Spectrophotometric Determination of Poldine Methylsulfate and Application to Pharmaceutical Preparations

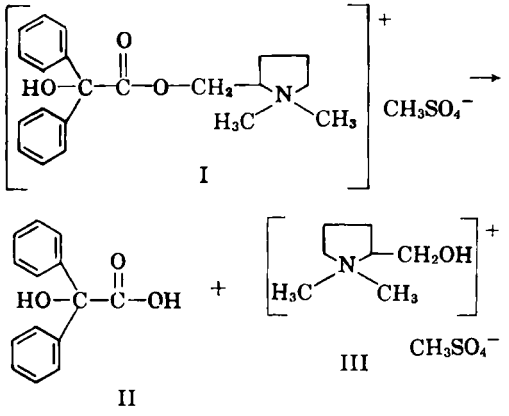
By JOHN W. POOLE and ALEXANDER A. MONTE

A spectrophotometric method for the determination of poldine methylsulfate is presented. The ultraviolet absorbance of methanol solutions of the compound is the analytical measurement utilized in the procedure. The quantitative separation of the drug from interfering substances and degradation products is accomplished through the formation of a reineckate derivative with the subsequent regeneration of the conjugate base by ion-exchange chromatography. The method is shown to be applicable to various pharmaceutical preparations and is useful for studying the hydrolytic degradation of the molecule.

PREVIOUSLY PUBLISHED METHODS for the determination of poldine methylsulfate[1] have employed infrared spectroscopy and colorimetric procedures. The infrared procedure of Rapson, *et al.* (1), was designed primarily for the examination of samples taken from bulk supplies of the pure material. Langley and his co-workers (2) employed a methyl orange technique for the determination of poldine methylsulfate in biological fluids; Singleton and Wells (3) utilized the ammonium cobaltothiocyanate complex for the colorimetric determination of this compound in pharmaceutical dosage forms. However, the colorimetric procedures cannot be applied to pharmaceutical formulations in many instances because of the presence of interfering substances.

The spectrophotometric assay described here might be expected to offer an advantage in that the absorption bands utilized correspond to the drug molecule being analyzed. The assay has been developed for the determination of the drug molecule in various pharmaceutical formulations alone and in combination with other compounds. In addition, it has been employed to follow the hydrolysis of this compound in aqueous systems under varying conditions of pH and temperature.

Poldine methylsulfate (2-benziloyloxymethyl-1,1-dimethylpyrrolidinium methylsulfate) (I) can hydrolyze to benzilic acid (II) and 2(α-hydroxymethyl) - 1,1 - dimethylpyrrolidinium methylsulfate (III). The ultraviolet absorption spectrum of poldine methylsulfate, shown in Fig. 1, is due almost entirely to the benzilic acid moiety. The development of a spectrophotometric assay for this compound resolved itself to the quantitative separation of the drug from interfering substances and its degradation product, benzilic acid.



Many organic bases react with ammonium reineckate to form derivatives which are useful in their identification and determination. Reineckate derivatives have been quantitatively determined gravimetrically, colorimetrically, and by titration procedures (4–8). However, in many instances, these methods suffer from a lack of specificity which may be overcome by regeneration of the base with the subsequent analysis of the pure material. Kapfhammer and Eck (9) utilized the treatment of acetone solutions of the reineckate derivative with silver sulfate, then with barium chloride as a means of regeneration of the basic compounds. More recently, Kum-Tatt (10) accomplished this regeneration by the use of a strong anion-exchange resin with acetone solutions of the reineckate derivatives.

In the present study, the formation of a reineckate derivative of the drug with the subsequent regeneration of the conjugate base by means of ion-exchange chromatography was the method used to accomplish the necessary quantitative separations. The ultraviolet absorption at 257 mμ of methanol solutions of the regenerated

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[1] Marketed as Nacton by McNeil Laboratories, Inc., Fort Washington, Pa.

compound was the analytical measurement employed in this procedure.

The purpose of this study was to develop an assay procedure for poldine methylsulfate useful for the determination of this drug in pharmaceutical preparations and in the presence of its hydrolysis products.

EXPERIMENTAL

A number of cationic and anionic ion-exchange resins were investigated as a means of regeneration of the conjugate base from the reineckate derivative of poldine methylsulfate. The only system studied which was found to give quantitative recovery of the basic compound with methanol solutions of the reineckate derivative was a weak anionic exchange resin. In the case of the strong cationic exchange resins, complete elution of the drug from the resin was not possible, while the intermediate and weak cationic exchange resins did not retain the drug quantitatively. The strong anionic exchange resins were efficient in removing the reineckate ion from the methanol solutions but also retained a considerable quantity of the drug molecule.

