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Chemical constituents from leaves and root bark of *Trichilia monadelpha* (Meliaceae)

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ABSTRACT

Two new limonoid derivatives designated, monadelphin A (**1**) and monadelphin B (**2**) and two new sesquiterpene derivatives named trichins A (**3**) and B (**4**) were isolated together with six known compounds (**5–10**) from the mixture of methylene chloride/methanol (1:1) extract of leaves and root bark of *Trichilia monadelpha* (Meliaceae) collected in Cameroon. The structures of the new compounds were unambiguously established by detailed spectroscopic analysis including 1D and 2D NMR data in conjunction with high resolution mass spectrometry data and by comparison of these data with those of related compounds described in the literature. Compounds **1–4** were screened for their cytotoxic potential. Compound **1** showed strong cytotoxicity against the mouse lymphoma L5178Y cell line with an IC_{50} value of 0.62 µg/mL. The biogenetic origin of trichin B (**4**) from trichin A (**3**) was also postulated.

1. Introduction

Trichilia, the largest genus of the Meliaceae family, consists of over 90 species which are widely distributed throughout the tropical and subtropical regions over the world (Xie et al., 1994). *Trichilia monadelpha* (Thonn) JJ De Wilde syn. *T. heudelotii* (Abbiw, 1990; Irvine, 1961), one of the thirteen species of this genus represented in Cameroon, is a towering tree with 0.4 m of diameter which grows up to 12–20 m high in the tropical rainforests in Africa (Irvine, 1961). The species of *Trichilia* genus have been used as timbers and herbal medicines by traditional healers in Cameroonian folk medicine for the treatment of various diseases such as abdominal pain, dermatitis, haemorrhoids, jaundice, gonorrhea, syphilis and skin inflammation (Pupo et al., 2002). Previous phytochemical investigations on some members of this genus reported the presence of a wide range of secondary metabolites, including phenolic acids (Aladesanmi and Odediran, 2000), terpenes (Aladesanmi and Odediran, 2000), steroids (Pupo et al., 1997) and limonoids (Adesida and Okorie, 1973; Okorie and Taylor, 1968; Tsamo et al., 2013), some of which display noteworthy biological properties, such as antimicrobial, anti-inflammatory, antiplasmodial, antioxidant, antimutagenic, cytotoxic and hepatoprotective activities (Aladesanmi and Odediran, 2000; Tsamo et al., 2016).

In the continuation of our effort in the search for bioactive secondary metabolites from Cameroonian medicinal plants (Tsamo et al., 2016, 2013), we have investigated the constituents of leaves and root bark of *T. monadelpha*. As a result, four new compounds, including two new limonoid derivatives, monadelphins A (**1**) and B (**2**), and two new sesquiterpenes, trichins A (**3**) and B (**4**), together with six known compounds (**5–10**) were isolated and structurally characterized. Herein, we describe the isolation and structure elucidation of these four new isolated compounds **1–4** as well as their cytotoxic potential. The plausible biogenetic origin of trichin B (**4**) from trichin A (**3**) was also postulated.

2. Results and discussion

The air-dried and powdered leaves (2.7 kg) and root bark (1.5 kg) of *T. monadelpha* were separately extracted by maceration at room

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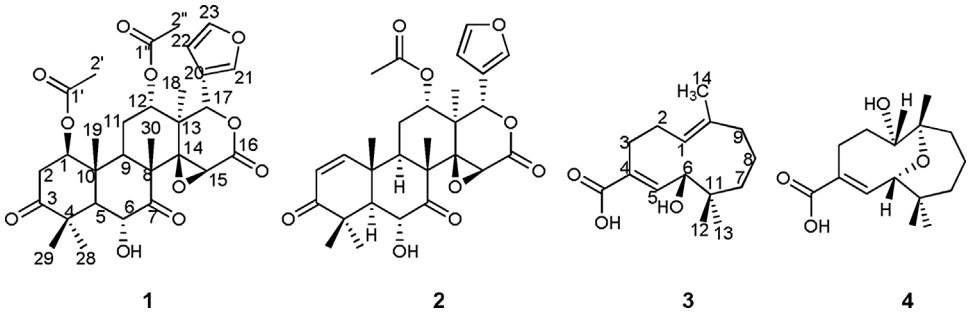


Fig. 1. Chemical structures of compounds **1–4.**

Table 1
^{1}H NMR spectroscopic data of compounds **1–4**.[a]

