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Krishnadimer A, an Axially Chiral Non-biaryl Natural Product: Discovery and Biomimetic Synthesis

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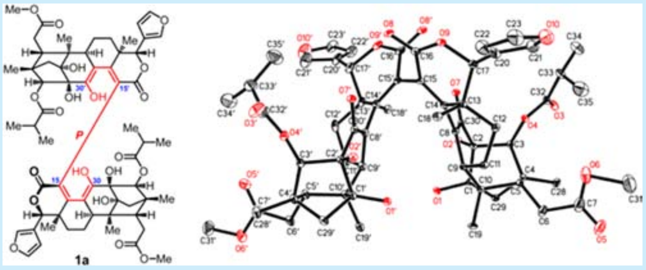
Ⓢ *Supporting Information*

ABSTRACT: Krishnadimer A (**1a**), a C_2-symmetric limonoid dimer representing a new class of axially chiral nonbiaryl natural products, was isolated and biomimetically semi-synthesized by atroposelective 2,3-dichloro-5,6-dicyanobenzoquinone-mediated dienol-oxidative coupling. Single-crystal X-ray diffraction and electronic circular dichroism spectral analysis on the synthesized **1a** unambiguously established its absolute configuration, including the *P*-configuration of the C15−C15′ central axis. Antitumor investigations of the synthesized dimers **1a**, **2a**, and **2b** revealed the large impact of axial chirality on bioactivities.



Axially chiral compounds, which can be classified into two categories, viz. biaryl and nonbiaryl atropisomers, usually refer to molecules with one or more rotationally hindered axes.[1] To date, more than 1000 axially chiral natural products have been reported.[1] Most of them are C,C-, C,N-, or N,N-coupled (hetero)biaryls,[1] such as the dimeric naphthylisoquinoline michellamine B,[2a] the bispyrroles marinopyrroles A and B,[2b] and the bisindolosesquiterpenes bixiamycins 6a/6b.[2c,d] Biosynthetic investigations validated that intermolecular oxidative phenol coupling is the main mechanism for the biosynthetic construction of chiral biaryl axes.[3] Besides multifaceted biaryl natural products, a limited number of axially chiral nonbiaryl natural products, such as bisnicalaterines A and B,[4a,b] ancistrocladinium A,[4c] streptorubin B,[4d,e] ustiloxin D,[4f] and abyssomicin C,[4g] have hitherto been discovered. To the best of our knowledge, all of the axially chiral nonbiaryl natural products except for abyssomicin C comprise an aryl unit that gives rise to one or two rotationally hindered axes. Because of their conformational characteristics and promising bioactivities, axially chiral natural products have triggered an upsurge of total syntheses in recent years.[5]

Trees of the genus *Xylocarpus* are mangroves harboring limonoids with plentiful chemodiversity.[6] Previous phytochemical investigations on the seeds of the Indian species *X. moluccensis*, collected in the Krishna mangrove swamps, had afforded a monomeric limonoid, moluccensin A (**3**).[6c] Herein, we report on the isolation and structural elucidation of the first dimeric limonoid, named krishnadimer A (**1a**) (Figure 1), featuring an unprecedented C_2-symmetric architecture with a *P*-configured central axis at the C15,C15′-positions of the monomeric units, on the synthesis of dimeric limonoids **1a**, **2a**, and *M*-diastereomer **2b** (Figure 1), and on the antitumor activities of **1a**, **2a**, **2b**, and **3**.

After recrystallization of **3**, the remaining mother liquor material was resolved on an RP-18 column to afford 60 subfractions. Further analysis of these subfractions by LC−DAD, with UV detection at a wavelength of 360 nm, yielded krishnadimer A (**1a**) (Figure 1).

Krishnadimer A (**1a**) was obtained as a yellow solid. The molecular formula $C_{62}H_{74}O_{20}$ with 26 degrees of unsaturation was established by the positive HRESIMS ion at *m*/*z* 1139.4844 ($[M + H]^+$, calcd 1139.4846). The ^1H and ^{13}C NMR spectra of **1a** exhibited half the number of proton and carbon signals, indicating the presence of a symmetric dimer.

