Pergamon

Tetrahedron 55 (1999) 7563–7572

TETRAHEDRON

Elucidation of the Absolute Configuration of Knipholone and Knipholone Anthrone by Quantum Chemical CD Calculations

Gerhard Bringmann*, Jürgen Kraus, Dirk Menche, and Kim Messer

Institut für Organische Chemie, Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany

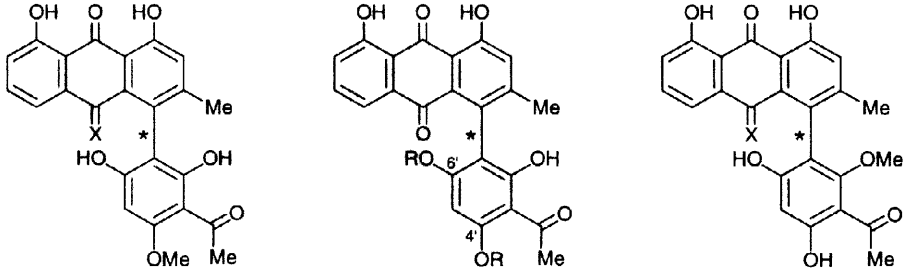
Received 2 April 1999; accepted 28 April 1999

Abstract: The absolute configuration of knipholone, an axially chiral phenylanthraquinone from *Kniphofia*, *Bulbine*, and *Bulbinella* species, has been established as *M* by quantum chemical CD calculations. For further evidence, the absolute stereostructure of the related compound knipholone anthrone, which has a significantly different chromophor, was likewise elucidated. The fact that both natural biaryls compounds were independently attributed the same absolute configuration, underlines the reliability of the results and the value of the applied method.

Keywords: Knipholone (anthrone); axial chirality; circular dichroism; quantum chemical calculations

INTRODUCTION

The phenylanthraquinone knipholone (**1**) (see Fig. 1) was first isolated by Dagne and Steglich in 1984,[1] as the main constituent of the roots of *Kniphofia foliosa* (Asphodelaceae), a widespread plant in the mountained regions of Central and Northern Ethiopia. Meanwhile, **1** has been found in numerous species of the genera *Bulbine*, *Bulbinella*, and *Kniphofia*.[2] Besides the major component knipholone (**1**)[1,3–5] and knipholone anthrone (**2**),[6] a set of similar compounds (see Fig. 1), *e.g.* 6'-*O*-methylknipholone (**3**),[3] 4'-*O*-demethylknipholone (**4**),[7] isoknipholone (**5**),[3,8] and its anthrone **6**,[8] were isolated from the same plants. All these compounds consist of an anthraquinone moiety, named chrysophanol (or its anthrone), and a smaller - but apparently likewise acetogenic - *O*-methylated acetylphloroglucinol part. Only little[4] is known about the biological activities of these constitutionally unsymmetric natural biaryls,[9] although the corresponding plants are widely used in folk



1 (X = O)
2 (X = H_2)

3 (R = Me)
4 (R = H)

5 (X = O)
6 (X = H_2)

Fig. 1. Knipholone (**1**), knipholone anthrone (**2**), and related natural biaryls **3** - **6** (* : configurationally stable axis).

*To whom correspondence should be addressed. FAX: +49 931 888 4755. E-mail: bringman@chemie.uni-wuerzburg.de

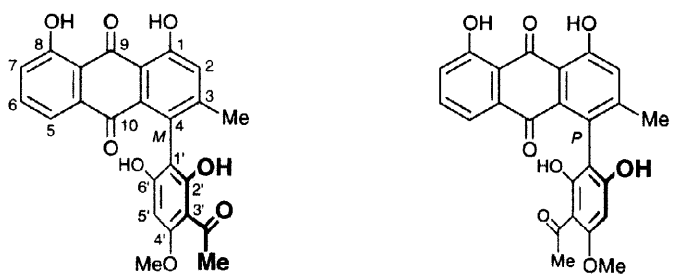
0040-4020/99/$ - see front matter
PII: S0040-4020(99)00391-9

medicine.[1,10] Very recently, knipholone (**1**) and some related compounds (**2** and **3**) have been found to exhibit considerable antimalarial *in vitro* activity against asexual erythrocytic stages of two strains of *Plasmodium falciparum*, with only little cytotoxicity.[11]

Knipholone (**1**) is optically active and thus chiral, due to a rotationally hindered, configurationally stable biaryl axis, but its absolute configuration is as yet unknown. For further studies, in particular for structure-activity relationship investigations and for the design of a directed, *i.e.* enantioselective first total synthesis, the unambiguous knowledge of the absolute stereostructure is essential. In this paper, we describe the elucidation of the absolute configurations of knipholone (**1**) and knipholone anthrone (**2**), by quantum chemical calculation of their circular dichroism (CD) spectra and comparison with the experimental ones, an efficient method recently improved by our group.[12–16]

RESULTS AND DISCUSSION

Natural knipholone (**1**) might in principle be *M*-configured and thus be represented by stereostructure **1a** (Fig. 2) or *P*-configured as in **1b**.

