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Research Article

Entiline E A New Limonoid Isolated from Root Bark of Antrocaryon Klaineanum Pierre (*Anacardiaceae*)

Yannick Fouokeng[1]*, Ahri Bernie Djamen Mbeunkeu[1], Stevine Claudiale Popwo Tameye[1], Sandrine Mewaba Goulefack1, Hartmut Laatsch[2] and Anatole Guy Blaise Azebaze[1]

[1]*Department of Chemistry, University of Douala, Faculty of Sciences, France*

[2]*Institute for Organic and Biomolecular Chemistry, University of Göttingen, Germany, France*

***Corresponding author:** Yannick Fouokeng, Department of Chemistry, University of Douala, Faculty of Sciences, France

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Abstract

One new pentacyclic triterpenoids, entiline E [1] together with ten known compounds were isolated from the methanol extract from the root barks of Antrocaryon klaineanum, a Cameroonian medicinal plant. The structures of all compounds were determined by comprehensive analyses of their 1D and 2D NMR, mass spectra (EI and ESI) data and comparison with previously known analogues.

Keywords: Antrocaryon Klaineanum; *Anacardiaceae*; Entiline E

Introduction

Antrocaryon klaineanum *(Anacardiaceae)* is an evergreen tree that usually grows up to 35 m high. It occurs in central Africa and is used in traditional medicine to treat chlamydia infections, wounds, back pain, liver diseases and female sterility [1-3]. Recent phytochemical investigations on bark of this specie revealed steroids, limonoids and phenolic compounds [4-6]. In this report, we describe the isolation and structural elucidation of one new limonoid entiline E [5] together with ten know compounds.

Experimental

General

The optical rotations were measured with a Perking-Elmer polarimeter, model 241, at the sodium D line (λ = 589 nm). Melting points were determined on a Melter FP61 melting point apparatus. The IR spectrum was recorded on a FT/IR-4100 Jasco spectrometer. UV/Vis's spectra were obtained on a Jasco V-650 spectrophotometer. The NMR spectra were recorded on a Varian Inova-500 NMR spectrometer at 600 MHz (1H) or 150 MHz (13C), respectively. Chemical shifts are given in δ values (ppm), and coupling constants are reported in [Hz]. HRESI mass spectra were obtained on a micrOTOF (Bruker) mass spectrometer. Open column chromatography was performed with silica gel (70-230 mesh). Thin-layer chromatography (TLC) was carried out on precoated silica gel 60 F254 plates (Merck), and the TLC spots were viewed at 254 nm and visualized by heating the plates at 80 0C for 10 minutes after spraying with 50% aqueous sulfuric acid.

Plant Material

The root bark of Antrocaryon klaineanum were collected at Mount Elounden, in the Center Region of Cameroon in November 2014 and identified by Mr Victor NANA, botanist at the National Herbarium of Cameroon (Yaounde), where a voucher specimen (N° 21247SRF/CAM) was deposited.

Extraction and Isolation

The air-dried and powdered root bark of A. klaineanum (7.5 Kg) was submitted twice to extraction with methanol at room temperature for 48 h. After evaporation with a rotary evaporator under reduced pressure at 40°C, 435.7 g of crude extract were obtained and were fractionated using vacuum chromatography into fractions A [hexane/ethyl acetate (1:0 and 1:1), 20.40 g], B [ethyl acetate, 170.35 g] and C [methanol, 180.25 g]. 20.1 g of fraction A was subjected to silica gel column chromatography and eluted with a gradient system of n-hexane/EtOAc (1:0 to 0:1); 185 sub-fractions (100 mL each) were collected and pooled based on their TLC profile to yield seven sub-fractions. Sub-fractions 20-33 obtained by eluting the column with n-hexane/EtOAc (97.5:2.5) afforded 5 (17.2 mg). Sub-fractions 54-73 obtained by eluting the column with n-hexane/EtOAc (97.5:2.5) was further chromatographed by silica gel column chromatography and eluted with a gradient of n-hexane/EtOAc (0:1 to 97:3). 65 sub-fractions (25 mL each) were collected and pooled based on their TLC profile leading in the order of their Rf values to give 2 (70.4 mg) and 3 (370.1 mg) respectively. Sub-fractions 74-113 (6.9 g) obtained by eluting the column with n-hexane/EtOAc (95:5) were further chromatographed by silica gel column chromatography and eluted with a gradient of n-hexane/EtOAc (1:0 to 95:5) 75 sub-fractions (10 mL each) were collected and pooled on the basis of their TLC profile leading in the order of their Rf values to give 4 (2155.6 mg), 1 (2.0 mg), 7 (1.3 mg) and 12 (10.1 mg) respectively. Sub-fractions 114-133 obtained with n-hexane/EtOAc (92.5: 7.5) yielded 6 (10.8 mg), while n-hexane/EtOAc (85:15) (134-160 sub-fractions) gave 9 (176.01 mg). 160.7 g of fraction B was subjected to silica gel column chromatography and eluted with a gradient system of n-hexane/EtOAc (7:3 to 0:1); 266 sub-fractions (100 mL each) were collected and pooled based on their TLC profile to yield seven sub-fractions. Sub-fractions 11-25 obtained by eluting the column with n-hexane/EtOAc (7: 3) was further chromatographed by silica gel column chromatography and eluted with a gradient of n-hexane/EtOAc (9:1 to 85:15). 45 sub-fractions (10 mL each) were collected and pooled based on their TLC profile leading in the order of their Rf values to give again 3 (70.4 mg) and 4 (370.1 mg) with 57.51 mg of 11. Sub-fractions 26-80 obtained by eluting the column with n-hexane/EtOAc (6: 4) afforded 3 (70.4 mg) and 9 (15.9 mg). Sub-fractions 184-237 obtained by eluting the column with n-hexane/EtOAc (4: 6) afforded 10 (160.01 mg). The fraction C is under examination.

