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Ivorenolide A, an Unprecedented Immunosuppressive Macrolide from *Khaya ivorensis*: Structural Elucidation and Bioinspired Total Synthesis

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Supporting Information

ABSTRACT: Ivorenolide A (**1**), a novel 18-membered macrolide featuring conjugated acetylenic bonds and five chiral centers, was isolated from *Khaya ivorensis*. The structure of **1** was fully determined by spectroscopic analysis, single-crystal X-ray diffraction, and bioinspired total synthesis. Both compound **1** and its synthetic enantiomer **2** showed potent and selective immunosuppressive activity.

Since the introduction of immunosuppressive agents into the study of experimental and clinical organ transplantation,[1,2] the past half century has witnessed rapid development in this area of research. In addition to their main application in organ grafts, immunosuppressive drugs are also used for the treatment of other immunity-associated disorders such as rheumatoid arthritis and multiple sclerosis.[3] Although currently used immunosuppressants (e.g., cortisol, cyclosporin A, FK506, and rapamycin) have provided undeniable clinical advantages, these drugs cause serious side effects, including liver and renal toxicity, increased susceptibility to infectious ailments, and decreased cancer immunosurveillance.[3] Therefore, the exploration of new-generation immunosuppression drugs having high efficacy but fewer adverse effects remains a pressing challenge.

Khaya ivorensis A. Chev. (Meliaceae), the well-known African mahogany, is mainly grown on the west coast of Africa from Sierra Leone to Cabinda,[4] although it is also cultivated in southern China. The crude extract of its stem bark has shown cytotoxic, anti-inflammatory, and antimalarial activities.[5] Previous chemical investigations of this plant have led to the isolation of a number of limonoids.[6] In the current study, a novel 18-membered macrolide, ivorenolide A (**1**), featuring conjugated acetylenic bonds and five chiral centers, was isolated as a major compound (as monitored by thin-layer chromatography) of the less polar fraction from the stem bark of *K. ivorensis*. We report herein the isolation, structural elucidation, and immunosuppressive activity of **1** and the bioinspired total synthesis of its enantiomer **2** (Figure 1).

Compound **1** was obtained as colorless prisms with $[\alpha]_D^{23}$ = 55.0 (*c* 0.095, MeOH). The molecular formula, $C_{18}H_{24}O_5$, with

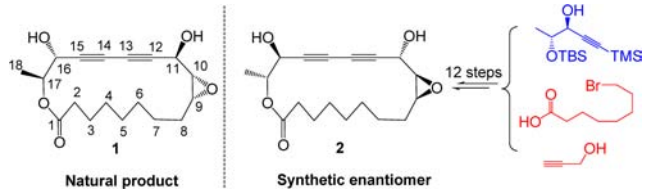

Figure 1. Structures of **1** and **2**.

seven double-bond equivalents (DBEs), was established by high-resolution positive-ion-mode electrospray ionization mass spectrometry [HR-ESI(+)-MS] at *m*/*z* 343.1530 $[M + Na]^+$ (calcd for $C_{18}H_{24}O_5Na$, 343.1521) and further secured by electron impact MS (EI-MS) at *m*/*z* 320 $[M]^+$. Its IR spectrum revealed the presence of hydroxyl (3421 cm^{-1}), alkynyl (2115 cm^{-1}, weak and sharp), and carbonyl (1705 cm^{-1}) groups.[7] In the ^1H NMR spectrum (Table S1 in the Supporting Information), two broad singlets at δ 8.37 and 8.44 that did not show any correlation in the heteronuclear single-quantum coherence (HSQC) NMR spectrum were assignable to the exchangeable protons of two hydroxyls. In accordance with the molecular formula, 18 carbon resonances were resolved in the ^{13}C NMR spectrum (Table S1) and were classified by distortionless enhancement by polarization transfer (DEPT) NMR experiments with the aid of the HSQC spectrum as a carbonyl (δ 172.9), a methyl (δ 17.6), seven sp^3 methylenes, five oxygenated sp^3 methines (δ 57.0, 61.0, 62.0, 65.4, and 72.7), and four sp quaternary carbons (δ 68.7, 70.3, 78.5, and 81.3). The aforementioned functionalities (a carbonyl and four sp quaternary carbons) accounted for five DBEs, and the remaining two DBEs required **1** to be bicyclic.

