FULL PAPER

Three New Derivatives and Others Constituents from the Roots and Twigs of *Trilepisium madagascariense* DC

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Three new compounds, trilepisflavene (**1**), trilepisdepsidone (**2**), and daturadiol stearate (**3**), together with nine known compounds, 2-hydroxy-4-[(4-hydroxy-2-methoxy-6-methylbenzoyl)oxy]-6-methylbenzoic acid (**4**), lichexanthone (**5**), naringenin (**6**), 3′,4′,5,7-tetrahydroxyflavanone (**7**), 2-hydroxybenzoic acid (**8**), methyl 2,4-dihydroxy-6-methylbenzoate (**9**), *β*-amyrin (**10**), eurothridiol palmitate (**11**), and *β*-sitosterol (**12**), were isolated from the AcOEt extract of the twigs and the roots of *Trilepisium madagascariense*. Acetylation of eurothridiol palmitate was carried out and a new acetylated derivative (**13**) was obtained. The structures of the isolated and acetylated compounds were elucidated on the basis of spectroscopic analysis. Antimicrobial activity of all these compounds was evaluated using *Mueller–Hinton* broth (MHB) and *Mueller–Hinton* agar (MHA) method. Trilepisdepsidone**,** 2-hydroxy-4-[(4-hydroxy-2-methoxy-6-methylbenzoyl)oxy]-6-methylbenzoic acid, 3′,4′,5,7-tetrahydroxyflavanone, and naringenin exhibited moderate to weak antimicrobial activity.

Keywords: *Trilepisium madagascariense*, Trilepisflavene, Trilepisdepsidone, Daturadiol stearate, Depsidone, Depside, Antimicrobial activity.

Introduction

Trilepisium (family: Moraceae) previously known as *Bosqueia* is a monotypic genus represented by *Trilepisium madagascariense* DC, a deciduous tree of up to 30 m height, of the middle storey of the high-forest, extending on to the borders of savanna [1]. *T. madagascariense* is found in Cameroon, Democratic Republic of Congo, and Madagascar [2]. The leaves of *T. madagascariense* are used as vegetable; other parts of the plant are traditionally used as pain killers and to treat venereal diseases, arthritis, rheumatism, diarrhea, dysentery, stomach troubles, malnutrition, debility, and cutaneous and subcutaneous parasitic infections [1] and this plant was reported to possess antidiarrheal and bacteriostatic activities [3]. Our previous investigations on the leaves and stem barks of *T. madagascariense* have led to the isolation and characterization of a new flavan (trilepisflavan) and a new ester derivative of caffeic acid (trilepisuimic acid) together with 10 other compounds [4]. In continuation of our systematic studies of plants from the Moraceae family, the twigs and the roots of *T. madagascariense* have been investigated.

Results and Discussion

The air-dried and powdered leafless twigs (500 g) and the dried powdered roots (400 g) of *T. madagascariense* were extracted with MeOH/CH_2Cl_2 1:1 at room temperature for 48 h and MeOH for 8 h. The combined extracts (25 g from the twigs) and (15 g from the roots), were separately subjected to extensive column chromatography to yield a new flavene, trilepisflavene (**1**), a new depsidone, trilepisdepsidone (**2**), a new triterpene, daturadiol stearate (**3**), together with nine known compounds: 2-hydroxy-4-[(4-hydroxy-2-methoxy-6-methylbenzoyl)oxy]-6-methylbenzoic acid (**4**) [5], lichexanthone (**5**) [6], naringenin (**6**) [7], 3′,4′,5,7-tetrahydroxyflavanone (**7**) [8], 2′-hydroxybenzoic acid (**8**) [9], methyl 2,4-dihydroxy-6-methylbenzoate (**9**) [10], *β*-amyrin (**10**) [11], eurothridiol palmitate (**11**) [12], and *β*-sitosterol (**12**) [13]. The structures of the isolated compounds are presented in *Fig. 1*. This paper describes the isolation and structure elucidation of the new compounds, as well as the evaluation of the antimicrobial activity of some of the pure isolated compounds and the acetylated derivative of eurothridiol palmitate.

