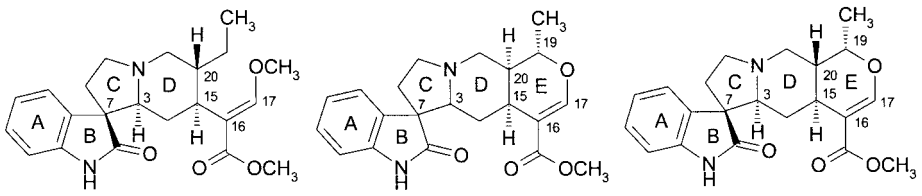
X-Ray Crystal Structure Analysis of Oxindole Alkaloids

by **Gerhard Laus***[a]), and **Klaus Wurst**[b])

[a]) Immodal Pharmaka GmbH, Bundesstrasse 44, A-6111 Volders
(phone: 0043 5224 57678; fax: 0043 5224 57646; e-mail: gerhardlaus@hotmail.com)
[b]) Institut für Allgemeine, Anorganische und Theoretische Chemie, Universität Innsbruck, Innrain 52, A-6020 Innsbruck

The single-crystal X-ray structures of speciophylline, mitraphylline, and rhynchophylline, oxindole alkaloids from the Peruvian climbing vine *Uncaria tomentosa* (Rubiaceae), were determined. The three compounds show N···H–N hydrogen bonding, which has not been observed in the crystal structures of the related alkaloids pteropodine and isopteropodine. In the tetracyclic alkaloid rhynchophylline, the side chain is rotated out of the ring plane into a position perpendicular to it. This is in contrast to the situation of the pentacyclic analogue mitraphylline, which possesses a conformationally rigid tricyclic core. This conformational difference possibly causes the competitive antagonism of these two types of alkaloids.

Introduction. – The genus *Uncaria* (Rubiaceae) is a rich source of oxindole alkaloids. During recent years, pharmacological activities of some of these alkaloids were investigated. The tetracyclic oxindole alkaloid rhynchophylline (**1**) blocks the release of calcium from intracellular storage and possesses hypotensive potency [1]. Effects of **1** on contraction of isolated rat uterus, contraction of rabbit aorta, and myocardial contractility in dogs and cats were reported [2]. Negatively chronotropic and inotropic effects of rhynchophylline (**1**) and isorhynchophylline were observed [3]. In contrast, pentacyclic oxindole alkaloids affect the cellular immune system. They stimulate endothelial cells *in vitro* to produce a lymphocyte-proliferation-regulating factor [4] and stimulate interleukin-1 and -6 production by alveolar macrophages [5]. Isopteropodine (**2**) and pteropodine (**3**) enhance the phagocytic activity of granulocytes [6]. A recent study showed that the tetracyclic alkaloids antagonize the stimulating effect of the pentacyclic alkaloids [7]. Therefore, it seemed to be of interest to examine their three-dimensional structures in detail. The results of single-crystal X-ray structure determinations of rhynchophylline (**1**), speciophylline (**4**), and mitraphylline (**5**), isolated from Peruvian *U. tomentosa* (WILLD.) DC root [8] are presented. Crystal structures were reported earlier for pteropodine hydrate [9], isopteropodine, and the $CHCl_3$ solvate of pteropodine [10], and the related isorhynchophyllic acid from *U. sinensis* (OLIV.) HAVIL. [11]. Mitraphylline was also detected in leaves of *U. quadrangularis* GEDDES (= *U. homomalla* MIQ.), *U. attenuata* KORTH., *U. orientalis* GUILL., *U. velutina* HAVIL., *U. perrottetii* (A. RICH.) MERR., in the root of *U. guianensis* (AUBL.) GMEL., and in the bark of *U. elliptica* R. BR. [12][13]. Speciophylline, pteropodine, and isopteropodine were also identified in the leaves of *U. homomalla*, *U. lanosa* var. *glabrata* (BL.) RIDSD., *U. sinensis*, and *U. orientalis* [12][14]. Rhynchophylline (**1**) was also found in the leaves of *U. borneensis* HAVIL., *U. longiflora* (POIR.) MERR., *U. guianensis*, *U. macrophylla* WALL., in aerial parts of *U. rhynchophylla* MIQ., and in the stem bark and hooks of *U. attenuata* [12][15].



