Kingianin A: A New Natural Pentacyclic Compound from *Endiandra kingiana*

Aurélie Leverrier, Marie Elise Tran Huu Dau, Pascal Retailleau, Khalijah Awang, Françoise Guéritte, and Marc Litaudon*

Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles, CNRS, 1, Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France, and Department of Chemistry, University of Malaya, 59100 Kuala Lumpur, Malaysia

marc.litaudon@icsn.cnrs-gif.fr

ABSTRACT
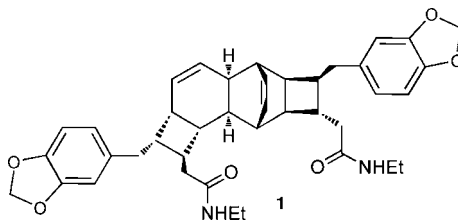
A new natural pentacyclic compound, named kingianin A, was isolated as a racemic mixture from the barks of *Endiandra kingiana* (Lauraceae). Its structure was elucidated by comprehensive analysis of NMR spectroscopic data, X-ray crystallography, and ECD calculations. The pentacyclic skeleton may be formed by a Diels−Alder reaction between two monomers having a bicyclo[4.2.0]octadiene backbone formed by a stereospecific electrocyclization of a linear compound of polyketide origin.

In continuation of our efforts to identify new bioactive compounds from Malaysian plants,[1] we investigated chemically the barks of *Endiandra kingiana* Gamble, a tree collected in the rain forest of Pahang State in Malaysia. The genus *Endiandra* belongs to the Lauraceae family and comprises about 100 species distributed in tropical parts of South East Asia, Australia, and the Western Pacific Ocean.[2] A previous phytochemical investigation of *Endiandra intorsa* resulted in the isolation of endiandric acids possessing an original skeleton formed by nonenzymatic electrocyclizations from nonchiral polyketide precursors.[3] Herein, we report the isolation and structure elucidation of kingianin A (1), the first member of a new chemical series having an unprecedented pentacyclic framework.

Air-dried barks of *E. kingiana* (1.5 kg) were powdered and extracted with EtOAc (3 × 1.5 L) followed by MeOH (3 × 1.5 L) at 40 °C and 100 bar using a static high-pressure, high-temperature extractor, Zippertex, developed at the ICSN pilot unit. The EtOAc extract was subjected to silica gel chromatography with a gradient elution of heptanes−dichloromethane−methanol (100:0:0 to 0:80:20) to give 12 fractions. Fraction 6 (0:92:8) was repeatedly purified by silica gel column chromatography and preparative C_{18} HPLC to afford 1 (26 mg).

Compound 1 was obtained as a white powder.[4] Its HRESIMS indicated a $[M - H]^-$ ion peak at m/z 649.3323, suggesting a molecular formula of $C_{40}H_{45}N_2O_6$ (calculated for $C_{40}H_{45}N_2O_6$ m/z 649.3278), from which 19 degrees of unsaturation can be deduced. The ^{13}C (Table 1) and DEPT-135 NMR spectra exhibited 40 signals for 2 methyl, 8 methylene, 22 methine, and 8 quaternary carbons. The full

(1) Litaudon, M.; Bousserouel, H.; Awang, K.; Nosjean, O.; Martin, M. T.; Tran Huu Dau, M. E.; Adi, H. A.; Boutin, J. A.; Sévenet, T.; Guéritte, F. *J. Nat. Prod.* **2009**, *72*, 480–483.

(2) Maberley, D. J. *The plant-book: a portable dictionary of the vascular plants*, 2nd ed.; Cambridge University Press: Cambridge & New York, 1997; p 858.

(3) Banfield, J. E.; Black, D.St.C.; Johns, S. R.; Willing, R. I. *Aust. J. Chem.* **1982**, *35*, 2247–2256.

