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BISABOLENE SESQUITERPENES AND FLAVONOIDS FROM *FRIESODIELSIA ENGHIANA*

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Key Word Index—*Freisodielsia enghiana*; Annonaceae; bisabolene sesquiterpenes; flavonoids; 2-hydroxyflavonoids; flavones; flavanones.

Abstract—An investigation of the stem bark of *Friesodielsia enghiana* led to the isolation of benzyl benzoate and benzyl 2-hydroxybenzoate, two bisabolene sesquiterpenes and nine flavonoids, two of which were new. The new compounds were identified by analysis of their spectroscopic data as 2,5-dihydroxy-7-methoxy-8-methylflavanone and 2,5-dihydroxy-7-methoxy-6-methylflavanone. Three of the co-occurring flavones also have 6- and/or 8-C-methylation. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Friesodielsia van Steenis is a genus of Annonaceae with about 60 species found in Africa and Asia [1]. Until 1948, species belonging to this genus were placed under *Oxymitra* [2]. The first phytochemical report on the genus was made in 1960, in which the presence of alkaloids in an unidentified species was noted [3]. Since then only two species have been investigated, *F. kingii* (syn. *Oxymitra kingii*) from which hexahydroxanthenic derivatives and a flavanone were isolated [4], and *F. velutina* (syn. *O. velutina*) which yielded flavonoids, phenylpropanoids, sterols and alkaloids [5]. In this report we present the results of a phytochemical investigation on the stem bark of *F. enghiana* (Diels) Verdc., a large woody climber found in the tropical rain forest of West Africa, from Sierra Leone to Zaire [1].

RESULTS AND DISCUSSION

The stem bark of *F. enghiana* was successively extracted with petrol (bp 60–80°) and $CHCl_3$. The concentrated petrol extract, after repeated column chromatography and preparative TLC on silica gel, yielded benzyl benzoate (**1**), benzyl 2-hydroxybenzoate (**2**) [6], two bisabolene sequiterpenes, β-bisabodol (**3**) [7] and gossonorol (**4**) [8], and the flavonoids 5-hydroxy-7-methoxy-8-methylflavone (**5**) [10], 5-hydroxy-7-methoxy-6-methylflavone (**6**) [10], 5-hydroxy-7-methoxyflavone (**7**) [11], 5-hydroxy-7-methoxy-6,8-dimethylflavone (**8**) [10], 5-hydroxy-7-methoxyflavanone (**9**) [12], 2,5-dihydroxy-7-methoxy-8-methylflavanone (**10**) and 2,5-dihydroxy-7-methoxy-6-methylflavanone (**11**). The $CHCl_3$ extract after similar treatment gave compounds **1**, **5**, **6**, **9**, **10** and **11**, and in addition 2,5-dihydroxy-7-methoxyflavanone (**12**) [13, 14] and 5,7-dihydroxy-6-methylflavanone (**13**) [15]. Compounds **1**, **5** and **6** formed the major constituents of the petrol extract; **10** and **11** the major constituents of the $CHCl_3$ extract. All the known compounds were identified by comparison of their spectroscopic data with those published.

Compounds **10** and **11** were isolated as a mixture (**10**:**11** = 1:0.94) as shown by the ^{1}H NMR. The HR-EIMS gave a single molecular ion peak at *m/z* 300.1006 which analysed for $C_{17}H_{16}O_5$. The UV analysis showed a strong absorption at 276 nm and a shoulder at 336 nm, which is typical of flavanones [10], and the IR spectrum indicated hydroxyl and carbonyl functions. The ^{1}H NMR spectrum of **10**, the major compound of the mixture, showed a singlet at δ 12.64, and aromatic multiplets at δ 7.43 (3H) and δ 7.68 (2H), suggesting a 5-hydroxyflavanone with an unsubstituted phenyl moiety (B-ring) which was supported by the fragment at *m/z* 77 in the mass spectrum. The ^{1}H NMR further showed an aromatic methyl singlet (8-Me), an aromatic proton singlet (H-6) and a methoxyl singlet (7-MeO). The positions of H-6 and 8-Me were unambiguously assigned from an HMBC experiment (Table 1) which showed ^3J couplings of the former with C-8 and C-10, and the latter with C-7 and C-9.

