Revision of the Structure of Dunnianin and Related Sesquiterpene Lactones from *Illicium* Species

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The sesquiterpene lactones dunnianin (**1**), debenzoyldunnianin (**2**), 7-deoxy-7-oxodunnianin (**3**), and pseudoanisatin (**4**) were isolated from fruits of *Illicium floridanum*. On the basis of the molecular structure of **4**, **1**−**3** had previously been assigned structures containing 11,14-ε-lactone rings (**1a**−**3a**). Due to inconsistencies in the ^{1}H-NMR spectra of **1**−**3**, when compared with the spectrum of **4**, their structures were reinvestigated by NMR spectroscopic analyses, and the molecular structure of **1** was determined by single-crystal X-ray diffraction. Compounds **1**−**3** were found to contain an 11,3-δ-lactone ring instead of the previously reported 11,14-ε-lactone, which required revision of their structures from **1a**−**3a** to **1b**−**3b**, respectively.

Members of the genus *Illicium* (Illiciaceae), produce a number of structurally unique sesquiterpene lactones (STL).[1−6] One subgroup of these compounds, represented by the unusual β-lactone anisatin, is reported to be strongly neurotoxic with a noncompetitive GABA antagonistic mechanism of action.[7] Besides the β-lactones, a number of STL with the same carbon skeleton but without a β-lactone moiety, for example, pseudoanisatin (**4**), have been isolated from the genus *Illicium*.[2−6] Compounds of the pseudoanisatin type have in the past received little attention from a pharmacological point of view except for their nontoxic properties in comparison with the strong toxin anisatin. Recent studies indicated that such compounds might be of interest due to neurotrophic activities.[6]

In search for a new source of such STL to be used for investigations of their possible pharmacological effects, we have recently initiated a chemical study of the North American species *I. floridanum* Ellis. This Louisiana native evergreen shrub with the common names Star Bush or American Star Anise had previously not been chemically analyzed.

Results and Discussion

From the fruits of *I. floridanum*, the known sesquiterpene lactones dunnianin (**1**, Scheme 1) and two derivatives, the nonesterified lactone **2** and the 7-oxo derivative **3**, as well as pseudoanisatin (**4**), were isolated, all of which had previously been obtained from other *Illicium* species.[2−5] The spectroscopic and physical data (^{1}H- and ^{13}C-NMR, MS, [α], mp) of **1**−**4** were in full agreement with data reported in the literature.[2−5]

The isolation of dunnianin (**1**) was first reported by Kouno *et al.*[2] It had been assigned structure **1a** on the basis of NMR spectroscopic data and by comparison with spectral data of pseudoanisatin **4**,[2] the structure of which had been unambiguously established by X-ray crystallography.[3] Accordingly, dunnianin should be identical with 7αH-pseudoanisatin 3-*O*-benzoate. Lac-

Scheme 1. Structures of Sesquiterpene Lactones **1**−**4**. The Correct Structures of **1**−**3** Are Represented by Formulas **1b**, **2b**, and **3b**.

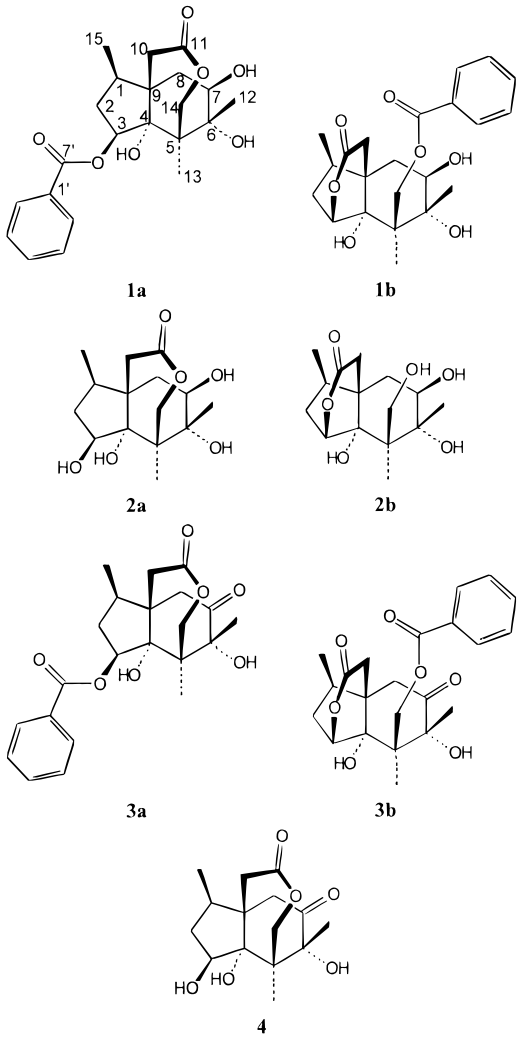

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Table 1. ^{1}H-NMR Data of Compounds **1**−**4** (500 MHz, TMS; Signal Assignments Confirmed by COSY and HMQC Experiments)

