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Alkaloids from *Dictyoloma vandellianum*: their chemosystematic significance

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Abstract

The dichloromethane extract from leaves of *Dictyoloma vandellianum* afforded five alkaloids 2-(14′-hydroxy-14′,15′-dimethylhexadecanyl)-4-quinolone, 2-(12′-hydroxy-12′-methyltridecanyl)-3-methoxy-4-quinolone, 2-(12′-hydroxy-12′-methyltridecanyl)-4-quinolone, 2-(14′-hydroxy-14′,15′-dimethylhexadecanyl)-3-methoxy-4-quinolone, 6-methoxydictyolomide A, besides the known alkaloid 8-methoxyflindersine and β-sitosterol. The presence of 2-alkyl-4(1*H*)-quinolones in *D. vandellianum* shows strong similarities with the Zanthoxyleae, which contains several 2-alkyl-4-quinolones. Thus, the Dictyolomatoideae apparently occupies a position between the proto-Rutaceae genera and the Spathelioideae, but close to the Zanthoxyleae.

Keywords: Rutaceae; *Dictyoloma vandellianum*; 2-Alkyl-4(1*H*)-quinolones; Chemosystematics

1. Introduction

Dictyoloma Juss. contains only two species, *D. vandellianum* Adr. Juss. (syn. *D. incanescens* DC), which occurs in Brazil, and *D. peruvianum* from Peru (Engler, 1874, 1931; Macbride, 1949). The taxonomy of this genus has been doubtful since its initial description in 1825, when Jussieu classified it in the Rutaceae, assigning it to the tribe Zanthoxyleae. According to the concept of Bentham and Hooker (1862), *Dictyoloma* belongs to the Simaroubaceae "ordo". Engler (1874) first included this genus in the Simaroubaceae, but later removed it to the Rutaceae, classifying *Dictyoloma* as the single genus in the subfamily Dictyolomatoideae (von Engler, 1931).

D. vandellianum is known to contain indole alkaloids, 2-quinolinone alkaloids, prenylated chromones, and limonoids (Vieira et al., 1988, 1990; Campos et al., 1987). The occurrence of these compounds was important for systematic classification of the genus in the Rutaceae family. In this paper the isolation and identification of five 2-alkyl-4(1*H*)-quinolones from the leaves of *D. vandellianum* are described. The chemosystematic significance of the presence of limonoids, alkaloids, and chromones in *Dictyoloma* in providing a better understanding of the phylgenetic relationships in the Rutales is also discussed.

2. Results and discussion

The dichloromethane extract from the leaves of *D. vandellianum* afforded five alkaloids **1**–**5**, besides the known alkaloid 8-methoxyflindersine (Auzi et al., 1997) and β-sitosterol.

Compound **1** showed the spectral characteristics of a 2-alkyl-4(1*H*)-quinolone (Tang et al., 1996). Elemental analysis and ESI-MS indicated the molecular formula to be $C_{27}H_{43}NO_2$. The IR spectrum showed a weak α,β-unsaturated carbonyl band at 1634 cm^{-1}, which can be explained by the carbonyl group being partially enolized. The UV absorption maximum at 236, 314 and 325 nm suggested that N in the 4(1*H*)-quinolone system was unsubstituted, when compared with 2-tridecyl-4(1*H*)-quinolone (**6**) and 1-methyl-2-dodecyl-4(1*H*)-quinolone (Tang et al., 1996). The ^1H NMR spectrum (Table 1), in

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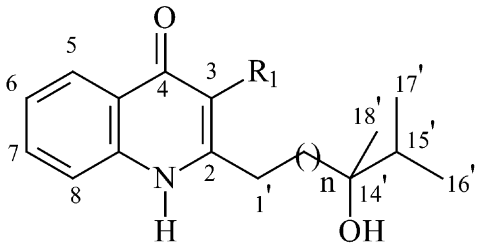

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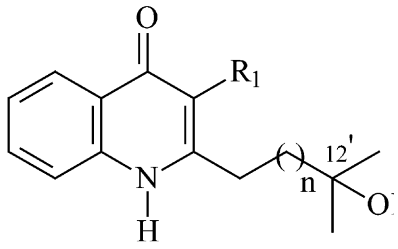

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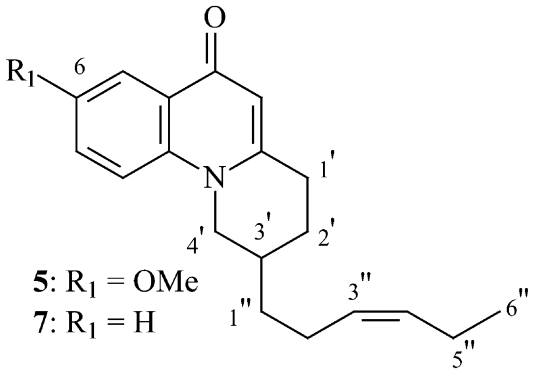

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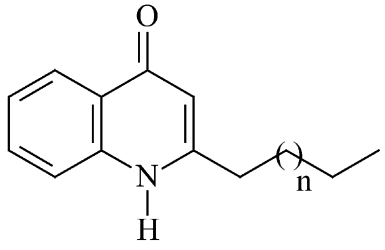

