Synthesis, Structural Determination of a New Ocotillol Derivative and its Epimer

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Received April 19, 2010: Revised September 14, 2010: Accepted December 06, 2010

Abstract Epimeric 20*S*, 24-epoxy-dammarane-*3β*, *6α*, *12β*, *25*-tetraol acetic ester was synthesized from 20(*S*)-protopanaxatriol in the presence of acetic anhydride and the product oxidated by *m*-CPBA. 20*S*, 24R-epoxy dammarane-3*β*, 6*α*, 12*β*, 25-tetraol (ocotillol derivative) and its epimer were synthesized by saponification in the presence of sodium hydroxide in 1:1 molar ratio. The structures of the two compounds were characterized by X-ray diffraction method. The results showed the configuration of C-24 of two epimers as S-form (**4**, ocotillol derivative) and R-form (**3**, epimer), respectively.

Keywords: Ocotillol, derivative, epimer, synthesis, structure determination.

INTRODUCTION

Both *Panax ginseng* and *Panax quinquefolium*, belonging to the Araliaceae, are well-known traditional medicinal herbs. They are used as tonics for the treatment of diseases, such as tumor and myocardial ischemia. *Panax ginseng* contains a number of saponins, called ginsenoside, including an oleanolic acid-type saponin in addition to the major protopanaxadiol and protopanaxatriol-type saponins [1]. However, *Panax quinquefolium* contains an ocotillol-type (20*S*, 24*R*-epoxyside) saponin with high anti-tumor activity [2], as well as oleanolic acid-type saponin, protopanaxadiol and protopanaxatriol-type saponins. Yu *et al*. have found that ocotillol, 20*S*, 24*S*-epoxy-dammarane-3*β*, 6*α*, 12*β*, 25-tetraol, possesses cardioprotective effect on myocardial injury induced by isoproterenol in rats [3]. However, ocotillol can only be degraded and isolated from *Panax quinquefolium L*. in poor yield. Therefore, to obtain the ocotillol-type saponin by modifying protopanaxatriol and protopanaxadiol-type saponins is an interesting study and has uses in diseases especially tumor and myocardial ischemia. Based on this, herein, we report the synthesis and characterization of ocotillol derivative **4** and its epimer **3** derived from 20(*S*)-protopanaxatriol. Epimeric acetic 20*S*, 24-epoxy-dammarane-*3β*, *6α*, *12β*, *25*-tetraol ester, **2**, was synthesized by reaction of 20 (*S*)-protopanaxatriol and acetic anhydride in pyridine at room temperature and oxidation of the resulting **1** using *m*-CPBA.

20*S*, 24S-epoxy dammarane-3*β*, 6*α*, 12*β*, 25-tetraol (ocotillol derivative) and its epimers are prepared by reaction of epimeric 20*S*, 24-epoxy-dammarane-*3β*, *6α*, *12β*, *25*-tetraol acetic ester **2** and sodium hydroxide in methanol and water in 1:1 molar ratio (Scheme **1**). The structures of ocotillol derivative and its epimer were confirmed by ESI-MS, 1H NMR, 13C NMR, ESI-MS, HSQC and/or HMBC, DEPT 90, DEPT 135 and X-ray diffraction. According to the literature, the difference of 20 (S)- and 20 (R)- isomer in ocotillol-type saponin may be observed from the carbon signal of C-21 (S: $\delta 28\pm1$; R: $\delta 20\pm1$) in 13C NMR spectra [4], The results showed that the configuration of C-24 of two epimers as S-form (**4**, ocotillol derivative) and R-form (**3**, epimer), respectively. Single crystals of **3** and **4** were obtained from acetone, and X-ray crystallography clearly showed that 20, 24-epoxy fraction and a pair of epimers had been synthesized [5]. The crystal data are illustrated in Tables **1** to **3**. ORTEP representations are shown in Figs. (**1** and **2**) together with the numbering scheme adopted. Hydrogen bonds for **3** and **4** were illustrated in Fig. (**3**). The X-ray crystal structure confirms the configuration of C-24 of two epimers as S-form (**4**, ocotillol derivative) and R-form (**3**, epimer), respectively.

