ORIGINAL ARTICLE

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Rec. Nat. Prod. 6:3 (2012) 242-254

records of natural products

Synthesis and Myocardial Ischemia Protective Effect of Ocotillol-Type Derivatives

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(Received March 12, 2011; Revised November 8, 2011; Accepted December 11, 2011)

Abstract: A series of ocotillol-type derivatives bearing a glycosyl moiety were synthesized. Among them, compound **3a** and **3b** led to a reduction in the increased CK, LDH, MDA activity and attenuate the decrease of SOD, GSH-PX and T-AOC compared with the iso group. In the heart section, compound **3a** and **3b** group showed nearly normal myofibrillar structure nearly without neutrophil granulocytes infiltration in the iso group. These results indicated that ocotillol-type derivatives may be the good lead compounds with protective property of myocardial ischemia.

Keywords: Ocotillol-type derivatives; myocardial ischemia; synthesis; ginsenoside.

1. Introduction

Ginseng (Panax ginseng C.A. Meyer) have been used in China for about 1000 years for the treatment of cardiovascular diseases and stroke. Ginsenosides are considered to be the main bioactive constituents. It is reported that gensenoside F11 and ocotillol derivatives have the potential clinical value in myocardial ischemia therapy.[1,2] In our previous study, we have obtain the ocotillol-type saponin and derivatives and proved their cardioprotective effects on myocardial injury induced by ISO in rats.[3] The source of octillol-type gensenoside is fewer than other type in natural world. So we design and synthesize a series of derivatives of octillol from 20(S)-Rh2, 20(S)-Rh1 and report their protective effect in myocardial injury induced by isoproterenol in rats.

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 03/25//2012 EISSN:1307-6167

2. Materials and Methods

2.1. Material

The material 20(S)-Rh2 and 20(S)-Rh1 were degraded and isolated from the crude product of ginsides in our laboratory (Rh1. m.p 191-194℃, lit. 192-194℃. Rh2. m.p 218-221℃, lit. 218-220℃). [4] The other reagents were bought from different companies in China. Melting points were determined using a digitzing melting point apparatus (WRS-1B) and were not corrected. All of the compounds synthesized were purified by column chromatography (CC) on silica gel (200-300 mesh) and thin-layer chromatography (TLC) on silica gel GF254 plates (Yantai Chemical Industry Research Institute, China). Subsequently, they were routinely analyzed by ^{1}H-NMR (Bruker VANCE-400), MS (Applied Biosystoms Mariner spectrometer).

2.2. Synthesis

(3β,12β)-12-acetyl-20-hydroxydammar-24-en-3-yl-β-D-(2',3',4',6'-tetra-acetyl)-glucopyranoside (**1a**). 20(S)-Rh2 (4.92g, 7.91mmol) and acetic anhydride (54.3ml, 47.25mmol) were dissolved in pyridine (55ml) and stirred for 2h in room temperature. After evaporation in vacuum and the residue was taken up in ethyl acetate and dilute hydrochloric acid. The organic phase was separated and washed with water and saturated sodium chloride solution, dried (Na_2SO_4), and then concentrated to yield a semi-solid. Flash chromatography (1:1 petroleum ether-ethyl acetate) gave the compound **1a** as a white solid (4.03 g, 62.0% yield, m.p 221-222℃, lit 223-225℃ (form EtOH)[5]). The results of ^{13}C-NMR are shown in Table 1.

(3β,12β)-12-acetyl-20S,24R-epoxy-dammar-24-en-3-yl-β-D-(2',3',4',6'-tetra-acetyl)-glucopyran oside (**2a**). A solution of compound **1a** (4.03 g, 4.90 mmol) in dichloromethane (30ml) was cooled to -3℃. Then a solution of *m*-CPBA (0.95 g, 5.50 mmol) in dichloromethane (10 ml) was added slowly and stirred for 1 h. The organic solution was washed with water and saturated sodium chloride solution and dried over Na_2SO_4. The dichloromethane was evaporated in vacuo to yield a white solid. The residue was chromatographed over silica gel (1:1 petroleum ether-ethyl acetate) and crystallized from ethyl acetate to get compound **2a** as white needle-like crystals (2.90 g, 73.2% yield, m.p 203-204℃). The results of ^{13}C-NMR are shown in Table 1.