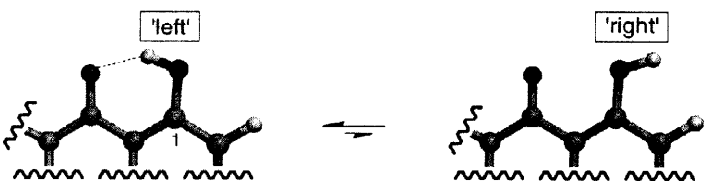


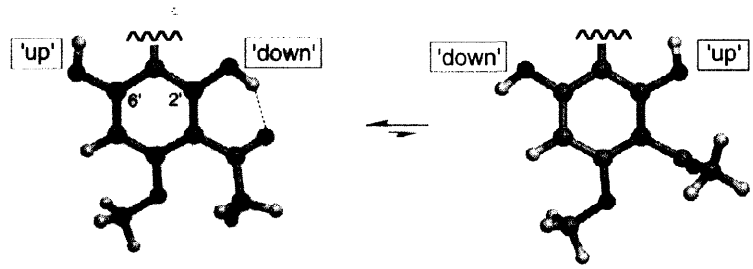
1a **1b**

Fig. 2. The two possible enantiomers of **1**, *M*-knipholone (**1a**) and *P*-knipholone (**1b**).

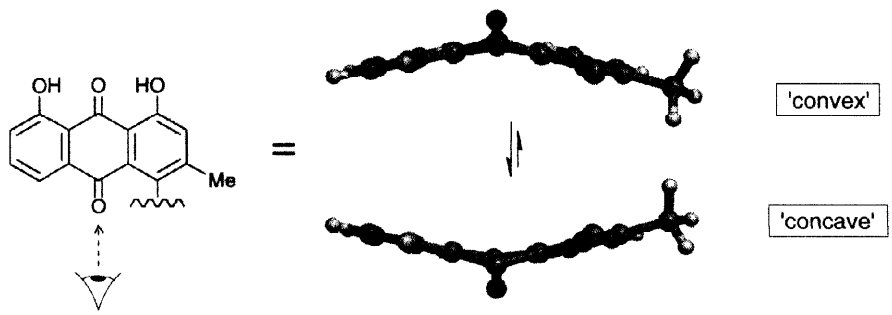
The CD calculations were arbitrarily started for the *M*-enantiomer, **1a**. For an exact knowledge of the conformational species relevant for its CD behavior, a comprehensive conformational analysis was performed using the semiempirical PM3 method, which revealed the following flexible parts of the molecule:

- All four hydroxy functions (at C-1, C-8, C-2', and C-6') were found to have two possible alignments each differing in their dihedral angles by approximately 180°. Orientations in which the hydrogen is directed towards a carbonyl group (left structures, exemplarily for the 1- and 2'-OH group), are energetically preferred.

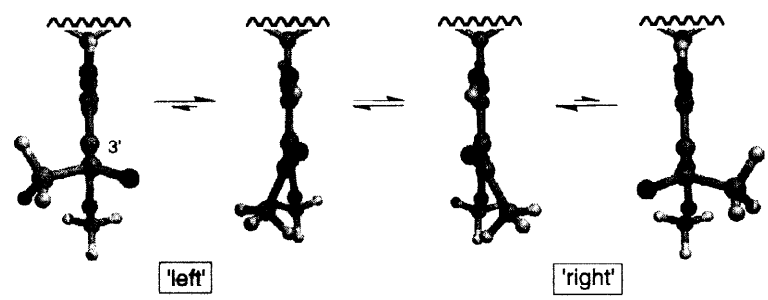




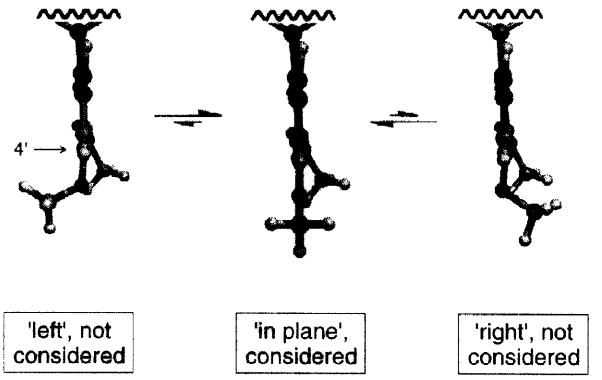
- The anthraquinone ring system has a convex or a (slightly preferred) concave curvature.



