CHRYSOSPORIN, A NEW INHIBITOR OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE PRODUCED BY *Chrysosporium pannorum*

Sir:

Cholesterol is synthesized *via* more than 20 enzymatic reactions. This pathway is mainly regulated by the enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase[1]. Recent clinical results have shown that HMG-CoA reductase inhibitors such as lovastatin[2] (monacolin K[3]) are safe and effective drugs for treatment of hypercholesterolemia[4].

We have previously reported that *Chrysosporium pannorum* M10539 produces an HMG-CoA reductase inhibitor, pannorin[5]. Further study of the metabolites produced by this strain led to the isolation of another novel inhibitor designated chrysosporin. The present communication deals with the isolation, physico-chemical properties and biological activity of chrysosporin.

Chrysosporin was produced by growing *C. pannorum* M10539 at 25°C for 10 to 12 days in a medium containing 20% potato extract and 2% glucose. The cultural conditions were identical to those described previously for production of pannorin by this microbe[5], except that production medium was inoculated with 2% (vol/vol) of seed culture. The cultue filtrate (5 liters) was adjusted to pH 2.5 with HCl and extracted three times with 2 liters of ethyl acetate. The organic extracts were combined and concentrated to 1 liter, followed by extracting three times with 1 liter of 5% $NaHCO_3$ (pH 8.2). The aqueous layer was pooled and adjusted to pH 2.5 with HCl. Precipitates formed were collected by centrifugation, washed with water and then lyophilized, giving 5.53 g of a brownish residue. The residue was suspended with 100 ml of water and extracted three times with 100 ml of *n*-butyl acetate at pH 3. The solvent extracts were dried over sodium sulfate and concentrated to give 1.88 g of a brownish powder. The residue was dissolved in 19 ml of 20 mM potassium phosphate, pH 7.4, and then was submitted to gel filtration in 19 batches over a Bio-Gel P-6 column (26 × 40 mm), which was equilibrated and developed with 20 mM potassium phosphate, pH 7.4, containing 0.3 M NaCl and 0.02% sodium azide. Active fractions were pooled and extracted three times with an equal volume of ethyl acetate at pH 3. The organic layer was dried over sodium sulfate and was concentrated to dryness, giving 310 mg of a brownish powder. The residue was dissolved in 15.5 ml of acetonitrile - 0.1% phosphoric acid (2 : 3) and was chromatographed using preparative HPLC on a silica ODS column (10 × 300 mm) in 75 batches. The column was developed with acetonitrile - 0.1% phosphoric acid (2 : 3) at a flow rate of 6 ml/minute. Active fractions were concentrated and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and was concentrated to dryness to give 35 mg of chrysosporin as a dark brown powder.

The physico-chemical properties of chrysosporin are summarized in Table 1. The molecular formula of chrysosporin was determined to be $C_{27}H_{18}O_{10}$ from the results of HRFAB-MS and ^{13}C NMR studies. The UV spectra of chrysosporin showed absorption maxima at 234, 284, 293 and 376 nm in methanol, which were strongly affected under alkaline conditions (Table 1). The IR, ^1H NMR and

Table 1. Physico-chemical properties of chrysosporin.

Appearance	Dark brown powder
Molecular formula	$C_{27}H_{18}O_{10}$
HRFAB-MS (*m/z*)	
Found:	501.0832 $(M-H)^+$
Calcd:	501.0827 for $C_{27}H_{17}O_{10}$
UV λ_{max} nm (ε)	
MeOH	234 (32,000), 284 (21,500), 293 (23,000), 376 (7,000)
MeOH - 1 M HCl (4 : 1)	232 (34,800), 284 (21,900), 292 (25,300), 374 (7,100), 420 (3,200)
MeOH - 1 M NaOH (4 : 1)	248 (26,300), 273 (23,400), 290 (21,500), 374 (9,900)
IR ν_{max} (KBr) cm^{-1}	3450, 2930, 1700, 1684, 1676, 1620, 1600, 1490, 1330, 1280, 1100, 1050, 840
^1H NMR[a] δ	2.34 (3H, s), 2.54 (3H, s), 2.61 (3H, s), 5.55 (1H, s), 6.67 (1H, s), 7.14 (1H, s), 7.57 (1H, s), 9.70 (1H, br s), 9.97 (1H, br s), 12.17 (1H, br s), 12.87 (1H, s)
^{13}C NMR[b] δ	19.04, 23.40, 31.55, 88.93, 102.39, 102.45, 106.61, 107.46, 112.81, 117.84, 120.21, 122.24, 130.44, 132.45, 136.21, 136.25, 141.61, 154.53, 156.33, 156.44, 156.71, 158.35, 161.07, 169.48, 180.30, 189.93, 203.07

[a] DMSO-d_6, 270 MHz.
[b] DMSO-d_6, 68 MHz.

Fig. 1. Chrysosporin inhibition of (A) *in vitro* sterol biosynthesis from [^{14}C]acetate and (B) HMG-CoA reductase activity.

Incorporation of [^{14}C]acetate into cholesterol (●) and lanosterol (○) and HMG-CoA reductase activity (■) were determined in the presence of the indicated concentrations of chrysosporin using a rat liver cholesterol biosynthetic system and microsomes, respectively. Each value represents the average of duplicate determinations.