(4) Kingianin A (1): UV (CHCl$_3$) λ_{max} (log ε) 289 (3.93), 240 (3.90) nm; IR ν_{max} 3287, 2921, 1640, 1549, 1503, 1488, 1441, 1245, 1039, 926, 812, 754 cm^{-1}; HRESIMS (pos.) m/z 673.3258 $[M + Na]^+$ (calcd for $C_{40}H_{46}N_2O_6Na$), HRESIMS (neg.) m/z 649.3323 $[M - H]^-$ (calcd for $C_{40}H_{45}N_2O_6$).

Table 1. NMR Spectroscopic Data of Kingianin A (1)[a]

position	δ_C	δ_H, mult (J in Hz)
1	43.9 d	2.03 m
2	33.4 d	2.46 m
3	125.1 d	5.54 br d (10.4)
4	132.4 d	5.64 br d (10.4)
5	38.3 d	2.22 m
6	38.1 d	1.68 br d (9.0)
7	42.6 d	1.89 m
8	42.6 d	2.00 m
9	36.0 d	2.55/2.44 m
10	135.5 s	–
11	109.0 d	6.61 d (1.2)
12	147.7 s	–
13	145.6 s	–
14	108.3 d	6.67 d (7.9)
15	121.2 d	6.54 dd (7.9, 1.2)
16	100.9 t	5.88[b] s
17	42.0 t	2.00 m
18	172.1 s	–
19	–	5.28 t (5.7)
20	34.5 t	3.19 qd (7.2, 5.7)
21	15.1 q	1.07 t (7.2)
1′	39.0 d	2.07 m
2′	44.5 d	2.24 m
3′	43.0 d	2.39 m
4′	132.6 d	6.09 dd (7.1, 7.6)
5′	135.0 d	6.20 t (7.1)
6′	38.6 d	2.50 m
7′	39.9 d	2.47 m
8′	43.9 d	2.26 m
9′	35.4 d	2.61/2.47 m
10′	135.7 s	–
11′	109.0 d	6.58 br s
12′	147.7 s	–
13′	145.6 s	–
14′	108.3 d	6.66 d (7.9)
15′	121.2 d	6.55 br d (7.9)
16′	100.9 t	5.86[b] s
17′	43.2 t	2.05/1.93 m
18′	172.1 s	–
19′	–	5.23 t (5.7)
20′	34.4 t	3.19 qd (7.2, 5.7)
21′	15.1 q	1.07 t (7.2)

[a] Data were recorded in CDCl$_3$ on a Bruker Avance 500 MHz spectrometer. [b] Values are interchangeable.

^{1}H and ^{13}C NMR assignments (Table 1) were established by [1]H−^{1}H COSY, [1]H−^{13}C HSQC, and [1]H−^{13}C HMBC experiments.

The IR spectrum of **1** showed absorption bands at ν_{max} 3287, 1640, and 1549 cm^{-1} for N−H, C=O elongations, and N−H deformation, respectively, indicating the presence of an amide group. A set of two *N*-ethylacetamide groups was suggested by the two triplets at δ_H 5.23 and 5.28 ppm, corresponding to NH-19′ and NH-19, respectively, exhibited in the ^{1}H NMR spectrum, and by cross peaks in the COSY spectrum between CH$_2$-20 (CH$_2$-20′) at δ_H 3.19 ppm and CH$_3$-21 (CH$_3$-21′) at δ_H 1.07 ppm and NH-19 (NH-19′). The HMBC cross peaks of H$_2$-17/C-18 (H$_2$-17′/C-18′), NH-19/C-18 (NH-19′/C-18′), and H$_2$-20/C18 (H$_2$-20′/C-18′) con-

firmed the presence of the two *N*-ethylacetamide substituents (Figure 1a). A set of two 1,2,4-trisubstituted benzene moieties
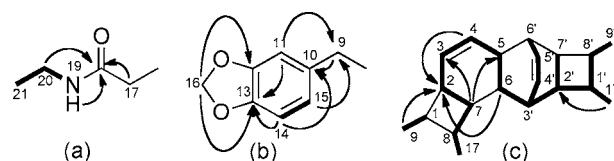

