# DIKETOCORIOLIN B, AN ACTIVE DERIVATIVE OF CORIOLIN B PRODUCED BY *CORIOLUS CONSORS*

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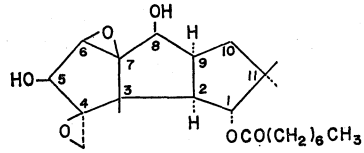
*Coriolus consors* was cultured under aeration, and from the mycelial cake coriolin B, a sesquiterpene compound, was extracted and purified. Coriolin B showed no antimicrobial activity. However diketocoriolin B which was obtained by the oxidation of coriolin B showed antibacterial and antitumor activities. Diketocoriolin B prolonged the survival period of mice inoculated intraperitoneally with mouse leukemia L-1210 cells or EHRLICH carcinoma cells.

In a previous paper we reported isolation of an antibiotic named coriolin from *Coriolus consors*[1]. Coriolin is found mainly in the cultured fluid, and from the mycelial cake a different but structurally related compound is obtained. This compound, named coriolin B, has no antibacterial activity, but gives a derivative, diketocoriolin B, which inhibits the growth of microorganisms and mammalian cells. As reported in another paper by KUNIMOTO *et al.*[2], diketocoriolin B inhibits Na-K-ATPase. In this paper we describe the isolation of coriolin B and the preparation and biological activities of diketocoriolin B.

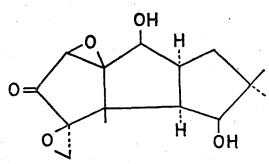
As described in a previous paper[1], *Coriolus consors* was cultivated in a wood dust medium at 27°C for 10~14 days. The resulting surface mycelium was then shaken-cultured or cultured in a fermenter under aeration. Coriolin B was extracted from the mycelial cake and crystallized by the following process: The cake was extracted with acetone and the extract was concentrated and dried under vacuum. The residue was extracted with ethyl acetate and the extract was concentrated and dried. Coriolin B in the residue was dissolved in a small amount of acetone and crystallized by addition of *n*-hexane.

Coriolin B (I), thin plate crystals, m.p. 215~216°C, is soluble in methanol, acetone, ethyl acetate, ether, chloroform and benzene, and insoluble in water, carbon tetrachloride and *n*-hexane. The infrared spectrum is shown in Fig. 1. The molecular weight of 408 is shown by the mass analysis and the formula $C_{23}H_{36}O_6$ is calculated from the result of the elemental analysis (found: C 67.36, H 8.88). The structure of coriolin B reported in a previous paper[3] was revised as follows[4].

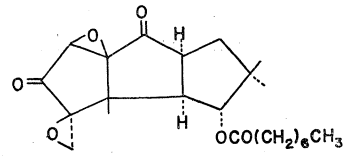



I

Coriolin B was oxidized by chromic anhydride in acetic acid at 20~30°C for 16~30 hours. The reaction mixture was then diluted with distilled water, and diketocoriolin B was extracted with ethyl acetate and purified by the following process: The ethyl acetate extract was washed with 0.1 N NaOH and distilled water, dehydrated with anhydrous sodium sulfate, and concentrated under vacuum, yielding diketocoriolin B crystals. Diketocoriolin B crystallizes as needles, m.p. 147~148°C. It is soluble in methanol, acetone, ether, ethyl acetate, chloroform and benzene, and insoluble in water, carbon tetrachloride, and *n*-hexane. It shows no maximum in the ultraviolet spectrum except the end absorption. Its infrared spectrum is shown in Fig. 2. The molecular weight shown by the mass analysis is 404. The formula, $C_{23}H_{32}O_6$, is calculated from the result of elemental analysis (found: C 69.02, H 8.08). As reported in another paper[4], the structures of coriolin (II) and diketocoriolin B (III) are as follows:




