New *allo*-Cedrane Type Sesquiterpene Hemiketals and Further Sesquiterpene Lactones from Fruits of *Illicium floridanum*

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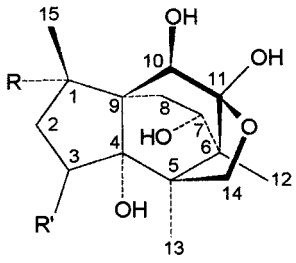
Three new tetracyclic sesquiterpene hemiketals possessing the very rare *allo*-cedrane carbon skeleton, debenzoyl-7-deoxo-1α,7α-dihydroxytashironin, debenzoyl-7-deoxo-7α-hydroxytashironin, and debenzoyl-7-deoxo-7α-hydroxy-3-oxotashironin (**1**−**3**), were isolated from the fruits of *Illicium floridanum* ELLIS (American Star Anise, Star bush). Their structures were elucidated by mass and NMR spectroscopic analyses. The molecular structure of **1**, including absolute stereochemistry, was determined by single-crystal X-ray diffraction of its monohydrate. Only one compound of this type, tashironin, has previously been isolated from *Illicium tashiroi*. Furthermore, a new sesquiterpene lactone of the *seco*-prezizaane type, 3,4-anhydro-13,14-dihydroxyfloridanolide (**4**), and the known anislactone B (**5**) were isolated. The occurrence of further *allo*-cedrane sesquiterpenes in another *Illicium* species confirms the hypothesis of previous authors that this type of compound is a biogenetic precursor of the typical *seco*-prezizaane sesquiterpene lactones found in this genus. Moreover, regarding their co-occurrence with anislactone B as reported here, they may also be considered precursors for the anislactone skeletal type.

Members of the genus *Illicium* (star anise, Illiciaceae) are characterized by the occurrence of a group of sesquiterpene lactones, such as the potent neurotoxin anisatin, which possess a unique carbon skeleton named *seco*-prezizaane.[1]

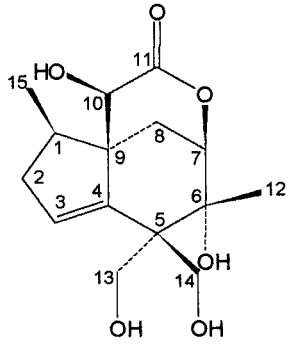
In continuation of an ongoing search for such sesquiterpenes in North American *Illicium* species,[2−4] the isolation and structure elucidation of three new sesquiterpenes **1**−**3**, possessing a hemiketal ring system of the very rare *allo*-cedrane type, from the fruits of *Illicium floridanum* ELLIS are now reported. Only one compound of this type has previously been described, tashironin from *Illicium tashiroi*.[5] Furthermore, a new *seco*-prezizaane sesquiterpene lactone, 13,14-dihydroxy-3,4-anhydrofloridanolide (**4**), and a known sesquiterpene dilactone, anislactone B (**5**),[6] were isolated from the same source.

Results and Discussion

Compound **1** possesses the molecular formula $C_{15}H_{24}O_6$, as established according to the mass spectrometric data (DCIMS: $[M + NH_4]^+$ at *m*/*z* 318 corresponding to M_r = 300) and the ^{13}C NMR spectrum (Table 1), in which 15 carbon resonances (three methyl, four methylene, two methine, and six quaternary carbons, assignment based on HMQC experiment) were observed. The ^1H NMR spectrum (see Experimental Section) showed a total of 19 protons attached to carbon, indicating the presence of five OH groups. Since the molecular formula contains four double-bond equivalents while neither olefinic nor carbonyl resonances could be observed in the ^{13}C NMR spectrum, the compound must be tetracyclic, with one of the rings containing the sixth oxygen atom. The resonance of a quaternary carbon at δ 105.9 clearly showed that a cyclic hemiketal must be present. The carbon skeleton hence must be tricyclic. The only tricarbocyclic sesquiterpene found in an *Illicium* species so far is tashironin from *I. tashiroi*.[5]



