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# SEVEN SESQUITERPENE LACTONES FROM *INULA BRITANNICA* VAR. *CHINENSIS*

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**Key Word Index**—*Inula britannica* var. *chinensis*; Inuleae; Compositae; eudesmanolides; guaianolides; pseudoguaianolides; xanthanolides; inuchinenolides A, B and C.

**Abstract**—Four known sesquiterpene lactones, tomentosin, ivalin, 4-*epi*-isoinuviscolide and gaillardin, together with three new lactones, inuchinenolides A, B and C, were identified in the whole plant of *Inula britannica* var. *chinensis*.

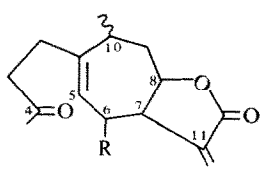
## INTRODUCTION

Sesquiterpene lactones of different skeletal types have been isolated from various *Inula* species [1]. Because of our interest in the natural distribution of such lactones, we have examined *Inula britannica* L. var. *chinensis* (Rupr.) Regel. (Japanese name *Oguruma*) [2], one of four *Inula* species growing in Japan. Along with known compounds (the xanthanolide, tomentosin (**1**) [3], the eudesmanolide, ivalin (**3**) [4], the guaianolides, 4-*epi*-isoinuviscolide (**8**) [3] and gaillardin (**9**) [5]), three new sesquiterpene lactones [inuchinenolides A (**2**), B (**5a**) and C (**7**)] were found. We report here isolation and structural elucidation of these new compounds.

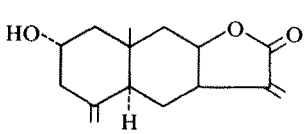
## RESULTS AND DISCUSSION

Inuchinenolide A (**2**) has the same absolute stereostructure as tomentosin (**1**) except for the presence of an acetoxyl group (UV(MeOH) 219.5 nm; CD curve $[\theta]_{295}$ + 422, $[\theta]_{284}$0, $[\theta]_{257}$ − 4432; IR($CHCl_3$) $cm^{-1}$: 1770 ($\alpha,\beta$-unsaturated $\gamma$-lactone), 1750 (ester), 1720 (C=O), 1665 (C=C); *m/e* 306 ($M^+$), 264, 246 ($M^+$ − HOAc), 228; $^1H$ NMR, see Experimental; $^{13}C$ NMR, Table 1).

The acetoxyl group is located at C-6, because in going from (**1**) to (**2**) a vinyl proton signal (H-5) changes from a doublet of doublets to a broad singlet and the H-7 signal changes from a multiplet to a quartet of doublets.



**1** R = H
**2** R = OAc



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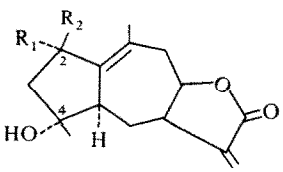
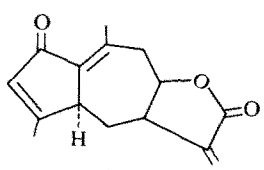


**4** $R_1 = R_2 = H$
**5a** $R_1$ = OAc, $R_2$ = H
**5b** $R_1$ = OH, $R_2$ = H
**5c** $R_1 = R_2 = O$



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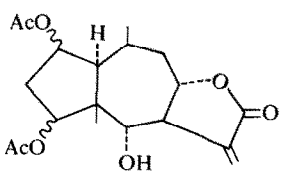

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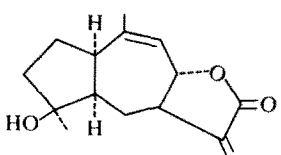

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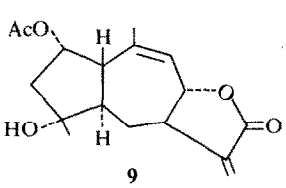

