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Specificity of Spectrophotometric Determination of Ephedrine and Other Phenalkanolamine Drugs as Benzaldehydes after Periodate Oxidation

LESTER CHAFETZ

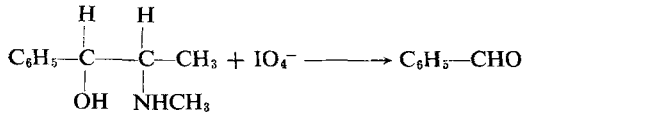
Abstract □ Determination of ephedrine and other phenalkanolamine drugs by spectrophotometry of benzaldehydes extracted after periodate oxidation was critically examined with respect to specificity. It is shown that the compounds must have the general structure: Ar—CHOH—CH(NHR)—R′, that Ar cannot be *o*-dihydroxyphenyl (catecholamines do not interfere), that the amine function must be basic and sterically unhindered, and that there is no mutual interference by phenolic compounds and benzaldehyde-forming drugs. It is demonstrated that *N*-acetylephedrine, as an example of an acylated phenalkanolamine, does not react with periodate under the assay conditions. The carbonyl compound analogs of ephedrine and phenylpropanolamine, *i.e.*, ephedrone and α-aminopropiophenone, respectively, are oxidized to benzoic acid by periodate and do not interfere in the assay for the amino-alcohols. A method for the determination of phenylephrine and phenylpropanolamine in the same solution is presented, where solvent extraction of the periodate oxidation products affords their differentiation. Because the structural requirements for periodate oxidation are so confining, the procedure affords very high selectivity for phenalkanolamine drugs together with greater sensitivity and facility than are provided by most extant methods for this important group of compounds.

Keyphrases □ Phenalkanolamines—determination □ Periodate oxidation—phenalkanolamines to benzaldehydes □ Specificity, periodate oxidation—phenalkanolamine determination □ UV spectrophotometry—analysis

UV spectrophotometric determination of benzaldehyde or substituted benzaldehydes formed by periodate oxidation of ephedrine and other drugs with vicinal hydroxyl and amine functions has provided simple and sensitive assay methods for them in dosage forms and in biological fluids. Ephedrine HCl, in dilute acid solution, exhibits a benzenoid UV spectrum with a molar absorptivity, ε, of about 190 l./mole cm. at its 258.5 nm. maximum: oxidation of it to benzaldehyde affords an ε-value of about 14,400 at its maximum at about 241 nm. in hydrocarbon solvents, about a 75-fold gain in sensitivity. Although the literature on this reaction provides abundant implications on its specificity, it has not been critically reviewed from this standpoint. The purpose of this article is to provide a synthesis of the relevant literature and of previously unreported observations from these laboratories on the specificity of the method.

Periodate oxidations are among the most elegant reactions used in organic chemistry, because they are often quantitative within minutes at room temperature in aqueous media. The extent of the reaction can be measured easily by titration of excess oxidant or determination of any of the reaction products. Malaprade (1) introduced periodic acid as a reagent for the oxidation of 1,2-glycols in 1928. Its use in structure determination and as a selective analytical reagent was reviewed by Jackson (2), Dyer (3), and Bunton (4).

Nicolet and Shinn (5) first reported the use of periodate for oxidation of ethanolamine derivatives. They found that ethanolamines with primary or secondary amine functions were rapidly and quantitatively cleaved to aldehydes and ammonia or a primary amine, while attack on compounds with tertiary amine or acylated amine functions was extremely slow. Wickstrøm (6) studied the rate of periodate oxidation of ephedrine as a function of pH, titrating excess oxidant iodometrically. He found that periodate consumption was too slow to measure at pH 3.0, very slow at pH 6.0, and stoichiometric within 10 min. at pH 7.5 or higher. Overconsumption of periodate, *i.e.*, further oxidation of the aldehyde reaction products, was negligible except at very high pH. [The oxidation potential of the reagent is irrelevant in this reaction. Periodate is a very strong oxidizing agent in acid solution, with the potential of the periodate–iodate couple estimated at −1.6 v. In alkaline solution, however, the potential is only about −0.7 v. (7). Moreover, other oxidizing reagents of comparable redox potentials do not selectively cleave glycols and ethanolamines.] Wickstrøm (6) showed that the ephedrine reaction products are benzaldehyde, acetaldehyde, and methylamine (Scheme



Scheme I

I). He proposed determination of ephedrine *via* colorimetry of acetaldehyde distilled from the reaction mixture.

