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Report

Phenolic Cyclization of Epinephrine, Metaproterenol, Metaraminol, Phenylephrine, and Terbutaline with Formaldehyde

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An exploratory study of the rates of cyclization of the title compounds, all 3-hydroxyphenalkanolamines, with formaldehyde to 1,2,3,4-tetrahydroisoquinolines (THIQs) was performed using high-performance liquid chromatographic (HPLC) methods. Reactions occur quantitatively and practically instantaneously at room temperature and neutral pH; thus, rates were measured at acid pH. Cyclization occurs *ortho* or *para* to the 3-phenolic function, so that all but the 3,5-dihydroxyphenyl derivatives, metaproterenol and terbutaline, gave two THIQs. Terbutaline reacted significantly slower than the other compounds. Formaldehyde occurs in pharmaceutical systems and it serves as a model for other aldehydes that occur in sugars and flavors. The pharmaceutical implications of the reaction are discussed.

KEY WORDS: phenolic cyclization; tetrahydroisoquinolines; epinephrine; metaproterenol; metaraminol; phenylephrine; terbutaline; 3-hydroxyphenethylamines.

INTRODUCTION

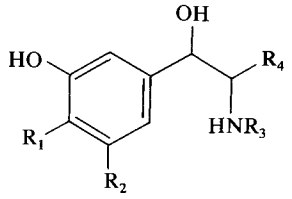
Cyclization of 3-hydroxyphenylethylamines with carbonyl compounds to form 1,2,3,4-tetrahydroisoquinolines (THIQs) under mild conditions has been found to be a very selective reaction. In 1951, Kovacs and Fodor (1) reported that only 3-hydroxyphenethylamine derivatives cyclize to THIQs with acetaldehyde. Although it is frequently called a Pictet–Spengler cyclization in the literature, the latter is acid catalyzed and not specific to 3-hydroxyphenethylamines. Kametani *et al.* (2) originated the term "phenolic cyclization."

The reaction has found use in synthesis (1–5), been implicated in biosynthesis and metabolism (6–8) and in drug decomposition (9–12), and been used in analysis (13,14). Because formaldehyde is the most reactive carbonyl compound, its reaction with 3-hydroxyphenethylamine derivatives could be considered a "worst-case" model for reactions of aldehydes with these compounds. Preliminary studies of the reactions of epinephrine, metaproterenol, and metaraminol, phenylephrine, and terbutaline are reported here. The structural relationships of these compounds are summarized in Table I, and the general reaction with formaldehyde is shown in Fig. 1.

MATERIALS AND METHODS

General Procedure. All reactions were conducted at controlled room temperature. Pilot experiments showed that the reactions were too fast to measure at neutral pH, and they were complicated by autoxidation of the presumed THIQs. The reaction of metaproterenol at neutral pH is described; however, only semiquantitative experiments were attempted with its analogues. Solutions were made to contain 1 mg/ml concentrations of epinephrine bitartrate, metaraminol bitartrate, metaproterenol sulfate, phenylephrine HCl, and terbutaline sulfate. All of the drugs met USP requirements. A 5-ml volume of each was pipetted into a 25-ml volumetric flask and 10 ml of buffer and 10 ml of 2%

Table I. Structural Relations of Hydroxyphenalkanolamines



Compound	R_1	R_2	R_3	R_4
Epinephrine	OH	H	CH_3	H
Metaproterenol	H	OH	$CH(CH_3)_2$	H
Metaraminol	H	H	H	CH_3
Phenylephrine	H	H	CH_3	H
Terbutaline	H	OH	$C(CH_3)_3$	H

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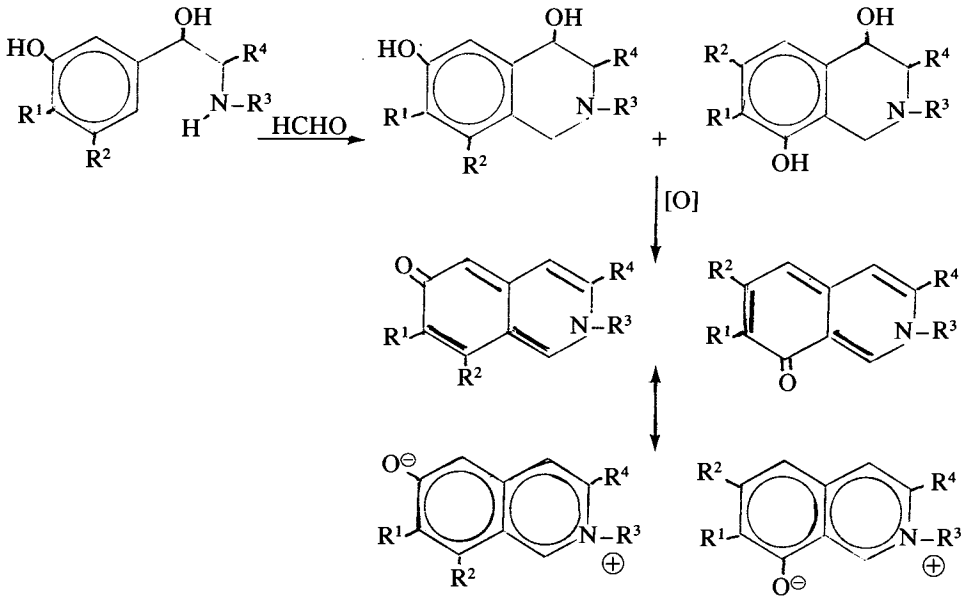