**9**

Table 1. $^{13}C$ NMR data (ppm) for inuchinenolide A (**2**), B (**5a**) and (**7**)

| Carbon*No. | 2 | 5a | 7 |
|---|---|---|---|
| 1 | 142.5 *s* | 135.7 *s* | 49.7 *d* |
| 2 | 30.0 *t* | 72.0 *d* | 75.6 *d* |
| 3 | 42.2 *t* | 46.8 *t* | 43.9 *t* |
| 4 | 207.0 *s* | 77.1 *s* | 75.6 *d* |
| 5 | 125.5 *d* | 52.1 *d* | 51.9 *s* |
| 6 | 70.0 *d* | 25.1 *d* | 74.7 *d* |
| 7 | 42.2 *d* | 41.9 *d* | 53.6 *d* |
| 8 | 76.7 *d* | 78.6 *d* | 74.2 *d* |
| 9 | 36.9 *t* | 36.7 *t* | 35.0 *t* |
| 10 | 35.0 *d* | 131.2 *s* | 30.1 *d* |
| 11 | 135.6 *s* | 138.2 *s* | 139.1 *s* |
| 12 | 169.5 *s* | 169.6 *s* | 169.3 *s* |
| 13 | 124.7 *t* | 121.7 *t* | 121.0 *t* |
| 14 | 20.6 *q* | 21.6 *q* | 17.1 *q* |
| 15 | 30.0 *q* | 22.6 *q* | 20.6 *q* |
| $\underline{C}H_3COO$ | 21.0 *q* | 20.9 *q* | 21.1 2 × *q* |
| $CH_3\underline{C}OO$ | 168.9 *s* | 170.3 *s* | 170.0 *s* |
| | | | 172.3 *s* |

Run in $CDCl_3$ on a JEOL FX-100 spectrometer with TMS as int. standard. *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet. Assignment establishment by frequency off-resonance decoupling.

Irradiation at the frequency of H-7 ($\delta$ 3.42) does not change the doublet ($J = 3$ Hz) of H-6, so H-6 exclusively couples with H-5 (dihedral angle from Dreiding models ~80 for $J_{6\alpha,7\alpha}$). Hence, the acetoxyl group at C-6 is $\beta$-oriented. The structure of inuchinenolide A should be represented by (**2**) except for the stereochemistry of C-10, which remains uncertain.

The second compound, inuchinenolide B (**5a**) shows the following spectral data: colourless oil; $[\alpha]_D$ − 57.2°; IR ($CHCl_3$) $cm^{-1}$: 3600, 3500, 1765, 1735; CD curve $[\theta]_{250}$ − 3477; *m/e* 306 ($M^+$, $C_{17}H_{22}O_5$), 264, 246 ($M^+$ − HOAc), 228 ($M^+$ − HOAc-$H_2O$), and has a $^1H$ NMR spectrum (see Experimental) superimposable on that of pseudoivalin (**4**) from *Ivа microcephala* [6], except for the signal of an acetoxyl group. A broad triplet at 5.42 (1H, $J = 8$ Hz) was assigned to H-2, since irradiation of C-10Me affected the proton attached to an acetoxyl group. Hydrolysis of **5a** with 2% aqueous KOH in dioxane gave the corresponding diol (**5b**) [IR ($CHCl_3$) $cm^{-1}$: 3620, 3450, 1765; *m/e* 264 ($M^+$)]. Oxidation of **5b** with PCC [7] in $CH_2Cl_2$ at room temperature afforded a cyclopentenone derivative (**5c**) [IR ($CHCl_3$) $cm^{-1}$: 1765, 1715, 1630; UV (MeOH) 210, 252 nm; m/e 262 ($M^+$)]. From the above physical data, **5c** is a methylenecyclopentenone. Furthermore, on dehydration with mesyl chloride and pyridine it gave the crossconjugated dienone (**6**) [IR ($CHCl_3$) $cm^{-1}$: 1765, 1720, 1685, 1640, 1620; UV (MeOH) 206, 255 nm; *m/e* 244 ($M^+$); $^1H$ NMR ($CDCl_3$): $\delta$ 6.09 (1H, *br. s*, H-3)]. These observations confirmed the presence of two hydroxyl groups at C-2 and C-4 in **5b**. The configuration of the hydroxyl group at C-2 was established by use of Horeau's method [8] and was shown to be $\alpha$. On the other hand, the diol (**5b**) formed a cyclic ethylidene derivative with acetaldehyde dimethylacetal and $H_2SO_4$, hence two hydroxyl groups are *cis*. These experiments and the physical data lead to the complete stereochemistry **5a** for inuchinenolide B.

