Cytotoxic clerodane diterpenoids from the roots of *Casearia barteri* Mast.†‡

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A study of diterpenoids as active ingredients against cancer from the active roots extract of *Casearia barteri* Mast. (IC$_{50}$ = 1.57 µg mL^{-1}) led to the isolation of six new clerodane diterpenoids, named as barterins A–F (1–6) alongside seven known compounds, caseamembrin A, caseamembrin E, casearlucin A, graveospene G, *N-trans*-feruloyltyramine, *N-cis*-feruloytyramine and sitosterol-3-*O*-β-*D*-(6-*O*-palmitoyl)-glucopyranoside. Their structures were elucidated based on NMR spectroscopic data and mass spectrometry. The absolute configurations of 1–6 were established by the time-dependent density functional theory (TDDFT), electronic circular dichroism (ECD) calculations and experimental data analysis. The cytotoxic effects of compounds 1–6 were evaluated against a human cervix carcinoma cell line *KB*-3-1. Barterins A–D (1–4) showed cytotoxic effects against the *KB*-3-1 cell line with IC$_{50}$ values ranging from 1.34–4.73 µM.

Introduction

Natural products have been a cornerstone of drug discovery given their considerable chemical diversity, which has proven invaluable in the discovery of new anti-cancer agents.[1] As the global cancer incidence continues to rise, the search for effective treatments remains a critical challenge. Among the promising candidates, clerodane-type diterpenes have been the subject of numerous works.[2–4] Belonging to the family *Flacourtiaceae*, the genus *Casearia* includes a group of about 180 plant species widely spread in tropical and subtropical areas of Africa, Asia, Australia, North and South America and the Pacific islands.[5] They are abundantly mentioned in traditional medicine for the treatment of diarrhea, skin lesions, ulceration, tropical leprosy, herpes, snake bite and fever.[6] Numerous investigations on species of the genus *Casearia* Jacq. have revealed the occurrence of several classes of secondary metabolites, including clerodane-type diterpenes having a zuelanin and isozuelanin skeleton as the predominant type, with more

than 150 already listed in the literature.[7–11] Moreover, biological assays have revealed that these diterpenes possess cytotoxic, antibacterial, antifungal and DNA-modifying properties.[5] *Casearia barteri* Mast. is a small to medium-size tree, up to 20(–40) m high, present in tropical forest of Cameroon and Nigeria. The twigs and stem barks are chewed for sore gum and teeth cleaning.[12] This high chemical and pharmacological potential as well as the contribution of the *Casearia* genus to traditional medicine motivated our choice of the study presented here.

Results and discussion

The crude extract derived from maceration of *C. barteri* roots in methanol led to further semi-pure extracts after fractionation with petrol ether–acetone solvent system of increasing polarity. These were purified using chromatographic columns with various stationary phases, leading to the isolation of thirteen compounds, including six new derivatives, barterins A–F (1–6) (Fig. 1) along with seven known compounds identified as caseamembrin A (7), caseamembrin E (8),[13] the mixture of casearlucin A (9)[14] and graveospene G (10),[11] *N-trans*-feruloyltyramine (11), *N-cis*-feruloytyramine (12),[15] (−)-β-sitosterol-3-*O*-β-*D*-(6-*O*-palmitoyl)glucopyranoside (13)[16] using their spectroscopic data by comparing with those reported in the literature.

Structure elucidation

Compound 1 was isolated as a colorless oil. Its molecular formula was deduced as C$_{31}$H$_{46}$O$_8$ by HRESIMS from the

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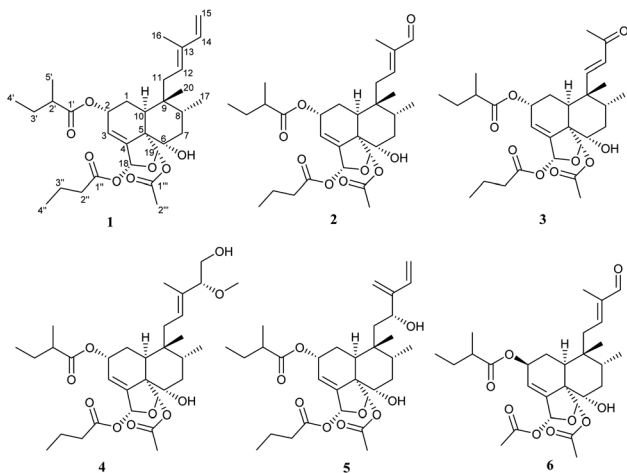‡ Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4ra04393f

Fig. 1   Structures of compounds **1**–**6** from *C. barteri*.

