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Scaphopetalone and scaphopetalumate, a lignan and a triterpene ester from *Scaphopetalum thonneri*

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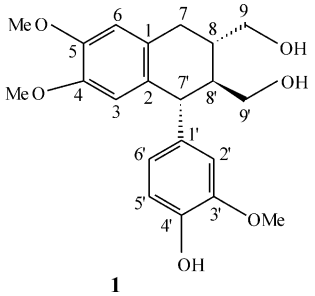
Abstract

From the methanol extract of the stem bark of *Scaphopetalum thonneri*, two new compounds, including one lignan, named scaphopetalone, one new ester of ferulic acid, named scaphopetalumate were isolated together with three known compounds including: two coumarins (scopoletin and scopolin), and one pentacyclic triterpene (oleanolic acid). The structure of the new compounds were elucidated by means of spectroscopic analyses.

Keywords: *Scaphopetalum thonneri*; Sterculiaceae; Scaphopetalone; Scaphopetalumate; Coumarins; Pentacyclic triterpene

1. Introduction

Scaphopetalum thonneri from the family Sterculiaceae is a small tree of 3–4 m height which grows in the rain forest region of Cameroon. Its stem bark is widely used in Cameroonian folk medicine for the treatment of kidney disease, wounds and stomach-ache (Bouquet, 1969). At the best of our knowledge, no phytochemical study has been reported on this plant. In the present paper, we wish to report the isolation and the structural elucidation of two novel compounds, one aryltetralin lignan derivative named scaphopetalone (**1**) and one new ester of ferulic acid, scaphopetalumate (**2**), along with three known compounds, scopoletin, scopolin, and oleanolic acid.



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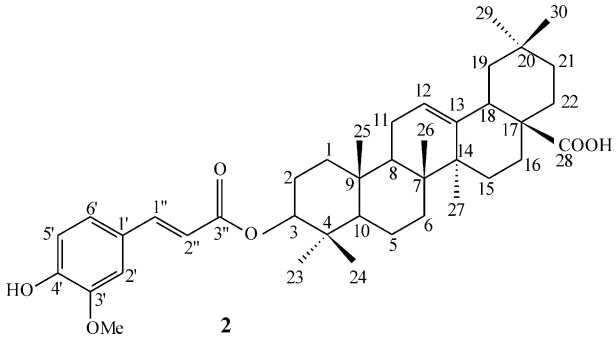

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2. Results and discussion

The methanol extract of the finely powdered stem bark of *S. thonneri,* on chromatographic separation, afforded a novel lignan named scaphopetalone (**1**), a novel ester of ferulic acid named scaphopetalumate (**2**), along with the known coumarins, scopoletin and scopolin and the known pentacyclic triterpen oleanolic acid. The known compounds were identified by direct comparison of their physical and spectral data (mp, ^{1}H and ^{13}C NMR) with authentic samples and corresponding published values (Sibanda et al., 1989;

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Rao et al., 1988; Mahato and Kundu, 1994; Spengel, 1996).