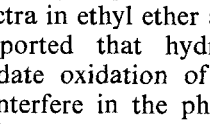
Pisano (8) proposed the determination of metanephrine and normetanephrine, the 3-*O*-methyl metabolites of epinephrine and norepinephrine, respectively, by spectrophotometry. He oxidized the compounds to 3-methoxy-4-hydroxybenzaldehyde (vanillin) with periodate and determined the UV absorption of an oxidized aliquot *versus* an unoxidized reagent blank. Tompsett (9) used the same procedure for the detection of ephedrine and phenylephrine, differentially determining the spectra of benzaldehyde and 3-hydroxybenzaldehyde, respectively.

In work on the pK requirement for the reaction, Chafetz (10) studied the periodate oxidation of ephedrine, phenylpropanolamine, phenyramidol, and the pyrimidyl and *s*-triazinyl analogs of phenyramidol. He found that all of the nonphenolic compounds, except the two phenyramidol analogs, were quantitatively oxidized to benzaldehyde in 10 min. in a bicarbonate medium, and he recommended extraction and spectrophotometry of benzaldehyde in hexane. He ascribed the failure of the reaction with the phenyramidol analogs to the lower availability of electrons on the exocyclic nitrogen, *i.e.*, lesser pKa values. Table I shows the structure of the three compounds and the pKa values attributed to them by Heitmeier and Gray (11). This observation is consistent with the findings of Wickstrøm (6) on the pH dependence of ephedrine oxidation by periodate.

Studying the effect of phenolic phenalkanolamines, several workers (8–10, 12, 13) described spectrophotometric methods for phenylephrine and other monophenolic phenalkanolamines after periodate oxidation. Heimlich *et al.* (13) determined the urinary excretion rate of phenylpropanolamine (norephedrine) by measuring the benzaldehyde spectra in ethyl ether after periodate oxidation. They reported that hydroxybenzaldehydes, formed by periodate oxidation of phenolic alkanolamines, do not interfere in the phenylpropanolamine assay because they are not extracted with ether. Chafetz (10) commented that hexane is preferable as an extraction solvent because its low polarity limits interference by polar chromophores.

The selectivity of the periodate oxidation method for the assay of phenylephrine will be the subject of a forthcoming report; however, it is worthwhile noting that phenylephrine and nonphenolic phenalkanolamines can be determined in the same sample without interference. This is illustrated in a method developed for a nose drop preparation declaring phenylpropanolamine HCl, phenylephrine HCl, thonzylamine HCl, thonzonium bromide, and cetylpyridinium chloride.

Table I—Structure of Phenethanol Derivatives of Aminoheterocycles



Compound	*Het*	pKa (11)
Phenyramidol	2-Pyridyl	6.9
Pyrimidyl analog	2-Pyrimidyl	3.5
Triazinyl analog	2-*s*-Triazinyl	2.9

EXPERIMENTAL

Standard Preparation—Dissolve accurately weighed quantities of about 50 mg. each of phenylephrine hydrochloride reference standard USP and of authentic phenylpropanolamine hydrochloride in water in a 100-ml. volumetric flask and dilute to volume. Dilute 4.0 ml. of the solution to 100 ml. with water to obtain concentrations of about 20 mcg./ml. for each amine.

Assay Preparation—Dilute 4.0 ml. of the solution to 500 ml. with water.

Procedure—Transfer 5.0-ml. portions of the assay preparation and the standard preparation to separate 60-ml. separators. Add 1 ml. of saturated sodium bicarbonate solution and 1 ml. of sodium metaperiodate solution (2 in 100) to each. Swirl, let stand 15 min., and then add 10.0 ml. of 1 *N* hydrochloric acid. After evolution of gas ceases, add exactly 15.0 ml. of hexane, previously shown to be transparent to UV light, shake the mixture for 30 sec., and withdraw most of the lower aqueous phase into a second separator. Retain the aqueous phase for phenylephrine assay. Wash the hexane with two 5-ml. portions of water, and add the washings to the aqueous phase.