sodium adduct ion peak at m/z 569.3084 [M + Na]$^+$ (calcd for $C_{31}H_{46}O_8Na$, 569.3084) corresponding to nine double bond equivalents. The FTIR spectrum displayed the typical stretching bands of carbonyl functions at 1728 and 1749 cm^{-1} as well as a large absorption band due to the presence of hydroxyl groups centered at 3468 cm^{-1}. The ^1H NMR spectrum of **1** showed signals of seven methyl groups [δ_H 1.88 (s), 1.67 (s), 1.15 (d, J = 6.9 Hz), 0.95 (d, J = 7.4 Hz), 0.97 (t, J = 7.4 Hz), 0.92 (t, J = 7.4 Hz), 0.84 (s)], five olefinic protons [δ_H 5.99 (dt, J = 4.4, 1.4 Hz), 5.51 (d, J = 8.0 Hz), 6.35 (dd, J = 17.3, 10.8 Hz), 5.09 (d, J = 17.4 Hz), 4.91 (d, J = 10.7 Hz)], and three oxygenated methine protons [δ_H 5.40 (m), 6.69 (dd, J = 1.6, 0.6 Hz), 6.47 (br s)] (Table 1). The decoupled broadband ^{13}C NMR spectrum of **1** exhibited resonances of 31 carbon atoms including those of 2-methyl-butanoyloxy, butanoyloxy, and acetyloxy groups established from the observation of the following carbon signals (δ_C 175.8, 41.9, 27.7, 17.1, 12.1; 173.1, 36.7, 18.9, 13.9; 169.6, 21.9) (Table 2). The remaining 20 carbons have been assigned to those of the diterpene-type skeleton with the help of DEPT and HMQC spectra having three double bonds including one terminal olefin (δ_C 121.7, 147.5, 136.0, 130.9, 111.0; 142.5) as well two acetal carbons (δ_C 96.4, 98.2). From these data, we suggested that compound **1** may possess a zuelanin type skeleton, a subgroup of clerodane diterpenes previously described from *Casearia* genus.[17–19] 2D homo- and hetero-nuclear correlations were further used to confirm the skeletal type of compound **1** and identify the position of its functional groups and substituents (Fig. 2). A tri-substituted double bound was located at position C-3/C-4 of the decalin ring system of diterpene from long range hetero-nuclear correlation of the proton signal at δ_H 5.99 (H-3) with carbons C-1 (δ_C 27.3), C-5 (δ_C 54.6) and C-18 (δ_C 96.4) and those of the proton at δ_H 5.40 (H-2) with C-10 (δ_C 37.7), C-3 (δ_C 121.7) and C-4 (δ_C 147.5). Similarly, a diene function was identified on the linear chain at C-12/C-13 and C-14/C-15 from correlations of the proton signal at δ_H 5.09 (H-15Z) with C-13 (δ_C 136.0) and C-14 (δ_C 142.5) and between the methyl signal at δ_H 1.67 (H-16) with the olefinic carbons C-12 (δ_C 130.9), C-13 (δ_C 136.0) and C-14 (δ_C 142.5). Furthermore, the butanoyl

substituent was inferred to be attached at position C-18 from the ^3J correlation between the proton signal at δ_H 6.69 (H-18) and the carbonyl at δ_C 173.1 (C-1″). In the same way, the correlation between the proton signal at δ_H 6.47 (H-19) and the carbonyl at δ_C 169.6 (C-1‴) was used to attach the acetoxyl group at position C-19. Following the same rationale, the 2-methyl-butanoyloxy was attached at position C-2 considering the de-shielding effect of the proton H-2 (δ_H 5.40) attributable to the attractive mesomeric effect of the ester function, in agreement with data reported for other diterpenoids from the genus *Casearia* in the literature.[2,10] An additional hydroxy group was located at C-6 using correlation between H-19 (δ_H 6.47) and C-6 (δ_C 72.9), and from H-10 (δ_H 2.44) to C-6 (δ_C 72.9). The E-configuration of the double bond C-12/C-13 as well as the transoid geometry of the conjugated double bond were established from NOESY interactions between H-11α,β/H-16/H-15 and H-8/H-12/H-14 and from the ^{13}C NMR chemical shift of the allylic carbon C-11 at δ_C 31.1.[11] Similarly, the relative configuration of the decalin ring system was discussed after examination of spatial correlations between H-17/H-10 and H-2/H-3/H-18/H-19/H-6/H-20/H-8. From these evidence, the decalin ring was established as *cis* fused with proton H-10 and methyl H-17 both α-oriented while protons H-2, H-6, H-18, H-19 and methyl H-20 adopted a β position (Fig. 3). The relative configuration (2R,5S,6S,8R,9R,10S,18R,19S) obtained from the analysis of spatial correlations was used as a model for the ECD calculations. The similarities between the negative Cotton effect at 215 nm of the experimental and theoretical ECD curves (Fig. 4) allow us to assign the absolute configuration 2R,5S,6S,8R,9R,10S,18R,19S for compound **1**. These observations were in agreement with the stereochemistry of grave-ospene A.[11]

Compound **2** was purified as a colorless oil. The molecular formula of compound **2** was deduced to be $C_{30}H_{44}O_9$ from the sodium adduct ion [M + Na]$^+$ observed on its HRESIMS spectrum at m/z 571.2878 (calcd for $C_{30}H_{44}NaO_9$, 571.2877) and its NMR data. The exhaustive analysis of the 1D NMR spectra revealed that compound **2** is analogous to compound **1**, notably due to the presence of the same substituents 2-methyl-butanoyloxy (δ_C 177.6, 42.4, 28.1, 17.2, 12.1), butanoyloxy (δ_C 174.4, 37.1, 19.3, 13.8) and acetyloxy (δ_C 170.8, 22.0) groups, as well the resonance of two acetal signals (δ_C 99.1, 97.1). Furthermore, it was established that the above-mentioned acyloxy groups as well as the hydroxyl function remain attached at positions 2, 18, 19 and 6, respectively, based on the observed HMBC and COSY correlations. The major difference is the absence of the resonance from the C-14/C-15 terminal double bond in conjunction with the appearance of a carbonyl signal (δ_C 196.1) attributable to an aldehyde function (δ_H 9.40). A putative oxidative cleavage of the double bond C-14/C-15 in **1** was thus suggested to establish the structure of compound **2**.

The position of the aldehyde function at C-14 was further conclusive after interpretation of the HMBC correlations of its proton (δ_H 9.40, H-14) with olefinic and methyl carbons respectively at δ_C 153.5 (C-12), 142.0 (C-13) and 9.5 (C-16) as well as those between methyl H-16 with carbon C-12, C-13, C-14 (Fig. 2). The double bond C-12/C-13 was identified as E-

Table 1 ^{1}H NMR data for compounds **1–6** (600 MHz, δ in ppm, J in Hz)

