X-ray Crystal Structure of Woodinine and Conformational Analysis by Semiempirical and ^{1}H-NMR Methods†

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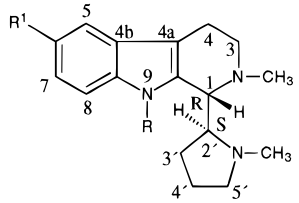
The molecular structure of (−)-woodinine (**1**), a carboline-based alkaloid with antibacterial and antimycobacterial activities, was investigated by X-ray crystallographic, NMR, and semiempirical quantum chemical methods. Compound **1** was prepared by reacting 5-bromotryptamine and (*S*)-(−)-*N*-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxyaldehyde in a Pictet−Spengler-type reaction. The debrominated analogue of **1** was methylated at the indole nitrogen with methyl methane sulfonate after deprotonation with *n*-BuLi. The X-ray crystal structure of **1** showed the indole ring in the expected planar conformation, the pyrrolidine ring in an envelope conformation, and a weak intramolecular hydrogen bond between the pyrrolidine nitrogen and the proton of the indole nitrogen. NMR experiments indicated that this hydrogen bond is not present in solution and that further differences exist between the crystal and the solution structures of **1**. By semiempirical quantum chemical methods, different local energy minimum conformations of **1**, resulting from inversions within the piperideine moiety and from rotation of the pyrrolidine ring, were calculated. The totality of all NOE signals can only be explained to originate from a population of some of these conformers, and additionally from different envelope and twisted conformations of the pyrrolidine ring.

The naturally occuring (−)-woodinine (**1**) is a tetrahydro-*β*-carboline-based alkaloid present in the tunicate *Eudistoma fragum* and was first isolated and characterized by Païs and co-workers.[1]

Eudistoma alkaloids are of medicinal interest because of their antitumor, antiviral, and antibacterial properties.[2−8] For example, compound **1** has both antibacterial and antimycobacterial activity.[1] The enantiomers of woodinine (**1**) have differing antimycobacterial potencies against *Mycobacterium tuberculosis* H37Ra[9] [MIC (−)-woodinine 8 *μ*g/mL, MIC (+)-woodinine 4 *μ*g/mL] (Mahboobi, S.; Meindl, U. Unpublished results). Based on laboratory syntheses, which involved the use of L-proline to introduce the pyrrolidine unit, the absolute configuration of **1** was established.[1,10−13] These conclusions, however, have not been confirmed by X-ray crystal analysis. This paper reports the first X-ray crystal structure of **1**.

To obtain insights for the design of more potent analogues of **1**, we focused next on determining the most favored orientation of the pyrrolidine ring relative to the carboline system in solution. However, NMR analysis was not straightforward because the C-1−methine proton of **1**, which would be expected to appear as a doublet due to the coupling to the C-2′−methine proton of the pyrrolidine ring, actually appears as a broad singlet in the ^{1}H-NMR spectrum;[1,10−13] thus, no reliable information about the preferred conformation can be obtained from the proton−proton coupling. In an effort to gain an indication for the most favored conformation of the piperideine relative to the pyrrolidine ring, X-ray analysis, semiempirical quantum chemical calculations, and nuclear Overhauser effect (NOE) experiments with **1** have been performed. This paper reports the results of these studies.



1 R = H R^1 = Br (-) - Woodinine
2 R = H R^1 = H
3 R = CH_3 R^1 = H

Results and Discussion

The crystal structure of **1** is shown in Figure 1. As expected, the indole system is planar, and the piperideine ring adopts a twisted conformation. The carbon atoms C-1 and C-4 are coplanar with the indole ring, while N-2 and C-3 are out of this plane by 0.348 Å and −0.469 Å, respectively. The pyrrolidine ring shows an envelope conformation. This structure indicates that a weak hydrogen bond between N-1′ and H-9 (distance 2.20 Å) stabilizes the position of the ring. Another strong hydrogen bond is formed between N-2 and the hydroxyl hydrogen of a solvent molecule.

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† Dedicated to Prof. Dr. W. Wiegrebe of the University of Regensburg on the occasion of his 65th birthday.