(3β,12β)-12-hydroxy-20S,24R-epoxy-dammar-24-en-3-yl-β-D—glucopyranoside (**3a**)[6]. Sodium hydroxide (2.00g, 50.00mmol) was added to the solution of compound **2a** (2.90 g, 3.58 mmol) in methanol (30 ml). The resulting mixture was stirred at room temperature for 2 h. The solvent was diluted with water 200 ml. After filtration, the residue was chromatographed over silica gel (ethyl acetate) and crystallized from ethyl acetate yielded compound **3a** as white pellet-like crystals (0.75g, 33.2% yield, m.p 184-185℃). HRMS for $C_{36}H_{62}O_9$ + H calcd 639.44666, found 639.44883.ESI-MS, *m/z*: 639.37[M + H]$^+$, 661.2[M + Na]$^+$. ^{1}H-NMR (C_6D_5N, 400 Hz) δ: 0.75 0.91 0.97, 1.24, 1.27，1.30, 1.30，1.45 (each 3H, s), 3.36 (1H, m) 3.70 (1H, m), 3.93 (1H, t, J=7.96Hz), 4.04 (1H, m), 4.25 (2H, m), 4.42(1H, t, J=5.36Hz), 4.60 (1H, m), 4.84 (1H, m), 4.94 (1H, d, J=7.76Hz). The results of ^{13}C-NMR, HMBC, HSQC, ^{1}H-^{1}H COSY are shown in Table 2.

(3β,6α,12β)-3,12-diacetyl-20-hydroxy-dammar-24-en-6-yl-β-D-(2',3',4',6'-tetra-acetyl)-glucopyr anoside (**1b**)**.** According to the procedure for the preparation of **1a**, treatment of 20(S)-Rh1 (9.25g, 14.50mmol) afforded **1b** as a white powder (8.88g, 68.1% yield, m.p 185-186℃). The results of ^{13}C-NMR are shown in Table 3.

(3β,6α,12β)-3,12-diacetyl-20S,24R-epoxy-dammar-24-en-6-yl-β-D-(2',3',4',6'-tetra-acetyl)-gluc opyranoside (**2b**)**.** According to the procedure for the preparation of **2a**, treatment of **1b** (8.88g, 9.86mmol) afforded **2b** as white solid (4.52g, 50.1% yield, m.p 187-188℃). The results of ^{13}C-NMR are shown in Table 3.

(3β,6α,12β)-3,12-dihydroxy-20S,24R-epoxy-dammar-24-en-6-yl-β-D-glucopyranoside (**3b**)[7]. According to the procedure for the preparation of **3a**, treatment of **2b** (4.52g, 4.94mmol) afforded **3b** as white needle-like crystals (1.12 g, 34.3% yield, m.p 224-225℃). HRMS for $C_{36}H_{62}O_{10}$ + H calcd 655.44157, found 655.44354. ESI-MS, *m/z*: 655.41 [M + H]$^+$, 677.39 [M + Na]$^+$. ^{1}H-NMR(C_6D_5N, 400Hz) δ: 0.75 (3H, s, CH_3-19)，1.00, 1.18, 1.24 , 1.24, 1.24, 1.27, 1.45 (each 3H, s), 3.52 (1H, d, J=10.28Hz), 3.68 (1H,m), 3.94 (1H, m), 4.08 (1H, d, J=7.48Hz), 4.23 (2H, m), 4.45 (3H, m), 4.82 (1H, s), 5.01 (1H, m). The results of ^{13}C-NMR, HMBC, HSQC are shown in Table 4.