According to the ^1H and ^{13}C NMR spectroscopic data (Tables S1 and S2), seven degrees of unsaturation were due to four C═C double bonds and three ester functions; thus, the monomeric unit of **1a** was hexacyclic. The NMR spectroscopic data of **1a** were closely related to those of moluccensin A (**3**),[6c] except for the presence of a 30-OH group and two conjugated double bonds (C8═C30 and C14═C15) in **1a** rather than the C8═C14 double bond, the C30 carbonyl, the C15 methylene group, and the 1-isobutyryloxy function in **3**. The replacement of the 1-isobutyryloxy function in **3** by a hydroxy group in **1a** was corroborated by HMBC correlations from the hydroxy singlet resonance at $\delta_H = 4.76$ ppm (recorded in DMSO-d_6) to C1, C2, and C10. The second hydroxy singlet resonance at $\delta_H = 4.72$ ppm (recorded in DMSO-d_6), which showed HMBC cross-peaks to C1, C2, and C30, was concluded to be the 2-OH group. HMBC correlations from the third hydroxy singlet

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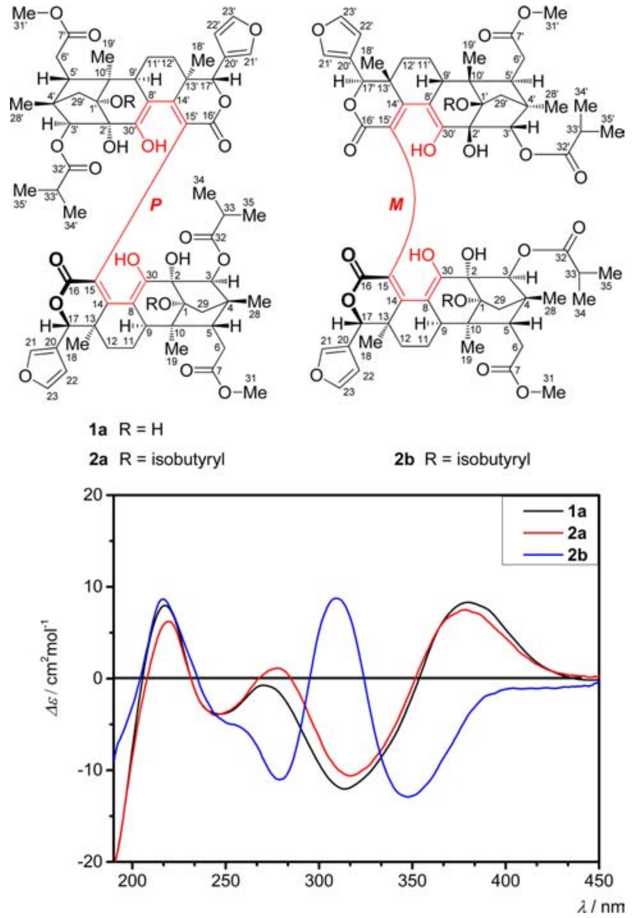


Figure 1. Structures and electronic circular dichroism (ECD) spectra of the dimers **1a**, **2a**, and **2b** (recorded in MeCN at 100 μg/mL).

resonance at δ_H = 6.83 ppm to C2, C30 [δ = 147.9 ppm (qC)], and C8 [δ = 104.4 ppm (qC)] (recorded in DMSO-d_6) assigned the presence of a 30-OH group and a C8═C30 double bond, whereas those from H9 [δ_H = 2.50 ppm (dd, J = 10.8, 4.0 Hz)] to C14 [δ = 157.7 ppm (qC)] and C15 [δ = 122.4 ppm (qC)] (recorded in $CDCl_3$) confirmed the existence of a C14═C15 double bond. On the basis of the above results, the gross structure of the monomeric unit of **1a**, i.e., a phragmalin-type limonoid, was clearly deduced (Figure 2a).

