Structure and Dynamic of Three Indole Alkaloids from the *Campylospermum* Genus (Ochnaceae)

by **Gaétan Bayiha Ba Njock**[a][b][c], **Trixie Ann Bartholomeusz**[a], **Dominique Ngono Bikobo**[c], **Mohammadali Foroozandeh**[b], **Rupali Shivapurkar**[b], **Philippe Christen**[a], **Dieudonné Emmanuel Pegnyemb***[c], and **Damien Jeannerat**[b]

[a]) Section des sciences pharmaceutiques, Université de Genève, Université de Lausanne, 30 quai Ernest-Ansermet, CH-1211 Genève 4

[b]) Département de chimie organique, Université de Genève, 30 quai Ernest-Ansermet, CH-1211 Genève 4

[c]) Département de chimie organique, Faculté des Sciences, Université de Yaoundé I, BP 812 Yaoundé, Cameroun (phone: +23777804176; fax: +23722221873; e-mail: pegnyemb@yahoo.com)

A new indole alkaloid, calanthumindole (**4**), and three known biflavonoids, amentoflavone, sequoiaflavone, and podoscarpusflavone B, were isolated from *Campylospermum calanthum* (Ochnaceae). Calanthumindole is a new indole alkaloid of the serotobenine family characterized by the presence of a C=C bond between atoms C(7′) and C(8′) of the furan ring. This is the first compound to have a fully unsaturated furan ring among the members of this family. The combination of NMR and DFT allowed the determination and comparison of the 3D structures and relevant conformational characteristics of serotobenine (**1**), flavumindole (**2**), and calanthumindole (**4**).

Introduction. – The Ochnaceae family comprises 28 genera and *ca.* 300 – 500 species of trees, shrubs, and herbs widespread in tropical and subtropical regions [1]. Seven genera are found in Cameroon: *Ochna*, *Lophira*, *Ouratea*, *Rhabdophyllum*, *Idertia*, *Sauvagesia*, and *Campylospermum* [2]. Sixty-five *Campylospermum* species occur mainly in tropical Africa and Madagascar as well as in Asia [2]. In Cameroon, *C. flavum* (SCHUM.) FARRON, *C. glaucum* (TIEGH.) FARRON, *C. mannii* (OLIV.) TIEGH., *C. calophyllum* (HOOK. *f.*) TIEGH., *C. elongatum* (OLIV.) TIEGH., and *C. excavatum* FARRON are frequently encountered. It has been reported that the MeOH extracts of roots of *C. densiflorum* (DE WILD. & T. DURAND) Farron and leaves of *C. zenkeri* (TIEGH.) FARRON are active against β-lactam-resistant *Gram*-positive bacteria [3]. The leaves and the stem bark of *C. mannii* have been reported to alleviate health problems such as digestive and cardiac disorders [4].

The genus *Campylospermum* is characterized by the presence of biflavonoids [4]. Nitrile glucosides have been reported to occur in *C. glaucum* [5]. More recently, two indole alkaloids, serotobenine (**1**) and flavumindole (**2**) have been isolated from the leaves and stem bark of *C. flavum* [6]. Compound **1** was first isolated from safflower seeds (*Carthamus tinctorius* L.) [7] and identified (but called 'moschamindole') in the seeds of *Centaurea moschata* L. [8]. It has also been reported in *Ouratea gilgiana* H. GILG. [9] and in *O. turnarea* (HOOK.) HUTCH & DALZ [5] and, together with decursivine (**3**), in *C. densiflorum* [10], three other species of the Ochnaceae family.

However, little is known about *C. calanthum* (GILG.) FARRON. This is a small tree with yellow flowers growing in the south part of Cameroon. In this article, we report the first occurrence of calanthumindole[1]) (**4**; *Fig. 1*) along with the three known biflavonoids amenthoflavone [11], sequoiaflavone [12], and podoscarpusflavone B [13].