Position	1^a	2^b	3^b	4^b	5^b	6^b
1α	2.06 m	2.11 dd (14.7, 4.5)	2.02 m	2.04 m	2.00 m	2.25 m
1β	1.89 m	1.95 m	1.53 m	1.88 m	1.31 m	1.78 m
2	5.40 m	5.45 m	5.43 m	5.41 m	5.38 br d (4.5)	5.59 m
3	5.99 dt (4.4, 1.4)	6.02 d (3.4)	5.97 d (4.2)	6.02 br d (4.2)	5.99 d (4.1)	5.91 br s
4	—	—	—	—	—	—
5	—	—	—	—	—	—
6	3.89 ddd (11.7, 7.3, 5.0)	3.84 m	3.88 dd (10.7, 3.7)	3.84 m	3.78 br d (7.4)	4.00 dd (11.6, 4.8)
7	1.73 m	1.70 m	1.78 m	1.65 m	1.76 m	1.74 m
8	1.88 m	1.93 m	1.90 m	1.87 m	1.77 m	1.95 m
9	—	—	—	—	—	—
10	2.44 m	2.44 m	2.14 m	2.42 m	2.18 dd (14.8, 3.2)	2.54 dd (13.9, 2.7)
11α	2.30 m	2.53 dd (17.8, 8.4)	7.06 d (16.4)	2.31 m	1.76 m	2.48 dd (18.1, 8.1)
11β	1.75 m	1.99 m	—	1.52 m	1.41 m	1.96 m
12	5.51 d (8.0)	6.64 m	6.14 d (16.4)	5.51 m	4.43 br d (6.4)	6.80 br d (4.3)
13	—	—	—	—	—	—
14	6.35 dd (17.3, 10.8)	9.40 s	—	3.54 m	6.33 m	9.43 s
15	5.09 d (17.4), H$_Z$	—	—	3.58 m	5.45 d (18.3)	—
15	4.91 d (10.7), H$_E$	—	—	3.45 m	—	—
16	1.67 s	1.69 s	2.24 s	1.54 s	5.11 d (15.3)	1.66 s
17	0.95 d (7.4)	0.99 s	0.89 d (6.0)	0.96 d (6.0)	1.07 d (6.0)	0.98 d (7.0)
18	6.69 dd (1.6, 0.6)	6.71 br s	6.71 t (1.6)	6.71 t (1.6)	6.69 br s	6.64 t (1.5)
19	6.47 br s	6.52 s	6.32 s	6.52 s	6.37 s	6.47 s
20	0.84 s	0.94 s	1.16 s	0.82 s	1.08 s	0.96 s
1'	—	—	—	—	—	—
2'	2.42 m	2.48 sext (6.9)	2.42 m	2.44 m	2.40 ddd (13.7, 6.9, 1.7)	2.40 sext (6.9)
3'α	1.67 m	1.70 m	2.15 m	1.63 m	1.61 m	1.72 m
3'β	1.54 m	1.55 m	2.01 m	1.52 m	1.50 m	1.51 dt (13.7, 7.2)
4'	0.97 t (7.4)	0.97 s	0.98 t (7.5)	0.97 t (7.2)	0.95 t (7.5)	0.93 t (7.0)
5'	1.15 d (6.9)	1.18 d (7.0)	1.15 d (7.5)	1.17 d (7.0)	1.16 d (7.0)	1.16 d (7.0)
1''	—	—	—	—	—	—
2''	2.32 t (7.4)	2.33 t (7.4)	2.31 t (7.2)	2.34 t (7.2)	2.30 t (7.2)	2.07 s
3''	1.61 sext (7.4)	1.65 sext (7.3)	1.63 sext (7.4)	1.66 sext (7.4)	1.62 sext (7.4)	—
4''	0.92 t (7.4)	0.96 t (7.3)	0.96 t (7.4)	0.95 t (7.3)	0.94 t (7.3)	—
1'''	—	—	—	—	—	—
2'''	1.88 s	1.88 s	1.84 s	2.06 s	1.08 s	1.89 s
OMe	—	—	—	3.20, s	—	—

^{a} Recorded in acetone-d_6. ^{b} Recorded in methanol-d_4.

configured according to the NOESY experiment where cross-peaks between H-11α,β/H-16 and H-14/H-12/H-17 were observed. Futher NOESY interaction between H-8/H-18/H-19 revealed the same relative configuration as found for compound **1**. Positive Cotton effects at 222 nm and 276 nm matched the simulated ECD curve (Fig. 4) and allow to assign the absolute configuration of compound **2** as 2*R*,5*S*,6*S*,8*R*,9*R*,10*S*,18*R*,19*S*, matching that of compound **1**. Compound **3** was obtained as a colorless oil. Its HRESIMS showed a sodium adduct pic at *m/z* 5557.2719 [M + Na]$^+$ (calcd for C$_{29}$H$_{42}$NaO$_9$, 557.2721), corresponding to a molecular formula C$_{29}$H$_{42}$NaO$_9$, bearing nine double bond equivalents. From the ^{1}H and ^{13}C NMR spectra, several similarities to compound **1** were apparent. Indeed, the 2-methylbutanoyloxy (δ_C 177.5, 42.5, 27.6, 17.2, 12.1), butanoyloxy (δ_C 174.4, 37.1, 19.4, 13.8), and acetyloxy (δ_C 171.2, 21.3) groups, as well as the resonance of two acetal signals (δ_C 98.7, 97.3) were evidenced. Furthermore, the position of these acyloxy groups as well as the hydroxyl function on the clerodane-type diterpene skeleton was identified by means of the HMBC spectrum in which

correlations between the protons at δ_H 5.43 (H-2), 6.71 (H-18) and 6.32 (H-19) with the carbonyl carbons at δ_C 177.5, 174.4, 171.2 and 72.9 demonstrated the 2-methylbutanoyloxy, butanoyloxy, acetoxy and hydroxy groups to be attached at C-2, C-18, C-19 and C-6 respectively. Beside these similarities with compound **1**, close analysis of the 1 and 2D NMR spectra indicated a difference in the side chain attached at position 9 of ring B. Indeed, resonances of an α,β-unsaturated methyl ketone at δ_C 201.4 (C-13), 154.6 (C-11), 132.1 (C-12), 27.1 (C-16) were observed and further corroborated from HMBC correlations between the methyl protons at δ_H 2.24 (H-16) with carbons C-13 and C-12 and also between the olefinic proton at δ_H 7.06 (H-11) and carbons C-13 and C-12. The stereochemistry of the double bond C-11/C-12 was inferred as *trans* from the coupling constant of both proton H-11 and H-12 (d, *J* = 16.4 Hz). The NOESY experiment disclosed cross-peaks between H-18/H-19/H-6/H-8/H-20 and H-10/H-17 as well as H-16/H-11α,β and H-12/H-20/H-2/H-6, in agreement with those found for compound **1**. The calculated ECD curve for the stereoisomer 2*R*,5*S*,6*S*,8*R*,9*R*,10*S*,18*R*,19*S* displays the same profile as the