Results and Discussion

The n-hexane/ethyl 1:1 fraction A and pure acetate fraction B obtained by chromatography of the crude A. klaineanum extract was further separated by a combination of column chromatography on silica gel and preparative thin layer chromatography, resulting in the isolation and characterization of eleven compounds. On the basis of detailed spectroscopic analyses and comparison with reported data, the structures of ten of them (Figure 1) were determined as entilin B (2)[7], entilin C (2)[8], antrocarine A (4), antrocarine C (6) [5], oleanoic acid (7)[9], 7α,20(S)-dihydroxy-4,24(28)-ergostadien-3-one (9)[10], β-sitosterolglucosid (10)[11], antrocarine F (11) [5], β-sitosterol and stigmasterol (12) [11]. Compound 1 was isolated as a white powder, which gave a positive test for limonoids with the ehrlich reagent (green) and had the molecular formula C26H34O8Na, as established by ESI-HRMS (m/z 497. 2146 [M+Na]+) regarding the ten degrees of unsaturation. The IR spectrum of 5 showed typical vibrations bands of hydroxyl group at 3499.2 and 3413.39 cm^{-1}, at 2924.52 and 2852.00 cm^{-1} an elongation band of C-H bond and at 1714.41 cm^{-1}, a large band characteristic of conjugated double bond. Analysis of the 1H-NMR data (Table 1) of compound 1, indicated resonances of aromatic protons at δ_H 6.29 (1H, d, J = 1.5 Hz), δ_H 7.29 (1H, s) and δ_H 7.36 (1H, d, J = 1.71) characteristic of furanic cycle β -substituted; a singlet at δ_H 5.52 (1H, s), two oxymetines protons at δ_H 5.22 (1H, d, 0.75 Hz) and δ_H 3.76 (1H, d, 1.7Hz), three hydroxyls protons at δ_H 3.60, 3.26 and 2.87, and four methyl's groups at δ_H 1.81 (3H). These findings agree with the furan limonoid skeleton especially entiline's type [7,12]. We also observed in the strong fields a methylene signal at δ_H 1.74 (2H, m) and an aliphatic methyl which appears in triplet at δ_H 1.04 ppm (3H, t).

