Novel Oligorhamnosides from the Stem Bark of *Cleistopholis glauca*

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A reinvestigation of the stem bark of *Cleistopholis glauca* yielded 14 compounds, of which seven were either novel or had not been previously reported from this species. These were identified as the farnesane sesquiterpene methyl-(2*E*,6*E*)-10-oxo-3,7,11-trimethyl-dodeca-2,6-dienoate (**1**); the azaanthracene alkaloid cleistopholine (**4**); two partially acetylated oligorhamnoside derivatives, 1-*O*-dodecanyl-2,3,4-tri-*O*-acetyl-α-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-rhamnopyranosyl-(1→4)-α-rhamnopyranoside (**6**) and 1-*O*-dodecanyl-2,4-di-*O*-acetyl-α-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-rhamnopyranosyl-(1→4)-α-rhamnopyranoside (**8**), for which the trivial names cleistetroside-7 and cleistetroside-6 were assigned, respectively; the dihydrobenzofuran neolignan *rel*-(2α,3*β*)-7-*O*-methylcedrusin (**12**); and the flavonoids dihydroquercetin (**13**) and quercetin (**14**). Structure assignments of all compounds were established by spectroscopic methods and comparison with published data. The chemosystematic significance of the occurrence of the isolated components is mentioned. Compounds **1**, **6**, and **8** are novel natural products.

Cleistopholis glauca Pierre ex Engl. & Diels (Annonaceae) is a canopy tree found in the tropical forests of west Central Africa.[1,2] The stem bark is used to treat stomach pain and bronchial diseases and for its emetic properties.[2−4] Previous phytochemical studies on this species have revealed the presence of lipids in the seeds,[5] and partially acetylated 1-*O*-dodecanyl trirhamnosides and tetrarhamnosides,[6,7] acyclic and monocyclic sesquiterpene methyl esters, *β*-sitosterol, and simple aromatic compounds[8] have been found in the stem bark. We now wish to report the isolation and identification of the following additional compounds from a stem-bark sample of this species collected in Cameroon; an acyclic sesquiterpene (**1**), an alkaloid (**4**), two partially acetylated oligosaccharide derivatives (**6**, **8**), a neolignan (**12**), and two flavonoids (**13**, **14**).

Results and Discussion

Fractionation of the petroleum ether extract by column chromatography afforded compounds **1**−**6**. From IR, UV, ^{1}H and ^{13}C NMR, and MS studies **1**−**3** were identified as acyclic farnesane sesquiterpenes. Two were confirmed as methyl-(2*E*,6*E*,10*ξ*)-10-hydroxy-3,7,11-trimethyldodeca-2,6,-11-trienoate (**2**) and methyl-(2*E*,6*E*,10*ξ*)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoate (**3**), both of which have been reported previously in the species.[8,9] Analysis of the spectroscopic data for **1** (Table 1) showed many similarities to **2** and **3**. The HREIMS exhibited a molecular ion at *m*/*z* 266, solving for $C_{16}H_{26}O_3$. Characteristic features in the ^{1}H NMR and JMOD ^{13}C NMR (direct H−C coupling assigned by the HC−COBI experiment) were an isopropyl unit, two methylenes with chemical shifts suggesting they were adjacent to a carbonyl and a double bond, respectively, and a second carbonyl resonance typical of a saturated ketone function (*δ* 214.5). The structure of compound **1** was confirmed by an HMBC experiment (Figure 1). The presence of the isopropyl, methyl ester, and ketone carbonyl groups were also indicated by EIMS (Figure 2).

Table 1. ^{1}H and ^{13}C NMR Spectral Data for **1** (in $CDCl_3$ at 400 and 100 MHz)

position	δ_H	δ_C
1		167.4
2	5.66 (b rs)	115.5
3		160.0
4	2.20−2.14 (m)	41.0
5	2.20−2.14 (m)	26.1
6	5.09 (br s)	123.5
7		135.3
8	2.24 (t, J = 7.7 Hz)	33.6
9	2.52 (t)	39.2
10		214.5
11	2.58 (m)	41.1
Me-12	1.09 (d, J = 6.9 Hz)	18.4
Me-13	1.09 (d, J = 6.9 Hz)	18.4
Me-14	1.61 (d)	16.3
Me-15	2.16 (d, J = 1.3 Hz)	19.0
OMe	3.69 (s)	51.0

The HREIMS of **4** gave a molecular ion at *m*/*z* 223 solving for $C_{14}H_9NO_2$. Comparison of IR, UV, and EIMS data with those published indicated this was the known alkaloid 1-aza-4-methylanthraquinone (cleistopholine).[9,10] The ^{1}H NMR assignments showed good agreement with those reported.[11] The ^{13}C NMR assignments had previously only been partially established,[9] or have incorrect assignments,[10,11] and these are corrected here. It had previously been determined[11] that the more deshielded carbonyl resonance was attributable to C-10 because of a nuclear Overhauser interaction between C-10 and the protons of the C-4 methyl. Through the HMBC experiment this permits the resonance for H-5 to be assigned and, consequently, the resonances for the other protons on that ring. Both H-5 and H-7 show ^3J coupling to a quaternary carbon at *δ* 134.1 that must be attributed to C-8a, and not (as it was previously been assigned[11]) at *δ* 127.1.