1 **2** (3*S*,7*S*) **5**
3 (3*S*,7*R*)
4 (3*R*,7*S*)

Results and Discussion. – Oxindole alkaloids are classified in stereochemical groups with *allo* (rings D/E *cis*, C(3) (*S*), *epiallo* (rings D/E *cis*, C(3) (*R*)), and *normal* (rings D/E *trans*, C(3) (*S*)) configurations [16]. The *pseudo* (rings D/E *trans*, C(3) (*R*)) configuration, although possible for indole alkaloids, is sterically not viable for oxindoles. All known compounds of this type possess (*S*)-configuration at C(15) due to their biogenetic origin from the common precursor strictosidine [17]. They occur as interconvertible pairs of C(7)-epimers [18] or even as C(3),C(7)-diastereoisomeric pairs of pairs [9]. The numbering system is based on that customarily used for the hetero-yohimbinoid alkaloids.

The tetracyclic alkaloid rhynchophylline (**1**) possesses the *normal* configuration with 18,19-seco ring. The five-membered ring C is nearest to an N(2) envelope, and the six-membered ring D is a chair. The side chain is rotated out of the ring plane into a position perpendicular to it (*Fig. 1*). A similar conformation is adopted by isorhynchophyllic acid [11]. There is N(2)···H–N bonding between chains of molecules stacked along the *a*-axis, with an N···N distance of 2.999 Å and an N···H distance of

