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Alkaloids from *Spathelia excelsa*: Their chemosystematic significance

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Abstract

The methanol extract from the leaves of *Spathelia excelsa* yielded six alkaloids: 2-(12-oxo-tridecanyl)-3-methoxy-4-quinolone, 2-(10-hydroxy-10-methyldodecanyl)-3-methoxy-4-quinolone, 2-(11-hydroxy-11-methyldodecanyl)-3-methoxy-4-quinolone, 2-(12-hydroxytridecanyl)-3-methoxy-4-quinolone, 7-hydroxy-2-(3-hydroxy-3-methylbutyl)-4-quinolone and 6-hydroxy-2-(3-hydroxy-3-methylbutyl)-4-quinolone, in addition to the known 3-*O*-β-D-glucopiranosylsitosterol and (−)-epicatechin. The 2-alkyl-4(1*H*)-quinolones in *S. excelsa* display strong similarities with those in Dictyolomatoideae, which contains several 2-alkyl-4-quinolones. The data reported herein thus provide firm support for placing Spathelioideae close to or within the Dictyolomatoideae.

Keywords: Rutaceae; *Spathelia excelsa*; 2-alkyl-4(1*H*)-quinolones; Chemosystematics

1. Introduction

The placement of the genera *Spathelia* L. and *Dictyoloma* Juss. has been doubtful since they were first described in Linnaeus (1762) and in de Jussieu (1825), who assigned them to the Rutaceae. According to Bentham and Hooker (1862), *Spathelia* and *Dictyoloma* belong to the order Simaroubaceae. Engler (1874) initially included these genera in the Simaroubaceae, but later moved them to the Rutaceae, placing *Spathelia* in the subfamily Spathelioideae and *Dictyoloma* in Dictyolomatoideae (von Engler, 1931).

In this paper, the isolation and identification of six 2-alkyl-4(1*H*)-quinolones from the leaves of previously uninvestigated *Spathelia excelsa* (K. Krause) Cowan and Brizicky (1960) (syn. *Sohnreyia* K. Krause) are described. Their chemosystematic significance is discussed in order to clarify the relationships between *Spathelia* and *Dictyoloma*.

2. Results and discussion

The methanol extract from *S. excelsa* leaves yielded six new alkaloids **1**–**6**, as well as the known 3-*O*-β-D-glucopiranosylsitosterol (Tandon et al., 1990) and (−)-epicatechin (Agrawal, 1989).

Compound **1** showed UV absorption maxima at 242, 247, 324 and 335 nm, which is characteristic of a 2-alkyl-4(1*H*)-quinolone (Tang et al., 1996). An elemental analysis and ESI-MS indicated the molecular formula $C_{23}H_{33}NO_3$. The IR spectrum showed a weak α,β-unsaturated carbonyl band at 1632 cm^{-1}, which can be explained by a partially enolized carbonyl group. In addition to signals typical of 4(1*H*)-quinolone with an unsubstituted A ring (four aromatic protons at δ 8.40, *brd*, $J = 7.5$ Hz; 7.28, *brt*, $J = 7.5$ Hz; 7.54, *brt*, $J = 7.5$ Hz; 7.73, *d*, $J = 7.5$ Hz), the ^{1}H NMR spectrum (Table 1) revealed a singlet at δ 11.12 for N–H, a singlet

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Table 1
^{1}H NMR chemical shifts for compounds **1**–**6**