Analysis of the HSQC and $^1H-^1H$ correlation spectroscopy (COSY) NMR spectra revealed the presence of two proton-bearing and spin-coupling structural units, **a** (C2−C11) and **b** (C16−C18) (drawn with thick bonds in Figure 2). The connectivity of the structural units **a** and **b**, the four sp quaternary carbons, and the ester carbonyl was readily elucidated by analysis of the heteronuclear multiple-bond correlation (HMBC) NMR spectrum (Figure 2). The ester

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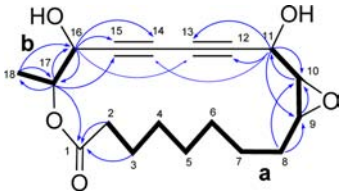


Figure 2. ^{1}H−^{1}H COSY (thick black lines) and selected HMBC H → C (blue arrows) correlations of **1**.

carbonyl C1 (δ 172.9) was shown to be attached to C2 by the HMBC correlations from H_2-2 and H_2-3 to C1. The connection of the carbonyl C1 to C17 via an oxygen atom to form a 1,17-lactone was revealed by the key HMBC correlation from H17 to C1, an assignment supported by the downfield shift of the H17 resonance (δ 5.45). An epoxy group was distinguished by the diagnostic chemical shifts of C9 (δ 57.0) and H9 (δ 3.10) and C10 (δ 61.0) and H10 (δ 3.53),[8] and it was finally assigned as a 9,10-epoxy group by the multiple HMBC correlations from H_2-8 to C9 and C10, H9 to C10, and H11 to C9 and C10. The two hydroxyls at C11 and C16 were identified by the chemical shifts of C11 (δ 62.0) and H11 (δ 4.77) and C16 (δ 65.4) and H16 (δ 4.74). The two loose ends of C11 and C16 were ultimately connected to furnish an 18-membered macrolactone by insertion of the four sp quaternary carbons (δ 68.7, 70.3, 78.5, and 81.3) to form a conjugated alkynyl moiety.[9] This was confirmed by the HMBC correlations from H11 to C12 (δ 78.5), C13 (δ 68.7), and C14 (δ 70.3) and from H16 to C13, C14, and C15 (δ 81.3). The rotating-frame Overhauser effect spectroscopy (ROESY) correlation between H9 and H10 and the small coupling constant ($J_{9,10}$ = 4.2 Hz) indicated that H9 and H10 are cis-configured. The relative configurations of the remaining three chiral centers (C11, C16, and C17) were not assigned by the available ROESY data in such a macrolide (Figure S6 in the Supporting Information). A single-crystal X-ray diffraction experiment was thus successfully performed to assign the relative stereochemistry of **1** unambiguously (Figure 3). However, the absolute configuration

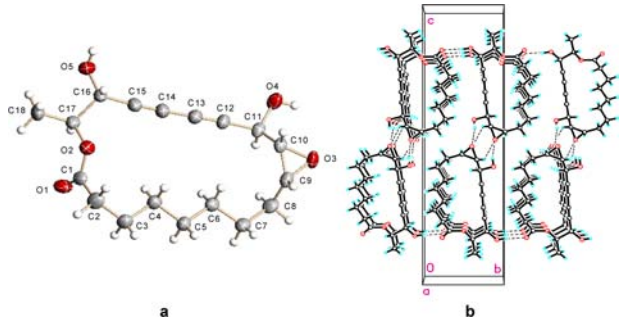


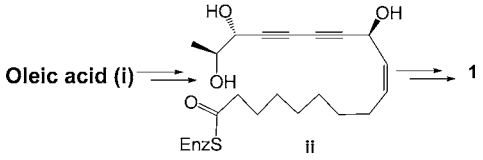
Figure 3. (a) X-ray structure of **1** and (b) assembly of **1** molecules in the crystal.

of **1** was left unassigned because of the poor absolute structure parameter [−10(10); see p S18 in the Supporting Information). **1** features an unprecedented 18-membered macrolide with conjugated acetylenic bonds and five chiral centers, which mold the molecule into a planar loop. These molecules show a very intriguing assembly in the crystal, in which two or three molecules are regularly overlapped and intermolecular hydrogen bonds are formed between the O16−H moieties and the C1 carbonyl groups as well as between the O11−H moieties and the 9,10-epoxy groups (Figure 3).