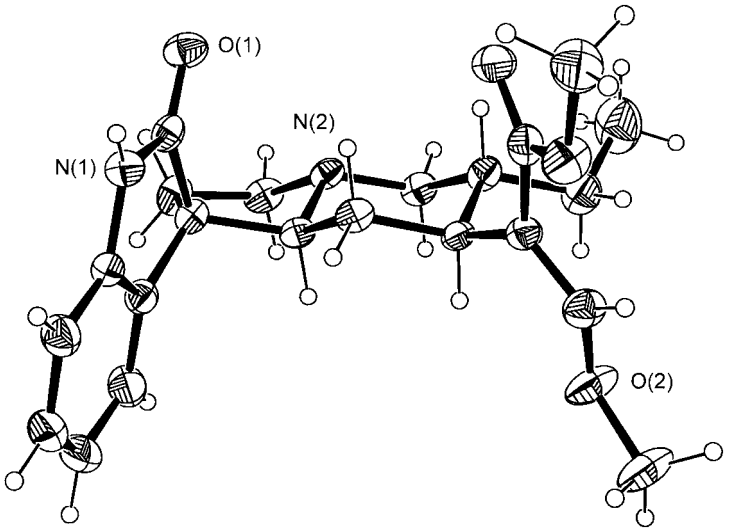


Fig. 1. *ORTEP View of* **1** *drawn with 30% displacement ellipsoids*

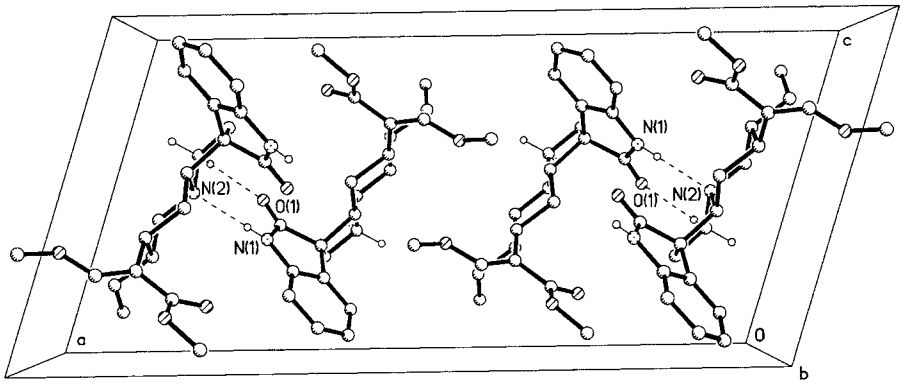


Fig. 2. *Content of unit cell showing H-bonding interactions of* **1**

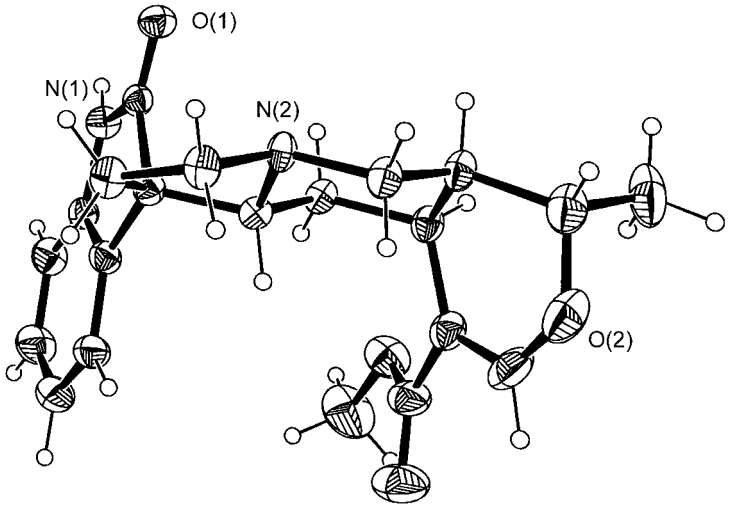


Fig. 3. *ORTEP View of* **4** *drawn with 30% displacement ellipsoids*

2.128 Å. A short contact is also observed between C=O(1) and H–C(5) with an O···C distance of 3.320 Å (*Fig. 2*).

Speciophylline (**4**) is the first example of the *epiallo* series for which a crystal structure has been determined. The five-membered ring C is twisted, and ring D is a chair. The heterocyclic ring E has a conformation in which C(15), C(16), C(17), and O(2) lie in a common plane, whereas atom C(19) lies below this plane by 0.324 Å, and C(20) lies above this plane by 0.424 Å (*Fig. 3*). The asymmetric unit contains three independent molecules. There is C=O(1)···H–N bonding along the *a*-axis with O···N distances of 2.840, 2.812, and 2.958 Å, respectively. The corresponding O···H distances are 2.078, 2.050, and 2.365 Å. Also, weak N(2)···H–N bonding with N···N distances of 3.221, 3.399, and 3.140 Å is observed. The N···H distances are 2.581, 2.976, and 2.437 Å. In addition, there are interactions between the ester C=O group and both H–C(15) and the aromatic H–C(10) (*Fig. 4*).

