A New Secokaurane Diterpenoid and its O-Glucoside from the Seeds of *Pentaclethra Macrophylla*

Gabriel N. FOLEFOC[a*], Jean Paul BISSECK[a], Zacharias T. FOMUM[a] AND Bernard BODO[b]

[a] Department of Organic Chemistry, University of Yaounde 1, P.O Box 812 Yaounde Cameroon.
[b] Laboratoire de Chimie URA 401 CNRS, Muséum National d'Histoire Naturelle, 63, rue Buffon 75005 Paris, France.

ABSTRACT

A new secokaurane diterpenoid, secopentaclethrolide **1** and its *O*-glucoside, secopentaclethroside **2**, were isolated from the seeds of *Pentaclethra macrophylla* together with the known alkaloid caffeoylputrescine **3**, and glyceryl monotetracosanoate **4**.
The structures of secopentaclethrolide and of secopentaclethroside were determined to be 16α,17-dihydroxy-6,7-seco-*ent*-kauran-19,6-β-olide and its 16-*O*-β-glucoside respectively, by spectral analysis.

Keywords: *Pentaclethra macrophylla*, Mimosaceae, Diterpene, Secopentaclethrolide, Secopentaclethroside.

RÉSUMÉ

Sécopentaclethrolide **1**, un nouveau diterpène de la classe de sécokaurane et son *O*-glucoside, sécopentaclethroside **2** ont été isolés des graines de *Pentaclethra macrophylla* ainsi que la caffeoylputrescine **3** et la 1-monotétracosanoate de glycérol **4** qui sont les métabolites sécondaires connus. Les structures de la sécopentaclethrolide et du sécopentaclethroside ont été établies sur la base de leur données spectroscopiques comme étant, respectivement, la 16α,17-dihydroxy-6,7-séco-*ent*-kauran-19,6-β-olide et son 16-*O*-β-glucoside.

Mots clés : *Pentaclethra macrophylla*, Mimosacées, diterpène, sécopentaclethrolide, sécopentaclethroside.

* Corresponding author: Tel: +237 751 02 81: Fax: + 237 222 13 20
E-mail: gfolefoc@yahoo.com (G.N. Folefoc)

INTRODUCTION

Pentaclethra macrophylla Benth. (Mimosaceae) is a large tree reaching 30 m or more in height and is widespread in the tropical West and Central African forest (Irvine, 1961). The roots and bark are used in Ghana as a laxative; the roots, bark and leaves are equally used as an enema for dysentery and as a liniment for itches, while the bark and seeds are used in parts of Ivory Coast, Congo Republics and Senegal to poison fish and as an ingredient in arrow poisons (Berhaut, 1975; Bouquet, 1969; Irvine, 1961).

Besides earlier reports stating the presence of a toxic alkaloid in the seeds (Berhaut, 1975; Bouquet, 1969; Irvine, 1961), very few phytochemical and pharmacological studies have been reported on *P. macrophylla*. Recently, triterpenes, a xanthone, a quinone, a quinol and hydroxyceratyl glycerols (Babady-Byla and Herz, 1996) have been recorded from this genus. As part of our continuing interest in Cameroonian plants of medicinal interests (Kamnaing et al., 1999; Ngadjui et al., 1999 and 2002), we have examined the extract of the seeds of *P. macrophylla*. This paper reports the isolation and structural elucidation of a new secokaurane diterpenoid, secopentaclethrolide **1** and its O-glucoside, secopentaclethroside **2**, together with previously known caffeoylputrescine **3** and 1-monotetracosanoate glycerol **4**.

EXPERIMENTAL

General

All melting points were determined on a Büchi Melting point apparatus B-540 and are uncorrected. IR spectra were realized with KBr disks. DIC-mass spectra were obtained using a Nermag R10-10C instrument. ^{1}H NMR (400 MHz) and ^{13}C NMR (75 MHz) spectra were recorded at room temperature with TMS as internal reference, and chemical shifts are given in δ values. 2D-NMR (^{1}H-^{1}H COSY, HMQC, HMBC and NOESY) experiments were performed with usual pulse-sequence and data processing was obtained with standard software.