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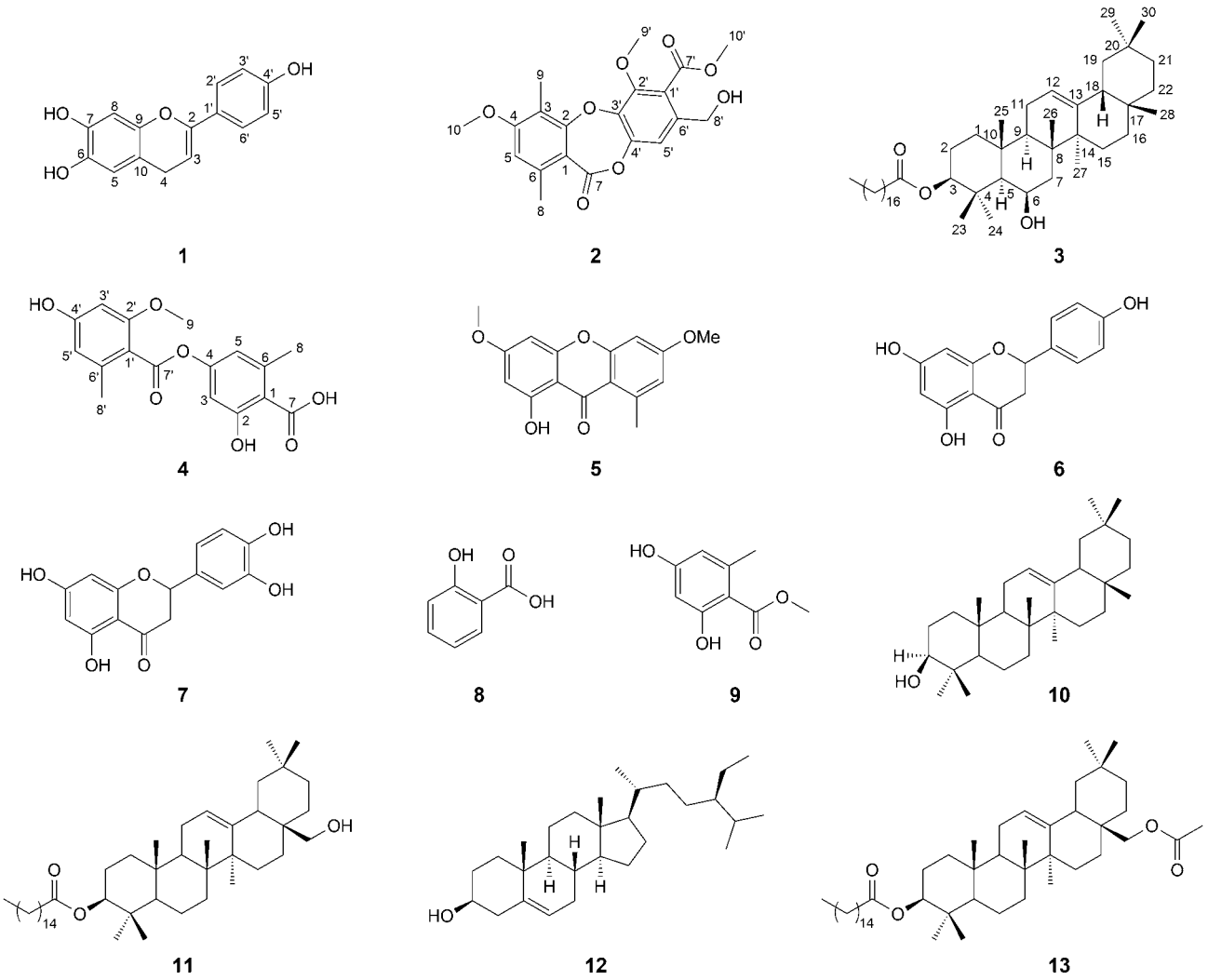

Fig. 1. Chemical structures of compounds **1** – **13**.

Trilepisflavene (**1**) was obtained as brown oil. Its molecular formula $C_{15}H_{12}O_4$ was deduced on the basis of HR-EI-MS, from the ion peak at *m/z* 256.0807 (calc. 256.0796). Its IR spectrum showed absorption bands at 3249 cm^{-1} (OH) and 1613 – 1513 cm^{-1} (aryl absorptions and overtones). The ^{1}H and ^{13}C-NMR data of **1** (*Table 1*) showed signals of one CH_2 group at (δ(H)/δ(C) 3.95 (br. *s*)/33.6) together with one 1,4-disubstituted (δ(H)/δ(C) 6.83 (*d*, 8.7)/115.3 and 7.15 (*d*, 8.7)/129.8) and one tetra-substituted (δ(H)/δ(C) 6.94 (*s*)/97.6 and 6.95 (*s*)/105.0) benzene rings suggesting, according to the coupling pattern and the chemical shifts, that **1** is a trisubstituted flavene [14]. Further analysis of the ^{13}C spectral data with two quaternary C-atom signals at δ(C) 141.9.4 and δ(C) 143.0 and HMBC between the H-atom signals at δ(H) 6.94 and 6.95 with these C-atoms was consistent with the fact that **1** is the 6-hydroxy derivative of 4′,7-dihydroxyflav-2-ene [14]. Therefore, **1** was identified as 4′,6,7-trihydroxyflav-2-ene, a new natural product to which the trivial name trilepisflavene is proposed.

