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MONOTERPENES FROM ASIASARI RADIX FROM *ASIASARUM* SP.

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Key Word Index—*Asiasarum sieboldi*; *Asiasarum heterotropoides*; Aristolochiaceae; Asiasari Radix; roots; monoterpenes; (±)-asarinol A; (±)-asarinol B.

Abstract—Two novel monoterpenes, named (±)-asarinol A, (±)-asarinol B, together with the known (±)-car-3-ene-2,5-dione, (−)-asarinin, (−)-sesamin, methyleugenol and elemicin have been isolated from the roots of asiasari Radix. On the basis of chemical and spectral analyses and X-ray crystallography, the structures of new compounds have been established as (±)-*rel*-(1*R*,5*S*,6*S*)-car-3-en-2-on-5-ol, (±)-*rel*-(1*R*,3*S*,4*S*,5*S*,6*S*)-4,5-epoxycaran-2-on-3-ol.

INTRODUCTION

Asiasari Radix ('Saishin' in Japanese) prepared from *Asiasarum sieboldi* F. Maekawa or *A. heterotropoides* F. Maekawa var. *mandshuricum* F. Maekawa (Aristolochiaceae) is one of the most important crude drugs in Chinese medicine, and it has been used as an anodyne or antitussive. Consequently the crude drug has long been the subject for extensive chemical investigations, and a number of essential oils [1], lignans [2] and amides [3] have been isolated. In this paper we report the isolation and structure elucidation of two new monoterpenes, named (±)-asarinol A (**2**), (±)-asarinol B (**3**), together with the known compounds (**1** and **4**–**7**) isolated from the methanolic extract of asiasari Radix.*

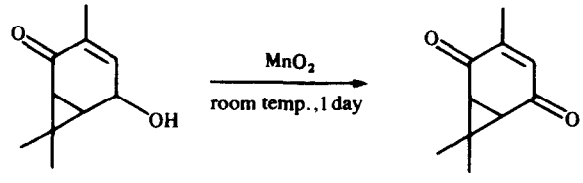
RESULTS AND DISCUSSION

A methanolic extract of the asiasari Radix yielded seven compounds (**1**–**7**) after chromatographic purification. Compounds **1** and **4**–**7** were identified as the known (±)-car-3-ene-2,5-dione (**1**) [4], (−)-asarinin (**4**) [5], (−)-sesamin (**5**) [6], methyleugenol (**6**) [1] and elemicin (**7**) [7], respectively, by direct comparison of its spectral data with an authentic published data.

(±)-Asarinol A (**2**) was isolated as needles, mp 80–82°, $[\alpha]_D^{24}$ 0°. Its molecular composition was found to be $C_{10}H_{14}O_2$ by HRMS. The IR absorption spectrum of **2** showed the presence of a hydroxy group (3456 cm^{-1}), a carbonyl group (1644 cm^{-1}) and a double bond (1632 cm^{-1}). The UV absorption suggested that **2** contained an α,β-unsaturated ketone moiety. The ^1H NMR spectrum of **2** (Table 1) showed the signals for two singlet methyl groups [δ1.04 (3H, *s*), δ1.20 (3H, *s*)], one vinyl methyl group [δ 1.72 (3H, *t* J = 1.3 Hz)], two methine protons [δ1.66 (1H, *dd*, J = 7.5, 1.6 Hz], δ1.68 (1H, *ddd*, J = 7.5, 1.8, 0.7 Hz)] and one vinyl proton [δ6.51 (1H, *dq*, J = 5.9, 1.3 Hz)]. Its ^{13}C NMR spectrum showed the presence of a carbonyl group (δ195.4), a double bond (δ136.5, δ143.3) and a methine carbon (δ62.0) carrying a hydroxyl group.

On oxidation, the keto alcohol **2** gave a diketone **1**, indicating that **2** corresponded to the dihydro compound of **1**. Therefore, its structure was assumed to be **2** or **2'** (Fig. 1). The ^1H–^1H COSY spectrum of **2** revealed some couplings shown in Fig. 2. A vinyl proton (δ6.51) coupled with a hydroxy methine proton (δ4.31) at 5.9 Hz indicated that the structure of (±)-asarinol A was **2**. Finally, the structure of (±)-asarinol A was determined to be (±)-*rel*-(1*R*,5*S*,6*S*)-car-3-en-2-on-5-ol (**2**) by X-ray crystallography employing **2b** (*p*-bromobenzoate of **2**) (Fig. 3).