	1[a]			2[a]			3[b]			4[c]		
H-	δ	mult	J (Hz)	δ	mult	J	δ	mult	J	δ	mult	J
1	2.585	m		2.596	m		2.67	m		2.647	m	
2α	2.786	ddd	5.1, 10.7, 13.9	2.764	ddd	6.6, 10.1, 13.5	2.67	m		2.75	*	
2β	1.431	dd	5.8, 13.9	1.528	dd	8.2, 13.5	1.42	*		1.510	ddd	2.8, 10.1, 13.7
3	4.846	d	5.1	4.603	d	6.6	4.517	d	4.7	4.810	dd	2.8, 7.7
7	4.355	m		4.560	m							
8a	2.402	dd	3.5, 14.2	2.652	d (br)	14.5	3.276	d	13.2	3.198	dd	1.6, 16.1
8b	1.898	dd	1.9, 14.2	1.794	dd	3.5, 14.5	2.164	d	13.2	2.759	d	16.1
10a	4.722	dd	1.9, 20.2	4.211	dd	1.9, 19.6	2.823	dd	1.0, 20.2	3.842	dd	1.6, 14.8
10b	2.962	d	20.2	2.901	d	19.6	2.624	d	20.2	2.717	d	14.8
12	1.889	s (3H)		1.850	s (3H)		1.413	s (3H)		1.729	s (3H)	
13	1.838	s (3H)		1.566	s (3H)		1.493	s (3H)		1.634	s (3H)	
14a	4.500	d	11.9	4.492	d	12.0	4.464	d	12.9	5.988	d	13.2
14b	4.469	d	11.9	4.045	d	12.0	4.350	d	12.9	3.962	d	13.2
15	0.965	d (3H)	6.9	0.948	d (3H)	6.9	0.973	d (3H)	6.3	0.879	d (3H)	7.6
2′/6′	8.365	dd (2H)	1.9, 8.8				7.923	dd (2H)	1.3, 8.2			
3′/5′	7.437	t (2H)	7.6				7.458	t (2H)	7.9			
4′	7.509	tt	1.9, 8.8				7.568	tt	1.3, 7.6			

[a] Recorded in pyridine-d_5. [b] Recorded in $CDCl_3$. [c] Recorded in pyridine-d_5 with 5% D_2O. * Multiplicity not determined because of signal overlap.

tone **2** and the most recently isolated **3** were assigned pseudoanisatin-like structures **2a** and **3a** with an 11,-14-ε-lactone ring.[4,5]

We found that the ^{1}H-NMR data of dunnianin (**1**) and its congeners **2** and **3** differ conspicuously in several respects from those expected for 11,14-ε-lactones of the pseudoanisatin type. In order to allow for a detailed comparison, the ^{1}H-NMR data of **1**−**4** are listed in Table 1. The geminal coupling constant of H-10a/H10b is in the range of 20−21 Hz, a value significantly larger than in **4** ($^2J_{10a,10b} = 15$ Hz). Moreover, in lactones **1**−**3** large differences in the chemical shift of H-14a/H-14b are observed, when compared with the values of the corresponding protons in **4**. The most significant discrepancy, however, is manifested in the coupling patterns of the cyclopentane ring protons, indicating a different geometry in this region of the molecule in **1**−**3**, when compared to **4**. In the ^{1}H-NMR spectra of **1**−**3**, H-3 appears as a sharp doublet with $^3J_{3,2\alpha} = 5.1$ Hz (**1**), 6.6 Hz (**2**), and 4.7 Hz (**3**), while $^3J_{3,2\beta} \approx 0$ in all three compounds. In **4**, determination of these coupling constants is more difficult due to line broadening of H-3, obviously caused by coupling with the OH-proton. Moreover, the signal for H-2α co-resonates with H-1 so that unambiguous measurement of the $^3J_{3,2}$ coupling constants is not possible. However, after H/D-exchange with D_2O, the H-3 signal of **4** appeared as a sharp doublet of a doublet with coupling constants of 2.8 and 7.7 Hz. The same coupling behavior could be expected for H-3 of **2a**, while for **1a** and **3a**, similar coupling constants would be expected, except that sharper spectral lines would occur due to the esterification.

