New Pseudoanisatin-like Sesquiterpene Lactones from the Bark of *Illicium dunnianum*

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Three new sesquiterpene lactones, 6-deoxypseudoanisatin (**4**), dunnianin (**5**), and 6-deoxydunnianin (**6**), have been isolated from the bark of *Illicium dunnianum*. The structure elucidation of these constituents is based on a detailed study of their high-field ^1H and ^{13}C n.m.r. spectra in comparison with those of pseudoanisatin (**1**). Two-dimensional homonuclear and heteronuclear correlation experiments were extensively used in the assignment of the n.m.r. spectra.

Anisatin (**2**)[1-3] and neoanisatin (**3**)[4] are well known as the convulsive constituents of *Illicium anisatum* (Illiciaceae), which is the sole species of this genus in Japan, and from which the non-toxic sesquiterpene pseudoanisatin (**1**) has also been isolated.[1,5] In continuation of our interest in *Illicium* plants,[5] we have made a detailed examination of the constituents of the bark of *I. dunnianum*, and in this paper we report the structures of three new compounds. This plant is distributed mainly in southern China, and is regarded as a toxic plant.

6-Deoxypseudoanisatin (**4**) was obtained as colourless prisms, m.p. 234—235 °C. The e.i. (electron impact) mass spectrum and elemental analysis indicated its molecular formula as $C_{15}H_{22}O_5$. The i.r. spectrum demonstrated the presence of ε-lactone ($\nu_{max.}$ 1 731 cm^{-1}) and ketone ($\nu_{max.}$ 1 702 cm^{-1}) carbonyls. Both ^{13}C and ^1H n.m.r. spectra showed strong resemblances to those of pseudoanisatin (**1**) as shown in Tables 1 and 2. Comparative analysis leads to the proposed structure (**4**).

The carbon and proton resonances of compound (**4**) correspond to those of pseudoanisatin (**1**), except for the methine signal at δ_C 48.8, the doublet methyl signal at δ_H 1.37, and the quartet methine signal at δ_H 3.21. As a long-range coupling, which was observed in the ^1H n.m.r. spectrum of (**1**), was also observed between 8-H_b and 10-H_b of (**4**), compound (**4**) should have the same comformation as that of compound (**1**). Moreover, the shape of signal at δ_H 4.85 (m, 1 H) of compound (**4**), assigned to 3-H, is closely similar to that of compound (**1**) (δ_H 4.80), which suggested an identical configuration at C-3. Compound (**4**) gave a monoacetate (**7**), m.p. 259—261 °C, whereas compound (**1**) forms a diacetate on acetylation.

All these findings indicated the structure of 6-deoxy-pseudoanisatin (**4**) for the new compound. The n.O.e. spectrum of the acetate (**7**) with irradiation at δ_H 3.85 (14-H_a) showed enhancements of the methyl signals at δ_H 1.16 (8%) and δ_H 1.09 (10%), thus indicating the β-configuration for C-6.

Dunnianin (**5**) was isolated as colourless needles, m.p. 245—246 °C, with the molecular formula $C_{22}H_{28}O_7$ allocated by the elemental analysis and supported by carbon and proton counts in the n.m.r. spectra. The e.i. m.s. showed an ($M^+ - H_2O$) ion at *m/z* 386, and a debenzoyl ion peak at *m/z* 282 [(M^+ − OH) − 105]. The benzoyl moiety in compound (**5**) was also confirmed by the signals of the ^{13}C n.m.r. spectrum [δ_C 166.6, 131.3, 129.9 (2-C), 128.8 (2-C), and 133.1].

The ^1H n.m.r. spectrum showed signals of two tertiary methyl signals at δ_H (1.84, 1.90, a doublet methyl signal at δ_H 0.94, and two sets for the isolated methylene groups (10- and 14-H_2) as shown in Table 3. All these data indicated the pseudoanisatin-like core, which was in accord with the ^{13}C n.m.r. spectrum of compound (**5**) although it lacks the ketone group at C-7. The assignment of the ^{13}C n.m.r. spectrum is based on the results obtained from the ^{13}C–^1H 2D (2-dimensional) correlation experiments.

The proton connectivity 15-H_3–1-H–2-H_2–3-H was established by the ^1H–^1H 2D correlation spectrum, which also exhibited the presence of a hydroxy group at C-7 [δ(7-H) 4.37, dd, *J* 3.3 and 2.0 Hz]. The configuration of C-7 was deduced as 7β-OH by the small coupling constants between 7-H and 8-$H_{a,b}$. Thus, compound (**5**) was considered as a benzoyl derivative of 7β-hydroxy-7-deoxopseudoanisatin. The long-range ^{13}C–^1H 2D correlation spectrum of compound (**5**) supported some proton and carbon connectivities for the pseudoanisatin structure, whose results are summarised in the Figure.