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Table 2 ^{13}C NMR Data for Compounds **1–6** (δ in ppm, 150 MHz)

Position	1^a		2^b		3^b		4^b		5^b		6^b	
1	27.3	CH$_2$	27.6	CH$_2$	28.0	CH$_2$	27.6	CH$_2$	27.6	CH$_2$	27.3	CH$_2$
2	67.2	CH	67.9	CH	67.7	CH	68.1	CH	68.1	CH	72.3	CH
3	121.7	CH	122.5	CH	122.2	CH	122.4	CH	122.3	CH	124.8	CH
4	147.5	C	147.4	C	147.3	C	147.7	C	147.7	C	146.3	C
5	54.6	C	54.9	C	54.9	C	55.2	C	55.4	C	54.9	C
6	72.9	CH	73.1	CH	72.9	CH	73.3	CH	73.6	CH	74.3	CH
7	38.0	CH$_2$	37.9	CH$_2$	37.7	CH$_2$	38.1	CH$_2$	38.0	CH$_2$	38.2	CH$_2$
8	37.3	CH	37.5	CH	36.2	CH	37.7	CH	38.3	CH	37.7	CH
9	38.5	C	39.0	C	42.4	C	39.3	C	39.6	C	39.5	C
10	37.7	CH	38.7	CH	43.0	CH	38.5	CH	42.0	CH	43.1	CH
11	31.1	CH$_2$	32.9	CH$_2$	154.6	CH	30.6	CH$_2$	40.8	CH$_2$	32.7	CH$_2$
12	130.9	CH	153.5	CH	132.1	CH	128.1	CH	68.5	CH	154.1	CH
13	136.0	C	142.0	C	201.4	C	135.6	C	151.9	C	142.0	C
14	142.5	CH	196.1	CH	—	—	90.5	CH	138.2	CH	196.7	CH
15	111.0	CH$_2$	—	—	—	—	64.8	CH$_2$	115.2	CH$_2$	—	—
16	12.0	CH$_3$	9.5	CH$_3$	27.1	CH$_3$	11.7	CH$_3$	114.7	CH$_2$	9.3	CH$_3$
17	15.9	CH$_3$	16.0	CH$_3$	16.5	CH$_3$	16.1	CH$_3$	16.4	CH$_3$	15.9	CH$_3$
18	96.4	CH	97.1	CH	97.3	CH	97.1	CH	96.9	CH	96.7	CH
19	98.2	CH	99.1	CH	98.7	CH	99.3	CH	99.7	CH	98.6	CH
20	25.4	CH$_3$	25.3	CH$_3$	24.9	CH$_3$	25.6	CH$_3$	24.9	CH$_3$	25.2	CH$_3$
1′	175.8	C	177.6	C	177.5	C	177.4	C	177.5	C	178.0	C
2′	41.9	CH	42.4	CH	42.5	CH	42.6	CH	42.6	CH	42.4	CH
3′	27.7	CH$_2$	28.1	CH$_2$	27.6	CH$_2$	28.2	CH$_2$	28.1	CH$_2$	27.9	CH$_2$
4′	12.1	CH$_3$	12.1	CH$_3$	12.1	CH$_3$	12.2	CH$_3$	12.1	CH$_3$	11.9	CH$_3$
5′	17.1	CH$_3$	17.2	CH$_3$	17.2	CH$_3$	17.2	CH$_3$	17.2	CH$_3$	17.0	CH$_3$
1″	173.1	C	174.4	C	174.4	C	174.5	C	174.4	C	171.9	C
2″	36.7	CH$_2$	37.1	CH$_2$	37.1	CH$_2$	37.2	CH$_2$	37.2	CH$_2$	21.1	CH$_3$
3″	18.9	CH$_2$	19.3	CH$_2$	19.4	CH$_2$	19.4	CH$_2$	19.4	CH$_2$	—	—
4″	13.9	CH$_3$	13.8	CH$_3$	13.8	CH$_3$	13.9	CH$_3$	13.8	CH$_3$	—	—
1‴	169.6	C	170.8	C	171.2	C	171.1	C	171.4	C	171.0	C
2‴	21.9	CH$_3$	22.0	CH$_3$	21.3	CH$_3$	22.1	CH$_3$	22.3	CH$_3$	22.0	CH$_3$
OMe	—		—		—		56.6	CH$_3$	—		—	

^{a} Recorded in acetone-d_6. ^{b} Recorded in methanol-d_4.

experimental one (Fig. 4) and this unambiguously led to the assignment of the absolute configuration for compound **3**.

Compound **4** was also isolated as a brownish oil. Its HRE-SIMS in positive mode having the sodium adduct peak at 617.3292 $[M + Na]^+$ (calcd for 617.3296 for $C_{32}H_{50}O_{10}Na$), suggested a molecular formula of $C_{32}H_{50}O_{10}$, corresponding to eight degrees of unsaturation. NMR data comparison revealed that compound **4** was similar to **1**, except for the presence of a methoxy (δ_H 3.20/δ_C 56.6), an oxymethylene (δ_H 3.58, 3.45/δ_C 64.8) and an oxymethine group (δ_H 3.54/δ_C 90.5) together with the loss of the signals from the terminal double bond C-14/C-15. Strong ^{1}H–^{1}H ^3J COSY correlations were observed between the oxymethine and the oxymethylene protons and their position at C-14 and C-15 were inferred from HMBC cross-peaks from the methyl protons at δ_H 1.54 (H-16) to carbons C-12 (δ_C 128.1), C-13 (δ_C 135.6) and C-14 (δ_C 90.5) and between the oxymethine proton at δ_H 3.58 (H-14) and carbon C-12, C-15. Furthermore, correlations of the oxymethine proton H-14 with the methoxy carbon at δ_C 56.6 evidenced the position of the methoxy at C-14. The same relative configuration for compound **4** as that of **1** was corroborated from H-20/H-8/H-11α/H-16/H-15α,β/MeO-14, H-2/H-3/H-18/H-19/H-6/H-1α. Similarly, the orientation of the proton H-14 in β position was deduced from NOESY cross peaks

from H-12/H-20/H-14. The experimental ECD curve was consistent with the calculated ECD (Fig. 4) for the configuration 2R,5S,6S,8R,9R,10S,14R,18R,19S establishing thus the absolute configuration of compound **4**.

