administered over 2 min. Such differences can result in correlation variations between pharmacological activity and blood concentration if the site of action resides in the tissue compartments. It would be reasonable to assume that the site of action probably will be, at least in part, in one or more of the tissue compartments, since the compartments simply represent time-dependent equilibration processes. Even if the organ on which the drug is acting can be clearly classified as part of the central compartment, such as the heart, the actual site of action may reside in tissue compartments because of the essential nature of time-dependent processes involved in the tissue equilibration.

From these data, it is clear that some degree of error will invariably result if an instantaneous input assumption is made. Therefore, all intravenous administrations should be considered as short-term zero-order inputs.

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Simple and Rapid Determination of a New Pharmaceutically Active Amine Hydrochloride

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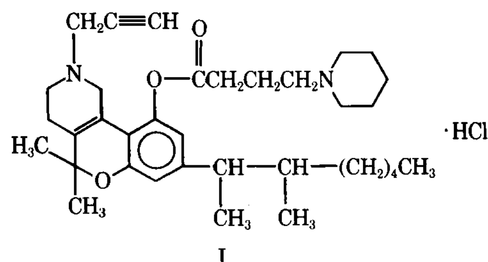
Abstract □ The quantitative analysis of a new pharmaceutically active amine hydrochloride is described. Samples are extracted with chloroform. A yellow amine-dye complex is formed by buffering a sample–bromthymol blue solution at pH 8.5 ± 0.1 and subsequently extracted with chloroform. The complex is treated with 0.01 *N* NaOH to convert it back to the sodium salt of bromthymol blue, which is then measured at 615 nm in the aqueous layer. The amount of complex extracted is linearly related to the amount of amine present, from 0.020 to 0.20 mg/ml. Under the selected conditions, the compound can be determined in the presence of degradation products and there is no interference from common pharmaceutical excipients. The method is suitable for stability studies.

Keyphrases □ Amine hydrochlorides—pharmaceutically active substituted benzopyranopyridine, complexation with bromthymol blue, colorimetric analysis □ Benzopyranopyridine, substituted—pharmaceutically active compound, colorimetric analysis □ Colorimetry—analysis, pharmaceutically active substituted benzopyranopyridine □ Bromthymol blue—use in colorimetric analysis of pharmaceutically active substituted benzopyranopyridine

A new biologically active compound with a benzopyran nucleus (I) was synthesized. Chemically, I is 5,5-dimethyl-10-[4-(1-piperidine)butyryloxy]-8-(3-methyl-2-octyl)-2-(2-propynyl)-1,2,3,4-tetrahydro-



5*H*-[1]benzopyrano[3,4-*d*]pyridine hydrochloride. It is readily soluble in water, chloroform, and ethanol, and its melting point[1] is 103–106°.

The method for the determination of amine salts, involving the formation of an ion-pair with indicator, is based on Prudhomme's (1) discovery that alkaloids form chloroform-soluble complexes with acid dyes such as eosin, the reaction being quantitative (2). The methods available for the determination of quaternary ammonium salts were reviewed previously (3). The extraction of the amine–bromthymol blue complexes of numerous compounds into methylene chloride was studied, and the optimum pH value was 7.4 (4). Thiamine was analyzed using bromthymol blue with a pH 6.6 buffer solution (5).

The indicator extraction method has the advantages of high sensitivity, rapidity, and partial selectivity and appeared to be of potential value for pharmaceutical preparations. The purpose of this investigation was to develop a stability method for the assay of a new pharmaceutically active amine hydrochloride (I) in various dosage forms.

EXPERIMENTAL

Instruments—The following were used: a UV–visible spectrophotometer[2] with 1-cm cells[3], a pH meter[4], a centrifuge[5], and an analytical balance[6].

[1] Thomas-Hoover, USP Class I.
[2] Beckman DU (or equivalent), Beckman Instruments, Fullerton, Calif.
[3] Bausch and Lomb.
[4] Beckman expandomatic instrument (or equivalent).
[5] International clinical centrifuge.
[6] Type H-51, Mettler Instrument Corp., Princeton, N.J.

