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Joziknipholones A and B: The First Dimeric Phenylanthraquinones, from the Roots of *Bulbine frutescens*

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Dedicated to Professor Berhanu M. Abegaz on the occasion of his 60th birthday

Abstract: From the roots of the African plant *Bulbine frutescens* (Asphodelaceae), two unprecedented novel dimeric phenylanthraquinones, named joziknipholones A and B, possessing axial and centrochirality, were isolated, together with six known compounds. Structural elucidation of the new metabolites was achieved by spectroscopic and chiroptical methods, by reductive cleavage of the central bond between the monomeric phenylanthraquinone and -anthrone portions with sodium dithionite, and by quantum chemical CD calculations. Based on the recently revised absolute axial configuration of the parent phenylanthraquinones, knipholone and knipholone anthrone, the new dimers were attributed to possess the *P*-configuration (i.e., with the acetyl portions below the anthraquinone plane) at both axes in the case of joziknipholone A, whereas in joziknipholone B, the knipholone part was found to be *M*-configured. Joziknipholones A and B are active against the chloroquine resistant strain K1 of the malaria pathogen, *Plasmodium falciparum*, and show moderate activity against murine leukemic lymphoma L5178y cells.

Keywords: antimalarial activity · chirality · joziknipholones · natural products · structure elucidation

Introduction

Bulbine frutescens (Asphodelaceae) is a South African plant species widely cultivated for aesthetic purposes.[1] Previous phytochemical studies have resulted in the isolation and characterization of the axially chiral phenylanthraquinones knipholone (**1**), 4′-*O*-demethylknipholone, gaboroquinones A and B, 4′-*O*-demethylknipholone-4′-β-D-glucopyranoside, and isoknipholone (**2**),[2,3] with the latter two compounds showing remarkable antiplasmodial activities. From the polar fractions of the same plant, the first 6′-*O*-sulfated phenylanthraquinones were reported,[3] making this species a rich source of novel bioactive compounds, thus warranting further investigations. In the present paper, we report on the isolation, structural elucidation, and biological activities of further unprecedented metabolites from *B. frutescens*, namely, the first phenylanthraquinone dimers, named joziknipholones A (**5**) and B (**6**), along with six known related compounds, isolated for the first time from this plant species. Based on the recent revision of the absolute configurations of the main atropisomers of both knipholone (**1**) and the related knipholone anthrone (**3**),[4] the absolute configurations at the biaryl axes and at the stereogenic center of

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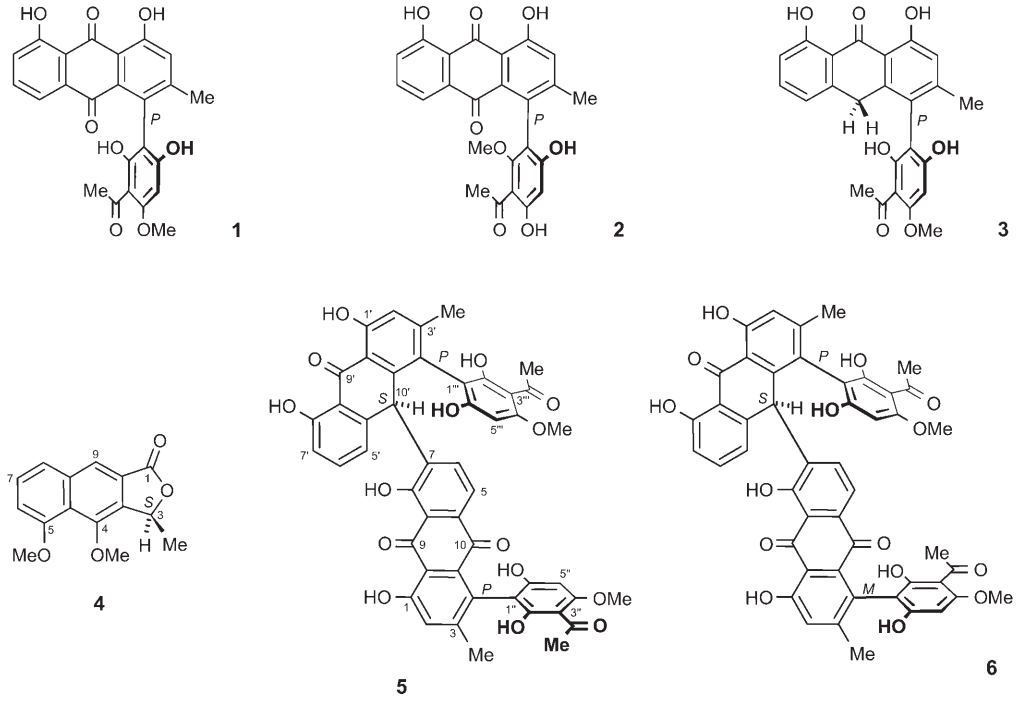
Supporting information for this article (Figures S1–S10) is available on the WWW under http://www.chemeurj.org/ or from the author.



the new dimers were determined by reductive cleavage of the central C7–C10′ linkage, followed by CD analysis of the obtained monomeric phenylanthraquinone and -anthrone products and by 2D NOESY experiments in agreement with hints from the results of quantum chemical CD calculations. We also report on the first documented occurrence of isoknipholone (**2**) in an enantiomerically enriched form.[5]

Results and Discussion

Column chromatography of the acetone extract of *B. frutescens* roots on Sephadex LH-20 and further purification by preparative TLC yielded two novel dimeric phenylanthraquinones (see below), along with three phenolic acids (*p*-coumaric acid, dihydro-*p*-coumaric acid, and vanillic acid)[6] and three naphthalene derivatives, namely, 5,8-dihydroxy-1-methylnaphtho[2,3c]furan-4,9-dione, 5,8-dihydroxy-1-hydroxymethyl-naphtho[2,3c]furan-4,9-dione,[7] and 4-*O*-methyl eleutherol (**4**).[8] This is the first report on the natural occurrence of **4**, which has previously been described only as a semisynthetic derivative of eleutherol.[8] The ^{13}C NMR data of this compound have never been reported, and are, therefore, included in the Experimental Section.

