# 6-Oxoferruginol and 6α-Acetoxyferruginol, New Abietane-Type Diterpenes from the Heartwood of *Juniperus formosana*

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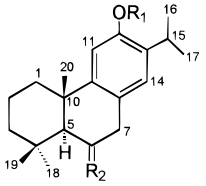
New abietane diterpenes, 6α-acetoxyferruginol and 6-oxoferruginol, were isolated from the heartwood of *Juniperus formosana* Hay. var. *concolor* Hay. together with the known compounds ferruginol, sugiol, xanthoperol, 5-*epi*-xanthoperol, and 6,12-dihydroxyabieta-5,8,11,13-tetraen-7-one. The structures of the new compounds were elucidated on the basis of spectral and chemical evidence.

Many interesting compounds including monoterpenes, sesquiterpenes, diterpenes, lignans, and tropolones have been identified as constituents of *Juniperus* species (Cupressaceae).[1] We have previously studied the chemical principles of five species of *Juniperus* indigenous in Taiwan, including the heartwood of *J. squamata* Lamb. var. *morrisonicola* Hay.[2] and of *J. formosana* Hay.,[3] the roots of *J. chinensis* Linn.,[4,5] and the barks of *J. chinense* Linn. var. *kaizuca* Hort. ex Endl[6] and of *J. formosana* Hay. var. *concolor* Hay.[7−9] This paper deals with the isolation and structure elucidation of two new ferruginol derivatives, 6α-acetoxyferruginol (**1**) and 6-oxoferruginol (**2**), isolated from the heartwood of *J. formosana* var. *concolor* together with five known abietane-type diterpenes, ferruginol (**3**),[10] sugiol,[3] xanthoperol,[10] 5-*epi*-xanthoperol,[11] and 6,12-dihydroxyabieta-5,8,11,13-tetraen-7-one.[12]




**1** $R_1$=H, $R_2$=α-OAc, β-H
**2** $R_1$=H, $R_2$=O
**3** $R_1$=H, $R_2$=$H_2$
**4** $R_1$=Ac, $R_2$=α-OAc, β-H
**5** $R_1$=H, $R_2$=α-OH, β-H
**6** $R_1$=H, $R_2$=α-H, β-OH

The structures of the new compounds **1** and **2** were established as follows. 6α-Acetoxyferruginol (**1**), an amorphous solid, has the molecular formula $C_{22}H_{32}O_3$ on the basis of exact mass (HRMS) at *m*/*z* 344.2354. It showed hydroxy (3422 $cm^{-1}$), ester (1724 and 1240 $cm^{-1}$), and aromatic group absorptions (3080, 1595, 1492 $cm^{-1}$). The $^1H$ NMR spectrum (Table 1) revealed that **1** has an isopropyl group attached to a phenyl group, three singlet methyl groups, an acetoxyl group, and two singlet *para* phenyl protons. Other signals at $\delta$ 1.48 (1H, d, $J$ = 7.8 Hz, H-5), 2.64 (1H, dd, $J$ = 16.8, 2.6 Hz), 3.28 (1H, dd, $J$ = 16.8, 6.6 Hz), and 5.39 (1H, ddd, $J$ = 7.8, 6.6, 2.6 Hz) are present. From the $^{13}C$ NMR (Table 1, assigned by HMQC) data and the typical observation of $H_\beta$-1 proton signal for ferruginol derivative at $\delta$ 2.09 (br d, $J$ = 12.3 Hz), compound **1** can be assigned as a derivative of ferruginol with an extra acetoxy group. On irradiation at $\delta$ 5.39 (H-6), the signals at $\delta$ 1.48 (H-5, d), 2.64 ($H_\beta$-7, dd), and 3.28 ($H_\alpha$-7, dd) were collapsed to a sharp singlet, a doublet ($J$ = 16.8 Hz), and a doublet ($J$ = 16.8 Hz), respectively. The signals at $\delta$ 2.64 and 3.28 were assigned as benzylic C-7 protons; therefore, the acetoxy group must be located at the C-6 position. The C-6 acetoxy group was assigned an α-equatorial orientation from the coupling constants of H-6 with H-5 and $H_\alpha$-7 ($J_{5,6}$ = 7.8 Hz, and $J_{6,7}$ = 6.6 Hz). The acetylation of **1** with $Ac_2O$ and pyridine afforded the new diacetate **4** [1751, 1728 $cm^{-1}$; $\delta$ 1.98 and 2.29 (each 3H, s)]. The saponification of **4** with NaOH in methanol gave **5** [3353 $cm^{-1}$; $\delta$ 1.15 (d, $J$ = 7.0 Hz, H-5), 4.28 (br s, H-6)].[13]