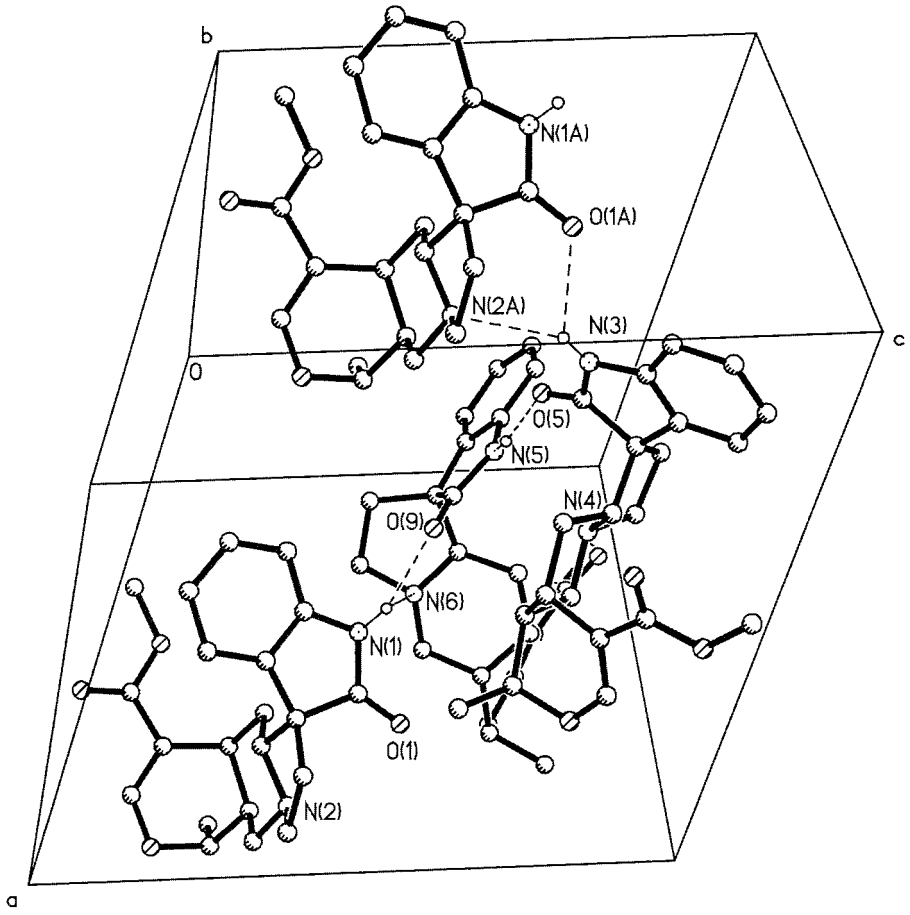


Fig. 4. *Intermolecular arrangement of part of the unit-cell content of* **4** *indicating H-bonding interactions along the* a*-axis*

Mitraphylline (**5**), a pentacyclic representative of the *normal* series, possesses a conformationally rigid tricyclic core due to the *trans* D/E ring junction. Ring C is nearest to an N(2) envelope. Again, atoms C(19) and C(20) lie 0.423 Å below and 0.218 Å above the common plane of C(15), C(16), C(17), and O(2), respectively (*Fig. 5*). No C=O(1)···H–N bonding is found. Instead, weak N(2)···NH bonding between chains of molecules stacked along the *b*-axis with an N···N distance of 3.284 Å and an N···H distance of 2.542 Å is observed. There are also short contacts of the type C=O(1)···H–C(12) and C=O(1)···H_{eq}–C(21) with O···C distances of 3.206 Å and 3.209 Å, respectively (*Fig. 6*). The H_2O molecule is disordered.

For comparison, the ring C in *allo*-isopteropodine (**2**) is nearest a C(3) envelope or twisted, and C=O···H–N bonding between three independent molecules along the *a*-axis has been observed [10]. In pteropodine (**3**) hydrate, also *allo*, ring C is nearest to an N(2) envelope, and ring D is a slightly twisted chair. An infinite chain of H-bonds along a twofold screw axis is formed between the alkaloid and the H_2O molecules [9].

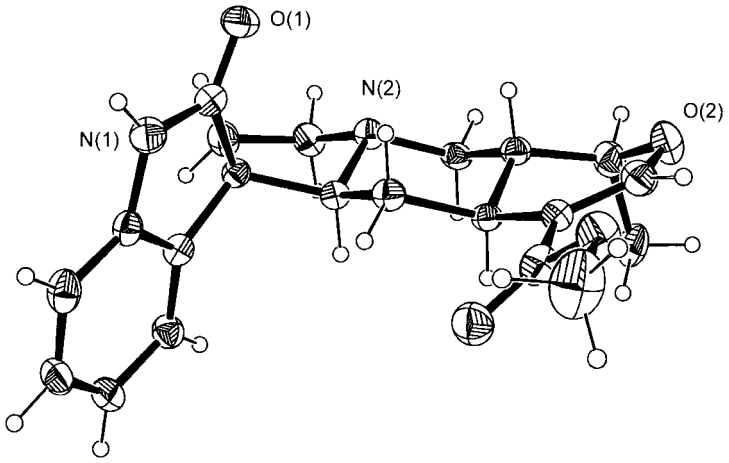


Fig. 5. *ORTEP View of* **5**. Displacement ellipsoids are shown at the 30% probability level.