Position	1 [b]δ_H (m, *J*, Hz)	2 [c]δ_H (m, *J*, Hz)	3 [c]δ_H (m, *J*, Hz)	4 [c]δ_H (m, *J*, Hz)
1	4.84, dd (10.1, 1.6)	7.07, d (10.0)	5.18, t (7.6)	3.54, dd (8.9, 2.9)
2a	3.33, dd (15.3, 10.1)	5.94, d (10.0)	2.33, m	2.10, dddd (13.8, 8.9, 6.5, 4.8)
2b	2.04, dd (15.3, 1.6)	–	2.18, m	1.85, dddd (13.8, 9.5, 6.2, 2.9)
3a	–	–	2.61, m	2.71, ddd (12.7, 9.5, 6.5)
3b			2.28, m	2.54, ddd (12.7, 6.2, 4.8)
5	1.97, d (11.4)	2.09, d (12.5)	6.88, d (10.3)	7.20, d (3.2)
6	4.74, dd (11.4, 3.6)	4.96, d (12.5)	4.04, d (10.3)	4.08, d (3.2)
7a	–	–	1.26, m	1.54, m
7b			1.07, m	1.44, m
8a	–	–	1.61, m	1.73, m
8b			1.31, m	1.55, m
9a	2.33, d (11.3)	2.42, d (12.2)	2.18, m	1.81, m
9b			1.44, m	1.57, m
11a	2.02, ddd (15.7, 11.3, 5.1)	2.22, ddd (15.5, 12.2, 5.6)	–	–
11b	1.93, d (15.7)	1,97, d (15.5)	–	–
12	4.77, t (5.1)	5.05, d (5.6)	0.94, s	1.11, s
13	–	–	0.96, s	0.93, s
14	–	–	1.54, s	1.23, s
15	3.66, s	3.81, s	–	–
17	5.47, s	5.64, s		
18	1.23, s	1.29, s		
19	1.14, s	1.30, s		
20	–	–		
21	7.39, d (1.5)	7.52, dd (1.6, 0.8)		
22	6.32, dd (1.5, 0.9)	6.46, dd (1.6, 0.8)		
23	7.38, d (1.5)	7.50, d (1.6)		
28	1.34, s	1.43, s		
29	1.40, s	1.33, s		
30	1.15, s	1.23, s		
6-OH	3.67, d (3,6)	–		
2′	2.00, s	–		
2″	1.73, s	1.72, s		

[a] Chemical shifts are expressed in δ (ppm) downfield from TMS and assigned by COSY, HSQC and HMBC experiments. *J* in Hz.
[b] Recorded in $CDCl_3$.
[c] Recorded in MeOD[5].

temperature for 48 h with a mixture of CH_2Cl_2/methanol (1/1, v/v). Filtration and evaporation of each resulting solution under reduced pressure led to a dark greenish leaf extract and brown root bark extract, respectively. Since both extracts showed strong cytotoxicity when assayed against the lymphoma cell line L5178Y, each of them was further fractionated into several fractions by vacuum liquid chromatography. The different fractions were purified by combination of silica gel, reversed-phase ODS column chromatography, and semi-preparative HPLC to give four new compounds named monadelphin A (**1**), monadelphin B (**2**), trichin A (**3**) and trichin B (**4**) together with six known compounds stigmasterol (**5**), β-sistoterol (**6**), ellagic acid (**7**), protocatechuic acid (**8**), coixol (**9**) and scopoletin (**10**). The structures of new compounds (Fig. 1) were elucidated by spectroscopic analysis using HRESIMS, 1D and 2D NMR experiments.

2.1. Characterization of the new compounds

Monadelphin A (1) was isolated as colorless crystals. It reacted positively, both to Liebermann-Burchard (red purple) and Erhlich (orange) tests suggesting its limonoidic nature. It was found to possess a molecular formula of $C_{30}H_{36}O_{11}$, from the sodiated molecular ion $[M + Na]^+$ at *m/z* 595.2134 (calcd. for $C_{30}H_{36}O_{11}Na$: 595.2155) in its HRESIMS requiring 13° of unsaturation. Its IR spectrum showed absorption bands at ν_{max} 3439 (-OH), 1734 and 1710 cm^{-1}, characteristic of hydroxyl, carbonyl and furan moieties (Benjamin et al., 2003), respectively. In accordance with its molecular formula, all the 30 carbon signals were well exhibited in the ^{13}C NMR spectrum (Table 2) of compound **1**, which were further sorted by HSQC experiments as 7 methyls, 2 methylenes, 10 methines (five oxygenated and three olefinic), and 11 quaternary carbons (five carbonyls, one oxygenated and one olefinic). The ^{1}H (Table 1) and ^{13}C NMR (Table 2) data of compound **1** exhibited resonances assignable to five carbonyl groups

Table 2
^{13}C NMR spectroscopic data of compounds **1–4**.[a]

Position	1[b]δ C	2[c]δ C	3 [c]δ C	4 [c]δ C	
1	77.9	156.5	123.4		76.7
2	42.1	126.0	26.8		38.8
3	212.8	205.9	26. 0		31.5
4	40.7	46.8	134.2		136.9
5	58.8	59.5	140.9		140.3
6	71.8	72.6	69.1		80.6
7	209.2	209.7	36.3		38.7
8	47.7	43.8	23.4		38.8
9	47.8	43.8	35.6		19.9
10	51.9	52.2	136.3		83.3
11	27.3	26.4	37.3		38.9
12	70.4	70.6	22.3		25.8
13	41.8	42.4	22.07		27.4
14	63.9	63.4	18.2		21.2
15	52.3	52.3	170.2		169.4
16	165.7	166.4			
17	76.7	76.2			
18	14.4	13.4			
19	13.4	20.7			
20	119.1	119.7			
21	141.4	141.8			
22	109.5	109.8			
23	143.2	142.7			
28	31.1	18.5			
29	18.9	30.4			
30	16.3	15.5			
1′	169.9	–			
2′	20.5	–			
1″	170.2	169.5			
2″	21.0	18.9			

[a] Chemical shifts are expressed in δ (ppm) downfield from TMS and assigned by HSQC and HMBC experiments. *J* in Hz.
[b] Recorded in $CDCl_3$.
[c] Recorded in MeOD[5].

