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# Crude Drugs from Aquatic Plants. I. On the Constituents of Alismatis Rhizoma. (1). Absolute Stereostructures of Alisols E 23-Acetate, F, and G, Three New Protostane-Type Triterpenes from Chinese Alismatis Rhizoma

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**From the less polar fraction of Chinese Alismatis Rhizoma [the dried rhizome of the aquatic plant *Alisma orientale* JUZEP. collected in Szechwan Province, China (Sentaku in Japanese)], three new protostane-type triterpenes named alisols E 23-acetate, F, and G were isolated together with four guaiane-type sesquiterpenes and several known triterpenes including 13,17-epoxyalisol A. The absolute stereostructures of alisols E 23-acetate, F, and G have been determined on the basis of chemical and physicochemical evidence, which included the chemical correlations of alisols E 23-acetate, F, and G with the known triterpenes alisols A and A 24-acetate, and the application of the modified Mosher's method.**

**Keywords** *Alisma orientale*; Alismatis Rhizoma; protostane-type triterpene; alisol E 23-acetate; alisol F; alisol G

The dried rhizome of the aquatic plant *Alisma orientale* JUZEPCZUK (Alismataceae) is used as a Chinese crude drug, Alismatis Rhizoma (Takusha in Japanese), which is prescribed for diuretic and antiinflammatory purposes in Chinese traditional medicine. In Japan, Chinese Alismatis Rhizoma is classified into two types named in Japanese as Kentaku (Alismatis Rhizoma from Fukien Province) and Sentaku (Alismatis Rhizoma from Szechwan Province) according to their regions of origin. In recent years, due to the poor supply of Japanese Alismatis Rhizoma, Chinese Alismatis Rhizoma, Sentaku, has been imported and commonly used in Chinese medicinal treatment in Japan.[1)]

In regard to chemical studies on the constituents of Alismatis Rhizoma,[1a,2)] the triterpene constituents, which are the principal constituents of this crude drug, have been investigated extensively. Five protostane-type triterpenes, alisols A (**1**), A monoacetate (**1a**), B, B monoacetate, and C monoacetate, were first isolated from Japanese Alismatis Rhizoma, and their absolute stereostructures were determined on the basis of chemical and physicochemical evidence, including the X-ray analysis of an alisol A derivative.[3)] Since then, several chemical investigations of Japanese and Chinese Alismatis Rhizoma have been carried out to discover more triterpenes[4)] such as 16$\beta$-methoxyalisol B monoacetate, 16$\beta$-hydroxyalisol B monoacetate, 11-deoxyalisol C, and alisol D, and sesquiterpenes.[5)]

In our search for biologically active constituents from natural occurring crude drugs originating from aquatic plants, we have investigated the chemical constituents of Alismatis Rhizoma.[6)] In a continuing study, we have isolated several new constituents from Chinese Alismatis Rhizoma Sentaku, namely three protostane-type triterpenes designated alisols E 23-acetate (**6a**), F (**7**), and G (**8**), four sesquiterpenes, orientalols A,[7)] B,[7)] C,[7)] and D,[7b)] and four water-soluble sesquiterpenes, sulfoorientalols a,[8)] b,[8)] c,[8)] and d,[8)] together with alisols A (**1**),[3)] A monoacetate (**1a**),[3)] B,[3)] and B monoacetate,[3)] 13,17-epoxyalisol A (**9**),[4f)] alismol[5,7)] and alismoxide.[5,7)] This paper presents a full account of the structure elucidations of alisols E 23-acetate (**6a**), F (**7**), and G (**8**), which were isolated from the less polar fraction of the constituents of Sentaku.

The less polar constituents from Chinese Alismatis Rhizoma were isolated through the procedure shown on Fig. 1. Thus, the methanolic extract of the rhizome was partitioned into an ethyl acetate and water mixture and the ethyl acetate-soluble portion was subjected to silica gel column chromatography to provide five fractions. Each fraction was further separated by silica gel and reversed-phase silica gel column chromatography finally to afford alisols E 23-acetate (**6a**), F (**7**), and G (**8**), and orientalols A, B, C and D, together with alisols A (**1**), A monoacetate (**1a**), B and B monoacetate, 13,17-epoxyalisol A (**9**), alismol and alismoxide.

**Alisol E 23-Acetate (6a)** Alisol E 23-acetate (**6a**) was obtained as colorless prisms of mp 167.5—169.0 °C. The molecular formula $C_{32}H_{52}O_6$ of **6a** was confirmed from the quasimolecular ion peak $(M+Na)^+$ at $m/z$ 555 in the positive FAB-MS and by high-resolution MS measurement. The IR spectrum of **6a** showed absorption bands due to hydroxyl, ester, and ketone moieties at 3440, 1735, and 1700 $cm^{-1}$. Treatment of **6a** with 5% sodium methoxide in methanol at room temperature provided the deacetylated product (alisol E, **6**), of which the molecular formula, $C_{30}H_{50}O_5$, was found to be identical with that of alisol A (**1**). The $^1$H-NMR spectrum of **6** showed the signals due to five tertiary methyls, two tertiary methyls geminal to a hydroxyl group and a secondary methyl, together with many other signals closely resembling those of **1**, except for the signals at $\delta$ 3.27 (d, $J=6$ Hz, 24-H) and 3.47 (t-like, 23-H). Detailed comparison of the $^{13}$C-NMR data for **6** with those for **1** led us to presume that **6** was the diastereoisomer of **1** at the C-23 or C-24-position. To verify this presumption, a chemical correlation of **1** and **6** was undertaken.

Thus, treatment of alisol A monoacetate (**1a**) with chloromethyl methyl ether (MOMCl) and subsequent deacetylation of the product gave 11,23,25-tri-*O*-methoxymethylalisol A (**2**) which was subjected to oxidation with pyridinium chlorochromate (PCC) followed by deprotection with *p*-toluenesulfonic acid (*p*-TsOH) to furnish the 24-keto derivative (**3**). Reduction of **3** with sodium borohydride ($NaBH_4$) in methanol at room temperature provided dihydroalisol A (**4**)[3b)] and the 24-diastereoisomer (**5**) in a 1:2 ratio. The latter (**5**) was found to be identical with dihydroalisol E, which was prepared by reduction of **6** with