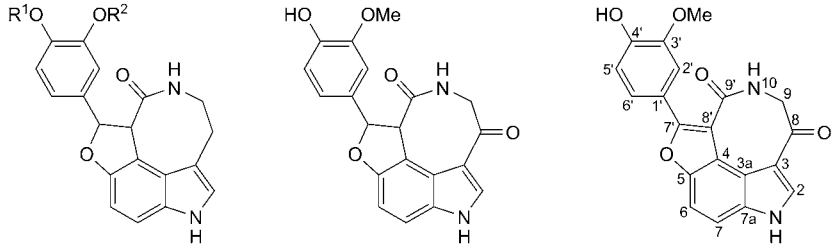

Fig. 1. *Serotobenine* (**1**), *flavumindole* (**2**), *decursivine* (**3**), *and calanthumindole* (**4**), *isolated from the* Campylospermum *genus*

Serotobenine (**1**) and decursivine (**3**) have been the object of some attention because of their antioxidant, antibacterial [14][15], and antimalaric [15] activities, respectively. The X-ray structure of **1** is known [7], and a total synthesis has been developed [16]. The importance of **1** and **3** justified a study of their structures and dynamics by density functional theory (DFT) and NMR techniques and a comparison with flavumindole (**2**) and the new compound calanthumindole (**4**).

Results and Discussion. – *Structure Elucidation of Calanthumindole* (**4**). Compound **4** was obtained as an orange powder soluble in DMSO and gave a positive response with *Ehrlich*'s reagent, revealing the presence of an indole structure. The HR-ESI-TOF-MS in negative-ion mode showed an $[M-H]^-$ signal at m/z 361.0801 in agreement with the molecular formula $C_{20}H_{14}N_2O_5$ giving 15 unsaturations. The ^{1}H- and ^{13}C-NMR spectra (*Table*) were highly similar to those of flavumindole (**2**) [6]. Compound **4** differed from **2** by ^{1}H- and ^{13}C-NMR signals indicating the presence of a C=C bond between C(7′) and C(8′) of the furan ring, instead of a single bond. This accounted for the presence of two additional quaternary unsaturated C-atoms at δ(C) 110.4 (C(8′)) and 159.7 (C(7′)) in the ^{13}C-NMR spectrum of **4**. The ^{13}C-NMR signals of two C=O, a CH_2, and an MeO group, and twelve aromatic and two remaining unsaturated C-atoms were also present and similar to those found in **2**. The ^{1}H-NMR spectrum of **4** clearly showed two *dd* at δ(H) 3.63 and 4.78 of the CH_2(9) group. The nonequivalence of these H-atoms is surprising because **4** has a plane of symmetry. Obviously, the structure is not necessarily flat, but the exchange between the two symmetrically related conformational enantiomers would impose the equivalence of these H-atoms provided it is faster than the NMR time scale. A study of the dynamics (see below) demonstrated that there are indeed two slowly exchanging atropisomers. For the rest, the ^{1}H-NMR spectrum of **4** showed three aromatic H-atoms in the low-

[1]) Arbitrary atom numbering; for the systematic name of **4**, see *Exper. Part*.

field region at δ(H) 6.88 (*d*, $J = 8.4$ Hz), 7.55 (*dd*, $J = 8.5$, 2.0 Hz), and 7.53 (*d*, $J =$ 2.0 Hz) with an *ABX* coupling pattern, four *s* corresponding to the indole H–N(1) at δ(H) 12.5, the phenol OH–C(4′) at δ(H) 9.56, the amide H–N(10) at δ(H) 8.55, and the MeO–C(3′) at δ(H) 3.82. The HMBC data (*Table*) clearly supported the proposed structure by the correlations between the CH_2(9) group and the two C=O groups at δ(C) 190.0 (C(8)) and 168.0 (C(9′)), between the amide H–N(10) and the quaternary sp^2 C-atom at δ(C) 110.4 (C(8′)), and between H–C(2′) and the quaternary sp^2 C-atom C(7′). The complete analysis of the HMBC spectrum in combination with additional NMR and computational data allowed the identification of calanthumindole (**4**) as a new indole derivative.

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; (D_6)DMSO) *of Calanthumindole*[1]) (**4**). δ in ppm, *J* in Hz.