6-Oxoferruginol (**2**), $C_{20}H_{28}O_2$, showed $^1H$ and $^{13}C$ NMR data similar to those of ferruginol (**3**).[10] The difference is the presence of an isolated ketone ($\nu_{max}$ 1703 $cm^{-1}$; $\delta$ 210.7). Thus, **2** was presumed to be a derivative of ferruginol with an extra oxo group. The location was deduced to be at the C-6 position from the observation of signals due to a methine [$\delta$ 2.37 (1H, s), H-5] and a methylene [$\delta$ 3.54 (2H, s), H-7], which are both vicinal to the carbonyl group. The reduction of 6-oxoferruginol (**2**) with sodium borohydride in methanol yielded two products, **5** and **6** (major).[13] Acid treatment of the latter gave 6,7-dehydroferruginol.[14]

## Experimental Section

**General Experimental Procedures.** Melting points were determined with a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. $^1H$ and $^{13}C$ NMR spectra were run on Bruker AM 300 spectrometer ($^1H$ and $^{13}C$ assignments by HMQC). MS (EI, HRMS) and specific rotation were taken on a JEOL-JMS-HX 300 spectrometer and a JASCO DIP-180 digital polarimeter, respectively.

**Plant Material.** The heartwood of *J. formosana* Hay. var. *concolar* Hay. was collected from Hua-Lian, Taiwan, in June 1990. The plant material was identified by Dr. Ih-Sheng Chen, School of Pharmacy, Kaohsiung Medical College, and a voucher specimen has been deposited at the Herbarium of the Department of Botany of the National Taiwan University, Taipei, Taiwan.

**Extraction and Isolation.** The dried heartwood of *J. formosana* Hay. var. *concolor* Hay. (2 kg) was

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**Table 1.** $^1$H and $^{13}$C NMR ($\delta$ values) Data for 1 and 2 (300 and 75 MHz in $CDCl_3$)

| | 1 | | | 2 | |
|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ | | $\delta_C$ | $\delta_H$ |
| 1 | 38.8 | 2.09 br d (12.3, $H_\beta$) | 1 | 38.5 | 2.21 br s (12.3, $H_\beta$) |
| 2 | 18.8 | | 2 | 19.0 | |
| 3 | 42.4 | | 3 | 42.2 | |
| 4 | 34.1 | | 4 | 40.2 | |
| 5 | 53.8 | 1.48 d (7.8) | 5 | 62.5 | 2.37 s |
| 6 | 71.6 | 5.39 ddd (7.8, 6.6, 2.6) | 6 | 210.7 | |
| 7 | 37.6 | 2.64 dd (16.8, 2.6, $H_\beta$) | 7 | 44.6 | 3.54 s |
| | | 3.28 dd (16.8, 6.6, $H_\alpha$) | | | |
| 8 | 125.3 | | 8 | 124.1 | |
| 9 | 148.0 | | 9 | 147.3 | |
| 10 | 35.5 | | 10 | 32.6 | |
| 11 | 109.7 | 6.61 s | 11 | 110.5 | 6.71 s |
| 12 | 151.4 | | 12 | 151.5 | |
| 13 | 131.4 | | 13 | 132.7 | |
| 14 | 126.3 | 6.83 s | 14 | 126.1 | 6.83 s |
| 15 | 28.8 | 3.10 sep (6.9) | 15 | 26.8 | 3.14 sep (6.8) |
| 16 | 22.5 | 1.21 d (6.9) | 16 | 22.5 | 1.22 d (6.8) |
| 17 | 22.6 | 1.21 d (6.9) | 17 | 22.8 | 1.23 d (6.8) |
| 18 | 33.5 | 1.05 s | 18 | 32.9 | 1.11 s |
| 19 | 21.9 | 0.85 s | 19 | 24.5 | 1.06 s |
| 20 | 21.8 | 1.15 s | 20 | 21.9 | 1.28 s |
| $CH_3C(O)$ | 22.4 | 1.98 s | OH | | 4.90 br s |
| $CH_3C(O)$ | 170.9 | | | | |
| OH | | 4.56 br s | | | |

