Phytochemistry 94 (2013) 184–191

Contents lists available at SciVerse ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Dichapetalins from *Dichapetalum* species and their cytotoxic properties

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ARTICLE INFO

Article history:
Received 29 November 2012
Received in revised form 27 March 2013
Available online 22 April 2013

Keywords:
Dichapetalum mombuttense
Dichapetalum leucosia
Dichapetalum ruhlandii
Dichapetalum zenkeri
Dichapetalum eickii
Dichapetalaceae
Dammarane
Cytotoxicity

ABSTRACT

Six dichapetalins named dichapetalins N–S were isolated from *Dichapetalum mombuttense*, *Dichapetalum zenkeri* and *Dichapetalum leucosia*. They were accompanied in the same plants by the known dichapetalins A, B, C, I, L and M. The structures of the compounds were elucidated by 1D and 2D NMR experiments and mass spectrometry. They all possessed the dammarane skeleton substituted at position C-3 by a C_6–C_2 unit forming a 2-phenylpyran moiety. All contained a lactone ring in the side chain except dichapetalins O, Q and R, in which this ring was replaced by a lactol. Dichapetalin Q and R were also the first dichapetalins bearing a tertiary methyl and a double bond instead of the cyclopropane of the dammaranes. All these compounds were assayed against cancer cell lines HCT116 and WM 266-4 and displayed cytotoxic and anti-proliferative activities in the 10^{-6} to 10^{-8} M range.

1. Introduction

The dichapetalins constitute a small family of secondary metabolites of mixed biosynthesis, composed of a dammarane part and a C_6–C_2 unit. Dichapetalin A was the first to be discovered and was isolated from the roots of *Dichapetalum madagascariense* (Achenbach et al., 1995; Osei-Safo et al., 2012). It presented a very potent cytotoxic effect against L1210 murine leukemia cells, with an EC90 below 1.7×10^{-10} M, but was four orders of magnitude less efficient towards KB carcinoma cells and murine bone marrow cells stimulated with GM-CSF (granulocyte-macrophage colony-stimulating factor). In 1996, Achenbach's group established the absolute configuration of dichapetalin A and identified seven new derivatives, dichapetalins B–H, presenting poor or no cytotoxic activities (Weckert et al., 1996; Addae-Mensah et al., 1996). In 2006, four new dichapetalin I–L were extracted from the stem bark of *Dichapetalum gelonioides*; these derivatives showed poor cytotoxic effects on a panel of nine (I, J) and five (K, L) cancer cell lines, with EC50s superior to 1.7×10^{-6} M, except on the SW626 human ovarian adenocarcinoma cell line where EC50s ranged from 3.3×10^{-7} to 8.3×10^{-7} M for dichapetalin I and J (Fang et al., 2006). Dichapetalin M was isolated in 2008 from *D. madagascariense* and its cytotoxicity, measured in a brine shrimp bioassay, was evaluated to an EC50 of 2.0×10^{-8} M (Osei-Safo et al., 2008). Finally, five new dichapetalin-derived triterpenoids, the acutissimatriterpenes, were isolated from *Phyllanthus acutissima*. These molecules were tested against a panel of six cancer cell lines and one of them (acutissimatriterpene E), presented a strong and selective effect on the P388 murine leukemia cell line (EC50 of 5.0×10^{-9} M) (Tuchinda et al., 2008).

Aiming at finding new cytotoxic natural products, we investigated a series of plants from the genus *Dichapetalum*, namely, *Dichapetalum mombuttense* Engl., *Dichapetalum leucosia* Engl., *Dichapetalum ruhlandii* Engl., *Dichapetalum zenkeri* Engl. and *Dichapetalum eickii* Ruhland. We focused on isolating dichapetalins, which give blue TLC spots upon vanillin/H_2SO_4 spray. Herein, we report the identification of six new compounds and their cytotoxic properties. Table 1 reports a summary of the isolation of the different dichapetalins in the genus *Dichapetalum*.

2. Results

2.1. Isolation and structural elucidation

All species but *D. leucosia*, originating from Madagascar, contained dichapetalin A (**1**), which was also the sole "blue" compound isolated from *D. ruhlandii*. It was identified by its spec-

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http://dx.doi.org/10.1016/j.phytochem.2013.03.023

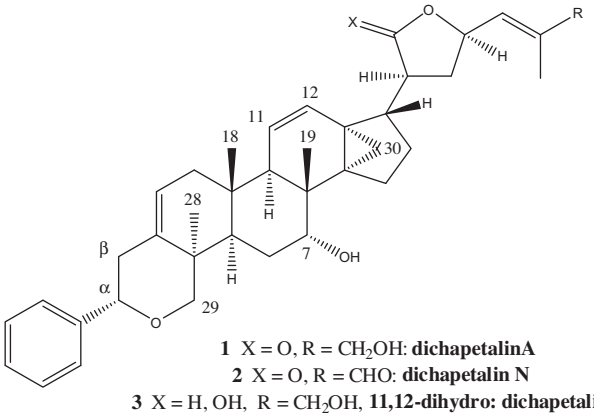
Table 1
Occurrence of the dichapetalins in *Dichapetalum* species.

Dichapetalin / *Dichapetalum*	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
madagascariense	√	√	√	√	√	√	√	√					√						
gelonoïdes	√								√	√	√	√							
ruhlandii	√																		
mombuttense	√											√		√					
leucosia			√						√							√			√
zenkiri	√	√										√			√	√	√	√	
eickii	√												√						

tral properties, which matched those published in the literature (Achenbach et al., 1995). Nevertheless, a severe discrepancy was observed in the rotation values between our samples ($[\alpha]^{20}_D$ −2.4 (*c* 0.9, $CHCl_3$)) and the original report ($[\alpha]^{20}_D$ 35 (*c* 1.5, $CHCl_3$)). To elucidate this point, a monocrystal suitable for X-ray diffraction was grown in absolute EtOH and the structure solved using direct methods.[1] It was found in all respects identical to the proposed one in the literature (Achenbach et al., 1995). The rotation value was measured three times on different instruments and compound **1** was always found levorotatory in contrast to what was previously described.

In addition to dichapetalin A, *D. eickii* from Kenya contained dichapetalin M, previously isolated from *D. madagascariensis* (Osei-Safo et al., 2008).

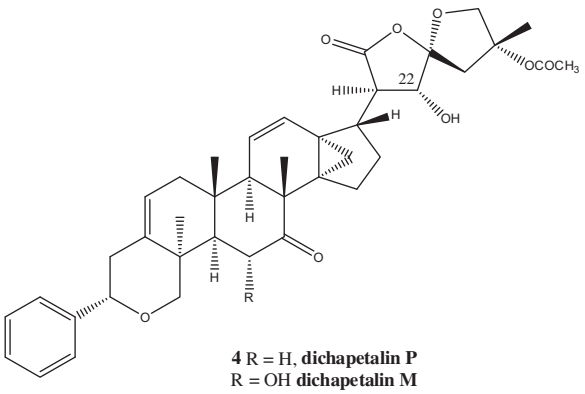
D. mombuttense collected in RDC (Democratic Republic of Congo) provided the known dichapetalins A and L (Fang et al., 2006), the abietic acid derivative pyracrenic acid (Otsuka et al., 1981) and a novel compound named dichapetalin N (**2**). The mass spectrum of **2** showed a molecular ion at *m*/*z* 582 corresponding to a $C_{38}H_{46}O_5$ formula. The differences in the ^1H and ^{13}C NMR spectra of compounds **1** and **2** were minor except for the signals belonging to the side chain. The presence of signals for an aldehyde and the absence of signals for CH_2OH-26 led us to propose structure **2**, in which the terminal primary alcohol is replaced by an aldehyde. A final structural proof was obtained by chemical transformation of **1** into **2** by MnO_2 oxidation.