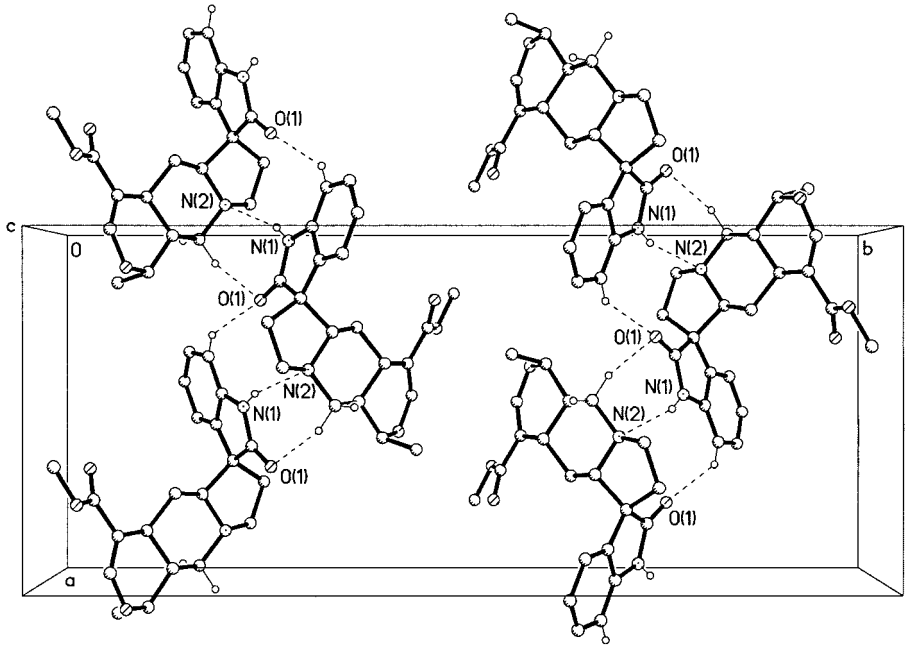


Fig. 6. *Intermolecular arrangement of part of the unit-cell content of* **5** *indicating H-bonding interactions* (the H_2O molecule is not shown)

Crystal data are summarized in the *Table*.

The tetracyclic alkaloids rhynchophylline (**1**) and isorhynchophylline are competitive antagonists towards the *allo*/*epiallo* as well as the *normal* pentacyclic alkaloids. Since the rhynchophylline isomers inhibit the effect of the mitraphylline isomers on

Table. *Summary of Crystal Data*

	Rhynchophylline (**1**) [76-66-4]	Speciophylline (**4**) [4697-68-1]	Mitraphylline (**5**) [52689-99-3]
Formula	$C_{22}H_{28}N_2O_4$	$C_{21}H_{24}N_2O_4$	$C_{21}H_{24}N_2O_4 \times 0.25\ H_2O$
M_r	384.46	368.42	372.93
T [K]	233(2)	233(2)	233(2)
Crystal description	Colorless prism	Colorless plate	Colorless plate
Crystal size [mm]	$0.29 \times 0.18 \times 0.05$	$0.4 \times 0.3 \times 0.05$	$0.25 \times 0.08 \times 0.03$
Crystal system	Monoclinic	Monoclinic	Orthorhombic
Space group	$C2$	$P2_1$	$P2_12_12$
a [Å]	23.096(1)	12.2177(6)	11.0779(9)
b [Å]	8.540(1)	19.392(2)	26.490(2)
c [Å]	10.984(1)	13.326(1)	6.4959(4)
α [°]	90	90	90
β [°]	107.622(7)	108.797(4)	90
γ [°]	90	90	90
V [Å^3]	2064.8(3)	2988.9(4)	1906.2(2)
Z	4	6	4
D_c [g cm^{-3}]	1.237	1.228	1.299
$F(000)$	824	1176	794
μ [mm^{-1}]	0.085	0.085	0.091
θ Range for data collection [°]	1.85 – 21.00	1.61 – 21.49	1.54 – 19.91
Index ranges	$-23 \le h \le 23$, $-8 \le k \le 8$, $-11 \le l \le 11$	$-12 \le h \le 12$, $-19 \le k \le 19$, $-13 \le l \le 13$	$-10 \le h \le 10$, $-25 \le k \le 25$, $-6 \le l \le 6$
Reflections measured	4093	11766	6821
Independent reflections	2195	6220	1765
R_{int}	0.0371	0.0483	0.0865
Reflections observed	1916	5217	1390
Parameters refined	260	747	265
Goodness-of-fit	1.071	1.065	1.015
R_1, wR_2 ($I > 2\sigma(I)$)	0.0401, 0.0883	0.0468, 0.1009	0.0467, 0.1037
R_1, wR_2 (all data)	0.0498, 0.0917	0.0610, 0.1064	0.0688, 0.1116
Largest diff. peak and hole [e nm^{-3}]	117, −122	331, −164	280, −144

