Structure of *trans*-Isoshinanolone in the Crystal and in Solution*

Gerhard Bringmann[a], Robert-Michael Pfeifer[a], Matthias Breuning[a],
Matthias Reichert[a], Kim Messer[a], Michaela Schraut[a], and Gabor Tóth[b,c]

[a] Institute of Organic Chemistry, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany
[b] Institute of General and Analytical Chemistry, Budapest University of Technology and Economics, Szt. Gellérttér 4, H-1111 Budapest, Hungary
[c] Present address: IVAX Drug Research Institute, H-1045 Budapest, Berlini u. 47-49, Hungary

Reprint requests to Prof. Dr. G. Bringmann. Fax: +49 931 888 4755.
E-mail: bringman@chemie.uni-wuerzburg.de

The first X-ray structure analysis of authentic, not derivatized (racemic) *trans*-isoshinanolone, a wide-spread acetogenic tetralone, is described. In the crystal, two slightly different conformers are found, along with the corresponding enantiomers. The 'homomeric' conformers differ by their H-bonding interactions with the respective neighboring molecules. Rows of a single enantiomeric species, but with the two conformers linked 'head-to-head', 'tail-to-tail', are surrounded by four analogous rows of the corresponding enantiomers. Apparently the interactions between these heterochiral rows are the reason for the enhanced crystallization tendency of the racemate as compared to that of enantiomerically pure isoshinanolone. The structures in the crystal are compared with those calculated (gas phase) and with the structure in solution.

Key words: Natural Products, Crystal Structure, Quantum Chemical Calculations, NMR Methods

Introduction

Trans-isoshinanolone (**1**) and its *cis*-isomer **2** (Fig. 1) are widely distributed naturally occurring tetralones isolated from a variety of different plant families [2 – 13], biosynthetically formed *via* the acetate-malonate pathway [14]. We have recently reported on their enhanced formation in cell tissue cultures of *Ancistrocladus heyanus* and *Triphyophyllum peltatum* upon chemical, physical, or biotic stress [14 – 16]. For the unambiguous identification of all four stereoisomers of isoshinanolone, *viz* the diastereomers **1** and **2** and their enantiomers, directly from crude plant extracts, we have established an efficient analytical device by HPLC coupled to circular dichroism (CD) spectroscopy [17]. The absolute configuration of these tetralones was established by CD spectroscopy on their dibenzoates using the exciton chirality method [2] and by degradative methods [18]. Due to the poor tendency of enantiomerically pure *trans*-isoshinanolone (**1**) to crystallize [2, 4, 5, 19], no
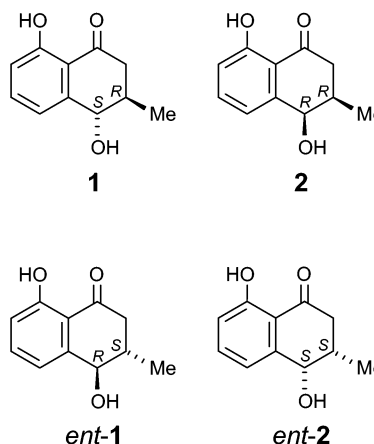

Fig. 1. *trans*-Isoshinanolone (**1**) and its *cis*-diastereomer **2**, and their enantiomers, *ent*-**1** and *ent*-**2**, respectively.

X-ray structure analysis of this secondary metabolite has yet been reported [20]. Racemic mixtures of **1**, by contrast, possess reasonable crystallization properties [2, 18], which allowed us to perform an X-ray diffraction analysis, as now reported in this paper. The crystal structure of **1** shows the presence of two slightly di-

---

* "Acetogenic Isoquinoline Alkaloids", Part 157. For Part 156, see: Lit. [1].
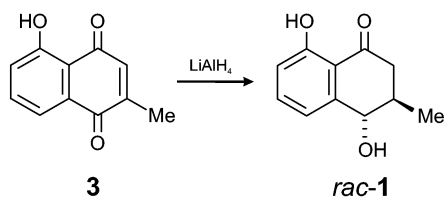
Scheme 1. Preparation of **1** from plumbagin (**3**).

vergent conformers, plus the respective enantiomers. These structures were found to match excellently with the calculated minimum structure. Furthermore, the conformational behavior of **1** in MeOH was examined by ^{1}H NMR spectroscopy.