Compound **5**, obtained as a brownish oil with a molecular formula $C_{31}H_{46}O_9$ based on the presence of a HRESIMS ion peak at m/z 585.3035 $[M + Na]^+$ (calcd for $C_{31}H_{46}NaO_9$, 585.3034). Compound **5** contains nine double bond equivalents and is like compound **1** described above with respect to the number of carbon atoms, but with an additional oxygen atom. In addition to the signals related to the identical acyl substituents present, a comparative analysis established that the basic skeleton of compound **5** belongs to isozuelanin, with two terminal double bonds at the level of the lateral chain of clerodane-type diterpenes. Indeed, the DEPT 135 of compound **5** spectrum exhibited resonances of two olefinic methylene carbons at δ_C 114.7 and 115.2. The typical β-monosubstituted diene of the isozuelanin skeleton was confirmed by interpretation of the HMBC correlations of both terminal protons at δ_H 5.11 (H-16) and 5.45 (H-15) with olefinic carbons at at δ_C 151.9 (C-13) and 138.2 (C-14). Beyond this aspect of position isomerism, compound **5** was found to have an additional oxygen atom as outlined above, which resulted in the presence of an
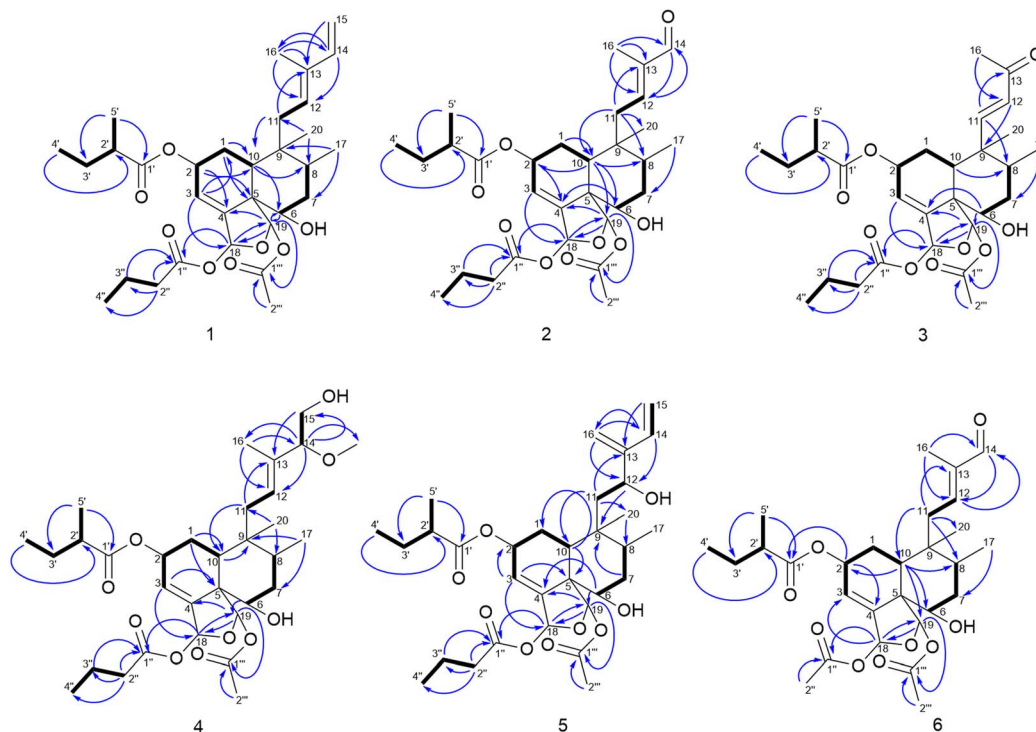
Fig. 2   COSY and HMBC correlations of compounds 1–6.

additional hydroxyl group compared to 1. Based on HMBC cross-peaks of protons H-16 and H-14 with carbon C-12, it was assigned unambiguously at position C-12. The cisoid configuration of the diene was deduced from the NOESY spatial correlation between proton H-15 and H-16. Similarly, the relative configuration of compound 5 was shown identical as compound 1 from analysis of spatial correlations with a β-oriented proton H-12 inferred from NOESY correlation between H-12 and H-8. This was further confirmed with the similar

Cotton effect at 223 nm on both experimental and calculated ECD curve for the stereoisomer 2R,5S,6S,8R,9R,10S,12R,18R,19S (Fig. 4).

The molecular formula, $C_{28}H_{40}O_9$, of compound 6 that was obtained as a colorless oil, could be inferred from the relevant HRESIMS ion peak at m/z 543.2557 $[M + Na]^+$ (calcd for $C_{28}H_{40}NaO_9$, 543.2564). Analysis of the 1D NMR spectra revealed that 6 had the same clerodane-like structure with three substituent groups, notably, a 2-methylbutanoyloxy group (δ_C
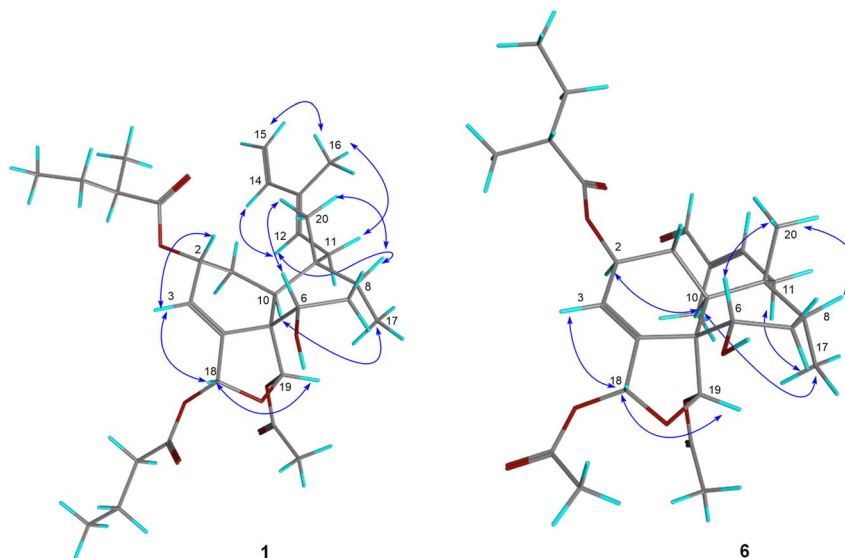