The above spectral discrepancies led to a re-analysis of the structures of **1a**−**3a**. Although long-range C/H-correlation NMR data were reported for dunnianin and some of its derivatives,[2,6] none of these data provided unambiguous evidence for the lactonic ring closure, which might have an alternative structure with an 11,3-lactone ring as represented by structures **1b**−**3b**.

Calculations of the $^3J_{2,3}$ coupling constants in molecular models of **2** with an 11,14-ε-lactone (**2a**) and the alternative 11,3-δ-lactone (**2b**) consistently yielded values that were in agreement with the experimental data for **2b**, while those of **2a** were closer to the experimental data of **4** (see Table 2).

Table 2. Experimental $^3J_{H,H}$-coupling Constants (Hz) of the Cyclopentane Ring Protons in **1**−**4** and Theoretical Values Calculated for AM1-Minimized Computer Models of Compound **2** as an 11,14-ε-Lactone (**2a**) and as an 11,3-δ-Lactone (**2b**)

$^3J_{H,H}$	**1**	**2**	**3**	**4**	**2a** (theor.)	**2b** (theor.)
1,2α	10.7	10.1	n.a.[a]	n.a.	7.5	9.9
1,2β	5.8	8.2	n.a.	10.1	10.3	7.2
2α,3	5.1	6.6	4.7	7.7	9.7	6.6
2β,3	<1	<1	<1	2.8	3.5	1.2

[a] n.a. = not accessible.

To prove this mode of lactone ring closure, the 2D-HMBC spectra **2** and **3** were recorded (Figure 1). As expected for both compounds, correlations were observed between H-3 and the lactone carbonyl C-11. Furthermore, a correlation of the C-14 methylene protons and the ester carbonyl C-7′ was observed in **3**. Figure 2 shows some of the HMBC correlations important for the structural assignments.

We therefore determined the molecular structure of dunnianin (**1**), which crystallized from EtOAc as colorless monoclinic prisms (space group $P2_1$) by single crystal X-ray diffraction analysis. The molecular structure of **1** is depicted in Figure 3, and the final atomic coordinates are given in Table 3.

As predicted from the magnitude of the ^{1}H-NMR coupling constants outlined above, the lactonic ring closure is indeed between C-11 and C-3, and the benzoyl ester group is attached to C-14−O. Due to the different mode of lactone ring closure, the conformation of the cyclopentane ring differs from that of **4**, in which this ring adopts a pure envelope geometry with C-9 as the out-of-plane carbon.[3] In **1**, this ring forms a somewhat distorted envelope with C-4 being out of plane. Thus, the dihedral angles between H-3 and H-2β/H-2α are 96 and −27°, respectively, which is in very good agreement with the observed ^{1}H-NMR coupling constants.

The crystal framework is constituted by two intermolecular H bonds, one between the lactone carbonyl and the OH at C-6 of another molecule [O-4···O-2 2.789-(2) Å], the other between the proton of OH at C-7 and the oxygen of the OH at C-3 of another molecule [O-5···O-3 2.719(2) Å]. Additionally, a strong intramolecular H bond exists between the two OH groups at C-4 and C-6, the first being the proton donor (O-3···O-4 2.549(2) Å]. The orientation of the phenyl ring of the