	δ(H)	δ(C)	HMBC (C → H)
H–N(1)	12.5 (*s*)	–	–
H–C(2)	8.17 (*s*)	132.0[b])	–
C(3)	–	116.0[a])	H_a–C(9)
C(3a)	–	118.0[a])	H–C(7)
C(4)	–	120.1[a])	H–C(6)
C(5)	–	149.2[a])	H–C(6), H–C(7)
H–C(6)	7.58 (*d*, $J = 8.9$)	107.6[b])	–
H–C(7)	7.42 (*d*, $J = 8.2$)	110.7[b])	–
C(7a)	–	133.7[a])	H–C(6), H–C(7)
C(8)	–	190.0[a])	H_a–C(9)
CH_2(9)	3.63 (*dd*, $J = 13.9$, 6.3), 4.78 (*dd*, $J = 13.9$, 9.3)	51.8[b])	–
H–N(10)	8.55 (*dd*, $J = 9.2$, 6.2)	–	–
C(1′)	–	122.7[a])	H–C(5′)
H–C(2′)	7.53 (*d*, $J = 2.0$)	113.0[b])	–
C(3′)	–	147.9[a])	*Me*O–C(3′), H–C(5′), OH–C(4′)
C(4′)	–	149.1[a])	H–C(2′)
H–C(5′)	6.88 (*d*, $J = 8.4$)	116.6[b])	OH–C(4′)
H–C(6′)	7.55 (*dd*, $J = 8.5$, 2.0)	122.7[b])	H–C(2′)
C(7′)	–	159.7[a])	H–C(6′), H–C(2′), H–N(10)
C(8′)	–	110.4[a])	H–N(10)
C(9′)	–	168.0[a])	H_a–C(9), H_b–C(9)
*Me*O–C(3′)	3.82 (*s*)	56.4[b])	–
OH–C(4′)	9.56 (*s*)	–	–

[a]) Chemical shifts based on the HMBC spectrum. [b]) Chemical shifts based on the HSQC spectrum.

Tridimensional Structures of Serotobenine (**1**), *Flavumindole* (**2**), *and Calanthumindole* (**4**). The 3D structures were obtained by DFT (*Fig. 2*). The structures of calanthumindole (**4**) and flavumindole (**2**), which differ from serotobenine (**1**) by the replacement of the CH_2(8) with a C(8)=O group, are nearly identical to the X-ray structure of **1** [7]. Compared to **1** and **2** where the aryl substituent at the saturated center C(7′) points up, the C(7′)=C(8′) bond of **4** forces the aryl group towards the plane of the indole part of the molecule. In all three structures, the 'chain' fragment between C(3) and C(8′) adopts a conformation where C(9) is completely out of the

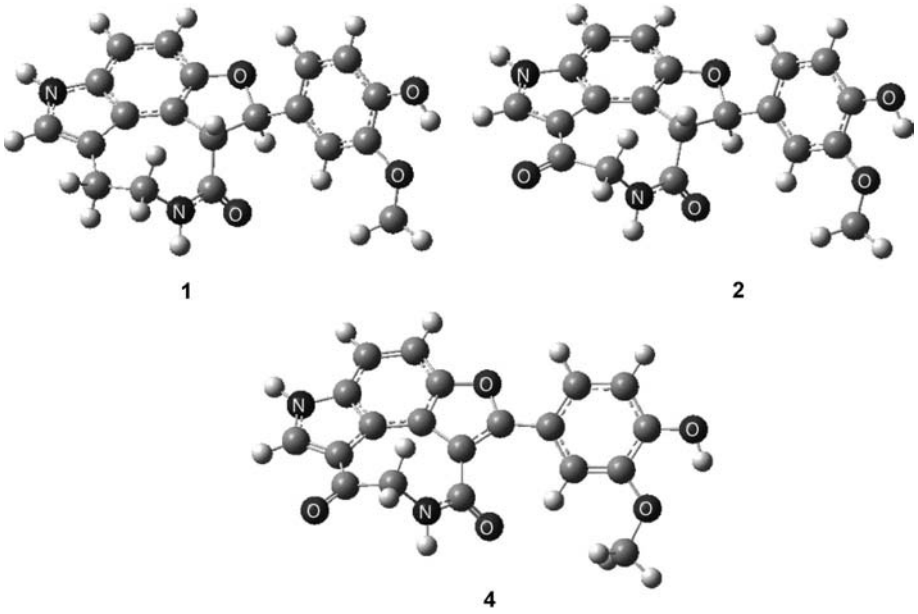

Fig. 2. *Lowest-energy conformations of serotobenine* (**1**), *flavumindole* (**2**), *and calanthumindole* (**4**), *obtained by DFT energy minimization with the B3LYP method and* the *6-31g* (d,p) *basis set*

plane, relative to the rest of the molecule and explains why the H-atoms of $CH_2(9)$ can be diastereotopic. With respect to the NMR of calanthumindole (**4**), the problem is that even if its geometry shows diastereotopic H-atoms at C(9), the mirror image relative to the plane of the indole moiety exchanges their positions. This means that a fast exchange should make the two H-atoms enantiotopic and therefore chemically equivalent. We, therefore, calculated the energy barrier involved in this conformational change.