1 X = O, R = CH_2OH: **dichapetalinA**
2 X = O, R = CHO: **dichapetalin N**
3 X = H, OH, R = CH_2OH, **11,12-dihydro: dichapetalin O**
7 X = H, OH, R = CH_2OH

D. zenkeri from Kenya yielded dichapetalin A and its 22-hydroxy derivative dichapetalin B as major compounds. They were accompanied by dichapetalin L and by four other compounds named here dichapetalins O, P, Q and R (**3**–**6**); compounds **3**, **5** and **6** appeared as unseparable mixtures of isomers. Dichapetalin O (**3**) displayed a molecular ion at *m*/*z* 611, which analyzed for $C_{38}H_{52}O_5Na$ (meas. 611.3717, calc. 611.3707), *i.e.*, 4 amu more than the reference compound **1**. The methyl count was identical in **1** and **3** but this latter compound was missing the C-11/C-12 double bond and the lactone carbonyl (no signal after 145 ppm in the ^{13}C NMR spectrum). A close examination of the ^1H and ^{13}C NMR spectra showed that most signals came as pairs of almost equal intensity, suggesting that **3** was a mixture. The observation of carbons at δ 102.1 and 98.2 was in favor of a hemiketal instead of the lactone. All attempts to separate the isomers were unsuccessful and led to recovery of the mixture. Signal overlap precluded configuration assignment using interproton couplings, but the high similarities in ^{13}C chemical shifts showed that **1** and **3** shared the same backbone configuration. Furthermore, when **1** was partially reduced to hemiketal **7**, also a mixture, the NMR spectra closely resembled those of **3**. The reduction was performed with a slight excess of Dibal and tetrol **9** was obtained as a by-product. Comparison of the NMR spectra of **3** and **7** indicated that the natural product **3** had same configuration of dichapetalin A.

Dichapetalin P (**4**) had a $C_{40}H_{48}O_8$ composition ($C_{40}H_{48}O_8Na$ meas. 679.3232, calc. 679.3241). Its NMR spectra strongly resembled those of dichapetalin M and particularly we observed signals for a ketone at δ 213.6 and for two isolated methylenes, as those present in the side chain of M. Since the composition of the two compounds differed only by an oxygen atom, it remained to determine which oxygen atom was missing in **4**. Comparison of the ^1H, ^{13}C and HSQC experiments showed that C-6 was now a methylene whose protons coupled with the C-7 ketone. Dichapetalin P was thus 6-deoxy dichapetalin M.



4 R = H, **dichapetalin P**
R = OH **dichapetalin M**

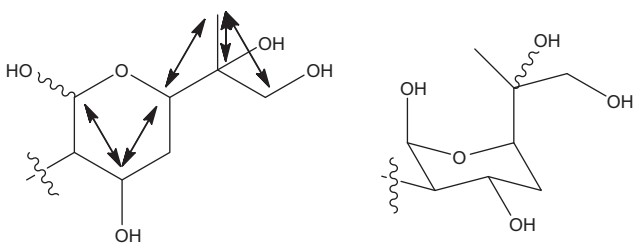
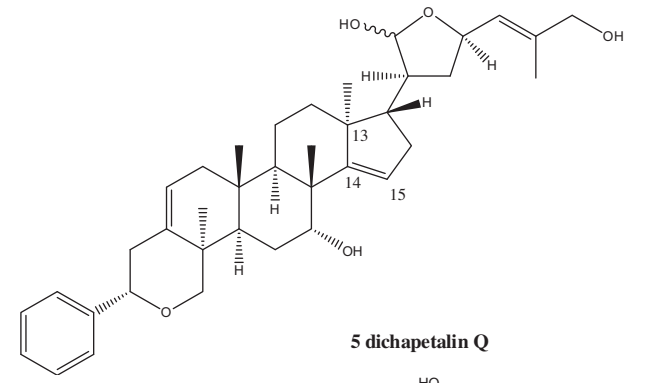
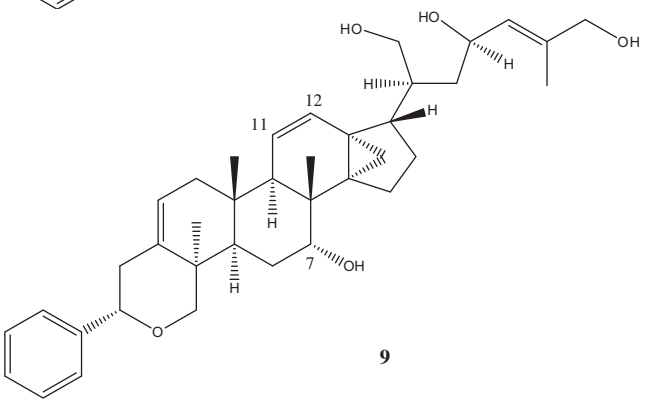


Fig. 1. HMBC correlations in the side chain of compound **6** (hydrogen atoms have been omitted for sake of clarity) and tentative stereochemical assignments.

[1] The crystal structure has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 910128.



5 dichapetalin Q



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Dichapetalin Q (**5**) was an isomer of dichapetalin O, with a $C_{38}H_{52}O_5$ analysis (meas. 611.3719, calc. 611.3707 for $C_{38}H_{52}O_5Na$). Its NMR spectra also displayed the typical signals for a 1:1 mixture of hemiketals presenting all the molecule backbone signals except those belonging to the C/D ring junction. Noteworthy was the absence of the protons of the methylene bridge of the cyclopropane, which appear at high field in all the dichapetalins, either directly observable in the ^1H spectrum or indirectly in the HSQC experiment in case of severe overlap. The missing methylene was replaced by a methyl on a quaternary carbon atom and a trisubstituted double bond was present between C-14 and C-15. In the dichapetalin series, cyclopropane ring opening was previously observed under acidic conditions (Tuchinda et al., 2008) and this was used to secure configurations in the side chain. Despite the fact that no acid was used during the extraction or purification stages, it cannot be totally ruled out that **5** derived from **3** and therefore had the same configurations, identical to the one determined for dichapetalins A and L (Fang et al., 2006).

Dichapetalin R (**6**) was the most oxygenated compound in the series with a $C_{38}H_{54}O_7$ composition ($C_{38}H_{54}O_7Na$ meas. 645.3753, calc. 645.3762). It was also a mixture of hemiketals in an approximate 80:20 ratio. The ^{13}C NMR spectrum showed that it had the same pentacyclic nucleus of compound **5** with a characteristic C-14 at δ 162. Signal assignment was done in a straightforward fashion up to C-21, which appeared at δ 97.3. A methyl (δ 22.1), two methylenes (δ 31.2 and 67.6), two methines (δ 76.1 and 77.8) and a quaternary carbon (δ 74.6) remained to be located. Fig. 1 reports some of the HMBC correlations, which allowed determination of the side chain. As far as relative configurations are

Table 2
^{13}C NMR of compounds **1–9** ($CDCl_3$, except for **7** and **9** measured in CD_3OD).