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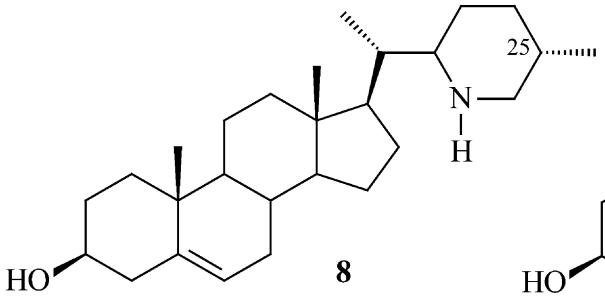

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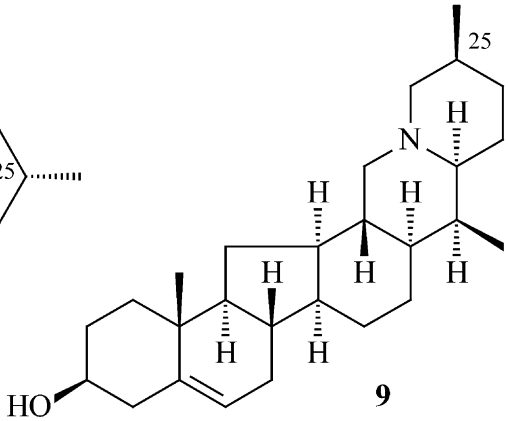

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addition to signals typical of a 4(1*H*)-quinolone (δ 6.22, *s*, H-3; four aromatic protons at δ 8.35, *d*, J = 8.0 Hz, H-5; 7.32, *dt*, J = 8.0 and 1.8 Hz, H-6; 7.56, *dt*, J = 8.0 and 1.5 Hz, H-7; 7.60, *dd*, J = 8.0 and 1.8 Hz, H-8; thus an unsubstituted A ring), revealed a methyl singlet at δ 1.40 and two methyl doublets at δ 1.20 and 1.12 (J = 7.0 Hz). The two methyl doublets were coupled to the ^{1}H signal at δ 2.45 (*sept*, J = 7.0 Hz), thus placing an isopropyl group at the end of the side chain. The ^{13}C NMR spectrum (Table 2) revealed resonances for C-2–C-8 in close agreement with those for 2-tridecyl-4(1*H*)-quinolone (**6**) (Tang et al., 1996), with an aliphatic chain of 16 carbon atoms. The two methyl doublets at δ 1.20 and 1.12 and the ^{1}H signal at δ 2.45 showed one-bond correlations (HSQC) with the ^{13}C signals at δ 19.0 and δ 35.0, permitting the assignment of these signals to isopropyl group as Me-16/Me-17 and CH-15, respectively. HSQC experiments also showed a correlation from methyl singlet at δ 1.40 to the ^{13}C signal at δ 26.1. Hydroxyl and methyl groups must be connected at the same carbon in the side chain, considering the observed signal for a hydroxytertiary carbon at δ 82.2. This was supported by the ESI-MS data which showed fragments at m/z 326 $[C_{27}H_{43}NO_2 + H - C_5H_{12}O]^+$ (40%) due to fission of the side chain between C-13′ and C-14′, thus indicating the hydroxyl and methyl groups to be located α (at C-14′) to the isopropyl group. The signals at δ 26.1 and δ 82.2 were then assigned to C-18′ and C-14′, respectively. The downfield signal at δ 2.64 (*t*, J = 7.8 Hz) from the methylene H_2-1′ was consistent with its situation being α to the 4(1*H*)-quinolone system. The observed correlation between this signal and the ^{13}C signal at δ 34.3 led to its assignment to C-1′. Moreover, the ^{13}C NMR spectrum also showed a downfield signal at δ 40.1, which was consistent for a methylene group with an electronegative element nearby; thus it was

assigned to C-13′. The slightly negative γ effect of the hydroxyl group permitted the assignment of C-11′ at δ 23.3. However, the signals at 28.8–29.6 for 10 methylenes (C-2′–C-10′ and C-12′) were less readily assignable; indeed, the assignments may be interchanged with each other. In addition, HSQC experiments showed correlation from the signal in the olefinic region at δ 6.22 (*s*) to the ^{13}C signal at δ 108.5, suggesting a methine proton adjacent to double bond of the 4(1*H*)-quinolone system, which were assigned to H-3 and C-3, respectively, since C-2 is more deshielded than C-3 because of the deshielding effect of the electronegative nitrogen. This evidence determined the position of the aliphatic chain at C-2. The new alkaloid was therefore identified as 2-(14′-hydroxy-14′,15′-dimethylhexadecanyl)-4-quinolone (**1**).

