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Alisolide, alisols O and P from the rhizome of *Alisma orientale*

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Abstract

A *nor*-protostane, alisolide (**1**), a rearranged protostane, alisol O (**2**), and a 2,3-*seco*-protostane triterpene, alisol P (**3**), were isolated from the rhizomes of *Alisma orientale*, along with eight known protostane triterpenes. The structures were elucidated to be (17*S*)-3,11-dioxo-23-*nor*-protost-12-en-23(17)-olide, 3-oxo-11β,23-dihydroxy-24,24-dimethyl-26,27-dinorprotost-13(17)-en-25-oic acid, and (20*R*,23*S*,24*R*)-23,24,25-trihydroxy-2,3-*seco*-protost-13(17)-en-3-oic acid 2,11β-lactone, respectively, by interpretation of spectroscopic data.

Keywords: *Alisma orientale*; Alismataceae; Protostane triterpenes; Alisolide; Alisol O; Alisol P

1. Introduction

Alisma orientale (Sam.) Juz. (Alismataceae) is distributed in China, Japan, Mongolia and Russia. The dried rhizome of this plant is a common traditional Chinese medicine known as Rhizoma Alismatis or Zexie, which is a diuretic agent used to ''remove dampness and promote water metabolism'' in the body according to Chinese medicinal principles (Sun, 1992). Previous studies have shown that it contains protostane triterpenes (Murata and Miyamoto, 1970; Murata et al., 1970; Fukuyama et al., 1988; Geng et al., 1988; Yoshikawa et al., 1993a,b, 1997, 1999; Nakajima et al., 1994; Zeng et al., 1995; Matsuda et al., 1999; Peng and Lou, 2001a; Peng et al., 2002a,b), guaiane sesquiterpenes (Yoshikawa et al., 1994a,b; Peng and Lou, 2001b; Peng et al., 2002c, 2003) and kaurane diterpenes (Nakajima et al., 1994; Yamaguchi et al., 1994; Peng and Lou, 2002). To date, all protostane triterpens occur only in *Alisma* plants, and they are considered to be chemotaxonomic markers of the genus. In the present study, alisolide (**1**), alisol O (**2**) and alisol P (**3**) (Fig. 1), were isolated from the rhizome of *A. orientale*, along with eight known protostane triterpenes, alisol A (Yoshikawa et al., 1993b), alisol B, alisol B 23-acetate, 25-*O*-methylalisol A (Nakajima et al., 1994), 25-anhydroalisol A 24-acetate, 25-anhydroalisol A (Peng et al., 2002b), alisol E 23-acetate (Yoshikawa et al., 1993b), as well as 13β,17β-epoxyalisol A (Peng et al., 2002a).

2. Results and discussion

The rhizomes of *Alisma orientale*, collected in Si-Chuan Province, China, were percolated with EtOH–H_2O (4:1, v/v). After removal of excessive EtOH, the extract separated into an oil and an aqueous layer. Alisolide (**1**), alisols O (**2**) and P (**3**) were purified from the organic portion by repeated column chromatography over silica gel and RP-18 gels.

Alisolide (**1**), $C_{26}H_{36}O_4$, displayed a quasi-molecular ion at *m/z* 413.27096 $[M+H]^+$ (calculated 413.26864). It showed IR absorption bands at 3447, 1782, 1705, and 1663 cm^{-1} assignable to hydroxyl, lactone, ketone, and