Dunnianin (**5**) gave an acetate (**8**) upon acetylation with acetic anhydride and DMAP (4-dimethylaminopyridine). Compound (**8**) was revealed to be a diacetate by the ^1H n.m.r. and e.i. m.s. As the ^1H n.m.r. of compound (**8**) showed the 7-H resonance shifted downfield by Δδ 2.25 p.p.m. compared with that of compound (**5**), the two acetoxy groups should be located

Table 1. ^{13}C N.m.r. data (δ_C/p.p.m. for [$^2H_5$]pyridine solutions) of compounds (**1**), (**4**), (**5**), and (**6**)

Carbon	(1)[a]	(4)[a]	(5)[a]	(6)
1	40.2d	40.8d	40.0d	40.0d
2	43.1t	43.3t	41.8t	42.0t
3	78.2d	78.5d	81.1d	81.1d
4	84.7s	83.1s	82.7s	81.3s
5	47.7s	47.9s	48.3s	46.6s
6	79.3s	48.8d	78.4s	41.7d
7	206.5s	209.5s	77.1d	72.3d
8	43.8t	47.1t	32.3t	36.7t[b]
9	48.8s	48.1s	46.3s	45.6s
10	35.2t	35.2t	36.3t	36.8t[b]
11	174.3s	174.2s	171.4s	171.1s
12	18.4q	18.0q	24.0q	21.8q
13	13.8q	8.2q	15.9q	15.0q
14	69.6t	69.5t	66.8t	67.8t
15	13.9q	13.9q	14.9q	13.7q
C=O			166.6s	166.8s
1′			131.3s	131.7s
2′,6′			129.9d	130.1d
3′,5′			128.8d	128.8d
4′			113.1d	133.0d

[a] Assignments were made by ^{13}C–^1H and long-range ^{13}C–^1H 2D correlation. [b] Assignments may be interchanged.

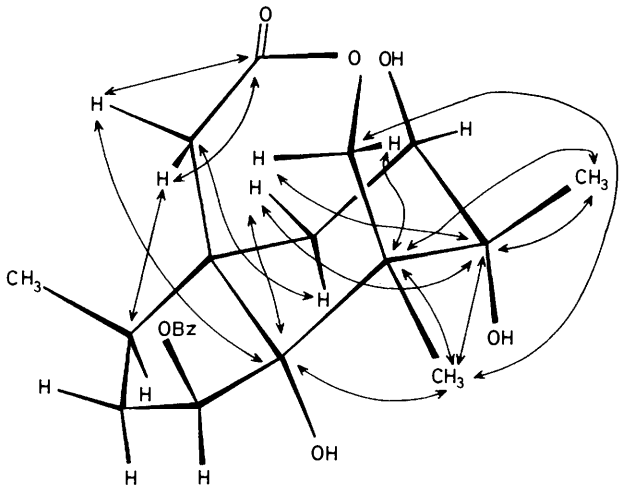


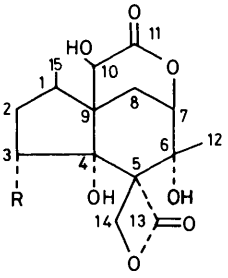
Figure. Long-range ^{13}C–^{1}H connectivity pattern for dunnianin (**5**)

Table 2. ^{1}H N.m.r. data (δ_H for [$^{2}H_5$]pyridine solutions) of compounds (**1**) and (**4**)

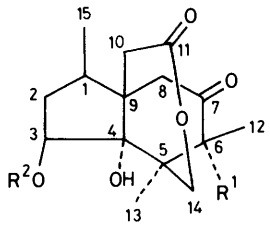
Proton	(**1**)	(**4**)
1	2.6—2.7m	2.75—2.8m
2a	1.50ddd (*J* 9.5, 6.6, 2.6)	1.55ddd (*J* 10.0, 8.1, 3.6)
2b	2.7—2.8m	2.65—2.75m
3	4.80m	4.85m
6		3.21q (*J* 7.0)
8a	2.79d (*J* 16.1)	2.82d (*J* 16.2)
8b	3.23dd (*J* 16.1, 1.8)	2.91dd (*J* 16.2, 1.8)
10a	2.74d (*J* 14.7)	2.69d (*J* 14.3)
10b	3.88dd (*J* 14.7, 1.8)	3.84dd (*J* 14.3, 1.8)
12	1.75s	1.37d (*J* 7.0)
13	1.64s	1.48s
14a	3.97d (*J* 13.2)	3.95d (*J* 13.2)
14b	6.03d (*J* 13.2)	5.82d (*J* 13.2)
15	0.88d (*J* 6.6)	0.88d (*J* 6.6)