including two keto carbonyls at *δ* 209.2 (C-7) and *δ* 212.8 (C-3), one carbonyl of a lactone group at *δ* 165.7 (C-16) and two other carbonyls of acetyl groups at *δ* 169.9 (C-1′) and *δ* 170.2 (C-1″). The presence of the two acetyl carbonyl groups was confirmed by two three protons singlets observed in the ^{1}H NMR at *δ* 2.00 and *δ* 1.73 which correlated in the HSQC spectrum with carbons at *δ* 20.5 and *δ* 21.0, respectively. The ^{1}H NMR spectrum of **1** also showed characteristic signals for a typical β-substituted furan ring at *δ* 6.32 (1H; t; H-22)/*δ* 109.5, *δ* 7.38 (1H; d; *J* = 1.5H_Z; 1·5H_Z; H-23)/*δ* 143.2 and *δ* 7.39 (1H; d; *J* = 1.5H_Z;class = 1·5H_Z; H-21)/*δ* 141.4, three oxymethine protons at *δ* 5,47 (1H; s)/*δ* 76.7, *δ* 4.84 (1H; dd; *J* = 10.1; 1.6 Hz)/*δ* 77.1 and *δ* 4.77 (1H; t; *J* = 5.1 Hz)/*δ* 77.1, one hydroxymethine proton at *δ* 4.74 (1H; dd; *J* = 11.4; 3.6 Hz)/*δ* 71.8, an epoxy moiety at *δ* 3.66 (1H; s; H-15)/*δ* 52.3, one exchangeable proton hydroxyl signal at *δ* 3.67 (1H) and five tertiary methyl groups at *δ* 1.14 (3H; s; H-19)/*δ* 13.4, *δ* 1.15 (3H; s; H-30)/*δ* 16.3, δ 1.23 (3H; s; H-18)/*δ* 14.4, *δ* 1.34 (3H; s; H-28)/*δ* 31.1 and *δ* 1.40 (3H; s; H-29)/δ 18.9. Deducting nine double bond equivalents accounted for by five carbonyl groups, an epoxy moiety and a furan ring, the remaining four degrees of unsaturation suggested a tetracyclic ring system in the core skeleton. The above-mentioned data suggested that compound **1** is a limonoid based on a khivorin skeleton, limonoid previously isolated from the timber of *Khaya ivorensis* (Bevan et al., 1963). This was evident from the HMBC spectrum on which the correlations were observed from H-15 (*δ* 3.66; s) to C-16 (δ 165.7) and from H-17 (*δ* 5.47; s) to C-13 (δ 41.8) and C-14 (*δ* 63.9), confirming the 14, 15-epoxylactone-substitution pattern of khivorin derivatives (Bevan et al., 1963). In fact, the comparison of the NMR data of compound **1** with those of khivorin revealed close similarity except for some few differences due to the presence in the structure of compound **1** of a hydroxyl and two ketone carbonyl functional groups. The hydroxyl group was located at C-6 position from the HMBC correlation observed between H-5 (*δ* 1.97) with carbon signal at *δ* 71.8 (C-6) and confirmed by COSY correlations between the proton signals H-6 (*δ* 4.74) and H-5, on one hand and between the proton signal H-6 and the proton of hydroxy signal (*δ* 3.67), on the other hand. Concerning the two-ketone carbonyl functional groups, their positions were determined to be at C-3 and C-7, respectively from the HMBC (Fig. 2) correlations observed between the proton at *δ* 1.97 (H-5) with carbon signals at *δ* 212.8 (C-3), *δ* 31.1 (C-28) and *δ* 18.9 (C-29), and between the same proton signal at *δ* 1.97 (H-5) with carbon signals at *δ* 71.8 (C-6) and *δ* 209.2 (C-7). It remained to us to determine the positions and the two acetyl groups, their relative stereochemistry as well as the one of the hydroxyl group. The positions of the two acetyl groups were determined to be at C-1 and C-12 from the multiplicity of protons H-1 (Connolly et al., 1972) and H-12 which appeared as doublet of doublet at *δ* 4·84 and as triplet at δH 4.77, respectively. The relative stereochemistry of the acetoxyl group at C-12 was deduced from the comparison of the value of the ^{1}H chemical shift of its methyl protons with those of 6, 12 *α*-diacetoxyangolensate, a limonoid isolated from the bark of *Guarea* species by Ibadan group in 1972 (Connolly et al., 1972) and which bears in its structure as compound 1 an acetoxyl group at C-12 position. From this comparison, the methyl of the acetyl group resonated at *δ* 2·00 was assigned to the acetyl group at C-1, whereas the methyl at *δ* 1.73 was assigned to the acetyl group at C-12. This unusual shielding of 12-acetate methyl group due to the effect of furan ring was also observed for 6, 12 *α*-diacetoxyangolensate. Thus, the relative stereochemistry of acetyl group at C-12 was assigned to have α-orientation. For the acetyl group at C-1, its relative stereochemistry was established from the values of the coupling constants and NOE difference experiments. The fact that in ^{1}H-NMR spectrum, proton H-1 appeared as doublet of doublet with coupling constants $^3J_{H1/H2a}$ (10.1 Hz) and $^3J_{H1/H2b}$ (1.6 Hz) indicated that at C-1, H-1 has an axial position (α-orientation) whereas the acetoxyl group occupied equatorial position (β-orientation). This was further confirmed by the NOE difference experiments (Fig. 3) in which enhancement of H-5 and H-9 signals was observed when proton H-1 was irradiated indicating clearly their close spatial proximity and confirming their α − orientation. These results are very close to those of 3, 7-dideacethyl-6α-hydroxykhivorin (Tchimene et al., 2006), a limonoid in