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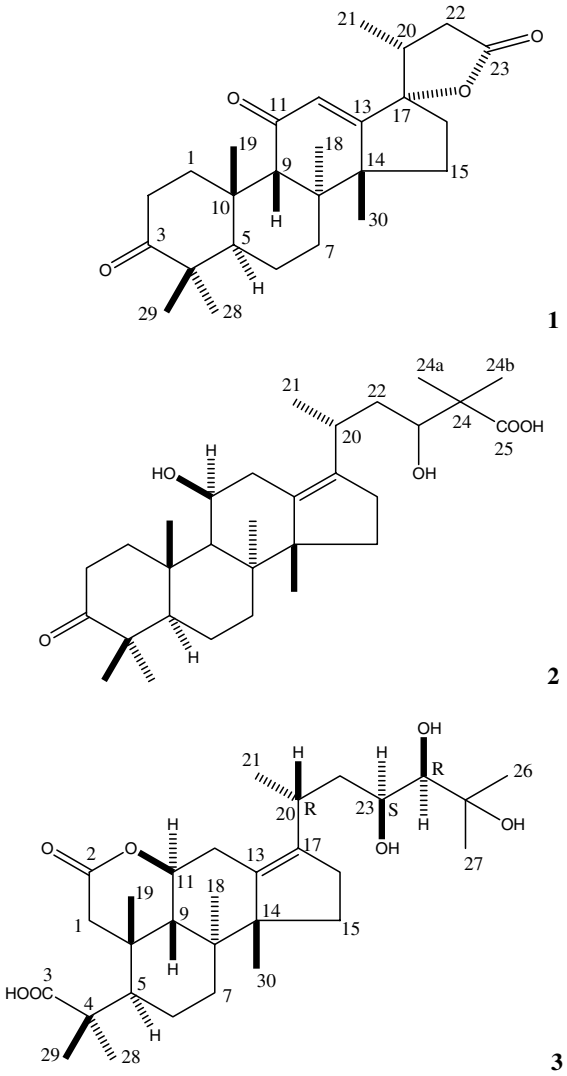

Fig. 1. Chemical structures of **1**–**3**.

enone functional groups, respectively. The ^{1}H and ^{13}C NMR spectroscopic data of **1** (Table 1) were in agreement with those of the A-, B-, and C-rings of alisol J 23-acetate (Zeng et al., 1995). It was noted, however, that signals for carbons C-24–C-27 were missing from the spectra of **1**. The compound was, therefore, a 23-*nor*-protostane bearing identical A–C rings as in alisol J 23-acetate. The structure of **1** was further established by interpretation of the COSY, DEPT, HMQC and HMBC data. That is, HMBC correlations were observed between the lactone carbon (δ_C 174.7) and H-20/H-22. A downfield shift of C-17 (δ_C 93.8) suggested a C-23(17) lactone structure. To the best of our knowledge, all known structures of protostane triterpenes have a 20*R* stereochemistry, **1** was therefore assumed to have the same stereochemistry at C-20 on the basis of biogenetic ground. The absolute configuration of C-17 was then determined to be *S* based on NOESY results, in particular the correlation observed between H-12 and CH_3-21 (Fig. 2). Finally, the structure of (17*S*)-3,11-dioxo-23-*nor*-protost-12-en-23(17)-olide was assigned to alisolide (**1**). In the literature, only three 23-*nor*-protostanes have been reported as oxidative reaction products (Murata et al., 1968; Lee et al., 2003). Alisolide (**1**) is therefore the first 23-*nor*-protostane structure reported from a natural source.

Alisol O (**2**), $C_{30}H_{48}O_5$, displayed a quasi-molecular ion peak at *m/z* 511.33999 $[M+Na]^+$ (calculated 511.33994) in the HRESIMS spectrum and the IR absorption bands at 3436 and 1706 cm^{-1} suggested the presence of hydroxyl and carboxyl groups. Analysis of the ^{1}H and ^{13}C NMR spectroscopic data of **2** (Table 1) resulted in structural similarity with alisol B (Nakajima et al., 1994), and the structure of the side-chain was determined by an HMBC analysis. Thus, correlation signals were observed between: H-23 (δ_H 3.47, *d*, $J = 10.4$ Hz) and C-20, C-22, C-24, C-24a, and C-25; CH_3-24a (δ_H 1.05) and C-25; CH_3-24b (δ_H 1.08) and C-25; CH_3-21 (δ_H 0.97, *d*, $J = 5.6$ Hz) and C-22, respectively. The stereostructure of **2** was then established by interpretation of the NOESY results (Fig. 2), in which correlations between CH_3-28 and H-5, CH_3-18 and H-5, CH_3-18 and H-11, CH_3-19 and H-9, as well as between CH_3-30 and H-9, were observed. The 11-OH was assigned to a β position due to the NOESY correlation observed between H-11 and CH_3-18. Consequently, alisol O (**2**) was elucidated to be 3-oxo-11β,23-dihydroxy-24,24-dimethyl-26,27-dinorprotost-13(17)-en-25-oic acid. It is a new rearranged skeleton of protostane, presumably biosynthesized from alisol B (Briggs et al., 1973).