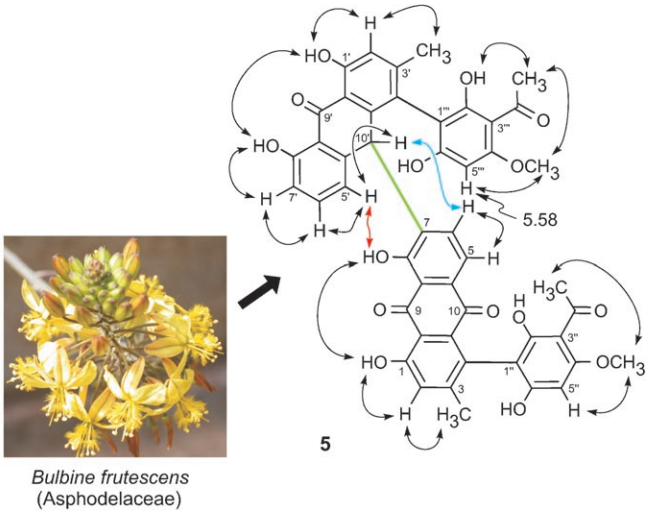
Dimeric phenylanthraquinones: The first of the dimeric phenylanthraquinones, compound **5**, was isolated as an orange amorphous powder. The ^{1}H NMR spectrum resembled that of knipholone (**1**),[9] except for the doubling of virtually all of its signals. Thus, the presence of six chelated hydroxy proton signals (δ = 11.95, 12.21, 12.47, 12.60, 14.07, and 14.45 ppm), two aromatic methyls (δ = 1.99 and 2.13 ppm), two methoxyls (δ = 3.73 and 3.93 ppm), two acetyl groups (δ = 2.62 and 2.72 ppm), and nine aromatic protons (Table 1) suggested that this compound could be a dimeric, yet unsymmetric, phenylanthraquinone. In support of this, the TOF-HREIMS spectrum showed an $[M+1]^+$ peak at m/z: 853.2117 corresponding to the molecular formula of $C_{48}H_{37}O_{15}$. Moreover, the ^{13}C NMR spectrum displayed the presence of five carbonyl groups (δ = 194.6, 193.3 ppm for C9′ and C9; 182.7 ppm for C10; and 204.1 ppm for the two acetyl carbonyls), thus being consistent with a mixed dimer of a phenylanthraquinone and a phenylanthrone. This was further confirmed by the UV spectrum (λ_{max} = 211, 231, 263, 291, 339, 439 nm), which indicated characteristic bands[9,10] of the two molecular moieties (ca. λ_{max} = 263 and 439 nm for a phenylanthraquinone and λ_{max} = 339 nm for a phenylanthrone). By detailed analysis of the 1D and 2D NMR data (^{1}H and ^{13}C NMR, HMBC, see Table 1, including COSY, ROESY, NOESY, and HSQC), the phenylanthraquinone moiety was identified as the known[9] compound knipholone. Proton signals in ring C (resulting from H5, H6, and H7), which usually appear as an ABX system in knipholone,[9] were now observed as two sets of doublets at δ = 7.24 and 6.91 ppm (J = 8.0 Hz) for H5 and H6, respectively, suggesting that the site of linkage in this moiety over to the other molecular portion is C7.

Comparison of the ^{1}H and ^{13}C NMR spectra of the other half of the molecule with those of knipholone anthrone (**3**)[10] showed identical features except for the proton resonance at C5‴ in this molecular half. This signal was shifted upfield (δ = 5.58 ppm) in comparison to that of free **3** (δ = 6.30 ppm),[10] which may be ascribed to the shielding effect of the neighboring anthraquinone system.[11] Furthermore, the methylene group at C10 in knipholone anthrone (**3**, δ_H = 4.07 and δ_C = 32.2 ppm)[10] was now replaced by a methine

Table 1. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) data together with HMBC (^{2}J and ^{3}J) correlations of joziknipholone A (**5**).

Atom no.	δ_H [ppm] (mult., J [Hz])	δ_C [ppm]	HMBC	Atom no.	δ_H [ppm] (mult., J [Hz])	δ_C [ppm]	HMBC
1	–	163.5	–	1″	–	107.6	–
2	7.29 (s)	126.0	1, 13, 3-CH_3, 4	2″	–	163.9	–
3	–	153.3	–	3″	–	106.5	–
4	–	126.2	–	4″	–	163.5	-
5	7.24 (d, 7.9)	120.3	7, 12, 10	5″	6.12 (s)	91.0	1″, 3″, 4″, 6″
6	6.91 (d, 8.0)	136.4	11, 8	6″	–	160.0	–
7	–	139.0	–	1‴	–	105.6	–
8	–	159.1	–	2‴	–	164.7	–
9	–	193.3	–	3‴	–	106.9	–
10	–	182.7	–	4‴	–	164.6	–
11	–	133.3	–	5‴	5.58 (s)	90.2	1‴, 3‴, 4‴, 6‴
12	–	116.1	–	6‴	–	161.0	–
13	–	115.7	–	3-CH_3	2.13 (s)	21.2	2, 3
14	–	133.4	–	3′-CH_3	1.99 (s)	21.0	2′, 3′, 4′
1′	–	163.6	–	4″-OCH_3	3.93 (s)	56.2	4″
1′a	–	115.7	–	4‴-OCH_3	3.73 (s)	56.2	4‴
2′	6.98 (s)	118.8	1′, 13′, 3′-CH_3, 4′	3″-$COCH_3$	2.62 (s)	33.5	3″, 3″-$COCH_3$
3′	–	150.8	–	3‴-$COCH_3$	2.72 (s)	33.5	3‴, 3‴-OCH_3
4′	–	121.7	–	3″-$COCH_3$	–	204.1	–
4′a	–	140.1	–	3‴-$COCH_3$	–	204.1	–
5′	6.87 (brd)	120.0	–	1-OH	12.47 (s)	–	1, 13, 2
6′	7.33 (t, 8.0)	137.4	–	8-OH	11.95 (s)	–	7, 8, 12
7′	6.81 (d, 8.2)	116.3	5′, 8′, 12′	1′-OH	12.60 (s)	–	1′, 13′, 2′
8′	–	163.2	–	8′-OH	12.21 (s)	–	7′, 8′, 12′
9′	–	194.6	–	2″-OH	14.07 (s)	–	–
10′	5.96	37.5	–	2‴-OH	14.45 (s)	–	1‴, 2‴, 3‴
11′	–	145.7	–				
12′	–	114.7	–				

group at C10′ in **5** (δ_H=5.96 and δ_C=37.5 ppm). This suggested that the knipholone portion was most probably attached to C10 of the knipholone anthrone moiety (i.e., to C10′ in **5**), thus forming an sp^2–sp^3 linkage between C7 and C10′ (indicated in green) of the two molecular halves of **5** (Figure 1). Key NOESY correlations were observed from H10′ to H6 and H5′, and very weak interactions between 8-OH and H5‴, and from 1-OH to 4‴-OCH_3 (the latter two are not shown). The new compound was therefore tentatively deduced to have the constitution **5**, that is, that of a mixed 7,10′-knipholone-knipholone anthrone "dimer" as shown in Figure 1.



