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Tirucallane triterpenes from *Dysoxylum macranthum*

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Abstract

Eleven new tirucallane-type triterpenes, dymacrins A–K, were isolated from the bark of *Dysoxylum macranthum* together with two known tetracyclic triterpenes and two known pregnane steroids. The structures of the new compounds were determined by spectral means, essentially 2D NMR experiments. Dymacrins B, C, H and J showed moderate cytotoxicity against KB cells (IC_{50} 5.6, 5.0, 8.3 and 1.0 μg/ml, respectively). © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Dysoxylum macranthum*; Meliaceae; Tirucallane triterpenoids; Structural elucidation

1. Introduction

Our systematic biological screening of New Caledonian plants using the cytotoxicity test against KB cells has led us to the chemical study of the ethanol extract from the bark of *Dysoxylum macranthum* C. DC (Meliaceae). Five known compounds were isolated: β-sitosterol, two pregnane steroids (2β,3β-dihydroxy-5α-pregnan-17,20-*Z*- and *E*-en-16-ones) (Inada, Murata, Inatomi, Naranishi & Darnaedi, 1997), a dammarane-type triterpene (dammarenediol-II) (Asakawa, Kasai, Yamasaki & Tanaka, 1977) and the tirucallane-type triterpene **1** (Guang-Yi, Gray & Waterman, 1988), together with 11 new tirucallanes, dymacrins A–K (**2–12**). Four of the new compounds, dymacrins B, C, H and J possessed moderate cytotoxic properties against KB cells. Cytotoxic triterpenes have been found previously in the genus *Dysoxylum*, especially the cumingianosides, 14,18-cycloapotirucallane glycosides from *D. cumingianum* (Kashiwada, Fujioka, Chang, Chen, Mihashi & Lee, 1992) and dysorone E, an apotirucallane-type triterpene isolated from *D. roseum* (Adesanya, Païs, Sévenet & Cosson, 1991).

2. Results and discussion

Dymacrin A (**2**) gave an $[M + H]^+$ peak at m/z 471.3488 in the HRCIMS corresponding to the molecular formula of $C_{30}H_{46}O_4$. The IR spectrum showed the absorptions of two keto groups at 1703 and 1679 cm^{-1}, the latter being α,β-unsaturated. In the ^{13}C NMR the signal of the carbonyls resonated at δ 216.4 and δ 201.4, respectively. The tirucallane skeleton was based on the presence of seven methyls (δ_H between δ 2.2 and 0.5) together with a hydroxymethyl group attached to a quaternary carbon and of an olefin with chemical shifts typical of a Δ^7 double bond (δ_C 118.1 and δ_C 146.1). One keto group was located at position 3 as shown by the characteristic ^{13}C NMR resonances of C-3 (δ 216.4), while the hydroxymethyl group was at the usual C-4 position (CH_2OH-29β : δ_C 65.8, Me-28α : δ_C 20.6). The structure of the fused ring moiety was entirely confirmed by 2D experiments (Table 1). The side chain contained the conjugated keto group and analysis of the NMR spectra (Table 1) allowed to formulate it as –CH(Me)CH(OH)COCH=C(Me)$_2$, which is similar to that of the known triterpene **1**. **1**,

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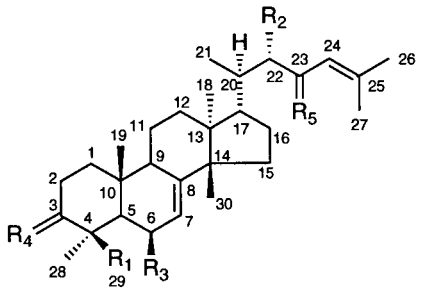
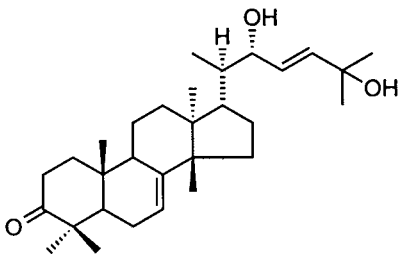


1 $R_1 = CH_3$, $R_2 = OH$, $R_3 = H$, $R_4 = R_5 = O$
2 $R_1 = CH_2OH$, $R_2 = OH$, $R_3 = H$, $R_4 = R_5 = O$
3 $R_1 = CH_3$, $R_2 = H$, $R_3 = OH$, $R_4 = R_5 = O$
4 $R_1 = CH_2OH$, $R_2 = R_3 = H$, $R_4 = R_5 = O$
5 $R_1 = CH_3$, $R_2 = R_3 = H$, $R_4 = R_5 = O$
6 $R_1 = CHO$, $R_2 = R_3 = H$, $R_4 = H$, α-OH, $R_5 = O$
7 $R_1 = CH_2OH$, $R_2 = R_3 = H$, $R_4 = R_5 = H$, α-OH



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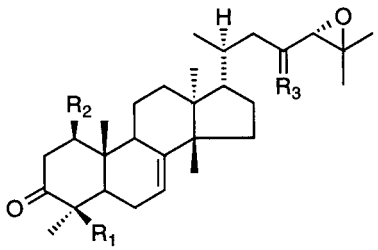
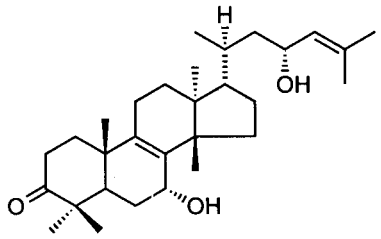