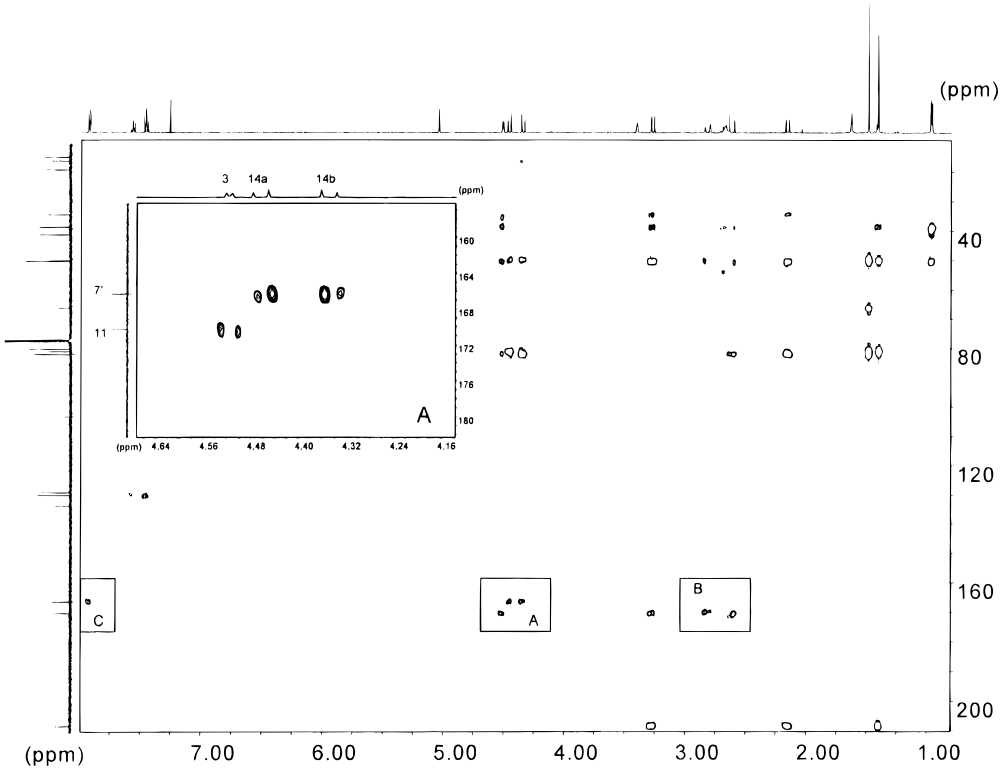

Figure 1. HMBC-spectrum of compd **3** in $CDCl_3$. (For assignment of the marked correlations see Figure 2).

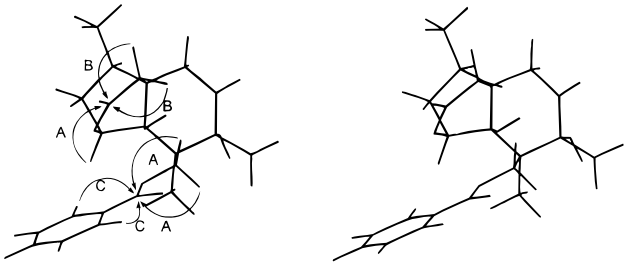

Figure 2. Stereoscopic representation of a molecular model of compound **3** showing the HMBC-correlations crucial for structure assignment as marked in Figure 1.

benzoyl ester group deviates slightly from a coplanar geometry (ω O-6/C-16/C-17/C-18 −19.5(3)°), which indicates that hydrophobic interactions between the phenyl rings also contribute to the stability of the crystal structure.

The above experiments provided unambiguous proof for the presence of 11,3-δ-lactone ring closures in compounds **1**−**3** so that their structures require revision from **1a**−**3a**[2,4,5] to **1b**−**3b**.

In conclusion, the occurrence of the very large coupling constant $^2J_{10,10'}$ near 20 Hz, on the one hand, and the appearance of H-3 as a doublet with essentially no coupling with H-2β, on the other, are diagnostic of the presence of a 3,11-δ-lactone moiety within this group of sesquiterpenes. Because the same spectral characteristics are exhibited by another dunnianin-type sesquiterpene lactone, isodunnianin, for which a neurotrophic activity has been reported,[6] isodunnianin most likely contains an analogous 3,11-δ-lactone ring representing the 14-*O*-acetyl-7-*O*-benzoyl derivative of **2b**.

Pharmacological activity studies of these compounds and a search for other constituents from the leaves and the fruits of *I. floridanum* are in progress.

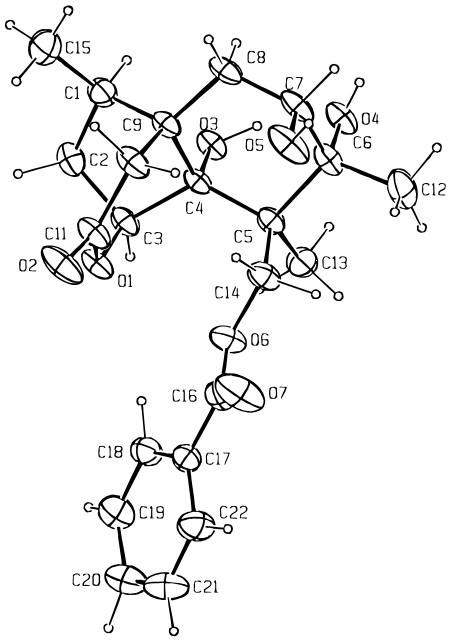

Figure 3. The molecular strucure of dunnianin (**1**).

