# *seco*-Prezizaane-type sesquiterpenes from *Illicium merrillianum*

Jian-Mei Huang [a], Chun-Shu Yang [b], Hironobu Takahashi [a], Yoshiyasu Fukuyama [a],*

[a]*Institute of Pharmacognosy, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan*
[b]*Faculty of Pharmaceutical Sciences, Beijing University of Chinese Medicine, Beijing 100029, China*

## Abstract

Four *seco*-prezizaane sesquiterpene lactones, 14-*O*-*n*-butyrylfloridanolide, 3,4-dehydrofloridanolide, 3,6-dideoxy-10-hydroxy-pseudoanisatin, and 2-*O*-*n*-butyrylpseudomajucin were isolated from the pericarps of *Illicium merrillianum*. Their structures were determined by spectroscopic methods. © 2000 Elsevier Science Ltd. All rights reserved.

*Keywords: Illicium merrillianum*; Illiciaceae; Pericarps; *seco*-Prezizaane; Sesquiterpene; Floridanolide; Pseudoanisatin; Pseudomajucin

## 1. Introduction

In a preceding paper, we reported five new *seco*-Prezizaane-type sesquiterpenes and the previously known anislactones A and B isolated from the pericarps of *Illicium merrillianum*, a plant species indigenous to southwestern China and Myanmar (Huang et al., 1999). Anislactones with two types of $\gamma$-lactone have a different carbon skeleton from *seco*-prezizaane-type sesquiterpenes found in the *Illicium* plants, and have been so far isolated only from *I. anisatum* as minor constituents (Kouno et al., 1990). We have found that these structurally rare sesquiterpenes are abundant in *I. merrillianum* (Huang et al., 1999). Our continuing studies on chemical constituents of the title plant have resulted in the isolation of four new *seco*-prezizaane-type sesquiterpene lactones 1–4, which belong to floridanolide-like, pseudoanisatin-like and pseudomajucin-like carbon skeletons, respectively. In this paper, the structural elucidation of these compounds is described.

## 2. Results and discussion

The methanol extract of the dried pericarps of *I. merrillianum* was subjected to silica gel, Sephadex LH-20 and reversed phase RP-8 chromatographies to give compounds 1–4.

Compound 1 had a molecular formula of $C_{19}H_{30}O_7$, as determined by high resolution (HR)-FAB-MS ($m/z$ 409.1643 $[M + K]^+$). The spectral data of 1 indicated the presence of a *n*-butyryloxy group [1740 cm$^{-1}$; $\delta_H$ 0.93 (3H, *t*, $J = 7.2$ Hz), 1.77 (2H, *qt*, $J = 7.3$ and 7.2 Hz) and 2.28 (2H, *t*, $J = 7.3$ Hz); $\delta_C$ 173.0], which was supported by the observation of the prominent fragment ion peak at $m/z$ 71 in the EI-MS. Moreover, the presence of three methyl groups, a $J = 12$ Hz for the coupling constant of H$_2$-14 and their AB-type proton signals (Table 1) suggested that 1 was either a floridanolide-type (Schmidt et al., 1998; Wang et al., 1994) or dunnianin-type sesquiterpene (Kouno et al., 1988; Schmidt et al., 1997), both of which differ in the lactone ring type on the C-7 or the C-3 position. The HMBC of 1 showed a cross peak between H-7 ($\delta_H$ 4.28) and the C-11 carbonyl ($\delta_C$ 175.2) in support of the lactone ring attached to C-7, and the H-14 oxygen-bearing methylene ($\delta_H$ 4.08 and 4.13) was correlated with the C-1′ ester carbonyl ($\delta_C$ 173.0) thereby indicating the *n*-butyryloxy group was connected to C-14. Thus 1 is a 14-*O*-butyryl derivative of a floridanolide-type sesquiterpene. Additionally, the relative configuration of 1 was identical with that of 13-acetoxy-14-butyryloxyfloridanolide (5) (Schmidt, 1998), as determined by analysis of the phase-sensitive nuclear Overhauser and exchange spectroscopy (NOESY) spectrum. The above spectral data thus established 1 to be 14-*O*-*n*-butyrylfloridanolide.