The third compound, inuchinenolide C (**7**) shows the following spectral data: colourless oil; $[\alpha]_D$ − 25.9°; CD curve $[\theta]_{271}$ + 538, $[\theta]_{248}$ 0; IR ($CHCl_3$) $cm^{-1}$: 3450, 1770, 1730; *m/e* 366 ($M^+$, $C_{19}H_{26}O_7$), 306 ($M^+$ − HOAc), 246 ($M^+$ − 2 × HOAc), 228 ($M^+$ − 2 × HOAc-$H_2O$); $^1H$ NMR, see Experimental; $^{13}C$ NMR, Table 1. Hence, inuchinenolide C is a $C_8$-pseudoguaianolide with a *trans*-fused lactone which contains two acetoxyl and one hydroxyl group. On acetylation with acetic anhydride and pyridine, it gave a triacetate [IR ($CHCl_3$) $cm^{-1}$: 1765, 1730; *m/e* 408 ($M^+$)]. A doublet of doublets at 3.52 (1H, $J = 4$, 8 Hz) in **7** collapsed into a doublet ($J = 8$ Hz) upon $D_2O$ addition. Furthermore, on irradiation at H-7 ($\delta$ 2.71) the signals of 13-H and 13′-H collapsed into two singlets and a doublet ($\delta$ 3.52) was converted into a broad singlet. These experiments showed that the hydroxyl group was located at C-6$\alpha$ ($J_{6\beta,7\alpha} = 8$ Hz). As irradiation at the frequency of either of the protons under the two acetoxyl groups did not affect the other, the acetoxyl groups are present at C-2 and C-4, respectively. On the other hand, no NOE was observed between C-5 Me and H-10. This supports the $\beta$-configuration of C-10 Me group because the C-5 Me group is probably $\beta$ on the basis of biogenetic considerations. Therefore, the structure of inuchinenolide C is assumed to be **7** except for the stereochemistry of the two acetoxyl groups.

## EXPERIMENTAL

Mps are uncorr. $^1H$ NMR (100 MHz) and $^{13}C$ NMR (25 MHz): $CDCl_3$, $\delta$ units relative to TMS. MS (70 eV) direct insertion. IR and $[\alpha]_D$: $CHCl_3$. UV and CD: MeOH.

*Extraction and separation.* The MeOH extract of fresh whole plant (15 kg) of *Inula britannica* var. *chinensis* collected at Komaki, Aichi prefecture, Japan, was divided into the *n*-hexane and the EtOAc-soluble fractions (100 g). The EtOAc-soluble fraction ($C_6H_6$–EtOAc, 3:1) gave tomentosin (**1**, 55 mg) and inuchinenolide A (**2**, 60 mg). The second fraction (1:1) gave ivalin (**3**, 1 g), inuchinenolide B (**5a**, 250 mg) and inuchinenolide C (**7**, 17 mg). The third fraction (1:3) gave 4-*epi*-isoinuviscolide (**8**, 35 mg) and gaillardin (**9**, 1.5 g).

*Tomentosin* (**1**). Colourless oil. $[\alpha]_D^{20}$ + 34.9° (*c* 0.55, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1765, 1720, 1665. UV $\lambda_{max}^{MeOH}$ nm: 219.5. CD curve $[\theta]_{290}$ + 228, $[\theta]_{284}$ 0, $[\theta]_{258}$ − 4692. MS *m/e*: 248 ($M^+$), 230, 215, 205, 190. $^1H$ NMR: $\delta$ 1.14 (3H, *d*, $J = 7$ Hz, C-10Me), 2.14 (3H, *s*, C-4Me), 3.28 (1H, *m*, C-7H), 4.62 (1H, *m*, C-8H), 5.40 (1H, *dd*, $J = 6$, 8 Hz, C-5H).

*Inuchinenolide A* (**2**). Colourless oil. $[\alpha]_D^{20}$ − 54.6° (*c* 0.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1770, 1750, 1720, 1665. UV $\lambda_{max}^{MeOH}$ nm: 219.5 (ε 5012). CD curve $[\theta]_{295}$ + 422, $[\theta]_{284}$ 0, $[\theta]_{255}$ − 4432. MS *m/e*: 306 ($M^+$, $C_{17}H_{22}O_5$), 264, 246, 228. $^1H$ NMR: $\delta$ 1.17 (3H, *d*, $J = 7$ Hz, C-10Me), 2.12, 2.16 (each 3H, *s*, C-4Me or OCOMe), 3.42 (1H, *m*(*qd*), C-7H), 4.64 (1H, *m*, C-8H), 5.08 (1H, *d*, $J = 3$ Hz, C-6αH), 5.60 (1H, *br. s*, C-5H), 5.71 (1H, *d*, $J = 3$ Hz, C-13H), 6.28 (1H, *d*, $J = 3$ Hz, C-13′H).