Compound **1**, to which we assigned the name scaphopetalone, was obtained as brown sticky oil whose elemental composition was determined to be $C_{21}H_{26}O_6$ corresponding to 9 degrees of unsaturation. The IR spectrum of **1** indicated the presence of hydroxyl group at 3420 cm^{-1} and aromatic rings at 1608 and 1500 cm^{-1}. The UV spectrum exhibited absorption maxima at 219 and 274 nm. The broad band decoupled ^{13}C NMR spectrum showed 21 carbon signals. The analysis of this spectrum by the aid of DEPT technique indicated the presence in the molecule of seven sp^2 quaternary carbons, eight methine with five sp^2 and three sp^3 carbons, three secondary sp^3 methylene carbons and three primary sp^3 methyl carbons. In the ^1H NMR spectrum analysed by ^1H–^1H COSY, a typical ABX spin system of three protons at δ 6.76 (*d*, $J=8.1$ Hz), 6.63 (*dd*, $J=8.1$, 1.9 Hz) and 6.69 ppm (*dd*, $J=1.9$ Hz), two singlets of one proton at δ 6.28 and δ 6.71 ppm were shown suggesting the presence of two aromatic rings in the molecule. From this evidence **1** was assigned as a derivative of an aryltetralin lignan (Zhang et al., 1999; Yuasa et al., 1996; Chang et al., 2000; Fonseca et al., 1978). The ^1H NMR spectrum of **1** also showed three singlets of three protons at δ 3.55, 3.77 and 3.88 ppm corresponding to three methoxyl groups, a set of signals consisting of one methylene multiplet at δ 3.68 (2 H, m), two 1 H double doublets at δ 3.42 (*dd*, $J=11.3$, 4.0 Hz) and 3.71 ppm (*dd*, $J=11.3$, 4.0 Hz) due to diastereotopic hydroxyl methyl protons. The ^{13}C NMR spectrum exhibited signals at δ 146.0, 148.2, 148.3 and 148.4 ppm corresponding to oxygenated aromatic carbons situated in *ortho* position in the two aromatic rings (Agrawal, 1989). As this molecule contained three methoxyl groups, the other oxygenated substituent in the aromatic ring is unequivocally the hydroxyl group. The placement of the methoxyl and hydroxyl groups on the two aromatic rings was established by HMBC spectra. Long range correlation through two bonds were observed on one hand between proton H-6 at δ 6.28 ppm with carbon C-5, and proton H-3 at δ 6.71 ppm with carbon C-4 at δ 148.2 ppm, and on the other hand between positions of methoxyl signals at δ 3.77 ppm with carbon C-5 at δ 148.3 ppm, and between proton at δ 3.80 ppm with carbon C-4, indicated that, two of these three methoxyl groups were located on aromatic rings at position C-4 and C-5, respectively. Thus, the third methoxyl group and hydroxyl were located on the aromatic ring B at adjacent positions. The C-3′ position of the third methoxyl group on this ring was determined from the HMBC correlation between proton H-2′ at δ 6.69 ppm with carbon C-3′ at δ 148.4 ppm, and methoxyl proton at δ 3.55 ppm with carbon C-3′. Therefore, the hydroxyl was placed at C-4′. Further evidence of the structure **1** was given by the analysis of ^1H–^1H ROESY spectrum which indicated spatial connectivities of H-2′ with OMe-3′, H-6 with OMe-5, H-3 with OMe-4. The relative stereochemistry of the tetrahydronaphtalene ring was inferred from the careful analysis of the ^1H NMR spectrum of compound **1**. In the matter of fact, the values of the coupling constants between H-8′ and H-8 protons ($J=7.0$ Hz), on one hand and between H-8′ and H-7′ protons ($J=10.2$ Hz), on the other hand, suggested that proton H-8 has *trans* axial–axial relationship with the other (H-7′ and H-8). This was corroborated by the ROESY spectrum which showed no correlation peaks between H-8′ and H-8 protons, but cross-peaks between H-8 and H-7′ suggesting their close proximity through the space. On these grounds compound **1** was deduced to be 9,4′,9′-trihydroxy 4,5,3′-trimethoxy aryltetralin lignan.

Compound **2**, named scaphopetalumate, was obtained as amorphous powder and has a molecular formula of $C_{40}H_{56}O_6$ determined by the high resolution electrospray-TOF mass spectrum (HRESIMS) which shows the pseudomolecular ion peak $(M+H)^+$ at *m*/*z* 633.4464 (Calc. for $C_{40}H_{56}O_6$, 633.4467). This formula indicated 13 degrees of unsaturation. The IR spectrum of **2** showed characteristic bands at 3423 (OH), 1700 (C=O), 1632 (olefinic C=C), 1509 (aromatic C=C) and 1280–1140 cm^{-1} (ether linkage). The UV spectrum showed absorption bands at λ_{max} (MeOH) (log ε): 204 (4.21), 236 (3.94), 327 (4.16) nm. The ^1H NMR spectrum of **2** displayed seven methyl groups in the region δ 0.82–1.31 ppm and a broad singlet at δ 5.40 ppm characteristic of a typical Δ^{12}-oleane skeleton (Ikuta and Itokawa, 1988; Conrad et al., 1998). This was confirmed in the ^{13}C NMR spectrum with the signals in the region of δ 15–34 ppm, at δ 122.4 and 144.0 ppm attributed respectively to seven methyl groups, to carbons 12 and 13 of the Δ^{12}-oleanan skeleton. The presence of this skeleton was also supported by the mass spectrum of **2** which showed prominent fragments ion peaks charateristic of RDA cleavage of B and C rings at *m*/*z* 248 and 203. The ion fragment at *m*/*z* 203 (248-COOH) indicated the presence of a carboxylic group in ring D.

