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Antifungal Activity of Oosponol, Oospolactone, Phyllodulcin, Hydrangenol, and Some Other Related Compounds[1]

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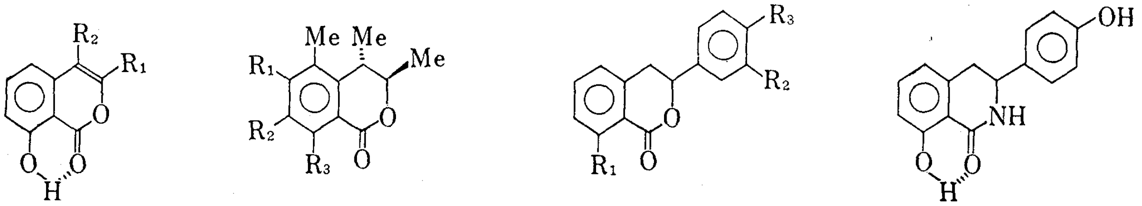
Various natural 8-hydroxyisocoumarin derivatives, *i.e.*, oosponol (**1**), oospolactone (**2**), *d*-phyllodulcin (*d*-**4**) and *dl*-hydrangenol (*dl*-**5**), were examined for antifungal activity. Several derivatives of *d*-**4** and *dl*-**5** were also examined.

It is very interesting that *d*-phyllodulcin, a sweetening component of *Hydrangea serrata* SERINGE var. *thunbergii* (Japanese name amacha), was found to possess antifungal activity.

Keywords——antifungal activity; 3,4-dihydroisocoumarins; 3,4-dihydro-8-hydroxyisocoumarins; 8-hydroxyisocoumarins; isocoumarins

In 1976, the present authors identified lenzitin,[2] a metabolite of a basidiomycete, *Gloeophyllum sepiarium*, as being identical with oosponol (**1**),[3] and, at the same time, reported the isolation of oospolactone (**2**)[4] from the same basidiomycete. It was also reported that these two isocoumarins possessed marked antifungal activity against *Candida albicans* and *Trichophyton asteroides*.[3,4] In this paper the authors report the antifungal activity of **1** and **2** against various plant-pathogenic molds (Table I). In addition, another natural isocoumarin possessing a hydroxyl group at the 8-position, *i.e.*, decarboxydihydrocitrinone (**3**), was also examined for antifungal activity (Table I). This compound (**3**) was derived, mostly according to the procedures of Brown *et al.*,[5] from citrinin, which we isolated recently in high yield from *Penicillium odoratum*[6] *via* the successive intermediates dihydrocitrinin, methyl O-dimethyldihydrocitrinin, and methyl O-dimethyldihydrocitrinone. These three intermediates showed no activity, whereas **3** showed antifungal activity.



1: $R_1=H$, $R_2=COCH_2OH$
2: $R_1=R_2=Me$

3: $R_1=R_3=OH$, $R_2=H$

4: $R_1=R_2=OH$, $R_3=OMe$
5: $R_1=R_3=OH$, $R_2=H$
6: $R_1=R_2=OAc$, $R_3=OMe$
7: $R_1=R_2=R_3=OH$
8: $R_1=OH$, $R_2=R_3=OMe$
9: $R_1=R_3=OAc$, $R_2=H$
10: $R_1=OH$, $R_2=H$, $R_3=OMe$

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Chart 1

Furthermore, we examined the antifungal activity of *d*-phyllodulcin (*d*-**4**) and hydrangenol (*dl*-**5**), constituents of *Hydrangea serrata* SERINGE var. *thunbergii* (Japanese name; amacha), and some other compounds structurally related to these two natural isocoumarins, namely *dl*-phyllodulcin (*dl*-**4**),[7] 8-acetoxy-3-(3-acetoxy-4-methoxyphenyl)-3,4-dihydroisocoumarins (*d*-**6** and *dl*-**6**),[7] 3,4-dihydro-3-(3,4-dimethoxyphenyl)-8-hydroxyisocoumarin (*dl*-**7**),[8] 3,4-

dihydro-3-(3,4-dimethoxyphenyl)-8-hydroxyisocoumarin (*d*-**8**),[9] 8-acetoxy-3-(*p*-acetoxyphenyl)-3,4-dihydroisocoumarin (*dl*-**9**),[10] 3,4-dihydro-8-hydroxy-3-(*p*-methoxyphenyl)isocoumarin (*dl*-**10**),[11] and 3,4-dihydro-8-hydroxy-3-(*p*-hydroxyphenyl)isocarbostyryl (*dl*-**11**) (Table I). The new compound (*dl*-**11**) was prepared by the reaction of *dl*-**5** with ammonia. This reaction is the first example of replacement of ring oxygen by nitrogen in a 3,4-dihydroisocoumarin nucleus. Other derivatives were prepared either from *d*-**4** or from *dl*-**5** by the procedures described in the literature.[7-11]

d-Phyllodulcin was found by Ueno *et al.* to possess an extremely weak antibacterial activity, inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus* at concentrations over 5000 μg per ml.[12] In the present work, we found that *d*-**4** and *dl*-**5** possessed antifungal activity, inhibiting the growth of the molds completely at concentrations from 25 to 200 μg per ml, as can be seen in Table I. In view of the fact that *d*-**4** is a well-known sweetening component of amacha, which is served as a modified tea or as a sweetening agent for diabetics, this finding is extremely significant.

