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Cyclombandakamines A_1 and A_2, Oxygen-Bridged Naphthylisoquinoline Dimers from a Congolese *Ancistrocladus* Liana

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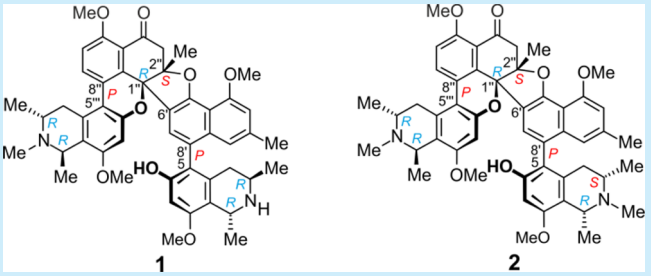
Supporting Information

ABSTRACT: Cyclombandakamines A_1 (**1**) and A_2 (**2**), both with an unprecedented pyrane−cyclohexenone−dihydrofuran sequence and six stereocenters and two chiral axes, are the first oxygen-bridged dimeric naphthylisoquinoline alkaloids. They were isolated from the leaves of an as yet unidentified Congolese *Ancistrocladus* species. Their stereostructures were established by spectroscopic, chemical, and chiroptical methods in combination with DFT and TDDFT calculations. They apparently originate from a cascade of oxidative cyclization reactions of "open-chain" naphthylisoquinoline dimers and exhibit significant antiprotozoal activities.



Naphthylisoquinoline alkaloids[1] from tropical Ancistrocladaceae and Dioncophyllaceae lianas are the first known tetrahydroisoquinoline natural products of polyketidic origin.[2] They consist of a naphthalene and an isoquinoline subunit connected by a biaryl axis, which is—in most cases—rotationally hindered. Their broad structural variety is further enlarged by the occurrence of dimeric representatives, with up to four stereogenic centers and three chiral axes.[3,4] Depending on the individual structures, some of them possess pronounced antiprotozoal and antiviral activities.[3−5]

From the leaves of a botanically as yet unidentified *Ancistrocladus* species growing in the northwestern part of the Democratic Republic of the Congo near the town of Mbandaka,[6] we have recently isolated two structurally remarkable antiplasmodial dimers, mbandakamines A (**3a**) and B (**3b**) (Figure 1).[4] They are the first 6′,1″-coupled naphthylisoquinoline dimers, thus possessing a constitutionally highly unsymmetric central biaryl axis, located in the *peri* position neighboring one of the outer axes. This close proximity of two bulky aryl substituents renders **3a** and **3b** the sterically most crowded naphthylisoquinoline alkaloids found to date in nature, making the search for further novel-type metabolites in this phytochemically so productive Congolese *Ancistrocladus* species a rewarding task.

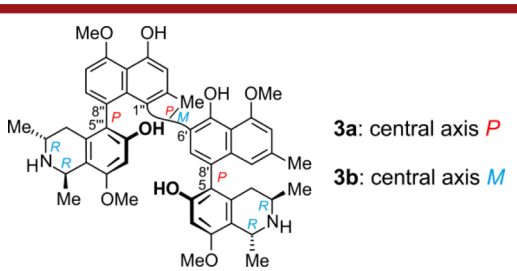


Figure 1. Structures of mbandakamines A (**3a**) and B (**3b**).

In this paper, we report on the isolation and structural elucidation of two novel dimeric naphthylisoquinoline alkaloids, cyclombandakamines A_1 (**1**) and A_2 (**2**), which feature, for the first time, oxygen bridges that link the "northwestern" naphthalene moiety to both the adjacent isoquinoline portion and the other "southeastern" naphthalene part, resulting in eight condensed rings. Compared with **3a** and **3b**, they possess only two chiral biaryl axes but have two additional stereogenic centers. Furthermore, we describe their anti-infective activities and discuss their presumable biosynthetic origin.