9 $R_1 = CH_2OH$, $R_2 = OH$, $R_3 = O$
10 $R_1 = CH_2OH$, $R_2 = H$, R_3= H, α-OH
11 $R_1 = CH_3$, $R_2 = OH$, $R_3 = H$, α-OH



12

which has been previously isolated from the resin of *Aucoumea klaineana*, was assumed to be a tirucallane triterpene rather than the corresponding euphane from biogenetic arguments. The configuration at C-22 had not been determined. Despite the fact that free rotation is possible at C-20, C-22 and C-23, NOEs were observed in the NOESY spectrum of **2** suggesting that the side chain has a preferred conformation (Fig. 1). The correlations Me-18/H-20, H-12β/Me-21 revealed an H-20α(C-20*R*) stereochemistry indicating a tirucallane triterpene. The 22*S* configuration was ascertained from the cross-peaks H-22/H-16αβ, H-22/H-20,H24 and H-20/H-24. The stereochemistry at C-20 and C-22 of **1** was identical owing to the similarity of all side chain NMR signals of compound **1** and **2**.

Dymacrin B (**3**) revealed an $[M + Na]^+$ peak at *m/z* 477.6871 in the HRFABMS corresponding to the molecular formula of $C_{30}H_{46}O_3$. The 1D and 2D NMR spectra of the fused rings showed a 3-keto tirucallane structure close to that of **3**. However, the hydroxymethylene group at C-29 was absent and replaced by a methyl group (Me-29β : δ_C 24.6). In addition an extra oxymethine group was observed (δ_C 67.1, δ_H 4.42). It was located at C-6 owing to the COSY spin system C_7–C_6–C_5. This was confirmed by the correlations observed in the HMBC spectra, essentially the cross peaks H-6/C-5,C-7,C-8,C-10. The H-6α configuration was deduced from the NOESY correlations H-6α/H-28 and H-6α/H-7. As for the side chain, the NMR spectra showed that the oxymethine at C-22 was replaced by a methylene. Such a chain formulated as –$CH(Me)CH_2COCH{=}C(Me)_2$ has been found previously in cycloartane triterpenes (Furlan, Roque & Filho, 1993). The H-20α configuration was indicated as for compound **2** by the NOESY correlations Me-18/H-20 and H-12β/Me-21.

Dymacrin C (**4**) revealed an $[M + H]^+$ peak at *m/z* 455.3554 in the HRCIMS corresponding to the molecular formula of $C_{30}H_{46}O_3$, which showed one oxygen less than for compounds **2** and **3**. All NMR data were close to those of the latter compounds indicating a structure for the fused ring and for the side chain similar to those of **2** and **3**, respectively.

Dymacrin D (**5**) gave an $[M + H]^+$ peak at *m/z*

Table 1
^{13}C (75 MHz) and ^{1}H NMR (400 MHz) data for dymacrins B (**2**) and C (**3**) ($CDCl_3$)[a]

Position	**2**				**3**			
	δ C	δ H (*J* Hz)	HMBC	NOESY	δ C	δ H (*J* Hz)	HMBC	NOESY
1	37.7	α 1.96 *m*	2,3,5	1β,2α	39.8	α 1.95 *m*	2,3,5,19	1β,2α
		β 1.48 *m*	2,3,5,9,19	19		β 1.48 *m*	2,3,5,9,19	19
2	35.7	α 2.34 *m*	1,3,4,10	2β	34.6	α 2.20 *m*	1,3,4,10	2β
		β 2.65 *ddd* (13,6,13)	1,3,10	19		β 2.80 *ddd* (13,6,13)	1,3,10	19,29
3	216.4				217.0			
4	53.4				48.8			
5	53.6	1.85 *m*	4,6,10,19,28,29	6α,9,28	56.0	1.46 *m*	4,6,10,19,28,29	6,9,28
6	24.8	α 2.14 *m*	4,5,7,8,10	6β,7,28	67.1	4.42 *m*	5,7,8,10	7,28
		β 1.99 *m*	4,5,7,10	19				
7	118.1	5.26 *br s*	5,9,14	15,30	121.5	5.45 *dd* (4,3)	5,6,9,14	15,30
8	146.1				146.1			
9	48.4	2.33 *m*	5,10,12	11,18	49.0	2.15 *m*	10,11,18	
10	35.1				34.8			
11	18.6	1.54 *m*	8,13		18.0	1.58 *m*	8,13,14	12β
12	33.5	α 1.52 *m*	11,13,14,18	18	33.1	α 1.62 *m*	11,13,14,18	18
		β 1.80 *m*	11,13,18	21,30		β 1.82 *m*	11,13,18	21,30
13	43.4				43.2			
14	51.5				51.2			
15	34.1	1.50 *m*	8,13,14,16,17,18		33.7	1.50 *m*	13,14,16,17	16α,β,30
16	28.1	α 1.38 *m*	13,15,17	22	28.0	α 1.26 *m*	13,15,17	16β,18,22b
		β 2.12 *m*	13,15,17	22		β 1.85 *m*	13,15,17	22b,30
17	49.1	1.98 *m*	13	21	52.8	1.52 *m*		21,22b
18	22.1	0.83 *s*	12,13,14,17	20	21.8	0.79 *s*	12,13,14,17	20
19	13.6	0.94 *s*	1,5,9,10	29b	15.6	1.21 *s*	1,5,9,10	
20	39.6	1.80 *m*	17,21	22,24	33.2	2.00 *m*	17,21	21,22b
21	12.1	0.59 *d* (7)	17,20,22		19.3	0.86 *d* (7)	17,20,22	22a
22	78.9	4.12 *s*	17,20,21,23	24	51.3	a 2.08 *m*	17,20,21,23	24
						b 2.45 *m*	17,20,21,23	24
23	201.4				201.2			
24	119.2	6.04 *s*	23,25,26,27	26	124.1	6.03 *br s*	23,26,27	26
25	159.7				155.0			
26	28.2	1.93 *s*	23,24,25,27		27.5	1.86 *s*	23,24,25,27	
27	21.5	2.18 *s*	23,24,25,26		20.7	2.11 *s*	23,24,25,26	
28	20.6	1.13 *s*	3,4,5,29	29a	23.8	1.20 *s*	3,4,5,29	
29	65.8	a 3.57 *d* (11)	3,4,5,28		24.6	1.49 *s*	3,4,5,28	
		b 4.03 *d* (11)	3,4,5,28					
30	27.7	1.02 *s*	8,13,14,15		26.4	1.05 *s*	8,13,14,15	

