Communications to the Editor

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THE ANTIFUNGAL ACTIVITY OF STILBENE DERIVATIVE

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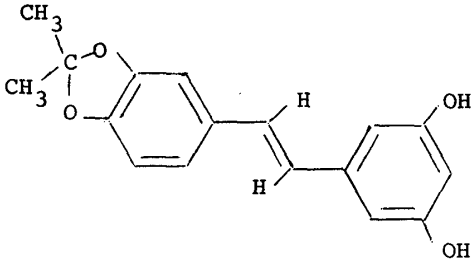
The purpose of the present study is to examine the antimicrobial activity of a new compound, 3,4-O-isopropylidene-3,3',4,5'-tetrahydroxystilbene (1) chemically derived from 3,3',4,5'-tetrahydroxystilbene (2) which was isolated from the heartwood of *Cassia garrettiana* CRAIB by the authors, and the antifungal activities of which were previously reported. It has become apparent that the antifungal activities of compound (1) are far stronger than those of compound (2), and that the acute toxicity of compound (1) is lower than that of compound (2). It should be emphasized that compound (1) shows its strong antifungal activity especially against *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

KEYWORDS ——— 3,4-O-isopropylidene-3,3',4,5'-tetrahydroxystilbene; 3,3',4,5'-tetrahydroxystilbene; stilbene derivative; antifungal activity; *Cassia garrettiana*

Previously, we examined the physiological activities of the components[1,2] of *Cassia garrettiana* CRAIB (Leguminosae) with antifungal activities and as a result, confirmed the antifungal, phytogrowth-inhibitory, and ichthyo-toxical activities of 3,3',4,5'-tetrahydroxystilbene (2),[3] one of stilbene derivatives. At that time, we synthesized 2-tetraacetate, 2-tetramethyl ether and 3,3',4,5'-tetrahydroxybibenzyl as other stilbene derivatives in order to examine the antifungal activities. Any of the activities, however, was far lower than that of the compound (2).

Subsequently, we synthesized new derivatives of compound (2) in order to examine the antifungal activities. Of these new compound, 3,4-O-isopropylidene-3,3',4,5'-tetrahydroxystilbene (1) (Chart 1) showed its antifungal activity more markedly than compound (2) (Chart 1). The toxicity of compound (1) was apparently lower than that of compound (2). In this paper, we wish to report the physico-chemical data, the antifungal activity and information on the acute toxicity of compound (1).

Compound (2) was heated with anhydrous acetone and P_2O_5. The reaction mixture was subjected to Sephadex LH-20 column chromatography using methanol as a solvent to afford the compound (1). Table I summarizes the physico-chemical data of the compound (1).



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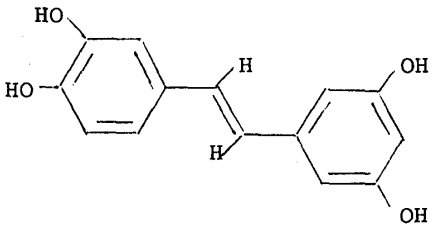

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Chart 1. Chemical Structures of 3,4-O-Isopropylidene-3,3',4,5'-tetrahydroxystilbene (1) and 3,3',4,5'-Tetrahydroxystilbene (2)

Table I. Physico-Chemical Data of 3,4-O-Isopropylidene-3,3',4,5'-tetrahydroxystilbene (1)