2.3. *Pharmaceutical Experiment*

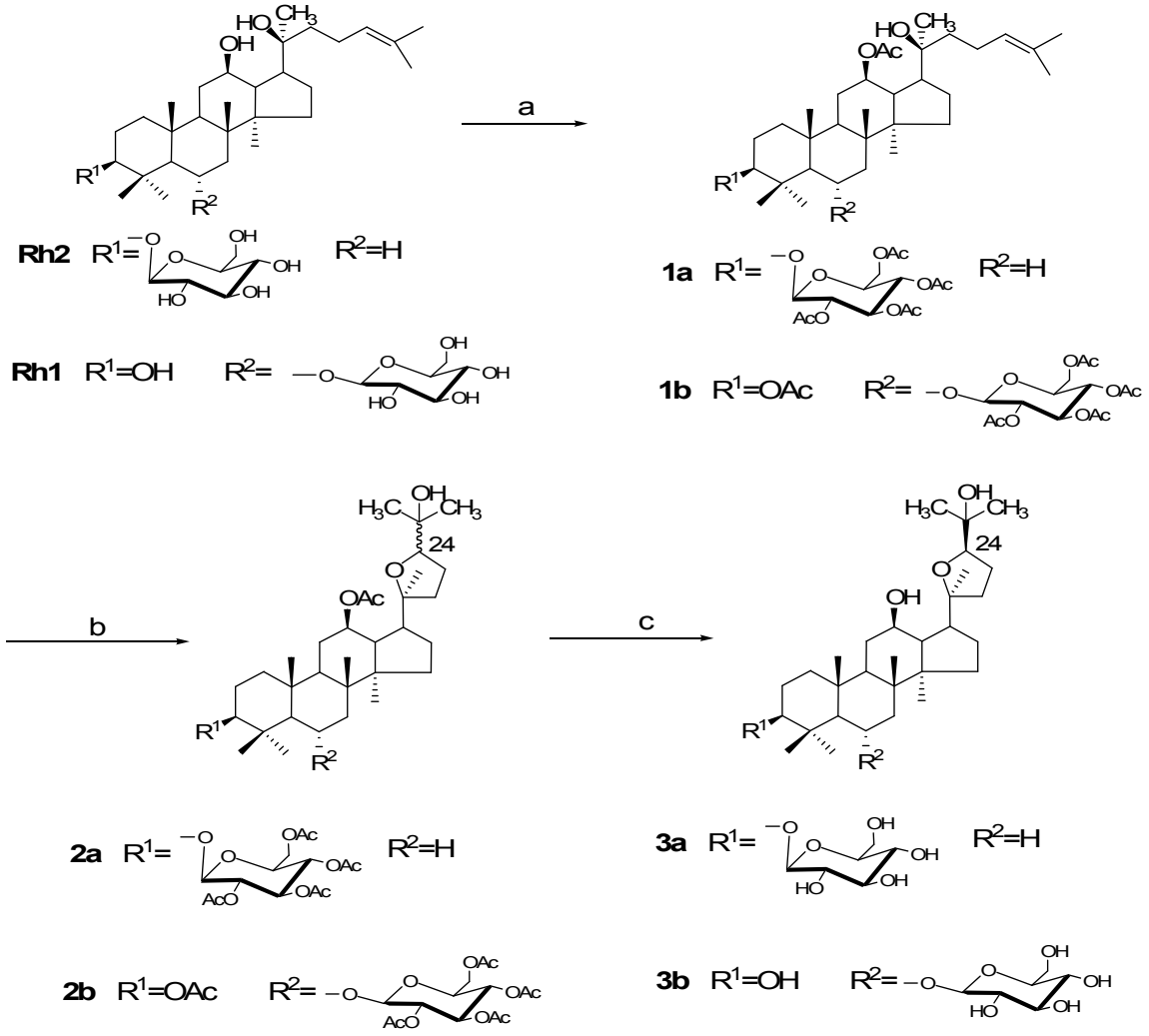
Except control group and isoproterenol alone group, rats in therapeutic groups were orally given Rh1（67.8 mg/kg），**3a**（69.5 mg/kg），Rh2（71.9 mg/kg），**3b**（73.6 mg/kg）once a day for 7 days, respectively. On the fifth day, except control the group, rats were subcutaneously injected with isoproterenol (20 mg/kg) once a day for 3 days. The experiment was stopped 2 h after the last injection of isoproterenol, and rats were anesthetized intraperitoneally with urethane (1 g/kg). A blood sample was drawn from abdominal aorta, centrifuged and the serum samples were assayed for LDH and CK activities. Then the rats were killed, and hearts were removed rapidly. The MDA，SOD、GSH-Px activity and T-AOC content in left ventricle homogenates were measured according to the kit instructions. In each group, two rat hearts was taken for pathological examination after hematoxylin–esosin staining. [8,9]

3. Results and Discussion

The mild saponification method of Ginsenoside has been explored. Panaxadiol saponins and panaxatriol saponins in DMSO were heated and degraded by treatment with sodium methoxide. 20(S)-Rh2 and 20(S)-Rh1 were obtained from panaxadiol saponins and panaxatriol saponins. Synthesis of derivatives of 20(S)-Rh2, 20(S)-Rh1 is shown in Scheme 1 and two target compounds are successfully obtained by acetylation, oxidation and saponification.

The structures of these ocotillol-type derivatives are elucidated by ^{1}H-NMR, ^{13}C-NMR, MS, DEPT, HMBC, HSQC or HRMS.