Results and Discussion

Synthesis

Racemic *trans*-isoshinanolone (*rac*-**1**) was prepared according to literature procedures [2, 18] by diastereoselective reduction of the commercially available (Aldrich) naphthoquinone plumbagin (**3**) with excessive LiAlH$_4$ at -20 °C to room temperature (Scheme 1). Despite these relatively harsh conditions, no substantial quantities of the *cis*-isomer, *rac*-**2**, were formed, nor was any further reduction of the remaining ketone function of **1** observed. This is certainly a consequence of the diminished carbonyl reactivity of the ketone, which, due to the *ortho*-OH group, can rather be addressed as a phenylogous carboxylic acid or, in its deprotonated form, as a phenylogous (lithium) carboxylate.

Structure of *rac*-**1** in the crystal

Crystalline material of racemic *trans*-isoshinanolone (**1**), suitable for an X-ray diffraction analysis, was obtained by slow crystallization from MeOH/water. The compound crystallized in the monoclinic space group $P2_1$/n with eight formula units in the unit cell. Crystal data are reported in Table 1; selected bond
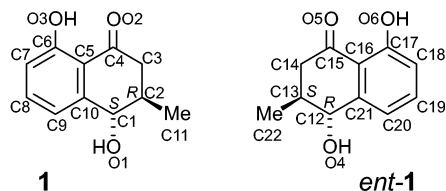
Fig. 2. Atom numbering for the crystal structure of racemic *trans*-isoshinanolone (*rac*-**1**).

Table 1. Crystal data and structure refinement for *rac*-**1**.

Empirical formula	C$_{11}$H$_{12}$O$_3$
Molecular mass	192.21
Crystal system	monoclinic
Space group	$P2_1$/n
Unit cell dimensions	$a = 915.96(4)$ pm
	$b = 1191.38(6)$ pm
	$c = 1763.47(8)$ pm
	$\beta = 101.611(10)°$
Formula units per cell	$Z = 8$
Unit cell volume (V)	$1885.2(15) \times 10^6$ pm^3
Calculated density	1.355 Mg/m^3
Crystal size	$500 \times 400 \times 200$ μm^3
Radiation, wavelength	Mo-K_α, 71.073 pm
Temperature of measurement	173(2) K
θ range	1.00° to 26.39°
Range in *hkl*	$-11 \leq h \leq +11$
	$-14 \leq k \leq +14$
	$-22 \leq l \leq +22$
Total no. reflections	14880
Independent reflections	3859 [R(int) = 0.0335]
Reflections with $I > 2\sigma(I)$	2944
Data / parameters	3859/268
Goodness-of-fit on F^2	1.037
Final R indices$^{a),b)}$ [$I > 2\sigma(I)$]	$R1 = 0.0492$
	$wR2 = 0.1343$
R Indices (all data)	$R1 = 0.0662$
	$wR2 = 0.1247$
g_1, g_2^{c}	0.070900, 0.479000
Largest diff. peak and hole	0.36/0.28 e 10^{-6} pm^{-3}

$^{a)}$ $R1 = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$; $^{b)}$ $wR2 = [\Sigma w(F_o^2 - F_c^2)^2 / \Sigma w(F_o^2)^2]^{1/2}$; $^{c)}$ $w = [\sigma^2(F_o^2) + (g_1 P)^2 + g_2 P]^{-1}$; $P = [\max(F_o^2, 0) + 2F_c^2]/3$.

Table 2. Selected bond lengths (pm) of *rac*-**1** in the solid state.

