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Improved Colorimetric Determination of Primary Aromatic Amines with 9-Chloroacridine: Application to Some Local Anesthetics

J. T. STEWART and D. M. LOTTI

Abstract □ Improvement in the stability of the 9-chloroacridine stock solution has been made in the colorimetric method for primary aromatic amines based on the reaction between the acridine and an amine. The improved procedure has been applied to some local anesthetics and local anesthetic mixtures. It has been found to be comparable in sensitivity to other local anesthetic determinations, particularly the popular diazotization-coupling procedures. Quantitative data from several systems reveal that use of this procedure permits the determination of local anesthetics in the presence of various drugs and other local anesthetics. Comparative analyses were performed with the method of Bratton and Marshall on procaine, metabutethamine, and nesacaine hydrochlorides.

Keyphrases □ Amines, primary aromatic—improved analysis □ 9-Chloroacridine solution—stabilization □ Tetrahydrofuran—9-chloroacridine solvent □ Colorimetric analysis—spectrophotometry

A colorimetric method for the determination of small quantities of primary aromatic amines with 9-chloroacridine and its use in the analysis of some sulfonamides have been previously reported by this laboratory (1, 2). Data presented in these papers showed that the sensitivity of the method rivals that of the commonly used diazotization-coupling procedures for primary aromatic amines and sulfonamides. It is necessary for the ethanolic 9-chloroacridine stock solution to be prepared immediately before use since the acridine undergoes rapid ethanolysis in ethanol (3). This fresh solution is permissible to use for approximately 0.5 hr. after preparation.

The objective of this paper is to report an improvement in the existing method by stabilization of the 9-chloroacridine stock solution, and application of the method to the analysis of several local anesthetics and local anesthetic mixtures. A comparative study of this improved technique was made with the procedure of Bratton and Marshall (4).

EXPERIMENTAL

Apparatus—Spectra and absorbance measurements were made with spectrophotometers (Perkin-Elmer, model 202, and Beckman, model DU). Matched cells with a 1-cm. optical path were used.

Reagents and Chemicals—9-Chloroacridine[1] was used as the chromogenic reagent. Powdered samples of propoxycaine hydrochloride,[2] butacaine sulfate,[3] butesin,[3] butethamine hydrochloride,[4] metabutethamine hydrochloride,[4] nesacaine hydrochloride,[5] benzocaine,[1] and procaine hydrochloride[6] were used in the analytical procedure for preparation of standard curves. Piperocaine,[7] lidocaine,[8] and tetracaine[9] hydrochlorides were also used in the analysis. All other chemicals used were the highest grade of the commercially available materials.

Solutions (4×10^{-4} *M*) were prepared by dissolving weighed amounts of benzocaine and butesin in ethanol and the remaining local anesthetics in water. The reagent solution of 9-chloroacridine (4×10^{-4} *M*) was prepared by dissolving a weighed amount in tetrahydrofuran[10] and storing in a light-resistant volumetric flask.[11]

Procedure—One milliliter of an ethanolic or aqueous solution of a local anesthetic (4×10^{-4} *M*) was placed in a 10-ml. volumetric flask. To this was added 1 ml. of a tetrahydrofuran solution of 9-chloroacridine (4×10^{-4} *M*). Then the pH was adjusted to approximately 4 with 10% v/v aqueous hydrochloric acid. The solution was shaken and allowed to sit for 15 min. at room temperature, followed by the addition of ethanol to volume, and absorbance was measured at 435 mμ. Absorbance measurements were corrected for reagent blanks in the procedure.

RESULTS AND DISCUSSION

The colorimetric method for primary aromatic amines using 9-chloroacridine has been improved by using tetrahydrofuran in place of ethanol as solvent to make the acridine stock solution. Data shown in Table I for some local anesthetics reveal that the addition of small amounts of tetrahydrofuran to the analytical procedure does not cause any significant change in the sensitivity. The ethanolic solution was only useful for about 0.5 hr. due to reaction between the acridine and ethanol, and it was a disadvantage to prepare new acridine stock solutions that often. There was a need for a solvent in which dissolution, but no reaction between the acridine and solvent, would occur. Miscellaneous solvents were investigated, but only tetrahydrofuran proved successful in meeting these requirements.

[1] Eastman Chemical Co.
[2] Sterling Drug Co., Rensselaer, N. Y.
[3] Abbott Laboratories, North Chicago, Ill.
[4] Novocol Chemical Manufacturing Co., Inc., Brooklyn, N. Y.
[5] Strasenburgh Laboratories, Rochester, N. Y.
[6] Purocaine Chemical Co., New York, N. Y.
[7] Eli Lilly and Co., Indianapolis, Ind.
[8] Astra Pharmaceutical Products, Worcester, Mass.
[9] Winthrop Laboratories, New York, N. Y.
[10] Mallinckrodt, analytical reagent grade.
[11] Low actinic volumetric flask (Corning No. 55640).

