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TRITERPENOIDS FROM THE RHIZOME OF *ALISMA PLANTAGO-AQUATICA*

GENG PEI-WU,* YOSHIYASU FUKUYAMA,† TOSHIHIDE YAMADA, WANG REI,* BAO JINXIAN* and KAZUYUKI NAKAGAWA

Tokushima Research Institute, Otsuka Pharmaceutical Co Ltd, 463-10 Kagasuno, Kawauchi-cho, Tokushima 771-01, Japan

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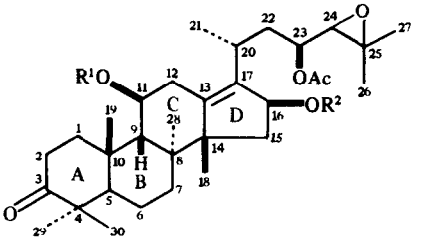
Key Word Index—*Alisma plantago-aquatica*, Alismataceae, protostane-type triterpene; 16β-methoxyalisol B monoacetate, 16β-hydroxyalisol B monoacetate; alisol B monoacetate, alisol C monoacetate.

Abstract—Two new protostane-type triterpenes have been isolated from the methanol extract of the rhizome of *Alisma plantago-aquatica* Their structures have been elucidated as 16β-methoxyalisol B monoacetate and 16β-hydroxyalisol B monoacetate mainly on the basis of spectroscopic data.

INTRODUCTION

A Chinese crude drug 'Zexie', Alismatic Rhizome, is composed of the rhizome of *Alisma plantago-aquatica* L. var *orientale* Samuelsson (Alismataceae), and it is well known as an important component in Oriental medicine. This medicinal herb has also been recognized as having diuretic action [1] The plant contains a series of unique triterpenoids, alisol A, alisol A monoacetate, alisol B, alisol B monoacetate, alisol C and alisol C monoacetate [2–5], which show a positive hypocholesterolemic action [6] In addition, these alisols, in particular, alisol A monoacetate and alisol B were identified as diuretic principles [7]. In the course of our continuing search for coronary vasodilating substances in this plant [8], we have isolated two new protostane-type triterpenes **1** and **2**, the structures of which are described in this paper.

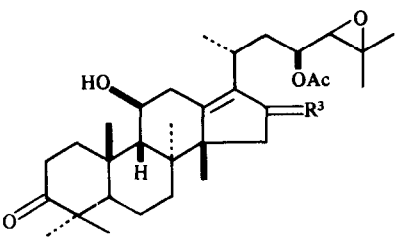


1 R^1 = H, R^2 = Me
1a R^1 = Ac, R^2 = Me
2 R^1 = H, R^2 = H
2a R^1 = Ac, R^2 = Ac

RESULTS AND DISCUSSION

From a methanol extract of the rhizome of *Alisma plantago-aquatica*, a combination of column chromatography and MPLC led to the isolation of compounds **1** and **2** along with the known alisol B monoacetate (**3**) and alisol C monoacetate (**4**).

Compound **1** had the molecular formula $C_{33}H_{52}O_6$(M^+ at *m/z* 544.3767) and displayed the presence of hydroxy (3500 cm^{-1}) and ester (1740 cm^{-1}) groups. The ^{13}C NMR spectrum of **1** (Table 1) was very similar to that of alisol B monoacetate (**3**) previously isolated from *A plantago-aquatica* except for the extra signals at δ56 09 and 86.20 assignable to a CHOMe



3 R^3 = H_2
4 R^3 = O

grouping, the ^1H NMR spectrum contained signals for seven tertiary methyls, a secondary methyl and a methoxy group. In addition, the mass spectrum of **1** fragmentation ions at *m/z* 150 and 122 characteristic of the A ring of alisols [5]. These spectral features disclosed that **1** would belong to a protostane-type triterpenoid [6] Extensive selective proton decoupling experiments (Table 2)

*Present address Beijing Institute of Pharmaceutical Industries, 12 Dong Da Qiao Road, Chao Yang Men Wai, Beijing, China.