Fig. 3   Key NOESY correlations of compounds 1 and 6.
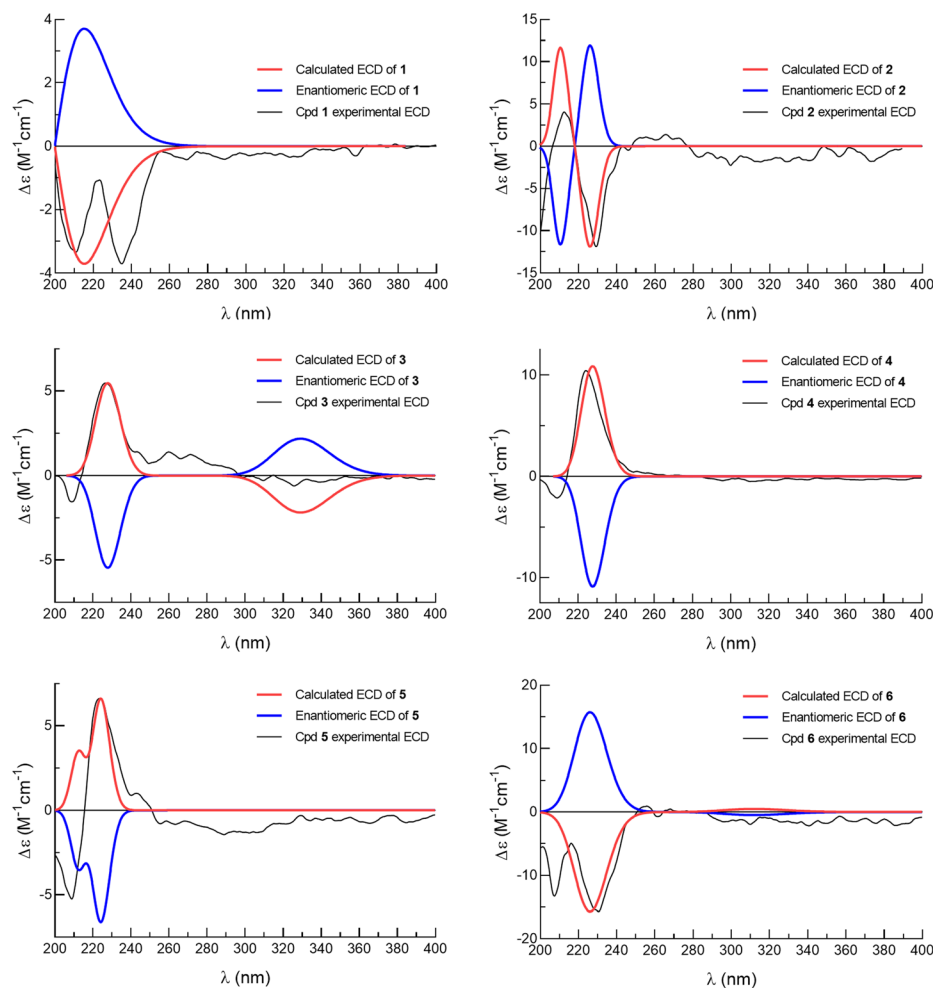
Fig. 4   Calculated and experimental ECD spectra of compounds **1–6**.

178.0, 42.4, 27.9, 11.9, 17.0) and two acetyloxy groups (171.9, 21.1, 171.0, 22.0). These conclusions were supported by analysis of the 2D NMR spectra crosspeaks. ^3J correlation of H-2 to the carbonyl group (δ_C 178.0) demonstrated that the 2-methyl-butyryloxy unit was positioned at C-2. Similarly, both acetyloxy placed at C-18 and C-19 were positioned from the corresponding HMBC couplings of protons H-18 (δ_H 6.64) and proton H-19 (δ_H 6.47) with ester carbonyls at δ_C 171.9 and 171.0. On the same basis, the hydroxy methine was placed at position 6 as it was for the compounds described above, based on the HMBC correlation between the proton H-19 and carbon at δ_C 74.3 (C-6). The presence of the aldehyde function at δ_C 196.7 brought us to compare the HMBC correlations of compound **6** to those of compound **2**. Indeed, this allowed us to identify the aldehyde at the same position C-14, notably through the correlations from proton H-14 to olefinic carbons C-12, C-13 and methyl C-16. It has, therefore, been established that compound **6** is distinct from compound **2** by the nature of the acyloxy substituent in position 2, specifically the 2-methylbutanoyloxy group replaced by the acetyloxy group. Furthermore, the spatial correlation of H-2 and H-10, H-17 were in agreement with an α orientation of H-2 unlike the β orientation it occupies in compound **1** (Fig. 3).

The absolute configuration 2*S*,5*S*,6*S*,8*R*,9*R*,10*S*,18*R*,19*S* was inferred from the comparison between theoretical and experimental ECD curves, with a negative Cotton effect at 228 nm (Fig. 4).

