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RESEARCH ARTICLE

Chemical compounds and pharmacological effects of *Rabdosia excisa*

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Abstract Many kinds of diterpenoids have been isolated from *Rabdosia* spp. Some of them have anti-microbial effects, counteract inflammation, and inhibit tumor progression activities. We conducted the present study in order to look for bioactive compounds in the medicinal plant *Rabdosia excisa*. In this study, five compounds were isolated from *R. excisa*; they were oridonin, isokamebakaurin, oleanolic acid, ursolic acid, and β-sitosterol. In order to identify the function of the extracts, the activity of antibiotics, anti-oxidation, and immunity test were carried out against these functions. Prospective results were observed in all of the tested items.

Keywords *Rabdosia excisa*, compound, pharmacological effects

1 Introduction

Rabdosia belongs to the *Labiatae* family, and there are more than 150 species in the genus. These species are widely distributed in East Asia and West Africa. There are 90 species and 25 varieties in China (Editorial board of Planta Sinica, 1977; Institute of Botany, 1994), and more than 30 of them are used as traditional medicines. The main functions are anti-tumor, anti-microbial, detoxification. Massive studies have been done on many species of the genus. Diterpenoids, triterpenoids, fatty acids, and steroids are found in them. More than 100 kinds are diterpenoids. Recently, some of them have been verified to have antibiotic, anti-inflammation, anti-oxidation, immunity regulation, and anti-tumor properties (Li et al., 2003; Sun et al., 2002; Wang et al., 2003). *Rabdosia excisa* is one of the species naturally distributed in the northeast of China (Fu et al., 1995). In order to identify activities of diterpenoids in it, we conducted the studies on its chemical compounds. Hereon, we report the isolation and characterization of five compounds. Furthermore, some pharmacological effects of the ethanol extracted/water soluble part were also studied. The activities of antibiotics, anti-inflammation, and anti-oxidation were observed from the experiments.

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2 Isolation and characterization of compounds

2.1 Materials and methods

Rabdosia excisa were collected from Zuojia District, Jilin Province, China (Editorial board of Planta Sinica, 1977). Ten kilograms of vegetable powder was eluted with 95% ethanol for three times. The extraction were combined and condensed, then were re-dissolved in methanol. Active carbon was added to de-stain the solution. After the methanol-fast substances were removed by filtration, the remaining solution was loaded on a silica gel column. The column was eluted with petroleum ether, chloroform, different ratio of chloroform/acetone mixture, and acetone. The different extractions were recrystallized, and 11 compounds were obtained from the procedures. In five of them, specific optical rotation, UV spectrum, IR spectrum, EIMS, HRMS, ^{1}H-NMR, and ^{13}C-NMR were analyzed.

2.2 Results and discussion

Compound 1: pale yellow needle-like crystal, melting temperature 245°C–247°C, $[\alpha]_D = +27.2°$ (MeOH, c 0.55); UV λ_{max}: 235 nm; IR (KBr) : 3 253, 2 879, 1 721, 1 641, 1 557, 1 446, 1 060 cm^{-1}; EIMS *m*/*z*: 364 $[M]^+$, 346, 328, 310, 299, 281, 235; ^{1}H NMR (400 MHz, C_5D_5N) δ: 3.83 (dd, 4.6, $J = 10.3$ Hz, H-1), 4.93 (dd, 4.4, $J = 12.1$ Hz, H-7),

3.41(m, H-13), 5.36 (br, s, H-14), 6.39 (s, H-17), 5.60 (s, H-17), 9.35 (s, CHO-18), 4.66(d, $J = 12.1$ Hz, H-20), 4.43 (d, $J = 12.1$ Hz, H-20); ^{13}C NMR δ: 80.9(d), 30.1(t), 32.5(t), 47.6(s), 44.7(d), 29.3(t), 73.2(d), 63.3(s), 57.6(d), 49.0(s), 22.5(t), 31.7(t), 49.8(d), 75.8(d), 210.0(s), 150.2(s), 117.1(t), 207.6(d), 14.9(q), 61.0(t). The MS analysis showed the formula $C_{20}H_{28}O_6$ (*m/z* 364 $[M]^+$). Compared with known compound kamebakaurin, most values were identical except the C-18 which was an aldehyde. The structure of the compound was concluded as *ent*-1α, 7α, 14β, 20-tetrahydroxy- kaur-16-ene-18-aldehyde-15-one (Sun et al., 1981; Guo et al., 2002), and it was nominated isokamebakaurin.