Isoproterenol is widely used as an agent to evaluate cardioprotective drugs and study myocardial consequences of ischemic disorders. The pharmaceutical experiment results are shown in Fig 1-7. The serum CK and LDH activity augmented in the Iso group as compared with that in the Con group. Administration with **3a** and **3b** led a reduction in the increased CK and LDH activity, respectively. The CK and LDH activity did not significantly decrease after treatment with 20(S)-Rh1, 20(S)-Rh2 in. After isoproterenol injection, the MDA levels increased in the heart tissues significantly. Treatment with **3a** and **3b**, MDA levels show a significant reduction in groups but not in Con group. MDA levels in 20(S)-Rh1, 20(S)-Rh2 group did not cause decrease as compared with Iso group. SOD, GSH-PX and T-AOC in heart tissues showed a significant decrease in Iso group. Treated with **3a** or **3b** attenuate the decrease of SOD, GSH-PX and T-AOC in the group. Administrated with 20(S)-Rh1, 20(S)-Rh2 did not led a reduction of SOD, GSH-PX and T-AOC. The heart sections in Con group showed a normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils. In Iso group the heart sections revealed extensive myofibrillar degeneration, which is related to infiltration with neutrophil granulocytes and interstitial edema. Treatment with 20(S)-Rh1, 20(S)-Rh2 group, the heart sections showed mild degenerative changes and discontinuity with adjacent myofibril. In **3a** and **3b** group, the heart sections showed nearly normal myofibrillar structure nearly without neutrophil granulocytes infiltration.



Scheme 1 Reagents and conditions: a) $(CH_3CO)_2O$, DMAP. Pyridine; b) *m*-CPBA, CH_2Cl_2; c) NaOH, CH_3OH, H_2O

4. Conclusion

The study indicated that compound **3a** and **3b** had the protective effectt of myocardial ischemia associated with their antioxidant activity. The octillol-type derivatives maybe the good lead compounds with protective property of myocardial ischemia. Further biological evaluation of these octillol-type derivatives and extensive lead optimizations are ongoing in our laboratory and more results will be reported in due time.

Table.1 ^{13}C NMR data of compounds **1a** and **2a**（C_6D_5N, δ, ppm）

No.	1a	2a	20(s)-Rh2
1	39.8	39.8	39.4
2	27.6	27.8	27.3
3	91.0	91.1	88.9
4	40.0	40.0	40.3
5	57.2	57.2	56.4
6	19.2	19.2	18.7
7	34.5	35.7	36.0
8	37.9	38.0	37.2
9	51.2	51.1	50.7
10	39.8	39.8	39.8
11	32.3	32.2	32.2
12	71.0	70.1	71.1
13	48.6	48.6	48.8
14	51.2	51.1	51.9
15	31.4	32.2	31.5
16	26.9	26.9	26.8
17	53.7	53.4	54.8
18	16.7	16.7	16.8
19	16.6	16.5	16.4
20	73.2	84.7	73.2
21	26.9	27.1	27.0
22	35.6	35.6	35.4
23	23.4	23.3	23.1
24	126.2	84.7	126.4
25	132.0	70.1	130.7
26	25.7	25.9	25.7
27	17.7	17.8	17.7
28	28.2	28.3	28.3
29	16.0	16.1	16.0
30	17.1	17.8	17.3
3-glc-1’	103.7	103.7	106.7
2	75.2	74.3	75.8
3	72.6	72.6	78.7
4	73.2	73.3	72.2
5	76.8	77.0	78.0
6	63.2	63.2	63.3

Table.2 NMR data for Compound **3a**

	δc		Correlated proton		
No.	**20(S-)Rh2**	**3a**	**HSQC**	**HMBC**	**1H-1H COSY**
1	39.4	39.2	1.52(1e), 0.79(1a)	19	1e, 2a
2	27.3	26.7	2.18(2e), 1.82(2a)		1a, 3, 2e
3	88.9	88.7	3.36(dd, J= 4.4, 4.3 Hz)	3-glc-1,28,29	2a, 2e
4	40.3	40.0	0.74	28,29	
5	56.4	56.4	0.66(d, J=10.0 Hz)	28,29,19	6a
6	18.7	18.4	1.51(6e), 1.39(6a)		5,7a
7	36.0	35.1	1.37(7e), 1.24(7a)	18	6e, 6a