	R	R'
1	OH	H
2	H	H
3	H	=O



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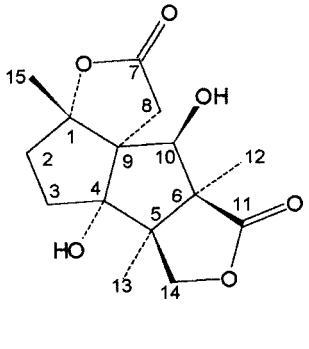

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The connectivity of carbon and hydrogen atoms was unambiguously deduced from the 2- and 3-bond H−C correlations observed in the HMBC spectrum (Figure 1A, Supporting Information), which were in full agreement with a tashironin type compound and led to the depicted structure. As the most characteristic feature, the presence of a C-11−C-6 ring closure clearly followed from an intense cross signal between *C*-11 and CH_3-12, while interactions between *C*-11 and CH_2-14 showed that the hemiketal

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Table 1. ^{13}C NMR Data of Compounds **1**−**4** (125 MHz, $CDCl_3$); All Assignments Were Confirmed by HMQC and HMBC Experiments

	δ_C (ppm)			
carbon	**1**[a]	**2**	**3**	**4**
1	80.4	39.0	35.0	44.5
2	40.4	31.3	44.9	39.7
3	31.5	33.2	214.0	135.3
4	87.3	86.7	81.8	137.2
5	49.2	49.8	47.2	49.8[b]
6	49.5	49.5	49.5	75.5
7	70.1	71.2	71.0	83.9
8	32.0	38.2	35.8	31.6
9	54.4	51.5	48.9	49.6[b]
10	77.6	77.6	77.1	71.8
11	105.9	106.4	105.5	175.2
12	12.7	13.2	12.6	22.2
13	15.8	16.9	17.3	60.9
14	72.8	73.2	71.5	64.4
15	20.7	14.0	12.9	13.8

[a] 20% CD_3OD. [b] Assignment interchangeable.

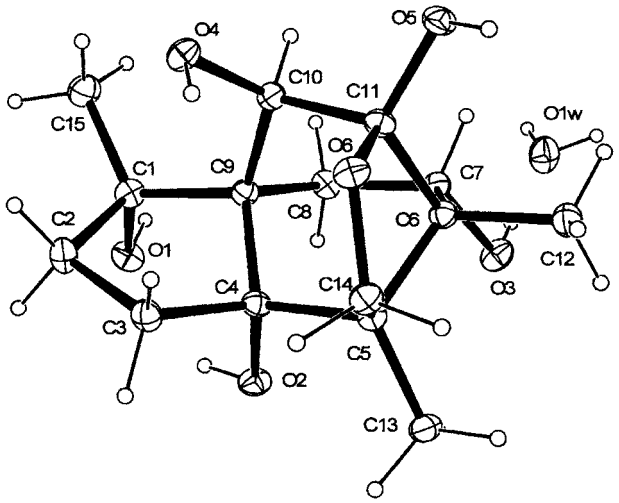

Figure 1. Structure of **1**·H_2O (debenzoyl-7-deoxo-1α,7α-dihydroxytashironin monohydrate) at 120 K, with ellipsoids at the 50% probability level.

moiety is part of a tetrahydrofuran ring closed between C-14−O and C-11.

The relative stereochemistry was deduced from the NOESY spectrum, in which all observed correlations were in agreement with the interatomic distances measured in a 3D molecular model (Figure 1B, Supporting Information).

The molecular structure of **1**, including the absolute configuration, was determined by X-ray crystallography (Figure 1 and Tables 2 and 3 of Supporting Information). The crystal was found to consist of the monohydrate, and the water molecule present in the unit cell contributes to an intricate network of inter- and intramolecular hydrogen bonds. All OH and H_2O hydrogen atoms donate to hydrogen bonds. Two of the OH groups, those at C-4 and C-10, form intramolecular hydrogen bonds. That at C-4 has the OH group at C-1 as its acceptor, with an O···O distance of 2.7142(11) Å and angle about H of 146(2)°. The OH group at C-10 forms a bifurcated hydrogen bond, with an intramolecular component to the tetrahydrofuran oxygen (O···O 2.6537(11) Å) and an intermolecular component to the OH at C-4 (O···O 2.9850(13) Å). The water molecule accepts one hydrogen bond from the OH group at C-7 (O···O 2.79923(15) Å) and donates two, to the OH groups at C-1 (O···O 2.8578(14) Å) and C-11 (O···O 2.9052(13) Å).