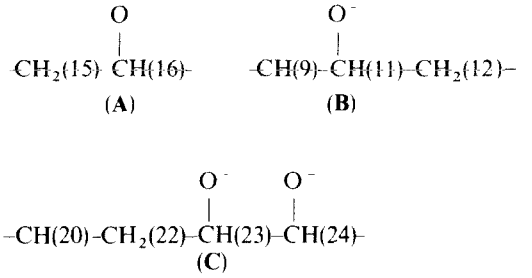
†Author to whom correspondence should be addressed

Table 1 ^{13}C NMR spectral data of compounds **1**, **2**, **3** and **4** ($CDCl_3$)

C	1	2	3	4
1	34 30	34 30	34 11	34 72
2	33 61	33 60	33 65	33 49
3	220 03	220 50	220 02	219 64
4	46 08	46 87	46 86	46 86
5	48 37	48 35	48 41	48 30
6	19 45	19 99	19 99	19 85
7	30 78	30 78	29 10	30 72
8	40 20	40 19	40 66	39 98
9	49 30	49 24	49 88	48 65
10	36 80	36 79	36 69	36 83
11	69 86	69 85	70 08	69 65
12	34 46	34 48	34 42	35 97
13	134 49	135 90	134 03	177 02
14	55 01	54 81	56 96	49 67
15	38 58	43 62	30 64	45 63
16	86 20	77 29	30 86	207 90
17	142 94	143 04	130 04	138 30
18	24 49	24 08	23 10	22 99
19	23 69	23 68	23 75	22 91
20	27 72	27 73	27 76	26 56
21	25 50	25 52	25 55	25 38
22	37 82	38 06	36 86	35 47
23	72 40	72 18	71 44	71 82
24	65 64	65 42	65 00	64 82
25	58 60	58 78	58 37	58 56
26	19 32[a]	19 57[b]	19 31[c]	19 62[d]
27	24 74[a]	24 71[b]	24.62[c]	24 57[d]
28	19 99	19 68	20.03	19 17
29	29 49	29 48	30 86	29 46
30	21 38	21 33	20 08	21 03
COMe	170 20	170 30	169 84	170 01
COMe	19 99	19 92	20 03	19 98
OMe	56 09			

[a–d] Assignment may be interchangeable

indicated the presence of the partial structures **A**, **B** and **C**

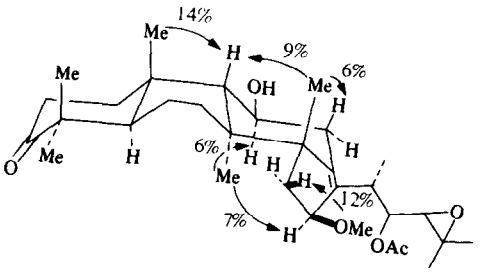


Among the protons on the carbons adjacent to the oxygen function, only the H-11 in the partial structure **B** gave rise to a large downfield shift (δ4 03→4 87) on acetylation of **1** This fact suggests that a hydroxy group must be bonded to C-11 in **B** and thus the remaining oxygen functions in **A** and **C** must be substituted On the other hand, the equatorial configuration of the C-11 hydroxyl was evident from the large J values (11 1 and 10 0 Hz) for H-11 From a consideration of the chemical shift values for H-23 (δ5 17) and H-24 (δ2 88) the oxygens on C-23 and C-24 in **C** should be incorporated into the acetoxy and epoxide moieties respectively, indicating that the partial structure **C** corresponds to the side chain of alisol **B** monoacetate (**3**) and C monoacetate (**4**) In fact, the ^1H NMR and ^{13}C NMR data for the partial structure (**C**) in **1** were in good accord with those for C-20 to C-27 in **3** and **4** Hence, **1** turned out to have the same side chain as that of alisol **B** monoacetate (**3**) Long-range coupling (J = 1 4 Hz) observed between H-12 in **B** and H-16 in **A** was ascribed to a homoallylic coupling through both the allylic protons next to the $\Delta^{13,\,17}$ double bond (δ134 4 and 142 9) This means that the partial structures (**B**) and (**A**) must be involved in the C and D rings, respectively, in the alisols It also suggested that the extra methoxy group would be linked to C-16 in **A** The

Table 2 ^1H NMR spectral data for compounds **1**, **2** and **4** (pyridine-d_5)