Alisol P (**3**), $C_{30}H_{48}O_7$, exhibited a quasi-molecular ion peak at *m/z* 543.32942 $[M+Na]^+$ (Calc. 543.32922) in its positive ion HRMS spectrum. The IR spectrum showed absorption bands at 3500–2500, 1748, and 1710 cm^{-1}, suggesting the presence of hydroxyl, lactone, and carboxyl functional groups, respectively. The ^{1}H NMR spectrum of **3** (Table 1) displayed signals due to seven tertiary methyls (δ_H 1.03, 1.15, 1.15, 1.22, 1.22, 1.23, 1.28) and a secondary methyl group (δ_H 1.03). Its ^{13}C DEPT spectra (Table 1) indicated the presence of thirty signals including two carbonyl carbons, two quaternary olefinic carbons, five sp^3 quaternary carbons (one oxygenated), six sp^3 methines (three oxygenated), seven sp^3 methylenes, and eight methyls. The NMR spectroscopic data of **3** were similar to those of a 2,3-*seco*-protostane-type triterpene such as alismalactone 23-acetate (Yoshikawa et al., 1997), except for the side-chain resonances. Compound **3** was, therefore, likely a 2,3-*seco*-protostane possessing identical A–D rings as for alismalactone 23-acetate. An analysis of the HMBC spectrum supported this assignment. The side-chain was determined to possess 23,24,25-trihydroxy substituents based on interpretation of the HMBC and ^{1}H–^{1}H COSY spectroscopic data. The stereostructure of **3** was then established on the basis of NOESY results (Fig. 2), in which NOE correlations were observed between: H-5 and CH_3-18/CH_3-28; CH_3-18 and H-11; CH_3-19 and H-9/CH_3-29; H-9 and CH_3-30; H-23 and H-24; H-23 and CH_3-27, and H-24 and CH_3-26/CH_3-27, respectively. Since H-11 exhibited a NOESY correlation with CH_3-18, it was assigned to

Table 1
^{1}H and ^{13}C NMR spectroscopic data for alisolide (**1**), alisols O (**2**) and P (**3**)[a]