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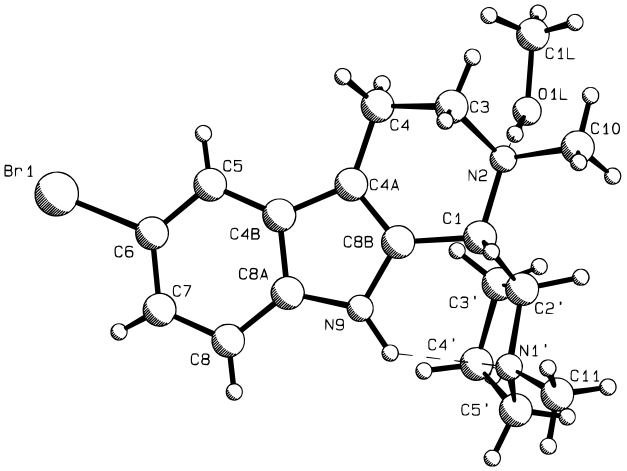
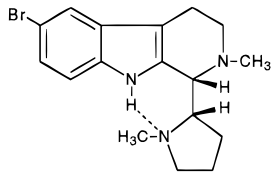


Figure 1. X-ray crystal structure of **1**.

^{1}H/^{1}H- and ^{1}H/^{13}C-NMR spectra were used in assigning the various protons of **1**. Neither H/D exchange nor protonation of the pyrrolidine- or piperideine-N by DCl altered the shape of the broad singlet of H-1. This observation is inconsistent with Still's hypothesis[10,11] that an intramolecular hydrogen bond exists between the indole N-H and the pyrrolidine nitrogen (see **4**). Moreover, between −80° and +100 °C the signal of H-1 remained unaltered in toluene-d_8, whereas that of the indole N-H broadened as the temperature was raised.



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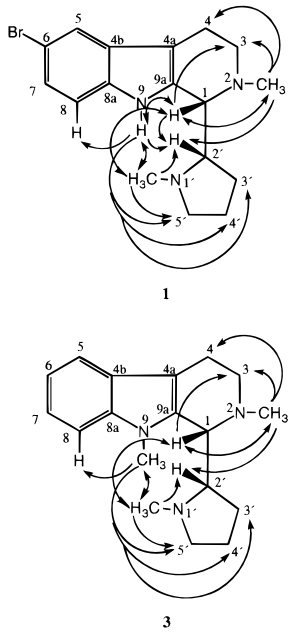
After deprotonation with *n*-BuLi,[14] the debrominated analogue of woodinine (**2**) was methylated at the indole nitrogen with methyl methane sulfonate to yield **3**. However, the ^{1}H-NMR signal of H-1 did not form a clear doublet, which indicated that the broadening of the H-1 singlet (4−6 Hz) is a result of the quadrupole effect of the neighboring nitrogen atom. Furthermore, the ^{1}H-NMR spectrum of **3**, where the possibility of forming an intramolecular hydrogen bond is eliminated, showed an identical signal for H-1.

Various organic solvents such as $CDCl_3$, C_6D_6, and DMSO-d_6 were examined for their effects on the NOE experiments; the best signal resolution was obtained by using C_6D_6 with **1** and $CDCl_3$ with **3**. The solvents were treated with basic Al_2O_3 in order to remove any traces of acid and H_2O and thus to guarantee that **1** and **3** were examined by NMR exclusively in their free-base form. The results of the NOE experiments are shown in Figure 2. For the indole N-CH_3 derivative **3**, the methyl groups of the indole and the pyrrolidine nitrogen are in proximity to each other. Furthermore, COSY showed only a weak coupling of H-1 and H-2′.

In the crystal structure of **1**, N-2 is above and C-3 below the indole plane. The methyl group at N-2 is in an equatorial position slightly below the plane; an axial position above the plane is energetically unfavorable because of repulsive interactions with the pyrrolidine ring; however, inversion of the piperideine ring leads to a conformation where N-2 is below and C-3 above the



1 **3**

Figure 2. Qualitative results showing the NOEs between the various protons of woodinine (**1**) and compound **3**.

indole plane. An axial position below the plane and an equatorial one above the plane are both possible for the methyl group at N-2. Thus, three different conformers of the methylpiperideine moiety must be considered (Figure 3).