Compound **2** gave rise to UV (239, 330 and 348 nm) and IR (a weak carbonyl band at 1658 cm^{-1}) spectra which indicated the presence of an 2-alkyl-4(1*H*)-quinolone nucleus. Elemental analysis and ESI-MS indicated the molecular formula to be $C_{24}H_{37}NO_3$, requiring the presence of an aliphatic chain shorter than C_{16}, when compared with **1**. In the NMR spectrum of **2**, four aromatic protons at δ 8.40 (*bd*, $J = 8.0$ Hz, H-5), 7.31 (*dt*, $J = 8.0$ and 1.5 Hz, H-6), 7.51 (*dt*, $J = 8.0$ and 1.2 Hz, H-7) and 7.59 (*dd*, $J = 8.0$ and 1.5 Hz, H-8) (Table 1), clearly indicated an A ring similar to **1**. The spectrum also revealed a singlet at δ 3.92 corresponding to one methoxyl group. Furthermore, it did not show the signal for the conjugated olefinic proton ca δ 6.22 (H-3), thereby locating the methoxyl group at C-3. These data resulted in the construction of a 3-methoxy-4(1*H*)-quinolone ($C_{10}H_8NO_2$). Hydroxyl and two methyl groups must be connected at the same carbon in the side chain, since signals for a hydroxytertiary carbon at δ 71.3 and two methyl singlets at δ_H 1.20 (*s*, 6H) and δ_C 29.2 in the ^{13}C (Table 2) and ^1H NMR spectra, respectively. The ESI-MS showed an ion at *m*/*z* 328 $[C_{24}H_{37}NO_3 + H - C_3H_8O]^+$, confirming the presence of an 12′-hydroxy-12′-methyltridecanyl chain ($C_{24}H_{37}NO_3$–$C_{10}H_8NO_2 = C_{14}H_{29}O$). ^{13}C NMR assignments for **2** were made using **1** as model. The downfield signals at δ 43.7 (CH_2, DEPT) and 29.2 (CH_3, DEPT), which were appropriate for a methylene and methyl groups with an electronegative element nearby, were thus assigned to C-11′ and C-13′/C-14′, respectively. Moreover, the slightly negative γ effect of the hydroxyl group permitted the assignment of C-9′ at δ 24.3. Thus, the structure of the new alkaloid was characterized as 2-(12′-hydroxy-12′-methyltridecanyl)-3-methoxy-4-quinolone (**2**).

Table 1
^1H NMR chemical shifts for compounds **1**–**5**

H	**1**	**2**	**3**	**4**	H	**5**
3	6.22 *s*	–	6.17 *s*	–	3	6.20 *s*
5	8.35 *d* (8.0)	8.40 *bd* (8.0)	8.30 *brd* (8.0)	8.40 *dd* (8.0; 1.0)	5	7.87 *d* (3.3)
6	7.32 *dt* (8.0, 1.8)	7.31 *dt* (8.0, 1.5)	7.26 *t* (8.0)	7.28 *t* (8.0)	6	
7	7.56 *dt* (8.0, 1.5)	7.51 *dt* (8.0, 1.2)	7.52 *t* (8.0)	7.55 *t* (8.0)	7	7.26 *dd* (10.0; 3.3)
8	7.60 *dd* (8.0, 1.8)	7.59 *dd* (8.0, 1.5)	7.71 *d* (8.0)	7.78 *d* (8.0)	8	7.52 *d* (10.0)
1′	2.64 *t* (7.8)	2.80 *t* (8.0)	2.60 *brt*	2.86 *t* (7.2)	1′a	2.97 *dt*(10.0; 5.0)
					1′b	2.94 *dt*(10.0; 5.0)
2′	1.70–1.80 *m*	1.28–1.71 *m*	1.10–1.80 *m*	1.68–1.72 *m*	2′a	2.07 *m*
					2′b	1.48 *m*
3′	1.20–1.30 *m*	1.28–1.71 *m*	1.10–1.80 *m*	1.68–1.72 *m*	3′	2.07 *m*
4′	1.20–1.30 *m*	1.28–1.71 *m*	1.10–1.80 *m*	1.68–1.72 *m*	4′a	3.55 *dd* (12.0; 10.2)
					4′b	4.26 *dd* (12.0; 6.3)
5′	1.20–1.30 *m*	1.28–1.71 *m*	1.10–1.80 *m*	1.68–1.72 *m*	1″	1.57 *dt* (10.0; 7.0)
6′	1.20–1.30 *m*	1.28–1.71 *m*	1.10–1.80 *m*	1.68–1.72 *m*	2″	2.21 *q* (7.2)
7′	1.20–1.30 *m*	1.28–1.71 *m*	1.10–1.80 *m*	1.68–1.72 *m*	3″	5.33 *dt* (10.6; 7.2)
8′	1.20–1.30 *m*	1.28–1.71 *m*	1.10–1.80 *m*	1.68–1.72 *m*	4″	5.45 *dt* (10.6; 7.2)
9′	1.20–1.30 *m*	1.28–1.71 *m*	1.10–1.80 *m*	1.68–1.72 *m*	5″	2.07 *m*
10′	1.20–1.30 *m*	1.28–1.71 *m*	1.10–1.80 *m*	1.68–1.72 *m*	6″	0.98 *t* (7.5)
11′	1.20–1.30 *m*	1.28–1.71 *m*	1.10–1.80 *m*	1.68–1.72 *m*		
12′	1.20–1.30 *m*	–	–	1.68–1.72 *m*		
13′	1.20–1.30 *m*	1.20 *s*	1.20 *s*	1.68–1.72 *m*		
14′	–	1.20 *s*	1.20 *s*	–		
15′	2.45 *sept* (7.0)			2.44 *sept* (7.0)		
16′	1.20 *d* (7.0)			1.10 *d* (7.0)		
17′	1.12 *d* (7.0)			1.10 *d* (7.0)		
18′	1.40 *s*			1.40 *s*		
OMe		3.92 *s*		3.90 *s*		3.93 *s*
NH	10.50 *brs*	10.10 *brs*	12.20 *brs*	11.60 *brs*		

Resonances were confirmed by $^1H/^1H$ and $^{13}C/^1H$ shift-correlated two-dimensional spectra. Coupling constants (Hz) in parentheses. Spectra of **1**–**5** run at 400 MHz in $CDCl_3$.

