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Anthocleistenolide B, a New Secoiridoid from *Anthocleista liebrechtsiana* De Wild & T. Durand

Francis T. Ngouafong[1], Roland T. Tchuenguem[2], Jonas Kühlborn[3], Billy T. Tchegnitegni[1], Beaudelaire K. Ponou[1], Rémy B. Teponno[1], Jean P. Dzoyem[2], Till Opatz[3], Léon A. Tapondjou[1*]

[1]Department of Chemistry, Faculty of Science, University of Dschang, Dschang, Cameroon
[2]Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon
[3]Institute of Organic Chemistry, Johannes Gutenberg University Mainz, Mainz, Germany
Email: *tapondjou2001@yahoo.fr

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Abstract

Drug resistance due to the extensive abuse and over-use of antibiotics has become an increasingly serious problem and researchers started to use plants as an alternative source of new antibiotics. The aim of the present study was to assess the antimicrobial properties of secondary metabolites isolated from *A. liebrechtsiana*. A new secoiridoid derivative, anthocleistenolide B (**1**), along with three known compounds, the monoterpene diol, djalonenol (**2**), the xanthone *O*-glycoside, decussatin 1-*O*-*β*-D-glucopyranoside and the fatty acid, dotriacontanoic acid (**4**) was isolated from the leaves and bark of this plant. Their structures were elucidated by extensive analysis of spectroscopic (1D and 2D NMR) and mass spectrometric data. The isolated compounds were screened for their antimicrobial properties against five strains of bacteria (two gram positive: *Staphylococcus aureus* 29213, *Enterococcus faecalis* ATCC 25922 and three gram negative: *Escherichia coli* ATCC51299, *Proteus mirabilis* (isolate), and *Pseudomonas aeruginosa* QC76110), but all were inactive.

Keywords

Anthocleista liebrechtsiana, Anthocleistenolide B, Antimicrobial Properties

1. Introduction

Antibiotics resistance is an increasing reality in modern medicine and it is emerging as a significant threat to public health. In order to find novel antibio-

tics with new mode of action, plants have been explored as sources of new and effective antimicrobial secondary metabolites [1]. These plants are widely used in ethnomedecine worldwide to treat many infections. *Anthocleista liebrechtsiana* De Wild & T. Durand (**Figure 1**) is a shrub belonging to the genus *Anthocleista* (Loganiaceae family) which contains fourteen species. It is found in tropical Africa, Madagascar, and the Comores [2]. *A. liebrechtsiana* is used in Nigeria and Cameroon traditional medicine to relieve ovarian problems and to treat fever [2] [3] [4]. We reported in our previous phytochemical investigation of the bark and leaves of *A. liebrechtsiana* the isolation and structure elucidation of an ergostane type steroid, (24*S*)-3*β*-hydroxy-7*β*-methoxyergost-5-ene, along with eleven known compounds: 7*α*-hydroxysterol, *β*-sitosterol, oleanolic acid, betulinic acid, lupeol, swertiaperennin, decussatin, tetracosanoic acid, *β*-sitosterol-3-*O*-D-glucopyranoside, (2*R*,3*S*)-2,3-dihydro-2-(3,4-dimethoxyphenyl)-3-hydroxymethyl-5-(2-formylvinyl)-7-hydroxybenzofuran, and acacetin 6-*C*-*β*-D-glucopyranoside [5]. The present paper deals with the isolation of a new secoiridoid, anthocleistenolide B, together with three known compounds, djalonenol, decussatin 1-*O*-*β*-D-glucopyranoside, and dotriacontanoic acid (**Figure 2**). Their structures were determined on the basis of spectroscopic and mass spectrometric data. The isolated compounds were screened for their antimicrobial properties against five bacterial strains.

