[Chem. Pharm. Bull. 28(2) 660—663 (1980)]

Phosphorimetric Determination of α-[(*tert*-Butylamino)methyl]-3,4-dihydroxybenzyl Alcohol 3,4-Di(*p*-toluate)

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(Received July 11, 1979)

A phosphorimetric method for the determination of α-[(*tert*-butylamino)methyl]-3,4-dihydroxybenzyl alcohol 3,4-di(*p*-toluate) (I) was established.

The method is based on the native phosphorescence of I in an alkaline extract after separation on a thin-layer chromatographic plate, and can be applied satisfactorily to the determination of I in the concentration range of 0.02—0.5 μg per spot.

Keywords——phosphorimetric determination; α-[(*tert*-butylamino)methyl]-3,4-dihydroxybenzyl alcohol 3,4-di(*p*-toluate); α-[(*tert*-butylamino)methyl]-3,4-dihydroxybenzyl alcohol; α-[(*tert*-butylamino)methyl]-3-methoxy-4-hydroxybenzyl alcohol; thin-layer chromatography

α-[(*tert*-Butylamino)methyl]-3,4-dihydroxybenzyl alcohol 3,4-di(*p*-toluate) (I), a derivative of catecholamine, is a bronchodilator. It was found to be metabolized successively to α-[(*tert*-butylamino)methyl]-3,4-dihydroxybenzyl alcohol (II) and α-[(*tert*-butylamino)methyl]-3-methoxy-4-hydroxybenzyl alcohol (III).[2]

Since low doses of I are employed for clinical treatment, a sensitive method is necessary for its determination in biological fluids.

The fluorescence and phosphorescence spectral characteristics of I, II, and III in ethanol were examined, and it was found that I showed intense phosphorescence in alkaline ethanol. This observation was successfully applied to the phosphorimetric microdetermination of I, and a procedure was established.

Experimental

Apparatus——Phosphorescence excitation and emission spectra, and intensity were measured with a Hitachi MPF-4 spectrofluorometer equipped with a Hitachi phosphoroscope attachment. After being frozen at 77°K in liquid nitrogen, the sample solution was held in a fused quartz microsample tube of 2 mm inner diameter. The resolution time of the phosphorimeter was kept constant at 1.5 msec and the lifetimes were measured with the same apparatus equipped with a Hitachi V-104 synchroscope.

Other apparatus used consisted of silica gel $60F_{254}$ plates, 20×20 cm (Merck), a Hamilton syringe (10 μl), and a chromatographic chamber (9×22×22, cm).

Reagents——The chemical structures of the compounds used are shown in Chart 1. These were obtained from Sterling-Winthrop Research Institute (New York).

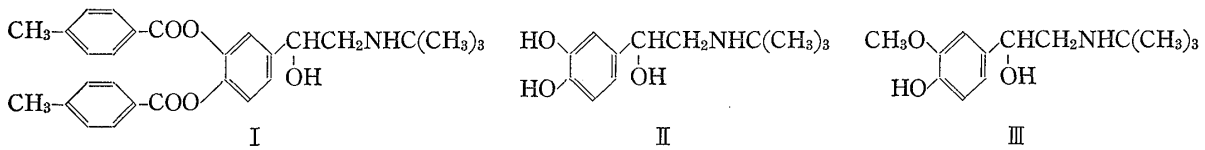

Chart 1. Chemical Structures of α-[(*tert*-Butylamino)methyl]-3,4-dihydroxybenzyl alcohol 3,4-di(*p*-toluate) (I), α-[(*tert*-Butylamino)methyl]-3,4-dihydroxybenzyl alcohol (II), and α-[(*tert*-Butylamino)methyl]-3-methoxy-4-hydroxybenzyl alcohol (III)

1) Location: *Sagisu, Fukushima-ku, Osaka, 553, Japan.*
2) Private communication from Sterling-Winthrop Research Institute (New York).

Stock Solution of I (II or III): Compound I (II or III) (20 mg) was dissolved in 50 ml of ethanol. This solution was stable for two weeks when stored in a refrigerator.

Working Solution of I: These were prepared by diluting the stock solution with ethanol to the desired concentrations before use.

