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Studies on the Constituents of the Higher Fungi of Korea(XI)

An Antibiotic Component and a Sterol of *Coriolus sanguineus* Fr.

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Abstract ☐ From the carpophores of *Coriolus sanguineus* Fr., a bright-red, wood-rotting fungus, which were collected at Suwon, an antibiotic component was isolated as red crystal and identified as cinnabarin by UV and IR spectra. The antibiotic activities against five bacteria and three fungi were determined. It showed considerably high activity against *Bacillus subtilis* ATCC 6633 and *Bacillus maceraus* and also against all the three fungi tested. And from this fungus a sterol was isolated and identified as ergosterol by cochromatographing with four authentic sterols.

Keyphrases ☐ *Coriolus sanguineus*–cinnabarin–ergosterol. GLC–TLC–cylinder plate method against *Bacillus, Staphylococcus, Sarcina* and *Escherichia* spp. and against *Trametes, Lentinus* and *Candida* spp.

As the results of current investigations on the constituents of higher fungi many useful constituents such as antibiotics and antineoplastic agents were found. The authors have been conducting studies on the components of Korean higher fungi.[1-10] However, no report of examinations for antibiotic constituents of the fungi has been made except that of Yoon in 1959.[11] In Yoon's report, the antibiotic constituents were not isolated, but only the various extracts of 81 species of mushrooms were screened for antibiotic activity. Therefore, we undertook this investigation on constituents of *Coriolus sanguineus* Fr., a bright red, wood-rotting fungus, and found that it contained an antibiotic constituent and a sterol.

EXPERIMENTAL METHODS

Fungal Material[12,13]

Carpophores of *Coriolus sanguineus* Fr., a member of Polyporaceae, were collected in

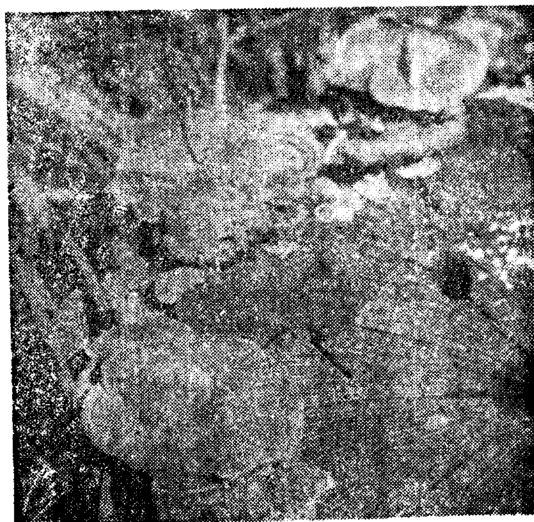


Fig. 1: Carpophores of *Coriolus sanguineus* Fr.

the Arboretum of College of Agriculture, Seoul National University at Suwon during the period from August to September, 1978. The mushrooms were dried in open shadow for four to five hours and then in an oven at 50°C for five hours. Some of the dried carpophores are preserved as voucher specimens at the Department of Microbial Chemistry, College of Pharmacy, Seoul National University. The observed characteristics of *Coriolus sanguineus* are as follows: pileus 2–8cm in diameter, more or less fan-shaped to semicircular shaped, characteristic vermilion-red to orange-red in color, with concentric figure and somewhat vanished appearance when old. Flesh tough, corky, pale red in color, 1–3mm in length, 5–7 pores/mm, absent near the margin (3–10mm from the margin), almost of the pore side is eaten by insect when old and only the flesh is left. Stipe lateral or sometimes absent, 2–5mm in length and 2–10mm in thickness. Spores white, smooth, somewhat bended ellipsoid, 7–8 x 2.5–3μ, almost all the spores are shot soon after the carpophores are in mature.