The $^1$H and $^{13}$C NMR spectra for compound 2, $C_{15}H_{22}O_5$ determined by HR-CI-MS ($m/z$: 283.1576 $[M + H]^+$), resembled those for 1. However, had new signals due to a trisubstituted olefin [$\delta_H$ 5.95 (1H, *dd*,

* Corresponding author. Tel.: + 86-88-6229611, ext. 5911; fax: 86-88-6553051.

*E-mail address:* fukuyama@ph.bunri-u.ac.jp (Y. Fukuyama).

**1** R = CH$_3$
**5** R = CH$_2$OAc

**2**

**3**

**4** R = COn-Pr
**6** R = H
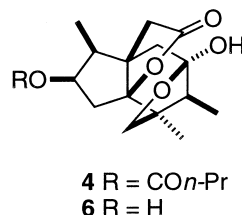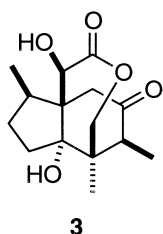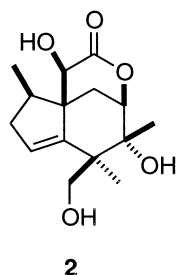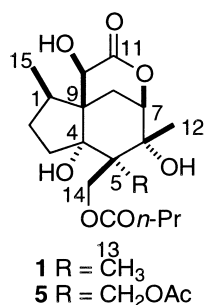
$J = 3.2$ and 1.3 Hz); $\delta_C$ 134.6 and 140.9] instead of signals due to the 14-O-n-butyryl group, the C-3 methylene and the C-4 oxygen-bearing quaternary carbon in **1**. Moreover, the AB doublet assignable to H-14 was shifted upfield from $\delta_H$ 4.03 and 4.13 in **1** to $\delta_H$ 3.09 and 3.34 in **2**, indicating the presence of a hydroxyl group at C-14. Detailed analyses of H–H COSY and HMQC enabled the olefinic proton to be assigned to H-3 in a

partial unit, C(15)H$_3$C(1) HC(2)H$_2$C(3)H = . Hence, the structure of **2** was deduced to be 3,4-dehydro-floridanolide, which was consistent with the structure obtained by analysis of the long-range C–H correlations observed in the HMBC of **2**. The NOESY spectrum of **2** gave the same relative stereochemistry as **1** and **5**. Thus, **2** was 3,4-dehydrofloridanolide.

Compound **3** possessed the molecular mass of 282.1495 obtained by HR–EI–MS, in accordance with the molecular formula C$_{15}$H$_{22}$O$_5$. The IR spectrum displayed absorptions at 3421 and 1716 cm$^{-1}$ attributable to hydroxyl and carbonyl groups. The NMR spectral data (Tables 1 and 2) for **3** showed the presence of an ester and a keto group ($\delta_C$ 172.6 and 205.0), three methyl groups [$\delta_H$ 1.03 (d, $J = 7.0$ Hz), 1.12 (s) and 1.19 (d, $J = 7.0$ Hz)], two isolated methylenes (C-8 and C-14) and a carbinol-like methine [C-10: $\delta_H$ 4.44 (d, $J = 4.8$ Hz); $\delta_C$ 77.4] coupled to a hydroxyl group [$\delta_H$ 2.67 (d, $J = 4.8$ Hz)], indicating that **3** was a 6-deoxypseudo-anisatin-type sesquiterpene lactone (Kouno et al., 1988). However, **3** was different from 6-deoxypeudoanisatin not only in the presence of a hydroxyl group at C-10 but also in the absence of a hydroxyl group at C-3. These comparative spectral data implied that **3** was 3-dehy-droxy-10-hydroxy-6-deoxypseudoanisatin. This was substantiated by the HMBC experiment, in which H-1 and H-10, and H-14 correlated to the C-4 and C-9 quaternary carbons, and C-4, C-5, C-6 and C-11, respectively. Finally, the NOESY spectrum showed cross-peaks