The formation of intramolecular hydrogen bonds in **1** seemed impossible because of the unfavorable stereochemistry. Aggregation of the molecules in solution (in pyridine-d_5) was thus suggested by the observation of two broad resonances for O11−H and O16−H (δ 8.44 and 8.37) in the ^{1}H NMR spectrum, with the aggregation considered to be stabilized by intermolecular hydrogen bonds similar to those observed in the solid state.

A plausible biogenetic pathway leading to **1** is proposed (Scheme 1). The biosynthetic precursor of **1** could be traced

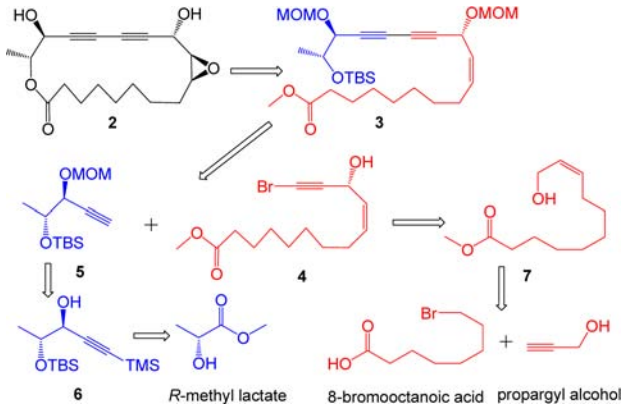
Scheme 1. Biosynthetic Pathway Proposed for 1



back to a C18 fatty acid [e.g., oleic acid (**i**)].[10] After a cascade of desaturation and oxidation reactions, **i** would provide a key enzyme-bound intermediate, **ii**, in which the conjugated acetylenic bonds and three hydroxylated chiral centers are present and the Δ^9 double bond in the *Z* geometry is retained to facilitate the macrolide formation. Intermediate **ii** would readily be transformed to **1** by a sequence of lactonization and epoxidation (Scheme 1).

To establish the absolute configuration of **1**, we carried out a bioinspired total synthesis via protected C18 fatty acid **3** as the key biomimetic intermediate, which afforded **2**, the enantiomer of **1**, in a high yield. The retrosynthetic analysis for **2** inspired by the proposed biosynthetic pathway (Scheme 2) involves

Scheme 2. Retrosynthetic Analysis



biomimetic lactonization of **3** via the Yamaguchi macrolactonization reaction as the key step. Intermediate **3** could be constructed by the assembly of bromoalkyne **4** and alkyne **5** via the Cadiot−Chodkiewicz coupling reaction. Compounds **4** and **5** could be prepared from commercially available 8-bromooctanoic acid and (*R*)-methyl lactate via intermediates **7** and **6**, respectively.

The synthesis of alkyne **5** commenced with the known alcohol **6**, which was prepared from (*R*)-methyl lactate in three steps.[11] **6** was converted to its methoxymethyl (MOM) ether **8**,

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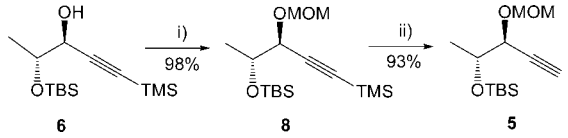
after which the trimethylsilyl (TMS) group was removed to afford the required product **5** (Scheme 3).[12]

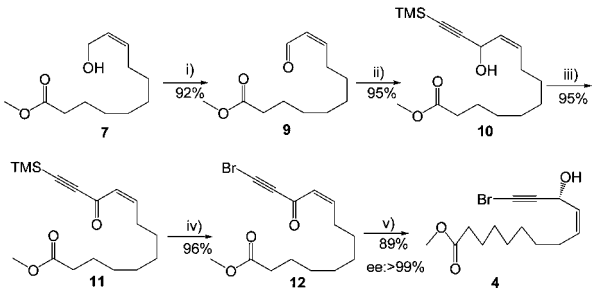
Scheme 3. Synthesis of 5[a]



[a] Conditions: (i) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, rt, 12 h; (ii) K_2CO_3, CH_3OH, rt, 2 h.

