Microbial Transformations of the Taxan Ring of 10-DAB by Some Strains of the Fungus *Curvularia lunata*: Formation of the Bis-abeotaxanes Wallifoliol and 4-Deacylwallifoliol

Alberto Arnone,[†] Adriana Bava,[†] Giovanni Fronza,[†] Luciana Malpezzi,[‡] and Gianluca Nasini*,[†]

CNR-ICRM, Department of Chemistry, Materials and Chemical Engineering, Politecnico di Milano, via Mancinelli, 7, I-20123, Milano, Italy, and Department of Chemistry, Materials and Chemical Engineering, Politecnico di Milano, via Mancinelli, 7, I-20123, Milano, Italy

Received November 27, 2009

The fermentation of 10-deacetylbaccatine III (10-DAB) (**1**) with *Curvularia lunata* afforded 4-deacylwallifoliol (**4**) and wallifoliol (**5**), the only natural taxoid with the unusual 5/6/6/6/4 ring system. The X-ray structure of compound **4** is reported. The skeletal rearrangements induced by the microbial enzymatic systems are also discussed.

Taxol, a potent antitumor agent, was originally isolated from *Taxus brevifolia*.[1] Its unusual biological activity, intriguing structure, and scarcity prompted extensive chemical and biological investigations. The most promising options for production of derivatives for pharmaceutical evaluation are semisynthesis from 10-deacetylbaccatine III (10-DAB, **1**) and biotransformation reactions. The whole cells of fungi possess a variety of enzymes, which can catalyze various chemical reactions and can be used for the derivatization of natural compounds. Thus, an enzymatic approach could directly produce potential alternatives for further chemical modification. When compared to chemical synthesis, biotransformation is environmentally friendly and usually performed under mild conditions. Biocatalysis provides access to derivatives not readily accessible by chemical synthetic means alone.[2] Surprisingly, few investigations have been conducted on biotransformations of taxanes by filamentous fungi.[3] On the other hand, it is clear that microbial enzymatic systems may serve as a useful tool to mimic some steps of taxoid biosynthesis in *Taxus* spp., such as extensive oxidation of the taxane skeleton.[4]

We previously reported that microbial transformation of **1** with *Trametes hirsuta* induced C-13 oxidation, whereas incubation with a strain of *Curvularia lunata* resulted in the isolation of 7-*epi*-10-DAB and 7-*epi*-10-oxo-10-DAB (**6**).[5]

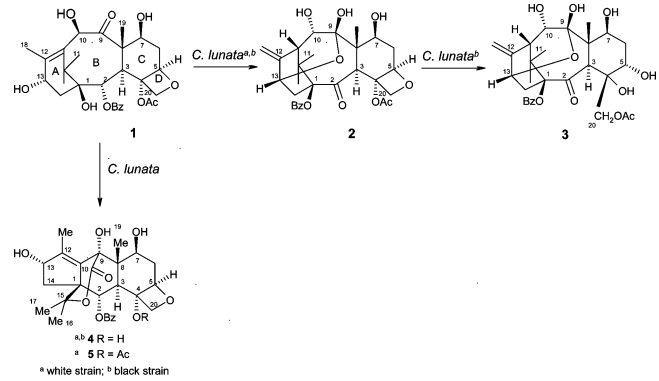
Recently, we continued our studies on the microbial transformation of 10-DAB (**1**) with *C. lunata*, increasing the time of incubation. The results of this work allowed us to identify two strains of the fungus *C. lunata* (176-ICRM and 198-ICRM) able to perform complex modifications of 10-DAB (**1**). In fact, from cultures of both strains it was possible to obtain products **2** and **3**, structurally similar to 10-DAB.[6]

Additional studies on the biotransformation of **1** with the same fungus led to new derivatives. We report herein the isolation and the structural identification of metabolites **4** and **5**. Their characterization was based on ^{13}C and ^{1}H NMR, including COSY, NOESY, and HMBC experiments, and X-ray analysis of **4**.

Results and Discussion

Incubation of compound **1** with *C. lunata* for two weeks afforded two new metabolites, **4** and **5**, in low yields (0.4% and 1.4%, respectively). The compounds were isolated from the culture medium CBS by extraction with EtOAc, and the extracts were fractionated by column chromatography and purified on preparative PLC.

Metabolite **4**, a white solid, exhibited a molecular peak at *m*/*z* 501 $[M + H]^+$, corresponding to the molecular formula $C_{27}H_{32}O_9$,



with an overall decrease of 44 mass units compared to the parent compound **1**. The structure of **4** was deduced via a ^{1}H and ^{13}C NMR study including accurate determination of the NOE enhancements and of the proton−carbon multiple-bond connectivities and finally confirmed by X-ray analysis. The ^{1}H and ^{13}C NMR data of **4** are shown in Tables 1 and 2, respectively. The long-range proton−carbon correlations are reported in Table 3 and the most significant NOEs in Table 4 (Supporting Information).