(±)-Asarinol B (**3**) was isolated as platelets, mp 92–94°, $[\alpha]_D^{24}$ 0°. Its molecular composition was found to be $C_{10}H_{14}O_3$ by HRMS. The IR absorption of **3** showed the presence of a hydroxy group (3472 cm^{-1}), a carbonyl group (1666 cm^{-1}) and an epoxy group (3008, 842 cm^{-1}). The ^1H NMR spectrum of **3** (Table 1) showed the signals for three singlet methyl groups [δ1.26 (3H, *s*), δ1.34 (3H, *s*), δ1.43 (3H, *s*)], two methine protons [δ1.71 (1H, *dd*, J = 7.2, 1.7 Hz), δ2.13 (1H, *dt*, J = 7.2, 1.4 Hz)] and two methine protons [δ3.31 (1H, *dd*, J = 3.9, 1.4 Hz), δ3.65 (1H, *ddd*, J = 3.9, 1.7, 1.4 Hz)] which were assumed to be associated with an epoxy moiety. Its ^{13}C NMR spectrum (Table 2) showed the presence of one carbonyl group (δ207.7) and no double bond indicating that **3** was a tricyclic compound. In the ^{13}C NMR spectra of **2** and **3**, some similar chemical shift values can be observed in C-1,6,7,8,9 indicating that **3** had a cyclopropane ring system



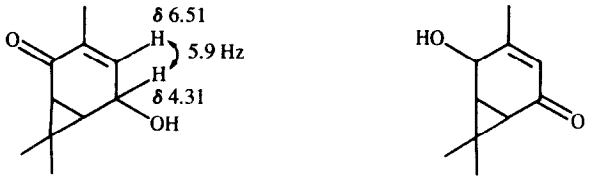
2 **1**

*We think that the plant material is *Asiasarum sieboldi* F. Maekawa, but an investigation to identify the plant more clearly is in progress.

Table 1. ^{1}H NMR spectral data of compounds **1**–**3** (500 MHz, TMS as int. standard)

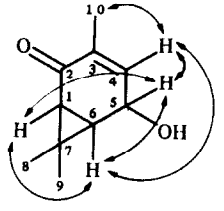
H	1*	2†	3*
1	2.34 *d* (6.5)	1.66 *dd* (7.5, 1.6)	1.71 *dd* (7.2, 1.7)
4	6.50 *dq* (1.6, 1.6)	6.51 *dq* (5.9, 1.3)	3.31 *dd* (3.9, 1.4)
5		4.31 *m*	3.65 *ddd* (3.9, 1.7, 1.4)
6	2.32 *dd* (6.5, 1.6)	1.68 *ddd* (7.5, 1.8, 0.7)	2.13 *dt* (7.2, 1.3)
8-Me	1.33 *s*	1.20 *s*	1.26 *s*
9-Me	1.32 *s*	1.04 *s*	1.34 *s*
10-Me	1.98 *d* (1.6)	1.72 *t* (1.3)	1.43 *s*

Coupling constants (*J*) in parentheses.
*In chloroform-*d*.
†In acetone-d_6.



2 **2'**

Fig. 1. Possible structures of compound **2**.



2

Fig. 2. The results of ^{1}H–^{1}H COSY spectrum of **2**.

Table 2. ^{13}C NMR spectral data of compounds **1**–**3** (50.1 MHz, TMS as int. standard)

C	1*	2†	3*
1	39.8	34.6	36.5
2	195.0[a]	195.4	207.7
3	150.0	136.5	70.8
4	137.7	143.3	59.4
5	194.4[a]	62.0	53.6
6	39.0	37.2	32.7
7	33.5	24.7	30.6
8	16.2[b]	28.9	28.9
9	15.4[b]	14.7	18.5
10	29.1	15.9	22.9

[a,b]Assignments may be interchanged.
*In chloroform-*d*.
†In acetone-d_6.