Table I—Effect of the Aqueous Phase pH on the Reagent Blank

pH of Aqueous Phase	Absorbance at 615 nm[a]
6.4	0.500
6.6	0.350
7.0	0.162
7.4	0.065
7.6	0.042
8.3	0.015
8.6	0.013
9.1	0.006
9.6	0.002
10.1	0.000

[a] The absorbance was obtained by comparing the reagent blank solution at each pH against distilled water as a blank.

Reagents and Solutions—All reagents were ACS grade.

Borate Buffer, pH 8.4—Dissolve 12.5 g of boric acid and 14.9 g of potassium chloride in water to make 1 liter (Solution A). Make a 0.8% sodium hydroxide solution (Solution B). Mix 500 ml of Solution A with 85.5 ml of Solution B and adjust to pH 8.4 ± 0.1, if necessary, with either Solution A or B.

Bromthymol Blue Solution—Use a 0.1% bromthymol blue sodium in 0.08% sodium hydroxide solution.

Buffer-Dye Mixture—On the day of use, mix 100 ml of borate buffer, pH 8.4, with 20 ml of bromthymol blue solution. The pH of the final solution is 8.5 ± 0.1. The pH of the solution decreases when allowed to stand overnight.

Standard Preparation—Weight accurately about 20 mg of reference standard, transfer to a 200-ml volumetric flask, dilute to volume with chloroform, and mix. This standard preparation should have a concentration of about 0.1 mg/ml.

Assay Preparation—Transfer an accurately weighed portion of the pharmaceutical preparation, equivalent to about 2 mg of I, to a 30-ml centrifuge tube. Add 20.0 ml of chloroform, shake the tube mechanically for 30 min, and then centrifuge for about 10 min. Filter the solution into a 30-ml centrifuge tube through a 0.45-μm silver membrane filter[7], using a 20-ml syringe attached to a suitable filter holder[8], and stopper immediately. The filtrate is the assay preparation.

Assay Procedure—Pipet 4.0 ml of the assay preparation and 4.0 ml of the standard preparation into separate 50-ml centrifuge tubes. Pipet 4.0 ml of chloroform into a similar 50-ml centrifuge tube to provide a blank. Add 20.0 ml of chloroform to each tube and mix. Taking one tube at a time, add 10.0 ml of buffer–dye mixture, shake the tube horizontally for 2 min, centrifuge for about 3 min, and then remove the upper aqueous layer by aspiration.

Pipet 10.0 ml of each chloroform layer into separate 30-ml centrifuge tubes. To each tube, add 10.0 ml of 0.04% sodium hydroxide; shake the tube horizontally for 2 min and then centrifuge for about 5 min. Concomitantly determine the absorbance of the upper layers obtained from the assay preparation (A_u) and the standard preparation (A_s) in 1-cm cells at 615 nm, with a suitable spectrophotometer, against the blank.

Calculate the milligrams of I as follows:

$$\text{mg of I per dose} = \frac{A_u}{A_s} CD \frac{F}{W} \qquad \text{(Eq. 1)}$$

where A_u = absorbance of the unknown, A_s = absorbance of the standard, C = concentration of the standard preparation (milligrams per milliliter), D = dilution factor, F = average dosage weight (grams per dose), and W = sample weight (grams).

RESULTS AND DISCUSSION

Compound I degrades in water, acid, and base by hydrolysis to 5,5-dimethyl-10-hydroxy-8-(3-methyl-2-octyl)-2-(2-propynyl)-1,-2,3,4-tetrahydro-5*H*-[1]benzopyrano[3,4-*d*]pyridine (II) and γ-piperidinobutyric acid hydrochloride (III). The degradation products were identified by TLC and compared with authentic samples of II and III. The type of plate used was silica gel GF_{254}, and the developing solvent was chloroform–methanol (80:20). Arsenomolybdate spray reagent was used for detection.

[7] FM-25, Selas Flotronics, Spring House, Pa.
[8] Swinnex-25, Millipore Corp., Bedford, Mass.