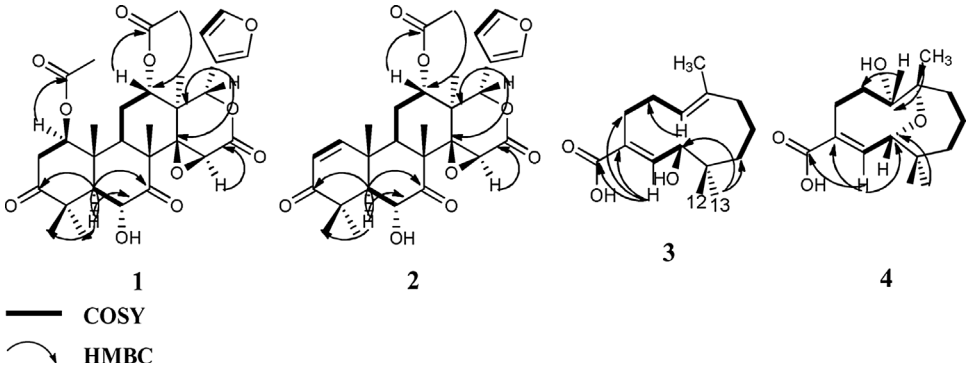


Fig. 2. Selected ^{1}H–^{1}H COSY and HMBC correlations of compounds **1–4.**

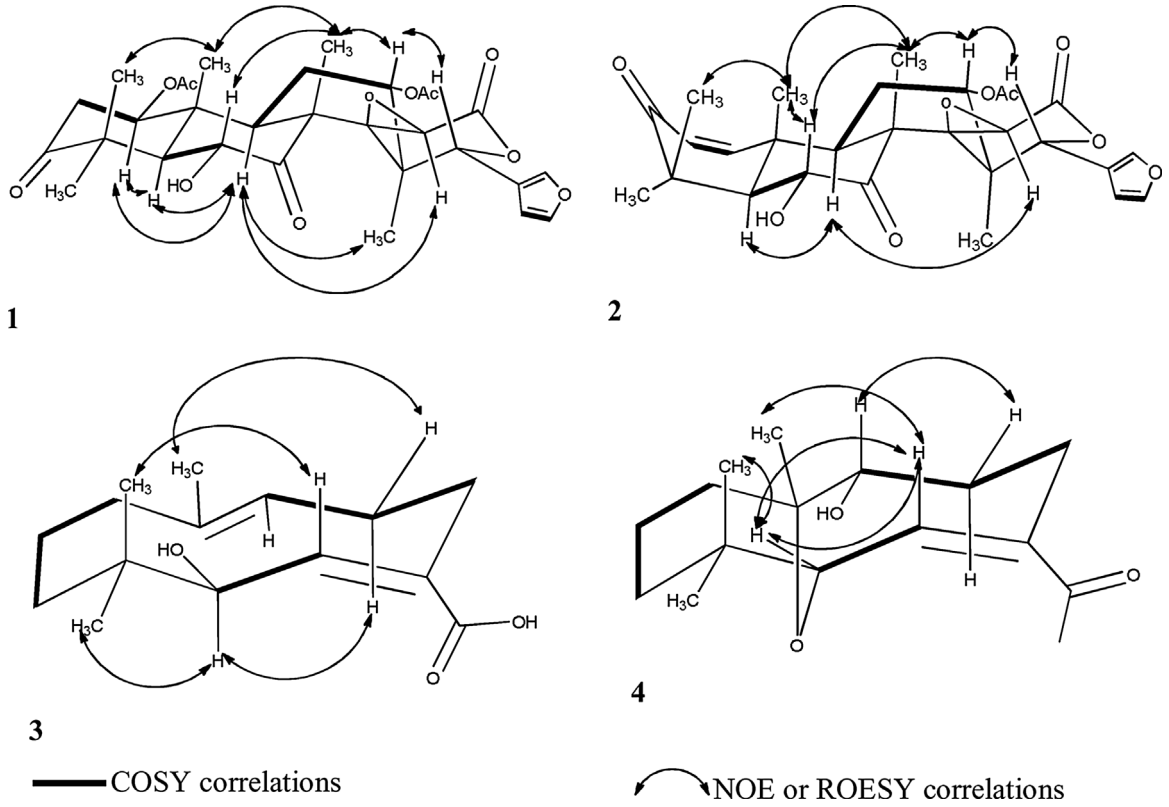
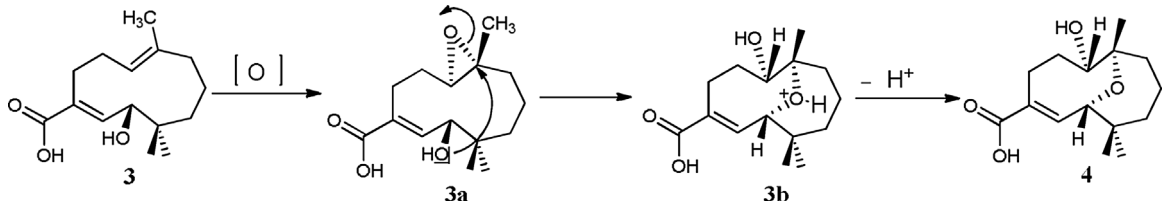


Fig. 3. NOE (compound **1**) and ROESY (compounds **2**, **3**, and **4**) key correlations.

which the A ring substitution pattern is similar to the one of compound **1**. Concerning the relative configuration of the 6-hydroxy group in ring B, it was determined to be α − oriented using, once more, NOE difference experiments. Thus, when proton H-6 was irradiated, increase of proton intensity signals H-29, H-30 and H-19 was observed while no enhancement was noticed for signals due to H-5 and H-9. From the above spectroscopic data, the structure of compound **1,** to which the trivial name monadelphin A was given, was therefore established as depicted (Fig. 1).

