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Full Length Research Paper

Synthesis, structural determination and protective effects on cultured anoxia/reoxygen injury myocardiocytes of ocotillol-type derivatives

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The octillol-type derivatives 3 and its epimer 4 had been designed and synthesized from 20(S)-panaxadiol. The structures of 3 and 4 were confirmed by ESI-MS, ^{1}H-NMR, ^{13}C-NMR and X-ray diffraction. The crystal data showed the configuration of C-24 of two epimers as R-form (3) and S-form (4, epimer) respectively. Compound 3 was evaluated *in vitro* for its protective effect on cultured myocardiocytes with anoxia/reoxygen injury and compound 4 had none of this activity.

Key words: Octillol-type derivative, epimer, X-ray diffraction, protective effect, cultured myocardiocytes with anoxia/reoxygen injury.

INTRODUCTION

Panax ginseng is a traditional Chinese medicine that is used to treat many diseases', such as cardiovascular diseases and stroke. *P. ginseng* contains numbers of ginsenosides, such as protopanaxadiol and protopanaxatriol-type saponins (Shibata et al., 1985; Takano et al., 1999). Ocotillol is one of the main components of *Panax quinquefolium* L. and has been proved to play a role in protective effect on myocardial ischemia injury. But Ocotillol only can be isolated from *P. quinquefolium* L. and the yield is too low, which restricts its use (Yu et al., 2007). 20(S)-panaxadiol is a well-known ginsengenin of *P. ginseng* and the source is relatively wide (Seiji et al., 1995). Now, we design to synthesize the ocotillol-type derivatives from 20(S)-panaxadiol in order to obtain the lead compounds with protective effect on cultured anoxia/reoxygen injury myocardiocytes.

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MATERIALS AND METHODS

General

Melting points were determined using a digitzing melting point apparatus (WRS-1B) and are reported directly. All 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 1H-NMR (Bruker VANCE-400), MS (Applied Biosystoms Mariner spectrometer), and Single Crystal Diffractometer (Enraf Nonius, Holand).

Synthesis of 3*β*, 12*β*-diacetyl-20 (*S*)-protopanaxadiol (1)

Acetic anhydride (14.4 ml, 12.53 mmol) was added to a stirred solution of 20 (*S*)-protopanaxadiol (1.22g, 2.65 mmol), *N*, *N*-dimethylamino- pyridine (0.32 g, 2.61 mmol) in pyridine (50 ml). The mixture was stirred for 24 h at room temperature. The solvent was removed in vacuo and the residue taken up in ethyl acetate and dilute hydrochloric acid. The organic phase was separated and washed with water and saturated sodium chloride solution, dried ($Na_2S_2O_4$) and then concentrated to yield a semi-solid. Flash

chromatography was carried out (1:1 petroleum ether-ethyl acetate) to give the product as white solid (0.83 g, 57.6% yield, m.p 172-173°C). ESI-MS, *m/z*: 1054.93 [2M - $2H_2O$ + 2H]$^{2+}$, 527.46 [M - H_2O + H]$^+$, 467.44 [M - H_2O - CH_3COOH + H]$^+$, 407.42 [M - H_2O - $2CH_3COOH$ + H]$^+$. 1H-NMR ($CDCl_3$) δ 0.83 (s, 3H*2), 0.86 (s, 3H), 0.93 (s, 3H), 0.99 (s, 3H), 1.11 (s, 3H) , 1.62 (s, 3H) , 1.69 (s, 3H), 2.20 (s, 3H*2), 4.47(dd, *J*=11.16 Hz, 4.36Hz, 1H), 4.71 (td, *J*=10.4Hz, 1H),.5.14 (t, *J*=7.0Hz, 1H).