(Table 2). The above data, coupled with the number of carbons (C=10) suggested that **3** was a monoterpene having the carane skeleton. The ^{1}H–^{1}H COSY spectrum of **3** revealed some couplings shown in Fig. 4 indicating that the protons (H-4, 5, 6, 1) were correlated as shown.

On acetylation, compound **3** gave a monoacetate **3a**. In the ^{1}H NMR spectra of **3** and **3a**, the methine (H-4) and methyl (10-Me) signals of **3a** (δ3.52 and δ1.57) appeared at lower field than those of **3** (δ3.31 and δ1.43), indicating that the hydroxyl group was linked to the C-3. Its structure and relative stereochemistry were determined by the NOESY spectrum (Fig. 5), namely, in the NOESY spectrum the NOEs are found in two places for **3**. One is found between H-1, H-6 and 8-Me, another is found between 3-OH, H-4 and 10-Me. On the basis of the above NOESY data, the relationship between H-1, H-6, H-5, H-4 and 3-OH can be derived as *cis*, *trans*, *cis*, *trans*, respectively. Therefore, its structure was suggested to be **3**. Finally, the structure of (±)-asarinol B was determined to be (±)-*rel*-(1*R*,3*S*,4*S*,5*S*,6*S*)-4,5-epoxycaran-2-on-3-ol (**3**) by X-ray crystallography (Fig. 6).

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR; 500 MHz spectrometer with TMS as int. standard. All the compounds were finally purified by HPLC on a C_{18} column using H_2O–MeOH or H_2O–MeCN solvent system [JASCO; BIP-I, column: YMC-S343 (10 μm, 25 mm i.d. × 30 cm), detection: UV at 254 nm].

Plant material. Plant material was purchased from Yamamoto Yakuhin Co., Ltd.

Extraction and isolation. The dried roots of asiasari Radix (100 kg) were extracted with MeOH under reflux (960 l × 2). The MeOH extract was concd under red. pres. and the residue (6.2 kg) suspended in H_2O. The suspension was extracted with *n*-hexane and then with *n*-BuOH. The *n*-BuOH extract, after concn (2.2 kg), was subjected to CC on Diaion HP-20, eluting with H_2O (70 l), 40% MeOH (100 l) and then MeOH (80 l). The MeOH eluates (324 g) were subjected repeatedly to chromatography on silica gel with $CHCl_3$–MeOH systems to furnish a fraction containing **1** (240 mg). Final purification of **1** was accomplished by recrystallization from EtOH. Initial purification of the 40% MeOH eluates (130 g) by silica gel with $CHCl_3$–MeOH systems resulted in an enriched fraction of compound **2** and **3**. This fraction was purified by HPLC (H_2O–MeOH 3:1, flow rate 5.0 ml min^{-1}) to give **2** (R_t 60 min, 3.4 g) and **3** (R_t 36 min, 730 mg). Final purification of **2** was accomplished by recrystallization from CH_2Cl_2–*n*-pentane system. Similarly **3** was purified by recrystallization from MeOH.

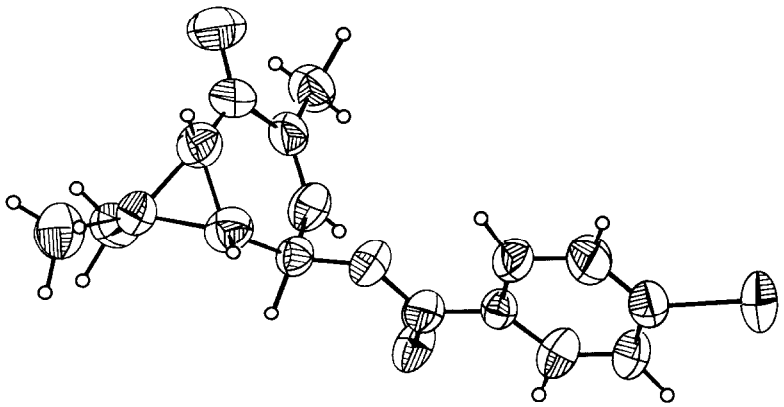
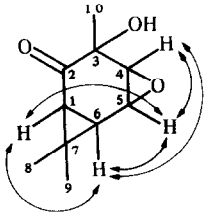
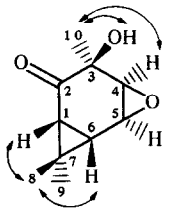


Fig. 3. ORTEP drawing of compound **2b**.