C1-O1	142.3 (2)	C16-C17	141.2 (2)
C1-C10	151.9 (2)	C16-C21	141.4 (2)
C1-C2	152.3 (2)	C15-C16	146.0 (2)
C2-C11	152.0 (2)	O6-C17	134.8 (2)
C2-C3	153.0 (2)	O5-C15	124.1 (2)
C3-C4	149.3 (2)	O4-C12	142.8 (2)
C4-O2	123.3 (2)	C20-C21	138.3 (2)
C4-C5	146.9 (2)	C21-C12	151.7 (2)
C5-C6	140.7 (2)	C18-C19	137.7 (2)
C5-C10	141.3 (2)	C18-C17	138.8 (2)
C6-O3	135.4 (2)	C19-C20	139.0 (2)
C6-C7	138.6 (2)	C14-C15	149.4 (2)
C7-C8	137.5 (2)	C13-C14	152.2 (2)
C8-C9	139.3 (2)	C12-C13	151.6 (2)
C9-C10	138.1 (2)	C13-C22	152.4 (2)

lengths are given in Table 2 according to the atom numbering shown in Fig. 2.

In the crystal the cyclohexenone moiety of **1** adopts a half-chair conformation with the substituents at the stereocenters – expectedly – occupying the energetically favored diequatorial positions (Fig. 3a and b).
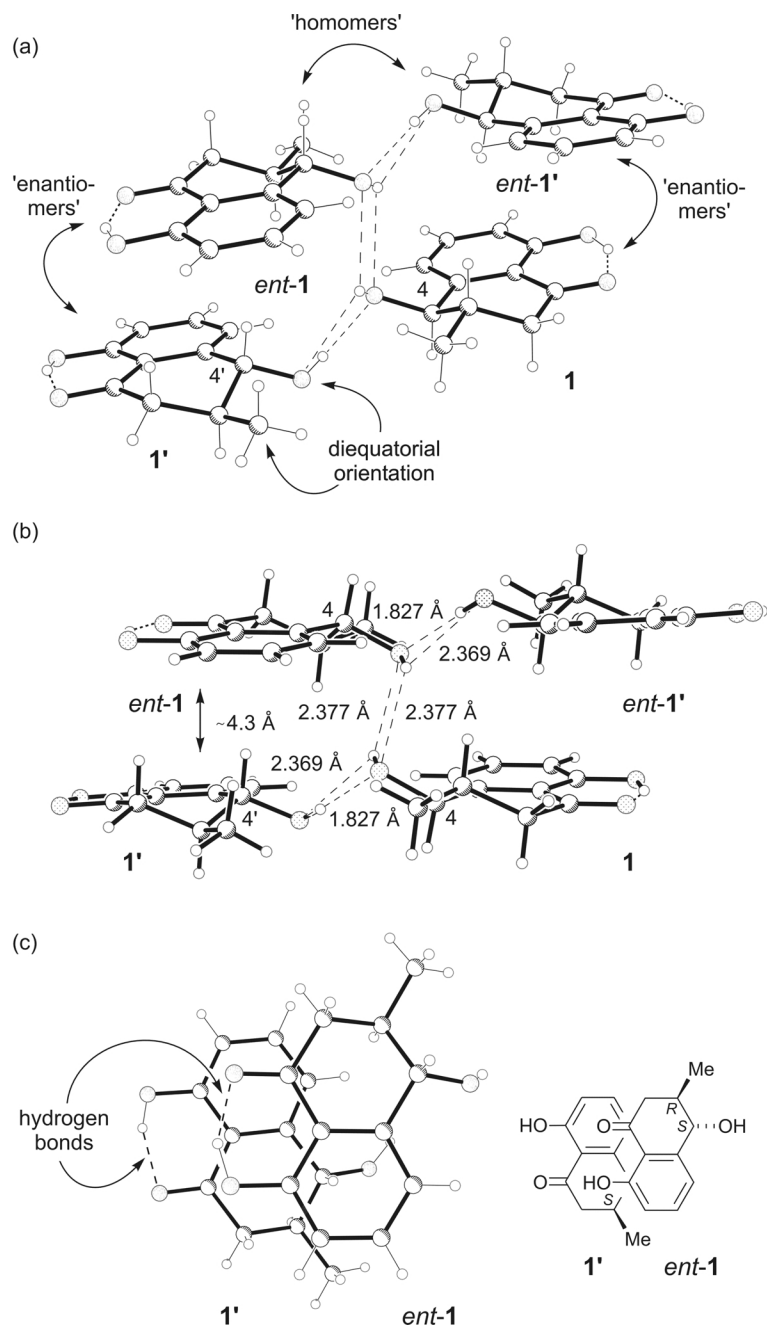
Fig. 3. Different (partial) views of the unit cell of *trans*-isoshinanolone.

