The flower buds of *Tussilago farfara* L. (Compositae), called “Farfarae Flos,” have been widely used for the treatment of coughs, bronchitis and asthmatic disorders in China. In previous papers, we reported the isolation and structural elucidation of the essential oil components, phenolic compounds, sesquiterpenoids and triterpenoids from the plant. Here, we report the isolation and structural elucidation of two new bisabolane-type sesquiterpenoids, (3\(R\),4\(R\),6\(S\))-3,4-epoxybisabola-7(14),10-dien-2-one (1) and (1\(R\),3\(R\),4\(R\),5\(S\),6\(S\))-1-acetoxy-8-angeloyloxy-3,4-epoxy-5-hydroxybisabola-7(14),10-dien-2-one, and a new oplopane-type sesquiterpenoid, 14(R)-hydroxy-7\(\beta\)-isovaleroyloxyoplop-8(10)-en-2-one (2), as well as three known compounds, (2)-cryptomerion (3), (2)-spathulenol (4) and hydroxytremetone (5). This is the first report of the isolation of 4—6 from Farfarae Flos. Extraction and isolation were carried out as described in the Experimental section.

Compound 1 was isolated as a colorless oil, \([\alpha]_D^22.3 \pm 22.0\). The molecular formula was determined to be \(C_{15}H_{22}O_2\) by high-resolution (HR)-MS, indicating five degrees of unsaturation. The IR spectrum showed the presence of a carbonyl group (1713 cm\(^{-1}\)). The \(^1H\)- (Table 1) and \(^13C\)-NMR spectra (Table 2), obtained with the aid of a 1H–1H shift correlation spectroscopy ("H–H COSY), \(^1H\)-detected heteronuclear multiple quantum coherence (HMQCC) and distortionless enhancement by polarization transfer (DEPT) spectra, showed signals due to a tertiary methyl group [\(\delta \ H\) 1.41 (3H, H-3-15); \(\delta \ C\) 14.9 (C-15)], two olefinic methyl groups [\(\delta \ H\) 1.60 (3H, H-3-12); \(\delta \ C\) 17.8 (C-13), 25.7 (C-12)], four methylenes [\(\delta \ H\) 2.00 (2H, H2-8), 2.01 (1H, H-5\(a\)), 2.10 (2H, H-9), 2.16 (1H, H-1\(b\)), 2.21 (1H, H-5\(b\)), 2.82 (1H, H-1\(b\)); \(\delta \ C\) 26.5 (C-9), 29.7 (C-5), 33.8 (C-8), 40.9 (C-1)], a methine [\(\delta \ H\) 2.58 (1H, H-6); \(\delta \ C\) 43.9 (C-6)], an exomethylene [\(\delta \ H\) 4.80 (1H, H-14a), 4.81 (1H, H-14b); \(\delta \ C\) 109.5 (C-14), 150.3 (C-7)], a trisubstituted double bond [\(\delta \ H\) 5.08 (1H, H-10); \(\delta \ C\) 123.6 (C-10), 132.1 (C-11)] and a carbonyl carbon [\(\delta \ C\) 208.4 (C-2)]. The presence of a trisubstituted epoxide was inferred from the chemical shifts [\(\delta \ H\) 3.45 (1H, H-4); \(\delta \ C\) 59.4 (C-3), 64.9 (C-4)] and the unsaturation degree. Detailed analysis of the "H–"H COSY spectrum of 1 implied connectivities for H2-1–H-6, H-4–H-5\(b\), H2-5–H-6, H2-8–H2-9 and H2-9–H-10 (Fig. 1). Interpretation of the "H-detected heteronuclear multiple bond connectivity (HBMC) spectrum revealed correlations from H-1 to C-2; H-2 and H-12 to C-10 and C-11; and H-15 to C-2, C-3 and C-4 (Fig. 1). Therefore, the planar structure of 1 was deduced to be 3,4-epoxybisabola-7(14),...
10-dien-2-one. The relative stereochemistry of 1 was determined as follows. The coupling pattern and the constants for H-6 (ddd, $J_{\alpha\beta}=13.9$ Hz, $J_{\alpha\gamma}=11.7$ Hz, $J_{\beta\gamma}=6.2$ Hz, and $J_{\gamma\delta}=2.6$ Hz) suggested that H-1$\alpha$ and H-6, and H-5$\alpha$ and H-6 were diaxially related and the side chain at C-6 was $\alpha$-equatorially oriented. The epoxide ring at C-3–C-4 must have been $\alpha$-oriented because of the small coupling constant of H-4 with H-5$\beta$ ($J_{\delta\beta}=4.4$ Hz). It was mentioned that the coupling constant of H-4 with H-5$\alpha$ was almost zero so their dihedral angle must be about 90° which results from the 3$\alpha$,4$\alpha$-epoxide ring. The configuration of the epoxide was supported by the nuclear Overhauser effect correlation spectroscopy (NOESY) cross peaks between H-4 and H-5$\beta$; and
H-4 and H-15 (Fig. 2). The absolute stereochemistry of 1 was determined by a circular dichroism (CD) spectrum. The empirical reversed octant rule can be applied to elucidate the absolute configuration of an epoxy-ketone ring system. 12) The CD spectrum of 1 showed a positive Cotton effect by the C-2 carbonyl group at 302.0 nm (Δε = +1.05), indicating that the absolute configurations at C-3, C-4 and C-6 were R, R and S, respectively. On the basis of the above data, the structure of 1 was determined to be (3R,4R,6S)-3,4-epoxybisabola-7(14),10-dien-2-one.