8	37.2	36.9		18,	
9	50.7	50.0	1.80	18,19	11e
10	39.8	39.7		19	
11	32.2	32.4	2.01(11e), 1.20(11a)	30	9,13,12
12	71.1	71.0	3.75 (m)		11a, 13
13	48.8	48.3	2.25(m)	21	11,12,17
14	51.9	50.7	1.46	18,30	
15	31.5	31.6	1.87(15e), 1.60(15a)	21	16
16	26.8	25.5	2.14(16e), 1.86(16a)	17	17,15a
17	54.8	52.1		18,30	13,16
18	16.8	16.7	0.97(s)	7	
19	16.4	16.5	0.75(s)		
20	73.2	86.7		21	
21	27.0	27.2	1.27(s)		
22	35.4	32.8	1.92(22e), 1.06(22a)	21	23a, 23e
23	23.1	28.1	2.16(23e), 1.84(23a)	27	22a, 22e
24	126.4	85.6	3.93(dd, J =7.0, 7.0 Hz)	26,27	23a, 23e
25	130.7	70.3		26,27	
26	25.7	27.6	1.24(s)		
27	17.7	26.9	1.45(s)		
28	28.3	28.1	1.30(s)	29,3	
29	16.0	15.5	0.96(s)	28,5	
30	17.3	18.3	0.91(s)		
3-glc-1’	106.7	106.7	4.95(d, J =8.0 Hz)	3	
2’	75.8	75.8	4.04		
3’	78.7	78.8	4.23		
4’	72.2	71.9	4.25	3’	
5’	78.0	78.4	4.00	4’	
6’	63.3	63.1	4.59,4.62		

Table.3 ^{13}C NMR data of compounds **1b** and **2b**（C_6D_5N,δ, ppm）

No.	1b	2b	20(S)-Rh1
1	39.24	39.22	39.38
2	27.49	27.97	26.55
3	76.34	76.66	78.30
4	40.41	40.41	40.06
5	61.34	61.31	61.14
6	77.80	76.66	77.83
7	45.41	45.43	44.95
8	41.88	41.83	40.81
9	50.51	50.37	49.50
10	39.01	39.22	39.11
11	30.84	30.85	30.94
12	69.72	69.70	70.74
13	48.36	48.35	47.96
14	51.20	50.37	51.34
15	32.22	32.15	31.43
16	26.00	26.21	26.73
17	53.40	53.17	54.47
18	17.28	17.27	17.38
19	17.30	17.21	17.34
20	72.75	87.00	72.70
21	26.43	26.93	26.73
22	35.60	35.6	35.53
23	21.75	23.3	22.70
24	126.21	84.80	126.03
25	131.98	70.1	130.48
26	25.88	25.9	25.52
27	17.27	17.8	17.09
28	32.21	28.3	31.78
29	17.58	16.1	16.50
30	17.68	17.8	16.08
6-glc-1’	102.44	102.42	105.73
2	75.13	75.09	75.18
3	80.77	80.77	79.76
4	72.75	72.75	71.58
5	82.47	82.47	79.35
6	63.51	63.50	62.79

Table.4 NMR data for Compound **3b**

		δc	Correlated proton	
NO.	**20(S)- Rh1**	**3b**	**HSQC**	**HMBC**
1	39.28	39.56	1.45(m), 0.84(m)	19
2	26.55	27.89	1.74(m), 1.82(m)	19
3	78.30	78.54	3.47(dd, J= 7.5, 11.3 Hz)	28,29
4	40.06	40.35		28,29
5	61.14	61.50	1.39(d, J=11.0 Hz)	28,29,19
6	77.83	78.14	4.66(dd, J= 3.0, 9.5 Hz)	28,29
7	44.95	45.08	1.98,2.5	18
8	40.81	40.99		18,30
9	49.90	49.42	1.54(d,7.0)	18,19
10	39.11	39.44		19
11	30.94	32.4	1.93(m),1.98(m)	18,19
12	70.74	71.16	3.69(m)	
13	47.96	48.37	1.54(m)	30
14	51.34	52.13	1.46	18,30
15	31.43	32.39	1.26(m), 1.60(m)	18,30
16	26.73	25.40	1.45(m), 1.74(m)	
17	54.47	50.52	2.08(m)	21
18	17.38	17.99	1.00(s)	
19	17.34	17.82	0.83(s)	28,29
20	72.70	86.67		21
21	26.73	27.66	1.27(s)	
22	35.53	32.68	1.92(m), 1.06(m)	21
23	252.70	28.75	2.16(m), 1.84(m)	26,27
24	126.03	85.58	4.16(dd, J =7.0, 7.0 Hz)	26,27
25	130.48	70.27		26,27
26	25.52	27.11	1.30(s)	27
27	17.09	26.90	1.45(s)	26
28	31.78	31.64	1.28(s)	29,19
29	16.50	17.04	1.11(s)	28
30	16.08	16.24	0.91(s)	
6-glc-1’	105.73	105.96	4.95(d, J =8.0 Hz)	3
2’	75.18	75.44	4.04	
3’	79.76	80.01	4.26	
4’	71.58	71.89	4.25	3’
5’	79.35	79.63	4.00	4’
6’	62.79	63.12	4.56,4.39	