Both ^{1}H and ^{13}C NMR spectra of **4** showed strong variations compared to those of **1**, and several of these were diagnostic for its structural elucidation. In particular the ^{13}C NMR chemical shifts of C-9 (83.2 ppm) and C-10 (173.0 ppm) of **4** are typical of carbons bearing a hydroxy and an ester group, respectively, indicating that the original C-9 carbonyl group of **1** (211.9 ppm) was reduced to a secondary alcohol, while the hydroxy group at C-10 (75.0 ppm)[6] was oxidized to the carbonyl of the ester group. This also implies the breaking of the C-10/C-11 bond of compound **1**. Analogously, the chemical shifts of C-1 (60.8 ppm) and C-15 (89.9 ppm) of **4** are strongly shifted with respect to C-1 and C-15 in compound **1** (79.0 and 42.8 ppm, respectively),[6] suggesting that the oxygen atom originally linked to C-1 in **1** underwent a migration to C-15 with the concomitant rupture of the C-11/C-15 bond and the formation of the new C-10/O-15 bond. The occurrence of such complex rearrangement on going from **1** to **4** was supported by the lack of ^{3}J proton−carbon correlation between C-11 and the methyl groups CH_3-16 and CH_3-17, clearly visible for **1**,[6] and the presence of a long-range correlation between CH_3-17 and the C-10 ester carbonyl carbon. This four-bond correlation is due to the favorable *W* disposition of the interacting nuclei, permitting assignment of CH_3-17 vs CH_3-16.

Collectively, these findings permitted recognition of the occurrence of the partial structure A in compound **4** (Scheme 1), showing that two bonds still remain to be completed. Intuitively, the most reasonable structure of **4** should be obtained by joining C-11 to C-1 and C-9.

* To whom correspondence should be addressed. Phone: +39-2-23993046. Fax: +39-2-23993180. E-mail: gianluca.nasini@polimi.it.
[†] CNR-ICRM, Milano.
[‡] DCMCE, Politecnico, Milano.

10.1021/np900765y
Published on Web 06/08/2010

Table 1. ^{1}H NMR Data (δ_H (*J* in Hz), 500 MHz, acetone-d_6) for **4** and **5**

atom	**4**	**5**
H-2	5.86, d (12.5)	5.88, d (120)
H-3	3.06, dd (12.5, 1.49)	2.80, dd (12.0, 1.4)
H-5	4.68, dd (7.3, 3.7)	4.82, dd (8.5, 1.1)
H-6α	2.15, ddd (15.7, 7.3, 5.0)	2.67, ddd (15.7, 7.8, 8.5)
H-6*β*	2.13, ddd (15.7, 3.7, 2.5)	1.75, ddd (15.7, 8.0, 1.1)
H-7	4.30, dd (5.0, 2.5)	4.31, dd (8.5, 8.0)
H-13	4.49, m	4.56, m
H-14α	2.48, dd (14.8, 7.0)	2.29, dd (14.8, 7.1)
H-14*β*	2.25, dd (14.8, 7.4)	2.17, dd (14.8, 6.8)
CH_3-16	1.20, s	1.21, s
CH_3-17	1.37, s	1.32, s
CH_3-18	1.98, br, s	2.06, br, d (0.9)
CH_3-19	1.43, s	1.65, s
H-20α	4.04, br, d (8.0)	4.09, br, d (8.2)
H-20*β*	4.56, br, d (8.0)	4.54, br, d (8.2)
Obz	8.08, m (2H)	8.07, m (2H)
	7.64, m, 1H)	7.70, m (1H)
	7.52, m (2H)	7.58, m (2H)
OAc-4		1.80, s
OH-4	4.46, br, s	
OH-7	3.15, d (4.5)	4.43 br, d (5.7)
OH-9	5.53, br, s	6.10, br, s
OH-13	4.35, br, s	4.97, br, s

Table 2. ^{13}C NMR Data (δ_C, mult., 500 MHz, acetone-d_6) for **4** and **5**

position	**4**	**5**
1	60.8, qC	60.3, qC
2	69.7, CH	68.9, CH
3	42.9, CH	44.6, CH
4	74.7, qC	80.1, qC
5	87.5, CH	84.4, CH
6	37.2, CH_2	38.3, CH_2
7	70.9, CH	71.2, CH
8	48.7, qC	47.5, qC
9	83.2, qC	85.2, qC
10	173.0, qC	172.9, qC
11	132.0, qC	131.4, qC
12	138.3, qC	141.0, qC
13	79.9, CH	79.2, CH
14	37.1, CH_2	37.1, CH_2
15	89.9, qC	88.8, qC
16	24.8, CH_3	24.9, CH3
17	22.5, CH_3	22.5, CH_3
18	11.1, CH_3	10.6, CH_3
19	14.0, CH_3	11.0, CH_3
20	78.6, CH_2	74.1, CH_2
OAc-4		169.4, qC
		21.0, CH_3
Obz	165.2, qC	165.2, qC
	133.6, CH	134.0, CH
	131.3, qC	130.7, qC
	130.2, CH	130.1, CH
	129.0, CH	129.2, CH