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SYNTHESIS

Synthesis of 3*β*, 6*α*, 12*β*-triacetyl-20 (*S*)-protopanaxatriol (1)

To a solution of 20 (*S*)-protopanaxatriol (7.2 g, 2.646 mmol), *N*, *N*-dimethylaminopyridine (0.192 g, 1.57 mmol) and pyridine (90 mL) in a round-bottomed flask, acetic anhydride (8.64 mL, 7.52 mmol) was added and the reaction was stirred for 24 h at room temperature. The solution was evaporated to dry under pressure, and the resulting solid was extracted with ethyl acetate. The organic phase was washed with dilute hydrochloric acid, water and saturated sodium chloride solution and dried over Na_2SO_4. The ethyl acetate was evaporated in vacuo, yielding a semi-solid. Flash chromatography (1:1 EtOAc-petroleum ether) yielded the product as colorless semi-solid (4.59 g, 57.63% yield). ESI-MS, *m/z*: 585.5 $[M\text{-}H_2O+H]^+$, 525.48 $[M\text{-}H_2O\text{-}CH_3COOH+H]^+$, 465.44 $[M\text{-}H_2O\text{-}2CH_3COOH+H]^+$, 405.40 $[M\text{-}H_2O\text{-}3CH_3COOH+H]^+$. Compared to spectra data of 20

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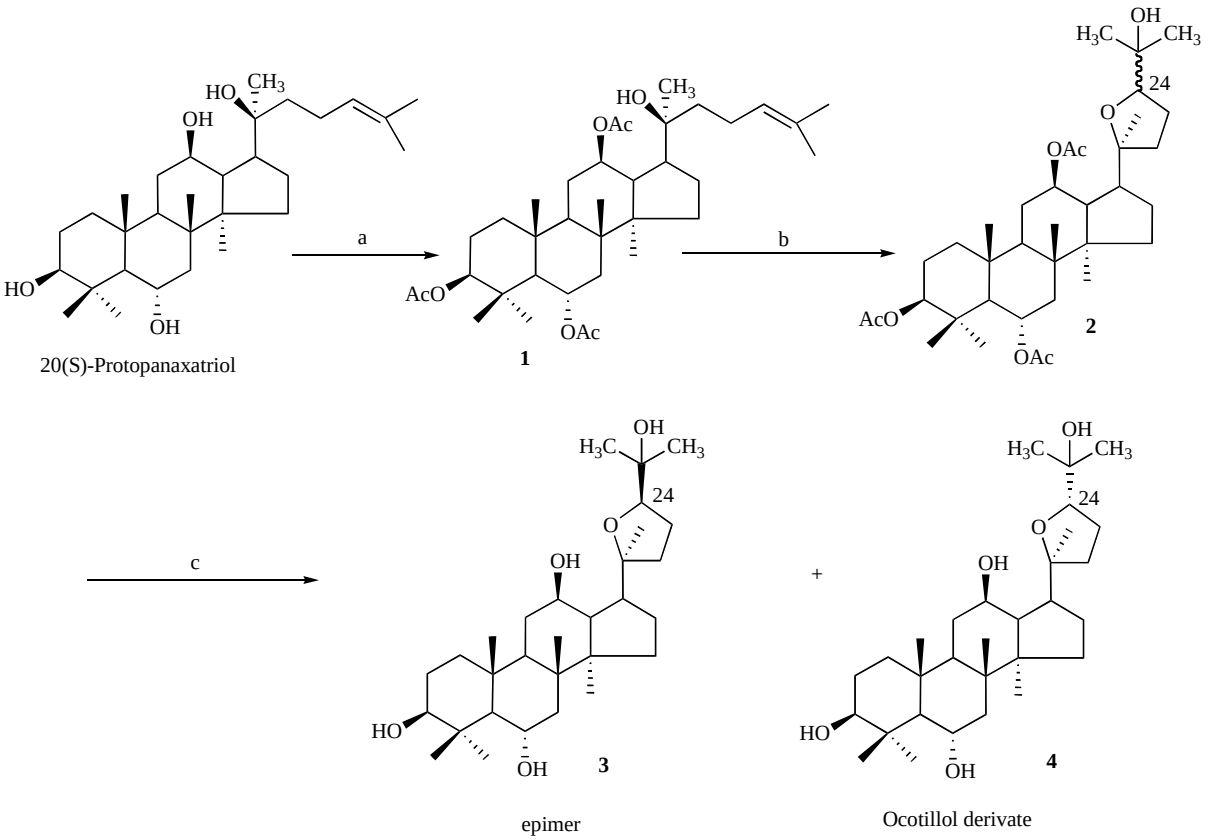