From MS, ^{1}H NMR (Table 3), and ^{13}C NMR (Table 4) data, **5** and **6** were identified as partially acetylated

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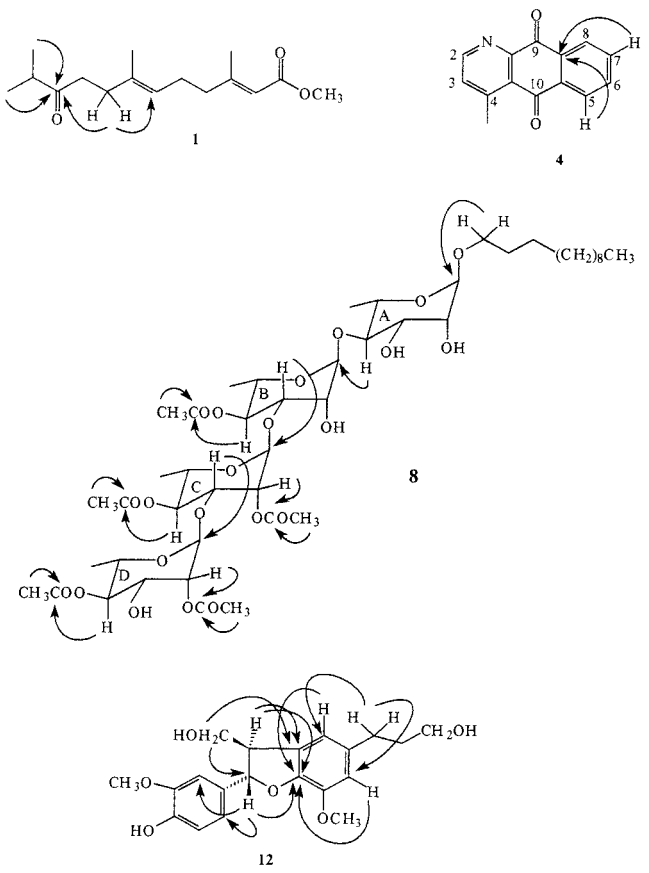


Figure 1. Some important HMBC correlations for compounds **1**, **4**, **8**, and **12**.

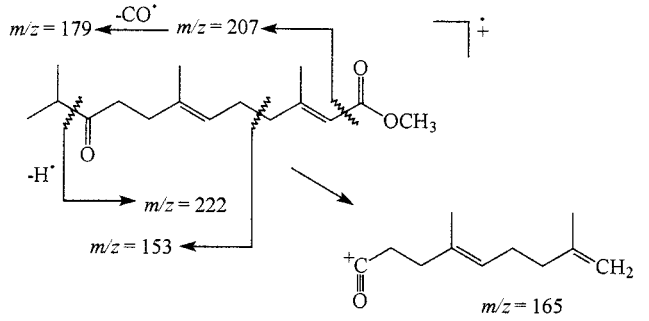


Figure 2. Putative major fragments in the electron impact mass spectrum of **1**.

dodecanyl-trirhamnoside- and -tetrarhamnoside derivatives, respectively. Compound **5** was confirmed as the trirhamnoside 1-*O*-dodecanyl 2,3,4-tri-*O*-acetyl-α-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-rhamnopyranosyl-(1→4)-α-rhamnopyranoside and showed physical and spectroscopic properties identical to those previously reported.[6,7] In a recent report on the occurrence of these oligosaccharides in *Cleistopholis patens*, we proposed the trivial names cleistriosides and cleistetrosides, respectively, for trimers and tetramers of this type.[12] Compound **5**, which is already known from *C. patens*, has been named cleistrioside-1.

The HRFABMS of **6** displayed a quasimolecular ion $[M + Na]^+$ at *m*/*z* 1045, indicating a molecular weight of 1022, corresponding to the molecular formula $C_{48}H_{78}O_{23}$. The ^1H and ^{13}C NMR spectra revealed signals typical of a tetrarhamnoside substituted by six acetoxyls. Acetylated positions were attributed to three H-4 positions, two H-2 positions, and one H-3 position through a combination of COSY and TOCSY experiments.[13]