Bulbine frutescens
(Asphodelaceae)

Figure 1. NOESY correlations providing evidence for the constitution of compound **5**.[13]

However, the possibility that the linkage between the two moieties could be as shown in Figure 2, that is, a biarylic

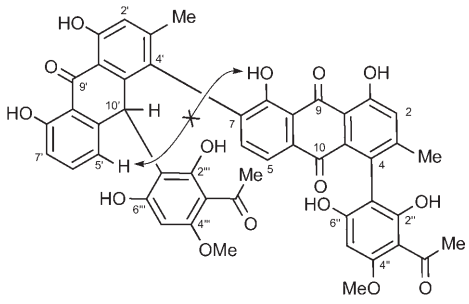


Figure 2. A likewise imaginable alternative coupling type of the dimeric phenylanthraquinone, with a biaryl-type sp^2–sp^2 axis between C7 and C4′, excluded by the NOESY interaction indicated.

sp^2–sp^2 axis from C7 to C4′, was not completely excluded at this point, as phenylanthraquinones composed of the acetylphloroglucinol coupled to C10 of an (additionally oxygenated) chrysophanthrone are also known in nature, like, for example, foliosone and isofoliosone.[12] Detailed analysis of the NOESY spectrum revealed a key connectivity from H5′ to OH8 (Figure 1, in red). This interaction is evidently inconsistent with the alternative structure shown in Figure 2.

The NOESY correlations (particularly the one from H10′ to H6) of **5** (Figure 1, in blue), together with the above mentioned upfield shift of H5‴ (δ=5.58 instead of 6.12 ppm, as,

for example, in the southern knipholone portion of **5**, H5″, which resonates at $\delta = 6.12$ ppm) and the deshielded signal of the acetyl group at 3‴ ($\delta = 2.72$ instead of 2.62 ppm) gave the first hints of the configuration in the northern part of **5**, namely, at the 4′–1‴ biaryl axis of the knipholone anthrone moiety relative to the stereogenic center, leaving either 4′*M*,10′*R* (i.e., **A**, see Figure 3) or its enantiomorphous version, 4′*P*,10′*S* (*ent*-**A**) as possible partial structures.

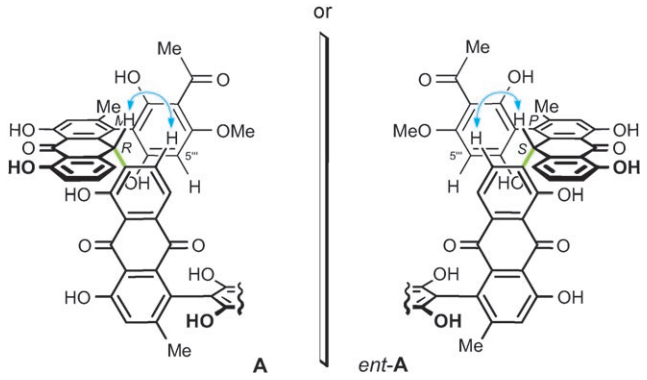
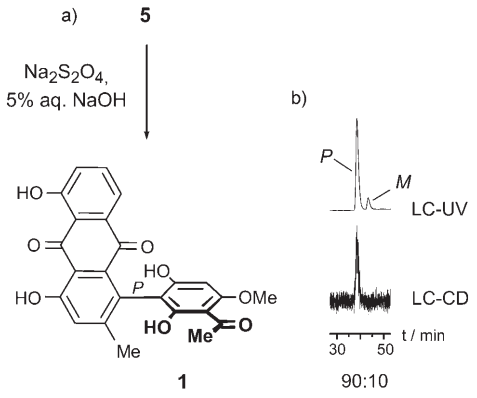


Figure 3. Two enantiomorphous partial structures, **A** and *ent*-**A** of **5**. The NOESY interaction indicative of the relative configuration in the northern part is illustrated in blue.

As for the configuration at the southern biaryl axis, namely, between C4 and C1″, chemical degradation experiments proved extremely helpful. However, first attempts to cleave the central C7–C10′ linkage under standard conditions as previously elaborated for the degradation of knipholone[9] by using sodium dithionite (see the Experimental Section) yielded only chrysophanol, acetylphloroglucinol, and knipholone (**1**; according to co-TLC and co-HPLC with authentic samples), initially leaving open the question as to whether the diagnostically valuable product **1** came exclusively from the 4–1″ axis or (also) from the 4′–1‴ linkage, as no traces of knipholone anthrone could be detected. HPLC analysis on a chiral phase with online CD detection revealed the resulting knipholone (**1**) to be *P*-configured,[4] that is, in accordance with the main naturally occurring enantiomer,[4] and nearly enantiomerically pure (>90:10 *P*/*M*; Scheme 1), that is, identical to an authentic sample from *B. frutescens* (60:40, likewise in favor of *P*[3]).

This, together with the observed NOESY correlations above and the upfield shift of H5‴, reduced the number of possible stereoisomeric structures from initially eight (due to the presence of three stereogenic elements, viz. the two biaryl axes and one stereogenic center) to only two remaining diastereomers, (4*P*,4′*M*,10′*R*) and (4*P*,4′*P*,10′*S*)-**5** (Figure 4).

For the assignment of the absolute configuration of **5**, CD spectra were calculated for both possible stereostructures, (4*P*,4′*M*,10′*R*)-**5** and (4*P*,4′*P*,10′*S*)-**5**. Despite their diastereomeric nature, these two structures possess pseudo-enantiomeric chromophoric frameworks (Figure 4), and therefore should provide nearly opposite CD spectra.



Scheme 1. a) Enantiomeric resolution of knipholone (**1**), obtained from reductive cleavage of **5**, on a chiral HPLC phase (Chiracel OD-H) with b) UV and CD detection monitored at 254 and 277 nm, respectively.