H	1	2	3	4	5	6
3					6.68 *s*	5.82 *s*
5	8.40 *brd* (7.5)	8.38 *dd* (7.2, 1.0)	8.38 *dd* (8.0, 1.2)	8.37 *dd* (8.0, 1.1)	7.90 *d*(9.0)	7.37 *d*(2.7)
6	7.28 *brt* (7.5)	7.27 *brt* (7.2)	7.27 *brt* (8.0)	7.28 *brt* (8.0)	7.43 *dd* (9.0, 2.5)	–
7	7.54 *brt* (7.5)	7.54 *brt* (7.2)	7.53 *brt* (8.0)	7.54 *brt* (8.0)	–	7.12 *dd* (8.8, 2.7)
8	7.73 *d*(7.5)	7.82 *d*(7.2)	7.82 *d*(8.0)	7.61 *d*(8.0)	7.47 *d*(2.5)	7.43 *d*(8.8)
1	2.84 *t*(7.6)	2.83 *t*(7.6)	2.84 *t*(7.7)	2.81 *t*(7.8)	2.91 *t*(8.3)	2.61 *t*(8.3)
2	1.70 *m*	1.67 *m*	1.68 *m*	1.70 *m*	1.80 *t*(8.3)	1.72 *t*(8.3)
3	1.30 *m*	1.25 *m*	1.28 *m*	1.19–1.25 *m*	–	–
4	1.21–1.28 *m*	1.25 *m*	1.25 *m*	1.19–1.25 *m*	1.17 *s*	1.15 *s*
5	1.21–1.28 *m*	1.25 *m*	1.25 *m*	1.19–1.25 *m*	1.17 *s*	1.15 *s*
6	1.21–1.28 *m*	1.25 *m*	1.25 *m*	1.19–1.25 *m*		
7	1.21–1.28 *m*	1.25 *m*	1.25 *m*	1.19–1.25 *m*		
8	1.21–1.28 *m*	1.35 *m*	1.25 *m*	1.19–1.25 *m*		
9	1.26 *m*	1.37 *m*	1.25 *m*	1.19–1.25 *m*		
10	1.54 *m*	–	1.43 *m*	1.30 *m*		
11	2.43 *t*(7.3)	1.45 *q*(7.4)	–	1.45 *m*		
12	–	0.87 *t*(7.4)	1.20 *s*	3.80 *sext* (6.2)		
13	2.14 *s*	1.12 *s*	1.20 *s*	1.19 *d*(6.2)		
OMe	3.92 *s*	3.91 *s*	3.90 *s*	3.91 *s*		
NH	11.12 *brs*	11.75 *brs*	12.00 *brs*	10.22 *brs*	10.50 *brs*	11.45 *brs*
3-OH					4.01 *brs*	4.42 *brs*
6-OH						9.65 *s*

Resonances were confirmed by COSY and HSQC experiments. Coupling constants (Hz) in parentheses. Spectra of **1**–**6** run at 400 MHz. Spectra of **1**–**4** run in $CDCl_3$ and **5**–**6** in DMSO-d_6.

at δ 3.92 corresponding to one methoxyl group and a methyl singlet at δ 2.14. The spectrum did not show the signal for the conjugated olefinic proton ca.δ 6.22 (H-3), thus locating the methoxyl group at C-3. Based on the HMBC experiments (Table 2), the correlation observed between the ^{1}H signal at δ 8.40 and the ^{13}C signals at δ 172.8, 131.1 (CH) and 138.6 led to their assignment as H-5, C-4, C-7 and C-8a, respectively.

Table 2
HMBC for compounds **1**–**6**

H	C					
	1	2	3	4	5	6
3					2,1,4a,4	1,2,4a
5	4,7,8a	7,8a	4,7,8a	4,7,8a	4,4a,7,8a	4,6,7,8a
6	4a,8	4a,8	4a,8	4a,8	7,8	
7	5,8a	5,8a	5,8a	5,8a		5,8a
8	4a,6		4a,6	4a,6	6,7,8a	4a,6
1	2,3		2,3,2	2,3,2	2,3,2,3	2,2
2					2,3,4	1,3
4					2,3	2,3
5					2,3	2,3
9		13				
10	11,12					
11	10,12	9,10,12,13				
12		10,11	10,11	10		
13	11,12	9,10,11	10,11	11,12		
OMe	3	3	3	3		
NH						3,4a
3-OH						2,3,4,5
6-OH						5,6,7

The latter resonance was coupled to the ^{1}H signal at δ 7.28, which showed cross peaks with the ^{13}C signals at δ 125.8 and 117.9, thus allowing these signals to be assigned to H-6, C-4a and C-8. The ^{1}H signals for H-5 and H-6 at δ 8.40 and 7.28 showed one bond correlations with the ^{13}C signals at δ 125.5 and 122.8, respectively. The H-7 signal was assigned to δ 7.54 by correlation with the ^{13}C signal of C-8a (δ 138.6). The remaining aromatic signal at δ 7.73 was then attributed to H-8. This led to the formulation of **1** as a 3-methoxy-4(1*H*)-quinolone ($C_{10}H_8NO_2$). The ^{13}C NMR spectrum (Table 3) revealed resonances for an aliphatic chain of thirteen carbon atoms containing a carbonyl group (δ 210.0). From the HMBC experiments (Table 2), the correlation found between the methyl proton at δ 2.14 (δ_C 30.0) and the ^{13}C signal at δ 210.0 and 43.8 (CH_2 by DEPT 135) led them to be assigned as H_3-13, C-12 and C-11, respectively, indicating that the carbonyl must be vicinal to C-13. The signals for H_2-11 and H_2-10 were established as δ 2.43 (*t*, $J = 7.3$ Hz) and 1.54 *m*, respectively, due to their correlation with the ^{13}C resonance for C-12 (δ 210.0). Moreover, the correlation between the H_2-11 signal and the ^{13}C resonance at δ 23.7 showed that δ 23.7 could be attributed to C-10 (^2J) or C-9 (^3J). The slightly negative γ effect of the carbonyl group