LC−MS investigations on further fractions obtained during the isolation of **3a** and **3b** hinted at the presence of another, minor dimeric alkaloid with a mass 12 mass units greater than that of **3a** and **3b** and a slightly different UV curve, especially in the range from 280 to 400 nm (Figure S1). Preparative HPLC on a reversed-phase column provided a beige amorphous powder. According to HR-ESI-MS (m/z 797.3783, $[M + 1]^+$), it had a molecular formula of $C_{49}H_{52}N_2O_8$, suggesting that it might be a

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Org. Lett. XXXX, XXX, XXX−XXX

dehydro derivative of **3a** or **3b** with an additional *N*- or *O*-methyl group.

Its ^{1}H NMR spectrum displayed a full set of signals indicative of an unsymmetric dimer, with four methoxy groups, as in **3a**/**b**, the additional methyl being attached to one of the nitrogen atoms (Table S1). In the aromatic region, only seven protons were observed, instead of eight or 10 as usual,[3,4] hinting at a novel-type structure. Likewise unprecedented was the presence of three (instead of two) methylene groups with diastereotopic protons and the observation of a quaternary carbon at δ_C 196.0 in the ^{13}C NMR spectrum, indicative of a tetralone carbonyl carbon.[7]

Similar to **3a**/**b**, the southeastern part of **1**, denoted as **1-A** (Figure 2, right) showed NMR signals typical of a 5,8′-coupled

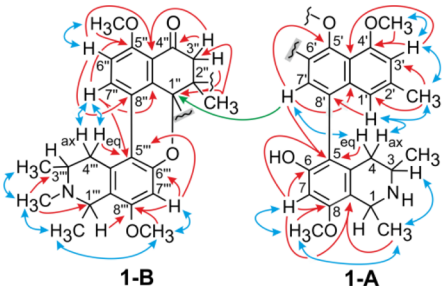

Figure 2. Key ROESY (double arrows) and HMBC (single arrows) interactions of **1** indicative of the constitutions of the southeastern moiety (**1-A**) and the northwestern part (**1-B**), including the key HMBC correlation between them (in green) that evidences their site of connection.

monomeric naphthylisoquinoline, with the other (northwestern) part, **1-B**, linked via C-6′.[4] The pattern included a *meta* coupling of the two aromatic protons, H-1′ [δ_H 6.51 (1H, *pt*, J = 1.1 Hz)] and H-3′ [δ_H 6.80 (1H, d, J = 1.2 Hz)], sequential ROESY interactions {H-7′ [δ_H 6.38 (1H, s)]−H-4$_{eq}$ [δ_H 2.33 (1H, dd, J = 17.9, 4.9 Hz)]−H-4$_{ax}$ [δ_H 1.94 (1H, dd, J = 17.9, 11.4 Hz)]−H-1′−CH$_3$-2′ [δ_H 2.29 (3H, *pd*, J = 0.3 Hz)]−H-3′−OCH$_3$-4′ [δ_H 4.00 (3H, s)]}, and HMBC correlations from H-7 [δ_H 6.41 (1H, s)], H-4$_{eq}$, and H-7′ to C-5 (δ_C 119.7). An HMBC interaction of H-7′ (colored in green in Figure 2) to a quaternary carbon (δ_C 85.3) belonging to **1-B** established C-6′ (δ_C 121.0) as the connection site of **1-A** to **1-B**.

The northwestern part **1-B** (Figure 2, left) is spectroscopically more complex. It possesses an *N*-methylated isoquinoline portion with a methoxy group [δ_H 3.72 (3H, s)] at C-8‴ (δ_C 158.0). This was evidenced by a series of ROESY correlations {CH$_3$-3‴ [δ_H 0.93 (3H, d, J = 6.7 Hz)]−CH$_3$-*N* [δ_H 2.92 (3H, s)]−CH$_3$-1‴ [δ_H 1.73 (3H, d, J = 6.6 Hz)]−OCH$_3$-8‴−H-7‴ [δ_H 6.47 (1H, s)]} together with HMBC correlations from CH$_3$-*N* to C-1‴ (δ_C 57.9) and C-3‴ (δ_C 55.1) and from H-1‴, H-7‴, and OCH$_3$-8‴ jointly to C-8‴.