II




III

Diketocoriolin B shows an activity similar to that of coriolin against microorganisms. The antibacterial spectrum was examined by the agar streak method using meat extract-peptone medium and is shown in Table 1. Diketocoriolin B is not soluble in water, and therefore, the crystals were ground, and suspended in physiological saline for injection into animals. The $LD_{50}$ by intraperitoneal injection into mice was 40 mg/kg, and the $LD_{50}$ by subcutaneous injection into mice was larger than 80 mg/kg. When diketocoriolin B was injected intraperitoneally daily for 10 days, 20 mg/kg/day caused death of all mice, but 10 mg/kg/day or 5 mg/kg/day caused no death. Thus, a total dose of 100 mg/kg given by daily injection did not cause death of mice and was larger than the acute $LD_{50}$. The daily subcutaneous injection of 20 mg/kg did not cause death of mice. Toxicity shown

Table 1. Antibacterial spectra of coriolin and diketocoriolin B

| Test organism* | MIC (mcg/ml) | |
|---|---|---|
| | Coriolin | Diketo-coriolin B |
| *Staphylococcus aureus* FDA 209P | 12.5 | 12.5 |
| 〃 Terajima | 25.0 | 12.5 |
| 〃 Smith | 12.5 | 12.5 |
| *Micrococcus flavus* FDA 16 | 12.5 | 12.5 |
| *Bacillus anthracis* | 12.5 | 12.5 |
| 〃 *subtilis* NRRL B-558 | 12.5 | 25.0 |
| *Escherichia coli* NIHJ | 100.0 | >100.0 |
| *Salmonella typhosa* | 100.0 | >100.0 |
| 〃 *enteritis* | 100.0 | 100.0 |
| *Shigella flexneri* 1a (Ew 8) | 50.0 | >100.0 |
| *Klebsiella pneumoniae* PCI 602 | 50.0 | >100.0 |
| *Pseudomonas aeruginosa* $A_3$ | >100.0 | >100.0 |

* The compounds were dissolved in a medium containing 1.0% peptone, 0.5% meat extract, and 0.3% NaCl, the organisms were steaked on the medium containing the compounds and the growth was examined after the incubation at 37°C for 42 hours.

Table 2. Effect of coriolin and diketocoriolin B on the growth of YOSHIDA sarcoma cells in tissue culture

| Concentration of coriolins (mcg/ml) | Inhibition (%) | |
|---|---|---|
| | Coriolin | Diketocoriolin B |
| 10 | 85.3 | 81.5 |
| 5 | 61.6 | 86.2 |
| 2.5 | 19.7 | 86.8 |
| 1.25 | 10.6 | 72.8 |
| 0.63 | 11.5 | 41.5 |
| 0.31 | — | 17.4 |

$ID_{50}$: Coriolin 4 mcg/ml, Diketocoriolin B 0.75 mcg/ml

Fig. 1. Infrared absorption spectrum of coriolin B (KBr)

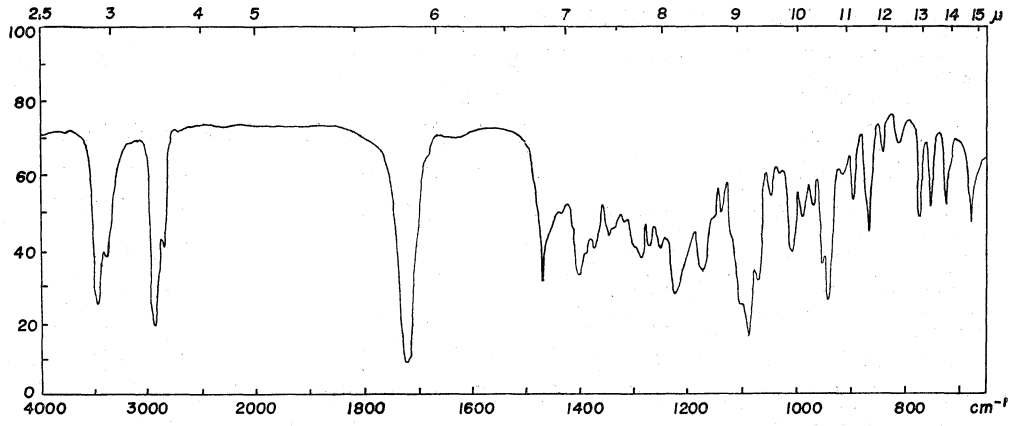



Fig. 2. Infrared absorption spectrum of diketocoriolin B (KBr)