Position	**1** ($CDCl_3$)		**2** ($CDCl_3$)		**3** ($CDCl_3$)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.92, 2.45	32.4 *t*	2.09, 2.22	31.0 *t*	2.56, *s*	42.9 *t*
2	2.31, 2.70	33.7 *t*	2.31, 2.66	33.7 *t*		173.9 *s*
3		218.9 *s*		220.2 *s*		183.5 *s*
4		46.9 *s*		47.0[b] *s*		45.2 *s*
5	2.20, *m*	48.3 *d*	2.08	48.4 *d*	2.67, *m*	44.8 *d*
6	1.55, *m*	20.2 *t*	1.23, 1.42	20.0 *t*	1.60, 1.75	18.8 *t*
7	1.40, 2.18	33.3 *t*	1.23, 2.01	34.2 *t*	1.40, 1.58	28.7 *t*
8		44.4 *s*		40.5 *s*		38.7 *s*
9	2.59, *m*	55.3 *d*	1.74, *d* (10.8)	49.3 *d*	1.73, *d* (11.0)	52.3 *d*
10		37.2 *s*		37.0 *s*		38.3 *s*
11		199.0 *s*	3.82, *m*	70.0 *d*	4.16, *dt* (5.2, 11.0)	76.7 *d*
12	5.82, *s*	124.2 *d*	2.78, 2.00	33.8 *t*	2.05, 3.03	30.1 *t*
13		166.3 *s*		138.6 *s*		136.3 *s*
14		51.3 *s*		57.0 *s*		56.6 *s*
15	1.45, 2.13	30.1 *t*	1.31, 1.85	30.5 *t*	1.37, 1.83	30.2 *t*
16	2.03, 2.17	35.9 *t*	2.11	28.9 *t*	2.22, *br s*	29.0 *t*
17		93.8 *s*		134.9 *s*		139.2 *s*
18	1.27, *s*	24.4 *q*	0.98, *s*	24.2 *q*	1.03, *s*	20.3 *q*
19	1.07, *s*	25.1 *q*	1.01, *s*	25.6 *q*	1.15, *s*	29.2 *q*
20	2.62, *m*	37.8 *d*	2.82, *m*	28.4 *d*	2.80, *m*	28.3 *d*
21	1.07, *d* (7.0)	15.8 *q*	0.97, *d* (5.6)	20.3 *q*	1.03, *d* (7.0)	20.3 *q*
22	2.84, *dd* (8.0, 17.6)	36.9 *t*	1.18, *m*	36.3 *t*	1.39, *m*	39.9 *t*
	2.41, *dd* (7.2, 17.6)		1.37, *m*		1.60, *m*	
23		174.7 *s*	3.47, *d* (10.4)	74.7 *d*	3.77, *dd* (3.5, 9.3)	69.6 *d*
24				46.9[b] *s*	3.00, *br s*	77.4 *d*
25				181.5 *s*		74.4 *s*
26/24a			1.05, *s*	22.9 *q*	1.28, *s*	27.2 *q*
27/24b			1.08, *s*	18.2 *q*	1.22, *s*	26.2 *q*
28	1.10, *s*	29.4 *q*	1.05, *s*	29.6 *q*	1.22, *s*	29.7 *q*
29	1.11, *s*	19.4 *q*	1.03, *s*	20.1 *q*	1.23, *s*	20.9 *q*
30	1.29, *s*	22.4 *q*	1.13, *s*	23.1 *q*	1.15, *s*	21.2 *q*

[a] *J* values (Hz) are in parentheses. Multiplicities of carbons were determined by a DEPT experiment. 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR.
[b] Assignments in same vertical column may be interchangeable.

the α position. The stereochemistry of the side-chain was further assigned as (20*R*, 23*S*, 24*R*) on the basis of the NOESY correlations observed among H-23, H-24, CH_3-26 and CH_3-27. Further support of such an assignment was obtained by comparing the NMR spectroscopic data of the side-chain in **3** to those of alisol A (20*R*, 23*S*, 24*R*) (Nakajima et al., 1994) (Table 2). Alisol E, which has a 20*R*, 23*S*, 24*S* stereostructure, exhibits different NMR results (Yoshikawa et al., 1993b) (Table 2). Finally, Alisol P (**3**) was elucidated to be (20*R*,23*S*,24*R*)-23,24,25-trihydroxy-2,3-*seco*-protost-13(17)-en-3-oic acid 2,11β-lactone.

During the course of this study, the X-ray crystallographic data of alisol B 23-acetate (**4**) (Fig. 3) was also obtained and is now reported for the first time. The compound was previously obtained from *Alisma orientale* as a secondary metabolite in Zexie (Nakajima et al., 1994).