Table II—Effect of the Aqueous Phase pH on Compounds I and II

pH of Aqueous Phase	*A* at 615 nm[a], Compound I	*A* at 615 nm[a], Compound II
8.1	0.910	0.035
8.3	0.735	0.010
8.5	0.525	0.000
8.7	0.260	0.000
8.9	0.068	0.000

[a] The absorbance was obtained from the standard solution (0.10 mg/ml in chloroform) of I or II through the procedure and compared against the reagent blank at each pH.

GLC cannot be used since the compound cannot be silylated without degradation. Also, direct UV spectrophotometry cannot be used as a stability method because I has a characteristic band at 300 nm (maximum) that is partially overlapped by II.

The described dye transfer method is a stability method. It has been used to assay I when formulated with various materials and compounded with approved excipients for pharmaceutical formulations.

A single chloroform extraction of the yellow amine–dye complex is made possible by buffering the dye solution to pH 8.5 ± 0.1. An amount of the dye proportional to the concentration of the drug was carried into the chloroform phase as a yellow complex compound. The complex appeared to form rapidly in the aqueous phase. The absorbance at 410 nm was obtained from the standard solution (0.10 mg/ml in chloroform) of I through the procedure using the buffer–dye mixture at pH 8.5 and compared against the reagent blank. The absorbances after 1, 2, 3, and 4 min of shaking were 0.261, 0.265, 0.264, and 0.265, respectively, indicating that the phase equilibrium was complete after 2 min of shaking.

Aqueous alkali produced dissociation of the ion-pair, resulting in the stable soluble sodium salt of bromthymol blue which was determined colorimetrically. The absorbance at 615 nm was obtained from the standard solution (0.10 mg/ml in chloroform) of I through the procedure using the buffer–dye mixture at pH 8.5 and compared against the reagent blank. The absorbances determined initially and after 1, 2, 3, and 4 hr at ambient temperature (25 ± 2°) were 0.520, 0.521, 0.520, 0.520, and 0.521, respectively. The conversion of the yellow ion-pair (ϵ_m = 9300 at 410 nm) to the blue color of the sodium salt of bromthymol blue (ϵ_m = 18,300 at 615 nm) makes it possible to assay lower concentrations of sample accurately because of the higher molar extinction and prevents interference caused by any yellow color imparted by the sample.

The effect of pH variation in the aqueous phase on the extraction of the complex into chloroform was studied (Tables I–III). The absorbance was measured after the complex was converted to the sodium salt of bromthymol blue and extracted.

Experimental results indicate that the optimum pH range of the aqueous phase is 8.4–8.6. Samples containing as little as 0.25 mg of active amine salt/dose were analyzed by this method. Beer's law is obeyed from 0.02 to 0.20 mg of I/ml of solution.

The validity of the single-extraction procedure was demonstrated

Table III—Effect of the Aqueous Phase pH on Compound III

pH of Aqueous Phase	Absorbance at 615 nm[a]
8.1	0.035
8.3	0.013
8.5	0.003
8.7	0.000
8.9	0.000

[a] The absorbance was obtained from the saturated solution of III through the procedure and compared against the reagent blank at each pH. The saturated solution of III used for dye transfer was obtained by shaking 10 mg of III in 200 ml of chloroform for 30 min and then filtering the solution through a 0.45-μm silver membrane filter, using a 20-ml syringe attached to a suitable filter holder. The solubility of III in chloroform at room temperature is about 0.02 mg/ml.

on nine assays conducted on 3 days. Mean and standard deviation values were 9.66 mg/g ± 0.12 mg for the powder. The relative standard deviation was ±1.3%.

On the basis of this investigation, it is concluded that the proposed method is a superior stability assay of this new pharmaceutically active amine hydrochloride.