2. Materials and Methods

2.1. General Experimental Procedures

Optical rotations were measured using the sodium D-line on a Perkin-Elmer 241 MC polarimeter at 23˚C. The IR absorption spectra were measured on a Bruker Tensor 27 FT-IR using a diamond ATR unit. Positive ion mode HRESI mass spectra were recorded on an Agilent 6320 Ion Trap Instrument. ^{1}H and ^{13}C NMR, COSY, HSQC, HMBC and NOESY spectra were recorded in deuterated MeOH (Merck) and chloroform (Merck) on a Bruker AVANCE III-600 MHz Spectrometer equipped with a 5 mm cryogenic TCI-probe head using standard gradient-selected pulse sequences. Column chromatography was performed using Sephadex LH-20 (Merck) and silica gel (Nacherey-Nagel Kieselgel 60 M 40 - 60 µm, 70 - 230 µm). TLC was carried out on precoated silica gel 60 F_{254} (Merck) plates developed with *n*-hexane-EtOAc and EtOAc-MeOH-H_2O. Substance zones were visualized under UV light (254 and 365 nm) and by spraying with 10% aqueous H_2SO_4 followed by heating.

2.2. Plant Material

The leaves and bark of *A. liebrechtsiana* were collected in Edea (Littoral region of Cameroon) in January 2016 with the location 3˚48'0"N, 10˚7'60"E and were authenticated at the National Herbarium of Cameroon, Yaoundé, where a voucher specimen (N˚55963/HNC) was deposited.

Figure 1. *Anthocleista liebrechtsiana* De Wild & T. Durand.

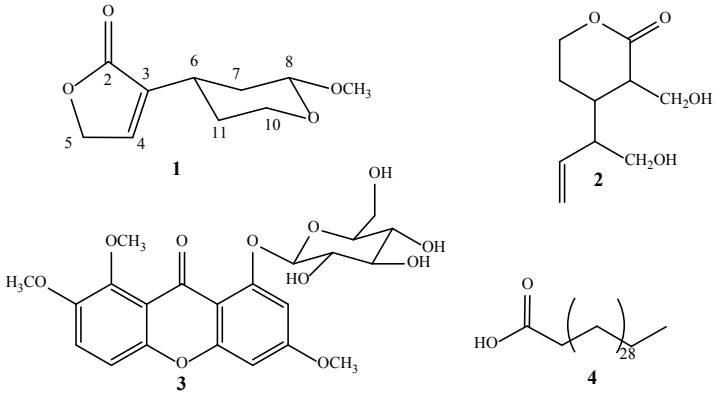

Figure 2. Chemical structures of compounds isolated from *A. liebrechtsiana*.

2.3. Extraction and Isolation

Air-dried and powdered leaves and bark of *A. liebrechtsiana* (3 kg) were extracted with MeOH for 72 h at room temperature. After evaporation of the filtrate under reduced pressure, a greenish residue (305 g) was obtained. Part of this extract (290 g) was triturated with EtOAc to yield an EtOAc fraction (100.4 g) after evaporation of solvent to dryness. The remaining residu was suspended in distilled water (1 L) and extracted with *n*-BuOH to yield after evaporation 83.3 g of extract. Portion of the EtOAc fraction (93 g) was subjected to column chromatography using silica gel 60 (70 - 230 µm) eluting with the mixture *n*-hexane-EtOAc (100:0 to 0:100) and EtOAc-MeOH (100:0 to 50:50) to yield five main fractions (A-E). Column chromatography of fraction B (5 g) under silica gel (40 - 60 µm) eluted with *n*-hexane-EtOAc (75:25) gave four sub-fractions (B_1-B_4). Compound **4** (18 mg) was obtained from sub-fraction B_1. Fraction E (10 g) was chromatographed on Sephadex LH-20 (MeOH) and by silica gel (40 - 60 µm) column chromatography using *n*-hexane-EtOAc (20:80) as eluent to furnish

three sub-fractions (E_{1-1}-E_{1-3}). Sub-fractions E_{1-1} (200 mg) and E_{1-3} (150 mg) were subjected to column chromatography on silica gel eluted with *n*-hexane-EtOAc (40:60) to give compounds **1** (15 mg) and **2** (16 mg) respectively. The *n*-BuOH extract (83.1 g) was chromatographed on silica gel (70 - 230 µm) using EtOAc-MeOH (100:0 to 40:60) as the eluent to afford six fractions (I-VI). Fraction III (10 g) was separated by Sephadex LH-20 column chromatography (MeOH) to give four sub-fractions (III_1-III_4). Sub-fraction III_1 (1 g) was rechromatographed on Sephadex LH-20 (MeOH) to yield sub-fractions III_{1-1}, III_{1-2} and III_{1-3}. Purification of sub-fraction III_{1-1} (100 mg) on silica gel column chromatography eluted with *n*-Hexane-EtOAc (45:55) yielded compound **3** (25 mg).