Fig. 1. Scheme for cyclization of 3-hydroxyphenethylamines to 6-hydroxy and 8-hydroxy THIQs followed by autoxidation to 6- and 8-isoquinolones, which may be depicted as zwitterions.

aqueous formalin solution (about 80 mg of formaldehyde) were added. The final pH of the solutions was noted. These were injected into a high-performance liquid chromatograph (HPLC) immediately after mixing and at timed intervals thereafter. Rates for the disappearance of the intact drug were calculated from HPLC peak responses. A "zero-reaction time" response could be determined by omitting formaldehyde from the solution.

Chromatography. The general procedure for catecholamines and their derivatives used in these laboratories employed a μ-Bondapak-C_{18} (Waters) column and a simple mobile phase of 1% acetic acid (0.17 *M;* 10 ml of glacial acetic acid diluted to 1 liter with water) with variation of flow rates to obtain convenient retention volumes for the analytes. This system proved applicable to three of the five compounds studied; however, incorporation of 15% (v/v) methanol in the mobile phase was necessary for terbutaline chromatography, and it was necessary to replace the Waters column with a Zorbax-TMS (DuPont) column in order to obtain satisfactory peak shapes with phenylephrine and terbutaline. In all instances a Waters Bondapak Corasil C_{18} guard column was used. Mobile-phase flow rates were 0.8 ml/min for epinephrine, 1.0 ml/min for metaproterenol and for estimation of the rate of autoxidation of its THIQ, 1.3 ml/min for metaraminol, 2.5 ml/min for phenylephrine, and 1.5 ml/min for terbutaline. A UV-spectrometric detector was set at 278 nm, and a Spectra-Physics SP-4100 computer integrator was used to determine peak responses. Waters and Perkin–Elmer chromatography pumps and other accessory equipment were used.

RESULTS AND DISCUSSION

Epinephrine Cyclization. Epinephrine eluted at 5.1 min, and the two presumptive THIQs at 6.1 and 6.6 min in preliminary trials at pH 2.80, 3.55, 4.05, and 7.0. The reaction rate increased with pH, as with all the compounds studied. A chromatogram is shown in Fig. 2, where, under the conditions used for measurement of intact drug, the two products were not separated.

Metaproterenol Cyclization. Under the conditions used, metaproterenol showed a retention time of about 5.4 min, with the single THIQ formed eluting at 7.5 min. At pH 2.1, 90% unchanged drug was evident after 75 min; 30% remained after overnight reaction. Data for estimate of rate were obtained at pH 2.97, using a phthalate buffer. At pH 7.16, the reaction is practically instantaneous, with no intact metaproterenol peak evident in the chromatogram; however, the THIQ is oxidized at this pH. At "zero time" about 2% of the total detected area was detected at a retention

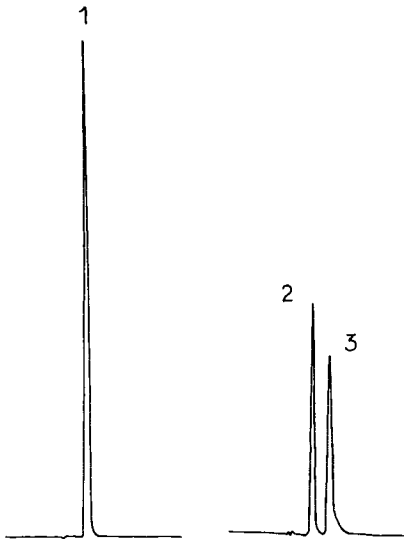

Fig. 2. Epinephrine cyclization at pH 2.80. Peaks 1 and 2 are epinephrine at RT 5.1. Peak 3 at RT 6.15, in a chromatogram recorded after 34 min of reaction, is a mixture of THIQ products.

time of 6.7 min. This increased to 38% in 40 min and 99% in 4.5 hr. The solution was yellow in 2 hr. At pH 12.7, obtained using 0.1 *N* sodium hydroxide instead of buffer, the solution turned yellow immediately. The only peak had a retention time of 6.8 min, with an area about half that of the metaproterenol control (without added formaldehyde) or its THIQ product. This is consistent with spectra reported for the THIQ oxidation products of the THIQs from phenylephrine, which have a maximum at about 350 nm and absorptivity at 278 nm about half that of the starting materials (14).

