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Cytotoxic sesquiterpenoids from Winteraceae of Caledonian rainforest

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ABSTRACT

One secobutanolide, two butanolides and six drimane sesquiterpenoids were isolated from the bark and leaves of *Zygogynum pancheri* and *Zygogynum acsmithii* (Winteraceae) along with six known drimanes, isodrimanial, 1β-*O*-*p*-methoxy-*E*-cinnamoyl-bemadienolide, 7-ketoisodrimenin, drimenin, polygodial and 1β-*E*-cinnamoyl-6α-hydroxypolygodial. Their structures were elucidated through analysis of spectroscopic data. Drimane sesquiterpenoids with a dialdehyde function exhibited significant inhibitory activities in the *in vitro* cytotoxic assays against KB, HL60 and HCT116 cancer cell lines.

1. Introduction

Drimane sesquiterpenoids are widespread in nature. They have been isolated from the sponge *Dysidea* sp. (Paul et al., 1997), from the nudibranch *Dendrodoris carbunculosa* (Sakio et al., 2001), from the fungus *Peniophora polygonia* (Ayer and Trifonov, 1992), from liverworts of the genus *Porella* (Asakawa and Aratani, 1976; Cullmann and Becker, 1999; Asakawa et al., 2001), from ferns such as *Thelipteris hispidula* (Socolsky et al., 2005) and from various higher plants including *Polygonum hydropiper*, Polygonaceae (Fukuyama et al., 1982), *Taxus cuspidata*, Taxaceae (Kiyota et al., 2002), *Warburgia ugandensis, Cinnamosma macrocarpa,* Canellaceae (Wube et al., 2005; Harinantenaina et al., 2007) and several *Drimys* species, Winteraceae (Brown, 1994; Vichnewski et al., 1986). These compounds possess a wide variety of biological activities, including antibacterial, antifungal, antifeedant, cytotoxic, molluscicidal, piscicidal, plant-growth regulatory and phytotoxic properties (Jansen and de Groot, 2004).

Zygogynum is one of the two genera of the family Winteraceae occurring on mainland Australia and offshore islands, the other being *Drimys*. Some forty one species of *Zygogynum* occur in Guinea, Australia and Caledonia. Of these, 18 species are endemic to New Caledonia (White and Vink, 1993). As far as we know, the chemical constituents of the genus *Zygogynum* have not been widely studied except in four papers dealing with leaf essential oil content in three species of Australian *Zygogynum* (Brophy et al., 1994), alkaloids from *Z. pauciflorum* (Ahond et al., 1990), drimane sesquiterpenoids isolated from *Zygogynum baillonii* (Fomekong Fotsop et al., 2008) and phenyl-3-tetralones isolated from four species of Caledonian *Zygogynum* in our group (Allouche et al., 2008). In continuation of our chemical and biological investigations on drimane sesquiterpenoids, we now report the study of *Zygogynum pancheri* subsp. *elegans* Vink, *Z. pancheri* subsp. *pancheri* (Baill.) Vink and *Zygogynum acsmithii* Vink. In this paper, we describe the isolation, structure elucidation and cytotoxicity of nine new compounds, one secobutanolide **13**, two butanolides **14** and **15**, six drimane sesquiterpenoids (**2–7**) along with six known drimanes (**1, 8–12**).

2. Results and discussion

Ground barks and leaves of *Z. pancheri* subsp. *elegans*, *Z. pancheri* subsp. *pancheri* and *Z. acsmithii* were extracted by ethyl acetate. A small amount of each crude extract was submitted to a rapid filtration on a polyamide cartridge in order to remove polyphenols and tannins (Bousserouel et al., 2005). The filtered extracts showed significant inhibition (55–65%) of KB cell growth at 1 μg/ml. Subsequent bioactivity-directed fractionation of the ethyl acetate extracts using different chromatographic methods allowed isolation of compounds **2, 4, 6, 11** and **12** from the bark of *Z. pancheri* subsp. *pancheri*, compounds **9** and **10** from the bark of *Z. pancheri*

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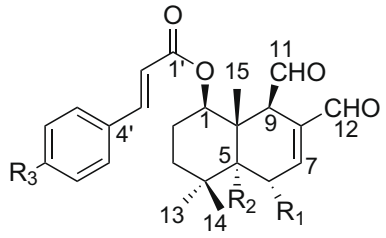
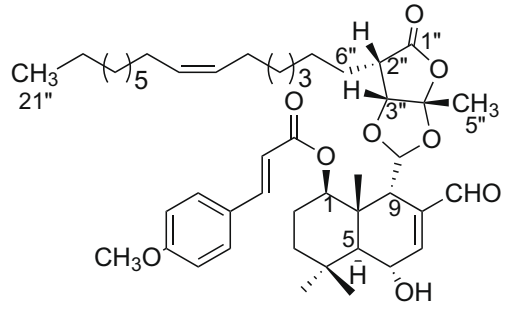
subsp. *elegans* and compounds **1, 5, 7, 8, 13–15** from the bark of *Z. acsmithii* (Fig. 1). Compound **3** was isolated from the leaves of *Z. pancheri* subsp. *pancheri* in a very good yield (0.18% w/w).

Compound **1,** obtained as a yellowish amorphous powder, possesses the same spectroscopic data (^{1}H NMR, ^{13}C NMR, IR, UV and MS) as those of isodrimanial isolated in our laboratory from the bark of *Z. baillonii* (Fomekong Fotsop et al., 2008). Accordingly, compound **1** is the 1β-*O*-(*p*-methoxy-*E*-cinnamoyl)-6α-hydroxypolygodial.

Compound **2**, obtained as a white amorphous powder, has the molecular formula $C_{24}H_{28}O_5$ as indicated by HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 419.1830 (calc. 419.1834). The ^{1}H and ^{13}C spectroscopic data indicated that compound **2** possesses the same polygodial moiety as compound **1**. NMR signals typical of a cinnamate ester group were recognised (Table 1) from previous work on **1** (Fomekong Fotsop et al., 2008). In the HMBC spectrum, correlations observed from the proton at δ_H 4.88 (1H, *dd*, *J* = 10.4, 4.6 Hz, H-1) to C-1′, C-2, C-3, C-9, C-10 and C-15 suggested that