Experimental Section

General Experimental Procedures. Optical rotation was measured with an IBZ Polar Monitor polarimeter at room temperature. Melting points (uncorr) were determined with a Leitz microscope type 350. Mass spectra were recorded in the direct inlet mode using chemical ionization with NH_3 as reactant gas [DCIMS-(NH_3)] on a Finnigan MAT INCOS 50 mass spectrometer. NMR spectra were recorded with a Bruker DRX 500 spectrometer at room temperature at 500.13 MHz with TMS as internal standard. Spectra of **1**, **2**, and **4** were obtained in pyridine-d_5, while those of **3** were obtained in $CDCl_3$. Gradient-selected Heteronuclear

Table 3. Table of Atomic Coordinates and Their Estimated Standard Deviations for the X-ray Structure of Compound **1**

atom	*x*	*y*	*z*	B_{eq}(Å^2)[a]
O1	0.1530(2)	0	0.18015(8)	3.98(3)
O2	0.3909(2)	0.1371(2)	0.1462(1)	6.20(4)
O3	0.0187(1)	−0.4066(1)	0.16421(7)	3.11(2)
O4	0.2571(2)	−0.6024(1)	0.22143(8)	4.00(3)
O5	0.6542(2)	−0.3507(2)	0.1904(1)	5.46(3)
O6	0.2795(2)	−0.0923(2)	0.32932(8)	4.34(3)
O7	0.5332(2)	0.0371(2)	0.3720(1)	7.46(5)
C1	0.1128(3)	−0.2535(2)	0.0400(1)	3.96(4)
C2	−0.0295(3)	−0.1503(2)	0.0813(1)	4.17(4)
C3	0.0427(2)	−0.1410(2)	0.1680(1)	3.32(3)
C4	0.1601(2)	−0.2890(2)	0.1791(1)	2.72(3)
C5	0.2703(2)	−0.3341(2)	0.2587(1)	3.14(3)
C6	0.3931(2)	−0.4817(2)	0.2415(1)	3.63(4)
C7	0.5114(2)	−0.4617(2)	0.1700(1)	3.98(4)
C8	0.3971(2)	−0.4176(2)	0.0949(1)	3.78(3)
C9	0.2742(2)	−0.2750(2)	0.1046(1)	3.17(3)
C10	0.3951(2)	−0.1270(2)	0.1087(1)	3.98(4)
C11	0.3122(3)	0.0128(2)	0.1447(1)	4.11(4)
C12	0.5152(3)	−0.5371(3)	0.3124(2)	5.66(5)
C13	0.1300(3)	−0.3762(3)	0.3212(1)	4.14(4)
C14	0.3984(3)	−0.2071(2)	0.2954(1)	3.97(4)
C15	0.1642(4)	−0.1953(3)	−0.0405(1)	6.19(6)
C16	0.3656(3)	0.0239(2)	0.3679(1)	4.13(4)
C17	0.2336(3)	0.1301(2)	0.4038(1)	3.79(4)
C18	0.0466(3)	0.1350(3)	0.3782(1)	4.25(4)
C19	−0.0734(3)	0.2344(3)	0.4144(1)	5.43(5)
C20	−0.0069(4)	0.3285(3)	0.4752(2)	6.23(6)
C21	0.1785(4)	0.3233(3)	0.5009(2)	6.52(6)
C22	0.3002(3)	0.2253(3)	0.4651(1)	5.24(5)
H3O	0.080(3)	0.495(3)	0.177(1)	5.2(5)
H4O	0.310(3)	−0.667(3)	0.201(1)	7.2(6)
H5O	0.765(3)	−0.381(3)	0.182(1)	6.1(5)

[a] $B_{eq} = 8\pi^2/3 \sum_i\sum_j U_{ij} a_i^* a_j^* \mathbf{a_i}\cdot\mathbf{a_j}$.

