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# CHEMISTRY IN THE ANNONACEAE, PART XXV. SESQUITERPENES FROM THE STEM BARK OF *CLEISTOPHOLIS GLAUCA*

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*Cleistopholis glauca* Pierre ex Engl. (Annonaceae) is a tree found in the tropical forests of west central Africa (1). It is reported to be used as an emetic (1). The only previous chemical investigation has revealed the presence of lipids (2), but other species of *Cleistopholis* have been found to produce bisbenzylisoquinoline (3), oxoaporphine (4,5) and azafluorenone (5) alkaloids, and acyclic and monocyclic sesquiterpene methyl esters (5).

After column chromatography of the $CHCl_3$-soluble fraction of the stem bark of *C. glauca*, seven bands were obtained, two of which were identified as β-sitosterol and the sesquiterpene methyl (+)-10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoate [**1**]. The latter was identical with material previously isolated from *Cleistopholis patens*, which had erroneously been reported to be levorotatory (5).

A third band was analyzed for $C_{16}H_{26}O_3$ and had many of the spectral characteristics of **1**. Significant differences in the $^1$H-nmr spectrum (Table 1) were the absence of a *gem*-dimethyl substituent and its replacement by resonances for an isopropenyl moiety, indicative of structure **2**, the 11,12-dehydro derivative of **1**. Comparison of the $^{13}$C-nmr spectrum (Table 2) with that previously published for **1** (5) supported this proposal. Sesquiterpene **2** appears to be a novel natural product. Absolute stereochemistry of **1** and **2** has not been assigned, but their dextrorotatory nature suggests that they are likely to have an *S* configuration at C-10. A seemingly comparable chiral center in a levorotatory farnesylacetone derivative isolated by Ravi *et al.* (6) was reported to have *R* configuration on the basis of Horeau's method.

A minor band (yield 0.02%) also analyzed for $C_{16}H_{26}O_3$. Careful examination of the high-field $^1$H-nmr spectrum revealed it to be a mixture of three compounds in a ratio of 6:2:1. The major component gave resonances identical with those recorded for methyl (1′ξ, 2*E*, 3′ξ)-3-methyl-5-(3′-hydroxy-2′,2′-dimethyl-6′-methylenecyclohex-1′-yl)-pent-2-enoate [**3**], which had also been isolated from *C. patens* (5). The remaining components both showed a $^1$H-nmr resonance for a vinylic methyl group. In the more abundant of these this methyl signal showed long-range coupling to an olefinic resonance at δ 5.30 indicating that this compound must be the 5′,6′-en derivative **4**. Absence of an olefinic signal for the minor isomer suggested that it was the 1′,6′-en compound **5**. The $^1$H-nmr spectra of **3-5** are given in Table 1. Attempts to separate the two minor components were unsuccessful.

A fifth band contained traces of another monocyclic sesquiterpene that on eims also analyzed for $C_{16}H_{26}O_3$. The $^1$H-nmr spectrum (Table 1) suggested a skeleton comparable with **3-5**,

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TABLE 1. $^1$H-nmr (250 MHz) Chemical Shifts for Sesquiterpenes **1**, **2**, **2**-Ac, **3**-**6**

