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ALISMOL AND ALISMOXIDE, SESQUITERPENOIDS OF *ALISMA* RHIZOMES*

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Key Word Index—*Alisma plantago-aquatica* var *orientale*, Alismataceae, sesquiterpenoid, guaiane skeleton, alismol, 1β,5β-guaia-6,10(15)-dien-4-ol, alismoxide, 4,10-epoxy-1β,5β-guai-6-ene

Abstract—From the crude drug 'takusha', which is the rhizomes of *Alisma plantago-aquatica* var *orientale*, two new sesquiterpenoids, alismol and alismoxide, have been isolated Their structures have been established on the basis of chemical and physical evidence to be 1β, 5β-guaia-6,10(15)-dien-4-ol and 4,10-epoxy-1β,5β-guai-6-ene, respectively

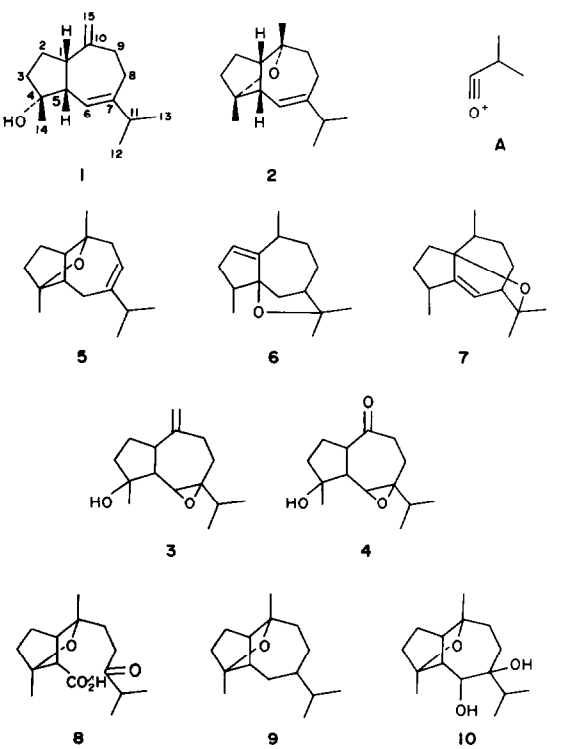
INTRODUCTION

The rhizome of *Alisma plantago-aquatica* L var *orientale* Samuelsson (Alismataceae) is known as the crude drug 'takusha' and it has been used for diuretic and antiinflammatory purposes in Oriental medicine A series of the protostane triterpenoids, alisol A and B, and the monoacetates of alismols A, B and C [1], have been isolated in addition to choline and sugars [2] In our survey, we have isolated from the methanol extract of the crude drug, two novel sesquiterpenoids, which are now termed alismol and alismoxide

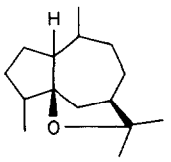
RESULTS AND DISCUSSION

Alismol has the molecular formula $C_{15}H_{24}O$ (HRMS *m/z* 220 1816 $[M]^+$) In the ^{13}C NMR spectrum, there were four signals attributable to olefinic carbons (δ154 3, *s*, 148 6, *s*, 123 2, *d* and 106 6, *t*) but no carbonyl carbon signal The ^1H NMR spectrum exhibited the presence of two secondary methyls (δ0 98) and a tertiary methyl on an oxygen-bearing carbon (δ1 24) Since it was found by double resonance experiments that the signal of the two secondary methyls was spin-coupled to a common hydrogen signal (*ca* δ2 3), these methyls were involved in an isopropyl group The chemical shifts and the splitting patterns of the ^1H NMR signals due to the olefinic hydrogens (δ5 52, *br s*, 4 75, *br s* and 4 68, *br s*), together with the splitting patterns of the olefinic carbon signals in the ^{13}C NMR spectrum, pointed to the presence of a trisubstituted ethylenic bond and a vinylidene The IR spectrum showed a strong band at 3370 cm^{-1} assignable to the hydroxyl Further, the hydroxyl group was revealed to be tertiary by the presence of a quaternary carbinyl carbon signal (δ79 6, *s*) Alismol is consequently a bicarbocyclic substance and dehydrogenation of alismol with Pd–C gave *S*-guaiazulene, demonstrating it to have the guaiane skeleton

Since the chemical shift of the signal attributed to the methine hydrogen in the isopropyl group (*ca* δ2 3) was shifted downfield compared with that due to a methine hydrogen in an isopropyl group connected to a saturated carbon [3], the trisubstituted double bond was assumed to be present at C-6–C-7 or C-7–C-8 in alismol Alismol was treated with *m*-chloroperbenzoic acid in chloroform to afford the monoepoxide (**3**) (mass spectrum *m/z* 236 $[M]^+$) whose ^1H NMR spectrum showed the absence of the trisubstituted double bond In the mass spectrum of the monoepoxide (**3**), a peak attributable to the fragment ion (**A**) occurred at *m/z* 71, supporting the above conclusion on the situation of the trisubstituted double bond