1: R_1 = OH; R_2 = H; R_3 = OCH_3
2: R_1 = OH; R_2 = R_3 = H; 9-*epi*
3: R_1 = R_3 = H; R_2 = OH
4: R_1 = R_2 = R_3 = H



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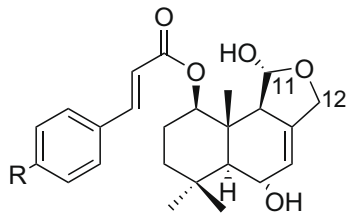
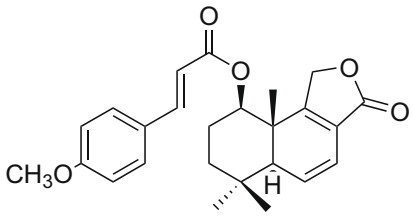
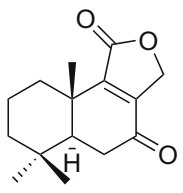


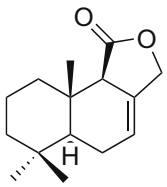
6: R = H
7: R = OCH_3



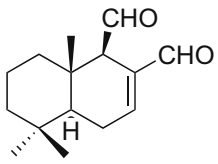
8: 1β-*E*-*O*-*p*-methoxycinnamoyl-bemadienolide



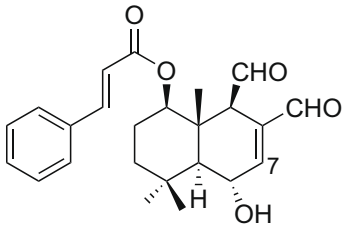
9: 7-ketoisodrimenin



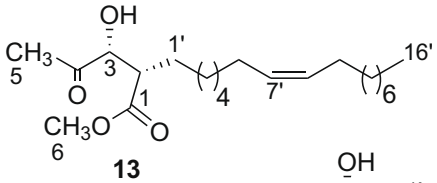
10: Drimenin



11: Polygodial



12: 1β-*E*-cinnamoyl-6α-hydroxypolygodial



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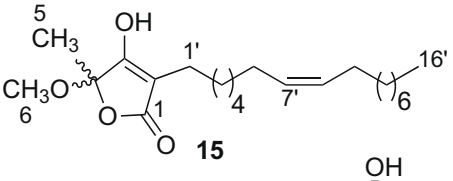

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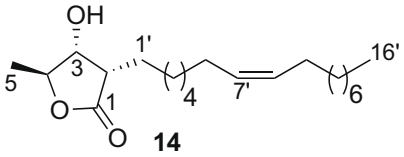

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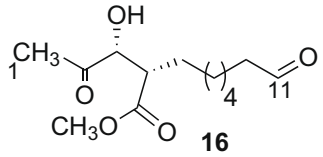

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Fig. 1. Structures of compounds **1**–**15** isolated from *Zygogynum* spp. and **16**.

Table 1
^{1}H NMR spectroscopic data (500 MHz, $CDCl_3$) for compounds **1**–**7**.

Position	**1**	**2**	**3**	**4**	**5**	**6**[a]	**7**
	δ_H (*J* in Hz)						
1	4.82 *dd* (11, 4)	4.88 *dd* (10.4, 4.6)	5.30 *dd* (11.6, 4.4)	4.86 *dd* (11, 4)	4.84 *dd* (11.2, 4.2)	4.73 *dd* (11, 4.2)	4.83 *dd* (11.4, 4.2)
2	1.79 *m*	1.75–1.86 *m*	1.67/1.94 *m*	1.67/1.87 *m*	1.79 *m*/2.02 *m*	1.67–1.80 *m*	1.69–1.86 *m*
3	1.52 *m*	1.45–1.53 *m*	1.32/2 *m*	1.54 *m*	1.52 *m*	1.47–1.59 *m*	1.45–1.62 *m*
5	1.41 *d* (10.2)	1.61 *d* (9.3)		1.40 *dd* (11, 5.4)	1.68 *d* (7.6)	1.37 *d* (10.7)	1.38 *d* (8.7)
6α			2.34 *ddd* (20, 5.6, 1.8)	2.31 *m*			
6β	4.68 *m*	4.52 *dd* (9.3, 2.9)	2.82 *ddd* (20, 2.7, 2.7)	2.51*m*	4.44 *m*	4.24 *m*	4.40 *bd* (8.7)
7	6.82 *m*	6.91 *d* (2.9)	6.87 *ddd* (5.6, 2.7, 1.5)	7.00 *m*	6.75 *d* (3.7)	5.30 *m*	5.61 *bs*
9	3.31 *d* (2.6)	3.65 *brs*	3.81 *m*	3.30 *d* (2.8)	2.98 *d* (4.4)	2.32 *bs*	2.46 *bs*
11	9.74 *d* (2.6)	9.85 *d* (1.6)	9.82 *d* (2.8)	9.78 *d* (2.8)	5.35 *d* (4.4)	5.48 *m*	5.57 *m*
12 α/β	9.35 *s*	9.46 *s*	9.33 *s*	9.31 *s*	9.51 *s*	4.13/3.76 *d* (11.6)	4.46/4.20 *d* (11.8)
13	1.20 *s*	1.19 *s*	1.02 *s*	0.94 *s*	1.20 *s*	1.16 *s*	1.19 *s*
14	1.15 *s*	1.12 *s*	1.19 *s*	0.99 *s*	1.09 *s*	1.08 *s*	1.12 *s*
15	1.08 *s*	1.15 *s*	1.13 *s*	1.05 *s*	1.04 *s*	0.97 *s*	1.06 *s*
2′	6.22 *d* (16)	6.50 *d* (16)	6.35 *d* (16)	6.35 *d* (16)	6.29 *d* (16)	6.40 *d* (16)	6.30 *d* (15.9)
3′	7.58 *d* (16)	7.74 *d* (16)	7.62 *d* (16)	7.62 *d* (16)	7.58 *d* (16)	7.60 *d* (16)	7.62 *d* (15.9)
5′, 9′	7.46 *d* (8.7)	7.56 *m*	7.51 *m*	7.51 *m*	7.49 *d* (8.7)	7.51 *m*	7.48 *d* (8.6)
6′, 8′	6.87 *d* (8.7)	7.41 *m*	7.36 *m*	7.36 *m*	6.92 *d* (8.7)	7.38 *m*	6.92 *d* (8.6)
7′		7.41 *m*	7.36 *m*	7.36 *m*		7.38 *m*	
5-OH			1.64 *bs*				
6-OH	1.97 *bs*	1.80 *bs*			1.97 *bs*	2.09 *bs*	1.97 *bs*
11-OH						4.56 *bs*	2.63 *bs*
OCH_3	3.81 *s*				3.85 *s*		3.85 *s*
2″					2.50 *ddd* (9.9, 5.4, 4.8)		
3″					4.31 *d* (5.4)		
5″					1.56 *s*		
6″					1.75/1.86 *m*		
7″					1.40 *m*		
11″, 14″					1.98 *m*		
12″, 13″					5.39 *m*		
8″–10‴, 13″–20″					1.20–1.30 *m*		
21″					0.89 *t* (7)		

[a] Chemical shifts obtained in $CDCl_3$ at 233 K on 600 MHz.