	1	2	3	3a	4	5	5a	6	7	7a	8	9
C												
1	40.0	40.1	40.2	40.3	40.1	39.5	39.2	39.5	41.4	41.4	40.0	41.4
2	117.8	117.7	118.3	118.3	117.8	118.4	118.4	118.4	119.5	119.5	117.8	119.5
3	140.0	140.2	139.7	139.7	139.9	139.7	139.7	139.8	141.3	141.3	140.0	141.3
4	38.3	38.3	38.5	38.6	39.2	38.4	38.4	38.4	39.7	39.7	38.3	39.6
5	43.7	43.8	43.9	43.9	53.4	44.6	44.6	44.7	45.2	45.2	43.7	45.2
6	24.1	24.2	24.0	24.0	35.9	23.4	23.4	23.4	26.3	26.4	24.1	26.3
7	72.3	72.3	74.2	74.1	213.6	72.0	72.0	72.0	73.5	73.6	72.3	73.5
8	36.4	36.3	38.7	38.8	48.4	44.3	44.3	44.3	39.2	39.2	36.2	37.5
9	45.7	45.8	42.4	42.6	52.5	40.3	40.3	40.3	47.1	47.1	45.7	47.1
10	36.2	36.4	36.8	36.8	36.4	37.2	37.2		37.5	37.5	36.4	37.5
11	124.1	124.4	16.8	16.9	120.6	16.3	16.3	16.4	123.3	123.7	124.0	123.8
12	128.9	128.7	25.8	25.8	131.5	32.8	33.2	33.2	132.6	133.4	128.9	132.8
13	30.0	30.1	28.5	28.9	31.5	47.2	46.9	46.8	32.7	33.5	30.0	31.5
14	35.1	35.4	37.3	37.3	36.2	162.4	162.1	162.1	37.7	37.8	35.1	37.6
15	24.9	25.0	26.1	26.2	27.1	119.4	119.9	119.9	25.8	26.1	24.9	26.1
16	22.7	23.0	26.5	27.6	23.7	34.9	35.3	35.2	28.2	29.9	22.8	26.6
17	40.9	41.1	44.9	48.5	40.6	58.0	52.9	53.0	46.0	43.0	40.8	45.3
18	17.4	17.5	19.7	19.8	17.2	27.3	27.3	27.6	18.6	18.6	17.4	18.4
19	18.1	18.2	16.8	16.9	17.9	16.2	16.2	16.4	18.8	18.8	18.1	18.8
20	42.1	41.7	50.2	51.6	47.4	48.5	44.6	45.2	52.0	52.8	41.2	41.9
21	178.3	177.2	98.1	102.0	174.4	102.4	97.4	97.4	99.1	103.3	177.7	65.8
22	31.3	30.5	37.6	34.8	72.1	39.2	35.4	77.8	36.1	38.3	35.0	37.6
23	75.1	74.4	75.4	74.0	111.5	75.3	73.9	31.2	75.3	76.8	114.2	68.3
24	122.0	147.7	127.7	124.8	45.9	127.8	124.6	76.2	126.2	129.3	51.3	129.6
25	141.7	140.7	138.2	139.5	85.3	139.8	138.4	74.6	140.1	138.5	78.2	138.4
26	67.2	193.7	68.0	70.2	78.7	68.0	68.0	67.6	68.1	68.3	80.2	68.4
27	14.2	9.8	14.0	14.3	22.2	14.0	14.3	22.1	13.9	14.2	24.7	14.4
28	23.8	23.8	24.1	24.1	23.9	24.1	24.1	24.0	24.4	24.4	23.8	24.4
29	72.5	72.5	72.8	72.8	72.2	72.9	72.9	72.9	73.8	73.8	72.5	73.8
30	14.9	15.1	13.9	14.1	14.5	19.7	20.2	20.2	16.7	17.3	14.9	17.9
α	81.8	81.9	82.0	82.0	82.1	82.0	82.0	82.0	83.5	83.5	81.8	83.5
β	40.7	40.7	40.9	40.9	40.6	40.9	40.9	40.9	42.0	42.0	40.7	42.0
1′	142.6	142.6	142.8	142.8	142.4	142.8	142.8	142.8	144.1	144.1	142.6	144.1
2′,6′	125.8	125.8	126.0	126.0	126.0	126.0	126.0	126.0	127.1	127.1	125.8	127.1
3′,5′	128.4	128.4	128.6	128.6	128.5	128.6	128.6	128.6	129.5	129.5	128.4	129.5
4′	127.5	127.5	127.7	127.7	127.9	127.7	127.7	127.7	128.8	128.8	127.5	128.7
$OCOCH_3$					170.7							
$OCOCH_3$					22.0							

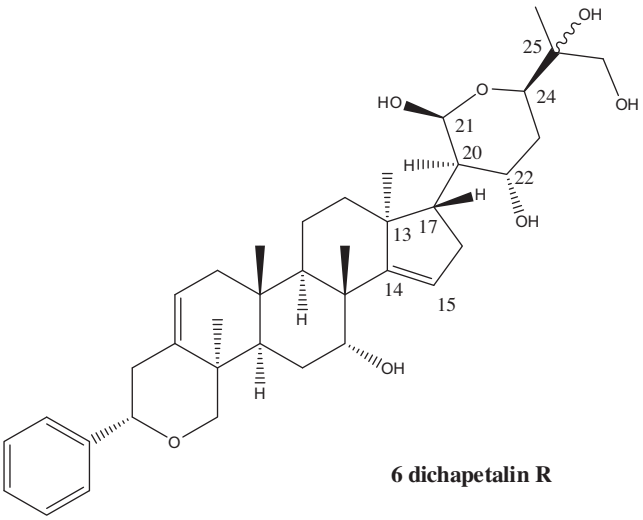
Table 3
Dichapetalin A chemosensitivity on 16 cancer cell lines.

