Lepadins F−H, New *cis*-Decahydroquinoline Alkaloids from the Australian Ascidian *Aplidium tabascum*

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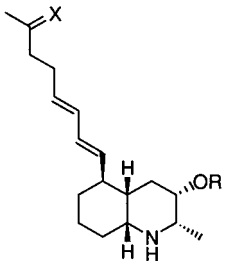
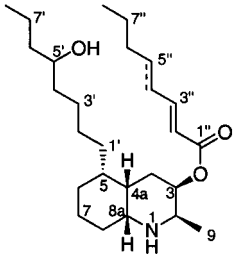
Chemical investigation of a Great Barrier Reef ascidian, *Aplidium tabascum*, has resulted in the isolation of three new *cis*-decahydroquinoline alkaloids, lepadins F−H (**4**−**6**). The three new compounds differ from the previously isolated lepadins A−C (**1**−**3**) in that they contain a fully saturated 5-hydroxyoctyl side chain attached at C-5, an unsaturated eight-carbon ester moiety attached to C-3, and opposite stereochemistry at C-5 and C-3. Lepadins G (**5**) and H (**6**) are epimers at C-2. NMR and molecular modeling studies indicated that the three new compounds adopt a chair−chair conformation in which the nitrogen equatorially substitutes the cyclohexyl ring. This contrasts with lepadins A−C (**1**−**3**), which adopt a chair−chair conformation in which the nitrogen axially substitutes the cyclohexyl ring.

Simple decahydroquinolines (DHQs) were first reported from the skins of dendrobatid frogs,[1] and to date approximately 30 *cis*- and *trans*-DHQs have been isolated from amphibian sources.[2] All of these alkaloids contain alkyl substituents attached at C-2 and C-5. 2,5-Dialkyl-DHQ alkaloids have also been isolated from ascidians,[3,4] marine flatworms,[3] and more recently myrmicine ants.[5−7] This discovery lends support to the hypothesis that, for chemical defense, dendrobatid frogs sequester this class of alkaloid from their arthropod diet, of which ants are a major item.[7] Interestingly, the isolation of the anticancer *cis*-DHQs, lepadins A−C (**1**−**3**) from both the flatworm *Prosthececeraeus villatus* and its ascidian prey *Clavelina lepadiformis*, also suggests the sequestering of these metabolites for biological defense purposes.[3] *cis*-DHQs have been reported to equilibrate between two chair−chair conformations in solution.[8] For the parent compound, *cis*-DHQ, the preferred conformation is one in which the nitrogen axially substitutes the cyclohexyl ring. However, the 2,5-dialkyl-*cis*-DHQs isolated from frog skins possess a twin chair conformation in which the nitrogen is situated either equatorially or axially relative to the cyclohexyl ring. The conformation adopted is dependent upon the stereochemistry of the substituents attached at C-2 and C-5.[7] As part of our continuing investigation of Great Barrier Reef ascidians, we report the isolation of three new 2-methyl-5-(5′-hydroxyoctyl)-DHQs, lepadins F−H (**4**−**6**), from *Aplidium tabascum* Kott (Polyclinidae). During the preparation of the manuscript we found that Wright et al. have also isolated lepadin F (**4**) as an antiplasmodial and antitrypanosomal compound from *Didemnum* sp. from the Great Barrier Reef.[9]

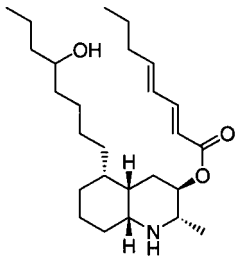
Results and Discussion

A freeze-dried sample of *A. tabascum* was exhaustively extracted with CH_2Cl_2. The CH_2Cl_2 extract was separated by centrifugal partition chromatography (CPC) using the solvent system heptane−CH_2Cl_2−CH_3CN (10:3:7) with the upper phase as the mobile phase. Fractions containing the lepadins were combined and further purified using NH_2-bonded silica flash chromatography followed by repeated purification using normal-phase NH_2 HPLC to yield lepadins F (**4**, 3.2 mg, 0.11% dry wt), G (**5**, 4.5 mg, 0.16% dry wt), and H (**6**, 21.0 mg, 0.72% dry wt).





