Synthesis and Crystal Structures of 3,6-diacetylated C24 epimeric 20(*R*)-Ocotillol-Type Saponins

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Summary: (20*R*,24*R*)-epoxy-dammar-3β,6α,12β,25-tetraol (**1**) and (20*R*,24*S*)-epoxy-dammar-3*β*, 6*α*,12*β*,25-tetraol (**2**) have been synthesized from 20(*R*)-protopanaxatriol with same polarity. In order to obtain optically pure **1** and **2**, (20*R*,24*R*)-3,6-diacetyl-20,24-epoxydammar-3*β*,6*α*,12*β*,25-tetraol (**3**) and (20*R*,24*S*)-3,6-diacetyl-20,24-epoxydammar-3*β*,6*α*,12*β*,25-tetraol (**4**) were designed and prepared to enhance the polarity difference of C24 epimers. Two suitable crystals of **3** and **4** were obtained and their structures were determined by ^{1}H NMR, ^{13}C NMR, HR-MS and X-ray single-crystal diffraction. The results indicated that the C-24 configuration of **3** and **4** are *R*-form and *S*-form, respectively. **3** has two intramolecular hydrogen bond. While there is only one in **4** and the crystal stacking displays that it generates a right-handed helically chiral channel viewing from the crystallographic b axis via classical O–H···O intermolecular hydrogen bond.

Keywords: 20(*R*)-protopanaxatriol; Ocotillol-type saponin; Epimer; Synthesis; Crystal structure.

Introduction

Ocotillol-type saponins share a tetrahydrofuran ring as the tetracyclic triterpenoids, which is rare existing in Panax quinquefolius L., Panax japonicus, Hana mina, and Vietnamese ginseng [1]. The previous study indicated that ocotillol-type saponins possessed neuroprotective effect [2], antimyocardial ischemia [3], anti-inflammatory [4], antibacterial [5] and antitumor activities [6].

In our previous study, the C24 epimeric ocotillol-type saponins were gained from 20(*S*)-Protopanaxadiol (20(*S*)-PPD), 20(*S*)-Protopanaxatriol (20(*S*)-PPT), 20(*S*)-Ginsenoside Rh1 (20(*S*)-Rh1), 20(*S*)-Ginsenoside Rg2 (20(*S*)-Rg2), 20(*S*)-Ginsenoside Rh2 (20(*S*)-Rh2) and 20(*S*)-Ginsenoside Rg3 (20(*S*)-Rg3), their cardioprotective effects were evaluated, and results showed that 24(*R*)-ocotillol type saponins exhibited significantly stronger myocardial protective effects than their corresponding 24(*S*)-epimers and ginsenosides, demonstrating distinct stereoselective activity [7-11].

In addition, the cytotoxicity of **1** and **2** was evaluated, **1** exhibited relatively better performance against A549 cell than **2**, and also indicated apparent stereoselectivity as a result of stereo-configuration at the C24 position [12]. It had been found that **1** could be achieved from 20(*R*)-pseudoginsenoside F11, but the yield was extremely lower so that 18mg/1kg 20(*R*)-pseudoginsenoside F11 was isolated from red American ginseng [13]. Moreover, the existence of 20(*R*)-ocotillol type saponins in nature is fewer than other saponins. In order to scale up prepare **1** and **2** for further pharmacological activity study, it's essential to establish optimizing chemical method to prepare 20(*R*)-ocotillol type saponins.

In this paper, two suitable crystals of (20*R*,24*R*)-3,6-diacetyl-20,24-epoxydammar-3*β*,6*α*,12*β*,25-tetraol (**3**) and (20*R*,24*S*)-3,6-diacetyl-20,24-epoxydammar-3*β*,6*α*,12*β*,25-tetraol (**4**) were obtained. And their stereo-configurations were confirmed by X-ray single-crystal diffraction. The synthetic route is showed as Scheme-1. The synthetic procedure and crystal structure characteristics are described as below.

