New Sesquiterpene Lactones from *Illicium floridanum*

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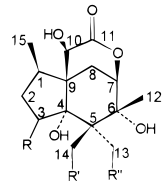
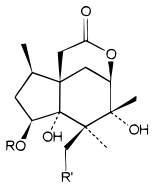
In continuation of our phytochemical investigation of *Illicium floridanum* Ellis (American star anise, star bush), three new sesquiterpene lactones possessing the anisatin−type carbon skeleton (8,9-*seco*-prezizaane skeleton), 14-acetoxy-3-oxofloridanolide (**1**), 13-acetoxy-14-(*n*-butyryloxy)floridanolide (**2**), and 3β-acetoxy-14-*n*-butyryloxy-10-deoxyfloridanolide (**3**), were isolated from fruits of this plant. Their structures were elucidated by 1D and 2D NMR measurements. The molecular structure of **1** was obtained by single crystal X-ray diffraction. The 11,3-δ-lactone structure of the compound previously described as debenzoyldunnianin in our previous communication, on grounds of NMR spectral evidence and X-ray crystallographic analysis is revised to a δ-lactone closed between C-11 and C-7 (compound **4**). The neurotoxic sesquiterpene lactone anisatin (**5**) and its isomer 2α-hydroxyneoanisatin (3-deoxy-2α-hydroxy-anisatin, **6**) were also isolated and identified by spectroscopic means. The presence of the neurotoxin **5** in relatively high amounts in the fruits and leaves confirms and explains early reports on the toxicity of this plant.

Species of the genus *Illicium* L. (Illiciaceae) occurring in eastern Asia are known to accumulate a variety of struturally unusual sesquiterpene lactones (STL),[1−10] some of which are held responsible for the neurotoxicity of most of these plants (e.g., see Kouno et al.[6]). The most prominent compound of this type is anisatin with a β-lactone structure. Anisatin is known to be a potent noncompetitive antagonist at GABA-dependent neurons in mammals and also in invertebrates, where it possesses a picrotoxin-like mechanism of action.[11] Several *Illicium*-STLs, such as pseudoanisatin and dunnianin, possess the same *seco*-prezizaane carbon skeleton as anisatin but lack the β-lactone structure and are reported to be nontoxic as compared with the strongly toxic anisatin. A dunnianin-type STL has recently been shown to possess a neurotrophic activity, as it enhances neurite sprouting in a fetal rat brain cell culture.[10]

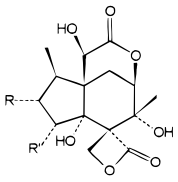
The search for STLs with potential activities on the nervous system and the complete lack of chemical data on species of *Illicium* of the western hemisphere have prompted us to investigate for the first time a North American species, *I. floridanum* Ellis. This reportedly toxic[12,13] species is commonly known as "star bush" or "American star anise" and occurs as an evergreen shrub in the southeastern United States from the Florida panhandle west to eastern Louisiana and southern Mississippi.[14] In a previous communication,[1] we reported on the isolation and structure analysis of several STLs from the fruits of *I. floridanum*, (compounds **4** and **7**−**9** in the present communication). The present work gives the results of our search for further STLs in the fruits of this species. Moreover, due to the fact that leaves of *Illicium* species have not been studied with respect to their chemical constituents to date, we extended our search for such metabolites to the leaves of *I. floridanum*.



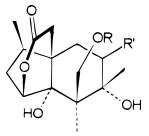
	1	**2**
R	=O	H
R'	O-Ac	O-*n*Bu
R''	H	O-Ac



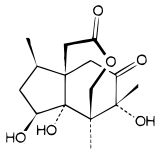
	3	**4**
R	Ac	H
R'	O-*n*Bu	OH



	5	**6**
R	H	OH
R'	OH	H



	7	**8**
R	O-Bz	O-Bz
R'	β-OH	=O



9

Results and Discussion

In addition to lactones **4** and **7**−**9**, reported in our previous communication,[1] three hitherto unknown sesquiterpene lactones (**1**−**3**) were isolated as pure substances from the CH_2Cl_2 extract of *I. floridanum* fruits. Compound **1** was found to possess a molecular mass of 356 {DCI(NH_3)−MS: [M + NH_4^+] at *m*/*z* 374}. Its ^{13}C-NMR spectrum (Table 1; data in $CDCl_3$ are referred to in the text; for data in pyridine-d_5, see Experimental Section) displayed 17 carbon signals, which by DEPT 135 were assigned to the resonances of seven quaternary, three CH, three CH_2, and four CH_3 carbon atoms, allowing for calculation of the molecular formula, $C_{17}H_{24}O_8$. An ester carbonyl and one of the methyls (δ

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Table 1. ^{13}C-NMR Data of Compounds **1**, **2**, and **3** (125.77 MHz, $CDCl_3$, TMS)[a]

C	**1**[b,c] δ (ppm)	**2**[c] δ (ppm)	**3** δ (ppm)
1	35.0	38.5	37.2
2	43.1	30.0	40.0
3	209.2	33.4	77.7
4	82.6	88.4	86.5
5	45.6	49.6	48.2
6	77.1	76.7	77.2
7	84.0	84.5	82.8
8	26.1	27.4	27.6
9	49.3	50.4	45.7
10	70.3	71.2	34.2
11	175.5	175.5	171.0
12	21.6	23.4	23.4
13	14.0	63.5	13.5
14	65.5	61.8	62.9
15	13.6	14.4	14.3
1′	171.6	170.1	169.6
2′	21.0	21.6	21.7
1″		172.9	173.1
2″		36.6	36.1
3″		18.7	18.3
4″		14.0	13.7

[a] All assignments were confirmed by 2D-HMQC and HMBC experiments. [b] Spectrum of **1** recorded in a 1:1 mixture of $CDCl_3$ with MeOH-d_4 due to poor solubility in pure $CDCl_3$. [c] For ^{13}C data recorded in pyridine-d_5, see Experimental Section.