Cytotoxic activity

Cervix cancer is the fourth most diagnosed cancer in women worldwide, with more than half a million new cases and 311 365 deaths in 2018, with nine-tenths of these being among women living in low- and middle-income countries.[20] A large number of plants and plant-derived principles has demonstrated anti-cancer activity through selective cytotoxicity towards tumour cells. To bring our contribution to the search for new anti-cancer agents, we evaluated the cytotoxic potential of diter-penes **1–6** and the crude extract towards the cervix carcinoma cell line *KB*-3-1 (a HeLa subclone) with griseofulvin as standard and using the MTT assay as previously described.[21] The crude extract exhibited strong cytotoxic activity with IC$_{50}$ of 1.58 μg mL^{-1}, in agreement with the range defined by the National Cancer Institute in US, which considers an extract to be active when the IC$_{50}$ value after incubation between 48 and 72 h, is less than 20 μg mL^{-1}.[22] This corroborates the results obtained on

Table 3 Cytotoxic activities

Samples	IC_{50}
Crude extract	1.58 µg mL^{-1}
Barterin A (**1**)	2.52 µM
Barterin B (**2**)	1.34 µM
Barterin C (**3**)	4.73 µM
Barterin D (**4**)	>200 µM
Barterin E (**5**)	>200 µM
Barterin F (**6**)	1.54 µM

other cancer cell lines such as mouse P388 lymphocytic leukemia cell line where extracts from various parts of *C. costulata*, *C. grayi*, *C. multinervosa*, *C. grewiifolia* have shown similar activity with IC_{50} ranging from 0.89 to 4.2 µg mL^{-1},[23] human breast adenocarcinoma cell line MCF-7 where the ethyl acetate leave extract of *C. capitellata* exhibited potent cytotoxicity (IC_{50} = 2.0 µg mL^{-1}). However, few studies have been done towards cervix cancer cell lines, such as the one performed by Silva, which indicated a moderate activity of the essential oil of *C. sylvestris* towards HeLa cells with IC_{50} = 63.3 µg mL^{-1}.[24] Compounds **1**, **2**, **3** and **6** also had pronounced activity with IC_{50} values of 2.52 µM, 1.34 µM, 4.73 µM, and 1.54 µM, respectively, compared to the control griseofulvin (IC_{50} = 19.3 µM) while compounds **4** and **5** were inactive (IC_{50} > 200 µM) (Table 3). It is noteworthy that these fulfil one of the criteria attributed to potential plant-derived anti-cancer candidates with an IC_{50} value less than 10 µM.[25] The high activity of compounds **2** and **6** could be related to the aldehyde function at position 14. However, further analysis needs to be carried out to better elucidate the influence of this chemical function, as no bioassay results or computational analysis on the cytotoxic activity of analogue compounds is available in the literature.

Experimental section

General experimental procedures

UV spectra were obtained using a Hitachi UV 3200 spectrophotometer, and IR spectra with a using JASCO 302-A spectrometer (Thermo Scientific, Waltham, MA, USA). ECD spectra were obtained on a JASCO J-715CD spectrometer (JASCO Corporation, Tokyo, Japan). Optical rotations were measured on a JASCO DIP-3600 digital polarimeter (JASCO, Tokyo, Japan) at 23 °C. ^{1}H, ^{13}C and 2D NMR spectra were recorded at room temperature using a Bruker DRX-600 spectrometer (125 MHz for ^{13}C and 500 MHz for ^{1}H, Bruker, Germany) to provide chemical shifts that are expressed in ppm relative to tetramethylsilane (TMS). ESI-Mass spectra were obtained with an Agilent 6220 TOF LCMS mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Column chromatography was performed using silica gel of 70–230 mesh (Merck, Darmstadt, Germany) and aluminum plates precoated with silica gel 60 F254 (Merck, Darmstadt, Germany) were used for thin layer chromatography. TLC spots were visualized under UV light at 254 or 365 nm, followed by spraying with 20% aqueous H_2SO_4 spray and heating. Normal phase medium pressure liquid chromatography

(MPLC) was run using Reveleris X2 Flash Chromatography System equipped with an UV-vis and ELSD detectors (Buchi, Switzerland).

Plant material

The roots of *Casearia barteri* Mast. were collected in March 2016 from Bangangté, West Province, Cameroon. The botanical identification was made by the botanist Mr Nana Victor at the National Herbarium of Cameroon, where a voucher specimen (no. 65823 HNC) were already available.

Extraction and isolation

The air-dried roots of *Casearia barteri* (1.86 kg) were powdered and macerated twice with methanol (2 × 10 L) during 48 H at room temperature. The filtrate was then evaporated using rotary evaporator under reduced pressure to afford a crude extract (77.1 g). The obtained extract was dissolved and fixed onto silica gel and subsequently fractioned using open silica gel column 230–400 mesh (Merck, Darmstadt, Germany) to yield F_1 (PE, 23.6 mg), F_2 (PE/acetone 1 : 1 v/v, 28.5 g), F_3 (acetone, 19.3 g), F_4 (acetone/MeOH 1 : 1 v/v, 10.2 g), F_5 (MeOH, 15.9 g) on the basis of their TLC profiles. Fraction was F_2 divided into two parts and separated by normal phase MPLC over silica gel eluting with a step gradient PE-acetone (5 : 95, 10 : 90, 15 : 85, 20 : 80, 30 : 70, 40 : 60, 50 : 50 v/v) to afford seven subfractions (F_{2-1}–F_{2-7}). F_{2-1} was purified using open column loaded with silica gel and eluted with DCM : MeOH (99 : 1) to yield the mixture of two inseparable compounds **9** + **10** (6.3 mg). Further elution on isocratic mode (DCM/MeOH (95 : 5)) of F_{2-2} over column silica gel let to partially purified mixtures labelled F_{2-2-A}–F_{2-2-F} that were purified repeatedly over sephadex column eluted with DCM/MeOH (1 : 1) to afford compound **1** (4.2 mg), **2** (5.9 mg), **3** (3.9 mg) and **7** (8.2 mg). Following the same procedure, F_{2-3} to F_{2-5} were treated separately to afford compound **4** (DCM/MeOH (85 : 15), 2.2 mg), **5** (DCM/MeOH (90 : 10), 3.1 mg), **6** (DCM/ MeOH (90 : 10), 3.2 mg), and **8** (DCM/MeOH (90 : 10), 8.2 mg). F_{2-6} was purified over column silica gel followed with sephadex column eluted with methanol yielded compound **11** (DCM/ MeOH (80 : 20), 5.2 mg) and **12** (DCM/MeOH (80 : 20), 6.3 mg). Compound **13** (DCM/MeOH (90 : 10), 3.1 mg) was obtained after elution of fraction F_3 with DCM/MeOH (70 : 30) over silica gel column.