*Ivalin* (**3**). Mp 131 ~ 134°. IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3625, 3500, 1765, 1670, 1650. Calc. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.21. Found: C, 72.32; H, 8.18%.

*Inuchinenolide B* (**5a**). Colourless oil. $[\alpha]_D^{20}$ − 57.2° (*c* 1.3, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3600, 3500, 1760, 1735. UV $\lambda_{max}^{MeOH}$ nm: 209 (ε 9876). CD curve $[\theta]_{250}$ − 3477. MS *m/e*: 306 ($M^+$, $C_{17}H_{22}O_5$), 264, 246, 228. $^1H$ NMR: $\delta$ 1.05 (3H, *s*, C-4Me), 1.68

(3H, *s*, C-10Me), 2.06 (3H, *s*, OCOMe), 3.30 (1H, *m*, C-7H), 4.84 (1H, *m*, C-8H), 5.42 (1H, *br. t*, $J = 8$ Hz, C-2H), 5.61 (1H, *d*, $J = 3$ Hz, C-13H), 6.26 (1H, *d*, $J = 3$ Hz, C-13'H).

*Hydrolysis of* **5a**. To a soln of 25 mg **5a** in 1.5 ml dioxane was added 4 ml 2% KOH, and the soln was allowed to stand overnight. This soln was acidified and extracted with EtOAc. The extract was evapd and the residue passed through a column of Si gel (1 g, $CHCl_3$) to give an oil (**5b**, 5 mg). $[\alpha]_D^{20} - 121.0°$ (*c* 0.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3620, 3450, 1765. UV $\lambda_{max}^{MeOH}$ nm: 210 ($\varepsilon$ 9029). MS *m/e*: 264 ($M^+$, $C_{15}H_{20}O_4$), 246 ($M^+ - H_2O$), 231, 228 ($M^+ - 2 \times H_2O$), 204. $^1$H NMR: $\delta$ 1.04 (3H, *s*, C-4Me), 1.85 (3H, *d*, $J = 2$ Hz, C-10Me), 3.28 (1H, *m*, C-7H), 3.70 (1H, *m*, OH), 4.52 (1H, *br. t*, $J = 8$ Hz, C-2H), 4.82 (1H, *m*, C-8H), 5.62 (1H, *d*, $J = 3$ Hz, C-13H), 6.30 (1H, *d*, $J = 3$ Hz, C-13'H).

*Oxidation of* **5b**. A soln of 12 mg **5b** in 5 ml $CH_2Cl_2$ was cooled with ice and treated with 20 mg pyridine chlorochromate. After 2 hr the solution was diluted with 5 ml $H_2O$ and extracted with $CHCl_3$. The extract was evapd to give an oil (**5c**, 8 mg). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3620, 1765, 1715, 1630. UV $\lambda_{max}^{MeOH}$ nm: 210 ($\varepsilon$ 1284), 252 ($\varepsilon$ 933). MS *m/e*: 262 ($M^+$, $C_{15}H_{18}O_4$), 247, 244, 204. $^1$H NMR: $\delta$ 1.16 (3H, *s*, C-4Me), 2.24 (3H, *d*, $J = 2$ Hz, C-10Me), 3.30 (1H, *m*, C-7H), 4.80 (1H, *m*, C-8H), 5.65 (1H, *d*, $J = 3$ Hz, C-13H), 6.32 (1H, *d*, $J = 3$ Hz, C-13'H).

*Dehydration of* **5c**. The oily oxidation product (**5c**, 2 mg was dissolved in 1 ml pyridine, and 1 drop mesyl chloride was added. The resulting solution was left overnight at 0°. The reactant was treated in the usual manner. Colourless oil (**6**, 1 mg). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1765, 1720, 1685, 1640, 1620. UV $\lambda_{max}^{MeOH}$ nm: 206, 255. MS *m/e*: 244 ($M^+$, $C_{15}H_{16}O_4$). $^1$H NMR: $\delta$ 2.13 (3H, *s*, C-4Me), 2.29 (3H, *s*, C-10Me), 4.88 (1H, *m*, C-8H), 5.58 (1H, *d*, $J = 3$ Hz, C-13H), 6.09 (1H, *br. s*, C-3H), 6.26 (1H, *d*, $J = 3$ Hz, C-13'H).