| Proton | Compounds | | | Proton | Compounds | | | |
|---|---|---|---|---|---|---|---|---|
| | **1** | **2**[a] | **2**-Ac | | **3** | **4** | **5** | **6** |
| H-2 | 5.65 q (1)[b] | 5.65 q (1) | 5.69 q (1) | H-2 | 5.66 q (1) | 5.70 q (1) | 5.70 q (1) | 5.70 q (1) |
| H-4/5 | 2.00-2.26 m | 2.00 m | 2.00-2.30 m | H-4/5 | 1.50-2.30 m | 2.00-2.30 m | 2.00-2.30 m | 1.50-2.40 m |
| H-6 | 5.15 br s | 5.11 t q (1,1) | 5.11 br s | H-1′ | 2.20 dd (5,1) | 2.20 dd (5,1) | — | 1.55 m |
| H-8 | 2.00-2.26 m | 1.95-2.10 m | 2.00-2.30 m | H-5′ | 2.00-2.30 m | 5.30 br s | 2.00-2.30 m | 1.20-1.80 m |
| H-9 | 1.48 m | 1.62 m | 1.64-1.80 m | H-4′ | 1.70 m | 2.15 m | 1.70 m | 1.20-1.80 m |
| H-10 | 3.30 dd (9,1) | 4.00 t (6.3) | 5.10 t (6) | H-3′ | 3.43 dd (9.3,4.2) | 3.49 dd (9,6) | 3.50 dd (8,4) | 3.32 dd (7.3,4.2) |
| Me-12[c] | 1.19 s | 4.82 d (1.5) | 4.82 br s | 2′-Me | 0.74 s | 0.85 s | 1.01 s | 0.79 s |
| | | 4.92 dd (1,2) | 4.90 br s | | | | | |
| Me-15[c] | 1.13 s | 1.71 t (1.1) | 1.71 br s | 2′-Me | 1.03 s | 0.97 s | 1.07 s | 1.04 s |
| Me-13 | 2.14 d (1) | 2.14 d (1) | 2.14 d (1) | 3-Me | 2.16 d (1) | 2.17 d (1) | 2.17 d (1) | 2.18 d (1) |
| Me-14 | 1.60 s | 1.59 d (1) | 1.60 d (1) | 6′-Me/ | 4.58 br s | 1.62 s | 1.71 s | 1.19 s |
| | | | | $=CH_2$ | 4.89 br s | | | |
| OMe | 3.67 s | 3.66 s | 3.66 s | OMe | 3.69 s | 3.69 s | 3.69 s | 3.69 s |
| Ac | | | 2.05 s | | | | | |

[a]Determined at 360 MHz.
[b]Coupling constants are in parentheses.
[c]Assignments are interchangeable.

TABLE 2. $^{13}$C-nmr Chemical Shifts for Sesquiterpenes **1** and **2**[a].

| Carbon number | **1**[b] | **2** |
|---|---|---|
| C-1 | 167.1 | 167.1 |
| C-2 | 115.3 | 115.3 |
| C-3 | 159.4 | 159.8 |
| C-4 | 40.6 | 40.7 |
| C-5 | 25.8 | 25.8 |
| C-6 | 123.4 | 123.2 |
| C-7 | 135.9 | 135.8 |
| C-8 | 36.5 | 35.5 |
| C-9 | 29.8 | 33.0 |
| C-10 | 77.9 | 75.4 |
| C-11 | 72.8 | 147.5 |
| C-12 | 22.3[c] | 110.8 |
| C-13 | 18.6 | 18.7 |
| C-14 | 15.8 | 15.9 |
| C-15 | 26.3[c] | 17.5 |
| OMe | 50.5 | 50.6 |

[a]Spectra run at 90.56 MHz in $CDCl_3$.
[b]Previously reported by Waterman and Muhammad (5).
[c]Assignments are interchangeable.

differing from **5** in that the 6′-methyl resonance was not vinylic but resonated as a singlet at δ 1.19, suggesting a *geminal* hydroxyl substituent (cf. **1**, H-12/H-15, Table 1). The presence of four oxygens including secondary and tertiary hydroxyls was confirmed by derivatization to give the mono perfluoropropionate ester and the bis trimethylsilyl ether, followed by gc-ms analysis. On this basis the compound was identified as **6**, which must undergo facile loss of the elements of $H_2O$ on eims to give an apparent molecular ion identical to **3-5**.