Table 3. ^{1}H N.m.r. data (δ_H for [$^{2}H_5$]pyridine solutions) of compounds (**5**), (**8**), and (**6**)

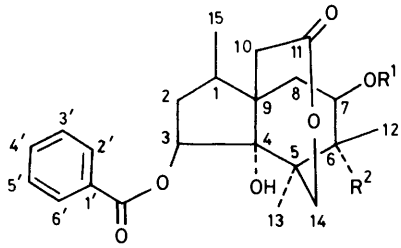
Proton	(**5**)	(**8**)	(**6**)
1	2.58qdd (*J* 7.0, 10.5, 5.5)	2.39qdd (*J* 7.3, 10.3, 5.5)	2.68qdd (*J* 6.9, 10.6, 5.9)
2a	2.79ddd (*J* 13.9, 10.5, 5.1)	2.71ddd (*J* 14.3, 10.3, 5.5)	2.82ddd (*J* 14.3, 10.6, 5.1)
2b	1.42dd (*J*13.9, 5.5)	1.37dd (*J* 14.3, 5.5)	1.51dd (*J* 14.3, 5.9)
3	4.85d (*J* 5.1)	4.81d (*J* 5.5)	5.03d (*J* 5.1)
6			2.64qd (*J* 7.3, 2.0)
7	4.37dd (*J* 3.3, 2.0)	6.62dd (*J* 3.6, 2.6)	4.21dd (*J* 3.5, 2.0)
8a	1.90br dd (*J* 14.3, 2.0)	1.79ddd (*J* 15.4, 2.6, 2.0)	1.94br dd (*J* 13.6, 2.0)
8b	2.39dd (*J* 14.3, 3.3)	2.08dd (*J* 15.4, 3.6)	2.07dd (*J* 13.6, 3.5)
10a	2.95d (*J* 21.5)	2.89d (*J* 20.5)	2.95d (*J* 20.5)
10b	4.72 br d (*J* 21.5)	3.73dd (*J* 20.5, 2.0)	4.76br d (*J* 20.5)
12	1.90s	1.89s	1.42 d (*J* 7.3)
13	1.84s	1.72s	1.64s
14a	5.45d (*J* 11.7)	5.21 d (*J* 11.4)	5.36d (*J* 12.7)
14b	5.48d (*J* 11.7)	5.30d (*J* 11.4)	5.42d (*J* 12.7)
15	0.94d (*J* 7.0)	0.88d (*J* 7.3)	0.97d (*J* 6.9)
2′,6′	8.36d (*J* 8.0)	8.25d (*J* 7.7)	8.40d (*J* 8.0)
3′,5′	7.45t (*J* 8.0)	7.48t (*J* 7.7)	7.41t (*J* 8.0)
4′	7.53t (*J* 8.0)	7.58t (*J* 7.7)	7.48t (*J* 8.0)
OH	6.97br s 8.04br s		6.41br s 6.74br d (*J* 3.0)
OAc		2.12s 2.06s	



(**2**) R = OH
(**3**) R = H



(**1**) R^1 = OH, R^2 = H
(**4**) R^1 = R^2 = H
(**7**) R^1 = H, R^2 = Ac



(**5**) R^1 = H, R^2 = OH
(**8**) R^1 = Ac, R^2 = OAc
(**6**) R^1 = R^2 = H
(**9**) R^1 = Ac, R^2 = H

Table 4. ^{1}H N.m.r. data (δ_H for $CDCl_3$ solutions) of compounds (**6**) and (**9**)