Table 1
$^1$H NMR spectral data for compounds **1–4**

| H | **1**[a] | **2**[b] | **3**[a] | **4**[c] |
|---|---|---|---|---|
| 1 | 2.43 m | 2.13 qdd (12.4, 10.1, 6.6) | 2.50 m | 2.27 qd (7.2, 4.2) |
| 2 | 1.98 m | 2.41 ddd (19.0, 12.4, 3.2) | 2.12 m | 5.11 ddd (4.2, 4.2, 1.0) |
|   | 1.52 m | 2.11 ddd (19.0, 10.1, 1.3) | 1.60 m | |
| 3 | 2.48 m | 5.95 dd (3.2, 1.3) | 2.54 m | 2.32 dd (15.0, 4.2) |
|   | 1.52 m | | 1.60 m | 2.20 dd (15.0, 1.0) |
| 6 | | | 2.88 q (7.0) | 1.89 q (7.2) |
| 7 | 4.28 dd (3.3, 2.2) | 4.31 dd (3.6, 1.8) | | |
| 8α | 2.50 dd (15.0, 2.2) | 2.19 dd (14.4, 1.8) | 2.27 d (16.5) | 1.82 d (13.8) |
| β | 1.77 dd (15.0, 3.7) | 1.99 dd (14.4, 3.6) | 2.74 d (16.5) | 2.24 d (13.8) |
| 10α | 4.15 s | 4.07 s | 4.44 d (4.8) | 2.74 d (18.6) |
| β | | | | 2.89 d (18.6) |
| 12 | 1.36 s | 1.31 s | 1.19 d (7.0) | 1.03 d (7.2) |
| 13 | 1.24 s | 1.28 d (1.2) | 1.12 s | 1.01 s |
| 14 | 4.08 d (12.4) | 3.09 d (11.4) | 3.86 d (13.2) | 3.68 d (9.0) |
|   | 4.13 d (12.4) | 3.34 dd (11.4, 1.2) | 4.88 d (13.2) | 3.80 d (9.0) |
| 15 | 1.04 d (6.8) | 1.12 d (6.6) | 1.03 d (7.0) | 0.99 d (7.2) |
| 2′ | 2.28 t (7.3) | | | 2.22 t (7.4) |
| 3′ | 1.77 qt (7.3, 7.2) | | | 1.60 qt (7.4, 7.4) |
| 4′ | 0.93 t (7.2) | | | 0.92 t (7.4) |
| 6-OH | 4.55 s | | | |
| 10-OH | 3.09 s | 3.31 s | 2.67 d (4.8) | |

[a] 400 MHz in CDCl$_3$.
[b] 600 MHz in CDCl$_3$.
[c] 600 MHz in CD$_3$OD. n-PrCO: CH$_3^{4'}$CH$_2^{3'}$CH$_2^{2'}$CO$^{1'}$.