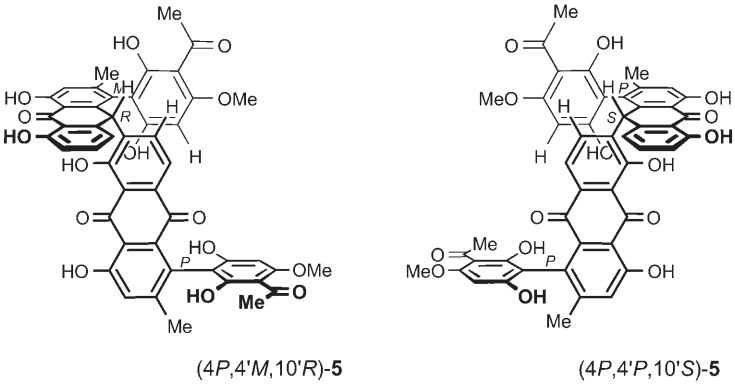
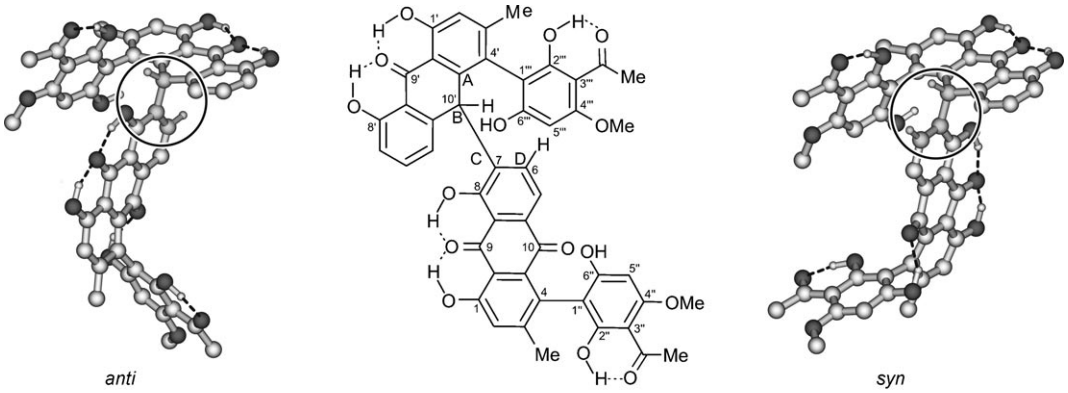


Figure 4. Two possible full stereostructures for compound **5**, (4*P*,4′*M*,10′*R*)-**5** and (4*P*,4′*P*,10′*S*)-**5**, possessing pseudo-enantiomeric core chromophores.

Starting with the (4*P*,4′*P*,10′*S*)-diastereomer, the conformational behavior of the dimeric phenylanthraquinone **5** was investigated by using the AM1[14] and BLYP/SVP[15,16] methods. The calculations for **5** revealed the same structural features as previously found for the monomers, knipholone and knipholone anthrone,[4] namely, showing hydrogen-bond formation in the anthraquinone and anthrone portions, and in the acetylphloroglucinol units. The orientations around the biaryl axes were likewise found to be identical to those of the global minima of the monomers.[4] Screening of the reaction coordinate for the central C7–C10′ bond of **5** revealed two minimum structures, one with a *syn* orientation of the protons at C6 and C10′ (Figure 5, right), and the other one with an *anti* arrangement (Figure 5, left). The calculations of these two minima both, in vacuum and in CH_2Cl_2, revealed the *anti* conformer to be energetically more favorable in both cases, namely, by 1.64 and 0.98 kcal mol^{-1}, respectively.

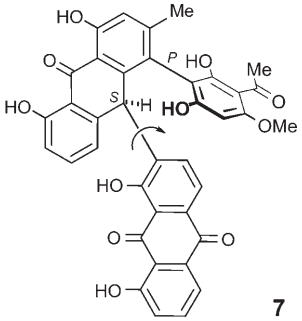
To investigate the stability of the central C7–C10′ bond of **5**, the rotation barrier was theoretically estimated by calculating that of **7** as a simplified model compound (Table 2), in which the acetylphloroglucinol unit and the methyl substituent of the knipholone portion of **5** were omitted (for details



	θ_{ABCD}	$\Delta\Delta H_f$ vacuum	$\Delta\Delta H_f$ CH_2Cl_2
anti	-48.4	0.00	0.00
syn	131.4	1.64	0.98

Figure 5. The two minimum conformers established for compound **5**, the decisive *anti*/*syn* array, that is, the (not stable but preferential) axial conformation at the central sp^2–sp^3 axis (C7–C10′) is indicated by a circle.

Table 2. Rotational barriers at the central sp^2–sp^3 axis (C7 to C10′) calculated (BLYP/3–21G) for **7** as a simplified model compound.



7

	$\Delta H^{\neq}$ [kJ mol^{-1}][a] TS1[b]	TS2[b]
from *syn* to *anti*	15.66	41.23
from *anti* to *syn*	31.76	57.33

[a] To get more accurate results, the rotational barriers were calculated by taking into account zero-point vibrational energies of the minimum conformers and transition states, which were furthermore scaled by a factor of 0.9945 as recommended for BLYP/3–21G-based calculations.[17] [b] For structures of the minimum conformers of a model compound and for the transition-state geometries, see Figure S3 in the Supporting Information.

see the Supporting Information, Figure S3) for the purpose of (at least slightly) reducing the size of the computed molecule, without changing the most important "core geometry" around the central C7–C10′ bond.

The obtained low rotational barrier values (Table 2) revealed that the central bond of **5** should be able to rotate rather freely, proving that the two global minimum structures, *syn* and *anti*, should both be present in solution and should, according to their population, have a substantial impact on the resulting circular dichroism (CD). Therefore, CD calculations, based on the time-dependent DFT (TDDFT) method, were performed for both basic conformers of **5**. An application of the more accurate hybrid B3LYP[15b,18] functional, which had shown good results in the case of knipholone (**1**) and knipholone anthrone (**3**),[4] was not possible for the dimer, because of the large size of the molecule and the resulting high computational costs, so the calculations were conducted by using the RI-BLYP/TZVP[15,19] method. The calculated single spectra were added up according to the Boltzmann statistics giving the overall CD curve predicted for (4*P*,4′*P*,10′*S*)-**5**. In the case of the (4*P*,4′*M*,10′*R*)-diastereomer of **5**, analogous conformers, *syn* and *anti*, were found, and the resulting CD spectrum was obtained as described above. Comparison of the experimental CD curve of **5** with the theoretically predicted ones showed that the first positive band (at $\lambda_{max} = 310$ nm) in the measured spectrum was reproduced by the curve calculated for the (4*P*,4′*P*,10′*S*)-diastereomer, whereas the one predicted for (4*P*,4′*M*,10′*R*)-**5** displayed negative peaks in this region (Figure 6). Therefore, although the TDDFT-based calculations could, unfortunately, not provide an unambigu-

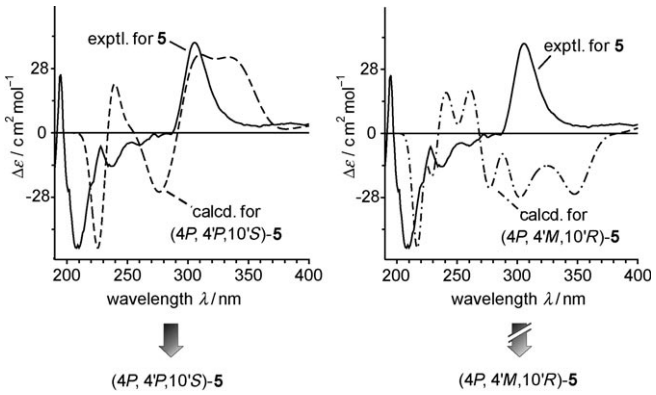


Figure 6. Comparison of the experimental CD spectrum of **5** with the spectra calculated for the two possible remaining stereoisomers, (4*P*,4′*P*,10′*S*)-**5** and (4*P*,4′*M*,10′*R*)-**5**.

ous full configurational assignment of **5**, due to the large size of the molecule, they still gave a preference for 4′*P*10′*S* rather than for 4′*M*10′*R*. Thus, for an additional confirmation of the absolute configuration of **5**, further experimental work was necessary.

