Novel *seco*-Prezizaane Sesquiterpenes from North American *Illicium* Species†

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Received September 4, 1998

Three novel *seco*-prezizaane sesquiterpenes (**1**−**3**) were isolated from leaves of *Illicium parviflorum* (swamp star anise, yellow star anise), a species occurring endemically in central Florida. Compound **1**, named cycloparvifloralone, possesses a hitherto unknown ring system with a cagelike acetal/hemiketal structure. Lactones **2** (cycloparviflorolide) and **3** (parviflorolide) which were obtained as an inseparable mixture, coexist in a hemiketal/keto equilibrium. It could be shown that a 4,7-hemiketal (**4**) occurs in an analogous fashion to pseudoanisatin **5**, a known constituent of other *Illicium* species. From the fruits of *Illicium floridanum* the novel ortholactone **6** was isolated. The structures of the new compounds were elucidated by interpretation of their 1D and 2D homo- and heteronuclear NMR spectroscopic data. The modes of cyclization observed in **1**, **2**, **4**, and **6** have not been described previously, and a biogenetic sequence is proposed for these compounds and further *seco*-prezizaane sesquiterpenes.

As a part of an ongoing phytochemical investigation of North American species of the genus *Illicium* (Illiciaceae),[1,2] the leaves of *Illicium parviflorum* Michaux ex Ventenat (swamp star anise, yellow star anise) were studied. This species is endemic to moist woods and swamps of central Florida. *I. parviflorum* is morphologically distinct from the second species occurring in North America, *Illicium floridanum*, which is distributed from northeastern Florida to western Louisiana. A. C. Smith in his systematic treatment of the genus *Illicium*[3] included *I. floridanum* and *I. parviflorum* in different taxonomic assemblies, namely, the section Badiana and the section Cymbostemon, respectively. While nine sesquiterpene lactones of the *seco*-prezizaane type were isolated from *I. floridanum*,[1,2] no previous reports on the chemical constituents of *I. parviflorum* exist. The occurrence of this structurally unique group of terpenoids is restricted to the genus *Illicium*. Compounds of this type are of pharmacological and toxicological interest because some representatives are highly potent inhibitors of GABA action in the central nervous system.[4]

Results and Discussion

From a column chromatographic fraction from the methylene chloride extract of *I. parviflorum* leaves, a hitherto unknown C_{15} compound (**1**) of molecular mass 300 (DCIMS (NH_3) and HRFABMS) and molecular formula $C_{15}H_{24}O_6$ was isolated.

The absence of a lactone structure as commonly found in the sesquiterpenes of *Illicium* followed from the ^{13}C NMR spectrum (Table 1) in which no carbonyl signals were observed. Instead, a methine and a quaternary carbon atom occurred at δ 97.4 and 99.2, respectively, and indicated the presence of an acetal and a ketal moiety. Further oxygenated sp^3 carbons (two quaternary, one methine, and one methylene) resonated at δ 89.6, 81.2, 70.5, and 68.6, which could be assigned to C-4, C-6, C-10, and C-14, respectively, in an anisatin-like *seco*-prezizaane skeleton.[2] HMQC and COSY experiments allowed assignment of the ^1H NMR resonances (Table 2). The acetal proton at δ 4.94 was coupled to the H-10 doublet (δ 3.74 ppm, $^3J_{10,11} = 5.4$ Hz), so that the acetal carbon must be C-11. The protons at C-8 appeared as sharp doublet signals at δ 2.15 and 1.35 ($^2J = 12.9$ Hz), showing that C-7 must be quaternary, which is only possible if the ketal function is located at this center. These assignments were confirmed by an HMBC experiment in which strong correlations between C-7 and H-11, C-11 and H-14b, and C-14 and H-11 proved the mode of cyclization proposed. The stereochemistry at C-10 was shown to be the same as in the anisatin series by a NOESY experiment, in which H-10 showed interactions with CH_3-15 and H-8β. Moreover, the coupling constant between H-10 and H-11 would be considerably smaller in case of the C-10 epimer. The theoretical values deduced from a force field molecular model were 4.6 Hz for the proposed structure ($\Phi_{H-10,H-11} = 42°$) and 1.5 Hz for the opposite configuration ($\Phi_{H-10,H-11} = 78°$). Thus, the structure of **1** was unambiguously assigned in the manner shown. As a result of the presence of the masked aldehyde and keto functions, the name cycloparvifloralone is proposed for this new compound.