Table 3
^{13}C NMR chemical shifts for compounds **1**–**6** and model compound **7**

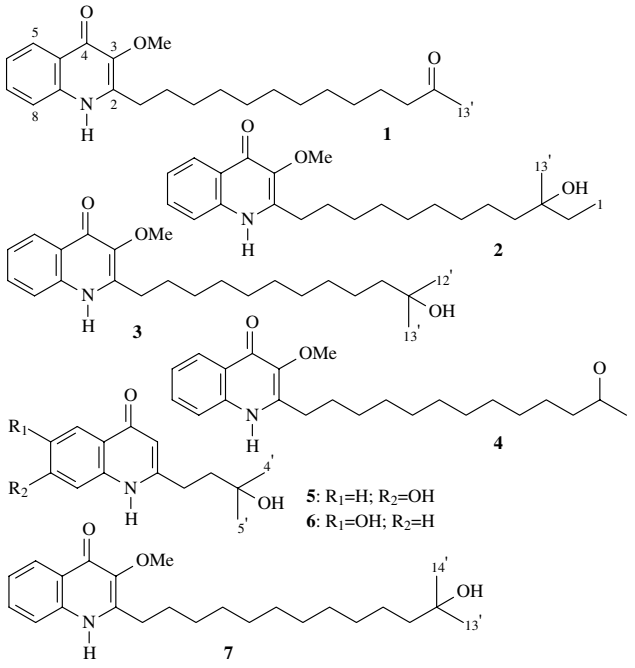
C	1	2	3	4	5	6	7
2	147.8	147.7	148.1	146.4	156.9	153.1	146.2[A]
3	140.2	140.3	140.2	140.2	105.1	105.7	138.4[A]
4	172.8	172.7	172.7	172.9	170.9	176.2	173.2
4[a]	125.8	125.8	125.8	125.8	122.8	125.9	125.9
5	125.5	125.2	125.2	125.6	120.8	107.3	125.8
6	122.8	122.9	122.9	122.8	124.4	153.2	122.9
7	131.1	131.0	131.0	131.1	156.8	121.4	135.1
8	117.9	118.4	118.6	117.6	105.7	119.2	117.5
8a	138.6	138.7	138.9	138.3	133.8	133.6	140.4
1	29.9	30.1	30.1	30.0	28.9	28.4	30.0
2	29.0	29.2	29.6	29.0	42.8	42.51	29.4
3	29.5	29.4[b]	29.7	29.4[d]	68.9	68.4	29.0
4	29.5[a]	29.3[b]	29.5[c]	29.4[d]	29.5	29.1	29.4
5	29.3[a]	29.3[b]	29.4[c]	29.4[d]	29.5	29.1	29.4
6	29.2[a]	29.3[b]	29.4[c]	29.4[d]			29.4
7	29.2[a]	29.2[b]	29.3[c]	29.5[d]			29.4
8	29.1[a]	23.6	29.3[c]	29.2[d]			29.4
9	29.7	41.1	24.3	29.2[d]			29.6[B]
10	23.7	73.1	43.9	25.7			24.3[B]
11	43.8	34.3	71.3	39.3			43.7
12	210.0	8.3	29.2	68.3			71.3
13	30.0	26.4	29.2	23.6			29.2
14							29.2
OMe	60.2	60.2	60.3	60.2			60.1

Spectra of **1**–**7** were run at 100.6 MHz. Spectra of **1**–**4** and **7** were run in $CDCl_3$ and **5**–**6** in DMSO-d_6. [a–d]Assignments may be interchanged in each column.

[A] Data obtained in this study suggest that these resonances were previously assigned incorrectly (Sartor et al., 2003).