3

Fig. 4. The results of ^{1}H–^{1}H COSY spectrum of compound **3**.



3

Fig. 5. NOE in the NOESY spectrum of compound **3**.

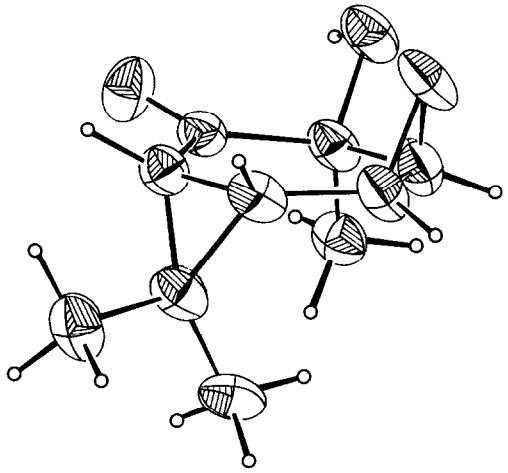


Fig. 6. ORTEP drawing of compound **3**.

(±)-*Car*-3-*ene*-2,5-*dione* (**1**). Pale yellow needles, mp 93–94°. $[\alpha]_D^{24}$ 0° ($CHCl_3$; *c* 0.57). IR ν_{max}^{KBr} cm^{-1}: 1662 (C=O), 1620 (C=C). UV λ_{max}^{MeOH} nm (log ε): 241 (3.99), 228 (3.94). ^{1}H NMR ($CDCl_3$): δ1.33 (6H, *s*, 8, 9-Me), 1.98 (3H, *d*, *J* = 1.6 Hz, 10-Me), 2.32 (1H, *dd*, *J* = 1.6, 6.5 Hz, H-6), 2.34 (1H, *d*, *J* = 6.5 Hz, H-1), 6.50 (1H, *dq*, *J* = 1.6, 1.6 Hz, H-4). ^{13}C NMR ($CDCl_3$): δ15.4 (*q*, C-8*), 16.2 (*q*, C-9*), 29.1 (*q*, C-10), 33.5 (*s*, C-7), 39.0 (*d*, C-6), 39.8 (*d*, C-1), 137.7 (*d*, C-4), 150.0 (*s*, C-3), 194.4 (*s*, C-2**), 195.0 (*s*, C-5**). *,**: may be interchanged. MS *m/z* (rel. int.): 164 ([M]$^+$, 27), 149 (50), 44 (100).

(±)-*Asarinol A*, (±)-rel-(1R,5S,6S)-*car*-3-*en*-2-*on*-5-*ol* (**2**). Needles, mp 80–82°. $[\alpha]_D^{24}$ 0° ($CHCl_3$; *c* 0.56). IR ν_{max}^{KBr} cm^{-1}: 3456 (OH), 1644 (C=O), 1632 (C=C). UV λ_{max}^{MeOH} nm (log ε): 231 (3.80). ^{1}H NMR (acetone-d_6): δ1.04 (3H, *s*, 9-Me), 1.20 (3H, *s*, 8-Me), 1.66 (1H, *dd*, *J* = 7.5, 1.6 Hz), 1.68 (1H, *ddd*, *J* = 7.5, 1.8, 0.7 Hz), 1.72 (3H, *t*, *J* = 1.3 Hz, 10-Me), 4.31 (1H, *m*, H-5), 6.51 (1H, *dq*, *J* = 5.9, 1.3 Hz, H-4). ^{13}C NMR (acetone-d_6): 14.7 (*q*, C-9), 15.9 (*q*, C-10), 24.7 (*s*, C-7), 28.9 (*q*, C-8), 34.6 (*d*, C-1), 37.2 (*d*, C-6), 62.0 (*d*, C-5), 136.5 (*s*, C-3), 143.3 (*d*, C-4), 195.4 (*s*, C-2). HRMS *m/z* Found, 166.0986 [M]$^+$. $C_{10}H_{14}O_2$ requires: 166.0993. (Found: C, 72.30; H, 8.39. $C_{10}H_{14}O_2$ requires: C, 72.26; H, 8.49%).