The relative configuration within the monomeric units of **1a** was established by diagnostic NOE interactions (recorded in $CDCl_3$, Figure 2b). Those between H29α/$H_3$19, H9/$H_3$18, and H9/$H_3$19 revealed their cofacial relationship, arbitrarily assigned as α-orientation. Similarly, NOE interactions between H5/H11β, H5/H12β, and H12β/H17 indicated that H5 and H17 were β-oriented. The NOEs between H3/$H_3$28 and H3/H29β, but not between H3/H5, established the α-orientation of H3 and the corresponding 3β-isobutyryloxy group. Due to the availability of only a small quantity of natural compound (4.2 mg) and its stereostructural complexity, the absolute configuration of **1a**, particularly the chirality of the C15−C15′ central axis, remained to be determined.

In order to gain a large enough quantity of **1a** for further structural confirmation, its biomimetic synthesis was investigated. Structurally, moluccensin A (**3**), of which the absolute configuration was determined by single-crystal X-ray diffraction (XRD) with Cu Kα radiation (see SI), is a potential precursor for a biomimetic synthesis of krishnadimer A (**1a**). With sufficient quantities of pure material at hand, and after numerous attempts and optimization (see the SI), the oxidative coupling of moluccensin A (**3**) was performed in MeOH with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, 1.2 equiv) as the oxidant under ambient conditions to afford two dimeric limonoids, **2a** and its *M*-atropo-diastereomer **2b**, with the molar ratio of 3:1, finally, after selective hydrolysis of **2a** with LiOH, providing 22.7 mg of pure **1a** with an overall yield of 13.4% (Scheme 1).[7] Surprisingly, the direct coupling of 1-*O*-deisobutyryl moluccensin A, which has a hydroxy function at C1 instead of the isobutyryloxy group, under the same reaction conditions, gave the desired dimer **1a** only in traces. After considerable efforts, crystals were obtained from the solvent mixture of $CHCl_3$/MeOH (1:2) suitable for a single-crystal XRD analysis of **1a**, conducted with Cu Kα radiation [Flack parameter of 0.06(5)], which unambiguously established the full absolute configuration of **1a**, including the *P*-configuration of the rotationally hindered C15−C15′ central axis. The absolute configuration of all 20 stereocenters of **1a** was clearly determined as 1*R*,2*R*,3*S*,4*R*,5*S*,9*R*,10*S*,13*R*,17*R*, 1′*R*,2′*R*,3′*S*,4′*R*,5′*S*,9′*R*,10′*S*,13′*R*,17′*R* (Figure 2c, CCDC 1505936).

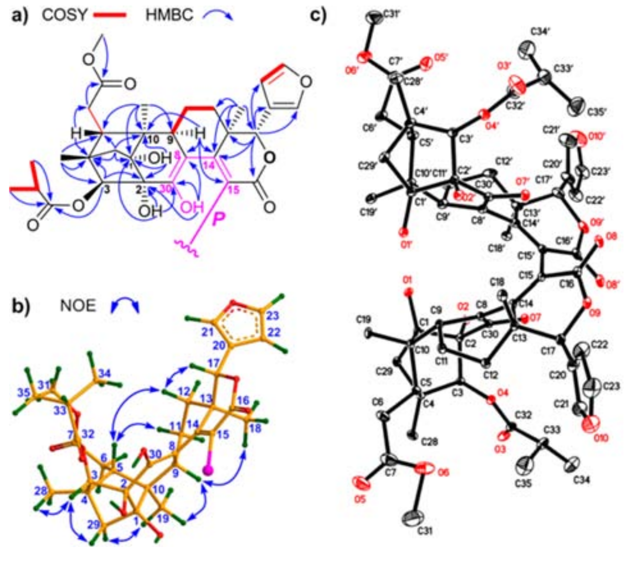
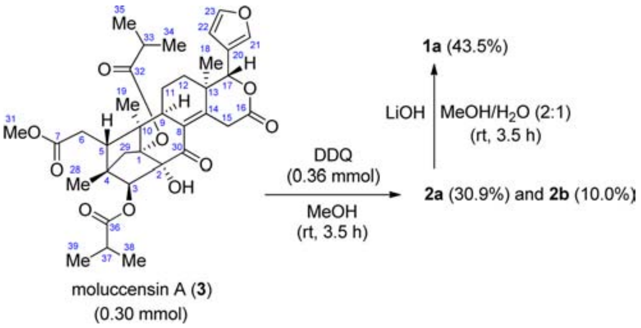