Trilepisdepsidone (**2**) was obtained as a brown amorphous powder. The ESI-MS (-ve) showed $[M - H]^-$ ion peak at *m/z* 387.1075 corresponding to the molecular formula $C_{20}H_{20}O_8$, containing 11 double-bond equivalent. The ^{1}H-NMR spectrum displayed two *singlet*s at δ(H) 6.49 (*s*, 1 H) and 6.66 (*s*, 1 H) for aromatic H-atoms. It also showed three *singlet*s of three H-atoms each at δ(H) 3.69, 3.92, and 3.97 for three MeO and two other *singlet*s for three H-atoms each at δ(H) 2.03 and 2.39 for two aromatic Me groups. The ^{13}C-NMR spectrum revealed the presence of 20 C-atoms of which two were ester C=O groups (δ(C) 167.0 and 168.1), two Me groups (δ(C) 8.7 and 20.2), three MeO groups (δ(C) 52.2, 55.8, and 56.3), and one CH_2 signal (δ(C) 65.9, DEPT). From HSQC and HMBC data (*Table 1*), the presence of two aromatic Ph groups was concluded. HMBC data indicated that the first Ph group (ring *A*) contained two Me groups (δ(H) 2.03 and 2.39) in correlation with C-atoms at δ(C) 117.0 (C(3)), 159.4 (C(4)), 151.5 (C(2)) and C-atoms at δ(C) 119.1 (C(1)), 109.4 (C(5)), 135.7 (C(6)). This ring also

Table 1. ^{1}H- (300 MHz), ^{13}C-NMR (75 MHz), and HMBC spectral data of **1** (in acetone) and **2** (in CD_3OD)

Position	**1**			**2**		
	δ(H)	δ(C)	HMBC (H → C)	δ(H)	δ(C)	HMBC (H → C)
1	–	–	–	–	119.1	
2	–	149.3	–	–	151.5	
3	6.26 (*d*, *J* = 0.9)	102.6	C(2), C(10)	–	117.0	
4	3.95 (br. *s*)	33.6	C(9)	–	159.4	
5	6.95 (*s*)	105.0	C(6), C(7)	6.49 (*s*, 1 H)	109.4	C(1), C(3), C(8)
6	–	143.0	–	–	135.7	
7	–	141.9	–	–	167.6	
8	6.94 (*s*)	97.6	C(6), C(7)	2.39 (*s*, 3 H)	20.2	C(1), C(5), C(6)
9	–	157.1	–	2.03 (*s*, 3 H)	8.7	C(1), C(3), C(4)
10	–	120.8	–	3.92 (*s*, 3 H)	55.8	C(4)
1′	–	128.7	–	–	105.8	
2′	7.15 (*d*, *J* = 8.7)	129.8	C(4′), C(9)	–	155.5	
3′	6.83 (*d*, *J* = 8.7)	115.3	–	–	136.5	
4′	–	156.0	–	–	152.9	
5′	6.83 (*d*, *J* = 8.7)	115.3	C(2′)	6.66 (*s*, 1 H)	100.2	C(3′), C(4′), C(1′)
6′	7.15 (*d*, *J* = 8.7)	129.8	C(4′), C(5′)	–	132.5	
7′	–	–	–	–	168.1	
8′	–	–	–	4.44 (*s*, 2 H)	65.9	
9′	–	–	–	3.97 (*s*, 3 H)	56.3	C(2′)
10′	–	–	–	3.69 (*s*, 3 H)	52.2	C(7′)

contained one MeO group, in correlation with C-atom at δ(C) 159.4 (C(4)). The second Ph group (ring *B*) was shown to bear only one single H-atom at δ(H) 6.66 (H–C(5′)) exhibiting long-range correlations with C-atoms at δ(C) 152.9 (C(4′)), 136.5 (C(3′)), 105.8 (C(1′)); one CH_2 group at δ(C) 132.5 (C(6′)) and one MeO group at δ(C) 155.5 (C(2′)). The presence of another C–O bond was deduced from the correlation observed for the H-atom at δ(H) 6.66 (H–C(5′)) with the quaternary C-atom at δ(C) 136.5 (C(3′)). This correlation together with a HMBC correlation between H–C(5′) and C(1′), and between H–C(10′) and C(7′) led to the complete deduction of the substitution pattern of ring *B*. Out of the all possible isomers with permutation of the substituents in ring *B*, only two types have been found in nature: the four angular depsidones with the skeleton of deoxystictic acid [15] were easily excluded on the basis of the HMBC pattern and chemical shifts. Our data for ring *B* were in agreement with excelsione isolated from *Phomopsis* sp. CAFT69 [16]. It should be mentioned that two different chemical shift assignments for C-atoms C(2′) and C(3′) have been published [1,17][18]. The chemical shift of C (3′) is in better agreement with our own measurements and predicted values. Our assignment (*Table 1*) and the linkage between the two units were proved by comparison with data published by *Talontsi et al*. [16]. This was also supported by comparison with previously reported NMR data [19 – 21]. Contrary to the downfield shift observed for the O_2-bonded carbons C(2), C(3′), and C(4′), an upfield shift was observed for C(1) (δ(C) 119.1), thus suggesting its connection to a C=O C-atom. Consequently, both Ph groups are connected by a seven-membered ring containing an ether linkage and an ester bridge [21], revealing compound **2** to be a new depsidone, to which the name trilepisdepsidone is proposed.