The absence of a double doublet in the region of δ 5.20 suggested the absence of an H-2 proton which normally couples with the H-3 protons in flavanones [11]. That this proton was substituted by an OH was supported by the presence of a doubly oxygenated sp^3 carbon signal at δ 101.8 (C-2) in the ^{13}C NMR

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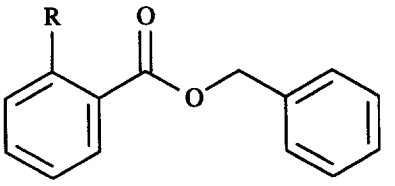
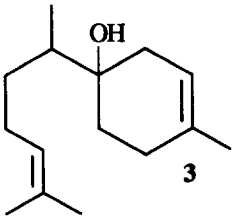


	R
1	H
2	OH



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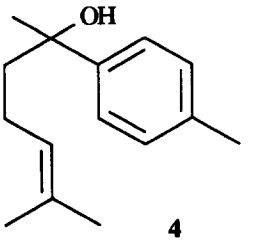

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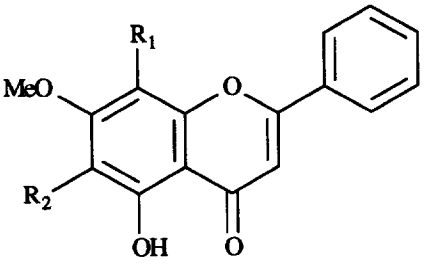
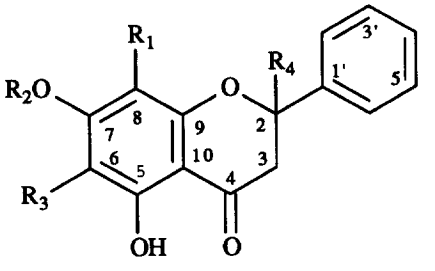


	R_1	R_2
5	Me	H
6	H	Me
7	H	H
8	Me	Me



	R_1	R_2	R_3	R_4
9	H	Me	H	H
10	Me	Me	H	OH
11	H	Me	Me	OH
12	H	Me	H	OH
13	H	H	Me	H

Table 1. Long-range ^1H–^{13}C correlations shown by **10** in HMBC experiment

H	^2J	^2J
H-3	101.8 (C-2), 195.9 (C-4)	
H-6	162.2 (C-5), 166.3 (C-7)	106.0 (C-8), 102.5 (C-10)
8-Me	106.0 (C-8)	166.3 (C-7), 155.7 (C-9)
H-2′/6′	142.4 (C-1′), 128.9 (C-3′)	101.8 (C-2), 129.4 (C-4′)

spectrum. The position of this carbon was established from ^3J coupling with the H-2′/6′ protons in the HMBC experiment. This 2-OH gave a doublet at δ 2.70 while the H-3 protons formed an *AB* system with resonance signals at δ 2.55 (H-3_{ax}) and δ 2.83 (H-3_{eq}). These data led to the assignment of the structure of **10** as 2,5-dihydroxy-7-methoxy-8-methylflavanone. The small J of 2.5 Hz for the 2-OH proton must be due to long range *W*-coupling with the 3-H_{ax} (Fig. 1) thus

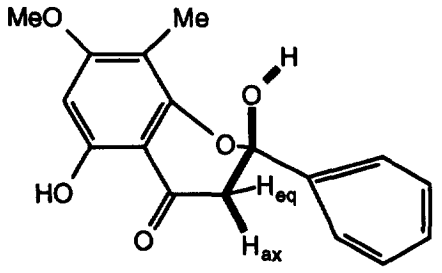


Fig. 1. *W*-coupling between H_{ax} and 2-OH.

suggesting a pseudo-equatorial configuration of the phenyl substituent.

Compound **11** gave signals similar to and sometimes overlapping with those of **10** in both the ^1H and ^{13}C NMR spectra (Table 2). Analysing the HC-COBI and HMBC experiments the structure of this compound was established as the corresponding 2,5-dihydroxy-7-methoxy-6-methylflavanone.

Although flavonoids are widespread in nature, the occurrence of such stable hemiacetals with hydroxyl substitution at position C-2, as in compounds **10**, **11** and **12**, is rare. Previously, only 7-methoxy-2,5-dihydroxyflavanone from *Populus nigra* [14] and *Uvaria rufas* [13], 7-glucosyloxy-2,5-dihydroxyflavanone from *Mallus* sp. [16] and 6-formyl-2,5,7-trihydroxy-8-methylflavanone and 8-formyl-2,5,7-trihydroxy-6-methylflavanone from *Unona lawii* [17], have been encountered. The biogenesis of these compounds remains unknown. However, they have been regarded as precursors of 5-hydroxyflavones because of the ease with which they are dehydrated to the latter [17]. In this regard, the co-occurrence of the 2,5-dihydroxyflavanones and their corresponding 5-hydroxyflavones in the stem bark of *F. enghiana* is noteworthy.