171.6 and 21.0, respectively) could be readily assigned to an *O*-acetyl moiety. The remaining 15 carbons were assigned by comparison of the ^{13}C- and DEPT 135 spectra with those of anisatin **4**[8] to be part of an anisatin-type sesquiterpene skeleton. One of the anisatin CH−O signals (C-3, δ 71.2) is replaced by a keto function at δ 209.2. Compound **1** lacks the typical lowfield signal of the quaternary sp^3 carbon in the β-lactone ring (C-5, δ 65.2), which occurs shifted upfield to δ 45.6 showing the absence of a β-lactone moiety. Moreover, an additional methyl group is observed at δ 14.0 instead of the β-lactone carbonyl (δ 169.6) of anisatin.

The ^{1}H-NMR spectrum (Table 2), in combination with a COSY experiment, confirmed the position of the keto group at C-3, since both C-2 protons appear as dd signals at relatively low field (2.60 and 2.16 ppm) possessing vicinal couplings only with H-1. This information led to establishment of structure **1**. The assignment of all proton and carbon resonances was confirmed by HMQC and HMBC experiments. The HMBC spectrum (Table 3), moreover, confirmed the position of the O-acetyl group and the closure of the lactone ring toward C-7. The relative stereochemistry at C-5 (i.e., β-orientation of the CH_2−O−Ac function) was unambiguously proven by a NOESY spectrum (Table 3), which shows an interaction between the methyl group at C-5 (CH_3-13) with the proton of the OH-group located at C-4α.

The molecular structure of **1** was also determined by single-crystal X-ray diffraction (Tables 4 and 6 and Figures 1 and 2).[15] The needle-shaped crystals, space group $P2_12_12_1$, obtained from EtOAc were found to consist of the hemi-solvate, formula $C_{17}H_{24}O_8 \cdot 1/2C_4H_8O_2$. The asymmetric unit cell consists of two sesquiterpene lactone molecules with slightly different conformations (A and B) and one solvent molecule. Figure 1 shows the molecular structure of conformers A and B of **1**; Figure 2 shows the crystal packing of the solvate. The two STL molecules within the asymmetric unit are connected by an intermolecular hydrogen bond from O-6(B)-H···O-2(A) (dist. 1.83 Å). These dimeric units are connected with each other via H-bonds from O-4(A)-H···O-5(A′) (dist. 1.87 Å) and from O-5(B)···O-3(B″) (dist. 1.94 Å). An intramolecular H-bond could be located between O-4(B)-H···O-5(B) (dist. 1.95 Å) and another one possibly exists between O-5(A)-H···O-4(A) (dist. O···O 2.638 Å; O5(A)-H not localized). To the best of our knowledge, **1** represents the first *Illicium* STL with an anisatin-type carbon skeleton possessing a keto group at C-3, which we have named 14-acetoxy-3-oxofloridanolide (**1**).

Compound **2** possesses a molecular mass of 428 [DCI-(NH_3)−MS]. Its ^{13}C-NMR [Table 1, data referred to in the text ($CDCl_3$); for data in pyridine-d_5, see Experimental Section) and DEPT 135 spectra revealed the presence of 21 carbon atoms, comprising four CH_3, seven CH_2, three CH, and seven quaternary nuclei. On the basis of this information, the molecular formula $C_{21}H_{32}O_9$ was calculated. Six of the carbon resonances indicated the presence of an acetoxy group, as in **1**, and another O-acyl moiety that could be identified as an *n*-butyryl group (δ 172.9, 36.6, 18.7, and 14.0). In comparison with **1**, **2** lacks the signal of the methyl group C-13 and, instead, shows an additional oxygen substituted CH_2 group at δ 63.5. Moreover, **2** does not show a keto carbonyl, which is replaced by a CH_2 at δ 33.4. In agreement with replacement of the C-3 keto group by CH_2, the signal for C-2 is shifted upfield by 13.1 ppm from δ 43.1 in **1** to 30.0 in **2**. The ^{1}H-NMR (Table 2) and COSY spectra are in full agreement with these observations, displaying typical resonances of an *n*-butyryl group, an additional CH_2O group at low field, and two signals for the protons at C-3 coupled to the C-2 protons whose resonances, consequently, appear as dddd signals in this case (stereochemical assignment of H-2a/b and H3a/b according to a molecular model). Hence, **2** was found to be a C-3 hydrogenated derivative of **1**, with an additional oxygen function, esterified with *n*-butyric acid.

Assignment of the positions of the ester groups was achieved by an HMBC and a NOESY experiment (Table 3). The CH_2−O group at lower field, assigned to bear the O-acetyl group by its HMBC correlation with the acetyl−carbonyl, shows a strong NOE with the proton of the hydroxyl group located at C-4α (HMBC correlations with C-3, -5, and -9). Hence the O-acetyl group must be located at C-13 (α-equatorial position at C-5), while C-14 bears the *n*-butyryloxy substituent. We named this new natural product 13-acetoxy-14-(*n*-butyryloxy)floridanolide (**2**).

Compound **3** [colorless resin, M_r = 412, DCI(NH_3)−MS)], like **2**, possesses 21 carbon atoms, six of which could be readily identified as belonging to an acetoxy- and an *n*-butyryloxy substituent (^{13}C-NMR data, see Table 1). The ^{1}H-NMR spectrum (Table 2) of **3**, however, resembles those of the pseudoanisatin- and dunnianin-type compounds.[1] The singlet signal for H-10 in the anisatin series is missing and, as found in the dunnianins, two signals with a large coupling constant of $^2J_{10a,10b}$ = 19.9 are observed at δ 2.94 (H-10β, dd; additional ^4J−w-coupling with H-8α) and δ 2.79 (H-10α, d). As stated in our previous communication,[1] this large coupling constant is indicative of a δ-lactone ring,

Table 2. ^{1}H-NMR Data of Compounds **1**, **2**, and **3** (500.13 MHz, $CDCl_3$, TMS)[a]