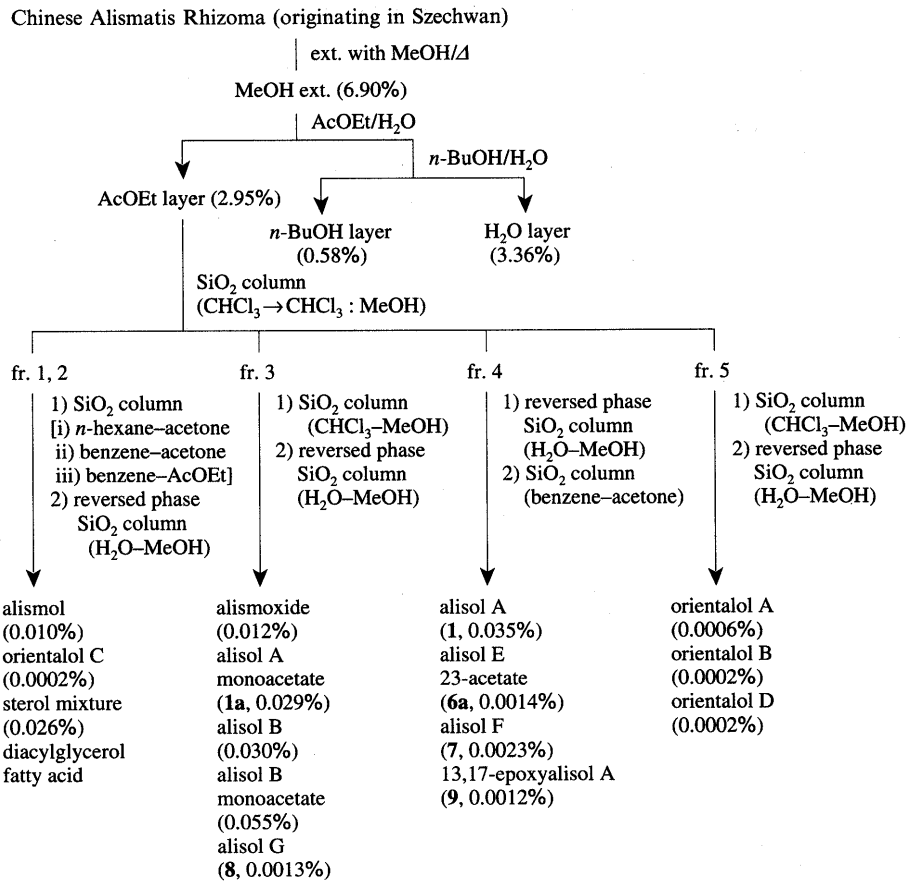


Fig. 1. Isolation Procedure for Sesquiterpenes and Triterpenes from Chinese Alismatis Rhizoma

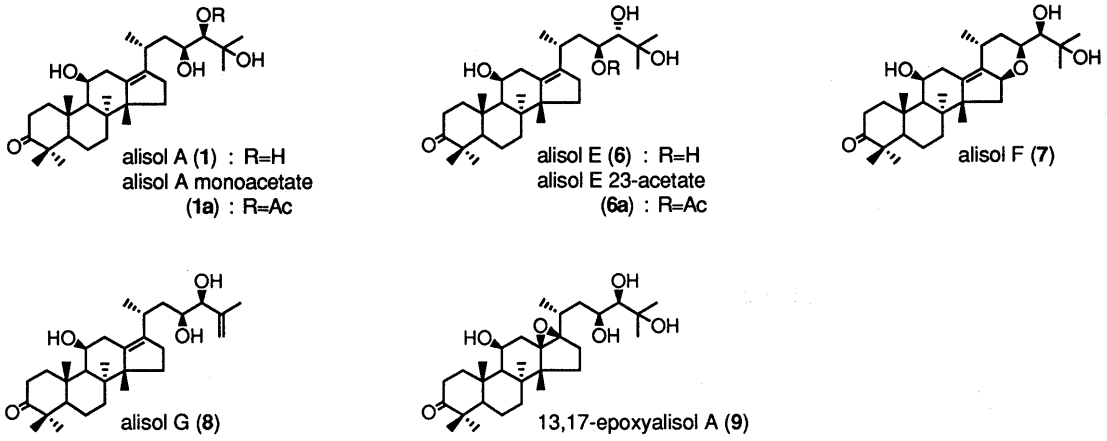


Chart 1

$NaBH_4$ in methanol. Consequently, the absolute stereostructure of alisol E (**6**) was concluded to be as shown.

The $^1$H-NMR spectrum of **6a** showed signals ascribable to an acetoxyl group at $\delta$ 2.07 (3H, s), an acetoxyl-bearing methine at $\delta$ 4.76 (m, 23-H) and two hydroxyl-bearing methines at $\delta$ 3.52 (br s, 24-H) and $\delta$ 3.81 (ddd, $J$=6, 11, 11 Hz, 11-H). In the $^{13}$C-NMR spectrum of **6a**, an acetylation shift[9] around the C-23 position was observed (Table I). Based on the above evidence, the structure of alisol E 23-acetate (**6a**) has been determined. Finally, the configuration at the C-24 position in **6a** has been further confirmed by $^1$H-NMR analysis (the modified Mosher's method)[10] of the 11,24-di-(−)-(*S*)- and (+)-(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetates (MTPA esters, **10** and **11**). As shown in Fig. 1, the signals due to protons attached to C-26 and C-27 in the (−)-(*S*)-MTPA ester (**10**) were observed at lower field as compared to those of the (+)-(*R*)-MTPA ester (**11**) [$\Delta\delta$: positive], while the signals due to protons on C-20, C-21, C-22 and C-23 in **10** were observed at higher fields as compared to those of **11** [$\Delta\delta$: negative]. Thus, the absolute configuration at C-24 in **6a** has been confirmed to be *S* and the absolute stereostructure of alisol E 23-acetate (**6a**) has been determined as shown.

**Alisol F (7)** Alisol F (**7**) was obtained as a white powder and its molecular formula, $C_{30}H_{48}O_5$, was clarified from the quasimolecular ion peak at $m/z$ 511 $(M+Na)^+$ observed

in the positive FAB-MS and by high-resolution MS measurement. In the IR spectrum of **7**, it showed the presence of hydroxyl and ketone moieties (3450, 1700 $cm^{-1}$). The $^1H$-NMR spectrum of **7** showed signals due to two hydroxyl-bearing methines [$\delta$ 3.05 (br s, 24-H), 3.79 (ddd, $J=6$, 11, 11 Hz, 11-H)] and two methines geminal to the oxide ring [$\delta$ 4.04 (br d, 23-H), 4.46 (dd, $J=5$, 8 Hz, 16-H)], together with seven tertiary methyls and a secondary methyl group in a protostane structure. The proton signals of the seven tertiary methyl groups of **7** could be assigned from the $^1H$–$^{13}C$ correlation spectroscopy ($^1H$–$^{13}C$ COSY), and nuclear Overhauser effect spectroscopy (NOESY) spectra and by comparisons with those of **1**. The carbon signals in the $^{13}C$-NMR spectrum of **7** were very similar to those of **1** except for some signals due to the 16,23-oxide ring moiety. Acetylation of **7** with acetic anhydride in pyridine at room temperature provided the diacetate (**7a**). Comparison of the $^1H$-NMR data for **7a** with those for **7** led us to presume the presence of 11, 24, and 25-hydroxyl groups and a 16,23-oxide ring in the protostane-type triterpene structure of **7**. The relative configuration of the 16,23-oxide ring in **7** was clarified by a NOESY experiment