The use of varying amounts of reagents and filtration of the insoluble reineckate derivative over a wide range of time showed that critical control of conditions was not necessary to obtain reliable and accurate results with this procedure. The requirements for quantitative formation of the reineckate derivative were found to be sufficiently acidic sample solutions and an excess of reineckate reagent. No variance in results was noted when the samples were stored at 5° for 24 hours or in an ice bath for 2 hours. The details of the procedure are stated below.

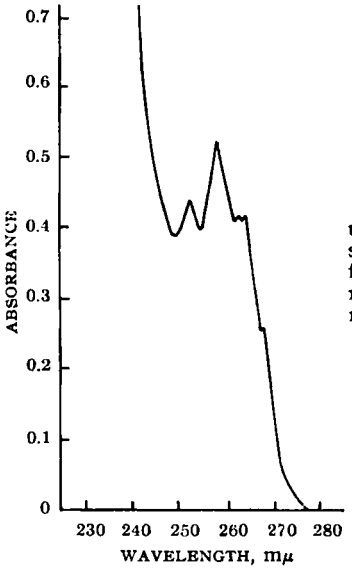

Fig. 1.—The ultraviolet absorption curve for poldine methylsulfate in methanol.

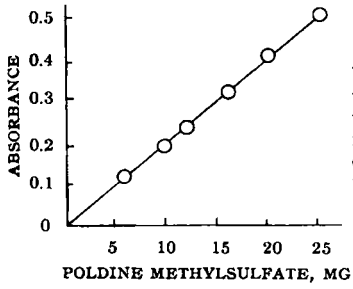

Fig. 2.—Calibration curve for the spectrophotometric determination of poldine methylsulfate.

Reagents.—*Ammonium Reineckate Solution.*—Approximately a 1% solution is prepared by dissolving 1 Gm. of ammonium reineckate in 100 ml. of distilled water. Shake for 10 minutes and filter through filter paper.

Ammonium Reineckate Wash Solution.—Add 2 ml. of ammonium reineckate solution to 1 L. of distilled water.

Methanol.—Analytical reagent grade is utilized.

Amberlite IR-4B Anionic Resin.—The resin is allowed to stand overnight in a 5% aqueous sodium hydroxide solution, then washed with distilled water until the effluent is neutral.

Procedure.—Add 10 ml. of ammonium reineckate solution to 20 ml. of a 5% sulfuric acid solution containing 10 to 50 mg. of poldine methylsulfate, and place this mixture in an ice bath for 2 hours. Filter the precipitate formed through a sintered-glass crucible, wash with 30 ml. of ammonium reineckate wash solution. Dry the precipitate in a vacuum oven for 1 hour at 50°, then dissolve the reineckate derivative in 30 ml. of absolute methanol. Prepare an ion-exchange column with a pledget of glass wool and 2 to 3 Gm. of Amberlite IR-4B anionic resin, previously treated as described above, and wash with 50 to 75 ml. of methanol. The methanol solution of the reineckate derivative is passed through the column at a rate of about 1 ml. per minute and collected in a 50-ml. volumetric flask. If color is present in the effluent, it is returned to the column or passed through a second column of the same type. The resin column is washed with sufficient methanol

Table I.—Assay of Poldine Methylsulfate in Aqueous Solutions Corresponding to a 10% Hydrolytic Degradation of the Drug

Sample	Poldine Methylsulfate, mg.	Benzilic Acid, mg.	2(α-Hydroxymethyl)-1,1-dimethylpyrrolidinium Iodide, mg.	Poldine Methylsulfate Found, mg.
1	45.0	2.5	2.5	45.7
2	45.0	2.5	2.5	45.5
3	45.0	2.5	2.5	44.3
4	45.0	2.5	2.5	45.6

Table II.—Determination of Poldine Methylsulfate in Tablets Containing a Combination of Poldine Methylsulfate and Butabarbital Sodium

Batch No.	Age, Mo.	Poldine Methylsulfate, mg./tablet— Declared	Found
271:43	3	4.0	4.10
	6	4.0	4.11
	7	4.0	4.01
278:31	3	4.0	4.00
278:35	2	4.0	3.81
	4	4.0	3.94
R1013	0	4.0	4.06
	2	4.0	3.96
R1017	2	4.0	4.05
	3	4.0	4.04

to bring the final volume to 50 ml. The absorbance of the methanol solution is measured at 257 mμ in a 1-cm. cell with a suitable spectrophotometer. The methanol wash from 2 to 3 Gm. of the Amberlite IR-4B anionic resin is used as a blank and the poldine methylsulfate content is determined from a standard curve. A Cary model 14 spectrophotometer was used for all absorbance measurements in this study.