Compound 2 was isolated as a colorless oil, [α]D = -32.0°. The molecular formula was determined to be C32H46O6 by HR-MS. The IR spectrum showed the presence of a hydroxyl group (3419 cm⁻¹), an α,β-unsaturated ester (1734, 1647 cm⁻¹) and a carbonyl group (1698 cm⁻¹). The 1H- and 13C-NMR spectra of 2 were similar to those of 1 except for the appearance of three oxygenated methine signals at C-1, C-5 and C-8 (δH 4.25 (1H, H-5), 4.72 (1H, H-8), 5.68 (1H, H-1); δC 71.4 (C-1), 73.7 (C-5), 78.4 (C-8)) instead of the methylene signals at C-1, C-5 and C-8 in 1. Furthermore, the signals ascribable to an acetyl group [δH 2.09 (3H, H3-4); δC 20.4, 169.5] and an angeloyl group [δH 1.91 (3H, H3-5), 2.00 (3H, H3-4), 6.20 (1H, H-3); δC 15.9 (C-4), 20.4 (C-5), 127.2 (C-2'), 140.6 (C-3'), 168.9 (C-1')] 13) were also observed in the 1H- and 13C-NMR spectra of 2. In the HMBC spectrum, a cross peak was observed between the 7a-H and C-5. The CD spectrum of 2a showed a negative Cotton effect by the C-2 carbonyl group at 303.0 nm (Δε = -2.37) as observed in tussilagone (7a), indicating that the absolute configuration at C-14 was R*.

The molecular formula was determined to be C22H30O7 by HR-MS. The IR spectrum showed the presence of a hydroxyl group (3419 cm⁻¹), a carbonyl group (1698 cm⁻¹) and a carbonyl carbon in the acetyl group at 1725X infrared spectrophotometer and UV spectra with a Beckman DU-64 spectrophotometer. 1H- and 13C-NMR spectra were recorded with a Perkin-Elmer FT-IR 1725X infrared spectrophotometer and UV spectra with a Beckman DU-64 spectrophotometer. 1H- and 13C-NMR spectra were recorded with JEOL JNM-LA 600 (600 and 100 MHz, respectively) and JEOL JNM-LA 400 (100 and 100 MHz, respectively) spectrometers. Chemical shifts are given in δH ppm). In the proton magnetic resonance (1H NMR) spectrum, a singlet was assigned to the C-2 carbonyl group at 296.0 nm (H-1). In the HMBC spectrum, a cross peak was observed between the H-7 at δH 5.57 and the C-1 at δC 172.1, confirming that the isovaleryl group was assigned to the oxygen at C-7. On acetylation, 3 gave a monoacetate (3a), whose 1H-NMR spectrum showed a downfield shifted oxygen proton assignable to H-14 at δH 5.10. The chemical shift value, coupling pattern and constants for H-14 (sq; J1,4 = 6.6 Hz, J4,5 = 3.3 Hz in 3a) were in accord with those of tussilagone (7a), whose relative stereochemistry has been established by the X-ray diffraction method 15) confirming the relative configuration at C-14 as R*.