Figure 2. (a) Key $^1H-^1H$ COSY and HMBC correlations of **1a**. (b) Diagnostic NOE interactions of **1a** (the second molecular moiety, attached at C15, is omitted for clarity). (c) ORTEP of the X-ray crystal structure of **1a** (ellipsoids are given at the 10% probability level).

Scheme 1. Semisynthesis of the Dimers 1a, 2a, and 2b from the Natural Monomer 3[a]



[a] Compound **2a** is the 1,1′-*O*-diisobutyryl analogue of **1a** (for details of the structure elucidation of **2a**, see the SI).

On the basis of the X-ray crystal structure of **1a** and the exciton chirality method,[8] the *P*-configuration of the C15−C15′ central axis in **1a** was corroborated by the positive couplet with a positive Cotton effect at 380 nm and a negative one at 314 nm in the electronic circular dichroism (ECD) spectrum of **1a** (see Figure S4).

The molecular formula of **2b** was determined to be the same as that of **2a** by HRESIMS (*m*/*z* calcd for $[M + Na]^+$ 1301.5503, found 1301.5527). The 1D NMR spectroscopic data (Tables S1 and S2) and 2D correlations ($^1H-^1H$ COSY, HMBC, and NOESY) of **2b** exhibited a high similarity to those of **2a**, revealing that both dimers possessed the same symmetric constitution and relative configuration of the monomeric units. However, the ECD spectra of the two compounds were nearly opposite to each other between 290 and 380 nm, showing a negative couplet in **2b** versus a positive one in **2a** (Figure 1), leading to the conclusion that these dimers were atropo-diastereomers (see Figure S4).[8] As expected, single-crystal XRD analysis of **2b**, conducted with Cu Kα radiation [Flack parameter of −0.001(10)], unambiguously established the *M*-configuration of the C15−C15′ central axis in **2b** (Figure 3, CCDC 1505937).

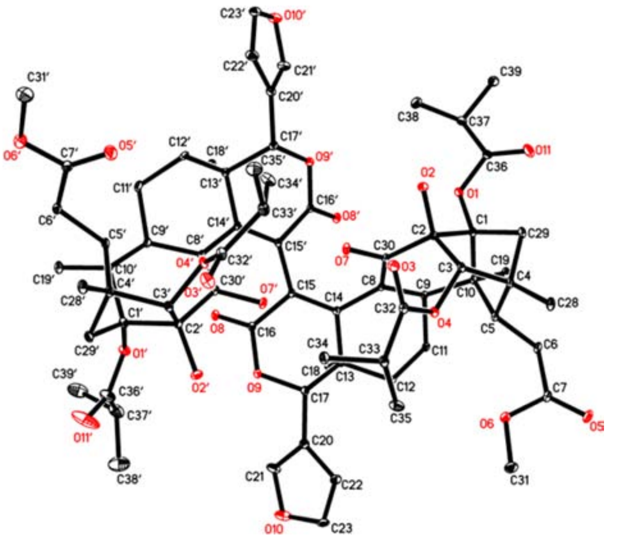
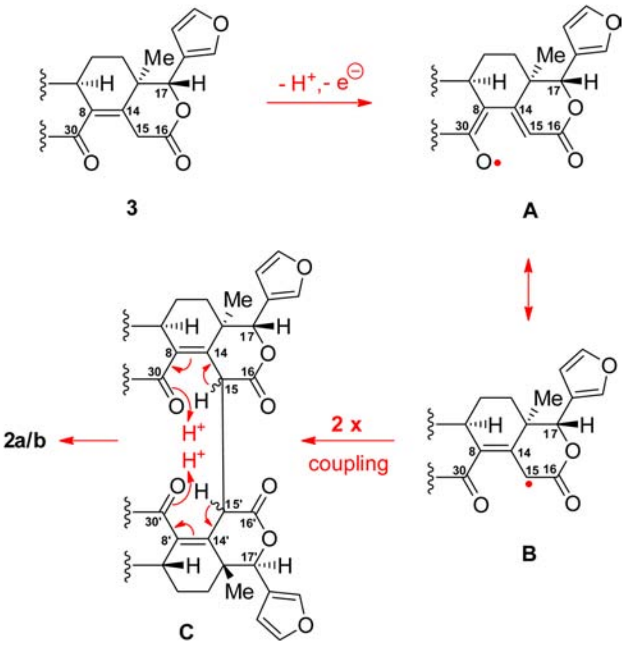