3. Concluding remarks

Protostane triterpenes are stereoisomers of dammaranes and characterized by the 8α-CH_3, 9β-H, 13α-H, and 14β-CH_3 structure. To date, all naturally occurring protostane triterpenes originate from the *Alisma* genus of plants and they are considered to be chemotaxonomic markers of this taxon. During the course of studying the chemical composition of a Chinese medicinal plant, *A. orientale*, we have obtained several protostane derivatives. Three of these secondary metabolites (**1–3**) are shown to be new structures, of which alisolide (**1**) is the first *nor*-protostane reported from a natural source.

4. Experimental

4.1. General

IR spectra were recorded on a Perkin–Elmer 16PC FT-IR spectrometer, whereas ^{1}H NMR and ^{13}C NMR spectroscopic data were obtained using a JEOL JNM-EX-400 FT-NMR spectrometer at 400 and 100 MHz, respectively, with TMS as internal standard. EI MS were determined on a Finnigan TSQ7000 triple quadrupole mass spectrometer, whereas APCI MS were determined on an Agilent HP 1100 series SL Trap MSD. The X-ray crystallography data were obtained using a Siemens P4-RA diffractometer. CC

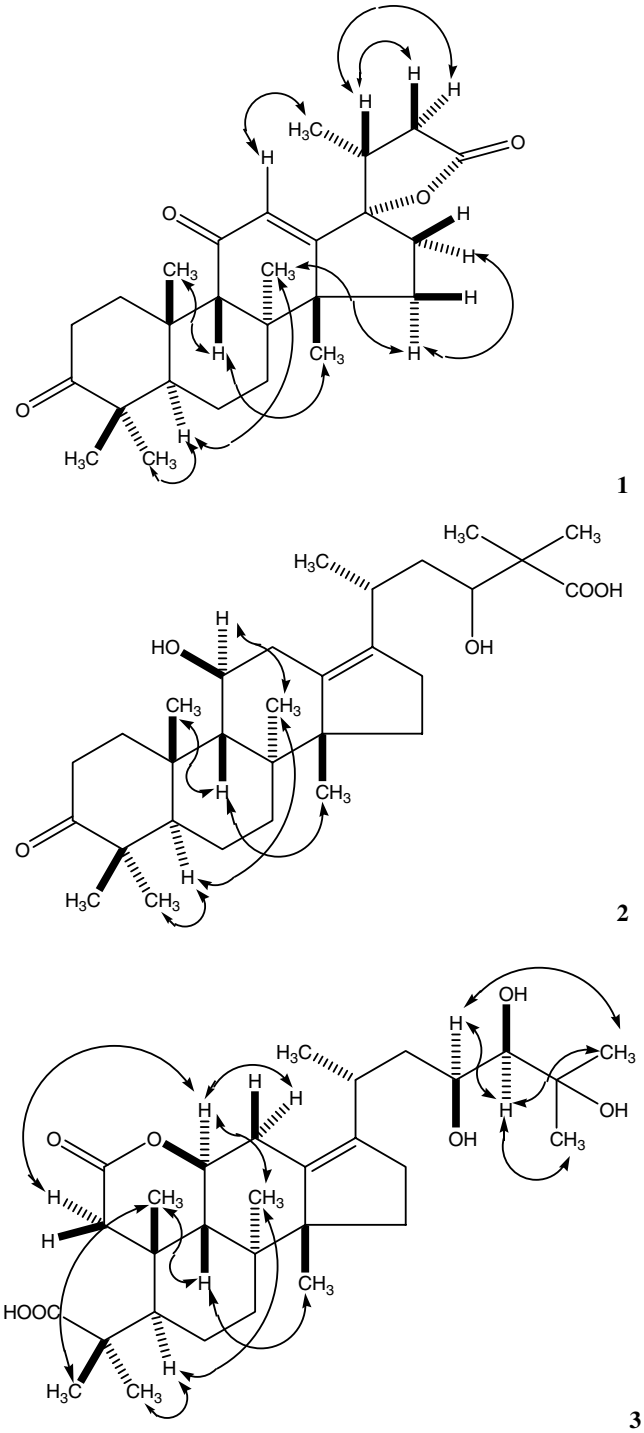

Fig. 2. Key NOESY correlations of **1**–**3**.

was carried out on silica gel (100–200 mesh, 230–400 mesh), RP-18 silica gel (40–63 μm) and Sephadex LH-20, respectively, and TLC was conducted on silica gel 60 F_{254} and RP-18 F_{254} plates.