Cell line	Tumor types	Dichapetalin A EC50 (M)
HCT-116	Human colorectal carcinoma	2.5×10^{-7}
NAMALWA	Human Burkitt's lymphoma	3.0×10^{-7}
SKOV-3	Human ovarian adenocarcinoma	8.6×10^{-7}
HOP-62	Human lung cancer	9.1×10^{-7}
A549	Human lung adenocarcinoma	1.2×10^{-6}
NCI-H460	Human prostate carcinoma	1.4×10^{-6}
T47D	Human breast ductal carcinoma	2.3×10^{-6}
BxPC3	Human pancreatic adenocarcinoma	3.0×10^{-6}
KM-12	Human colon carcinoma	8.7×10^{-6}
OVcAR-3	Human ovarian adenocarcinoma	1.0×10^{-5}
HL-60	Human acute promyelocytic leukemia	1.1×10^{-5}
Colo-205	Human colorectal adenocarcinoma	1.2×10^{-5}
TK10	Human renal adenocarcinoma	1.2×10^{-5}
MDAMB-231	Human breast adenocarcinoma	1.3×10^{-5}
DU145	Human prostate carcinoma	1.3×10^{-5}
WM 266-4	Human melanoma	1.7×10^{-5}

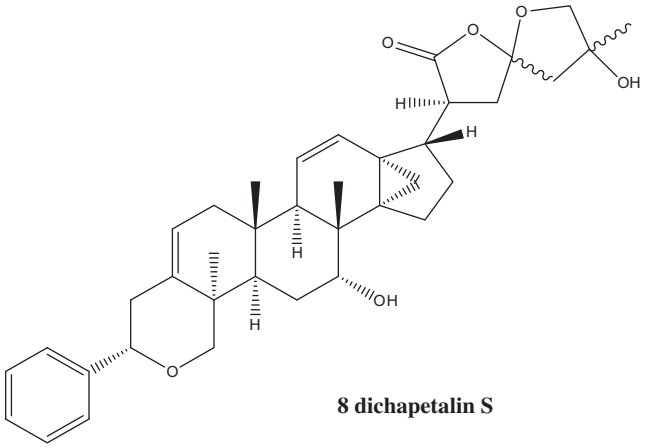
Table 4
Dichapetalins chemosensitivity on HCT116 and WM 266-4 cell lines reported as the concentration at which 50% of cell proliferation is inhibited.

Dichapetalin derivatives	HCT116 EC50 (M)	WM 266-4 EC50 (M)
A (**1**)	2.5×10^{-7}	1.7×10^{-5}
B	8.1×10^{-8}	3.4×10^{-7}
C	5.0×10^{-7}	2.5×10^{-6}
I	2.8×10^{-7}	1.0×10^{-5}
L	6.8×10^{-7}	3.1×10^{-6}
M	9.9×10^{-9}	7.8×10^{-8}
N (**2**)	9.2×10^{-8}	1.5×10^{-6}
O (**3**)	8.9×10^{-7}	8.4×10^{-6}
P (**4**)	5.8×10^{-8}	2.3×10^{-7}
Q (**5**)	2.5×10^{-6}	2.7×10^{-5}
R (**6**)	4.2×10^{-6}	3.1×10^{-5}
S (**8**)	4.5×10^{-7}	1.3×10^{-6}
7	4.7×10^{-7}	7.1×10^{-6}
9	3.0×10^{-6}	1.8×10^{-5}
Doxorubicin	3.4×10^{-8}	4.1×10^{-8}
Camptothecin	3.5×10^{-9}	4.9×10^{-9}

concerned, interproton coupling constants measurement (or linewidth) indicated that H-21 and -24 were equatorial and H-22 axial. Even if it was not possible to assign with certainty the configuration of C-25, we assumed it to be 25R as in all dichapetalins studied so far.



6 dichapetalin R



8 dichapetalin S

D. leucosia was a source of dichapetalins C and I, previously isolated from *D. madagascariensis* and *D. genoloides*, of the new dichapetalin Q (**5**) and of a new one named here dichapetalin S (**8**). This compound had a $C_{38}H_{46}O_6$ composition according to HRMS ($C_{38}H_{46}O_6Na$ meas. 679.3232, calc. 679.3241). The hexacyclic part of the molecule was very similar to the one of dichapetalin A and the ^1H NMR spectrum showed the typical signals of the pyran ring, of the two double bonds, of the cyclopropane and of hydroxylation at C-7. The originality therefore was in the side chain, which showed ^{13}C signals for a lactone and for a quaternary carbon atom at δ 114.2. This latter signal was reminiscent of C-23 in the spiroketal moiety of compound **4** and of dichapetalin M (Osei-Safo et al., 2008). It differed however by the absence of an alcohol on C-22, and the tertiary acetate on C-25 was replaced by an alcohol. Paucity of material did not allow full structural elucidation in the side chain.

2.2. Biological activities of isolated compounds

Before evaluating the biological activities of the new dichapetalin derivatives, we profiled dichapetalin A cytotoxicity on a set of 16 cancer cell lines (Table 3). HCT116 (human colorectal carcinoma) was found to be the most sensitive to dichapetalin A (EC50 = 2.5×10^{-7} M) and WM 266-4 (human melanoma) the most resistant (EC50 = 1.7×10^{-5} M): HCT116 chemosensitivity being 68-fold higher than the WM 266-4 one. Because of this significant difference, we used the HCT116 and WM 266-4 cell line couple to analyze the cytotoxic and anti-proliferative properties of dichapetalins B, C, I, L, M, O, P, Q, R and S as well as of two hemisynthetic derivatives (Table 4). All new derivatives were found active on HCT116 cancer cells and new dichapetalin P (EC50 = 4.8×10^{-8} M) was 4-fold more active than dichapetalin A (EC50 = 2.5×10^{-7} M). Dichapetalin P was also more potent than dichapetalin A on WM 266-4 cells (EC50 = 2.3×10^{-7} M). Interestingly, derivative P contains the intact lactone ring, a constraint moiety in the side chain and a carbonyl group in position 7. Its 6α-hydroxy derivative, dichapetalin M, is the most potent cytotoxic agent measuring an EC50 of 9.9×10^{-9} and 7.8×10^{-8} M in HCT116 and WM 266-4 cell lines, respectively. The lactone is clearly important for activity, as seen for compounds R, Q, **9**, O and L, as well as the tricyclic motif (R and Q being the least active on both cell lines).

3. Conclusions

Six dichapetalins are here described for the first time (named N, O, P, Q, R and S): three of them possess a partially reduced form of

the side chain lactone and two the genuine skeleton of the dammaranes without the cyclopropane ring.

In the literature, the biological activity profile of the dichapetalin derivatives is mainly based on cytotoxicity measurements in various cancer cell lines or brine shrimps assays. Here, we first profiled dichapetalin A on 16 cancer cell lines finding EC50 values in agreement with previous reports. Achenbach et al. found an EC90 below 1.7×10^{-10} M on L1210 murine leukemia cells, whereas KB carcinoma cells and murine bone marrow cells stimulated with GM-CSF were found four orders of magnitude less sensitive (Achenbach et al., 1995). We found an EC50 of 3×10^{-7} M in human Burkitt's lymphoma (Namalwa) and of 1.1×10^{-5} M in human acute promyelocytic leukemia HL60 cells. Similar micromolar cytotoxicity levels were found for dichapetalin A in KB, KB-V+ and KB-V− cell lines (Weckert et al., 1996; Addae-Mensah et al., 1996) and in seven other cancer cell lines, excepted in the SW626 human ovarian adenocarcinoma cell line 10 times more sensitive with an EC50 of 3.4×10^{-7} M (Fang et al., 2006). In agreement, human ovarian adenocarcinoma SKOV-3 cells in our study gave an EC50 of 8.6×10^{-7} M for dichapetalin A, while human ovarian adenocarcinoma OVcAR-3 was 12-fold less sensitive (EC50 1.1×10^{-5} M). We measured a subnanomolar cytotoxicity in human colorectal carcinoma HCT116 cells (EC50 2.5×10^{-7} M), while the human melanoma WM 266-4 cells was 68-fold more resistant. Therefore, we used the HCT116 and WM 266-4 cell line couple to evaluate the cytotoxic and anti-proliferative activities of the other dichapetalin derivatives. New derivative dichapetalin P, constraint on the side chain, resulted 4-fold more active than dichapetalin A on HCT116 and 74-fold more active on WM 266-4. Its hydroxy derivative, dichapetalin M, was the most potent cytotoxic compound. Dichapetalin B, initially isolated by Addae-Mensah et al. but never described in terms of biological activity, was found nearly as active as dichapetalin P on the two cell lines. Newly described compound N was also very potent, while new dichapetalin derivatives O, Q and R, lacking the lactone, showed a decreased toxicity on the two cell lines. The hemisynthetic compounds **7** and **9** were tested o and **7** was found 10-fold more active than **9** on both cell lines, showing how limited structural modifications can lead to an important difference in biological activity.

