Simple Determination of Some Acid Salts of Organic Bases

By TATJANA BIĆAN-FIŠTER and ANTONIJA ANDREC

The present official methods of analyses for preparations containing acid salts of organic bases, when based on the determination of the isolated base, are time consuming and tedious. An extraction procedure based on the basic hydrolysis of the organic salt in the presence of magnesium oxide was used to convert the salt to the corresponding free base, which was then titrated directly with an acid. The method was applied to tablets containing certain alkaloids or some other organic salts.

THE ASSAY of acid salts of organic bases official in the majority of pharmacopeias is based on the determination of the liberated base which involves prior extraction from the salt carried out by the classical procedure in alkaline media with organic solvents using separators. Although these procedures are satisfactory assay procedures, they have several disadvantages. The number of manipulations involved not only makes the procedure tedious and time consuming, but also allows for more sources of error.

The purpose of this study was to develop a rapid, accurate, and simple method for the determination of acid salts of organic bases which could be applied to the control of tablets containing such salts. In the procedure developed in this study the salts were determined by using a simple extraction procedure based on the basic hydrolysis of the salt in the presence of magnesium oxide, making possible a direct titration of the liberated base with standard acids.

EXPERIMENTAL

Reagents

Chloroform, ethylacetate, ether, glacial acetic acid, and ethanol (96%) reagent grade, were employed. Magnesium oxide was used for chromatography (Merck AG, Darmstadt). U.S.P. Crystal violet indicator solution and methyl red indicator solution and B.P. methyl red-methylene blue indicator solution were employed. Codeine phosphate, papaverine hydrochloride, chlorpromazine hydrochloride, and amphetamine sulfate were commercial samples of U.S.P. quality.

Determination of Salts

Codeine Phosphate.—Accurately weigh approximately 0.3 Gm. of codeine phosphate, transfer into a glass crucible $G_4\sqrt{}$;[1] add 0.5 Gm. magnesium oxide and moisten only slightly with a few drops of water. Mix well with the aid of a glass rod and extract the alkaloid base with four 10-ml. portions of chloroform. Evaporate the chloroform from the filtrate to dryness by heating on a steam bath, dissolve the residue in 10 ml. glacial acetic acid, and titrate the liberated base with 0.1 *N* perchloric acid to a distinct blue color using crystal violet as indicator. Perform a blank determination on the solvents and make any correction necessary.

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[1] √porosity 2r = 5-λ5μ.

TABLE I.—DETERMINATION OF SALTS

Salt	Pharmacopeia Method	Proposed Method
Codeine phosphate	99.1	102.2
		101.7
		101.8
Papaverine hydrochloride	97.3	101.2
		101.0
		101.4
Chlorpromazine hydrochloride	99.0	98.9
		99.2
		98.7
Amphetamine sulfate	98.7	99.8
		99.5
		100.1

TABLE II.—DETERMINATION OF TABLETS

Tablets	Theoretical Content mg./Tablet	Recovery mg./Tablet	Recovery %
Codeine phosphate	300.0	296.0	98.7
		295.0	98.4
		302.0	100.4
Papaverine hydrochloride	40.0	39.7	99.25
		39.6	99.0
		39.2	97.4
Chlorpromazine hydrochloride	250.0	253.0	101.2
		256.0	101.8
Amphetamine sulfate	75.0	73.8	98.8
		73.5	98.0

Papaverine Hydrochloride.—Papaverine hydrochloride was determined in the same way as described for *Codeine Phosphate*.

Chlorpromazine Hydrochloride.—Approximately 0.2 Gm. of chlorpromazine hydrochloride is extracted in the same way as described for *Codeine Phosphate*, with five portions each of 10 ml. ether. Evaporate the ether from the filtrate, dissolve the residue in 2 ml. ethanol, add 20 ml. of 0.1 *N* hydrochloric acid and 15 ml. of water, and titrate the excess of acid with 0.1 *N* sodium hydroxide using the mixed indicator solution methyl red–methylene blue. The titration of the extracted base can be carried out also in nonaqueous media (glacial acetic acid solution) with 0.1 *N* perchloric acid using crystal violet as indicator.

Amphetamine Sulfate.—Approximately 75 mg. of amphetamine sulfate is extracted in the same manner as described for *Codeine Phosphate*, with four portions each of 10 ml. ethylacetate. After adding to the filtrate 40 ml. of glacial acetic acid, the liberated base is titrated with 0.1 *N* perchloric

acid to a distinct blue color, using crystal violet as indicator.

For purposes of comparison, codeine phosphate, chlorpromazine hydrochloride, and amphetamine sulfate were assayed by the methods official in B.P., while papaverine hydrochloride by that official in Ph.Dan. Results of these experiments are recorded in Table I.