4
5 = $\Delta^{4"}$



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Lepadin G (**5**) was obtained as an unstable red gum. A pseudomolecular ion in the positive high-resolution liquid secondary ion mass spectrum [(+)-HRLSIMS] at *m*/*z* 420.3490 (Δ 2.9 ppm) allowed a molecular formula of $C_{26}H_{45}NO_3$ to be assigned to **5**. Strong absorption bands at 3375, 2928, and 1709 cm^{-1} in the IR spectrum suggested that **5** contained hydroxyl, amine, and unsaturated ester carbonyl functionalities. The ^{13}C NMR spectrum (Table 1) displayed signals for all 26 carbons, including an ester carbonyl carbon at δ 166.6. A DEPT spectrum indicated that **5** contained three methyl, 12 methylene, and 10 methine carbons. ^1H NMR signals (Table 2) at δ 7.59 (1H, dd, J = 15.6, 10.8 Hz), 6.00 (1H, d, J = 15.6 Hz), 5.97 (1H, dd, J = 15.0, 10.8 Hz), and 5.75 (1H, dt, J = 15.0, 7.2 Hz) together with an UV absorption band at 263 nm indicated the presence of a conjugated (*E*,*E*) diene. The gCOSY

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Table 1. ^{13}C NMR Data for Lepadins F−H (**4**−**6**)[a]

C	**4**	**5**	**6**
2	47.6 (d)[b]	47.6 (d)	52.2 (d)
3	71.2 (d)	71.3 (d)	72.6 (d)
4	23.9 (t)	23.9 (t)	23.3 (t)
4a	33.2 (d)	33.0 (d)	33.4 (d)
5	39.8 (d)	39.8 (d)	39.0 (d)
6	26.9 (t)	27.0 (t)	27.8 (t)
7	25.7 (t)	25.8 (t)	23.0 (t)
8	25.3 (t)	25.2 (t)	29.8 (t)
8a	55.8 (d)	55.7 (d)	55.1 (d)
9	18.3 (q)	18.3 (q)	19.1 (q)
1′	33.5 (t)	33.5 (t)	33.0 (t)
2′	27.4 (t)	27.3 (t)	27.7 (t)
3′	26.2 (t)	26.2 (t)	26.1 (t)
4′	38.1 (t)	38.1 (t)	38.1 (t)
5′	71.2 (d)	71.1 (d)	71.2 (d)
6′	40.3 (t)	40.3 (t)	40.3 (t)
7′	19.2 (t)	19.2 (t)	19.2 (t)
8′	14.4 (q)	14.4 (q)	14.3 (q)
1″	166.0 (s)	166.6 (s)	166.5 (s)
2″	121.9 (d)	120.2 (d)	120.0 (d)
3″	149.5 (d)	145.4 (d)	146.0 (d)
4″	32.3 (t)	129.0 (d)	129.1 (d)
5″	27.9 (t)	141.2 (d)	144.6 (d)
6″	31.6 (t)	35.1 (t)	35.0 (t)
7″	22.7 (t)	22.1 (t)	22.0 (t)
8″	14.1 (q)	13.7 (q)	13.6 (q)

[a] Spectra were recorded at 150 MHz in C_6D_6 at 30 °C. [b] Implied multiplicity determined by DEPT (s = C, d = CH, t = CH_2, q = CH_3).