Synthesis of 20*S*, 24-epoxy-dammarane-*3β*, *12β*, *25*-triol acetic ester (2)

A solution of 3*β*, 12*β*-diacetyl-20 (*S*)-protopanaxadiol (0.83 g, 1.53 mmol) in dichloromethane (20 ml) was cooled to -3°C. Then a solution of *m*-CPBA (0.52 g, 3.06 mmol) in dichloromethane (10 ml) was added slowly and stirred for 2 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 and yield a white solid. The residue was chromatographed over silica gel (1:1 petroleum ether-ethyl acetate) and crystallized from ethyl acetate to get white needle-like crystal (0.66 g, 76.6% yield, m.p 191-192°C). ESI-MS, *m/z*: 561.[M + H]$^+$, 501.41 [M - CH_3COOH + H]$^+$, 407.42 [M - $2CH_3COOH$ + H]$^+$。1H-NMR ($CDCl_3$) δ 0.83 (s, 3H*2), 0.86 (s, 3H), 0.93 (s, 3H), 0.99 (s, 3H), 1.11 (s, 3H), 1.62 (s, 3H), 1.69 (s, 3H), 2.20 (s, 3H*2), 3.64(m, 3H).

Synthesis of 20*S*, 24R-epoxy-dammarane-*3β*, *12β*, *25*-triol (3) and 20*S*, 24S-epoxy-dammarane-*3β*, *12β*, *25*-triol (4)

Potassium hydroxide (0.2g, 3.54mmol) was added to the solution of 20*S*, 24-epoxy-dammarane-*3β*, *12β*, *25*-triol acetic ester 2 (0.66 g, 1.18 mmol) in methanol (10 ml). The resulting mixture was stirred at room temperature for 2 h. The solvent was diluted with water (200 ml). After filtration, the solid was washed with water, and dried in vacuo. The residue was chromatographed over silica gel (ethyl acetate) and crystallized from ethyl acetate yielded compound 3 as white pellet-like crystal (0.25g, 45.2% yield, m.p 167-169°C), and acetone yielded compound 4 as white lump-like crystal (0.17 g, 30.6% yield, m.p 224-225°C).

Compound 3: ESI-MS, m/z: 953.8 [2M + H]$^+$, 477.3 [M + H]+, 459.3 [M - H_2O + H]$^+$. 1H-NMR ($CDCl_3$) δ 0.78(s, 3H), 0.88(s, 3H), 0.91 (s, 3H), 0.97 (s, 3H), 1.01 (s, 3H), 1.10 (s, 3H) , 1.24 (s, 3H) , 1.27 (s, 3H) ,2.20 (s, 3H*2), 3.19 (t, 1H), 3.54 (td, *J*=10.12Hz, 1H), 3.89 (t, 1H).

Compound 4: ESI-MS, m/z: 477.41 [M + H]$^+$, 441.39 [M - $2H_2O$ + H]$^+$. 1H-NMR ($CDCl_3$) δ 0.76(s, 3H), 0.85(s, 3H), 0.89 (s, 3H), 0.96 (s, 3H), 0.97 (s, 3H), 1.09 (s, 3H) , 1.26 (s, 3H) , 1.27 (s, 3H) ,2.20 (s, 3H*2), 3.17 (t, *J*=4.88Hz , 1H), 3.50 (td, *J*=10.48 Hz, 4.6Hz, 1H), 3.83 (t, *J*=5.80Hz , 1H).

The ^{13}C NMR data of compounds 1- 4 are shown in (Table 1). The crystal data of compounds 3 and 4 are illustrated in (Table 2). ORTEP representations are shown in Figures 1 and 2 together with the numbering scheme adopted. The X-ray crystal structure confirms the configuration of C-24 of two epimers as R-form (compound 3) and S-form (compound 4), respectively.