Bromoalkyne **4** was synthesized from the known starting material **7**, which was prepared by coupling of 8-bromooctanoic acid with propargyl alcohol under lithium amide conditions followed by methyl esterification and Lindlar's catalytic hydrogenation.[13] Oxidation of **7** with 2-iodoxybenzoic acid (IBX) afforded allyl aldehyde **9**,[14] which was converted into **10** in excellent yield (95%) by reaction with TMS-acetylene (TMSA) in the presence of *n*-butyllithium in tetrahydrofuran (THF) at −78 °C for 3 h (Scheme 4). **10** was oxidized with

Scheme 4. Synthesis of 4[a]

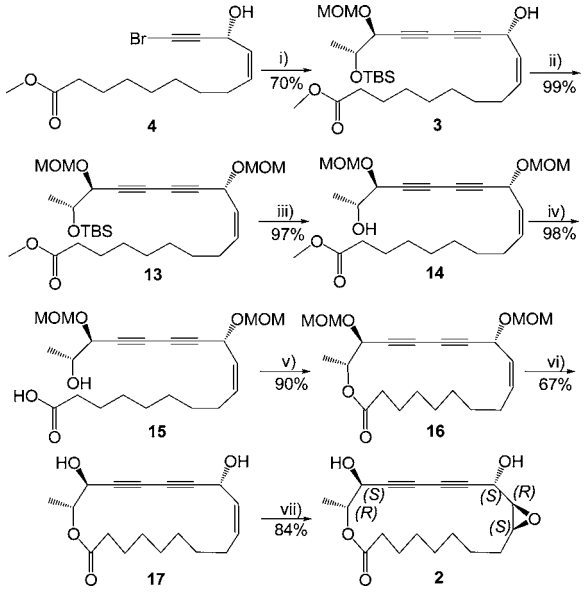


[a]Conditions: (i) IBX, DMSO, CH_2Cl_2, 0 °C, 24 h; (ii) TMSA, *n*-BuLi, THF, −78 °C, 3 h; (iii) DMP, CH_2Cl_2, rt, 14 h; (iv) NBS, $AgNO_3$, acetone, 3 h; (v) CBS, BH_3·SMe, THF, −40 °C, 40 min.

Dess−Martin periodinane (DMP) to give ketone **11**,[14a] which was transformed into **12** in 96% yield using *N*-bromosuccinimide (NBS) and $AgNO_3$.[15] **12** was subjected to reduction with (*R*)-2-methyl-CBS-oxazaborolidine (CBS) to afford the key intermediate **4** in good yield (89%) with the required stereoselectivity (>99% ee).[16]

With **4** and **5** in hand, we next focused on their coupling. The desired product **3** was obtained in moderate yield in the presence of Cu(I) via Cadiot−Chodkiewicz coupling (Scheme 5).[14a,17] Protection of the remaining hydroxyl group of **3** as the MOM ether provided **13**. Removal of the *tert*-butyldimethylsilyl (TBS) group and hydrolysis of the methyl ester under basic conditions[11a,18] afforded **14** and **15**, respectively, in excellent yields. Carboxylic acid **15** underwent the Yamaguchi macrolactonization reaction[19] to produce 18-membered macrolactone **16** in good yield (90%), and removal of the MOM ethers[20] afforded diol **17**. Subsequent epoxidation of **17** under the established Sharpless asymmetric epoxidation conditions was attempted, but unfortunately, no reaction products were detected. Interestingly, oxidation of **17** with *m*-chloroperoxybenzoic acid (*m*-CPBA) afforded **2** (84% yield) as the sole product; is assumed that the oxidant *m*-CPBA approached the double bond from the sterically less hindered face of the molecule.[21] The ^1H and ^{13}C NMR data of **2** matched those of