Figure 1. Key COSY (−) and HMBC (→) correlations of *N*-ethylacetamide (a) and methylenedioxyphenyl (b) substituents and of the framework (c) of **1**.

was suggested by signals at δ_H 6.61 (d, J = 1.2 Hz, H-11), 6.67 (d, J = 7.9 Hz, H-14), and 6.54 (dd, J = 7.9, 1.2 Hz, H-15) observed in the ^{1}H NMR spectrum, the chemical shifts for H-11′, H-14′, and H-15′ being almost identical. In addition, the two singlets at δ_H 5.88 and 5.86 ppm (CH$_2$-16 and CH$_2$-16′, respectively) showed HMBC correlations with two sp2 quaternary carbons at δ_C 145.6 and 147.7 (C-13 and C-13′ and C-12 and C-12′, respectively), which confirmed the presence of two methylenedioxyphenyl substituents.

The ^{1}H and ^{13}C NMR spectra showed signals for two additional double bonds. Considering the 12 unsaturations attributed for the amide and methylenedioxyphenyl groups, we can assume that the skeleton of **1** possesses five rings containing two double bonds. A careful examination of the NMR spectra allowed a pentacyclic carbon skeleton to be proposed as depicted in Figure 1c. Finally, the connectivities between the four substituents and the pentacyclic skeleton were elucidated by HMBC and COSY correlations.

In the HMBC spectrum, cross peaks were observed between H-11 and H-15 (H-11′ and H-15′) and C-9 (C-9′). In the COSY spectrum, cross peaks between CH$_2$-9 and H-1, CH$_2$-9′ and H-8′, on one hand, and CH$_2$-17 and H-8, and CH$_2$-17′ and H-1′, on the other hand, allowed the two acetamido groups to be located at positions 8 and 1′ and the two benzene moieties at positions 1 and 8′.

The relative configuration of kingianin A could be deduced from a NOESY experiment (Figure 2). Cross
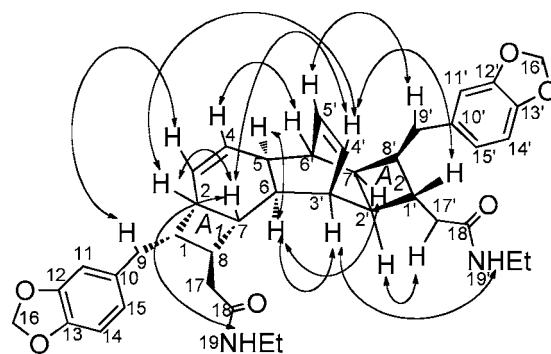
Figure 2. Key NOESY correlations of **1**.

peaks between H-6, H-5, H-3′, and H-7′, which in turn were correlated with H-2′, indicated that junctions 5−6 and 2′−7′ are *cis* and that H-5, H-6, H-2′, and H-7′ are α-oriented. This necessarily implied the β-position for the bridge formed by the C-4′−C-5′ double bond and for the cyclobutane A$_2$ formed by C-1′−C-2′−C-7′−C-8′. Other NOESY correlations between H-4′, H-2, and H-7 indicated that H-2 and H-7 are *cis* and β-oriented. Thus, the second cyclobutane ring A$_1$ is positioned on the α-face. Cross peaks between CH$_2$-9′ and H-5′ and between H-1′ and H-4′ indicated that H-1′ was β-oriented and H-8′ α-oriented. Consequently, the phenyl substituent at C-8′ is *anti* to the *N*-ethylacetamide side chain on the cyclobutane ring A$_2$. This was confirmed by the correlation between NH-19′ and H-3′. Finally, NOESY correlations between CH$_2$-9 and H-3 and between NH-19 and H-7 fixed the two substituents in an *anti* position on the second cyclobutane A$_1$. Therefore, the relative configuration of kingianin A was determined to be 1*S**, 2*S**, 5*R**, 6*R**, 7*R**, 8*S**, 1′*S**, 2′*R**, 3′*R**, 6′*S**, 7′*S**, 8′*S**.