	1			**2**			**3**		
H	δ (ppm)	mult	J (Hz)	δ (ppm)	mult	J (Hz)	δ (ppm)	mult	J (Hz)
1	2.75	ddq	7.0 (q), 9.3, 9.7	2.20	ddq	6.9 (q), 8.7, 9.2	2.40	ddq	7.3(q), 9.3, 9.5
2α	2.60	dd	9.3, 18.7	1.97	dddd	6.6, 9.2, 10.3, 12.7	2.67	ddd	7.6, 9.5, 14.8
2β	2.16	dd	9.7, 18.7	1.52	dddd	2.8, 8.7, 2 × ~12	1.06	ddd	2.3, 9.3, 14.8
3α	n.a.[c]			1.62	[d]		5.13	dd	2.3, 7.6
3β	n.a.			2.50	ddd	6.6, 11.7, 13.9	n.a.		
7	4.33	dd	2.2, 3.4	4.23	dd	2.5, 3.5	4.36	dd	2.1, 3.7
8α	2.60	dd	2.2, 15.0	2.50	dd	2.5, 15.1	2.35	ddd (dt)	2.5(t)[e], 14.5(d)
8β	1.94	dd	3.4, 15.0	1.79	dd	3.5, 14.8	1.80	dd	3.8, 14.5,
10 α	4.30	s		4.16	s		2.68	d	19.9
10 β	n.a.			n.a.			2.94	dd	2.8[e], 19.9
CH_3-12	1.40	s (3H)		1.48	s		1.52	s (3H)	
CH_3-13	1.49	s (3H)		n.a.			1.17	s (3H)	
13a	n.a.			4.76	d	11.7	n.a.		
13b	n.a.			4.59	d	11.7	n.a.		
14a	4.43	d	12.6	4.29	d	12.6	4.81	d	12.3
14b	3.96	d	12.6	4.12	d	12.6	4.16	d	12.3
CH_3-15	1.10	d (3H)	7.0	1.03	d (3H)	6.9	0.94	d (3H)	7.3
CH_3-2′	2.04	s (3H)		2.03	s (3H)		2.11	s (3H)	
2″	n.a.			2.53	t (2H)	7.4	2.25	dt (2t)[f]	7.4
3″	n.a.			1.62	sx (2H)	7.4	1.63	sx (2H)	7.4
CH_3-4″	n.a.			0.93	t (3H)	7.4	0.94	t (3H)	7.4
4-OH[b]	3.75	s		3.33	s		4.15	s	
6-OH[b]	3.21	s		4.72	s		3.35	s	
10-OH[b]	3.27	br s		3.18	s		n.a.		

[a] All assignments were confirmed by heteronuclear 2D-experiments as well as COSY and NOESY experiments. [b] δ-Values are concentration dependent. [c] n.a. = not applicable. [d] Signal overlapped with sextet of CH_2-3″, multiplicity not determined. [e] Additional signal splitting caused by ^4J-w-coupling H-8α, H-10β. [f] Signal shape dt results from non first-order AB-system with $\delta\nu_0 \sim 5$ Hz.

Table 3. HMBC and NOESY Correlations of Compounds **1**, **2**, and **3**

	1		**2**		**3**	
H	HMBC, corr. with C	NOESY, corr. with H[a]	HMBC, corr. with C	NOESY, corr. with H[a]	HMBC, corr. with C	NOESY, corr. with H[a]
1	2,15	2α,2β,15	2,9,10	2α,8α,15,4-OH		2α,15
2α	1,3,9	2β	9	2β	1,4	2β,3,15
2β	1,3,15	15	4	3β,15	1,15	
3α	n.a.[b]	n.a.	4,9	13a	2,9,1′	2α,13,4-OH
3β	n.a.	n.a.	2	3α,14a	n.a.	n.a.
7	5,6,9	8α,8β,12	5,6,9,11	8α,8β,12	5,9,11	8α,8β,12,6-OH
8α	4,9,10	8β,4-OH	9,10	8β, 6-OH	9,10	4-OH, 6-OH, 8β
8β	4,6,7,9,10	10,15	4,6,7,9,10	10,15	4,6,7,9,10	
10 α	1,4,9,11	15	1,4,8,9	15, 10-OH	4,8,9,11	15
10 β	n.a.	n.a.	n.a.	n.a.	1,4,9,11	10α,14a,14b
CH_3-12	5,6,7	13,14b,6-OH	5,6,7	13b, 14b, 6-OH	5,6,7	14b, 6-OH
CH_3-13	4,5,6,14	14a, 14b, 4-OH	n.a.	n.a.	4,5,6,14	14a,14b,4-OH, 6-OH
13a	n.a.	n.a.	4,5,6,14,1′	13b,4-OH	n.a.	n.a.
13b	n.a.	n.a.	4,5,6,14,1′	4-OH	n.a.	n.a.
14a	4,5,6,13,1′	14b	4,5,6,13,1″	14b	4,5,6,13,1″	14b, 2′
14b	4,5,6,13,1′		4,5,6,13,1″		4,5,6,13,1″	
CH_3-15	1,2,9		1,2,9		1,2,9	
CH_3-2′	1′		1′,13		1′	
2″	n.a.	n.a.	1″,3″,4″	3″	1″,3″,4″	3″
3″	n.a.	n.a.	1″,2″,4″	4″	2″,4″	4″
CH_3-4″	n.a.	n.a.	2″,3″		3″,2″	
4-OH	[c]	6-OH	3,4,9		3,4,9	
6-OH	[c]		5,6,12		5,6,7,12	
10-OH	[c]		9,10		n.a.	n.a.

[a] NOESY correlations denoted only once. [b] n.a. = not applicable. [c] OH signals not localized in HMBC of **1** (solvent $CDCl_3$ + MeOH-d_4 1:1); NOESY was recorded in $CDCl_3$.

inasmuch as this coupling is only 15 Hz in the ϵ-lactone ring of pseudoanisatin. In the same context, we reported that the coupling constants of H-3 differ significantly in the pseudoanisatin- and dunnianin-type compounds due to the different geometry of the cyclopentane ring; $^3J_{3,2\alpha}$ and $^3J_{3,2\beta}$ are 7.7 and 2.8 Hz in pseudoanisatin and 5−7 and <1 Hz, respectively, in the dunnianin series. In **2**, the signal for H-3 appears as a pseudoanisatin-like dd signal with $^3J_{3,2\alpha}$= 7.6 Hz and $^3J_{3,2\beta}$= 2.3 Hz. Hence, **3** comprises spectral properties of both types of STL. The presence of both, a pseudoanisatin-like geometry of the five-membered ring and a coupling constant $^2J_{10a,10b}$ as large as 19.9 Hz cannot be explained on the basis of either a dunnianin- or a pseudoanisatin-like structure. These spectral characteristics would be in agreement with a hitherto unknown structural type of STL containing a δ-lactone ring closed from C-11 to C-7 as in the anisatin series and a β-oriented oxygen function at C-3, which is not part of the lactone ring, as in pseudoanisatin.