Table 1. ^{13}C NMR Data of Compounds **1**−**4** and **6** (δ (ppm), 125 MHz, TMS)[a,b]

carbon	**1**[c]	**2**[d]	**3**[d]	**4**[e]	**6**[c,d]	
1	39.9	46.0	41.5	44.2	36.4	35.9
2	30.8	32.4	31.2	44.4	44.5	44.1
3	31.5	27.5	33.0	73.0	212.3	209.9
4	89.6	95.8	89.0	91.9	84.0	83.4
5	47.0	51.9	47.5	51.7	48.1	47.7
6	81.2	79.8	78.6	79.8	79.0	78.8
7	99.2	109.2	206.0	109.5	79.2	78.4
8	37.6	38.5	42.9	39.7	29.8	29.6
9	51.8	55.2	51.7	52.6	49.7	48.7
10	70.5	75.7	77.1	33.7	75.7	75.5
11	97.4	172.4	171.4	≈175[f]	113.7	113.3
12	16.0	18.8	18.2	18.9	21.0	21.5
13	15.5	17.7	14.5	16.8	14.8	14.7
14	68.6	69.0	69.6	71.5	67.8	67.4
15	13.4	13.6	14.0	14.1	13.6	13.6

[a] Assignments of **1**−**3** and **6** confirmed by HMQC and HMBC experiments. Assignments for **4** based on those of **5**[6] in analogy to **2** and **3**. [b] Data for **4** and **5** at 100 MHz. [c] Spectrum recorded in methanol-d_4. [d] Recorded in acetone-d_6. [e] Recorded in pyridine-d_5. [f] Assignment tentative due to poor signal/noise ratio.

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† Dedicated to Prof. Dr. Günter Willuhn, Heinrich-Heine-Universität Düsseldorf, Germany, on the occasion of his 65th birthday.

10.1021/np980382a CCC: $18.00
Published on Web 04/21/1999

Table 2. ^{1}H NMR Data of Compounds **1**−**4** and **6** (500 MHz, TMS)