H	1	2	4
9	1 95 (*d*, 10 0)*	1 83 (*d*, 10 4)	2 06 (*d*, 10 6)
11	4 03 (*dddd*, 11 1, 10 0, 6 5, 5 6)	4 07 (*dddd*, 10 4, 10 0, 7 0, 5 4)	4 23 (*dddd*, 10 6, 10 2, 6 5, 5 6)
12β	2 44 (*ddd*, 13 4, 11 1, 1 4)	2 55 (*ddd*, 14 0, 10 0, 1 7)	3 23 (*dd*, 13 8, 10 2)
12α	2 89 (*dd*, 13 4, 5 6)	2 96 (*dd*, 14 0, 5 4)	2 76 (*dd*, 13 8, 5 6)
15	1 29 (*dd*, 14 3, 3 7)	1 58 (*dd*, 13 8, 4 4)	1 94 (*d*, 18 0)
	2 24 (*dd*, 14 3, 7 9)	2 50 (*dd*, 13 8, 8 1)	2 59 (*d*, 18 0)
16	4 35 (*ddd*, 7 9, 3 7, 1 4)	5 17 (*dddd*, 8 1, 4 7, 4 4, 1 7)	
20	2 82 (*qdd*, 6 9, 14 8, 4 2)	3 01 (*qdd*, 6 4, 15 0, 6 0)	2 85 (*qdd*, 6 9, 13 4, 4 2)
21	1 15 (*d*, 6 9)	1 31 (*d*, 6 4)	1 31 (*d*, 6 9)
22	1 77 (*ddd*, 14 8, 10 6, 4 2)	2 44 (2H, *m*)	1 86 (*ddd*, 13 4, 11 6, 4 2)
	2 05 (*td*, 14 8, 2 3)		2 06 (*td*, 13 4, 2 3)
23	5 17 (*ddd*, 10 6, 8 3, 2 3)	5 39 (*ddd*, 11 1, 8 1, 2 7)	4 94 (*ddd*, 11 6, 8 2, 2 3)
24	2 88 (*d*, 8 3)	2 94 (*d*, 8 1)	2 92 (*d*, 8 2)
Me	1 44, 1 36, 1 36, 1 26, 1 18, 1 10, 0 99 (each s)	1 54, 1 46, 1 37, 1 23, 1 18, 1 09, 1 03 (each s)	1 38, 1 38, 1 36, 1 23, 1 18, 1 09, 0 98 (each s)
OMe	3 19 (s)		
OH	5 87 (*d*, 6 5)	5 83 (*d*, 7 0)	6 34 (*d*, 6 5)
		6 02 (*d*, 4 7)	
Ac	1 96 (s)	1 99 (s)	1 95 (s)

* Coupling constants (J in Hz) are given in parentheses



location of the methoxy group was verified by comparison between the ^{13}C NMR data of **1** and **3**. An oxygen-bearing methine carbon signal (δ86.20) replaced the C-16 methylene carbon (δ30 86) seen in the spectrum of **3**. The signals for C-15 and C-17 in **1** were observed in fields lower by +8 and +12 ppm respectively, than the corresponding ones in **3**. These downfield shifts were rationalized by a β-effect from the neighbouring methoxy group [9], which therefore must be located at C-16. Thus, the structure of **1** was elucidated as alisol **B** 23-monoacetate bearing the methoxy substituent at C-16 Its configuration on the D ring could be clarified by the following NOE experiments Upon selective irradiation at δ1 18 (18-Me), and δ1.26 (19-Me) NOEs were observed for H-9 (9%) and H-12β (6%), and for H-9 (14%), respectively, supporting the fact that the B ring must adopt a boat conformation like all previously known protostane triterpenoids [10]. The crucial detection of NOEs (6 and 7%) for H-11 and H-16 upon irradiation at δ0 99 (28-Me) clearly substantiated a β-configuration of the methoxy substituent at C-16. Accordingly, the structure of **1** was proposed to be 16β-methoxyalisol B 23-monoacetate on the basis of the above mentioned spectral data and the analogy of its congeners