extracted with MeOH (20 L) at room temperature (7 days × 3). The MeOH extract was evaporated *in vacuo* to give a black residue (189 g) that was chromatographed on Si gel (Merck 3379) (2 kg) with hexane/EtOAc, EtOAc, and EtOAc/MeOH gradient solvent systms. The 20% EtOAc in hexane eluate gave a residue (6.5 g), a part of which (3.2 g) was purified by repeated Si gel column chramatography. Two new diterpenes, 6α-acetoxyferruginol (**1**) (8 mg), 6-oxoferruginol (**2**) (19 mg), and five known abietane type diterpenes, ferruginol (**3**) (8 mg), sugiol (**32** mg), xanthoperol (12 mg), 5-*epi*-xanthoperol (16 mg), and 6,12-dihydroxyabieta-5,8,11,13-tetraen-7-one (7 mg), were isolated in that order (gradient elution with 10−20% AcOEt in hexane). The known compounds were identified by comparison with authentic samples or by their physical and spectroscopic data with literature reports.

**6α-Acetoxyferruginol (1):** amorphous solid; $[\alpha]^{30}_D$ +91.7° (*c*, 0.5, $CHCl_3$); IR (KBr) $\nu_{max}$ 3422 (−OH), 3080, 1724, 1595, 1492, 1453, 1372, 1240, 1175, 1020, 967, 911, 864, 802, 732, 647; $^1$H and $^{13}$C NMR data, see Table 1; EIMS (70 eV) *m/z* $[M]^+$ 344 (25), 318 (10), 300 (10), 284 ($M^+$ − AcOH, 100), 202 (20), 149 (20), 136 (10), 69 (25); HRMS exact mass for $C_{22}H_{32}O_3$ *m/z* 344.2351, found 344.2354.

**6-Oxoferruginol (2):** amorphous solid; $[\alpha]^{30}_D$ +86.8° (*c* 1.0, $CHCl_3$); IR (dry film) $\nu_{max}$ 3389 (−OH), 3060, 1703 (ketone), 1601, 1580, 1495, 1296, 1267, 803 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table 1; EIMS (70 eV) *m/z* $[M^+]$ 300 (100), 285 (36), 257 (15), 243 (12), 229 (5), 149 (45), 69 (30); HRMS exact mass for $C_{20}H_{28}O_2$ requires *m/z* 300.2089, found 300.2095.

**Acetylation of 1 with Acetic Anhydride and Pyridine.** A solution of **1** (5 mg) in pyridine (0.5 mg) and acetic anhydride (0.5 mL) was left at room temperature overnight. The reaction mixture was poured onto crushed ice and was extracted with ether (20 mL × 3). The combined organic layers were washed with 3 N HCl, saturated aqueous $NaHCO_3$, and brine and dried ($Na_2SO_4$). Evaporation of the solvent under reduced pressure produced **4** (5 mg): amorphous solid; IR (dry film) $\nu_{max}$ 3035, 1751, 1728, 1620, 1489, 1239, 1206, 1015, 969 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 0.98, 1.07, 1.17 (3H each, s, H-18, H-19, H-20), 1.15, 1.17 (3H each, d, $J$ = 6.8 Hz, H-16, H-17), 1.50 (1H, d, $J$ = 7.8 Hz, H-5), 1.98 and 2.29 (3H each, s, −OAc), 2.69 (1H, dd, $J$ = 17.1, 2.4 Hz, $H_\beta$-7), 2.90 (1H, sep, $J$ = 6.8 Hz, H-15), 3.34 (1H, dd, $J$ = 17.1, 7.8 Hz, $H_\alpha$-7), 5.41 (1H, td, $J$ = 7.8, 2.4 Hz, H-6), 6.78 and 6.93 (1H each, s, H-11, 14).

