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Favolon B, a New Triterpenoid Isolated from the Chilean *Mycena* sp. Strain 96180

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Abstract A new biologically active triterpenoid, favolon B (**1**), was isolated from fermentation broths of *Mycena* sp. strain 96180. Favolon B showed antifungal activities towards *Botrytis cinerea*, *Mucor miehei*, *Paecilomyces variotii* and *Penicillium notatum*. No activities were observed against bacteria and yeasts. The structure of favolon B was elucidated by spectroscopic techniques.

Keywords terpenoids, fungi, basidiomycetes, bioactive metabolites

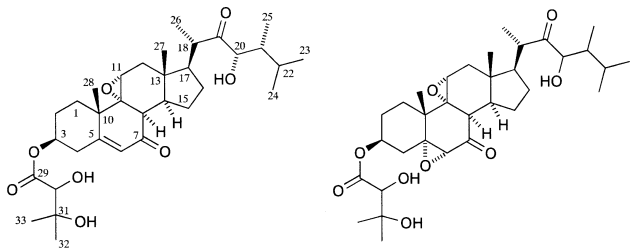
Introduction

In our search of novel and biologically active metabolites from Chilean basidiomycetes, we previously reported four new bioactive compounds, the himanimides, from *Serpula himantoides* [1]. In the course of further screenings, a new antifungal and cytotoxic triterpenoid, favolon B (**1**), related to favolon (**2**) [2] (see Figure 1 for structures), was isolated from fermentations of *Mycena* sp. strain 96180. This paper describes the fermentation of *Mycena* sp. strain 96180 and the isolation and chemical as well as biological characterization of favolon B.

Results and Discussion

Mycena 96180 was found on endemic forest soil from Contulmo, near to Concepción, Chile. Mycelial cultures were produced from spore prints of fruiting bodies, which were then grown in a YMG medium composed of yeast extract 0.4%, malt extract 1%, glucose 0.4%, and agar 1.5%, pH 5.5. Small sections of the stock culture were cut under sterile conditions and transferred to a 5-liter Erlenmeyer flask containing 2500 ml of YMG. This flask was incubated at 22°C on a rotary shaker (120 rpm). After 20 days of fermentation, the culture broth was extracted with EtOAc (2.5 liters). Evaporation of the organic phase yielded a crude extract (915 mg), which was applied onto a column containing silica gel (Merck 60, 0.063~0.2 μm; column 3×30 cm). Elution with ethyl acetate : methanol (3 : 7) yielded 33 mg of an enriched product containing the active compound **1**.

Final purification was achieved by preparative HPLC (Jasco model PU-980 with a diode array detector; column: Macherey and Nagel, 250×21.2 mm containing 7 μm



1 **2**

Fig. 1 Relative structures of favolon B (**1**) and favolon (**2**).

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Table 1 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of favolon B

