

STUDIES ON RUBESCENSINE B: A NEW ANTITUMOR PRINCIPLE ISOLATED FROM *DONGLINCAO*

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A Chinese herb called *Donglincao* was identified as *Rabdosia rubescens* (Hemsl) Hara (Labiatae). It is widely distributed in the Yellow River valley and used in folk medicine for the treatment of esophageal carcinoma. Experiments showed that the water and alcoholic extracts of this herb exhibited cytotoxic, antitumor and antibacterial activities^[1,2]. Clinically, it is also effective in the treatment of esophago-cardial carcinoma, primary liver carcinoma and breast cancer^[3,4]. For this reason, we studied the chemical constituents of this plant and isolated two antitumor diterpenoids, rubescensine A and B, from the ether extract of its dry leaves. Since the chemical structure and pharmacological properties of rubescensine A were already reported^[5], we present in this paper the isolation, identification and pharmacological activities of rubescensine B.

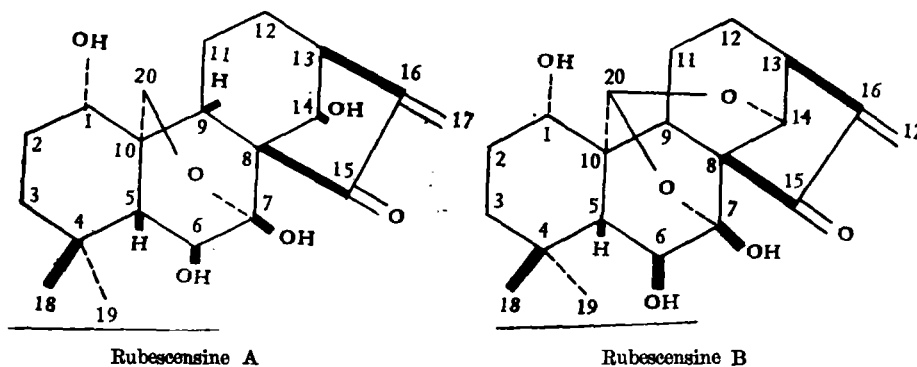
I. ISOLATION

The dry leaves of this plant had been put in ether before the crude extract was dissolved in methanol, treated with activated charcoal, concentrated and allowed to stand at room temperature overnight. Then it was filtrated. The filtrates chromatographed on silica gel column and eluted with chloroform-acetone mixture would yield rubescensine A (0.49%) and rubescensine B (0.067%).

II. IDENTIFICATION

When recrystallized from methanol, rubescensine B looks like yellowish platelets, its properties are as follows: m.p. 238—241°C, $[\alpha]_D^{25}$ —107 (C=0.12, pyridine); $C_{30}H_{46}O_6$ (Found: C 65.91%, H 7.39%, M^+ 362.177; but $C_{30}H_{46}O_6$ requires C 66.28%, H 7.23%, M 362.173); UV λ_{max}^{EtOH} 230 nm (log 3.71), IR ν_{max}^{KBr} cm^{-1} : 3350(—OH), 1730($>C=O$), 1640, 850($>C=CH_2$), 1080, 1070, 1060; NMR(C_6D_6N , TMS as internal standard, WH-90) σ : 6.21 and 5.31(each 1 H, br S, $C_{17}-H_2$), 5.94 (1 H, br S, $C_{20}-H$), 5.06 (1 H, d,

$J=6$ Hz, $C_{14}-H$), 4.24 (1 H, br s, C_6-H), 3.82 (1 H, t, $J=8$ Hz, C_1-H), 3.24—3.1 (1 H, m, $C_{15}-H$), 1.60 (1 H, br s, C_3-H), 1.09 and 0.95 (each 3 H, s, C_4-Me_3). Rubescensine B acetylated with acetic anhydride and pyridine by usual method gave a mixture of 1, 6-diacetate and 1, 6, 7-triacetate. The latter was the main product, which might be isolated by column chromatography on silica gel and recrystallized from methanol to give a colorless prism, m.p. 235—237°C. IR ν_{max}^{KBr} cm^{-1} : 1770, 1740 1730, 1640, 1230—1240. All the data mentioned above were identical with those of *ponicidin*^[6].