Phenylpropanolamine HCl—Filter the washed hexane layers through dry filter paper, and determine their absorbances in 1-cm. cells at the absorption maximum at about 242 nm. in a suitable spectrophotometer, using hexane as the blank. Calculate the concentration, in milligrams per milliliter, of $C_9H_{13}NO \cdot HCl$ from the formula $0.125C(A_U/A_S)$, where C is the concentration of phenylpropanolamine HCl in micrograms per milliliter in the standard preparation, and A_U and A_S are the absorbances of the solutions from the assay preparation and the standard preparation, respectively.

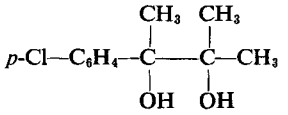
Phenylephrine HCl—Extract the retained aqueous phase with four 15-ml. portions of chloroform, and collect the extracts in a 125-ml. separator. Add exactly 15.0 ml. of 0.1 *N* sodium hydroxide, shake the mixture for 30 sec., and then allow the layers to separate. Discard the chloroform layer, and filter the alkali extract through dry paper. Concomitantly determine the absorbances of the solutions in 1-cm. cells in a suitable spectrophotometer at the wavelength of maximum absorbance at about 237 nm., using sodium hydroxide solution as the blank. Calculate the concentration, in milligrams per milliliter, of $C_9H_{13}NO_2 \cdot HCl$ in the sample taken from the formula $0.125C^1(A_U^1/A_S^1)$, where C^1 is the concentration in micrograms per milliliter of phenylephrine HCl in the standard preparation, and A_U^1 and A_S^1 are the absorbances of the solutions from the assay preparation and the standard preparation, respectively.

Six trials of the method provided a recovery of 101.0 ± 1.6% for phenylpropanolamine HCl and of 99.6 ± 2.6% for phenylephrine HCl. The foregoing procedure is adapted directly from the procedures described by Chafetz (10); recent improvements will be reported.

DISCUSSION

Interference by Other Amine Drugs—Wallace (14) recently described a similar method to that described by Chafetz (10). He described methods for estimation of ephedrine, pseudoephedrine, phenylpropanolamine, and phenyramidol in biological samples. Despite the reports, *inter alia*, by Wickstrøm (6), Heimlich *et al.* (13), and Chafetz (10) showing that the reaction is quantitative within 10 min. at room temperature, Wallace (14) conducted the reaction at the temperature of boiling hexane, about 69°, with a reaction time

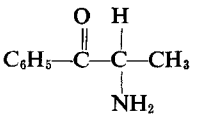
of 30 min. After separating the hexane layer, he washed it with dilute acid and determined benzaldehyde spectrophotometrically either as such or after conversion to its semicarbazone. He reported the absorbance values obtained with more than 80 different drugs, without the phenalkanolamine structure, which had been tested in the procedure; none of them showed any significant degree of interference even under the unnecessarily rigorous oxidation conditions employed. The highest absorbance reported was for phenaglycodol (I), about 10% that obtained with ephedrine; however, phenaglycodol is a 1,2-glycol which could be oxidized to 4-chloroacetophenone and acetone to some extent under Wallace's conditions. Moreover, the intact drug has a strong absorption band in hexane at 222 nm.



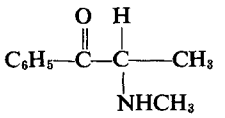
phenaglycodol

I

Possible Interference by Ketone Analogs—Although degradation of ephedrine or phenylpropanolamine *via* oxidation of the secondary alcohol function to the corresponding ketone has never been encountered here or, to the author's knowledge, reported in the literature, the availability of samples of the aminoketones, α-aminopropiophenone and α-methylaminopropiophenone (ephedrone), led the author to study the effect of periodate oxidation on these compounds. These aminoketones (II, III), because they have carbonyl groups in conjugation with the benzene ring, have UV spectra closely similar to that of benzaldehyde. They can easily be distinguished from benzaldehyde, however, by reaction with periodate under the conditions described by Chafetz (10) for ephedrine assay. Benzaldehyde is unaffected, while the spectra of the aminoketones are destroyed. By TLC of benzene extracts of the reaction products of ephedrone and α-aminopropiophenone oxidation, the aromatic reaction product was determined to be benzoic acid. Benzoic acid was identified by comparison with standards on two different thin-layer systems:



II



III

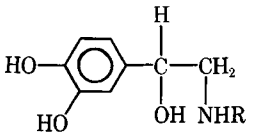
System 1: *n*-butyl alcohol–dehydrated alcohol–concentrated ammonia–water (60:60:60:15) with a 20 × 20-cm. plate consisting of a 0.25-mm. layer of microcrystalline cellulose on glass (Analtech).