TABLE I. $^{13}C$-NMR Data for **1**, **6**, **6a**, **7**, **8**, and **9**

| Carbon | 1 | 6 | 6a | 7 | 8 | 9 |
|---|---|---|---|---|---|---|
| 1 | 31.0 | 31.0 | 30.9 | 30.6 | 31.1 | 30.9 |
| 2 | 33.7 | 33.7 | 33.7 | 33.8 | 33.8 | 33.5 |
| 3 | 220.6 | 220.1 | 220.4 | 220.0 | 220.6 | 220.1 |
| 4 | 46.9 | 47.0 | 46.9 | 46.9 | 47.0 | 46.9 |
| 5 | 48.5 | 48.5 | 48.4 | 48.1 | 48.5 | 48.7 |
| 6 | 20.0 | 20.1 | 20.0 | 19.2 | 20.1 | 20.1 |
| 7 | 34.3 | 34.3 | 34.1 | 33.5 | 34.3 | 34.7 |
| 8 | 40.3 | 40.6 | 40.7 | 40.4 | 40.6 | 40.3 |
| 9 | 49.6 | 49.8 | 49.9 | 49.5 | 49.6 | 48.9 |
| 10 | 36.9 | 36.9 | 36.9 | 36.9 | 37.0 | 36.9 |
| 11 | 69.7 | 70.1 | 70.2 | 70.3 | 69.9 | 68.4 |
| 12 | 34.4 | 34.6 | 34.5 | 33.8 | 34.5 | 35.3 |
| 13 | 137.6 | 137.6 | 137.8 | 136.9 | 137.9 | 73.3 |
| 14 | 57.0 | 57.0 | 57.0 | 55.3 | 57.0 | 50.0 |
| 15 | 30.5 | 30.6 | 30.7 | 39.4 | 30.6 | 30.6 |
| 16 | 29.5 | 29.3 | 29.4 | 80.1 | 29.1 | 26.5 |
| 17 | 135.5 | 135.8 | 134.8 | 132.9 | 135.2 | 78.5 |
| 18 | 23.0 | 23.2 | 23.4 | 24.3 | 23.3 | 19.2 |
| 19 | 25.6 | 25.6 | 25.6 | 25.4 | 25.7 | 25.7 |
| 20 | 28.2 | 28.0 | 28.1 | 26.5 | 28.3 | 30.7 |
| 21 | 20.1 | 20.4 | 20.1 | 18.2 | 20.4 | 17.5 |
| 22 | 40.0 | 38.5 | 33.2 | 34.8 | 38.3 | 38.7 |
| 23 | 69.4 | 73.2 | 74.2 | 72.6 | 70.8 | 68.6 |
| 24 | 77.6 | 79.1 | 78.4 | 77.2 | 79.9 | 76.9 |
| 25 | 74.2 | 71.6 | 71.9 | 73.3 | 144.7 | 73.6 |
| 26 | 26.2 | 25.9 | 24.9 | 26.5 | 17.8 | 26.6 |
| 27 | 27.3 | 26.4 | 28.4 | 26.9 | 114.1 | 26.7 |
| 28 | 29.5 | 29.6 | 29.5 | 29.5 | 29.6 | 29.6 |
| 29 | 20.0 | 20.0 | 20.0 | 19.9 | 20.1 | 19.9 |
| 30 | 24.1 | 24.0 | 23.8 | 23.5 | 24.0 | 24.6 |