Instruments

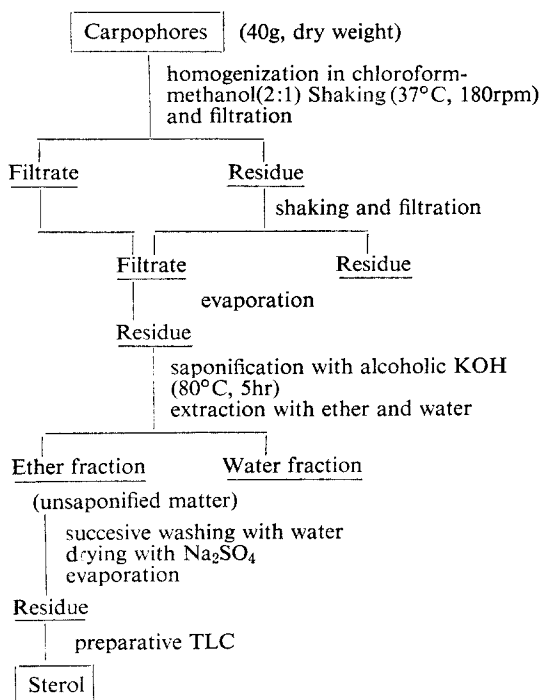
GLC was performed using Shimadzu Gas Chromatograph Model GC-4BM under the condition in Table I. UV spectra were obtained using Hitachi Recording Spectrophotometer, and IR spectrum using Beckmann IR 20A.

Table I: Measurement condition of GLC

Column	3% OV-17 (80–100 mesh shimalite)
	3mm $\phi\chi$ 2m boronsilicate glass column
Temperature	Injection port : 250°C
	Column : 227°C (constant)
	Detector : 300°C
Flow Rate	N_2 : 40ml/min
	H_2 : 60ml/min (0.8kg/cm^2)
	Air : 880ml/min (1.2kg/cm^2)
Attenuation	10^2MΩ
	8 × 0.01–32 × 0.01 V

Isolation of Sterol

Forty grams of the dried carpophore were homogenized with 1500 ml of chloroform-methanol(2:1) in Waring blendor. The homogenized mixture was divided into six 500 ml Erlenmeyer flasks and extraction was carried out for 24 hours using Gallenkamp orbital incubator, setting incubation temperature at 37°C and shaker at 180 rpm. After filtration, the residue was extracted again for 48 hours with 1200 ml of the same solvent under the same condition, and then filtered. From the filtrate, dried residue was obtained after evaporation of the solvent with Gallenkamp thin film evaporator at low temperature (50–60°C). Saponification of the residue was proceeded with 350 ml of alcoholic KOH on a water bath (80–85°C) for five hours. To the reaction mixture were added 250 ml of ether and 250 ml of distilled water. From this, the water layer was separated. The ether layer containing unsaponified matter was washed with water three times and then dried with anhydrous sodium sulfate. Pale yellow residue was obtained from the ether layer after evaporation. This residue was positive to Liebermann-Bürchard reaction. The Rf values of the constituents on TLC were obtained. Then preparative TLC was performed and the constituents were detected as dull bands when methanol was sprayed. The sterol, extracted with ether from silica gel after preparative TLC, was applied to GLC with four authentic

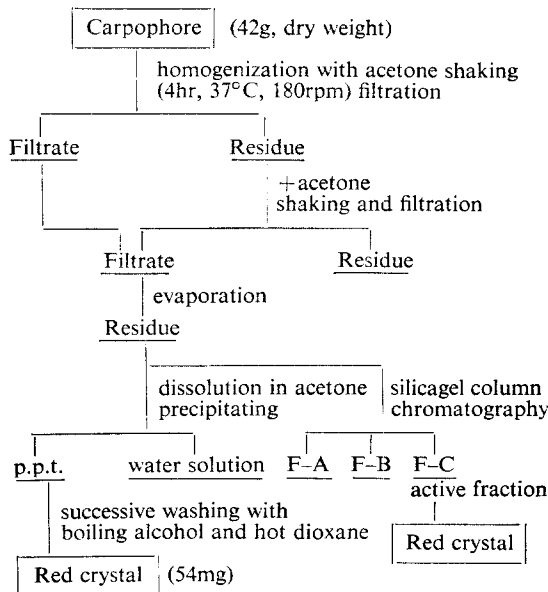


Scheme I: Isolation of sterol

sterols.