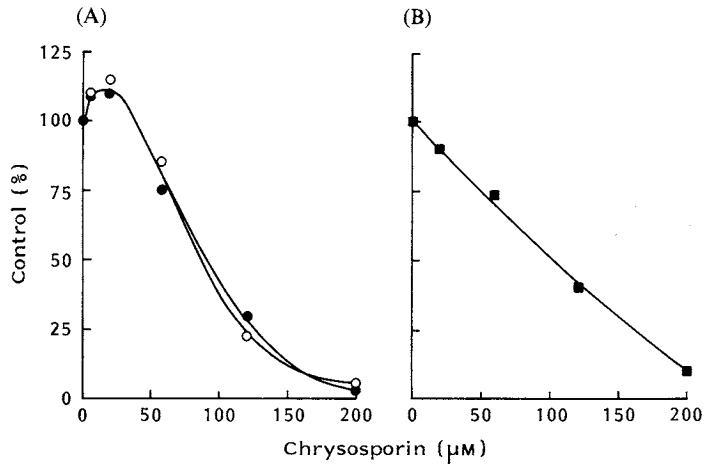
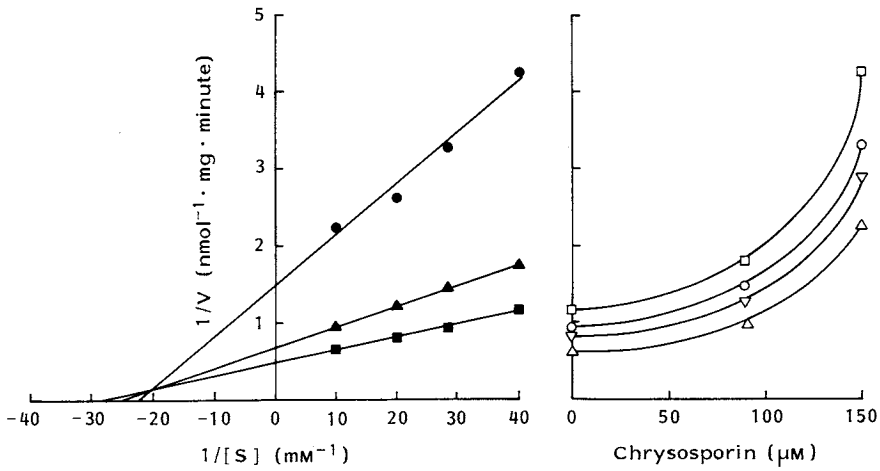


Fig. 2. Double reciprocal plots and Dixon plots (inset) for the inhibition of HMG-CoA reductase by chrysosporin.

Microsomal HMG-CoA reductase activity was determined in the presence of chrysosporin at concentrations of 0 (■), 90 (▲) and 150 μM (●). In the inset, concentrations of [^{14}C]HMG-CoA were 25 (□), 35 (○), 50 (▽) and 100 μM (△). Each value represents the average of duplicate determinations.



^{13}C NMR data are shown in Table 1. These data suggested that chrysosporin is a novel compound closely related to pannorin, another HMG-CoA reductase inhibitor produced by *C. pannorum*[5]. Structure of chrysosporin is under investigation and will be reported elsewhere.

In vitro sterol synthesis and HMG-CoA reductase activities were determined using rat liver preparations as described previously[6]. Chrysosporin inhibited [^{14}C]acetate incorporation into both cholesterol and lanosterol by 50% at a concentration of 80 μM (Fig. 1A). Microsomal HMG-CoA

Table 2. Irreversible inhibition of HMG-CoA reductase by chrysosporin.

Chrysosporin (μM)	HMG-CoA reductase activity (pmol/minute/mg)	
	Before dialysis	After dialysis
0	853 (100%)	530 (100%)
50	277 (32%)	268 (51%)
100	132 (16%)	135 (25%)
160	66 (8%)	92 (17%)

Rat liver microsomes were incubated at 37°C for 10 minutes in 100 mM potassium phosphate, pH 7.4, in the presence or absence of chrysosporin. HMG-CoA reductase activity in microsomes was determined before and after dialysis at 4°C for 20 hours against 100 mM potassium phosphate, pH 7.4, 10 mM EDTA and 2 mM dithiothreitol. Each value represents the average of duplicate determinations.

reductase activity was inhibited 50% by 100 μM chrysosporin (Fig. 1B).

Double reciprocal plots[7] for HMG-CoA reductase inhibition by chrysosporin showed that the inhibition was the mixed type with respect to HMG-CoA, and Dixon plots[7] curved upwards (Fig. 2), suggesting that the inhibition is cooperative or time-dependent. Reversibility of the inhibition was determined by dialysis. As shown in Table 2, HMG-CoA reductase activity was inhibited 70~90% after 10-minute preincubation with 50~160 μM chrysosporin, and did not recover significantly from inhibition even after extensive dialysis of the microsomes. This result demonstrated that chrysosporin is an irreversible inhibitor of HMG-CoA reductase.

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JONG KOO PARK
KAORU SAKAI
HARUO OGAWA
KEIJI HASUMI
AKIRA ENDO

Department of Applied Biological Science,
Tokyo Noko University,
Fuchu, Tokyo 183, Japan

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