The ^1H NMR of **2** exhibited besides the presence of a *trans* double bond by signals at δ 6.38 and 7.65 ppm with a coupling constant of $J=16.0$ Hz, a typical ABX spin system in the aromatic ring at δ 6.97 (*d*, $J=8.5$ Hz), 7.18 (*dd*, $J=8.5$ and 2.1 Hz) and 7.10 ppm (*d*, $J=2.1$ Hz) with *ortho*, *ortho*/*meta* and *meta* coupling constant respectively, a singlet of three protons at δ 3.98 ppm corresponding to a methoxyl group and a broad signal exchangeable with D_2O corresponding to a hydroxyl group. This indicated the presence of a feruloyl or isoferuloyl unit in compound **2**. This was supported by ^{13}C NMR spectrum which showed signals at δ 168.6 ppm due to the carbonyl group of an ester function. Further confirmation of this skeleton came from the mass spectrum of **2** which showed significant fragment at *m*/*z* 177

characteristic of a feruloyl or isoferuloyl moiety (Nkengfack et al., 1997). The positions occupied by the hydroxyl and methoxyl groups on aromatic ring were established through NOESY spectral analysis which showed spatial connectivities of H-2′ at δ 7.10 ppm with OMe at δ 3.98 ppm. This indicated that H-2′ is in close spatial proximity with respect to methoxyl group. Thus the methoxyl is attached at the C-3′ position, the hydroxyl at the C-4′ position in the aromatic ring. From the above spectroscopic evidence the structure of **2** was established as 3β-*O*-(*E*)-feruloyl oleanolic acid.

3. Experimental

3.1. General experimental procedures

1D and 2D homo and heteronuclear NMR spectra were recorded on a Varian unity INOVA-500 spectrometer: 500 MHz (^{1}H) and 75.42 MHz (^{13}C) in CD_3OD and $CDCl_3$. IR spectra were recorded on a SHIMADZU 408 spectrophotometer in KBr disks. UV spectra were obtained on a Beckman Model 25 spectrophotometer. EIMS (ionization voltage 70 eV) was measured in a Varian MAT 311A spectrometer, and HRESI-TOF mass spectra were taken on a APCI QSTAR pulsar spectrometer. Silica gel 60GF 254 plates (Merck) was used for TLC. Preparative HPLC was done on RP18 Li chrospher 100 (250×16 mm), with flow rate and detection wavelenght 5 ml mn^{-1} and 254 nm, respectively.

3.2. Plant material

Stem bark of *S. thonneri* was collected in July 1999 at Kribi (Cameroon). A Voucher specimen documenting the collection was identified at the National Herbarium,Yaounde, Cameroon and is on deposit there.

3.3. Extraction and isolation

The air dried powdered stem bark of *S. thonneri* (6 kg) was successively extracted with petroleum ether (30–60°), EtOAc and MeOH yielding 9, 18 and 70 g of crude extracts respectively at room temperature. The MeOH extract was subjected to column chromatography over Si gel 60 (70–230 mesh, ASTM; Merck) eluting with $CHCl_3$–(MeOH–H_2O 9:1) mixture with increasing polarity. A total of 56 fractions of ca. 250 ml each were collected and combined on the basis of TLC analysis leading to 10 series (A–J). Serie A (3 g) eluted with $CHCl_3$, was column chromatographed over Si gel using petroleum ether–EtOAc mixture of increasing polarity. A total of 194 fractions of ca. 100 ml each were collected. Fractions 81–166 (1 g), eluted with petroleum ether–EtOAc (4:1), were subjected to column chromatography over Si-gel, with petroleum ether–EtOAc (7:1) to yield 80 fractions of ca. 10 ml each.