This hypothesis could be confirmed by the long-range H−C correlations observed in the HMBC spectrum of **3**

Table 4. Fractional Coordinates for **1**

	x	*y*	*z*	B_{eq}[a] (Å^2)
O1A	0.4683 (4)	0.4785 (3)	0.58178 (9)	3.78 (8)
O2A	0.6386 (5)	0.3796 (3)	0.61192 (9)	4.95 (9)
O3A	1.0658 (4)	0.6360 (3)	0.53165 (9)	3.92 (9)
O4A	0.7130 (4)	0.6403 (3)	0.49431 (8)	2.68 (7)
O5A	0.4411 (4)	0.7103 (3)	0.52568 (8)	3.31 (8)
O6A	0.8963 (4)	0.4119 (3)	0.56677 (9)	4.01 (8)
O7A	0.7402 (5)	0.7168 (3)	0.61434 (9)	5.1 (1)
O8A	0.9410 (8)	0.6687 (5)	0.6475 (1)	12.2 (2)
C1A	0.8029 (7)	0.4342 (4)	0.4939 (1)	3.2 (1)
C2A	0.9792 (7)	0.4846 (4)	0.4983 (1)	3.7 (1)
C3A	0.9567 (6)	0.5787 (4)	0.5211 (1)	3.0 (1)
C4A	0.7657 (6)	0.5944 (4)	0.5266 (1)	2.6 (1)
C5A	0.7002 (6)	0.6663 (4)	0.5557 (1)	2.4 (1)
C6A	0.5005 (6)	0.6550 (4)	0.5553 (1)	3.0 (1)
C7A	0.4387 (6)	0.5409 (4)	0.5509 (1)	3.4 (1)
C8A	0.5084 (6)	0.4820 (4)	0.5200 (1)	3.1 (1)
C9A	0.6996 (6)	0.4786 (4)	0.5244 (1)	2.7 (1)
C10A	0.7276 (7)	0.4083 (4)	0.5554 (1)	3.4 (1)
C11A	0.6093 (7)	0.4236 (4)	0.5852 (1)	3.7 (1)
C12A	0.4107 (7)	0.7058 (5)	0.5853 (1)	4.0 (1)
C13A	0.7850 (6)	0.6368 (4)	0.5897 (1)	3.3 (1)
C14A	0.7494 (7)	0.7838 (4)	0.5487 (1)	3.4 (1)
C15A	0.8038 (9)	0.3132 (5)	0.4890 (2)	5.1 (2)
C16A	0.8217 (9)	0.7224 (5)	0.6425 (2)	5.8 (2)
C17A	0.759 (1)	0.8077 (6)	0.6650 (2)	6.7 (2)
O1B	−0.0131 (5)	0.2848 (3)	0.64270 (9)	5.1 (1)
O2B	0.1982 (7)	0.2732 (4)	0.60728 (9)	6.9 (1)
O3B	0.4835 (5)	0.2458 (3)	0.73825 (9)	4.55 (9)
O4B	0.1346 (4)	0.3580 (3)	0.75130 (9)	4.06 (9)
O5B	−0.1553 (5)	0.2664 (4)	0.7310 (1)	5.7 (1)
O6B	0.4196 (5)	0.3211 (3)	0.66083 (9)	4.19 (9)
O7B	0.1810 (5)	0.0267 (3)	0.6902 (1)	4.37 (9)
O8B	0.4444 (6)	−0.0138 (4)	0.6793 (2)	9.3 (2)
C1B	0.2951 (8)	0.4898 (4)	0.7083 (2)	4.5 (1)
C2B	0.4398 (7)	0.4340 (5)	0.7273 (2)	4.6 (2)
C3B	0.3944 (7)	0.3175 (4)	0.7299 (1)	3.4 (1)
C4B	0.2043 (6)	0.3121 (4)	0.7208 (1)	3.2 (1)
C5B	0.1195 (6)	0.2012 (4)	0.7142 (1)	2.9 (1)
C6B	−0.0664 (7)	0.2259 (5)	0.7021 (1)	4.1 (1)
C7B	−0.0803 (7)	0.3164 (5)	0.6757 (2)	4.6 (1)
C8B	0.0024 (8)	0.4209 (5)	0.6867 (2)	5.1 (1)
C9B	0.1905 (7)	0.3993 (4)	0.6928 (1)	3.5 (1)
C10B	0.2608 (8)	0.3686 (4)	0.6577 (1)	4.0 (1)
C11B	0.1479 (9)	0.3041 (5)	0.6344 (1)	4.7 (2)
C12B	−0.1586 (7)	0.1285 (6)	0.6887 (2)	5.7 (2)
C13B	0.2245 (7)	0.1377 (4)	0.6884 (1)	3.6 (1)
C14B	0.1185 (7)	0.1372 (5)	0.7471 (2)	4.7 (2)
C15B	0.362 (1)	0.5741 (5)	0.6844 (2)	7.3 (2)
C16B	0.3044 (8)	−0.0414 (4)	0.6848 (2)	4.8 (2)
C17B	0.251 (1)	−0.1536 (5)	0.6875 (2)	6.6 (2)
O1S	0.8347 (6)	0.0340 (3)	0.5989 (1)	5.7 (1)
O2S	0.7529 (9)	0.1514 (4)	0.5609 (1)	9.9 (2)
C1S	0.724 (1)	0.1003 (5)	0.5858 (2)	6.2 (2)
C2S	0.561 (1)	0.0935 (7)	0.6041 (2)	9.0 (3)
C3S	0.999 (1)	0.0318 (6)	0.5827 (2)	7.5 (2)
C4S	1.104 (1)	−0.0474 (8)	0.5994 (2)	9.0 (3)