Isolation of the Antibiotic Component, Cinnabarin

Forty-two grams of the dried carpophore were homogenized with 1200 ml of acetone in Waring blendor. The homogenized mixture was divided into five 500 ml Erlenmeyer flasks and extraction was carried out for four hours using Gallenkamp orbital incubator by setting the temperature at 37°C and the shaker at 180 rpm. After the filtration the residue was extracted again for 24 hours with 1000 ml of acetone under the same condition and then filtered. From the filtrate, the crude extract consisting of dried residue and some thick oily substances was obtained after evaporation of the solvent. Some of the crude extract was loaded on a column (2.2 × 30cm) of silica gel (Kiegel gel 60. E. Merck Co.) in chloroform-methanol(100 : 0.3), and eluted with the same solvent. Two yellow colored bands were eluted, collected and named F-A (the first fraction) and F-B (the second fraction), respectively. The third fraction, F-C, was dissoluted with acetone from the red colored silica gel of the upper part of the column. When each residue from F-A, F-B, and F-C was tested for its antibiotic activity by sylinder-plate method using *Bacillus subtilis* ATCC 6633 strain as a test organism, only the fraction F-C was found to be active(Table II). The antibiotic component of F-C was obtained as red needle crystals (M.P. 280°, decomposition) when crystalized from pyridine solution. The rest of the crude extract was dissolved with small volumes of acetone (3 × 10ml). To the acetone solution, 60 ml of distilled water were added. After the evaporation of acetone, dark red precipitate was obtained



Scheme II: Isolation of the antibiotic component

Table II: The antibiotic activity of each fraction

Fraction	Concentration of test solution (μg/ml)	Result
F-A	100	—
F-B	100	—
F-C	20	+

from water on glass filter. From this precipitate red needle crystals were obtained after successive washing with boiling alcohol (4 × 10ml) and hot dioxane (4 × 5ml). This crystaline component and that from silica gel column were found to be the same compound by comparing UV and IR spectra.

Antibiotic Activities

To determine antibacterial activities, five bacteria, *Bacillus subtlis* ATCC 6633, *Bacillus maceraus, Staphylococcus albus, Sarcina lutea,* and *Escherichia coli* were used, and to determine antifungal activities, three fungi, *Trametes versicolor, Lentinus edodes* and *Candida albicans* were used. The incubation temperatures were 37°C for bacteria and 25°C for fungi. For preliminary test of antibacterial activities, cylinder plate method was used. The medium was nutrient agar (Difco nutrient broth 8.0g, Meer Corp. Agar Lot No. 83279 15 g per liter). To determine minimum inhibitory concentration (=MIC), nutrient broth medium (Difco nutrient broth 8.0 g/1) was used. For inoculum one loop of each bacterium was diluted in 10 ml of nutrient broth and incubated at 37°C for three hours in case of *E. coli, S. albus* and *S. lutea* and for twenty-four hours in case of *B. subtilis* and *B. maceraus*. After incubation 0.1 ml was used as inoculum in each case. After inoculation the result was observed after 24 hours in case of *E. coli, S. lutea, S. albus* and after 48 hours in case of *B. subtilis* and *B. maceraus*. As for preliminary test of antifungal activity, effects of the test compound on the growth of *Candida albicans* and *T. versicolor* on agar plate containing 20 μg of test compound /ml and on control agar plate after incubation of seven days were observed. In case of *L. edodes*, the fungus was grown on PDA medium (potato 200 g, dextrose 20 g, agar 15 g per liter) containing 10 and 30 μg of test compound /ml and on control PDA plate at 25°C for ten days, and then the diameters of the fungal colonies were measured.

RESULTS AND DISCUSSION

1) TLC of sterol-containing residue: When the unsaponified matter was subjected to TLC with a solvent system of benzene-acetone (4:1) and sprayed with c-H_2SO_4, eight spots appeared. The Rf values and colors of the spots together with authentic cholesterol are shown in Table III.