Compound **1** crystallized from methanol in a noncentrosymmetric orthorhombic space group, Pca21, with one molecule in the asymmetric unit. X-ray analysis confirmed the relative configuration of **1** but also brought further evidence of crystallization of a racemate (Figure 3, Supporting Information).
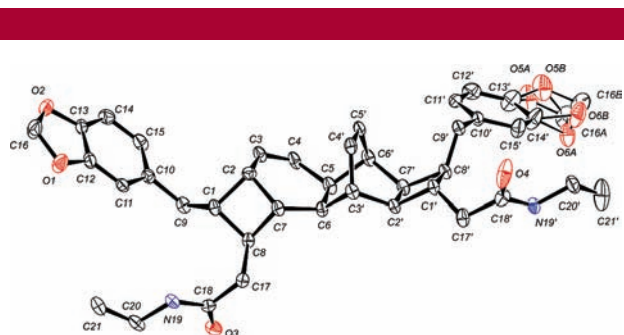
Figure 3. X-ray structure of kingianin A (**1**).

Indeed, the optical rotation value of **1** was found to be zero, which led us to postulate that kingianin A was obtained as a racemic mixture, as was the case for the endiandric acids isolated from *Endiandra intorsa*.[3] The mixture (26 mg) was subjected to chiral preparative HPLC (HPLC conditions are detailed in Supporting Information) to afford two enantiomers, (+)-kingianin A (11.0 mg) and (−)-kingianin A (11.5 mg), having specific optical rotation values of +48 and −44, respectively (*c* 0.2, CHCl$_3$).[5]

Kingianin A could arise from a possible spontaneous Diels−Alder reaction between two bicyclo[4.2.0]octa-2,4-diene monomers. Such a unit could be formed by a conrotatory 8π *e* electrocyclization of the unstable arylpolyene **2** followed by a disrotatory 6π *e* electrocyclization of the previously formed cyclooctatriene (Figure 4).[3] Electro-

cyclizations could explain the racemic character of **1** and the *anti* position of the two side chains on each cyclobutane ring. Our biogenetic hypothesis is in accordance with the biogenesis of endiandric acids,[3] which was supported by the biomimetic total synthesis of Nicolaou.[6] Interestingly, polyketides with such a bicyclo[4.2.0]octadiene backbone have been recently isolated from marine organisms.[7]

The absolute configurations of (−)- and (+)-kingianin A could be assigned by comparaison of experimental and calculated CD spectra. Therefore, 1000 conformations of one enantiomer were generated by the random search Monte Carlo method[8] and optimized by the PRCG minimization method[9] using the Macromodel (version 5.5) program[10] with the MM2 force field[11] and GB/SA chloroform solvation. Among the possible conformations within 12.5 kJ/mol (3 kcal/mol) from the global minimum, the conformer consistent with NOESY NMR experimental data was retained (Figure 5). Then, using time-dependent density functional theory (TD-DFT),[12] ECD calculations