Compound **2** had the molecular formula $C_{32}H_{50}O_6$ (M^+ at *m/z* 530 3602), and contained hydroxy (3450 cm^{-1}), ester (1740 cm^{-1}) and ketone (1695 cm^{-1}) groups Its mass spectrum exhibited fragment peaks at *m/z* 150 and 122 characteristic of alisols. The ^1H NMR spectrum of **2** showed the presence of seven tertiary methyls and a secondary methyl group disclosing its protostane triterpenoid nature Detailed selective proton decoupling experiments (Table 2) indicated the presence of the partial structures **A**, **B** and **C** found in compound **1** However, no signal due to a methoxy group was observed and two new signals at δ6 02 (*d*, *J* = 4 7 Hz) and 5.83 (*d*, *J* = 7 0 Hz) appeared These signals disappeared with D_2O exchange and were assigned to hydroxyl protons, which were coupled with the multiple signals at δ5 17 and 4 07 presumably assignable to H-16 and H-11, respectively Acetylation of **2** gave a diacetate [δ2.04 (6 H)] confirming the presence of the two hydroxy groups. These data implied that the structure of **2** was closely related to that of **1**, the methoxy substituent at C-16 in **1** being replaced with the hydroxy group. The ^{13}C NMR data of **2** (Table 1) corresponded well to those of **1** except for the signals for C-15, C-16 and C-17 and the loss of the methoxy signal It could be reasonably explained by a substituent effect on methylation of the hydroxyl [9] that the C-16, C-15 and C-17 signals were found to be located at higher field by −9 ppm, and lower fields by +5 and +1 ppm, respectively, than the corresponding signals in the spectrum of compound **1** These facts verified the hydroxy group was located at C-16. A β-configuration of the hydroxy group could be assigned by the observations of the NOEs for H-16 (7%) and H-11 (7%) upon irradiation at δ1 03 (28-Me) Accordingly, the structure of **2** was elucidated as 16β-hydroxyalisol B 23-monoacetate

From the rhizome of *Alisma plantago-aquatica* nine protostane triterpenoids, including **1** and **2**, and sesquiterpenoids [11] have so far been isolated However, we have already shown that these compounds exhibit no coronary vasodilating activity [12] and thus the active substances of this plant have not yet seen isolated.

EXPERIMENTAL

Mps uncorr, ^1H NMR (400 MHz) and ^{13}C NMR (62 9 MHz) $CDCl_3$, TMS as int standard, CC silica gel (Wakogel C-300), neutral alumina (Merck), TLC precoated silica gel plates F_{254} and RP-8 plates F_{254} (Merck, 0 25 mm) Spots were visualized by UV (254 nm) and 40% $CeSO_4$–H_2SO_4

Plant material Alisma plantago-aquatica was collected in China and identified by Wang Rei A voucher specimen has been deposited at Beijing Institute of Pharmaceutical Industries

Exatraction and isolation Air-dried and powdered rhizome (5 kg) of *A plantago-aquatica* was extracted with MeOH (30 l) at room temp for 29 days The MeOH extract was evapd *in vacuo* to give a crude extract (400 g), 170 g of which was divided by CC on silica gel using CH_2Cl_2–MeOH gradient into 11 fractions fr 1 (CH_2Cl_2), frs 2–4 (CH_2Cl_2–MeOH, 19 1), frs 5–7 (CH_2Cl_2–MeOH, 9 1), frs 8,9 (CH_2Cl_2–MeOH, 4 1), fr 10 (CH_2Cl_2–MeOH, 7 3), fr 11 (CH_2Cl_2–MeOH, 1 1) Fr 5 (26 g) was chromatographed on Sephadex LH-20 (CH_2Cl_2–MeOH, 1 1), followed by CC on silica gel (CH_2Cl_2–EtOAc, 10 1→7 3) to give 8 fractions The fifth fraction (3 05 g) afforded alisol B monoacetate (**3**) (114 mg) and alisol C monoacetate (**4**) (38 mg) The sixth fraction (2 4 g) was further divided by MPLC [column Lichroprep RP-8, type C, solvent· MeOH–H_2O, 4 1] to afford **1** (16 mg) as a crystal, which was recrystallized from CH_2Cl_2–MeOH On the other hand, the third fraction was purified by CC on silica gel ($CHCl_3$–MeOH, 60 1) to afford **2** (11 mg) as crystals, recrystallized from CH_2Cl_2–MeOH

16β-Methoxyalisol B monoacetate (**1**) Colourless prisms, mp 164–166°, $[\alpha]_D^{25}$ 89 4 ($CHCl_3$, *c* 0 92); MS *m/z* (rel int) 544 3767 $[M]^+$ (calc 544 3764 for $C_{33}H_{52}O_6$, 3), 526, 484, 150 (31), 122 (21), IR ν_{max}^{KBr} cm^{-1} 3500 (OH), 1740 (ester), 1700 (C=O) ^1H NMR and ^{13}C NMR see Tables 1 and 2