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

Table 5. Final Atomic Coordinates of **4**

	x	*y*	*z*	B_{eq}[a] (Å^2)
O1	0.3027 (1)	0.2761 (1)	0.33660 (9)	1.12 (2)
O2	0.4056 (1)	0.1309 (1)	0.41455 (9)	1.22 (2)
O3	0.6387 (1)	0.4428 (1)	0.55082 (9)	1.15 (2)
O4	0.5089 (1)	0.6318 (1)	0.35182 (9)	1.11 (2)
O5	0.2495 (1)	0.5955 (1)	0.30843 (8)	1.19 (2)
O6	0.4041 (1)	0.4482 (1)	0.63787 (8)	1.27 (2)
C1	0.6891 (2)	0.4640 (2)	0.3005 (1)	1.04 (3)
C2	0.7460 (2)	0.5274 (2)	0.3975 (1)	1.30 (3)
C3	0.6307 (2)	0.5358 (2)	0.4762 (1)	1.06 (3)
C4	0.5039 (2)	0.5217 (1)	0.4086 (1)	0.83 (3)
C5	0.3593 (2)	0.5129 (1)	0.4578 (1)	0.97 (3)
C6	0.2569 (2)	0.4871 (1)	0.3663 (1)	0.99 (3)
C7	0.3061 (2)	0.3933 (2)	0.2893 (1)	0.99 (3)
C8	0.4441 (2)	0.4188 (2)	0.2432 (1)	0.97 (3)
C9	0.5450 (2)	0.4224 (1)	0.3314 (1)	0.83 (3)
C10	0.5464 (2)	0.2948 (1)	0.3741 (1)	0.95 (3)
C11	0.4145 (2)	0.2284 (1)	0.3764 (1)	0.98 (3)
C12	0.1140 (2)	0.4564 (2)	0.4016 (1)	1.41 (3)
C13	0.3217 (2)	0.6312 (2)	0.5089 (1)	1.23 (3)
C14	0.3422 (2)	0.4174 (2)	0.5425 (1)	1.05 (3)
C15	0.7842 (2)	0.3719 (2)	0.2565 (1)	1.37 (3)
H3O	0.716 (2)	0.420 (2)	0.567 (1)	3.2 (5)
H4O	0.431 (2)	0.652 (2)	0.326 (2)	3.2 (5)
H5O	0.204 (2)	0.590 (2)	0.262 (2)	2.2 (4)
H6O	0.483 (2)	0.458 (2)	0.626 (1)	2.6 (5)

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

Table 6. Crystal Data and X-ray Data Collection Parameters

compound	**1**	**4**
formula	$C_{17}H_{24}O_8\cdot{}^1/_2C_4H_8O_2$	$C_{15}H_{24}O_6$
FW	400.4	300.4
crystal syst	orthorhombic	orthorhombic
space grp	$P2_12_12_1$	$P2_12_12_1$
cell constants		
a, Å	7.9326 (8)	9.962 (6)
b, Å	12.5862 (11)	11.321 (2)
c, Å	39.447 (4)	13.072 (3)
V, Å^3	3937.5 (11)	1474 (2)
Z	8	4
D_c, g cm^{-3}	1.351	1.353
μ, cm^{-1}	8.7	1.0
temperature, °C	24	−173
cryst size, mm	0.03 × 0.05 × 0.30	0.38 × 0.45 × 0.63
radiatn	Cu Kα (λ = 1.54184 Å)	Mo Kα (λ = 0.71073 Å)
θ limits, deg	2−75	2−27.5
min. transmission, %	95.5	
octants collected	$h, k, \pm l$	$h, \pm k, \pm l$
unique data	5821	1947
obsd data	4239	1869
criterion	$I > 1\sigma(I)$	$I > 1\sigma(I)$
variables	506	287
R	0.069	0.031
R_w	0.061	0.036
resid. density, e$^-$ Å^{-3}	0.50	0.35
extinction	3.0 (3) × 10^{-7}	8.5(6) × 10^{-7}
hydrogen Atoms	not refined	refined iso

(Table 3). As expected, H-7 shows a ^3J correlation with the lactone carbonyl C-11 (assigned by its correlations with H-10α and 10β) proving the position of lactone ring closure. The ester carbonyl of the acetoxy group shows a correlation with H-3, while that of the *n*-butyryloxy group correlates with the characteristic doublet signals of the CH_2−O group. As in **2**, the orientation of the CH_2−O-*n*-butyryl group at C-5 could be assigned by the nuclear Overhauser effects observed in a NOESY spectrum of **3** (Table 3). The proton of the CH_2−O group resonating at lower field (H-14a) shows an NOE with H-10β, while the CH_3 group at C-5 shows an effect with the OH proton of the C-4α hydroxyl. Thus, as in **2**, the *n*-butyryloxy group is located at C-14 (i.e., it is attached to the carbon β-oriented at C-5). Compound **3** represents a new type of *Illicium* STL, being the first compound with the anisatin carbon skeleton and mode of lactone ring closure (i.e., lactone closed toward C-7) lacking the C-10-hydroxyl function and possessing a β-oriented oxygen function at C-3. This new sesquiterpene lactone was named 3β-acetoxy-14-*n*-butyryloxy-10-deoxyfloridanolide.

The finding that a large geminal coupling between the protons at C-10 is not exclusively a diagnostic feature in the ^{1}H-NMR spectra of the 11,3-δ-lactonized dunnianins but may also occur in compounds with an 11,7-fused δ-lactone ring, as in **3**, prompted us to reinspect the NMR spectra of the three dunnianin derivatives described in our previous communication.[1]

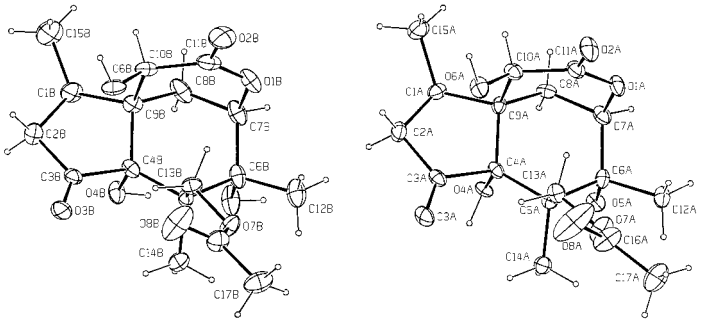

Figure 1. Molecular structure of conformers A and B of **1** present in the asymmetric unit cell.