Proton	(**6**)	(**9**)
1	2.37qdd (*J* 7.0, 11.5, 5.9)	2.41qdd (*J* 7.3, 10.6, 5.9)
2a	2.61ddd (*J* 14.7, 11.6, 5.1)	2.63ddd (*J* 14.7, 10.6, 5.7)
2b	1.47dd (*J* 14.7, 5.9)	1.49dd (*J* 14.7, 5.9)
3	4.57d (*J* 5.1)	4.59d (*J* 5.7)
6	2.26qd (*J* 7.3, 2.9)	2.42qd (*J* 7.3, 2.9)
7	4.01br d (*J* 2.9)	5.18dd (*J* 3.7, 2.9)
8a	1.78d (*J* 2.9)	1.89dd (*J* 15.4, 3.7)
8b	1.78d (*J* 2.9)	1.79dd (*J* 15.4, 1.8)
10a	2.69d (*J* 20.5)	2.69d (*J* 20.1)
10b	4.01dd (*J* 20.5, 1.8)	3.45dd (*J* 20.1, 1.8)
12	1.21d (*J* 7.3)	1.10d (*J* 7.3)
13	1.31s	1.35s
14a	4.88d (*J* 12.6)	4.92d (*J* 12.5)
14b	4.62d (*J* 12.6)	4.54d (*J* 12.5)
15	1.01d (*J* 7.0)	1.00d (*J* 7.3)
2′,6′	8.07d (*J* 7.3)	8.06d (*J* 7.3)
3′,5′	7.43t (*J* 7.3)	7.45t (*J* 7.3)
4′	7.54t (*J* 7.3)	7.56t (*J* 7.3)

at C-6 and C-7. In the case of forced acetylation of compounds (**1**) and (**2**), the 6-*O*-acetyl compound was reported.[5,6] Therefore, the benzoyl group should be at C-3.

6-Deoxydunnianin (**6**) was obtained as colourless needles, m.p. 222—223 °C. The elemental analysis and e.i. m.s. indicated the molecular formula as $C_{22}H_{28}O_6$, one oxygen atom less than that of dunnianin (**5**). The ^{1}H n.m.r. spectrum of compound (**6**) showed a singlet methyl signal at δ_H 1.64, and two doublet methyl signals at δ_H 0.97 and 1.42. This evidence was in agreement with the ^{13}C n.m.r. spectrum, which closely

resembles that of dunnianin (**5**). The differences from compound (**5**) were similar to those between compounds (**1**) and (**4**); *i.e.* a doublet methyl signal (12-H_3) at δ_H 1.42 was coupled with the methine signal (6-H) at δ_H 2.64, which was also coupled with 7-H (δ_H 4.21), as revealed by the ^1H–^1H 2D correlation spectrum. The doublet carbon signal at δ_C 41.7 (C-6) for (**6**) was seen instead of the singlet ^{13}C signal (δ_C 78.4) of a carbon attached to oxygen in dunnianin (**5**).

Acetylation of compound (**6**) with acetic anhydride and DMAP yielded the monoacetate (**9**). The 7-H signal in the ^1H n.m.r. spectrum of (**9**) (Table 4) was shifted downfield by $\Delta\delta_H$ 1.17 p.p.m. (in $CDCl_3$) compared with that of compound (**6**). Thus, we conclude that compound (**6**) is the 6-deoxy derivative of dunnianin. The configuration of C-6 was established as 6β-Me by the n.O.e. enhancement of the signal at δ 5.36 ([$^2H_5$]-pyridine) (14-H_a) (8%) upon irradiation at δ 1.42 (12-H_3).

Pseudoanisatin (**1**) was not obtained from *I. dunnianum*, and it is notable that none of the anisatin-like sesquiterpenes was obtained from the bark of *I. dunnianum*. The toxicity of 6-deoxypseudoanisatin (**4**) will be reported elsewhere, but the other products have not yet been examined because of the limited quantity available.

Experimental

M.p.s were determined on a Yanagimoto hot-stage apparatus and are uncorrected. I.r. spectra were recorded for KBr discs on a JASCO IR-810 spectrometer. Mass spectra were taken on a JEOL JMS-DX-303 spectrometer.

N.m.r. spectra were recorded on a JEOL GX-400 spectrometer operating at 399.65 MHz for ^1H and 100.40 MHz for ^{13}C nuclei. N.O.e. and 2-dimensional experiments were performed on the same apparatus. Chemical shifts are reported in p.p.m. relative to tetramethylsilane as internal standard.

Optical rotations were taken with a JASCO DIP-181 spectrometer. Merck silica gel 60 (particle size 0.063—0.200 nm) was used for column chromatography.

Extraction and Isolation.—The bark (1.19 kg) of *I. dunnianum* was extracted at ambient temperature with methanol (6 l). The extract was evaporated to give a brown gum (180 g), which was dissolved in water and partitioned between EtOAc and water. The EtOAc-soluble part was subjected to counter-current distribution using the solvent system of EtOAc–water (1:1) to give fractions of 1—68 (each 20 ml). The residue from fractions 41—55 was chromatographed on silica gel [$CHCl_3$–MeOH (97:3)] to give 6-deoxypseudoanisatin (**4**) (500 mg). The residue from fractions 21—40 was chromatographed on silica gel to give dunnianin (**5**) (37 mg) and 6-deoxydunnianin (**6**) (40 mg).