Oxidation of **2**. Compound **2** (166 mg) was dissolved in $CHCl_3$ (2 ml) and MnO_2 (350 mg) added and the mixt. kept at room temp. for 24 hr. The reaction mixt. was chromatographed over silica gel. Elution with C_6H_6–EtOAc (20:1) yielded **2a** (115 mg) which was identical with compound **1** in all respects (TLC, IR, ^{1}H NMR and mmp).

p-*Bromobenzoylation of* **2**. Compound **2** (45 mg) was dissolved in pyridine (2 ml), *p*-bromobenzoyl chloride (100 mg) added and the mixt. kept at room temp. for 3 hr. The usual work-up followed by crystallization from EtOAc afforded platelets (**2b**, 43 mg).

Compound **2b** (p-*bromobenzoate of* **2**). Prisms, mp 136–138°. $[\alpha]_D^{24}$ 0° ($CHCl_3$; *c* 0.31). IR ν_{max}^{KBr} cm^{-1}: 1716, 1658, 1642, 1590, 1266, 1114, 1106, 938, 754. UV λ_{max}^{MeOH} nm (log ε): 246 (4.52). ^{1}H NMR ($CDCl_3$): δ1.17 (3H, *s*, 9-Me), 1.26 (3H, *s*, 8-Me), 1.64 (1H, *dd*, *J* = 7.5, 1.6 Hz, H-1), 1.77 (1H, *ddd*, *J* = 7.5, 1.8, 0.7 Hz, H-6), 1.87 (3H, *t*, *J* = 1.3 Hz, 10-Me), 5.76 (1H, *m*, H-5), 6.50 (1H, *dq*, *J* = 5.9, 1.3 Hz, H-4), 7.60 (2H, *dd*, *J* = 8.7, 2.0 Hz, H-3′, 7′), 7.93 (2H, *dd*, *J* = 8.7, 2.0 Hz, H-4′, 6′). ^{13}C NMR ($CDCl_3$): 14.8 (*q*, C-9), 16.2 (*q*, C-10), 25.3 (*s*, C-7), 28.9 (*q*, C-8), 33.0 (*d*, C-1), 34.0 (*d*, C-6), 65.5 (*d*, C-5), 128.7 (*s*, C-2′), 128.9 (*s*, C-5′), 131.5 (*d*, C-4′, 6′), 132.0 (*d*, C-3′, 7′), 136.8 (*d*, C-4), 140.1 (*s*, C-3), 165.3 (*s*, C-1′), 195.3 (*s*, C-2). MS *m/z* 348 ([M]$^+$, $C_{17}H_{17}{}^{79}BrO_3$, $C_{17}H_{17}{}^{81}BrO_3$). (Found: C, 58.40; H, 4.75. $C_{17}H_{17}BrO_3$ requires: C, 58.47; H, 4.91%).

X-Ray analysis of **2b**. The crystals were monoclinic, space group $P2_1/n$ with $a = 7.8715(5)$, $b = 19.846(2)$, $c = 10.102(1)$ Å and $d_{calc} = 1.47\ g\,cm^{-3}$ for $Z = 4$. The size of the crystal used for data collection was *ca* $0.3 \times 0.3 \times 0.2$ mm. No absorption correction was necessary ($\mu = 36.4\ cm^{-1}$). A total of 3311 reflections were measured for $2° \leqq 2\theta \leqq 140°$ of which 2883 reflections were considered to be observed [$I > 3\sigma(I)$]. The final discrepancy indices were $R = 4.6\%$. The final difference Fourier map was essentially featureless, the highest residual peaks having densities of $0.52\ e\,Å^{-3}$.