Compound **3** had NMR spectra (Tables 1 and 2) similar to **2**, except for the absence of a signal for a methoxyl group, and the signal for H-3 was visible as a singlet at δ 6.17. Elemental analysis and ESI-MS indicated the molecular formula to be $C_{23}H_{35}NO_2$, confirming that the difference between **3** and **2** is the absence of one methoxyl group at C-3. The presence of a 4(1*H*)-quinolone nucleus was also suggested by its UV (238, 325 and 335 nm) and IR (1657 cm^{-1}) spectra. The observed signals for a hydroxytertiary carbon at δ 71.2 and two methyl singlets at δ_H 1.20 (*s*, 6H) and δ_C 29.1 in the ^{13}C (Table 2) and ^1H NMR spectra, together with the ion at *m*/*z* 298 $[C_{23}H_{35}NO_2+H-C_3H_8O]^+$ in the ESI-MS, confirmed the presence of an 12′-hydroxy-12′-methyltridecanyl chain. The complete assignment of chemical shifts for all carbons are given in Table 2. Using compounds **1** and **2** as models, the structure of **3** was elucidated as 2-(12′-hydroxy-12′-methyltridecanyl)-4-quinolone.

Compound **4**, $C_{28}H_{45}NO_3$ (EA/ESI-MS), also showed the UV (237, 288 and 335 nm) and IR (1636 cm^{-1}) spectral characteristics of an 2-alkyl-4(1*H*)-quinolone. The ^1H and ^{13}C NMR spectra showed signals typical of a 3-methoxy-4-quinolone (δ_H 8.40, *dd*, *J* = 8.0 and 1.0 Hz, H-5; 7.28, *t*, *J* = 8.0 Hz, H-6; 7.55, *t*, *J* = 8.0 Hz, H-7; 7.78, *d*, 8.0 Hz, H-8; 3.90, *s*, OMe; δ_C = 147.6; C-3) very close related to those in **2**, and of a 14′-hydroxy-14′,15′-dimethylhexadecanyl side chain (δ_H 2.86, *t*, *J* = 7.2 Hz, H-1′; 1.68–1.72, *m*, H-2′–H–13′; 2.44, *sept*, *J* = 7.0 Hz, H-15; 1.10, *d*, *J* = 7.0 Hz, Me-16′/Me-17′; 1.40, *s*, Me-18′; δ_C 82.1, C-14′); as in **1**. This was supported by the mass spectrum, which showed fragments at *m*/*z* 356 $[C_{28}H_{45}NO_3+H-C_5H_{12}O]^+$ (80%) due to fission of the side chain between C-13′ and C-14′. The structure of this alkaloid was thus established as 2-(14′-hydroxy-14′,15′-dimethylhexadecanyl)-3-methoxy-4-quinolone (**4**).

Table 2
^{13}C NMR chemical shifts for compounds **1**–**5** and model compounds **6** and **7**

C	1	2	3	4	6	C	5	7
2	154.3	138.4	155.2	138.8	156.9	2	150.7	152.1
3	108.5	146.2	108.2	147.6	107.1	3	108.9	109.7
4	179.1	173.2	179.0	177.0	–	4	176.5	176.7
4a	125.1	125.9	124.9	125.9	124.6	4a	128.2	126.5
5	125.7	125.8	125.4	125.4	124.9	5	106.9	126.8
6	123.6	122.9	123.6	122.8	123.5	6	156.3	124.0
7	131.8	135.1	131.8	131.0	132.4	7	122.4	132.0
8	117.9	117.5	118.4	118.3	119.0	8	116.5	114.8
8a	140.3	140.4	140.6	140.3	140.3	8a	136.0	141.3
1′	34.3	30.0	34.2	30.0	34.3	1′	29.7	30.0
2′	28.9	29.4	29.0	29.0	28.2	2′	25.3	25.0
3′	28.8	29.0	29.0	29.0	29.3	3′	33.7	33.8
4′	29.0	29.4	29.0	29.3	29.3	4′	51.5	51.2
5′	29.0	29.4	29.0	29.0	29.5	1″	34.1	34.0
6′	29.0	29.4	29.0	29.0	29.5	2″	23.9	24.1
7′	29.0	29.4	29.0	29.0	29.6	3″	127.7	127.8
8′	29.0	29.4	29.0	29.0	29.8	4″	133.0	133.0
9′	29.0	24.3	24.0	29.0	29.8	5″	20.8	20.6
10′	29.0	29.6	30.1	29.0	29.8	6″	14.5	14.5
11′	23.3	43.7	43.7	23.4	31.9			
12′	29.6	71.3	71.2	29.4	22.7			
13′	40.1	29.2	29.1	40.3	14.1			
14′	82.2	29.2	29.1	82.1				
15′	35.0			34.9				
16′	19.0			19.0				
17′	19.0			19.0				
18′	26.1			26.0				
Ome		60.1		60.1			56.0	

Spectra of **1**–**5** run at 100.6 MHz and **6**–**7** at 75 MHz. All spectra run in $CDCl_3$.