Monadelphin B (**2**) was isolated as yellow oil. As compound **1**, it also gave a positive reaction to both Liebermann-Burchard and Erhlich tests suggesting its limonoidic nature. Its molecular formula was deduced as $C_{28}H_{32}O_9$ from HRESIMS which showed the protonated molecular ion at *m/z* 513.2123 $[M + H]^+$ (calcd for $C_{28}H_{33}O_9$: 513.2125) with 13 double-bond equivalents. Comparison of NMR data (Tables 1 and 2) of compound **2** with those of **1** showed very close similarities due to the presence of characteristic signals of the furan ring moiety at *δ* 6.46 (1H; dd; *J* = 1.6; 0.8; H-22)/*δ* 109.5, *δ* 7.50 (1H; d; *J* = 1.6; H-23)/*δ* 143.2 and *δ* 7.52 (1H; dd; *J* = 1.6; 0.8; H-21)/*δ* 141.4; one singlet proton H-17 at *δ* 5.64 (1H; s; H-17)/*δ* 76.2 belonging to a butyro-lactone moiety, one singlet proton at *δ* 3.81 (1H; s; H-15/*δ* 52.3 due to H-15 of oxiran moiety, five singlet methyls at *δ* 1.23 (3H; s; H-30)/*δ* 15.5, *δ* 1.29 (3H; s; H-18)/*δ* 13.4, *δ* 1.30 (3H; s; H-19)/*δ* 20.7, *δ* 1.33 (3H; s; H-29)/*δ* 30.4 and *δ* 1.43 (3H; s; H-28)/*δ* 18.4 and one singlet of three protons due to a methyl of an acetyl group at C-12 at *δ* 1.72 (3H; s; H-2″)/*δ* 18.9. The main difference between these two compounds is the disappearance of the acetyl group at C-1 position in compound **2**, which was replaced by an AB spin system of two *cis* olefinic protons at δ_H 7.07 (1H; d; *J* = 10 Hz; H-1)/*δ* 156.5 and *δ* 5.94 (1H; d; *J* = 10 Hz; H-2)/*δ* 126.0 corresponding to an α,*β*-unsaturated carbonyl moiety. The relative configuration of compound **2** was assigned as being identical with that of **1** by comparing their NMR data, the coupling patterns of all relevant protons and also by ROESY experiments (Fig. 3). Thus, from the above spectroscopic data, the structure of compound **2** was elucidated as depicted. The trivial name monadelphin B was given to this compound.

Trichin A (**3**) was obtained as a yellow oil. Its molecular formula $C_{15}H_{24}O_3$ was established on the basis of HRESIMS at *m/z* 253.2123 $[M + H]^+$ (calcd for $C_{15}H_{25}O_3$: 253.1803) requiring 4 double-bond equivalents. The ^{1}H NMR spectrum of compound **3** coupled with its HSQC and HMBC spectra (Tables 1 and 2) exhibited resonances due to two trisubstituted double bonds at *δ* 6.88 (1H; d; *J* = 10.3; H-5)/*δ* 140.9 and *δ* 5.18 (1H; t; *J* = 7.6; H-1)/*δ* 123.4, an oxymethine proton at *δ* 4.04 (1H; d; *J* = 10.3; H-6)/*δ* 69.1, four quarternary carbons comprising the signal of a carbonyl belonging to a carboxylic acid at *δ* 170.2 (C-15), a signal due to two sp^2 carbons at *δ* 136.3 (C-10) and 134.2 (C-4) and one sp^3 carbon signal at *δ* 37.3 (C-11). Furthermore, three singlet methyl groups at *δ* 1.54 (3H; s; H-14)/*δ* 18.2, *δ* 0.96 (3H; s; H-13)/*δ* 22.1 and *δ* 0.94 (3H; s; H-12)/*δ* 22.3 and five sp^3 methylene groups were observed. All these functionalities account for 3 out of 4 ° of unsaturation present in this molecule. The only remaining unsaturation implied one monocyclic core in compound **3**. The substructure consistent with the above data is an α-humulenoid type-skeleton for compound **3.** This was confirmed by the COSY spectrum which showed a correlation between H-1 at *δ* 5.18 (1H; t; *J* = 7.6; H-1) and a methylene allylic proton H-2 at *δ* 2.33 (2H; m; H-2), which also correlated with H-3a at *δ* 2.61 (1H; m; H-3). Furthermore, ^{1}H–^{1}H COSY correlations were also observed between H-5/H-6, H_2-7/H_2-8, and H_2-8/H_2-9. The presence of α-humulenoid type-skeleton was also supported by comparison of the above data with those reported for kurubaschic acid angelate, a sesquiterpenoid isolated from *Ferula haussknechtii* (Apiaceae) (Mahmut et al., 1987). The only difference between them is the absence of signals of angelate group in the ^{1}H NMR spectrum of compound **3**. The relative configuration of **3** was assigned from the ROESY spectrum and also by comparing its NMR data with those of kurubaschic acid angelate (Mahmut et al., 1987). The methyl group at C-10 was assigned with a β-configuration because of the correlations between H-2b and Me-14. The other stereocenters of compound **3** were



Scheme 1. Plausible biosynthetic route from **3** to **4**.

identical to those of kurubaschic acid angelate. Thus, compound **3** was assigned as depicted and was given the trivial name trichin A.