An AB spin system {H-7″ [δ_H 8.04 (1H, d, J = 9.0 Hz)], H-6″ [δ_H 7.35 (1H, d, J = 9.0 Hz)]}, ROESY interactions of H-6″ with OCH$_3$-5″ [δ_H 3.97 (3H, s)], and HMBC correlations from H-7″ to C-5″ (δ_C 160.6) and C-9″ (δ_C 138.3), from OCH$_3$-5″ to C-5″, and from H-6″ to C-10″ (δ_C 119.4) proved the position of OCH$_3$-5″ and thus evidenced the aforementioned carbonyl group to be at C-4″ (Figure 2, **1-B**). This assignment was corroborated by the low-field shifts of the diastereotopic protons [δ_H 3.12 (1H, dd, J = 0.7, 14.6 Hz) and δ_H 3.03 (1H, d, J = 14.6 Hz)] at C-3″, by their ROESY interactions with CH$_3$-2″ [δ_H 1.85 (3H, *pd*, J = 0.3 Hz)], and by their HMBC correlations with CH$_3$-2″, C-4″, and C-10″. From the HMBC correlations from H-7″, H-7‴, and H-4$_{eq}$‴ [δ_H 3.06 (1H, dd, J = 17.1, 4.51 Hz)] to C-5‴, the northwestern half **1-B** was found to be 5‴,8‴-coupled, like the southeastern part **1-A** (Figure 2). Joint HMBC correlations from H-7′, CH$_3$-2″, and both protons at C-3″ to a quaternary carbon (δ_C 85.3) proved this C atom to be C-1″ (Figure 2), hence indicating that the two molecular portions **1-A** and **1-B** are coupled through C-6′ and C-1″, respectively, as in **3a**/**b**.[4]

In contrast to **3a** and **3b** and all other known naphthylisoquinoline alkaloids, ROE interactions between H-7″ and both diastereotopic protons, H-4$_{eq}$‴ (for the NMR data, see Table S1) and H-4$_{ax}$‴ [δ_H 3.84 (1H, dd, J = 17.1, 5.4 Hz)], were observed (Figure 2, **1-B**), suggesting that the two moieties in that northwestern half are not orthogonal but much closer to one another than usual, firmly pressed against each other, apparently by being part of a tight ring. This is due to an ether bond between O-6‴ and C-1″, which furthermore explains the observed downfield shift of this carbon (δ_C 85.3). In a similar way, the chemical shift of C-2″ (δ_C 92.3) indicated that this atom is involved in an ether bond with the spatially close oxygen, O-5′, like the above-mentioned linkage between C-1″ and O-6‴, thus establishing the northern part of **1-B** to be a tetralone subunit. Hence, the isolated dimer was found to be the first cyclized (since twofold-oxygen-bridged) *N*-methyl analogue of **3a** and **3b**. It was therefore named cyclombandakamine A$_1$.

This novel-type naphthylisoquinoline dimer is also stereochemically thrilling: it possesses not only the usual two stereocenters in each of the isoquinoline moieties and one stereogenic axis in the southeastern half but also, for the first time, two additional stereocenters in the tetralone moiety. Furthermore, the *C,C* axis linking the two northwestern bicyclic aryls (C-5‴ to C-8″) remains a further potential stereogenic element.

The relative configurations at the stereocenters in the two isoquinoline moieties were determined to be *trans* from ROESY interactions between CH$_3$-1 and H-3, between CH$_3$-1‴ and H-3‴ (Figure 3), and between CH$_3$-3‴ and H-1‴. The latter, quite

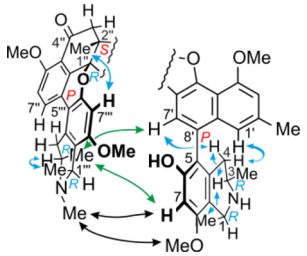

Figure 3. Key ROESY interactions indicating the relative and (given the results of oxidative degradation for C-3 and C-3‴) absolute configurations of **1**. The blue arrows concern the correlations within the molecular halves and the black and green ones the interactions between them.