[B] The signal for C-10 was established as δ 24.3 by the existence of a negative γ effect of the hydroxy group; however, it was previously assigned incorrectly as C-9 (Sartor et al., 2003); hence, the ^{13}C signals for C-9 and C-10 were interchanged.

allowed C-10 to be assigned to δ 23.7. Unambiguous ^{13}C NMR assignments were also made to C-1 to C-3 and C-9 to C-11 based on COSY and HSQC experiments. However, the signals at δ 29.1–29.5 for five methylenes (C-4–C-8) were not unequivocal; hence, it is possible they may be reassigned. The mass spectrum showed a fragment at *m/z* 175 (100%) due to fission of the side-chain between C-2 and C-1, resulting in a stable odd-electron ion $[C_{23}H_{33}NO_3 + H - C_{13}H_{25}O]^+$, 3-methoxy-4(1*H*)-quinolone. The downfield signal at δ 2.84 (*t*, $J = 7.6$ Hz; δ_C 29.9) from the methylene H_2-1 was consistent with it is allylic connected in the 4(1*H*)-quinolone system. The methoxyl proton at δ 3.92 showed a cross-peak with the ^{13}C signal at δ 140.2, and was therefore assigned to C-3. Moreover, a correlation between the methylene signal at δ 2.84 assigned to H_2-1 and the ^{13}C resonance at δ 140.2 and 147.8 confirmed the presence of a methylene substituent at C-2, allowing for the assignment of the latter ^{13}C signal at δ 147.8 to C-2. The new alkaloid was therefore identified as 2-(12-oxo-tridecanyl)-3-methoxy-4-quinolone (**1**). The structural assignment was also supported by comparing the ^{13}C NMR spectrum with that of 2-(12-hydroxy-12-methyltridecanyl)-3-methoxy-4-quinolone (**7**), which was isolated for the first time from *Dictyoloma vandellianum* (Sartor et al., 2003). HMBC experiments on **1** led to a minor correction previously made in the ^{13}C NMR assignments for **7**; in the latter, the ^{13}C signals of C-2 and C-3 had to be exchanged.



The remaining compounds **2**–**6** gave rise to UV and IR spectra, indicating the presence of a 2-alkyl-4(1*H*)-quinolone nucleus. The ^1H NMR spectra of **2**–**4** clearly indicated A and B rings similar to **1**. The HSQC and HMBC experiments on **2**–**4** permitted the assignment of all hydrogen and carbon atoms of the 3-methoxy-4(1*H*)-quinolone system (Tables 2 and 3). In compound **2**, elemental analysis indicated the molecular formula $C_{23}H_{35}NO_3$, requiring the presence of an aliphatic chain of thirteen carbon atoms when compared with **1**. Hydroxyl, methyl and ethyl groups must be connected to the same carbon in the side-chain due to the observed signals for a hydroxytertiary carbon at δ 73.1, a methyl singlet at δ_H 1.12, a methyl triplet at δ 0.87 ($J = 7.4$ Hz) and a methylene quartet at δ 1.45 ($J = 7.4$ Hz) in the ^{13}C and ^1H NMR spectra (Tables 1 and 3). The methyl signal at δ 0.87 showed cross-peaks with the ^{13}C resonances at δ 73.1 and 34.3 (CH_2, DEPT). The methyl signal at δ 1.12 showed a long-range correlation with two methylene resonances at δ 34.3 and 41.1. These correlations indicated the presence of a structural unit, $–CH_2$-$C(CH_3)(OH)$-CH_2-CH_3, at the end of the side-chain. The signals at δ 41.1 (δ 1.37 *m*, by HSQC), 73.1 and 34.3 (δ 1.45 *q*, by HSQC) were then assigned to C-9, C-10 and C-11 , respectively. The correlation observed between the signals for H_2-11 and H_2-9 and the ^{13}C res-

onances at δ 8.3 and 26.4, respectively, led to their assignment to Me-12 and Me-13 . The slightly negative γ effect of the hydroxy group permitted the assignment of C-8 at δ 23.6. The ESI-mass spectrum showed ions at *m/z* 356 $[C_{23}H_{35}NO_3 + H - H_2O]^+$ and *m/z* 342 $[C_{23}H_{35}NO_3 + H - OH{-}Me]^+$, confirming the presence of a 10-hydroxy-10-methyldodecanyl chain. Thus, the structure of the new alkaloid was characterized as 2-(10-hydroxy-10-methyldodecanyl)-3-methoxy-4-quinolone (**2**).