Finally, our study constitutes the more exhaustive analysis of dichapetalin molecules based on both cytotoxic activity and molecular diversity. Dichapetalins M and P remained the most potent representatives of this class of dammarane-type triterpenoids. The structure activity relationships determined here inspires the road to simplify the compounds and keeping the pharmacological activity.

4. Experimental section

4.1. General experimental procedures

Optical rotations were determined in $CHCl_3$ solution on a Perkin-Elmer 341 automatic polarimeter. UV spectra were obtained in MeOH using a UV MC^2 Safas spectrophotometer. A FT-IR Bruker Tensor 27 spectrophotometer was used for scanning IR spectroscopy. The NMR spectra were recorded on a Bruker Avance II spectrometer equipped with a ^{13}C cryoprobe at 500 MHz for ^{1}H and 125 MHz for ^{13}C; 2D experiments were performed using standard Bruker programs. The ESIMS and MS/MS were performed using a Bruker Esquire-LC ion trap mass spectrometer; the samples were introduced by infusion in a solution of MeOH. HRESIMS were obtained on a Bruker MicrOTOF. TLC was carried out on precoated silica gel $60F_{254}$ (Merck) with CH_2Cl_2–MeOH (95:5) and spots were visualized by heating after spraying with 3% H_2SO_4 + 1% vanillin. Column chromatography (CC) was carried out on prepacked cartridge Kieselgel (40–60 μm) with CH_2Cl_2–MeOH. Analytical HPLC was performed on a Merck–Hitachi apparatus equipped with an L-7200 automated sample injector, a L-7100 pump, a L-7450 diode array detector, a D-7000 interface and EZChrom software. A prepacked C_{18} reversed-phase column (Lichrospher 100 RP-18, 4 × 125 mm, 5 μm) was used for analytical HPLC with a binary gradient elution (solvent A: H_2O and solvent B: MeCN) and a flow rate of 1 mL min^{-1}. Semipreparative HPLC was performed on an apparatus equipped with a VWR International LaPrep pump P110, a VWR LaPrep P314 Dual λ absorbance detector and EZChrom software. A prepacked C_{18} reversed-phase column (Hibar-Lichrospher 100 RP-18, 25 × 250 mm, 5 μm) was used for semipreparative HPLC with a binary gradient elution (solvent A: H_2O and solvent B: MeCN), a flow rate of 30 mL min^{-1} and the chromatogram was monitored at 210 and 320 nm.

4.2. Plant material

D. ruhlandii and *D. zenkeri* were collected near Kaya Muhaka (Kenya), *D. eickeii* was collected in the Ngaongao Forest (all in the Coast Province, Kenya) by one of us (P. B. C. M.) in March and April, 2010 for the first two species and in May, 2003 for the third one. They were authentified by comparison with authentic samples. Specimens have been deposited at the University of Nairobi Herbarium under respective references 2010/059, 2010/101 and 2003/0152. *D. mombuttense* was collected near Isiro (RDC) in November 1987 by René Bellé (Pierre Fabre Research Institute). It was identified by R. Bellé and an herbarium specimen was deposited under reference RBL-068 in the Pierre Fabre Botanical Conservatory in Cambounet-sur-le-Sor (France). *D. leucosia* was collected near Ampasy Naompoana (Madagascar) by Raymond Gérold in November 2001; an herbarium specimen was deposited under reference SEAR1122 in the Pierre Fabre Botanical Conservatory.

4.3. Extraction and isolation of compounds

The dried roots of *D. mombuttense* (100 g) were powdered and extracted with EtOAc at room temperature for 24 h. After filtration, the organic solvent was concentrated under reduced pressure. The crude extract (1.55 g) was further subjected to silica gel CC (70 g, 40 × 100 mm) using a CH_2Cl_2–MeOH gradient [1:0 to 8.5:1.5; 15 mL each], to give 60 fractions. All the fractions were analyzed by TLC on silica gel using the solvent mixture CH_2Cl_2–MeOH (95:5) and pooled according to TLC into seven fractions (frs.1–7). The spots of fraction 2 on the TLC plate were blue, as detected by heating after spraying with 3% H_2SO_4 + 1% vanillin. Fraction 2 (749 mg) was purified by repeated semi-preparative HPLC RP-18 chromatography, eluting with a linear gradient (70–100% B) to give **1** (146.7 mg) and an impure fraction (17.0 mg). This fraction was further purified by extensive preparative TLC on silica gel using the solvent mixture CH_2Cl_2–MeOH (98:2) to afford **1** (0.2 mg), dichapetalin L (3.3 mg) and **2** (1.7 mg).

The dried roots of *D. leucosia* (100 g) were powdered and extracted with EtOAc at room temperature for 24 h. After filtration, the organic solvent was concentrated under reduced pressure. The crude extract (2.22 g) was further subjected to silica gel CC (70 g, 40 × 100 mm) using a CH_2Cl_2–MeOH gradient [1:0 to 8.5:1.5; 15 mL each], to give 60 fractions. All the fractions were analyzed by TLC on silica gel using the solvent mixture CH_2Cl_2–MeOH (95:5) and pooled according to TLC into height fractions (frs.1–8). The spots of fraction 4 on the TLC plate were blue, as detected by heating after spraying with 3% H_2SO_4 + 1% vanillin. Fraction 4 (646 mg) was purified by repeated semi-preparative HPLC RP-18 chromatography, eluting with a linear gradient (50–80% B) to give dichapetalin I (6.4 mg), dichapetalin C (8.0 mg), dichapetalin S **8** (12.0 mg) and an impure fraction (10.4 mg). This fraction was further purified by preparative TLC on silica gel using the sol-

vent mixture CH_2Cl_2–MeOH (98:2) to afford dichapetalin Q **5** (2.4 mg).

The dried roots of *D. ruhlandii* (200 g) were powdered and extracted with EtOAc at room temperature for 24 h. After filtration, the organic solvent was concentrated under reduced pressure. The crude extract (2.14 g) was further subjected to silica gel CC (70 g, 40 × 100 mm) using a CH_2Cl_2–MeOH gradient [1:0 to 8.5:1.5; 15 ml each], to give 60 fractions. All the fractions were analyzed by TLC on silica gel using the solvent mixture CH_2Cl_2–MeOH (95:5) and pooled according to TLC into seven fractions (frs.1–6). The spots of fraction 3 (1.04 g) on the TLC plate were blue, as detected by heating after spraying with 3% H_2SO_4 + 1% vanillin. Fraction 3 (100 mg) was purified by semi-preparative HPLC RP-18 chromatography, eluting with a linear gradient (60–100% B) to give dichapetalin A (2.4 mg).