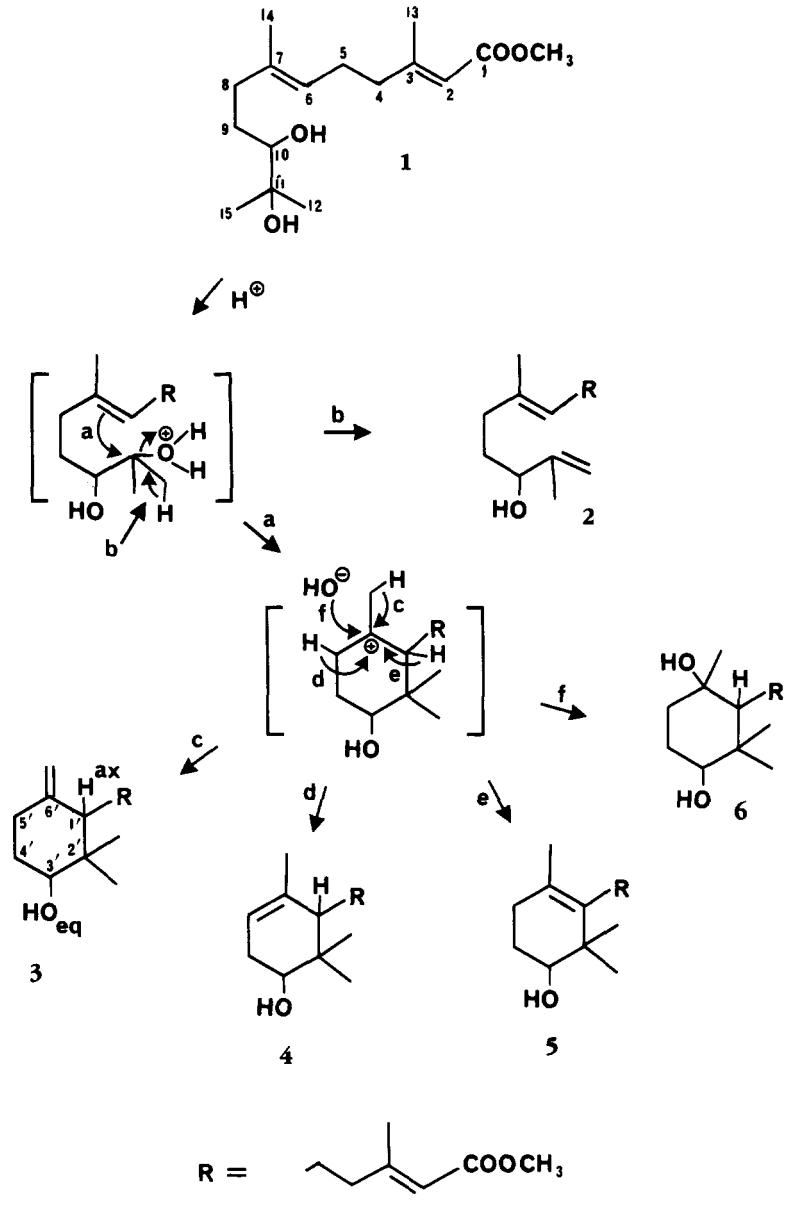
The components of the remaining two bands were characterized as 3-methoxy-4-hydroxy-*trans*-cinnamaldehyde [**7**] (7) and 5-hydroxymethyl-2-furancarboxaldehyde [**8**] (8), both of which have been recorded previously as natural products but not from the Annonaceae.

In its capacity to produce simple sesquiterpenes *C. glauca* is similar to *C. patens* (5). The compounds isolated appear to represent a parent diol **1**, its dehydration product **2**, and four monocyclic derivatives that could be formed from **1** by acid-catalyzed processes (Scheme 1). However, **2-6** could be detected in the petrol extract prior to column chromatography and are not readily formed from **1** during passage through a Si gel column, implying that they are not artifacts of the separation process. No trace of alkaloids could be detected in the *C. glauca* material, but it should be noted that in *C. patens* alkaloids were found primarily in the root bark.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were measured in MeOH and ir spectra as KCl discs. $^1$H-nmr spectra were run at 250 or 360 MHz and $^{13}$C-nmr spectra at 90.56 MHz in $CDCl_3$ using TMS as internal standard. High resolution eims were obtained on an AEI MS902 double focusing instrument by direct probe insert at elevated temperatures and at 70 eV. Gc-ms was obtained on a Hewlett-Packard 5890/5988A instrument connected to a 1000E series data processing system; column HP-1, 25 m×0.20 mm i.d. fused silica, He carrier gas, source temperature 140°, injection port temperature 250°. Optical rotations were measured at 25°. Petrol refers to petroleum ether (bp 60-80°).

