aminopropane (VII)[2], and 1-(2,5-dimethoxy-4-methyl)-2-aminopropane (IX)[2] were all established as R(−) and S(+). Consequently, it is tempting to assume a correlation between retention time and absolute configuration. Consistent with such an assumption is the observation that the amide of R-(+)-α-methylbenzylamine (X*b*) has a shorter retention time than the amide of S-(−)-α-methylbenzylamine (X*a*). However, until more information on the molecular nature of the interactions of such diastereomers with column materials is available, this suggestion must remain speculative. Similar differences in retention times with configuration of a series of camphorsulfonamides (17) of α-methoxy-α-methylpentafluorophenylacetamides (18) and of *N*-trifluoroacetylprolylamides (19) have been reported.

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[2] The absolute configurations of these compounds were established by optical rotatory dispersion and circular dichroism analyses of the resolved amines (*via o*-nitrotartranilate salts). The details of these studies will be submitted for future publication.

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Degradation of Bronchodilator Agents in Oxymix System

GEORGE H. KRONBERG▲ and BERTIL H. TAKMAN

Abstract □ Known amounts of isoproterenol, phenylephrine, and epinephrine were added to the aqueous oxymix system and thereby exposed to potential redox destruction. Thirty minutes later, recoveries of isoproterenol and phenylephrine were 89–100% and 89–108%, respectively, while the recovery of epinephrine was 53–79%.

Keyphrases □ Bronchodilator agents—degradation in oxymix system, clinical implications of concurrent use □ Oxymix–isoproterenol, phenylephrine, or epinephrine—degradation of bronchodilators, clinical implications of concurrent use □ Fluorometry—determination of stability of isoproterenol, phenylephrine, and epinephrine in aqueous oxymix system

Oxymix[1] is a mixture of ascorbic acid, cupric sulfate, sodium percarbonate, a buffer system, and excipients and is essentially a redox system. It has mucolytic properties and has been clinically administered as an aerosol in the treatment of several pathologic pulmonary conditions (1). Because bronchodilating agents are often used for the same indications, knowledge of the compatibility of representative bronchodilators and oxymix was considered necessary, particularly since concurrent administration might be preferred. Since no investigations of the stability of catecholamines or catecholamine-like compounds under such conditions had been reported previously, the stability of epinephrine, isoproterenol, and phenylephrine in the oxymix system was determined using fluorometric assay methods.

[1] Ascoxal (Gum-ox, Ascumist), marketed for oral hygiene by Astra Läkemedel, Södertälje, Sweden.

EXPERIMENTAL

Apparatus—The fluorometric measurements were performed with two types of fluorometers[2]. The ion-exchange columns were of the same design and dimensions as described by Kelly and Auerbach (2) but contained 50 × 5-mm. resin beds.

[2] G. K. Turner Associates, model 111: primary filter No. 110-812 (405 nm.) and secondary filter No. 110-825 (65A) (495 nm.). Aminco-Bowman, model 4-8202: excitation 270 nm., emission 305 nm., slit arrangement 5, xenon lamp 416-992, photomultiplier 10-213, meter multiplier 0.001, and sensitivity setting 46.

