BASIDALIN, A NEW ANTIBIOTIC FROM BASIDIOMYCETES

Sir:

In the course of screening for new antibiotics, a new antibiotic, which had a very weak antibacterial activity and antitumor activity against L1210 mouse leukemia, was isolated as crystals from the culture filtrate of *Leucoagricus naucina* (Fr.) Sing. We named it basidalin. The structure was determined to be (*Z*)-4-amino-5-(formylmethylene)-2(5*H*)-furanone (I) by X-ray crystallographic analysis. In this communication, the production, isolation, chemical and physical properties, and structure of basidalin are reported.

The basidalin-producing strain (the strain number in Institute of Microbial Chemistry, NZ157) was isolated from the fruit body of a mushroom collected in New Zealand and classified as *Leucoagricus naucina* (Fr.) Sing[1]. The mycelium (*ca.* 0.25 m²) grown on a slant culture (2.0% glucose, 0.5% dried yeast and 1.5% agar, pH 5.8) was inoculated into a medium containing 2.0% glucose, 0.5% peptone, 0.3% KH_2PO_4, 0.3% yeast extract and 0.1% $MgSO_4 \cdot 7H_2O$. It was cultured in a stationary mode at 28°C for 10 days followed by shake-culture on a reciprocal shaker (130 strokes per minute) at 28°C for one day. Five ml of this culture was inoculated to a 500-ml Sakaguchi flask containing 125 ml of the above medium, and it was shake-cultured at 28°C for 4 days.

The culture filtrate (9.4 liters) thus obtained was adjusted to pH 6.0 and extracted with *n*-butyl acetate. The extract was concentrated under reduced pressure to give yellow brownish powder (1.2 g). It was dissolved in hot methanol and kept in refrigerator to yield 870 mg of crude crystals of basidalin. It was recrystallized from hot methanol [mp 142~149°C (decomp.)].

Basidalin is soluble in hot methanol and dimethylsulfoxide, and slightly soluble in ethyl acetate, chloroform, benzene, hexane and water. It shows positive color reactions with 2,4-dinitrophenylhydrazine, triphenyltetrazolium chloride, potassium permanganate and RYDON-SMITH reagents, but does not react with ninhydrin and ferric chloride.

The molecular formula of basidalin was established to be $C_6H_5NO_3$ (MW 139.11) by high resolution mass spectrometry (M^+ *m/z* 139.0257, Calcd. 139.0268) and elemental analysis (Calcd.: C 51.80, H 3.62, N 10.07, O 34.51. Found: C 52.05, H 3.58, N 9.82, O 34.12). The UV spectra showed absorption maxima at 220 nm (ε 7800) and 277 nm (ε 16000) in MeOH, at 251 nm (ε 16700) and 308 nm (ε 9500) in 0.01 N HCl, and at 260 nm (ε 20600) in 0.01 N NaOH. The IR spectrum (KBr) showed absorptions at 3350, 2850~2000 (broad), 1760, 1670, 1635, 1575, 1395, 1325, 1265, 1185, 1135, 1080, 1020, 920, 830 and 805 cm^{-1}. After the structure determination by X-ray crystallographic analysis, the signals of the ^1H NMR spectrum measured in deutero-dimethylsulfoxide at 100 MHz (internal TMS reference) were assigned as follows: 7-H δ 10.03 (d, 8 Hz), 4-NH_2 7.68 (br. s), 6-H 6.12 (d, 8 Hz), 3-H 4.97 (s). The ^{13}C NMR signals measured in deutero-dimethylsulfoxide at 25.2 MHz (internal TMS reference) were also assigned by selective proton decoupling and gated decoupling experiments: 2-C δ 168.2, 3-C 81.9, 4-C 158.6, 5-C 159.5, 6-C 102.8 and 7-C 189.2.

The results of the X-ray crystallographic analysis are as follows. A very thin platy crystal of approximate dimensions 0.9×0.15×0.03 mm was grown in ethyl acetate solution and was used for the diffraction study. The X-ray measurements were carried out on a Philips PW1100 diffractometer using CuKα radiation monochromated by a graphite plate. Crystal data are listed in Table 1. Of the total of 1288 reflections within the 2θ range of 6°~156°, 817 (63%) could be measured as above the 2σ(I) level. The structure was solved by direct methods[2] and refined to an R value of 0.037 by least-squares procedures with block-diagonal matrix approximations*. All hydrogen atoms were located on a difference electron-density map and included in the least-squares calculations assuming isotropic thermal vibrations. The molecular structure is illustrated in Fig. 1 with bond lengths and angles. As expected from its chemical structure, the molecule is nearly planar except for the exocyclic amino hydrogen atoms. The deviations

Table 1. Crystal data.

Basidalin, $C_6H_5NO_3$, MW=139.11, Monoclinic $P2_1/a$, a=9.344(5), b=12.671(6), c=5.286(3)Å, β=99.81(5)°, U=616.7Å³, Z=4, D_{calc}=1.499 g/cm³

* A list of atomic parameters was sent to Cambridge Crystallographic Data Centre.

Fig. 1. Molecular structure of basidalin.