For this purpose, the degradation cleavage of **5** was investigated more closely. In the literature,[11,20] the reductive cleavage of (simpler) anthraquinone–anthrone or anthrone–anthrone dimers with $Na_2S_2O_4$ in alkaline solution is known to yield only one product, namely, the respective anthraquinone, not only from the quinoid portion of the dimer, as expected, but also from the anthrone part, by aerial oxidation under basic conditions. This may explain why knipholone anthrone had not been detected under the above reaction conditions and would indicate that the *P*-configuration found for knipholone formed in the degradation would probably have resulted from *both* axes, C4–C1″ and C4′–C1‴, thus excluding the presence of two differently configured biaryl axes (i.e., *P* and *M*). This would leave only the 4*P*,4′*P*,10′*S* diastereomer as a possible stereostructure for the "mixed dimer" **5**. Still, a confirmation of this result seemed desirable, and the availability of a method for the stereoanalysis of dimers with possibly two differently configured biaryl axes was also of interest, because, in that case, the degradation would lead to (partially or fully) racemic knipholone (**1**), leaving open which axis was *M* and which *P* (like, the second dimer isolated, see below). Therefore, the method was further optimized, which was first attempted by using model compounds (knipholone and knipholone anthrone) to ensure that under the reaction conditions, knipholone anthrone (**3**) was not converted to knipholone (**1**) nor, vice versa, **1** to **3**. Although oxidation of knipholone anthrone to knipholone could not be completely avoided, even when working under nitrogen, it was minimized down to a ratio of 98:2 (according to HPLC). When applied to compound **5**, these conditions now gave both, knipholone (**1**) and knipholone anthrone (**3**), which were successfully separated on an X-Terra RP_{18} analytical column (see the Experimental Section). Offline CD measurements of these products with subsequent comparison to the CD spectra of authentic samples (Supporting Information, Figure S4) now unambiguously revealed both, knipholone (**1**) and knipholone anthrone (**3**) to be *P*-configured. These findings further proved our above assignment (Figure 6) that **5** should have the 4*P*,4′*P*,10′*S*-configuration. Compound **5** was given the trivial name joziknipholone A according to the Swahili word *jozi* (=a pair).

The elemental composition of the second new isolated compound, **6**, was established to be $C_{48}H_{37}O_{15}$, by its TOF-HREIMS spectrum as shown by the $[M+1]^+$ peak at *m*/*z*: 853.2117, indicating that this product was isomeric to **5** and probably a diastereomer. In agreement with this, the UV spectrum (λ_{max}=219, 267, 291, 351, and 439 nm) again showed the presence of a phenylanthraquinone and a phenylanthrone chromophore.

The ^1H and ^{13}C NMR spectroscopic data of this compound showed structural features identical to those of **5**, with only marginal differences (Table 3), suggesting **6** to be

Table 3. ^1H (600 MHz) and ^{13}C NMR (150 MHz) data together with HMBC (^2J and ^3J) correlations of joziknipholone B (**6**).

Atom no.	δ_H [ppm] (mult., *J* [Hz])	δ_C [ppm]	HMBC	Atom no.	δ_H [ppm] (mult., *J* [Hz])	δ_C [ppm]	HMBC
1	–	163.7	–	1″	–	107.6	–
2	7.30 (s)	125.9	13, 3-CH_3, 4	2″	–	163.9	–
3	–	153.4	–	3″	–	106.5	–
4	–	126.1	–	4″	–	163.6	–
5	7.24 (d, 7.9)	120.4	7, 12, 10	5″	6.09 (s)	91.1	1″, 3″, 4″, 6″
6	6.90 (d, 7.9)	136.1	11, 8	6″	–	159.8	–
7	–	139.6	–	1‴	–	106.9	–
8	–	159.1	–	2‴	–	164.7	–
9	–	193.3	–	3‴	–	106.9	–
				4‴	–	164.5	–
10	–	182.5	–	5‴	5.57 (s)	90.2	1‴, 3‴, 4‴, 6‴
11	–	133.2	–	6‴	–	160.9	–
12	–	115.9	–	3-CH_3	2.13 (s)	21.3	2, 3
13	–	115.7	–	3′-CH_3	1.99 (s)	21.1	2′, 3′, 4′
14	–	133.2	–				
1′	–	163.6	–	4″-OCH_3	3.92 (s)	56.2	4″
1′a	–	115.7	–	4‴-OCH_3	3.69 (s)	56.2	4‴
2′	6.97 (s)	118.8	1′, 13′, 3′-CH_3, 4′	3″-$CO\mathit{C}H_3$	2.63 (s)	33.5	3″, 3″-$COCH_3$
3′	–	151.0	–	3‴-$COCH_3$	2.71 (s)	33.5	3‴, 3‴-$COCH_3$
4′	–	121.9	–	3″-$COCH_3$	–	204.3	–
4′a	–	142.9	–	3‴-$COCH_3$	–	204.1	–
5′	6.92 (br)	119.9	–	1-OH	12.51 (s)	–	1, 13, 2
6′	7.32 (t, 8.0)	137.4	11′, 8′	8-OH	12.01 (s)	–	7, 8, 12
7′	6.80 (d, 7.9)	116.3	5′, 8′, 12′	1′-OH	12.58 (s)	–	1′, 13′, 2′
8′	–	163.2	–	8′-OH	12.23 (s)	–	7′, 8′, 12′
9′	–	194.6	–	2″-OH	14.11 (s)	–	–
10′	6.06	37.3	–	2‴-OH	14.45 (s)	–	–
11′	–	147.1	–				
12′	–	114.7	–				

another, structurally closely related mixed "dimer", again composed of a knipholone portion and a knipholone anthrone part interlinked at positions C7 and C10′, indicated in green (Figure 7a).