III. PHARMACOLOGICAL STUDIES

1. Cytotoxic Actions

Ehrlich ascitic carcinoma cells were removed under aseptic condition from ECA-bearing mice 7—9 days after implantation. Tumor cells suspending in nutrient medium kept a concentration of 10^6 cells/ml. Then tested drugs were put into the cell suspension which had been already incubated at 37°C for 24 hr. A drop of the suspension was placed on a slide and a bit of 0.5% trypan blue was added in order to examine the staining and morphologic changes of the cells microscopically. The tumor cells stained blue were killed, while others not stained alive. The blue-staining rate was thus calculated. Our experiments showed that the blue-staining rate for rubescensine A at the concentration of 5, 2 and 1 mmg/ml was 97%, 85% and 35% and that for rubescensine B at the concentration of 5, 1, 0.5 and 0.2 mmg/ml was 93%, 83%, 56% and 9% respectively.

2. Therapeutic Effect on Animal Tumors

The effect of rubescensine B on Ehrlich carcinoma (ECA), sarcoma 180 (S_{180}), hepatoma (HCA), ascitic lymphoma (L_1), reticulo-cell sarcoma (ARS) and sarcoma 37 (S_{37}) in mice was investigated. One day after five millions of tumor cells were inoculated into each mouse, the drugs were given intraperitoneally once daily for 7—10 days. The effectiveness was justified by the prolongation of survival time in mice with ascitic tumors and the inhibition of tumor weight in mice with solid tumors. For these animals inoculated with ECA, HCA, S_{180} and L_1 intraperitoneally, rubescensine B at the dose of 20 mg/kg/day markedly prolonged the survival time in the tumor-bearing mice (Table 1). For the solid forms of ARS, S_{37} and HCA, rubescensine B at the dose

Table 1
Effect of Rubescensine B on the Ascitic Tumors

| Group | Dose (mg/kg) | Tumor | No. of Survivals | M. S. D ^{a)} (days) | I. L. S. ^{b)} (%) | P value |
|-------------|--------------|------------------|------------------|------------------------------|----------------------------|---------|
| Control | | ECA | 0/10 | 17.2 | | |
| Rubescen. B | 10 | ECA | 3/10 | >34.7 | >102.7 | <0.01 |
| Control | | ECA | 0/10 | 18.4 | | |
| Rubescen. B | 20 | ECA | 1/8 | >42.4 | >129.1 | <0.01 |
| Control | | S ₁₈₀ | 0/10 | 12.0 | | |
| Rubescen. B | 20 | S ₁₈₀ | 2/10 | >39.9 | >232.5 | <0.001 |
| Control | | S ₁₈₀ | 0/10 | 14.8 | | |
| Rubescen. B | 10 | S ₁₈₀ | 0/10 | 18.3 | 23.6 | >0.05 |
| Control | | ECA | 0/10 | 17.7 | | |
| Rubescen. B | 20 | HCA | 1/9 | >29.2 | >64.5 | <0.01 |
| Control | | L ₁ | 0/10 | 18.0 | | |
| Rubescen. B | 20 | L ₁ | 3/10 | >42.5 | >136.1 | <0.01 |

a) M. S. D.—mean survival days; b) I. L. S.—increase in life span.

of 20 mg/kg injected intraperitoneally once daily for 10 days inhibited 58.6%, 29.9% and 39.2% of the tumors respectively. By intratumorous injection, rubescensine B at the dose of 10 mg/kg once every other day for 5 times was able to inhibit 58.6% of the tumor.

3. Toxicity

Experiments revealed that the acute LD₅₀ value of rubescensine B injected intraperitoneally was 45.1 ± 6.7 mg/kg in mice.