For these conformers, the rotational barriers and local minima of the rotatable bond between the pyrrolidine and the piperideine rings were calculated by means of semiempirical quantum chemical methods (MOPAC 6.0, AM1 Hamiltonian, using the Molecular Modeling software SYBYL 6.1, Tripos Associates, St. Louis, MO, on a SG Indigo XS 24). The torsion angle N-2−C-1−C-2′−N-1′ served as reference. It was varied from 0° to 330° with an increment of 30°.

All other inner coordinates, except those dihedral angles defining the given piperideine conformers, were freely optimized in MOPAC using the GNORM = 0.01 keyword. The resulting potential map is presented in Figure 4. The general properties of a C·sp3−C·sp3 bond with a favored staggered conformation are preserved. As expected, the global minimum of each curve corresponded to a nearly antiperiplanar orientation of nitrogens N-2 and N-1′. These global minimum conformations are drawn in Figure 3. The lowest energies belong to conformation C, inasmuch as there is no repulsive interaction between the axial methyl group at N-2 and the rotating pyrrolidine ring. The highest rotational barriers occur if the pyrrolidine atom C-3′ passes an equatorial methyl group at N-2, and especially if this methyl group is above the indole plane (conformation B).

Although the pyrrolidine ring was freely optimized in each step of the reaction coordinates, it did not change its conformation, and the methyl group at N-1′ did not invert. The standard deviations of the inner dihedral angles of the ring were not greater than 12°. Compared to the crystal structure with angles C-5′−C-4′−C-3′−C-2′ of −22.3° and N-1′−C-5′−C-4′−C-3′ of 39.5°, the AM1-optimized form is relatively flat (averages of these angles of 5.5° and −6°, respectively). Because this flattening is typical of the calculation of heterocycles

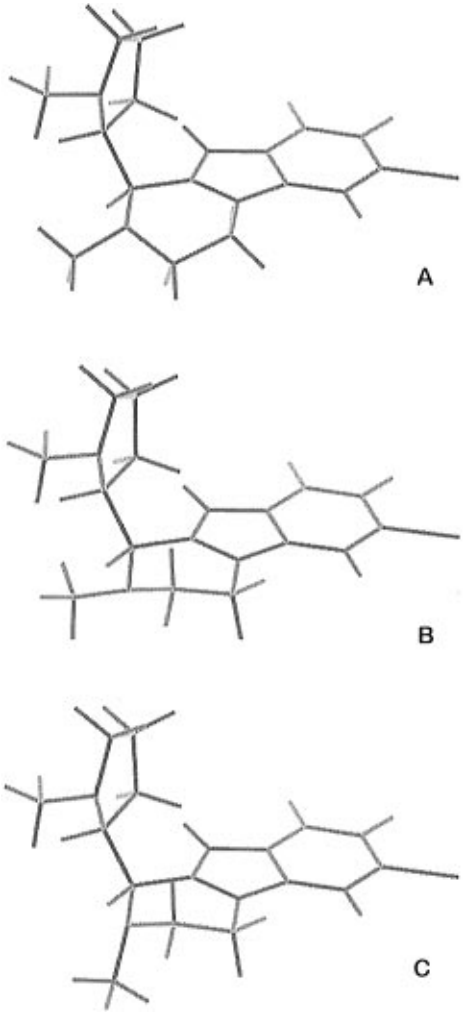


Figure 3. MOPAC minimum energy geometries of woodinine with regard to the possible *N*-methylpiperideine conformations. **A**: X-ray crystal structure where N-2 is above the indole plane, CH_3 equatorial, heat of formation (HF) 67.70 kcal/mol, torsion angle N-2−C-1−C-1′−N-1′ 171.8°. **B**: N-2 below the indole plane, CH_3 equatorial, heat of formation (HF) 61.17 kcal/mol, torsion angle 168.1°. **C**: N-2 below the indole plane, CH_3 axial, heat of formation (HF) 65.38 kcal/mol, torsion angle 164.9°.

with semiempirical quantum chemical methods, we have not systematically checked the different twist and envelope pyrrolidine conformations.