Determination of Salts in Tablets

Codeine phosphate, papaverine hydrochloride, chlorpromazine hydrochloride, and amphetamine sulfate in tablets are determined in the same manner as directed in the assay of the salts, the quantity of the powdered tablets being equivalent to the stated amount of the corresponding salt. Results of these determinations are recorded in Table II. Experiments were carried out with standard tablets prepared in this laboratory.

SUMMARY

A simple method for the determination of certain acid salts of organic bases, based on the basic hydrolysis of the salt in the presence of magnesium oxide has been described. The proposed method proved to be a rapid, simple, and accurate one that makes it especially useful for routine work.

Examinations on the possibilities of the application of this procedure to other pharmaceutical preparations will be carried out and reported in a subsequent communication from this laboratory.

Antimicrobial Properties of Aliphatic Thiosemicarbazones

By M. MANOWITZ and G. WALTER

A series of thiosemicarbazones of saturated and α,β-unsaturated aliphatic aldehydes was prepared and tested *in vitro* for activity against various bacteria, yeasts, and molds. Antimicrobial potency was found to depend on chain length and was greatly enhanced by the presence of an unsaturated linkage. Thiosemicarbazones derived from unsaturated aldehydes with chain lengths C-10 to C-12 were the most active against the microorganisms tested.

THE TUBERCULOSTATIC PROPERTIES of thiosemicarbazones have been investigated extensively since the initial publication by Domagk and coworkers (1). A large number of thiosemicarbazones have been prepared and tested for these properties; however, their effect on other microorganisms has received only limited consideration (2–5). Benns, *et al.* (6), determined the antifungal activity of 40 thiosemicarbazones against *Aspergillus niger* and *Chaetomium globosum* and found the most effective compounds were derived from aliphatic aldehydes. Preliminary observations in our laboratories have demonstrated interesting antibacterial properties for certain aliphatic thiosemicarbazones; a further study of the antimicrobial spectrum of a more extensive series of these compounds was indicated. For this purpose, thiosemicarbazones of saturated and unsaturated aliphatic aldehydes were prepared and tested against various bacteria, yeasts, and molds.

EXPERIMENTAL

The thiosemicarbazones of the C-4 through C-12 straight chain saturated aldehydes and of the C-4 through C-13 α,β-unsaturated aldehydes, with the omission of the C-5 unsaturated compound, were included in this investigation. The thiosemicarbazones were prepared by usual methods described in the literature (3).

Antimicrobial Tests.—The antimicrobial properties of the compounds were determined by agar dilution technique employing the following organisms: *Staphylococcus aureus*, ATCC 6538; *S. epidermidis*, ATCC 155; *Bacillus subtilis*, ATCC 9372; *Escherichia coli*, ATCC 11229; *Proteus vulgaris*, ATCC 9920; *Pseudomonas aeruginosa; Bacterium ammoniagenes*, ATCC 6871; *Pityrosporum ovale; Candida albicans*, ATCC 10231; *Trichophyton mentagrophytes*, ATCC 9129; and *Microsporum audouini*, ATCC 11347.

Twofold serial dilutions of the compounds were prepared in alcohol (S.D.30) and 0.2-ml. aliquots of each dilution added to 20-ml. tubes of molten agar. The contents of the tubes were thoroughly mixed and poured into sterile Petri plates. Dextrose tryptone extract agar was employed for the bacteria and Sabouraud's dextrose agar was used for the yeasts and molds.

Bacterial inoculum consisted of 1–100 distilled water dilution of a 24-hour, tryptic soy broth culture grown at 35°. Yeast inoculum was prepared by washing a 3-day-old slant of the organism with 10 ml. of distilled water and diluting the suspended cells 1–100 with distilled water. Mold inoculum consisted of a conidial suspension from the surface growth of a 7-day slant of the organism in 20 ml. of distilled water. Plates were inoculated by placing 1 drop (0.007 ml.) of the inocula on the surface of the hardened agar media with the aid of an Accu-Drop dispenser.[1] Inoculated plates were incubated at 35° for bacterial tests and at 30° for yeasts and molds. Examination of the plates for the presence of growth was made after incubation periods of 48 hours for the bacteria, 4 days for the yeasts, and 14 days for the molds.

RESULTS

Results of the microbiological tests are summarized in Table I. None of the compounds was active against the Gram-negative bacteria (*E. coli, Ps. aeruginosa, Pr. vulgaris*) and therefore these organisms were not included in the table. The data

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[1] Scientific Products, Flushing, Long Island, N. Y.