Multiple Bond Correlation (HMBC)[8] spectra of **2** and **3** were obtained with an inverse multinuclear probehead equipped with actively shielded *z*-gradient coil and a GREAT 1/10 gradient unit. Acquisition parameters were relaxation delay 1.4 s, delay for evolution of long-range coupling 65 ms, delay for creation of antiphase magnetization 3.45 ms. Sinusoidal-shaped field gradients were used with gradient strength ratio 5:3:4. In all, 2048 data points were collected in t_2, with 128 FIDs in t_1. Typical sweep widths were 8−10 ppm in F_2 and 220 ppm in F_1. The spectra were transformed into a 2048 × 512 matrix by zero filling, with both time domains in each data set multiplied by sine-bell functions before Fourier transformation. X-Ray diffraction data[9] for compound **1** were collected on an Enraf-Nonius CAD4 diffractometer equipped with Cu K_α (λ = 1.541 84 Å) radiation and graphite monochromator. Friedel-related data were collected. Data reduction included corrections for background, Lorentz, polarization, and absorption effects. Absorption corrections were based on ψ scans. The structure was solved by direct methods and refined using the MolEN programs.[10] Refinement was by full-matrix least squares, with neutral-atom scattering factors and anomalous dispersion corrections. Weights were $w = 4\ F_o^2[\sigma^2(I) + (0.02F_o^2)^2]^{-1}$. All non-hydrogen atoms were refined anisotropically, while hydrogen atoms were refined isotropically. Crystal data, final *R* values, and other details are included in Table 4. Refinement with the reported (expected) absolute configuration for **1** yielded R = 0.0362, R_w = 0.0429, while the opposite configuration yielded R = 0.0364, Rw = 0.0432.

Molecular models were created using MMX Force field preminimized structures (PCModel 4) that were subsequently minimized using the AM1 method as implemented with MOPAC v. 6.0. Coupling constants were calculated with PCModel 4.

Table 4. Crystal Data and Summary of Intensity Data Collection and Structure Refinement for Compound **1**

formula	$C_{22}H_{28}O_7$
crystal shape	colorless lath
formula weight	404.5
crystal system	monoclinic
space group	$P2_1$
T, °C	26
a, Å	7.1577(9)
b, Å	8.6433(5)
c, Å	16.960(2)
β, °	93.833(9)
cell volume, Å^3	1046.9(3)
Z	2
D_{calc}, g cm^{-3}	1.283
μ_{calc}, cm^{-1}	7.49
radiation	Cu Kα
cryst dimens, mm	0.48 × 0.28 × 0.08
decay of standards	<1%
reflns measured	7088
2θ range, deg	4 − 150
range of h,k,l	8, ±10, ±21
unique data	4271
observed data	3794
criterion for obsd data	$I > 1\sigma(I)$
no. of parameters	374
R	0.036
R_w	0.043
max, final diff. map	0.38 eÅ^{-3}

Plant Material. Fruits of *I. floridanum* Ellis were collected in St. Helena Parish, near Montpelier, LA, in October 1995 (voucher No. Sch-IF-1, Herbarium of the Institut für Pharmazeutische Biologie, Universität Düsseldorf).

Extraction and Isolation. *I. floridanum* fruits (750 g) were dried at ambient temperature, powdered, and extracted ehaustively with CH_2Cl_2 to give 8 g of extract. Column chromatography on 400 g Sephadex LH20/MeOH yielded 6 fractions, A−F. Column chromatography of fraction D (2 g) on 200 g silica with EtOAc−*n*-hexane mixtures of increasing polarity (see below) yielded 32 fractions (6/4: fractions 1−10, 4/6: fractions 8−20, 2/8: fractions 21−27, 0/1: fractions 28−32). **1**−**4** crystallized from fractions 16 (**1**), 25 (**2**), 10 (**3**), and 12 (**4**). They were further purified by washing with hexane to yield 9, 67, 39, and 136 mg of pure compounds **1**−**4**, respectively.

The ^{13}C- and ^{1}H-NMR data of all compounds were identical with literature data[2,4,5] (^{1}H-NMR data are given in Table 1 for easier comparison).

Dunnianin (**1**): colorless prisms (EtOAc); mp 222 °C; $[\alpha]_D$ +32° (*c* 0.08, MeOH); DCIMS(NH_3) *m/z* 422 $[M + NH_4]^+$.

Debenzoyldunnianin (**2**): colorless prisms (EtOAc−*n*-hexane); mp 209 °C; $[\alpha]_D$ −64°(*c* 0.47, MeOH); DCIMS(NH_3) *m/z* 318 $[M + NH_4]^+$.

7-Deoxy-7-oxodunnianin (**3**): colorless needles (EtOAc); mp 197 °C; $[\alpha]_D$ −81°(*c* 1.9, MeOH); DCIMS(NH_3) *m/z* 420 $[M + NH_4]^+$.

Pseudoanisatin (**4**): colorless plates (EtOAc); mp 196 °C; $[\alpha]_D$ +8°(*c* 1.1, MeOH); DCIMS(NH_3) *m/z* 316 $[M + NH_4]^+$.

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