[a] Assignments based on 2D experiments (COSY, HMQC, HMBC, NOESY).

439.3563 in the HRCIMS corresponding to the molecular formula of $C_{30}H_{46}O_2$. The NMR spectra revealed that the side chain was again similar to that of compound **3** and **4**. The only difference with compound **4** lay in absence of the hydroxymethylene group at C-4 replaced by a methyl group with classical ^{13}C resonances for Me-28 and Me-29 (δ_C 24.7 and 21.7).

Dymacrin E (**6**) showed an $[M + Na]^+$ peak at 477.6970 in the HRFABMS corresponding to the molecular formula of $C_{30}H_{46}O_3$. The side chain was similar to those of compounds **3–5**. The hydroxymethylene group of **3** was replaced by an aldehyde function, which resonated at δ_H 9.98 and δ_C 208.3. Instead of the 3-keto group, there was C-3β-hydroxymethine, as shown from the typical ^{1}H NMR coupling pattern of H-3 (δ_H 3.25, *dd*, $J = 4$, 10 Hz) and the ^{13}C chemical shifts of both C-3 (δ 77.7) and C-2 (δ 29.8).

Dymacrin F (**7**) gave an $[M + H]^+$ peak at *m*/*z* 457.3687 in the HRCIMS corresponding to the molecular formula of $C_{30}H_{48}O_3$. The spectra of the fused ring were similar to those of compounds **2** and **4**. The side chain showed an allylic hydroxyl group instead of the conjugated keto group at C-23 present in compounds **3–5**. This type of side chain is already known in other tetracyclic triterpenes, but not the configuration at C-23 (Furlan et al., 1993). The latter was deduced from the NOESY spectrum, which showed that the side chain adopted a preferred conformation (Fig. 1). The usual correlations Me-18/H-20 and H12β/Me-21 revealed the tirucallane stereochemistry,

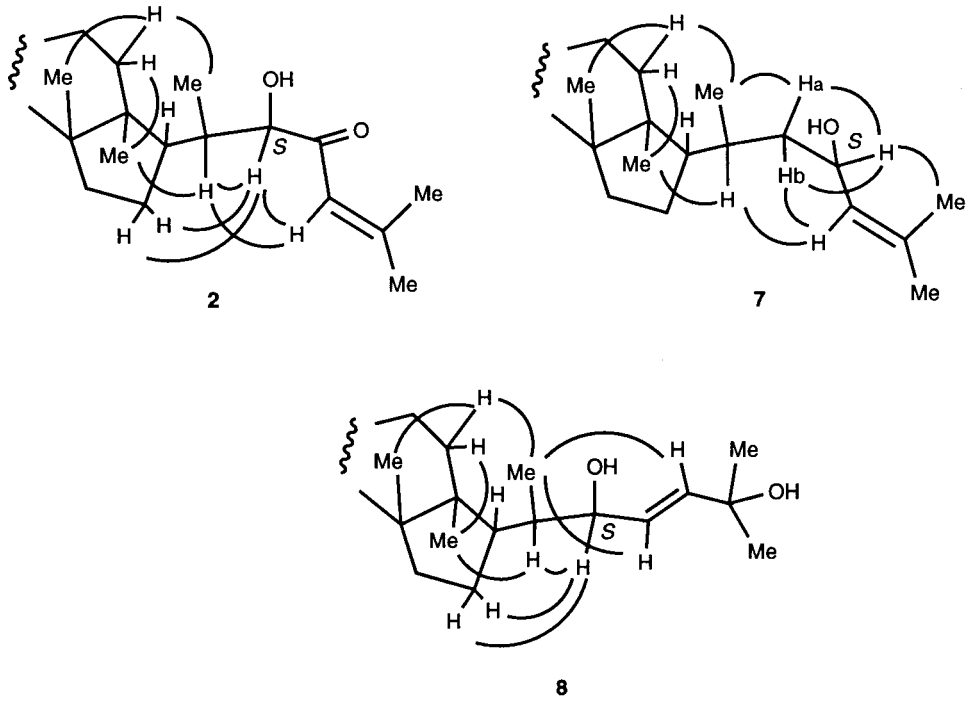


Fig. 1. Main NOESY correlations for dymacrins A (**2**), F (**7**) and H (**8**) (ring D and side chain).

while the cross peaks H-20/H-24, H-21/H-22a,H-23, H-22a/H-23, H-22b/H-23,H-24 and H-23/Me-27 indicated the C-23*S* configuration.