2.4. Methodology of Antibacterial Essay

The antibacterial activity of the crude extract, fractions and compounds was assessed by determining the minimum inhibitory concentration (MIC) using the broth microdilution method as previously described [6]. Five bacterial strains, (two Gram positive and three Gram negative) were used: *Staphylococcus aureus* 29213*, Enterococcus faecalis* ATCC 25922*, Escherichia coli* ATCC51299*, Proteus mirabilis* (isolate), and *Pseudomonas aeruginosa* QC76110. These strains were obtained from the American Type Culture Collection (ATCC).

3. Results and Discussion

The structures of compounds **2**, **3**, and **4** were determined on the basis of the spectroscopic and mass spectrometric data as djalonenol [7] [8], decussatin 1-*O*-*β*-D-glucopyranoside [9], and dotriacontanoic acid [10], respectively.

Compound **1** was isolated as brown oil and its high-resolution electrospray ionization mass spectrum (HRESIMS) showed a pseudomolecular ion peak $[M + Na]^+$ at *m*/*z* 221.0789 (calcd 221.0784) consistent with the molecular formula $C_{10}H_{14}O_4$, indicating four degrees of unsaturation. The infra-red (IR) spectrum indicated the presence of carbonyl group due to absorption band at 1750 cm^{-1}. Its ^{1}H-NMR spectrum exhibited a signal at δ 4.42 (*dd*, 1H, J = 2.2, 9.2 Hz, H-8) characteristic of an acetalic proton. In addition, this spectrum showed an olefinic proton signal at δ 7.40 (*m*, 1H, H-4), one methoxy group at δ 3.48 (8-OCH_3) while those at δ 2.67 (*m*, 1H, H-6), 4.07 (*m*, 1H, H-10eq), 3.61 (*m*, 1H, H-10ax), 1.84 (*m*, 1H, H-11eq), 1.52 (*m*, 1H, H-11ax), 2.07 (*m*, 1H, H-7eq), 1.39 (*m*, 1H, H-7ax), 4.85 (*m*, 2H, H-5) were respectively attributed to one methine and four methylenes. The ^{13}C-NMR spectrum of compound **1** combined with the HSQC experiment indicated the presence of one ester carbonyl (δ 174.5), two olefinic carbons (δ 145.8, 135.5), one acetal carbon at δ 102.6 which is confirmed by the correlation (HSQC) between carbon C-8 and the proton at δ 4.42 (*dd*, 1H, J = 9.2; 2.2 Hz, H-8). This spectrum also exhibited four methylenes (δ 70.8, 64.3, 35.5, 29.6), one methine (δ 31.3), and a methoxy group (δ 55.1). The ^{2}J and ^{3}J correlations observed on ^{1}H-^{1}H COSY and HMBC spectra allowed to determine and assign the ^{1}H and ^{13}C resonances of compound **1** which was shown to have a