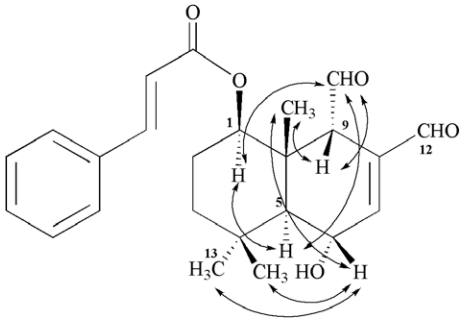

Fig. 2. Significant NOESY correlations observed for compound **2**.

the cinnamoyl group is located at C-1. The relative configuration of the five asymmetric carbons was established by analysis of the coupling constants and NOESY spectrum (Fig. 2): between H-1 and H-5, which showed these to be on the same face of the molecule and between H-6, Me-14 and Me-15, on the opposite face. This stereochemistry was also supported by the axial-axial coupling constants shown by both H-1 and H-6 (Table 1). In addition, the α-position of the aldehyde group at C-9 could be easily assigned by the observation of correlations between the aldehyde proton H-11 and protons H-1 and H-5, and between protons H-9 and Me-15 (Fig. 2). It is interesting to note that in contrast to compounds having a β-oriented aldehyde group, and similarly to compounds having a H-9 β-oriented as in compound **5**, strong correlations of proton H-9 with C-5, C-7, C-8, C-10, C-11, C-12 and C-15 were observed in the HMBC spectrum. Moreover a negative $[\alpha]_D$ value (−117° for **2**) was obtained for compounds having a β-oriented H-9, whereas a positive value is generally observed for compounds having an α-oriented H-9. Based on these data, compound **2** was established as 1β-*O*-*E*-cinnamoyl-6α-hydroxy-9-*epi*-polygodial.

Compound **3**, obtained as a yellow oil, has the same molecular formula ($C_{24}H_{28}O_5$) as compound **2** supported by HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 419.1830 (calc. 419.1834). From the analysis of the spectral data (UV, IR, ^{1}H and ^{13}C NMR), we deduced that compound **3** possessed the same skeleton as that of **2**. The main differences in the ^{1}H NMR spectrum of **3** were that the OH signal shifted from δ_H 1.80 in **2** to 1.64 in **3** and a methylene group at δ_H 2.34/2.82 replaced the oxymethine proton at δ_H 4.52 previously observed in **2**. The presence of signals for a quaternary carbon at δ_C 78.0 and a methylene group at δ_C 32.1 corresponding to C-5 and C-6, respectively, observed in the ^{13}C NMR spectrum, suggested that the hydroxyl group is located at C-5 in compound **3**. In the HMBC spectrum, long range correlations between the Me-13, Me-14 and Me-15 protons with H-6 to C-5 (δ_C 78.0), and from OH-5 to C-4, C-5, C-6 and C-10, confirmed the location of the hydroxyl group at C-5. In the NOESY spectrum, cross peaks between H-9, H-1 and OH-5 indicated that they are all axial and α-oriented. Other HMBC and NOESY correlations, which were very close to those of drimanial (Malheiros et al., 2001), confirmed the location and the relative configurations of the other groups. Compound **3** was thus established as 1β-*O*-*E*-cinnamoyl-5α-hydroxypolygodial.

Compound **4,** obtained as a brown oil, exhibits the molecular formula $C_{24}H_{28}O_4$ supported by HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 403.1879 (calc. 403.1885) suggesting the absence of the hydroxyl group at position 6 when compared with **2**. The ^{1}H and ^{13}C NMR spectroscopic data of compound **4** were similar to those of compound **2** except for the methine at C-5 (δ_H 1.40, *dd*, *J* = 11, 5.4 Hz, δ_C 48.8) and the presence of a methylene group at C-6 (δ_H 2.31/2.51, δ_C 24.4). Other HMBC and NOESY correlations confirmed

that compound **4** possessed the same chemical groups and the same relative configuration as compound **1**. Consequently, compound **4** was established as the 1β-*O*-*E*-cinnamoylpolygodial.

Compound **5** was obtained as a yellow oil. Its HRESIMS exhibits a pseudomolecular ion peak at *m/z* 785.4536, consistent with a molecular formula of $C_{46}H_{66}O_9Na$ (calc. 785.4605). The IR spectrum of **5** showed the characteristic absorption bands due to the presence of hydroxy (3440 cm^{-1}), lactone (1790 cm^{-1}), and ester (1698 cm^{-1}) groups. Parts of the ^1H and ^{13}C NMR spectroscopic data of compound **5** were almost identical to those of compound **1** indicating that **5** has the same drimane moiety as compound **1** but possesses a different side-chain located at position 9. The ^1H NMR data (Table 1) showed additional signals for two olefinic protons at δ_H 5.39 (2H, *m*, H-12″ and H-13″), two oxymethine protons at δ_H 4.31 (1H, *d*, *J* = 5.4 Hz, H-3″) and δ_H 5.35 (*d*, *J* = 4.4 Hz, H-11), one methine proton at δ_H 2.50 (1H, *ddd*, *J* = 9.9, 5.4, 4.8 Hz, H-2″), methylene groups at δ_H 1.40 (2H, *m*, H-7″), δ_H 1.751.86 (2H, *m*, H-6″), δ_H 1.98 (4H, *m*, H-11″ and H-14″) and δ_H 1.20–1.30 (18H, *m*, H-8″–H-10″, H-15″–H-20″), and methyl groups at δ_H 1.56 (3H, *s*, H-5″) and δ_H 0.89 (3H, *t*, *J* = 7 Hz, H-21″). Twenty two signals: one carbonyl (δ_C 175.0, C-1″), two olefinic carbons (δ_C 129.7, C-12″ and C-13″), one methine and one oxymethine carbons (δ_C 45.3 and 81.3, C-2″ and C-3″, respectively), and two acetal carbons, one methine and one quaternary carbons (δ_C 104.5 and 110.5, C-11 and C-4″, respectively), 13 methylene groups and two methyl groups at δ_C 14.1 and 20.2, C-21″ and C-5″, respectively, were observed in the ^{13}C NMR and DEPT spectra. The connectivities of these groups were derived from an analysis of the ^1H–^1H COSY, HMQC and HMBC NMR spectra. In the ^1H–^1H COSY spectrum, the signal at δ_H 2.50 (H-2″) showed cross peaks with H-3″ and the methylene group H_2-6″. In the HMBC spectrum (Fig. 3), correlations from proton H-3″ to C-1″, C-2″, C-4″, C-5″ and C-11, from H-2″ to C-6″ and C-7″, and from the methyl group H_3-5″ to C-3″ and C-4″ revealed the connectivities from C-1″ to C-7″ and suggested the presence of a trisubstituted γ-lactone. In addition, correlations from H-9 to C-5, C-7, C-8, C-10, C-11 and C-15 and from H-11 to C-9 and C-10 suggested that the trisubstituted γ-lactone is attached to C-11 forming an additional dioxolane ring. A 16 carbon side-chain linked to the C-2″ position of the lactone ring was deduced from the NMR (^1H, ^{13}C and 2D) and mass spectra (Ming et al., 2002). The position of a *cis* double bond between C-12″ and C-13″ in the side-chain, was deduced from biogenetic considerations. It is postulated that compound **5** could be formed by the condensation of the secobutanolide **13** having a *cis* Δ7′ (see below and Fig. 1), with 1β-(*p*-methoxycinnamoyl)-6α-hydroxy-9*epi*-polygodial. The 9α-orientation of the substituted γ-lactone moiety was confirmed by observation of NOESY correlations between protons H-1, H-5 and H-11, and between proton H-9 and the methyl group H_3-15. Finally, cross peaks between protons H-3″ and H-2″ and H_3-5″ suggested a *cis*-junction for the 3,4-dioxo-γ-lactone bicycle and an α-orientation for the alkenyl chain. Based on the above discussion, the structure of compound **5** was established as depicted in Fig. 1.