Ethanol: Reagent-grade EtOH (1000 ml) was distilled after dissolving 10 g of Na metal in it.

Methanol, formic acid, chloroform, and potassium hydroxide were of reagent grade.

Procedure——Sample solution (10 μl) containing I (0.02—0.5 μg) was spotted on a plate and developed with a mixture of methanol, formic acid, and chloroform (2: 1: 7 v/v) for 1 hr in a chromatographic chamber saturated with solvent vapor. After drying the plate for 15 min in air at room temperature, a dark spot ($Rf \fallingdotseq 0.7$) under ultraviolet illumination (254 nm) was scraped off, and the material was extracted with 5 ml of 0.05 N KOH–95% EtOH by shaking for 5 min followed by centrifugation for 5 min. Within 80 min, the phosphorescence intensity of the extract was measured at 400 nm with excitation at 250 nm.

The calibration curve was obtained by plotting the intensities *vs.* the concentrations of working solutions of I. The concentration of I in the sample solution was then obtained by interpolation.

Results and Discussion

Phosphorescence Spectral Data

The phosphorescence spectral characteristics of I, II, and III in 95% EtOH at 77 °K were examined. As shown in Table I, these compounds phosphoresced in all media tested

Table I. Phosphorescence Spectral Data for I, II, and III in 95%EtOH at 77°K[a]

Compound	Medium	Ex_{max}(nm)[b]	Em_{max}(nm)[b]	RPI[c]	τ(sec)[d]
I	0.01 N H_2SO_4–95%EtOH	258	403	5.6	2.73
		277 sh			
	95%EtOH	254	407	6.6	2.58
		276 sh			
	0.01 N KOH–95%EtOH	250	375	41.5	3.29
		285	389 sh		
			400		
			410		
			421 sh		
			436 sh		
			455 sh		
II	0.01 N H_2SO_4–95%EtOH	237	434	5.2	0.37
		287			
	95%EtOH	253	420	9.6	0.42
		294			
	0.01 N KOH–95%EtOH	250	414	25.6	0.46
		296			
III	0.01 N H_2SO_4–95%EtOH	240	436	9.2	0.30
		284			
	95%EtOH	255	424	12.4	0.46
		286			
	0.01 N KOH–95%EtOH	251	418	80.4	0.54
		294			

a) The concentration of sample solutions was 2×10^{-5} M.
b) Maxima used for excitation (emission) are underlined.
c) Relative phosphorescence intensity. For comparison of phosphorescence intensity, that of carbazole solution (2×10^{-5} M in EtOH) was taken as 100 (at 340/437 nm).
d) Mean lifetime.
sh=shoulder.

and gave the maximum intensity in alkaline ethanolic solution. The intensities of II and III at 400 nm with excitation at 250 nm in alkaline ethanolic solution were 0.2 and 24% of that of I, respectively. Therefore, for the determination of I in the presence of II and III, the separation of I from II and III was necessary.

Selection of Developing Solvent

Two combinations of the methanol–formic acid–chloroform solvent system were examined. As shown in Table II, effective separation of I was obtained using a mixture of methanol–formic acid–chloroform (2 : 1 : 7, v/v).

Furthermore, in this solvent system, epinephrine and norepinephrine were also separated from I, II, and III with *Rf* values of about 0.17 and 0.10, respectively.

Table II. Effect of Developing Solvent on the Separation of I, II, and III on TLC Plates[a)]

Solvent system ($MeOH:HCOOH:CHCl_3$)	*Rf*		
	I	II	III
15 : 10 : 75	0.56—0.58	0.23—0.25	0.40—0.43
20 : 10 : 70	0.66—0.76	0.35—0.40	0.58—0.62

a) Ten microliter portions of mixed sample solution containing 0.2 μg each of I, II, and III were treated according to the standard procedure.