Compound **3**, obtained as a white amorphous powder gave a positive test with Liebermann–Burchard reagent characteristic of triterpenes. Its HR-ESI-MS showed the pseudomolecular ion $[M + Na]^+$ peak at *m*/*z* 731.6286 corresponding to the molecular formula $C_{48}H_{84}O_3$. The ^{13}C-NMR spectrum (*Table 2*) of compound **3** showed methine and quaternary C-sp^2 signals (δ(C) 121.6 and 145.2) characteristic of oleanene triterpenes [22]. Detailed analyses of this spectrum indicated that the triterpene moiety was esterified with a fatty acid. This observation was supported by the presence in this spectrum of peaks of an additional methyl (δ(C) 14.1) and of acyl groups (*ca.* δ(C) 179.9) whose C=O group was downshielded (Δδ *ca.* 3.2) relative to 3β-acetylamyrin. The C(2) of the triterpene moiety in this compound was shielded (Δδ 4), while the oxymethine C-atoms showed downshielding effect in comparison to C(3) of β-amyrin [22]. In addition, the C(3) signal appeared at higher frequency (δ(C) 81.4) than that observed for β-amyrin (δ(C) 78.9). The molecular ion recorded on the MS at *m*/*z* 708 was conclusive of the esterified nature of **3**. The ^{1}H-NMR spectrum of **3** exhibited one characteristic triplet at δ(H) 5.10 corresponding to an olefinic H-atom and characteristic of the Δ^{12} H-atom in the pentacyclic triterpenes [23], and two doublets of doublets at δ(H) 4.48 and δ(H) 3.92 revealing the presence of two oxymethine. The observation of the additional oxymethine C-atom peak at δ(H) 68.7 and comparison of the ^{1}H and ^{13}C-NMR data of this compound with the data described for methylsumaresinolate [23] allowed us to establish the β relative configuration for the OH group at C(6) in compound **3** [22]. The

Table 2. ^{1}H- (300 MHz) and ^{13}C-NMR (75 MHz) Spectral data (in $CDCl_3$) of **3**. δ in ppm, J in HZ.

Position	δ(H)	δ(C)
1		41.6
2		28.4
3	4.48 (*dd*, *J* = 1.8, 9.0)	81.4
4	–	37.8
5		55.2
6	3.92 (*dd*, *J* = 4.5, 11.6)	68.2
7	2.42 – 2.47 (*m*), 2.29 – 2.37 (*m*)	41.7
8	–	39.8
9		47.6
10	–	36.9
11		23.5
12	5.24 (*t*, *J* = 3.3)	121.6
13	–	145.2
14	–	39.8
15		27.0
16		22.8
17	–	36.6
18		38.2
19		46.8
20	–	31.1
21		32.6
22		32.0
Me(23)	0.83 (*s*)	28.1
Me(24)	0.83 (*s*)	16.8
Me(25)	1.06 (*s*)	15.6
Me(26)	1.06 (*s*)	16.8
Me(27)	1.11 (*s*)	26.0
Me(28)	0.78 (*s*)	26.1
Me(29)	0.89 (*s*)	33.3
Me(30)	0.89 (*s*)	23.6
1′	–	172.9
2′	2.29 – 2.37 (*m*)	34.7
3′		31.9
4′		22.7
5′ – 16′	1.18 (br. *s*)	29.6 – 29.7
17′		22.7
18′	0.88 (*t*, *J* = 7.1)	14.1

position of the OH group at C(6) was confirmed by the HR-EI-TOF-MS with the ion peak at *m*/*z* 218.2035 (100%, $C_{16}H_{26}^{+\cdot}$) arising from a retro *Diels–Alder* cleavage of ring *C* (*Fig. 2*). The mass spectrum recorded for the transesterification product of **3** made it possible to recognize stearic acid esterified with –OH at the C(3) position of the triterpene through the molecular ion at *m*/*z* 298 (methyl stearate). This transesterification of **3** also confirmed the structure of the triterpene moiety as 3*β*,6*β*-dihydroxyolean-12-ene (*m*/*z* 442) known as daturadiol and firstly isolated from *Datura innoxa* by *Kocor et al.* in 1973 [24]. Consequently, compound **3** was identified as (6*β*)-hydroxyolean-12-en-3-yl stearate, a new esterified triterpenoid to which the name daturadiol stearate is proposed.