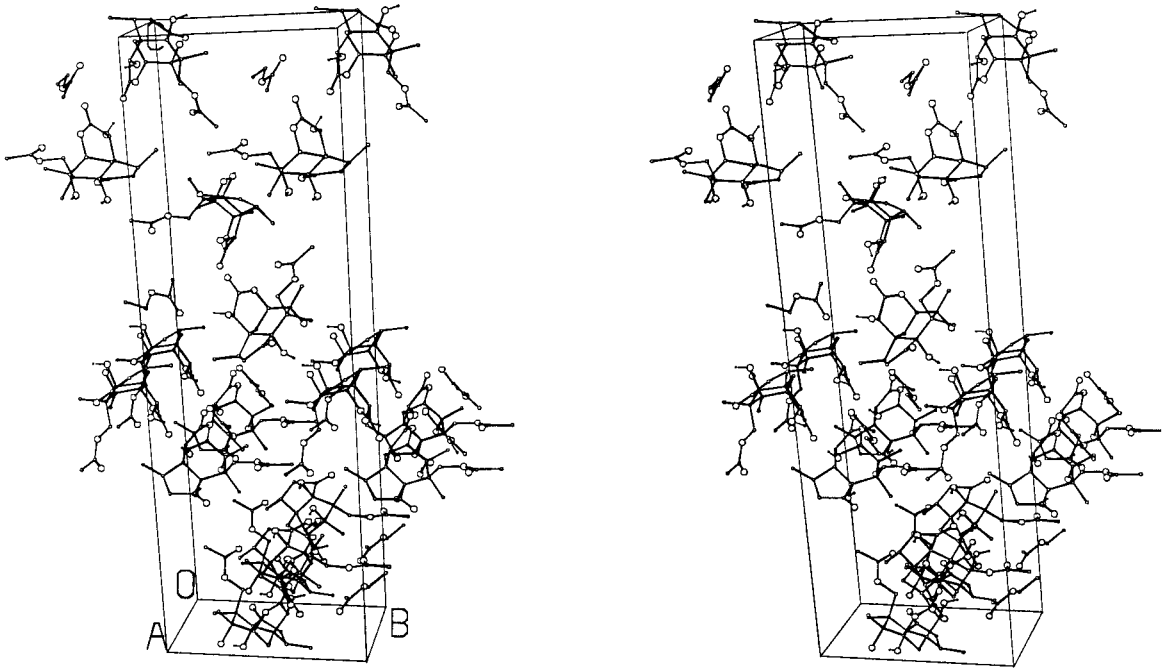

Figure 2. Stereoscopic view of the spatial arrangement in the $(\mathbf{1})_2$·EtOAc crystal.

In the case of compound **4** (compound **2** in Schmidt et al.[1]), we detected an error in our assignment of the HMBC signals. A cross signal between H-7 and C-11 had been mistaken to result from an interaction of this carbon atom with H-3, which resonates only 0.04 ppm downfield from H-7. Hence, the lactone ring in this compound, originally revised from an 11,14-ϵ-lactone structure to an 11,3-δ-lactone,[1] is actually closed between C-11 and C-7, as described above for compound **3**. The ^3J coupling constant between H-3 and H-2β of $\sim$ 0 Hz, however, was not in agreement with such a structure (see Schmidt et al.,[1] and compare with compound **3**).

We, therefore, undertook an X-ray crystallographic analysis of **4**, which unambiguously proved the mode of lactone ring closure toward C-7. The molecular structure of **4** is depicted in Figure 3, and details are given in Tables 5 and 6. The above-mentioned spectral discrepancy, most noteworthy, could be attributed to a strong intramolecular hydrogen bond between the OH groups attached to C-14 and C-3 [distance O-6···O-3 = 2.599 (2) Å and angle about H 156 (2)°]. This interaction is obviously strong enough also in solution to stabilize a conformation of the cyclopentane ring that results in a dihedral angle between H-3 and H-2β of ca. 90°, (91° in the crystal structure), explaining the absence of a detectable ^{1}H-NMR coupling between these protons. The

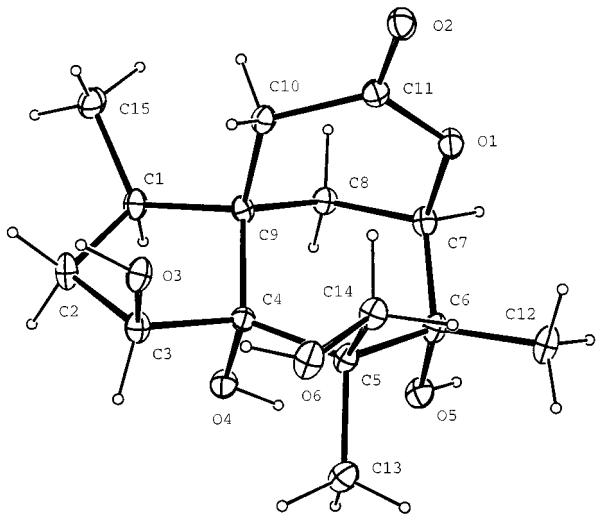

Figure 3. Molecular structure of compound **4**.

OH groups at C-4 and C-6 also form an intramolecular hydrogen bond, with O-4···O-5 distance 2.677 (2) Å and angle about H 142 (2)°. Hydroxy groups O-3 and O-5 also engage in intermolecular hydrogen bonds, O-3···O-2 with distance 2.824 (2) Å and angle about H 173 (2)°, and O-5···O-6 with distance 2.749 (2) Å and angle about H 171 (2)°.