*Part 58 in the series, "Sesquiterpenoids" For Part 57 see Hikino, H, Taguchi, T and Endo, K (1981) *Nippon Toyoigaku Kaishi* **31**, 85



11 1β-H, 4β-Me, 10β-Me
12 1β-H, 4β-Me, 10α-Me
13 1β-H, 4α-Me, 10β-Me
14 1β-H, 4α-Me, 10α-Me
15 1α-H, 4β-Me, 10β-Me
16 1α-H, 4β-Me, 10α-Me
17 1α-H, 4α-Me, 10β-Me
18 1α-H, 4α-Me, 10α-Me

[4] Furthermore, in the ^{1}H NMR spectrum of the monoepoxide (**3**), a signal assignable to a hydrogen on the carbon bearing an oxygen atom of the epoxide group appeared as a doublet (J = 7 Hz), indicating the location of the trisubstituted double bond at C-6–C-7

Ozonolysis of the monoepoxide (**3**) yielded the ketone (**4**) (mass spectrum m/z 238 $[M]^+$) whose ^{1}H NMR spectrum exhibited no signal attributable to the terminal methylene hydrogens An IR band at 1704 cm^{-1} demonstrated the formation of a carbonyl in a six- or larger-membered ring Consequently, the situation of the terminal methylene at C-10–C-15 was clarified The location of the trisubstituted double bond at C-6–C-7 in alismol was substantiated as follows Provided that the trisubstituted double bond was located at C-7–C-8, the ^{1}H NMR signal due to the methylene hydrogens on C-9 between two double bonds, would appear at a lower field region (*ca* δ2 9) [5] However, no methylene signal below δ2 6 was observed in the ^{1}H NMR spectrum of alismol, confirming the above conclusion

On the basis of the above evidence, the structure (without regard to the stereochemistry) of alismol was concluded to be **1**

Alismoxide possesses the molecular formula $C_{15}H_{24}O$ (HRMS m/z 220 1816 $[M]^+$) An oxygen atom was shown to form an ether linkage by the fact that the IR spectrum showed the absence of both hydroxyl and carbonyl groups, and that signals due to two quaternary carbons carrying oxygen atoms were present (δ80 2, *s* and 75 3, *s*) in the ^{13}C NMR spectrum The ^{1}H NMR spectrum indicated the presence of two secondary methyls (δ0 97) and two tertiary methyls on carbons attached to oxygen atoms (δ1 22 and 1 28) The presence of a trisubstituted ethylenic linkage was revealed by two olefinic carbon signals (δ149 5, *s* and 121 3, *d*) in the ^{13}C NMR spectrum and an olefinic hydrogen signal (δ5 50) in the ^{1}H NMR spectrum Therefore, alismoxide is tricyclic and dehydrogenation of alismoxide furnished *S*-guaiazulene, indicating it to have the guaiane skeleton

The accumulated data demonstrated that the ether function was linked between C-4 and C-10, C-1 and C-11, or C-5 and C-11 When the strain of the molecule involving the trisubstituted double bond was taken into consideration, the structures **2** and **5**–**7** would be possible for alismoxide However, the possibility of structure **7** was excluded by the fact that the IR spectrum of the ozonolysis product (**8**) of alismoxide showed a band at 1708 cm^{-1} due to a carbonyl in a six- or larger-membered ring

Catalytic hydrogenation of alismoxide in the presence of platinum oxide in methanol–acetic acid yielded the saturated product **9** (mass spectrum m/z 222 $[M]^+$) Since the ^{1}H NMR spectrum of the product (**9**) was not identical with those of the eight stereoisomers (**11**–**18**) [6–12] derived from the ethers (**6**), it was concluded that structure **6** was not appropriate for alismoxide (Table 1)

Table 1 ^{1}H NMR spectral data of the ethers **9** and **11**–**18** ($CDCl_3$)