The results of the conformational analysis were useful in interpreting the NMR spectra and NOE signals. Our calculations predicted an approximate Maxwell−Boltzmann distribution at 20 °C *in vacuo* of 92.5% of conformation C, 4.5% of conformation B, and 2% of conformation A (the crystal-like one), each representing the N-2−N-1′ antiperiplanar orientation. All other local minima could be neglected. Although the distribution of conformers in solution could be markedly different from the calculated one, it is likely that the strongly dominating conformation C with the axial methyl group at N-2 would occur to a significant extent in the NMR probes.

To investigate the similarities and differences between theoretical and experimental structures, a comparison of interatomic distances of the three calculated minimum conformations and of the X-ray structure with maximum distances resulting from NOE signals was necessary.

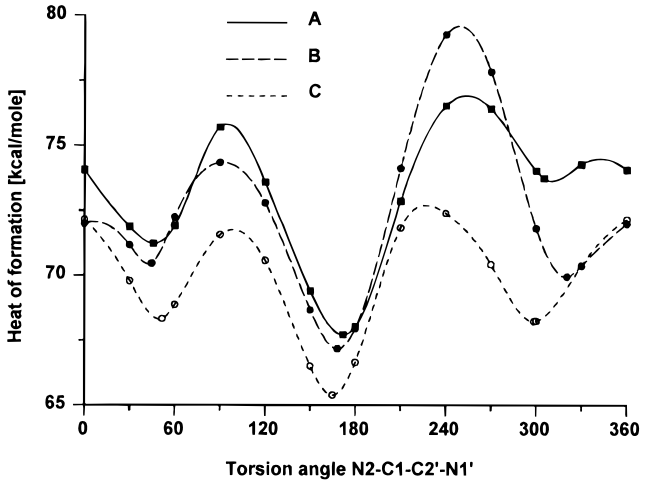


Figure 4. MOPAC potential maps for the rotation of the bond between the *N*-methylpiperideine and the pyrrolidine rings. **A**: N-2 above the indole plane, CH_3 equatorial. **B**: N-2 below the indole plane, CH_3 equatorial. **C**: N-2 below the indole plane, CH_3 axial.

Table 1. Comparison of the Strengths of the NOE Signals with the Proton−Proton Distances (in Å) Obtained by Either X-Ray Crystal or Molecular Orbital Methods

distance	NOE[a]	X-ray[b] (Å) (Figure 1)	MO[b] (Å) (Figure 3)
indole-H and H-3′	moderate	3.19	A: 3.77
			B: 3.65
			C: 3.55
indole-H and H-4′	moderate	2.61	A: 4.03
			B: 4.13
			C: 4.02
indole-H and H-5′	moderate	3.04	A: 2.64
			B: 2.72
			C: 2.67
H-1 and H-3	moderate	2.55	A: 3.14
			B: 3.87
			C: 3.81
N-CH_3 (piperideine) and H-4	strong	>4	A: 4.31
			B: 4.12
			C: 2.58

[a] For only one of the possible protons. [b] Shortest distance between the respective protons.

Table 1 summarizes some of these distances. For example, the X-ray crystal analysis showed that of the H-3′, H-4′, and H-5′ protons (i.e., one proton for each methylene group), only the H-4′ one is closer than 3 Å to the indole proton. This is due to the envelope conformation of the pyrrolidine ring. In the NMR analysis, however, NOEs were observed between the indole proton and H-3′, H-4′, and H-5′. This means that in solution the pyrrolidine ring exists in different conformations that give rise to these NOEs. Assuming an equilibrium of various twisted and enveloped conformations, the signals arising from H-4′ and H-5′ were consistent with an antiperiplanar pyrrolidine−piperideine orientation. The signal from H-3′, however, suggests a *gauche* conformation.

The observations made on the piperideine ring also led to contrasting results. On account of the measured NOEs, both the distance between H-1 and H-3, on the one hand, and the distance between one of the H-4 and one of the NCH_3 (piperideine) hydrogens, on the other, must be less than 3 Å. In this case, the semiempirical quantum chemical calculations showed that the position of the methyl group at the piperideine nitrogen is critical. If the methyl group assumes an equatorial