experiment provided evidence that the diene was attached to a propyl group. HMBC correlations from the olefinic protons, δ 6.00 and 7.59, to the ester carbonyl carbon at δ 166.6 allowed the octa-2,4-dienoyl group to be assigned. Isolated signals in the ^{1}H NMR spectrum at δ 2.82 (1H, ddd, J = 12.9, 4.8, 3.7 Hz) and 2.86 (1H, qd, J = 6.4, 1.0 Hz) were assigned to protons on carbons [C-8a (δ 55.7) and C-2 (δ 47.6)] adjacent to the nitrogen, following HMQC analysis. A HMQC spectrum allowed all of the protonated carbons to be assigned, and apart from the four olefinic methines and two nitrogenated methines, a further two isolated proton resonances at δ 5.02 (1H, ddd, J = 3.7, 3.7, 1.0 Hz) and 3.42 (1H, tt, J = 6.1, 6.1 Hz) could be assigned to oxygenated methines. COSY correlations were observed from the signal at H-2 to a methyl signal at δ 1.08 (3H, d, J = 6.4 Hz) and the oxygenated methine signal at δ 5.02. This latter resonance (H-3) showed a HMBC correlation to the ester carbonyl carbon at δ 166.6, confirming that C-3 was the point of attachment of the octadienoyl side chain. COSY correlations were observed from H-3 to methylene protons δ 1.35 and 1.83 and from these protons to a methine proton δ 2.19 (H-4a). H-4a showed COSY correlations to the other nitrogenated methine H-8a and to a methine proton at δ 1.34 (H-5). HMBC correlations observed between H-2 and C-8a and between H-8a and C-2 confirmed a nitrogen bridge between C-2 and C-8a. COSY correlations from δ 2.82 and 1.34 allowed all of the remaining proton signals around the cyclohexyl portion of the DHQ ring system to be assigned as shown in Table 2. A 4.8 Hz coupling between H-4a and H-8a indicated that the DHQ contained a cis ring junction. This assignment was supported by the lack of Bohlmann bands in the IR spectrum.[10] The remaining unassigned proton signals were a methyl triplet at δ 0.90 and a complex band between δ 1.10 and 1.40, representing 12 methylene protons. HMBC correlations were observed between the oxygenated methine proton at δ 3.42 and methylene carbons at δ 19.1, 26.1, 38.1, and 40.3. COSY correlations were observed from the protons δ 1.30 and 1.40 attached to the carbon at δ 19.2, to the methyl triplet, while the protons δ 1.31 attached to the carbon at δ 26.1 showed a correlation at δ 1.28, which in turn correlated to methylene protons at δ 1.10. The methylene protons at δ 1.10 showed HMBC correlations to the DHQ ring carbons C-5 and C-6 and COSY correlations to the methine proton at δ 1.32 (H-5). These correlations indicated that a 5-hydroxyoctyl side chain was attached to C-5 of the DHQ.

Cis-fused DHQs can adopt either of two twin chair conformations.[8] The most stable conformation for *cis*-DHQ is one in which the nitrogen is situated axially with respect to the cyclohexyl group. However it is only in the less stable conformation where the nitrogen is orientated equatorially with respect to the cyclohexyl ring that one observes strong hydrogen−hydrogen interactions between the axial protons H-2, H-4α, and H-8α. A ROESY experiment and detailed analysis of the proton coupling constants allowed the conformation of the DHQ system and the relative stereochemistry of the substituents to be assigned for lepadin G (**5**). Strong 1,3 and 1,4 hydrogen−hydrogen correlations in the ROESY spectrum were observed between H-2 and H-4α, and H-2 and H-8α, respectively. This indicated that the DHQ of **5** adopted a chair−chair conformation in which the nitrogen was situated equatorially with respect to the cyclohexyl ring. A large coupling (12.9 Hz) between H-5 and H-6α indicated that H-5 was axial. Finally a small coupling (1.0 Hz) between H-2 and H-3 and a ROESY correlations from H-2 to H-3 indicated that the ester substituent attached at C-3 was axially orientated. As the side chain hydroxyl was an extended distance away from the rest of the chiral centers in the molecule, the relative stereochemistry at this carbon (C-5′) could not be assigned.

The major metabolite, lepadin H (**6**), was isolated as an unstable red gum. The molecular formula $C_{26}H_{45}NO_3$ was determined by interpretation of the $[MH]^+$ ion at m/z 420.4481 (Δ 0.89 ppm) in the (+)-HRLSIMS. Comparison of the ^{13}C and ^{1}H NMR data of **6** with **5** (Tables 1 and 2) showed few differences. The only major differences were associated with the signals for C-2 and C-8, suggesting a difference in stereochemistry at C-2. Full 2D NMR analysis confirmed that **6** had the same gross structure as **5**. However ROESY correlations observed between the methyl protons H-9 attached to C-2 and H-4α and H-8α indicated that lepadin H (**6**) was a C-2 epimer of lepadin G (**5**).

Lepadin F (**4**) was isolated as an unstable red gum that gave a pseudomolecular ion in the (+)-HRLSIMS at m/z 422.3630 (Δ 0.89 ppm) appropriate for a molecular formula $C_{26}H_{47}NO_3$. Comparison of the NMR data for lepadin F (**4**) with the NMR data for lepadin G (**5**) (Tables 1 and 2) revealed that the two molecules had identical DHQ ring systems. The 5′-hydroxyloctyl group was also common to both molecules. Replacement of two of the olefinic methines by an extra two methylenes in the spectra of **4** indicated that the octa-2,4-dienoyl ester moiety of **5** was replaced in **4** by an octa-2-enoyl ester moiety.