Scheme 1. synthetic route for the preparation of the two epimers. Reagents: a) $(CH_3CO)_2O$, DMAP. Pyridine; b) *m*-CPBA, CH_2Cl_2; c) NaOH, CH_3OH, H_2O.

Table 1. Selected Crystal Data for Compound 3 and 4

Parameter	3	4
Empirical formula	$C_{30}H_{52}O_5$	$C_{30}H_{52}O_5$
Formula weight	492.72	492.72
Crystal size /mm^3	0.54×0.50×0.50	0.38×0.20×0.18
Crystal system	Orthorhombic	Orthorhombic
Space group	*P*2 (1) 2 (1) 2 (1)	*P*2 (1) 2 (1) 2 (1)
a/nm	1.27918 (6)	1.29341 (18)
b/nm	1.37842 (7)	1.37677 (19)
c/nm	1.60902 (8)	1.6074 (2)
α/(°)	90	90
β/(°)	90	90
γ/(°)	90	90
V/nm^3	2.8371 (2)	2.8623 (7)
D_x/($g·cm^{-1}$)	1.154	1.143
F (000)	1088	1088
Absorportion coefficiention/mm^{-1}	0.076	0.075
Θ for data collection/(°)	1.95~26.02	1.95~25.50
Final *R* indices	0.0416	0.0528
wR_2	0.1107	0.0991
S	1.046	0.979

Table 2. Hydrogen-Bonds Geometry for 3 (Å,˚)

D-H...A	D-H	H...A	D...A	D-H…A
O(2)-H(2)...O(5)	0.82	1.95	2.697(2)	152.1
O(1)-H(1A)...O(2)	0.82	2.41	2.882(3)	117.4

Symmetry code: x, y, z+1.

Table 3. Hydrogen-Bonds Geometry for 4 (Å,˚)

D-H...A	d (D-H)	d (H...A)	d (D...A)	D-H…A
O2-H2...O5 [i]	0.82	1.90	2.693 (3)	163.7
O1-H1*A*...O2 [ii]	0.82	1.95	2.763 (2)	169.6
O2-H2*A*...O4	0.82	1.87	2.671 (2)	166.0
O1-H1...O3[iii]	0.82	2.04	2.858 (2)	172.2

Symmetry codes: (i) x, y, z +1; (ii) -x, y-1/2, -z +3/2; (iii) x-1/2, -y +1/2, -z +1.