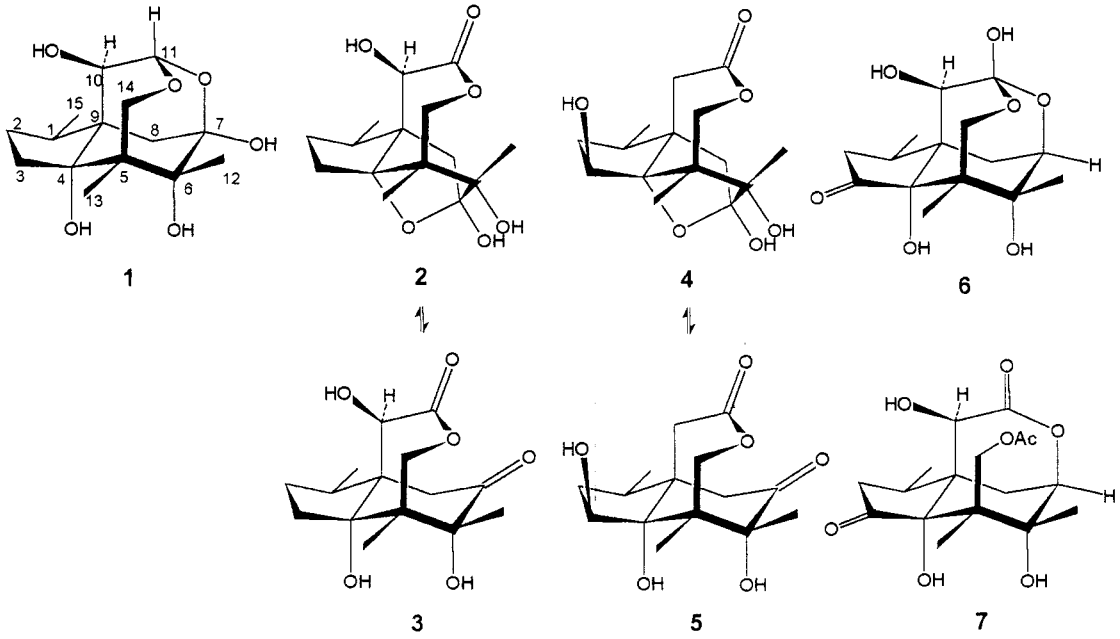
	1[a]			**2**[b]			**3**[b]			**4**[c]			**6**[b]		
H	δ (ppm)	mult	*J* (Hz)	δ (ppm)	mult	*J* (Hz)	δ (ppm)	mult	*J* (Hz)	δ (ppm)	mult	*J* (Hz)	δ (ppm)	mult	*J* (Hz)
1	2.06	ddq	10,10,7	2.56	*f*		2.56	*f*		2.65	*f*		2.48	A [g]	
2α	1.85	dddd	7,10,10,13	1.91	dddd	4,9,10,12	2.10	*f*		2.75	*f*		2.46	B [g]	
2*β*	1.3−1.4	*f*		1.60	dddd	4,11,12,12	1.56	*f*		1.62	*f*		1.97	X [g]	
3α	1.3−1.4	*f*		1.71	ddd	4,12,14	1.75	*f*		4.71	dd	3,7			
3*β*	2.19	ddd	7,12,12	2.01	ddd	4,10,14	2.53	*f*							
7													3.61	dd	2,3
8α	2.15	d	13	1.66	d	14	2.98	d	16	2.22	d	14	2.28	dd	2,14
8*β*	1.35	d	13	1.55	d	14	2.12	d	16	2.12	d	14	1.69	dd	3,14
10a	3.74	d	5.4	4.54	d	5	4.35	d	6	3.51	d	15	3.89	d	3
10b										2.85	d	15			
11	4.94	d	5.4												
12	1.12	s (3H)		1.27	s (3H)		1.29	s (3H)		1.74	s (3H)		1.42	s (3H)	
13	0.96	s (3H)		0.94	s (3H)		1.17	s (3H)		1.61	s (3H)		1.28	s (3H)	
14a	3.86	d	12	5.01	d	13	4.94	d	13	5.12	d	13	4.36	d	12
14b	3.35	d	12	3.98	d	13	3.87	d	13	4.17	d	13	3.33	d	12
15	0.93	d (3H)	7	1.00	d (3H)	7	1.05	d (3H)	7	0.88	*f*		1.11	d (3H)	7
4-OH				–			5.63	s[d]					4.96	s[e]	
6-OH				3.85	s[d]		6.12	s[d]					5.39	s[e]	
7-OH				5.01	s[d]										
10-OH				5.54	d[d]	5	5.80	d[d]	6				4.40	d	3

[a] Recorded in methanol-d_4. [b] Recorded in acetone-d_6. [c] Recorded at 400 MHz in pyridine-d_5 + 5% D_2O (see ref 1). [d] Assignment of the OH signals in acetone determined by HMBC correlations. [e] Assignment interchangeable. [f] Multiplicity not determined (signal overlap). δ values and assignments from cross peaks in 2D spectra. [g] Non-first-order ABX system with $^3J_{AB} \approx {}^3J_{AX} \approx 10$, $^2J_{BX} \approx -20$ Hz.

From the column chromatographic fraction immediately preceding that containing compound **1**, 6 mg of a crystalline mixture were isolated. The components were inseparable by chromatographic methods applying normal and reversed phase (RP18) silica gel. Both possess the same molecular weight of 298 as determined by DCIMS (NH_3) from which the elemental formula $C_{15}H_{22}O_6$ was derived. The NMR spectra (Tables 1 and 2) revealed the presence of two closely related isomeric sesquiterpenes (**2** and **3**) in a ratio of approximately 4:1. The ^{13}C NMR spectrum (Table 1) showed that both compounds are lactones (δ 172.4 and 171.4, C-11). The major component **2** contains a quaternary sp^3 carbon atom resonating at δ 109.2 which lies in the range expected for a ketal or hemiketal structure. Assignment of the ^{1}H NMR data (Table 2) followed from the COSY and HMQC experiments. As in **1**, the hemiketal carbon must be C-7, since the protons at C-8 showed only a geminal coupling with each other. This assignment, as well as those of all other quaternary carbon atoms, was confirmed by an HMBC experiment. Also, from this experiment it could be deduced that the hemiketal ring must occur between C-4 and C-7. Although no long range H/C-correlation over the oxygen bridge could be observed, since both C-4 and C-7 are quaternary, the oxygen function at C-4 is the only one capable of attacking a C-7 keto group to form the hemiketal. This is in agreement with the marked downfield shift of C-4 resonating at δ 95.8.