Compound **5**, $C_{20}H_{25}NO_2$ (EA/ESI-MS), belonged to the same class of molecules as **1**–**4.** The 4-quinolone skeleton was recognized on the basis of UV (240, 331 and 347 nm) and IR (1648 cm^{-1}) spectra. In the ^1H and ^{13}C NMR spectra the absence of signals for N–H ca. δ 10–12 and N–Me ca. δ_C 34, suggested the presence of a third ring. Compound **5** exhibited similar NMR spectra to dictyolomide A (**7**) (Lavaud et al., 1995). The ^1H NMR revealed the presence of a methoxyl group (δ_H 3.93) and of three aromatic protons (δ_H 7.87, *d*, *J* = 3.3 Hz; 7.26, *dd*, *J* = 10.0 and 3.3 Hz; 7.52, *d*, *J* = 10.0 Hz). From HMBC experiments (see Experimental), the observed correlation between the ^1H signal at δ 7.87 (*d*, *J* = 3.3 Hz) and the ^{13}C signal at δ 176.5 (^3J) led to their assignment as H-5 and C-4, respectively, thus indicating the methoxyl group to be located at C-6 and permitting the assignments of the signals at δ 7.26 (*dd*, *J* = 10.0 and 3.3 Hz) and 7.52 (*d*, *J* = 10.0 Hz) to H-7 and H-8, respectively. Moreover, the existence of a correlation between the ^1H signal at δ 6.20, assigned to H-3, and the ^{13}C signal at δ 29.7 suggested a methylene substituent at C-2. This correlation, COSY experiments and the chemical shift of a methylene at δ_C 51.5 indicated the presence of a structural unit, –CH_2–CH_2–CH–CH_2–N– in ring B (δ_H 2.97, *dt*, *J* = 10.0 and 5.0 Hz, H-1′a, 2.94, *dt*, *J* = 10.0 and 5.0 Hz, H-1′b, δ_C1′ 29.7; 2.07, *m*, H-2′a, 1.48, *m*, H-2′b, δ_C2′ 25.3; 2.07, *m*, H-3′, δ_C3′ 33.7; δ_H 3.55, *dd*, *J* = 12.0 and 10.2 Hz, H-4′a, 4.26, *dd*, *J* = 12.0 and 6.3 Hz, H-4′b, δ_C4′ 51.5), confirming a third ring as a piperidine substituent. The methylene signal at δ 3.55 showed a long-range correlation (^3J) with the methylene signal at δ 34.1, suggesting the presence of a methylene at C-3′ of piperidine ring. This methylene signal was coupled to another methylene signal at δ 2.21, which was coupled to the olefinic proton signal at δ 5.33. The latter resonance was coupled to the ^1H signal at δ 5.45, which showed a one-bond correlation with the ^{13}C signal at δ 133.0. The methyl proton at δ 0.98 showed cross peaks with the ^{13}C signals at δ 133.0 (^3J) and 20.8 (^2J; CH_2 by HSQC and DEPT). These correlations resulted in the construction of a C_6H_{11} linear chain containing a disubstituted double bond between the third (C-3″) and fourth (C-4″) carbons. The *cis*-configuration of the double bond was deduced from the small coupling

constant [$J = 10.6$ Hz; J (*cis*) = 7-11 Hz], and the chemical shift of the terminal methylene (δ 20.6) shielded as in the *cis*-hex-3-ene at δ 20.8 (Lavaud et al., 1995). The ESI-MS showed ions at m/z 228 $[C_{20}H_{25}NO_2 + H - C_6H_{12}$ (alkenyl chain)$]^+$ (30%) and 189 $[C_{20}H_{25}NO_2 + H - C_9H_{15}]^+$ (100%), confirming the presence of a piperidine ring and a alkenyl chain at C-3′. These data were consistent with the structure of 6-methoxydictyolomide A (**5**).

A model shows that in the piperidine moiety, H-3′ is not rigidly antiperiplanar with respect to one of the protons 2H-4′. In the isomer in which H-3′β is antiperiplanar to H-4′α (J is large), the alkenyl chain is in a pseudoequatorial conformation (α). However, the large coupling constant of the C-4′ methylene protons to H-3′ is also consistent with their situation in a second isomer in which H-3′α is antiperiplanar to H-4′β (J is large), requiring the alkenyl chain to be in a pseudoequatorial conformation (β). The NOESY experiments showed correlations of H-4′ at δ 3.55 ($J_{4'\text{-}3'} = 10.2$) with H-2′b at δ 1.48. However, a spatial proximity of one H_2-4′ to H_2-2 is possible for each of the isomers discussed above. When one of the H-4′a and H-2′a protons are in spatial proximity on the β-side, H-3′ is on the α-side of the molecule, then the alkenyl chain is also on the β-side, but in a pseudoequatorial conformation. If it is presumed that H-3′ is on the β-side in a pseudoaxial conformation, the other H-4′bα and H-2′bα are also in a spatial proximity. Therefore, these observations do not allow determination of the C-3′ stereochemistry. However, compound **5** was presumed to be 3′-(*S*)-6-methoxydictyolomide A based on the chemical shift of C-3′ (δ_C 33.7) and its comparison with those of oblonginine (**8**, 25α-Me, δ_C 32.8), and shinonomenine (**9**, 25β-Me, δ_C 28.0) (Kadota et al., 1995). As a further confirmation of the assignments, the synthesis of **5**, under chiral control at C-3′, is in progress.