Fraction 50 (200 mg), eluted with petroleum ether–EtOAc (7:1) was further purified by column chromatography using petroleum ether–EtOAc mixture as elution gradient to afford 70 fractions of ca. 10 ml each. Fraction 30 was purified by PTLC using petroleum ether–acetone (8:2) to yield compound **5** (oleanolic acid, 20 mg).

Fraction 60 (100 mg), eluted with petroleum ether–EtOAc (7:1) was chromatographed over Si gel to give 70 fractions of ca. 10 ml each. Fraction 50 (25 mg) was further purified by PTLC using petroleum ether–acetone 77.5:22.5 to give scaphopetalumate (**2**, 10 mg).

Serie B (2 g) eluted with $CHCl_3$–(MeOH–H_2O 9/1) (8/2), was subjected to column chromatography of increasing polarity using petroleum ether–EtOAc (17:3). Fifty-two fractions of 100 ml each were collected. Fractions 21–34 (100 mg) eluted with petroleum ether–EtOAc (3:1) were purified by column chromatography using petroleum ether–EtOAc (77.5:22.5). Fifty fractions of 10 ml each were collected. Fraction 40 (20 mg) was further purified by PTLC using petroleum ether–EtOAc–MeOH (9:4:1) to yield scopoletin (**3**, 10 mg).

Serie E eluted with $CHCl_3$–(MeOH–H_2O 9:1) (7.3), was column chromatographed over Si gel using $CHCl_3$–MeOH (92.5:7.5). Forty fractions of ca. 25 ml each were collected. Fraction 30 was subjected to preparative RP 18 HPLC using gradient 20–100% CH_3CN–H_2O + 0.05 TFA in 40 min. Fractions 2–3 were subjected to another RP 18 HPLC semi preparative using 9–100% CH_3CN–H_2O + 0.05 TFA. Fraction 5 obtained with 9% CH_3CN–H_2O + 0.05 TFA gave scopolin (**4**, 8 mg).

Serie F (3.5 g) eluted with $CHCl_3$–(MeOH–H_2O 9/1) (7:3), was chromatographed on a Sephadex LH 20 column with MeOH as eluent to afford 34 fractions of 25 ml each. Fractions 22–26 (150 mg) were subjected to preparative RP 18 HPLC using gradient 20–100% CH_3CN–H_2O + 0.05 TFA in 30 mn, 19 fractions were collected. Fraction 14 (17 mg) was further purified by semi preparative RP 18 HPLC using CH_3CN–H_2O + 0.05 TFA 18% to afford scaphopetalone (**1**, 10 mg).

3.3.1. 9,4′,9′-Trihydroxy 4,5,3′-trimethoxy aryltetralin lignan (scaphopetalone, 1)

Brown sticky oil, UV λ_{max} nm (MeOH) (log ε): 219(2.32), 274(0.45); IR ν_{max} (KBr) cm^{-1}: 3420, 1608, 1500, 1470, 1290 and 1195; ^{1}H NMR (500 MHz,CD_3OD) see Table 1, ^{13}C NMR (75,42 MHz, CD_3OD) see Table 1, HREIMS *m*/*z* 374.17296 (calcd. for $C_{21}H_{26}O_6$, 374.17298; EIMS *m*/*z*: 374 [M$^+$] (100), 356 (16), 325 (60), 298 (18), 255 (69), 201 (14), 189 (22), 137 (25), 115 (9), 69 (16), 44 (28), 29 (38).