The minor constituent **3** was identified as the corresponding C-7 ketone, in which HMBC correlations of H-8a, H-8b, and CH_3-12 with a keto carbonyl at δ 206.0 (C-7) were observed. In this case, C-4 resonated at δ 89.0 which is in the range normally observed for the hydroxyl-substituted C-4 in this type of compound,[2] and as seen for compound **1**. The new sesquiterpene lactone **3** is an isomer of pseudoanisatin (**5**) which was previously isolated from *I. floridanum*[1,2] and is a known constituent of some other *Illicium* species.[5,6] From this lactone (**5**), **3** differs in the position of one hydroxyl group, being hydroxylated at C-10, while **5** is hydroxylated at C-3. In the course of the identification of **5** from *I. floridanum*,[1,2] we became aware of an impurity (about 10%) observed in the NMR spectra of this compound. These spectra were reexamined, and it was found that the hemiketal **4**, analogous to **2**, was present also in this case. Based on the detailed spectral knowledge obtained on the pair **2**/**3** where the hemiketal is the major component, all carbon resonances of **4** were assigned (Table 1; for ^{13}C NMR data of **5** see ref 6).

In the course of the reinvestigation of the mother liquor remaining after the crystallization of **5** during its isolation from *I. floridanum* fruits,[1] another unknown C_{15} constituent, **6**, was isolated which had originally been overlooked because of the high concentration of **5** in this fraction. The DCIMS (NH_3) of **6** revealed a molecular mass of 314 consistent with an elemental formula of $C_{15}H_{22}O_7$, which was confirmed by HRFABMS. Its ^{13}C NMR spectrum (see Table 1, data referred to in the text were obtained in acetone-d_6), closely resembled that of 14-acetoxy-3-oxofloridanolide **7**.[2] However, **6** lacked the signals of an acetoxy group and a signal for a lactone carbonyl. Instead, the resonance of a quaternary carbon at δ 113.3 similar to the signal of the hemiketal carbon in **2** was observed. In this case, however, the protons at H-8 (dd at δ 2.28 and 1.69) in the ^{1}H NMR and COSY spectra showed couplings with a doublet of doublets at δ 3.61 which was assigned to H-7. The corresponding methine carbon, C-7, resonated at δ 78.4 as assigned by HMQC. Thus, C-7 is apparently substituted by a single oxygen atom and, therefore, cannot be part of a ketalized keto carbonyl group. The H-10 signal in acetone-d_6 appeared as a sharp doublet at δ 3.89 coupled only to the OH proton at δ 4.40 (H-10 in methanol-d_4: s, 3.75 ppm) and did not show any further couplings in the COSY spectrum. Accordingly, it was deduced that C-11 is quaternary. Since a lactone carbonyl did not exist, C-11 could only be assigned the above-mentioned signal at δ 113.3, which in this case must be a sp^3 carbon substituted by three oxygen atoms, so that it must form an intramolecular orthocarboxylic acid diester ("ortholactone") group. This can only be achieved by attack of the hydroxyl group at C-14 on the lactone carbonyl of a normal (11)7-δ-lactone or by attack of a C-7*β* hydroxyl function on an (11)14-ε-lactone, respectively. The structural assignment for **6** was confirmed unambiguously by an HMBC experiment, in which correlations of C-11 with H-14b and H-7 proved the connectivity of the ortholactone group. The structure of **6** is deducible from the deacetylation product of **7**, i.e., 14-hydroxy-3-oxofloridanolide. Consequently, an alkaline hy-



drolysis was performed on **7**. The sole product obtained was found to be identical with the isolated compound **6**. Interestingly, the normal lactone could not be detected either by TLC or in the NMR spectra. To confirm this also for a protic solvent, spectra in methanol-d_4 were recorded, which, apart from minor shift differences, showed the same characteristics. It was concluded that compound **6** in solution is considerably more stable in the (11)7,14-ortholactone form than in the normal (11)14- or (11)7-lactone form. We are currently investigating such equilibria in previously isolated lactones.