Barterin A (**1**): colorless oil; $[\alpha]_D^{20}$ + 32.1 (*c* = 1.0, MeOH); ECD (CH$_3$CN) 210 (Δε − 3.6), 240 (Δε − 3.9) nm; IR (film) ν$_{max}$ 2965, 2933, 1748, 1721, 1459, 1370, 1221, 1170, 946, 735 cm^{-1}; ^{1}H NMR (600 MHz, acetone-d_6) and ^{13}C NMR (150 MHz, acetone-d_6) data, see Tables 1 and 2; HRESIMS *m/z* 569.3084 [M + Na]$^+$ (calcd for C$_{31}$H$_{46}$O$_8$Na, 569.3084).

Barterin B (**2**): colorless oil; $[\alpha]_D^{20}$ + 17.5 (*c* = 2.0, MeOH); ECD (CH$_3$CN) 212 (Δε − 2.2), 245 (Δε − 2.1) nm; IR (film) ν$_{max}$ 2965, 2930, 1749, 1728, 1369, 1222, 1172, 1062, 947 cm^{-1}; ^{1}H NMR (600 MHz, methanol-d_4) and ^{13}C NMR (150 MHz, methanol-d_6) data, see Tables 1 and 2; HRESIMS *m/z* 571.2878 [M + Na]$^+$ (calcd for C$_{30}$H$_{44}$NaO$_9$, 571.2877).

Barterin C (**3**): colorless oil; $[\alpha]_D^{20}$ − 5.4 (*c* = 1.0, MeOH); ECD (CH$_3$CN) 209 (Δε − 2.8), 232 (Δε − 3.6) nm; IR (film) ν$_{max}$ 2965,

2933, 1748, 1721, 1459, 1370, 1221, 1170, 946, 735 cm^{-1}; ^{1}H NMR (600 MHz, methanol-d_4) and ^{13}C NMR (150 MHz, methanol-d_6) data, see Tables 1 and 2; HRESIMS m/z 5557.2719 [M + Na]$^+$ (calcd for $C_{29}H_{42}NaO_9$, 557.2721).

Barterin D (**4**): colorless oil; [α]$_D^{20}$ + 62.7 (c = 1.0, MeOH); ECD (CH$_3$CN) 230 ($\Delta\varepsilon$ + 8.9) nm; IR (film) v_{max} 2964, 2929, 1749, 1730, 1457, 1372, 1225, 1147, 1063, 1010, 947, 669 cm^{-1}; ^{1}H NMR (600 MHz, methanol-d_4) and ^{13}C NMR (150 MHz, methanol-d_6) data, see Tables 1 and 2; HRESIMS m/z 617.3292 [M + Na]$^+$ (calcd for 617.3296 for $C_{32}H_{50}O_{10}Na$).

Barterin E (**5**): colorless oil; [α]$_D^{20}$ + 22.9 (c = 2.0, MeOH); ECD (CH$_3$CN) 210 ($\Delta\varepsilon$ − 1.8), 241 ($\Delta\varepsilon$ + 2.1) nm; IR (film) v_{max} 2961, 2924, 1728, 1373, 1226, 1061, 946, 669 cm^{-1}; ^{1}H NMR (600 MHz, methanol-d_6) and ^{13}C NMR (150 MHz, methanol-d_4) data, see Tables 1 and 2; HRESIMS m/z 585.3035 [M + Na]$^+$ (calcd for $C_{31}H_{46}NaO_9$, 585.3034).

Barterin F (**6**): colorless oil; [α]$_D^{20}$ − 66.2 (c = 2.0, MeOH); ECD (CH$_3$CN) 210 ($\Delta\varepsilon$ − 3.6), 240 ($\Delta\varepsilon$ − 3.9) nm; IR (film) v_{max} 2968, 2929, 1751, 1732, 1685, 1372, 1229, 1179, 1003, 962 cm^{-1}; ^{1}H NMR (600 MHz, methanol-d_4) and ^{13}C NMR (150 MHz, methanol-d_4) data, see Tables 1 and 2; HRESIMS m/z 543.2557 [M + Na]$^+$ (calcd for $C_{28}H_{40}NaO_9$, 543.2564).

ECD calculations

The calculated ECD spectra of new compounds were performed as previously reported.[26,27] Systematic conformational searches were performed firstly using MOE software and conformers under 3.0 kcal mol^{-1} were optimized using the DFT method at the B3LYP/6-31+G(d,p) level (Gaussian 09).[28] A second optimization was performed using the DFT method at the B3LYP/6-311+G(d,p) level, and a polarizable continuum model (IEFPCM, solvent: acetonitrile) was applied to mimic the effects of the solvent used in the experimental ECD spectra. Theoretical ECD spectra were simulated for those conformers falling above 1% population threshold after applying Boltzmann distribution. Time Dependant Density Functional Theory (TDDFT) was used at the CAM-B3LYP/6-31+G(d) level of theory (IEFPCM, solvent: acetonitrile). The ECD curves were extracted using SpecDis 1.61 software.[29] The overall ECD curves of all the compounds were weighted by Boltzmann distribution after UV correction.

Conclusions

Six new clerodane-type diterpenoids, barterins **1–6** as well as four knowns were described from the roots extract of *Casearia barteri*. These isolates were found to share the same skeleton as the previous ones reported from the genus *Casearia*, enhancing their character as chemotaxonomic markers of this genus. Besides, their structural diversity, the reported diterpenoids are also endowed with significant cytotoxic activity, such as barterin B and E which presented the pronounced inhibition activity (IC$_{50}$ = 1.34, 1.54 μM respectively) while the crude extract exhibited also a strong activity (IC$_{50}$ = 1.58 μg mL^{-1}). These observations suggest that *Casearia barteri* should be given considerable attention, especially to better understand the mode of action in the perspective of the search for alternative treatment for cervical cancer.

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and ESI.‡

Conflicts of interest

The authors declare no conflict of interest that could have appeared to influence the work reported in this paper.

Acknowledgements

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