Table I—Comparison of Absorbance Readings of Reaction Products Formed During the Analytical Procedure, with Ethanol and/or Tetrahydrofuran (THF) as Solvent for 9-Chloroacridine

Local Anesthetic	Concn., $M \times 10^{-5}$	Absorbance — Analytical Reaction With Ethanol	Absorbance — Analytical Reaction With THF
Propoxycaine hydrochloride	4.00	0.305	0.310
Butacaine sulfate	4.00	0.325	0.320
Butethamine hydrochloride	4.00	0.305	0.305

There was, however, limited success with *tert*-butyl alcohol. The tetrahydrofuran solution should be stored in a light-resistant volumetric flask, since it has been observed in this laboratory that light caused some decomposition of the 9-chloroacridine, possibly through a free radical reaction (5). Stock solutions of the acridine in tetrahydrofuran have been stable up to 1 month after preparation when properly stored.

tert-Butyl alcohol has some limitations in its employment as a solvent for 9-chloroacridine. The alcohol is very difficult to handle since it freezes at 25.5° (6). The observed reaction time for the analytical procedure is 30–45 min. longer with *tert*-butyl alcohol present than with tetrahydrofuran, and the stock solution of the acridine is stable for only 1 week. It has been possible in this laboratory to overcome the disadvantage of handling due to freezing by insulating the volumetric flask containing the acridine stock solution, but the observed slower reaction time for the analysis and instability of the reagent solution for periods longer than 1 week make tetrahydrofuran the preferred solvent for the acridine.

The improved method using the tetrahydrofuran–acridine solution was then applied to the analysis of some local anesthetics and local anesthetic mixtures. The results indicated that the reaction between local anesthetics containing a primary aromatic amino group and 9-chloroacridine to yield highly colored aminoacridine hydrochlorides can be utilized as a suitable assay procedure for local anesthetics. The absorption curve in the visible spectrum for a typical sample of benzocaine is shown in Fig. 1; the absorption maximum occurs at 435 mμ. Reagent blank readings at this wavelength are very low.

In comparing absorption curves of the colored solutions obtained with equimolar concentrations of the various local anesthetics containing primary aromatic amino groups, it was noted that the curves were almost identical. Compounds such as propoxycaine, butacaine, butesin, butethamine, metabutethamine, and procaine all produce color that absorbs at the same wavelength and with essentially the same intensity as does benzocaine. Absorbance values for these anesthetics were between 0.30–0.35 absorbance units at 435 mμ as exemplified by benzocaine and metabutethamine (Fig. 1).

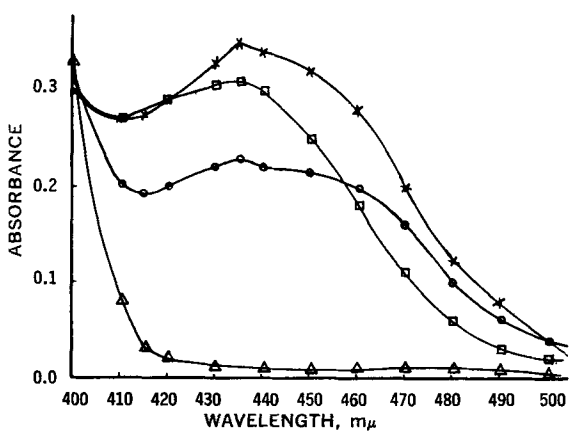

Figure 1—*Visible absorbance curves of aminoacridine derivatives of local anesthetics. Key: ×, benzocaine; □, metabutethamine hydrochloride; ○, nesacaine hydrochloride; and △, reagent blank.*

Table II—Analysis of Known Local Anesthetic Mixtures for Local Anesthetic

Mixture	Components, Concn. of 4.00×10^{-5} M	Local Anesthetic — Found, $M \times 10^{-5}$	Local Anesthetic — Yield, %
I[a]	Procaine hydrochloride Tetracaine hydrochloride	3.976	99.4
II[b]	Propoxycaine hydrochloride Piperocaine hydrochloride	3.960	99.0
III[c]	Butacaine sulfate Lidocaine hydrochloride	3.988	99.7
IV	Benzocaine Benzyl alcohol 8-Hydroxyquinoline	3.992	99.8
V	Butethamine hydrochloride Pentobarbital sodium Aminophylline	3.972	99.3
VI	Butesin Menthol Camphor Isopropyl alcohol	3.988	99.7

[a] Mixture analyzed for procaine content. [b] Mixture analyzed for propoxycaine content. [c] Mixture analyzed for butacaine content.

Structurally all of these compounds have a primary aromatic amino group in the position *para* to the ester linkage except metabutethamine, in which the amino function is *meta* to the ester linkage. Nesacaine shows somewhat diminished intensity values at the same wavelength (Fig. 1), due presumably to a less-than-quantitative yield of reaction with 9-chloroacridine caused by the inductive effect of the chloro group *meta* to the primary amino group in the local anesthetic.