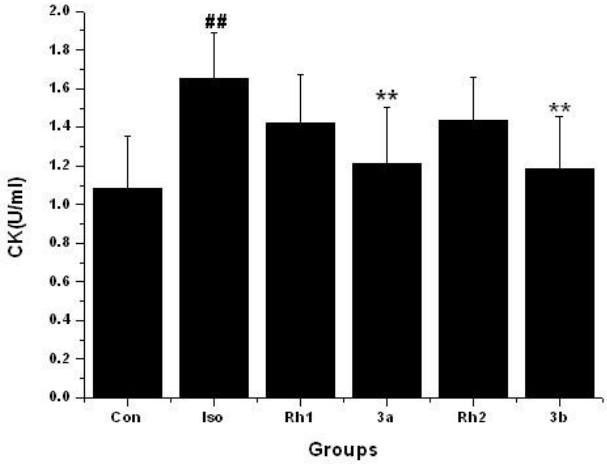


Figure.1 Effects of 20(S)-Rh1, 20(S)-Rh2 and its derivatives on CK activity in isoproterenol-induced myocardial injury in rats. Data was expressed as the mean ± S.D. (n = 6 to 8). Statistical significances were determined using unpaired two-tailed Student t-test or one-way analysis of variance (ANOVA) followed by Dunnett's contrast. ##P<0.01 compared with control group; **P<0.01 compared with isoproterenol group.

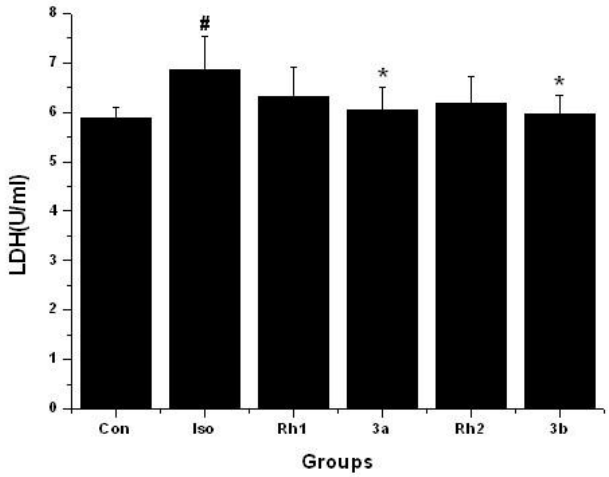


Figure.2 Effects of 20(S)-Rh1, 20(S)-Rh2 and its derivatives on LDH activity in isoproterenol-induced myocardial injury in rats. Data was expressed as the mean ± S.D. (n = 6 to 8). Statistical significances were determined using unpaired two-tailed Student t-test or one-way analysis of variance (ANOVA) followed by Dunnett's contrast. #P<0.05 compared with control group; *P<0.05compared with isoproterenol group.

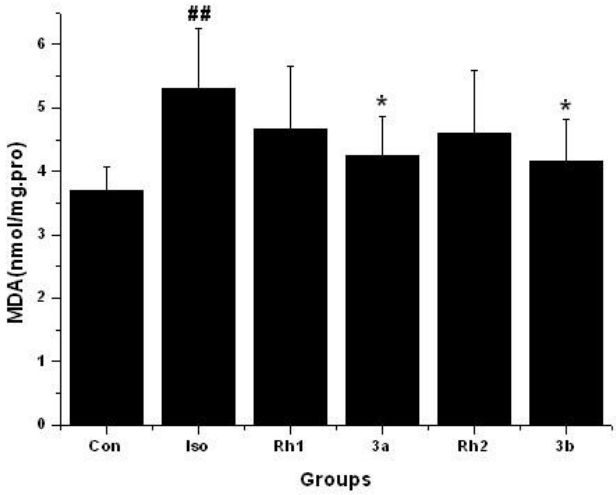


Figure. 3 Effects of 20(S)-Rh1, 20(S)-Rh2 and its derivatives on MDA in isoproterenol-induced myocardial injury in rats. Data was expressed as the mean ± S.D. (n = 6 to 8). Statistical significances were determined using unpaired two-tailed Student t-test or one-way analysis of variance (ANOVA) followed by Dunnett's contrast. ##P<0.01 compared with control group; *P<0.05 compared with isoproterenol group.