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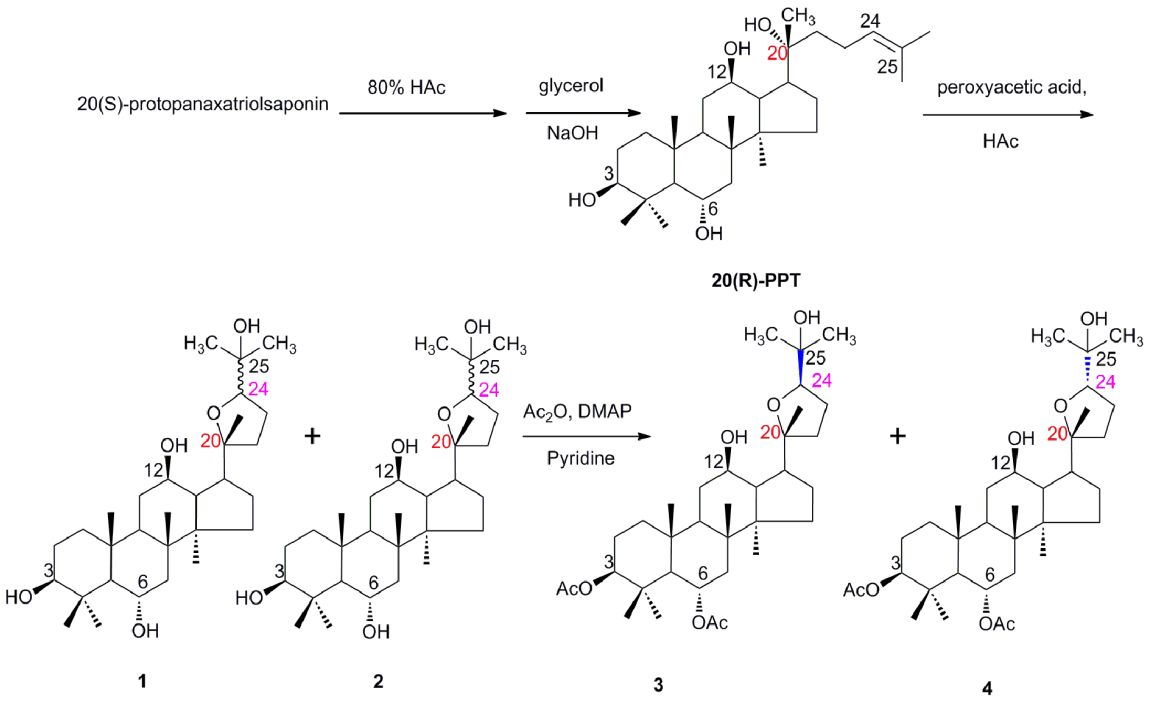

Scheme-1: The synthetic routes of **3** and **4.**

Experimental

Synthesis and crystallization

Preparation of 20(R)-PPT

The natural protopanaxatriol saponins (40.0 g) were dissolved with 80% acetic acid (400 ml) and stirred vigorously at 60 ºC for 3 h. Glycerol (250 ml) and sodium hydroxide (40.0 g) were mixed with the crude product above and heated at 180 ºC for 12 h. After cooled to room temperature, the residue was diluted with water (300 mL) and extracted with ethyl acetate (800 mL). The organic layer was washed with water and brine, and dried over sodium sulfate. After filtration, the ethyl acetate was concentrated in vacuo, yielding a brown solid. Purified by silica gel column chromatography (petroleum ether/ethyl acetate, 4/1, *v/v*) and recrystallized from ethyl acetate to afford white solid (2.5 g).