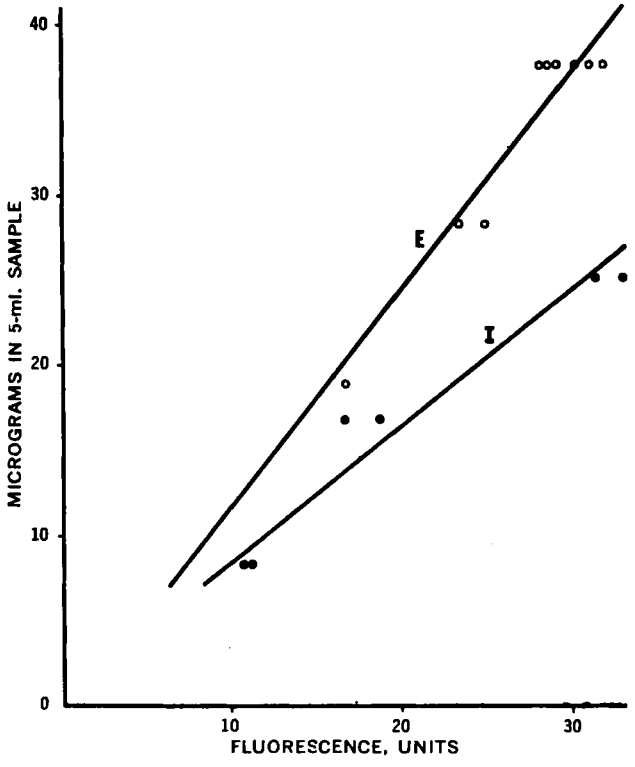

Figure 1—*Standard curves for isoproterenol (I) and epinephrine (E): fluorescence (units)* versus *amount in 5-ml. sample (micrograms of base form).*

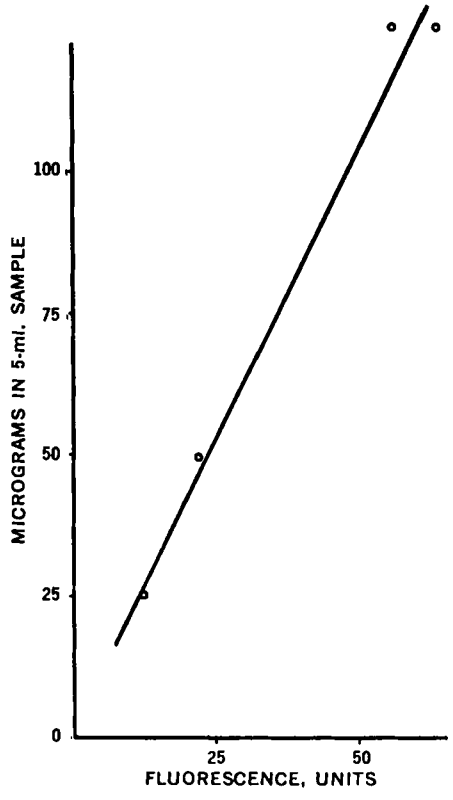

Figure 2—*Standard curve for phenylephrine: fluorescence (units)* versus *amount in 5-ml. sample (micrograms of base form).*

Reagents and Chemicals—The following were used: Amberlite CG 120 (type 2, 200 mesh, Na^+ form); bromthymol blue, 0.04% in ethanol; potassium carbonate, 2 *N* solution; phosphate buffer, 0.2 *M*, pH 6.5; zinc sulfate, 0.25% solution; potassium ferricyanide, 0.25% solution; sodium hydroxide, 5 *N* solution; ascorbic acid, 2% solution (prepared fresh daily); sodium hydroxide–ascorbic acid solution (9:1 v/v, freshly prepared within a few minutes of use); hydrochloric acid, 0.1 and 2 *N* solutions; oxymix (granulate) with a quantitative composition (percent) of: ascorbic acid 33.33, sodium percarbonate 19.67, cupric sulfate 0.07, polyvinylpyrrolidone 7.67, erythrosin 0.003, tartaric acid 16.00, sodium bicarbonate 21.51, mannitol 1.27, menthol 0.08, and saccharin 0.40; epinephrine bitartrate USP[2]; isoproterenol hydrochloride USP[2]; phenylephrine hydrochloride USP[2]; and sodium edetate, 0.1% solution (I), prepared by treating reagent grade ethylenediaminetetraacetic acid with 0.02 *N* NaOH to form a solution of pH 7.

Stock Solutions—Stock solutions of epinephrine, isoproterenol, and phenylephrine were made to contain the equivalent of 1 mg. base/ml. in 0.1 *N* HCl and were kept in amber bottles at 4° for subsequent dilutions. They were found to be quite stable for the duration of these experiments.

Ion-Exchange Columns—The ion exchangers were prepared by stirring about 20 g. of the resin with 100 ml. of 2 *N* NaOH solution (containing 1% ethylenediaminetetraacetic acid) for 15 min. Excess alkali was decanted and the resin was washed with distilled water. This procedure was repeated using 100 ml. 3 *N* HCl. The resin was allowed to settle and the fines were removed by decantation. The prepared resin was then slurried into columns (5 mm. i.d.) to form beds 50 mm. long. Further washing of the beds with distilled water compacted the resin to give flow rates varying from 10 to 20 ml./hr. Small plugs of glass wool were placed on top of the resin beds. At this point, the columns were ready for use.