Compound	Methyl hydrogen signals
9	0 86 (6H, *d*, J = 7 Hz), 1 15 (3H, *s*), 1 20 (3H, *s*)
11	0 91 (3H, *d*, J = 5 4 Hz), 0 96 (3H, *d*, J = 5 4 Hz), 1 15 (3H, *s*), 1 30 (3H, *s*)
12	0 95 (3H, *d*, J = 6 Hz), 0 98 (3H, *d*, J = 6 5 Hz), 1 18 (3H, *s*), 1 36 (3H, *s*)
13	0 87 (3H, *d*, J = 4 5 Hz), 0 93 (3H, *d*, J = 6 5 Hz), 1 16 (3H, *s*), 1 32 (3H, *s*)
14	0 85 (3H, *d*, J = 6 Hz), 0 90 (3H, *d*, J = 6 5 Hz), 1 21 (3H, *s*), 1 33 (3H, *s*)
15	0 93 (6H, *d*, J = 5 5 Hz), 1 15 (3H, *s*), 1 33 (3H, *s*)
16	0 85 (3H, diffuse *d*), 0 95 (3H, *d*, J = 6 Hz), 1 19 (3H, *s*), 1 25 (3H, *s*)
17	0 90 (3H, diffuse *d*), 0 98 (3H, *d*, J = 6 5 Hz), 1 15 (3H, *s*), 1 30 (3H, *s*)
18	0 87 (3H, *d*, J = 5 5 Hz), 0 89 (3H, *d*, J = 7 5 Hz), 1 18 (3H, *s*), 1 23 (3H, *s*)

Double resonance experiments in the ^{1}H NMR spectrum of alismoxide clarified that the hydrogen signal of the two secondary methyls was spin-coupled to the same hydrogen signal, indicating the presence of an isopropyl group Furthermore, the chemical shift of the signal assignable to the methine hydrogen in the isopropyl group (*ca* δ2 2) showed that the isopropyl group was directly bonded to the trisubstituted double bond These observations demonstrated that the structure of alismoxide is represented either by formula **2** or **5**

Treatment of alismoxide with *m*-chloroperbenzoic acid in chloroform resulted in concomitant hydrolysis of the epoxide to afford the diol (**10**) whose mass spectrum exhibited a peak due to the fragment ion (**A**) at m/z 71 In the ^{1}H NMR spectrum of the diol (**10**), the carbinyl hydrogen signal (δ3 79) appeared as a doublet (J = 9 Hz) These spectral data indicated that the trisubstituted double bond was located at C-6–C-7 The structure of alismoxide was thus concluded to be **2**

In order to clarify the relationship between alismol and alismoxide, alismol was treated with sulfuric acid to yield alismoxide Consideration of this reaction revealed that the C-1 hydrogen is *cis* to the C-5 hydrogen which in turn is *trans* to the C-4 hydroxyl group

Hence, the relative stereostructures of alismol and alismoxide are represented by formulae **1** and **2** and they are 1β,5β-guaia-6,10(15)-dien-4-ol and 4,10-epoxy-1β,5β-guai-6-ene, respectively

EXPERIMENTAL

Isolation of alismol and alismoxide The crude drug 'takusha' (5 3 kg) i e the rhizomes of *Alisma plantago-aquatica* var *orientale*, was extracted with MeOH (100 l × 4) for 4 days (each extraction) at room temp to give the MeOH extract (410 g) which was suspended in H_2O (4 l) and extracted with EtOAc (3 l × 3) The EtOAc solubles were concd *in vacuo* to afford the EtOAc fraction (120 g) which was chromatographed over Si gel (1 kg) Elution with hexane–EtOAc (3 1) furnished alismol (**1**) (310 mg) as a colorless oil, $[\alpha]_D$ +8 7° ($CHCl_3$, *c* 0 29), HRMS *m/z* 220 1816 $[M]^+$, MS *m/z* 220, 205, 202, 177, 162, 159, 119 (base peak), 107, 105, 93, 91, 81, 79, 43, IR ν_{max}^{neat} cm^{-1} 3370, 865, ^1H NMR (100 MHz, $CDCl_3$) δ0 98 (6H, *d*, *J* = 6 5 Hz, H-12 and H-13), 1 24 (3H, *s*, H-14), 4 68, 4 75 (each 1H, *br s*, H-15), 5 52 (1H, *br s*, H-6), ^{13}C NMR (25 MHz, C_5D_5N) δ21 4 (*q*), 21 7 (*q*), 24 6 (*q*), 25 3 (*t*), 30 3 (*t*), 37 5 (*t*), 37 6 (*d*), 40 6 (*t*), 47 3 (*d*), 55 4 (*d*), 79 6 (*s*), 106 6 (*t*), 123 2 (*d*), 148 6 (*s*), 154 3 (*s*)