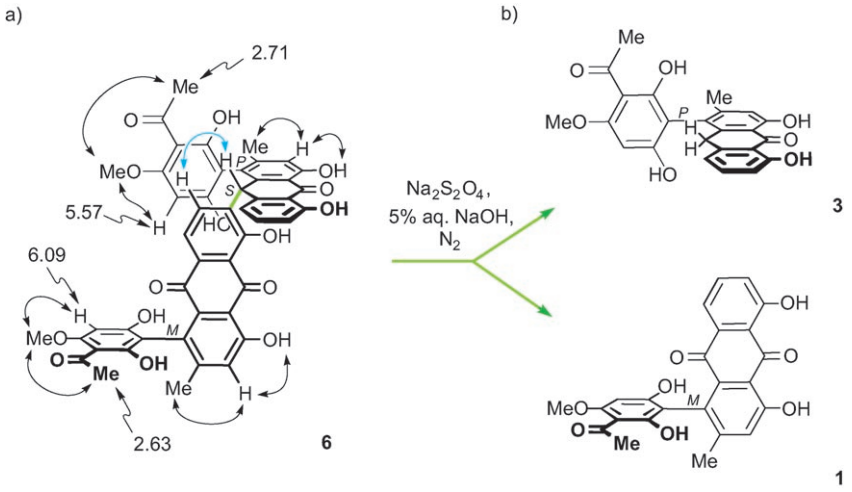


Figure 7. Joziknipholone B (**6**): a) Full absolute stereostructure with diagnostically significant ^{1}H NMR spectroscopic chemical shifts and NOESY interactions and b) degradation to (*P*)-knipholone anthrone (**3**) and (*M*)-knipholone (**1**), by reductive cleavage of the C7–C10′ linkage of **6** (in green).

knipholone (**2**, 0.12 μgmL^{-1})[3] and knipholone anthrone (**3**, 0.15 μgmL^{-1}).[22] It was thus less active than the standard drug chloroquine in the same system only by a factor of 3, while the activity of joziknipholone B (**6**, IC_{50} = 0.23 μgmL^{-1}) was slightly weaker than that of joziknipholone A (**5**). Both compounds, **5** and **6**, exhibited only low cytotoxicities against rat skeletal myoblast (L6) cells. Further investigations against other pathogens of tropical diseases (Table 4) revealed that these novel "mixed dimers" **5** and **6** are not active against the trypanosomes and *L. donovani*, and thus specifically act against the malarial parasite (Table 4).

Furthermore, joziknipholones A (**5**) and B (**6**) showed low antitumoral activities against murine leukemic lymphoma L5178y cells (Table 4).

In addition, the NOESY correlations at the central linkage were exactly the same as those observed for joziknipholone A (**5**). Upon treatment with $Na_2S_2O_4$ under the conditions optimized for **5** (Figure 1), compound **6** furnished knipholone anthrone (**3**), which, as for **5**, was again shown to be *P*-configured by CD analysis, while the knipholone (**1**) now formed was found to have the *M*-configuration (Supporting Information, Figure S5). In analogy to joziknipholone A (**5**), the *P*-configuration in the anthrone moiety of **6**, in combination with the observed upfield shift of H5‴ (δ = 5.57 ppm, directly indicated the *S*-configuration at the stereogenic center, thus deducing the second compound to have the full stereostructure **6** as shown in Figure 7. Compound **6**, which is the 4,1″-epimer of **5**, that is, with two heterochiral axes, was given the trivial name joziknipholone B.

Table 4. Antiprotozoal activities of **5** and **6** against *P. falciparum* (K1 strain), *T. cruzi*, *T. brucei rhodesiense*, *L. donovani* (axenic amastigotes), and cytotoxicities against rat skeletal myoblast cells (L6) and, antitumor activities against murine leukemic lymphoma L5178y cells.

Compound	IC_{50} [μgmL^{-1}]					
	P. falciparum	*T. cruzi*	*T. brucei rhodesiense*	*L. donovani*	L6 cells (cytotoxicity)	L5178y cells (lymphoma)
Standard	0.041[a]	0.276[b]	0.0026[c]	0.158[d]	0.007[e]	0.8[f]
5	0.142	>30	33.9	12.5	16.3	10
6	0.23	>30	14.4	9.06	17.4	8.7

[a] Chloroquine. [b] Benznidazole. [c] Melarsoprol. [d] Miltefosine. [e] Podophyllotoxin. [f] Bleomycin.

Biological activities of joziknipholones A (5) and B (6): Given the promising antimalarial bioactivities of "normal", that is, monomeric phenylanthraquinones,[2,3,21,22] the new dimeric compounds were evaluated for their potency against the chloroquine-resistant strain K1 of *Plasmodium falciparum*. Joziknipholone A (**5**) exhibited good antiplasmodial activity (IC_{50} = 0.14 μgmL^{-1}), that is, comparable to those of the two most active monomeric phenylanthraquinones, iso-

Conclusion

Joziknipholones A (**5**) and B (**6**) are the first members of a structurally unique novel class of dimeric phenylanthraquinones. The elucidation of the constitution and of the configuration at the stereogenic center at C10′ relative to one of the biaryl axes was achieved by NMR spectroscopic techniques. An unambiguous establishment of the absolute stereostructures succeeded by the optimization and application of an improved method for the reductive cleavage of the central C7–C10′ linkage to give stereochemically known[4] monomeric phenylanthraquinones and phenylanthrones, thus avoiding their interconversion, which had initially hampered a differentiated interpretation. This was achieved by elaborating mild reaction conditions in the absence of air oxygen for the reductive cleavage of the C7–C10′ axis. These novel-type metabolites **5** and **6**, which apparently originate through a mixed phenol-oxidative coupling of *P*-

knipholone anthrone (**3**) with *P*- and *M*-knipholone (**1** and *ent*-**1**), respectively, possess good antimalarial activities and are thus potential lead compounds for antimalarial drug discovery.

Experimental Section

General: All reactions were carried out under a nitrogen atmosphere. 5% NaOH was degassed for 30 min prior to use. Melting points were measured on a Reichert–Jung Thermovar hot-plate and are uncorrected. UV/Vis spectra were obtained on a Cary 50 Conc spectrometer (Varian). IR spectra were recorded on a Jasco FTIR-410 spectrophotopolarimeter. For joziknipholones A and B, NMR spectroscopic experiments (in CD_2Cl_2) were performed on a Bruker Avance DMX 600 (600 MHz) instrument by using a 5 mm DCH cryoprobe head with z-gradient and ATM unit. In the case of 4-*O*-methyl eleutherol, the NMR spectra were recorded on a Bruker Avance DMX 500 (500 MHz). Chemical shifts (δ) are given in parts per million (ppm). HPLC separations were carried out by using an X-Terra RP_{18} column (Waters, 4.6×250 mm). Preparative HPLC was achieved on a Chromolith RP_{18} column (100×10 mm). For stereoanalytical separations, a chiral stationary phase employing a Chiralcel OD-H HPLC column (4.6×250 mm; particle size: 5 μm; Daicel Chemical Industries, Tokyo, Japan) was used. Optical rotations were taken on a JASCO P-1020 polarimeter. CD spectra were recorded in MeOH on a J-715 spectrometer (JASCO Deutschland, Gross-Umstadt, Germany) at room temperature by using a 0.05 cm standard cell. EIMS was carried out by using a direct inlet, 70 eV on a SSQ 710, Finnigan MAT spectrometer. Analytical TLC was performed on Merck precoated silica-gel 60 F_{254} plates. The R_f reported values refer to TLC. Column chromatography was achieved on oxalic acid-impregnated silica gel 60 (70–230 mesh) or on silica gel (0.063 mm, Merck).