2) Preparative TLC: When methanol was sprayed on the developed preparative TLC

Table III: Rf value and color of each spot

Spot	Rf value	Color
A	0.85	blue
B	0.65	green-orange
Cholesterol	0.66	orange red
C	0.50	
D	0.41	blue
E	0.33	blue
F	0.25	
G	0.12	
H	0.11	
I	0.08	

Table IV: Rf value of each band and reaction to L–B test (preparative TLC)

Band	Rf value (approximate)	L–B test
B–1 (sterol)	0.7–0.5	+
B–2	0.4–0.1	—
B–3	0.1	—

Table V: Retention times of sterols

Sterol	Retention time (min)	
ergosterol	23.5	
stigmasterol	24.8	
sitosterol	22.8	28.3
cholesterol	17.5	
sample	23.5	
sample + ergosterol	23.5	

plate, three distinguishable bands appeared (Table IV). Constituents of each band were extracted with ether and tested for sterol using L–B reaction. Of these, only B–1 was found to be positive.

3) GLC of Sterols: The sterol sample isolated from *C. sanguineus* and four authentic sterols were subjected to GLC. The sample had retention time of 23.5 min. This value, when compared with those of authentic sterols (Table V), is the same as that of authentic ergosterol. Moreover the mixture containing approximately same quantities of sample and authentic ergosterol showed one single peak (Fig II) and the retention time was also 23.5 min. These results confirm that *C. sanguineus* has ergosterol as its sterol constituent.

4) Physicochemical Properties of Antibiotic Component: From 42 g of the dried carpophores, 54 mg of a red crystalline component was obtained. Its crystal from pyridine was needle (Fig III) and sometimes starlike when gathered. And the

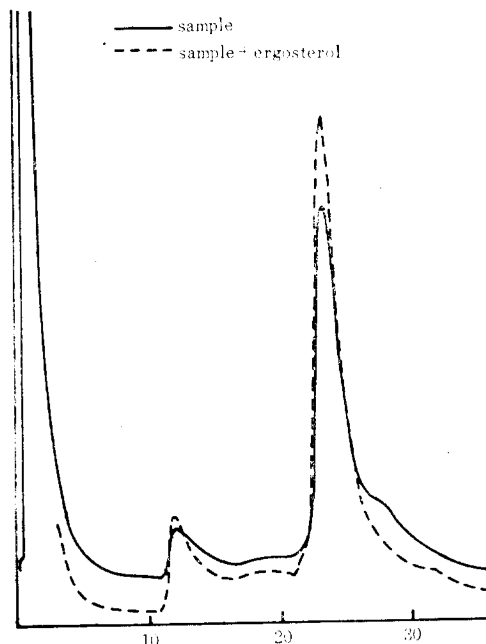


Fig. 2: Gas chromatogram of sample and a mixture of standard ergosterol and sample.

Fig. 3: A microphotograph of the red needle crystal of cinnabarin from pyridine solution

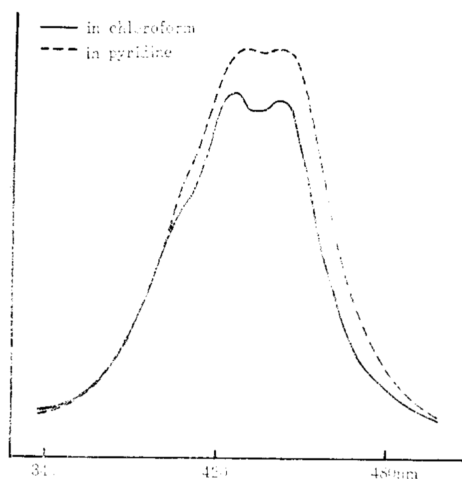


Fig. 4: UV spectra of the crystalline component, cinnabarin

crystal from acetone and chloroform was somewhat irregular plate. It decomposed above 280°C to yield a blackish brown compound. Table VI shows Rf values on TLC with two solvent systems. Its UV spectra (Fig IV) in chloroform and in pyridine show maxima at 434, 457 nm and at 439, 457 nm, respectively (Table VII). Figure V shows its IR spectrum, which is almost completely consistent with that of cinnabarin reported by Cavill.[17] All these physicochemical properties of the red crystalline component isolated from Korean strain of *C. sanguineus* showed that it is cinnabarin.