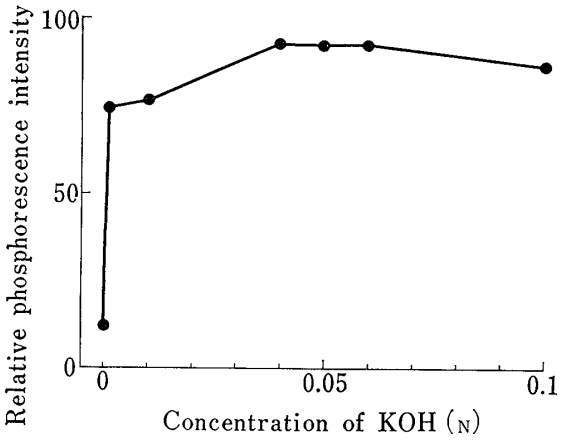

Fig. 1. Effect of KOH Concentration on the Phosphorescence of I in 95%EtOH

Ten microliter portions of sample solution containing 0.2 μg of I were treated according to the standard procedure.

Effect of KOH Concentration

Figure 1 shows the effect of KOH concentration on the phosphorescence intensity of I in 95%EtOH. The intensity was constant in the concentration range from 0.04 to 0.06 N and the spectra scarcely changed over the concentration range tested. Thus, 0.05 N KOH–95% EtOH was selected as a suitable solvent for the procedure.

Phosphorescence Stability

The phosphorescence stability of I in 0.05 N KOH–95%EtOH was examined under room light and room temperature. As shown in Fig. 2, the phosphorescence intensity was stable for 80 min, from 10 to 90 min after adding 0.05 N KOH solution, with a coefficient of variation of 4%.

Calibration Curve

Figure 3 shows the calibration curve for I in the concentration range of 0.02—0.5 μg per spot. A linear relationship was observed between the phosphorescence intensity and the concentration with a correlation coefficient of 0.998.

Regression Analysis

Regression analysis for the determination of I in the concentration range of 0.051—0.512 μg was examined using ten mixed sample solutions in which II and III were present in ratios of 1 : 1 to 10 : 1 relative to I.

As shown in Table III, the calculated relation between the theoretical (x) and the experimental (y) values gave a correlation coefficient of 0.966.

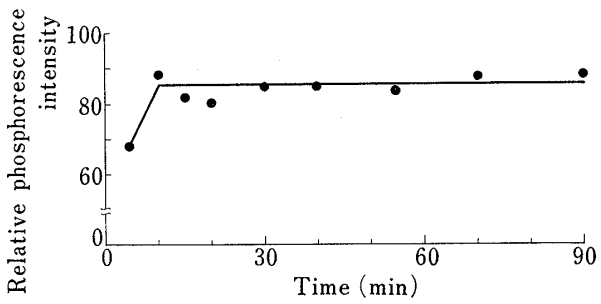

Fig. 2. Stability of the Phosphorescence of I in 0.05 N KOH–95% EtOH

Ten microliter portions of sample solution containing 0.2 μg of I were treated according to the standard procedure.

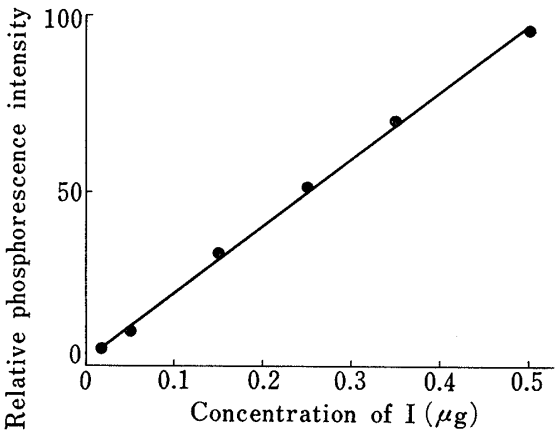

Fig. 3. Calibration Curve for I

TABLE III. Regression Analysis for the Assay of I in the Presence of II and III[a)]

Sample No.	Components in 10 μl of mixed sample solution (μg)			Found (μg)
	I(x)	II	III	I (y)
1	0.051	0.520	0.520	0.031
2	0.153	0.520	0.520	0.145
3	0.156	0.520	0.520	0.187
4	0.252	0.520	0.520	0.240
5	0.256	0.520	0.520	0.282
6	0.260	0.520	0.520	0.281
7	0.307	0.520	0.520	0.291
8	0.353	0.520	0.520	0.295
9	0.358	0.520	0.520	0.414
10	0.512	0.520	0.520	0.486

a) Regression equation : $y=0.9641x+0.009$, $s=0.035$, $r=0.966$.