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PROTOTANE-TYPE TRITERPENES FROM THE RHIZOMES OF *ALISMA PLANTAGO-AQUATICA*

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SUMMARY

*Three terpenes with Protostane type were isolated from the rhizomes of Alisma plantago-aquatica. The chemical structures of isolated compounds were characterized as 11β,23S,24R,25-tetrahydroxyprotost-13(17)-en-3-one (alisol A, **1**), 11β,23S,25-trihydroxyprotost-13(17)-en-3-one-24R-yl acetate (alisol A acetate, **2**), and 11β,23S,24S-trihydroxyprotost-13(17),25-dien-3-one (alisol G, **3**), by detailed analysis of the 1D- and 2D-NMR spectra such as ^{1}H-, ^{13}C-NMR, DEPT 90, DEPT135, HSQC, HMBC, ^{1}H-^{1}H COSY, and by the Electronspray Ionization (ESI) mass spectrum. This is the first report of alisol G from Alisma plantago-aquatica.*

I - INTRODUCTION

The dried rhizome of *Alisma plantago-aquatica* L. var. *orientalis* Samuelsson is a crude drug, and has been used as a folk medicine for diabetes and swellings [1]. From the phytochemical investigations including its physiological active principles, it was reported to contain protostane-type triterpenoids, e.g. alisol A and its 24-acetate, alisol B and its 23-acetate and alisol C and its 23-acetate [2 - 5] and many other components isolated from fresh rhizome *Alismatis orientale* and the crude drug Alismatis rhizoma of Japanese and Chinese origins [6]. Here, we report the isolation and structural determination of three terpenes with Protostane type as 11β,23*S*,24*R*,25-tetrahydroxyprotost-13(17)-en-3-one (alisol A, **1**), 11β,23*S*,25-trihydroxyprotost-13(17)-en-3-one-24*R*-yl acetate (alisol A acetate, **2**), and 11β,23*S*,24*S*-trihydroxyprotost-13(17),25-dien-3-one (alisol G, **3**) from the rhizomes of this plant.

II - EXPERIMENTAL

1. General experimental procedures

The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer using TMS as the internal standard. The Electronspray Ionization (ESI) mass spectrum was obtained using a AGILENT 1100 LC-MSD Trap spectrometer. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70 - 230 mesh and 230 - 400 mesh, Merck) or YMC RP-18 resins (30 - 50 μm, FuJisilisa Chemical Ltd). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F254 (Merck 1.05715) or RP18 F254s (Merck) plates.

2. Plant material

The rhizomes of *Alisma plantago-aquatica* L. var. *orientalis* Samuelsson were collected in Tam Dao Mountain, Vinh Phuc Province in January, 2006 and were identified by Dr Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnamese Academy of Science and Technology.

3. Extraction and isolation

Air-dried and powdered rhizomes of *Alisma plantago-aquatica* L. (6.0 kg) were extracted with methanol to get the residue (150 g), which was then suspended in water and extracted sequentially using hexane, chloroform and ethyl acetate to yield hexane (53 g), $CHCl_3$ (64 g), EtOAc (13 g) extracts, and water layer (20 g). Repeated chromatography of the $CHCl_3$ extract (64 g) on a silica gel or YMC column with the suitable solven systerms to get compounds **1** (250 mg), **2** (130mg) and **3** (54 mg) as white crystals.