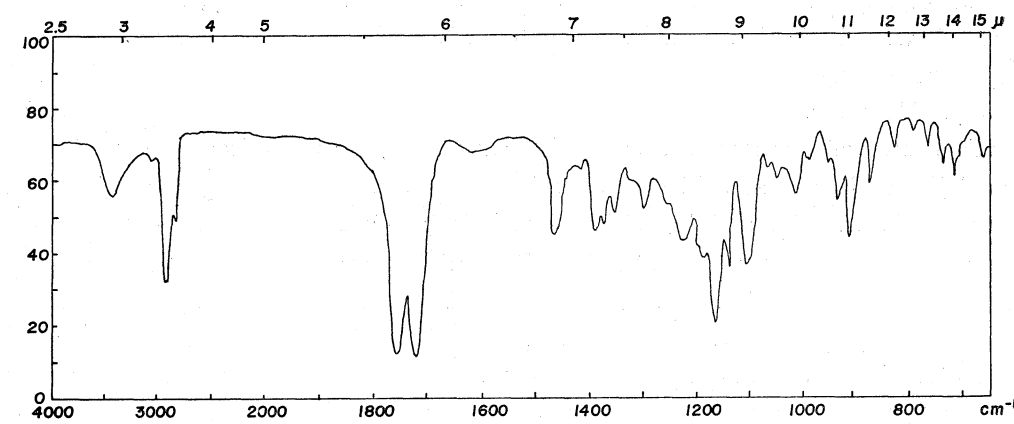



Table 3. Activity of coriolin and diketocoriolin B prolonging the survival period of mice inoculated with L-1210 cells

| Dose mcg/mouse/day×10 | anti-L-1210 (T/C×100) | |
|---|---|---|
| | Coriolin | Diketocoriolin B |
| 100 | 140* | 173* |
| 50 | 139.8 | 162 |
| 25 | 123.3 | 162 |
| 12.5 | 112.9 | 162 |
| 6.25 | 102.1 | 137 |
| 3.12 | — | 118 |

One hundred thousand cells were inoculated, and the first injection of the compounds was made on the day of the inoculation.
* The value means the percent of the survival period of the treated mice to the control.

Table 4. The effect of diketocoriolin B on EHRLICH ascites carcinoma

| Dose mg/kg/mouse/day (for 10 days) | Mean days of the survived after the inoculation of the tumor cells | % of the survival days to the control |
|---|---|---|
| 0 | 18 (14～23) | 100 |
| 0.19 | 16.2 (11～18) | 90 |
| 0.39 | 21.4 (12～50) | 119 |
| 0.78 | 34.5 (15～50) | 192 |
| 1.56 | 41.5 (33～50) | 231 |
| 3.12 | 39.6 (29～50) | 220 |
| 6.25 | 43.5 (24～50) | 242 |
| 12.5 | 46.3 (39～50) | 257 |
| 25.0 | 5 ( 4～ 6) | 28 |

Two million EHRLICH carcinoma cells were intraperitoneally inoculated, and survival period was observed up to 50 days.

by daily oral administration suggested its possible absorption. Thus, daily oral administration of 20 mg/kg for 10 days caused death of 50 % of the mice, and no death was observed in the case of 10 mg/kg/day.

Diketocoriolin B inhibits growth of YOSHIDA rat sarcoma cells. The growth of the cells was determined by the content of nucleic acid by the method described by HORI *et al.*[5] The results are shown in Table 2. Diketocoriolin B and coriolin showed similar antibacterial activities, but the former was more active than the latter against YOSHIDA rat sarcoma cells *in vitro* and also against mouse leukemia L-1210 and EHRLICH ascites carcinoma as shown in Tables 3 and 4. Daily intraperitoneal injection of 12.5~100 mcg/mouse/day of diketocoriolin B prolonged the survival period of mice inoculated with L-1210 cells, this period being 162~173 % of the control. With mice bearing EHRLICH ascites carcinoma, daily intraperitoneal injection of 0.78~12.5 mg/kg/day caused prolongation of the survival period, but the daily intraperitoneal injection of 25 mg/kg was toxic, causing death of all mice on the 4~6 th day.