It appears noteworthy that the 7-keto-11-aldehyde *seco*-prezizaane (upper right structure in the box marked in Scheme 1) underlying the structure of cycloparvifloralone **1** might represent a key intermediate in the biogenesis of the *seco*-prezizaane-type sesquiterpene lactones found in *Illicium* species. On mechanistic grounds it is speculated that **1** could be derived directly from a hypothetical prezizaane precursor by a retro-aldol-type reaction (Scheme 1, top). This type of reaction is widespread in nature. An analogous C−C cleavage occurs, for example, in glycolysis, where degradation of fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehyde-3-phosphate is catalyzed by aldolase.[7] The α-hydroxyaldehyde structure (reductone) underlying **1** could be oxidized easily to the corresponding carboxylic acid whose lactone **3** (parviflorolide), also found in *I. parviflorum*, might be the biogenetically most simple representative of the C-10 hydroxylated lactone types commonly found in *Illicium* species (Scheme 1, right).

While the C-10 hydroxylated lactones can thus be formed by oxidation, the 10-deoxy derivatives of the pseudoanisatin/dunnianin series could be derived by dehydration of the 10-hydroxy group from the (11)14-hemiacetal form of **1**, which then forms the tautomer of a pseudoanisatin-type (11)14-lactone (Scheme 1, left). Within this group of 10-deoxy compounds, representatives with (11)14-, (11)7-, and (11)3-lactonization have been isolated.[1,2] They are probably interrelated with each other via ortholactone intermediates analogous to **6**.

Experimental Section

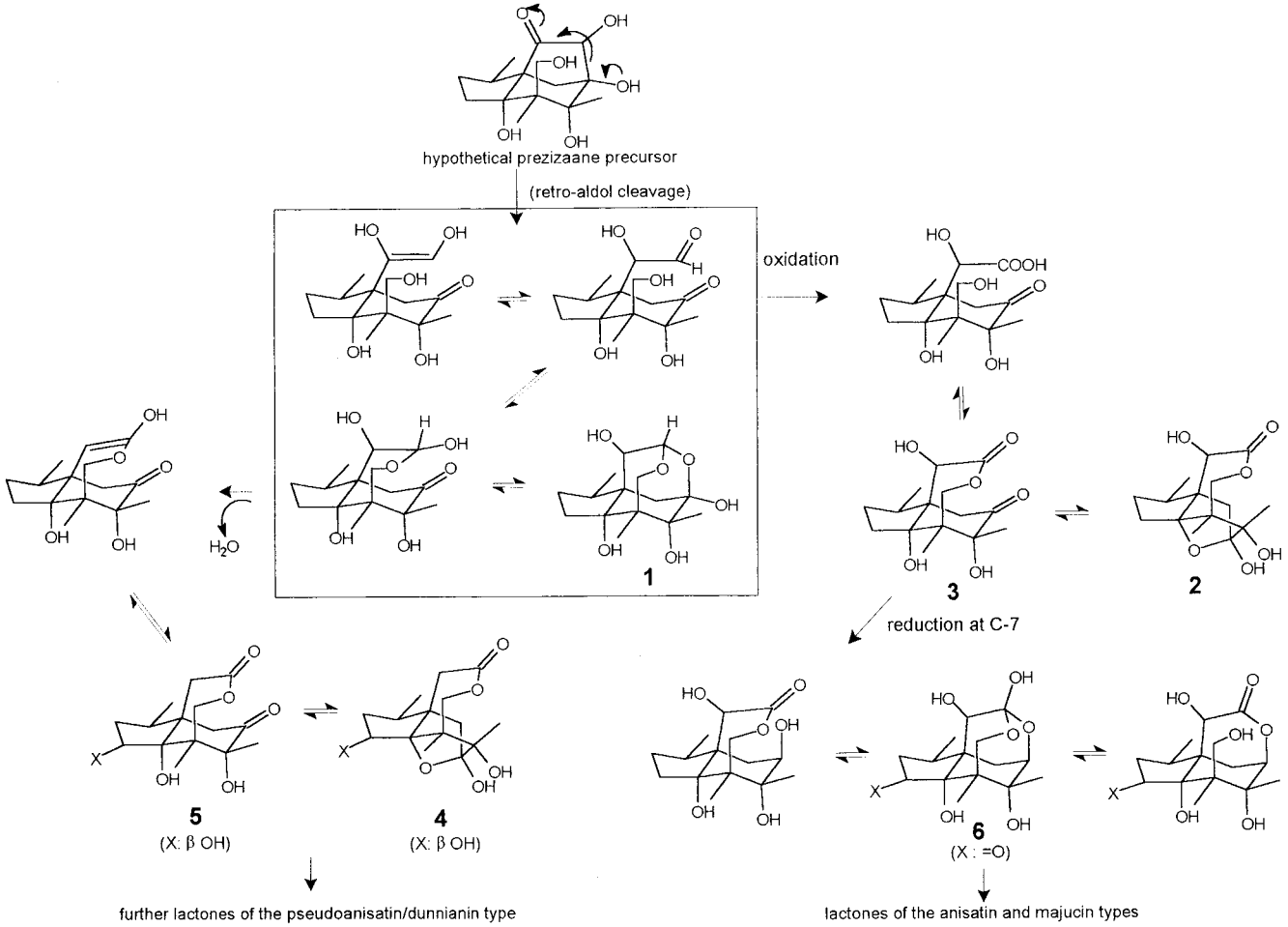
General Experimental Procedures. Melting points (uncorrected) were determined with a Leitz microscope type 350. Optical rotations were measured with a Perkin-Elmer 343 LC polarimeter in a 10 cm/0.35 mL microcuvette at 20 ± 0.5 °C. UV absorbance was investigated on a Beckman DB-G UV/vis spectrophotometer in MeOH. IR spectra were taken with a Perkin-Elmer 297 IR spectrometer in KBr.