Figure 3. ORTEP of the X-ray crystal structure of **2b** (ellipsoids are given at the 10% probability level).

For the biomimetic construction of the two new dimeric architectures of **2a** and **2b** from moluccensin A (**3**), we suggest an oxidative coupling as shown in Scheme 2. Thus, deprotonation of the enone function (C30, C8, and C14) of **3** and trapping of an electron (in our synthesis by DDQ) would generate a dienoxy radical **A** with spin density not only on the oxygen atom, but also on C15 (see the mesomeric structure **B**). Two molecules of **B** could then undergo a homocoupling to give the dimer **C**, which would afford **2** as its two atropo-diastereomers, **2a** and **2b**, after keto−enol tautomerism.

Antitumor activities of **1a**, **2a**, **2b**, and **3** were tested by the MTT cytotoxity assay against eight human tumor cell lines, including ovarian A2780 and A2780/T, melanoma A375, lung A549, colorectal HCT-8 and HCT-8/T, cervical Hela, and breast MDA-MB-231. Cisplatin was used as the positive control. The *M*-configured dimer **2b** exhibited potent, and selective, cytotoxicities against A2780, A2780/T, A375, A549, HCT-8, and HCT-8/T cells with IC_{50} values of 1.73, 12.96, 2.68, 6.72, 5.47, and 11.31 μM, respectively, whereas the *P*-configured dimers, **1a** and **2a**, and the monomeric analogue, **3**, were inactive (Table S3).

Scheme 2. Proposed Reaction Mechanism for the DDQ-Mediated Semisynthesis of 2a and 2b



In conclusion, krishnadimer A (**1a**), isolated from the seeds of an Indian mangrove, *X. moluccensis*, represents a new class of axially chiral nonbiaryl natural products. The semisynthetic construction of its unique molecular architecture succeeded by DDQ-mediated dimerization through oxidative dienol coupling of **3**. It is noteworthy that the two atropo-diastereomeric products, **2a** and **2b**, were not formed in equal amounts, but with a 3:1 ratio, making an atropo-diastereoselectivity of 75%. This biomimetic synthesis successfully afforded a large enough quantity of **1a** to permit unequivocal assignment of its dimeric architecture, including the absolute configuration, by single-crystal XRD and ECD spectral analyses.

The discovery of krishnadimer A as the first dimeric limonoid featuring an unprecedented architecture with axial chirality represents a milestone during decades of work on natural limonoids. Most notably, the likewise synthesized unnatural dimer **2b**, with an *M*-configurationally central axis, showed potent and selective antitumor activities, whereas its natural atropo-diastereomer **2a** was completely inactive. This shows the impact of axial chirality on the bioactivity and makes the synthesis of stereochemically pure limonoid dimers with further improved bioactivities by directed, atroposelective coupling, a rewarding task. Additional work is in progress.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03479.

Details of the structure elucidation of **2a**, experimental details, ^1H and ^{13}C NMR data, and Figures S1−S4 (PDF)

HRESIMS, 1D and 2D NMR spectra of **1a**, **2a**, and **2b**, and HPLC, ESIMS, and ^{1}H NMR spectra of the synthesized **1a** (PDF)
Crystallographic data for **1a** (CIF)
Crystallographic data for **2b** (CIF)
Crystallographic data for **3** (CIF)

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Notes

The authors declare no competing financial interest.

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