(6) Nicolaou, K. C.; Petasis, N. A.; Zipkin, R. E.; Uenishi, J. *J. Am. Chem. Soc.* **1982**, *104*, 5555–5557.
(7) Wei, H.; Itoh, T.; Kinoshita, M.; Kotoku, N.; Aoki, S.; Kobayashi, M. *Tetrahedron* **2005**, *61*, 8054–8058.
(8) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379–4386.
(9) Polak, E.; Ribiere, G. *Rev. Fr. Inf. Rech. Oper.* **1969**, *16-R1*, 35–44.
(10) Mohamadi, F.; Richards, N. J. G.; Guida, W. C.; Liskamp, R.; Lipton, M. C.; Caufield, M.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440–467.
(11) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134.
(12) (a) Stratmann, R. E.; Scuseria, G. E.; Frisch, M. J. *J. Chem. Phys.* **1998**, *109*, 8218–8224. (b) Bauernschmitt, R.; Ahlrichs, R. *Chem. Phys. Lett.* **1996**, *256*, 454–464. (c) Casida, M. E.; Jamorski, C.; Casida, K. C.; Salahub, D. R. *J. Chem. Phys.* **1998**, *108*, 4439–4449. (d) Stephens, P. J.; Devlin, F. J.; Cheeseman, J. R.; Frisch, M. J. *J. Phys. Chem. A* **2001**, *105*, 5356–5371. (e) Mennucci, B.; Tomasi, J.; Cammi, R.; Cheeseman, J. R.; Frisch, M. J.; Devlin, F. J.; Gabriel, S.; Stephens, P. J. *J. Phys. Chem. A* **2002**, *106*, 6102–6113. (f) Grimme, S. *Chem. Phys. Lett.* **2001**, *339*, 380–388. (g) Grimme, S.; Furche, F.; Ahlrichs, R. *Chem. Phys. Lett.* **2002**, *361*, 321–328. (h) Autschbach, J.; Patchkovskii, S.; Ziegler, T.; van Gisbergen, S. J. A.; Baerends, E. J. *J. Chem. Phys.* **2002**, *117*, 581–592. (i) Ruud, K.; Helgaker, T. *Chem. Phys. Lett.* **2002**, *352*, 533–539. (j) Furche, F.; Ahlrichs, R.; Wachsmann, C.; Weber, E.; Sobanski, A.; Vogtle, F.; Grimme, S. *J. Am. Chem. Soc.* **2000**, *122*, 1717–1724. (k) Autschbach, J.; Ziegler, T.; van Gisbergen, S. J. A.; Baerends, E. J. *J. Chem. Phys.* **2002**, *116*, 6930–6940.
(13) (a) Binkley, J. S.; Pople, J. A.; Hehre, W. J. *J. Am. Chem. Soc.* **1980**, *102*, 939–947. (b) Gordon, M. S.; Binkley, J. S.; Pople, J. A.; Pietro, W. J.; Hehre, W. J. *J. Am. Chem. Soc.* **1982**, *104*, 2797–2803. (c) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652. (d) Petersson, G. A.; Al-Laham, M. A. *J. Chem. Phys.* **1991**, *94*, 6081–6090. (e) Petersson, G. A.; Bennett, A.; Tensfeldt, T. G.; Al-Laham, M. A.; Shirlet, W. A.; Mantzaris, J. *J. Chem. Phys.* **1988**, *89*, 2193–2218.
(14) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Vreven, T., Jr.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T. A.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *GAUSSIAN 03*; Gaussian, Inc.: Wallingford, CT, 2004.

(5) (+)- and (−)-Kingianin A: mp 78−82 °C.
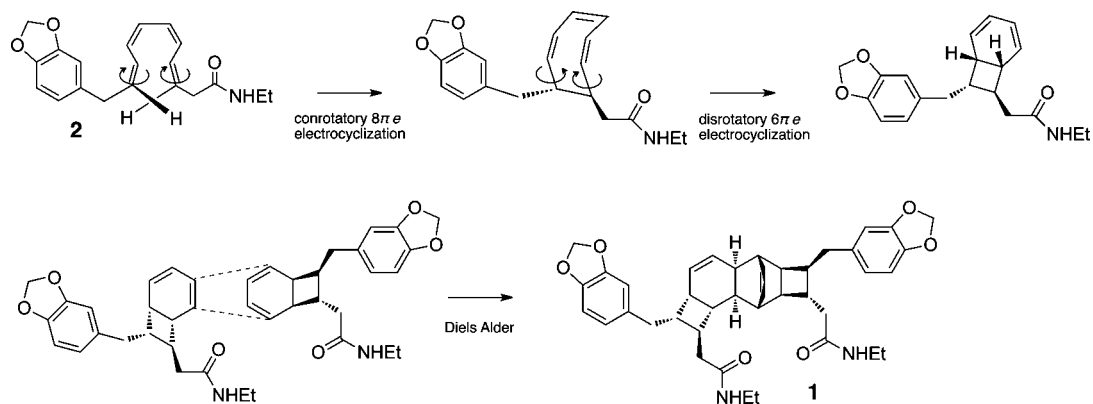
Figure 4. Hypothesis for the biogenesis of kingianin A (**1**).