NMR spectra were recorded with a Bruker DRX 500 spectrometer (500.13 /125.77 MHz), and those of **4** and **5** with a Bruker AM 400 spectrometer (400.13/100.61 MHz). All NMR spectra were taken at room temperature and referenced to TMS. Coupling constants for compound **1** were calculated with PCModel 4 from a molecular model generated and geometry optimized with the same program. DCI mass spectra (DCIMS) were recorded in the direct inlet mode using chemical ionization with NH_3 as reactant gas on a Finnigan-MAT INCOS 50 mass spectrometer. High-resolution FAB mass spectra (HR-FABMS) were taken with a Finnigan-MAT HSQ 30 mass spectrometer equipped with an IonTech FAB gun using Xe as FAB gas. TLC was performed using silica 60 plates (Merck) with cyclohexane/EtOAc (40:60) as solvent and anisaldehyde/H_2SO_4 as visualizing agent, with heating at 200 °C.

Plant Material. Leaves of *I. parviflorum* Michaux ex Ventenat were provided by Dr. R. P. Wunderlin, Institute for Systematic Botany, Department of Biology, University of South Florida (USF), Tampa, FL. A voucher specimen is deposited at the USF Herbarium (reg. no. R. P. Wunderlin 10708). For the origin of *I. floridanum* fruits, see refs 1 and 2.

Extraction and Isolation. The dried and powdered leaves of *I. parviflorum* (500 g) were extracted exhaustively with CH_2Cl_2. The residue (69 g) was macerated with 5 × 100 mL MeOH at room temperature. The methanol-soluble part (12 g) was separated by column chromatography on Sephadex LH-20 (400 g) and eluted with MeOH to yield six fractions. Fraction 3 (4.6 g) was further separated by column chromatography on 450 g silica gel with mixtures of *n*-hexane, EtOAc, *i*-PrOH, and MeOH, of increasing polarity, to yield 38 fractions. From fraction 29 (EtOAc/*i*-PrOH, 4:1, 56 mg), 36 mg of colorless needles of **1** was obtained upon standing in the eluent. Column chromatography of the mother liquor on 6 g of silica gel with CH_2Cl_2 gave an additional quantity of **1**, leading to a total yield of 50 mg of pure crystalline **1**. From fraction 28 (100% EtOAc, 30 mg), 11 mg of a crystalline substance was obtained in the eluent after standing, which was further purified by column chromatography on 3 g of silica gel with CH_2Cl_2 to yield 6 mg of colorless needles of **2** and **3** as an inseparable mixture. Compound **4** was detected in the NMR spectra of **5**, a constituent of *I. floridanum*.[1,2] It was inseparable from **5** by TLC. Compound **6** was isolated from the mother liquor remaining after crystallization of **5** from *I. floridanum* fruits[1] (90 mg). By column chromatography on 18 g silica gel with