The results provide firm support for including the genus *Dictyoloma* in the Rutaceae. An examination of the systematic distribution of alkaloids, coumarins, limonoids, flavonoids and chromones in the Rutales led Waterman (1983) to propose the biochemical evolution of the order. Limonoids and quassinoids, two biosynthetically related compounds, are found in the Simaroubaceae, Meliaceae, Rutaceae, and Cneoraceae (da Silva et al., 1984, 1987). There are fairly consistent differences between the limonoids of the Rutaceae and those of the Meliaceae, the quassinoids being restricted to the Simaroubaceae. According to Waterman (1983), a consideration of other classes of secondary metabolites leads to the conclusion that Meliaceae, Cneoraceae, and Simaroubaceae have all evolved from proto-rutaceous stock. Furthermore, the enormous variety of alkaloids and coumarins in the Rutaceae, not found in the other families, is taken to suggest that substantial chemical evolution must have occurred in the diversification of this large family after Cneoraceae, Meliaceae, and Simaroubaceae lines split off. In the Simaroubaceae, *Harrisonia* is exceptional (da Silva et al., 1984, 1987), since no quassinoids have so far been isolated from it. Its limonoid types, however, suggest strong affinity with rutaceous genera such as *Spathelia*. Besides, both *Harrisonia* and *Spathelia* contain chromones which have not been found in other Simaroubaceae or Rutaceae, but do occur in the Ptaeroxylaceae and Cneoraceae (Waterman, 1983). These deductions were incorporated by Waterman (1983) into a phylogenetic diagram (Fig. 1). In 1983, the only phytochemical knowledge of *Dictyoloma* was the record of a simple indole (*N,N*-dimethyl-5-methoxytryptamine; Mester, 1983), of little value for the purpose of a taxonomic position of Dictyolomatoideae into Waterman's phylogenetic diagram.

The 2-quinolone alkaloids, limonoids, and chromones that have recently been isolated from *Dictyoloma vandellianum* provide firm support for including the subfamily Dictyolomatoideae near the allied taxa Spathelioideae, Ptaeroxylaceae, Cneoraceae, and *Harrisonia* (Vieira et al., 1988, 1990). Now, the finding of 2-alkyl-4(1*H*)-quinolones in *D. vandellianum* showed strong similarities with Zanthoxyleae [*Platydesma* and *Tetradium* (*T. ruticarpum* = *Euodia rutaecarpa*)], Ruteae (*Haplophyllum* and *Ruta*), Boronieae (*Boronia*), Cusparieae (*Raulinoa*), and Toddalieae (*Acronychia*,*Vepris* and*Ptelea*), which contain several 2-alkyl-4-quinolones (Mester, 1983; Biavatti et al., 2002). *Dictyoloma*,*Tetradium*, *Raulinoa,* and *Vepris* also produce limonoids (Vieira et al., 1988, 1990; Ng et al., 1987; Biavatti et al., 2001; daSilva et al., 1984, 1987), suggesting a relative advance. These phytochemical data are in line with the view of Waterman (1987) who consider genera of the Rutaceae, which produce the full range of advanced rutalean metabolites (furoquinoline alkaloids, coumarins and limonoids), as arising from a progenitor with the metabolic profile of *Tetradium*. Interestingly, de Jussieu (1825) classified the genus *Dictyoloma* in the same tribe of *Tetradium*, Zanthoxyleae. Thus, in Waterman's phylogenetic diagram (Fig. 1), Dictyolomatoideae apparently occupies a position between the proto-Rutaceae genera and the Spathelioideae, but close to Zanthoxyleae.

This interpretation of chemical data is consistent with available DNA sequences. The analysis of DNA sequence data from members of the Cneoraceae, Meliaceae, Ptaeroxylaceae, Rutaceae, and Simaroubaceae, showed that the Rutaceae are paraphyletic, with *Spathelia* and *Dictyoloma* (Rutaceae), *Harrisonia* (Simaroubaceae), *Cneorum* (Cneoraceae), and *Ptaeroxylon* (Ptaeroxylaceae) forming a clade sister to all other Rutaceae. Circumscription of Rutaceae to include all of these taxa was recommended. This analysis also indicated that the Simaroubaceae and Meliaceae are the outgroups closest to Rutaceae (Chase et al., 1999; Scott et al., 2000).