Compound **6** was obtained as an amorphous white powder. Its HRESIMS exhibits a pseudomolecular ion peak at *m/z* 421.1995, consistent with an elemental formula of $C_{24}H_{30}O_5Na$ (calc. 421.1991). The IR spectrum of **6** showed the presence of hydroxyl groups (broad band absorption at 3400 cm^{-1}) and one ester carbonyl function (1708 cm^{-1}). The ^1H and ^{13}C NMR spectroscopic data of compound **6** were highly comparable to those of **4** except for absence of signals related to the aldehyde groups. In the ^1H NMR spectrum, compound **6** exhibited an isolated asymmetrical oxymethylene group at δ_H 4.13 (H-12α) and 3.76 (H-12β) (each 1H, *d*, *J* = 11.6 Hz), an oxymethine group at δ_H 5.48 (1H, *m*, H-11) and hydroxyl group at δ_H 4.56 (1H, *bs*, 11-OH), which suggested the presence of a tetrahydrofuran-2-ol ring instead of the two dialdehyde groups observed in **4**. The presence of a hemiacetal carbon at C-11 was further supported by the downfield resonance at δ_C 99.7 observed in the ^{13}C NMR spectrum. The ^1H–^1H coupling observed between H-9 and H-11 in the COSY spectrum and correlations from H_2-12 to C-7 and C-8 in the HMBC spectrum confirmed that the additional ring is fused to the drimane moiety as depicted in Fig. 1. The α-orientation of the hydroxyl group at C-11 was deduced from correlations between H-11 and H_3-15 and between OH-11 and H-9 observed in the NOESY spectrum, obtained in $CDCl_3$ at a temperature of 233 K and using a mixing time of 100 ms (Fig. 4). In addition, the H-12α and H-12β protons were unambiguously assigned by the presence of a cross peak between protons H-9 (δ_H 2.32) and H-12α (δ_H 4.13). The relative configuration of **6** is coincident with the relative configuration of isodrimeninol (Fukuyama et al., 1982) and dendrocarbin C (Sakio et al., 2001). Thus, compound **6** is the 1β-*O*-*E*-cinnamoyl-6α-hydroxy-isodrimeninol.

Compound **7** was obtained as a colorless oil. Its molecular formula was determined as $C_{25}H_{32}O_6$ from the $[M+Na]^+$ molecular ion peak at *m/z* 451.2069 (calc. 451.2097) in the HRESIMS suggesting the presence of an additional methoxy group when compared with compound **6**. The ^1H and ^{13}C NMR spectroscopic data of compound **7** were similar to those of compound **6** except for the appearance of signals at δ_H 3.85 (3H, *s*) and at δ_C 55.3 in the ^1H and ^{13}C NMR spectra, respectively, confirming the presence of the methoxy group. The downfield shift of the C-7′ signal from δ_C 130.3 in **6** to δ_C 161.4 in **7** supported the position of the methoxy group at C-7′. These data, together with other results of COSY, NOESY and HMBC analysis, confirmed the structure of compound **7**, which was deduced to be 1β-*O*-*p*-methoxy-*E*-cinnamoyl-6α-hydroxy-isodrimeninol.

The spectroscopic data of compound **8** were similar to those of 1β-*E*-*O*-*p*-methoxycinnamoyl-bemadienolide recently isolated from *Z. bailloni* (Fomekong Fotsop et al., 2008). As has been suggested for *Z. bailloni*, the lactone **8** may be formed from compound **1** by a dehydration process followed by a hydride transfer in a Tish-

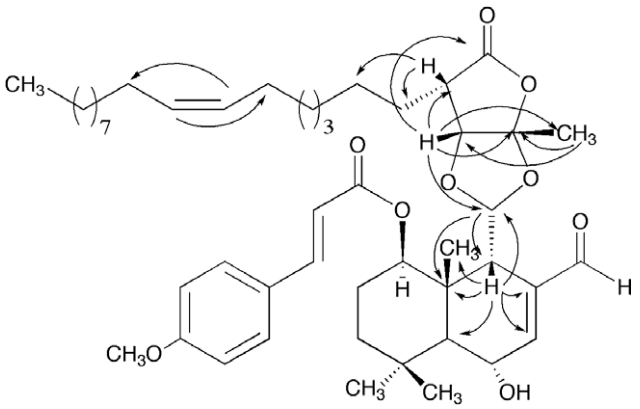

Fig. 3. Significant HMBC correlations observed for compound **5**.

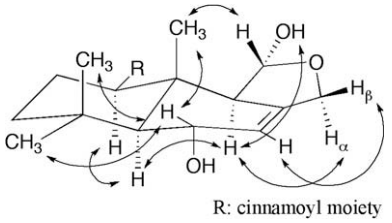

Fig. 4. Significant NOESY correlations observed for compound **6**.

chenko-type reaction (Fomekong Fotsop et al., 2008). This reaction may also occur in the plant.

