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# Spirokermeline: A Macrocyclic Spirolactone from *Kermadecia elliptica* Brongn. & Gris

Mays Khazem,[a] Claire Jolly,[e] Thomas Gaslonde,*[a] Stéphane Massip,[b] Philippe Negrier,[c] Philippe Espeau,[d] Sylvie Michel,[a] and Marc Litaudon*[e]

**Abstract:** Spirokermeline, a new macrocyclic compound derived from resorcinol, was isolated from *Kermadecia elliptica*, an endemic species of New Caledonia. The structure of spirokermeline was elucidated on the basis of spectroscopic analysis and X-ray single-crystal diffraction analysis. This is the first time that a 3*H*,3′*H*-spiro[benzofuran-2,1′-isobenzofuran]-3,3′-dione was isolated from higher plants and only the second time that this scaffold was found in nature. A possible biosynthetic pathway is proposed.

## Introduction

The genus *Kermadecia* (Proteaceae) is well represented in New Caledonia with four endemic species. From the bark of *K. elliptica* and *K. rotundifolia*, Litaudon and colleagues reported the isolation of twelve derivatives with a (14-*p*,0-*o*)cyclophane skeleton, which were given the trivial name "kermadecin".[1,2] Kermadecins resulted from the cyclization of bisresorcinol derivatives in cyclophane, leading to turriane-type kermadecin[3] (**1**) exemplified by kermadecin A (**3**), and robustol-type kermadecin (**2**) exemplified by kermadecin D (**4**), Figure 1.[4] Kermadecins A and B showed significant cytotoxic activities against KB and L1210 cancer cell lines, whereas kermadecins D and J and isokermadecin D exhibited a good inhibitory effect on acetylcholinesterase.[1,2] Recently, a series of derivatives of kermadecin A has been synthesized from the natural product to conduct structure–activity relationship studies.[5] These studies required the extraction of 16 kg of barks of *K. elliptica* leading to the isolation of 450 mg of kermadecin A after several purification steps. The investigation of one of the apolar chromatographic fractions has led to the isolation of a minor metabolite of this plant possessing a 2,9-dioxo-1,6-dioxaspiro[4,4]nona-3,7-dien moiety described for the second time in nature. To the best of our knowledge, nidulal, an inducer of differentiation of human HL-60 promyeocytic leukemia cells isolated from the basidiomycete *Nidula candida* was the only natural product possessing such a spirobicyclic moiety.[6] However, the first synthesis of a compound possessing such a spiro substructure took place more than 35 years ago.[7] This subunit can be found in the structure of fluorescamine, a reagent that reacts with primary amines to yield intensely fluorescent substances.[8] Also, this subunit is present in various spiro lactones,[9] some of which have shown interesting antiviral properties against influenza viruses.

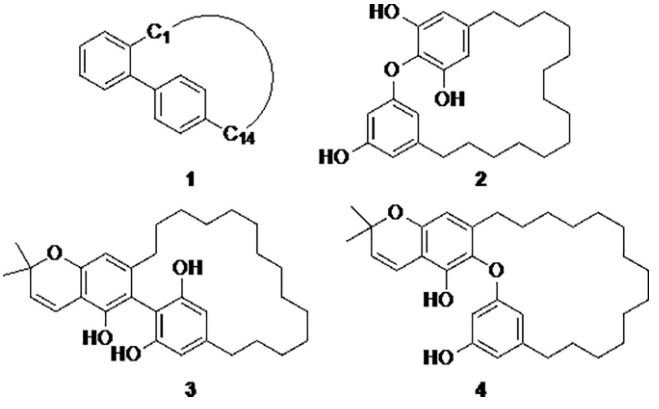



Figure 1. Turriane ring system (**1**), robustol (**2**), kermadecin A (**3**), and kermadecin D (**4**).

## Results and Discussion

The bark (16 kg) of *Kermadecia elliptica* Brongn. & Gris was collected in 2008 in New Caledonia at "Forêt plate" (UTM 0511686/7663347, elevation 328 m), and was extracted with ethyl acet-

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ate at 40 °C under a pressure of 100 bars. The corresponding voucher specimen POU-0289 is kept at the Herbarium of the IRD Center, Nouméa, New Caledonia. The chemical investigation of this extract led to the isolation of a new macrocyclic compound, named spirokermeline (**5**), together with 13 known compounds, β-sitosterol and β-sitostenone, *trans*-docosanyl and *trans*-cosanyl ferulate,[10] vanillin, lignoceric acid, kermadecins A–F, and isokermadecin D.[1,2] The structures of the known compounds were unambiguously determined by $^{1}H$ and $^{13}C$ NMR analysis, and through comparison with spectroscopic data reported in the literature.