Dymacrin G (**8**) exhibited an $[M + H]^+$ peak at *m/z* 457.3652 in the HRCIMS, which corresponded to the molecular formula of $C_{30}H_{48}O_3$. The fused ring moiety was again similar to the one of compounds **2**, **4** and **7**. The NMR spectral data showed that the side chain has the structure –CH(Me)CH(OH)CH=CHC(OH)Me_2 with an *E* geometry of the double bond. The same side chain was present in a cycloartane triterpene saponin isolated from *Thalictrum minus* (Gromova, Lutskii, Zinchenko, Ganenko & Semenov, 1993). The stereochemistry at C-22 had not been determined. The NOESY spectrum indicated again a preferred conformation for the side chain (Fig. 1). The C-20*R* and the C-22*S* stereochemistry were deduced from the observed NOEs (Fig. 1 and Section 3).

Dymacrin H (**9**) showed an $[M + H]^+$ peak at *m/z* 487.3435 in the HRCIMS corresponding to the molecular formula of $C_{30}H_{46}O_5$. The 1D and 2D NMR spectra of the fused rings were close to those of **2** and **4** with however, a straightforward difference for position 1. The usual resonances of CH_2-1 were absent and replaced by the resonances of a C-1 oxymethine (δ_C 76.2, δ_H 3.80, $J = 5$, 10 Hz) as shown by the downfield shift of C-2 (45.8) and the HMBC correlations H-1/C-9,C-19 (Table 2). The H-1α-axial configuration was obvious from the observed coupling pattern and the NOESY correlations H-1/H-5,H-9. The side chain was assigned the structure depicted in **9** showing a keto group at C-23 and a 24,25-epoxide. This type of chain has been described previously in cimicidanol-3-arabinoside, a cycloartenol glycoside recently isolated from *Cimicifuga foetida* and the configuration at C-24 has been determined as *R* by chemical means (Kadota, Li, Tanaka & Namba, 1995). In the case of compound **9**, the NOESY spectrum indicated a C-20*R* configuration (cross peaks H-20/H-18, and H-12α/Me-21), which is opposite to the one of cimicidanol-3-arabinoside, but the configuration at C-24 could not be deduced from the observed NOEs. Similar ^{13}C NMR resonances (in pyridine-d_5, see Section 3) were observed for the side chains of both compound **9** and cimicidanol-3-arabinoside. Hence, the 24*S* stereochemistry was tentatively assigned to **9**.

Dymacrin I (**10**) and J (**11**) gave [M + Na] peaks at *m/z* 495.7036 and *m/z* 495.7021, respectively, in the HRFABMS, which corresponded to the molecular formula of $C_{30}H_{48}O_3$. The structure of the fused ring of **10** was similar to that of compounds **2**, **4** and **7**. In the case of **11**, the 1D NMR spectra showed a 3-ketotirucallane with a dimethyl group at position 4 and an OH-1β group. The latter feature was deduced from the resonances of CH-1 (δ_C 77.6, δ_H 3.77, $J = 5$, 12 Hz) and C-2 (δ_C 45.2) similar to those of compound **9**. Both **10** and **11** have the same side chain as depicted, with again a 24,25-epoxide group as in the preceding compound **9**. However, the keto group at C-23 was replaced by an hydroxyl function. This type of chain

Table 2
^{13}C (75 MHz) and ^{1}H NMR (400 MHz) data for dymacrins I (**9**) and K (**12**) ($CDCl_3$)[a]

Position	**9**				**12**			
	δ C	δ H (*J* Hz)	HMBC	NOESY	δ C	δ H (*J* Hz)	HMBC	NOESY
1	76.2	3.80 *dd* (5,10)	2,3,9,19	5,9,11α	34.9	α 1.57 *m*	2,3,10	1β,2
						β 1.98 *m*	2,3,5,10	2,19
2	45.8	α 2.56 *dd* (13,3)	1,3,10	5	34.4	2.53 *m*	1,3	28
		β 2.77 *dd* (13,10)	1,3,4,10	19				19,29
3	216.6				217.0			
4	53.5				46.7			
5	50.2	1.67 *m*	1,3,4,6,9,10,19,28,29	6α,9,28	44.5	2.19 *m*	4,6,7,10,28,29	28
6	24.6	α 2.21 *m*	5,7,8,10	6β,7	24.3	α 2.19 *m*	7,8	6β,7,28
		β 2.08 *m*	5,7,8,10	19,29a		β 1.45 *m*	5,10	7,19,29
7	118.4	5.26 *br s*	5,6,9,14	15,30	79.5	4.39 *br s*	5,8,9	15,30
8	146.5				131.4			
9	48.8	2.46 *m*		11α,18	144.0			
10	41.0				38.1			
11	20.9	α 2.00 *m*	12,13	11β	22.2	2.08 *m*	12	18,19
		β 1.61 *m*	12,13	30				
12	33.9	α 1.62 *m*	13,18	12β,18,21	30.9	1.74 *m*		18,21,30
		β 1.76 *m*	13,14,18	21,30				
13	43.2				44.1			
14	51.4				50.1			
15	34.4	1.46 *m*	8,13,14,16,18		29.8	1.48 *m*		30
16	28.3	α 1.20 *m*	17,20	16β,18	28.5	α 1.26 *m*		18
		β 1.86 *m*	13,17	17,22b,30		β 2.00 *m*	15	30
17	53.0	1.47 *m*	12,13,14,16	21,30	51.0	1.48 *m*		
18	22.2	0.80 *s*	13,14,17	20	15.8	0.71 *s*	12,13,14,17	20
19	8.7	0.95 *s*	1,5,9,10	29b	18.8	0.99 *s*	1,5,9,10	
20	32.6	1.98 *m*		21,22b	34.1	1.25 *m*	23	21,24
21	19.6	0.85 *d* (7)	17,20,22	22a	19.7	0.92 *d* (7)	17,20,22	23
22	48.1	a 2.25 *m*	17,20,23,24	22b,24,26	44.6	a 1.33 *m*		23,24
		b 2.50 *m*	17,20,21,23,24	24,26		b 1.57 *m*		23,24
23	207.1				67.4	4.45 *dt* (4,9)		24,27
24	65.7	3.29 *s*	23,25,27	27	128.5	5.10 *d* (9)		26
25	61.2				135.9			
26	18.6	1.21 *s*	24,25,27		25.8	1.74 *s*	24,25,26	
27	24.8	1.38 *s*	24,25,26		18.5	1.70 *s*	24,25,27	
28	20.1	1.08 *s*	3,4,5,29	29a	21.3	1.15 *s*	3,4,5,29	
29	65.3	a 3.55 *d* (11)	3,4,5,28		27.2	1.08 *s*	3,4,5,28	
		b 4.04 *d* (11)	3,4,5,28					
30	27.7	0.98 *s*	8,13,14,15		26.0	0.90 *s*	8,13,14,15	