7-Ketoisodrimenin (**9**) (Harrigan et al., 1993), drimenin (**10**) (Brown, 1994), polygodial (**11**) (Mashimbye et al., 1999) and 1-β-*O*-*E*-cinnamoyl-6α-hydroxy-polygodial (**12**) (Larsen et al., 2007) were identified by spectroscopic methods (^{1}H NMR, ^{13}C NMR, IR, UV and MS) and by comparison with data reported in the literature.

Compound **13** was obtained as a colorless oil. Its HRESIMS exhibited a pseudomolecular ion peak at *m/z* 391.2805, consistent with a molecular formula of $C_{22}H_{40}O_4Na$ (calc. 391.2824). The IR spectrum showed the presence of hydroxyl groups (broad band absorption at 3446 cm^{-1}), one ester carbonyl function (1732 cm^{-1}) and a ketone group (1714 cm^{-1}) suggesting that compound **13** is a secobutanolide (Chen et al., 2005). The ^{1}H NMR data of **1** (Table 3) exhibited signals for a hydroxymethine proton at δ_H 4.13 (1H, *dd*, *J* = 6.5, 2.8 Hz, H-3), a methine proton at δ_H 2.94 (1H, *ddd*, *J* = 7.5, 7.5, 2.8 Hz, H-2), a hydroxyl group at δ_H 3.69 (1H, *d*, *J* = 6.5 Hz), a methoxy group at δ_H 3.65 (3H, *s*, H-6), and an acetyl group at δ_H 2.29 (3H, *s*, H-5). The connectivities of these groups were derived from an analysis of the ^{1}H–^{1}H COSY, HMQC and HMBC NMR spectra. In the ^{1}H–^{1}H COSY, the double doublet at δ_H 4.13 (H-3) showed cross peaks with the protons at δ_H 3.69 (OH-3) and at δ_H 2.94 (H-2). The latter also coupled with the two multiplets at δ_H 1.70 and 1.90 (H_2-1′). In the HMBC spectrum, correlations from the methoxy protons H_3-6 to C-1, from the methyl protons H_3-5 to C-4, and from the proton H-2 to C-1, C-3, C-4, C-1′ and C-2′ confirmed that compound **13** is a secobutanolide. The superimposable signals at δ_H 5.34/5.35 (2H, *m*) in the ^{1}H NMR spectrum and the presence of signals at δ 129.6 and 130.0 in the ^{13}C NMR spectrum indicated that a double bond was present in the side-chain. A coupling constant $J_{H7'-H8'}$ of 10.9 Hz was observed for the olefinic protons by a decoupling experiment indicating a *cis* geometry for Δ7′. The (*Z*) configuration was confirmed by the chemical shifts of the methylene carbons flanking the double bond C-6′ and C-9′ (δ_C 27.2) which compared favorably with the literature values (Anderson et al., 1992). Ozonolysis of compound **13** was undertaken and allowed localization of the double bond on the side-chain. The ozonolysis was followed by a reduction using dimethyl sulfide resulting in the formation of the carbonyl compound **16** (see experimental part). Its HRESIMS exhibited a pseudomolecular ion peak at *m/z* 281.1357, consistent with the molecular formula $C_{13}H_{22}O_5Na$ (calc. 281.1365) indicating the presence of respectively six and seven methylene groups on either each side of the double bond in compound **13**. The 1D and 2D NMR spectroscopic data of compound **16** unambiguously confirmed the location of the double bond at C-7′ in compound **13**. Finally, assignment of the relative stereochemistry of the two asymmetric carbons was accomplished by analyses of the coupling constant and NOESY spectrum. The coupling constant J_{H2-H3} (2.8 Hz) as well as the presence of a cross peak between protons H-2 and H-3 suggested a *cis* relationship between them. Accordingly, compound **13** was assigned the structure (2S^*,3R^*)-methyl 3-hydroxy-2-((*Z*)-hexadec-7-enyl)-4-oxopentanoate.

Compound **14**, obtained as a colorless oil, had the molecular formula $C_{21}H_{38}O_3$ as indicated by HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 361.2742 (calc. 361.2719). Analysis of the IR spectrum of **14** suggested the presence of a five-membered lactone (1750 cm^{-1}) and a hydroxyl group (3450 cm^{-1}). The EIMS fragmentation and the ^{1}H and ^{13}C spectroscopic data indicated that compound **14** possessed the same side-chain as that observed in compound **13**. On the other hand, the ^{1}H NMR spectrum of **14** displayed signals for two oxymethine protons at δ_H 4.20 (1H, *dd*, *J* = 5.5, 5.5 Hz, H-3) and δ_H 4.52 (1H, *qd*, *J* = 6.7, 5.5 Hz, H-4) and a methyl group at δ_H 1.35 (3H, *d*, *J* = 6.7 Hz, H-5). These signals were similar to those found in the γ-lactone of the butanolides isolated from *Iryanthera* and *Virola* species (Lopes et al., 1998; Ming et al., 2002). The analysis of the ^{1}H-^{1}H COSY, HMQC and HMBC NMR spectra confirmed that the secobutanolide moiety observed in compound **13** was changed into a trisubstituted γ-lactone in the case of compound **14.** The relative configuration of **14** was determined by comparison of its ^{13}C NMR with those described in the literature (Lopes et al., 1998). It was established that the chemical shift of the methyl group at C-4 is approximately δ_C 13.8 ± 0.2 when it is *cis* to the hydroxyl group at C-3, and approximately δ_C 18.0 ± 0.2 when it is *trans*. The same result was deduced from the chemical shift of CH_2 at C-1′, the signal appearing at approximately δ_C 23.2 ± 0.2 when it is *cis* to the hydroxyl group and at about δ_C 27.8 ± 0.6 when it is *trans*. It can be concluded that the C-5 methyl (δ_C 17.9) and the C-1′ methylene (δ_C 23.1) are *trans* and *cis* to the hydroxyl group, respectively, for compound **14**. This relative configuration was further confirmed by correlations between H-3, H-2 and the H_3-5 methyl in the NOESY spectrum. Based on the above discussion, compound **14** was identified as (2S^*, 3R^*, 4S^*)-2-hexadec-7(*Z*)-enyl-3-hydroxy-4-methyl-butanolide.