Compound **5**, obtained as a white solid (9 mg), showed a protonated molecule ion at *m/z* 479.279259 (calcd. 479.279201) in HRESI(+)MS, corresponding to the molecular formula, $C_{30}H_{38}O_5$. When compared with the formula of almost all kermadecins, we noticed that compound **5** had one less carbon atom. The IR spectrum of **5** showed absorption bands at 1610, 1721, and 1789 $cm^{-1}$ for α,β-unsaturated ketone and α,β-unsaturated γ-lactone functions. The $^{1}H$ NMR and $^{13}C$ NMR spectroscopic data (Table 1) revealed the presence of a penta-substituted aromatic ring (ring A), a long carbon chain, and signals assignable to a 2,2-dimethylpyran ring (ring B). In the HMBC spectrum, correlations observed from the vinylic proton H-26 at $\delta_H$ = 6.46 ppm (d, *J* = 10.6 Hz) to carbon atoms C-21 ($\delta_C$ = 167.6 ppm), C-22 ($\delta_C$ = 104.5 ppm), C-23 ($\delta_C$ = 162.4 ppm), and C-28 ($\delta_C$ = 79.1 ppm), and from H-26 and H-27 to C-28, which bears two equivalent methyl groups at $\delta_C$ = 29.0 ppm, suggested that the dimethylpyran ring system is fused to the aromatic ring A as depicted in Figure 2.

Table 1. $^{1}H$ (300 MHz) and $^{13}C$ (75 MHz) data of spirokermeline (**5**) in $CDCl_3$.

| Position | $\delta_H$ [ppm], mult. (*J* in Hz) | $\delta_C$ [ppm] |
|---|---|---|
| 1a | 2.57, m | 31.9 |
| 1b | 3.07, m | |
| 2 | 1.55, m | 30.1 |
| 3 to 12 | 1.24–1.36, m | 27.2–30.0 |
| 13 | 1.70, m | 30.6 |
| 14a | 2.35, m | 25.2 |
| 14b | 2.55, m | |
| 15 | – | 140.4 |
| 16 | 6.69, s | 140.3 |
| 17 | – | 105.9 |
| 18 | – | 170.5 |
| 19 | – | 188.7 |
| 20 | – | 110.1 |
| 21 | – | 167.6 |
| 22 | – | 104.5 |
| 23 | – | 162.4 |
| 24 | 6.36, s | 113.5 |
| 25 | – | 148.0 |
| 26 | 6.46, d (10.6) | 114.4 |
| 27 | 5.61, d (10.6) | 129.2 |
| 28 | – | 79.1 |
| 29 | 1.47, s | 29.0 |
| 30 | 1.48, s | 29.0 |

Long-range correlations from H-24 to C-1, C-19, C-20, C-22, and C-23 in the HMBC spectrum, confirmed the location of a ketone function ($\delta_C$ = 188.7 ppm, C-19) at C-19, and an aliphatic chain to C-25. The presence of a 2,9-dioxo-1,6-dioxaspiro[4,4]-

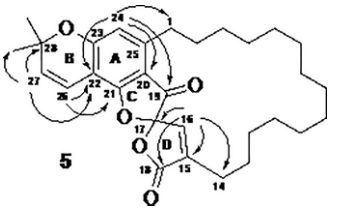



Figure 2. Key HMBC correlations for spirokermeline (**5**).

nona-3,7-dien moiety can be deduced from long-range correlations between the vinylic proton at $\delta_H$ = 6.69 ppm (H-16) with C-14, C-15, C-17, and C-18, and from the chemical shift of C-17 at $\delta_C$ = 105.9 ppm, typical of a hemiketal carbon. From these data, it can be proposed that the furanone ring C is fused to the benzopyran unit through C-20 and C-21, and that a 14-carbon aliphatic chain is attached to ring A at C-25. However, the substitution of the lactone ring D at the alpha or beta position with the long aliphatic chain still needs to be established.