Thus the structure of this new natural product was unabmbiguously established as debenzoyl-7-deoxo-1α,7α-dihydroxytashironin (**1**). At the same time the absolute stereochemistry determined for **1** supports the stereochemistry postulated for tashironin by previous authors.[5]

Compounds **2** and **3** were obtained as an inseparable mixture (1:0.8, deduced from intensity of ^1H NMR signals) using several normal phase and reversed phase chromatographic systems so that their structures had to be solved from the mixture. The DCIMS showed quasimolecular ions $[M + NH_4]^+$ at *m/z* 302 and 316, revealing molecular masses of 284 and 298, for **2** and **3**, respectively. This information, in combination with the ^{13}C NMR and HMQC spectra, which allowed clear distinction between all carbon and proton signals of **2** and **3**, led to the molecular formulas $C_{15}H_{24}O_5$ and $C_{15}H_{22}O_6$, respectively.

The ^1H NMR signals of **2** were very similar to those of **1**. As the major difference, the signal for CH_3-15 was shifted upfield and split into a doublet coupled to a multiplet for H-1. In the ^{13}C NMR spectrum (Table 1) the signals of C-1 and C-15 were shifted upfield by 41 and 7 ppm, respectively, in comparison with **1**. These findings indicated that **2** was the 1-deoxy derivative of **1**. This was confirmed by the 2- and 3-bond proton−carbon correlations observed in the HMBC spectrum, which showed that the atomic connectivity was essentially the same as in **1**.

The NMR signals of **3** were very close to those of **2** in most respects, except for the absence of one CH_2 group, which was replaced by a keto-carbonyl (δ_C 214.0). The resonances for C-1/H-1 and C-2/H-2 were markedly shifted downfield in comparison with the corresponding signals of compound **2**, so that the keto group was expected to be at C-3. This was confirmed by the HMBC spectrum in which the keto carbonyl showed correlations exclusively with H-2a, H-1, and H-2b. Moreover, all further long-range proton−carbon connectivities observed between the resonances of **3** were the same as in compound **2**. Thus, the structure of **3** was unambiguously established as the 3-oxo-derivative of **2**. The relative stereochemistry of both **2** and **3** was assigned on the basis of a NOESY experiment, which showed that both compounds had the same characteristic NOEs as observed for **1**. Thus, the structures of the new natural products **2** and **3** correspond to debenzoyl-7-deoxo-7α-hydroxytashironin and debenzoyl-7-deoxo-7α-hydroxy-3-oxotashironin, respectively.

The structure of compound **4** ($C_{15}H_{22}O_6$) was solved by analysis of its NMR spectra (^1H, ^{13}C, DEPT, COSY, HMQC, HMBC) in comparison with those of compounds isolated previously from the same source.[2−4] Its spectra differed from those of 13-acetoxy-14(*n*-butyryloxy)floridanolide[3] by the absence of signals for the acyl moieties and the presence of olefinic resonances for a double bond between C-3 and C-4, so that the structure of this new sesquiterpene lactone was unambiguously established as 13,14-dihydroxy-3,4-anhydrofloridanolide.