Compound **3** displayed a similar NMR spectrum to that of 2-(12-hydroxy-12-methyltridecanyl)-3-methoxy-4-quinolone (**7**) (Sartor et al., 2003). The HSQC and HMBC experiments on **3** allowed for the assignment of all the hydrogen and carbon atoms. Elemental analysis and ESI-MS indicated the molecular formula $C_{23}H_{35}NO_3$, confirming that **3** differed from **7** ($C_{24}H_{37}NO_3$) only by removal of one CH_2 group in the side-chain. Moreover, a correlation between the methylene signal at δ 2.84 assigned to H_2-1 , and the ^{13}C resonances at δ 140.2 and 148.1 permitted the assignment of the latter ^{13}C signal at δ 148.1 to C-2. These correlations for a homolog skeleton (**3**) confirm that in **7** the ^{13}C signals for C-2 and C-3 must be interchanged. The complete assignments of chemical shifts for all carbons are given in Table 3. Compounds **1**, **2**, and **7** were used as models. The structure of **3** was thus elucidated as 2-(11-hydroxy-11-methyldodecanyl)-3-methoxy-4-quinolone.

The ^{13}C NMR spectrum of **4** (Table 3) revealed resonances for an aliphatic chain of thirteen carbon atoms containing a hydroxyl group (δ_C 68.3). Moreover, the ^1H NMR spectrum showed an oxymethine proton at δ 3.80 (*sext*, $J = 6.2$ Hz), which was coupled to the methyl doublet at δ 1.19 ($J = 6.2$), indicating the hydroxyl group was located vicinal to C-13 and an unbranched side-chain. HMBC experiments showed correlations of the signal of H_3-13 with the resonance of C-12 (δ 68.3) and the ^{13}C signal at 39.3 (CH_2 by DEPT), leading to its assignment to C-11. The oxymethine proton at δ 3.80 showed cross peaks with the ^{13}C signal at δ 25.7 (CH_2 by DEPT), which was then assigned to C-10. From ESI-MS the ion observed at *m/z* 356 $[C_{23}H_{35}NO_3 + H - H_2O]^+$ confirmed the hydroxyl group in the side-chain. These data were consistent with the structure of 2-(12-hydroxytridecanyl)-3-methoxy-4-quinolone (**4**).

Compound **5**, $C_{14}H_{17}NO_3$ (EA), exhibited signals typical of a 4-quinolone with a substituted A-ring. The ^1H NMR spectrum revealed the presence of three aromatic protons (δ_H 7.90, *d*, $J = 9.0$ Hz; 7.43, *dd*, $J = 9.0$ and 2.5 Hz; 7.47, *d*, $J = 2.5$ Hz). From HMBC experiments, the correlation observed between the ^1H signal at δ 7.90 and the ^{13}C resonance at δ 170.9 (^3J) led to their assignment as H-5 and C-4, respectively, indicating the hydroxyl group is located at C-7 and permitting the assignments of the signals at δ 7.43 and 7.47 to H-6 and H-8, respectively. The HSQC experiments allowed for the assignments of all CH atoms of the 4(1*H*)-quinolone system (Table 3). Moreover, the correlation between the ^1H signal at δ 6.68 (δ_C 105.1 by HSQC), assigned to H-3, and the ^{13}C resonances at δ 28.9 (CH_2 by DEPET) and 156.9 suggested a methylene substituent at C-2 and led to the assignment of the former ^{13}C signal at δ 28.9 to C-1 and the latter δ 156.9 to C-2. The signals for C-4a and C-8a were established as δ 122.8 and 133.8, respectively, due to correlations of the first ^{13}C resonance δ 122.8 and the ^1H signals for H-5 and H-3 and the second δ 133.8 with the ^1H signal for H-5. The methylene signal at δ 2.91 (*t*, $J = 8.3$; δ_C 28.9), assigned to H_2-1, showed a long-range correlation with the ^{13}C resonances at δ 42.8 (CH_2 by DEPET) and 68.9, which were attributed to C-2 and C-3 , respectively, suggesting the presence of a hydroxytertiary carbon for C-3 . Thus, hydroxyl and two methyl groups must be connected at C-3 due to the signals for two methyl singlets at δ_H 1.17 (*s*, 6H, δ_C 29.5) in the ^1H NMR spectrum. The ESI-mass spectrum showed ions at *m/z* 230$[C_{14}H_{17}NO_3 + H - H_2O]^+$ and *m/z* 174 $[C_{14}H_{17}NO_3 - C_4H_9O]^+$, confirming the presence of an 3-hydroxy-3-methylbutyl chain. The structure of the new alkaloid was therefore characterized as 7-hydroxy-2-(3-hydroxy-3-methylbutyl)-4-quinolone (**5**).