III - RESULTS AND DISSCUSSION

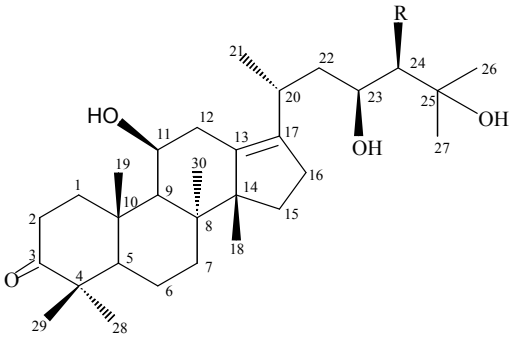
Compounds **1** - **3** were obtained as white crystals from the methanolic extract. The ^{1}H-NMR spectrum of **1** showed 7 singlets of the quaternary methyl groups (δ 1.00, 1.05, 1.06, 1.07, 1.13, 1.27, 1.21) and a doublet at δ 1.01 (3H, d, J = 7.0 Hz, H_3-21), three protons of the oximethine carbons at δ 3.88 (1H, ddd, J = 5.8, 10.7, 10.7 Hz, H-11), 3.76 (1H, d, J = 9.0 Hz, H-23) and 3.76 (1H, d, J = 9.0 Hz, H-24). All signals of the ^{1}H-NMR spectrum suggested that **1** is a triterpenoid. The ^{13}C-NMR and DEPT spectra of **1** exhibited the signals of 30 carbons including 8 methyl, 8 methylene, 6 methine and 8 quaternary carbons. The carbonyl group was assigned at δ 220.5, a double bond without olefinic protons was confirmed at δ 137.6 and 135.5, four carbons bearing oxygen atom including three oximethine at δ 69.4, 69.9, 77.6 and a quaternary carbons at δ 74.1. The side chain of **1** was connected from the spin-system of the ^{1}H-^{1}H COSY and was further confirmed by the long-range correlations in the HMBC spectrum as shown in table 1. All the NMR data suggested the Protostane type of **1** resembling those of alisol A. The hydroxyl group with β configuration was at C-11 confirmed by H-C long-range correlations between H-11 and C-13 (δ 137.6)/C-9 (δ 49.6)/C-10 (δ36.9) in the HMBC spectrum and by the spin-coupling of proton H-11 (δ 3.88, ddd, J = 5.8, 10.7, 10.7 Hz) [6]. The carbonyl group was assigned to C-3 from the cross peaks of protons H-23/H-24 and carbon C-3 in the HMBC spectrum. Furthermore, the ESI spectrum of **1** exhibited ion peaks at m/z 473 $[M\text{-}H_2O+H]^+$, 455 $[M\text{-}2H_2O+H]^+$, 437 $[M\text{-}3H_2O+H]^+$ and 419 $[M\text{-}4H_2O+H]^+$, corresponding to the molecular formula of $C_{30}H_{50}O_5$. Consequently, **1** was identified as 11β,23*S*,24*R*,25-tetrahydroxy-protost-13(17)-en-3-one (alisol A).

Table 1: The NMR data of compound **1**

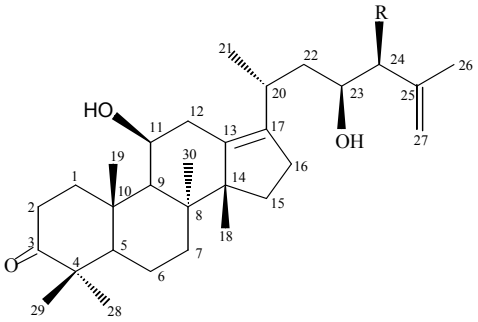
C	δ_C [#]	δ_C [a,c]	δ_H [b,c] (J in Hz)	HMBC (H to C)
1	31.3 t	31.0 t	2.13 m; 2.26 m	
2	33.9 t	33.7 t	2.34 m; 2.70 m	
3	219.2 s	220.5 s	-	
4	47.1 s	46.9 s	-	
5	48.8 d	48.5 d	2.11*	
6	20.3 t	20.0 t	1.32 m; 1.42 m	
7	34.6 t	34.9 t	1.24 m; 2.03 m	
8	40.8 s	40.4 s	-	
9	50.0 d	49.6 d	1.77 d (10.6)	11
10	37.2 s	36.9 s	-	

C	δ_C #	δ_C a,c	δ_H b,c (*J* in Hz)	HMBC (H to C)
11	70.1 d	69.9 d	3.88 ddd (5.8, 10.7, 10.7)	9, 10, 13
12	34.8 t	34.4 t	2.80 dd (5.8, 13.2)	9, 11, 13, 14
13	137.2 s	137.6 s	-	
14	57.2 s	56.9 s	-	
15	30.8 t	30.5 t	1.34 m; 1.90 m	
16	29.5 t	29.1 t	2.17 m	
17	135.4 s	135.5 s	-	
18	23.4 q	23.0 q	1.13 s	13
19	25.8 q	25.6 q	1.05 s	5, 10, 9
20	28.7 d	28.3 d	2.77 m	
21	20.3 q	20.1 q	1.01 d (7.0)	17
22	40.4 t	40.0 t	1.39 m; 1.67 ddd (4.2, 9.3, 13,9)	
23	69.5 d	69.4 d	3.76 d (9.0)	
24	77.6 d	77.6 d	3.01 br s	
25	74.1 s	74.1 s	-	
26	27.6 q	27.3 q	1.27 s	24, 25
27	26.4 q	26.2 q	1.21 s	24, 25
28	29.8 q	29.5 q	1.07 s	4, 3, 5
29	20.4 q	20.0 q	1.06 s	4, 3, 5
30	24.3 q	24.1 q	1.00 s	7, 8, 9, 14

