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Short Communication

Photochemical hydroxylation of phenylephrine to epinephrine (adrenaline)

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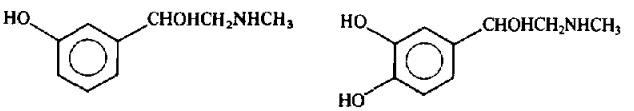
Introduction

Phenylephrine is (−)-m-hydroxy-α[(methylamino)methyl]benzyl alcohol, a synthetic analog of epinephrine, which differs from it in structure only in having a 3,4-dihydroxy or catechol moiety (Scheme 1). Like epinephrine, phenylephrine solutions are subject to autoxidation, evidenced by a brown discolouration and, ultimately, formation of a dark brown precipitate, a melanin pigment. Discolouration is accelerated by light, but it occurs eventually even in light-protected solutions. Luduena *et al.* [1] deduced that epinephrine was among the photochemical oxidation products of phenylephrine solutions in UV light, basing this conclusion on biological assay of the increase in pressor effect and on spectrofluorometry. Al Taii *et al.* [2] studied this reaction many years later. They inferred that epinephrine was a reaction product from the similarity of spectra of the melanin pigments from it and from phenylephrine. They did not actually demonstrate the presence of epinephrine, and they provided no quantitative data on its formation. Using a method selective for the secondary amine function [3], they estimated that 88.5% of the initial phenylephrine content remained after irradiation for 8 weeks. They indicated that free radical oxidation is the mechanism — as would be expected in autoxidative processes — by showing similar TLC mobilities and reactions to spray reagents of photolysed phenylephrine and its solutions treated with hydrogen peroxide and Fenton's reagent.

Scheme 1



phenylephrine epinephrine

Hydroxylation of phenols to polyphenols is an exotic reaction *in vitro*. That a 3-hydroxyphenyl compound is hydroxylated to a 3,4-dihydroxyphenyl is especially remarkable, since autoxidative attack generally occurs at points of low electron density, and the 4-position is relatively electron rich. The reaction is thus interesting from the standpoint of the organic chemist.

Because of their sensitivity to oxidation, usual pharmaceutical practice is to fill solutions of catecholamines and their monophenolic analogues under nitrogen to displace most of the air, using bisulphite to scavenge residual oxygen and label solutions with the caution that they are not to be used if they are discoloured or contain a precipitate. If epinephrine is detectable only in photolysed phenylephrine solutions at the point where discolouration has occurred, the significance of the reaction would be merely academic — in the pejorative sense of the term. If, however, significant amounts of epinephrine are present in solutions with indiscernible colour, the reaction would have medical importance owing to the much greater potency of epinephrine as compared with phenylephrine. The intention of the experiments described below was to reproduce the original irradiation procedure of Luduena *et al.* [1] as closely as possible in order to obtain a direct comparison of results, but high-performance liquid chromatography (HPLC) was used to obtain a quantitative estimate of epinephrine and phenylephrine with time and a qualitative picture of other products formed.

Experimental

A solution of phenylephrine HCl in distilled water, 1 mg ml^{-1}, was divided among several glass petri dishes, 100- × 15-mm, using 15-ml in 1- and 25-ml volumes in the others. A shortwave UV light source was rigged by removing the cover of a chromatographic view box fitted with six 15-W G15T8 tubular lamps (Sylvania and General Electric) and suspending it inverted 20-cm above the dishes. After irradiation for periods of 1–5 h, the solutions became tan with a pink tinge and had lost about 20% of their original volumes by evaporation. (The original report [1] described the irradiated solutions as having a "strong tea colour" and replacement of volume lost by evaporation with distilled water.) The solutions were reconstituted to their original volumes with water and assayed for phenylephrine and epinephrine content by HPLC, using the external standard method for quantification. The HPLC system comprised a Waters Associates Model 6000A pump, a Perkin–Elmer LC-420 autosampler fitted with a 20-μl loop, a duPont Zorbax CN column, 4.6-mm × 25-cm, a Perkin–Elmer LC-75 detector set at 280 nm and a Spectra-Physics SP-4100 integrator. Mobile phase was 1% acetic acid in water (0.017 M, prepared by diluting 10-ml of glacial acetic acid to 1-l with water) at a flow rate of 1.5 ml min^{-1}. Additional evidence of the identity of epinephrine was adduced by gas chromatography. A portion of one solution was evaporated, the residue silylated with BSA and injected into a Perkin–Elmer Model 900 instrument fitted with a 6 ft × 0.25 in glass column packed with 5% OV-1 on 100/200 mesh Gas Chrom Q, column temperature 100°C, injector and FID 275°C, helium being used as the carrier gas. Silylated phenylephrine eluted at 9 min and silylated epinephrine at 21 min, these times corresponding exactly to those for similarly-treated authentic materials.