Various preliminary manipulations were employed for the determination of poldine methylsulfate in the several pharmaceutical dosage forms investigated. Solutions such as parenterals and syrups were appropriately diluted with sulfuric acid and analyzed as described. For the analysis of tablets, 20 tablets were accurately weighed and reduced to a powder in a mortar and pestle. A suitable weight of powder equivalent to 50 mg. of poldine methylsulfate is extracted with 50 ml. of a 5% sulfuric acid solution, filtered, and a 20-ml. aliquot of the filtrate used for the assay.

TABLE III.—DETERMINATION OF POLDINE METHYLSULFATE IN SYRUPS

Sample	Poldine Methylsulfate, mg./5 ml. Declared	Found
A	4.0	4.22
B	4.0	4.25
C[a]	4.0	3.91
D[a]	4.0	3.84

[a] These samples contained butabarbital sodium, 15 mg./5 ml.

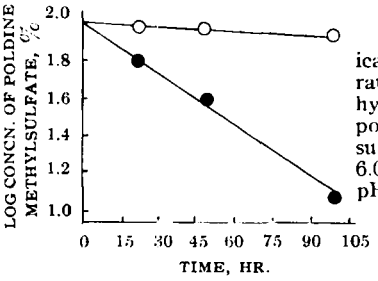

Fig. 3.—Typical first-order rate plots for the hydrolysis of poldine methylsulfate at pH 6.0 ○ and at pH 7.0 ●.

RESULTS AND DISCUSSION

Figure 2 illustrates a standard curve obtained with a series of aqueous poldine methylsulfate solutions carried through the complete analytical procedure. The precision of the analytical method was tested on a number of simple aqueous solutions of the drug, and the standard deviation in a series of 27 assays was found to be 1.84%.

Aqueous solutions of benzilic acid and 2(α-hydroxymethyl)-1,1-dimethylpyrrolidinium iodide alone and in combination showed no absorbance at 257 mμ when carried through the entire analytical procedure. To determine the reliability of the method for poldine methylsulfate in the presence of these products, synthetic mixtures, corresponding to a 10% hydrolytic breakdown of the drug, were prepared and analyzed. The results of this study are shown in Table I.

Table II shows the results of assays on compressed tablets containing a combination of poldine methylsulfate and butabarbital sodium.[2] The results obtained on several batches of syrups containing the drug alone and in combination with the barbiturate are given in Table III. None of the ingredients normally used in the formulation of such products showed interference in the assay procedure.

The stability of aqueous solutions of poldine methylsulfate under varying conditions of pH and temperature were studied employing the assay procedure described. Standard solutions containing 2.0 mg./ml. of the drug were prepared utilizing 0.2 *M* biphthalate or 0.2 *M* phosphate buffers, and the pH adjusted between 3.0 and 8.0 with hydrochloric acid or sodium hydroxide. These solutions were maintained at a constant temperature of 40°, 60°, or 80° and aliquots removed and assayed at various times. Some typical apparent first-order plots for the hydrolysis of poldine methylsulfate are shown in Fig. 3. The temperature dependency of the hydrolysis reaction, under the conditions of the study, is illustrated by the Arrhenius plots of Fig. 4. The heat of activation was determined from this data to be 20 Kcal./mole. Table IV summarizes the apparent first-order rate constants observed in this study. No hydrolytic degradation was detected in solutions buffered at pH 3.0 or 3.5 after 80 days at 40°.

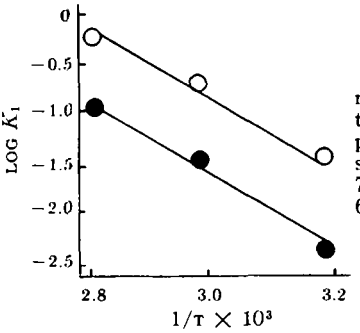

Fig. 4.—Arrhenius plots for the hydrolysis of poldine methylsulfate at pH 7.0 ○ and at pH 6.0 ●.