endothelial cells, and both types of alkaloids have the *normal* configuration, it then follows that the side chain perpendicular to the ring plane in rhynchophylline and possibly the additional Me group are the features that are responsible for the antagonistic behavior. It is conceded that conformations in the solid state do not necessarily match those in solution. Nevertheless, these results are a firm experimental basis for further investigations, *e.g.* molecular modeling, to understand the pharmacological mechanism of action.

Experimental Part

Alkaloids. Peruvian *U. tomentosa* root was extracted with supercritical CO_2 (saturated with H_2O, 300 bar and 60° with a flow ratio of 30 kg CO_2/kg drug), followed by conventional acid-base workup. Column chromatography on silica gel (*Merck*) with gradient elution from AcOEt/hexane 9 : 1 to AcOEt/MeOH 9 : 1 yielded the pure alkaloids, which were characterized by conventional 2D-NMR techniques. All data were in complete accordance with the literature [19].

Crystallography. Single crystals were obtained by slow evaporation of solns. of **1** in MeOH/H_2O 95 : 5 and **4** in AcOEt. Compound **5** crystallized from many solvents as fine needles, too small for structure determination.

However, suitable crystals were obtained from acetone/hexane 1:1. Diffraction intensitiy data were measured *via* ϕ and ω scans with a *Nonius Kappa CCD* diffractometer and graphite-monochromated MoK_α radiation ($\lambda =$ 0.71073 Å); structure solution by direct methods (SHELXS-86) and refinement on F^2 (SHELXL-97). Positions of H-atoms on C-atoms were calculated after each refinement cycle, and H-atoms on N-atoms were located in difference-electron-density maps and refined with isotropic displacement parameters. The three structures crystallize in chiral space groups, but the data sets did not allow the determination of their absolute structures by using *Flack*'s *x*-parameter refinement [20]. CCDC 189178 (for **1**), 189179 (for **4**), 189180 (for **5**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

REFERENCES

[1] J. S. Shi, G. X. Liu, Q. Wu, Y. P. Huang, X. D. Zhang, *Acta Pharmacol. Sin*. **1992**, *13*, 35; J. S. Shi, G. X. Liu, Q. Wu, W. Zhang, X. N. Huang, *Chin. J. Pharmacol. Toxicol*. **1989**, *3*, 205.

[2] A. S. Sun, G. X. Liu, X. Y. Wang, W. Zhang, X. N. Huang, *Chin. J. Pharmacol. Toxicol*. **1988**, *2*, 93; W. Zhang, G. X. Liu, X. N. Huang, *Acta Pharmacol. Sin*. **1987**, *8*, 425; W. Zhang, G. X. Liu, *Acta Pharmacol. Sin*. **1986**, *7*, 426.

[3] Y. Zhu, G. X. Liu, X. N. Huang, *Chin. J. Pharmacol. Toxicol*. **1993**, *7*, 117.