*Configuration of* C-2 *in* **5b**. A soln of 4 mg **5b** and 9 mg (±)-2-phenylbutanoic anhydride in 0.5 ml pyridine was kept at room temp. overnight. After working up the reaction mixture by the usual procedure 8 mg 2-phenylbutanoic acid was recovered. $[\alpha]_D^{20} - 5.6°$ (*c* 0.53, $C_6H_6$).

*Ethylidene product*. To a soln of **5b** (5 mg) in 3 ml acetaldehyde dimethylacetal was added with cooling 1 drop of conc $H_2SO_4$. After standing overnight at room temp., the mixture was poured into ice and ether. The organic layer was washed with water and dried. Oil (2 mg). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1765, 1155. MS *m/e*: 290 ($M^+$). $^1$H NMR: $\delta$ 1.06 (3H, *s*, C-4Me), 1.28 (3H, *d*, $J = 6$ Hz, $CH_3$-CH), 1.90 (3H, *s*, C-10Me), 5.20 (1H, *q*, $J = 6$ Hz, Me-CH).

*Inuchinenolide* C (**7**). Colourless oil. $[\alpha]_D^{20} - 25.9°$ (*c* 1.13, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3450, 1770, 1730. UV $\lambda_{max}^{MeOH}$ nm: 209 ($\varepsilon$ 6889). CD curve $[\theta]_{271} + 538$, $[\theta]_{248}0$. MS *m/e*: 366 ($M^+$, $C_{19}H_{26}O_7$), 306, 246, 228. $^1$H NMR: $\delta$ 0.92 (3H, *s*, C-5Me), 0.98 (3H, *d*, $J = 6$ Hz, C-10Me), 1.90 (1H, *dd*, $J = 2$, 6 Hz, C-10H), 2.04, 2.14 (each 3H, *s*, C-2,4 OCOMe), 2.17 (1H, *m*, 7-H), 3.52 (1H, *dd*, $J = 4$, 8 Hz, C-6H), 4.42 (1H, *m*, C-8H), 4.80 (1H, *d*, $J = 4$ Hz C-6 OH), 4.90 (1H, *br. t*, $J = 8$ Hz, C-2H), 5.58 (1H, *dd*, $J = 9$, 11 Hz, C-4H), 5.95 (1H, *d*, $J = 3$ Hz, C-13H), 6.17 (1H, *d*, $J = 3$ Hz, C-13'H).

*Acetylation of* **7**. **7** (5 mg) was treated with $Ac_2O$ (1 ml) and pyridine (1 ml) at room temp. for 2 days to give triacetate (4 mg).

4-Epi-*isoinuviscolide* (**8**). Mp 143 ~ 146°. $[\alpha]_D^{20} - 59.2°$ (*c* 0.5, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3620, 1770, 1670, 1660. UV $\lambda_{max}^{MeOH}$ nm: 205.5. CD curve $[\theta]_{237} + 818$, $[\theta]_{226}0$. MS *m/e*: 248 ($M^+$), 230, 215. $^1$H NMR: $\delta$ 1.24 (3H, *s*, C-4Me), 1.84 (3H, *s*, C-10Me), 4.70 (1H, *br. d*, $J = 8$ Hz, C-8H), 5.46 (1H, *d*, $J = 3$ Hz, C-13H), 5.83 (1H, *br. s*, C-9H), 6.16 (1H, *d*, $J = 3$ Hz, C-13'H).

*Gaillardin* (**9**). Mp 199 ~ 201°. $[\alpha]_D^{20} - 9.7°$ (*c* 1.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3600, 1770, 1740, 1660. UV $\lambda_{max}^{MeOH}$ nm: 210 ($\varepsilon$ 11 200). CD curve $[\theta]_{250} + 977$, $[\theta]_{235}0$. MS *m/e*: 306 ($M^+$), 288, 246, 228. $^1$H NMR: $\delta$ 1.24 (3H, *s*, C-4Me), 1.80 (3H. *s*, C-10Me), 2.06 (3H, *s*, OCOMe), 4.44 (1H, *br. d*, $J = 8$ Hz, C-8H), 5.24 (1H, *br. s*, C-2H), 5.46 (1H, *d*, $J = 3$ Hz, C-13H), 5.84 (1H, *br. s*, C-9H), 6.12 (1H, *d*, $J = 3$ Hz, C-13H).

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