Acetylation of **1** The acetate of **1** (2 mg) was prepared by treatment with Ac_2O (0 1 ml) and pyridine (0 4 ml) The usual work-up yielded **1a** (2 1 mg) as an amorphous gum MS *m/z* (rel int) 586 $[M]^+$ (1), 526 (12), 466 (5), ^1H NMR δ0 90 (3 H, *s*), 0 98 (3 H, *s*), 1 05 (3 H, *s*), 1 07 (3 H, *s*), 1 10 (3 H, *d*, *J* = 6 9 Hz, H-21), 1.24 (3 H, *s*), 1 28 (3 H, *s*), 1 32 (3 H, *s*), 1 68 (1 H, *ddd*, *J* = 13 9, 11 2, 5 1 Hz, H-22), 1 85 (1 H, *ddd*, *J* = 13 9, 11 2, 3 1 Hz, H-22), 1 93 (1 H, *ddd*, *J* = 13 9, 10.5, 1 7 Hz, H-12), 2 01 (6 H, *s*, 2 x OAc), 2 03 (1 H, *d*, *J* = 10 9 Hz, H-9), 2 11 (1 H, *dd*, *J* = 13 9, 7 1 Hz, H-15), 2 68 (1 H, *d*, *J* = 8 5 Hz, H-24), 2 70 (1 H, *dd*, *J* = 13 9, 6 1 Hz, H-12), 3 20 (3 H, *s*, OCH_3), 4 27 (1 H, *ddd*, *J* = 7 1, 3 4, 1 7 Hz, H-16), 4 87 (1 H, *ddd*, *J* = 10 9, 10 9, 6 1 Hz, H-11), 4 65 (1 H, *ddd*, *J* = 11 2, 8 5, 3.1 Hz, H-23).

16β-Hydroxyalisol B monoacetate (**2**) Colourless needles, mp 196 5–198°, $[\alpha]_D^{25}$ 11 0 ($CHCl_3$, *c* 0 32), MS *m/z* (rel int) 530 3602 $[M]^+$ (calc. 530.3607 for $C_{32}H_{50}O_6$, 5), 512 (3), 494 (5), 452 $[M-18-60]^+$ (36), 434 $[M-36-60]^+$ (11), 150 (41), 122 (51), IR ν_{max}^{KBr} cm^{-1} 3450 (OH), 1740 (ester), 1695 (C=O), ^1H NMR and ^{13}C NMR: see Tables 1 and 2

Acetylation of **2**. The acetate of **2** (4 mg) was prepared by treatment with Ac_2O (0.1 ml) and pyridine (0.4 ml) The usual work-up afforded **2a** (3 5 mg) as an amorphous material MS

m/z (rel int) 614 $[M]^+$ (1), 554 (4), 494 (32), 434 (33), ^{1}H NMR δ0 93 (3 H, s) 0 99 (3 H, *s*), 1 05 (3 H, *s*), 1 07 (3 H, *s*), 1 08 (3 H, *d*, *J* = 7 0 Hz, H-21), 1 19 (1 H, *dd*, *J* = 14 9, 3 6 Hz, H-15), 1.26 (3 H, *s*), 1 30 (3 H, s), 1 37 (3 H, s), 1 71 (1 H, *td*, *J* = 9 2, 5 3 Hz, H-22), 1 99 (1 H, *ddd*, *J* = 13 8, 10 7, 1 4 Hz, H-12), 2 04 (1 H, *d*, *J* = 10 7 Hz, H-9), 2 02 (3 H, *s*, OAc), 2 04 (6 H, s, 2 × OAc), 2 47 (1 H, *dd*, *J* = 14 9, 8 2 Hz, H-15), 2 68 (1 H, *m*, H-20), 2 72 (1 H, *d*, *J* = 9 2 Hz, H-24), 2 75 (1 H, *dd*, *J* = 13 8, 5 7 Hz, H-12), 4 66 (1 H, *td*, *J* = 9 2, 4 3 Hz, H-23), 4 87 (1 H, *ddd*, *J* = 10 7, 10 7, 5 7 Hz, H-11), 5 65 (1 H, *ddd*, *J* = 8 2, 3 6, 1 4 Hz, H-16)

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