The 'Northern' part ('head') of the molecule is characterized by a strong chelating intramolecular hydrogen bond between the phenolic OH-group at C8 and the neighboring carbonyl oxygen at C1, so that in principle only the 'Southern' (in particular the 'Southeastern' part) of the molecule ('tail'), with its free hydroxy function at C4 (and with its two stereogenic centers at C4 and C3) is open for *intermolecular* hydrogen bonding. This is realized by a 'tail-to-tail' interaction of two formally homochiral (but conformationally not fully identical, see below) molecules, *e.g.* between **1** and **1'** (see Fig. 3a and b), linked *via* a strong hydro-

gen bond C4–O···H–O–C4' (1.827 Å) – plus a significantly weaker bond C4–O–H···O–C4' (2.369 Å). As already evident from the space group, the same hydrogen bonding interaction is observed between *ent-1* and *ent-1'*. Given the presence of a racemate and the existence of two conformationally slightly divergent forms for each of the two enantiomers of *trans*-isoshinanolone, there are, altogether, four structural species in the crystal. Two of these molecules, *viz* **1** and its enantiomer *ent-1*, constitute the electron-donating partners in the strong hydrogen bondings, while **1'** and *ent-1'* act as the hydrogen donors.

Each of the 'homodimers' **1···1'** mentioned above is connected to another, enantiomeric 'homodimer', *ent-1'···ent-1*, *via* weak OH-bonds (2.377 Å), yet only between the oxygen at C4 of **1** with the hydrogen of the hydroxy group at C4 in *ent-1* and, *vice versa*, of the oxygen at C4 of *ent-1* with the hydrogen of the hydroxy function of C4 of **1** – but not through interactions between **1'** and *ent-1'*, since these molecules are located too far from each other. This leads to a kind of cluster consisting of four molecules of different structures, *ent-1*, *ent-1'*, **1'**, and **1**, with the distances *within* this array being significantly shorter than from these four molecules to any other molecules outside the cluster.

Due to the symmetry properties of the space group, the 'Northern' ('head') parts of the molecules, *e.g.* of **1**, with their chelated keto/phenol functionality, are located *vis-à-vis* to the respective 'head' portion of the nearest molecule in the neighboring cluster, thus leading to a not quite linear row of molecules ···(**1···1'**)·······(**1'···1**)·······(**1···1'**)··· (with **1···1'** being linked tail-to-tail and **1······1** and **1'······1'** head-to-head), with only 'homochiral' (though alternatingly slightly modified) species existing within one row. Each such row of 'homomers' (*e.g.* one consisting of **1** and **1'**) is neighbored by four enantiomeric rows (*i.e.* built up from *ent-1* and *ent-1'*), one row 'before' and one 'behind' this row each in the same 'layer', and one each stacking 'above' and 'below' (as seen, in part, in Fig. 3b and c).