Molecular modeling studies using Macromodel were performed on *cis*-DHQ, 2-methyl-*cis*-DHQ, and lepadins A (**1**), G (**5**), and H (**6**). The minimum energy conformation for the parent compound, *cis*-DHQ, was confirmed to be the twin chair conformation in which the nitrogen axially substituted the cyclohexyl ring. The energy difference between this conformer and the other twin chair conformation was 4.8 kJ/mol. Significantly the addition of a methyl substituent at C-2 led to the twin chair conformation with the nitrogen equatorially substituting the cyclohexyl ring being the lowest energy conformation. The energy difference between the two twin chair conformers was 4.6 kJ/

Table 2. ^{1}H and HMBC NMR Data for Lepadins F−H (**4**−**6**)[a]

	lepadin F (**4**)		lepadin G (**5**)		lepadin H (**6**)	
H	^{1}H (mult., *J* in Hz)	HMBC[b]	^{1}H (mult., *J* in Hz)	HMBC	^{1}H (mult., *J* in Hz)	HMBC
2	2.84 (qd, 6.6, 1.8)	3, 9, 8a	2.86 (qd, 6.4, 1.0)	3, 4, 8a, 9	3.19 (qd, 6.4, 4.8)	3, 4, 8a, 9
3	5.00 (br s)	2, 4a, 4, 9, 1″	5.02 (ddd, 3.7, 3.7, 1.0)	1″, 4a, 4	4.96 (ddd, 4.6, 4.6, 4.8)	4a, 9, 1″
4α	1.35 (m)	4a	1.35 (m)	4a, 8a	1.66 (ddd, 14.9, 10.8, 4.6)	3, 4a, 5, 8a
4*β*	1.82 (ddd, 13.8, 4.0, 3.0)	2, 3, 4a, 8a	1.83 (ddd, 14.5, 3.7, 3.7)	3, 8a, 2, 4a	1.58 (ddd, 14.9, 4.6, 4.6)	5, 8a
4a	2.17 (dddd, 13.8, 4.0, 4.0, 4.6)	5, 8, 8a	2.19 (dddd, 13.2, 4.8, 4.4, 4.4)	4, 5, 8a	2.28 (dddd, 10.8, 4.6, 4.8, 5.4)	3, 5, 6, 8, 8a
5	1.35 (m)		1.32 (m)	4a	1.37 (m)	
6α	0.80 (dddd, 13.2, 13.2, 13.2, 3.6)	5, 7	0.80 (dddd, 12.9, 12.9, 12.9, 3.7)	5, 7	0.96 (m)	
6*β*	1.24 (m)	1′	1.26 (m)	1′	1.19 (m)	
7α	1.65 (m)	5	1.66 (ddddd, 12.9, 4.1, 3.7, 3.7, 3.7)	5, 8a	1.52 (m)	6
7*β*	1.13 (m)	5	1.15 (m)		1.02 (m)	6
8α	1.58 (dddd, 13.2, 13.2, 12.6, 4.2)	5, 7, 8a	1.58 (dddd, 12.9, 12.9, 12.9, 4.1)	7, 8a	1.36 (m)	
8*β*	1.48 (m)		1.48 (dddd, 12.9, 4.4, 3.7, 3.7)	6, 8a	1.72 (dddd, 12.6, 4.8, 4.6, 4.8)	8a
8a	2.81 (ddd, 12.6, 4.6, 4.2)	2, 4	2.82 (ddd, 12.9, 4.8, 3.7)	2	3.04 (ddd, 10.2, 4.8, 4.8)	2, 7, 8, 4a, 5
9	1.08 (d, 6.6)	2, 3	1.08 (d, 6.4)	2, 3	1.13 (d, 6.4)	2, 3
1′	1.10 (m)	2′	1.10 (m)		1.32 (m)	
2′	1.21 (m)	4′	1.28 (m)	1′	1.25 (m)	1′, 3′
3′	1.38 (m)	2′, 4′, 5′	1.31 (m)		1.41 (m)	
4′	1.32 (m)		1.32 (m)		1.40 (m)	
5′	3.42 (tt, 6.5, 5.4)	3′, 6′, 7′	3.42 (tt, 6.1, 6.1)	3′, 6′, 7′	3.49 (tt, 6.1, 6.1)	3′, 4′, 6′, 7′
6′	1.30 (m)		1.32 (m)	5′, 7′, 8′	1.35 (m)	−
7′	1.30 (m)		1.30 (m)	8′	1.34 (m)	−
	1.43 (m)	5′, 6′, 8′	1.44 (m)	5′, 6′, 8′	1.47 (m)	5′, 6′, 8′
8′	0.90 (t, 6.6)	6′, 7′	0.90 (t, 7.0)	6′, 7′	0.92 (t, 7.0)	6′, 7′
2″	6.00 (dt, 15.6, 1.2)	1″, 4″	6.00 (d, 15.6)	1″, 3″, 4″	6.02 (d, 15.6)	1″, 3″, 4″
3″	7.19 (dt, 15.6, 7.2)	1″, 2″, 4″, 5″	7.59 (dd, 15.6, 10.8)	1″, 2″, 4″	7.61 (dd, 15.6, 10.8)	1″, 2″, 4″, 5″
4″	1.89 (ddt, 1.2, 7.2, 7.2)	2″, 3″, 5″, 6″	5.97 (dd, 15.0, 10.8)	2″, 3″, 6″	6.00 (dd, 15.0, 10.8)	2″, 3″, 5″, 6″
5″	1.18 (m)	4″, 7″	5.75 (dt, 15.0, 7.2)	3″, 6″, 7″	5.86 (dt, 15.0, 7.2)	3″, 4″, 6″, 7″
6″	1.08 (m)	5″, 7″	1.80 (dt, 7.2, 7.0)	4″, 5″, 7″	1.82 (dt, 7.2, 7.2)	7″, 8″
7″	1.13 (m)	5″, 6″, 8″	1.18 (qt, 7.0, 7.0)	5″, 6″, 8″	1.20 (qt, 7.2, 7.2)	
8″	0.81 (t, 6.5)	6″, 7″	0.74 (t, 7.0)	6″, 7″	0.76 (t, 7.2)	6″, 7″