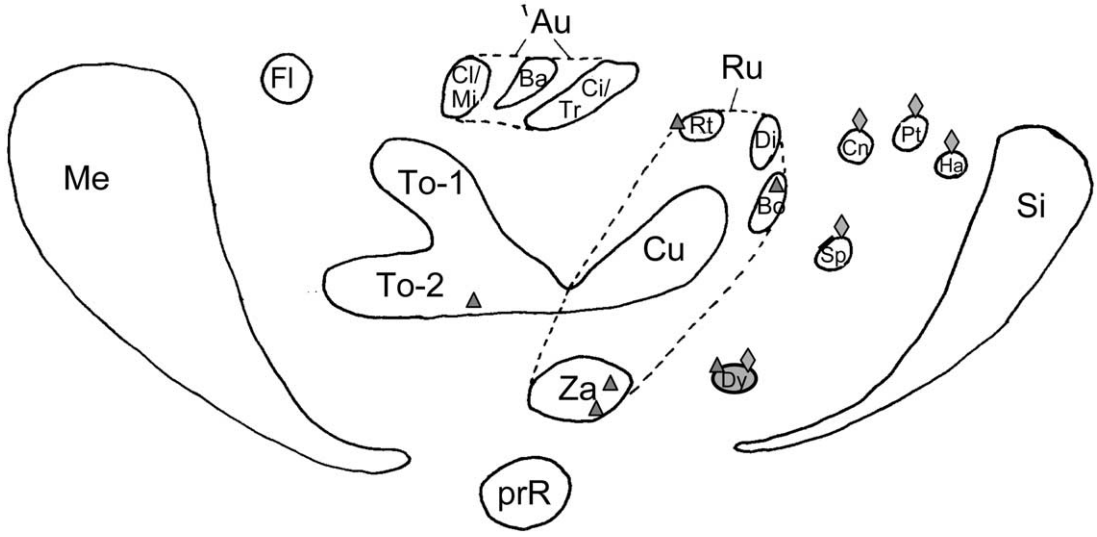

Fig. 1. Waterman's phylogenetic diagram of the Rutales, based on the distribution of secondary metabolites, is used herein to place the Dictyolomatoideae subfamily, which was previously not included by Waterman due to limited phytochemical data. A = Aurantioideae, Ba = Balsamocitrinae, Bo = Boronieae, Ci = Citrinae, Cl = Clauseninae, Cn = Cneoraceae, Cu = Cusparieae, Di = Diosmeae, Fl = Findersioideae, Ha = *Harrisonia* (Simaroubaceae), Me = Meliaceae, Mi = Micromelinae, prR = proto-Rutaceae genera, Pt = Ptaeroxylaceae, Rt = Rutinae, Ru = Rutoideae, Si = Simaroubaceae, Sp = Spathelioideae, To-l = (coumarin containing) Toddalioideae, To-2 = (acridone containing) Toddalioideae, Tr = Triphasiinae, Za = Zanthoxyleae (Waterman, 1983); Dy = Dictyolomatoideae. ◆ Distribution of chromones, ▲ distribution of 2-alkyl-4(1*H*)-quinolones.

3. Experimental

3.1. General

NMR: on a Brüker DRX 400, with TMS as int. standard; ESI-MSMS: low resolution on a triple quadrupole Micromass Quattro LC instrument, equipped with a "Z-spray" ion source; IR: Bomen-Ft/IR; UV: Perkin-Elmer; Elemental analysis: on a EA 1108, CHNS-O (Fisons); $[\alpha]_D$: Perkin-Elmer 241 instrument.

3.2. Plant material

D. vandellianum was collected in Campinas, SP, Brazil, and identified by J.R. Pirani (Universidade de São Paulo). A voucher (SPF 81-317) is deposited in the Herbarium of Instituto de Biociências, USP, São Paulo.

3.3. Isolation of compounds

Ground leaves (300 g) were extracted with hexane, then CH_2Cl_2 and finally with MeOH. The conc. CH_2Cl_2 extract was subjected to CC over silica gel. Elution with hexane, followed by a CH_2Cl_2–EtOAc–Me_2CO–MeOH gradient yielded eight frs. Fr. 2 was subjected to cellulose chromatography eluting with a hexane–CH_2Cl_2–EtOAc gradient to afford 8-methoxyflindersine (3 mg) and additional frs. Fr. 2.1 was applied twice (Sephadex LH-20, EtOAc; then silica gel, hexane–CH_2Cl_2–EtOAc gradient) to give β-sitosterol (13 mg). Fr. 5 was subjected twice to Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) yielding new frs., with Fr. 5.2, following flash chromatography on silica gel eluting with a CH_2Cl_2–EtOAc–MeOH gradient, affording new frs. Fr. 5.2.1 was applied to Sephadex LH-20 (EtOAc) to give **1** (8 mg) and a new fr. containing **2**. The latter was purified by prep. TLC (silica gel, CH_2Cl_2–EtOAc, 6:4) to give 20 mg of **2**. Fr. 5.3 was subjected three times to Sephadex LH-20 ($CHCl_3$–MeOH, 1:1; MeOH; EtOAc) to afford a new fr., which was purified by prep. TLC (silica gel; CH_2Cl_2–MeOH, 1:1) to yield **3** (5 mg) and **4** (5 mg). Fr. 6 was applied to Sephadex LH-20 eluting with MeOH, affording a fr. containing **5**. It was then purified by prep. TLC (silica gel; $CHCl_3$–MeOH, 95:5) to give 12 mg of **5**.

3.3.1. 2-(14′-Hydroxy-14′,15′-dimethylhexadecanyl)-4-quinolone (*1*)

Yellow gum; $[\alpha]_D + 3^\circ$ ($CHCl_3$; *c* 0.2); UV λ_{max} (MeOH) nm: 236, 314, 325; IR ν_{max} ($CHCl_3$) cm^{-1}: A weak carbonyl band at 1634 cm^{-1} can be explained by the carbonyl group being present partially enolized; ^1H NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C NMR/DEPT (100 MHz, $CDCl_3$): see Table 2; HSQC (400/100 MHz, $CDCl_3$); COSY (400 MHz, $CDCl_3$). EA: Found: C, 78.38; H, 10.50, N, 3.40. Calc. for $C_{27}H_{43}NO_2$: C, 78.40, H, 10.48, N, 3.39, O, 7.74%; ESI-MSMS, *m*/*z* (rel. int.): 414 $[M+H]^+$ (25), 386 (98), 326 (40), 298 (100).