- The acetyl group at C-3' is twisted out of the phenyl plane in both possible directions up to a perpendicular position if no hydrogen bond to the acetyl oxygen is present (see the two outer structures).



- The 4'-methoxy group shows three principal orientations, but to keep the sum of relevant conformers to be calculated in an adequate scope, only those conformers were considered for further calculations in which the substituent at the 4'-position lies in the plane of the acetylphloroglucinol moiety. Conformers with a nearly perpendicular left or right alignment of the 4'-methoxy group were neglected, because they show higher heats of formation and the position of the methoxy group has no significant influence on the theoretical CD spectrum.



All of the six chiroptically relevant flexible parts (the four OH groups, the anthraquinone ring, and the acetyl moiety) were found to be independent of each other, resulting in $2^6 = 64$ possible input geometries, which were used for further semiempirical optimizations. For the calculation of the chiroptical properties, only those 12 conformers whose energies are no higher than 3 kcal/mol compared to the global minimum (see Table 1), were chosen. Two of these conformers are shown in Fig. 3.

Of these 12 energetically favored conformers, single CD spectra were calculated and added up following the Boltzmann statistic, *i.e.* according to their heats of formation. Subsequent 'UV correction'[12] led to the calculated overall CD spectrum for *M*-knipholone (**1a**), which, apart from the absence of the band at 280 nm, shows a very good agreement with the experimental one for (+)-knipholone as isolated from *Bulbine frutescens* (see Fig. 4, left), whereas the spectrum calculated for *P*-knipholone (**1b**), as obtained by reflection of the

Table 1. Selected parameters of all conformers used for the calculation of the circular dichroism of **1a**. Heats of formation ΔH_f [kcal/mol], relative heats of formation $\Delta\Delta H_f$ [kcal/mol], the curvature of the anthraquinone ring, and the orientation of the acetyl group at C-3' and the hydroxy functions at C-6' and C-2' (for a definition of these stereo parameters, see the beginning of the RESULTS AND DISCUSSION section). In all of the conformers listed below, both of the OH groups of the anthraquinone ring show hydrogen bonding to the carbonyl oxygen (*i.e.* 'left' for 1-OH and 'right' for 8-OH)

conformer	ΔH_f	$\Delta\Delta H_f$	curvature	3'-COMe	6'-OH	2'-OH
1a-*conf1*	-254.924	≡ 0.000	concave	left	up	down
1a-*conf2*	-254.851	0.073	concave	right	up	down
1a-*conf3*	-254.116	0.808	convex	left	up	down
1a-*conf4*	-253.757	1.167	convex	right	up	down
1a-*conf5*	-253.675	1.249	convex	left	up	up
1a-*conf6*	-253.589	1.335	concave	left	up	up
1a-*conf7*	-253.474	1.450	concave	right	up	up
1a-*conf8*	-252.924	2.000	convex	right	up	up
1a-*conf9*	-252.563	2.361	concave	left	down	down
1a-*conf10*	-252.539	2.385	concave	right	down	down
1a-*conf11*	-252.405	2.519	convex	left	down	up
1a-*conf12*	-252.260	2.664	convex	left	down	down

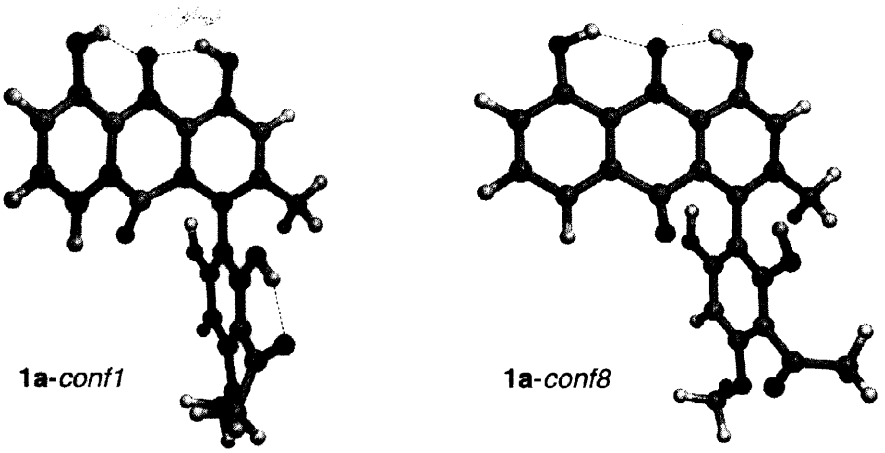


Fig. 3. 3D structures of two selected calculated conformers of **1a** (see also Table 1); hydrogen bonds indicated by dotted lines.