4.2. Plant material

The dried rhizomes of *Alisma orientale* (Sam.) Juzep. were collected in Si-chuan Province, China, in 2002, and identified by Prof. Rui-Chao Lin of the Beijing Institute of Control of Biological and Pharmaceutical Products, Bei-jing. Voucher specimens (No. SCM072002) have been deposited in the School of Chinese Medicine, The Chinese University of Hong Kong.

Table 2
Comparison of NMR spectroscopic data of alisol P (**3**), alisol A and alisol E [a]

	Alisol P (**3**)	Alisol A [b] (20*R*, 23*S*, 24*R*)	Alisol E [c] (20*R*, 23*S*, 24*S*)
C-20	28.3	28.7	28.0
C-21	20.3	20.3	20.4
C-22	39.9	40.4	38.5
C-23	69.6	69.5	73.2
H-23	3.75 (*dd*, $J = 3.6$, 9.6 Hz)	3.77 (*dd*, $J = 3.5$, 9.3 Hz)	3.47 (*t*-like)
C-24	77.5	77.6	79.1
H-24	3.00 (*br s*)	3.00 (*br s*)	3.27 (*d*, $J = 6$ Hz)
C-25	74.4	74.1	71.6
C-26	27.2	27.6	25.9
C-27	26.2	26.4	26.4

[a] Chemical shifts expressed in ppm.
[b] Data extracted from Nakajima et al. (1994).
[c] Data extracted from Yoshikawa et al. (1993b).

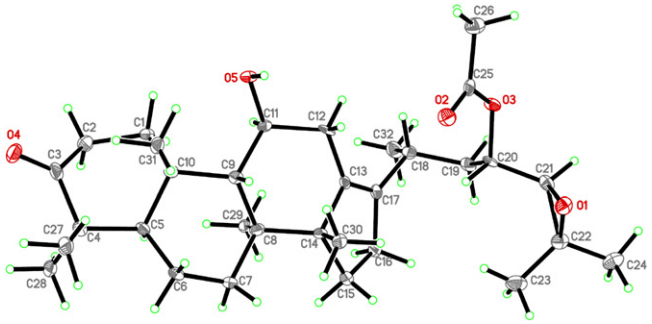

Fig. 3. X-ray structure of alisol B 23-acetate (**4**).

4.3. Extraction and isolation

Dried rhizomes of *Alisma orientale* (2.5 kg) were powdered and percolated with EtOH–H_2O (4:1, v/v) at room temperature until the percolate became colorless. After removal of excessive EtOH under reduced pressure, the remaining solution was allowed to stand for several hours to separate into an oil and an aqueous layer. The aqueous layer was then further extracted with EtOAc (2 L × 3 times). The oil layer was subjected to silica gel CC eluted with a petroleum ether–EtOAc gradient (9:1 → 1:1) to afford three fractions (Fr. I–III). Fr. I was fractionated by silica gel CC using mixtures of petroleum ether–EtOAc (10:0 → 8:2) as mobile phase to yield thirty fractions (Fr. I_1–I_{30}). Fr. I_{15} was subjected to further separation on a RP-18 column eluted with MeOH–H_2O (4:1, v/v) to yield alisolide (**1**, 8 mg). Fr. I_{20} yielded a crop of alisol B 23-ace-