Compound 2: colorless needle crystal, melting point 248°C–250°C, molecular formula by MS: $C_{20}H_{28}O_6$, EIMS *m/z* : 364 $[M]^+$; UV λ_{max}: 238 nm; IR (KBr): 3 400, 3 200, 1 705, 1 645, 1 095, 1 080, 1 069 cm^{-1}; ^{1}H NMR (400 MHz, C5D5N) δ:6.92 (1H, d, $J = 10.0$), disappearing after D_2O exchange, showing β-OH; 6.31 (1H, s, 17-H_2), 5.53(1H, s, 17-H_2), 5.86 (1H, br s, OH), 5.35 (1H, s, 14α-H), 4.78 and 4.42 (1H each, ABd, $J = 10.0$, 20-H_2), 4.29 (1H, dd, $J = 10.0$, 7.0, 6α-H), 3.65 (1H, t, $J = 8.0$, 1β-H),1.20 and 1.13(each 3H, s, 2CH_3); ^{13}C NMR δ: 74.5 (d), 30.4 (t), 39.2(t), 33.9(s), 60.6(d), 73.4(d), 98.2(s), 62.8(s), 54.1(d), 41.6(s), 20.2(t), 30.8(t), 43.8(d), 73.0(d), 208.9(s), 153.1(s), 119.0(t), 33.1(q), 22.1(q), 63.8(t). The data were identical to the reported (Fujita et al, 1976) oridonin.

Compound 3: white granule crystal (MeOH), melting point 285°C–286°C, EIMS *m/z*: 456$[M]^+$, 439$[M-OH]^+$, 438$[M-H_2O]^+$, 411 $[M-COOH]^+$, 393, 300, 248(base peak), 207, 203. IR (KBr): 3440 (OH), 1695 (COOH), 1459 cm^{-1}. ^{1}H NMR (C_5D_5N, TMS) δ: 5.51 (1H, t, 12-H), 3.52 (1H, dd, $J = 10.3$ Hz, 10.2 Hz, 3-H), 2.67 (1H, d, $J = 11.3$ Hz, 18-H), 1.25 (CH_3, s), 1.23 (CH_3, s), 1.08 (CH_3, s), 1.03 (CH_3, s), 1.01 (CH_3, d), 0.97 (CH_3, d), 0.90 (CH_3, s). The shift ratio of TLC was identical to oleanolic acid standard; and the melting point of their mixture did not drop. This compound was characterized as oleanolic acid.

Compound 4: white granule crystal (MeOH), melting point 285°C–286°C; EIMS *m/z*: 456$[M]^+$, 439$[M-OH]^+$, 438$[M-H_2O]^+$, 411$[M-COOH]^+$, 393, 300, 248 (base peak), 207, 203. IR (KBr): 3440 (OH), 1695 (COOH), 1459 cm^{-1}. ^{1}H NMR (C_5D_5N, TMS) δ: 5.51 (1H, t, 12-H), 3.52 (1H, dd, $J = 10.3$ Hz, 10.2 Hz, 3-H), 2.67 (1H, d, $J = 11.3$ Hz, 18-H), 1.25 (CH_3, s), 1.23 (CH_3, s), 1.08 (CH_3, s), 1.03 (CH_3, s), 1.01 (CH_3, d), 0.97 (CH_3, d), 0.90 (CH_3, s). The shift ratio of TLC was identical to ursolic acid standard; and the melting point of their mixture did not drop. This compound was characterized as ursolic acid.

Compound 5: Melting point 137°C–138°C, EIMS *m/z*: 414 $[M]^+$, 396, 381, 329, 273, 255; molecular formula was $C_{29}H_{50}O$, IR (KBr): 3 410, 3 385, 1 640 cm^{-1}; ^{1}H NMR 1δ: 6.32, 5.45, 1.62, 1.27, 0.92. The shift ratio of TLC was identical to β-sitosterol standard, and the melting point of their mixture did not drop. This compound was characterized as β-sitosterol (Cheng, 1982).

3 Pharmacology study

3.1 Materials

The ethanol extracted / vacuum condensed extraction was re-dissolved in hot water, and made 1 g/mL liquor solution with the original material.

The animal for research was Kunming mouse, and their body weights ranged from 18 to 22 g.

The bacterial strains were *Escherichia coli* (#44103), *Staphylococcus aureus* (#26001-19), *Pseudomonas aeruginosa* (#10102) and *Bacillus subtilis* (#63501).

3.2 Experimental methods

3.2.1 Anti-oxidation

Forty mice were randomly grouped into four groups. The experiment used 10 mL/kg normal saline, 5 g / kg (small dosage), 10 g / kg (medium dosage), 20 g / kg (large dosage) of extraction. They were given to the mice in the different groups respectively twice a day for 7 days. The mice were euthanized 1 h after the final administration. Their hearts, livers, and kidneys were rapidly collected. Five percent tissue homogenates were made with normal saline. One milliliter of homogenate of each animal in each group were incubated at 37°C for 2 h (1 mL of 20% trichloroacetic acid is added to one sample of each animal/organ), 1 mL of 20% trichloroacetic acid was added to each sample after incubation to terminate the oxidation. The samples were spun for 10 min at 1000 r/ min and 1 mL supernatant was recovered. We added 0.5 mL 0.67% TBA and boiled the solution for 10 min. Finally, we measured the absorbance at 532 nm after cooling.