The name "debenzoyldunnianin" hence cannot be retained for this STL, since it should be reserved for an 11,3-lactone such as dunnianin (**7**) itself. We, therefore, propose, in analogy with the new lactone **3**, the name 3β,14-dihydroxy-10-deoxyfloridanolide for compound **4**.

The sesquiterpene dilactones anisatin (**5**) and 2α-hydroxyneoanisatin (**6**) were isolated from further fractions of the CH_2Cl_2 extract of *I. floridanum* fruits (see experimental section). Both compounds were identified by their physical properties and ^{13}C- and ^{1}H-NMR spectral data in comparison with literature data.[8,16] Compounds **1**, **5**, and **9** were also isolated from the CH_2Cl_2 and MeOH extracts of *I. floridanum* leaves and identified by comparison of their chromatographic behavior as well as their ^{1}H- and ^{13}C-NMR spectra with the same compounds isolated from the fruits. All other sesquiterpene lactones isolated from the fruits (**2**−**4**, **6**−**8**) were identified in fractions of the leaf extracts by direct TLC comparison with authentic compounds from the fruits in several solvent systems.

The β-lactone anisatin (**5**) is known to be a potent neurotoxin with an LD_{50} of 1 mg/kg (mouse, ip).[6] The presence of this compound and some of its toxic congeners in the fruits of *I. anisatum* L. (= *I. religiosum* Sieb. et Zucc., Japanese star anise or Shikimi) has been the cause of poisonings when they were mistakingly used as a spice or medicinal herb instead of those of *I. verum* Hook. f. (Chinese star anise). It has been speculated on grounds of structural similarity, that **6**, the isomer of **5**, might also be toxic.[8] Although an exact quantitative statement on the amounts in which **5** is present in *I. floridanum* fruits and leaves cannot be made on grounds of the amounts in which it was isolated, the high preparative yields of this toxin account for the plant's reported toxicity.[12,13] Although no case of acute poisoning with *I. floridanum* is known to us, it has to be emphasized that the fruits are morphologically relatively similar to those of *I. verum*. Erroneous misuse by unaware persons might result in poisonings similar to those mentioned with *I. anisatum*.

Due to its neurotoxic activity, **5** has received attention in a number of neuropharmacological studies.[11,17−20] It has been demonstrated to block GABA induced depolarization of GABAergic neurons in the vertebrate CNS even more effectively than picrotoxinin.[11] As does picrotoxinin, **5** exerts its effect on the GABA receptor by a noncompetitive mechanism.[11] Taking into consideration that some structural similarity between these STLs exists, it may be speculated that **5** binds to a similar site at the GABA-receptor-coupled chloride ionophore as does picrotoxinin.[21,22] This speculation will have to be proven in further studies. Because no data on possible toxicological or pharmacological effects on the other STLs isolated from *I. floridanum* exist, a pharmacological investigation of these compounds has been initiated and will be subject of a subsequent communication.

Experimental Section

General Experimental Procedures. Optical rotation was measured with Perkin−Elmer 241 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 [DCI-(NH_3)−MS] on a Finnigan MAT INCOS 50 mass spectrometer. NMR spectra of **1**, **2**, **3**, and **6** were recorded with a Bruker DRX 500 (500.13/125.77 MHz); those of **5**, with a Bruker AM 400 spectrometer (400.13/100.61 MHz). All NMR spectra were taken at room temperature with TMS as internal standard. Gradient-selected HMBC and NOESY spectra of **1**−**3** were obtained with the 500 MHz instrument using an inverse multinuclear probehead equipped with actively shielded z-gradient coil and a GREAT 1/10 gradient unit.

X-Ray diffraction data[15] for compounds **1** and **4** were collected on Enraf−Nonius CAD4 diffractometers equipped with Cu Kα or Mo Kα radiation and graphite monochromators. Friedel-related data were collected to $\theta = 50°$ for **1**, and a hemisphere of data was collected at 100 K for **4**. Unit-cell parameters at 299 K for **4** are $a = 9.9603$ (6), $b = 11.3980$ (6), $c = 13.1443$ (10) Å, $V = 1492.2$ (3) Å^3. Data reduction included corrections for background, Lorentz, polarization, and absorption (for **1**) effects. Absorption corrections were based on ψ scans. The structures were solved by direct methods and refined using the MolEN programs.[23] Refinement was by full-matrix least squares, with neutral-atom scattering factors and anomalous dispersion terms. Weights were $w=4F_o^2[\sigma^2(I) + (0.02F_o^2)^2]^{-1}$. All nonhydrogen atoms were refined anisotropically. Hydrogen atoms were refined isotropically for **4**, but were not refined for **1**. The H atom on hydroxy group O5A of **1** was not located. Crystal data, final *R* values, and other details are included in Table 6.

A molecular model of compound **2** was generated using the program PCModel 4 (MMX force field). Coupling constants were calculated with the same program.

Plant Material. Leaves of *I. floridanum* Ellis were collected in May 1995, from the same population as the fruits.[1] The plants were identified in comparison with authentic voucher samples at the herbarium, Department of Botany, Louisiana State University. A voucher sample of this collection (Sch-IF-1) is deposited at the herbarium of the Institut für Pharmazeutische Biologie, Universität Düsseldorf.

Extraction and Isolation. *I. floridanum* (750 g) fruits were extracted and separated on Sephadex LH20 as described previously.[1] VLC of fraction C (7.5 g) in three portions of 2.5 g on 100 g of silica with petroleum ether, *n*-hexane, EtOAc mixtures yielded 14 fractions (C1 petroleum ether; C2 hexane−EtOAc 6:4; C3 1:1; C4, C5 4:6; C6, C7 3:7; C8, C9 2:8; C10 1:9; C11−C14 EtOAc). Column chromatography of C7 (412 mg) on 40 g of silica with *n*-hexane−EtOAc 8:2 yielded nine subfractions C7.1−C7.9. From C7.4 (209 mg), 112 mg of pure colorless needles of 14-acetoxy-3-oxofloridanolide (**1**) crystallized upon standing in the eluent.