tate (**4**) (50 mg) upon purification over RP-18 silica gel. Fr. II was subjected to further silica gel CC eluted with petroleum ether–EtOAc (8:2 → 6:4) to afford 25 fractions (Fr. II_1–II_{25}). From Fr. II_6, alisol B (15 mg) was obtained after chromatography on RP-18 silica gel. Fr. II_{12} was further purified on a RP-18 column (MeOH–H_2O, 5.5:4.5 → 7.5:2.5) followed by silica gel chromatography ($CHCl_3$–MeOH, 10:0 → 9.8:0.2) to afford alisol O (**2**, 10 mg). Fr. $II_{14–16}$ and Fr. $II_{18–21}$ were subjected to silica gel CC eluted with mixture of $CHCl_3$–MeOH (10:0 → 9.7:0.3), respectively, to afford alisol A (16 mg), 25-*O*-methylalisol A (11 mg), 25-anhydroalisol A 24-acetate (10 mg), and 25-anhydroalisol A (15 mg). Fr. II_{23} was purified over a RP-18 column (MeOH–H_2O, 6:4 → 6.5:3.5, as mobile phase) to obtain alisol E 23-acetate (12 mg). Lastly, Fr. III and the EtOAc-soluble portion of the aqueous layer were combined and purified over a silica gel column using $CHCl_3$–MeOH (10:0 → 7:3) as mobile phase to afford twenty fractions (Fr. $III_{1–20}$). Fr. $III_{10–15}$ was applied to a silica gel column (eluted with $CHCl_3$–MeOH, 9:1 → 8:2) and then on a RP-18 column (eluted with MeOH–H_2O, 5.5:4.5 → 6.5:3.5) to afford alisol P (**3**, 15 mg) and 13β,17β-epoxyalisol A (15 mg), respectively.

4.4. Alisolide (*1*)

Colorless crystal, $C_{26}H_{36}O_4$; IR (KBr) ν_{max}: 3447, 2980, 1782, 1705, and 1663 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data, see Table 1; APCIMS *m/z* 413 $[M+H]^+$; HRMS Calc. for $C_{26}H_{37}O_4H$ $[M+H]^+$ 413.26864, found *m/z* 413.27096.

4.5. Alisol O (*2*)

White powder, $C_{30}H_{48}O_5$; IR (KBr) ν_{max}: 3436, 1706 cm^{-1}; For ^1H and ^{13}C NMR spectroscopic data, see Table 1; HRESIMS Calc. for $C_{30}H_{48}O_5Na$ $[M+Na]^+$ 511.33994, found *m/z* 511.33999.

4.6. Alisol P (*3*)

Colorless crystal, $C_{30}H_{48}O_7$; IR (KBr) ν_{max}: 3500–2500, 1748 and 1710 cm^{-1}. For ^1H and ^{13}C NMR spectroscopic data, see Table 1; APCIMS *m/z* 520 $[M]^+$; HRMS Calc. for $C_{30}H_{48}O_7Na$ $[M+Na]^+$ 543.32922, found *m/z* 543.32942.

4.7. X-ray data for alisol B 23-acetate (*4*)

Colorless prisms, $C_{32}H_{50}O_5$. Crystal size $0.40 \times 0.35 \times 0.30$ mm^3; The space group is $P2_12_12_1$; The crystals belong to the orthorhombic system with cell parameters $a = 7.5492(6)$ Å, $b = 14.5156(11)$ Å, $c = 26.888(2)$ Å, $V = 2946.4(4)$ Å3, $Z = 4$, $D_{calcd} = 1.160$ mg/m^3, $\mu = 0.076$ mm−1, $F(000) = 1128$. The structures were refined by full-matrix least-squares on F^2 using SHELEXL-97. Final discrepancy indices of $R_1 = 0.0565$, $wR_2 = 0.1156$ and GOOF = 1.007 for observed data with $I > 2\sigma(I)$. The final difference electron density map contains maximum and minimum peak heights of 0.232 and -0.209 e $Å^{-3}$. Crystallographic data (excluding structure factors) for the structural analysis have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 612128. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033; e-mail: deposit@ccdc.camac.uk].

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