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Stereochemistries of Geometric Isomers of 4-(2-Bromo-1,2-diphenylvinyl)phenol, 4-(2-Bromo-1,2-diphenylvinyl)anisole, and 2-[p-(2-Bromo-1,2-diphenylvinyl)phenoxy]triethylamine: Corrections of the Literature

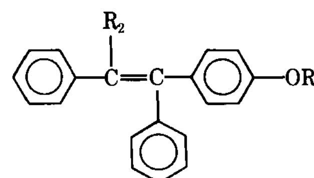
A. RICHARDSON, Jr. *, H. D. BENSON *, and G. HITE ‡ˣ

Abstract □ The stereochemistries of geometric isomers of 4-(2-bromo-1,2-diphenylvinyl)phenol, 4-(2-bromo-1,2-diphenylvinyl)anisole, and 2-[*p*-(2-bromo-1,2-diphenylvinyl)phenoxy]triethylamine were determined by conversion of the phenolic analog to the ethers and subsequent comparison of physical properties with those of 2-[*p*-(2-chloro-1,2-diphenylvinyl)phenoxy]triethylamine of known stereochemistry.

Keyphrases □ Stereochemistry—geometric isomers of various substituted 1,2-diphenylvinyl compounds determined, literature correction □ Geometric isomers—various substituted 1,2-diphenylvinyl compounds, stereochemistry determined, literature correction □ Isomers, geometric—various substituted 1,2-diphenylvinyl compounds, stereochemistry determined, literature correction □ 1,2-Diphenylvinyl compounds, substituted—various, stereochemistry of geometric isomers determined, literature correction

The more estrogenic geometric isomers of 2-[*p*-(2-chloro-1,2-diphenylvinyl)phenoxy]triethylamine, clomiphene[1] (I) (1), and of 2-[*p*-(1,2-diphenyl-1-butenyl)phenoxy]-*N*,*N*-dimethylethylamine (II) (2) were designated *trans-(E)* (3) and *cis-(Z)* (4), respectively. Single-crystal X-ray diffraction studies on isomers of I and II (5) established the *cis*-stereochemistry for the more estrogenic isomers, permitted a consistent correlation of the biological data with structure and stereochemistry, and provided insight into the molecular mechanisms of action (6). Numerous conflicting aspects of the structural determinations of I and II prompted this report, which will review the chemical and physical lines of evidence, place them in perspective, and record the correct stereochemistries and physical properties of 4-(2-bromo-1,2-diphenylvinyl)anisole (III), 2-[*p*-

[1] Clomid, Merrell-National Laboratories, Division of Richardson-Merrell Inc., Cincinnati, Ohio.



cis-configuration

I: $R_1 = CH_2CH_2N(CH_2CH_3)_2$, $R_2 = Cl$
II: $R_1 = CH_2CH_2N(CH_3)_2$, $R_2 = CH_3CH_2$
III: $R_1 = CH_3$, $R_2 = Br$
IV: $R_1 = CH_2CH_2N(CH_2CH_3)_2$, $R_2 = Br$
V: $R_1 = H$, $R_2 = Br$

(2-bromo- 1,2- diphenylvinyl)phenoxy]triethylamine (IV), and 4-(2-bromo-1,2-diphenylvinyl)phenol (V), which played the dominant role in the earlier incorrect assignments of stereochemistry to geometric isomers of I (5, 7).

EXPERIMENTAL

(*Z*)-4-(2-Bromo-1,2-diphenylvinyl)phenol (V*a*)—This compound was prepared as described by Longfellow and Jackson (8), mp 145–147° [lit. (8) mp 147–149°].

(*Z*)- and (*E*)-4-(2-Bromo-1,2-diphenylvinyl)anisoles (III*a* and III*b*)—These isomers, previously identified incorrectly as *E* and *Z*, respectively, were prepared by the method described by Koelsch (9): (*Z*), mp 117.5–119° [lit. (9) mp 118–120°, lit. (10) mp 118.5–119.5°], and by Curtin *et al.* (10): (*E*), mp 93–94.5° [lit. (10) mp 97–98°]. In addition, III*a* was obtained by treatment of a solution of V*a* with methyl iodide and base.

(*Z*)- and (*E*)-2-[*p*-(2-Bromo-1,2-diphenylvinyl)phenoxy]-triethylamines (IV*a* and IV*b*)—These compounds were prepared as described by Palopoli *et al.* (3). Their physical properties are summarized in Table I.