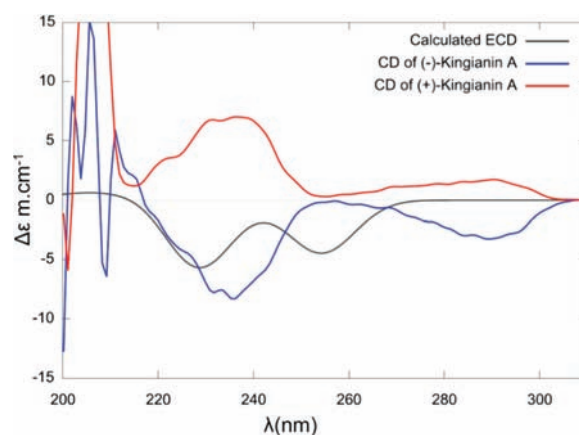were performed on DFT-B3LYP/631G(d,p)[13] optimized geometry of the retained conformer by the Gaussian03 program.[14]
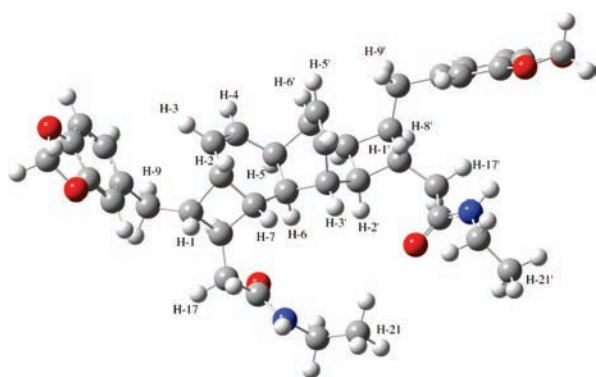
Figure 5. DFT-optimized structure found for the conformer of (−)-kingianin A in accordance with NOESY correlations.

Figure 6. Experimental CD spectra of the two enantiomers of kingianin A and calculated ECD spectra for (−)-kingianin A.

The experimental CD spectra of both (−)- and (+)-kingianin A showed strong opposite Cotton effects at λ_{max} 235 nm (Figure 6). The comparison between the experimental CD spectra of (−)-kingianin A and the calculated spectra of the selected conformer (Figure 6) allowed the absolute configuration of (−)-kingianin A to be assigned as 1*S*, 2*S*, 5*R*, 6*R*, 7*R*, 8*S*, 1′*S*, 2′*R*, 3′*R*, 6′*S*, 7′*S*, 8′*S*.

The calculation of the molecular orbital energy indicated that the CD was mainly dependent on the two methylene-dioxyphenyl groups (λ_{max} 235 nm), which show a stable spatial position regardless of the conformations generated, and to a lesser extent on the position of the two *N*-ethylacetamide groups, which differed from one conformer to another.

From the barks of *Endiandra kingiana*, a new pentacyclic compound kingianin A (**1**) with a unique carbon skeleton was isolated as a racemic mixture. Its structure was elucidated by extensive spectroscopic analysis, and the absolute configurations of the enantiomers were assigned by comparing experimental and calculated CD spectra. The possible implication of a Diels−Alder reaction in the biosynthetic pathway could explain the formation of both enantiomers. Kingianin A is the first member of a new chemical series comprising 13 other derivatives, some having interesting biological properties, that will be reported later.

Supporting Information Available: Experimental procedures, copies of NMR spectra, and X-ray crystallographic data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL101427M