Table 2. ^1H and ^{13}C NMR Spectral Data for **5** (in $C_5D_5N-CD_3OD$ at 400 and 100 MHz)[a]

position	δ_H	δ_C
1A	4.93 (br s)	101.7
2A	4.11 (dd)	73.0
3A	4.16 (dd, *J* = obsc, 8.6 Hz)	73.6
4A	3.96 (t, *J* = 8.8 Hz)	81.4
5A	3.88 (m)	68.1
Me-6A	1.41 (d, *J* = 5.9 Hz)	19.3
1B	5.76 (br s)	103.7
2B	4.57 (br s)	71.8
3B	4.21 (dd, *J* = obsc, 9.8 Hz)	79.9
4B	5.56 (t, *J* = 9.6 Hz)	73.6
5B	4.08 (m)	68.6
Me-6B	1.15 (d, *J* = 7 Hz)	18.2
1C	5.11 (br s)	100.4
2C	5.40 (br s)	71.4
3C	5.45 (dd, *J* = 3.3, 10 Hz)	70.3
4C	5.22 (t, *J* = 10 Hz)	72.1
5C	4.33 (m)	67.8
Me-6C	1.08 (d, *J* = 6.2 Hz)	17.9
*Me*CO	2.14 (s) (H-4B)	21.3 (H-4B)
	1.98 (s) (H-2C)	21.0 (H-2C)
	1.92 (s) (H-4C)	21.0 (H-4C)
	1.87 (s) (H-3C)	20.9 (H-3C)
*CO*Me		171.2 (H-4B)
		171.0 (H-2C)
		170.9 (H-4C)
		170.6 (H-3C)
OCH_2	3.67/3.36 (m)	68.4
$CH_2\,\beta$	1.52 (m)	30.5
$CH_2\,\gamma$	1.26 (m)	27.1
$(CH_2)_6$	1.17 (br s)	30.5−30.2
CH_2	1.17 (br s)	32.8
CH_2	1.17 (br s)	23.6
Me	0.80 (t, *J* = 6.2 Hz)	14.8

[a] Nonequivalent oxymethylene signals are listed as Ha/a′ in each column.

The location of interglycosidic links and the positions of the ether side chain and the acetoxyl substituents were elucidated unambiguously by an HMBC experiment (cf. Figure 1). The unit bearing the ether side chain was named unit A, and the following linked units, B, C, and D. The placement of the acetylated positions was established through ^3J interactions between each of the six deshielded oxymethines, now identified as H-4B/H-2C/H-4C/H-2D/H-3D and H-4D, and their corresponding acetoxycarbonyls. Assignments of the acetoxymethyl groups were established through ^2J interactions to their corresponding carbonyls. The α-configuration of the rhamnose residues was established on the basis of $^1J_{C-1,H-1}$ values obtained from gated spectra,[12,14,15] the observation of a ^5J coupling between H-1 and Me-6 protons in LR−COSY experiments,[16] and the absence of NOE interactions between H-1/H-3 and H-1/H-5.[6,7] Consequently, compound **6** was identified as the new oligorhamnoside 1-*O*-dodecanyl-2,3,4-tri-*O*-acetyl-α-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-rhamnopyranosyl-(1→3)-4-*O*-acetyl-α-rhamnopyranosyl-(1→4)-α-rhamnopyranoside and was named cleistetroside-7.

Chromatographic fractionation of the EtOAc extract yielded compounds **7**−**10**. Compound **7** was readily characterized as 4-hydroxy-3-methoxy-(*E*)-cinnamylaldehyde (feruladehyde) by comparison with literature data.[8,17] From their HRFABMS and ^1H and ^{13}C NMR spectra (Tables 3 and 4) **8**−**10** were indicated to be further partially acetylated dodecanyl tetrarhamnoside derivatives. The physical and spectroscopic properties of **9** (cleistetroside-3) and **10** (cleistetroside-4) complied with those reported previously.[6,7]

The HRFABMS of **8** gave a quasimolecular ion $[M + Na]^+$ at *m*/*z* 1003, indicating a molecular weight of 980, corresponding to the molecular formula $C_{46}H_{76}O_{22}$. The ^1H

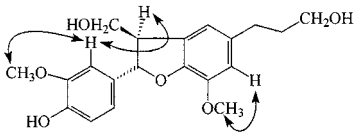


Figure 3. Important interactions observed in the NOESY spectrum of **12**.

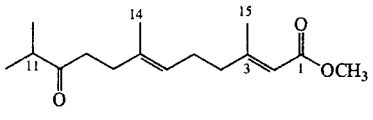
(Table 3) and ^{13}C NMR (Table 4) spectra showed signals typical of a tetrarhamnoside, substituted by five acetoxymethyls. Acetylated positions were attributed to three H-4 positions and two H-2 positions. A combination of COSY and TOCSY experiments established ^{1}H NMR assignments for each rhamnose unit, while an HMBC experiment (Figure 1) again identified the interglycosidic links as (1→3), (1→3), and (1→4) and placed the dodecanyl ether and acetoxyl substituents, with the α-configuration of the rhamnose units determined in the same manner as for **6**. This led to the identification of **8** as the new 1-*O*-dodecanyl-2,4-di-*O*-acetyl-α-rhamnopyranosyl-(1→3)-2,4-di-*O*-acetyl-α-rhamno-pyranosyl-(1→3)-4-*O*-acetyl-α-rhamnopyranosyl-(1→4)-α-rhamnopyranoside, which was named cleistetroside-6.