Compound **4** was obtained as a brown amorphous powder. The ESI-TOF-MS (+ve) showed $[M + Na]^+$ ion peak at *m*/*z* 355.0788 corresponding to the molecular formula $C_{17}H_{16}O_7$. The ^{1}H-NMR spectrum displayed four *doublet*s at δ(H) 6.59 (1 H, *d*, *J* = 2.1), 6.54 (1 H, *d*, *J* = 2.1), 6.38 (1 H, *d*, *J* = 2.1), and 6.33 (1 H, *d*, *J* = 2.1) accounting for aromatic H-atoms. It also showed one *singlet* at δ(H) 3.85 for MeO group and two *singlet*s at δ (H) 2.35 and 2.62 for two aromatic Me groups. The ^{13}C-NMR spectrum revealed ester C=O groups at δ(C) 164.8 and an acid carbonyl group at δ(C) 168.1. Based on ^{1}H-NMR, ^{13}C-NMR, HMBC, and mass spectrum, compound **4** was identified as 2-hydroxy-4-[(4-hydroxy-2-methoxy-6-methylbenzoyl)oxy]-6-methylbenzoic acid. This compound isolated for the first time from natural source belongs to the depside family and, was previously synthesized by *Elix et al.* [5].

Compounds **2** and **4** belong to the group of depside and depsidones, which are typically found in lichens [25] [26]. Only a few depsidones have been isolated from non-lichen sources, such as auranticins A and B, from the mangrove fungal *Preussia aurantiaca* [27], emeguising from the ascomycete *Emericella unguis* [20], depsidones garcinisidone B – F from two species of *Garcinia* plants [28], and Botryorhodines A – D from *Botryosphaeria rhodina*, an endophyte of the medicinal plant *Bidens pilosa* [29]. Some few years ago, depsidone from non-lichens and non-endophytic plant have been reported [30].

Part of erythrodiol palmitate (**11**) obtained in bulk amount was acetylated in order to study the effect of acetylation on antimicrobial activity. A new acetylated eurothridiol palmitate derivative (**13**) was obtained.

The results of the antimicrobial activity summarized in *Table 3* showed that compounds **5** and **9** were able to prevent the growth of all the eight studied microbial strains, including bacteria and fungi, within a *MIC* range of 64 – 512 μg/ml. Other compounds showed selective or no activity. Compound **11** was previously tested and was shown to display moderate antimicrobial activities against *Klebsiella pneumoniae* (*MIC* of 512 μg/ml), *Salmonella typhi* (*MIC* of 512 μg/ml), *Escherichia coli* AG100 (*MIC* of 512 μg/ml), *Staphylococcus aureus* (*MIC* of 256 μg/ml), and *E. coli ATCC8739* (*MIC* of 128 μg/ml) [4]. We are not aware of any previous report on the antimicrobial activities of compounds **2, 3, 4, 5,** and **13**. The activity recorded for active samples could be considered moderate to weak, compared to chloramphenicol or nystatin. It can be observed that terpenoids compounds mostly showed poor activity than phenolics. When regarding the structure–activity relationship, acetylation seems to decrease both the antibacterial and anticandidal activity as the results obtained for compounds **11** and **13** shows.

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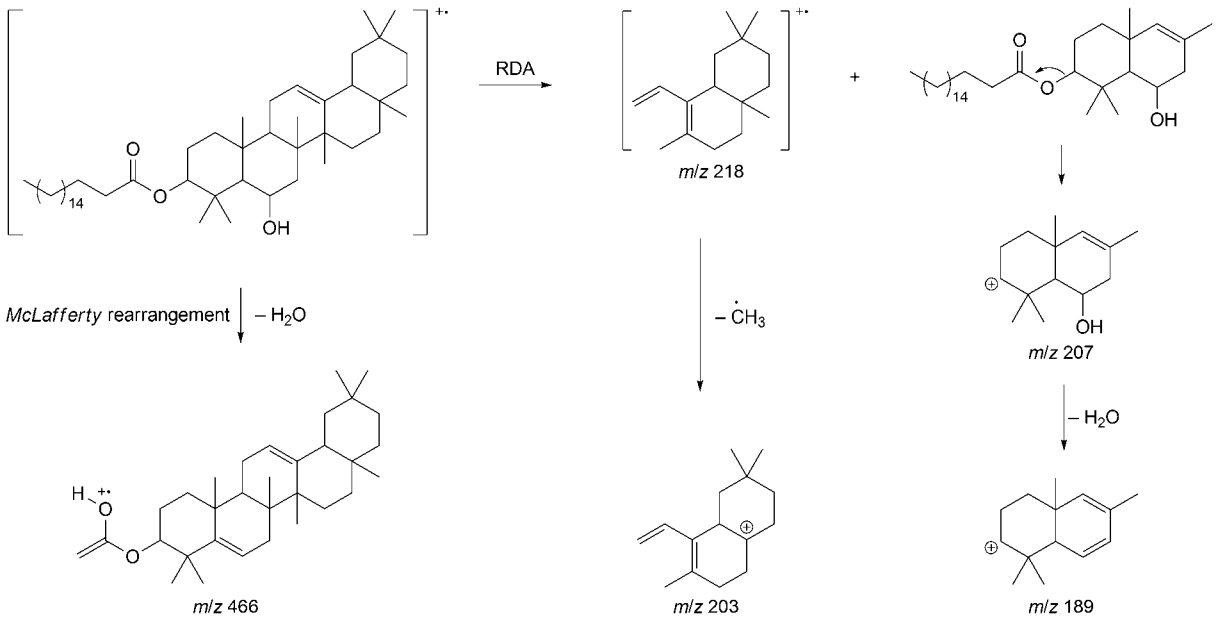

Fig. 2. Mass fragmentation of **3**.