calculated overall spectrum of *M*-knipholone at the zero line, is nearly opposite to the experimental one (see Fig. 4, right). From this, the structure of natural (+)-knipholone can unambiguously be assigned as **1a**, *i.e.* with *M*-configuration.

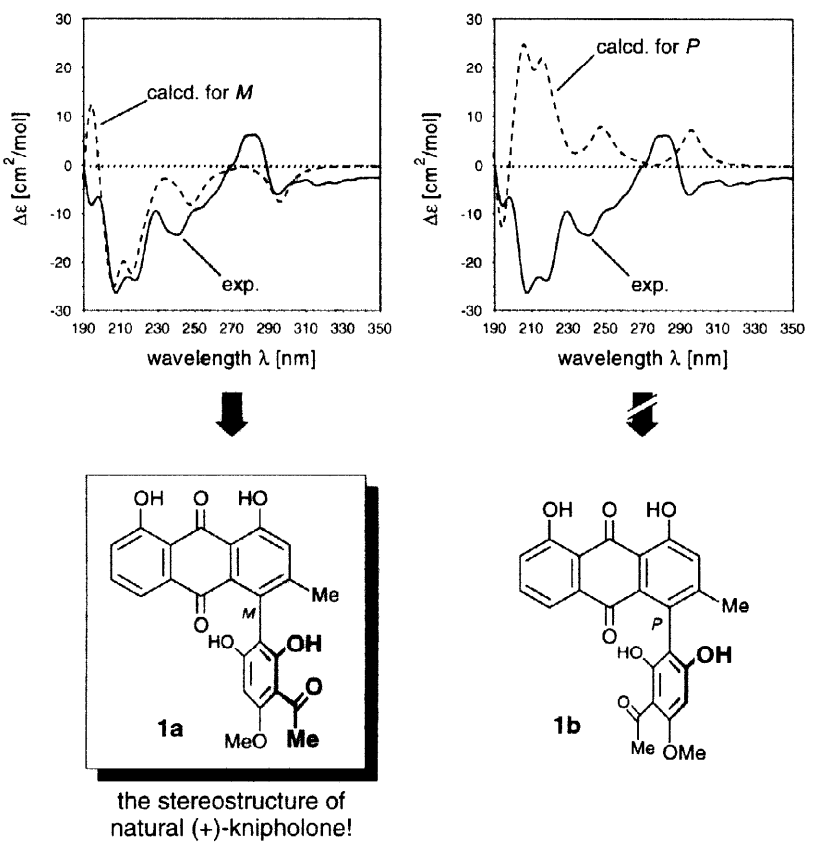


Fig. 4. Attribution of the absolute configuration of (+)-knipholone (**1a**).

To get further evidence for this result, the absolute configuration of the reduced analog of **1a**, knipholone anthrone (**2**) (see Fig. 1) was separately determined in a similar but independent way. This natural product, first isolated in 1993 from the stems of *Kniphofia foliosa*,[6] was available by reduction of **1a** and was found to be chiroptically identical with a sample kindly provided by E. Dagne and B. M. Abegaz from previous isolation work.[6] Due to the close structural relationship of **1** and **2**, the 64 input geometries of knipholone, again arbitrarily starting with the *M*-enantiomer **2a**, were modified by replacing the oxygen atom at C-10 (see Fig. 2) by two hydrogen atoms. The species thus generated were then used as input geometries for the further semiempirical conformational analysis of knipholone anthrone (**2**). For **2**, in contrast to knipholone, the optimization of some of the input geometries led to identical (and thus fewer) joint minimum structures for **2**, so that only six conformers (see Table 2) were detected for knipholone anthrone within the energetic cut-off of 3 kcal/mol.