Fractionation of the MeOH extract afforded **11**−**14**. From ^{1}H and ^{13}C NMR data compound **11** was confirmed as 5-hydroxymethyl-2-furaldehyde.[8,17] The ^{1}H NMR of **12** displayed signals for an AMX spin system of a 1′,3′,4′-trisubstituted aromatic A-ring and a singlet (2H) at δ 6.72 for a tetrasubstituted aromatic ring with two equivalent methines. Other resonances indicated two methoxyl substituents, an *n*-propanol, and −O−CH−CH−CH_2O− spin systems. The HREIMS displayed a molecular ion at *m*/*z* 360, solving for the formula $C_{20}H_{24}O_6$, and the fragmentation pattern confirmed hydroxymethyl and arylmethoxyl groups.

The ^{13}C NMR spectrum, with direct H−C coupling assigned by the HC−COBI experiment, consisted of 20 resonances. These included seven quaternary carbons, of which four were oxygenated aromatics; five aromatic and two aliphatic methines, one of which was an oxymethine; four methylenes, of which two were oxygenated; and two relatively shielded arylmethoxyls (δ 56.9 and 56.5), each adjacent to at least one free *ortho* position.[18] Comparison of the $[\alpha]_D$, IR, UV, and MS data for **12** showed them to be identical to those quoted for the dihydrobenzofuran neolignan *rel*-(2α,3*β*)-7-*O*-methylcedrusin.[19,20] The previously incomplete NMR assignments were assigned unambiguously by HMBC (Figure 1) and NOESY (Figure 3) experiments. The latter allowed the unambiguous placement of the arylmethoxyls. The relative configuration as C-2α,3*β* was also indicated by the NOESY experiment and confirmed by the ^{1}H NMR chemical shift values and coupling constant of H-2, H-3.[21] Assignments of the C-4 and C-6 resonances were made on the basis of the influence of the OMe-7[22] and by comparison with values previously reported for a glycoside derivative of **12**.[23]

Comparison of the physical and spectroscopic properties of compounds **13** and **14** showed them to be identical to those reported, respectively, for the flavonoids dihydroquercetin[24−27] and quercetin.[26,27] Identification of both compounds was confirmed by co-TLC with authentic samples.

Compound **1** is a novel acyclic sesquiterpene clearly closely related biogenetically to **2** and **3**, both previously reported in the same sample of *C. glauca*[8] and in the root and stem barks of *Cl. patens*.[9] Their presence is noteworthy, as few farnesane sesquiterpenes have been recorded



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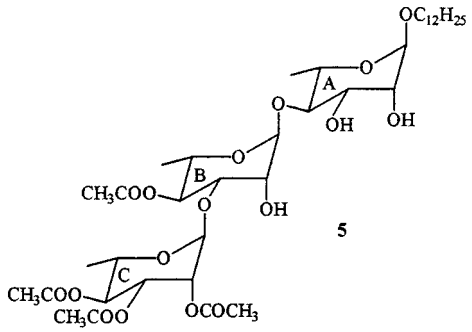

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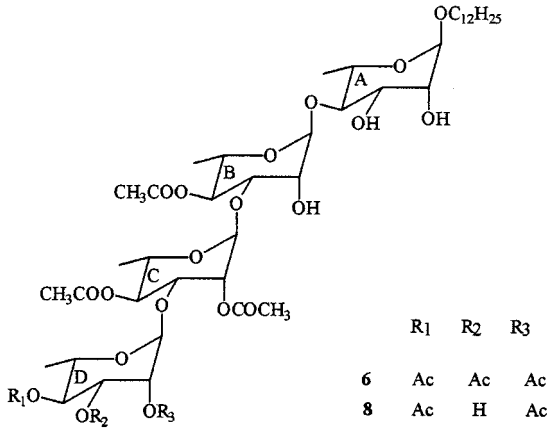
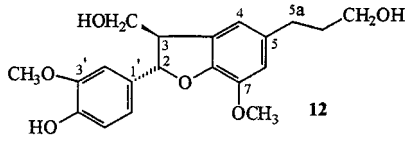


	R_1	R_2	R_3
6	Ac	Ac	Ac
8	Ac	H	Ac
9	H	H	Ac
10	H	H	H



12

in the Annonaceae. Likewise, the two novel partially acetylated 1-*O*-dodecanyl tetrarhamnosides **6** and **8** are allied to similar compounds (**5**, **9**, and **10**) previously reported from the same sample.[6,7] Related oligorhamnosides have been reported in the family only from other *Cleistopholis* species and from *Mezzettia leptopoda*.[13,28,29]

The isolation of **12** in *C. glauca* is the first record of this compound in the Annonaceae. This may be of chemotaxonomic value because such lignans are more frequently encountered in the neighboring Magnoliaceae, Myristicaceae, Eupomatiaceae, and Lauraceae.[30] On the other hand, the isolation of **12** from *C. glauca* conforms with what is already known of neolignans in the family, which seem to be exclusively dihydrobenzofurans.[30,31]