Compound **15** was obtained as a colorless oil. Its molecular formula was determined as $C_{22}H_{38}O_4$ from the pseudomolecular ion peak $[M+Na]^+$ at *m/z* 389.2747 (calc. 389.2668) in the HRESIMS. The IR spectrum showed absorption bands for a hydroxyl group at 3400 cm^{-1} and an α,β-unsaturated-γ-lactone at 1730 cm^{-1}. The presence of the α,β-unsaturated γ-lactone moiety was further supported by a strong UV absorption at 242 nm (Chen et al., 2005). The ^{1}H and ^{13}C NMR spectroscopic data of compound **15** were close to those of **14** indicating that this compound has a butanolide skeleton with the same alkenyl side-chain. However, H-2 and H-3 signals were absent in the ^{1}H NMR spectrum of **15** and the C-2 and C-3 chemical shifts changed from δ_C 43.5 and 73.7 in **14** to 104.9 and 168.5 in **15**, in the ^{13}C NMR spectrum (Table 3), suggesting the presence of an additional double bond in the γ-lactone moiety of compound **15**. Moreover, the presence of an additional methoxy group is suggested by signals at δ_H 3.23 (3H, *s*) and at δ_C 50.8 displayed in the ^{1}H and ^{13}C NMR spectra, respectively. The long range correlations from the methyl protons H_3-5 and the methoxy protons H_3-6 to C-4 and from the protons H_2-1′ and H_3-5 to C-3 in the HMBC spectrum allowed localization of both methyl and methoxy groups on C-4. The relative configuration at C-4 remained unknown. The above spectroscopic evidence strongly support the structural assignment of compound **15** to be (*E*)-3-(hexadec-7-enyl)-4-hydroxy-5-methoxy-5-methylfuran-2(5*H*)-one.

2.1. Biological activity

Compounds **1–15** were subjected to a cytotoxic assay against KB, HL60 and HCT116 cancer cell lines. As shown in Table 4, only compounds **1–4**, **11** and **12** were active against the cell lines (IC_{50} around 1 μM). These results confirm that the presence of the dialdehyde function is essential for a strong cytotoxicity.

3. Conclusion

Results of the current study and those previously reported by our group indicate that drimane sesquiterpenoids are the main constituents present in the bark and the leaves of various species of the genus *Zygogynum*. Although these molecules have been encountered occasionally in other plants or animals, they could be considered as chemical markers of this primitive family, related to the Magnoliale order. To the best of our knowledge, this is the first time that natural drimanes bearing cinnamoyl groups and having a β-oriented H-9 have been isolated and identified from higher plants. Secobutanolide **13** and butanolides **14** and **15** consti-

tute the first report of aliphatic γ-lactones in a species of the Winteraceae family.

4. Experimental

4.1. General experimental procedures

The NMR spectra were recorded with a Bruker 500 and 600 MHz (Avance 500 and 600) spectrometer. Samples were dissolved in $CDCl_3$ and values are reported in δ (ppm) downfield from TMS. ESIMS was obtained on a Navigator Mass Thermoquest apparatus. HRESIMS were run on a MALDI-TOF spectrometer (Voyager-De STR; Perspective Biosystems). A Kromasil preparative C-18 column (250 × 21.2 mm I.D., 5 μm, Thermo®) was used for preparative HPLC separations using a Waters autopurification system equipped with a binary pump (Waters 2525), a UV–vis diode array detector (190–600 nm, Waters 2996) and a PL-ELS 1000 ELSD detector Polymer Laboratory. IR spectra were obtained on a Nicolet FTIR 205 spectrophotometer. The UV spectra were recorded on a Perkin-Elmer Lamba 5 spectrophotometer. Optical rotations and CD spectra at 22 °C were measured on a JASCO P-1010 polarimeter and a JASCO J-810 spectrophotometer, respectively. Silica gel 60 (35–70 μm) and analytical TLC plates (Si gel 60 F-254) were purchased from SDS (France). Preparative TLC plates (Silica gel 60 F-254) were purchased from Merck. All other chemicals and solvents were analytical grade and used without further purification.

4.2. Plant material

The barks and leaves of *Z. pancheri* subsp. *pancheri, Z. pancheri* subsp. *elegans* and *Z. acsmithii* were collected in various rain forests of the main island of New Caledonia (“Nodela” and “Tchingou”, North Province; “Goro”, South Province, respectively) by one of us (V.D.). The corresponding voucher specimens (DUM-578, DUM-220, DUM-197, respectively) are kept at the Herbarium of the Botanical and Tropical Ecology Department of the IRD Center, Noumea, New Caledonia.

4.3. Extraction and isolation

The milled and dried barks of *Z. pancheri* subsp. *pancheri* (440 g), *Z. pancheri* subsp. *elegans* (200 g) and *Z. acsmithii* (200 g) were extracted by maceration in EtOAc (3 × 2 l) at room temperature. The combined extracts related to each species were concentrated *in vacuo* at 40 °C to yield 17 g (extract A), 4.2 g (extract B) and 3.3 g (extract C) of residues, respectively. The leaves of *Z. pancheri* subsp. *pancheri* (540 g) were extracted using the same procedure to afford 8.5 g of extract D. Extract A was subjected to a flash chromatography on reversed-phase (VersaPak™, C-18 Cartridge 40 × 75 mm) using a gradient mobile phase consisting of H_2O-MeCN 80:20 to 0:100 at 10 ml/min to give 10 fractions (A1–A10). Extract C was submitted to silica gel chromatography (35–70 μm) using a gradient of *n*-heptane-EtOAc from 100:0 to 0:100 to give 14 fractions (C1–C14). The same procedure was used on 0.85 g of extract B to give 19 fractions (B1–B19). The combined fractions A3 + A4 (3.59 g) were submitted to silica gel column chromatography (35–70 μm) using a gradient of *n*-heptane-EtOAc from 100:0 to 0:100, to give compounds **2** (65 mg, 0.014% w/w), **6** (55 mg, 0.012% w/w), polygodial **11** (63 mg, 0.014% w/w) and 1β-*E*-cinnamoyl-6α-hydroxypolygodial **12** (1.88 g, 0.42% w/w). Fraction A5 (1.03 g) was subjected to preparative HPLC (C-18 Kromasil column, 250 × 21.2 mm I.D., 5 μm) using an isocratic elution by H_2O/MeCN (4:6) at a flow rate of 21 ml/min, leading to the isolation of compound **4** (210 mg, 0.047% w/w, t_R 25 min). Fraction C6 (100 mg) was purified by preparative TLC (*n*-heptane-EtOAc, 6:4) to yield **13** (80 mg, 0.04% w/w, R_f 0.67). Fraction C7 (65 mg) was purified by preparative TLC (*n*-heptane-EtOAc, 6:4) to yield **8** (31 mg, 0.015% w/w, R_f 0.57). Fraction C8 (91 mg) was purified by preparative TLC (*n*-heptane-EtOAc, 5:5) to yield **14** (16 mg, 0.008% w/w, R_f 0.55). Preparative HPLC using an isocratic elution by H_2O/MeCN (5:5) at a flow rate of 21 ml/min yielded compound **5** (9 mg, 0.0045% w/w, t_R 20 min) from C9 (0.41 g) and compound **1** (324 mg, 0.16% w/w, t_R 24 min) from C11 (775 mg). Fraction C10 (142 mg) was purified by preparative TLC (*n*-heptane-EtOAc, 5:5) to yield **15** (7 mg, 0.0035% w/w, R_f 0.40). Fraction C12 (62 mg) was purified by preparative TLC (*n*-heptane-EtOAc, 2:8) to yield **7** (4 mg, 0.002% w/w, R_f 0.46). Fractions B4 (15 mg) and B6 (95 mg) were subjected to preparative HPLC using an isocratic elution by H_2O/MeCN (5:5) at a flow rate of 21 ml/min, leading to the isolation of compounds **10** (drimenin, 4.5 mg, 0.011% w/w, t_R 17.8 min) and **9** (7-ketoisodrimenin, 5 mg, 0.012% w/w, t_R 20 min), respectively. Extract D was subjected to flash chromatography on reversed-phase (VersaPak™, C-18 Cartridge 40 × 75 mm) using a gradient mobile phase consisting of H_2O-MeCN 80:20 to 0:100 at 10 ml/min to give seven fractions (D1–D7). Fraction D5 (1.39 g) was submitted to silica gel column chromatography (35–70 μm), using a gradient of *n*-heptane- EtOAc from 100:0 to 0:100, to give compound **3** (935 mg, 0.18% w/w).