The molecular formula of trichin B (**4**) was assigned as $C_{15}H_{24}O_4$ on the basis of the HRESIMS which showed the quasimolecular ion peak at *m/z* 269.1743 $[M + H]^+$ (calcd for $C_{15}H_{25}O_4$: 269.1753), implying 4 double-bond equivalents. The ^{1}H NMR spectrum of compound **4** (Table 1) coupled with its HSQC and HMBC spectra displayed signals due to one trisubstituted double bond at δ 7.20 (1H; d; J = 3.2; H-5)/δ 140.3 and δ 136.9, two oxymethines at δ 4.08 (1H; d; J = 3.2; H-6)/δ 80.6 and δ 3.54 (1H; dd; J = 8.9; 2.9; H-1)/δ 76.7, a signal of a carbonyl belonging to a carboxylic acid at δ 169.4 (C-15). Further signals included three methyls at δ 25.8 (C-12), 27.4 (C-13) and 21.2 (C-14), five sp^3 methylenes, two sp^3 quaternary carbons at δ 83.3 (C-10) and 38.9 (C-11) and one sp^2 quaternary carbon at δ 136.9 (C-4). By comparing the NMR data (Tables 1 and 2) of compound **4** with those of compound **3**, it is evident that they share similar core structure except for two major differences. The first one is the disappearance of $\Delta^{1,10}$ double bond with its olefinic proton in the ^{1}H NMR spectrum of compound **4**, which was replaced by hydroxymethine at δ 3.54 (1H; dd; J = 8.9; 2.9; H-1)/δ 76.7. The second difference is the presence of the ether-bridge between C-6 and C-10. The relative configuration of compound **4** was deduced from ROESY correlations (Fig. 3) and by comparing its NMR data with those of compound **3**. Thus, from the above spectroscopic data, the structure of compound **4** was elucidated as depicted. The trivial name trichin B was given to this compound. From their structures, it's clearly appears that trichin B might derived biogenetically from trichin A by a series of transformation involving epoxidation, following by the opening of the oxiran ring through nucleophily attack of the hydroxy group to give trichin B *via* intermediate (**3b**) as shown in Scheme 1. This was supported by the nearly identical configurations of all chiral centers in compounds **3** and **4**.

The known isolated compounds were identified by comparison of their spectroscopic and MS data with those reported in the literature. They include stigmasterol (**5**) (Habib et al., 2007), β-sitosterol (**6**) (Habib et al., 2007), ellagic acid (**7**) (Duc et al., 1990), protocatechuic acid (**8**) (Aladesanmi and Odediran, 2000), coixol (**9**) (Venkateswarlu et al., 1999) and scopoletin (**10**) (Yuan et al., 2007).

Limonoid and sesquiterpene derivatives have been isolated frequently from *Trichilia* species (Ivo et al., 2014), and are known to exibit a number of biological properties including antiplasmodial (Krief et al., 2004), antitumoral (Kubo and Klocke, 1982), antiviral (Tsamo et al., 2013), antibacterial (Germanò et al., 2005) and cytotoxic (Maminata et al., 2007) activities. Given the traditional use of *Trichilia* species to treat infectious diseases and the fact that decoctions of *T. monadelpha* are used for gastrointestinal pains and cough, some of the isolated compounds were screened for their cytotoxic potential.

2.2. Biological activity

Compounds **1–4** were evaluated for their effects on the growth of the mouse lymphoma L5178Y cell line. Compound **1** possesses cytotoxic activity, with an IC_{50} value of 0.62 µg/mL, while the other tested compounds were inactive. Many previous studies provided evidence of the cytotoxicity of limonoids against cancer cells (Ejaz et al., 2006).

3. Conclusion

Two new limonoid (**1-2**) and two new sesquiterpene derivatives (**3-4**) were isolated from leaves and root bark of *T. monadelpha*. Their structures were established based on extensive spectroscopic experiments. The new limonoid derivative monadelphin A (**1**) exhibited in *vitro* a remarkable cytotoxicity against the mouse lymphoma L5178Y cell line. Therefore, monadelphin A should be considered as a promising drug candidate that could be developed as a therapeutic agent against cancer. In the current study, however, most of the tested compounds were inactive in the assay employed. Thus, to explore the bioactivities of these compounds in other bioassays could be promising.