The dried roots of *D. eickii* (110 g) were powdered and extracted with EtOAc at room temperature for 24 h. After filtration, the organic solvent was concentrated under reduced pressure. The crude extract (360 mg) was further subjected to silica gel CC (30 g, 30 × 100 mm) using a CH_2Cl_2–MeOH gradient [1:0 to 8.5:1.5; 15 mL each], to give 30 fractions. All the fractions were analyzed by TLC on silica gel using the solvent mixture CH_2Cl_2–MeOH (95:5) and pooled according to TLC into seven fractions (frs.1–8). The spots of fraction 4 (138 mg) on the TLC plate were blue, as detected by heating after spraying with 3% H_2SO_4 + 1% vanillin. Fraction 4 was purified by semi-preparative HPLC RP-18 chromatography, eluting with a linear gradient (20–100% B) and by repeated preparative TLC on silica gel using the solvent mixture CH_2Cl_2–MeOH (98:2) to afford dichapetalin A (0.8 mg) and dichapetalin M (0.2 mg).

The dried roots of *D. zenkeri* (1 kg) were powdered and extracted with EtOAc at room temperature for 24 h. After filtration, the organic solvent was concentrated under reduced pressure. The crude extract (6.90 g) was further subjected to silica gel CC (190 g, 40 × 250 mm) using a CH_2Cl_2–MeOH gradient [1:0 to 8.5:1.5; 15 mL each], to give 400 fractions. All the fractions were analyzed by TLC on silica gel using the solvent mixture CH_2Cl_2–MeOH (95:5) and pooled according to TLC into seven fractions (frs.1–7). The spots of fraction 4 (3200 mg) and 5 (755 mg) on the TLC plate were blue, as detected by heating after spraying with 3% H_2SO_4 + 1% vanillin. Fraction 4 (400 mg) was purified by semi-preparative HPLC RP-18 chromatography, eluting with a linear gradient (60–100% B) and by repeated preparative TLC on silica gel using the solvent mixture CH_2Cl_2–MeOH (97:3) to give **1** (14.8 mg), dichapetalin B (1.4 mg), dichapetalin L (0.7 mg) and **4** (2.8 mg). Fraction 5 (300 mg) was purified by semi-preparative HPLC RP-18 chromatography, eluting with a linear gradient (60–100% B) and by repeated preparative TLC on silica gel using the solvent mixture CH_2Cl_2–MeOH (97:3) to give dichapetalin B (4.8 mg), **3** (1.7 mg), **5** (12.3 mg) and **6** (2.6 mg).

4.4. Dichapetalin A (*1*) X-ray analysis

Data were collected at low temperature (180 K) on an Agilent Gemini diffractometer using a graphite-monochromated Cu-Kα radiation (λ = 1.54184 Å) and equipped with an Oxford Instrument Cooler Device. The final unit cell parameters have been obtained by means of a least-squares refinement. The structure has been solved by direct methods using SIR92 (Altomare et al., 1993) and refined by means of least-squares procedure on F^2 with the aid of the program SHELXL97 (Sheldrick, 2008) included in the software package WinGX (Farrugia, 1999). The atomic scattering factors were taken from the International Tables for X-ray Crystallography[2]. All hydrogens atoms were geometrically placed and refined by using a riding model, excepted for the two hydrogens of the water molecule, which were located by Fourier differences. Although the studied Dichapetalin is an organic compound, it was possible to determine its absolute configuration after recording the data under Cu-Kα instead of Mo-Kα radiation. All non-hydrogens atoms were anisotropically refined, and in the last cycles of refinement, a weighting scheme was used, where weights were calculated from the following formula: $w = 1/[\sigma^2(Fo^2)+(aP)^2 + bP]$ where $P = (Fo^2 + 2Fc^2)/3$. Drawing of the molecule was performed with the program ORTEP32 (Farrugia, 1997) with 30% probability displacement ellipsoids for non-hydrogen atoms.

[2] International Tables for X-ray Crystallography, 1974, Vol IV, Kynoch Press, Birmingham, United Kingdom.

4.5. Dichapetalin N (*2*)

Yellow gum, $[\alpha]^{20}_D$ +12 (*c* 0.33, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 226 (4.0) nm; IR (film) ν_{max} 1770, 1692 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.48 (1H, *s*, H-26), 7.39–7.32 (4H, *m*, H-2′, -3′, -5′, -6′), 6.46 (1H, *dq*, *J* = 7, 1 Hz, H-24), 6.16 (1H, *dd*, *J* = 10, 3 Hz, H-12), 5.49 (1H, *dd*, *J* = 10, 2.5 Hz, H-11), 5.41 (1H, *br d*, *J* = 7 Hz, H-2), 5.31 (1H, *dt*, *J* = 11, 6 Hz, H-23), 4.27 (1H, *dd*, *J* = 11.5, 2.5 Hz, H-α), 3.95 (1H, *br t*, *J* = 2.3 Hz, H-7), 3.77 (1H, *d*, *J* = 10.5 Hz, H-29), 3.61 (1H, *d*, *J* = 10.5 Hz, H-29), 3.15 (1H, *ddd*, *J* = 12.5, 8.5, 5 Hz, H-20), 2.65 (2H, *m*, H-β/H-17), 2.54 (1H, *ddd*, *J* = 14, 8, 6 Hz, H-22), 2.21 (1H, *dd*, *J* = 13, 2.5 Hz, H-β), 1.84 (3H, *br d*, *J* = 1 Hz, CH_3-27), 1.33 (3H, *s*, CH_3-28), 1.22 (1H, *d*, *J* = 5 Hz, H-30), 1.09 (3H, *s*, CH_3-19), 0.93 (3H, *s*, CH_3-18), 0.78 (1H, *d*, *J* = 5 Hz, H-30); ^{13}C NMR, see Table 2; HRESIMS *m/z* 608.3228 (calcd for $C_{38}H_{46}O_5$Na: 608.3237).

4.6. Dichapetalin O (*3*)

Yellow gum, $[\alpha]^{20}_D$ +18 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (4.0) nm; IR (film) ν_{max} 3375, 1563 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.29 (2 × 4H, *m*, H-2′, -3′, -5′, -6′), 5.50 (1H, *d*, *J* = 8 Hz, H-24), 5.42 (1H, *d*, *J* = 8 Hz, H-24), 5.37–5.33 (4H, *m*, 2 × H-21, 2 × H-12), 4.86 (1H, *ddd*, *J* = 13, 9, 5 Hz, H-23), 4.73 (1H, *dt*, *J* = 6.5, 9.5 Hz, H-23), 4.24 (2 × 1H, *dd*, *J* = 11.5, 2.5 Hz, H-α), 4.0 (2 × 2H, *d*, *J* = 2 Hz, H-26), 3.79 (2 × 1H, *m*, H-7), 3.73 (2 × 1H, *d*, *J* = 10.8 Hz, H-29), 3.56 (2 × 1H, *d*, *J* = 10.8 Hz, H-29), 2.60 (2 × 1H, *t*, *J* = 12.5 Hz, H-β), 1.71 (3H, *br s*, CH_3-27), 1.69 (3H, *br s*, CH_3-27), 1.30 (3H, *s*, CH_3-28), 1.04 (3H, *s*, CH_3-18), 1.03 (3H, *s*, CH_3-18), 1.00 (3H, *s*, CH_3-19), 0.72 (1H, *d*, *J* = 5 Hz, H-30), 0.65 (1H, *d*, *J* = 5 Hz, H-30), 0.47 (2 × 1H, *m*, H-30); ^{13}C NMR, see Table 2; HRESIMS *m/z* 611.3717 (calcd for $C_{38}H_{52}O_5$Na: 611.3707).