*3.3.2. 2-(12′-Hydroxy-12′-methyltridecanyl)-3-methoxy-4-quinolone (**2**)*

Yellow gum; UV λ_{max} (MeOH) nm: 239, 330, 348; IR ν_{max} ($CHCl_3$) cm^{-1}: A weak carbonyl band at 1658 cm^{-1} can be explained by the carbonyl group being present partially enolized; ^1H NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C NMR/DEPT (100 MHz, $CDCl_3$): see Table 2; HSQC (400/100 MHz, $CDCl_3$); COSY (400 MHz, $CDCl_3$). EA: Found: C, 74.40; H, 9.64, N, 3.60. Calc. for $C_{24}H_{37}NO_3$: C, 74.38, H, 9.62, N, 3.61, O, 12.38%; ESI-MSMS, *m*/*z* (rel. int.): 388 $[M+H]^+$ (20), 357 (100), 328 (50).

*3.3.3. 2-(12′-Hydroxy-12′-methyltridecanyl)-4-quinolone (**3**)*

Yellow gum; UV λ_{max} (MeOH) nm: 238, 325, 335; IRν_{max} ($CHCl_3$) cm^{-1}: A weak carbonyl band at 1657 cm^{-1} can be explained by the carbonyl group being present partially enolized; ^1H NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C NMR/DEPT (100 MHz, $CDCl_3$): see Table 2; HSQC (400/100 MHz, $CDCl_3$); COSY (400 MHz, $CDCl_3$). EA: Found: C, 77.26; H, 9.88, N, 3.90. Calc. for $C_{23}H_{35}NO_2$: C, 77.27, H, 9.87, N, 3.92, O, 8.95%; ESI-MSMS, *m*/*z* (rel. int.): 358 $[M+H]^+$ (60), 357 (100), 298 (20).

*3.3.4. 2-(14′-Hydroxy-14′,15′-dimethylhexadecanyl)-3-methoxy-4-quinolone (**4**)*

Yellow gum; $[\alpha]_D+4^\circ$ ($CHCl_3$; *c* 0.3); UV λ_{max} (MeOH) nm: 237, 288, 335; IRν_{max} ($CHCl_3$) cm^{-1}: A weak carbonyl band at 1636 can be explained by the carbonyl group being present partially enolized; ^1H NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C NMR/DEPT (100 MHz, $CDCl_3$): see Table 2; HSQC (400/100 MHz, $CDCl_3$); COSY (400 MHz, $CDCl_3$). EA: Found: C, 75.79; H, 10.23, N, 3.17. Calc. for $C_{28}H_{45}NO_3$: C, 75.80, H, 10.22, N, 3.16, O, 10.82%; ESI-MSMS, *m*/*z* (rel. int.): 444 $[M+H]^+$ (20), 416 (70), 356 (80), 328 (100).

*3.3.5. 6-Methoxydictyolomide A (**5**)*

Yellow powder; $[\alpha]_D+22.0^\circ$ ($CHCl_3$; *c* 0.7); UV λ_{max} (MeOH) nm: 240, 331, 347; IRν_{max} ($CHCl_3$) cm^{-1}: 2951, 2841, 1648, 1444, 1403, 1017, 782; ^1H NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C NMR/DEPT (100 MHz, $CDCl_3$): see Table 2; HSQC (400/100 MHz, $CDCl_3$); COSY (400 MHz, $CDCl_3$), HMBC (400/100 MHz, $CDCl_3$): δ 7.87 (H-5) → 176.5 (C-4, CO), 136.0 (C-8a), 122.4 (C-7); 7.52 (H-8) → 156.3 (C-6), 128.2 (C-4a); 7.26 (H-7) → 156.3 (C-6), 136.0 (C-8a); 6.20 (H-3) → 150.7 (C-2), 128.2 (C-4a), 29.7 (CH_2, C-1′); 3.55 (H-10′a) → 34.1 (CH_2, C-4′); 0.98 (Me-9′) → 133.0 (CH, C-7′), 20.8 (CH_2, C-8′); 3.93 (OMe) → 156.3 (C-6). NOESY (400 MHz, $CDCl_3$): δ 7.87 (H-5) → 3.93 (OMe); 7.52 (H-8) → 4.26 (H-10′b), 3.55 (H-10′a); 6.20 (H-3) → 2.97 (H-1′a), 2.94 (H-1′b); 4.26 (H-10′b) → 3.55 (H-10′a), 2.07 (H-2′a/ ou H-3′); 3.55 (H-10′a) → 1.57 (H-4′), 1.48 (H-2′b); 2.97–2.94 (H-1′a/H-1′b) → 2.07 (H-2′a/ ou H-3′); 2.07 (H-8′) → 0.98 (Me-9′). EA: Found: C, 77.13; H, 8.10, N, 4.52. Calc. for $C_{20}H_{25}NO_2$: C, 77.14, H, 8.09, N, 4.50, O, 10.27%; ESI-MSMS, *m*/*z* (rel. int.): 312 $[M+H]^+$ (30), 228 (30), 189 (100).

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