butenolide and a 2-methoxytetrahydropyran-4-yl moiety [11]. The presence of a butenolide was further confirmed by important correlations observed in the HMBC spectrum between the protons at δ 7.40 (*m*, 1H, H-4) and carbons at δ 174.5 (C-2), 135.5 (C-3), 70.8 (C-5) and the proton at δ 4.85 (H-3) and carbon at δ 174.5 (C-2). Furthermore, the butenolide was attached to the 2-methoxytetrahydropyran at C-6 using the HMBC correlations from H-4 (δ 7.40) to C-6 (δ 31.3) and H-6 (δ 2.67) to C-3 (δ 135.5) (**Figure 3**). The NOESY experiment allowed to assign the relative configuration of the chiral centers by the correlation depicted between H-8/H-6. From the above spectroscopic data compared to those of Anthocleistenolide [11], compound **1** was established as a new secoiridoid to which the trivial name anthocleistenolide B was given. It was reported that extraction of plant material by methanol can led to the artifacts containing methoxyl groups [12]. Since compound **1** contains a methoxyl group, we therefore change the solvent extraction of the plant material by using EtOAc. The thin layer chromatography analysis of the methanol and EtOAc extracts revealed the presence of **1** proving that it is not an artifact. This metabolite is related to the rearranged nor-secoiridoid anthocleistenolide previously isolated from the stem bark of *Anthocleista vogelii* [11]. The only difference being the lack of the C-10 methoxyl group. Anthocleistenolide was reported to derive from the 7,8-secoiridoid precursor by considerable oxidative modification [11].

The isolated compounds were screened for their antimicrobial properties against five bacterial strains (two Gram positive: *Staphylococcus aureus* 29213, *Enterococcus faecalis* ATCC 25922 and three Gram negative: *Escherichia coli* ATCC51299, *Proteus mirabilis* (isolate), and *Pseudomonas aeruginosa* QC76110), but none of them showed significant activity.

4. Spectroscopic Data of Compounds 1-4

Compound (1)—Brown oil, $[\alpha]_D^{28}$: −0.3 (*c* 0.7, MeCN). IR ν_{max} cm^{-1}: 2956, 2927, 1750, 1447, 1392, 1252, 1124; ^{1}H NMR (CD_3OD, 600 MHz): ^{13}C NMR (CD_3OD, 150 MHz): see **Table 1**. HRESIMS *m*/*z*: 221.0789 $(M + Na)^+$ (calcd for $C_{10}H_{14}O_4$, 221.0784).

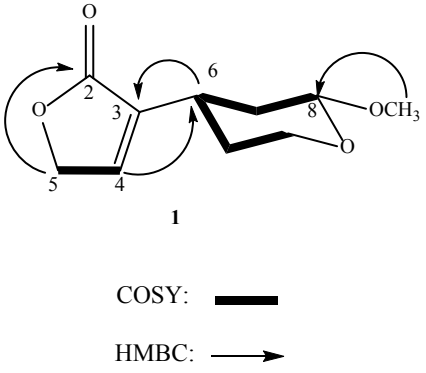

Figure 3. Selected ^{1}H-^{1}H COSY and HMBC correlations of anthocleistenolide B (**1**).

Table 1. ^{1}H and ^{13}C-NMR data (600 and 150 MHz, respectively, CD_3OD) of compound **1**.

Position	1		
	δ_H	δ_C	HMBC
1	-	-	-
2	-	174.5	-
3	-	135.5	C-5
4	7.40 (*m*)	145.8	C-2, 3, 5, 6
5	4.85 (*m*)	70.8	C-2
6	2.67 (*m*)	31.3	C-3
7eq, 7ax	2.07 (*m*), 1.39 (*m*)	35.5	
8	4.42 (*dd*, *J* = 9.2, 2.2 Hz)	102.6	OCH_3
9	-	-	
10eq, 10ax	4.07 (*m*), 3.61 (*m*)	64.3	C-8
11eq, 11ax	1.84 (*m*), 1.52 (*m*)	29.6	
OCH_3	3.48 (*s*)	55.1	C-8