PLANT MATERIAL.—Stem bark of *C. glauca* was collected in the Korup National Park, Cameroon. A voucher specimen (Thomas 2561) has been deposited at the Herbarium of the Missouri Botanic Gardens.

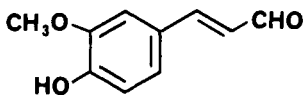



SCHEME 1. Sesquiterpenes from *Cleistopholis glauca*; potential formation through acid-catalyzed rearrangements of **1**.

EXTRACTION AND ISOLATION OF COMPOUNDS.—Ground stem bark (300 g) was extracted successively with petrol, $CHCl_3$, and MeOH. Tlc analysis of petrol and $CHCl_3$ extracts revealed them to be identical, and they were combined, concentrated, and subjected to column chromatography over Si gel eluting with toluene containing increasing amounts of EtOAc. From 1% EtOAc a mixture was obtained, which was separated by centrifugal preparative tlc (Si gel, $C_6H_6$-$Me_2CO$, 4:1) to give β-sitosterol (10 mg) and **2** (40 mg). Elution with 15% EtOAc gave a mixture of **3-5** (60 mg) that was not further separated. Elution with 20% EtOAc gave a mixture that was then separated by centrifugal preparative tlc ($CHCl_3$-MeOH, 19:1) to give **7** (20 mg) followed by **1** (150 mg). Elution with 30% EtOAc gave mainly **8** (45 mg) followed by **6** (10 mg).

*Methyl* (+)-(2E, 6E, *10ξ*)-*10,11-dihydroxy-3,7,11-trimethyldodeca-2,6-dienoate* [**1**].—Oil, $[\alpha]_D + 13°$ ($c = 0.6$, MeOH); uv, ir, eims as previously reported (5); $^1H$ nmr see Table 1.

*Methyl* (+)-(2E, 6E, *10ξ*)-*10-hydroxy-3,7,11-*



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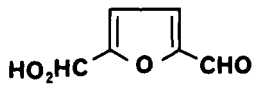

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*trimethyldodeca-2,6,11-trienoate* [**2**].—Oil, $[\alpha]_D$ +5° ($c$ = 0.3, $CHCl_3$); uv λ max 230 nm; ir ν max 3450, 3080, 1720, 1660 $cm^{-1}$; $^1$H-nmr see Table 1; $^{13}$C-nmr see Table 2; eims (*m/z*, rel. int.) 266.1890 ($M^+$, 35%) (calcd for $C_{16}H_{26}O_3$, 266.1882), 246 (10%), 234 (16%), 206 (12%), 193 (10%), 189 (23%), 180 (27%), 109 (58%). Compound **2** (16 mg) was dissolved in pyridine (5 ml) and $Ac_2O$ (2 ml) added. After 24 h the reaction mixture was diluted with $H_2O$ and extracted into $Et_2O$ to give the acetate (15 mg) as an oil, ir ν max 1740, 1720 $cm^{-1}$; $^1$H-nmr see Table 1; eims (*m/z*, rel. int.) 308.2007 ($M^+$, 3%), (calcd for $C_{18}H_{28}O_4$, 308.1987).

*Mixture of methyl* (*1'ξ, 2E, 3'ξ*)*-3-methyl-5-*(*3'-hydroxy-2',2'-dimethyl-6'-methylenecyclohex-6'-en-1'-yl*)*-pent-2-enoate* [**3**], *methyl* (*1'ξ, 2E, 3'ξ*)*-3-methyl-5-*(*3'-hydroxy-2',2',6'-trimethylcyclohex-5'-en-1'-yl*)*-pent-2-enoate* [**4**], *methyl* (*2E, 3'ξ*)*-3-methyl-5-*(*3'-hydroxy-2',2',6'-trimethylcyclohex-6'-en-1-yl*)*-pent-2-enoate* [**5**].—Oil, uv λ max 230 nm; $^1$H-nmr see Table 1; eims (*m/z*, rel. int.) 266.1885 ($M^+$) (calcd for $C_{16}H_{26}O_3$, 266.1882).