Compound **5** was found to be identical with anislactone B, previously isolated from *I. anisatum*, by its mass and NMR spectral data (DCIMS and ^1H, ^{13}C, HMQC, HMBC, and NOESY NMR) in comparison with the literature data.[6]

The occurrence of *allo*-cedrane[7] sesquiterpenes in *Illicium* species is of high interest with respect to the biogenetic origin of the typical sesquiterpenes of this genus. It has been proposed that this carbon skeleton, which is still intact with respect to the isoprene rule, could be a biogenetic precursor for terpenoids with an anisatin-type (*seco*-prezizaane) skeleton (such as **4** and many others).[5] Moreover, co-occurrence of **1**−**3** with anislactone B (**5**) indicates that the biogenetic origin of the anislactone skeleton might

also lie in *allo*-cedrane precursors. While the anisatin type compounds may be directly formed from *allo*-cedrane precursors by scission of the C-11−C-6 bond[5] (which would mean that their "*seco*-prezizaane" skeleton should actually be termed *seco*-*allo*-cedrane skeleton), the anislactone skeleton could arise from analogous scission between C-7 and C-6 followed by rearrangement under formation of the bond between C-6 and C-10 (Figure 3, Supporting Information). The problem that an inversion of configuration at C-9 would have to take place, if anislactone were derived from a *seco*-prezizaane compound of the majucin type as proposed by earlier authors,[6] would not arise in this case.

Experimental Section

General Experimental Procedures. Optical rotation was measured with a Perkin-Elmer 241LC polarimeter at 20 °C using a microcuvette (0.35 mL). Melting points (uncorrected) were determined with a Leitz microscope type 350. Mass spectra were recorded on a Finnigan MAT INCOS 50 mass spectrometer in the direct inlet mode using chemical ionization with NH_3 as reactant gas (DCIMS) or electron impact ionization (EIMS, 70 eV). All NMR spectra were recorded at room temperature with a Bruker DRX 500 spectrometer at 500.13/125.77 MHz. Spectra were referenced to solvent signals (^{1}H NMR $CHCl_3$ at δ 7.270; ^{13}C NMR $CDCl_3$ at δ 77.20).

X-ray diffraction data for compound **1**·H_2O[8] were collected at 120 K on an Nonius KappaCCD area-detector diffractometer equipped with Mo Kα (λ = 0.71073 Å) radiation, to a maximum θ of 35°. Data reduction was by DENZOSMN[9] and SCALEPAK.[9] The structure was solved by direct methods and refined by full-matrix least-squares using SHELXL.[10] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms bonded to O were refined isotropically, while those on C were constrained in calculated positions. Atomic coordinates, crystal data, final *R* values, and other details are included in Tables 2 and 3, Supporting Information. The absolute configuration was determined by measuring intensity data from the same crystal at 298 K using Cu Kα radiation on an Enraf-Nonius CAD4 diffractometer, to a maximum θ of 75° (1402 Friedel pairs). The Flack parameter[11] refined to a value of −0.04(11), confirming the correctness of the absolute configration reported for the low-temperature Mo Kα determination. Unit-cell dimensions at 298 K are a = 7.5240(4) Å, b = 11.0790(4) Å, c = 8.9360(4) Å, β = 100.711(4)°, V = 731.91(6) Å^3, R = 0.029 for 2992 observed data and 231 variables.

Plant Material. Fruits of *I. floridanum* Ellis were collected in 1995.[2] The plants were identified by 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. Compounds **1**, **4**, and **5** were isolated from the MeOH extract (113 g) of 750 g of *I. floridanum* fruits obtained after extraction with CH_2Cl_2.[2] The EtOAc-soluble part of this extract (3.1 g, obtained by maceration with 4 × 100 mL EtOAc) was separated by column chromatography (CC) on Sephadex LH-20 with MeOH to yield fractions I−XIII. Fraction IV was further separated on silica with *n*-hexane/EtOAc mixtures of increasing polarity to yield 26 fractions (hexane/EtOAc 1:1 = fractions 1−5; 4:6 = fractions 6−19; 3:7 = fractions 20−26). From fraction 7, 5.8 mg of colorless **5** crystallized upon standing in the eluent. Fraction 15 was further separated by CC on silica with CH_2Cl_2/EtOAc/MeOH (8:1:1) to yield four fractions, of which fraction 2 yielded 4 mg of colorless amorphous **4** after purification on a silica column with EtOAc as eluent. Fraction 16 was separated on silica with CH_2Cl_2/EtOAc/MeOH (8:1:1) to yield three fractions, of which fraction 3 was separated by PTLC (silica, eluent as above) to yield 2 mg of colorless amorphous **1**, which crystallized in the form of colorless monohydrate prisms after a solution in acetone had been evaporated and the residue treated with CH_2Cl_2.