Scheme 5. Synthesis of 2[a]



[a](i) **5**, CuCl, *n*-$BuNH_2$, H_2O, NH_2OH·HCl, CH_2Cl_2, 30 min; (ii) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, rt, 12 h; (iii) *n*-Bu_4NF, THF, 0 °C, 18 h; (iv) LiOH (2.0 M), *t*-BuOH, rt, 12 h; (v) 1,3,5-$Cl_3C_6H_2COCl$, Et_3N, THF, 0 °C, 45 min, then toluene, DMAP, 75 °C, 12 h; (vi) HCl (3.0 M), CH_3CH_2OH, 80 °C, 2 h; (vii) *m*-CPBA, CH_2Cl_2, 12 h.

the natural isolate **1** very well (Table S1 and Figures S38 and S39). However, its specific rotation $\{[\alpha]_D^{23} = -50.9$ (*c* 0.157, MeOH)} and CD spectrum (Figure 4) were opposite those of **1**, indicating that the synthesized compound **2** is the enantiomer of **1**.

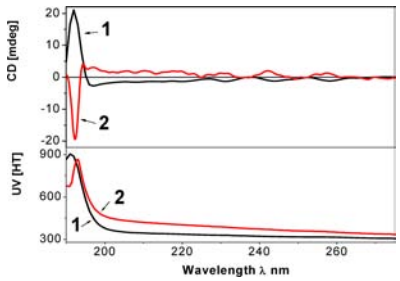


Figure 4. CD spectra of **1** and **2**.

Compounds **1** and **2** were tested for immunosuppressive activity and exhibited remarkable inhibition of ConA-induced T-cell proliferation and LPS-induced B-cell proliferation (Table 1). In particular, these compounds showed selectivity indexes (SIs) comparable to or better than those of the positive controls cyclosporin A (CsA) and periplocoside A (PSA),[22] the latter of which is the active component of *Periploca sepium* extract, a traditional Chinese medicine used clinically to treat rheumatoid arthritis.

In summary, this communication has presented an unprecedented immunosuppressive 18-membered macrolide obtained from *K. ivorensis*, ivorenolide A (**1**), that features conjugated acetylenic bonds and five chiral centers. The bioinspired asymmetric total synthesis of its enantiomer **2** was achieved in 12 steps with a groundbreaking 22% overall yield, thereby allowing the complete assignment of the molecule's absolute stereochemistry. Cadiot−Chodkiewicz

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Table 1. Immunosuppressive Effects of 1 and 2 on Murine Lymphocyte Proliferation Induced by ConA (5 μg/mL) or LPS (10 μg/mL)[a]

		ConA-induced T-cell proliferation		LPS-induced B-cell proliferation	
cmpd	CC_{50} (μM)	IC_{50} (μM)	SI	IC_{50} (μM)	SI
1	>100	4.80	>20.83	8.12	>12.32
2	>100	2.86	>34.97	4.55	>21.98
CsA	4.79	0.11	44.80	0.33	14.50
PSA	18.85	0.68	27.72	2.17	8.69

[a]The selectivity index (SI) is defined as the ratio of the concentration of the compound that reduced cell viability to 50% (CC_{50}) to the concentration of the compound needed to inhibit the proliferation by 50% relative to the control value (IC_{50}).

coupling and Yamaguchi macrolactonization were used as the key steps to construct the scaffold of the macrolide. Both **1** and its synthetic enantiomer **2** showed potent and selective immunosuppressive activities, with **2** being more potent than **1**. This finding provides a new structural template for the development of immunosuppressive agents.

■ ASSOCIATED CONTENT

Supporting Information

General experimental procedures; plant materials; extraction and isolation, X-ray crystal data, and a CIF for **1**; total synthesis of **2**; immunosuppressive activity tests; and ^{1}H, ^{13}C, and 2D NMR, MS, and IR spectra of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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