Scheme 1. Proposed Biogenetic Interrelations of *Illicium* Sesquiterpenes



n-hexane/EtOAc mixtures of increasing polarity, 7 mg of pure crystalline **6** were obtained with *n*-hexane/EtOAc (30:70).

Partial Synthesis of Compound 6. A quantity (15 mg) of 14-acetoxy-3-oxofloridanolide (**7**)[2] (42 μmol) was dissolved in 3 mL of 1 N aqueous NaOH and stirred for 1 h at 60 °C. After adjustment to pH 6 with 1 N HCl, the solution was extracted with 3 × 30 mL of EtOAc. On evaporation of the solvent, the crystalline product (9 mg, 29 μmol) was analyzed by TLC and found to contain one pure compound with the same R_f value as **6**. The ^{1}H NMR data were identical with those of **6** (Table 2).

Cycloparvifloralone (1): mp 191 °C (EtOAc), sublimed at ca. 160 °C; $[\alpha]^{20}_D$ −3° (*c* 7.2, MeOH); UV (MeOH) no significant absorbance >215 nm; IR (KBr) ν_{max} 3390 (br), 2950, 2870 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; DCIMS (NH_3) *m/z* 318 $[M + NH_4]^+$ (100), 300 $[M + NH_4 - H_2O]^+$ (63); HRFABMS *m/z* 283.1535 ± 0.0022 $[M + H - H_2O]^+$ (calcd for $C_{15}H_{23}O_5$, 283.15455); TLC R_f 0.18, violet.

Cycloparviflorolide (2) and parviflorolide (3): (data on 4:1 equilibrium mixture) mp 196 °C (acetone) sublimed at ca. 165 °C; $[\alpha]^{20}_D$ +83° (*c* 1.2, EtOAc); UV (MeOH) no significant absorbance >215 nm; IR (KBr) ν_{max} cm^{-1}) 3420 (br), 2940, 2870, 1715, 1700 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; DCI(NH_3) MS *m/z* 316 $[M + NH_4]^+$ (100); TLC R_f 0.30, reddish brown.

4,7-Hemiketal of pseudoanisatin (4): ^{1}H and ^{13}C NMR data, see Tables 1 and 2.

(11)7,14-Ortholactone of 14-hydroxy-3-oxofloridanolide (6): mp 210 °C (acetone); $[\alpha]^{20}_D$ −33° (*c* 1.4, MeOH); UV (MeOH) no significant absorbance >215 nm; IR (KBr) ν_{max} 3400 (br), 3300 (br), 2930, 2880, 1740 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; DCIMS (NH_3) *m/z* 332 $[M + NH_4]^+$ (100); HRFABMS *m/z* 315.1450 ± 0.0030 $[M + H]^+$ (calcd for $C_{15}H_{23}O_7$, 315.1444); TLC R_f 0.16, orange-brown.

Acknowledgment. The author thanks Ms. Eva Müller for excellent technical assistance, Dr. R. P. Wunderlin, Institute for Systematic Botany, University of South Florida, Tampa, FL, for supplying the leaves of *I. parviflorum*, Dr. U. Matthiesen, Institut für Klinische Chemie und Laboratoriumsdiagnostik, Universität Düsseldorf, for recording the DCI mass spectra, and the NMR service staff at the Insitut für Anorganische Chemie und Strukturchemie, Universität Düsseldorf, for recording the NMR spectra. The help of Prof. Dr. H. Budzikiewicz, Institut für Organische Chemie, Universität zu Köln, Germany, in obtaining high-resolution mass data is most gratefully acknowledged.

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NP980382A