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were determined on a Gallenkamp apparatus. Optical rotations were measured on a Bellingham & Stanley ADP 220 polarimeter. IR spectra were recorded as KBr disks or liquid films on a Mattson Galaxy 5000 FT-IR spectrometer. UV spectra were recorded on a Unicam UV 4-100 UV/vis spectrophotometer in EtOH or MeOH. NMR spectra (both 1D and 2D) were obtained on a Bruker AMX-400 (400 MHz for ^{1}H) spectrometer, using the residual solvent peaks as internal

Table 3. ^{1}H NMR Spectral Data for **6**, **8**−**10** (in C_5D_5N−CD_3OD at 400 MHz)[a]

position	6	8	9	10
1A	4.93 (d, J = 1.6 Hz)	4.97 (d)	5.04 (d)	4.95 (d)
2A	4.10 (dd, J = 1.6, 3.4 Hz)	4. 15 (dd, J = 1.6, 3.4 Hz)	4.22 (dd)	4.13 (dd)
3A	4.16 (dd, J = 3.6, 9 Hz)	4.22 (dd)	4.30 (dd)	4.17 (dd)
4A	3.96 (t, J = 9.1 Hz)	4.02 (t, J = 9.2 Hz)	4.11 (t, J = 9.3 Hz)	3.99 (t, J = 9.1 Hz)
5A	3.89 (m)	3.93 (m)	3.98 (m)	3.90 (m)
Me-6A	1.43 (d, J = 6 Hz)	1.45 (d, J = 6.1 Hz)	1.49 (d, J = 6.1 Hz)	1.43 (d, J = 5.6 Hz)
1B	5.76 (d, J = 1.6 Hz)	5.83 (d)	5.93 (d, J = 1.1 Hz)	5.80 (d)
2B	4.55 (dd)	4.60 (dd)	4.69 (dd)	4.57 (dd)
3B	4.19 (dd, J = 3.3, 10 Hz)	4.22 (dd)	4.28 (dd)	4.19 (dd, J = 3.4, 9.7 Hz)
4B	5.55 (t, J = 9.9 Hz)	5.61 (t, J = 9.8 Hz)	5.70 (t, J = 9.8 Hz)	5.58 (t, J = 9.8 Hz)
5B	4.10 (m)	4.13 (m)	4.20 (m)	4.12 (m)
Me-6B	1.19 (d, J = 6.2 Hz)	1.20 (d, J = 6.3 Hz)	1.24 (d, J = 6.2 Hz)	1.19 (obsc)
1C	5.08 (d)	5.08 (d)	5.15 (d)	5.09 (d)
2C	5.31 (dd, J = 1.6, 3.5 Hz)	5.30 (dd, J = obsc, 3.5 Hz)	5.38 (dd, J = 1.3, 3.3 Hz)	5.34 (obsc)
3C	4.27 (dd, J = 3.4, 9.8 Hz)	4.24 (dd)	4.26 (dd)	4.26 (dd, J = 3.5, 9.9 Hz)
4C	5.24 (t, J = 10 Hz)	5.24 (t, J = 9.9 Hz)	5.29 (t, J = 9.9 Hz)	5.21 (t, J = 9.9 Hz)
5C	4.27 (m)	4.29 (m)	4.34 (m)	4.28 (m)
Me-6C	1.12 (d, J = 6.2 Hz)	1.10 (d, J = 6.2 Hz)	1.12 (d, J = 6.2 Hz)	1.08 (d, J = 6.2 Hz)
1D	4.78 (d, J = 1.4 Hz)	4.69 (d)	4.73 (d)	4.92 (d)
2D	5.25 (dd)	5.17 (dd)	5.27 (dd)	4.06 (dd)
3D	5.35 (dd, J = 3.4, 10.2 Hz)	4.22 (dd)	4.21 (dd)	3.99 (dd)
4D	5.24 (t, J = 10 Hz)	5.27 (t, J = 9.8 Hz)	3.85 (t, J = 9.4 Hz)	3.84 (t)
5D	4.08 (m)	3.99 (m)	3.94 (m)	3.88 (m)
Me-6D	1.27 (d, J = 6.3 Hz)	1.31 (d, J = 6.3 Hz)	1.54 (d, J = 6.1 Hz)	1.43 (d, J = 5.6 Hz)
*Me*CO	2.20 (s) (H-4B)	2.23 (s) (H-4B)	2.29 (s) (H-4B)	2.23 (s) (H-4B)
	2.08 (s)[a] (H-2C)	2.09 (s) (H-4C)	2.11 (s)[a] (H-2C)	1.89 (s)[a] (H-2C)
	2.01 (s)[a] (H-2D)	1.96 (s) (H-4D)	2.03 (s)[a] (H-2D)	1.88 (s)[a] (H-4C)
	2.01 (s)[a] (H-4C)	1.94 (s)[a] (H-2C)	1.90 (s)[a] (H-4C)	
	1.97 (s)[a] (H-4D)	1.93 (s)[a] (H-2D)		
	1.90 (s) (H-3D)			
OCH_2	3.69/3.38 (m)	3.71/3.39 (m)	3.73/3.42 (m)	3.68/3.38(m)
CH_2 β	1.53 (m)	1.53 (m)	1.56 (m)	1.52 (m)
CH_2 γ	1.27 (m)	1.27 (m)	1.31 (m)	1.27 (m)
$(CH_2)_8$	1.18 (br s)	1.17 (br s)	1.18 (br s)	1.18 (br s)
Me	0.81 (t, J = 6.7 Hz)	0.81 (t, J = 6.8 Hz)	0.82 (t, J = 6.7 Hz)	0.80 (t, J = 6.6 Hz)