Djalonenol (2)—Amorphous powder, $[\alpha]_D^{28}$: 0.9 (*c* 0.4, MeCN). IR ν_{max} cm^{-1}: 3408, 2921, 1715, 1473, 1402, 1312, 1207, 1073; ^{1}H-NMR (CD_3OD, 600 MHz) δ; 5.79 (*m*, 1H, H-7), 5.19 (*m*, 2H, H-9), 4.35 (*m*, 1H, H-6b), 4.33 (*m*, 1H, H-6a), 3.98 (*dd*, 1H, *J* = 3.9, 10.8 Hz, H-11b), 3.75 (*dd*, 1H, *J* = 3.9, 10.8 Hz, H-11a), 3.64 (*m*, 2H, H-10), 2.71 (*m*, 1H, H-3), 2.31 (*m*, 1H, H-4), 2.30 (*m*, 1H, H-8), 2.00 (*m*, 1H, H-5b), 1.75 (*m*, 1H, H-5a); ^{13}C-NMR (CD_3OD, 150 MHz) δ, 179.6 (C=O), 137.5 (C-7), 117.0 (C-9), 67.6 (C-6), 64.2 (C-11), 62.7 (C-10), 51.2 (C-3), 48.5 (C-3), 35.2 (C-4), 25.8 (C-5); HRESIMS *m*/*z*: 223.0946 (calcd for $C_{10}H_{16}O_4$, 223.0941).

Decussatin 1-*O*-*β*-D-glucopyranoside (3)—yellow powder. ^{1}H-NMR (CD_3OD, 600 MHz) δ; 7.54 (*d*, 1H, *J* = 9.2 Hz, H-6), 7.29 (*d*, 1H, *J* = 9.2 Hz, H-5), 6.77 (*d*, 1H, *J* = 2.4 Hz, H-2), 6.75 (*d*, 1H, *J* = 2.4 Hz, H-4), 4.92 (*d*, 1H, *J* = 7.7 Hz, H-1'), 3.88 (*s*, 3H, 3-OCH_3), 3.86 (*s*, 3H, 7-OCH_3), 3.81 (*s*, 3H, 8-OCH_3), 3.74 (*m*, 1H, H-6'a), 3.48 (*m*, 1H, H-6'b), 3.41 (*m*, 1H, H-5'), 3.40 (*m*, 1H, H-2'), 3.31 (*m*, 1H, H-3'), 3.18 (*m*, 1H, H-4'),^{13}C-NMR (CD_3OD, 150 MHz) δ, 175.0 (C=O), 164.6 (C-3), 159.6 (C-1), 158.2 (C-4a), 149.7 (C-7), 149.5 (C-5a), 147.8 (C-8), 120.0 (C-6), 117.9 (C-8a), 112.7 (C-5), 107.9 (C-9a), 103.3 (C-1'), 100.8 (C-2), 95.3 (C-4), 78.1 (C-5'), 76.5 (C-3'), 73.9 (C-2'), 70.3 (C-4'), 61.3 (C-6'), 61.2 (8-OCH_3), 57.0 (7-OCH_3), 56.5 (3-OCH_3).

Dotriacontanoic acid (4)—White powder. ^{1}H-NMR (CD_3OD, 600 MHz) δ; 2.50 (*t*, 2H, H-2), 1.78 (*m*, 2H, H-3), 1.30 (*m*, 2H, H-4), 1.25 (*m*, 2H, H-5), 1.29 (*s*, 48H, $(CH_2)_{12}$), 1.18 (*m*, 2H, H-30), 1.22 (*m*, 2H, H-31), 0.83 (*m*, 2H, H-32), ^{13}C-NMR (CD_3OD, 150 MHz) δ, 175.9 (C=O), 34.7 (C-2), 25.4 (C-3), 29.4 (C-4), 29.6 (C-5), 29.8 ($(CH_2)_{24}$), 29.4 (C-30), 22.7 (C-31), 14.1 (C-32).

5. Conclusion

In summary, this study presents the isolation and characterization of a new secoiridoid derivative, anthocleistenolide B from the leaves and bark of *A. liebrechtsiana* together with the monoterpene diol, djalonenol, the xanthone *O*-glycoside decussatin 1-*O*-*β*-D-glucopyranoside and the fatty acid dotriacontanoic acid. Their structures were elucidated by extensive analysis of spectroscopic (1D and 2D NMR) and mass spectrometric data. The isolated compounds were screened for their antimicrobial properties against five strains of bacteria but none of them showed significant activity. Based on the results of our previous and present investigations on the bark and leaves of *A. liebrechtsiana*, further studies need to be undertaken in view of isolating the antimicrobial active principles of this plant since its EtOAc extract had shown a significant antimicrobial activity [3].

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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