Table 2. Selected parameters of all conformers used for the calculation of the circular dichroism of **2a**. Heats of formation ΔH_f [kcal/mol], relative heats of formation $\Delta\Delta H_f$ [kcal/mol], the orientation of the acetyl group at C-3', and the hydroxy functions at C-6' and C-2' (for a definition of these stereo parameters, see the beginning of the RESULTS AND DISCUSSION section). In all of the conformers listed below, both of the OH groups of the anthraquinone ring show hydrogen bonding to the carbonyl oxygen (*i.e.* 'left' for 1-OH and 'right' for 8-OH)

conformer	ΔH_f	$\Delta\Delta H_f$	3'-COMe	6'-OH	2'-OH
2a-*conf1*	-234.592	≡ 0.000	right	up	down
2a-*conf2*	-234.561	0.031	right	up	up
2a-*conf3*	-234.524	0.068	left	up	down
2a-*conf4*	-234.425	0.167	left	up	up
2a-*conf5*	-232.259	2.333	right	down	up
2a-*conf6*	-232.151	2.441	left	down	up

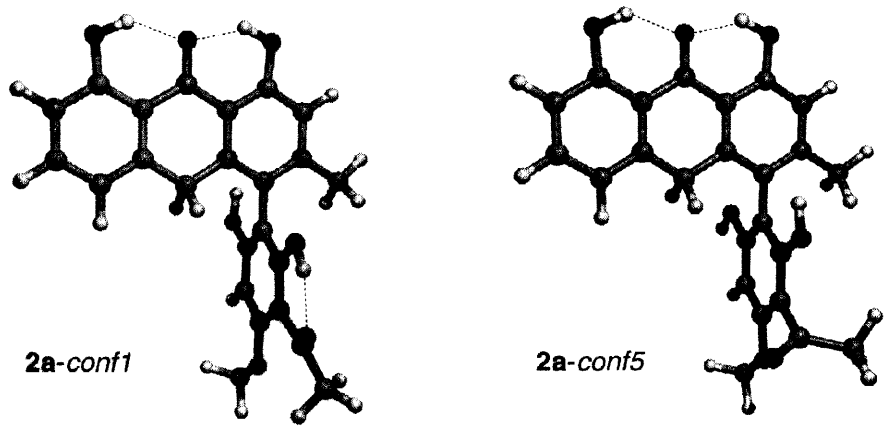


Fig. 5. 3D structures of two selected calculated conformers of **2a** (see also Table 2); hydrogen bonds indicated by dotted lines.

It is remarkable that in this case, the relative heats of formation of *conf1* to *conf4* lie within only 0.167 kcal/mol (see Table 2), showing that all of these main conformers should play a significant role in solution. They should thus, all of them, contribute to the CD-behavior of knipholone anthrone, which again underlines the necessity of a Boltzmann-weighted consideration of all of the species. Another difference to knipholone (**1a**) consists in the geometry of the anthracene-derived moiety of the molecule. In contrast to the strongly distorted anthraquinone system in **1**, the curvature of this tricyclic part is largely reduced for the anthrone moiety of **2**.

Although this ring system is not fully planar in **2**, the curvature is too small to justify a differentiation into 'convex' and 'concave'. As for **1**, the single CD spectra for *M*-**2** and, analogously, for *P*-**2**, were calculated, Boltzmann-weighted, and 'UV-corrected' to give the overall CD spectra, which were then compared with the experimental one obtained for (+)-knipholone anthrone.

Unexpectedly, the experimental CD spectrum of the natural anthrone initially gave significant differences, which did not allow an unambiguous attribution of the absolute configuration with sufficient confidence. As a possible reason for this, the chemical sensitivity of knipholone anthrone as compared to its more stable anthraquinone analog **1** and possibly resulting chiroptically active impurities originating from storage of **2** were assumed. By HPLC-CD coupling,[17] a significantly improved CD spectrum (especially in the diagnostically deciding region of 200 to 230 nm) was attained for the main UV peak, assigned to knipholone anthrone. This made it rewarding to perform a preparative follow-up of the HPLC experiment under the same conditions, with a subsequent off-line CD investigation of the freshly purified knipholone anthrone, which permitted to further enlarge the limited measuring range of the HPLC-CD experiment (only > 200 nm) to reach wavelengths down to 190 nm. Now, a near-perfect agreement with the spectrum calculated for the *M*-enantiomer (see Fig. 6, left) was obtained, whereas the theoretical spectrum for *P* (see Fig. 6, right) is virtually opposite to the experimental one. Thus, knipholone anthrone is clearly attributed the full absolute stereostructure **2a**, *i.e.* with *M*-configuration at the axis.