Compound **6** exhibited a similar NMR spectrum to that of **5** (Tables 1 and 3). In addition to the signal described for the side-chain of **5**, the ^1H NMR spectrum revealed the presence of three aromatic protons (δ_H 7.37, *d*, $J = 2.7$ Hz; 7.12, *dd*, $J = 8.8$ and 2.7 Hz; 7.43, *d*, $J = 8.8$ Hz). From HMBC experiments, the correlation observed between the ^1H signal at δ 7.37 and the ^{13}C resonance at δ 176.2 (^3J) led to their assignment as H-5 and C-4, respectively, indicating that the hydroxyl group is located at C-6 and allowing for the signals at δ 7.12 and 7.43 to be assigned to H-7 and H-8, respectively. The HSQC and HMBC experiments on **6** permitted the assignment of all the hydrogen and carbon atoms (Tables 1 and 3). The ESI-mass spectrum also showed ions at *m/z* 230 $[C_{14}H_{17}NO_3 + H{-}H_2O]^+$ and at *m/z* 174 $[C_{14}H_{17}NO_3 - C_4H_9O]^+$, confirming that **6** differed from **5** only by the position of the hydroxyl group in the A ring. The structure of **6** was thus defined as 6-hydroxy-2-(3-hydroxy-3-methylbutyl)-4-quinolone.

Spathelia and *Dictyoloma* are known to contain 2-quinolone alkaloids, prenylated chromones and limonoids (Burke et al., 1972; Mester, 1983; Gray, 1983; Diaz et al., 1983; Suwanborirux et al., 1987; Vieira et al., 1988, 1990; Campos et al., 1987; Sartor et al., 2003). However, prenylated chromones have only been reported from the genera *Spathelia, Dictyoloma* and *Harrisonia*, as well as from the Cneoraceae, and Ptaeroxylaceae (Gray, 1983). *Harrisonia* is a member of the Simaroubaceae, although it differs from other genera

by its lack of quassinoids. Its limonoid constituents suggest a strong affinity with rutaceous genera such as *Spathelia* (da Silva et al., 1984, 1987). Chromones have not been found in other Simaroubaceae or Rutaceae. Thus, according to Waterman (1983), the co-occurrence of chromones in these taxa is phylogenetically significant by segregating them into a distinct group near the associated large family Rutaceae. 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 (Chase et al., 1999; Scott et al., 2000). This analysis also indicated that the Simaroubaceae and Meliaceae are the outgroups closest to Rutaceae (Chase et al., 1999; Scott et al., 2000). The 2-alkyl-4(1*H*)-quinolone alkaloids that have recently been isolated from *Dictyoloma vandellianum* showed strong similarities to the Zanthoxyleae, which contain several 2-alkyl-4-quinolones. These data strongly supported Chase and Scott's taxonomic conclusions (1999, 2000) and permitted Sartor et al. (2003) to include Dictyolomatoideae in Waterman's phylogenetic diagram (1983) in a position between the proto-Rutaceae genera and the Spathelioideae, but close to the Zanthoxyleae (Sartor et al., 2003). These taxa, as separated small figures (an expression of the number of species in the taxon), occupy a position between the advanced Rutaceae and Simaroubaceae (Fig. 1). Now, the finding of 2-alkyl-4(1*H*)-quinolones in *Spathelia excelsa* shows strong similarities with Zanthoxyleae [*Platydesma* and *Tetradium* (*T. ruticarpum* = *Euodia rutaecarpa*)], Ruteae (*Haplophyllum* and *Ruta*), Boronieae (*Boronia*), Cusparieae (*Raulinoa*), Toddalieae (*Acronychia, Vepris* and *Ptelea*) and Dictyolomatoideae (*Dictyoloma vandellianum*), which contain several 2-alkyl-4-quinolones (Mester, 1983; Biavatti et al., 2002; Sartor et al., 2003). Thus, these data provide firm support for moving Spathelioideae close to or within the Dictyolomatoideae (Fig. 1).

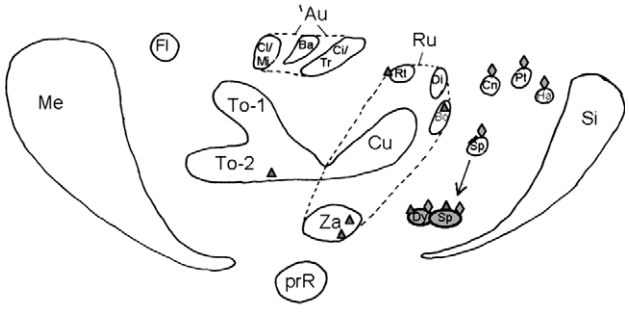


Fig. 1. Waterman's phylogenetic diagram of the Rutales, based on the distribution of secondary metabolites, is modified to remove the Spathelioideae subfamily, shifting it to close to the Dictyolomatoideae. A, Aurantioideae; Ba, Balsamocitrinae; Bo, Boronieae; Ci, Citrinae; Cl, Clauseninae; Cn, Cneoraceae; 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-1, (coumarin containing) Toddalioideae, To-2, (acridone containing) Toddalioideae, Tr, Triphasiinae; Za, Zanthoxyleae (Waterman, 1983); Dy, Dictyolomatoideae (Sartor et al., 2003). (♦) Distribution of chromones, (▲) Distribution of 2-alkyl-4(1*H*)-quinolones.