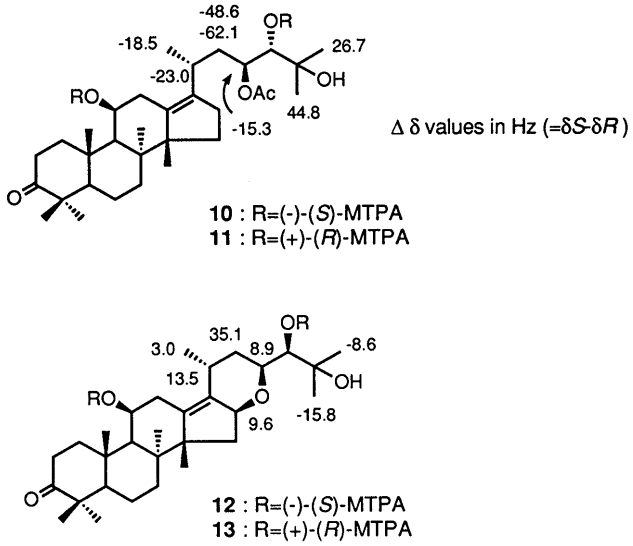


Fig. 2

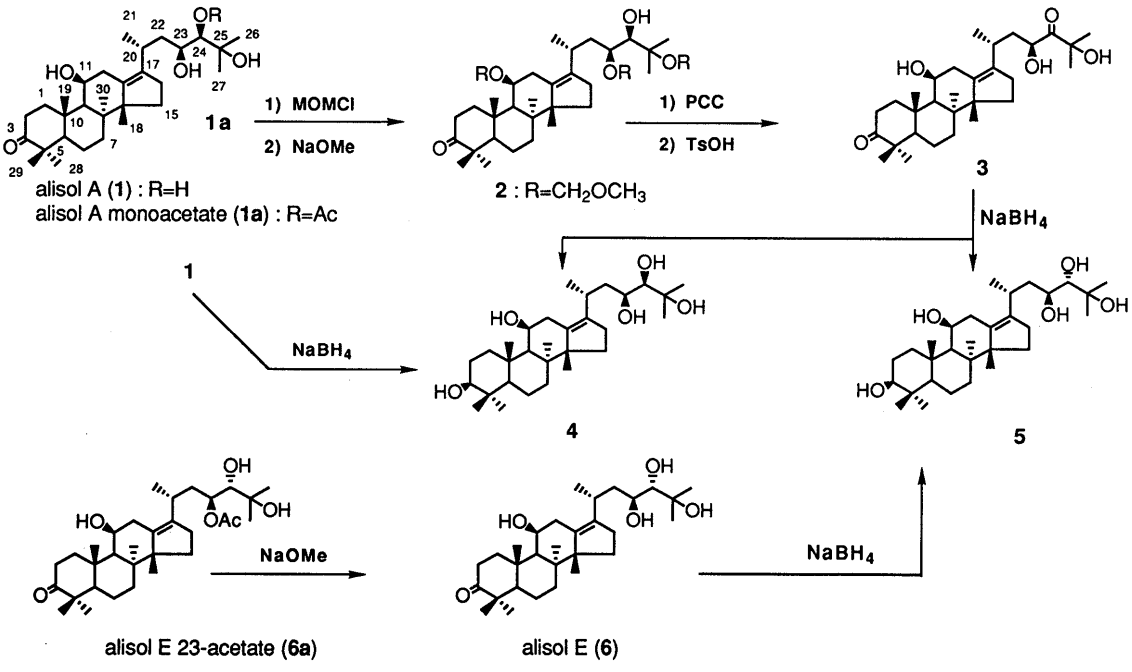


Chart 2

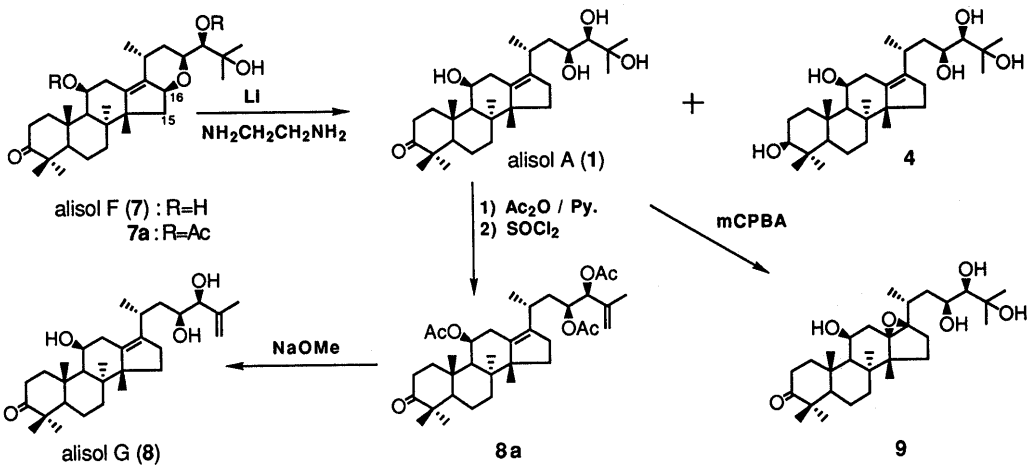


Chart 3

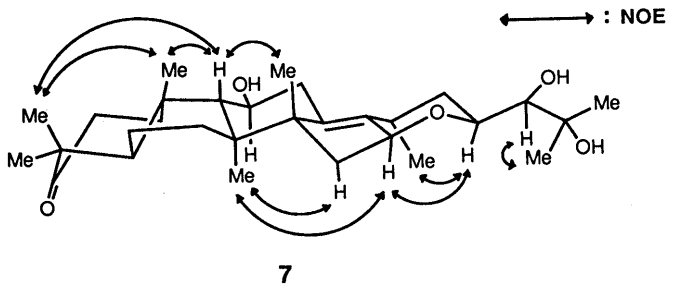


Fig. 3

as depicted in Fig. 3 and also by comparison of the $^1H$–$^1H$ coupling constants with those reported for related protostane-type triterpenes.[4)]

The absolute configuration at C-24 in **7** has been confirmed by the application of the modified Mosher's method. As shown in Fig. 2, the signals due to the protons on C-26 and C-27 in the (−)-(*S*)-MTPA ester (**12**) were observed at higher field as compared to those of the (+)-(*R*)-MTPA ester (**13**), while the signals due to the protons of C-16, C-20, C-21, C-22 and C-23 in **12** were observed at lower field as compared to those of **13**. Consequently, the absolute configuration at C-24 in **7** has been elucidated as *R*. Furthermore, in order to confirm the absolute stereostructure of **7**, a chemical conversion of **7** to **1** was undertaken. Thus, it was found that reduction of **7** with lithium in ethylenediamine furnished **1** and its dihydro derivative **4**. Based on the above-mentioned evidence, the absolute stereostructure of alisol F (**7**) was determined to be as shown.

**Alisol G (8)** Alisol G (**8**), which was obtained as colorless plates of mp 151.5—152.5 °C, showed absorption bands of hydroxyl, ketone, and *exo*-olefin groups. The molecular formula, $C_{30}H_{48}O_4$, was clarified from the quasimolecular ion peaks $(M+Na)^+$ and $(M+H)^+$ observed in the positive FAB-MS and by high-resolution MS measurement. The $^1H$-NMR spectrum of **8** showed signals assignable to the isopropenyl moiety at $\delta$ 1.66 (3H, s, 26-$H_3$) and $\delta$ 4.93, 4.96 (1H each, both br s, 27-$H_2$) together with the signals due to five tertiary methyls, a secondary methyl and three hydroxy-bearing methines. The carbon signals in the $^{13}C$-NMR spectrum of **8** were superimposable on those of alisol A (**1**) except for several signals assignable to the isopropenyl moiety. Based on these findings, **8** has been presumed to be the 25-dehydroxy derivative of **1**.[4a,11)] In order to verify this presumption, a chemical conversion of **1** to **8** was undertaken. Thus, ordinary acetylation of **1** with acetic anhydride in pyridine and subsequent dehydroxylation of the product with thionyl chloride ($SOCl_2$) in pyridine furnished **8a**, which was subjected to deacetylation with 5% sodium methoxide in methanol to give **8** in 60% yield. Consequently, the absolute stereostructure of alisol G (**8**) has been clarified to be as shown.

**13,17-Epoxyalisol A (9)** 13,17-Epoxyalisol A (**9**), obtained as colorless prisms of mp 138.5—140.0 °C, was shown to possess hydroxyl and ketone groups by its IR spectrum. The positive FAB-MS of **9** showed a quasimolecular ion peak $(M+Na)^+$ at *m/z* 529 and the high-resolution MS measurement revealed the molecular formula to be $C_{30}H_{50}O_6$. The $^1H$-NMR spectrum of **9** showed the presence of seven tertiary methyls and a secondary methyl group as well as three methine protons on carbon bearing a hydroxyl group. Detailed comparisons of the $^1H$- and $^{13}C$-NMR data (Table I) for **9** with those for **1** and alisol D[4e)] led us to presume that **9** was the 13$\beta$,17$\beta$-epoxide derivative of **1**. 13,17-Epoxyalisol A, isolated from Alismatis Rhizoma, has been prepared from **1** by epoxidation with *m*-chloroperbenzoic acid (mCPBA).[4f)] In order to confirm the identification, **1** was subjected to epoxidation with mCPBA in dichloromethane, and the resultant epoxide, 13,17-epoxyalisol A, was found to be identical with **9**.