4.3.1. Compound **2** (1β-O-E-cinnamoyl-6α-hydroxy-9-epi-polygodial)

White amorphous powder; $[\alpha]_D^{22°}$: −117° (*c* 0.25, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3445, 2952, 1708, 1682, 1635 cm^{-1}; UV ($CHCl_3$) λ_{max} ($\log\varepsilon$) 223 nm (4.6), 275 nm (4.6); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2, respectively. HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 419.1830 (calc. 419.1834).

Table 2
^{13}C NMR spectroscopic data (125 MHz, $CDCl_3$) for compounds **1–7**.

Position	1	2	3	4	5	6	7
1	80.8	76.3	77.3	81.6	75.4	80.7	80.3
2	23.9	24.0	24.0	24.1	23.6	24.5	24.6
3	40.5	40.0	34.3	39.2	39.8	40.6	40.6
4	33.2	33.1	38.0	32.7	33.3	33.2	33.2
5	55.9	51.2	78.0	48.8	51.6	57.6	57.7
6	68.1	67.9	32.1	24.4	67.7	68.0	68.2
7	153.4	152.4	148.1	151.9	149.1	120.1	120.0
8	139.7	137.0	141.2	140.2	138.4	139.5	139.9
9	58.4	54.0	55.0	59.2	45.1	60.1	60.2
10	44.9	44.3	46.6	42.2	42.0	42.4	42.0
11	199.8	201.6	201.2	200.2	104.5	99.7	99.6
12	192.3	192.6	192.1	192.2	192.9	68.0	68.1
13	35.9	34.8	27.1	32.5	34.5	35.2	35.2
14	22.8	22.5	25.5	22.1	22.5	22.4	22.3
15	11.4	17.2	13.5	10.6	17.9	10.8	10.8
1′	166.2	166.2	166.0	166.0	166.0	166.2	166.4
2′	114.9	117.7	117.8	117.8	115.9	118.5	115.9
3′	145.6	145.7	145.7	145.6	144.1	144.4	144.1
4′	128.6	134.1	134.1	134.1	127.1	134.3	127.0
5′,9′	130.0	128.2	128.2	128.2	129.7	128.7	129.7
6′,8′	114.3	128.9	128.8	128.8	114.3	128.8	114.3
7′	161.6	130.5	130.4	130.4	161.3	130.3	161.4
OCH_3	55.3				55.3		55.3
1″					175.0		
2″					45.3		
3″					81.3		
4″					110.5		
5″					20.2		
6″					24.5		
7″					27.9		
8″–10″					29.2–29.7		
11″,14″					27.3		
12″,13″					129.7		
15″–18″					29.2–29.7		
19″					31.9		
20″					22.7		
21″					14.1		

*4.3.2. Compound **3** (1β-O-E-cinnamoyl-5α-hydroxypolygodial)*

Yellow oil; $[\alpha]_D^{22°}$: +71.1° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3440, 2950, 1710, 1668, 1639 cm^{-1}; UV ($CHCl_3$) λ_{max} ($\log\varepsilon$) 236 nm (4.07), 282 nm (4.16); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2, respectively. HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 419.1830 (calc. 419.1834).

*4.3.3. Compound **4** (1β-O-E-cinnamoylpolygodial)*

Brown oil; $[\alpha]_D^{22°}$: +53.3° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2952, 1708, 1681, 1636 cm^{-1}; UV ($CHCl_3$) λ_{max} ($\log\varepsilon$) 236 nm (4.01), 282 nm (4.18); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2, respectively. HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 403.1879 (calc. 403.1885).

*4.3.4. Compound **5***

Yellow oil; $[\alpha]_D^{22°}$: −74° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3440, 2920, 1790, 1698 cm^{-1}; UV ($CHCl_3$) λ_{max} ($\log\varepsilon$) 240 nm (4.03), 311 nm (4.32); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2, respectively. HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 785.4536 (calc. 785.4605).

*4.3.5. Compound **6** (1β-O-E-cinnamoyl-6α-hydroxy-isodrimeninol)*

Amorphous white powder; $[\alpha]_D^{22°}$: +54.3° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3409, 2949, 1708, 1637 cm^{-1}; UV ($CHCl_3$) λ_{max} ($\log\varepsilon$) 235 nm (4.06), 280 nm (4.15); ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2, respectively. HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 421.1995 (calc. 421.1991).

*4.3.6. Compound **7** (1β-O-p-methoxy-E-cinnamoyl-6α-hydroxy-isodrimeninol)*

Colorless oil; $[\alpha]_D^{22°}$: +28.2° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3452, 1705, 1603 cm^{-1}; UV ($CHCl_3$) λ_{max} ($\log\varepsilon$) 240 nm (3.79), 311 nm (4.8); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2, respectively. HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 451.2069 (calc. 451.2097).

*4.3.7. Compound **13** (2S*,3R*)-methyl 3-hydroxy-2-((Z)-hexadec-7-enyl)-4-oxopentanoate*

Colorless oil; $[\alpha]_D^{22°}$: −59.8° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3446, 1732, 1714 cm^{-1}; UV ($CHCl_3$) λ_{max} ($\log\varepsilon$) 242 nm (3.12), 275 nm (2.52); ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR, ($CDCl_3$, 150 MHz) see Table 3. HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 391.2805 (calc. 391.2824).