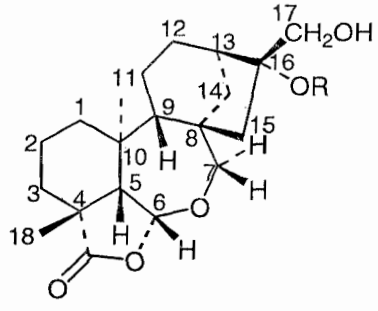
Plant material

The seeds of Pentaclethra macrophylla Benth. were collected in Ebolowa, South Province of Cameroon in October 1997. A voucher specimen (No. 29043/SRF/CAM) documenting the collection is deposited at the National Herbarium, Yaounde, Cameroon.

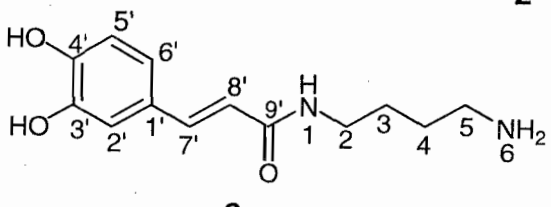
Extraction and Isolation

Air-dried, powdered seeds of Pentaclethra macrophylla (3 kg) were extracted with MeOH. The MeOH extract was filtered and evaporated under reduced pressure in a rotatory evaporator to obtain a fatty brown mass.(170 g) which was then re-extracted with hexane to give finally 120 g of MeOH extract and 40 g of hexane extract.

The methanol extract (120 g) was subjected to column chromatography (CC) over silica gel and eluted with hexane followed by hexane-CH_2Cl_2 and CH_2Cl_2-MeOH gradients. A total of 210 fractions of 200 ml each were collected and combined on the basis of TLC analyses leading to three main series (A-C).



1 R=H
2 R=Glc



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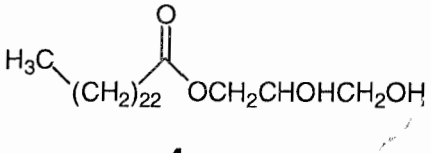

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Series A resulting from the combination of fractions (141-156) eluted with CH_2Cl_2-MeOH (95:5) afforded secopentaclethrolide **1** (35 mg) after recrystallization with $CHCl_3$ as white solid. Series B (fractions 171-175) was rechromatographed over a silica gel column. The elution of this column with EtOAc-MeOH (9:1) yielded secopentaclethroside **2** (22 mg) as white needles. Series C (fractions 198-206) was also rechromatographed over a silica gel column. Initial elution with CH_2Cl_2 and then CH_2Cl_2-MeOH (7:3) gave caffeoylputrescine **3** (52 mg) as a yellow powder.

The hexane extract (40 g) was equally subjected to chromatographic fractionations over silica gel column eluted with hexane and then hexane-EtOAc gradient. Glyceryl monotetracosanoate **4** (24 mg) was obtained from fractions eluted by hexane-EtOAc (65:35) as white crystals. Known compounds (3 and 4) were identified by comparison (mp, ^1H, ^{13}C NMR) with published information.

Secopentaclethrolide **1**

White non-crystalline solid (35 mg); mp: 290-291°C; IR ν_{max}^{KBr} cm^{-1}: 3500-3400 (OH), 2977 (C-H), 1777 (C= O), 1038 (C-O); DIC-MS m/z (rel.int.%): 351 (100) [M+H], 336 (4), 334 (4), 319 (8), 109 (15). ^1H NMR (400 MHz, DMSO) & ^{13}C NMR (75 MHz, DMSO) see Table 1.

Secopentaclethroside **2**

White needles (22 mg); mp: 318-319°C; IR ν_{max}^{KBr} cm^{-1}: 3500-3400 (OH), 2977 (C-H), 1777 (C = O), 1038 (C-O); DIC-MS m/z (rel.int.%): 513 (28) [M + H], 351(100), 319 (21), 287 (8), 180 (15), 127 (36), 109 (55). ^1H NMR (400 MHz, DMSO) & ^{13}C NMR (75 MHz, DMSO) see Table 1.