4.7. Dichapetalin P (*4*)

Yellow gum, $[\alpha]^{20}_D$ −29 (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (4.47) nm; IR (film) ν_{max} 3367, 1769, 1749, 1706 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.31 (4H, *m*, H-2′, -3′, -5′, -6′), 6.26 (1H, *dd*, *J* = 10, 3 Hz, H-12), 5.42 (1H, *br d*, *J* = 7 Hz, H-2), 5.39 (1H, *dd*, *J* = 10, 2.5 Hz, H-11), 4.30 (1H, *d*, *J* = 10 Hz, H-26), 4.27 (1H, *dd*, *J* = 9, 2.5 Hz, H-α), 4.16 (1H, *t*, *J* = 9.7 Hz, H-22), 4.07 (1H, *d*, *J* = 10 Hz, H-26), 3.79 (1H, *d*, *J* = 10.5 Hz, H-29), 3.53 (1H, *d*, *J* = 10.5 Hz, H-29), 2.95 (1H, *dd*, *J* = 10, 5.5 Hz, H-20), 2.82 (1H, *d*, *J* = 15 Hz, H-24), 2.52 (1H, *m*, H-17), 2.48 (1H, *d*, *J* = 15 Hz, H-24), 2.41 (1H, *d*, *J* = 10 Hz, OH), 2.34 (1H, *dd*, *J* = 13, 2.5 Hz, H-7), 2.22 (1H, *dd*, *J* = 13.5, 2.5 Hz, H-β), 2.00 (3H, *s*, OAc), 1.76 (1H, *dd*, *J* = 14.7, 2.6 Hz, H-5), 1.67 (3H, *s*, CH_3-27), 1.63 (1H, *br d*, *J* = 15 Hz, H-1), 1.29 (3H, *s*, CH_3-28), 1.26 (3H, *s*, CH_3-19), 1.22 (1H, *d*, *J* = 6 Hz, H-30), 1.16 (3H, *s*, CH_3-18), 0.95 (1H, *d*, *J* = 6 Hz, H-30); ^{13}C NMR, see Table 2; HRESIMS *m/z* 679.3232 (calcd for $C_{40}H_{48}O_8$Na: 679.3241).

4.8. Dichapetalin Q (**5**)

Yellow gum, $[\alpha]^{20}_D$ 0 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (4.34) nm; IR (film) ν_{max} 3374, 1601 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.40–7.32 (2 × 4H, *m*, H-2′, -3′, -5′, -6′), 5.52 (1H, *dq*, *J* = 9, 1.3 Hz, H-24), 5.49 (2 × 1H, *m*, H-15), 5.44 (1H, *dq*, *J* = 8.5, 1.3 Hz, H-24), 5.39 (2 × 1H, *d*, *J* = 7 Hz, H-2), 5.36 (1H, *m*, H-21), 5.33 (1H, *t*, *J* = 3 Hz, H-21), 4.90 (1H, *ddd*, *J* = 10.5, 9, 5 Hz, H-23), 4.79 (1H, *dt*, *J* = 6.5, 9.5 Hz, H-23), 4.27 (2 × 1H, *dd*, *J* = 11.8, 2.5 Hz, H-α), 4.03 (2 × 2H, *br s*, H-26), 3.96 (2 × 1H, *br s*, H-7), 3.78 (2 × 1H, *d*, *J* = 10.5 Hz, H-29), 3.62 (2 × 1H, *d*, *J* = 10.5 Hz, H-29), 2.63 (2 × 1H, *br t*, *J* = 12 Hz, H-β), 2.58 (1H, *br s*, OH), 2.40 (1H, *m*, H-20), 1.74 (3H, *br d*, *J* = 1 Hz, CH_3-27), 1.73 (3H, *br d*, *J* = 1 Hz, CH_3-27), 1.33 (2 × 3H, *s*, CH_3-28), 1.12 (3H, *s*, CH_3-30), 1.09 (2 × 3H, *s*, CH_3-18), 1.05 (2 × 3H, *s*, CH_3-30), 1.04 (2 × 3H, *s*, CH_3-19); ^{13}C NMR, see Table 2; HRESIMS *m/z* 611.3719 (calcd for $C_{38}H_{52}O_5Na$: 611.3707).

4.9. Dichapetalin R (**6**)

Yellow gum, $[\alpha]^{20}_D$ −4 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (4.28) nm; IR (film) ν_{max} 3353, 1575 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.29 (4H, *m*, H-2′, -3′, -5′, -6′), 5.47 (1H, *br t*, *J* = 2.2 Hz, H-21), 5.37 (1H, *br d*, *J* = 7 Hz, H-2), 5.31 (1H, *d*, *J* = 3.8 Hz, H-21), 4.37 (1H, *t*, *J* = 8.7 Hz, H-22), 4.24 (1H, *dd*, *J* = 11.8, 2.2 Hz, H-α), 3.94 (1H, *br s*, H-7), 3.76 (1H, *d*, *J* = 11 Hz, H-29), 3.67 (1H, *d*, *J* = 12 Hz, H-26), 3.59 (1H, *d*, *J* = 11 Hz, H-29), 3.51 (1H, *d*, *J* = 12 Hz, H-26), 3.38 (1H, *br s*, H-24), 2.60 (1H, br *t*, *J* = 12 Hz, H-β), 1.30 (3H, *s*, CH_3-28), 1.16 (3H, *s*, CH_3-27), 1.73 (3H, *br d*, *J* = 1 Hz, CH_3-27), 1.12 (3H, *s*, CH_3-30), 1.09 (3H, *s*, CH_3-18), 1.02 (3H, *s*, CH_3-30), 1.01 (3H, *s*, CH_3-19); ^{13}C NMR, see Table 2; HRESIMS *m/z* 645.3753 (calcd for $C_{38}H_{54}O_7Na$: 645.3762).