unusual interaction hinted at the presence of a second, less common conformation in the western isoquinoline part, presumably as a consequence of the repulsive van der Waals interactions of the tightly pressed H-4‴ and H-7″. Ruthenium-mediated oxidative degradation[8] established the absolute configuration at both C-3 and C-3‴ to be *R*, which, with respect to the above-assigned relative *trans* configuration, evidenced *R* configurations at C-1 and C-1‴ too. From the ROESY interactions of H-4$_{ax}$ with H-1′ and of H-4$_{eq}$ with H-7′ (Figure 3) and on the basis of the above-established absolute *R*

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Org. Lett. XXXX, XXX, XXX−XXX

configurations at C-3 and C-1, the axis in the southeastern naphthylisoquinoline half, **1-A**, was determined to be *P*-configured, as in **3a**/**b**.

In the tetralone subunit, the linkage of O-6‴ with C-1″ sterically fixes the western axis, making its absolute configuration and that of C-1″ mutually dependent: a *P* configuration at the western biaryl axis and *R* at C-1″ entailing each other, like *M* implying *S*. This phenomenon reduced the number of remaining possible stereoisomers from eight (as expected for three independent stereogenic elements) to only four, two of them with a *cis* array of the dihydrofuran−cyclohexenone system and two with a *trans* junction, which, however, would be severely strained (Figure S2a). Indeed, the ROE interaction between CH_3-2″ and H-7‴ (Figures 3 and S2) indicated the dihydrofuran and cyclohexenone rings to be *cis*-fused, thus further reducing the number of possible diastereomers to only two, with 1″*R*,2″*S*,8″*P* and 1″*S*,2″*R*,8″*M* configurations (Figure S2b).

These two remaining isomers were distinguished by the ROESY interactions observed between CH_3-3‴ and H-7′ and between CH_3-3‴ and H-7 (Figure 3, green arrows), which showed that C-1″, C-2″, and the C-8″−C-5‴axis are *R*-, *S*-, and *P*-configured, respectively. In the other possible diastereomer, with the 1″*S*,2″*R*,8″*M* configuration, CH_3-3‴ would point in the opposite direction (Figure S3) and thus would be far away from H-7′ and H-7, so that no spatial interactions between these nuclei would be expected. Consequently, cyclombandakamine A_1 (**1**) is 1*R*,3*R*,5*P*,1″*R*,2″*S*,8″*P*,1‴*R*,3‴*R*-configured, i.e., with the full absolute stereostructure presented in the abstract graphic.

To further verify our experimental assignment, both 1″*R*,2″*S*,8″*P*- and 1″*S*,2″*R*,8″*M*-configured diastereomers were subjected to a B97-D3/def2-TZVP-based conformational analysis. From the resulting lowest-energy conformers, one for each diastereomer was chosen. The experimentally observed ROE interactions matched well only with the ones predicted for the optimized structure of the 1″*R*,2″*S*,8″*P* isomer. Moreover, the electronic CD (ECD) spectrum calculated for the 1″*R*,2″*S*,8″*P* diastereomer at the TDCAM-B3LYP/def2-SVP level showed good agreement with the experimental spectrum of the isolated product (Figure 4, left), while that of the

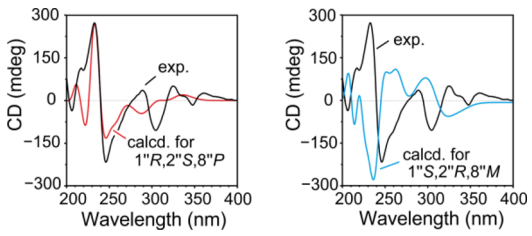

Figure 4. Comparison of the experimental ECD spectrum of **1** with the ones calculated for (left) 1″*R*,2″*S*,8″*P*- and (right) 1″*S*,2″*R*,8″*M*-configured isomers.

1″*S*,2″*R*,8″*M* diastereomer was virtually opposite (Figure 4, right). This confirmed the above-established full absolute stereostructure of **1** and indicated that its CD curve is largely dominated by the tetralone chromophore (see the Supporting Information).