Biological activity

H9C2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 5% equine serum. When the cultures reached 80% confluence, cells were sub-cultured using trypsin and were seeded in 96-well culture plates at a density of 8×103~1×104 cells/well (control group). To induce oxygen and glucose-deprivation, the culture media were replaced with glucose-free Earle's solution of 100 µl for 30 min, and then added sodium dithionite ($Na_2S_2O_4$) with final concentration of 2 mmol/l. After 1 h, $Na_2S_2O_4$ solution were removed (model group), and the compound of interest were added with the final concentration of 1 µg/ml for 24 h at 37°C in a humidified 5% CO_2 incubator (20(S)-panaxadiol (compounds 3 and 4 group). After 24 h, 25 µl precooling 50% TCA (w/v) (final concentration of 10% TCA) was added into each well. Kept the mixtures at 4°C for 1 h, then discarded the fixing solution and washed the wells with deionized water for 5 times. After drying, 100 µl SRB was added into each well to stain the cells. After 10 min of staining, the staining solution was discarded, the wells were washed with 1% acetic acid for 5 times and the samples were dried in the air. At the last step, bound stain is solubilized with 150 µl Tris solution in each well so as to prepare samples for cell density measurement (OD 540) and the cell viability was calculate (Zhu et al., 2009; Jian et al., 2009; Yu et al., 2010):

Cell viability = OD/OD(control group)

RESULTS

Chemistry

Compounds 3 and 4 were prepared as shown in (Scheme 1). 20(S)-panaxadiol was converted to the intermediate 1 with *N*, *N*-dimethylamino-pyridine and acetic anhydride in pyridine.

Through epoxidation of 1 with meta-chloroperbenzoic acid (m-CPBA), the compound 2 was obtained. Then 2 was degradated with potassium hydroxide to give compounds 3 and 4.

Biological activity

The synthesized compounds 3 and 4 were evaluated for their protective effect on cultured myocardiocytes in anoxia/reoxygen injury. The results reported in Figure 3 showed that compound 3 exhibited potent protective effect on cardiac muscle cells apoptosis, which was superior to 20(S)-panaxadiol. And the epimer 4 had no this pharmaceutical activity, but contain some cytotoxity.

DISCUSSION

Compounds 3 and 4 had been designed and synthesized. Compound 3 had been proved to have protective effect on cultured myocardiocytes with anoxia/reoxygen injury and the epimer 4 had none of this activity. Maybe the protective effect is related to the form of C-24.

Further studies of the pharmaceutical activity, structure-activity relationship of ocotillol-type derivatives are underway.

Table 1. ^{13}C NMR data of compounds 1~4 ($CDCl_3$, δ ppm).

No.	1	No.	2	No.	3	No.	4
1	38.5	1	38.5	1	38.9	1	38.9
2	23.5[a]	2	23.6	2	28.5	2	23.6
3	80.5	3	80.6	3	78.8	3	78.8
4	37.8	4	37.9	4	38.8	4	37.9
5	55.9	5	55.9	5	55.9	5	55.9
6	18.1	6	18.1	6	18.2	6	18.1
7	34.5	7	34.4	7	34.8	7	34.4
8	39.7	8	39.6	8	39.7	8	39.6
9	49.9	9	49.6	9	50.4	9	49.6
10	37.0	10	37.0	10	37.1	10	37.0
11	28.3	11	28.4	11	31.1[a]	11	28.4
12	79.5	12	75.5	12	70.9	12	70.6
13	44.9	13	46.4	13	49.3	13	46.4
14	52.6[d]	14	52.4	14	51.9	14	52.4
15	31.4	15	30.9	15	32.6	15	30.9
16	27.1	16	27.6	16	27.4	16	27.6
17	52.9[d]	17	50.2	17	47.9	17	50.2
18	15.6	18	15.5[a]	18	15.3	18	15.5[a]
19	16.2[e]	19	17.4[a]	19	16.3	19	17.4[a]
20	73.6	20	85.5	20	86.4	20	87.2
21	26.2	21	26.9	21	27.5	21	26.9
22	36.1	22	39.8	22	31.3	22	39.8
23	22.2[a]	23	24.0	23	24.9	23	24.0
24	125.2	24	84.5	24	85.3	24	87.4
25	131.2	25	70.5	25	70.0	25	69.9
26	25.7	26	25.7	26	27.9	26	25.7
27	17.6[e]	27	22. 8	27	26.1	27	22.8
28	27.9	27	27.9	28	27.9	28	27.9
29	16.4[e]	29	16.0[a]	29	15.2	29	16.0[a]
30	16.4[e]	30	16.5[a]	30	18.1	30	16.5[a]
3-1’	170.7[c]	3-1’	170.8[a]				
3-2’	21.5[b]	3-2’	21.8[b]				
12-1’	169.5[c]	12-1’	170.5[a]				
12-2’	21.2[b]	12-2’	21.2[b]				

Table 2. Crystal data for compounds 3 and 4.