Conformational Study of Serotobenine (**1**)*, Flavumindole* (**2**)*, and Calanthumindole* (**4**). The study of the energy profile of the flip of the $CH_2(9)$ moiety relative to the plane of the indole part is shown in *Fig. 3*. For calanthumindole (**4**), DFT calculations estimated it to be *ca.* 17 kcal. At this level, the exchange rate was predicted to be *ca.* 2 s^{-1}, at 22°, a rate which is not fast enough to manifest itself in the ^{1}H-NMR spectrum. Replacing the solvent with DMSO and heating to *ca.* 90° resulted in the broadening of the signals of the $CH_2(9)$ H-atoms of **4**, the first manifestation of exchange. A precise experimental value of ΔG could not be determined because of experimental limitations but it was estimated to be close to 20 ± 1 kcal, a value reasonably close to the value predicted by DFT given the precision of this type of calculations. For comparison, the energy profiles of the CH_2 flip for serotobenine (**1**) and flavumindole (**2**) were also calculated. These molecules being chiral (unlike **4**), the profiles were not symmetrical with respect to the sign of the dihedral angles α and β, and might have revealed two stable conformations, something that may be relevant with respect to the biological activity. It turned out that if flavumindole (**2**) indeed showed a local minimum (see

Fig. 3) for a second conformation (for $\alpha \approx +95$), but it was too high (12.6 kcal above the lowest energy conformation) to have a significant probability to be accessed. In the case of serotobenine (**1**), the profile showed a plateau for conformations with $\alpha = 0-30°$, but this occurred 6 kcal above the minimum and could also be neglected. We can, therefore, safely conclude that, relative to the CH_2 position, the geometries of **1**, **2**, and **4** shown in *Fig. 2* are the only relevant ones. The energy profile of the rotation of the aryl group at C(7′) (angle γ in *Fig. 3*) was also calculated (results not shown). A 180° rotation of the aryl group required *ca.* 5 kcal activation energy and resulted in structures *ca.* 0.3 kcal higher in energy for **1** and **2**; thus the two rotamers were equally relevant at room temperature. For **4**, the energy difference was 1.4 kcal which means that the higher-energy rotamer had a relative population of 10% at 25°. This larger difference in energy in the case of **4** is probably due to the close contact of the H-atoms in *ortho* position of the aryl group (H–C(2′) and H–C(6′)) with the O-atom of the C(9′)=O moiety which seems to favor H–C(2′) over H–C(6′). In **1** and **2**, this distance is too large to permit this weak differentiation.