Table 2
$^{13}$C NMR spectral data for compounds **1–4**

| C | 1[a] | 2[b] | 3[a] | 4[c] |
|---|------|------|------|------|
| 1 | 38.8 | 44.4 | 41.1 | 53.9 |
| 2 | 29.4 | 39.4 | 30.4 | 78.5 |
| 3 | 32.8 | 134.6 | 33.6 | 41.6 |
| 4 | 89.6 | 140.9 | 85.8 | 102.0 |
| 5 | 46.6 | 46.3 | 47.1 | 51.7 |
| 6 | 77.2 | 74.0 | 47.7 | 44.4 |
| 7 | 83.7 | 83.5 | 205.0 | 107.5 |
| 8 | 27.4 | 32.2 | 45.6 | 54.8 |
| 9 | 49.3 | 49.3 | 50.7 | 49.9 |
| 10 | 71.1 | 71.6 | 77.2 | 41.0 |
| 11 | 175.2 | 175.1 | 172.6 | 178.8 |
| 12 | 21.6 | 20.9 | 8.5 | 8.5 |
| 13 | 14.6 | 19.7 | 18.8 | 14.4 |
| 14 | 65.1 | 63.9 | 69.0 | 71.8 |
| 15 | 14.0 | 13.8 | 14.3 | 9.9 |
| 1′ | 173.0 | | | 174.6 |
| 2′ | 36.3 | | | 37.1 |
| 3′ | 18.3 | | | 19.3 |
| 4′ | 13.7 | | | 13.9 |

[a] 100 MHz in CDCl$_3$.
[b] 150 MHz in CDCl$_3$.
[c] 150 MHz in CD$_3$OD. *n*-PrCO: CH$_3^{4'}$CH$_2^{3'}$CH$_2^{2'}$CO$^{1'}$.

between H-15 and H-10, and H-12 and H-14 in support of the relative configuration at C-1, C-6 and C-10 as depicted in **3**. Thus, **3** was 3,6-dideoxy-10-hydroxy-pseudoanisatin.

Compound **4** had the molecular formula C$_{19}$H$_{28}$O$_6$, as established by HR–FAB–MS (*m/z* 353.1974 [M + H]$^+$). Its spectral data indicated the presence of a *n*-butyryloxy group [1725 cm$^{-1}$; $\delta_C$ 174.6, 37.1, 19.3 and 13.9; $\delta_H$ 2.22 (*t*), 1.60 (*qt*), 0.92 (*t*); *m/z* 71]. This butyryloxy group must be placed at C-2 based on the HMBC correlation between H-2 ($\delta_H$ 5.11) and the butyl ester carbonyl. Additionally, the IR spectrum had an absorption at 1757 cm$^{-1}$ typical of a γ-lactone. Its $^1$H NMR spectrum contained signals (Table 1) due to a tertiary methyl and two secondary methyl groups, and three isolated methylene groups, one (H-14) of which had a small geminal coupling constant (9.0 Hz) indicative of a methylene functionality present in a five-member ether ring. These spectral features, together with 15 carbons as characterized by a DEPT experiment, indicates that **4** was closely related to pseudomajucin (**6**) (Kouno et al., 1989). In fact, the $^{13}$C NMR spectral data (Table 2) of **4** corresponded well to those of **6**, except for the C-1, C-2 and C-3 resonances, suggesting the *n*-butyryloxy group to be placed at C-2. Additionally, this tentative structure was not in contradiction to analyses of all 2 D NMR spectra (H–H COSY, HMQC and HMBC). The relative configurations at C-1, C-5, C-6 and C-9 were verified to be the same as **6** based on analysis of the NOESY spectrum as shown in Fig. 1. On the other hand, the small vicinal coupling constant (1.0 Hz) between H-2 and H-3β ($\delta_H$ 2.20) suggested the
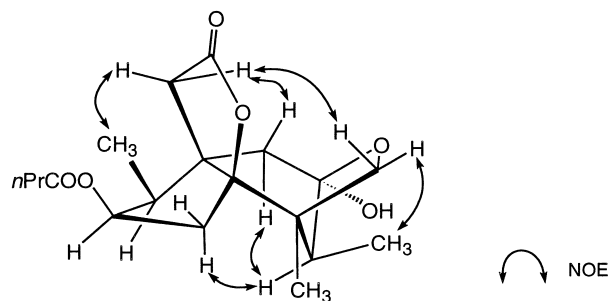


Fig. 1. Selected correlations observed in the NOESY spectrum of **4**.

dihedral angle to be close to 90°, which could account for the *n*-butyryloxy group taking a β orientation on C-2. Accordingly, **4** was 2-*O*-*n*-butyrylpseudomajucin.