[a] Assignments based on 2D experiments (COSY, HMQC, HMBC, NOESY).

was known before in tirucallane (Silvanand, Hoffmann, Schram & Cole, 1981; Mulholland & Taylor, 1988; Su et al., 1990) and protostane-type triterpenes (Nakaima, Satoh, Katsumata, Tsujiyama, Ida & Shoji, 1994), as well as in cumingianoside F, a 13,18-cycloapotirucallane triterpene glycoside isolated from *D. cumingianum* (Kashiwada et al., 1992). A 23*R* and 24*S* configuration has been established for cumingianoside F by X-Ray analysis (Kashiwada et al., 1995). By comparison of the ^{13}C NMR data of the latter compound and both **10** and **11** the same chain stereochemistry as for cumingianoside F was tentatively assigned to compounds **10** and **11**.

Dymacrin K (**12**) showed an $[M + H]^+$ peak at *m/z* 457.3655 in the HRCIMS corresponding to the molecular formula of $C_{30}H_{48}O_3$. The ^{13}C NMR spectrum of the fused rings (Table 2) indicated a tetracyclic triterpene, but the resonances of the Δ^7 double bond were absent. Instead, the signals of a tetrasubstituted olefin were observed. The chemical shift values were in agreement with those described for the Δ^8 double bond of triterpenes having an oxymethine at position 7. The usual signal of a C-3 keto group and a dimethyl at C-4 were also present. The ^{1}H pattern of the C-7 oxymethine (δ 4.39 *br s*) indicated an Hβ-equatorial position. The 2D spectra (Table 2) confirmed the latter structural features and showed that compound **12** was a tirucallane-type triterpene, since the NOESY correlations H-7/H-30, H-16β/H30, H-16α/H-18 were observed. The NMR resonances of the side chain indi-

cated a structure similar to the one of **7**. The observed NOESY cross peaks were also similar revealing the same H-20α and C-23*S* configuration as for compound **7** (Table 2).

Tirucallane triterpenes have been found previously in the genus *Dysoxylum* (Mulholland & Nair, 1992; Benosman, Richomme, Sévenet, Hadi & Bruneton, 1994). Among the 11 tirucallanes isolated here only dymacrin B, C, H and J showed moderate cytotoxicity against KB cells (IC_{50} 5.6, 5.0, 8.3 and 1.0 μg/ml, respectively). Cytotoxic activity at about the same level has also been reported for previously isolated tirucallanes (Itokawa, Kishi, Morita & Takeya, 1992), but others are inactive (Benosman et al., 1994; Benosman, Richomme, Sévenet, Perromat, Hadi & Bruneton, 1995). No structure activity studies have been achieved in this series, since the cytoxicity level is too low for justifying such studies. Consequently, the structural features necessary for the cytotoxic properties are unknown. However, the tirucallane skeleton itself appears clearly as insufficient for activity. Cytoxicity at the same level has been found for the apotirucallane dysorone E from *D. roseum* (Adesanya et al., 1991) and other apotirucallanes also are moderately cytotoxic (Mohamad et al., 1999). The 14,18-cycloapotirucallane glycosides isolated from *Dysoxylum cumingianum* showed a potent cytotoxic activity (IC_{50} 0.0062 μg/ml against MOLT-4 human leukemia cells for cumingianoside A) (Kashiwada et al., 1992). Some preliminary structure activity studies have been performed in these series of triterpenes (Kashiwada et al., 1997).

3. Experimental

3.1. General

IR: $CHCl_3$; ^1H NMR: 400 MHz; ^{13}C NMR: 75 MHz; 2D experiments: 400 MHz; CC: Merck Silica gel 60 230-400 mesh or 60 H.

3.2. Plant material

Leaves of *D. macranthum* D. CD (Meliaceae) were collected at the Réserve Spéciale de Faune du Col d'Amieu, New Caledonia by one of us (M. L.). Voucher specimens (Lit 0251) are deposited in the Herbarium of the Centre ORSTOM, Noumea, New Caledonia.