Results and Discussion

Epinephrine content of the irradiated phenylephrine solutions, determined by HPLC

and GC, was about 2% of the initial content. This value is in agreement with the estimate of Luduena *et al.* [1]. The relative constancy of this value over a 5 h period suggests that epinephrine found at any time is a vector of the amounts being formed and undergoing further oxidation. Irradiation of a 3 $\mu g\ ml^{-1}$ solution of epinephrine HCl under the same experimental conditions for 3 h resulted in conversion of about 80% of it to an unknown with HPLC retention time of about 6.9 min. Thus, as expected, photo-oxidation of epinephrine proceeds much faster than for phenylephrine.

Whether epinephrine is formed in significant concentration before the solutions darken or, more importantly, can be detected in ostensibly colourless commercial phenylephrine solutions would be of great interest for further work. The identity of some of the major peaks in Fig. 1, which constitute the majority of the reaction products, would also be interesting. One may speculate some of these may be dihydroxyphenyl analogues other than 3,4-dihydroxy. Photochemical oxidation of hydroxyphenalkanolamines may well be a general phenomenon. Shepard and West [4] noted similar discolouration of solutions of norphenylephrine and its 4-hydroxyphenyl analogue after UV irradiation.

Table 1
Residual phenylephrine and epinephrine formed in irradiated phenylephrine HCl solutions

Hours irradiated	% Initial phenylephrine	% Epinephrine
1	76.1	1.7
2	78.1	1.5
3	75.4	2.4
5	71.5	2.7

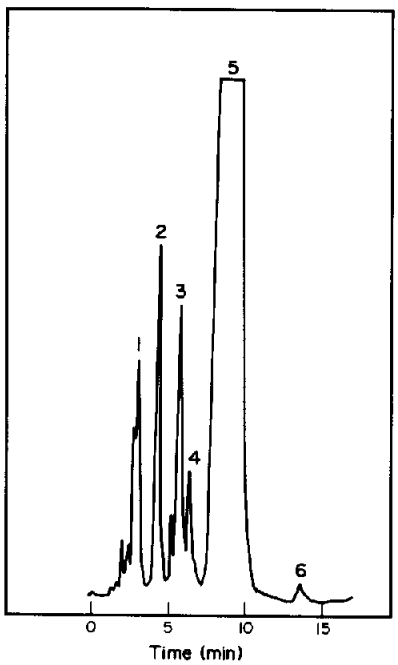


Figure 1
Chromatogram of UV-irradiated phenylephrine solution. Peak 3, at 6.0 min retention time, corresponds to authentic epinephrine, and Peak 5, eluting at 8.8 min is phenylephrine. The remaining peaks were not identified.

Phenylephrine is generally stable in its pharmaceutical preparations; however, its phenol function may be oxidised, its hydroxyl and secondary amine functions have been reported to be acetylated by co-formulated aspirin [3], and it may react with carbonyl compounds to form tetrahydroisoquinolines [5]. The findings reported herein confirm and extend those reported originally [1]. Whether or not they have pharmaceutical importance is a question requiring further work.

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