Parameter	3	4
Crystal size /mm^3	0.46×0.38×0.28	0.46×0.38×0.28
Formula weight	476.72	476.72
Crystal system	Orthorhombic	Orthorhombic
Space group	*P*2 (1) 2 (1) 2 (1)	*P*2 (1) 2 (1) 2 (1)
a/nm	0.73964 (11)	0.76752 (11)
b/nm	1.3960 (2)	1.30682 (19)
c/nm	2.7212 (4)	2.8070 (4)
α/(°)	90	90
β/(°)	90	90
γ/(°)	90	90
V/nm^3	2.8096 (7)	2.8154 (7)

Table 2. Contd.

D_c/(g·cm^{-1})	1.127	1.125
F (000)	1056	1056
Absorportion coefficiention/mm^{-1}	0.072	0.072
Θ for data collection/(°)	1.50~25.50	2.13~25.50
Final R indices	0.0542	0.0678
wR_2	0.1201	0.1315
S	1.036	1.036

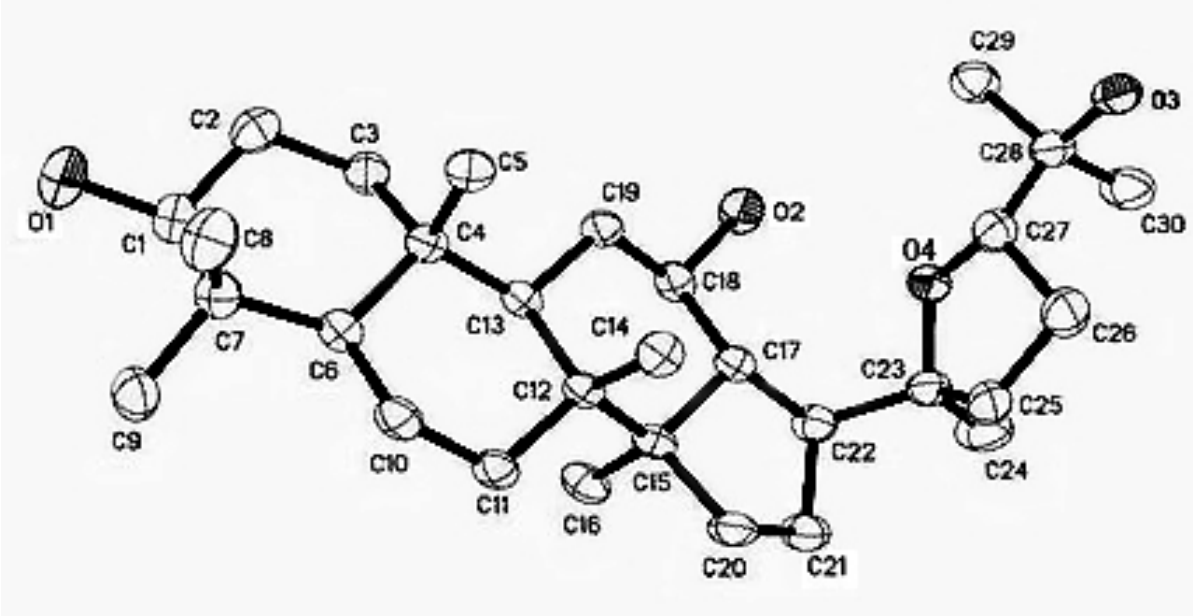


Figure 1. ORTEP of 3 with thermal ellipsoids shown at 30% probability.