[a] Assignments interchangeable within the same column.

standard. All experiments were run using pulse sequences in the standard Bruker library. *J*-modulated 1D spectra were acquired with a d_1 of 4 s. HMBC spectra were optimized for a long-range J_{H-C} of 7 Hz (d_6 = 0.07 s), and HC−COBI spectra were acquired with the hxcobi sequence as previously described.[32] HREIMS were run on a JEOL JMS-AX505HA double-focusing instrument at 70 eV. Positive-ion FABMS were run on the same instrument, using a nitrobenzoyl alcohol matrix. Vacuum-liquid chromatography (VLC) was carried out using Merck Si gel 60 H. Column chromatography was carried out using Merck Si gel 60 (70−230 mesh) or Sephadex LH-20 (Pharmacia). Flash chromatography was performed using the Flash 40 (Biotage) system with KP-Sil silica (32−63 μm) (Biotage) in pre-packed polyethylene cartridges. Pressurization was obtained with nitrogen (ca. 0.5 bar), for an average flow rate of 25 mL/min. Analytical TLC was performed on precoated normal-phase Merck Si gel 60 PF_{254} plates. The plates were visualized under UV light (λ 254 and 366 nm) and by spraying with 1% vanillin−H_2SO_4 or 0.5% anisaldehyde−H_2SO_4 reagents, both followed by heating. Modified Dragendorff's reagent was used for the detection of alkaloids.

Plant Material. The original stem bark sample of *C. glauca* was collected in the Korup National Park, Cameroon, in April 1979, and a resupply was made from the same source in April 1997. A voucher specimen (Thomas 2561) has been deposited at the Herbarium of the Missouri Botanic Garden.

Extraction and Isolation. The dried, ground plant material (500 g) was extracted (Soxhlet) sequentially with petroleum ether (bp 40−60 °C), followed by EtOAc and then MeOH. VLC fractionation of the petroleum ether extract (11 g) on Si gel was carried out, eluting with petroleum ether−EtOAc mixtures of increasing polarity. The fraction eluted with 5% EtOAc was further subjected to column chromatography over Si gel to afford **1** (6.3 mg) (3% EtOAc in petroleum ether). The fraction eluted with 20% EtOAc in petroleum ether was further subjected to passage over Sephadex LH-20 ($CHCl_3$) followed by column chromatography over Si gel to yield **2** (12.5 mg) (15% EtOAc in petroleum ether). The fraction eluted with 50% EtOAc in petroleum ether was further subjected to gel filtration on Sephadex LH-20 ($CHCl_3$) to afford **3** (91.4 mg). The fraction eluted with 60% EtOAc in petroleum ether was subjected to column chromatography over Si gel yielding **4** (6.7 mg) (60% $CHCl_3$ in petroleum ether). The fraction eluted with 70% EtOAc in petroleum ether was subjected to flash chromatography. Elution with $CHCl_3$−EtOAc−MeOH (85:7.5:7.5) yielded **5** (293 mg) and then **6** (44.1 mg).

VLC fractionation of the EtOAc extract (30 g) on silica gel was carried out, eluting with petroleum ether−EtOAc and EtOAc−MeOH mixtures of increasing polarity. The fraction eluted with 40% EtOAc in petroleum ether was subjected to column chromatography over Si gel to afford **7** (5.4 mg) (35% EtOAc in petroleum ether). The fractions eluted with EtOAc and 3% MeOH in EtOAc were subjected to flash chromatography, eluting with $CHCl_3$−EtOAc−MeOH (82:9:9) and then $CHCl_3$−EtOAc−MeOH (78:11:11) to yield **8** (72.2 mg) and **9** (38 mg). The fraction eluted with 20% MeOH in EtOAc was subjected to gel filtration over Sephadex LH-20 ($CHCl_3$) to afford **10** (335.7 mg).

The MeOH extract (40 g) was partitioned between EtOAc, *n*-BuOH, and H_2O. The EtOAc residue (2.9 g) was submitted to VLC over Si gel, eluting with $CHCl_3$−MeOH mixtures of increasing polarity. The fractions eluted with 1% and 5% MeOH in $CHCl_3$ were each further subjected to gel filtration over Sephadex LH-20 ($CHCl_3$) to give, respectively, **11** (9.8 mg) and **12** (11.1 mg). The fraction eluted with 20% MeOH in $CHCl_3$ was submitted to gel filtration over Sephadex LH-20 ($CHCl_3$−MeOH mixtures of increasing polarity). Elution with 40 and 50% MeOH in $CHCl_3$, respectively, gave **13** (267.5 mg) and **14** (4.4 mg).