**Experimental**

The following instruments were used to obtain physical data: melting points, Yanagimoto micro-melting point apparatus (values are uncorrected); specific rotation, Horiba SEPA-200 digital polarimeter ($l$=0.5 dm); IR spectra, Shimadzu FT-IR DR-8000 infrared spectrometer; $^1H$-NMR spectra, JEOL EX-270 (270 MHz) FT-NMR spectrometer (with tetramethylsilane as an internal standard); $^{13}C$-NMR spectra, JEOL EX-270 (67.5 MHz) FT NMR spectrometer; EI-MS, Hitachi M-80 mass spectrometer; FAB-MS and high-resolution MS measurement, JEOL JMS SX-102 double-beam high-resolution mass spectrometer (Xe gas) equipped with a JMA DA-6000 data system. The following experimental conditions were used for chromatography: HPLC, Shimadzu LC-10AS; differential refractometer, Shimadzu RID-6A; column chromatography, Silica gel BW-200 (Fuji-Devison) or Silica gel 60 silanised (Merck) as the absorbent; TLC, pre-coated TLC plates, Silica gel 60 $F_{254}$ (0.25 mm) or RP-18 $F_{254s}$

(0.25 mm) with detection by spraying 1% $Ce(SO_4)_2$–10% aqueous $H_2SO_4$ followed by heating.

**Isolation of Triterpenes and Sesquiterpenes from the Less Polar Fraction of Chinese Alismatis Rhizoma** The dried rhizomes (Chinese Alismatis Rhizoma, Sentaku, 10 kg, purchased from Kireido, Kyoto) were cut finely and extracted with MeOH under reflux three times. Evaporation of the solvent under reduced pressure gave the MeOH extract (690 g), which was partitioned into a AcOEt–$H_2O$ mixture. Removal of the solvent from the AcOEt-soluble phase under reduced pressure yielded the AcOEt extract (295 g). The AcOEt extract (290 g) was subjected to silica gel column chromatography [$CHCl_3$, $CHCl_3$–MeOH (20:1—10:1)] to furnish five fractions. Evaporation of the solvent under reduced pressure gave fr. 1 (mainly fatty acid and diglycerides, 32 g), fr. 2 (85.6 g), fr. 3 (124 g), fr. 4 (20.8 g), and fr. 5 (22.3 g).

Fraction 2 (85.3 g) was purified by repeated silica gel column chromatography [*n*-hexane–acetone(7:1), benzene–acetone (5:1), and benzene–AcOEt (10:1)] and reversed-phase silica gel column chromatography (33% aqueous MeOH) to give alismol (1.0 g), a mixture of sterols (2.6 g), and orientalol C (24 mg). Silica gel column chromatography [$CHCl_3$–MeOH (20:1)] of fraction 3 (124 g) followed by reversed-phase silica gel column chromatography (50% aqueous MeOH) afforded alismoxide (1.2 g), alisol A monoacetate (**1a**, 2.9 g), alisol B (3.0 g), alisol B monoacetate (5.5 g) and alisol G (**8**, 129 mg). Fraction 4 (20.1 g) was subjected to reversed-phase silica gel column chromatography (50% aqueous MeOH) and subsequent silica gel column chromatography [benzene–acetone (3:1)] to give alisol A (**1**, 3.5 g), 13,17-epoxyalisol A (**9**, 120 mg), alisol E 23-acetate (**6a**, 137 mg), and alisol F (**7**, 230 mg). Silica gel column chromatography [$CHCl_3$–MeOH (15:1)] of fraction 5 (22.0 g) followed by reversed-phase silica gel column chromatography (60% aqueous MeOH) gave orientalols A (63 mg), B (21 mg) and D (18 mg). Alisols A (**1**), A monoacetate (**1a**), B, and B monoacetate, alismol, and alismoxide were identified by comparison with authentic samples[3,6] ($^1$H-NMR, $^{13}$C-NMR, IR, and $[\alpha]_D$ data). The water-soluble portion was extracted with 1-BuOH and removal of the solvent from the 1-BuOH-soluble portion under reduced pressure gave the 1-BuOH extract (58 g). The procedure for separation of the 1-BuOH extract will be reported in detail in our forthcoming paper.[12]

Alisol E 23-Acetate (**6a**): mp 167.5—169.0 °C (colorless prisms from *n*-hexane–AcOEt), $[\alpha]_D^{22}$ +77.1° ($c$=1.8, MeOH). High-resolution MS: Found, 555.3661; Calcd for $C_{32}H_{52}O_6Na$ [$(M+Na)^+$], 555.3617. IR (KBr): 3440 (OH), 1735 (OAc), 1700 (C=O), 1245 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.98 (3H, s, 30-$H_3$), 1.01 (3H, d, $J$=8 Hz, 21-$H_3$), 1.04, 1.05, 1.07, 1.15, 1.23, 1.27 (3H each, all s, 19, 29, 28, 18, 26, 27-$H_3$), 1.72 (1H, d, $J$=11 Hz, 9-H), 1.75 (1H, m, 22-H), 2.07 (3H, s, OAc), 2.50 (1H, m, 22-H), 2.63 (1H, m, 20-H), 3.52 (1H, br s, 24-H), 3.81 (1H, ddd, $J$=6, 11, 11 Hz, 11-H), 4.76 (1H, m, 23-H). $^{13}$C-NMR ($CDCl_3$) $\delta_C$: 170.9, 21.4 ($CH_3CO$–) and other signals as given in Table I. FAB-MS: $m/z$ 555 $(M+Na)^+$.

Alisol F (**7**): White powder, $[\alpha]_D^{22}$ +67.8° ($c$=0.5, MeOH). High-resolution MS: Found, 511.3381; Calcd for $C_{30}H_{48}O_5Na$ [$(M+Na)^+$], 511.3316. IR (KBr): 3450 (OH), 1700 (C=O), 1460, 1375 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.88, 1.05, 1.06, 1.07 (3H each, all s, 30, 19, 29, 28-$H_3$), 1.17 (3H, d, $J$=7 Hz, 21-$H_3$), 1.24, 1.24, 1.30 (3H each, all s, 18, 26, 27-$H_3$), 1.27, 1.33 (1H each, both m, 22-$H_2$), 1.76 (1H, d, $J$=11 Hz, 9-H), 2.86 (1H, m, 20-H), 3.05 (1H, br s, 24-H), 3.79 (1H, ddd, $J$=6, 11, 11 Hz, 11-H), 4.04 (1H, br d, 23-H), 4.46 (1H, dd, $J$=5, 8 Hz, 16-H). $^{13}$C-NMR: see Table I. FAB-MS: $m/z$ 511 $(M+Na)^+$.