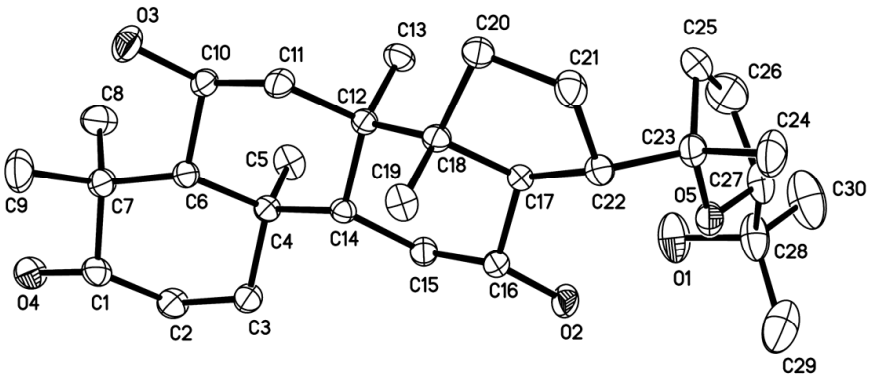

Fig. (1). ORTEP of **3** with thermal ellipsoids shown at 30% probability.

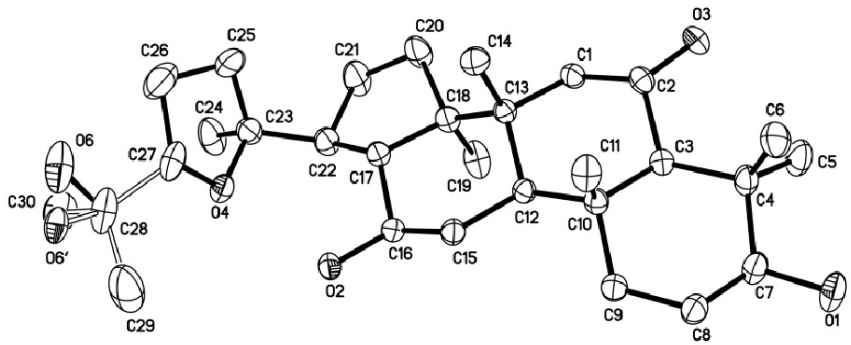

Fig. (2). ORTEP of **4** with thermal ellipsoids shown at 30% probability.

(*S*)-protopanaxatriol reported in literature [6], the assignments based on 1H NMR and 13C NMR data showed that hydroxyls connected to C-3, C-6, C-12 had been acetylated, 20 (S)-hydroxyl had not been acetylated at all.

Synthesis of epimeric 20*S*, 24-epoxy-dammarane-*3β, 6α, 12β, 25*-tetraol acetic ester (2)

A mixture of 3*β*, 6*α*, 12*β*-triacetyl-20 (*S*)-protopanaxatriol (3.830 g, 6.362 mmol) in methylene chloride was cooled to -3°C, and then *m*-CPBA was

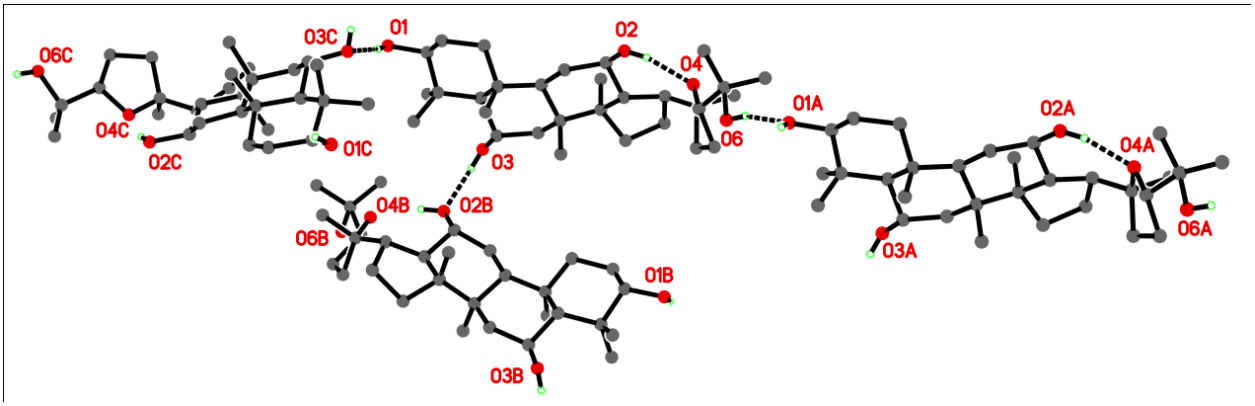

Fig. (3). Hydrogen bonds for **3** and **4** (dashed lines).