Subacute toxicity test was also carried out in rats. Rubescensine B at the dose of 10 and 20 mg/kg injected intraperitoneally once daily for 10 days. No changes in body weight, blood cell counts, liver and kidney functions were noticed. Two weeks after stopping the drug administration, animals were sacrificed in order to examine the pathological manifestations. But there were no significant pathological findings except a slight hepatic and renal congestion.

4. Influence of Rubescensine B on Immunologic Functions in Mice

(1) *Influence on hemolysin formation.* Sheep red blood cells (sRBC) had been washed 3 times with 0.85% NaCl prior to intraperitoneal injection with 0.5 ml of their 10% (v/v) suspension in male CFW mice. Animals in both groups were bled and sera collected. By means of hemolysin assay, the function of humoral-mediated immunity was monitored. The maximal hemolytic titer was considered as a criterion of this assay. In control group it was 1:256, while in group of rubescensine B injected intraperitoneally at the dose of 10 and 20 mg/kg/day for four days it increased to 1:512. Evidently rubescensine B had some stimulant effect on the humoral-mediated immunity.

(2) *Influence on graft versus host reaction*^[5,7]. F₁ hybrid mice (CFW♂ × 615

mice ♀) were used for this experiment. Rubescensine B at the dose of 20 mg/kg was injected intraperitoneally in male CFW mice for 5 successive days. Parental spleen cells taken from the untreated as well as treated mice were used to make a suspension of 10^8 cells/ml, 0.1 ml of which was given intraperitoneally to 10-day old F_1 hybrid mice, being killed 8 days later for the measurement of spleen index. It was considered effective in enhancing the cellular-mediated immunity if the agent caused the value of spleen index in the treated animals much higher than that of the control ones, and *vice versa*. The spleen index of the control group was 1.524 and 1.375 while that of the rubescensine B group 1.707 and 1.84 (Table 2).

Table 2
Effect of Rubescensine B on the Graft Versus Host Reaction

| Group | Dose (mg/kg/d) | No. of Animals | A. B. W. ^{a)} (g) | A. S. W. ^{b)} (g) | Spleen (mg) | Spleen index | P value |
|-------------|----------------|----------------|----------------------------|----------------------------|-----------------|--------------|---------|
| | | | | | Body wt. (100g) | | |
| Normal | | 8 | 7.94 | 26.22 | 327.5 | | |
| Untreated | | 6 | 8.81 | 44.0 | 499.4 | 1.524 | |
| Rubescen. B | 20 × 5 ip | 6 | 8.62 | 48.2 | 559.1 | 1.707 | >0.05 |
| Normal | | 7 | 7.04 | 21.0 | 398.2 | | |
| Untreated | | 6 | 8.0 | 32.8 | 410.0 | 1.375 | |
| Rubescen. B | 20 × 5 ip | 7 | 9.9 | 53.3 | 588.2 | 1.84 | <0.05 |

a) A. B. W.—average body weight; b) A. S. W.—average spleen weight.

IV. SUMMARY

Rubescensine B is a new antitumor active principle isolated from *Rabdosia rubescens* Hara. Chemically, it belongs to the ent-kaurene type of diterpene. The data obtained by means of infrared, ultraviolet and magnetic resonance spectra showed an identity of rubescensine B with *ponicidin*. Our experiments proved that rubescensine B had marked cytotoxic effect on Ehrlich carcinoma cells *in vitro*, and possessed an antitumor activity against a number of animal tumors such as ECA, HCA, S₁₈₀, L₁ and ARS etc. The subacute toxicity test revealed no noticeable effect of rubescensine B on the bone marrow, liver and kidney functions. However, by hemolysin assay method and graft versus host reaction test to monitor the humoral- and cellular-mediated immunity respectively, rubescensine B was observed to have a slight stimulant effect. This differs from rubescensine A which had some depressant action on humoral-mediated immunity but not had any effect on cellular-mediated immunity.

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