Alisol G (**8**): mp 151.5—152.5 °C (colorless plates from *n*-hexane–AcOEt), $[\alpha]_D^{22}$ +68.2° ($c$=1.8, MeOH). High-resolution MS: Found, 495.3431; Calcd for $C_{30}H_{48}O_4Na$ [$(M+Na)^+$], 495.3366. IR: 3410 (OH), 1700 (C=O), 1650, 1460, 1375, 900 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.99 (3H, s, 30-$H_3$), 1.00 (3H, d, $J$=6 Hz, 21-$H_3$), 1.06 (6H), 1.07, 1.14, 1.66 (3H each) (all s, 19, 29, 28, 28, 26-$H_3$), 1.75 (1H, d, $J$=10 Hz, 9-H), 3.34 (1H, ddd, $J$=3, 7, 10 Hz, 23-H), 3.77 (1H, d, $J$=7 Hz, 24-H), 3.86 (1H, ddd, $J$=6, 10, 10 Hz, 11-H), 4.93, 4.96 (1H each, both br s, 27-$H_2$). $^{13}$C-NMR: see Table I. FAB-MS: $m/z$ 495 $(M+Na)^+$.

13,17-Epoxyalisol A (**9**): mp 138.5—140.0 °C (colorless plates from *n*-hexane–isopropyl ether), $[\alpha]_D^{22}$ +122.1° ($c$=0.5, MeOH). High-resolution MS: Found, 529.3514; Calcd for $C_{30}H_{50}O_6Na$ [$(M+Na)^+$], 529.3505. IR (KBr): 3430 (OH), 1700 (C=O), 1465, 1380 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.05 (3H, s), 1.06—1.09 (12H, m) (*tert*-$CH_3$ × 4, 21-$H_3$), 1.12, 1.27, 1.31 (3H each, all s, *tert*-$CH_3$ × 3), 3.10 (1H, br s, 24-H), 4.06 (1H, ddd, $J$=6, 11, 11 Hz, 11-H), 4.15 (1H, dd, $J$=4, 10 Hz, 23-H). EI-MS (%): $m/z$ 506 ($M^+$, 0.5), 470 ($M^+-2H_2O$, 1), 199 (100). FAB-MS: $m/z$ 529 $(M+Na)^+$.

**Alkaline Hydrolysis of Alisol E 23-Acetate (6a)** A solution of **6a** (28.7 mg) in 5% NaOMe–MeOH (1 ml) was stirred at 20 °C under an $N_2$ atmosphere for 1 h. The reaction mixture was neutralized with Dowex 50w × 8 ($H^+$) and filtered to remove the resin. Evaporation of the solvent from the filtrate under reduced pressure gave a product, which was purified by silica gel column chromatography [benzene–acetone (2:1)] to give alisol E (**6**, 25.1 mg).

Alisol E (**6**): White powder, $[\alpha]_D^{22}$ +42.1° ($c$=0.4, MeOH). High-resolution MS: Found, 513.3572; Calcd for $C_{30}H_{50}O_5Na$ [$(M+Na)^+$], 513.3556. IR (KBr): 3450 (OH), 1700 (C=O), 1460, 1375 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.99 (3H, s, 30-$H_3$), 1.02 (3H, d, $J$=7 Hz, 21-$H_3$), 1.03 (3H), 1.05 (6H), 1.13, 1.21, 1.22 (3H each) (all s, 19, 29, 28, 18, 26, 27-$H_3$), 3.27 (1H, d, $J$=6 Hz, 24-H), 3.47 (1H, t-like, 23-H), 3.88 (1H, ddd, $J$=6, 11, 11 Hz, 11-H). $^{13}$C-NMR: see Table I. EI-MS (%): $m/z$ 472 ($M^+-H_2O$, 6), 150 (100), 122 (40). FAB-MS: $m/z$ 513 $(M+Na)^+$.

**Preparation of 2 from Alisol A Monoacetate (1a)** A solution of **1a** (51.1 mg) in $CH_2Cl_2$ (2 ml) was treated with chloromethylmethyl ether (21 $\mu$l) in the presence of diisopropylethylamine (98 $\mu$l) and the whole mixture was stirred at 20 °C under an $N_2$ atmosphere for 12 h. The reaction mixture was neutralized with 5% aqueous HCl and the whole was extracted with AcOEt. The AcOEt extract was washed successively with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. After removal of the solvent from the AcOEt extract under reduced pressure, the product was purified by silica gel column chromatography [*n*-hexane–AcOEt (3:1)] to furnish the methoxymethyl derivative (44.9 mg). This derivative was successively dissolved in 5% NaOMe–MeOH (1 ml) and the solution was stirred at 20 °C under an $N_2$ atmosphere for 1.5 h. The reaction mixture was neutralized with Dowex 50w × 8 ($H^+$) and filtered to remove the resin. Evaporation of the solvent from the filtrate under reduced pressure gave **2** (35.8 mg).

**2**: White powder, $[\alpha]_D^{22}$ +55.6° ($c$=0.4, MeOH). IR (KBr): 3500 (OH), 1710 (C=O), 1465, 1375 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.99 (3H, s, 30-$H_3$), 1.01 (3H, d, $J$=7 Hz, 21-$H_3$), 1.05, 1.06, 1.07, 1.15, 1.22, 1.29 (3H each, all s, 19, 29, 28, 18, 26, 27-$H_3$), 3.23 (1H, dd-like, 24-H), 3.29 (1H, d, $J$=6 Hz, 24-OH), 3.36, 3.38, 3.40 (3H each, all s, $OCH_3$), 3.56 (1H, m, 23-H), 3.65 (1H, ddd, $J$=6, 11, 11 Hz, 11-H), 4.61—4.70 (6H, m, $-CH_2O-$ × 3). EI-MS (%): $m/z$ 528 (6), 496 (10), 150 (100), 122 (30).

**Preparation of 3 from 2** A solution of **2** (63.3 mg) in $CH_2Cl_2$ (2 ml) was treated with PCC (61 mg) and the whole mixture was stirred at 20 °C under an $N_2$ atmosphere for 5 h. The product was purified by Florisil column chromatography (ether) to give the ketone derivative (53 mg). A solution of the ketone derivative in 90% aqueous acetone (1 ml) was treated with *p*-TsOH·$H_2O$ (8 mg) and the whole mixture was stirred at 55 °C for 5 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave **3** (13.4 mg).