4.10. Dichapetalin S (**8**)

Yellow gum, $[\alpha]^{20}_D$ +50 (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 212 (4.26) nm; IR (film) ν_{max} 1762 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.40–7.32 (4H, *m*, H-2′, -3′, -5′, -6′), 6.16 (1H, *dd*, *J* = 10, 3 Hz, H-13), 5.46 (1H, *dd*, *J* = 10, 2.5 Hz, H-11), 5.41 (1H, *br d*, *J* = 7 Hz, H-2), 4.27 (1H, *dd*, *J* = 11.6, 2.7 Hz, H-α), 3.94 (1H, *br s*, H-7), 3.91 (1H, *d*, *J* = 9.5 Hz, H-26), 3.89 (1H, *d*, *J* = 9.5 Hz, H-26), 3.77 (1H, *d*, *J* = 11 Hz, H-29), 3.61 (1H, *d*, *J* = 11 Hz, H-29), 3.79 (1H, *d*, *J* = 10.5 Hz, H-28), 3.53 (1H, *d*, *J* = 10.5 Hz, H-28), 3.38 (1H, *ddd*, *J* = 12, 8, 5 Hz, H-20), 2.48 (1H, *dd*, *J* = 12, 8 Hz, H-14), 2.47 (1H, *d*, *J* = 14 Hz, H-24), 2.34 (1H, *d*, *J* = 14 Hz, H-24), 1.50 (3H, *s*, CH_3-27), 1.33 (3H, *s*, CH_3-28), 1.20 (1H, *d*, *J* = 5 Hz, H-30), 1.08 (3H, *s*, CH_3-19), 0.91 (3H, *s*, CH_3-18), 0.76 (1H, *d*, *J* = 5 Hz, H-30); ^{13}C NMR, see Table 2; HRESIMS *m/z* 623.3343 (calcd for $C_{38}H_{48}O_6Na$: 623.3343).

4.11. Oxidation of dichapetalin A (**1** to **2**)

Dichapetalin A (8 mg) was dissolved in 1 mL CH_2Cl_2 and 40 mg (large excess) of MnO_2 was added in one portion. After 24 h of stirring at room temperature, the mixture was filtered on a Celite plug in a Pasteur pipette and eluted with 10 mg of CH_2Cl_2. After evaporation, the residue was purified by HPLC, which yielded 3.4 mg (42%) of pure **2** as a pale waxy solid.

4.12. DIBAL reduction of dichapetalin A (**1** to **7** and **9**)

Dichapetalin A (37 mg) was dissolved in a mixture of 2 mL CH_2Cl_2 and 0.2 mL dry THF cooled at 0 °C. A 1 M solution of DIBAL in hexane (800 μL) was added slowly and the reaction mixture was stirred at 0 °C for three hours. A 1 M solution of Rochelle salt (10 mL, excess) was then added and the mixture was stirred for an hour at room temperature; the organic phase was separated, dried over Na_2SO_4 and evaporated. The crude oil (45 mg) was purified by HPLC to yield **7** (5 mg) and **9** (15 mg) as amorphous solids. Compound **7** (1:1 mixture of isomers): UV (MeOH) λ_{max} (log ε) 206 (4.2) nm; IR (film) ν_{max} 3355, 1647 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 7.38–7.23 (2 × 4H, *m*, H-2′, -3′, -5′, -6′), 6.34 (1H, *dd*, *J* = 10.2, 3.3 Hz, H-12), 6.18 (1H, *dd*, *J* = 10.3, 3 Hz, H-13), 5.53 (1H, *dq*, *J* = 8,1.3 Hz, H-24), 5.45 (1H, *br d*, *J* = 7 Hz, H-2), 5.42 (1H, *dq*, *J* = 8, 1.3 Hz, H-24), 5.39–5.30 (6H, *m*, 2 × H-21, 2 × H-11), 4.88 (1H, *m*, H-23), 4.76 (1H, *ddd*, *J* = 10, 9, 6 Hz, H-23), 4.27 (2 × 1H, *dd*, *J* = 11.5, 2.5 Hz, H-α), 3.93 (2 × 2H, *m*, H-26), 3.93 (2 × 1H, *m*, H-7), 3.73 (2 × 1H, *d*, *J* = 10.8 Hz, H-29), 3.57 (2 × 1H, *d*, *J* = 10.8 Hz, H-29), 2.62 (2 × 1H, *t*, *J* = 12.5 Hz, H-β), 1.71 (3H, *d*, *J* = 1.2 Hz, CH_3-27), 1.69 (3H, *d*, *J* = 1.2 Hz, CH_3-27), 1.32 (2 × 3H, *s*, CH_3-28), 1.11 (2 × 3H, *s*, CH_3-19), 0.92 (3H, *s*, CH_3-18), 0.91 (3H, *s*, CH_3-18), 0.85 (1H, *d*, *J* = 5 Hz, H-30), 0.84 (1H, *d*, *J* = 5 Hz, H-30); ^{13}C NMR, see Table 2; HRESIMS *m/z* 609.3540 (calcd for $C_{38}H_{50}O_5Na$: 609.3550). Compound **9:** UV (MeOH) λ_{max} (log ε) 206 (4.5) nm; IR (film) ν_{max} 3343, 1646 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 7.41–7.26 (4H, *m*, H-2′, -3′, -5′, -6′), 6.18 (1H, *dd*, *J* = 10.2, 3 Hz, H-12), 5.49 (1H, *br d*, *J* = 7 Hz, H-2), 5.42–5.38 (2H, *m*, H-24, H-11), 4.53 (1H, *dt*, *J* = 9, 7 Hz, H-23), 4.30 (1H, *dd*, *J* = 11.6, 2.6 Hz, H-α), 3.98 (2H, *s*, H-26), 3.94 (1H, *t*, J = 2.5 Hz, H-7), 3.84 (1H, *d*, *J* = 10.8 Hz, H-29),3.67 (1H, *dd*, *J* = 5.7, 10.7, H-21), 3.60 (2H, *m*, H-21, H-29), 2.65 (1H, *br t*, *J* = 12.5 Hz, H-β), 1.75 (3H, *d*, *J* = 1.2 Hz, CH_3-27), 1.34 (3H, *s*, CH_3-28), 1.14 (3H, *s*, CH_3-19), 0.93 (3H, *s*, CH_3-18), 0.90 (1H, *d*, *J* = 5.5 Hz, H-30); ^{13}C NMR, see Table 2; HRESIMS *m/z* 611.3705 (calcd for $C_{38}H_{52}O_5Na$: 611.3707).

4.13. Cell cytotoxicity assay

The cytotoxicity activities of all tested molecules were measured *in vitro* using the ATP quantification method as described elsewhere (Long et al., 2012). Briefly, cells were seeded in 96-well plates and incubated during 24 h for adhesion in RPMI1640 medium, 10% FBS, 2 mM glutamine, 50 U/mL penicillin/streptomycin and 1.25 μg/mL fungizone. Different concentrations of dichapetalin derivatives or vehicle were added and cells were incubated for 72 h at 37 °C in humidified 5% CO_2 atmosphere. Cell viability was evaluated by determining the level of ATP released by viable cells after molecule contact. ATP was measured with the ATPlite assay (Perkin Elmer) according to the manufacturer conditions. EC50 values were determined with curve fitting analysis method (non linear regression model with a sigmoid dose response, variable Hill slope coefficient) provided by the Prism Software (GraphPad). Results were expressed as average EC50 values (concentration of tested compound that inhibits 50% of the maximum effect for the considered compound).

Acknowledgment

The authors are indebted to Dr Bruno David (IRPF) for the botanical sample collections.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2013.03.023. These data include MOL files and InChiKeys of the most important compounds described in this article.

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