Table 3. Antimicrobial activity of the tested compounds and reference antibiotics

Samples[a])	Microorganisms[b]), strains, and *MIC* [μg/ml]							
	P. stuartii	*P. aeruginosa*	*K. pneumoniae*	*S. aureus*	*S. typhi*	*E. coli*		*C. albicans*
	ATCC29916	PA01	ATCC11296	*ATCC25922*	*ATCC6539*	AG100	ATCC8739	ATCC 9002
4	512	–[c])	256	256	–	512	256	–
2	> 512	512	> 512	> 512	512	512	512	64
3	–	–	–	–	–	–	–	–
5	512	128	256	128	128	512	256	256
12	–	–	–	–	–	–	–	–
13	512	–	512	512	–	–	512	512
9	256	512	256	128	256	512	256	512
RA	32	64	4	4	4	4	4	16

[a]) Samples (RA or reference antibiotics: chloramphenicol for bacteria and nystatin for *C. albicans*). [b]) Microorganisms (*Providencia stuartii* (*P. stuartii*); *Pseudomonas aeruginosa* (*P. aeruginosa*); *Klebsiella pneumoniae* (*K. pneumoniae*); *Staphylococcus aureus* (*S. aureus*); *Salmonella typhi* (*S. typhi*); *Escherichia coli* (*E. coli*); *Candida albicans* (*C. albicans*)). [c]) MIC > 512 μg/ml.

Supplementary Material

Supporting Information for this article is available on the WWW under http://dx.doi.org/10.1002/hlca.201600073.

Experimental Part

General

TLC: Precoated plates of silica gel *GF254*; spots were detected under UV light and spraying with anilsaldehyde/sulfuric acid in EtOH or H_2O/sulfuric acid. Column chromatography (CC): silica gel *60* (SiO_2, 0.04 – 0.063 mm; *Merck*, Darmstadt, Germany) and *Sephadex LH-20* (*Sigma*, Dorset, UK). UV Spectra: *Shimadzu UV-210 IPC* spectrophotometer (*Shimadzu Corp.*, Kyoto, Japan); λ_{max} (log ε) in nm. IR Spectra: *Shimadzu FTIR-8700* and *Shimadzu 8900* infrared spectrometer with KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker Avance* 300 (*Bruker Daltonics Inc.*, Billerica, MA, USA) at 300 MHz (^1H) and 75 MHz (^{13}C) and *Bruker Avance* 600 at 600 MHz (^1H) and 150 MHz (^{13}C), with the residual solvent peaks as internal references; δ in ppm, J in Hz. GC/MS: GC chromatograph *Varian* mod. *Saturn II* (*Varian Inc.*, Walnut Creek, CA, USA) coupled to an ion trap mass detector, employing a *DB1-MS* column (30 m × 0.32 mm × 1 μm); in *m/z*. HR-ESI-MS: *micrOTOF* mass spectrometer (*Bruker Daltonik GmbH*, Bremen, Germany); in *m/z*. EI-TOF-MS (pos.): *Finnigan SSQ-7000* spectrometer (*Finnigan Corp.*, San Jose, CA, USA) by direct inlet (70 eV); in *m/z* (rel. %).

Plant Material

The twigs and the roots of *T. madagascariens*e DC were collected at Nkolnkoumou, Yaoundé, Center region of Cameroon in April 2012, and was identified by Mr. *Victor Nana* of the National herbarium, Yaoundé, Cameroon, where a voucher specimen (number 1778/SRFK) is deposited.