3. Experimental

3.1. General

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

3.2. Plant material

Spathelia excelsa was collected in the Forest Reserve Adolpho Ducke, Amazonas, Brazil, and identified by J.R. Pirani (Universidade de São Paulo). A voucher specimen (4227) is deposited in the Herbarium of the Instituto Nacional de Pesquisa da Amazônia (INPA), Manaus, AM.

3.3. Isolation of compounds

Ground leaves (2940 g) were extracted with hexane, then CH_2Cl_2 and finally with MeOH. The concentrated MeOH extract was subjected to CC over silica gel. Elution with hexane, followed by a CH_2Cl_2–MeOH gradient, yielded 16 frs. Fr. 14 was applied to silica gel, CC eluted with a CH_2Cl_2–MeOH gradient to afford additional frs (Fr.14A–C). Fr. 14A was subjected to further Si gel CC eluted with gel, CH_2Cl_2, CH_2Cl_2–MeOH 1:1, MeOH, and then Si gel CC using CH_2Cl_2–EtOAc as eluant to give additional frs. Fr. 14A.5 was reapplied to silanized silica (EtOAc), yielding a new fr. containing **1**, with the latter purified by prep. TLC (silica gel, CH_2Cl_2–EtOAc, 6:4) to give **1** (10 mg). Fr. 14A.6 was subjected to flash chromatography on silica gel eluted with a CH_2Cl_2–EtOAc–MeOH gradient to afford **2** (14 mg), **3** (26 mg) and **4** (3 mg), respectively. Fr. 14B was applied to successive Si gel CC (eluant, CH_2Cl_2, CH_2Cl_2–MeOH 1:1, MeOH and then again on silica gel, CH_2Cl_2–EtOAc gradient) to give 3-*O*-β-D-glucopyranosylsitosterol (8 mg). Fr. 14C was also subjected to successive Si gel CC (Florisil), eluted with a CH_2Cl_2–MeOH gradient, and then silica gel, using CH_2Cl_2–

EtOAc as a gradient to yield (−)-epicatechin (7 mg), **5** (5 mg) and **6** (26 mg).

*3.3.1. 2-(12-Oxo-tridecanyl)-3-methoxy-4-quinolone (**1**)*

White gum; UV λ_{max} (MeOH) nm (ε): 242, 247, 324, 335; IR ν_{max} ($CHCl_3$) cm^{-1}: 1705, a weak carbonyl band at 1632 cm^{-1} can be explained by the carbonyl group being present in partially enolized form; ^1H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR/DEPT (100 MHz, $CDCl_3$), see Table 3; HSQC (400/100 MHz, $CDCl_3$); COSY (400 MHz, $CDCl_3$); HMBC (400/100 MHz, $CDCl_3$), see Table 2. EA: Found: C, 74.34; H, 8.92, N, 3.76. Calc. for $C_{23}H_{33}NO_3$: C, 74.36; H, 8.95; N, 3.77; O, 12.92%; ESI-MSMS, *m/z* (rel. int.): 372 $[M+H]^+$ (70), 230 (50), 202 (30), 175 (100).

*3.3.2. 2-(10-Hydroxy-10-methyldodecanyl)-3-methoxy-4-quinolone (**2**)*

Colorless oil; $[\alpha]_D + 5.4^0$ ($CHCl_3$; c 0.02); UV λ_{max} (MeOH) nm: 241, 248, 325, 334; IR ν_{max} ($CHCl_3$) cm^{-1}: 3326, a weak carbonyl band at 1632 cm^{-1} can be explained by the carbonyl group being present in partially enolized form; ^1H NMR 400 MHz, $CDCl_3$, see Table 1; ^{13}C NMR/DEPT 100 MHz, $CDCl_3$, see Table 3; HSQC (400/100 MHz, $CDCl_3$); COSY (400 MHz, $CDCl_3$); HMBC 400/100 MHz, $CDCl_3$, see Table 2. EA: Found: C, 73.94; H, 9.45, N, 3.74. Calc. for $C_{23}H_{35}NO_3$: C, 73.96; H, 9.44; N, 3.75; O, 12.85%; ESI-MSMS, *m/z* (rel. int.): 356 $[M+H-H_2O]^+$ (20), 342 $[M+H-OH-Me]^+$ (100), 175 (40).

