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Turrianes from *Kermadecia rotundifolia* as new acetylcholinesterase inhibitors

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ABSTRACT

Four new kermadecins, together with the known kermadecins A, B and D have been isolated from the *Kermadecia rotundifolia* ethyl acetate bark extract. These compounds are derivatives of the (20-membered-*o*,-*p*)cyclophane skeleton and belong to the turriane family. The structures were elucidated by mass spectrometry, extensive one- and two-dimensional NMR spectroscopy and through comparison with data reported in the literature. Isokermadecin D (**2**) and kermadecins D and J (**7** and **4**) possess significant inhibitory effect on acetylcholinesterase.

1. Introduction

In the course of automated screening for small-molecules for acetylcholinesterase inhibitory activity, a significant activity was observed for kermadecin D (**7**), a cyclophane-type compound isolated from the bark of *Kermadecia elliptica* (Jolly et al., 2008). With the aim to discover analogues of kermadecin D, we performed a chemical investigation of *Kermadecia rotundifolia* Brongniart & Gris, an endemic species to New Caledonia with very similar morphological characteristics to *K. elliptica*.

The genus *Kermadecia* (Proteaceae), contains twelve species, of which four are endemic to New Caledonia (Virot, 1968). No report is mentioned regarding their utilisation by traditional healers. *K. rotundifolia*, may reach 20 m in height, possesses large orbiculate to ovalate leaves, and white to yellowish small flowers are organized in branched racemes 15–40 cm long. This rare species is mainly distributed in the Northern part of the main highland. Until now *K. elliptica* is the only species that has been investigated chemically and biologically (Jolly et al., 2008).

The isolated compounds are new bisresorcinol derivatives having a 20-membered-*o*,-*p*-cyclophane skeleton or a biaryl-ether containing macrocycle in their structure. We wish to report in this paper the isolation and characterization of four new kermadecins (**1**–**4**) together with their acetylcholinesterase inhibitory activities and those of three other known kermadecins (**5**–**7**).

2. Results and discussion

This study was accomplished with the aid of HPLC, LC–APCI-MS and NMR analysis, and led to the isolation and identification of four new kermadecins (**1**–**4**) together with the known kermadecins A, B and D (**5**–**7**). In order to determine the presence of common fragments in this chemical series, we applied the LC/MS–MS method developed in our previous study on *K. elliptica* (Jolly et al., 2008). In APCI positive-ion mode, LC/MS–MS analysis of compounds **1**–**4** indicated the presence of ions resulting from the systematic loss of fragments 182 ($C_{13}H_{26}$) (**1**, **2** and **4**) or 180 ($C_{13}H_{24}$ for compound **3**), mass units in favour of a 14 carbons aliphatic chain, with an additional double bond in the case of **3**. In negative-ion mode, LC/MS–MS analyses of the quasimolecular peak $[M-H]^-$ of **2** and **3** showed the presence of an ion at $m/z = 369$ corresponding to the loss of a fragment of 108 mass units, suggesting the loss of a dimethylpyran ring.

Kermadecins A, B and D (**5**–**7**), previously isolated from *K. elliptica* (Jolly et al., 2008) were identified by comparison of their spectroscopic data (HR-MS, ^1H and ^{13}C NMR).

The HRESIMS of compound **1** indicated an ion peak m/z 491.2789 $[M-H]^-$ (calcd. for 491.2797) giving the molecular formula $C_{31}H_{40}O_5$. The IR spectrum of **1** showed absorption bands at 3600 cm^{-1} for hydroxyl groups, 1608 and 1424 cm^{-1} for an

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Table 1
^{13}C NMR spectroscopic data (125 MHz) in $CDCl_3$ for compounds **1**–**3** and CD_3OD for compound **4**.

Carbon	1	2	3	4
1	34.5	30.7	32.9	31.2
2	31.3	31.2	30.8	31.7
3	27–29	27–30	27.7	31.3
4	27–29	27–30	130.9	28–30
5	27–29	27–30	131.1	28–30
6	27–29	27–30	29.0	28–30
7–11	27–29	27–30	27–29	28–30
12	27–29	27–30	27–29	28–30
13	27.0	27–30	29.4	31.8
14	27.7	35.8	35.1	36.8
15	145.5	147.1	144.4	146.4
16	135.8	107.9	107.4	108.5
17	183.7	160.5	153.8	161.6
18	116.3	99.5	107.3	100.0
19	152.4	156.2	153.8	159.3
20	183.5	108.5	107.4	109.5
21	109.1	134.5	108.4	134.6
22	149.0	148.0	153.3	151.8
23	108.4	108.7	108.2	102.6
24	154.6	150.0	154.2	156.0
25	110.7	108.9	109.2	108.6
26	126.0	137.5	146.1	139.0
27	116.6	116.5	115.7	
28	128.7	128.8	129.2	
29	76.3	76.5	76.3	
30	28.2	28.0	27.8	
31	28.1	28.0	27.8	