Methyl-(2*E*,6*E*)-10-oxo-3,7,11-trimethyldodeca-2,6-dienoate (1): yellow oil; IR ν_{max} (film) 2966, 2948, 2934, 2875,

Table 4. ^{13}C NMR Spectral Data for **6**, **8**−**10** (in $C_5D_5N-CD_3OD$ at 100 MHz)[a]

position	6	8	9	10
1A	101.7	101.7	101.8	101.7
2A	73.0	73.0	73.1	73.0
3A	73.6	73.6	73.6	73.8
4A	81.4	81.4	81.4	81.4
5A	68.1	68.1	68.2	68.1
Me-6A	19.3	19.3	19.4	19.3
1B	103.6	103.6	103.6	103.6
2B	72.1	72.1	72.1	72.1
3B	80.1	80.3	80.4	80.2
4B	73.7	73.7	73.7	73.6
5B	68.5	68.5	68.6	68.5
Me-6B	18.1	18.1	18.2	18.2
1C	100.7	100.8	100.8	100.8
2C	72.8	73.2	73.4	73.5
3C	76.1	75.9	76.2	75.9
4C	73.4	73.5	73.5	73.8
5C	68.0	67.9	67.1	67.8
Me-6C	17.9	17.9	18.0	18.0
1D	100.0	100.6	101.2	104.4
2D	71.1	74.1	74.4	72.7
3D	70.0	67.9	70.4	72.6
4D	71.8	75.2	74.0	73.8
5D	68.3	68.3	70.9	71.0
Me-6D	17.9	18.0	18.5	18.5
*Me*CO	21.4 (H-4B)	21.4 (H-4B)	21.5 (H-4B)	21.4 (H-4B)
	21.1[a] (H-2C)	21.3[a] (H-4C)	21.3[a] (H-2C)	21.0 (H-2C)
	21.0[a] (H-2D)	21.2[a] (H-4D)	21.2[a] (H-2D)	21.0 (H-4C)
	21.0[a] (H-4C)	21.1[a] (H-2C)	21.0[a] (H-4C)	
	20.9[a] (H-4D)	21.0[a] (H-2D)		
	20.9[a] (H-3D)			
*CO*Me	171.4 (H-4B)	171.4 (H-2D)	171.5 (H-4B)	171.4 (H-4B)
	171.4 (H-2C)	171.4 (H-2C)	171.4[a] (H-2C)	171.3 (H-2C)
	171.2 (H-2D)	171.3 (H-4B)	171.3[a] (H-2D)	170.8 (H-4C)
	171.1[a] (H-4C)	171.3 (H-4D)	171.2[a] (H-4C)	
	171.0[a] (H-4D)	171.2 (H-4C)		
	170.8 (H-3D)			
OCH_2	68.4	68.4	68.4	68.4
CH_2 β	30.5	30.4	30.4	30.3
CH_2 γ	27.2	27.1	27.1	27.1
$(CH_2)_6$	30.6−30.2	30.5−30.2	30.6−30.2	30.6−30.2
CH_2	32.8	32.8	32.7	32.8
CH_2	23.6	23.5	23.5	23.5
Me	14.7	14.7	14.7	14.7

[a] Assignments interchangeable within the same column.

1717, 1648, 1436, 1384, 1358, 1224, 1148, 1075 cm^{-1}; UV λ_{max} (log ϵ) (EtOH) 218 (4.41), 262 (3.69) nm; ^1H and ^{13}C NMR (see Table 1); HREIMS *m/z* 266.1911 (calcd for $C_{16}H_{26}O_3$, 266.1882); EIMS *m/z* 266 $[M]^+$ (27), 234 (25), 222 (32), 204 (93), 207 (20), 189 (37), 179 (31), 161 (100), 153 (50), 135 (54), 121 (82), 109 (98), 105 (72), 95 (100), 81 (100).

Methyl-(2*E*,6*E*,10*ξ*)-10-hydroxy-3,7,11-trimethyldodeca-2,6,11-trienoate (2): yellow oil; $[\alpha]_D$, IR, UV, ^1H and ^{13}C NMR, and EIMS data in agreement with literature.[8]

Methyl-(2*E*,6*E*,10*ξ*)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoate (3): yellow oil; $[\alpha]_D$ in agreement with literature;[8] IR, UV, ^1H and ^{13}C NMR, and EIMS data in agreement with literature.[9]

Cleistopholine (4): pale yellow amorphous solid; IR, UV, EIMS data in agreement with literature;[9,10] ^1H NMR data in agreement with literature;[11] ^{13}C NMR in $CDCl_3$ δ 153.6 (C-2), 131.4 (C-3), 151.7 (C-4), 129.4 (C-4a), 127.6 (C-5), 134.4 (C-6), 134.8 (C-7), 127.4 (C-8), 134.1 (C-8a), 185.0 (C-9), 150.4 (C-9a), 182.1 (C-10), 132.8 (C-10a), 23.1 (Me-4).