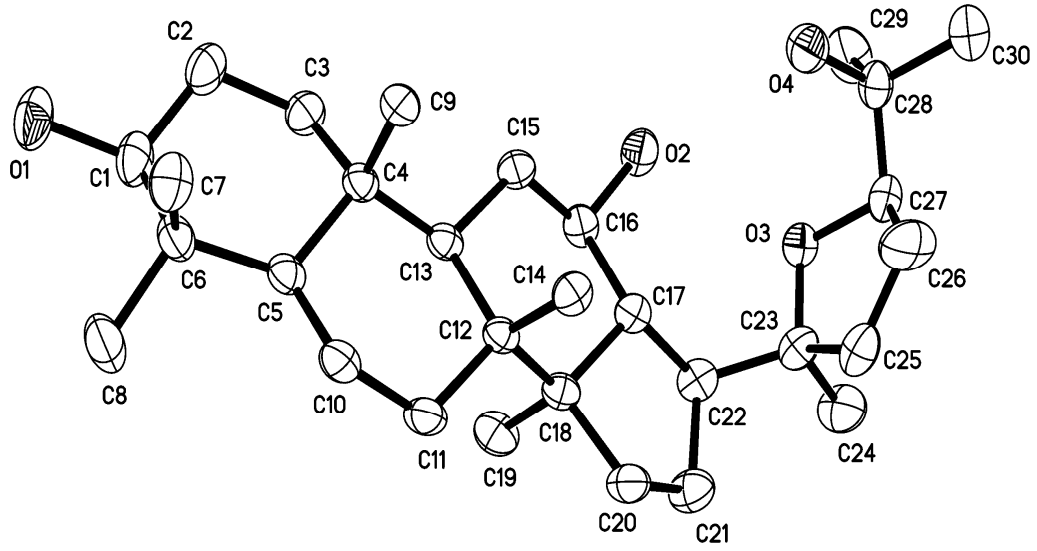
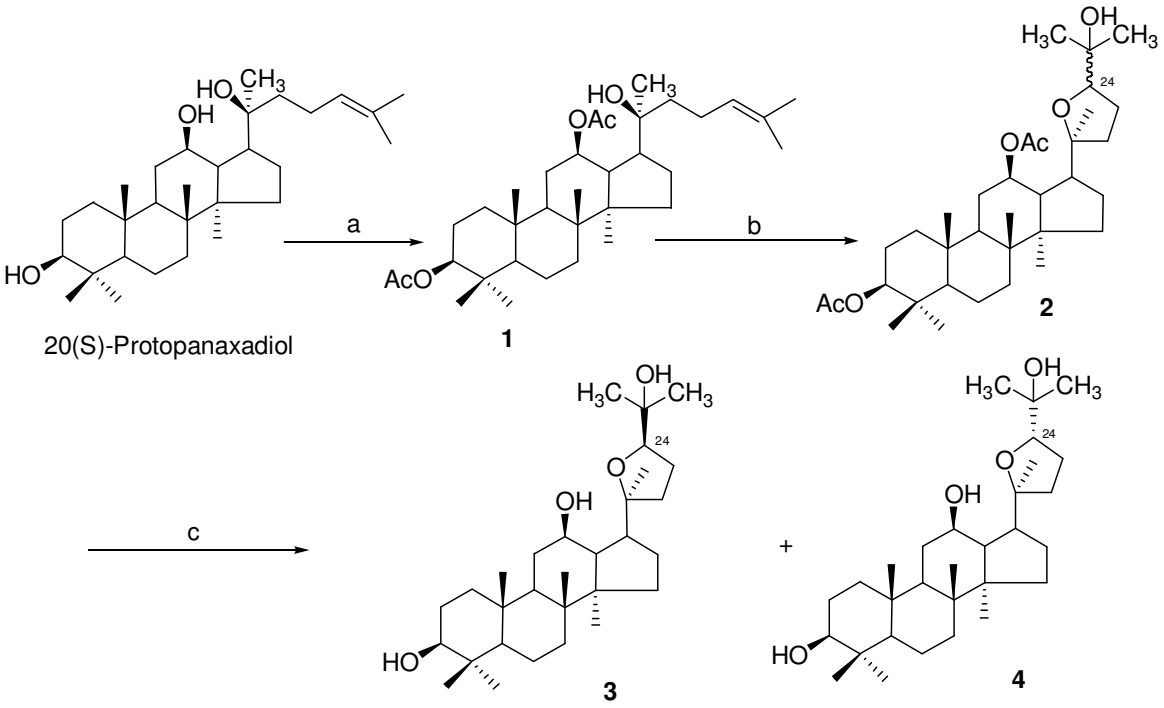


