Note

Chrysodin, an Antifungal Antimetabolite

Hiroyuki HARAGUCHI, Makoto TANIGUCHI,*
Kazuhiko MOTOKA,* KOZO SHIBATA,*
Susumu OI* and Kensuke HASHIMOTO

Faculty of Engineering, Fukuyama University,
Higashimura-cho, Fukuyama 720-02, Japan

* Faculty of Science, Osaka City University,
Sumiyoshi-ku, Osaka 558, Japan

Received March 5, 1990

During the course of screening for antifungal antimetabolites,¹⁾ the culture filtrate of a filamentous fungus was found to inhibit the growth of *Candida albicans* in a synthetic medium. The inhibitory effect could be reversed by adding lipids extracted from the *C. albicans* cells to the medium. In this paper, we report the isolation of chrysodin as an active principle from the culture broth and describe its antifungal antimetabolite properties. The filamentous fungus could not be identified because it did not form spores in any of the culture media tested. Chrysodin has been reported to be produced by *Sepedonium chrysospermum*,²⁾ but its biological properties are not reported.

The unidentified fungus was cultured with shaking at 25°C in a medium consisting of 3% lactose, 0.2% yeast extract, 0.1% KH₂PO₄, 0.05% KCl, 0.05% MgSO₄·7H₂O, and 0.001% FeSO₄·7H₂O (pH 6.0). After cultivation for 7 days, the broth filtrate was put on a Diaion HP-20 column chromatography and the active eluate was obtained with 95% methanol. The eluate was concentrated *in vacuo* and extracted with ethyl acetate. Further purification of the crude extract was done by column chromatography on silica gel, eluting with CHCl₃. The active substance thus obtained was crystallized from (CH₃)₂CO and yielded compound **1** as red cubic crystals.

Compound **1**, [α]_D²² −408 (*c*=0.025, CH₂Cl₂, lit.²⁾ −388° (*c*=0.06, CH₂Cl₂), showed the M⁺ ion at *m/z* 326 and the base peak ion (M−C₂H₅O)⁺ at *m/z* 284 in the mass spectrum, agreeing with the results of the elemental analysis (Found: C, 69.78%; H, 5.62%, Calcd. for C₁₉H₁₈O₅: C, 69.92%; H, 5.56%). The absorption spectra of compound **1** had absorption maxima at λ_{max} (CH₂Cl₂) 244 (log ε 3.99), 309 (log ε 4.12) and 382 nm (log ε 4.61) (lit.²⁾, 243 (3.99), 308.5 (4.12) and 380 (4.61)) and ν_{max} (nujol) 1740, 1720, 1625 and 1601 cm^{−1} (lit.²⁾, 1740, 1717, 1630, 1603 cm^{−1}). From these data and the ¹H- and ¹³C-NMR data shown in Table I compound **1** was identified as chrysodin.²⁾

The antifungal activity of chrysodin against several

Table I. CHEMICAL SHIFTS (ppm from TMS in ¹H NMR and from CDCl₃ 77.03 in ¹³C NMR) IN CDCl₃ OF CHRYSODIN (¹H NMR, 400 MHz; ¹³C, 100 MHz)

Assign- ment	¹³ C	¹ H	Lit. ²⁾
1	18.89	1.84 (d, <i>J</i> =6.1 Hz)	1.88 (d, <i>J</i> =5 Hz)
2	134.66	5.93 (dq, 14.6, 6.1)	5.7–7.3 (7H)
3	131.70	6.15 (dd, 14.6, 10.7)	
4	139.61	6.49 (dd, 14.6, 10.7)	
5	128.14	6.20 (dd, 14.6, 11.2)	
6	136.37	6.95 (dd, 15.1, 11.2)	
7	120.05	6.02 (d, 15.1)	
8	155.48		
9	107.57	5.57 (d, 1.0)	5.6 (s)
10	142.19		
11	109.46	6.14 (s)	
12	192.61		
13	84.34		
14	191.88		
15	114.61		
16	153.02	7.89 (d, 1.0)	7.90 (s)
17	20.42	1.54 (s)	1.55 (s)
CH ₃ CO	22.70	2.17 (s)	2.18 (s)
	169.52		

