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Cytotoxic turrianes of *Kermadecia elliptica* from the New Caledonian rainforest

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Abstract

In the course of an automated screening for small molecules presenting cytotoxic activity, eight new cyclophanes named kermadecins A–H (**1–8**), have been isolated from the bark of a New Caledonian plant, *Kermadecia elliptica,* Proteaceae. A LC/APCI-MS study performed on kermadecins A, B and C, provided a reliable method for the detection of other analogues existing in small quantities in the extract. This led to the isolation of five other members of this chemical series. The structures were elucidated by extensive mono- and bi-dimensional spectroscopy and mass spectrometry. The cytotoxic activity of four of them was evaluated on various cell lines.

Keywords: *Kermadecia elliptica*; Proteaceae; Kermadecin; Turriane; Cyclophane; Cytotoxicity; LC/APCI-MS

1. Introduction

With the objective to discover new bioactive compounds from the New Caledonian flora, *Kermadecia elliptica* Brongniart & Gris, (Proteaceae) was selected for a phytochemical study following its potent cytotoxicity against KB cells. The EtOAc extract of the bark exhibited 100% and 44% inhibition of the cell growth at 10 and 1 μg ml^{-1}, respectively. Bioassay and LC/MS-directed fractionations of the EtOAc extract provided eight new cyclophanes, named kermadecins A–H (**1–8**). These compounds are derivatives of the (14-*p*,0-*o*)cyclophane skeleton (**9**) and belong to the turriane family. Turrianes were isolated first from two Australian Proteaceae, *Grevillea striata* (Ridley et al., 1970) and *G. robusta* (Cannon et al., 1973; Chuang and Wu, 2007). Those turrianes were shown to be potent DNA cleaving agents under oxidative conditions when evaluated in the presence of copper ion and butylamine (Furstner et al., 2002). The skeleton of kermadecin D (**4**) was also found in grevirobstol B isolated from *Grevillea robusta* (Ahmed et al., 2000). A literature survey revealed that the *Kermadecia* species had never been studied for their secondary metabolite content.

The Proteaceae are an archaic family of tropical plants that are mostly represented in Africa and Australia. There are more than 1300 species all over the world. The genus *Kermadecia* is well represented in New Caledonia with 4 endemic species. No report is mentioned regarding their utilisation by traditional healers. Naphthoquinones (Mock et al., 1973), tropane alkaloids (Bick et al., 1979; Butler et al., 2000; Lounasmaa et al., 1980), phenols (Cannon and Metcalf, 1971) and β-sitosterol (Ritchie et al., 1965) have been isolated from other Proteaceae. The structures of compounds **1** to **8** were identified by NMR spectroscopic

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and mass spectroscopic analysis. Four of the eight new turrianes were submitted to cytotoxicity assays. Two of them (**1–2**) showed significant cytotoxicity against KB and L1210 cancer cell lines.

2. Results and discussion

Kermadecia elliptica was collected in the rain forest "Forêt Plate" in the west central chain of New Caledonia, known as "Grande Terre". The air-dried bark was extracted successively with EtOAc and MeOH. The EtOAc extract was found to be the most cytotoxic, producing 100% and 44% of inhibition of the KB cell growth at 10 and 1 μg ml^{-1}, respectively. Bioassay-guided fractionation of the active fraction led to the isolation of three new compounds: kermadecins A–C **(1–3).** A LC/MS method was then used to detect and to direct further purifications leading to the isolation of kermadecins D–H **(4–8)**. Based on NMR data obtained from ^{1}H, ^{13}C, COSY, HSQC and HMBC experiments, the compounds could be identified as turrianes (Fig. 1).