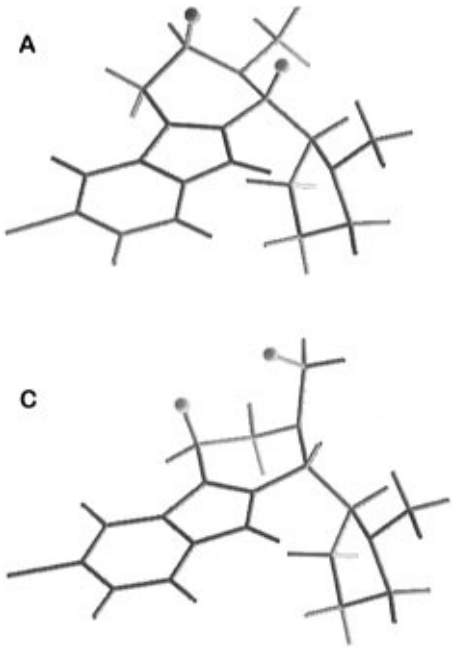


Figure 5. Illustration of distances < 3 Å within the *N*-methylpiperideine ring resulting from NOEs. Conformation **A** (N2 above the indole plane, CH_3 equatorial) explains the short distance between H-1 and H-3, conformation **C** (N-2 below the indole plane, CH_3 axial) that between N-2−CH_3 and H-4.

position as in the case with the X-ray crystal structure, then the distance between H-1 and H-3 is less than 3 Å (see Table 1 and Figure 5A). If, however, the methyl group is in an axial position, then the distance between one of the H-4 and one of the N-CH_3 (piperideine) hydrogens becomes less than 3 Å (see Table 1 and Figure 5C).

In the X-ray crystal structure of **1**, a weak hydrogen bond between the indole proton and the pyrrolidine nitrogen is present; however, the NMR studies give no indication for such an interaction. It should be noted that the crystal structure represents a single conformation, while NMR data represent a variety of conformations in solution. It can be concluded that the broad singlet observed with H-1 is not, as postulated by Still,[4] caused by a hydrogen bond but is rather a result of the energetically most favored-conformations in solution. In these conformations, the pyrrolidine nitrogen adopts an antiperiplanar orientation to the piperideine nitrogen and approaches the indole proton. As a result, the distances between the indole proton and H-4′ and H-5′, respectively, are less than 3 Å. The orientation of N-1′ and the indole proton, however, is not optimal for forming a typical hydrogen bond.[15] Finally, this work illustrates that, for the determination of the solution conformations of even relatively simple molecules, a variety of methods must be employed.

Experimental Section

General Experimental Procedures. Melting points were measured with a Büchi 512, Reichert hot-stage microscope. ^{1}H- and ^{13}C-NMR data were obtained with a Bruker ARX 400 (400 MHz). Mass spectrometry was performed on a Varian MAT 112 S/SS, 70 eV. The specific rotation was measured on a Perkin-Elmer 241 MC polarimeter (length: 1 cm). X-ray data were acquired on an Enraf-Nonius CAD4 diffractometer. All reactions were carried out under nitrogen that had been dried over self-indicating Si gel, concentrated H_2SO_4, and KOH.

(−)-Woodinine (1): prepared as described;[12,13] mp 112 °C; $[\alpha]^{23}_D$ −81° (*c* 0.6, MeOH); ^{1}H NMR (benzene) δ 1.10 (1H, m, H-4′), 1.30−1.41 (2H, m, H-4′, H-3′), 1.56−1.68 (1H, m, H-3′), 1.72−1.84 (1H, m, H-5′), 2.13 [3H, s, N-CH_3 (pyrrolidine)], 2.23 [3H, s, N-CH_3 (piperideine)], 2.31−2.45 (2H, m, H-3, H-4), 2.48−2.55 (1H, m, H-2′), 2.68−2.84 (3H, m, H-3, H-4, H-5′), 3.34 (1H, br s, H-1), 6.84−6.88 (1H, d, J = 7.7 Hz, H-8), 7.26−7.30 (1H, d, J = 7.7 Hz, H-7), 7.78 (1H, s, H-5), 9.87 [1H, br s, NH (indole)]; ^{13}C NMR δ 21.52 (C-4), 23.07 (C-4′), 25.90 (C-3′), 40.93 [N-CH_3 (pyrrolidine)], 43.67 [N-CH_3 (piperideine)], 54.48 (C-3), 57.80 (C-5′), 61.05 (C-1), 67.20 (C-2′), 109.82 (C-4a/C-6), 112.55 (C-4a/C-6), 112.66 (C-8), 121.05 (C-5), 123.99 (C-7), 129.27 (C-4b), 134.72 (C-9a), 136.17(C-8a).