The structure of compound **5** was finally confirmed by single-crystal X-ray diffraction (deposition number: CCDC 1822347). The asymmetric unit obtained from X-ray analysis is presented in Figure 3. This study permitted us to confirm that the 14-carbon aliphatic chain was attached to C-15, in the alpha position of the carbonyl group. In addition, asymmetric carbon atom 17 found in the structure solved in the $P2_1/c$ centrosymmetric space group indicates that *R* and *S* enantiomers are present in the unit cell. Consequently, the natural spirokermeline occurs as a racemic mixture, as confirmed by the specific rotation value of **5** $[\alpha]_D^{22} = 0$ (*c* = 0.65, $CHCl_3$). The details of this study are presented in the Supporting Information, including the crystal data and the structure refinement of the racemic compound.

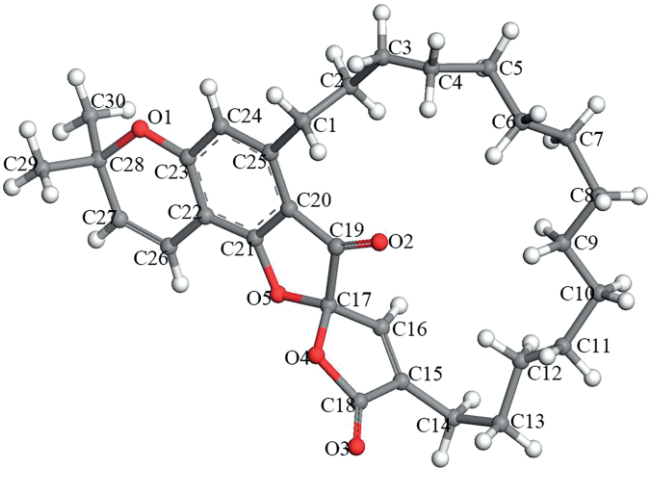
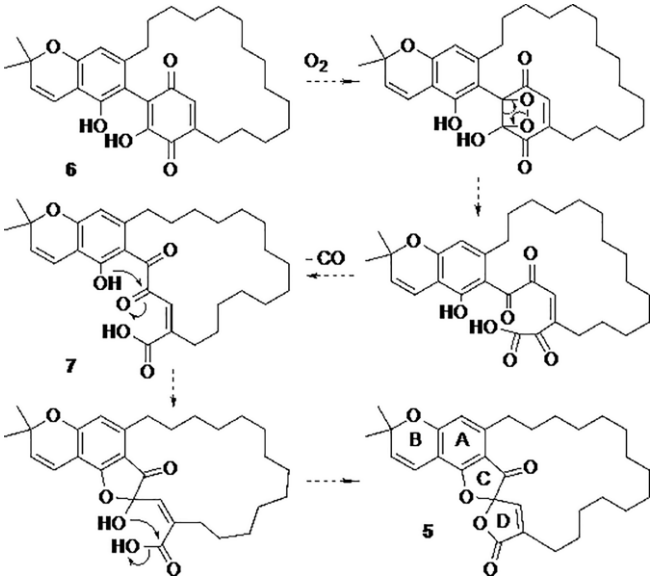



Figure 3. Asymmetric unit of of spirokermeline (**5**) with atom numbering obtained from X-ray diffraction.

A plausible biosynthetic pathway for spirokermeline was postulated (Scheme 1). Isokermadecin F (**6**), which results from a first oxidation of kermadecin A to quinone, could give a 1,2-dioxetane intermediate in the presence of singlet oxygen. A spontaneous rearrangement of the dioxetane ring leads to a pyruvic acid derivative easily decarboxylated and oxidized under oxidiz-

ing (or enzymatic) conditions into the corresponding carboxylic acid derivative **7** as depicted in Scheme 1. The mechanism of decarboxylation of pyruvic acid into carboxylic acid in the presence of $H_2O_2$ has been studied by Lopalco and co-workers.[11] Afterwards, attack of the phenol group onto the ketone carbonyl group would form ring C and then the butyrolactone D as indicated previously by Letcher and co-workers.[12]




Scheme 1. Plausible biosynthesis of spirokermeline (**5**) from isokermadecin F (**6**).