Investigations of fresh, genetically identical plant material collected at the same site resulted in the isolation of a second, similar compound. It had a molecular formula of $C_{50}H_{55}N_2O_8$, as evidenced by HR-ESI-MS (*m*/*z* 811.3952, $[M + 1]^+$), thus showing a molecular weight 14 units greater than that of **1**. Its ^1H and ^{13}C spectra revealed close similarities to those of **1**, including the presence of seven aromatic protons and a carbonyl function, but with one additional CH_3 group (δ_H 2.96 and δ_H 41.5). The 1D and 2D NMR data (Figure 5a and Table S2) proved that this methyl group is linked to the nitrogen of the southeastern naphthylisoquinoline half. This is the only constitutional difference compared with **1**.

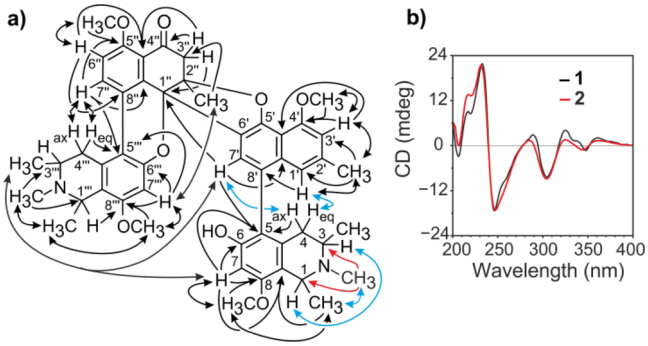

Figure 5. (a) ROESY (double arrows) and HMBC (single arrows) interactions of cyclombandakamine A_2 (**2**) relevant for its constitution. Colored correlations (in red and blue) highlight differences in the eastern part of **2** compared with that of **1**. (b) Comparison of the ECD spectra of **1** and **2**.

Stereochemical differences between the two alkaloids were found in the southeastern half of the second dimer, too (Figure 5a). In this part, the proton at C-3, which is axial, as is obvious from the coupling constants of H-4_{ax} [δ_H 2.31, (1H, dd, *J* = 17.5, 11.9 Hz)] and H-4_{eq} [δ_H 1.97 (1H, dd, *J* = 17.5, 2.9 Hz)], showed a ROESY interaction with H-1, thus indicating a *cis* configuration at C-1 versus C-3 instead of *trans* as in the respective part of **1**. In addition, this alkaloid displayed ROESY interactions between H-4_{ax} and H-7′ and between H-4_{eq} and H-1′ (Figure 5a), opposite to the ones observed for **1-A** (Figure 2). In the northwestern half, the relative configurations of all stereogenic elements were identical to those in **1-B**, i.e., *trans* configuration for both C-3‴ versus C-1‴ and C-1″ relative to C-2″. The oxidative degradation provided a ca. 1:1 ratio of *R*- and *S*-configured *N*-methyl-3-aminobutyric acid as a consequence of the opposite configurations at C-3 and C-3‴, leaving open the question of whether the molecule is 3*R*,3‴*S*- or 3*S*,3‴*R*-configured. Considering the aforementioned relative configurations of all stereogenic elements and the ROESY interactions observed between its two halves, among them H-7 with CH_3-3‴ and H-7′ with CH_3-3‴ (Table S2 and Figure 5a), a 3*R*,3‴*S* configuration would lead to the overall 1*S*,3*R*,5*M*,1″*S*,2″*R*,8″*M*,1‴*S*,3‴*S* configuration, while 3*S*,3‴*R* would give a total 1*R*,3*S*,5*P*,1″*R*,2″*S*,8″*P*,1‴*R*,3‴*R* configuration, i.e., the enantiomer. The close similarity of the ECD curves of the second compound with that of **1** (Figure 5b) established that the new alkaloid possesses the absolute stereostructure **2**, as shown in the abstract graphic, i.e., with the 1*R*,3*S*,5*P*,1″*R*,2″*S*,8″*P*,1‴*R*,3‴*R* configuration. It was henceforth named cyclombandakamine A_2.