**3**: White powder, $[\alpha]_D^{22}$ +147.0° ($c$=0.2, MeOH). IR (KBr): 3450 (OH), 1705 (C=O), 1465, 1375 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 1.01 (3H, s, 30-$H_3$), 1.05 (3H, d, $J$=7 Hz, 21-$H_3$), 1.06 (6H), 1.07, 1.18, 1.30, 1.40 (3H each) (all s) (29, 19, 28, 18, 26, 27-$H_3$), 1.75 (1H, d, $J$=11 Hz, 9-H), 3.79 (1H, ddd, $J$=6, 11, 11 Hz, 11-H), 4.33 (1H, br d, 23-H). EI-MS (%): $m/z$ 410 (0.1), 150 (100), 122 (28).

**$NaBH_4$ Reduction of 3** A solution of **3** (8.7 mg) in MeOH (0.5 ml) was treated with $NaBH_4$ (5.0 mg) and the whole mixture was stirred at 20 °C under an $N_2$ atmosphere for 15 min. The reaction mixture was poured into ice-5% aqueous HCl and the whole was extracted with $CH_2Cl_2$. The $CH_2Cl_2$ extract was washed with brine and then dried over $MgSO_4$. After removal of the solvent from the $CH_2Cl_2$ extract, the product was purified by HPLC (column: Chromatorex 10.0 mm i.d. × 250 mm, mobile phase: 80% MeOH) to give dihydroalisol A (**4**, 2.3 mg) and **5** (5.4 mg). Dihydroalisol A thus obtained was shown to be identical with an authentic sample, which was prepared from **1** by $NaBH_4$ reduction, based on comparison of $^1$H-NMR, IR, EI-MS, and $[\alpha]_D$ data and TLC behavior.

**5**: White powder, $[\alpha]_D^{22}$ +11.4° ($c$=0.3, MeOH). High-resolution MS: Found, 515.3724; Calcd for $C_{30}H_{52}O_5Na$ [$(M+Na)^+$], 515.3712. IR (KBr): 3400 (OH), 1460, 1375 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.81, 0.95, 1.00 (3H each, all s, *tert*-$CH_3$ × 3), 1.03 (3H, d, $J$=7 Hz, 21-$H_3$), 1.11, 1.17, 1.23, 1.25 (3H each, all s, *tert*-$CH_3$ × 4), 3.26 (2H, m, 3, 24-H), 3.48 (1H, t-like, 23-H), 3.84 (1H, m, 11-H). FAB-MS: $m/z$ 515 $(M+Na)^+$.

**Preparation of the 11,24-Di-(−)-(*S*)-MTPA Ester (10) from 6a** A solution of **6a** (4.6 mg) in $CH_2Cl_2$ (0.5 ml) was treated with (−)-(*S*)-MTPA (23.6 mg) in the presence of DCC (29.0 mg) and DMAP (9.2 mg), and the whole mixture was stirred at 20 °C under an $N_2$ atmosphere for 15 min. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner

gave a product which was purified by silica gel column chromatography [*n*-hexane–acetone (5 : 1)] to furnish **10** (6.0 mg).

**10**: White powder. $^1$H-NMR ($CDCl_3$) $\delta$: 0.44 (3H, s, 19-$H_3$), 0.89 (3H, d, $J$=7 Hz, 21-$H_3$), 0.93, 0.97, 0.98, 1.11, 1.17, 1.24 (3H each, all s, 29, 30, 28, 18, 27, 26-$H_3$), 1.29, 1.72 (1H each, both m, 22-$H_2$), 1.90 (OAc), 2.49 (1H, m, 20-H), 3.33 (3H, d, $J$=1 Hz, OMe), 3.56 (3H, s, OMe), 4.80 (1H, br d, 23-H), 5.01 (1H, ddd, $J$=6, 11, 11 Hz, 11-H), 5.12 (1H, d, $J$=2 Hz, 24-H), 7.36—7.54 (6H, m), 7.59 (4H, m).

**Preparation of the 11,24-Di-(+)-(*R*)-MTPA Ester (11) from 6a** A solution of **6a** (5.0 mg) in $CH_2Cl_2$ (0.5 ml) was treated with (+)-(*R*)-MTPA (29.6 mg) in the presence of DCC (31.0 mg) and DMAP (10.1 mg), and the whole mixture was stirred at 20 °C under an $N_2$ atmosphere for 15 min. The reaction mixture was worked up as described above to give an AcOEt extract, which was purified by silica gel column chromatography [*n*-hexane–acetone (5 : 1)] to furnish **11** (6.3 mg).

**11**: White powder. $^1$H-NMR ($CDCl_3$) $\delta$: 0.47, 0.91, 0.96 (3H each, all s, 19, 29, 30-$H_3$), 0.96 (3H, d, $J$=7 Hz, 21-$H_3$), 0.97, 1.08, 1.16, 1.21 (3H each, all s, 28, 27, 18, 26-$H_3$), 1.47 (1H, m, 22-H), 1.94 (3H, s, OAc), 1.95 (1H, m, 22-H), 2.57 (1H, m, 20-H), 3.59 (3H, d, $J$=1 Hz, OMe), 3.69 (3H, d, $J$=2 Hz, OMe), 4.85 (1H, br d, 23-H), 4.94 (1H, ddd, $J$=6, 11, 11 Hz, 11-H), 5.16 (1H, d, $J$=2 Hz, 24-H), 7.36—7.52 (6H, m), 7.59 (4H, m).

**Acetylation of Alisol F (7)** A solution of **7** (5.0 mg) in pyridine (1.0 ml) was treated with $Ac_2O$ (0.5 ml) and the whole mixture was stirred at room temperature under an $N_2$ atmosphere for 10 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product, which was purified by silica gel column chromatography [*n*-hexane–acetone (4 : 1)] to furnish **7a** (5.7 mg).

**7a**: White powder, $[\alpha]_D^{25}$ +39.2° ($c$=0.4, $CHCl_3$). IR (KBr): 3450 (OH), 1740 (OAc), 1710 (C=O), 1240 cm$^{-1}$. $^1$H-NMR ($CDCl_3$) $\delta$: 0.94, 1.00, 1.06, 1.08, 1.12 (3H each, all s, *tert*-$CH_3$ × 5), 1.20 (3H, d, $J$=7 Hz, 21-$H_3$), 1.26, 1.27 (3H each, both s, *tert*-$CH_3$ × 2), 2.04, 2.18 (3H each, both s, OAc × 2), 4.27 (1H, br d, 23-H), 4.47 (1H, dd-like, 16-H), 4.74 (1H, d, $J$=2 Hz, 24-H), 4.88 (1H, ddd, $J$=6, 11, 11 Hz, 11-H). EI-MS (%): *m/z* 494 (18), 434 (20), 381 (100).