C	^{1}H (C_6D_6)	^{13}C (C_6D_6)	^{1}H ($CDCl_3$)	^{13}C ($CDCl_3$)
1α	1.28 (m)	30.9 (t)	2.02 (m)	30.7 (t)
1β	0.98 (m)		1.66 (m)	
2α	1.77 (m)	27.4 (t)	2.11 (m)	26.8 (t)
2β	1.47 (m)		1.90 (m)	
3	4.63 (m)	75.4 (d)	4.85 (m)	75.2 (d)
4α	2.10 (dd, *J*=4.7, 11.6 Hz)	37.4 (t)	2.63 (m)	37.3 (t)
4β	2.18 (ddd, *J*=1.3, 11.4, 11.6 Hz)		2.67 (m)	
5	–	156.4 (s)	–	156.9 (s)
6	5.65 (d, *J*=1.3 Hz)	127.2 (d)	5.81 (s)	126.6 (d)
7	–	197.2 (s)	–	197.6 (s)
8	2.86 (d, *J*=7.3 Hz)	44.1 (d)	3.35 (m)	43.6 (d)
9	–	64.6 (s)	–	64.4 (s)
10	–	43.8 (s)	–	43.6 (s)
11	2.81 (d, *J*=2.6 Hz)	56.9 (d)	3.36 (m)	56.9 (d)
12α	1.11 (d, *J*=14.7 Hz)	40.3 (t)	1.69 (m)	39.8 (t)
12β	2.02 (dd, *J*=2.6, 14.7 Hz)		2.40 (dd, *J*=2.3, 14.9 Hz)	
13	–	42.5 (s)	–	42.0 (s)
14	1.01 (m)	47.3 (d)	1.48 (m)	46.9 (d)
15α	1.45 (m)	22.5 (t)	1.50 (m)	21.7 (t)
15β	2.81 (m)		2.54 (m)	
16α	1.56 (m)	27.8 (t)	1.59 (m)	27.0 (t)
16β	1.27 (m)		1.30 (m)	
17	1.44 (m)	55.6 (d)	1.53 (m)	55.0 (d)
18	2.60 (dq, *J*=10.2, 6.7 Hz)	44.3 (d)	2.68 (m)	43.6 (d)
19	–	217.0 (s)	–	216.9 (s)
20	4.41(s)	80.4 (d)	4.31 (s)	79.8 (d)
21	1.58 (m)	41.7 (d)	1.63 (m)	41.0 (d)
22	1.85 (m)	32.2 (d)	1.76 (m)	31.4 (d)
23	1.09 (d, *J*=6.9 Hz)	21.3 (q)	1.07 (d, *J*=6.9 Hz)	20.9 (q)
24	0.99 (d, *J*=6.9 Hz)	21.1 (q)	0.99 (d, *J*=6.9 Hz)	20.7 (q)
25	0.79 (d, *J*=6.8 Hz)	11.7 (q)	0.70 (d, *J*=6.7 Hz)	11.1 (q)
26	1.02 (d, *J*=6.7 Hz)	16.3 (q)	1.10 (d, *J*=6.9 Hz)	15.7 (q)
27	1.10 (s)	16.3 (q)	0.99 (s)	15.7 (q)
28	0.73 (s)	16.4 (q)	1.08 (s)	16.3 (q)
29	–	173.1 (s)	–	172.3 (s)
30	3.92 (s)	78.0 (d)	3.97 (s)	77.1 (d)
31	–	72.4 (s)	–	72.1 (s)
32	1.32 (s)	26.6 (q)	1.32 (s)	25.9 (q)
33	1.26 (s)	25.9 (q)	1.24 (s)	25.0 (q)

Nucleosil C18, flow rate: 5 ml/minute). Elution with water - methanol gradient 23 : 77 v/v yielded 11 mg of compound (**1**).

The structure was elucidated by spectra of ^{1}H NMR (500 MHz, $CDCl_3$, C_6D_6) and ^{13}C NMR (125 MHz, $CDCl_3$, C_6D_6). EIMS. (70 eV), *m/z* (rel. int.): 574 (100%, M^+), 556 (40%) 440 (60%) 423 (20%). HRFABMS observed 575.3589 for $M+H^+$ (calculated for $C_{33}H_{51}O_8$ 575.3584). IR (KBr): 3433, 2961, 2925, 1701, 1652, 1617, 1380, 1200, 1144 and 1094 cm^{-1}. UV (MeOH), λ_{max} 235 nm. NMR data are given in Table 1.

The elemental composition suggested by the high resolution MS data show that the molecule has 9 unsaturations, and with one carbon - carbon double bond and three carbonyl groups (suggested by the 1D NMR data) the compound should consequently be pentacyclic. The

Table 2 Antifungal activity of favolon B in the agar diffusion assay

Organism	Diameter of inhibition zone (mm)		
	μg/disc*		
	0.1	1	10
Absidia glauca (+)	–	–	–
A. glauca (–)	–	–	–
Alternaria porri	–	10di	26di
Aspergillus ochraceus	8di	15di	20di
Botrytis cinerea	20	24	42
Cladosporium cladosporoides	–	–	–
Epicoccum purpurascens	–	–	–
Fusarium fujikuroi	–	–	–
F. oxysporum	–	–	–
Mucor miehei	15	17	22
Nematospora coryli	–	–	–
Paecilomyces varioti	20	25	30
Penicillium islandicum	–	–	–
P. notatum	10	12	18
Pythium ultimum	–	–	–
Rhodotorula glutinis	–	–	–
Saccharomyces cerevisiae is **1****	–	–	–
S. cereviseae α288c	–	–	–
Ustilago nuda	–	9	16
Zygorhynchus moelleri	–	–	–
Bacillus brevis ATCC 9999	–	–	–
B. subtilis ATCC 6633	–	–	–
Enterobacter dissolvens	–	–	–
Sarcina lutea	–	–	–

* diameter=6 mm; –=no inhibition zone; di=diffuse inhibition zone; **=Gift from Prof. Lacrouter, Strasbourg, France.