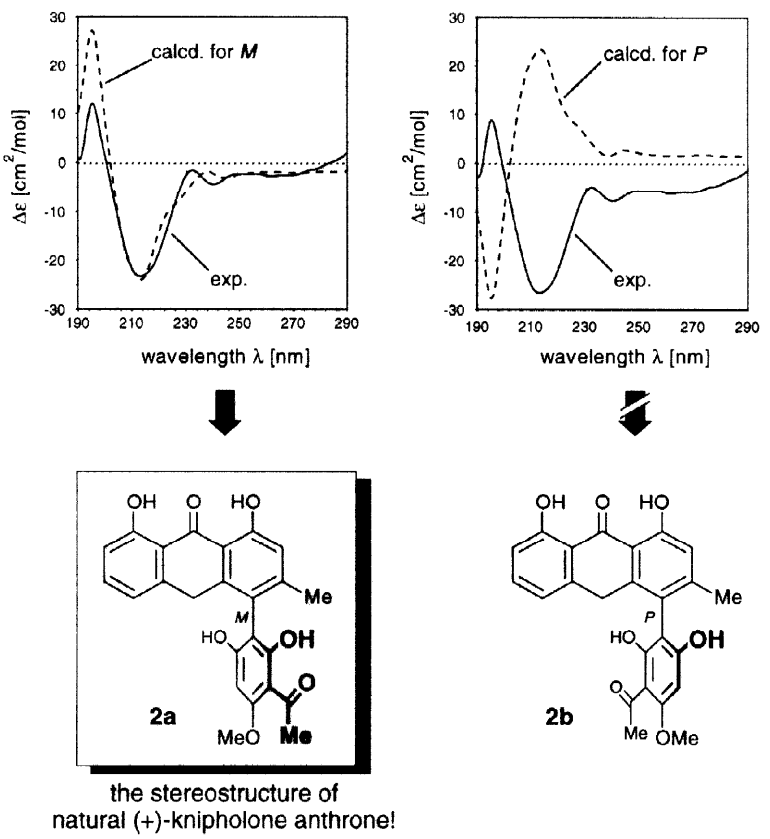
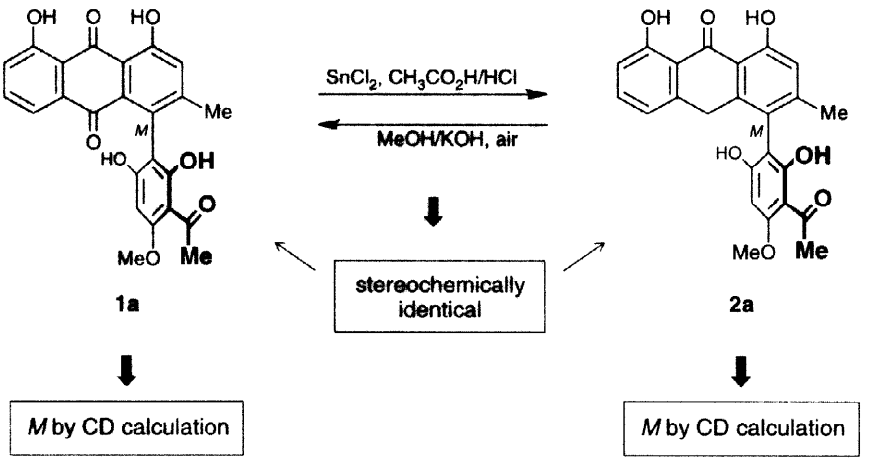


Fig. 6. Elucidation of the absolute configuration of (+)-knipholone anthrone (**2a**).

Experimentally, (+)-knipholone and (+)-knipholone anthrone have the same axial configuration, which is evident from the (already above mentioned) reduction of knipholone to knipholone anthrone and, *vice versa*,



Scheme 1. Interconversion and thus stereochemical identity of (+)-knipholone (+)-and knipholone anthrone.

from the oxidative transformation of knipholone anthrone into knipholone, with all the partial synthetic compounds being chiroptically fully identical with the authentic natural products (see Scheme 1). The fact that these hence stereochemically identical natural products with their different chromophors were independently found to have, both of them, *M*-configuration by the CD calculations, underlines the reliability of the method, which here permitted the first determination of the absolute stereostructures of these two interesting, constitutionally unsymmetric biaryl compounds.

COMPUTATIONAL AND EXPERIMENTAL SECTION

Conformational Analyses. Conformational analyses were performed on Silicon Graphics IRIS 4D and INDIGO (R4000) workstations. For the PM3[18] calculations the program package VAMP6.1[19] was used, starting from geometries preoptimized by the TRIPOS[20] force field.