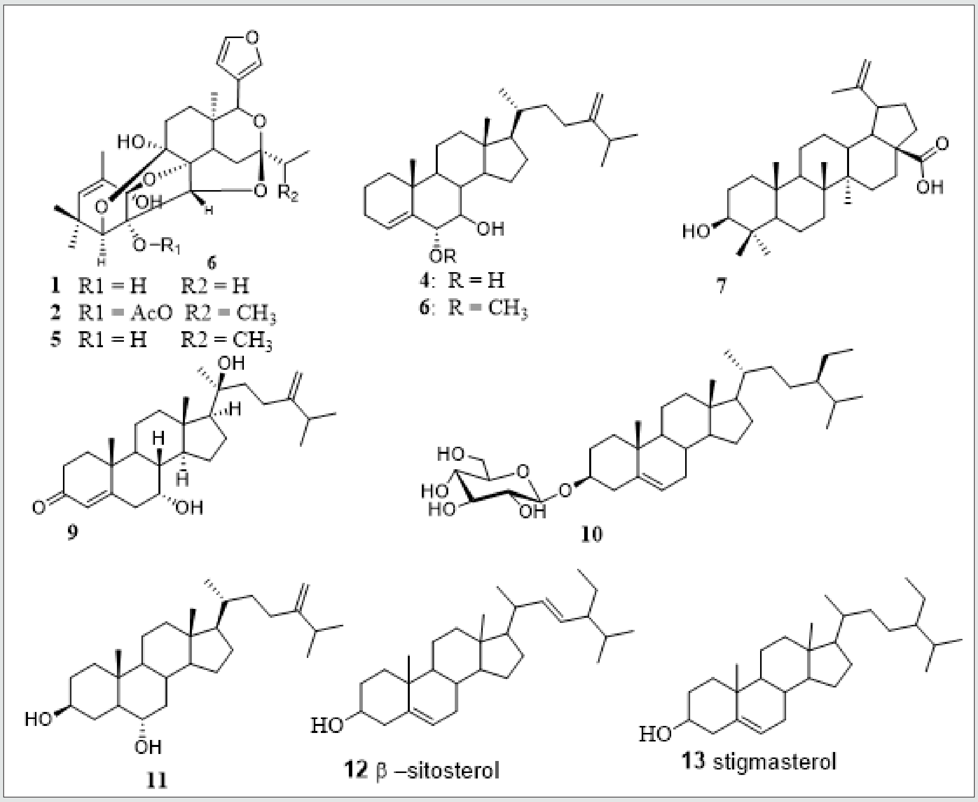

Figure 1: Chemical structures of compounds 1-13 isolated from Antrocaryon klaineanum.

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Table 1: ^{1}H-NMR and ^{13}C NMR data of entiline E in $CDCl_3$.

Attributions					
Positions	**^{13}C**	**1H (Mult; J(Hz))**	**Positions**	**^{13}C**	**1H (Mult; J(Hz))**
1	98.6	-	17	72.7	5.52 (1H, s)
2	77.5	-	18	24.5	0.80 (3H; s)
3	81	3.76 (1H; d; 1.7)	19	16.5	1.82 (3H; s)
4	38.1	-	20	124.2	-
5	133.8	5.22 (1H; d; 0.75)	21	139.6	7,29 (1H; s)
6	-	-	22	109.7	6.29 (1H; dd; 1.5)
7	-	-	23	142.6	7.36 (1H; d; 1.71)
8	79.6	-	28	27. 9	1.17 (3H; s)
9	97.6	-	29	27.8	1.08 (3H; s)
10	127.6	-	30	79	4.63 (1H; s)
11	30.9	1.85; 2.00 (2H, t)	1’	98.9	-
12	32.2	1.5 (2H; t)	2’	34.4	1.74 (2H; q)
13	35.3	-	3’	7.9	1.04 (3H; d; 7.0)
14	38.3	2.06 (1H; t; 3.0)	1-OH	-	3.60 (1H; s)
15	22.5	1.93; 2.03 (2H; t)	2-OH	-	3.26 (1H; s)
16	-	-	9-OH	-	2.87 (1H; s)

This base skeleton is confirmed on its 13C NMR spectrum which reveals the presence of 26 carbons atoms including: Four olefinic carbon characteristics at δ_C 142.6 (C-23); 139.7 (C-21); 124.6 (C-20) and 109.7 (C-22) of the furanic cyle as well as those of a double-bond tri-substituted at δ_C 132.5 (C-5) and 128.6 (C-10). The C-17 carbon signal of furolimonoids is observed at δ_C 72.52. We also observe the presence of six other sp^3 carbon atoms linked to oxygen including, three ketals at δ_C 98.9 (C-1 ‘); 98.6 (C-1) and C 97.6 (C-9), a quaternary at δ_C 77.5 (C-2) and oxymethyl at δ_C 79.0 (C-30) and δ_C 81.0 (C-3). Also, five methyl signals are observed, thus the gem dimethyl at δ_C 27.9 (C-28) and δ_C 27.8 (C-29) as well as those δ_C 24.5 (C-18) and 16.5 (C -19). We finally observed another aliphatic methyl at δ_C 7.9 (C-3’). The presence of ethyl group was confirmed in COSY spectrum where we observed the correlations between protons H-22 and H-23, H-11 and H-12, H-3 and H-14, another correlation between aliphatic methyl protons (H-3’) with methylene (H-2’) was observed. In other to determine the position of ethyl group, 2D experiments were used. The HSQC spectrum showed that the carbon C-1’ is quaternary and suggested that it would be the seat of junction. This is confirmed on the HMBC spectrum in which we observed the correlations of H-3’ (δ_H 1.04) with C-2’ (δ_C 34.4) and C-1’ (δ_C 98.9) in 2J and 3J respectively, with H-2 ‘ (δ_H 1.92) and C-1’ (δ_C 98.9) and C-15 (δ_C 22.5). To determine the relative configuration around the C-1’ carbon, we used the NOESY spectrum on which we observed the correlation of protons of methylene H-3’ with proton H-14 in alpha. There are also other couplings with proton H-2’ and H-15 in alpha. This makes it possible to assign the configuration S to the carbon C-1’. All these data compared to those of the literature confirm that the compound 1 is a new tetranortriterpenoide described here for the first time that we have named trivially entiline E.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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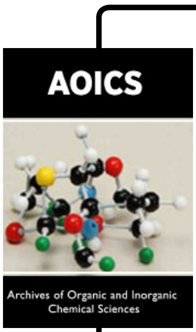

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