**Saponification of 4 with Sodium Hydroxide in Methanol.** Compound **4** (5 mg) was added to 3 mL of 0.5 N NaOH/methanol solution for 5 h at room temperature under argon atmosphere. The reaction mixture was poured into 30 mL of water and then acidified to pH 3. The product was extracted with ether (20 mL × 3) and then purified on Si gel to produce **5**[13] (4 mg): amorphous solid; IR (dry film) $\nu_{max}$ 3353, 3045, 1608, 1500, 1417, 1231, 1166, 994, 907, 733 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.07, 1.08, 1.14 (3H each, s, H-18, H-19, H-20), 1.22, 1.23 (3H each, d, $J$ = 6.8 Hz, H-16, H-17), 1.45 (1H, d, $J$ = 7.0 Hz, H-5), 2.06 (1H, br d, $J$ = 12.5 Hz, $H_\beta$-1), 2.65 (1H, dd, $J$ = 15.9, 3.2 Hz, $H_\beta$-7), 3.10 (1H, sep, $J$ = 6.8 Hz, H-15), 3.23 (1H, dd, $J$ = 15.9, 6.6 Hz, $H_\alpha$-7), 4.28 (1H, br s, $W_{1/2}$ = 15.3 Hz, H-6), 6.58, 6.89 (1H each, s, H-11, 14).

**Reduction of 2 with Sodium Borohydride in MeOH.** Excess $NaBH_4$ was added to a solution of 6-oxoferruginol (**2**) (18 mg) in MeOH (3 mL), and the reaction mixture was left at room temperature for 4 h. The reaction was evaporated *in vacuo* at room temperature to give a residue, and then 20 mL of water was added. The aqueous solution was acidified to pH 3 and then extracted with ether (30 mL × 3). The product was purified on Si gel to yield **5**[12] (4 mg) and **6** (11 mg): amorphous solid; IR (dry film) $\nu_{max}$ 3327, 3030, 1611, 1505, 1413, 1372, 1226, 1082, 1036, 1000, 863 cm$^{-1}$; $^1$H NMR ($CDCl_3$ 300 MHz) $\delta$ 1.02, 1.25, 1.52 (3H each, s, H-18, H-19, H-20), 1.37 (1H, br s, H-5), 1.21, 1.22 (3H each, d, $J$ = 6.8 Hz, H-16, H-17), 2.06 (1H, br d, $J$ = 12.6 Hz, $H_\beta$-1), 2.83 (1H, br d, $J$ = 17.0 Hz, $H_\beta$-7), 3.08 (1H, dd, $J$ = 17.0, 4.5 Hz, $H_\alpha$-7), 3.10 (1H, sep, $J$ = 6.8

Hz, H-15), 4.65 (1H, br s, $W_{1/2}$ = 7.8 Hz, H-6), 6.66, 6.82 (1H each, s, H-11, 14)].

**Dehydration of 6 with *p*-Toluenesulfonic Acid in Acetone.** Compound **6** (7 mg) was treated with *p*-toluenesulfonic acid (10 mg) in acetone (3 mL) at room temperature for 1 day. The reaction mixture was neutralized with saturated methanolic $NaHCO_3$ solution and then subjected to Si gel column chromatography to yield 6,7-dehydroferruginol (4 mg).[14]

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