*3.3.3. 2-(11-Hydroxy-11-methyldodecanyl)-3-methoxy-4-quinolone (**3**)*

Colorless oil; UV λ_{max} (MeOH) nm: 241, 248, 326, 336; IR ν_{max} ($CHCl_3$) cm^{-1}: 3347, a weak carbonyl band at 1660 cm^{-1} can be explained by the carbonyl group being present in partially enolized form; ^1H NMR (400 MHz, $CDCl_3$): see Table 1; ^{13}C NMR/DEPT (100 MHz, $CDCl_3$), see Table 3; HSQC (400/100 MHz, $CDCl_3$); COSY (400 MHz, $CDCl_3$); HMBC (400/100 MHz, $CDCl_3$), see Table 2. EA: Found: C, 73.95; H, 9.45, N, 3.74. Calc. for $C_{23}H_{35}NO_3$: C, 73.96; H, 9.44; N, 3.75; O, 12.85%; ESI-MSMS, *m/z* (rel. int.): 374 $[M+H]^+$ (100), 356 $[M+H-H_2O]^+$ (30), 125 (40).

*3.3.4. 2-(12-Hydroxytridecanyl)-3-methoxy-4-quinolone (**4**)*

Colorless oil; $[\alpha]_D + 20.7^0$ ($CHCl_3$; c 0.02); UV λ_{max} (MeOH) nm: 240, 248, 326, 335; IRν_{max} ($CHCl_3$) cm^{-1}: 3269, a weak carbonyl band at 1632 can be explained by the carbonyl group being present in partially enolized form; ^1H NMR (400 MHz, $CDCl_3$), see Table 1; ^{13}C NMR/DEPT (100 MHz, $CDCl_3$), see Table 3; HSQC (400/100 MHz, $CDCl_3$); COSY (400 MHz, $CDCl_3$); HMBC (400/100 MHz, $CDCl_3$), see Table 2. EA: Found: C, 73.95; H, 9.45, N, 3.74. Calc. for $C_{23}H_{35}NO_3$: C, 73.96; H, 9.44; N, 3.75; O, 12.85%; ESI-MSMS, *m/z* (rel. int.): 374 $[M+H]^+$ (90), 356 $[M+H-H_2O]^+$ (30), 139 (70), 125 (100).

*3.3.5. 7-Hydroxy-2-(3-hydroxy-3-methylbutyl)-4-quinolone (**5**)*

Yellow powder; UV λ_{max} (MeOH) nm: 240, 331, 347; IRν_{max} (MeOH) cm^{-1}: 3452, a weak carbonyl band at 1632 can be explained by the carbonyl group being present in partially enolized form; ^1H NMR (400 MHz, DMSO-d_6), see Table 1; ^{13}C NMR/DEPT (100 MHz, DMSO-d_6), see Table 3; HSQC (400/100 MHz, DMSO-d_6); COSY (400 MHz, DMSO-d_6), HMBC (400/100 MHz, DMSO-d_6), see Table 2. EA: Found: C, 68.03; H, 6.93, N, 5.64. Calc. for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66; O, 19.41%; ESI-MSMS, *m/z* (rel. int.): 230 $[M+H-H_2O]^+$ (30), 174 $[M-C_4H_9O]^+$ (100).

*3.3.6. 6-Hydroxy-2-(3-hydroxy-3-methylbutyl)-4-quinolone (**6**)*

Yellow powder; UV λ_{max} (MeOH) nm: 240, 332, 346; IR ν_{max} (MeOH) cm^{-1}: 3454, a weak carbonyl band at 1632 can be explained by the carbonyl group being present in partially enolized form; ^1H NMR (400 MHz, DMSO-d_6), see Table 1; ^{13}C NMR/DEPT (100 MHz, DMSO-d_6), see Table 3; HSQC (400/100 MHz, DMSO-d_6); COSY (400 MHz, DMSO-d_6), HMBC (400/100 MHz, DMSO-d_6), see Table 2. EA: Found: C, 68.00; H, 6.92, N, 5.67. Calc. for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66; O, 19.41%; ESI-MSMS, *m/z* (rel. int.): 230 $[M+H-H_2O]^+$ (30), 174 $[M-C_4H_9O]^+$ (100).

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