*Methyl* (+)-(*1'ξ, 2E, 3'ξ, 6'ξ*)*-3-methyl-5-*(*3',6'-dihydroxy-2',2',6'-trimethylcyclohex-1'-yl*)*-pent-2-enoate* [**6**].—Oil, $[\alpha]_D$ +10° ($c$ = 0.01, $CHCl_3$); uv λ max 230 nm; ir ν max 3500, 1720, 1640 $cm^{-1}$. $^1$H-nmr see Table 1; eims (*m/z*, rel. int.) 266 (17%), 253 (2%), 248 (30%), 109 (24%).

*Preparation of pentafluoropropionyl* (*PFP*) *ester.*—Excess PFP-imidazole (10 μl) was added to a solution of **6** (50 μg) in MeCN (20 μl). After 30 min the reaction mixture was diluted to 1 ml with hexane and passed through a 1 cm bed of Sephadex LH-20. The eluate was subjected to gc-ms (oven temperature 80° rising to 250° at 10° min). Electron capture negative chemical ionization ms using $CH_4$ reagent gas and 200 eV gave for the peak $R_t$ 15.0 min, $M^-$ 430.2 (1.6%) for $C_{19}H_{27}O_5F_5$, 410.2 $[M\text{-}HF]^-$ (100%).

*Preparation of the bis*(*trimethylsilyl*) (*TMSi*) *ether.*—An excess of TMSi-imidazole (20 μl) was added to **6** (50 μg) and heated for 15 min to 80°. The reaction mixture was diluted with EtOAc (200 μl) followed by hexane (800 μl) and treated as for the PFP ester. The product was subjected to gc-ms (oven temperature 100° rising to 280° at 15°/min). Eims of the peak at $R_t$ 11.1 min gave $M^+$ 428.30 (0.2%) for $C_{22}H_{44}O_4Si_2$.

*3'-Hydroxy-4'-methoxy-*trans*-cinnamaldehyde* [**7**].—Brown oil; uv λ max 250, 290, 335; (+NaOH) 242, 250, 290, 335, 412 nm; ir ν max 3400, 1660, 1600, 1500 $cm^{-1}$; $^1$H-nmr δ 9.65 (d, 1H, $J$=7.7 Hz, 1-H), 7.42 (d, 1H, $J$=15.9 Hz, 3-H), 7.13 (dd, 1H, $J$=8.4, 1.8 Hz, 6'-H), 7.07 (d, 1H, $J$=1.8 Hz, 2'-H), 6.97 (d, 1H, $J$=8.4 Hz, 5'-H), 6.53 (dd, 1H, $J$=15.9, 7.7 Hz, 2-H), 3.95 (s, 3H, 4'-OMe); eims (*m/z*, rel. int.) 178.0633 ($M^+$, 100%) (calcd for $C_{10}H_{10}O_3$, 178.0630), 177 (18%), 161 (6%), 147 (19%).

*5-Hydroxymethyl-2-furancarboxaldehyde* [**8**].—Brown oil, uv λ max 278 nm; ir ν max 3400, 1660, 1180 $cm^{-1}$; $^1$H-nmr δ 9.55 (s, 1H, 2-CHO), 7.20, 6.50 (ABq, 2H, $J$=3.5 Hz, 4-H, 3-H), 4.70 (s, 2H, 5-$CH_2$), 2.90 (s, 1H, OH); eims (*m/z*, rel. int.) 126.0310 ($M^+$, 41%) (calcd for $C_6H_6O_3$, 126.0317), 125 (23%), 109 (13%), 97 (100%).

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