1-[(*S*)-*N*-Methylpyrrolidin-2-yl]-2,9-dimethoxy-1,2,3,4-tetrahydro-*β*-carboline (3). To a solution of 135 mg (0.5 mmol) of **2** in 5 mL of dry THF cooled to −78 °C, 0.37 mL of a 15% solution of *n*-BuLi in hexane was slowly injected. After stirring for 1 h at −78 °C, the mixture was allowed to warm to room temperature. A solution of 0.05 mL methyl methane sulfonate (0.55 mmol) in 3 mL of dry THF was added, the mixture was stirred for 48 h at room temperature, and the reaction was stopped by addition of 1 g of ice. After separation of the THF phase, the aqueous layer was extracted twice with 5 mL of Et_2O. The combined organic phases were washed with 8 mL of H_2O, dried with Na_2SO_4, and evaporated *in vacuo*. The residue was separated from starting material by column chromatography (alumina, column 10 × 1 cm, CH_2Cl_2−Et_2O 1:1) to give 42 mg of colorless crystals, mp 40.5 −42 °C; $[\alpha]^{23}_D$ −97.6° (*c* 0.06, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.16−1.40 (1H, m, H-4′), 1.40−1.57 (2H, m, H-4′, H-3′), 1.61−1.72 (1H; m, H-3′), 2.08−2.17 (1H, m, H-5′), 2.42 [3H, s, N-CH_3 (pyrrolidine)], 2.49 [3H, s, N-CH_3 (piperideine)], 2.54−2.65 (1H, m, H-4), 2.65−2.73 (1H, m, H-2′), 2.85−2.99 (3H, m, H-3, H-4, H-5′), 3.18−3.29 (1H, m, H-3), 3.76 (1H, br s, H-1), 3.77 [3H, s, N-CH_3 (indole)], 7.08−7.14 (1H, m, H-6/H-7), 7.14−7.22 (1H, m, H-6/H-7), 7.27−7.31 (1H, m, H-8), 7.49−7.54 (m, 1H, H-5); ^{1}H NMR (benzene) δ 1.26−1.43 (2H, m, H-4′, H-3′), 1.55−1.69 (2H, m, H-4′, H-3′), 1.89−1.97 (1H, m, H-5′), 2.11 [3H, s, N-CH_3 (pyrrolidine)], 2.40 [3H, s, N-CH_3 (piperideine)], 2.49−2.57 (1H, m, H-4), 2.63−2.69 (1H, m, H-2′), 2.71−2.82 (2H, m, H-5′, H-3), 2.82−2.92 (1H, m, H-4), 3.35−3.42 (1H, m, H-3), 3.39 [3H, s, N-CH_3 (indole)], 3.58 (1H, br s, H-1), 7.16−7.21 (1H, m, H-8), 7.25−7.33 (2H, m, H-6, H-7), 7.66−7.71 (1H, m, H-5); ^{13}C NMR ($CDCl_3$) δ 17.42 (C-4), 23.74 (C-4′), 28.39 (C-3′), 30.91 [N-CH_3 (indole)], 41.08 [N-CH_3 (pyrrolidine)], 42.47 [N-CH_3 (piperideine)], 46.66 (C-3), 56.84 C-5′), 61.03 (C-1), 71.15 (C-2′), 107.26 (C-4a), 108.97 (C-8), 117.85 (C-5), 118.57 (C-6/C-7), 120.68 (C-6/C-7), 126.90 (C-4b), 134.76 (C-8a), 137.52 (C-9a); EIMS m/z 283 $[M^\circ]^+$ (6), 199 $[M - C_5H_{10}N]^+$ (62), 84 $[C_5H_{10}N]^+$ (100).