#δ_C of alisol A [6], a125 MHz, b500 MHz, cMeasured in $CDCl_3$ *Overlap signals, Chemical shift are given in ppm. Assignments were confirmed by COSY, 1D-TOCSY, HMQC, and HMBC experiments.



1 R = OH; **2** R = $OCOCH_3$



3 R = OH

Figure 1: The structures of compounds **1** - **3**

The NMR spectra of compound **2** were very similar to those of **1** except for the more appearence of an acetate group in the NMR spectra of **2** (δ_C 170.8/20.7 and δ_H 2.20). This evidence suggested that **2** was an acetyl derevative of **1**. In the other hand, the ESI spectrum of **2** exhibited ion peaks at *m/z* 515 $[M\text{-}H_2O+H]^+$, 497 $[M\text{-}2H_2O+H]^+$, 479 $[M\text{-}3H_2O+H]^+$, corresponding to the molecular formula of $C_{32}H_{52}O_6$. The NMR assignments of **2** were made from the comparison with those of **1**, and were further confirmed by HSQC and HMBC spectra of **2**. The H-C long-range correlation were

observed between H-24 (δ 4.61) and carbon carbonyl C-31 at δ 170.8, confirming that the acetate group was connected to C-24. All NMR data of **2** were in good agreements with those of alisol A 24-acetate. Accordingly, **2** was determined as 11β,23*S*,25-trihydroxyprotost-13(17)-en-3-one-24*R*-yl acetate (alisol A 24-acetate).

Table 2: The NMR data of compound **2**

C	δ_C [#]	δ_C [a,c]	δ_H [b,c] (*J* in Hz)	HMBC (H to C)
1	31.3 t	30.9 t	2.15 m; 2.30 m	
2	34.0 t	33.7 t	2.36 m 2.73 m	
3	219.2 s	220.5 s	-	
4	47.1 s	47.0 s	-	
5	48.8 d	48.6 d	2.12*	
6	20.3 t	20.0 t	1.32 m; 1.49 m	
7	34.6 t	34.3 t	1.28 m; 2.05 m	
8	40.7 s	40.4 s	-	
9	50.0 d	49.5 d	1.77 d (10.6)	11
10	37.2 s	36.9 s	-	
11	70.0 d	69.8 d	3.88 ddd (5.8, 10.7, 10.7)	
12	34.8 t	34.3 t	2.89 dd (5.8, 13.2)	9, 11, 13, 14
13	137.7 s	138.3 s	-	
14	57.2 s	57.0 s	-	
15	30.8 t	30.4 t	1.35 m; 1.92 m	
16	29.4 t	28.9 t	2.18 m	
17	135.0 s	135.0 s	-	
18	23.5 q	23.0 q	1.16 s	13
19	25.8 q	25.5 q	1.10 s	5, 10, 9
20	28.2 d	27.8 d	2.77 m	
21	20.2 q	19.9 q	1.00 d (7.0)	17
22	40.0 t	39.6 t	1.39 m; 1.67 ddd (4.2, 9.3, 13.9)	
23	69.1 d	69.0 d	3.88 d 9.0	
24	78.8 d	78.7 d	4.61 br s	31
25	73.9 s	73.9 s	-	
26	27.6 q	27.2 q	1.18 s	24, 25
27	26.9 q	26.7 q	1.34 s	24, 25
28	29.7 q	29.5 q	1.11 s	4, 3, 5
29	20.4 q	20.0 q	1.01 s	4, 3, 5
30	24.3 q	24.1 q	1.02 s	7, 8, 9, 14
31	170.5 s	170.8 s	-	
32	21.0 q	20.7 q	2.20 s	31

[#]δ_C of alisol A 24-acetate [6], [a]125 MHz, [b]500 MHz, [c]Measured in $CDCl_3$ *Overlap signals and chemical shift are given in ppm. Assignments were confirmed by COSY, 1D-TOCSY, HMQC, and HMBC experiments.