CD Calculations. The wavefunctions for the calculation of the rotational strengths for the electric transitions from the ground state to excited states were obtained by CNDO/S-CI calculation[21] with a CI expansion including 576 singly occupied configurations and the ground state determinant. These calculations were carried out on LinuX PPro workstations by the use of the BDZDO/MCDSPD[22] program package. All single CD spectra were then added up following the Boltzmann statistic, *i.e.* according to their heats of formation, to give the theoretical overall CD spectrum. For a better visualization, the rotational strengths were transformed into $\Delta\epsilon$ values and superimposed with a Gaussian band shape function.

CD Spectra. The experimental CD spectra of knipholone (**1a**) and knipholone anthrone (**2a**) were measured at room temp. in ethanol and acetonitrile-water (62:38), respectively, using the J-715 CD spectrometer from JASCO Deutschland (Gross-Umstadt, Germany). The HPLC-CD experiment was performed using a Guard Pak Symmetry® C-18 column (3.9 x 20 mm) and a Symmetry® C-18 column (5 μm, 4.6 x 250 mm) from Waters (Eschborn, Germany). As the mobile phase (1 ml/min), acetonitrile-water (62:38) acidified with 0.1%

trifluoroacetic acid (TFA, Merck, Darmstadt, Germany) was used. Furthermore, a LG-980-025 Ternary Gradient Unit, a PU1580 pump (JASCO Deutschland, Gross-Umstadt, Germany), a Rheodyne 7725i injection valve, and the Borwin chromatographic software from JASCO Deutschland were used for the HPLC-CD coupling. For CD measurements, the flow was manually detached from the pump, using a Rheodyne 7010 injection valve connected to a Besta Motorventil "H" (Besta, Wilhelmshafen, Germany). The preparative chromatographic separation of knipholone anthrone (**2a**) was performed using a Guard Pak C-18 column (3.9 x 20 mm), a Nova Pak C-18 column (4 μm, 3.9 x 150 mm) from Waters (Eschborn, Germany), a 600E pump (Waters, Eschborn, Germany), a Rheodyne 7725i injection valve, and the Millenium Software 2.10 from Waters. As the mobile phase (0.5 ml/min), a methanol-water mixture (70:30) acidified with 0.1% trifluoroacetic acid (TFA, Merck, Darmstadt, Germany) was used.

Plant Material. The roots of *Bulbine frutescens* (Asphodelaceae) were collected from the Botanical Gardens of the University of Würzburg in December 1998. A voucher specimen is filed under the number 34 in Herbarium Bringmann, Würzburg.

Extraction and Isolation. Dried and powdered roots of *B. frutescens* were extracted in acetone (22 °C)[2] and (+)-knipholone (**1a**) was isolated according to a method previously elaborated.[4] All chiroptical and spectral data were identical to literature values,[4] and identity of the compound was confirmed by comparison with an authentic sample kindly provided by Prof. B. M. Abegaz.

Conversion of (+)-Knipholone (1a) to (+)-Knipholone Anthrone (2a). The reduction was performed according to a method by Auterhoff and Scherff.[23] To a solution of 2.12 mg of (+)-knipholone (**1a**, 4.88 μmol) in 2 ml glacial acetic acid, 2 ml of 40% $SnCl_2$ solution in conc. HCl were added with heating (120 °C). The resulting solution was refluxed for 2 h, cooled and poured into 20 ml of brine, thoroughly extracted with ethyl acetate, dried over Na_2SO_4, and the solvent evaporated. By chromatography on a short silica gel column (eluent: CH_2Cl_2 / methanol = 100:0.5), 1.32 mg (3.14 μmol, 64%) of (+)-knipholone anthrone (**2a**) were obtained, fully identical spectroscopically and chromatographically to an authentic sample of knipholone anthrone generously provided by Prof. B. M. Abegaz and Prof. E. Dagne.

Conversion of (+)-Knipholone Anthrone (2a) to (+)-Knipholone (1a). (+)-Knipholone anthrone (**2a**) was oxidized to (+)-knipholone (**1a**) by a method previously described by Dagne and Yenesew.[6] A solution of (+)-knipholone anthrone (**2a**, 4.82 mg, 11.5 μmol) in 10 ml 5% methanolic KOH was stirred overnight in the presence of air. The resulting red solution was acidified with 2 N HCl, diluted with water and extracted thoroughly with ethyl acetate. Purification of the extract by column chromatography on silica gel with CH_2Cl_2 / methanol (100:0.5) as the eluent afforded (+)-knipholone (**1a**, 1.83 mg, 4.13 μmol, 36%) as a red solid, identical with authentic knipholone (**1a**) obtained by isolation (see above).