Table 1: ^1H (400 MHz, DMSO) and ^{13}C (75 MHz, DMSO) – NMR Assignments for Compounds 1 & 2

N°C	1			2		
	δ_C	δ_H	J(Hz)	δ_C	δ_H	J(Hz)
1	37.4	1.75		36.2	1.65	
2	18.6	1.55		17.7	1.38	
3	31.1	2.03		30.0	1.90	
4	44.9	-		43.8	-	
5	57.5	2.17	(d, 4.5)	55.7	2.15	(d, 4.5)
6	104.3	5.93	(d, 4.5)	103.2	5.91	(d, 4.5)
7	75.1	3.45 (α) 3.65 (β)	(d,11.0) (d,11.0)	73.4	3.45 (α) 3.65 (β)	(d,11.0) (d,11.0)
8	50.4	.		49.4	-	
9	58.3	1.33		56.4	1.23	
10	40.1	-		39.0	-	
11	19.3	1.88		18.3	1.77	
12	26.8	1.61		25.6	1.65	
13	45.3	2.16		41.7	2.10	
14	39.0	0.95		37.6	0.85	
15	48.8	1.45		45.3	1.43	
16	82.1	-		89.4	-	
17	66.3	3.50 (α) 3.60 (β)	(d, 11.3) (d, 11.3)	61.6	3.58 (α) 3.68 (β)	(d,11.3) (d,11.3)
18	27.3	1.26		26.5	1.18	
19	178.2	-		178.0	-	
20	19.9	1.23		19.1	1.10	
1'				96.9	4.26	(d,8.0)
2'				73.5	3.98	
3'				76.9	4.96	
4'				70.1	4.48	
5'				76.7	4.93	
6'				61.1	5.32	

RESULTS AND DISCUSSION

Secopentaclethrolide **1** was isolated as a white non-crystalline solid from a methanol extract of the dried powdered seeds as described in section 3. It gave a Libermann Burchard positive test for terpenes. Its molecular formula was determined as $C_{20}H_{30}O_5$ from NMR spectroscopy and DIC mass spectrometric measurements. It showed IR absorption bands for hydroxyl groups at ν_{max} 3500-3400 cm^{-1} and for a γ-lactone carbonyl at ν_{max} 1777 cm^{-1}. The ^{13}C NMR spectrum of **1** showed typical 16?,17-dihydroxy-*ent*-kaurane signals (Ohtani et al., 1992; Yamasaki et al., 1976) at δ57.5 ppm (C-5), 50.4 ppm (C-8), 58.3 ppm (C-9), 45.4 ppm (C-13), 39.0 ppm (C-14), 82.1 ppm (C-16), 66.3 ppm (C-17) and 178.2 ppm (C-19 carbonyl). The ^1H NMR spectrum of **1** also indicated the presence of two tertiary methyl groups at δ 1.23 and 1.26 (3H each, s).

However, the chemical shifts of C-6 and C-7 in **1** were observed very far down field δ 104.3 and 75.1 respectively) compared to those (δ 22.9 and 42.7 respectively) in 16α,17-dihydroxy-*ent*-kaurane diterpenoids (Ohtani et al., 1992; Yamasaki et al., 1976) suggesting the presence of electron-attracting O-atoms on them. In fact, the absence of a ^1H-^1H COSY correlation between H-6 and H-7α or H-7β confirmed the presence of an O-atom between C-6 and C-7 thus indicating the presence of a seco-ring B (Nagashima et al., 1994 and 1996) in the structure of **1**. Moreover, a strong ^1H-^1H COSY correlation between H-5 and H-6, together with a slight NOE effect between H-5 and H-7β were observed. Furthermore, the ^1H NMR spectrum of **1** displayed a low field doublet signal (δ 5.93, J = 4.5 Hz) characteristic of a dioxy-methine proton, due to the proton at C-6, which coupled with the proton at C-5 (δ 2.17, d, J = 4.5 Hz). Therefore, C-6 is attached to two O-atoms, one of which presumably forms a 5-membered ring γ-lactone with the observed carbonyl at C-19, and the other which is the O-atom of the seco-ring B.