Figure 2. ORTEP of 4 with thermal ellipsoids shown at 30% probability.



Scheme 1. synthetic route for the preparation of the two epimers. Reagents: a) $(CH_3CO)_2O$, DMAP. Pyridine; b) *m*-CPBA, CH_2Cl_2; c) KOH, CH_3OH, H_2O.

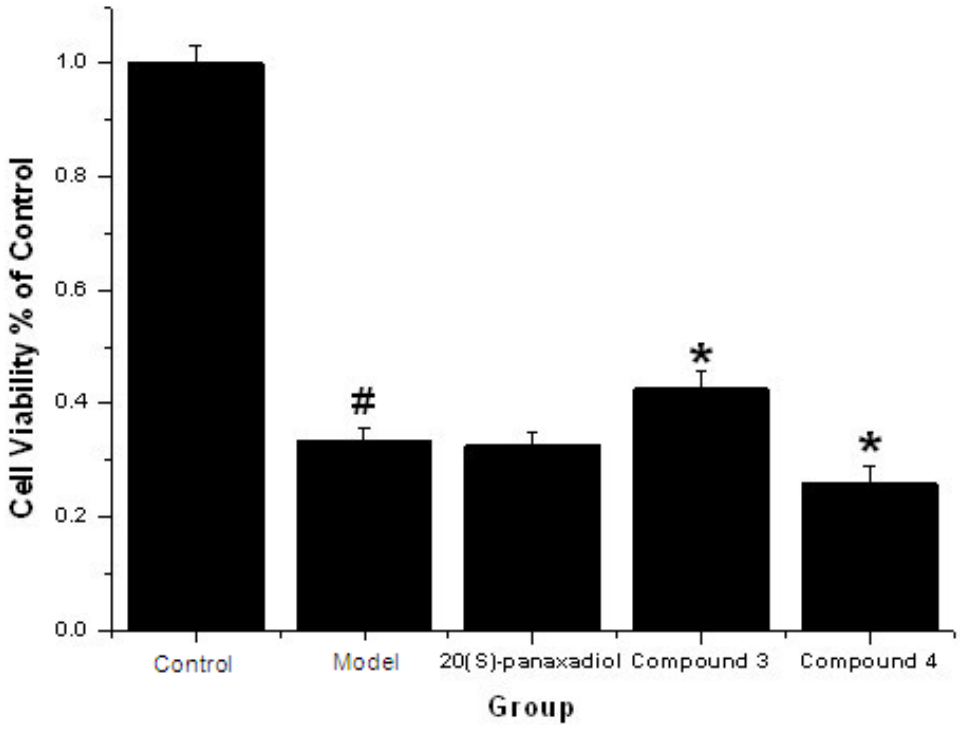


Figure 3. Protective Activity of 20(S)-panaxadiol, compounds 3 and 4 on cultured anoxia/reoxygen injury myocardiocytes Data was expressed as the mean ± S.D. (n = 6 to 8), Statistical significances were determined using unpaired two-tailed Student t-test or one-way analysis of variance (ANOVA) followed by Dunnett's contrast. # $p<0.01$ compared with control group, *$p<0.01$ compared with model group.

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