(±)-*Asarinol B*, (±)-rel-(1R,3S,4S,5S,6S)-4,5-*epoxycaran*-2-*on*-3-*ol* (**3**). Prisms, mp 92–94°. $[\alpha]_D^{24}$ 0° ($CHCl_3$; *c* 0.39). IR $\nu_{max}^{KBr}\ cm^{-1}$: 3472 (OH), 1666 (C=O), 3008, 842 (C–O–C). ^1H NMR ($CDCl_3$): δ1.26 (3H, *s*, 8-Me), 1.34 (3H, *s*, 9-Me), 1.43 (3H, *s*, 10-Me), 1.71 (1H, *dd*, $J = 7.2$, 1.7 Hz, H-1), 2.13 (1H, *dt*, $J = 7.2$, 1.4 Hz, H-6), 3.26 (1H, *s*, OH), 3.31 (1H, *dd*, $J = 3.9$, 1.4 Hz, H-4), 3.65 (1H, *ddd*, $J = 3.9$, 1.7, 1.4 Hz, H-5). ^{13}C NMR ($CDCl_3$): δ18.5 (*q*, C-9), 22.9 (*q*, C-10), 28.9 (*q*, C-8), 30.6 (*s*, C-7), 32.7 (*d*, C-6), 36.5 (*d*, C-1), 53.6 (*d*, C-5), 59.4 (*d*, C-4), 70.8 (*s*, C-3), 207.7 (*s*, C-2). HRMS *m/z* Found, 182.0943 $[M]^+$. $C_{10}H_{14}O_3$ requires: 182.0945. (Found: C, 65.63; H, 7.65. $C_{10}H_{14}O_3$ requires: C, 65.91; H, 7.74%).

Acetylation of **3**. Compound **3** (45 mg) was acetylated with Ac_2O–pyridine (1 ml, 1:1), 4-dimethylaminopyridine (5 mg) at room temp. overnight. The usual work-up followed by crystallization (EtOH) gave needles (**3a**, 37 mg).

Compound **3a** (*acetate of* **3**). Needles, mp 96–98°. $[\alpha]_D^{24}$ 0° (MeOH; *c* 0.11). IR $\nu_{max}^{KBr}\ cm^{-1}$: 1744 (C=O), 1698 (C=O). ^1H NMR ($CDCl_3$) δ: 1.22 (3H, *s*, 8-Me), 1.26 (3H, *s*, 9-Me), 1.57 (3H, *s*, 10-Me), 1.86 (1H, *dd*, $J = 7.2$, 1.7 Hz, H-1), 2.02 (1H, *ddd*, $J = 7.2$, 1.2, 1.2 Hz, H-6), 2.06 (3H, *s*, 2′-Me), 3.52 (1H, *dd*, $J = 3.9$, 1.2 Hz, H-4), 3.60 (1H, *ddd*, $J = 3.9$, 1.7, 1.2 Hz, H-5). ^{13}C NMR ($CDCl_3$): δ18.0 (*q*, C-9), 20.9 (*q*, C-2′), 21.4 (*q*, C-10), 28.6 (*q*, C-8), 29.1 (*s*, C-7), 29.5 (*d*, C-6), 36.6 (*d*, C-1), 53.2 (*d*, C-5), 57.9 (*d*, C-4), 77.0 (*s*, C-3), 169.5 (*s*, C-1′), 201.6 (*s*, C-2). FDMS *m/z*: 225 $[M + H]^+$.

X-ray analysis of **3**. The crystals were monoclinic, space group $P2_1/C$ with $a = 6.750$ (2), $b = 9.439$ (1), $c = 14.759$ (1) Å and $d_{calc} = 1.184\ g\,cm^{-3}$ for $Z = 4$. The size of the crystal used for data collection was *ca* $0.25 \times 0.2 \times 0.2$ mm. No absorption correction was necessary ($\mu = 6.1\ cm^{-1}$). A total of 1713 reflections were measured for $2° \leqq 2\theta \leqq 120°$ of which 1388 reflections were considered to be observed [$I > 3\sigma(I)$]. The final discrepancy indices were $R = 7.7\%$. The final difference Fourier map was essentially featureless, the highest residual peaks having densities of $0.30\ e\,Å^{-3}$.

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