5) Antibiotic Activities of Cinnabarin: Table VIII shows the antibiotic spectrum of

Table VI: Chromatographic behaviour of crystalline component in two solvent systems

Solvent system	Rf value
acetone-dioxane 2:1	0.74
acetone-chloroform 2:1	0.58

Table VII: UV data of crystalline component

Solvent	λ max (nm)	
chloroform	434	458
pyridine	439	457

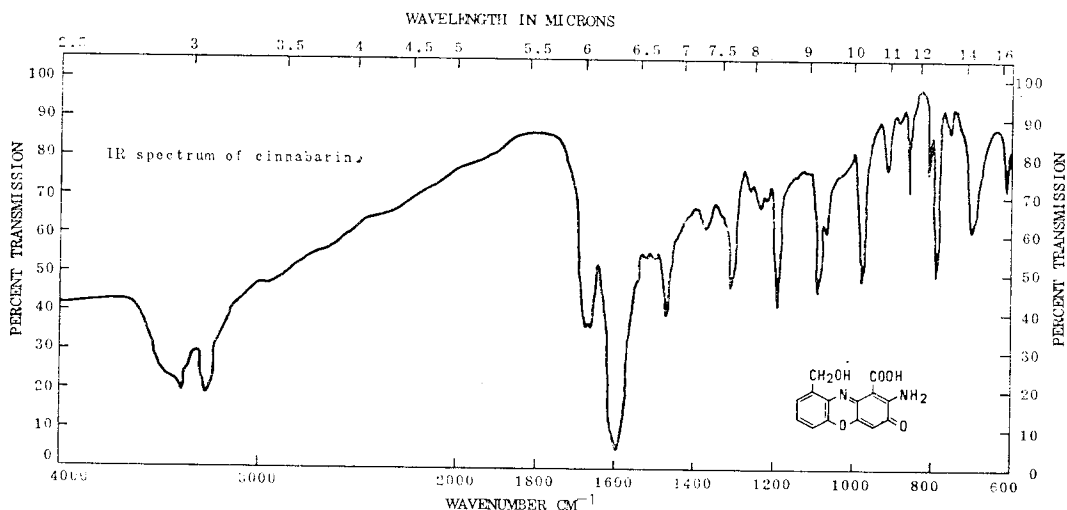


Fig. 5: IR spectrum of cinnabarin in KBr

Table VIII: Antibiotic activities of cinnabarin

Test organism	Preliminary test	MIC (μg/ml)
Bacteria:		
B. subtilis	++	6.3–20
E. coli	—	20–100
B. maceraus	+	<6.3
S. albus	+	50
S. lutea	—	>50
Fungi:		
C. albicans	+	
L. edodes	+	
T. versicolor	+	

cinnabarin. In preliminary tests, cinnabarin was found to be active against *B. subtilis, B. maceraus* and *S. albus*, that is, three of five bacterial strains and also to be active against three fungi, *C. albicans, L. edodes* and *T. versicolor*. The MIC (ug/ml) was 6.3–20 against *B. subtilis*, less than 6.3 against *B. maceraus*, 25–100 against *E. coli*, 50 against *S. albus*, and more than 50 against *S. lutea*. It showed considerably high activity against *B. subtilis* and *B. maceraus*. In case of *L. edodes* the diameter of fully grown colony on control PDA plate was 5.5 cm and that on PDA plate containing 30 μg of cinnabarin was 2.8 cm. While that on PDA plate containing 8 μg of authentic gentamicin was 4.3 cm.

CONCLUSION

From the carpophores of *Coriolus sanguineus* Fr. (*Gan-beoseot*) which was collected in Korea, ergosterol and an antibiotic component cinnabarin were isolated and identified. The antibiotic activity of cinnabarin was found to be considerably high to *Bacillus subtilis* and *Bacillus maceraus* and also to inhibit the growth of three fungi, *Candida albicans, Lentinus edodes* and *Trametes versicolor*.

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