Compounds **2** and **3** were isolated from fraction D 13 of the CH_2Cl_2 extract, which was obtained as described previously.[2] Fraction D 13 (36 mg) was separated by CC on silica with *n*-hexane/EtOAc (2:8), followed by MPLC on silica with an *n*-hexane/EtOAc gradient and PTLC (silica, hexane/EtOAc, 2:8) to yield 5 mg of **2** and **3** as a colorless glassy mixture.

Debenzoyl-7-deoxo-1α,7α-dihydroxytashironin (**1**): colorless prisms (monohydrate); loss of solvent at 115 °C, mp of remaining solid 183 °C; $[\alpha]^{20}_D$ −8° (*c* 0.46, MeOH); ^{1}H NMR ($CDCl_3$ + 20% CD_3OD, 500 MHz) δ 3.74 (1H, d, J = 9.5 Hz, H-14a), 3.66 (1H, dd, J = 1.5, 9.5 Hz, H-7), 3.57 (1H, d, J = 9.5 Hz, H-14b); 3.38 (1H, s, H-10), 2.15 (1H, ddd, J = 4.5, 12.0, 13.2 Hz, H-2β), 2.04 (1H, dd, J = 1.5, 15.1 Hz, H-8α), 2.03 (1H, ddd, J = 5.0, 12.0, 13.9 Hz, H-3β), 1.93 (1H, ddd, J = 5.0, 9.5, 13.2 Hz, H-2α), 1.70 (1H, ddd, J = 4.5, 9.5, 13.9 Hz, H-3α), 1.57 (1H, dd, J = 9.5, 15.1 Hz, H-8β), 1.22 (3H, s, H-15, CH_3-15), 1.09 (3H, s, CH_3-12), 1.04 (3H, s, CH_3-13); DCIMS *m*/*z* 318 $[M + NH_4]^+$ (40), 300 $[M + NH_4 - H_2O]$ (100); EIMS *m*/*z* 300 $[M]^+$ (<1), 282 $[M - H_2O]^+$ (4), 267 $[M - H_2O - CH_3]^+$ (5), 264 $[M - 2H_2O]^+$ (17), 249 $[M - 2H_2O - CH_3]^+$ (5), 246 $[M - 3H_2O]^+$ (5), 235 (5), 229 (21), 217 (9), 207 (13), 199 (21), 193 (23), 189 (24), 175 (31), 165 (39), 161 (44), 147 (47), 138 (56), 126 (80), 123 (68), 113 (83), 109 (85), 99 (77), 79 (59), 67 (69), 55 (84), 43 (85), 41 (100).

Debenzoyl-7-deoxo-7α-hydroxytashironin (**2**): colorless glassy mixture with **3**; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.76 (1H, d, J = 9.5 Hz, H-14a), 3.64 (1H, br d, J ≈ 10 Hz, H-7), 3.61 (1H, d, J = 9.5 Hz, H-14b), 3.45 (1H, s, H-10), 2.10 (1H, m, H-1), 2.08 (1H, m, H-3β), 2.00 (1H, m, H-2β), 1.84 (1H, dd, J = 9.5, 14.8 Hz, H-8β), 1.62 (1H, dd, J = 1.6, 14.8 Hz, H-8α), 1.52 (1H, dddd, J ≈ 3, 9, 12, 13 Hz, H-2α), 1.45 (1H, ddd, J = 3.5, 9.2, 13.0 Hz, H-3α), 1.11 (3H, s, CH_3-12), 1.09 (3H, s, CH_3-13), 1.05 (3H, d, J = 6.9 Hz, CH_3-15); DCIMS *m*/*z* 302 $[M + NH_4]^+$ (98), 284 $[M + NH_4 - H_2O]^+$ (100).

