

SOME PHYSICO-CHEMICAL PROPERTIES OF 2,4,3',5'-TETRAHYDROXYSTILBENE

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Summary

The purification and some physico-chemical properties of the antifungal compound 2,4,3',5'-tetrahydroxystilbene were studied. These include ultraviolet absorption spectra, fluorescence spectra, solubilities and partition coefficients in various organic solvents.

Introduction

2,4,3',5'-Tetrahydroxystilbene (THS) is a phenolic compound isolated from aqueous extracts of several plants¹. The crude aqueous extract of *Artocarpus lakoocha* Roxb. heart wood contains up to 70% in THS and has been used in Thailand as anthelmintic² and antipruritic. THS exhibits antifungal activity against many saprophytic fungi which are responsible for wood decomposition³ and against six major pathogenic dermatophytes⁴. It is also a potent inhibitor of mitochondrial oxidative phosphorylation¹ and of mitochondrial adenosinetriphosphatase⁵. However, many physicochemical properties which are useful in pharmacological and pharmaceutical studies have not been reported. We report here the purification and some of these properties of THS.

Materials and Methods

Materials

Methanol, ethanol, diethyl ether, 1,4 dioxane and chloroform (all Spectro-grade^R) were obtained from E. Merck. Isobutanol (Spectranalyzed^R) was from Fisher Scientific Co. 1-Hexanol and 1-octanol (reagent grade) were from BDH Chemical Ltd. Ultraviolet absorption spectra were determined on a Unicam model SP-800 spectrophotometer. Excitation and emission spectra were determined on a Aminco-Bowman spectrofluorometer, model 4-8202 SPF.

Purification procedure.

The crystalline THS obtained as described by Mongolsuk *et al*⁶ was found to contain minor impurities. These impurities were removed by chromatography of the

preparation on silica gel column using ethyl acetate-petroleum ether (1:1) as eluent. THS, which was the main and the only fluorescent component, eluted in the second peak. Preparative thin layer chromatography of this component on silica gel PF 254 using ethyl acetate-petroleum ether (1:1) resulted in THS dihydrate after recrystallization of the fluorescence band in water. Anhydrous THS was obtained after heating the dihydrate at 80°C under vacuum. The dihydrate needle-shaped crystal of THS was used in this study.

Results and Discussion

Ultraviolet Absorption

Ultraviolet absorptions of THS in various organic solvents obey Beer's Law upto 20 $\mu\text{g/ml}$ with absorption maxima and extinction coefficients at 25°C as shown in Table I. Two isobestic points are observed between pH 5-9 in aqueous solution, one at 340 nm (ϵ $1.784 \times 10^4 \text{ M}^{-1}/\text{cm}$) and the other at 273 nm (ϵ $0.831 \times 10^4 \text{ M}^{-1}/\text{cm}$).

TABLE I: ULTRAVIOLET ABSORPTION MAXIMA AND EXTINCTION COEFFICIENTS OF THS IN VARIOUS SOLVENTS.

Solvent	λ_{max} (nm)	$10^{-4} \epsilon$ (M^{-1}/cm)
water	324	2.462
methanol	324	2.446
ethanol	326	2.266
1-hexanol	328	2.220
1-octanol	328	2.201
1, 4 dioxane	325	2.334
isobutanol	327	2.201
chloroform	327	2.010
diethyl ether	325	2.656

Fluorescence Properties.

Fluorescence spectra and relative fluorescence intensities of THS in some organic solvents are shown in Fig. 1. The excitation and emission maxima of THS in various solvents are shown in Table II.

The order of relative fluorescence intensity at 1 $\mu\text{g/ml}$ THS is 1-octanol \sim 1,4-dioxane $>$ 1-hexanol $>$ diethyl ether $>$ isobutanol $>$ ethanol $>$ methanol $>$ chloroform \sim water. Thus, THS tends to exhibit a fluorescence hypsochromic shift and increase in fluorescence intensity as the polarity of the solvents decrease, especially from water to dioxane (Table II). It has also been found that fluorescence measurement is a sensitive method for quantitative determination of THS in solution. As low as 0.1 $\mu\text{g/ml}$ THS in ethanol could easily be detected. The range of linearity in such determination is between 0.1 to 1.5 $\mu\text{g/ml}$.