Extraction and Isolation

The air-dried and powdered leafless twigs (500 g) of *T. madagascariense* were extracted with $MeOH/CH_2Cl_2$ 1:1 at r.t. for 48 h and MeOH for 8 h. The combined extracts (25 g) were subjected to CC over SiO_2 *60* (100 g) and eluted with petroleum ether (PE)/$CHCl_3$ and $CHCl_3$/MeOH of increasing polarity. Thirty fractions of 200 ml each were collected, concentrated, monitored by TLC and similar ones pooled to give a total of four fractions (*Frs. A – D*). *Fr. B* (6 g, PE/$CHCl_3$ 50:50 – 0:100), were resubjected to CC on SiO_2 (40 g) eluted with PE/$CHCl_3$ (1:0 – 3:7) and the obtained fractions were sometimes passed through *Sephadex LH-20* eluted with $CHCl_3$/MeOH 70:30 and AcOEt/MeOH 90:10 to give daturadiol stearate (**3**; 10 mg), trilepisdepsidone (**2**; 15 mg), lichexanthone (**5**; 25 mg), eurothridiol palmitate (**11**; 150 mg), *β*-sitosterol (**12**; 25 mg), and *β*-amyrin (**10**; 12 mg). *Fr. C* (5 g, $CHCl_3$/MeOH 95:5 to 85:15), was resubjected to CC on SiO_2 (40 g) eluted with $CHCl_3$/MeOH (100:0 to 80:20) and the obtained fractions passed through *Sephadex LH-20* eluted with $CHCl_3$/MeOH 70:30 to give 2-hydroxy-4-(4-hydroxy-2-methoxy-6-methylbenzoyloxy)-6-methylbenzoic acid (**4**; 15 mg), 2-hydroxybenzoic acid (**8**; 10 mg). *Fr. A* (7 g, PE/$CHCl_3$ 100:0 to 50:50) and *Fr. D* (5 g, $CHCl_3$/MeOH 80:20 – 70:30) were found to contain mostly hydrocarbons or tannins and were not investigated further.

The dried powdered roots (400 g) of *T. madagascariense* were extracted with $MeOH/CH_2Cl_2$ 1:1 at r.t. for 48 h and MeOH for 8 h. The combined extracts (15 g) were subjected to CC over SiO_2 *60* (80 g) and eluted with PE/$CHCl_3$ and $CHCl_3$/MeOH of increasing polarity. Twenty fractions of 150 ml each were collected, concentrated, monitored by TLC and similar ones pooled to give a total of five fractions (*Frs. A – E*). *Fr. C* (2.5 g, PE/$CHCl_3$ 40:60 – 0:100), was passed through *Sephadex LH-20* eluted with $CHCl_3$/MeOH 70:30 to give trilepisflavene (**1**; 65 mg). *Fr. E* (2 g, $CHCl_3$/MeOH 95:5 to 85:15), was passed through *SephadexLH-20* eluted with $CHCl_3$/MeOH 70:30 to give methyl 2,4-dihydroxy-6-methylbenzoate (**9**; 5 mg), 3′,4′,5,7-tetrahydroxyflavanone (**7**; 7 mg) and naringenin (**6**; 14 mg).

Acetylation of Eurothridiol Palmitate (**11**)

Eurothridiol palmitate (**11**) (60 mg) was placed in a round-bottomed flask equipped with a magnetic stirrer. Ac_2O (30 ml) and pyridine (30 ml) were added to the flask. The mixture was magnetically stirred at r.t. for 6 h. After completion of acetylation, toluene was added and the soln. was concentrated under vacuum. Evaporation of the solvent gave the corresponding acetate derivative (**13**) which is a new acetylated eurothridiol palmitate derivative.

Transesterification of Compound **3**

Compound **3** was refluxed in dry MeOH (20 ml) with sodium methoxide (20 mg) for 1 h. The reaction product was extracted with H_2O and CH_2Cl_2. The org. phase was separated, dried (Na_2SO_4), and evaporated. The Me ester of stearic acid was obtained (7.6 mg). Addition of HCl (1%) to the remaining aq. phase, followed by extraction with CH_2Cl_2 yielded the triterpene moieties 3*β*,6*β*-dihydroxyolean-12-ene. Transesterification products were characterized by GC analysis.

Trilepisflavene (= **4′,6,7-Trihydroxyflavene**; **2-(4-Hydroxyphenyl)-4*H*-1-benzopyran-6,7-diol**; **1**). Brown oil. UV (acetone): 329 (1.13), 326 (1.11). IR (KBr): 3447, 3249, 1613, 1513. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-EI-MS: 256.0807 (M^+, $C_{15}H_{12}O_4^+$; calc. 256.0736).

Trilepisdepsidone (= **Methyl 8-(Hydroxymethyl)-3,6-dimethoxy-1,4-dimethyl-11-oxo-11*H*-dibenzo[*b*,*e*][1,4]dioxepine-7-carboxylate**; **2**). Brown amorphous powder. UV (acetone): 217.8 (1412). IR (KBr): 3264.3 (H–O), 2922.0, 1740.1 (C=O), 1601.9, 1492 (C=C), 1326.5, 1151.6, 1065.3. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 387.1075 ($[M - H]^-$, $C_{20}H_{19}O_8^-$; calc. 387.1085).