aromatic ring and a strong band at 1632 cm^{-1} suggesting a paraquinone moiety, confirmed in the ^{13}C NMR spectrum by the presence of signals at 187.3 and 183.5 ppm for two ketones at C-17 and C-20, respectively. The spectral data of **1** were very similar with those of kermadecin F (**8**) (Jolly et al., 2008). In the 1H NMR, two singlets at δ 6.38 (H-25) and 6.64 (H-16) suggested the presence of one pentasubstituted aromatic and one trisubstituted paraquinone ring. In addition, the presence of a dimethylpyran ring fused to a phenol ring was confirmed with ^1H and ^{13}C NMR data (Table 1). In the HMBC spectrum, correlations from H_2-1 to C-21, C-25 and C-26, H-16 to C-14, C-17, C-18 and C-20, and from H_2-14 to C-16 and C-20 implied that the trisubstituted paraquinone was located as shown in structure **1**. Other HMBC correlations were similar to those observed for kermadecin A (**5**), indicating that the dimethylpyran ring was fused to the aromatic ring in a similar way to **5**. This was confirmed by the correlation between H-27 (δ 6.57, *d*, *J* = 10 Hz) and the OH-22 (δ 4.80) observed in the NOESY spectrum, obtained in $CDCl_3$ at room temperature. Compound **1** was named isokermadecin F. Isokermadecin F, which possesses a chiral biaryl axis arising from the presence of the asymmetric paraquinone moiety, showed optical activity $[\alpha]_D$ +24 (*c* = 1, $CHCl_3$), of opposite sign to kermadecin F (**8**).

Compound **2** was assigned the formula $C_{31}H_{42}O_4$ based on HRESIMS. Most NMR signals of compound **2** were very similar to those of kermadecin D (**7**) suggesting the presence of the same substituted aromatic rings as in **7**: one dimethylpyran ring fused to one aromatic ring and a long carbon chain attached to both aromatic rings. The ether linkage was suggested by the shift in the down field region of C-21 at δ 134.5 (Ahmed et al., 2000), which in turn give a correlation to H_2-1 in the HMBC spectrum. In addition, the chemical shifts of the two methyl groups at δ 1.25, similar to the methyl signals in kermadecin B (**6**), and HMBC correlations between H-27 and C-22, C-23 and C-24, indicated that the dimethylpyran ring was fused to the aromatic ring in a similar way to **6**. Thus compound **2**, named isokermadecin D, possesses the structure depicted in Fig. 1.

The molecular formula of **3**, named kermadecin I, was $C_{31}H_{40}O_4$ as indicated by HRESIMS. The ^1H and ^{13}C NMR spectra were very similar to those of kermadecin B (**6**) revealing symmetrical tetrasubstituted and pentasubstituted aromatic rings linked by a monounsaturated C_{14} aliphatic chain, and a dimethylpyran ring fused to an aromatic ring. In addition the presence of multiplet signals at δ 5.22 and 5.34 (H-4 and H-5) in the ^1H NMR spectrum and signals at δ 130.9 and 131.1 (C-4 and C-5) in the ^{13}C NMR spectrum were compatible with an additional double bond in the

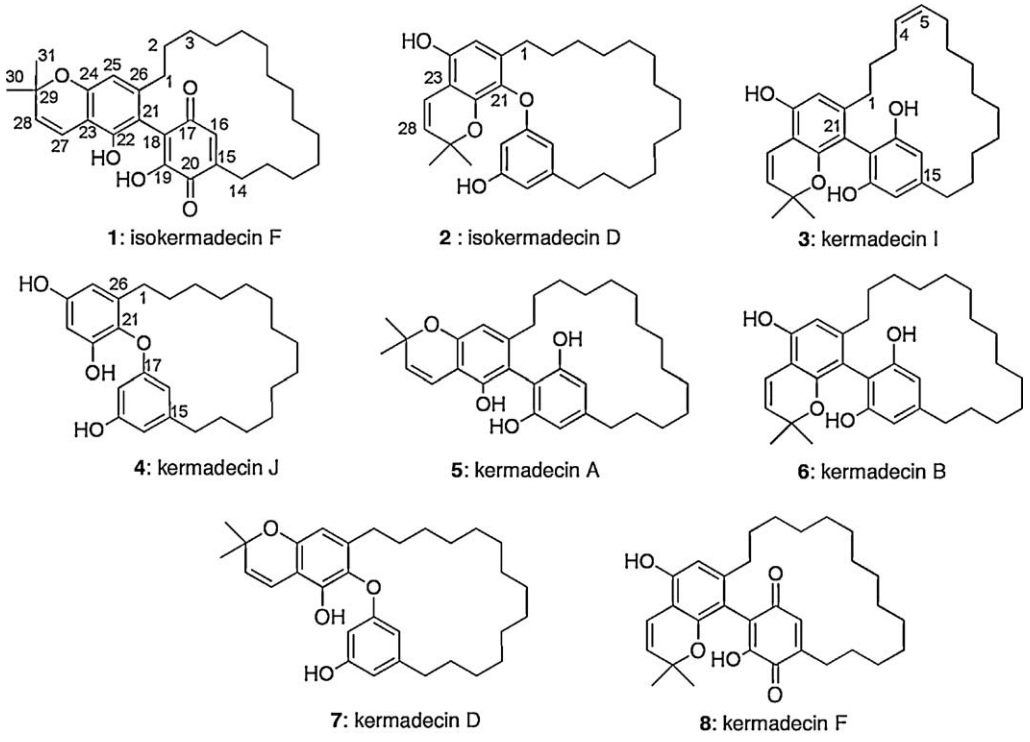

Fig. 1. Structures of compounds **1**–**8**.