All modifications of the structure of phyllodulcin (*d*-**4**), that is, acetylation of the two hydroxyl groups (*d*-**6** and *dl*-**6**), demethylation of the methoxyl group (*dl*-**7**), or methylation of the hydroxyl group at the 3′-position (*dl*-**8**), resulted in a decrease of its antifungal activity. Similarly, structural modification of *dl*-hydrangenol, *i.e.*, methylation of the hydroxyl group at the 4′-position (*dl*-**10**) or the replacement of the ring oxygen by an imino group (*dl*-**11**), was also found to cause a lowering of the antifungal activity (Table I). Optically active isomers (*d*-**4** and *d*-**6**) showed stronger activity than the racemates (*dl*-**4** and *dl*-**6**) (Table I).

Table I. *In Vitro* Antifungal Activity of Isocoumarins

Compd. No.	MIC,[a] μg/ml					
	Alternaria maritima	*Cochliobolus miyabeanus*	*Fusarium splendens*	*Giberella zeae*	*Helminthosporium maydis*	*Penicillium expansum*
1	25	6.25	25	ND[b]	6.25	12.5
2	12.5	50	100	ND[b]	50	50
3	100	ND[b]	200	100	ND[b]	ND[b]
d-**4**	25	100	25	25	25	50
dl-**4**	50	N[c]	50	50	50	200
dl-**5**	50	25	50	50	200	200
d-**6**	(200)	N[c]	25 (12.5)	(12.5)	50 (12.5)	(12.5)
dl-**6**	(12.5)	N[c]	50 (12.5)	(12.5)	(12.5)	N[c]
dl-**7**	(50)	100 (25)	100 (50)	(12.5)	100 (25)	(200)
d-**8**	(12.5)	(25)	(25)	(12.5)	(25)	(50)
dl-**9**	(12.5)	N[c]	50 (12.5)	(12.5)	50 (12.5)	N[c]
dl-**10**	(12.5)	N[c]	(12.5)	(12.5)	ND[b]	N[c]
dl-**11**	N[c]	ND[b]	(12.5)	(25)	ND[b]	N[c]

[a] Minimum inhibitory concentration (MIC) is the lowest concentration of the compound that prevents visible growth of molds completely after 73 h of incubation at 27°C. The numbers in parenthesis are the lowest concentrations at which inhibition of growth was more or less apparent.

[b] ND: not determined.

[c] N: No inhibition of growth was observed at the concentration of 400 μg/ml.

Experimental

Melting points are uncorrected. Infrared (IR) spectra were taken with a Hitachi model 215 grating spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained on a JEOL JNM FX-100 FT NMR spectrometer at 100 MHz, with tetramethylsilane as an internal standard. Mass spectra were obtained on a JEOL JMS D-300 spectrometer.

3,4-Dihydro-8-hydroxy-3-(*p*-hydroxyphenyl)isocarbostyryl (*dl*-11)——A mixture of *dl*-5 (0.50 g) and ethanol (10 ml) saturated with ammonia was heated at 100°C for 17 h in an autoclave. The residue obtained after concentration was mixed with dil.HCl and filtered. The crude product obtained was washed, dried and recrystallized from EtOH to give *dl*-11 as colorless needles of mp 202°C; yield, 0.32 g (64%). *Anal.* Calcd for $C_{15}H_{13}NO_3$: C, 70.58; H, 5.13; N, 5.49. Found: C, 70.48; H, 5.27; N, 4.95. IR ν_{max}^{KBr} cm^{-1}: 1652, 3070, 3170 (CONH). PMR (in acetone-d_6) δ: 3.13 (2H, d, $J=3$ Hz, CH_2), 4.83 (1H, t, $J=3$ Hz, $-C\underline{H}NH-$), 6.6—7.4 (9H, m, aromatic-H, NH, and OH), 8.41 (1H, s, OH). MS *m/e*: 255 (M^+).

Antifungal Test——The antifungal activity was determined on agar plants by the two-fold dilution method. The experimental details were as reported in our previous paper.6)

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References and Notes

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Lactams. XVIII.1) Oxidation of 1-Substituted 3-*tert*-Butylpiperidine with Mercuric Acetate–EDTA

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1-(3,4-Dimethoxyphenyl)-2-(3-*tert*-butylpiperidino)ethanol (7) was prepared from 3-*tert*-butylpyridine (5) through the quaternary salt **6**. The mercuric acetate–EDTA oxidation of **7** produced the 6-piperidone **10** and the 2-piperidone **13** in a ratio of 98: 2. The former piperidone was chemically correlated with the known 6-pyridone **8** through the lactam **9**, and **9** was converted into the benzoquinolizidine **11** by cyclization and reduction of the resulting iminium salt **12**.

Keywords——1,3-disubstituted piperidine; piperidone; benzoquinolizidine; quaternization; catalytic reduction; mercuric acetate–EDTA oxidation; *tert*-butyl group; steric effect; regioselectivity; stereoselectivity

One of the most important aspects of our recent chiral syntheses2) of the Ipecac and *Alangium* alkaloids was the generation of the lactam carbonyl function at the 6-position of cincholoipon ethyl ester [(+)−1], a degradation product of the major *Cinchona* alkaloids,