[4] M. Wurm, L. Kacani, G. Laus, K. Keplinger, M. P. Dierich, *Planta Med*. **1998**, *64*, 701.

[5] I. Lemaire, V. Assinewe, P. Cano, D. V. C. Awang, J. T. Arnason, *J. Ethnopharmacol.* **1999**, *64*, 109.

[6] H. Wagner, B. Kreutzkamp, K. Jurcic, *Planta Med*. **1985**, *51*, 419.

[7] K. Keplinger, G. Laus, M. Wurm, M. P. Dierich, H. Teppner, *J. Ethnopharmacol*. **1999**, *64*, 23.

[8] G. Laus, D. Brössner, K. Keplinger, *Phytochemistry* **1997**, *45*, 855.

[9] G. Laus, D. Brössner, G. Senn, K. Wurst, *J. Chem. Soc., Perkin Trans. 2* **1996**, 1931.

[10] I. Muhammad, I. A. Khan, N. H. Fischer, F. R. Fronczek, *Acta Crystallogr., Sect. C* **2001**, *57*, 480.

[11] H. Liu, X. Feng, *Phytochemistry* **1993**, *33*, 707.

[12] J. D. Phillipson, S. R. Hemingway, C. E. Ridsdale, *Lloydia* **1978**, *41*, 503.

[13] P. Tantivatana, D. Ponglux, S. Wongseripipatana, J. D. Phillipson, *Planta Med.* **1980**, *40*, 299; P. Tantivatana, D. Ponglux, V. Jirawongse, Y. Silpvisavanont, *Planta Med.* **1979**, *35*, 92; J. D. Phillipson, S. R. Hemingway, *Phytochemistry* **1975**, *14*, 1855.

[14] D. Ponglux, P. Tantivatana, S. Pummangura, *Planta Med.* **1977**, *31*, 26; J. D. Phillipson, S. R. Hemingway, *Phytochemistry* **1975**, *14*, 1855.

[15] I. Sakakibara, H. Takahashi, S. Terabayashi, M. Yuzurihara, M. Kubo, A. Ishige, M. Higuchi, Y. Komatsu, M. Okada, M. Maruno, C. Biqiang, H. X. Jiang, *Phytomedicine* **1998**, *5*, 83; N. Aimi, T. Shimizu, H. Sada, H. Takayama, S. Sakai, S. Wongseripipatana, D. Ponglux, *J. Chem. Soc., Perkin Trans. 1* **1997**, 187; G. Laus, H. Teppner, *Phyton (Austria)* **1996**, *36*, 185; T. S. Kam, K. H. Lee, S. H. Goh, *Phytochemistry* **1992**, *31*, 2031.

[16] M. Shamma, R. J. Shine, I. Kompis, T. Sticzay, F. Morsingh, J. Poisson, J. L. Pousset, *J. Am. Chem. Soc*. **1967**, *89*, 1739.

[17] T. M. Kutchan, *Phytochemistry* **1993**, *32*, 493; M. Rueffer, N. Nagakura, M. H. Zenk, *Tetrahedron Lett*. **1978**, *18*, 1593.

[18] G. Laus, *J. Chem. Soc., Perkin Trans. 2* **1998**, 315.

[19] H. Toure, A. Babadjamian, G. Balansard, R. Faure, P. J. Houghton, *Spectrosc. Lett.* **1992**, *25*, 293; D. Arbain, M. M. Putri, M. V. Sargent, M. Syarif, *Aust. J. Chem.* **1993**, *46*, 863; H. Seki, H. Takayama, N. Aimi, S. Sakai, D. Ponglux, *Chem. Pharm. Bull.* **1993**, *41*, 2077.

[20] H. D. Flack, G. Bernadinelli, *J. Appl. Crystallogr.* **2000**, *33*, 1143; H. D. Flack, G. Bernadinelli, *Acta Crystallogr., Sect. A* **1999**, *55*, 908; H. D. Flack, *Acta Crystallogr., Sect. A* **1983**, *39*, 876.

Received August 2, 2002