EXPERIMENTAL

Mps: uncorr.; UV-MeOH; IR-$CHCl_3$; NMR experiments were run on a Bruker AMX 400 instrument; HR-EIMS were obtained on a AEI MS 902 double

Table 2. ^1H and ^{13}C NMR data for **10** and **11**

	δ ^1H*			δ ^{13}C†	
Position	**10**	**11**	**12**	**10**	**11**
2				101.8 *s*	101.3 *s*
3_{eq}	2.83 *d* (*J* = 17 Hz)	2.81 *d* (*J* = 17 Hz)	2.77 *d* (*J* = 17 Hz)	48.4 *t*	48.7 *t*
3_{ax}	2.55 *dd* (*J* = 2.6, 17 Hz)	2.60 *dd* (*J* = 2.4, 17 Hz)	2.56 *dd* (*J* = 2.6, 17 Hz)		
4				195.0 *s*	194.6 *s*
5				162.2 *s*	160.3 *s*
6	6.02 *s*		6.13 *d* (*J* = 2.0 Hz)	92.8 *d*	106.7 *s*
7				166.3 s	165.9 s
8		5.99 *s*	6.19 *d* (*J* = 2.0 Hz)	106.0 *s*	91.9 *d*
9				155.7 *s*	158.0 *s*
10				102.5 *s*	102.5 *s*
1′				142.4 *s*	142.1 *m*
2′/6′	7.43 *m*	7.39 *m*		125.2 *d*	125.2 *d*
3′/5′	7.14 *m*	7.09 *m*		128.9 *d*	128.9 *d*
4′	7.14 *m*	7.09 *m*		129.4 *d*	129.4 *d*
6-Me		2.22 s			7.1 *q*
7-OMe	3.16 *s*	3.25 *s*	3.13 *s*	56.1 *q*	56.0 *q*
8-Me	2.28 *s*			8.0 *q*	
2-OH	2.70 *d* (*J* = 2.7 Hz)	3.02 *d* (*J* = 2.6 Hz)	2.84 *d* (*J* = 2.6 Hz)		
5-OH	12.64 *s*	12.57 *s*	12.56 *s*		

*Solution in C_6D_6 referenced to C_6D_6 at δ 7.16 (400 MHz).
†Solution in $CDCl_3$ referenced to $CHCl_3$ at δ 77.23 (100 MHz).

focusing spectrometer using direct probe insertion at 120° and 70 eV.

Plant material. The stem bark of *F. enghiana* was collected from the Bobre Forest Reserve, Ghana, in August 1993, and was identified by comparison with herbarium specimens at the Forest Herbarium of the forestry Department, Kumasi.

Extraction and isolation of compounds. Oven-dried (40°) powdered stem bark (500 g) was Soxhlet extracted successively with petrol (bp 60–80°) and $CHCl_3$. The extracts were evaporated at 30° under red. pres. to give 11.08 and 10.51 g extract, respectively. The concd petrol extract gave a ppt. which was filtered and recrystallized from $CHCl_3$ to give **6** (64 mg). The residual extract (4.6 g) was repeatedly subjected to CC over silica gel (230–400 mesh) eluting with petrol (40–60°), followed by petrol–EtOAc mixtures and EtOAc to give a series of compounds which were purified by PTLC using toluene, toluene–EtOAc (9:1; 8:2) and $CHCl_3$–MeOH (98:2) to give: **1** (206 mg), **2** (14 mg), **3** (38 mg), **4** (4 mg), **5** (137 mg), **6** (60 mg), **7** (19 mg), **8** (6 mg), **9** (38 mg) and **10/11** (35 mg). The concd $CHCl_3$ extract (6 g) was subjected to VLC over silica gel eluting with petrol, petrol–EtOAc mixtures, EtOAc and EtOAc–MeOH mixtures to give 4 frs labelled A–D. Fr. A was CC over silica gel, eluting with toluene and toluene–EtOAc mixtures. The early fractions after PTLC (toluene–EtOAc, 9.5:0.5) gave **1** (15 mg). Fr. B was similarly treated and after PTLC (toluene–EtOAc, 8:2) gave **5** (72 mg), **6** (34 mg) and **7** (30 mg). Frs C and D gave ppts which were filtered and recrystallized from $CHCl_3$ to give **10/11** (553 mg). Further PTLC of the supernatant using $CHCl_3$–MeOH (95:5) gave more **10/11** (94 mg), **12** (5 mg) and **13** (14 mg).

2,5-*Dihydroxy*-7-*methoxy*-8-*methlyflavanone and* 2,5-*dihydroxy*-7-*methoxy*-6-*methylflavanone* **10** *and* **11** (*in* 1:0.94 *mixture*). Pale yellow crystalline powder; mp 134–136°; $[\alpha]_D$ +20.7 ($c = 0.105$, $CHCl_3$); UV: λ_{max}: 277, 334 (sh) nm; IR ν_{max} cm^{-1}: 3346 (OH), 2916, 1639 (C=O), 1448, 1317, 1205, 1155, 1126, 767; HR-EIMS: *m/z* (rel. int.): 300.1006 [M^+] (100) (calcd 300.0998), 283 (41), 282 (99), 281 (30), 154 (23), 181 (16), 77 (11); ^1H NMR and ^{13}C NMR (Table 2).

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