3.2.2 Immunoloregulation

Forty mice were grouped and treated with the same method as the previous experiment. The mice were injected with 0.1 mL/20 g India ink after 24 h of the final administration. Blood samples were taken from their eye socket vein at 2 min (t_1) and 20 min (t_2) after the injection for 40 μL each time. The blood samples were dissolved in 4 mL 0.1% sodium carbonate. The absorbance at 680 nm for the samples at t_1 and t_2 were measured as A_1 and A_2. We calculated the clearance index with the following formula:

$$K = (\log A_1 - \log A_2) / (t_2 - t_1)$$

After taking and weighing the liver and spleen, we calculated the phagocytic activity with the following formula:

$$A = K^{1/3} \times \text{body weight} / (\text{liver weight} + \text{spleen weight})$$

3.2.3 *In vitro* antibacterial action

We doubly diluted the original extract solution with common broth, to 1:1 and 1:128 (v:v), 1 mL each vial. We added

0.05 mL of different prepared bacterial solutions to the vials (*Escherichia coli* 3.2×10^8, *Staphylococcus aureus* 1.0×10^{10}, *Pseudomonas aeruginosa* 9.5×10^8, *Bacillus subtilis* 6.0×10^5). They were incubated at 37°C for 48 h. The clear vials were regarded as bacteriostatic concentration at 18 h, and the concentrations with no bacterial growth at 48 h were regarded as bacteriocidal concentration.

3.3 Results

3.3.1 Anti-oxidation activity

The dissolved part *in vivo* anti-oxidation activities of *R. excisa* ethanol extraction/water were show in Table 1.

3.3.2 Immunoloregulation

The effects of *R. excisa* ethanol extracted/water dissolved part on mice carbon particle clearance ratios are shown in Table 2.

The *R. excisa* extraction largely affected the carbon particle clearance ratio of mice. The dosage and clearance index were apparently negatively correlated.

3.3.3 Bacteriocidal activity

The bacteriocidal activities of different dilutions of *R. excisa* extraction are shown in Table 3.

4 Discussion

Five compounds were isolated from *R. excisa*, and for the first time isokamebakaurin is characterized from this plant. Extraction of *R. excisa* strongly represses the formation of hyper-oxidation of mouse organs *in vitro*. It shows compound(s) in the extraction repressing the oxidase activity on unsaturated fatty acid and protecting the cells from oxidation. There are evidences on the anti-inflammation and anti-tumor activity of diterpenoids (Cheng et al., 1985), but

Table 1 *In vivo* anti-oxidation activities

Organ	Group	Dosage (g / kg)	Absorbance(A_{532})	Decrease rate / %
	Control	0	0.1120 ± 0.0073	–
Heart	Small	5	0.0689 ± 0.0049***	38.48
	Medium	10	0.0526 ± 0.0085***	53.04
	Large	20	0.0471 ± 0.0065***	57.95
	Control	0	0.4722 ± 0.0572	–
Liver	Small	5	0.1936 ± 0.0246***	58.96
	Medium	10	0.1401 ± 0.0213***	70.33
	Large	20	0 1124 ± 0.0198***	76.20
	Control	0	0.2742 ± 0.0263	–
Kidney	Small	5	0.1641 ± 0.0174***	40.15
	Medium	10	0.1239 ± 0 0128***	54.81
	Large	20	0.1091 ± 0.0072***	60.21

$N = 10$; ***: $P < 0.001$

Table 2 Effect on mouse carbon particle clearance ratio ($x \pm$ s.d.; $n = 10$)

Group	Dosage / ($g \cdot kg^{-1}$)	Phagocytic index /K	Phagocytic activity / α
Control	0	0.043 7 ± 0.004 6	8.937 8 ± 0.527 7
Small	5	0.039 5 ± 0.004 7*	8.769 5 ± 0.593 2
Medium	10	0.036 2 ± 0.003 7**	8.512 1 ± 0.276 2
Large	20	0.033 8 ± 0.003 1**	8.123 5 ± 0.334 0

$n = 10$; *: $P < 0.05$; **: $P < 0.01$

Table 3 Bacteriostatic / bacteriocidal effect of extraction

Strain	Incubation / h	Dilution factor of the extraction								
		1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	control
E. coli	18	–	–	–	–	+	+	+	+	+
	48	–	–	–	+	+	+	+	+	+
P. aeruginosa	18	–	–	–	–	+	+	+	+	+
	48	–	–	–	–	+	+	+	+	+
S. aureus	18	–	–	–	–	–	+	+	+	+
	48	–	–	–	–	–	+	+	+	+
B. subtilis	18	–	–	–	–	–	+	+	+	+
	48	–	–	–	–	–	+	+	+	+

–: no growth; +: growth

the content of diterpenoids in the ethanol extracted/water dissolved is relatively low, and the anti-oxidation activity of the solution may not be from the diterpenoids. The basic active compounds are still unknown. By comparing the speed alternation of mouse carbon particle clearance, a conclusion that *R. excisa* extraction significantly depresses reticuloendothelial system (RES) can be obtained. The polysaccharides in the extraction should reinforce the RES activity, and the diterpenoids do little on RES (Wang et al., 2002). However, the RES activity is actually depressed, probably because there is something unknown that is strongly repressing cellular immunity. The *R. excisa* extraction shows inhibition on several clinical pathogens, and the activity is a little stronger than that of berberine. It is an evidence of the presentation of strong bacteriostatic compound (Zhang et al., 2000).

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