Solution Systems—

1. *Reagent Blank*—Five milliliters of distilled water containing 0.5 ml. of I was used.

2. *Oxymix Blank (Aged 30 min.)*—To a 400-ml. tall-form beaker containing 75 ml. of distilled water, 12 g. of oxymix granulate was added as rapidly as allowed by the marked foaming that occurred during mixing. Cooling of the mixture suppressed the foaming. As soon as the first portion of oxymix was added, timing was begun. The mixture was stirred for 15 min., then transferred to a 100-ml. volumetric flask, diluted to the mark with distilled water, and centrifuged. A 1-ml. aliquot of this solution was transferred to a 10-ml. volumetric flask. At 30 min., 1 ml. of I was added and the mixture was diluted to the mark with distilled water.

3. *Pure Drug (Standard Solution)*—The requisite amount of drug (either from acid stock solution or from the solid powder form of its salt) was diluted to 100 ml. with distilled water in a volumetric flask. A 2.5-ml. aliquot of this solution was then transferred to a 25-ml. volumetric flask, followed by 2.5 ml. of I, and diluted to the mark with distilled water. If the drug was taken from its acid stock solution, the pH was adjusted to 6–7 with sodium hydroxide before the final dilution was completed.

4. *Drug in 30-min.-old Oxymix*—Oxymix granulate (12 g.) was dissolved in 50 ml. of distilled water and allowed to stir for 30 min. before the drug (salt form) was added. After transfer of the drug quantitatively by rinsing, the mixture was diluted to 100 ml. Aliquots of this solution were mixed with equal parts of I and the mixture was diluted fivefold. No pH adjustment was necessary.

5. *Oxymix Granulate Added to Drug Solution*—A quantity of drug (as the salt) corresponding to the concentration desired was accurately weighed and dissolved in 50 ml. of distilled water contained in a 400-ml. tall-form beaker. To this solution, 12 g. of oxymix granulate was added and the mixture was allowed to stir for 15 min. from the time that the first portion of oxymix was added. The mixture was then transferred and diluted in a 100-ml. volumetric flask at 30 min. Final dilutions were prepared as described for Solution System 4.

6. *Drug Added to Fresh Oxymix Solution*—In this solution system, 12 g. of oxymix granulate was dissolved in 50 ml. of distilled water and the salts of the drugs were then added quantitatively. Aging for 30 min. was again timed from the beginning of the addition of oxymix. In this case, the drug was actually introduced into the active oxymix system within 1–3 min.; loss in activity of the redox system could be considered almost negligible under these conditions. After the mixture had stirred for 15 min., it was transferred to

[2] Winthrop.

Table I—Stability of Bronchodilators in the Presence of Oxymix

Drug	Solution System	Amount Added, mg.	Number of Samples	Recovery, %
Isoproterenol	4	3.33	1	100
	5	3.33	2	89, 97
	6	3.33	2	89
Phenylephrine	4	5, 25	2	108, 93
	5	5–25	7	89–100
Epinephrine	4	7.5	5	73–79, 98[a]
	5	7.5	2	53, 65
	6	7.5	2	65, 71

[a] For unknown reasons, a discordant value of 98% was obtained on one sample.

a 100-ml. volumetric flask and diluted to the mark with distilled water. Thirty minutes later, final dilutions were prepared as described for Solution System 4.

Ion-Exchange Chromatography—The reagent blank (5 ml.) was pipeted onto an ion-exchange column, allowed to pass through, eluted with 15 ml. of 2 *N* HCl into a 50- or 100-ml. volumetric flask, and diluted to the mark with distilled water.

For the oxymix blank and for the other four solution systems, the above procedure was employed, except that the 5-ml. aliquot was allowed to seat properly on the column, washed with 3 × 10-ml. portions of distilled water, eluted with 15 ml. acid, and diluted to proper volumes. The volume of acid used in eluting the columns was sufficient to regenerate automatically the resin beds (to the H^+ form). Washing with water then removed excess acid so that the ion exchangers were ready for subsequent experiments. No loss of drug occurred on water washing the columns after the samples had been applied to the resin beds (prior to elution with acid).