Metaraminol Cyclization. Metaraminol had a retention time of about 5.3 min, and the THIQs formed from it were well separated from each other at retention times of 5.8 and 8.7 min. Reaction mixtures at pH values of 2.2, 2.6, 2.9, 3.6, and 4.1 were allowed to stand several hours until cyclization was complete. No further change was evident in chromatograms from these solutions recorded over several days. The "5.8-min peak" was 83.7% of the total peak area at pH 2.2 and 71.0% at pH 4.1, with proportional decreases at intermediate pH values. Retention times and total detected areas were constant within the precision of the method. The rate was determined using a chloride buffer which gave a pH of 2.62. In chromatograms recorded at intervals from 13 to 156 min, corresponding to 15 to 86.5% reaction of the intact compound, the earlier-eluting THIQ was found to remain constant at about 83% of the total THIQ areas.

Phenylephrine Cyclization. Solutions at pH values of 3.4, 5.0, and 7.2 showed nearly complete conversion to THIQs immediately after reaction. At pH 5.0, using a mobile phase containing 15% methanol and a flow rate of 1.5 ml/min, intact phenylephrine eluted at 3.4 min and the two THIQs were resolved at retention times of 4.9 and 5.5 min. The later peak has the same retention time as authentic 1,2,3,4-tetrahydro-2-methyl-4,6-dihydroxyisoquinoline, synthesized as described in Ref. 3. At this pH, however, the rate of cyclization was too fast to measure. The THIQ peaks were not resolved in a wholly aqueous mobile phase, appearing as a peak at 5.5 min with an earlier-eluting shoulder.

Terbutaline Cyclization. The rate of cyclization of terbutaline with formaldehyde was measured at pH 4.30, a much lower acidity than could be used for the other compounds. Intact terbutaline showed a retention time of 3.2 min, and its single THIQ product eluted at 5.4 min. Authentic 1,2,3,4-tetrahydro-2-t-butyl-4,6,8-trihydroxyisoquinoline, synthesized as described by Svensson (12), showed identical chromatographic properties. He had reported it as a minor decomposition product of the forced decomposition of terbutaline solutions in the presence of air.

UV Spectra. All chromatograms were monitored at 278 nm, the absorbance maximum for hydroxyphenalkanolamines. One of the THIQs from phenylephrine and the THIQ from terbutaline were identified by comparison with authentic compounds synthesized by literature methods, but the identities of the remaining THIQs and their oxidation products were presumed from evidence in the literature. Although the THIQs have molar absorptivities closely similar to those of the starting 3-hydroxyphenalkanolamines, their maxima are displaced bathochromically (4). Further, the 6-hydroxy THIQs have maxima at higher wavelengths than the 8-hydroxy THIQs (5). Thus, the use of percentages of the total detected area in comparing the relative amounts of isomeric THIQs formed is an approximation with apparently good precision but doubtful accuracy. The absorption maximum difference between 6- and 8-hydroxy THIQs suggests that the use of an instrument equipped with a spectrometer which provided spectra of the peaks could distinguish *para* cyclization from *ortho*. In the experiment with metaproterenol at pH 7.2, the oxidation product found to be 38% of the total detected area likely represents double that concentration.

Table II. Observed First-Order Rates at Room Temperature

Compound	pH	k_1/min
Epinephrine	2.80	0.0246
Metaproterenol	2.97	0.0155
THIQ from metaproterenol	7.16	0.01
Metaraminol	2.62	0.0136
Phenylephrine	2.22	0.0271
Terbutaline	4.30	0.0212

Comparison of Rates. Because the work reported here was a pilot study, no attempt was made to control the reaction temperature rigorously; however, the good fit of the data to the first-order rate equation shows that temperature variation during the experiments was not significant. A summary of the rates calculated from the data is presented in Table II. Direct comparison of cyclization rates cannot be derived from the data because different pH values and buffers were used. Phenylephrine, showing the fastest reaction at the lowest pH, appears to be the most reactive of the series, and terbutaline the least reactive. The steric effect of the t-butyl group of terbutaline may account for its relative stability. Metaraminol, on the other hand, is a primary amine, and it exhibited less reactivity than its structural isomer, phenylephrine. One might speculate that this is due to the steric effect of the C-methyl group. Metaraminol is the only compound of those studied with two asymmetric centers. The stereochemistry of its cyclization was not studied.

Reaction Products. Metaproterenol and terbutaline, because they are 3,5-dihydroxyphenethylamines, can cyclize only to one THIQ, the *ortho* and *para* positions being equivalent. Each of the other compounds give two THIQs. In all instances, one of them predominated, and the ratio of the two varied with the pH of the reaction. With phenylephrine, the THIQ peak with the longer retention time and larger area was identified as the product of cyclization *para* to the phenolic hydroxyl group. Whether or not this is general with its analogues is a question to be resolved in further studies.

Pharmaceutical Implications. The facile autoxidation of metaproterenol THIQ at neutral pH suggests that the fluorometric method for phenylephrine described by Kaito *et al.* (14) could be simplified by omitting the oxidant and would be generally applicable to this group of compounds. A study of reaction rate as a function of pH might well shorten the time for the determination of the optical rotation of levodopa as its THIQ (13). It would be interesting to determine if cyclization to THIQs is a significant factor in the stability of such products as phenylephrine and metaproterenol syrups.

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