3.3. Extraction and isolation

Dried ground leaves (1 kg) of A. *D. macranthum* were extracted exhaustively with EtOH at room temperature. The extract (53.6 g) was repeatedly chromatographed on silica gel yielding dymacrin D (**5**) (35 mg, 1. CC CH_2Cl_2–MeOH 99.5 : 0.5, 2. CC heptane–EtOAc 9 : 1), compound **1** (38 mg, 1. CC CH_2Cl_2–MeOH 99.5 : 0.5, 2. CC heptane–EtOAc 90 : 10), dammarane (48 mg 1. CC CH_2Cl_2–MeOH 99.5 : 0.5, 2. CC heptane–EtOAc 9 : 1, 3. PTLC heptane–EtOAc 40 : 60), dymacrin B (**3**) (25 mg, 1. CC CH_2Cl_2–MeOH 99.5 : 0.5, 2. CC heptane/EtOAc 85 : 15, 3. PTLC heptane–EtOAc 60 : 40), dymacrin E (**6**) (9 mg, 1. CC CH_2Cl_2–MeOH 99.5 : 0.5, 2. CC heptane–EtOAc 85 : 15, 3. PTLC heptane–EtOAc 60 : 40), dymacrin K (**12**) (6 mg, 1. CC CH_2Cl_2–MeOH 99.5 : 0.5, 2. CC, heptane–EtOAc 85 : 15, 3. PTLC heptane–EtOAc 60 : 40), dymacrin C (**4**) (550 mg, 1. CC CH_2Cl_2–MeOH 99.5 : 0.5, 2. CC heptane–EtOAc 8 : 2), dymacrin A (**2**) (730 mg, 1. CC CH_2Cl_2–MeOH 99.5 : 0.5, 2. CC heptane–EtOAc 6 : 4), dymacrin F (**7**) (60 mg, 1. CC CH_2Cl_2–MeOH 95.5, 2. CC heptane–EtOAc 8 : 2, 3. PTLC heptane–EtOAc 40 : 60), dymacrin G (**8**) (21 mg, 1. CC CH_2Cl_2–MeOH 95.5, 2. CC heptane–EtOAc 8 : 2, 3. PTLC heptane–EtOAc 40 : 60), dymacrin I (**10**) (35 mg, 1. CC CH_2Cl_2–MeOH 95.5, 2. CC heptane–EtOAc 8 : 2, 3. PTLC heptane–EtOAc 40 : 60), dymacrin J (**11**) (27 mg, 1. CC CH_2Cl_2–MeOH 95.5, 2. CC heptane–EtOAc 8 : 2, 3. PTLC heptane–EtOAc 40 : 60), 2β,3β-dihydroxy-5α-pregnan-17,20-*Z*-en-16-one (5 mg, 1. CC CH_2Cl_2–MeOH 95.5, 2. CC heptane–EtOAc 8 : 2, 3. PTLC heptane–EtOAc 40 : 60), 2β,3β-dihydroxy-5α-pregnan-17,20-*E*-ene-16-one (9 mg, 1. CC CH_2Cl_2–MeOH 95.5, 2. CC heptane–EtOAc 8 : 2, 3. PTLC heptane–EtOAc 40 : 60), dymacrin H (**9**) (2.2 g, CC CH_2Cl_2–MeOH 90–10).

3.4. Dymacrin A (*2*)

$[\alpha]_D + 31^\circ$ (*c* 1, $CHCl_3$); IR ν cm^{-1} 1703, 1679; ^1H NMR and ^{13}C NMR see Table 1; HRCIMS *m/z* 471.3488, $[M + H]^+$ ($C_{30}H_{47}O_4$, Δ 1.43 mmu).

3.5. Dymacrin B (*3*)

$[\alpha]_D - 17^\circ$ (*c* 1, $CHCl_3$); IR ν cm^{-1} 3518, 1703, 1678; ^1H NMR and ^{13}C NMR see Table 1; HRFABMS *m/z* 477.6871, $[M + Na]^+$ ($C_{30}H_{46}O_3Na$, Δ −0.7 mmu).

3.6. Dymacrin C (*4*)

$[\alpha]_D + 16^\circ$ (*c* 1, $CHCl_3$); IR ν cm^{-1} 3518, 1703, 1678; ^1H NMR ($CDCl_3$): δ 2.25 (1H, *m*, H-2α), 2.75 (1H, *ddd*, *J* = 13,6, 13 Hz, H-2β), 5.25 (1H, *br s*, H-7), 2.25 (1H, *m*, H-9), 0.86 (3H, *s*, Me-18), 1.00 (3H, *s*, Me-19), 0.88 (3H, *d*, *J* = 7 Hz, Me-21), 2.08 (1H, *m*, H-22a), 2.50 (1H, *dd*, *J* = 14, 2 Hz, H-22b), 6.10 (1H, *s*, H-24), 1.88 (3H, *s*, Me-26), 2.13 (3H, *s*, Me-27), 1.10 (3H, *s*, Me-28), 3.58, 4.08 (2 × 1H, 2*d*, *J* = 11, CH_2-

Table 3
^{13}C (75 MHz) data for dymacrins C (**4**), D (**5**), E (**6**), F (**7**), G (**8**), I (**10**) and J (**11**) ($CDCl_3$)