The structure of secopentaclethrolide **1** was thus assigned as 16α,17-dihydroxy-6,7-seco-*ent*-kauran-19,6-β-olide. This structure was confirmed by both the ^{13}C NMR spectrum and by the DIC mass spectrum. The DIC mass spectrum showed fragments at m/z: 335, 333 and 319 corresponding to $[M\text{-}CH_3]^+$, $[M\text{-}OH]^+$ and $[M\text{-}CH_2OH]^+$ respectively. The ^{13}C NMR (Tables 1 & 2) was fully assigned using JMOD spectra and by comparison of measured values with those reported for *ent*-kaurane (Ohtani et al., 1992; Yamasaki et al., 1976) and secokaurane diterpenoids (Nagashima et al., 1994 and 1996).

Compound **2**, secopentaclethroside was isolated as white needles. It reacted positively to the Libermann Burchard test for terpenes and to the Molish test for glucosides. It was formulated as $C_{26}H_{40}O_{10}$ on the basis of its DIC mass spectrum and ^{13}C NMR analysis. Like secopentaclethrolide **1**, compound **2** also showed IR absorption bands for hydroxyl groups at ν_{max}3500-3400 cm^{-1} and for a γ-lactone carbonyl

Table 2: ^1J (From HMQC), ^2J and ^3J gradient HMBC correlations for compound 1

Proton	Position	^1J correlated carbon	^{2-3}J correlated carbons
1.23	Me – 20	19.9	58.3 (C-9); 57.5 (C-5); 40.1 (C-10); 27.4 (C-1)
1.45	15	48.8	66.3 (C-17); 58.3 (C-9); 50.4 (C-8); 45.3 (C-13); 39.0 (C-14)
0.95	14	39.0	58.3 (C-9)
2.16	13	41.7	82.1 (C-16); 50.4 (C-8); 48.8 (C-15); 26.8 (C-12); 19.3 (C-11)
1.61	12	25.6	58.3 (C-9)
1.33	9	58.3	75.1 (C-7); 50.4 (C-8); 40.1 (C-10); 39.0 (C-14); 19.3 (C-11)
3.45 (α) 3.65 (β)	7	75.1	104.3 (C-6); 58.3 (C-9); 50.4 (C-8) 104.3 (C-6); 58.3 (C-9); 50.4 (C-8)
5.93	6	104.3	40.1 (C-10)
2.17	5	57.5	104.3 (C-6); 58.3 (C-9); 19.3 (C-11)
2.03	3	31.1	57.5 (C-5); 18.6 (C-2)
1.75	1	36.2	58.3 (C-9); 40.1 (C-10)

group at ν_{max} 1777 cm^{-1}.

Besides the chemical shift signals of **1**, the NMR spectra of **2** showed additional signals characteristic of a glucose skeleton (Table 1) [δ_H 4.26 (1H,d, J = 8Hz, H-1'), δ_C 96.9 (C-1'), 73.5 (C-2'), 76.9 (C-3'), 70.1 (C-4'), 76.7 (C-5'), 61.1 (C-6')]. The presence of one glucoside linkage was established by the observation of only one anomeric resonance (δ 96.9) in the δ 90-112 ppm region of the ^{13}C NMR of **2**, and the β-configuration of the linkage was deduced from the coupling constant (J = 8 Hz) of the doublet signal of H-1' (Thomson, 1985). The appearance of the anomeric carbon (C-1') signal at high field (δ 96.9) in the ^{13}C NMR spectrum of **2** suggests that C-1' is bound to the tert-OH at C-16 (Ohtani et al., 1992; Yamasaki et al., 1976). In fact, the observation of a strong ^3J correlation (HMBC) between C-16 and H-1' together with the significant low-field shift of the C-16 signal [from δ 82.1 in **1** to δ 89.4 in **2**] lends strong support to the glucoside linkage at the tert-OH at C-16.

Hence, the structure of compound **2** was assigned as the 16-*O*-β-glucoside of **1**. Both the ^{13}C NMR and the DIC mass spectra also confirmed this structure. The DIC mass spectrum showed important fragments at m/z: 351 and 180 corresponding to secopentaclethrolide **1** and a glucoside residue respectively. The ^{13}C NMR of **2** (Table 1) was fully assigned as in **1**. Secopentaclethrolide and secopentaclethroside are reported here for the first time.

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