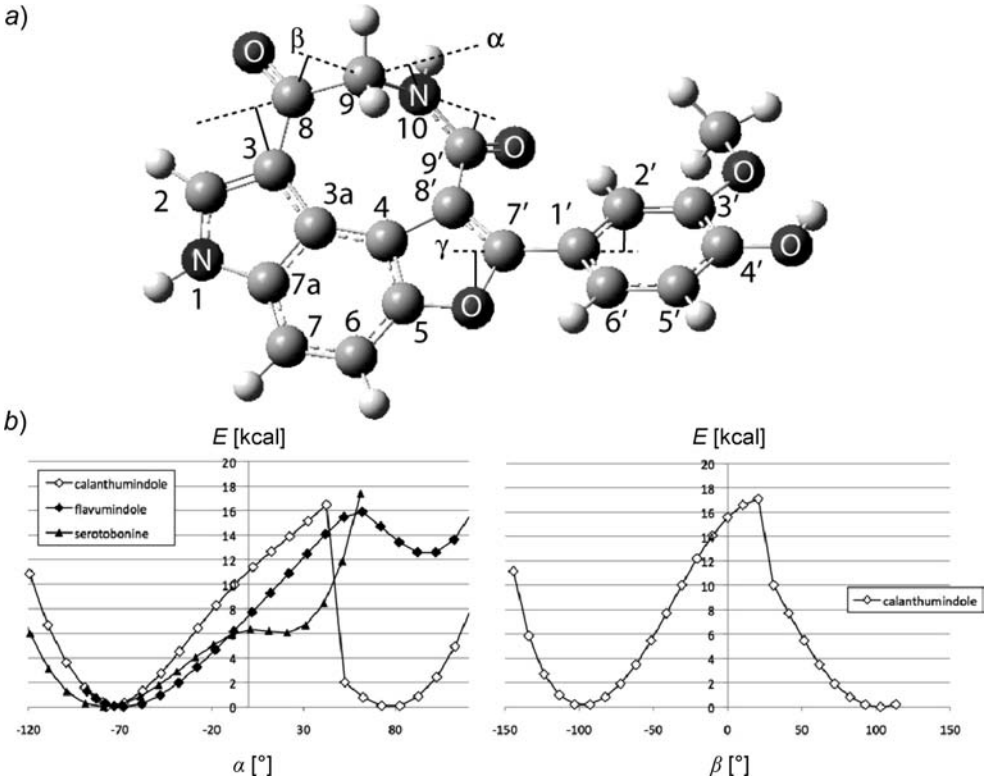

Fig. 3. *a*) *Dihedral angles in calanthumindole* (**4**) *for which the geometry minimizations were constrained to calculate the energy profile of conformational changes. b*) *Energy profiles obtained by geometry optimization as a function of the imposed dihedral angle* α (left) *and* β (right). The minima correspond to $\alpha = -78.96°$ for **1**, $-68.1°$ for **2**, and $-77.6°$ for **4**. The transition states (local energy maxima) are 6.4, 15.9, and 16.9 kcal for **1**, **2**, and **4**, respectively (*Fig. 3*,*b*, left) and 17.1 kcal for **4** (*Fig. 3*,*b*, right). DFT calculations were performed with B3LYP/6-31g (d,p).

Conclusions. – Indole alkaloids were previously isolated from the genus *Campylospermum*; the isolation of a new derivative, calanthumindole (**4**), represents a new addition to the chemotaxonomic markers of this genus which is closely related to the genus *Ouratea*. The structure of this new compound was elucidated on the basis of a computational analysis and compared to other members of the serotobenine family.

D. J. thanks Dr. *Joseph Thierry Ndongo* for stimulating discussions concerning the presence of indole alkaloids in Ochnaceae, *Jiri Mareda* for advice on the DFT calculations, and the *Département de l'Instruction Publique* (DIP), Geneva, and the *Swiss National Science Foundation* (No. 200020-135089 and 206021-128746) for funding. *André Pinto* is acknowledged for the acquisition of many NMR spectra, and we thank the students of the group of Prof. *Kündig* for some sample preparation. *Gaétan Bayiha Ba Njock* is grateful to the *Swiss Department of Foreign Affairs* for an international scholarship and to Dr. *Turibio Tabopda* for assistance.

Experimental Part

General. TLC: aluminium sheets silica gel *60 F_{254}*. Column chromatography (CC): silica gel (SiO_2; 70–230 mesh) and *Sephadex LH-20.* Semiprep. HPLC: *Shimadzu-LC-8A* apparatus; *X-Bridge*® column (250 × 10 mm); flow rate 10 ml/min; detection at 210 nm with a UV *LKB-Bromma-2151* detector and an *LKB-2210* recorder. NMR Spectra: *Bruker* instrument, equipped with a 5 mm ^{1}H- and ^{13}C-probe operating at 500 and 125 MHz, resp.; in (D_6)DMSO; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz; assignments by 2D-HSQC and HMBC experiments. HR-ESI-MS: *Micromass-LCT*-Premier TOF spectrometer (*Waters*); at 70 eV; in *m*/*z*.

Plant Material. Fresh leaves of *Campylospermum calanthum* were harvested at Ebolowa in the south region of Cameroon in 2009 and identified by a senior botanist, *Victor Nana*. A voucher specimen (N° 51945/HNC) was deposited with the National Herbarium in Yaoundé, Cameroon.

