effective in the selection of a suitable acid for the assay of a wide variety of alkoids in addition to those discussed, including methapyrilene, codeine, pheniramine, antazoline, thonzylamine, methorphan, hexylcaine, narcotine, and others. Thus, the need to explore the behavior of each alkoid vis-à-vis a variety of acids over a wide range of concentrations is obviated. It is to be expected, of course, that acids other than these three will be required for some individual alkoids; the screening will indicate this situation.

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Potential Radiation Protective Agents IV. Sulfur Analogs Related to Norephedrine

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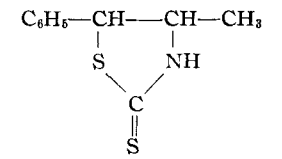
Condensation of 2-amino-1-chloro-1-phenylpropane with potassium ethyl xanthate gave 4-methyl-5-phenylthiazolidine-2-thione. Reaction of 2-amino-1-chloro-1-phenylpropane with sodium thioacetate gave 2-acetamido-1-mercapto-1-phenylpropane, which oxidized in air to the corresponding disulfide. The amide group of the latter compound, bis-(2-acetamido-1-phenyl-1-propyl) disulfide, was cleaved by refluxing with acid.

IN A CONTINUATION of work reported previously on mercapto analogs related to ephedrine (1, 2), similar analogs corresponding to norephedrine were investigated also. *dl*-Norephedrine reacted with thionyl chloride to produce 2-amino-1-chloro-1-phenylpropane hydrochloride (3). This salt was converted to the free base, and its reaction with two different sulfur nucleophiles was studied. Upon reaction of the free base with potassium ethyl xanthate, cyclization occurred to produce 4-methyl-5-phenylthiazolidine-2-thione (I). Upon condensation of the free base with sodium thioacetate, migration of



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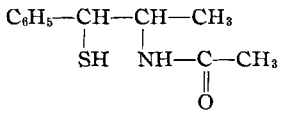

II

the acetyl group from sulfur to nitrogen occurred to produce 2-acetamido-1-mercapto-1-phenylpropane (II). During isolation, the mercaptan was partly air-oxidized to the corresponding disulfide.

Hydrolysis of the acetyl group of bis-(2-acetamido-1-phenyl-1-propyl) disulfide was hindered, but it could be forced under drastic conditions. The amide was recovered unchanged after refluxing for 6 hr. in a mixture of 30 ml. of ethanol and 20 ml. of concentrated hydrochloric acid. The amide group was cleaved, however, by refluxing for 48 hr. in concentrated hydrochloric acid. Originally, bis-(2-amino-1-phenyl-1-propyl) disulfide dihydrochloride was obtained, but because solvent of crystallization gave difficulty in securing a satisfactory analytical sample, this compound was converted through the free base to the hydrobromide salt.

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EXPERIMENTAL

4-Methyl-5-phenylthiazolidine-2-thione (I).—To a solution of 20.6 Gm. (0.1 mole) of 2-amino-1-chloro-1-phenylpropane hydrochloride, m.p. 201–202° (3), in 200 ml. of anhydrous methanol was added 23 ml. of methanolic sodium methoxide (10% w/v sodium) to liberate the free base. Potassium ethyl xanthate (16 Gm., 0.1 mole) was added, and the mixture was refluxed for 4 hr. After cooling, the mixture was filtered, and the solvent was evaporated under reduced pressure in a rotary evaporator. The white gum which remained was crystallized from isopropyl alcohol and cyclohexane to give, after several recrystallizations from the same mixture, a yield of 5.6 Gm. (27%), m.p. 97–98°.

Anal.—Calcd. for $C_{10}H_{11}NS_2$: C, 57.38; H, 5.30; N, 6.69; S, 30.64. Found: C, 57.44; H, 5.27; N, 6.73; S, 30.94.

2-Acetamido-1-mercapto-1-phenylpropane (II).—To a solution of 35.5 Gm. (0.172 mole) of 2-amino-1-chloro-1-phenylpropane hydrochloride (3) in 200 ml. of absolute methanol was added 39.5 ml. of methanolic sodium methoxide (10% w/v sodium) to liberate the free base. A solution of sodium thioacetate, prepared from 12.5 ml. (0.172 mole) of thioacetic acid in 39.5 ml. of methanolic sodium methoxide (10% w/v sodium), was added; the mixture was refluxed for 3 hr. with stirring. After