basic steroid structure could be established by the COSY and HMBC correlations (in C_6D_6), for example the HMBC correlations from 28-H_3 to C-1, C-5, C-9 and C-10, from 27-H_3 to C-12, C-13, C-14 and C-17, from 26-H_3 to C-17, C-18 and C-19, from 6-H, 8-H and 14-H to C-7, from 12-H_2 to C-9 and C-11, and from 8-H to C-9. The position of the ester group is shown by the HMBC correlation from 3-H to C-29. The epoxide function is supported by the chemical shifts as well as the correlations observed, and in addition by the coupling constant between 11-H and C-11 (171 Hz). The relative configuration of favolon B was suggested by the correlations observed in NOESY experiments (in C_6D_6). The C-10 methyl group gives NOESY correlations with the axial protons 2-Hβ and 4-Hβ, as well as to 11-H. 3-H correlates with 2-Hα and 4-Hα, and to the axial 1-Hα, and 1-Hβ gives as expected a correlation to 11-H. 8-H gives a strong NOESY to 1-Hα, showing that it is alfa, and the closeness of the two protons is revealed by a Dreiding model of the suggested structure, and in addition to 14-H. 27-H_3 give NOESY correlations to 15-Hβ and 18-H, while 17-H correlates to 12-Hα, 16-Hα and 26-H_3. The correlations between 18-H and 16-Hα as well as 21-H, together with those already mentioned, demonstrate the configuration of C-18. 20-H correlates to 16-Hβ and 18-H, but not to 25-H_3, and must consequently be on the upper side of the molecule. A favourable conformations allowing for an intermolecular hydrogen bond between 20-OH and 19=O, the NOESY correlations for 20-H discussed above and the NOESY correlation between 18-H and 21-H is obtained if the configurations of C-20 and C-21 are as suggested in Figure 1, but not in other diastereomers. 30-H only gives NOESY correlations to 32-H_3 and 33-H_3, and it is not possible to propose the relative configuration of C-30 based on NMR data.

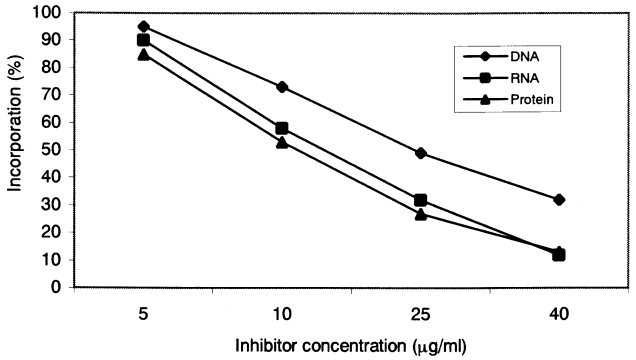

Fig. 2 Inhibitory effect of favolon B on incorporation of radio-labelled precursors into macromolecules HL-60 cells. [^{14}C] thymidine into DNA, control 61267 cpm; [^{14}C] uridine into RNA, control 124829 cpm; [^{14}C] leucine into proteins, control 18837 cpm.

The assays for antimicrobial [3] and cytotoxic activities [4] were carried out towards HL-60 (ATCC CCL 240) and Colon 320 (DSMZ ACC-144) cells. The incorporation of the precursors [2-^{14}C thymidine into DNA, 2-^{14}C uridine into RNA, and 1-^{14}C leucine into proteins] was assayed with HL-60 cells [5].

The compound's antifungal and antibacterial activities are compared in Table 2. Flavolon B (**1**) exhibits higher antifungal activities towards *Botrytis cinerea*, *Mucor miehei*, *Paecilomyces variotii*, and *Penicillium notatum*. No antibiotic activities were observed against bacteria and yeasts.

Favolon B (**1**) also showed moderate cytotoxic activity towards HL-60 (ATCC CCL 240), with IC_{50}'s of 10 μg/ml, whereas lower cytotoxic effects (25 μg/ml) were observed with Colon 320 (DSMZ ACC-144) cells. The incorporation of ^{14}C-labelled precursors into macromolecules in HL-60 showed a preferential inhibition of RNA and protein biosynthesis starting from 5~10 μg/ml (Fig. 2).

Favolon B (**1**) differs from favolon (**2**) due to the presence of a double bond in the B ring, which replaces the original favolon's epoxide-group. The structure of favolon B is confirmed by the presence of the proton C-6 in the ^{1}H NMR as a singlet at 5.65.

This difference apparently confers a selective and reduced spectrum of sensitive fungal strains and positive cytotoxic activities.

It is interesting to note that the only active compound produced by *Mycena* sp. strain 96180 was favolon B and not the antifungal compounds strobilurins and/or oudemansins, the typical active substances of the *Mycena* genus [6].

This compound is probably ecologically significant in nature, where the *Mycena* species uses the isopentyl pyrophosphate pathway, an alternative pathway to shikimic acid, thereby producing favolon B, an antifungal compound that gives the species competitive advantages against potential competitors [7].

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