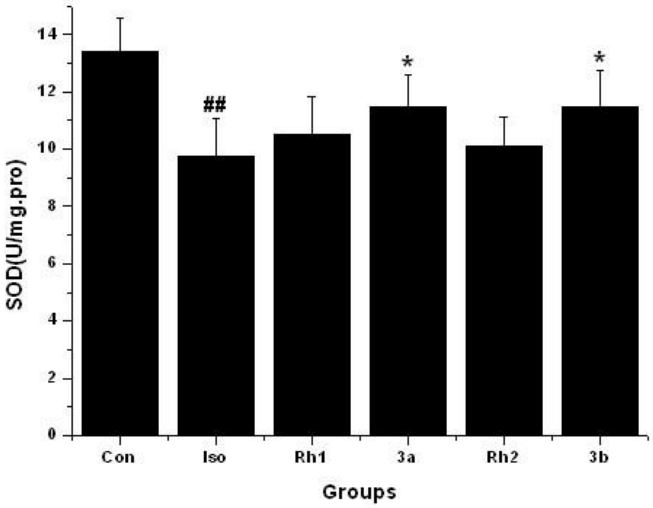


Figure.4 Effects of 20(S)-Rh1, 20(S)-Rh2 and its derivatives on SOD activity in isoproterenol-induced myocardial injury in rats. Data was expressed as the mean ± S.D. (n = 6 to 8). Statistical significances were determined using unpaired two-tailed Student t-test or one-way analysis of variance (ANOVA) followed by Dunnett's contrast. ##P<0.01 compared with control group; *P<0.05 compared with isoproterenol group.

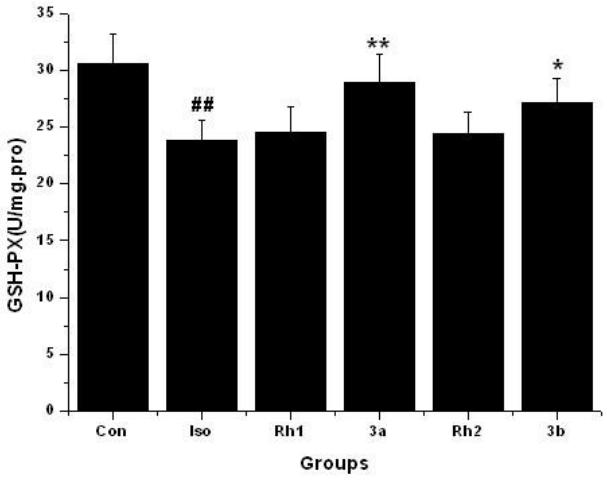


Figure.5 Effects of 20(S)-Rh1, 20(S)-Rh2 and its derivatives on GSH-PX activity in isoproterenol-induced myocardial injury in rats. Data was expressed as the mean ± S.D. (n = 6 to 8). Statistical significances were determined using unpaired two-tailed Student t-test or one-way analysis of variance (ANOVA) followed by Dunnett's contrast. ##P<0.01 compared with control group; *P<0.05, **P<0.01 compared with isoproterenol group.