Fluorometric Assay Procedures (3–5)—After epinephrine and isoproterenol had been collected from the ion-exchange columns, 2-ml. aliquots were transferred to 16 × 150-mm. test tubes, followed by addition of 1 ml. of distilled water to each sample. One drop of bromthymol blue indicator was added, and the solution was adjusted with vigorous stirring or shaking to approximately pH 6 by the dropwise addition of 2 *N* potassium carbonate. This was followed by addition of 0.5 ml. of 0.2 *M* phosphate buffer. Oxidation was then performed by addition, with thorough mixing, of 0.1 ml. of 0.25% zinc sulfate and 0.1 ml. of 0.25% potassium ferricyanide. After exactly 3 min., 1 ml. of the freshly prepared sodium hydroxide–ascorbic acid reagent was added and mixed thoroughly.

The fluorometer was blanked on the dummy cell. The solutions were transferred to 12 × 75-mm. cells and were read, at a range selector setting of ×10, 10–20 min. after the last reagent was added.

For the determination of phenylephrine, the acid eluate was first adjusted to pH 1 by addition of 5 *N* NaOH solution and was then diluted to a final volume of 25 ml. with 0.1 *N* HCl. The natural fluorescence of the drug was then measured[4] by merely introducing a few milliliters of the solution into the sample cell and reading its "percent transmission" on the meter, using 0.1 *N* HCl as a blank. (See also *Reference 6.*)

The reagent blanks (Solution Systems 1 and 2) gave fluorescence readings in the following ranges: in the epinephrine experiments, 0.8–0.9 fluorescence unit; and in the isoproterenol experiments, 1.1–1.2. In the phenylephrine experiments, blank readings of 1.3–1.8 were obtained.

RESULTS AND DISCUSSION

An arbitrary time of 30 min. was chosen at which to measure residual drug activity, since it was known that the clinical treatment is usually completed well inside this time span and that potential redox destructibility of the oxymix system decays to a minimum value during 15–30 min.[5]. Two methods of addition (drug to oxymix and oxymix to drug) were investigated, since in clinical situations both procedures may be used.

The standard solutions (System 3) gave the data presented in Figs. 1 and 2. The lines were constructed from the experimental data by the method of least squares (7).

The results of the investigation of the stability of the bronchodilators in the presence of oxymix are compiled in Table I. It was evident that a reduction in epinephrine concentration occurred, even in a 30-min. old oxymix solution, and to a somewhat greater extent in the freshly prepared solutions[6]. However, of more importance from a clinical point of view, at least 50% of the initially added amount of epinephrine remained unchanged in all solutions. No significant degradation could be observed in the experiments with isoproterenol and phenylephrine.

Aerosols containing 6–12% of oxymix have been used clinically (1). In the present study, all experiments were performed at or initially even somewhat above the high limit of this range. This is probably adequate because of the extremely high molar ratio of redox components to bronchodilator in all clinical compositions (>100:1). From the fact that a loss of epinephrine was also observed when it was combined with oxymix that had been kept in water solution for 30 min., it is clear that a reaction occurs with some component(s) of the aged system and that isoproterenol and phenylephrine do not undergo this reaction.

The stability of phenylephrine in a pharmaceutical composition containing, *inter alia*, ascorbic acid was reported (10). Even when such a mixture had been kept at 70° for 36 hr., almost no change in the phenylephrine content was observed. The authors also found that, under certain conditions, phenylephrine can be changed in its side chain in such a manner that this structural change could not be observed spectroscopically. However, by the use of an ion-exchange method, these types of formed derivatives could be separated completely, thus allowing quantitative measurement of the intact phenylephrine.

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[4] Aminco-Bowman fluorometer.

[5] P. Levins and N. Adler, Arthur D. Little, Inc., Cambridge, Mass., personal communication.

[6] In water solutions of epinephrine alone, the degradation is very slow in the acid and neutral pH range (8, 9); during a 30-min. period, no significant change in epinephrine concentration can be observed.