Carbon	4	5	6	7[a]	8[a]	10	11
1	37.7	38.6	37.3	37.6	38.7	37.6	77.6
2	35.6	35.0	29.8	35.7	35.2	35.7	45.2
3	216.3	216.1	77.7	216.0	216.0	216.4	213.3
4	53.4	48.0	52.6	53.4	48.1	53.4	48.2
5	53.5	53.3	53.2	53.6	52.5	53.6	53.5
6	24.7	24.7	23.9	24.8	24.5	24.8	24.5
7	118.0	118.0	117.0	118.0	118.1	118.2	118.1
8	145.9	145.9	146.1	146.2	146.0	146.6	145.9
9	48.2	48.5	47.6	48.4	48.6	48.4	48.5
10	35.0	35.1	35.1	35.1	35.1	35.2	35.8
11	18.5	18.3	18.7	18.4	18.4	18.7	21.5
12	33.5	33.6	33.7	33.8	33.7	33.7	34.1
13	43.6	43.7	43.7	43.6	43.6	43.4	43.3
14	51.3	51.4	51.4	51.4	51.4	51.4	51.4
15	34.0	34.1	34.1	34.1	34.3	34.1	34.6
16	28.3	28.5	28.5	28.6	27.8	27.6	28.8
17	53.1	52.4	52.4	53.7	49.2	53.4	49.4
18	22.1	22.1	22.1	22.1	22.0	22.0	22.0
19	13.5	12.9	13.2	13.7	12.9	13.7	7.7
20	33.5	33.6	33.6	33.7	41.8	33.7	33.8
21	19.4	19.5	19.1	19.3	11.8	20.0	20.1
22	51.5	51.6	51.6	44.4	73.6	40.8	40.9
23	201.5	201.5	201.6	67.3	129.8	69.4	69.5
24	124.3	124.4	124.5	128.5	137.8	68.6	68.7
25	154.8	154.8	154.8	135.8	70.9	61.5	60.5
26	27.7	27.8	27.8	26.0	30.1	20.0	20.0
27	20.7	20.8	20.8	18.7	30.1	25.0	25.5
28	20.5	24.7	19.6	20.7	24.7	20.7	24.4
29	65.7	21.7	208.3	65.9	21.8	65.9	21.2
30	27.5	27.5	27.4	27.6	27.7	27.6	27.6

[a] Assignments based on 2D experiments (COSY, HMQC, HMBC, NOESY).

29), 1.00 (3H, *s*, Me-30); ^{13}C NMR see Table 3; HRCIMS *m/z* 455.3554, $[M + H]^+$ ($C_{30}H_{47}O_2$, Δ 2.95 mmu).

3.7. Dymacrin D (**5**)

$[\alpha]_D - 36^\circ$ (*c* 1, $CHCl_3$); IR ν cm^{-1} 1705, 1782; ^1H NMR ($CDCl_3$): δ 2.25 (1H, *m*, H-2α), 2.75 (1H, *ddd*, *J* = 13,6, 13 Hz, H-2β), 5.30 (1H, *br s*, H-7), 2.28 (1H, *m*, H-9), 0.86 (3H, *s*, Me-18), 0.98 (3H, *s*, Me-19), 0.88 (3H, *d*, *J* = 7 Hz, Me-21), 2.08 (1H, *m*, H-22a), 2.50 (1H, *dd*, *J* = 14,2 Hz, H-22b), 6.10 (1H, *s*, H-24), 1.88 (3H, *s*, Me-26), 2.13 (3H, *s*, Me-27), 1.05 (3H, *s*, Me-28), 1.12 (3H, *s*, Me-29), 1.00 (3H, *s*, Me-30); ^{13}C NMR (see Table 3); HRCIMS *m/z* 439.3563, $[M + H]^+$ ($C_{30}H_{47}O_2$, Δ −1.31 mmu).

3.8. Dymacrin E (**6**)

$[\alpha]_D + 4^\circ$ (*c* 1, $CHCl_3$); IR ν cm^{-1} 3535, 1704, 1782; ^1H NMR ($CDCl_3$): δ 3.25 (1H, *dd*, *J* = 10, 4 Hz, H-3), 5.25 (1H, *br s*, H-7), 2.30 (1H, *m*, H-9), 0.86 (3H, *s*, Me-18), 1.25 (3H, *s*, Me-19), 0.88 (3H, *d*, *J* = 7 Hz, Me-21), 2.08 (1H, *m*, H-22a), 2.50 (1H, *dd*, *J* = 14, 2 Hz, H-22b), 6.08 (1H, *s*, H-24), 1.88 (3H, *s*, Me-26), 2.13 (3H, *s*, Me-27), 0.68 (3H, *s*, Me-28), 9.98 (1H, *s*, H-29), 0.98 (3H, *s*, Me-30); ^{13}C NMR (see Table 3); HRFABMS *m/z* 477.6970, $[M + Na]^+$ ($C_{30}H_{46}O_3Na$, Δ −0.8 mmu).

3.9. Dymacrin F (**7**)

$[\alpha]_D - 27^\circ$ (*c* 1, $CHCl_3$); IR ν cm^{-1} 3500, 1703; ^1H NMR ($CDCl_3$): δ 2.35 (1H, *m*, H-2α), 2.65 (1H, *ddd*, *J* = 13, 6, 13 Hz, H-2β), 5.25 (1H, *br s*, H-7), 2.32 (1H, *m*, H-9), 1.60 (1H, *m*, H-12α), 1.84 (1H, *m*, H-12β), 0.76 (3H, *s*, Me-18), 0.93 (3H, *s*, Me-19), 0.85 (3H, *d*, *J* = 7 Hz, Me-21), 1.30 (1H, *m*, H-22a), 1.56 (1H, *m*, H-22b), 4.42 (1H, *dt*, *J* = 4, 9, H-23), 1.71 (3H, *s*, Me-26), 1.68 (3H, *s*, Me-27), 1.14 (3H, *s*, Me-28), 3.58, 4.02 (2 × 1H, 2*d*, *J* = 11, CH_2-29), 0.97 (3H, *s*, Me-30); ^{13}C NMR see Table 3; main NOESY correlations: H-12α/Me-18, H-12β/H-21,Me-30, Me-18/H-20, H-20/H-24, H-21/H-22a,H-23, H-22a/H-23, H-22b/H-23,H-24, Me-27 and H-24/Me-26. HRCIMS *m/z* 457.3627, $[M + H]^+$ ($C_{30}H_{49}O_3$, Δ 0.2 mmu).