Daturadiol Stearate (= **(3*β*,6*β*)-6-Hydroxyolean-12-en-3-yl Octadecanoate**; **3**). White amorphous powder. IR (KBr): 2922.9, 2853.1, 1715.7 (C=O), 1603.8, 1463 (C=C), 1045.5, 756.0. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-EI-TOF-MS (pos.): 466.3784 (68, $C_{32}H_{50}O_2^{+\cdot}$), 218.2035 (100, $C_{16}H_{26}^{+\cdot}$), 207.0329 (78, $C_{14}H_{23}O_2^+$), 203.1808 (56, $C_{15}H_{23}^+$), 189.1640 (40, $C_{14}H_{21}^+$). HR-ESI-MS: 731.6286 ($[M + Na]^+$, $C_{48}H_{84}NaO_3^+$; calc. 731.6313).

2-Hydroxy-4-[(4-hydroxy-2-methoxy-6-methylbenzoyl)oxy]-6-methylbenzoic Acid (**4**). Brown amorphous powder. UV (acetone): 209.7 (5248). IR (KBr): 3229.1 (H–O), 1714 (C=O), 1599.3, 1517.2, 1441.5 (C=C), 1239.2 (O–C=O). ^{1}H-NMR (300 MHz, CD_3OD): 6.33 (*d*, ^3J(H,H) = 1.2, H–C(3′)); 6.38 (*d*, ^3J(H,H) = 1.2, H–C(5′)); 6.54 (*d*, ^3J(H,H) = 2.1, H–C(4)); 6.59 (*d*, ^3J(H,H) = 1.2, H–C(6)); 2.62 (*s*, Me); 2.35(*s*, Me); 3.85 (*s*, MeO). ^{13}C-NMR (75 MHz, CD_3OD): 116.5 (*s*, C(1)); 161.6 (*s*, C(2)); 108.6 (*d*, C(3)); 155.3 (*s*, C(4)); 114.9 (*d*, C(5)); 140.1 (*s*, C(6)); 168.1 (*s*, C(7)OOH); 114.5 (*s*, C(1′)); 160.6 (*s*, C(2′)); 97.9 (*d*, C(3′)); 161.6 (*s*, C(4′)), 110.3 (*d*, C(5′)), 116.5 (*s*, C(6′)), 164.8 (*s*, C(7′)); 56.4 (*s*, MeO); 20.0 (*s*, Me); 23.6 (*s*, Me). HR-ESI-MS: 355.0788 ($[M + Na]^+$, $C_{17}H_{16}NaO_7^+$; calc. 355.0788). ESI-MS (-ve): 331.1 ($[M - H]^-$).

Antimicrobial Assay

Microbial Strains and Culture Media. The studied microorganisms included reference (ATCC) and multi-

drug-resistant strains of *Providencia stuartii* (ATCC299 16), *Pseudomonas aeruginosa* (PA01), *Klebsiella pneumoniae* (ATCC11296), *Escherichia coli* (ATCC 8739 and AG100), *Salmonella typhi* ATCC6539, and *Candida albicans* ATCC 9002 obtained from the American type Culture Collection. They were maintained on agar slant at 4 °C and subcultured on fresh appropriate agar plates 24 h prior to any antimicrobial test. Nutrient Agar and Sabouraud Glucose Agar were used for the activation of bacteria and fungi, resp. The *Mueller–Hinton* broth (MHB) was used for the determination of the minimal inhibitory concentrations (*MIC*) [31].

Chemicals for Antimicrobial Assay

Chloramphenicol and nystatin (*Sigma–Aldrich*, St. Quentin Fallavier, France) were used as reference antibiotics (RA), resp., against bacteria and *C. albicans*. *p*-Iodonitrotetrazolium chloride (INT; *Sigma–Aldrich*) was used as microbial growth indicator [32][33].

MIC *Determinations*

The *MIC* determinations on bacteria was conducted using rapid INT colorimetric assay according to described methods [32][33] with some modifications. Briefly, the test sample was first of all dissolved in 10% (*v*/*v*) DMSO/MHB to give a final concentration of 1024 μg/ml and serially diluted twofold to obtain concentration ranges. A quantity of 100 μl of each concentration was added in a well (96-well microplate) containing 95 μl of MHB and 5 μl of inoculum (standardized at 1.5×10^6 CFU/ml by adjusting the optical density to 0.1 at 600 nm SHIMADZU UV-120-01 spectrophotometer) [34]. The final concentration of DMSO in the well was less than 3% (preliminary analyses with 3% (*v*/*v*) DMSO do not alter the growth of the test organisms). The negative control well consisted of 195 μl of MHB and 5 μl of the standard inoculum [35]. The plates were covered with a sterile plate sealer, then agitated to mix the contents of the wells using a plate shaker, and incubated at 37 °C for 24 h. The assay was repeated three times in triplicate. The *MIC* of samples was detected following addition (40 μl) of 0.2 mg/ml INT and incubation at 37 °C for 30 min [32][33]. Viable microorganisms reduced the yellow dye to a pink color. *MIC* was defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of bacterial growth [35].

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