Plant material: The roots of *Bulbine frutescens* were collected from Chiromo Campus Garden in June 2004. The plant was identified at the University Herbarium, Department of Chemistry, University of Nairobi, where a voucher specimen (SGM-AYT-2004–27) is deposited.

Extraction and isolation: Air-dried and powdered roots (200 g) were extracted with acetone (2×1 L) by cold percolation giving the crude extract (15 g), which was filtered over Sephadex LH-20 by using $CHCl_3$/MeOH (1:1) affording three fractions (I, II, and III). Fraction II (3.5 g) was subjected to column chromatography on silica gel (100 g), eluting first with $CHCl_3$ and then with increasing amounts of MeOH. This furnished 25 fractions, which were combined into ten major fractions (A to J) based on TLC analysis.

Fraction C (eluted with 1% MeOH in CH_2Cl_2) was purified by PTLC (CH_2Cl_2/*n*-hexane 2:3) to give 5,8-dihydroxy-1-methylnaphtho[2,3c]furan-4,9-dione (7 mg).

Fraction D (eluted with 2% MeOH in CH_2Cl_2) formed a deep-red precipitate of knipholone (**1**, 137 mg, identified by co-TLC with an authentic sample). Further purification of the mother liquor by PTLC ($CHCl_3$/EtOAc 9:1) gave 4-*O*-methyl eleutherol (**4**, 12 mg) and 5,8-dihydroxy-1-hydroxymethylnaphtho[2,3c]furan-4,9-dione (16 mg). Crystallization of fraction E (eluted with 3% MeOH in CH_2Cl_2) yielded further quantities of **1** (126 mg). Purification of the mother liquor by PTLC ($CHCl_3$/MeOH 15:1) gave isoknipholone (**2**, 5 mg).

Fraction F (eluted with 4% MeOH in CH_2Cl_2) was further separated by column chromatography on Sephadex LH-20 ($CHCl_3$/MeOH 1:1) followed by PTLC ($CHCl_3$/MeOH 15:1) giving **5** (13 mg) and **6** (8 mg). Fraction G (eluted with 5% MeOH in CH_2Cl_2) was similarly treated to yield 4′-*O*-demethylknipholone (129 mg).

Fraction H (eluted with 8% MeOH in CH_2Cl_2) was applied to Sephadex LH-20 ($CHCl_3$/MeOH 1:2) followed by PTLC ($CHCl_3$/EtOAc 1:1) furnishing a mixture of *p*-coumaric acid and dihydro-*p*-coumaric acid (9 mg). Fraction I (eluted with 10% MeOH in CH_2Cl_2) was similarly treated to give vanillic acid (4 mg), whereas fraction J (eluted with 15% MeOH in CH_2Cl_2) furnished 4′-*O*-demethylknipholone-4′-*O*-β-D-glucopyranoside (173 mg).

Joziknipholone A (5): Red amorphous powder; R_f=0.29 (CH_2Cl_2/MeOH 96:4); m.p.-85–88 °C (dec); $[\alpha]_D^{20}$=+380 (c=0.03 in CH_2Cl_2); UV/Vis (MeOH): λ_{max} (logε)=439 (3.74), 339 (3.96), 291 (4.41), 263 (4.33), 231 (4.55), 211 nm (4.61); CD (MeOH): $\Delta\varepsilon_{195}$=+25.4, $\Delta\varepsilon_{208}$=−50.1, $\Delta\varepsilon_{228}$=−5.9, $\Delta\varepsilon_{238}$=−14.5, $\Delta\varepsilon_{252}$=−4.3, $\Delta\varepsilon_{260}$=−5.5, $\Delta\varepsilon_{272}$=−0.5, $\Delta\varepsilon_{276}$=−1.7, $\Delta\varepsilon_{282}$=−0.3, $\Delta\varepsilon_{306}$=+39.2 $cm^2 mol^{-1}$; IR (KBr): $\tilde{\nu}$=3422, 2923, 2851, 1616, 1458, 1430, 1364, 1281, 1208, 1112 cm^{-1}; ^1H NMR (600 MHz, CD_2Cl_2) and ^{13}C NMR (150 MHz, CD_2Cl_2): see Table 1; EIMS: m/z (%): 853 (100) $[M+1]^+$, 835 (10), 784 (3), 687 (3), 589 (5), 491 (8), 393 (10), 295 (13); HRMS (TOF): m/z: calcd for $C_{48}H_{37}O_{15}$: 853.2132; found: 853.2130.

Joziknipholone B (6): Red-colored amorphous powder: R_f=0.34 (CH_2Cl_2/MeOH 96:4); m.p. 68–71 °C (dec); $[\alpha]_D^{20}$=+46 (c=0.03 in CH_2Cl_2); UV/Vis (MeOH): λ_{max} (logε)=439 (3.36), 351 (3.61), 291 (4.04), 267 (4.01), 219 nm (4.34); CD (MeOH): $\Delta\varepsilon_{195}$=+20.9, $\Delta\varepsilon_{212}$=−18.4, $\Delta\varepsilon_{228}$=+0.4, $\Delta\varepsilon_{238}$=−5.6, $\Delta\varepsilon_{262}$=+0.7, $\Delta\varepsilon_{282}$=−8.8, $\Delta\varepsilon_{306}$=+22.0 $cm^2 mol^{-1}$; IR (KBr): $\tilde{\nu}$=3427, 2925, 2853, 1617, 1459, 1377, 1281, 1208, 1112 cm^{-1}; ^1H NMR (600 MHz, CD_2Cl_2) and ^{13}C NMR (150 MHz, CD_2Cl_2): see Table 3; EIMS: m/z (%): 853 (100) $[M+1]^+$, 687 (20), 597 (8), 588 (28), 491 (35), 432 (14), 393 (46), 295 (39); HRMS (TOF): m/z: calcd for $C_{48}H_{37}O_{15}$: 853.2132; found: 853.2117.