A preliminary LC/APCI-MS study of kermadecins A (**1**), B (**2**) and C (**3**) revealed to be particularly efficient due to the low polarity of this kind of compounds and the presence of phenols which gave reliable ionisations in both positive- and negative-ion modes. The LC/MS-MS study was then performed on all the fractions with the aim to find minor compounds of the same family. This led to the isolation of kermadecins D–H (**4–8**). The presence of common fragments was systematically observed in MS–MS. In negative-ion mode, LC/MS-MS analyses of the quasimolecular peak $[M–H]^-$ of kermadecins A and B (**1** and **2**) showed the presence of an ion at $m/z = 369$ corresponding to the loss of a fragment of 108 amu, suggesting the loss of the dimethylpyran ring as shown in Fig. 2. The presence of this ion was also observed

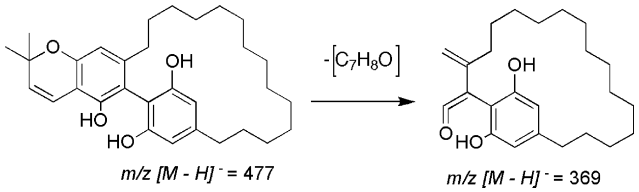

Fig. 2. Hypothesis of fragmentation of kermadecin A in APCI negative-ion mode.

in the mass spectrum of kermadecins D, E, and G (**4**, **5**, **7**). It is interesting to note that an ion at m/z 107 is also systematically observed in the MS spectra for compounds **1**, **2**, **4** and **6** having the dimethylpyran fused to the phenol moiety. In the case of kermadecin E (**5**), a fragment of 110 amu was lost, corresponding to the open heterocycle; this time an ion at m/z 109 was observed. Finally, it is worthy to note that the fragmentation did not occur for kermadecin C (**3**), which possesses a paraquinone moiety fused to the heterocycle. In APCI positive-ion mode, LC/MS–MS analysis of kermadecins A–C (**1–3**) indicated the presence of another ion at $m/z = 297$, resulting of the loss of a 182 amu fragment, which was supposed to be a 14 carbons long chain. This fragmentation, which could correspond to two kinds of ions (Fig. 3), was systematically observed for all the compounds of the series, indicating that they all have a 14 carbon aliphatic chain. In the case of kermadecin H (**8**), a fragment of 180 amu was lost, suggesting the presence of a double bond in the aliphatic chain.

Compound **1** has a molecular formula $C_{31}H_{42}O_4$ supported by HRESIMS showing a $[M + Na]^+$ ion peak at 501.3071 (calcd. 501.2981). The IR spectrum of **1** showed absorption bands at 3518 cm^{-1} for hydroxy groups and at 1616 and 1426 cm^{-1} for an aromatic ring suggesting that compound **1** was a phenolic compound. The UV absorption λ_{max} (MeOH) at 280 nm (log ε 4.15) was indicative

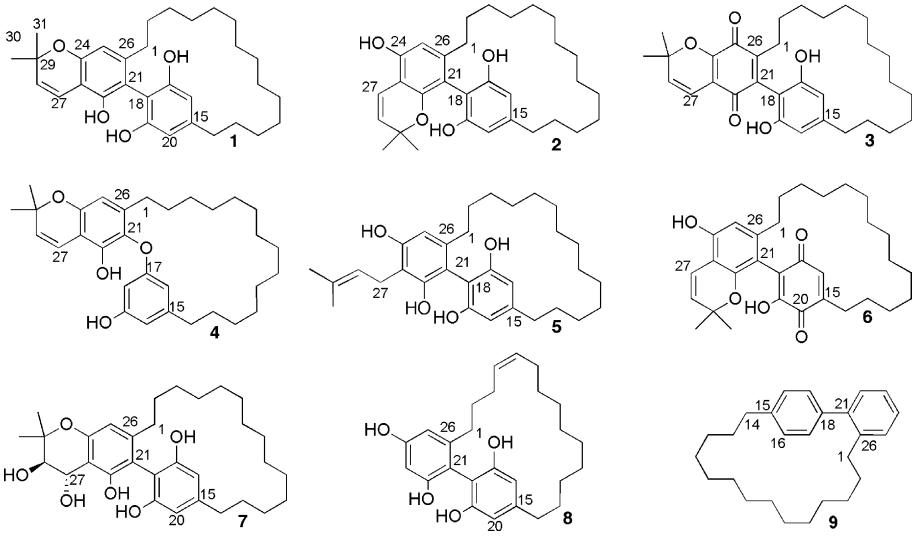

Fig. 1. Structures of kermadecins A–H (**1–8**) and (14-*p*,0-*o*)cyclophane skeleton (**9**).