The stacking perpendicular to the plane of the bicycle is further stabilized by dipole-dipole interactions (∼ 4.3 Å), since the rings are arranged in a way that the carbonyl function at C1 (*e.g.* of *ent-1*, Fig. 3a) is oriented above the electron-rich aromatic ring of the neighboring enantiomer (here of **1'**) and the phenolic OH-function (here of *ent-1*) is nearly in the approach vector of the carbonyl group (here of **1'**), while the
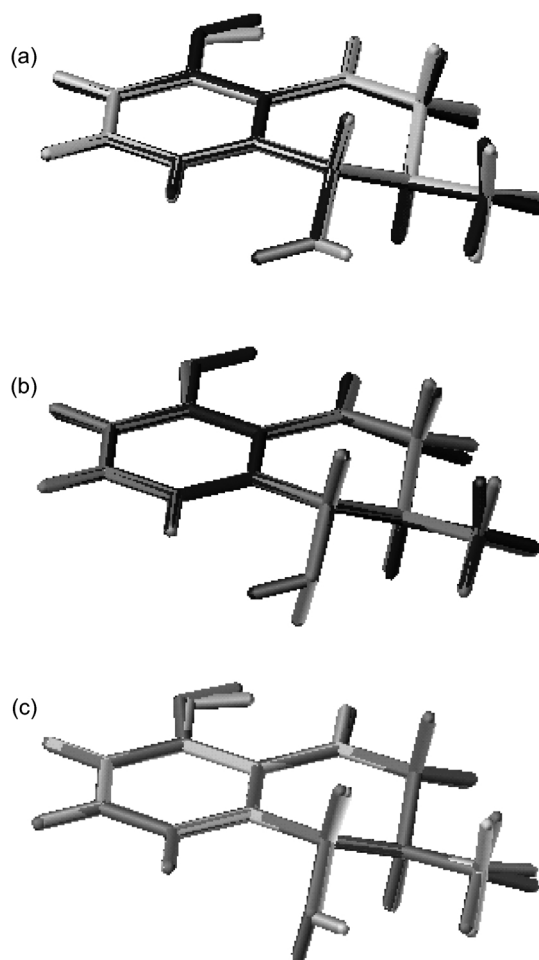

Fig. 4. Matchplots of the two conformational geometries of *trans*-isoshinanolone, **1** (light gray) and **1'** (dark gray) in the crystal (a), and of each of these two experimental structures in comparison to the calculated structure (medium gray) of this natural product (b).

overlap of the bicyclic ring systems is only marginal. These interactions between the heterochiral rows – the H-bridging between **1** and *ent-1* and the likewise mentioned dipole-dipole interactions – might be the reason why the racemate crystallizes much better than the enantiopure compound.

The slightly different geometries of the two isoshinanolone conformers in the crystal, **1** and **1'** (and thus also of their enantiomers, *ent-1* and *ent-1'*), can be visualized by a matchplot as seen in Figure 4a, the major (still very small) difference consisting in the – expectedly divergent – array in the proximity of the 'Southern' OH-group at C4, which gives rise to two hydrogen

bonding partners in **1**, while it is only one for **1'** (see above). In order to investigate in how far the two conformational arrays of these two isoshinanolone geometries in the crystal correspond to the global energetic minimum or whether they are (possibly both of them) a result of packing effects, we calculated the minimum structure of *trans*-isoshinanolone using the semiempirical AM1 method [21]. As seen from the matchplot in Figure 4b, both of the two conformational forms of isoshinanolone, **1** and **1'** (and thus of course also the enantiomers) found in the crystal, fit with the calculated structure nearly perfectly; an exception are the dihedral angles at the OH-groups, which, however, differ also between the two experimental structures. This good agreement excludes any major crystal related distortions and, simultaneously, demonstrates the predictive value of this theoretical method.

NMR investigations

In solution at room temperature the cyclohexenone moiety of **1** can appear in an equilibrium of the two rapidly interconverting half-chair conformations **A** and **B** (Scheme 2). Conformer **A**, which was found to be more stable in the AM1 calculations and also occurs in the solid state (see above), should be energetically favored also in solution, due to the diequatorial orientation of both, the methyl substituent at C-3 and the OH-group at C-4. The preference of the 4-OH substituent for the equatorial position appears remarkable, because owing to the unfavorable steric interaction (1,3-allylic strain [22]) between the substituent and the fused aromatic ring, this arrangement is destabilized. Due to the solvatation of 4-OH, this effect should be further enhanced in methanol solution.