4-*O*-Methyl eleutherol (4): Colorless amorphous powder, blue fluorescence in dichloromethane: R_f=0.73 ($CHCl_3$/EtOAc 9:1); m.p. 90–93 °C (dec) (lit.[8b] 124 °C, for synthetic material); $[\alpha]_D^{20}$=+16 (c=0.11 in $CHCl_3$) (lit.[8a] $[\alpha]_D$=+37 (c=0.94 in $CHCl_3$), lit.[8b] $[\alpha]_D^{20}$=+35.6 (c=0.77 in $CHCl_3$)); UV/Vis (MeOH): λ_{max} (logε)=249 (4.40), 271 (3.57), 302 (3.60), 313 (3.80), 328 (3.60), 357 nm (3.82); ^1H NMR (500 MHz, $CDCl_3$): δ=1.71 (d, J=6.5 Hz, 3H; $CH_3$3), 3.87 (s, 3H; OCH_3), 3.99 (s, 3H; OCH_3), 5.72 (q, J=6.5 Hz, 1H; H3), 6.95 (d, J=7.5 Hz, 1H; H6), 7.41 (t, J=8.0 Hz, 1H; H7), 7.54 (d, J=8.0 Hz, 1H; H8), 8.11 ppm (s, 1H; H9); ^{13}C NMR (125 MHz, $CDCl_3$): δ=20.1 ($CH_3$3), 56.2 ($OCH_3$5), 62.6 ($OCH_3$4), 77.0 (C3), 108.1 (C6), 122.6 (C8 or C9), 122.9 (C9 or C8), 123.1 (C4a), 125.1 (C3a), 127.2 (C7), 135.9 (C8a), 138.1 (C9a), 151.4 (C4), 156.1 (C5), 170.0 ppm (C1); EIMS: m/z (%): 258 (88) $[M]^+$, 243 (96) $[M-CH_3]^+$, 228 (7) $[M-2CH_3]^+$, 215 (100) $[M-CH_3-CO]^+$, 200 (4), 199 (8), 187 (31), 171 (21), 127 (22), 115 (25); EIMS: m/z: calcd for $C_{15}H_{14}O_4$: 258.0892; found: 258.0885.

Initial procedure for the reductive cleavage of joziknipholone A (5) to give knipholone (1): This reaction was carried out under atmospheric conditions as previously elaborated for the degradation of knipholone.[9] $Na_2S_2O_4$ (2.5 mg, 14.4 μmol) was added to a solution of **5** (3.3 mg, 3.87 μmol) in 5% NaOH (1 mL) and the reaction mixture was heated to 70–80 °C for 15 min. After this time, the reaction was quenched with 3% HCl and exhaustively extracted with EtOAc. Drying of the organic phase over $MgSO_4$ and removal of the solvent under reduced pressure followed by purification of the obtained crude material by column chromatography on silica gel eluted with petroleum ether/EtOAc (4:1; 3:2; 1:4) and finally CH_2Cl_2/MeOH (1:1) gave knipholone (**1**, 1 mg, 30%), chrysophanol, and acetylphloroglucinol. HPLC-CD analysis of **1**, in comparison to an authentic specimen, revealed the product to be *P*-configured (i.e., 90:10 *P*/*M*).

Optimized reductive cleavage of joziknipholone A (5) to give both knipholone (1) and its anthrone (3): Degassed 5% NaOH (1 mL) was added to a sample of **5** (2.2 mg, 2.58 μmol) and $Na_2S_2O_4$ (1.9 mg, 10.9 μmol) under nitrogen. The mixture was stirred at 40–50 °C (by using a preheated oil bath) for 5 min and the reaction was immediately quenched with 3% HCl, still under an N_2 atmosphere. The resulting solution was extracted with EtOAc and the dried ($MgSO_4$). The organic phase was removed under reduced pressure. A solution of the residue in MeOH was purified by analytical HPLC by using an X-Terra RP_{18} column (4.6×250 mm, 5 μm) employing the following gradient: H_2O (A)/CH_3CN (C): 0 min 40% C, 10 min 40% C, 20 min 100% C, 30 min 100% C, 32 min 40% C, 40 min 40% C, at a flow rate of 1 mL min^{-1}. This yielded pure knipholone anthrone (**3**, 0.1 mg, 9%, t_R=22 min). Knipholone (**1**) coeluted with chrysophanol at t_R=20 min; further resolution of this fraction was thus achieved by conventional column chromatography on silica gel

eluted with CH_2Cl_2 followed by CH_2Cl_2/MeOH (96:4) giving knipholone (**1**, 0.1 mg, 9%). Both, knipholone anthrone (**3**) and knipholone (**1**) were identical with authentic samples (co-TLC and co-HPLC). Analysis of the products by offline CD measurements and subsequent comparison of the obtained spectra with those of authentic samples (Supporting Information, Figure S4) revealed knipholone anthrone and knipholone to be *P*-configured at the axes.

Reductive cleavage of joziknipholone B (6): Compound **6** (2.2 mg, 2.58 μmol) was subjected to the same optimized reductive cleavage conditions. Subsequent purification gave knipholone anthrone (**3**, 0.20 mg, 19%) and knipholone (**1**, 0.23 mg, 21%). Their configurations were determined to be *P* and *M*, respectively, as deduced from offline CD measurements and subsequent comparison with CD spectra of authentic samples (Supporting Information, Figure S5).

Biological experiments: Antiprotozoal activities against *P. falciparum* (K1 strain), *T. cruzi*, *T. brucei rhodesiense*, *L. donovani*, and cytotoxicities (against rat skeletal myoblast L-6 cells), as well as antitumor activities (against murine leukemic lymphoma L5178y cells) were performed as described earlier.[23,24]

Computational: The conformational analysis of joziknipholone A (**5**) was performed by using the semiempirical AM1[14] method and the DFT-based RI-BLYP/SVP[15,16] approach within the Gaussian 03[25] and Turbomole[26] program packages, respectively. The single-point BLYP/SVP calculations of the two global minima of **5** in the presence of a solvent (CH_2Cl_2) were done by applying a polarizable continuum (PCM) model[27] as implemented in Gaussian 03.

For calculations of the rotational barrier of the central C7–C10′ bond in **5**, the transition state structures of the model compound were located and optimized by using the STQN[28] method at the BLYP/3–21G[14,29] level. The transition states and minimum isomers were verified by frequency calculations, which were also used to get zero-point corrected energies (zero-point vibrational energies were scaled by a factor of 0.9945[17]).

The CD and UV spectra of **5** were calculated by a time-dependent DFT approach by using the BLYP functional and a TZVP[19] basis set. The oscillator and rotatory strengths were computed by using the dipole-velocity formalism.[30] The overall CD and UV curves were simulated as sums of Gaussian functions centered at the wavelengths of the corresponding electronic transitions and multiplied by the respective overall oscillator or rotatory strengths—transformed into absorption and ε values, respectively.

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