The CD spectrum of 3a showed a negative Cotton effect by the C-2 carbonyl group at 303.0 nm (Δε = -2.37) as observed in tussilagone (7a), indicating that the absolute configuration at C-14 was R. On the basis of this evidence, the structure of 3 was determined to be 14(R)-hydroxy-7β-isovaleroyloxyoplop-8(10)-en-2-one. Compound 3 is the first example of the isolation of an olepane derivative with an isovaleroyl group at the C-7 position from Farfarae Flos.

**Experimental**

**General Procedures** Optical rotations were determined with a JASCO DIP-360 digital polarimeter. CD spectra were performed on a JASCO J-720 spectropolarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1725X spectrophotometer and UV spectra with a Beckman DU-64 spectrophotometer. 1H- and 13C-NMR spectra were recorded with JEOL JNM-LA 600 (600 and 150 MHz, respectively) and JEOL JNM-LA 400 (100 and 100 MHz, respectively) spectrometers. Chemical shifts are given in δH ppm). A 1H- and 13C-NMR spectrum, a singlet was assigned to the C-2 carbonyl group at 296.0 nm (H-1). In the HMBC spectrum, a cross peak was observed between the H-7 at δH 5.57 and the C-1 at δC 172.1, confirming that the isovaleryl group was assigned to the oxygen at C-7. On acetylation, 3 gave a monoacetate (3a), whose 1H-NMR spectrum showed a downfield shifted oxygen proton assignable to H-14 at δH 5.10. The chemical shift value, coupling pattern and constants for H-14 (sq; J1,4 = 6.6 Hz, J4,5 = 3.3 Hz in 3a) were in accord with those of tussilagone (7a), whose relative stereochemistry has been established by the X-ray diffraction method 15) confirming the relative configuration at C-14 as R*.

**Plant Material** The dried flower buds of Tussilago farfara, “Farfarae Flos,” were purchased from Tochimoto Tenkaido Co., Ltd., Osaka, Japan in 1990.

**Extraction and Isolation** The dried flower buds of T. farfara (5.0 kg) were extracted with Et2O at room temperature for one week. The Et2O extract was subjected to steam distillation to give an essential oil (7.7 g) and residue (64.5 g). The essential oil (7.7 g) was separated to neutral oil (7.0 g) and acidic oil (0.7 g). The neutral oil (7.0 g) was placed on a silica gel column and developed with n-hexane, benzene and Et2O to afford n-hexane elution part (4.5 g), benzene elution part (1.3 g) and Et2O elution part (1.2 g). The benzene elution part (1.3 g) was purified by preparative GC [column, 10% silicone SE-52 on Chromosorb W (AW) (60—80 mesh); flow rate, 45 ml/min; detector, TCD] to give I (1.5 mg), II (1.3 mg) and III (1.1 mg). The Et2O elution part (1.2 g) was purified by preparative GC [column, 3% silicone SE-52 on Chromosorb W (AW) (60—80 mesh); flow rate, 45 ml/min; detector, TCD] to give IV (2.91 mg).

The residue of the part of steam distillation (25.3 g) was placed on a silica gel column and developed with n-hexane—AcOEt (4:1—1:4). AcOEt and MeOH to afford 19 fractions (frs. 1—19). Fraction 8 was separated by preparative HPLC [MeOH—H2O (3:2); flow rate, 2.0 ml/min; column temp., 40 °C] to give a mixture of 2 and 3. The mixture of 2 and 3 was purified by preparative HPLC [MeOH—H2O (3:2); flow rate, 2.0 ml/min; column temp., 40 °C] to give 2 (3.9 mg) and 3 (2.5 mg).