System 2: ethyl acetate–dioxane–water (40:90:90) with a 20 × 20-cm. plate consisting of a 0.25-mm. layer of silica gel GF on glass (Analtech).

The reaction mixture was acidified with hydrochloric acid, and the benzene extract was filtered through paper and concentrated to about 1 ml. on the steam bath. The extract and a standard of authentic benzoic acid in benzene, 5 mg./ml., were spotted in 10- and 15-μl. volumes at equidistant points 3 cm. from the bottom of the plates. The chromatograms were developed in standard chambers, lined on three sides with filter paper, and previously equilibrated with the developing solvent mixture at least 30 min. before use. The chromatograms were developed until the solvent front ascended about 12 cm. from the starting line. The plate developed in System 1 was dried at 105° for 1 hr. and then sprayed with a freshly prepared mixture of equal volumes of 8% potassium iodide, 2% potassium iodate, and 1% amylose as described by Chafetz and Penner (15). Benzoic acid was revealed as a dark-blue spot on a light-blue background at about R_f 0.8. The plate developed in System 2 was air dried at room temperature and examined under shortwave UV light. Benzoic acid was revealed as a single spot at R_f about 0.9 along with two spots of lesser intensity. Spraying the plate with 0.1% alcoholic bromcresol purple revealed a single yellow spot for benzoic acid on a purple-brown background. In both systems, using detection reagents selective for acids, the extract of the periodate oxidation products of the aminoketones revealed spots identical in R_f and reaction with authentic benzoic acid.

Benzoic acid is a much weaker UV chromophore than benzaldehyde. Moreover, its partition coefficient between aqueous and hexane phases is much less favorable than with the less polar aldehyde; thus, if present, the corresponding aminoketones will not interfere in periodate assays of ephedrine and related drugs.

Effect of Catecholamines (IV)—Epinephrine (R = CH_3—), norepinephrine (R = H), isoproterenol [R = $(CH_3)_2CH$—], and several other natural and synthetic pressor compounds are 3,4-dihydroxyphenethanolamine derivatives, which might be expected to undergo periodate oxidation to 3,4-dihydroxybenzaldehyde (protocatechualdehyde). These compounds, however, are preferentially



catecholamine structure

IV

oxidized by cyclization to adrenochrome and other indole derivatives by periodate, iodate, and any number of mild oxidizing agents. Under the Chafetz (10) conditions, the compounds are immediately oxidized to red-colored compounds in the aqueous phase, and these are not extractable with hexane. Kaistha (16) reported the periodate oxidation of isoproterenol to protocatechualdehyde at acid pH, using a large excess of periodate and a reaction time of 18 hr. at room temperature. His data indicate that the extent of conversion of isoproterenol HCl to protocatechualdehyde is only about 10% at best.

Acylated Phenalkanolamines—Troup and Mitchner (17) showed that phenylephrine may be acetylated by aspirin in dosage forms. The literature (5) and pK considerations indicate that acylated phenalkanolamines should not be affected by periodate. As a *punctilio*, to demonstrate the lack of interference in the assay by such products of incompatibility, the author prepared *N*-acetylephedrine by the method of Welsh (18) and compared it with ephedrine HCl in the following experiment.

Aqueous solutions were prepared containing, respectively, 20 mcg./ml. of ephedrine HCl and of *N*-acetylephedrine, m.p. 84.0–84.5° (uncorrected). Volumes of 5.0 ml. of each solution were transferred to separate 60-ml. separators and treated with 1.0 ml. of saturated sodium bicarbonate and 1.0 ml. of sodium metaperiodate (2 in 100). The solutions were swirled, allowed to stand for 10 min., and then extracted with exactly 20.0 ml. of hexane. Spectra of the hexane extracts were scanned in 1-cm. cells in a Cary model 14 recording spectrophotometer *versus* hexane from 300 nm., using hexane as the blank. The spectra of the extract of the ephedrine HCl oxidation product showed the typical pattern of benzaldehyde; the solution from the *N*-acetylephedrine showed no absorbance, confirming that acylated compounds do not interfere.