The NMR spectra of compound **3** were also similar to those of **1**, except for the more appearence of the signals of a double bond at δ_C 144.7 (s), 114.1 (t) /δ_H 4.94 (br s) and 4.98 (br s), instead of of the signals of a quaternary carbon at δ 74.1, and the methyl group at δ_C 26.2/δ_H 1.21 as shown in the NMR spectra of **1**. This evidence suggested that the double bond must be at C-25 and C-27. All the NMR assignments of the Protostane skeleton of **3** were made by comparison with those of **1**. In the HMBC spectrum, H-27 δ_H 4.94 (br s) and 4.98 (br s) correlated with C-24 δ 79.9/C-25 (δ 144.7)/C-26 (δ 17.8) confirming that the double bond was at C-25 and C-27, and that compound **3** must be alisol G. Furthermore, the ESI spectrum of **1** exhibited the ion peaks at *m/z* 473 $[M+H]^+$, 455 $[M-H_2O+H]^+$, 437 $[M-2H_2O+H]^+$ and 419 $[M-3H_2O+H]^+$, corresponding to the molecular formula of $C_{30}H_{48}O_4$. Oviously, compound **3** was identified as 11β,23*S*,24*S*-trihydroxyprotosta-13(17),25-dien-3-one. The stereochemistry of this compound at C-11 was further confirmed by ROESY spectrum. The NOEs correlation between H-11 and H-30 was observed confirming that the hydroxyl group was *axial*. This is the first report of **3** from *Alisma plantago-aquatica* L.

Table 3: The NMR data of compound **3**

C	δ_C [#]	δ_C [a,c]	δ_H [b,c]	HMBC (H to C)	ROESY
1	31.0 t	31.1 t	2.11 m; 2.25 m		
2	33.7 t	33.8 t	2.26 m; 2.69 m		
3	220.3 s	220.6 s	-		
4	46.9 s	47.0 s	-		
5	48.5 d	48.5 d	2.12 m		
6	20.0 t	20.1 t	1.30 m; 1.46 m		
7	34.3 t	34.3 t	1.25 m; 2.03 m		
8	40.6 s	40.6 s	-		
9	49.6 d	49.6 d	1.75 d (10.5)	8, 11, 30	
10	36.9 s	37.0 s	-		
11	70.0 d	69.9 d	3.88 ddd (5.8, 10.7, 10.7)		H-30
12	34.5 t	34.5 t	2.81 dd (5.8, 13.2); 2.83 m		H-11
13	137.7 s	137.9 s	-		
14	57.0 s	56.0 s	-		
15	30.6 t	30.6 t	1.23 m; 1.81 m		
16	29.1 t	29.1 t	2.16 m		
17	135.4 s	135.2 s	-		
18	23.3 q	23.3 q	1.14 s	8, 13, 14, 15	
19	25.7 q	25.6 q	1.05 s	5, 9, 10	
20	28.3 d	28.3 d	2.88 m		
21	20.3 q	20.4 q	1.01 d (7.0)	17, 20	
22	38.3 t	38.3 t	1.39 m		

C	δ_C #	δ_C a,c	δ_H b,c	HMBC (H to C)	ROESY
23	70.7 d	70.8 d	3.49 d (7.5)		H-24
24	79.7d	79.9 d	3.78 d (7.0)	23, 26, 27	H-23
25	144.6 s	144.7 s	-		
26	17.9 q	17.8 q	1.67 s	24, 25, 27	
27	113.9 t	114.1 t	4.94 br s 4.98 br s		
28	29.5 q	29.6 q	1.07 s	3	
29	20.1 q	20.1 q	1.06 s	3	
30	24.0 q	24.0 q	1.00 s		H-11

#δ_C of alisol G [7], a125 MHz, b500 MHz, cMeasured in $CDCl_3$ *Overlap signals and **c**hemical shift are given in ppm. Assignments were confirmed by COSY, 1D-TOCSY, HMQC, and HMBC experiments.

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