4. Experimental

4.1. General experimental procedures

Melting points were determined on a Buchii melting point apparatus. IR spectra were recorded on a Bruker Fourier transform/infrared (ATR) spectrophotometer. Optical rotations were recorded on a Perkin–Elmer-241 MC polarimeter. Mass spectra (ESI–MS) were obtained with a Thermofinnigan LCQ DECA mass spectrometer and HRESIMS spectra were measured with a FTHRMS-Orbitrap (Thermo-Finnigan) mass spectrometer. 1D and 2D NMR spectra were recorded on either Bruker ARX 500 or AVANCE DMX 600 NMR spectrometers. Chemical shifts (δ) are quoted in parts per million (ppm) with tetramethylsilane (TMS) as internal standard, and the coupling constants (J) are given in Hz. Solvents were distilled prior to use. Spectral grade solvents were used for spectroscopic measurements while analytical grade solvents were used for HPLC. Column chromatography was performed using Merck MN silica gel 60 M (0.04–0.063 mm) or Sephadex LH-20 (40–75 µm) as stationary phases. Thin-layer chromatography (TLC) was performed on aluminium or glasses silica gel 60 F_{254} (Merck) precoated plates (0.2 mm layer thickness). HPLC analysis was performed with a Dionex P580 system coupled to a photodiode array detector (UVD340S). Routine detection was at 235, 254, 280 and 340 nm. The separation column (125 mm x 4 mm, 5 µm) was prefilled with Europhere-10 C18 (Knauer). HPLC separation was performed on a semi-preparative HPLC system of Lachrom-Merck Hitachi (Pump L-7100 and UV detector L-7400) using a C-18 column (Knauer, 300 × 8 mm ID, prefilled with C-18 Eurosphere, flow rate 5 mL/min, UV detection at 280 nm). The solvent system consisted of a linear gradient of HPLC grade MeOH and ultra-pure H_2O. Spots were detected on TLC under UV lamp (254 and 366 nm) or by heating after spraying with 20% H_2SO_4 (v/v) solution. Different mixtures of *n*-hexane, EtOAc, CH_2Cl_2 and MeOH were used as eluting solvents.

4.2. Plant material

The leaves and root bark of *T. monadelpha* were collected in June 2014 and June 2015, respectively, in Mbankomo (Eloumden Mont) locality situated in the Central Region of the Republic of Cameroon. Plant material was identified by Dr. Nole, plant taxonomist at the Institute of Medical Research and Medicinal Plants Studies (IMPM). A voucher specimen was deposited at the National Herbarium of Cameroon under the reference number 66909/HNC.

4.3. Extraction and isolation

The air-dried and powdered leaves (2.7 kg) and root bark (1.5 kg) of *T. monadelpha* were separately extracted by maceration at room temperature for 48 h with a mixture of CH_2Cl_2/methanol (1/1, v/v). The suspension was filtered, and the resulting solution was concentrated under vacuum, using a rotary evaporator to afford a dark greenish residue (314 g) for the leaves, and a brown residue (204 g) for the root.

300 g of the leaf extract was subjected to flash chromatography over silica gel using *n*-hexane/EtOAc gradient to afford four main fractions labelled F_1 (32 g; *n*-hexane/EtOAc 4:1), F_2 (15 g; *n*-hexane/EtOAc 1:1), F_3 (5 g; pure EtOAc) and F_4 (134 g; MeOH).

Fraction F_1 (32 g), obtained from the elution with *n*-hexane/EtOAc (4:1), was further chromatographed on a silica gel column eluting with a gradient of *n*-hexane/EtOAc to give a total of 227 fractions of 175 mL each, which were combined based on TLC analysis to afford 12 main series (S_1 (1–11), S_2 (12–28), S_3 (29–44), S_4 (45–103), S_5 (104–115), S_6 (116–120), S_7 (121–129), S_8 (130–143), S_9 (144–166), S_{10} (167–195), S_{11} (196–218) and S_{12} (219–227). Series S_1, mainly low polar compounds, yielded **5** (25 mg) and **6** (20 mg). S_7 crystallized at room temperature, and after filtration gave **8** (10.8 mg). Then, S6 crystallized at room temperature, and after filtration **1** (40 mg) was obtained.

Fraction F_2 (15 g) was subjected to vacuum liquid chromatography (VLC) on a silica gel column eluted with a step gradient of *n*-hexane/EtOAc and then DCM/MeOH yielding 14 fractions (S1-S14). Subfraction S6 (1.1 g), obtained by elution with a mixture of *n*-hexane/EtOAc (3:2) was further chromatographed on silica gel eluting with a gradient of *n*-hexane/EtOAc to give a total of 100 fractions of 100 mL each, which were combined on the basis of their TLC analysis and analytic HPLC profiles to 10 main series ($S_{6\text{-}10}$ (1–9), $S_{6\text{-}20}$ (10–22), $S_{6\text{-}30}$ (23–31), $S_{6\text{-}40}$ (32–53), $S_{6\text{-}50}$ (54–72), $S_{6\text{-}60}$ (73–88), $S_{6\text{-}80}$ (89–91) and $S_{6\text{-}100}$ (91–100). Series $S_{6\text{-}40}$ crystallized at room temperature and after filtration **7** (8 mg) was obtained. $S_{6\text{-}50}$ (149.7 mg) was then subjected to reversed-phase ODS (octadecylsilyl) column chromatography and eluted with a gradient of MeOH/H_2O to yield 3 subfractions: $S_{6\text{-}50\text{-}A}$: MeOH/H_2O (3:2), $S_{6\text{-}50\text{-}B}$: MeOH/H_2O (3:7) and $S_{6\text{-}50\text{-}C}$: MeOH. Subfraction $S_{6\text{-}50\text{-}A}$ (46.1 mg) was further purified by semi-preparative HPLC and eluted with a step wise of MeOH/H_2O to yield **10** (3.1 mg) with MeOH/H_2O (16:9) as eluant and **2** (1.1 mg) with MeOH/H_2O (1:2) as elution solvent.