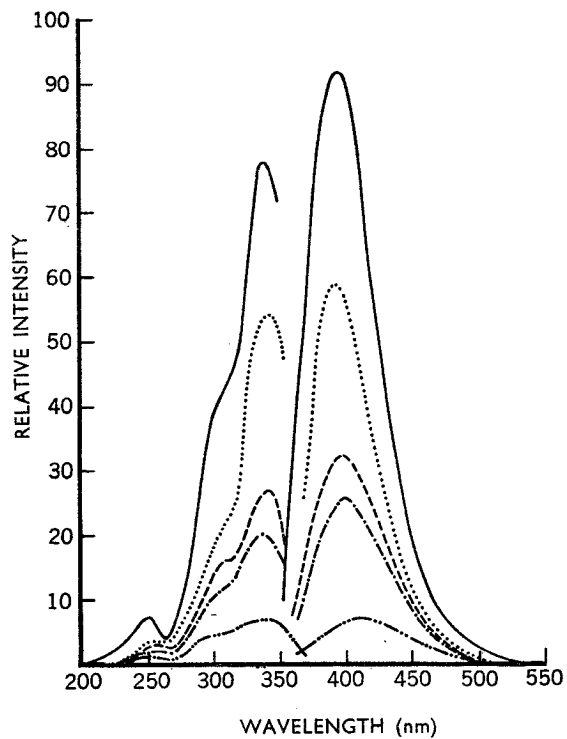


Fig. 1. Excitation and emission spectra of THS in 1,4 dioxane (—————), diethyl ether (.....), ethanol (-----), methanol (-.-.-.-) and water (..-.-.-). The corresponding spectra of the solvents have been subtracted. The concentration of THS was 1.0 $\mu\text{g/ml}$ in all cases.

TABLE II: EXCITATION AND EMISSION MAXIMA OF THS IN VARIOUS SOLVENTS.

Solvent	Excitation maximum(nm)	Emission maximum(nm)
water	334	410
methanol	335	397
ethanol	337	395
isobutanol	340	393
diethyl ether	340	390
1, 4 dioxane	335	387
chloroform	335	390
1-hexanol	340	400
1-octanol	340	400

Alkaline lability.

Alkaline conditions have been extensively used for extractions and fluorescence studies of many phenolic compounds. It was found that the excitation and emission maxima of THS shift from 334 and 410 nm at pH 6.6 in water to 375 and 465 nm at pH 10.5 in 0.01 N NaOH with a higher quantum yield. Of practical importance is the finding that THS decomposes rapidly under alkaline conditions. Fluorescence studies reveal that the decomposition follows first-order reaction and that at pH 10.5 (in 0.01 N NaOH) the half-life of THS is 70 minutes when the solution is kept in the dark and is 18 minutes when the solution is irradiated at 375 nm (excitation maximum). Both values were obtained at 21°C. This observation explains the low yield or the failure to obtain THS by extraction of *A. lakoocha* heart wood with 1% NaOH even in the presence of antioxidant as compared to extraction with ether⁷. The decomposition product(s) have not been identified but are most likely quinones resulted from air oxidation of the phenolates. The fluorescence intensity of THS in ethanol remains unchanged under irradiation at 337 nm (excitation maximum) for at least one hour at this temperature.

Solubility and partition coefficients.

The solubilities of THS in various solvents have been determined by UV absorption and are as follows: water, 0.640 mg/ml (2.285 mM); methanol, 406.6 mg/ml (1.450 M); ethanol, 328 mg/ml (1.171 M); 1,4 dioxane, 415.6 mg/ml (1.484 M); diethyl ether, 355.5 mg/ml (1.269 M); isobutanol, 173.5 mg/ml (0.62 M); 1-hexanol, 114.1 mg/ml (0.41 M) and 1-octanol, 69.76 mg/ml (0.25 M). These values were obtained at 25°C except in the case of diethyl ether where the study was made at 5°C. The solubility of THS in carbon tetrachloride is extremely low and could not be determined accurately even by the fluorescence technique. Starting with 10 µg/ml THS in organic solvents the partition coefficients of THS (the ratio of THS concentration in organic solvent to THS concentration in water at equilibrium, K_D) are as follows: chloroform, 0; isobutanol, 5.6; 1-hexanol, 7.8; 1-octanol, 48. It was found that diethyl ether quantitatively extracted all the THS from the aqueous phase.

Because of the alkaline lability of THS, we have found that the extraction of THS from biological fluids such as plasma and urine by the procedure described by Brown⁸ for estriol, estrone and estradiol gave very poor recovery of THS. However, if the sodium hydroxide washing step of this procedure is omitted, over 95% of THS could be recovered from Tyrode's solution.

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บทคัดย่อ

2,4,3',5'-Tetrahydroxystilbene เป็นสารที่สกัดได้จากยาสมุนไพรพวกเห็ด สารเคมีตัวนี้มีคุณสมบัติทางชีวเคมีและเภสัชวิทยาหลายอย่างด้วยกัน ในบทความนี้ได้รายงานถึงวิธีทำให้บริสุทธิ์ และคุณสมบัติทางเคมี-ฟิสิกส์ บางประการของสารตัวนี้โดยศึกษาคุณสมบัติในการดูดอมแสงอุลตราไวโอเล็ตและในทางฟลูออเรสเซนส์ การละลายตัวในน้ำ การละลายและ partition coefficients ในตัวละลายต่างๆ