X-Ray Structure Analysis of 1: empirical formula $C_{17}H_{22}Br{\cdot}CH_3OH$, molecular mass 380.33, T = 300 K, graphite monochromated Cu K_α radiation, wave length 1.54178 Å, measuring device CAD4 (Enraf-Nonius) with rotating anode, crystal system monoclinic, space group $P2_1$, unit cell: a = 8.2221 (3) Å, b = 10.8888 (4) Å, c =

10.5821 (3) Å, $\beta = 101.256(2)°$, $V = 929.19(6)$ Å^3, cell parameter refined using 75 reflections between $65° < \theta < 70°$, $Z = 2$, density (calculated) 1.359 Mg/m^3, absorption coefficient 3.07 mm^{-1} correction with δ-F refinement using DIFABS[17] $t_{min} = 0.201$, $t_{max} = 1.0$, $F(000) = 396$, crystal size 0.2 × 0.4 × 0.5 mm, $\omega = 0.7 + 0.14$, tan(θ). A total of 2516 reflections were collected (partially with Friedel pairs), of which 2312 are independent ($R_{int} = 0.033$). The θ-range was $1.5° < \theta < 70°$, index ranges $0 \le h \le 10$, $-13 \le k \le 0$, $-12 \le l \le 12$. Lorentz and polarization corrections were applied. The structure was solved by direct methods using SIR92 and refined with SHELXL-93[18] by full-matrix least-squares on F^2. Some of the hydrogen atoms were found on a difference Fourier map and refined isotropic, assuming a riding-motion model. The total number of refined parameters was 228, compared with 2312 data. All reflections were included in the refinement. The absolute configuration could be determined using the Flack parameter $x = -0.02(3)$. Goodness-of-fit on F^2 was 1.048, final R value for [I > 2σ(I)] $R1 = 0.0395$, wR = 0.1109, the largest differential peak and hole were 0.32 and −0.54 Å^3, respectively, located near the bromine atom.[16]

References and Notes

(1) Païs, M.; Debitus, C. ; Laurent, D. *J. Nat. Prod.* **1988**, *51*, 779−801.
(2) Rinehart, K. L., Jr.; Kobayashi, J.; Harbour, G. C.; Hughes, R. G.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1984**, *105*, 1524−1526.
(3) Rinehart, K. L., Jr.; Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Mascal, M.; Holt, T. G.; Shield, L. S.; Lafargue, F. *J. Am. Chem. Soc.* **1987**, *109*, 3378−3387.
(4) Blunt, J. W.; Lake, R. J.; Munro, M. H. G. *Tetrahedron Lett.* **1987**, *28*, 1825−1826.
(5) Lake, R. J.; Blunt, J. W.; Munro, M. H. G. *Tetrahedron Lett.* **1988**, *29*, 2255−2256.
(6) Lake, R. J.; Blunt, J. W.; Munro, M. H. G. *Aust. J. Chem.* **1989**, *42*, 1201−1206.
(7) Kobayashi, J.; Cheng, J. F.; Ohta, T.; Nozoe, S.; Ohizumi, Y.; Sasaki, T. *J. Org. Chem.* **1990**, *55*, 3666−3670.
(8) Davidson, B. S. *Chem. Rev.* **1993**, *93*, 1771−1791.
(9) Meindl, W. *Arch. Pharm. (Weinheim)* **1988**, *321*, 473−476.
(10) Still, I. W.; McNulty, J. *Tetrahedron Lett.* **1991**, *32*, 4875−4878.
(11) Still, I. W.; McNulty, J. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1329−1337.
(12) Mahboobi, S. *Arch. Pharm. (Weinheim)* **1992**, *325*, 249−250.
(13) Mahboobi, S.; Burgemeister, T.; Wiegrebe, W. *Arch. Pharm. (Weinheim)* **1993**, *326*, 33−37.
(14) Epling, G. A. ; Kumar, A. *Synlett* **1991**, 347−348.
(15) Pauling, L. *The Nature of the Chemical Bond*; Cornell University Press: Ithaca, NY, 1960; p 453.
(16) Atomic coordinates, thermal parameters, bond lengths, and bond angles have been deposited with number CSD-406635 at the Fachinformationszentrum Karlsruhe, 76344 Eggenstein-Leopoldshafen.
(17) Walker, N.; Stuart, D. *Acta Crystallogr.* **1983**, *A39*, 158−166.
(18) Sheldrick, G. M. *SHELXL93. Program for Crystal Structure Refinement*; University of Göttingen, Germany, 1993.

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