Extraction and Isolation. The dried and powered leaves of *Campylospermum calanthum* (1kg) were extracted for 48 h with MeOH (3 × 2 l) at r.t. After filtration and evaporation, the crude MeOH extract (120 g) was suspended in MeOH/H_2O 4:1 (500 ml), defatted with hexane (400 ml) and extracted with AcOEt (400 ml). The AcOEt residue (20 g) was subjected to CC (SiO_2, CH_2Cl_2/MeOH 1:0 → 0:1): *Fractions C_1–C_4* (TLC monitoring). Each fraction was repeatedly purified by CC (*Sephadex LH-20*, MeOH (C_1 and C_2) or AcOEt (C_3)) and by isocratic semi-prep. HPLC (MeOH/H_2O 4:6): *calanthumindole* (=*4,5-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3*H-*furo[2,3,4*-kl*]pyrrolo[4,3,2*-fg*][3]benzazocine-3,6(8*H*)-dione*; **4**; 1.0 mg), amentoflavone [11] (3.5 mg), sequoiaflavone [12] (5.5 mg), and podoscarpusflavone B [13] (10.2 mg).

REFERENCES

[1] R. K. Brummitt, 'Vascular Plant Families and Genera', Lubrecht & Cramer Ltd., Kew, 1992.
[2] G. Bayiha Ba Njock, 'Détermination structurale de quelques métabolites secondaires isolés de *Campylospermum calanthum* et *Ouratea gilgiana* par la RMN 2D à haute résolution par repliement spectral', Ph.D. Thesis, Yaoundé, 2011.
[3] J. Gangoue-Pieboji, N. Eze, A. N. Djintchui, B. Ngameni, N. Tsabang, D. E. Pegnyemb, L. Biyiti, P. Ngassam, S. Koulla-Shiro, M. Galleni, *J. Infect. Dev. Ctries.* **2009**, *3*, 671.
[4] S. S. Elo Manga, R. Tih, R. T. Ghogomu, A. Blond, B. Bodo, *Biochem. Syst. Ecol.* **2009**, *37*, 402.
[5] A. Abouem a Zintchem, D. N. Bikobo, A. d. T. Atchade, J. N. Mbing, J. Gangoue-Pieboji, R. G. Tih, A. Blond, D. E. Pegnyemb, B. Bodo, *Phytochemistry* **2008**, *69*, 2209.
[6] J. T. Ndongo, M. Shaaban, J. N. Mbing, D. N. Bikobo, A. d. T. Atchade, D. E. Pegnyemb, H. Laatsch, *Phytochemistry* **2010**, *71*, 1872.
[7] H. Sato, H. Kawagishi, T. Nishimura, S. Yoneyama, Y. Yoshimoto, S. Sakamura, A. Furusaki, S. Katsuragi, T. Matsumoto, *Agric. Biol. Chem.* **1985**, *49*, 2969.
[8] S. D. Sarker, T. Savchenko, P. Whiting, V. Sik, L. N. Dinan, *Nat. Prod. Lett.* **1997**, *9*, 189.

[9] G. Bayiha Ba Njock, T. A. Bartholomeusz , M. Foroozandeh, D. E. Pegnyemb, P. Christen, D. Jeannerat, *Phytochem. Anal.* **2012**, *23*, 126.
[10] D. S. Ngono Bikobo, J. L. Nkot, P. Mosset, A. T. Atchade, J. T. Ndongo, R. Pemha, D. E. Pegnyemb, *Rasayan J. Chem.* **2011**, *4*, 753.
[11] V. M. Chari, M. Ilyas, H. Wagner, A. Chen, Y. Lin, *Phytochemistry* **1977**, *16*, 1273.
[12] M. Kamil, M. Ilyas, W. Rahman, N. Hasaka, M. Okigawa, N. Kawano, *J. Chem. Soc., Perkin Trans. 1* **1981**, 553.
[13] H. Miura, T. Kihara, N. Kawano, *Chem. Pharm. Bull.* **1969**, *17*, 150.
[14] Y. Kumarasamy, M. E. Fergusson, L. Nahar, S. D. Sarker, *Pharm. Biol.* (*Lisse*, *Neth.*) **2002**, *40*, 307.
[15] H. Zhang, S. Qiu, P. Tamez, G. T. Tan, Z. Aydogmus, N. Van Hung, N. M. Cuong, C. Angerhofer, D. D. Soejarto, J. M. Pezzuto, H. H. S. Fong, *Pharm. Biol.* (*Lisse*, *Neth.*) **2002**, *40*, 221.
[16] Y. Koizumi, H. Kobayashi, T. Wakimoto, T. Furuta, T. Fukuyama, T. Kan, *J. Am. Chem. Soc.* **2008**, *130*, 16854.

Received June 26, 2012