TABLE IV.—APPARENT FIRST-ORDER RATE CONSTANTS FOR THE HYDROLYSIS OF POLDINE METHYLSULFATE UNDER VARIOUS CONDITIONS OF pH AND TEMPERATURE

pH	Temp., °C.	Apparent First-Order Rate Constant (K_1) (hr.$^{-1}$ × 10²)
4.0	40	0.01
4.5	40	0.02
5.0	40	0.04
	60	0.33
	80	3.00
6.0	40	0.21
	60	2.80
	80	15.00
7.0	40	2.20
	60	9.20
	80	46.00
8.0	40	14.00

In the course of this investigation, it was noted that the amine formed by the hydrolysis of poldine methylsulfate forms a reineckate derivative which is insoluble in methanol. This suggests the possibility of the separation of amines by selective solution of derivatives of this type.

The disadvantages of the analytical method reported here are that it is rather time consuming and relatively large quantities of drug are required for the assay. However, it is applicable to a wide

[2] Marketed as Nactisol by McNeil Laboratories, Inc., Fort Washington, Pa.

variety of complex samples and unusual techniques or critical control of the conditions are not necessary to obtain reliable and accurate results.

SUMMARY

A spectrophotometric method is described for the determination of the quaternary ammonium compound, poldine methylsulfate. The method is based on the ultraviolet absorbance of methanol solutions of the drug after separation of interfering substances by a reineckate derivative and regeneration of the conjugate base through ion-exchange chromatography. The method has been shown to be applicable to the determination of the compound in pharmaceutical preparations and in the presence of its hydrolytic degradation products. The stability of the compound in buffered aqueous systems under various conditions of pH and temperature was investigated utilizing the spectrophotometric procedure presented.

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Methandrostenolone

Mechanism of Hydrochloric Acid Induced Fluorescence

By F. TISHLER and S. M. BRODY

The main products formed when methandrostenolone (17α-methyl-17β-hydroxyandrosta-1,4-dien-3-one) is heated with methanolic hydrochloric acid have been isolated by preparative thin-layer chromatography and identified by application of infrared, ultraviolet, and nuclear magnetic resonance spectra. A mechanism for the hydrochloric acid induced fluorescence is postulated.

IN A PREVIOUS PAPER by Tishler, *et al.* (1), a fluorometric procedure was described for the determination of methandrostenolone[1] (Compound I, Fig. 1) based on the fluorogen formed when the steroid was heated with a methanolic solution of hydrochloric acid at 100°. A number of related steroids were studied to determine the selectivity of the reaction. Under the conditions employed, the reaction appeared to be selective for $\Delta^{1,4}$-dien-3-one or $\Delta^{1,3,5(10)}$-trien-3-ol steroids which had both a 17β-hydroxy and a 17α-alkyl or alkyne substitution.

With the aid of preparative thin-layer chromatography it has been possible to isolate the main products formed during the reaction. Based upon the identification of these products, a mechanism for hydrochloric acid-induced fluorescence with methnadrostenolone and structurally related steroids is postulated.

DISCUSSION

A preparative thin-layer technique as described by Korzun, *et al.* (2), was used to isolate the desired compounds. Figure 2 shows a typical separation after the entire plate has been sprayed with a modified LeRosen reagent (3). A summary of the isolated fractions is given in Table I.

The total recovery was approximately 80%. Material was lost due to the development of a guide strip on the plates and due to strongly adsorbed material which was not eluted. The three major components, Compounds IV, V, and VIII (Fig. 1), were further purified by chromatographing them separately on a small alumina column. Figure 3 shows the purified steroids as chromaotgraphed on a standard thin-layer plate together with the reaction mixture.

It was evident from the ultraviolet spectrum (Fig. 4) of the reaction mixture that methandrostenolone had undergone a change in its structure. Since the reaction was selective for those steroids which had the structure previously described above, one could predict that the changes had occurred solely in ring A or D or in both rings.

Infrared and nuclear magnetic resonance (N.M.R.) data of Compounds IV, V, and VIII showed the loss of the hydroxyl group originally present in ring D of methandrostenolone. The N.M.R. spectra for the above compounds showed one or two bands between 55 and 61 cycles per second, a region characteristic of the $C_{17,17'}$ dimethyls (4). The integrated area of the bands indicated the presence of six hydrogens which is consistant for two methyl groups. It was apparent that the loss of the 17-hydroxyl group was followed by the migration of

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[1] Marketed as Dianabol by Ciba Pharmaceutical Co., Summit, N. J.