*4.3.8. Compound **14** (2S*, 3R*, 4S*)-2-hexadec-7(Z)-enyl-3-hydroxy-4-methyl-butanolide*

Colorless oil; $[\alpha]_D^{22°}$: +63.2° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3450, 1750 cm^{-1}; UV ($CHCl_3$) λ_{max} ($\log\varepsilon$) 240 nm (2.96), 312 nm (3.01); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR, ($CDCl_3$, 125 MHz) see Table 3. HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 361.2742 (calc. 361.2719).

*4.3.9. Compound **15** (E)-3-(hexadec-7(Z)-enyl)-4-hydroxy-5-methoxy-5-methylfuran-2(5H)-one*

Colorless oil; $[\alpha]_D^{22°}$: −26.8° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3400, 1768, 1730, 1668 cm^{-1}; UV ($CHCl_3$) λ_{max} ($\log\varepsilon$) 242 nm (4.26), 276 nm (3.86), 380 nm (3.73); ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR, ($CDCl_3$, 125 MHz) see Table 3. HRESIMS of the $[M+Na]^+$ ion peak at *m/z* 389.2747 (calc. 389.2668).

*4.3.10. Compound **16** methyl 2-(1-hydroxy-2-oxopropyl)-9-oxononanoate*

Compound **13** (4.6 mg; 0.0125 mmol) was dissolved in 5 ml of MeOH and the solution was cooled to −78 °C. Ozonolysis (Ozone generator BMT 802×, flow rate 0.1 Nl/min) was continued until compound **13** had completely reacted (Sudan III from red to colorless, approximatively 2 min). An excess of dimethyl sulfide (100 μl, 78 mg; 1.25 mmol) was then added and the reaction mixture was stirred for 20 h at room temperature. The solvent was evaporated under reduced pressure. The residue was then dissolved in MeOH and purified on a C-18 cartridge (Chromabond® C-18) to afford compound **16** (1.8 mg); $[\alpha]_D^{22°}$: −9.3° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz): 1.3–1.4 (H_2-6, H_2-7, H_2-8, *m*); 1.6–1.7 (H_2-9, *m*); 1.7, 1.9 (H_2-5, *m*); 2.30 (H_3-1, *s*); 2.4 (H_2-10, *td*, *J* = 7.5, 1.6 Hz); 2.94 (H-4, *td*, *J* = 7.3, 2.7 Hz); 3.66 (H_3-13, *s*); 3.70 (OH, *brs*); 4.10 (H-3, *d*, *J* = 2.7 Hz); 9.78 (H-11, *t*, *J* = 1.6 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): 21.9 (C-9); 25.7 (C-1); 27.3 (C-6); 28.3 (C-5); 28.9 (C-8); 29.2 (C-7); 43.8 (C-10); 47.8 (C-4); 51.9 (C-13); 77.5

Table 4
In vitro cytotoxic activity of compounds **1**–**15** (IC50 in μM).

	1	**2**	**3**	**4**	**6**	**11**	**12**	**13**	**5, 7–10, 14, 15**
KB	0.5	0.5	0.4	0.4	10	1	0.4	4	>10
HL60	0.5	0.3	0.3	0.2	8	1.2	0.3	NT	NT
HCT116	0.2	0.2	0.1	0.1	3	0.7	0.1	NT	NT

Table 3
^1H NMR and ^{13}C NMR data for compounds **13**–**15**[a].

Position	**13**		**14**		**15**	
	δ_H (mult., *J*/Hz)	δ_C	δ_H (mult., *J*/Hz)	δ_C	δ_H (mult., *J*/Hz)	δ_C
1	–	173.3	–	176.9	–	171.6
2	2.94 (*ddd*, 7.5, 7.5, 2.8)	47.8	2.61 (*ddd*, 9.9, 5.5, 5.5)	43.5	–	104.9
3	4.13 (*dd*, 6.5, 2.8)	77.4	4.20 (*dd*, 5.5, 5.5)	73.7	–	168.5
4	–	208.8	4.52 (*qd*, 6.7, 5.5)	82.1	–	103.1
5	2.29 (*s*)	25.6	1.35 (*d*, 6.7)	17.9	1.65 (*s*)	22.5
6	3.65 (*s*)	51.8	–	–	3.23 (*s*)	50.8
1′	1.70/1.90 (*m*)	28.3	1.66/1.81 (*m*)	23.1	2.22 (*t*, 7.7)	21.2
2′	1.39 (*m*)	27.4	1.46 (*m*)	27.4	1.53 (*m*)	27.6
6′, 9′	2.02 (*m*)	27.2	2.03 (*m*)	27.0	1.98 (*m*)	27.2
7′	5.34[b] (*m*)	129.6[b]	5.35 (*m*)	129.4[b]	5.34 (*m*)	129.6[b]
8′	5.35[b] (*m*)	130.0[b]	5.35 (*m*)	129.9[b]	5.34 (*m*)	130.0[b]
3′–5′, 10′–13′	1.24/1.38 (*m*)	29.0–29.7	1.24/1.32 (*m*)	28.8–29.5	1.24/1.32 (*m*)	29–29.7
14′	1.24 (*m*)	31.9	1.24 (*m*)	31.7	1.24 (*m*)	31.8
15′	1.29 (*m*)	22.6	1.31 (*m*)	22.5	1.24 (*m*)	22.6
16′	0.88 (*t*, 6.8)	14.0	0.88 (*t*, 6.9)	13.9	0.89 (*t*, 7)	14.0
OH	3.69 (*d*, 6.5)	–	–	–	–	–

[a] Spectra were recorded in $CDCl_{13}$; chemical shifts are reported as δ values (ppm) from TMS at 500 MHz for 1H and 125 MHz for ^{13}C. (compounds 14 and 15) and at 600 MHz for 1H and 150 MHz for ^{13}C (compound 13).

[b] Assignments could be interchanged.

(C-3); 173.3 (C-12); 202.7 (C-11); 208.7 (C-2). HRESIMS 221.1357 (calc. for $C_{13}H_{22}O_5Na$, 281.1365).

4.4. Cell culture assay for cytotoxicity activity

The human KB (mouth epidermoid carcinoma), HCT116 (colon) and HL60 (promyelocytic leukemia) cancer cell lines were originally obtained from the ATCC. The cytotoxicity assays were performed according to a published procedure (Tempete et al., 1995). Taxotere was used for a control experiment.

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