Table II. ANTIFUNGAL ACTIVITY OF CHRYSODIN

Fungus tested	MIC (μg/ml)
<i>Saccharomyces cerevisiae</i> IFO 0203	1.56
<i>Schizosaccharomyces pombe</i> IFO 0342	1.56
<i>Candida albicans</i> IFO 1061	0.78
<i>Rhodotorula rubra</i> IFO 0001	1.56
<i>Mucor mucedo</i> IFO 7648	3.13
<i>Rhizopus chinensis</i> IFO 4745	3.13
<i>Aspergillus niger</i> ATCC 6275	12.5
<i>Penicillium chrysogenum</i> IFO 4626	12.5

kinds of fungi was measured by a two-fold serial broth dilution method³⁾ using 2.5% malt extract medium. After incubation at 25°C for 2 days, fungal growth was examined with the naked eye. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of chrysodin at which there was no visible growth. As shown in Table II, chrysodin had broad antifungal activity against all of the yeasts and filamentous fungi tested. *C. albicans* IFO 1061 was the most sensitive among them; the MIC was 0.78 μg/ml. Both Gram-positive and Gram-

Table III. REVERSION OF THE ANTIFUNGAL ACTIVITY OF CHRYSODIN BY FATTY ACIDS IN *C. albicans*

Fatty acid ^a	Diameter of growth inhibitory zone (mm)	
	Chrysodin ($\mu\text{g}/\text{disc}$)	
	10	20
None	16	19
Lauric acid (C12:0)	16	18
Myristic acid (C14:0)	15	17
Palmitic acid (C16:0)	16	17
Palmitoleic acid (C16:1)	15	18
Stearic acid (C18:0)	16	18
Oleic acid (C18:1)	9	11
Linoleic acid (C18:2)	0	0
Linolenic acid (C18:3)	0	0

^a 100 $\mu\text{g}/\text{ml}$.

negative bacteria were insensitive to this antifungal substance, with the MIC for bacteria being more than 100 $\mu\text{g}/\text{ml}$ (data not shown).

The reversing effect of nutrients on the antifungal activity of chrysodin against *C. albicans* IFO 1061 was examined by a disc-agar plate method⁴⁾ using a synthetic medium^{5,6)} consisting of 2% glucose, 0.3% $(\text{NH}_4)_2\text{SO}_4$, 0.3% KH_2PO_4 , 0.025% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.025% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1% monosodium glutamate, 0.005% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.01% $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$. A paper disc with 10 or 20 μg of chrysodin was placed on the seeded

agar medium with addition of 100 $\mu\text{g}/\text{ml}$ of fatty acids or other metabolites. The agar plate was incubated at 25°C for 2 days, and the diameter of the inhibition zone was measured. The results are shown in Table III. The antifungal activity of chrysodin was markedly reversed by linoleic acid and linolenic acid, and to a certain extent by oleic acid, but not by other fatty acids. Besides fatty acids, it was slightly reversed by ergocarciferol and L-cysteine.

These results, indicated that chrysodin is an antifungal antimetabolite which can affect the biosynthesis of unsaturated fatty acids in *C. albicans*. Further studies on its action mechanism in the yeast are now in progress.

Acknowledgments. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 63560112) to M. T. from the Ministry of Education, Science and Culture of Japan.

References

- 1) D. L. Pruess and J. P. Scannell, *Adv. Appl. Microbiol.*, **17**, 19 (1974).
- 2) A. Closse and D. Hauser, *Helv. Chim. Acta*, **56**, 2649 (1973).
- 3) Y. Yano, M. Taniguchi, E. Tada, T. Tanaka, S. Oi, H. Haraguchi, K. Hashimoto and I. Kubo, *Agric. Biol. Chem.*, **53**, 1525 (1989).
- 4) S. Nomura, T. Horiuchi, S. Omura and T. Hata, *J. Biochem.*, **71**, 783 (1972).
- 5) A. H. Rose and W. J. Nicherson, *J. Bacteriol.*, **72**, 324 (1956).
- 6) G. Kessler and W. J. Nicherson, *J. Biol. Chem.*, **234**, 2281 (1959).