*Illicium merrillianum* is phytochemically similar to *I. floridanum*, American Star Anise (Schmidt et al., 1997, 1998; Schmidt, 1999). Both elaborate a variety of common *seco*-prezizaane-type sesquiterpenes of the anisatin-type, floridanolide-type, pseudoanisatin-type, and cyclo-parvifloralone-type. However, anislactone-type sesquiterpenes, which are the main components in *I. merrillianum*, have not been isolated from *I. floridanum*. Thus, this diversity of secondary metabolites in *I. merrillianum* has attracted our interest in the chemical studies of this plant.

## 3. Experimental

### 3.1. General

Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were measured on a Jasco FT–IR 5300 infrared spectrophotometer. NMR spectra were recorded on a Varian Unity 600 or 400 instrument. Chemical shifts were given as δ (ppm) with TMS as internal standard. The MS were recorded on a JEOL AX-500 instrument. Column chromatography (CC) was carried out on Kiselgel 60 (70–230 mesh and 230–400 mesh), Sephadex LH-20 and Cosmosil 75C$_{18}$-OPN.

### 3.2. Collection, extraction and isolation

The ripe fruits of *I. merrillianum* A.C. Smith were collected in Yunnan, China in September 1998 and a voucher specimen (94041) is available in Beijing University of Chinese Medicine.

The dried pericarps of *I. merrillianum* (3.7 kg) were powdered and extracted with methanol at room temperature to give 1 kg of pale yellow extract. The extract (430 g) was applied to a 400 g silica gel column (70–230 mesh) eluted successively with CH$_2$Cl$_2$, CH$_2$Cl$_2$–EtOAc (9:1, 1:1), EtOAc, EtOAc–MeOH (7:3) and MeOH to yield seven fractions (A–G). Fraction B was further

subjected to silica gel column chromatography with *n*-hexane–EtOAc (3:2) as eluent to give 7 fractions. Fr. 6 was subjected to column chromatography on Sephadex LH-20 with MeOH to afford fractions 8–12. Of these fractions, 14-*O*-*n*-butyrylfloridanolide (**1**) (5 mg) was obtained from fr. 11 by a combination of chromatography on silica gel [CHCl$_3$–EtOAc (3:2)] and medium pressure liquid chromatography [Lichroprep RP-8 (40–63 µm) B, MeOH–H$_2$O (3:2; 2 ml/min)]. Fr. 12 was separated by TLC on silica gel [CH$_2$Cl$_2$–EtOAc (2:3)] and RP-8 [MeOH–H$_2$O (1:1)] to yield 3,6-dideoxy-10-hydroxypseudoanisatin (**3**) (1.5 mg). Fraction C was divided by chromatography on silica gel eluting with hexane–EtOAc (1:1) into fractions 13–23. Fr. 19 was chromatographed by CC on 75C$_{18}$-OPN [MeOH–H$_2$O (2:3)], followed by CC on AgNO$_3$ impregnated silica gel [CH$_2$Cl$_2$–EtOAc (1:2)], to give 3,4-dehydrofloridanolide (**2**) (15 mg). Fr. 20 was also separated by CC on 75C$_{18}$-OPN using MeOH–H$_2$O (2:3) as eluent to give fractions 24–27. 2-*O*-*n*-Butyrylpseudomajucin (**4**) (7 mg) was obtained from fr. 27 by TLC on RP-8 [MeOH–H$_2$O (9:11)].