Arylglycolates—Salts and esters of arylglycolates, such as methenamine mandelate, magnesium pemoline, and homatropine HBr, are oxidized to the corresponding carbonyl compounds by periodic acid as illustrated for mandelic acid (Scheme II). Chafetz (19) used

Scheme II

this reaction for determination of these compounds, although he showed that ceric ion is preferable as the oxidant. He showed that acid pH and heat are necessary. The compounds are not oxidized in bicarbonate solution. Thus, they will not interfere, if present, in the determination of ephedrine.

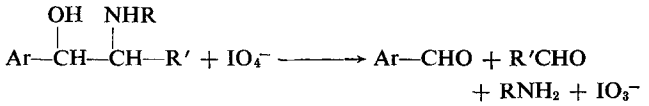
Limitations of the Method—Spectrophotometry of the aromatic aldehyde produced by periodate oxidation of phenalkanolamines will not distinguish between compounds such as ephedrine and phenylpropanolamine, both of which afford benzaldehyde, or phenylephrine and metaraminol, both of which give 3-hydroxybenzaldehyde. It will not discriminate stereoisomers such as ephedrine (*erythro*-configuration) and pseudoephedrine (*threo*-configuration).

These limitations are common to most other methods described for these drugs. Since they are rarely combined with each other in therapy, the limitations are more theoretical than real in dosage form assay. However, ephedrine is metabolized to norephedrine in part, and the method will not distinguish between the two in biological fluids.

A preliminary extraction of the phenalkanolamine drug is necessary in the presence of high concentrations of sugars, as in pharmaceutical syrups. Certain reducing agents, such as iodide, interfere by destroying periodate. In general, the limitations of the method are easily negated by use of suitable adjunctive procedures.

SUMMARY AND CONCLUSIONS

Evidence from the literature and previously unreported observations from these laboratories has been critically examined with respect to the specificity of the periodate method for ephedrine and related phenalkanolamine drugs. The reaction used may be generalized as shown in Scheme III. The method is based on spectrophotometric determination of Ar—CHO after its extraction with an immiscible solvent. Obviously, ethanolamine derivatives that are oxidized to compounds without UV chromophores will not interfere in the method.



Scheme III

The reaction proceeds quantitatively within a few minutes at room temperature and in neutral or alkaline solution where Ar is phenyl or monohydroxyphenyl, R is hydrogen or alkyl, and R′ is hydrogen or alkyl. The reaction does not proceed at any appreciable rate with compounds bearing electron-withdrawing substituents on the amine function, *e.g.*, where R is acyl, 2-pyrimidyl, or 2-*s*-triazinyl.

The accepted mechanism for periodate oxidation of ethanolamines involves formation of a cyclic intermediate comprising the iodine atom of periodate and the amine and hydroxyl functions. Probably because of steric requirements, compounds with tertiary amine functions are not cleaved by periodate under the assay conditions. Preliminary results in these laboratories on periodate oxidations of compounds of the type Ar—O—$CH_2CH(OH)$—CH_2—NHR support this viewpoint; compounds that have the bulky *tert*-butyl substituent for R are not affected by periodate under vigorous reaction conditions, but where R = methyl or isopropyl, the reaction proceeds smoothly.

Specificity in an assay method is a function of both the measurement and isolation procedures. Substitution on the aryl ring will affect both the UV spectra (measurement) and the extraction characteristics of Ar—CHO (isolation).

Because their oxidation products are not extracted by the nonpolar solvents used for the nonphenolic analogs, monophenolic phenalkanolamines do not interfere in the determination of benzaldehyde-producing compounds. A procedure for the determination of phenylpropanolamine and phenylephrine in the same solution has been described where the oxidation products are separately determined after simple solvent extraction procedures. Catecholamines do not interfere in the determination of other phenalkanolamines, because they are preferentially oxidized to adrenochrome derivatives. Aminoketone analogs of phenalkanolamines are oxidized to benzoic acids, which do not interfere in the determination.

Overall, the method requires that a basic compound of circumscribed structural characteristics be oxidized to a nonpolar neutral compound with a characteristic and intense UV spectrum. Since very few drugs or excipient materials satisfy these criteria, one must conclude the method has very high specificity for phenalkanolamines. With respect to specificity, the periodate oxidation procedure is clearly superior to the methods official for ephedrine preparations in the compendia, which imply only the presence of an ether-soluble amine.

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