Synthesis of (20R,24R)-20,24-epoxydammar-3β,6α, 12β,25-tetraol (1) and (20R, 24S)- 20,24-epoxydammar-3β,6α,12β,25-tetraol **(2)**

20(*R*)-protopanaxatriol (20(*R*)-PPT) (2.005 g, 4.198 mmol) was added to 140 mL acetic acid. Then peroxyacetic acid (0.83 mL, 12.594 mmol) was added slowly and stirred at -4 ºC for 1.5 h, isopropanol (1.0 mL, 12.967 mmol) was added and stirred for another 30 min. The organic phase was washed with saturated sodium bicarbonate solution, water and brine, respectively and dried over sodium sulfate. The organic phase was evaporated in vacuo, white solid product was yielded. After silica gel column chromatography purification (petroleum ether/ethyl acetate, 3/1, *v/v*), mixed **1** and **2** (1.798 g) was yielded.

Synthesis of (20R,24R)-3,6-diacetoxy-20,24-epoxydammar-12β,25-diol (3) and (20R,24S)-3,6-diacetoxy-20,24-epoxydammar-12β,25-diol **(4)**

Acetic anhydride (3.5 ml, 31.662 mmol) were added to mixture of **1** and **2** (1.5 g, 3.046 mmol) in pyridine (30 ml) and stirred overnight at room temperature. The solution was evaporated in vacuo and the residue was extracted with ethyl acetate. The organic phase was washed with dilute hydrochloric acid, water and brine, respectively and dried over anhydrous sodium sulfate. Then it was evaporated in vacuo. After silica gel column chromatography purification (1:10 ethyl acetate-petroleum ether), **3** and **4** was obtained as white solid. In addition, **3** was crystallized in ethyl acetate as columnar crystal (0.752 g) in yield 43.0%, m.p.: 484-485 K., **4** was obtained as white granular crystal (0.749 g) in

yield 42.8% by crystallization in acetone, m.p.: 534-535 K.

Analytical data for **3**

Compound **3**: m.p. 484-485 K. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.35 (td, 1H, *J* =10.5, 4.5 Hz, H-6), 4.46 (dd, 1H, *J* =11.5, 5.2 Hz, H-3), 3.89 (t, 1H, *J* =7.3 Hz, H-24), 3.57 (td, 1H, *J* =10.3, 5.2 Hz, H-12), 2.05 (s, 3H, 34-CH_3 or 32-CH_3), 2.03 (s, 3H, 34-CH_3 or 32-CH_3), 1.23 (s, 3H, 29-CH_3), 1.19 (s, 3H, 26-CH_3), 1.13 (s, 6H, 27-CH_3 and 21-CH_3), 1.03 (s, 3H, 19-CH_3), 1.02 (s, 3H, 18-CH_3), 0.92 (s, 3H, 30-CH_3), 0.91 (s, 3H, 28-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.05, 170.28, 86.25, 85.44, 80.38, 71.13, 70.70, 70.26, 58.86, 51.50, 50.53, 49.48, 48.76, 42.64, 40.89, 39.44, 38.37, 38.17, 37.85, 31.37, 30.55, 30.42, 29.81, 27.34, 26.90, 25.53, 25.46, 23.40, 22.11, 21.38, 19.33, 17.30, 17.04, 16.91, 16.87. HR-MS calcd for $C_{34}H_{56}O_7$ $[M+H]^+$ 577.40946; found 577.40988.

Analytical data for **4**

Compound **4**: m.p. 534-535 K. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 5.36 (m, 1H, H-6), 4.46 (dd, 1H *J* = 11.1, 5.3 Hz, H-3), 3.86 (dd, 1H, *J* =9.4, 4.7 Hz, H-24), 3.55 (td, 1H, *J* =10.2, 5.0 Hz, H-12), 2.05 (s, 3H, 32-CH_3 or 34-CH_3), 2.04(s, 3H, 32-CH_3 or 34-CH_3), 1.24 (s, 3H, 29-CH_3), 1.20 (s, 3H, 26-CH_3), 1.13 (s, 3H, 27-CH_3), 1.12 (s, 3H, 21-CH_3), 1.03 (s, 6H, 18-CH_3 and 19-CH_3), 0.92 (s, 3H, 30-CH_3), 0.91 (s, 3H, 28-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.08, 170.28, 86.66, 86.42, 80.39, 70.71, 70.40, 70.35, 58.92, 51.54, 50.72, 49.60, 48.75, 42.67, 40.92, 39.44, 39.19, 38.40, 37.87, 31.44, 30.49, 30.42, 29.82, 28.03, 26.87, 26.02, 24.87, 23.41, 22.12, 21.48, 21.40, 17.36, 16.98, 16.86. HR-MS calcd for $C_{34}H_{56}O_7$ $[M+H]^+$577.40942; found 577.40988.