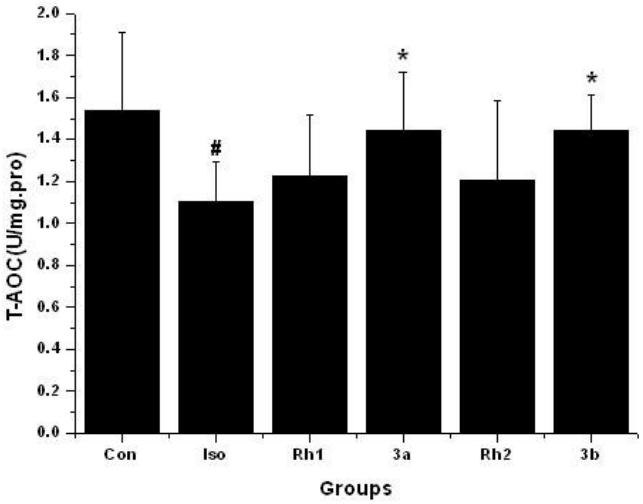
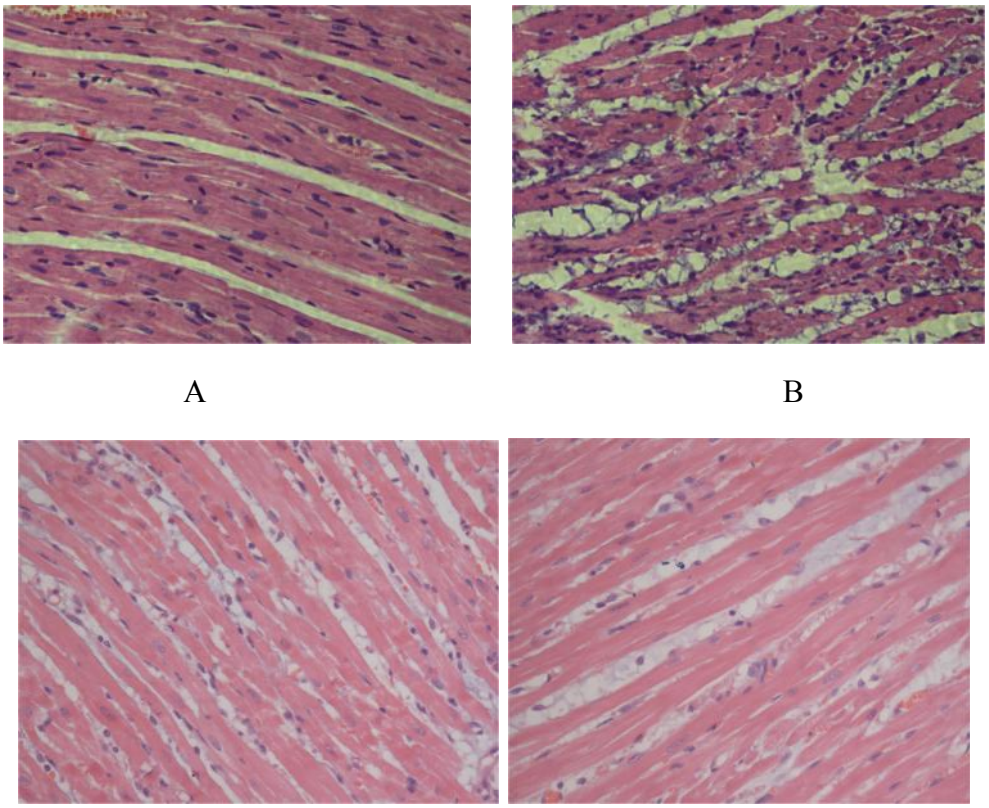
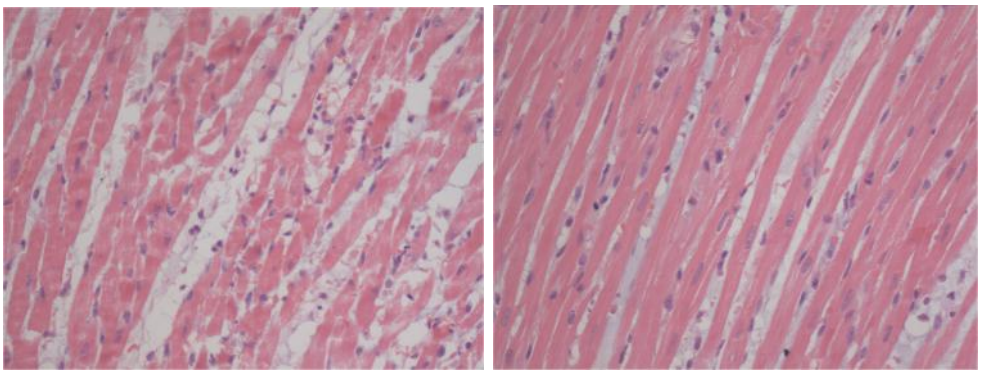


Figure. 6 Effects of 20(S)-Rh1, 20(S)-Rh2 and its derivatives on T-AOC in isoproterenol-induced myocardial injury in rats. Data was expressed as the mean ± S.D. (n = 6 to 8). Statistical significances were determined using unpaired two-tailed Student t-test or one-way analysis of variance (ANOVA) followed by Dunnett's contrast. #P <0.05 compared with control group; *P<0.05 compared with isoproterenol group.



A B

C D



E F

Figure.7 Effects of 20(S)-Rh1, 20(S)-Rh2 and its derivatives on histological change in isoproterenol-induced myocardial injury in rats.
A. Haematoxylin and eosin stained heart section of control group (×200).
B. Haematoxylin and eosin stained heart section of Iso group (×200).
C. Haematoxylin and eosin stained heart section of 20(S)-Rh1 group (×200).
D. Haematoxylin and eosin stained heart section of **3a** group (×200).
E. Haematoxylin and eosin stained heart section of 20(S)-Rh2 group (×200).
F. Haematoxylin and eosin stained heart section of **3b** group (×200).

Acknowledgments

The authors are grateful to National Natural Science Foundation of China (No.81001358), A Project of Shandong Province Higher Educational Science and Technology Program (No. BS2010YY073)，Scientific Reasearch Foundation of the Higher Education Institutions of Shandong Province, China (No. J07WE26), Shandong Provincial Natural Science Foundation, China （No. Y2007C138） for financial support. We also gratefully acknowledge Mr. Jiyong Chen and Ms. Li Shen for the measurement of ESI-MS and NMR and Mr. Guangzhi Shan for HRMS

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