Subsequent elution with hexane–EtOAc (1 1) afforded alismoxide (**2**) (220 mg) as a colorless oil, $[\alpha]_D$ +3 1° ($CHCl_3$, *c* 0 63), HRMS *m/z* 220 1816 $[M]^+$, MS *m/z* 220, 205, 162 (base peak), 149, 147, 134, 119, 107, 93, 71, 43, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 2953, 1461, 1380, 1203, 1096, ^1H NMR (100 MHz, $CDCl_3$) δ0 97 (6H, *d*, *J* = 7 Hz, H-12 and H-13), 1 22, 1 28 (each 3H, *s*, H-14 and H-15), 5 50 (1H, *d*, *J* = 2 5 Hz, H-6), ^{13}C NMR (25 MHz, $CDCl_3$) δ21 1 (*q*), 21 3 (*q*), 21 4 (*q*), 21 5 (*t*), 22 5 (*q*), 25 0 (*t*), 37 3 (*d*), 40 4 (*t*), 42 6 (*t*), 50 2 (*d*), 50 6 (*d*), 75 3 (*s*), 80 2 (*s*), 121 3 (*d*), 149 5 (*s*)

Dehydrogenation of alismol Alismol (60 mg) was heated with 10% Pd–C (30 mg) under N_2 at 320–330° for 5 min The product was extracted with petrol and chromatographed over Al_2O_3 (3 g) Elution with petrol gave *S*-guaiazulene as a blue oil UV λ_{max}^{EtOH} nm 233, 245, 285, 290, 305, 604, 630, 658, 730 The trinitrobenzene adduct, prepared in the customary manner, crystallized from MeOH as maroon needles, mp 132–134 5° The identity was confirmed by the usual criteria

Epoxidation of alismol Alismol (75 mg) was treated with *m*-chloroperbenzoic acid (56 mg) in $CHCl_3$ (30 ml) under ice-cooled conditions for 1 5 hr The product isolated in the usual way was chromatographed over Si gel (5 g) Elution with C_6H_6–EtOAc (4 1) afforded alismol monoepoxide (**3**) (12 mg) as a colorless oil MS *m/z* 236 $[M]^+$, ^1H NMR (100 MHz, $CDCl_3$) δ0 87 (3H, *d*, *J* = 5 Hz), 0 94 (3H, *d*, *J* = 5 Hz), 1 36 (3H, *s*), 2 82 (1H, *d*, *J* = 7 Hz), 4 83 (2H, *br s*)

Ozonolysis of alismol monoepoxide Alismol monoepoxide (5 mg) in MeOH (5 ml) was ozonized at −40° for 5 min The mixture was hydrogenated over 10% Pd–C (2 mg) and worked-up in the usual manner to afford the ketone (**4**) (2 mg) as a colorless oil MS *m/z* 238 $[M]^+$, IR ν_{max}^{KBr} cm^{-1} 3425, 1704

Dehydrogenation of alismoxide Alismoxide (17 mg) and 10% Pd–C (10 mg) were heated at 320–330° for 5 min The petrol soluble product was chromatographed over Al_2O_3 (3 g) Elution with petrol gave *S*-guaiazulene as a blue oil UV λ_{max}^{EtOH} nm 233, 245, 285, 290, 305, 604, 630, 650, 730 The identity was confirmed by the usual criteria

Ozonolysis of alismoxide Alismoxide (7 mg) in MeOH (10 ml) was ozonized at −40° for 5 min and then the reaction mixture was hydrogenated over 10% Pd–C (2 mg) to give the ketone (**8**) (3 mg) as a colorless oil MS *m/z* 268 $[M]^+$, IR ν_{max}^{KBr} cm^{-1} 1708

Epoxidation of alismoxide Alismoxide (5 mg) was treated with *m*-chloroperbenzoic acid (8 mg) in $CHCl_3$ (2 ml) at room temp for 2 5 hr The product was isolated in the usual way and chromatographed over Si gel (5 g) Elution with $CHCl_3$–MeOH (9 1) yielded the diol (**10**) (2 mg) MS *m/z* 254 $[M]^+$, ^1H NMR (100 MHz, $CDCl_3$) δ1 03 (6H, *d*, *J* = 7 Hz), 1 22, 1 33 (each 3H, *s*), 3 79 (1H, *d*, *J* = 9 Hz)

Hydrogenation of alismoxide Alismoxide (3 mg) was hydrogenated over PtO_2 (3 mg) in MeOH–HOAc (50 1) at room temp for 10 hr The product was isolated in the usual manner and chromatographed over Si gel (1 g) Elution with hexane–EtOAc (2 3) gave dihydroalismoxide (**9**) (1 mg) MS *m/z* 222 $[M]^+$, ^1H NMR (100 MHz, $CDCl_3$) δ0 86 (6H, *d*, *J* = 7 Hz), 1 15, 1 20 (each 3H, *s*)

Acid treatment of alismol Alismol (9 mg) and 4 N H_2SO_4 (2 ml) in H_2O were left at room temp for 2 days The $CHCl_3$ extract was chromatographed over Si gel (3 g) Elution with $CHCl_3$–MeOH (9 1) afforded alismoxide (1 mg) Identification was performed by the usual criteria

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