Results and Discussion

Synthesis and determination of the absolute configuration

20(*R*)-PPT was prepared from C20 epimeric protopanaxatriol saponins, which was transformed with 80% HAc. The raw material was purified over silica gel column and recrystallized from ethyl acetate. (20*R*,24*R/S*)-dammar-20,24-epoxy-3*β*,6*α*,12*β*,25-tetraol (**1**/**2**) were prepared from 20(*R*)-PPT by oxidation in HAc at -4 °C. Due to the same polarity, it's very difficult to obtain optically pure **1** and **2**, so **3** and **4** were designed and prepared to enhance the polarity difference of C24 epimers. After isolation over silica gel (petroleum ether:ethyl acetate=10/1), suitable crystals of **3** and **4** were obtained from ethyl acetate and acetone, respectively. Their structures were identified by HR-MS, ^{1}H NMR, ^{13}C NMR and X-ray single-crystal diffraction. The NMR data of **3** and **4** showed extremely high similarity except for the slightly differences in signals appearing at the side chain, exhibiting the similar characteristic in the structure, and both of record were similar to those of 20(*S*)-PPT, indicating that their structures were deemed to the ocotillol-type. The carbon signals of C21 at δ 20±1 ppm intimated the *R*-configuration at C20 for **3** and **4**, and the stereochemistry of C24 in 20(*R*)-ocotillol type saponins could be discriminated between carbon signals of C24(*S*: δ 86.66; *R*: δ 86.25). Finally, **3** was identified as (20*R*,24*R*)-3,6-diacetyl-20,24-epoxydammar-3*β*,6*α*,12*β*, 25-tetraol and **4** was (20*R*,24*S*)-3,6- diacetyl-20, 24-epoxydammar-3*β*,6*α*,12*β*,25-tetraol.

Crystal Packing Arrangements

Crystals **3** was crystallized via solvent evaporation in ethyl acetate. As shown in Fig. 1, X-ray single-crystal diffraction analysis shows that **3** crystallizes in a orthorhombic space group *P2(1)2(1)2(1)*, and asymmetric unit contains only one independent molecule. Two intramolecular hydrogen bond O(5)–H(5)···O(6) and O(7)–H(7)···O(6) are formed in a molecule (Table-2). The *C20–O4–C24–C25* torsion angle is –140.412 (310) °, which suggests that the C24 configuration is *R*-form.

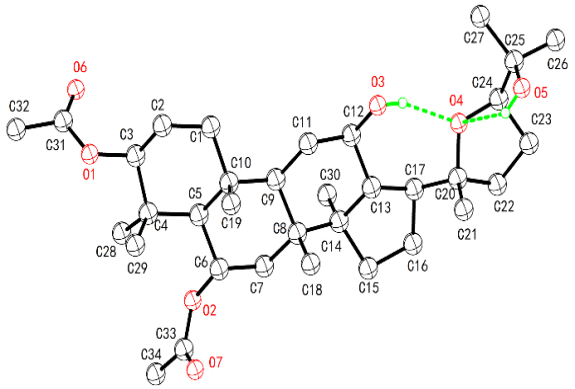

Fig. 1: A partial packing diagram for (**3**), showing the atom-labelling scheme. Dashed lines indicate hydrogen bonds. Displacement ellipsoids are drawn at the 50% probability level.

Table-1: Experimental details.