Cleistrioside-1 (5): gummy solid; $[\alpha]^{23.3}_D$ (*c* 0.47, MeOH) −59.1° in agreement with literature;[7] IR ν_{max} (film) 3478, 2977, 2925, 2854, 1748, 1371, 1227, 1134, 1076, 1047 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRFABMS *m/z* 815.3997 (calcd for $C_{38}H_{64}O_{17}Na$, 815.4041); FABMS *m/z* 815 $[M + Na]^+$ (16), 461 (7), 273 (100).

Cleistetroside-7 (6): gummy solid; $[\alpha]^{23}_D$ (*c* 0.24, MeOH) −58.8°; IR ν_{max} (film) 3490, 2977, 2925, 2856, 1748, 1372, 1224, 1137, 1076, 1044 cm^{-1}; ^1H and ^{13}C NMR, see Tables 3 and 4; HRFABMS *m/z* 1045.4768 (calcd for $C_{48}H_{78}O_{23}Na$, 1045.4831); FABMS *m/z* 1045 $[M + Na]^+$ (90), 919 (32), 801 (26), 771 (28), 729 (24), 565 (27), 503 (100), 467 (49), 323 (29).

Feruladehyde (7). brown oil; IR, UV, ^1H and ^{13}C NMR and EIMS data in agreement with literature.[8,17]

Cleistetroside-6 (8): gummy solid; $[\alpha]^{23.8}_D$ (*c* 0.474, MeOH) −54.8°; IR ν_{max} (film) 3474, 2979, 2926, 2855, 1745, 1375, 1230, 1136, 1076, 1045 cm^{-1}; ^1H and ^{13}C NMR, see Tables 3 and 4; HRFABMS *m/z* 1003.4729 (calcd for $C_{46}H_{76}O_{22}Na$, 1003.4726); FABMS *m/z* 1003 $[M + Na]^+$ (33), 961 (77), 919 (100), 877 (28), 801 (25), 697 (20), 571 (25), 461 (18), 419 (25), 377 (33), 341 (15).

Cleistetroside-3 (9): gummy solid; $[\alpha]^{23.2}_D$ (*c* 0.256, MeOH) −54.7° in agreement with literature;[7] IR ν_{max} (film) 3461, 2977, 2925, 2855, 1742, 1375, 1233, 1137, 1076, 1047 cm^{-1}; ^1H and ^{13}C NMR, see Tables 3 and 4; HRFABMS *m/z* 961.4722 (calcd for $C_{44}H_{74}O_{21}Na$, 961.4620); FABMS *m/z* 961 $[M+Na]^+$ (93), 919 (83), 801 (40), 697 (30), 645 (30), 571 (35), 419 (40), 383 (30), 341 (30).

Cleistetroside-4 (10): gummy solid; $[\alpha]^{21.2}_D$ (*c* 0.176, MeOH) −79.5° in agreement with literature;[7] IR ν_{max} (film) 3424, 2976, 2925, 2854, 1742, 1377, 1233, 1136, 1075, 1047 cm^{-1}; ^1H and ^{13}C NMR, see Tables 3 and 4; HRFABMS *m/z* 919.4449 (calcd for $C_{42}H_{72}O_{20}Na$, 919.4515); FABMS *m/z* 919 $[M + Na]^+$ (80), 877 (32), 835 (30), 731 (30), 607 (52), 565 (100).

5-Hydroxymethyl-2-furaldehyde (11): brown oil; IR, UV, ^1H and ^{13}C NMR and EIMS data in agreement with literature.[8,17]

***rel*-(2α,3*β*)-7-*O*-Methylcedrusin (12):** pale amorphous solid; $[\alpha]_D$, IR, UV, EIMS data in agreement with literature;[19,20]

^{1}H NMR in CD_3OD δ 5.49 (1H, d, J = 6.3 Hz, H-2), 3.47 (dt, J = 6.2, 6.3 Hz, H-3), 3.83/3.76 (m, H-3a/a′), 6.72 (s, H-4), 2.62 (t, J = 7.7 Hz, H-5a), 1.81 (tt, J = 6.5, 7.7 Hz, H-5b), 3.58 (t, J = 6.5 Hz, H-5c), 6.72 (s, H-6), 6.95 (d, J = 1.8 Hz, H-2′), 6.76 (d, J = 8.1 Hz, H-5′), 6.82 (dd, J = 1.8, 8.2 Hz, H-6′), 3.85 (s, OMe-7), 3.81 (s, OMe-3′); ^{13}C NMR δ 89.1 (C-2), 55.6 (C-3), 65.1 (C-3a/a′), 118.1 (C-4), 130.1 (C-4a), 137.1 (C-5), 33.0 (C-5a), 35.9 (C-5b), 62.4 (C-5c), 114.3 (C-6), 145.4 (C-7), 147.7 (C-7a), 135.0 (C-1′), 110.7 (C-2′), 149.2 (C-3′), 147.6 (C-4′), 116.3 (C-5′), 119.9 (C-6′), 56.9 (OMe-7), 56.5 (OMe-3′).

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