Cyclombandakamines A_1 (**1**) and A_2 (**2**) are the first examples of (twofold) *O*-bridged naphthylisoquinoline dimers. As outlined in Scheme 1, the direct biogenetic precursor of **1** might be an *N*-methylated derivative of **3a**/**b**, like compound **8**. This dimer should arise from three oxidative coupling steps, viz., those linking **4** and **5** to give the two monomers **6** and **7** (blue axes), differing only in their *O*- and *N*-methylation patterns, and one oxidative cross-coupling of **6** and **7** to yield the mbandakamine analogue **8** (red axis). Compound **8** could then be further

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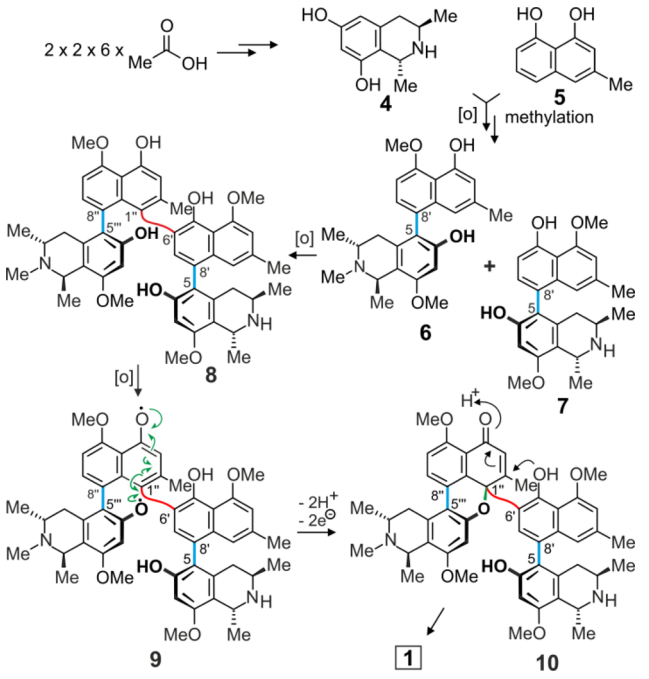
Scheme 1. Proposed Biosynthetic Pathway of 1



oxidized to give enone **10** through another phenol-oxidative activation via phenoxy radical **9**, now leading to an intramolecular C−O coupling (green C−O bond). In this step, a *P* configuration at the northwestern axis would create the observed *R* configuration at C-1″. Subsequent intramolecular Michael addition of the phenolic oxygen function at C-5′ (inducing the observed *cis* array and hence the *S* configuration at C-2″) would then give **1**. Thus, cyclombandakamine A_1 (**1**) would arise from four consecutive oxidative coupling steps (forming the bonds highlighted in blue, red, and green)—the highest total number for a naphthylisoquinoline alkaloid to date. In a similar manner, **2** might originate from a doubly *N*-methylated 3-*epi*-derivative of **3a**.

This thrilling cyclization behavior, which has never been observed before in naphthylisoquinoline alkaloids,[9,10] is certainly a consequence of the highly condensed structure of the mbandakamines, in which the phenolic parts are tightly pressed against each other, thus facilitating the domino-type reaction sequence depicted in Scheme 1.

Cyclombandakamines A_1 (**1**) and A_2 (**2**) displayed significant inhibitory activities in vitro against *Plasmodium falciparum* NF54, with IC_{50} values of 0.043 and 0.055 μM, respectively. In addition, **2** was very active against *Trypanosoma brucei rhodesiense*, with an IC_{50} of 0.01 μM (selectivity index >1000), making it a promising candidate for further investigations. Compound **1**, by contrast, was much less active (IC_{50} = 1.09 μM; Table S3).

In summary, cyclombandakamines A_1 (**1**) and A_2 (**2**) are the first oxygen-bridged naphthylisoquinoline dimers, and the first such compounds to possess eight stereogenic elements (six centers and two axes, one of them being thermodynamically dictated). Their unprecedented structures will trigger more-in-depth biosynthetic, synthetic, and pharmaceutical investigations. This 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.7b00209.

Experimental details and additional data (PDF)

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

Dedicated to Professor Dr. Dr. h.c. Lutz F. Tietze, University of Göttingen, on the occasion of his 75th birthday.

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(10) For (non-natural) overoxidized products of monomeric naphthylisoquinoline alkaloids, see ref 7.

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