Among sesquiterpene fungus products, hirsutic acid C has a skeleton similar to that of coriolins[6]. Hirsutic acid C has no antimicrobial activity, but hirsutic acid N, which was obtained by treatment of hirsutic acid C with the homogenate of the fungus producing these compounds, has been reported to have weak antibacterial activity. However, the present paper describes the first observation that members of this group of compounds show antitumor activity. Comparison of the structures of coriolin, coriolin B and diketocoriolin B suggest that the 5-keto group is important for this activity. The activity of 5-ketocoriolin B will be reported in another paper.

As reported by KUNIMOTO *et al.*[2], diketocoriolin B causes efflux of intracellular amino acids and potassium ion and inhibits influx of amino acids and potassium ion into cells. The mechanism of the inhibition of Na-K-ATPase was analyzed and it was shown that the inhibition was reversed by phosphatidyl serine. Thus, from the point of view of its mode of action diketocoriolin B is an interesting antitumor compound. This unique mechanism of action suggests that it would be worthwhile to study of this compound in combination with other antitumor compounds.

## Experimental

Would dust was boiled for 1 hour and, after separation from the water, was washed with water and dried. Three g of the dust thus prepared were placed in 15 ml of a medium containing 2.0 % glucose and 0.5 % dried yeast in 100-ml flask. It was sterilized at 120°C for 20 minutes. This wood dust medium was inoculated with the mycelium of *Coriolus consors* which had been grown on an agar medium and the culture grown at 27°C for 10 days. Fifty ml of the medium described above was added to the growth in the wood dust medium in a flask and shaken, and 10 ml of the suspension thus prepared was used to inoculate 125 ml of the production medium in a 500-ml flask. The medium used for production of coriolin B was as follows: 5.0 % glucose, 0.2 % peptone, 0.2 % $KH_2PO_4$, 0.1 % $MgSO_4 \cdot 7H_2O$, 1.6 % $CaCO_3$, 0.5 % dried yeast, sterilized at 120°C for 20 minutes.

The shaken culture was kept at 27°C for 9 days, and the contents of the flasks were combined and filtered. From 3 liters of the culture filtrate, about 100 g of solids were obtained. The solids were extracted with 300 ml of acetone, the extract was concentrated

under vacuum, and the residue was extracted twice with 100 ml of ethyl acetate. The ethyl acetate extract thus obtained was concentrated under vacuum and dried, yielding a brown residue of 4 g. It was dissolved in a small amount of acetone and *n*-hexane was added until the solution became turbid. After storage in the cold room, crystals of coriolin B (1.5 g) were collected by filtration.

In another experiment, 3 liters of the mycelial suspension which was obtained from the growth in the wood dust medium was used to inoculate 40 liters of the production medium described above, supplemented with 0.01 % silicone oil in a 70-liter fermenter. Cultivation was carried out at 27°C with aeration of 20 liters of sterile air per minute and 200 r.p.m. stirring. After 10 days of fermentation, solids weighing 1.2 kg were obtained by filtration of the culture. From these solids, 18 g of coriolin B crystals were obtained by the method described above.

Preparation of diketocoriolin B: 1.5 g of coriolin B was dissolved in 65 ml of acetic acid, and 650 mg of anhydrous chromic acid was added. It was kept at 25°C for 24 hours. Thereafter, 600 ml of distilled water was added, and the mixture was extracted with 300 ml of ethyl acetate three times. The ethyl acetate extracts were combined and washed with 300 ml of 0.1 N NaOH and 300 ml of distilled water successively. It was dehydrated with anhydrous sodium sulfate, concentrated, and dried. The white powder thus obtained was dissolved in a small amount of ether, and *n*-hexane was added until the solution became turbid. It was kept in cold room, and 700 mg of white needle crystals of diketocoriolin B was obtained.

## Reference

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