The population of the minor conformer **B** with the two substituents in diaxial position was examined by NMR spectroscopy, using the coupling constants $J_{2,3}$ and $J_{3,4}$ between the protons 2-H/3-H, and 3-H/4-H, respectively, as the probe. Applying the modified Karplus equation, taking into account also the electronegativity of the substituents [23, 24], the J value calculated for antiperiplanar-arranged 2-H/3-H hydrogens is 12.4 Hz, whereas for the gauche arrangement it is 3.2 Hz. In the spectrum of **1**, a value of 10.8 Hz was measured for the *trans* 2-H/3-H protons, indicating a conformational 81:19 equilibrium of **A** and **B**. The corresponding values calculated for the 3-H/4-H coupling constant were 10.4 Hz and 3.4 Hz, respectively, while the measured value is 8.9 Hz. On the basis of these data
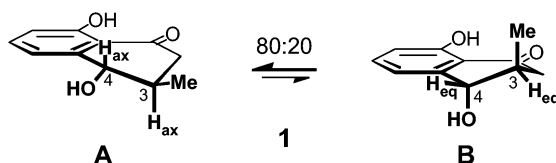
Scheme 2. Experimentally observed conformers **A** and **B** of **1**.

the equilibrium should be ca. 79:21, which is in good agreement with the above results.

Experimental and Computational Section

*Synthesis of racemic trans-isoshinanolone (rac-**1**)*

Racemic *trans*-isoshinanolone (**1**) was prepared according to literature procedures [2, 18]. Repeated slow crystallization from MeOH/water delivered colorless needles, m.p. 114 – 115 °C, suitable for an X-ray diffraction analysis.

Crystal structure determination

A crystal of **1** was glued on the tip of a glass fibre and used for intensity data collection on a BRUKER Smart-APEX with a D8-goniometer, employing Mo-K_α radiation in an ω-scan mode. The data were integrated with SAINT [25], and an empirical adsorption correction was applied (SADABS II) [26]. The structure was solved by direct methods (SHELXS-97) [27] and refined by full matrix least-squares calculations on F^2 (SHELXL-97) [28]. All non-hydrogen atoms in **1** were located by difference Fourier syntheses and were refined anisotropically. All hydrogen atoms were placed in idealized calculated positions and allowed to ride on their corresponding carbon atoms with fixed isotropic contributions. Further information on crystal data and data collection are summarized in Table 1. Interatomic distances are given in Table 2. For details on the labelling of the atoms see Fig. 1. All other information on the crystal structure analysis has been deposited with the Cambridge Crystallographic Data Centre. The data are available free of charge as a CIF file upon request on quoting CCDC No. 220317, the author names, the journal name, and the page numbers.

Computational methods

Quantum chemical calculations were performed on i686-LinuX workstations using the VAMP 6.5 [29] program package. Input geometries were obtained using the TRIPOS force field as implemented within SYBYL 6.7.1 [30] on a Silicon Graphics OCTANE (R10000).

NMR studies

The NMR spectra were taken on a Bruker Avance 500 spectrometer at room temperature in methanol-d$_4$. Chemical shifts are given on the δ-scale.

[1] G. Bringmann, C. Schneider, U. Möhler, R.-M. Pfeifer, R. Götz, L. Aké Assi, E.-M. Peters, K. Peters, Z. Naturforsch. **58b**, 577 (2003).

[2] M. Tezuka, C. Takahashi, M. Kuroyanagi, M. Satake, K. Yoshihira, S. Natori, Phytochemistry **12**, 175 (1973).

[3] V. Kumar, K. M. Meepagala, S. Balasubramaniam, Phytochemistry **24**, 1118 (1985).