Fraction C5 (753 mg) was further separated by column chromatography on 200 g of silica with *n*-hexane−EtOAc 6:4 to yield seven subfractions, C5.1−C5.7; C5.5 (323 mg) containing **2** and **3** was separated by two further column chromatography steps on silica with *n*-hexane−EtOAc 4:6 and with CH_2Cl_2−EtOAc 8:2 to yield pure crystalline 13-acetoxy-14-(*n*-butyryloxy)floridanolide (**2**, 73 mg), and pure 3β-acetoxy-14-*n*-butyryloxy-10-deoxyfloridanolide (**3**, 11 mg, colorless resin).

Column chromatography of fraction D (2 g) on 200 g of silica with EtOAc−*n*-hexane mixtures of increasing polarity yielded 32 fractions (6:4, D1−D10; 4:6, D8−D20; 2:8, D21−D27; 0:1, D28−D32). In addition to compounds **4** and **7**−**9** (isolation from D25, D16, D10, D21, respectively, as reported previously[1]), anisatin (**5**) and 2α-hydroxyneoanisatin (**6**) were isolated from fractions D11 and D12 (**5**) and D23 (**6**). Compound **5** crystallized from fractions D11 (64 mg) and D12 (153 mg) in a total yield of 144 mg. Pure **6** (13 mg) crystallized from fraction D23 (18 mg).

The leaves of *I. floridanum* were dried at ambient temperature and powdered; 500 g were exhaustively percolated with CH_2Cl_2 and, subsequently, with MeOH. The CH_2Cl_2 extract (24 g) was macerated with 200 mL of MeOH and the MeOH-soluble part (10 g) separated by VLC on 200 g of silica with hexane−EtOAc and EtOAc−*i*-PrOH mixtures of increasing polarity yielding eight fractions, A−H (A, 10:0; B, 9:1; C, 7:3; D, 5:5; E, 3:7; F, 1:9; G, 0:10; H, EtOAc−*i*-PrOH 75:25).

Fraction E (1.7 g) was further separated by VLC on 20 g of silica with hexane−EtOAc mixtures of increasing polarity to yield eight subfractions, E1 to E8. From fraction E6 (247 mg), eluted with hexane−EtOAc 3:7 and 2:8, 110 mg needles of anisatin (**5**) crystallized upon standing in the eluent. The mother liquor of E6 and fraction E5 (866 mg, 4:6) contained further **5** along with compounds **2**, **3**, and **8**. Fraction F (680 mg) contained **5** along with **1**. F was separated by VLC on 12 g silica with hexane−EtOAc mixtures to yield a fraction (F6, 38 mg, 3:7 and 2:8) from which **1** crystallized in a yield of 24 mg. The preceding fraction, F5, (70 mg, 4:6) contained compound **5** and a trace amount of **7**. Separation of fraction G (890 mg) by VLC on 17 g of silica with hexane−EtOAc mixtures of increasing polarity yielded 347 mg of a subfraction G5 eluted with hexane−EtOAc 2.5:7.5 from which 244 mg of colorless pseudoanisatin (**9**) crystallized upon standing in the eluent. Fraction G6 (44 mg, 1:9) contained compound **6** along with further **9**. Fraction H (1.1 g), upon VLC separation on 20 g of silica with hexane−EtOAc and EtOAc−*i*-PrOH yielded a subfraction H3 (495 mg, EtOAc−*i*-PrOH 6:4) in which compound **4** was detected.

The MeOH extract (86 g) was divided into an EtOAc-soluble and an EtOAc-insoluble part. From the EtOAc soluble part (13 g), 8 mg of **1**, 230 mg of **5**, and 252 mg of **9** were isolated in pure crystalline form in a similar manner as described above. All STLs isolated from the fruits were detected by TLC analysis in several systems in fractions of the leaf extracts in direct comparison with the authentic compounds.

14-Acetoxy-3-oxofloridanolide (1): colorless needles; mp 197 °C (EtOAc); $[\alpha]_D$ −28° (*c* 0.41, MeOH); DCI-(NH_3)−MS *m/z* 374 $[M + NH_4]^+$ (100%); ^{13}C NMR (125 MHz, pyridine-d_5, TMS) δ 35.3 (C-1), 43.7 (C-2), 208.5 (C-3), 83.0 (C-4), 46.2 (C-5), 77.2 (C-6), 84.0 (C-7), 26.3 (C-8), 49.7 (C-9), 70.3 (C-10), 174.9 (C-11), 22.4 (C-12), 14.9 (C-13), 65.6 (C-14), 13.9 (C-15), 170.2 (C-1′), 20.8 (C-2′).

13-Acetoxy-14-(*n*-butyryloxy)floridanolide (2): colorless needles; mp 172 °C (EtOAc); $[\alpha]_D$ −12° (*c* 0.51, MeOH); DCI(NH_3)−MS *m/z* 446 $[M + NH_4]^+$ (100%); ^{13}C NMR (125 MHz, pyridine-d_5, TMS) δ 39.0 (C-1), 31.3 (C-2), 33.2 (C-3), 88.9 (C-4), 49.7 (C-5), 76.8 (C-6), 84.2 (C-7), 27.8 (C-8), 51.0 (C-9), 71.1 (C-10), 175.7 (C-11), 23.8 (C-12), 64.2 (C-13), 62.5 (C-14), 14.4 (C-15), 170.6 (C-1′), 20.9 (C-2′), 172.5 (C-1″), 36.3 (C-2″), 18.5 (C-3″), 13.7 (C-4″).

3β-Acetoxy-14-*n*-butyryloxy-10-deoxyfloridanolide (3): colorless resin; $[\alpha]_D$ +6° (*c* 0.11, MeOH); DCI-(NH_3)−MS *m/z* 430 $[M + NH_4]^+$ (100%).

Anisatin (5): colorless needles; mp 206 °C (EtOAc); $[\alpha]_D$ −22° (*c* 0.41, MeOH); DCI(NH_3)−MS *m/z* 346 $[M + NH_4]^+$ (100).

2α-Hydroxyneoanisatin (6): colorless needles; mp 219 °C (EtOAc); $[\alpha]_D$ −3° (*c* 0.10, MeOH); DCI(NH_3)−MS 346 *m/z* $[M + NH_4]^+$ (100). For data of **4** and **7**−**9** see Schmidt et al.[1]

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