(dissolved in 10 mL methylene chloride) added slowly. After 2 h, the organic solution was washed with water and saturated sodium chloride solution and dried over Na_2SO_4. The CH_2Cl_2 was evaporated in vacuo, yielding a white solid. Flash chromatography (1:1 EtOAc-petroleum ether) and crystallization from EtOAc yielded the product as white needle-like crystal (3.20 g, 81.38% yield). mp 205~206 ^oC. ESI-MS, *m/z*: 619.54 $[M+H]^+$, 559.50 $[M-CH_3COOH+H]^+$, 499.45 $[M-2CH_3COOH+H]^+$, 481 $[M-2CH_3COOH-H_2O+H]^+$, 439.40 $[M-3CH_3COOH+H]^+$, 421.39 $[M-3CH_3COOH-H_2O+H]^+$. Chemical shift values of C-24, C-25 in compounds **1** and **2** were quite different [7], showing that C-24 and C-25 of compound **2** were connected to hydrogen atom, and C-24 and C-25 of compound **2** were alkene atom.

Synthesis of 20*S*, 24S-epoxy-dammarane-*3β, 6α, 12β, 25*-tetraol (4) and 20*S*, 24R-epoxy-dammarane-*3β, 6α, 12β, 25*-tetraol (3)

The epimeric 20*S*, 24-epoxy-dammarane-*3β*, *6α*, *12β*, *25*-tetraol acetic ester **2** (2.480 g, 4.53 mmol) and methanol 24 ml were stirred at room temperature, and pH was adjusted 12 with 1 mol/L potassium hydroxide solution. After 2 h, the resulting mixture was diluted with water 200 mL, the precipitate formed and filtered off, washed with water, and dried in vacuo. Flash chromatography (EtOAc) and crystallization from acetone yielded compound **3** as white pellet-like crystal (0.80 g, 34.90% yield), mp 249~250 ^oC and compound **4** as white lump-like crystal (0.778 g, 34.62% yield), mp 263~264 ^oC.

ACKNOWLEDGEMENTS

The authors are grateful to the Faculty of Pharmacy of Yantai University, National Natural Science Foundation of China (No.81001358), 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. The authors thank Mr. Liandong Liu (School of Chemistry, Shandong Normal University, Jinan, 250014 P R China) for his invaluable support in X-ray data collection. We also gratefully acknowledge Mr. Jiyong Chen and Ms. Li Shen for the measurement of ESI-MS and NMR.

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[5] Data collection

Bruker SMART CCD area-detetor diffractometer	4474 independent reflections
Ψ and ω scans	4355 reflections with $I > 2\sigma(I)$
Absorption correction: multi-scan	R_{int}=0.026
(*SADABS*; Breker, 2000)	θ_{max}= 25.0˚
T_{min}=0.513, T_{max}=0.872	h= -12 → 13
8908 measured reflections	k= -12 →14
	l= 0 → 14
Refinement	
Refinement on F^2	H-atom parameters constrained
$R[(F^2>2\sigma(F^2)]$=0.100	w= $1/[\sigma^2(F_o^2) + 102.4837P]$
$wR(F^2)$=0.226	where $P = (F_o^2 + 2F_c^2)/3$
S = 1.15	$(\Delta/\sigma)_{max}$< 0.001
4474 reflections	$\Delta\rho_{max}$ = 2.49e Å $^{-3}$
483 parameters	$\Delta\rho_{min}$ = -1.23e Å $^{-3}$

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[7] C-24: 125.0 (**1**), 85.5 (**2**); C-25: 131.2 (**1**), 70.2 (**2**).