	3	4
	Crystal data	
Chemical formula	$C_{34}H_{56}O_7$	$C_{34}H_{56}O_7$
M_r	576.79	576.78
Crystal system, space group	Orthorhombic, $P2_12_12_1$	Orthorhombic, $P2_12_12_1$
Temperature (K)	153	173
a, b, c (Å)	6.5867 (13), 14.145 (3), 35.673 (7)	6.6068 (9), 13.5827 (19), 36.019 (5)
V (Å^3)	3323.6 (11)	3232.3 (8)
Z	4	4
Radiation type	Mo $K\alpha$	Mo $K\alpha$
μ (mm^{-1})	0.08	0.08
Crystal size (mm)	0.24 × 0.18 × 0.14	0.45 × 0.27 × 0.14
	Data collection	
Diffractometer	Bruker P4	*MM007*-HF CCD(Saturn 724+)
Absorption correction	–	Multi-scan *CrystalClear* (Rigaku Inc., 2007)
T_{min}, T_{max}	0.983,0.989	0.773, 1.000
No. of measured, independent and observed [I > 2σ(I)] reflections	64868, 6141, 4760	21078, 7420, 7083
R_{int}	0.042	0.039
(sin θ/λ)$_{max}$ (Å^{-1})	0.606	0.650
	Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.062, 0.199, 1.09	0.044, 0.105, 1.05
No. of reflections	6141	7420
No. of parameters	382	382
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement	H-atom parameters constrained
Δρ$_{max}$, Δρ$_{min}$ (e Å^{-3})	0.42, −0.30	0.25, −0.17
Absolute structure parameter	−0.6 (15)	0.3 (4)

Computer programs: Bruker *XSCANS*, *CrystalClear* (Rigaku Inc., 2007), Bruker *SHELXTL*, '*SHELXT* (Sheldrick, 2015)', *SHELXS97* (Sheldrick, 1990), *SHELXL* (Sheldrick, 2015), *SHELXL97* (Sheldrick, 1997), Olex2 (Dolomanov et al., 2009), CIFTAB (Sheldrick, 2013) [14-16].

Table-2: Hydrogen bond geometry (Å, º) for (**3**).

D–H···*A*	*D*–H	H···*A*	*D*···*A*	*D*–H···*A*
O(3)–H(3)···O(4)	0.82	1.94	2.72	160.0
O(5)–H(5)···O(4)	0.82	2.42	2.84	112.8

Table-3: Hydrogen bond geometry (Å, º) for (**4**).

D–H···*A*	*D*–H	H···*A*	*D*···*A*	*D*–H···*A*
O(3)–H(3)···O(4)	0.82	1.93	2.73	163.0
O(5)–H(5)···O(7)^{i}	0.82	2.10	2.92	174.6

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

In contrast to **3**, the crystal of **4** was grown in acetone. As shown in Fig. 2, though there is also one molecule in an asymmetric unit and it also crystallizes in a orthorhombic space group *P2(1)2(1)2(1)*. In **4**, the similar intramolecular hydrogen bond O(5)–H(5)···O(6) with **3** is included. The O(5)–H(5)···O(6) angle is 145.345(404)º (Table-3). The *C20–O4–C24–C25* torsion angle is 140.934 (665)º, indicating that the C24 position is *S*-form, which is different from **3**. As exhibited in Fig. 3, the molecules are a stretch of an infinite one-dimensional network with classical intermolecular O–H···O hydrogen bonding, atom H(5) on O(5) plays a donor role in the hydrogen-bond and atom O(7)^{i} plays an acceptor role in it. A right-handed helical chain was formed along the crystallographic b axis in the molecules (Fig. 4). The period of the helix and the translation vector in the column is equal to the crystallographic b axis (ca 13.6 Å). (Symmetry code: (i) −x, y+1/2, −z+3/2)

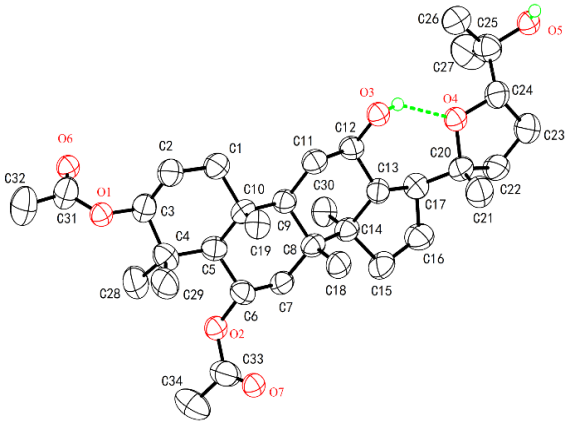

Fig. 2: A perspective view of (**4**), showing the atom-labelling. Dashed lines indicate hydrogen bonds. Displacement ellipsoids are drawn at the 50% probability level.