[a] Spectra were recorded at 600 MHz in C_6D_6 at 30 °C. [b] Carbons that correlate to the proton resonance.

mol. The minimum energy conformations for both compounds **5** and **6** showed the nitrogen equatorially substituting the cyclohexyl ring, and this correlated well with the experimental NMR data. No structures possessing the other twin chair conformation were detected. It is interesting to note that lepadin A (**1**) adopts the twin chair conformation in which the nitrogen axially substitutes the cyclohexyl ring (Figure 1). It would appear that the most stable conformation for all of the lepadins is one in which the side chain attached at C-5 is equatorial.

The occurrence of DHQs and the structurally related quinolizidine, alkyl pyrolidine, and indolizidine alkaloids from several ascidian families (Clavelinidae, Polycitoridae, and Polyclinidae) indicates that ascidians, together with frogs and ants, represent a major source of these biologically active substances. As no optical rotation data have been published for the frog and ant compounds, it therefore still remains to be determined whether these compounds from such diverse origins all possess the same absolute configuration.

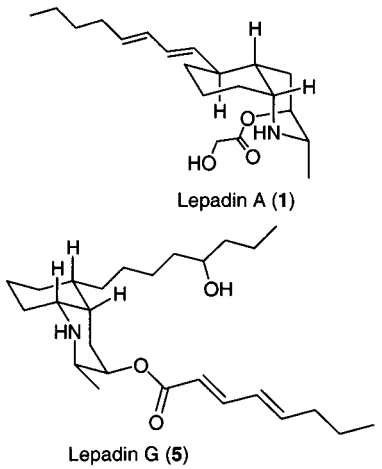

Figure 1. Minimum energy conformers for lepadins A (**1**) and G (**5**).

Experimental Section

General Experimental Procedures. NMR spectra were recorded at 30 °C on a Varian 600 MHz Unity INOVA at 599.926 MHz for ^{1}H and 149.98 MHz for ^{13}C. The ^{1}H and ^{13}C chemical shifts were referenced to the solvent peak (C_6H_6−C_6D_6) at δ 7.16 and 128.00 ppm, respectively. HRLSIMS was recorded on a Kratos Analytical Concept ISQ mass spectrometer instrument using a *m*-nitrobenzyl alcohol matrix. FTIR and UV spectra were recorded on a Perkin-Elmer 1725X spectrophotometer and a GBC UV/vis 916 spectrophotometer, respectively. Optical rotations were measured on a JASCO P-1020 polarimeter. A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler were used for HPLC. A Sanki Model LLB-M (total cell volume, 230 mL) was used for CPC. Alltech Davisil 40−60 μm 60 Å NH_2-