3.10. Dymacrin G (**8**)

$[\alpha]_D - 17^\circ$ (*c* 1, $CHCl_3$); IR ν cm^{-1} 1705; ^1H NMR ($CDCl_3$): δ 2.25 (1H, *m*, H-2α), 2.73 (1H, *ddd*, *J* = 13, 6, 13 Hz, H-2β), 5.30 (1H, *br s*, H-7), 2.25 (1H, *m*, H-9), 1.58 (1H, *m*, H-12α), 1.82 (1H, *m*, H-12β), 1.37 (1H, *m*, H-16α), 2.06 (1H, *m*, H-16β), 1.87 (1H, *m*, H-17), 0.81 (3H, *s*, Me-18), 0.99 (3H, *s*, Me-19), 0.82 (3H, *d*, *J* = 7 Hz, Me-21), 4.25 (1H, *d*, *J* = 5, H-22), 5.70 (1H, *dd*, *J* = 16, 5 Hz, H-23), 5.80 (1H, *d*, *J* = 16 Hz, H-24), 1.32 (6H, *s*, Me-26, Me-27), 1.03 (3H, *s*, Me-28), 1.10 (3H, *s*, Me-29), 1.04 (3H, *s*, Me-30); ^{13}C NMR see Table 3; main NOESY correlations: H-12β/Me-21,Me-30, H-16αβ/H-22, H-17/H-22, Me-18/H-20, H-20/H-22,23, Me-21/H-23,H-24, H-23/Me-26,Me-27 and H-24/Me-26,Me27. HRCIMS *m/z* 457.3652, $[M + H]^+$ ($C_{30}H_{49}O_3$, Δ −2.97 mmu).

3.11. Dymacrin H (**9**)

$[\alpha]_D + 17^\circ$ (*c* 1, $CHCl_3$); IR ν cm^{-1} 1711; ^1H NMR and ^{13}C NMR ($CDCl_3$), see Table 2; ^{13}H NMR (side chain, pyridine-d_5): 18.1 (H-21), 46.6 (H-22), 204.9 (H-23), 64.1 (H-24), 59.3 (H-25), 18.7 (Me-26), 22.9 (Me-27); HRCIMS *m/z* 487.3435, $[M + H]^+$ ($C_{30}H_{47}O_5$, Δ 1.14 mmu).

3.12. Dymacrin I (**10**)

$[\alpha]_D - 31^\circ$ (*c* 1, $CHCl_3$); IR ν cm^{-1} 3500, 1700; ^1H NMR ($CDCl_3$): δ 2.35 (1H, *m*, H-2α), 2.70 (1H, *ddd*,

$J = 13$, 6, 13 Hz, H-2β), 5.25 (1H, *br s*, H-7), 2.35 (1H, *m*, H-9), 0.82 (3H, *s*, Me-18), 0.94 (3H, *s*, Me-19), 0.92 (3H, *d*, $J = 7$ Hz, Me-21), 3.55 (1H, *m*, H-23), 2.65 (1H, *d*, $J = 8$ Hz, H-24), 1.30 (6H, *s*, Me-26, Me-27), 1.15 (3H, *s*, Me-28), 3.60, 4.15 (2×1H, 2*d*, $J = 11$, CH_2-29), 1.00 (3H, *s*, Me-30); ^{13}C NMR ($CDCl_3$), see Table 3; ^{3}H NMR (side chain, pyridine-d_5): 20.8 (Me-21), 42.1 (H-22), 70.1 (H-23), 69.8 (H-24), 59.0 (H-25), 20.4 (Me-26), 25.3 (Me-27); HRFABMS *m/z* 495.7036 $[M + Na]^+$ ($C_{30}H_{48}O_4Na$, Δ 0.5 mmu).

3.13. Dymacrin J (*11*)

$[\alpha]_D + 30^\circ$ (*c* 1, $CHCl_3$); IR ν cm^{-1} 1700; ^{1}H NMR ($CDCl_3$): δ 3.77 (1H, *ddd*, $J = 12$, 5 Hz, H-1), 5.25 (1H, *br s*, H-7), 0.80 (3H, *s*, Me-18), 0.98 (3H, *s*, Me-19), 0.95 (3H, *d*, $J = 7$ Hz, Me-21), 3.55 (1H, *m*, H-23), 2.65 (1H, *d*, $J = 8$ Hz, H-24), 1.31, 1.32 (2×3H, 2*s*, Me-26, Me-27), 1.03 (3H, *s*, Me-28), 1.12 (3H, *s*, Me-29), 1.02 (3H, *s*, Me-30); ^{13}C NMR see Table 3. HRFABMS *m/z* 495.7021 $[M + Na]^+$ ($C_{30}H_{48}O_4Na$, Δ −1.0 mmu).

3.14. Dymacrin K (*12*)

$[\alpha]_D - 5^\circ$ (*c* 1, $CHCl_3$); IR ν cm^{-1} 3415, 1705; ^{1}H NMR and ^{13}C NMR see Table 2; HRCIMS *m/z* 457.3655, $[M + H]^+$ ($C_{30}H_{49}O_3$, Δ −2.67 mmu).

3.15. Cytotoxicity assays

The assays were performed according to a published technique (Tempête, Werner, Favre, Roja & Langlois, 1995). The control used for comparison is doxorubicin (IC_{50} 0.058 μg/ml)

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