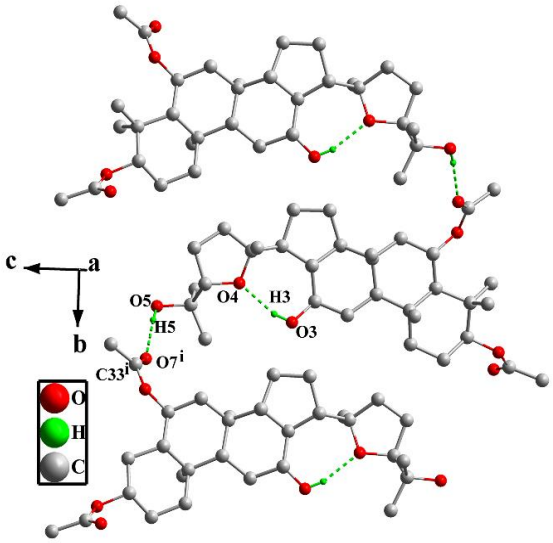

Fig. 3: A perspective view of (**4**). showing the formation of the one-dimensional chain. Dashed lines indicate hydrogen bonds. Symmetry codes: (i) i:-x,y+1/2,-z+3/2.

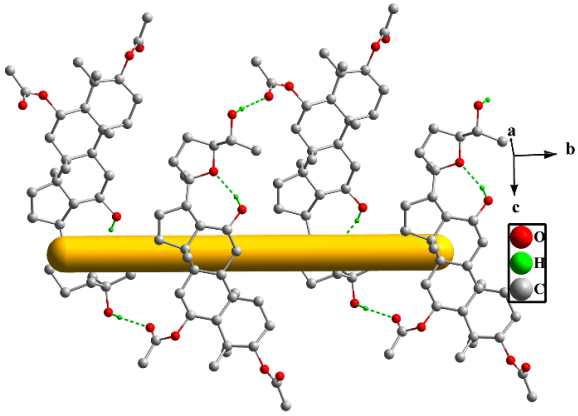

Fig. 4: The helical structure of the one-dimensional hydrogen-bonded chain of **4**. Dashed lines indicate hydrogen bonds.

Structurally, for **3**, the configuration of C24 is *R*-form, intramolecular O4–H5···O5 hydrogen bonds are easy formed due to the close distance between O(4) and H(5). But in **4**, it is difficult to create intramolecular O5–H5···O4 hydrogen bond. So, the broad-spectrum O(5)–H(5) hydrogen-bond donors can provide more opportunities for intermolecular interactions. It is well known that molecular polarity can be bound up with intramolecular hydrogen bonds. Therefore, **3** and **4** display different molecular polarity, it can be identified by R_f values in Petroleum ether:Ethyl acetate (3:1), which are 0.5 and 0.4, respectively.

Conclusion

Two C-24 epimers, (20*R*,24*R*)-3,6-diacetyl-epoxydammar-3*β*,6*α*,12*β*,25-tetraol (**3**) and (20*R*,24*S*)-3,6-diacetyl-epoxydammar-3*β*,6*α*,12*β*,25-tetraol (**4**), were synthesized and their structures were identified by HR-MS, ^{1}H NMR, ^{13}C NMR and X-ray single-crystal diffraction. Moreover, it is worth to make efforts to further study stereo pharmacological activity of **1** and **2**, and the original synthetic route discovered in this work facilitated the amply preparation problem.

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