The local anesthetics, piperocaine, lidocaine, and tetracaine, which do not contain a primary aromatic amino group, give no color formation with this procedure.

Standard curves can be prepared by plotting observed absorbance readings *versus* the volumes taken of equimolar concentrations of various local anesthetics. In all cases, Beer's law holds for this system.

Quantitative data from several systems shown in Table II reveal that use of this improved procedure permits the determination of local anesthetics containing a primary aromatic amino group in the presence of other local anesthetic derivatives, such as piperocaine, lidocaine, and tetracaine, and in the presence of other compounds which are found in various combinations with local anesthetics in commercially available products. It was shown from earlier studies that primary, secondary, and tertiary aliphatic amines; secondary and tertiary aromatic amines; heterocycles; and carbonyl-containing compounds also do not interfere with this method (1).

The analytical method is essentially a microprocedure, and sensitivity is in the range of 10^{-5} M of local anesthetic, which makes it comparable to other local anesthetic determinations, particularly the popular diazotization-coupling procedures.

Table III—Determination of 2.00×10^{-5} M Solutions of Procaine, Nesacaine, and Metabutethamine Hydrochlorides by the 9-Chloroacridine Method and the Method of Bratton and Marshall

	9-Chloroacridine Method — Mean % of Concn. Employed	9-Chloroacridine Method — SD of Mean, %	Bratton-Marshall Method — Mean % of Concn. Employed	Bratton-Marshall Method — SD of Mean, %
Procaine hydrochloride	99.30	0.29	99.43	0.28
Nesacaine hydrochloride	99.60	0.17	99.75	0.23
Metabutethamine hydrochloride	99.55	0.23	99.20	0.29

A favorable characteristic of the analysis is that the absorbance of the product formed is stable and does not fade over a 24-hr. period. This is an advantage over the colorimetric method of Bratton and Marshall. In the latter method, absorbance readings must be made within 15 min. after color development, due to precipitation of the azo dyes in the method (7). The 9-chloroacridine method also does not involve diazotization. Thus, it eliminates the need for freshly prepared sodium nitrite and ammonium sulfamate solutions required with the Bratton-Marshall technique. Control of pH is required in both methods.

The improved method of analysis for local anesthetics by the 9-chloroacridine approach was carried out for some representative local anesthetics, and comparative analysis were performed using the colorimetric procedure of Bratton and Marshall. Assays were performed on procaine, nesacaine, and metabutethamine hydrochlorides.

The procedure outlined by Connors was used for the analysis by the Bratton-Marshall method (8).

Four determinations by each method were performed for each local anesthetic. The mean percent of concentration employed and the percent standard deviation of the mean for each local anesthetic are shown in Table III for both methods (9).

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Qualitative and Quantitative Determination of 1,2- and 1,3-Diglycerides by Nuclear Magnetic Resonance Spectroscopy

R. J. WARREN and J. E. ZAREMBO

Abstract □ An NMR procedure is presented for the qualitative and quantitative analysis of 1,2- and 1,3-diglycerides alone or in combination. The method provides a rapid, accurate quantitative analysis, as well as serving as a specific identification of the two isomers. The determination can be carried out on sample sizes in the range 20–50 mg.

Keyphrases □ 1,2- and 1,3-Diglycerides—determination □ NMR spectroscopy—analysis

One of the more difficult problems in glyceride analysis is the differentiation and quantitative determination of 1,2- and 1,3-diglycerides in the presence of one another. Chemical methods are tedious and time consuming. IR spectra are of little value when trying to determine low percentages of one isomer in a mixture of the two. Near-IR spectroscopy (1) has been used to differentiate the 1,2- and 1,3-diglycerides and might have some value. The major drawbacks to using this technique are the large amounts of sample required for a determination, overlap of absorption bands, and relatively small differences in absorptivity values.

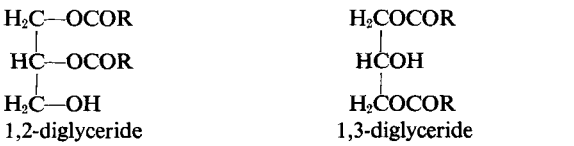
The purpose of this study was to establish the feasibility of using NMR for differentiating between the two isomers and for quantitative analysis of the two isomers.

EXPERIMENTAL

All spectra were recorded on a JEOLCO C60H spectrometer. Deuterated chloroform with 3% $CHCl_3$ added was used as solvent. The spectra were recorded at room temperature at a concentration of 80 mg./ml. Chemical shifts were measured relative to trimethylsilane (TMS).

The 1,2- and 1,3-diglycerides used were 1,2- and 1,3-distearins of reference standard quality.[1]

RESULTS AND DISCUSSION



The NMR spectra of the 1,2- and 1,3-diglyceride isomers differ markedly in the region 220–260 c.p.s. (3.6–4.4 p.p.m.) (Fig. 1). The 1,3-isomer has a singlet absorption at 249 c.p.s. due to the

[1] Supelco and Applied Science Laboratories, State College, PA 16801