**Preparation of the 11,24-Di-(−)-(*S*)-MTPA Ester (12) from 7** A solution of **7** (7.5 mg) in $CH_2Cl_2$ (0.5 ml) was treated with (−)-(*S*)-MTPA (43.0 mg) in the presence of DCC (46.5 mg) and DMAP (15 mg), and the whole mixture was stirred at 20 °C under an $N_2$ atmosphere for 15 min. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. After work-up of the AcOEt extract in the usual manner, the product was purified by silica gel column chromatography [*n*-hexane–acetone (5 : 2)] to furnish **12** (8.7 mg).

**12**: White powder. $^1$H-NMR ($CDCl_3$) $\delta$: 0.47, 0.91, 0.97, 1.03, 1.08 (3H each, all s, 19, 30, 29, 28, 26-$H_3$), 1.20 (3H, d, $J$=7 Hz, 21-$H_3$), 1.21, 1.31 (3H each, both s, 18, 27-$H_3$), 1.56 (2H, m, 22-$H_2$), 2.77 (1H, m, 20-H), 3.37, 3.59 (3H each, both s, OMe × 2), 4.26 (1H, br d, 23-H), 4.46 (1H, dd, $J$=5, 8 Hz, 16-H), 4.88 (1H, d, $J$=2 Hz, 24-H), 5.01 (1H, ddd, $J$=6, 11, 11 Hz, 11-H), 7.21—7.72 (10H, m).

**Preparation of the 11,24-Di-(+)-(*R*)-MTPA Ester (13) from 7** A solution of **7** (6.2 mg) in $CH_2Cl_2$ (0.5 ml) was treated with (+)-(*R*)-MTPA (37.0 mg) in the presence of DCC (38.0 mg) and DMAP (13.0 mg), and the whole mixture was stirred at 20 °C under an $N_2$ atmosphere for 15 min. The reaction mixture was worked up as described above to furnish an AcOEt extract, which was purified by silica gel column chromatography [*n*-hexane–acetone (5 : 1)] to furnish **13** (9.0 mg).

**13**: White powder. $^1$H-NMR ($CDCl_3$) $\delta$: 0.44, 0.87, 0.92, 0.98, 1.13, 1.18 (3H each, all s, 19, 30, 29, 28, 26, 18-$H_3$), 1.19 (3H, d, $J$=7 Hz, 21-$H_3$), 1.34 (3H, s, 27-$H_3$), 1.43 (2H, m, 22-$H_2$), 2.72 (1H, m, 20-H), 3.58 (3H, s, OMe), 3.69 (3H, d, $J$=2 Hz, OMe), 4.23 (1H, br d, 23-H), 4.42 (1H, dd, $J$=5, 8 Hz, 16-H), 4.90 (1H, d, $J$=2 Hz, 24-H), 4.91 (1H, m, 11-H), 7.32—7.76 (10H, m).

**Reduction of 7 with Lithium** A solution of **7** (3.0 mg) in ethylenediamine (0.5 ml) was treated with Li (1 block, i.d. 2 mm) and the whole mixture was stirred at 20 °C under an $N_2$ atmosphere for 1.5 h. The reaction mixture was treated with MeOH and the whole was neutralized with Amberlite IR C-76 ($H^+$). After removal of the resin, evaporation of the filtrate under reduced pressure gave a product, which was purified by preparative TLC [$CHCl_3$–MeOH (8 : 1)] to furnish alisol A (**1**, 1.8 mg) and dihydroalisol A (**4**, 0.7 mg). Alisol A (**1**) and dihydroalisol A, thus obtained, were identified by comparison with authentic samples ($^1$H-NMR and IR data and TLC behavior).

**Preparation of 8a from Alisol A (1)** A solution of **1** (99.0 mg) in pyridine (3 ml) was treated with $Ac_2O$ (2 ml) and the whole mixture was stirred at 20 °C under an $N_2$ atmosphere for 12 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product, which was purified by silica gel column chromatography [*n*-hexane–acetone (2 : 1)] to furnish triacetylalisol A (114.4 mg). A solution of triacetylalisol A (5.0 mg) in pyridine was treated with $SOCl_2$ (0.1 ml). The reaction mixture was stirred at 0 °C under an $N_2$ atmosphere for 19 h, then poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was worked up in the usual manner to give a product, which was purified by silica gel column chromatography [*n*-hexane–acetone (2 : 1)] to yield **8a** (3.6 mg).

**8a**: White powder, $[\alpha]_D^{22}$ +31.5° ($c$=0.2, MeOH). IR (KBr): 3450 (OH), 1740 (OAc), 1710 (C=O), 1655, 1475, 1375, 900 cm$^{-1}$. $^1$H-NMR ($CD_3OD$) $\delta$: 0.99 (3H, s, *tert*-$CH_3$), 1.00 (3H, d, $J$=7 Hz, 21-$H_3$), 1.02, 1.05, 1.07, 1.19, (3H each, all s, *tert*-$CH_3$ × 4), 1.73 (3H, s, 27-$H_3$), 2.02, 2.03, 2.06 (3H each, all s, OAc × 3), 4.94, 4.97 (1H each, both br s, 27-$H_2$), 4.89 (2H, m, 11, 23-H), 5.05 (1H, d, $J$=6 Hz, 24-H).

**Deacetylation of 8a** A solution of **8a** (2.0 mg) in 5% NaOMe–MeOH (1 ml) was stirred at 20 °C under an $N_2$ atmosphere for 24 h. The reaction mixture was neutralized with Dowex 50w × 8 ($H^+$) and filtered to remove the resin. Removal of the solvent from the filtrate under reduced pressure gave alisol G (**8**, 1.2 mg). Alisol G (**8**) was shown to be identical with an authentic sample, which was isolated from Chinese Alismatis Rhizoma, by comparisons of the $^1$H-NMR and IR data and TLC behavior.

**Oxidation of Alisol A (1) with mCPBA** A solution of **1** (50.0 mg) in $CH_2Cl_2$ (20 ml) was treated with mCPBA (35 mg). The reaction mixture was stirred at 0 °C under an $N_2$ atmosphere for 1 h, then poured into ice-water, and the whole was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Work-up of the $CHCl_3$ extract in the usual manner gave 13,17-epoxyalisol A (41.0 mg). 13,17-Epoxyalisol A thus obtained was shown to be identical with **9**, which was isolated from Chinese Alismatis Rhizoma, by comparisons of the $^1$H-NMR, $^{13}$C-NMR and $[\alpha]_D$ data and TLC behavior.

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