In a similar way, 194 g of root bark extract was subjected to vacuum liquid chromatography (VLC) on a silica gel column eluting with a gradient of *n*-hexane-EtOAc (1:0, 4:1, 3:2, 2:3, 1:4, 0:1) and then DCM-MeOH (1:0, 19:1, 4:1, 1:1, 0:1) to give a total of 11 fractions (F1-F11), which were combined based on their TLC analysis and analytic HPLC profiles. F3 (9.0 g) was subjected to vacuum liquid chromatography (VLC) on a silica gel column. Elution started with a gradient of *n*-hexane/EtOAc (S1-S9), then DCM/MeOH gradient was applied until pure MeOH (S10-S12). F3-S3 (1.9 g) was subjected to column chromatography over Sephadex LH-20 eluted with MeOH to give six subfractions F3-S3-(a–f) on the basis of their TLC and HPLC profiles. Subfraction F3-S3-f (5.8 mg) was evaporated to afford **9** (5.7 mg). F3-S4 (182.6 mg), obtained from elution with *n*-hexane/EtOAc (3:1), was first subjected to chromatography over Sephadex LH20 using MeOH as eluting solvent to give eight subfractions F3-S4-(a–h). Subfraction F3-S4-h (37.5 mg) was then purified by semi-preparative HPLC to yield **4** (2.1 mg) with MeOH/H_2O (9:1) as eluent. F3-S9 (100.6 mg) was purified in a similar manner by semi-preparative HPLC to yield **3** (1.59 mg) with MeOH/H_2O (5:1) as elution solvent.

4.3.1. New compounds informations

*4.3.1.1. Monadelphin A (**1**).* Colorless crystal ($CDCl_3$); $[\alpha]_D^{20}$ −7.3 (*c* 0.08, MeOH); mp 230–232 °C; IR (KBr)ν_{max}: 3439, 2950, 2877, 1706, 1734, 1710, 873 cm^{-1}; ^1H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS *m/z* 595.2134 $[M + Na]^+$ (calcd. for $C_{30}H_{36}O_{11}Na$: 595.2155).

*4.3.1.2. Monadelphin B (**2**).* Yellow oil (MeOH); $[\alpha]_D^{20}$ +31.9 (c 0.07, MeOH); UV (MeOH) λ_{max} 214 nm; ^1H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS *m/z* 513.2123 $[M + H]^+$ (calcd. for $C_{28}H_{33}O_9$: 513.2125), *m/z* 535.1943 $[M + Na]^+$ (calcd. for $C_{28}H_{32}O_9Na$: 535.1944).

*4.3.1.3. Trichin A (**3**).* Yellow oil (MeOH); $[\alpha]_D^{20}$ −5.2 (c 0.07, MeOH); UV (MeOH) λ_{max} 218 nm; ^1H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS *m/z* 275.1613 $[M + Na]^+$ (calcd. for $C_{15}H_{24}O_3Na$: 275.1623).

*4.3.1.4. Trichin B (**4**).* Yellow oil (MeOH); $[\alpha]_D^{20}$ +3.3 (c 0.05, MeOH); UV (MeOH) λ_{max} 219 nm; ^1H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS *m/z* 269.1743 $[M + H]^+$ (calcd. for $C_{15}H_{25}O_4$: 269.1753).

4.4. Biological activity

4.4.1. Cytotoxicity assay

Cytotoxicity against the L5178Y mouse lymphoma cells (source: mouse lymphoma (leukemia) cell (Combes et al., 1995)) was determined using the microculture tetrazolium (MTT) assay and compared to that of untreated controls (Carmichael et al., 1987). Stock solutions of test samples were prepared in 96% EtOH (v/v). Exponentially growing cells were harvested, counted and diluted appropriately. Fifty microliters cell suspension containing 3750 cells was pipetted into 96-well microtiter plates. Cells suspensions were prepared in RPMI Media 1640 (GIBCO) from Sigma (Taufkirchen, Germany) plus 10% horse serum (Sigma) and the final cell concentration in the well was: 3500 cells/mL. Subsequently, 50 µL of the test sample solution were added to each well. The final concentration was 3–10 µg/mL. The small amount of EtOH present in the wells did not affect the experiments. The test plates were incubated at 37 °C with 5% CO_2 for 72 h. A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was prepared at 5 mg/mL in phosphate buffered saline (PBS: 1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl; pH 7.4), and from this solution, 20 µL was pipetted into each well (the cells needed not to be washed). MTT, was added to each well and the plates were incubated for 3 h at 37 °C. Then, 100 µl of lysis buffer (20% sodium dodecyl sulfate [SDS] in 50% N, N-dimethylformamide, containing 0.5% [v:v] 80% acetic acid and 0.4% [v:v] 1N HCL) was added to each well and incubated overnight (16 h). After thorough mixing, the absorbance was measured at 520 nm using a scanning microtiter-well-spectrometer. The colour intensity is correlated with the number of healthy living cells. All experiments were carried out in triplicates and repeated three times. As negative control, media with 0.1% EGMME-DMSO were included in the experiments. The IC_{50} was computed as described by Boubaker-Elandalousi et al. (2014). The depsipeptide kahalalide F, isolated from *Elysia grandifolia* was used as positive control (IC_{50} 6.3 µg/l). After verification that the respective values follow a standard normal Gaussian distribution and that the variances of the respective groups are equal, the results were statistically evaluated using the independent two-sample Student's *t*-test (Petrie and Watson, 2013) Data were expressed as percent viability compared with control (mean ± SD, $n = 3$).

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.phytol.2017.11.020.

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