Some spiro lactones were shown to exhibit potent antiviral activities against influenza B virus,[9] therefore, spirokermeline **5** was evaluated for its antiviral activity against influenza A/H1N1, influenza A/H3N2, influenza B, HIV-I (IIIB strain), and HIV-2 (ROD strain) viruses, but was found inactive at a concentration up to 100 μM on all viruses tested (see the Supporting Information). In addition, spirokermeline was subjected to cytotoxic assays against HCT116 colon, K562 leukemia, and U87 glioblastoma human cancer cell lines, and to an antibacterial assay against a panel of 52 Gram-positive and Gram-negative strains (see the Supporting Information). Spirokermeline did not show any cytotoxic or antibacterial activities at a concentration up to 10 μM and 50 mg/L, respectively.

## Conclusions

Spirokermeline (**5**), a new rearranged macrocyclic cyclophane was isolated from the bark of *Kermadecia elliptica*. This compound is the second example of a naturally occurring 3*H*,3′*H*-spiro[benzofuran-2,1′-isobenzofuran]-3,3′-dione derivative, and the first example from higher plants. Although we cannot rule out an artifactual origin, it is likely that spirokermelin is a genuine natural product. Indeed, two different chemical investigations were conducted in 2008 and 2016 from 3 and 16 kg of barks,[1,5] respectively, leading to the isolation of spirokermelin in comparable yields (≈ 0.000033 % and 0.000056 %, respectively).

## Experimental Section

**Plant Material:** The bark of *Kermadecia elliptica* was collected in 2008 at "Forêt plate" (New Caledonia). The corresponding voucher specimen POU-0289 has been deposited at the Herbier IRD de Nouméa (NOU), New Caledonia.

**Extraction and Isolation:** Air-dried material (16 kg) was extracted with EtOAc (2 × 4.5 L, 1 h each) at 40 °C under 100 bars and concentrated under vacuum at 40 °C. The EtOAc extract (70 g) was subjected to a silica gel column chromatography using a gradient of cyclohexane/$CH_2Cl_2$ (100:0 to 0:100) and $CH_2Cl_2$/MeOH (100:0 to 80:20) to give 26 fractions (fractions 1 to 26) according to their TLC profile.

Fraction 10 (400 mg) was subjected to a centrifugal partition chromatography using the biphasic system cyclohexane/AcOEt/MeOH/$H_2O$ (5:6:5:6), to give 9 fractions (fractions 10-1 to 10-9). Fraction 10-3 was finally subjected to preparative TLC purification (silica, cyclohexane/AcOEt, 8:2) to afford spirokermeline (**5**) (9 mg).

**Spirokermeline (5):** Colorless crystalline powder. $[\alpha]_D^{22} = 0$ ($c = 0.65$, $CHCl_3$). UV (MeOH) $\lambda_{max}$ [log($\varepsilon$/m$^{-1}$ cm$^{-1}$)] = 239 [4.6], 249 [4.1], 271 nm [3.6], 323 [2.6]. IR: $\tilde{\nu}_{max}$ = 1138, 1372, 1573, 1610, 1721, 1789 cm$^{-1}$. $^1$H NMR and $^{13}$C NMR, see Table 1. HRMS (ESI): calcd. for $C_{30}H_{38}O_5$ [M + H]$^+$ 479.279201, found 479.279259, $\Delta m$ = 5.6 ppm.

**X-ray Crystallographic Data of (5):** ($M$ = 478.60 g/mol): space group $P2_1/c$, $a$ = 24.313(2) Å, $b$ = 5.6049(4) Å, $c$ = 22.152(2) Å, $\beta$ = 111.707(11)°, $V$ = 2804.6(5) Å$^3$, *Dcalc* = 1.133 g/cm$^3$, 30081 reflections measured, 5438 unique ($R_{int}$ = 0.0708). $R_1$ = 0.0980, w$R_2$ = 0.1640 [$I > 2\sigma(I)$], and $R_1$ = 0.2115, w$R_2$ = 0.1997 (all data). Largest diff. peak and hole 0.132 and –0.097 e Å$^{-3}$.

Crystallographic data of compound **5** have been deposited in the Cambridge Crystallographic Data Centre (deposit no. CCDC 1822347).

CCDC 1822347 (for **5**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

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**Keywords:** *Kermadecia elliptica* · Lactones · Spiro compounds · Macrocycles · Natural products

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