6-*Deoxypseudoanisatin* (**4**).—*Prisms*, m.p. 234—235 °C (from EtOAc) (Found: C, 63.9; H, 7.7. $C_{15}H_{22}O_5$ requires C, 63.81; H, 7.85%); $[\alpha]_D^{16}$ +16° (*c* 2 in EtOH); $\nu_{max.}$ 3 500, 3 440 (OH), 1 731, and 1 702 cm^{-1} (CO); *m/z* 282 (20%, M^+).

Acetylation of Compound (**4**).—Compound (**4**) (15 mg) was dissolved in a mixture of dry pyridine (1 ml) and Ac_2O (1 ml) and the solution was left overnight at room temperature, then poured onto ice, and the precipitate was collected and purified by silica gel chromatography to give the monoacetate (**7**) as needles (16 mg), m.p. 259—261 °C; *m/z* 324 (15%, M^+); $\delta_H(CDCl_3)$ 2.55 (1 H, m, 1-H), 1.23 and 2.81 (2 × 1 H, m, 2-H_2), 5.17 (1 H, dd, *J* 7.7 and 2.9 Hz, 3-H), 2.85 (1 H, q, *J* 5.8 Hz, 6-H), 2.52 (1 H, d, *J* 16.5 Hz, 8-H_a), 2.63 (1 H, br d, *J* 16.5 Hz, 8-H_b), 2.53 (1 H, d, *J* 15.5 Hz, 10-H_a), 2.98 (1 H, br d, *J* 15.5 Hz, 10-H_b), 1.16 (3 H, d, *J* 5.8 Hz, 12-H_3), 1.09 (3 H, s, 13-H_3), 3.85 and 4.92 (2 × 1 H, d, *J* 13.6 Hz, 14-H_a and 14-H_b), and 0.96 (3 H, d, *J* 7.0 Hz, 15-H_3).

Dunnianin (**5**).—*Needles*, m.p. 245—246 °C (from $CHCl_3$) (Found: C, 65.6; H, 6.8. $C_{22}H_{28}O_7$ requires C, 65.33; H, 6.98%); $[\alpha]_D^{24}$ +61° (*c* 0.1 in dioxane); $\nu_{max.}$ 3 510 (OH), 1 710 (CO), 1 455, and 720 cm^{-1} (benzene); *m/z* 386 ($M^+ - H_2O$) and 282 ($M^+ - OH - 105$).

Acetylation of Dunnianin (**5**).—Dunnianin (**5**) (2 mg) was dissolved in Ac_2O (1 ml) and DMAP (5 mg) was added. After 2 days at 70 °C, the solution was evaporated under reduced pressure, and the residue was purified by silica gel chromatography to afford the diacetate (**8**) as an oil (2 mg); *m/z* 488 (5%, M^+) and 446 ($M^+ - 42$).

6-*Deoxydunnianin* (**6**).—*Needles*, m.p. 222—223 °C (from hexane–$CHCl_3$) (Found: C, 67.8; H, 7.2. $C_{22}H_{28}O_6$ requires C, 68.02; H, 7.27%); $[\alpha]_D^{24}$ +34° (*c* 0.1 in dioxane); $\nu_{max.}$ 3 500 (OH), 1 710, 1 700 (CO), 1 455, and 710 cm^{-1} (benzene); *m/z* 388 (5%, M^+) and 266 ($M^+ - OH - 105$).

Acetylation of 6-*Deoxydunnianin* (**6**).—6-Deoxydunnianin (**6**) (3 mg) was treated under the same conditions as for the acetylation of dunnianin (**5**) and gave the monoacetate (**9**) as an oil (2 mg); *m/z* 430 (10%, M^+) and 388 ($M^+ - 42$).

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References

1 J. F. Lane, W. T. Koch, N. S. Leeds, and G. Gorin, *J. Am. Chem. Soc.*, 1952, **74**, 3211.
2 Y. Yamada, S. Takada, S. Nakamura, and Y. Hirata, *Tetrahedron Lett.*, 1965, 4797.
3 K. Yamada, S. Takada, S. Nakamura, and Y. Hirata, *J. Chem. Soc. Jpn.*, 1967, **88**, 653.
4 S. Takada, S. Nakamura, K. Yamada, and Y. Hirata, *Tetrahedron Lett.*, 1966, 4739.
5 I. Kouno, H. Irie, and N. Kawano, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2511.
6 K. Yamada, S. Takada, S. Nakamura, and Y. Hirata, *Tetrahedron*, 1968, **24**, 1267.

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