Debenzoyl-7-deoxo-7α-hydroxy-3-oxotashironin (**3**): colorless glassy mixture with **2**; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.92 (1H, d, J = 9.8 Hz, H-14a), 3.64 (1H, br d, J ≈ 10 Hz, H-7), 3.60 (1H, s, H-10), 3.55 (1H, d, J = 9.8 Hz, H-14b), 2.51 (1H, m*, H-2β), 2.38 (1H, m*, H-1), 2.33 (1H, m*, H-2α), 1.97 (1H, dd, J = 9.2, 14.8 Hz, H-8β), 1.71 (1H, dd, J = 1.6, 14.8 Hz, H-8α), 1.25 (3H, s, CH_3-13), 1.14 (3H, d, J = 6.9 Hz, CH_3-15), 1.09 (3H, s, CH_3-12), *non-first-order spin system with $^2J_{2\alpha,2\beta} \approx -18$, $^3J_{1,2\beta} \approx {}^3J_{1,2\alpha} \approx 9-10$, $^3J_{1,15}$ = 6.9 Hz; DCIMS *m*/*z* 316 $[M + NH_4]^+$ (100), 298 $[M + NH_4 - H_2O]^+$ (50).

13,14-Dihydroxy-3,4-anhydrofloridanolide (4): colorless amorphous solid; $[\alpha]^{20}_D$ +49° (*c* 0.66, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.93 (1H, m*, H-3), 4.16 (1H, dd, J = 2.0, 4.0 Hz, H-3), 4.16 (1H, dd, J = 2.0, 4.0 Hz, H-7), 4.10 (1H, d, J = 12.0, Hz, H-14a), 4.04 (1H, s, H-10), 3.94 (1H, d, J = 12.0 Hz, H-14b), 3.21 (1H, d, J = 12.3 Hz, H-13a), 2.97 (1H, br d, J = 12.3 Hz, H-13b), 2.42 (1H, m*, H-2β), 2.23 (1H, dd, J = 2.0, 14.2 Hz, H-8α), 2.16 (1H, m*, H-2α), 2.12 (1H, m*, H-1), 1.30 (3H, s, CH_3-12), 1.09 (3H, d, J = 6.9 Hz, CH_3-15), *non-first-order spin system with $^2J_{2\alpha,2\beta} \approx -18$, $^3J_{1,2\beta} \approx 8$, $^3J_{1,2\alpha} \approx 10$, $^3J_{2\beta,3} \approx 3$, $^3J_{2\alpha,3} \approx 2$, $^3J_{1,15}$ = 6.9 Hz; DCIMS *m*/*z* 430 $[M + NH_4]^+$ (100); EIMS *m*/*z* 298 $[M - H_2O]^+$ (<1), 265 $[M - H_2O - CH_3]^+$ (1), 262 $[M - 2H_2O]^+$ (1), 250 $[M - H_2O - 2CH_3]^+$ or $[M - H_2O - HCHO]^+$ (23), 232 (5), 221 (7), 205 (8), 187 (14), 175 (100), 161 (44), 147 (50), 131 (44), 117 (39), 105 (39), 93 (38), 79 (42), 69 (34), 55 (38), 43 (45), 41 (46).

Anislactone B (5): colorless prisms (MeOH/EtOAc); mp 278−281 °C; $[\alpha]^{20}_D$ −8° (*c* 1.14, MeOH); DCIMS *m*/*z* 314 $[M + NH_4]^+$ (100).

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Supporting Information Available: Table 2: Atomic coordinates and their estimated standard deviations for the X-ray structure of compound **1**. Table 3: Crystal data and summary of intensity data collection and structure refinement for compound **1**. Figure 2: 2- and 3-bond proton→carbon correlations and representative NOE effects observed in the HMBC and NOESY spectra of debenzoyl-7-deoxo-1α,7α-dihydroxytashironin A (**1**). Figure 3: Possible role of a*llo*-cedrane type compounds as precursors for other *Illicium*-sesquiterpenes. This material is available free of charge via the Internet at http://pubs.acs.org.

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