Table 1
^1H CD_3OD (500 MHz) and ^{13}C CD_3OD (75.42 MHz) assignments for 9,4′,9′-trihydroxy 4,5,3′-trimethoxy aryltetralin lignan (**1**)

Position	^{13}C	^1H (*J* Hz)
1	130.0	–
2	134.0	–
3	112.8	6.71 (*s*)
4	148.2	–
5	148.3	–
6	114.6	6.28 (*s*)
7	33.5	2.80 (*br d*, *J* = 7.5)
		2.81 (*br d*, *J* = 7.5)
8	40.0	2.01 (*br dd*, *J* = 3.8, 7.0)
9	65.9	3.68 (*m*)
1′	138.5	–
2′	113.8	6.69 (*d*, *J* = 1.9)
3′	148.4	–
4′	146.0	–
5′	116.0	6.76 (*d*, *J* = 8.1)
6′	123.2	6.63 (*dd*, *J* = 1.9, 8.1)
7′	46.0	3.88 (*d*, *J* = 10.2)
8′	47.0	1.78 (*br ddd*, *J* = 10.2, 7,0, 3.9)
9′	62.1	3.42 (*dd*, *J* = 4.0, 11.3)
		3.71 (*dd*, *J* = 4.0, 11.3)
4-OMe	56.4	3.80 (*s*)
5-OMe	56.4	3.77 (*s*)
3′-OMe	56.5	3.55 (*s*)

3.3.2. 3β-O-E-feruloyl oleanolic acid (scaphopetalumate, 2)

Amorphous powder, UV λ_{max} nm (MeOH) (log ε): 204 (4.21), 236 (3.94), 327 (4.16); IR ν_{max} (KBr) cm^{-1}: 3423, 1700, 1632, 1509, 1280 and 1140; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.65 (1H, *d*, *J* = 16.0 Hz, H-1″), 6.38 (1H, *d*, *J* = 16.0 Hz, H-2″), 7.18 (1H, *dd*, *J* = 2.1, 8.5 Hz, H-6′), 7.10 (1H, *d*, *J* = 2.1 Hz, H-2′), 6.97 (1H, *d*, *J* = 8.5 Hz, H-5′), 5.85 (1H, *s*, exch D_2O, OH-4′), 5.40(1H, broad singlet, H-12), 3.98 (3H, *s*, OMe-3′), 4.7 (1H, *t*, *J* = 8.7 Hz, H-3), 2.94 (1H, *dd*, *J* = 4.0, 14.0 Hz, H-18),1.31,1.18, 1.02, 0.98, 0.97, 0.96, 0.86 (each, 3H, *s*, 7×CH_3), ^{13}C NMR (75.42 MHz, $CDCl_3$) δ: 38.1(C-1), 26.1(C-2), 80.6 (C-3), 37.4 (C-4), 55.7 (C-5), 18.3 (C-6), 32.5 (C-7), 39.6 (C-8), 47.8 (C-9), 37.4 (C-10), 24.1 (C-11), 122.4 (C-12), 144.0 (C-13), 41.8 (C-14), 28.0 (C-15), 24.2 (C-16), 46.1 (C-17), 41.4 (C-18), 47.5 (C-19), 30.8 (C-20), 33.5 (C-21), 33.2 (C-22), 28.4 (C-23), 17.1 (C-24), 15.6 (C-25), 17.5 (C-26), 26.1 (C-27), 180.5 (C-28), 33.8 (C-29), 23.7 (C-30), 127.6 (C-1′), 115.4 (C-2′), 147.9 (C-3′), 147.1 (C-4′), 116.2 (C-5′), 122.8 (C-6′), 144.0 (C-1″), 110.0 (C-2″), and 168.6 (C-3″). ESIMS: *m*/*z* 633.4464 $[M+H]^+$ (calcd. for $C_{40}H_{56}O_6$, 633.4467); EIMS *m*/*z* (rel. int.): 586 $[M^+–COOH–H]$ (2), 552 (2), 509 (3), 471 (1), 438 (12), 423 (8), 384 (40), 353 (2), 285 (3), 248 (87), 203 (80), 191 (58), 177 (100), 145 (26), 119 (22), 69 (37), 43 (41), 28 (16).

Scopoletin, scopolin (Sibanda et al., 1989; Rao et al., 1988), oleanolic acid (Mahato and Kundu, 1994; Spengel, 1996) were identified by comparison of their spectral data (^1H NMR, ^{13}C NMR and MS) with those published in the literature.

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