(3R,4R,6S)-3,4-Epoxybisabola-7(14),10-dien-2-one (1): Colorless oil. [α]D20 +23.2° (c = 0.2, CH2Cl2). IR νmax (CHCl3) cm⁻¹: 1713. UV λmax (MeOH) nm (log ε): 202 (3.9). CD (c = 1.2,1×10⁻⁵ MeOH, Δε (nm)): +1.05 (302.0), -6.63 (206.5). HR-MS m/z: 234.1646 (M⁺, Calcd for C14H23O2; 234.1647).
Acetylation of 2 Compound 2 (3.7 mg) was treated overnight with 0.5 ml of acetic anhydride and 0.5 ml of pyridine. The products were purified by HPLC [MeOH-H2O (4:1); flow rate, 1.2 ml/min; column temperature, 40 °C], giving 2a (2.6 mg).

(1R,3R,4R,5S,6S)-1-Acetoxy-8-angeloyloxy-3,4-epoxy-5-hydroxybicyclo[7.1.0]dec-10-en-2-one (2a): Colorless oil. [α]D20 = -57.6° (c=0.2, CHCl3). IR νmax (CHCl3) cm⁻¹: 3511, 1726. HR-MS m/z: 448.2078 (M⁺, Calcd for C₂₀H₂₄O₄: 448.2098). ¹H-NMR (600 MHz, CDCl3); see Table 1. ¹³C-NMR (100 MHz, CDCl3); see Table 2.

Acetylation of 3 Compound 3 (2.3 mg) was treated overnight with 0.5 ml of acetic anhydride and 0.5 ml of pyridine. The products were purified by HPLC [MeOH-H2O (4:1); flow rate, 1.2 ml/min; column temperature, 40 °C], giving 3a (1.8 mg).

(1R,3R,4R,5S,6S)-Acetoxy-7-β-isovaleroyloxybicyclo[8.10.0]trideca-3,6-dien-2-one (3a): Colorless oil. [α]D20 = -39.8° (c=0.2, CHCl3). IR νmax (CHCl3) cm⁻¹: 3511, 1726. HR-MS m/z: 336.2283 (M⁺, Calcd for C₂₀H₂₄O₃: 336.2230). ¹H-NMR (600 MHz, CDCl3); see Table 1. ¹³C-NMR (150 MHz, CDCl3); see Table 2.

Acetylation of 4 Compound 4 (2.3 mg) was treated overnight with 0.5 ml of acetic anhydride and 0.5 ml of pyridine. The products were purified by HPLC [MeOH-H2O (4:1); flow rate, 1.2 ml/min; column temperature, 40 °C], giving 4a (1.7 mg).

(1R,3R,4R,5S,6S)-Acetoxy-7-β-isovaleroyloxyoxypentalen-8-(10)-en-2-one (4a): Colorless oil. [α]D20 = -13.8° (c=1.30×10⁻², MeOH). IR νmax (MeOH) cm⁻¹: -3.27 (303.0). HR-MS m/z: 378.2409 (M⁺, Calcd for C₂₃H₂₅O₇: 378.2406). ¹H-NMR (600 MHz, CDCl3) δ: 0.77 (3H, d, J=6.6 Hz, H-13), 0.94 (6H, d, J=6.6 Hz, H-4'), 0.97 (3H, d, J=7.0 Hz, H-12), 1.22 (3H, d, J=6.6 Hz, H-15), 2.11 (3H, s, COCH₃), 2.30 (1H, m, H-11), 2.39 (1H, dd, J=16.5, 5.9 Hz, H-1β), 2.50 (1H, dd, J=11.0, 3.3 Hz, H-3), 2.55 (1H, m, H-9), 4.78 (1H, d, J=1.1 Hz, H-10a), 5.10 (1H, qd, J=6.6, 3.3 Hz, H-14), 5.12 (1H, s, H-10b), 5.36 (1H, dd, J=2.9, 2.9 Hz, H-7). The sign of optical rotation and spectral data of 4 and 5 were identical with those of the reported values.

(−)-Spathulenol (5): [α]D20 = -2.9° (c=1.3, CHCl3) [lit., 3) [α]D20 = -7.5° (c=1.48, CHCl3)].

Hydroxytremetone (6): [α]D20 = -26.8° (c=0.1, MeOH) [lit., 4) [α]D20 = -50.7° (EtOH)].

Acknowledgements We are grateful to Mrs. S. Sato and T. Matsuki of this university for measurement of the mass and NMR spectra.

References and Notes