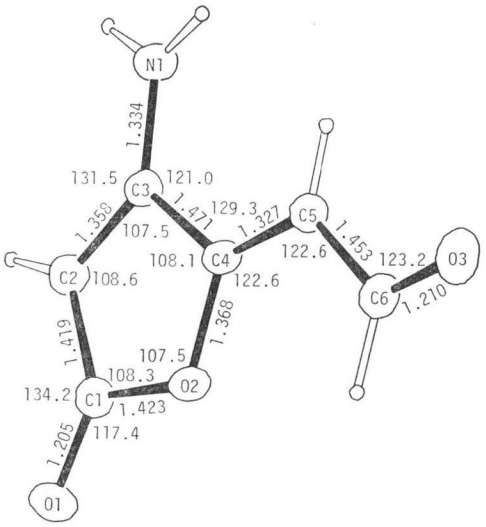

Table 2. Deviations of atoms from the least-squares plane through all the non-hydrogen atoms.

C1	0.009(2)Å	C6	0.017(2)Å	H(C5)	−0.05(1)Å
C2	0.025(2)	N1	0.004(2)	H(C2)	0.08(1)
C3	−0.004(2)	O1	0.003(1)	H(C6)	0.01(1)
C4	−0.021(2)	O2	−0.027(1)	H(N1)	0.05(1)
C5	−0.021(2)	O3	0.016(1)	H′(N1)	−0.15(1)

of atoms from the least-squares plane formed by all the heavier atoms are listed in Table 2. The amino nitrogen N1 lies above the basal plane through the atoms C3, H(N1) and H′(N1) by 0.04(1)Å and thus exhibits a shallow pyramidal configuration. This plane twists out from the five-membered ring plane by about 6°. N1 forms hydrogen bonds by donating H(N1) and H′(N1) to O3 and O1 of the neighboring two molecules with the N---O distances 2.888(3) and 3.342(3)Å and O---N–H angles of 12.7° and 5.9° respectively.

Reduction of basidalin with $NaBH_4$ afforded dihydrobasidalin (**II**), melting at 191~192°C (Fig. 2). Catalytic reduction of basidalin or **II** gave tetrahydrobasidalin (**III**), melting at 144~146°C.

The *E*-isomer of basidalin was isolated, as a minor component, from the mother liquor of the crystallization of basidalin by column chromatography on silica-gel developed with chloroform. It melted at 116~124°C (decomp.). The ^1H NMR spectrum measured in deutero-dioxane at

Fig. 2 Reduction of basidalin.

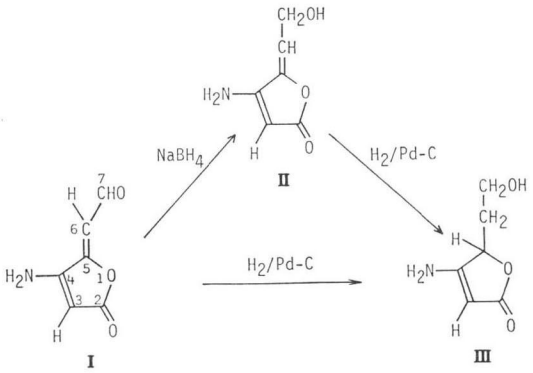

Table 3. Effect of basidalin on macromolecular synthesis and cell growth of cultured L1210 cells.

	IC_{50} (μg/ml)
Cell growth	0.11
RNA synthesis*	0.60
DNA 〃 *	0.40
Protein 〃 *	0.42

* Cell density: 5×10^5 cells/ml in 10% calf serum-RPMI 1640 medium.
Incorporation: ^{14}C-Precursor (thymidine, uridine and leucine), 0.05~0.1 μCi/ml for 60 minutes, 37°C.

Table 4. The effect of basidalin on mouse leukemia L1210.

Dose (μg/mouse/day×10)	T/C×100*
25	191
12.5	151
6.25	125
3.13	105
1.56	125

L1210 cells (10^5) were inoculated, and the first injection of basidalin was made on the day of the inoculation.
Animal: CDF_1 mouse (female); control (n=8), treated (n=4).
* The value means the percent of the survival period of the treated mice to control.

100 MHz (internal TMS reference) showed signals at δ 9.50 (CHO, d, 5 Hz), 7.70 (NH_2, broad s), 6.38 (=C*H*–CHO, m, 5 and 6 Hz) and 5.0 (=CH–, d, 5 Hz). Catalytic reduction of the *E*-isomer gave tetrahydrobasidalin (**III**).

Basidalin showed weak antibacterial activity against the following bacteria on nutrient agar:

Aeromonas salmonicida ATCC14174 (MIC 100 μg/ml) and *Vibrio anguillarum* NCMB6 (100). Other bacteria so far tested were not inhibited at 100 μg/ml.

As shown in Table 3, basidalin inhibited the synthesis of protein, RNA and DNA in cultured L1210 cells; the concentrations of 50% inhibition were 0.4~0.6 μg/ml.

The intraperitoneal administration of basidalin to CDF_1 mice (female, 6~7 weeks old) to which 10^5 cells of mouse leukemia L1210 were inoculated prolonged the survival time (Table 4). The LD_{50} (i.p.) of basidalin in mouse was in the range of 6.25~12.5 mg/kg.

The antibacterial and antitumor activities of basidalin were lost by reduction of the formyl group.

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