### 3.2.1. 14-O-n-Butyrylfloridanolide (1)

Colorless amorphous; $[\alpha]_D^{20} -14°$ (CHCl$_3$, *c* 0.65); IR (film, cm$^{-1}$): 3404 (OH), 1740 (CO), 1722 (CO); HR–FAB–MS *m/z* 409.1643 [M + K]$^+$ (calc. for C$_{19}$H$_{30}$O$_7$K: 409.1629); EI–MS *m/z* (rel. int.): 282 (5), 264 (37), 177 (51), 71 (65); $^1$H and $^{13}$C NMR: see Tables 1 and 2.

### 3.2.2. 3,4-Dehydrofloridanolide (2)

Colorless amorphous; $[\alpha]_D^{20} +44°$ (CHCl$_3$, *c* 1.90); IR (film, cm$^{-1}$): 3412 (OH), 1722 (CO). HR–CI–MS *m/z* 283.1576 (calc. for C$_{15}$H$_{23}$O$_5$: 283.1546); $^1$H and $^{13}$C NMR: see Tables 1 and 2.

### 3.2.3. 3,6-Dideoxy-10-hydroxypseudoanisatin (3)

Colorless amorphous; IR (film, cm$^{-1}$): 3431 (OH), 1716 (CO). HR–EI–MS *m/z* 282.1495 (calc. for C$_{15}$H$_{22}$O$_5$: 282.1468); EI–MS *m/z* (rel. int.): 282 (5), 264 (10), 189 (24), 113 (100); $^1$H and $^{13}$C NMR: see Tables 1 and 2.

### 3.2.4. 2-O-n-Butyrylpseudomajucin (4)

Colorless powder; $[\alpha]_D^{20} -61.9°$ (CH$_3$OH, *c* 0.65); IR (film, cm$^{-1}$): 3389 (OH), 1757 (CO). HR–FAB–MS *m/z* 353.1974 [M + H]$^+$ (calc. for C$_{19}$H$_{29}$O$_6$: 353.1964); EI–MS *m/z* (rel. int.): 234 (22), 165 (26), 113 (100),71 (69); $^1$H and $^{13}$C NMR: see Tables 1 and 2.

## References

Huang, J.-M., Yang, C.-S., Wang, H., Wu, Q.-M., Wang, J.-L., Fukuyama, Y., 1999. Structures of novel sesquiterpenes from the pericarps of *Illicium merrillianum*. Chemical and Pharmaceutical Bulletin 47, 1749–1752.

Kouno, I., Kawano, N., Yang, C.-S., 1988. New pseudoanisatin-like sesquiterpene lactones from the bark of *Illicium dunnianum*. Journal of the Chemical Society Perkin Transactions I, 1537–1539.

Kouno, I., Baba, N., Hashimoto, M., Kawano, N., Yang, C.-S., Sato, S., 1989. A new sesquiterpene lactone and its glucoside from the pericarps of *Illicium majus*. Chemical and Pharmaceutical Bulletin 37, 2427–2430.

Kouno, I., Mori, K., Okamoto, S., Sato, S., 1990. Structures of anislactone A and B; novel type of sesquiterpene lactones from the pericarps of *Illicium anisatum*. Chemical and Pharmaceutical Bulletin 38, 3060–3063.

Schmidt, T.J., Peters, W., Fronczek, F.R., Fischer, N.H., 1997. Revision of the structure of dunnianin and related sesquiterpene lactones from *Illicium* species. Journal of Natural Products 60, 783–787.

Schmidt, T.J., 1998. New sesquiterpene lactones from *Illicium floridanum*. Journal of Natural Products 61, 230–236.

Schmidt, T.J., Schmidt, H.M., Müller, E., Peters, W., Fronczek, F.R., Truesdale, A., Fischer, N.H., 1999. Novel *seco*-prezizaane sesquiterpenes from North America *Illicium* species. Journal of Natural Products 62, 684–687.

Wang, J.-L., Yang, C.-S., Yan, R.-N., Yao, B., Yang, X.-B., 1994. Chemical studies on the fruits of *Illicium miwanense*. Acta Pharmaceutical Sinica 29, 693–696.