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 347 'Selektive Reaktionen Metall-aktivierter Moleküle'). We thank Prof. B. M. Abegaz, University of Botswana (Gaborone, Botswana) and Prof. E. Dagne, Addis Ababa University (Ethiopia), for samples of authentic (+)-knipholone (**1a**) and (+)-knipholone anthrone (**2a**), Dr. J. Schlauer for helpfully supporting the isolation work, and JASCO Deutschland for generous technical support.

REFERENCES

1. Dagne, E.; Steglich, W. *Phytochemistry* **1984**, *23*, 1729-1731.
2. van Wyk, B.-E.; Yenesew, A.; Dagne, E. *Biochem. Syst. Ecol.* **1995**, *23*, 277-281.
3. Bezabih, M.; Motlhagodi, S.; Abegaz, B.M. *Phytochemistry* **1997**, *46*, 1063-1067.
4. van Staden, F.; Drewes, S.E. *Phytochemistry* **1994**, *35*, 685-686.
5. Alemayehu, G.; Hailu, A.; Abegaz, B.M. *Phytochemistry* **1996**, *42*, 1423-1425.
6. Dagne, E.; Yenesew, A. *Phytochemistry* **1993**, *34*, 1440-1441.
7. Bezabih, M.; Abegaz, B.M. *Phytochemistry* **1998**, *48*, 1071-1073.
8. Yenesew, A.; Dagne, E.; Müller, M.; Steglich, W. *Phytochemistry* **1994**, *37*, 525-528.
9. Bringmann, G.; Günther, C.; Ochse, M.; Schupp, O.; Tasler, S. in *Progress in the Chemistry of Organic Natural Products*, Eds. Herz, W.; Falk, H.; Kirby, G.W.; Moore, R.E.; Tamm, C., Springer Verlag Wien, New York, in preparation.
10. Watt, J.M.; Breyer-Brandwijk, M.G. in *The Medicinal and Poisonous Plants of Southern and East Africa*, Eds. Livingstone, E. and S., Edinburgh, 1962, p. 695.
11. Bringmann, G.; Menche, D.; Bezabih, M.; Abegaz, B.M.; Kaminsky, R. *Planta Med.*, submitted.
12. Bringmann, G.; Busemann, S. in *Natural Product Analysis*, Eds. Schreier, P.; Herderich, M.; Humpf, H.U.; Schwab, W., Vieweg, Braunschweig, 1998, pp. 195-212.
13. Bringmann, G.; Busemann, S.; Krohn, K.; Beckmann, K. *Tetrahedron* **1997**, *53*, 1655-1664.
14. Bringmann, G.; Günther, C.; Busemann, S.; Schäffer, M.; Olowokudejo, J.D.; Alo, B. *Phytochemistry* **1998**, *47*, 37-43.
15. Linker, T.; Rebien, F.; Tóth, G.; Kraus, J.; Bringmann, G. *Chem. Eur. J.* **1998**, *4*, 1944-1951.
16. Tochtermann, W.; Kuckling, O.; Meints, C.; Kraus, J.; Bringmann, G. *Tetrahedron: Asymmetry* **1999**, *10*, 21-24.
17. Bringmann, G.; Messer, K.; Wohlfarth, M.; Kraus, J.; Dumbuya, K.; Rückert, M. *Anal. Chem.*, **1999**, in press.
18. Steward, J.J.P. *J. Comput. Chem.* **1989**, *10*, 209-264.
19. Rauhut, G.; Chandrasekhar, J.; Alex, A.; Beck, B.; Sauer, W.; Clark, T., VAMP 6.1; available from Oxford Molecular Ltd., The Medewar Centre, Oxford Science Park, Sandford-on-Thames, Oxford, OX4 4GA, England.
20. SYBYL: Tripos Associates, 1699 St. Hanley Road, Suite 303, St. Louis, MO, 63144.
21. Del Bene, J.; Jaffé, H.H. *J. Chem. Phys.* **1968**, *48*, 1807-1813.
22. Downing, J.W., Program Packet BDZDO/MCDSPD, Department of Chemistry and Biochemistry, University of Colorado, Boulder, USA; modified by Fleischhauer, J.; Schleker, W.; Kramer W.; ported to LinuX by Gulden, K.-P.
23. Auterhoff, H.; Scherff, F. C. *Arch. Pharm.* **1969**, *293*, 918-925.