bonded Si packed into an open glass column (40 mm × 45 mm) was used for flash chromatography. Rainin Microsorb 3 μm 100 Å NH_2 column (10 mm × 50 mm) was used for HPLC semipreparative separations. All solvents used for HPLC, UV, $[\alpha]_D$, and MS were Merck Omnisolv grade, and the H_2O used was Millipore Milli-Q PF filtered. Standard parameters were used for the 2D NMR spectra obtained, which included gradient COSY, HMQC, and HMBC and phase-sensitive ROESY. Molecular modeling studies were performed using MacroModel version 6.0 on a Silicon Graphics workstation. Monte Carlo searching with MM2 force-fields was employed for all molecules.

Animal Material. A specimen of *A. tabascum* was collected by scuba diving (−24 m) off Gannet Cay, Swains Reefs, Great Barrier Reef, and kept frozen prior to freeze-drying and extraction. Voucher specimen QMG305408 has been deposited at the Queensland Museum, South Brisbane, Queensland, Australia.

Extraction and Isolation. The freeze-dried ascidian (2.9 g dry weight) was extracted exhaustively with CH_2Cl_2, then concentrated under vacuum to yield a dark red gum (133 mg). This extract was separated by CPC using the solvent system heptane/CH_2Cl_2/CH_3CN (10:3:7) run in the ascending mode at a flow rate of 3 mL/min. Fractions containing the lepadins were combined (124 mg) and chromatographed on a NH_2-bonded Si flash column using a 1% stepped gradient from 100% hexane to 6% i-PrOH/94% hexane to yield a fraction containing a mixture of lepadins H and F (41 mg) and a later eluting fraction containing impure lepadin G (36 mg). Repeated purification of these fractions by semipreparative NH_2 HPLC with isocratic conditions of 5% i-PrOH (containing 1% NH_3)/95% hexane at a flow rate of 4 mL/min yielded pure lepadin H (**6**, 21.0 mg, 0.72% dry wt), pure lepadin G (**5**, 4.5 mg, 0.16% dry wt), and a fraction containing impure lepadin F. This later fraction was further purified by semipreparative NH_2 HPLC employing isocratic conditions of 8% i-PrOH (containing 1% NH_3)/92% hexane at a flow rate of 4 mL/min to yield lepadin F (**4**, 3.2 mg, 0.11% dry wt).

Lepadin F (4): unstable red gum; $[\alpha]_D$ +5.55° (*c* 0.12, CH_2Cl_2); UV (MeOH) λ_{max} 262 nm (ϵ, 3000); IR ν_{max} (NaCl disk) 3375, 2928, 2856, 1718, 1655, 1461, 1264, 1202, 1173, 1127, 998 cm^{-1}; ^{13}C and ^1H NMR data, see Tables 1 and 2; (+)-HRLSIMS *m/z* 422.3630 (calcd for $C_{26}H_{48}NO_3$ $[MH]^+$, 422.3634).

Lepadin G (5): unstable red gum; $[\alpha]_D$ +12.5° (*c* 0.31, CH_2Cl_2); UV (MeOH) λ_{max} 263 nm (ϵ, 24 000); IR ν_{max} (NaCl disk) 3375, 2928, 2857, 1709, 1642, 1248, 1137, 1001 cm^{-1}; ^{13}C and ^1H NMR data, see Tables 1 and 2; (+)-HRLSIMS *m/z* 420.3490 (calcd for $C_{26}H_{46}NO_3$ $[MH]^+$, 420.3478).

Lepadin H (6): unstable red gum; $[\alpha]_D$ +8.20° (*c* 0.75, CH_2Cl_2); UV (MeOH) λ_{max} 261 nm (ϵ, 16 000); IR ν_{max} (NaCl disk) 3400, 2929, 2861, 1709, 1674, 1643, 1464, 1250, 1201, 1180, 1135, 1002 cm^{-1}; ^{13}C and ^1H NMR data, see Tables 1 and 2; (+)-HRLSIMS *m/z* 420.3481 (calcd for $C_{26}H_{46}NO_3$ $[MH]^+$, 420.3478).

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