mp	118 - 119°C (colorless crystalline powder)
Anal.	$C_{17}H_{16}O_4$ Calcd : C 71.80, H 5.68 Found : C 71.60, H 5.84
TLC *Rf* (solvent system)[a]	0.11 (hexane:AcOEt = 3:1) 0.34 (benzene:AcOEt = 3:1) 0.32 ($CHCl_3$:MeOH = 10:1)
UV λ_{max}^{EtOH} nm (log ε)	302 (4.61), 328 (4.76)
IR ν_{max}^{KBr} cm^{-1}	3300 (OH), 1610, 1590 (aromatic ring)
PMR ($CDCl_3$, δ ppm)	1.66 (6H,s), 6.34 (1H,d, *J*= 3 Hz, aromatic H), 6.51 (2H,d, *J*= 3 Hz, aromatic H), 6.66 (1H,d, *J*= 8.5 Hz, aromatic H), 6.71, 6.95 (1H,each d, *J*= 17 Hz, -CH=CH-), 6.87 (1H,dd, *J*= 8.5 and 3 Hz, aromatic H), 6.92 (1H,d, *J*= 3 Hz, aromatic H), 8.20 (2H, br s, OH x 2)

a) TLC plate : Kiesel gel 60 (Merck Co., Ltd.)

Next, the antimicrobial activities of compound (1) were examined by agar dilution method. As shown in Table II, the compound (1) showed its antifungal activity more markedly than the original substance (2). Especially, compound (1) showed such a strong antifungal activity that the minimal inhibitory concentration (MIC) levels against *Trichophyton rubrum* and *Trichophyton mentagrophytes* came to 8 μg/ml and 6 μg/ml, respectively (the MIC levels of substance (2)[3] against *Trichophyton rubrum* and *Trichophyton mentagrophytes* were 50 μg/ml and 60 μg/ml, respectively). However, the antibacterial activity of compound (1) was lower than that of compound (2).

Table II. Antimicrobial Activities of 3,4-O-Isopropylidene-3,3',4,5'-tetrahydroxystilbene (1)

Microorganism	MIC (μg/ml)
Fungi	
Trichophyton mentagrophytes IFO-5811	6
Trichophyton rubrum IFO-5467	8
Mucor racemosus IFO-4581	10
Aspergillus niger IFO-4414	20
Candida albicans IAM-4966	20
Trichoderma longibrachiatum IFO-4847	30
Cladosporium cladosporioides IFO-6348	30
Saccharomyces cerevisiae IFO-0203	30
Penicillium thomii IFO-7002	50
Aspergillus terreus IFO-6346	50
Bacteria	
Staphylococcus aureus 209-P	>500
Bacillus subtilis PCI-219	>500
Escherichia coli IFO-12734	>500
Proteus vulgaris IFO-3851	>500
Proteus mirabilis IFO-3849	>500
Serratia marcescens IFO-3735	>500

Culture conditions: Fungi--- 27°C, 7 days (*Saccharomyces cerevisiae*: 5 days, *Candida albicans*: 2 days). Bacteria--- 37°C, 18 h. Media: Fungi--- Potato Dextrose Agar (*Saccharomyces cerevisiae*: Malt Agar, *Candida albicans*: Sabouraud Glucose Agar). Bacteria--- Nutrient Agar. Method: Agar dilution method.

The general conditions and the acute toxicity in mice following the administration of compound (1) were examined. When 1100 mg/kg of the compound (1) was administered, convulsions developed at approximately 5 minutes after the administration and all the mice died within 5 h. In the group given 800 mg/kg, reduction in spontaneous movements began approximately 20 minutes after the administration. Then the animals showed crouching associated with eye-closing and apparently began to die approximately 10 h after the administration. The LD_{50} value of compound (1 for the mice was 574.9 mg/kg (intraperitoneal injection, Van der Waerden method). These results suggest that the acute toxicity of compound (1) is far lower than that of compound (2)[3] (LD_{50} of 217 mg/kg: intraperitoneal injection to mice, Van der Waerden method).

The aforementioned findings clearly indicate that the antifungal activity of compound (1) is stronger than that of compound (2).

In addition to compounds (1) and (2), there are resveratrol[4] (isolated from the root of *Polygonum cuspidatum*, Polygonaceae), pinosylvin and its methyl ether[5] (the phytoalexin isolated from *Pinus resinosa*), and pterostilbene,[6] which have hydroxyl groups or methoxyl groups in the 3', 5' position, belong to stilbene derivatives, and show the antimicrobial activities.

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