[4] G. M. K. B. Gunaherath, A. A. L. Gunatilaka, M. U. S. Sultanbawa, S. Balasubramaniam, Phytochemistry **22**, 1245 (1983).

[5] J. Bhattacharyya, V. R. Carvalho, Phytochemistry **25**, 764 (1986).

[6] K. Likhitwitayawuid, R. Kaewamatawong, N. Ruangrungsi, J. Krungkrai, Planta Medica **64**, 237 (1998).

[7] M. Lavault, J. Bruneton, Planta Medica (Suppl.), 17 (1980).

[8] S. W. Hanson, M. Crawford, D. P. J. Thanasingh, Phytochemistry **20**, 1162 (1981).

[9] G. Bringmann, C. Schneider, L. Aké Assi, Planta Medica (Suppl. 2) **57**, 10 (1991).

[10] N. H. Anh, H. Ripperger, A. Porzel, T. V. Sung, G. Adam, Phytochemistry **44**, 549 (1997).

[11] S.-M. Zhong, P. G. Waterman, J. A. D. Jeffreys, Phytochemistry **23**, 1067 (1984).

[12] M. Bin Zakaria, J. A. D. Jeffreys, P. G. Waterman, S.-M. Zhong, Phytochemistry **23**, 1481 (1984).

[13] P. Richomme, B. Papillon, P. Cabalion, J. Bruneton, Pharm. Acta Helv. **66**, 88 (1991).

[14] G. Bringmann, M. Wohlfarth, H. Rischer, M. Rückert, J. Schlauer, Tetrahedron Lett. **39**, 8445 (1998).

[15] G. Bringmann, J. Schlauer, M. Rückert, B. Wiesen, K. Ehrenfeld, P. Proksch, Plant Biology **1**, 581 (1999).

[16] G. Bringmann, H. Rischer, M. Wohlfarth, J. Schlauer, L. Aké Assi, Phytochemistry **53**, 339 (2000).

[17] G. Bringmann, K. Messer, W. Saeb, E.-M. Peters, K. Peters, Phytochemistry **56**, 387 (2001).

[18] G. Bringmann, M. Münchbach, K. Messer, D. Koppler, M. Michel, O. Schupp, M. Wenzel, A. M. Louis, Phytochemistry **51**, 693 (1999).

[19] G. Bringmann, M. Rückert, K. Messer, O. Schupp, A. M. Louis, J. Chromatogr. A **837**, 267 (1999).

[20] For the crystal structure of the 5,7-dibromo derivative of *cis*-isoshinanolone, see lit. [17].

[21] M. J. S. Dewar, E. G. Zoebisch, E. Healy, J. J. P. Stewart, J. Am. Chem. Soc. **107**, 3902 (1985).

[22] F. Johnson, Chem. Rev. **68**, 375 (1968).

[23] C. A. G. Haasnoot, F. A. A. M. de Leeuw, C. Altona, Tetrahedron **36**, 2783 (1981).

[24] SpecTool software, Chemical Concepts in E. Pretsch, J. T. Clerc, Spectra Interpretation of Organic Compounds, VCH, Weinheim (1997).

[25] Bruker-AXS Inc., SAINT-NT, Madison WI (2000).

[26] G. M. Sheldrick, SADABS 2.0, University of Göttingen (2000).

[27] G. M. Sheldrick, Acta Crystallogr. Sect. A **461**, 467 (1990).

[28] G. M. Sheldrick, Program for the refinement of crystal structures, Göttingen (1997).

[29] G. Rauhut, J. Chandrasekhar, A. Alex, B. Beck, W. Sauer, T. Clark, VAMP 6.5, available from Oxford Molecular Ltd., The Medawar Centre, Oxford Science Park, Sandford-on-Thames, Oxford, OX4 4GA, England.

[30] SYBYL: Tripos Associates, 1699 St. Hanley Road, Suite 303, St. Louis, MO, 63144.