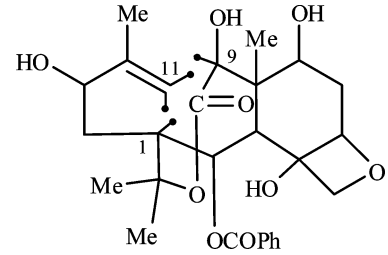
NOE experiments provided information about the relative configuration of compound **4** (Table 4, Supporting Information). The NOE effects observed between CH_3-19, assumed as *β*-disposed, and H-2 (18%), H-2 and CH_3-17 (2%), H-13 and CH_3-16 (1%), and H-14*β* at 2.22 ppm and both CH_3-16 and CH_3-17 (0.5%) indicate that all these protons are on the *β*-face of the molecule. The NOEs observed between H-3 and both H-14α at 2.49 ppm (2%) and H-7 (1.5%) suggest that these protons are α-oriented. Finally, the biotransformation of compounds **1** to **4** was accompanied by the loss of the acetyl group, leaving a free hydroxy group at C-4 of **4**. Compounds **4** and **5** differ in the acetyl group at OH-4. Interestingly, the acetylation of OH-4 induces large variations of the chemical shifts and coupling constants of the protons of ring C in **5** compared to **4** (Table 1). This behavior suggests that the conformation of ring C is strongly affected by the presence of the acetoxy group at C-4, due either to its bulkiness compared to the free OH group or to the potential rupture of intramolecular H bonds.

Table 3. Long-Range Proton−Carbon Correlations for Compounds **4** and **5** in Methanol-d_4[a]

	correlated protons	
carbon	**4**	**5**
1	H-2,H-3,CH_2-14,CH_3-16,CH_3-17	H-2,H-3,CH_2-14,CH_3-16,CH_3-17
2	H-3,H-14α	H-3,H-14α
3	H-2,H-5,H-7,CH_3-19,CH_2-20	H-2,H-5,H-7,CH_3-19,CH_2-20
4	H-3,H-5,CH_2-6,CH_2-20	H-3,H-5,H-6*β*,CH_2-20
5	CH_2-6,H-7,H-20*β*	CH_2-6,H-7,H-20*β*
6	H-7	H-7
7	H-3,H-5,CH_2-6,CH_3-19	H-3,H-5,CH_2-6,CH_3-19
8	H-2,H-3,CH_2-6,H-7,CH_3-19	H-2,H-3,H-6α,H-7,CH_3-19
9	CH_3-19	H-3,H-7,CH_3-19
10	CH_3-17	CH_3-17
11	H-13,H-14*β*,CH_3-18	H-13,H-14*β*,CH_3-18
12	H-13,H-14*β*,CH_3-18	H-13,H-14*β*,CH_3-18
13	H-14α,CH_3-18	H-14α,CH_3-18
14	H-2	H-2
15	H-2,CH_2-14,CH_3-16,CH_3-17	H-2,CH_2-14,CH_3-16,CH_3-17
16	CH_3-17	CH_3-17
17	CH_3-16	CH_3-16
19	H-3	H-3,H-7
20	H-3	H-3
2-OCOPh	H-2′,H-6′	H-2′,H-6′
4-OCOMe		H of OCO*Me*

[a] HMBC correlations are optimized for 8 Hz.

Scheme 1. Partial Structure A



X-ray structural analysis was carried out in order to confirm the molecular structure of compound **4** and to obtain more detailed information about the molecular conformation. Crystallization from acetone/water yielded single crystals suitable for X-ray analysis. The compound was found to crystallize with a water molecule in a 1:1 ratio. We also obtained single crystals of the product by crystallization from acetone. In this case, the compound was found to crystallize with an acetone solvent molecule, in the same 1:1 ratio. The molecular structure of the compound obtained by crystallization from acetone is very similar to the one obtained from acetone/water: the crystalline unit cell is still orthorhombic, space group $P2_12_12_1$, with axes *a* and *b* nearly unchanged, while axis *c* appears elongated, owing to a different crystal packing.[7]

A view of the water-solvated compound with the relative atomic numbering scheme is shown in Figure 1. The molecular structure and conformation found in the solid state by crystallographic analysis matched completely the one assigned using NMR results. The main body of the molecule consists of a 5/6/6/6/4 saturated ring skeleton fused by hedge sharing. A benzyloxy group is connected as a side chain. The nonaromatic six-membered rings adopt a conformation more or less distorted with respect to the canonical chair-boat conformation. In particular, the B-ring assumes a twist boat conformation, while rings C and E show half-chair conformations. The cyclopentane A-ring adopts an envelope conformation with C-14 as apex atom. The bond distances and angles within the molecule are in agreement with the expected values. The unusual C-8−C-9 bond length of 1.625 Å may be ascribed to the strain resulting from the condensed-ring molecular conformation.

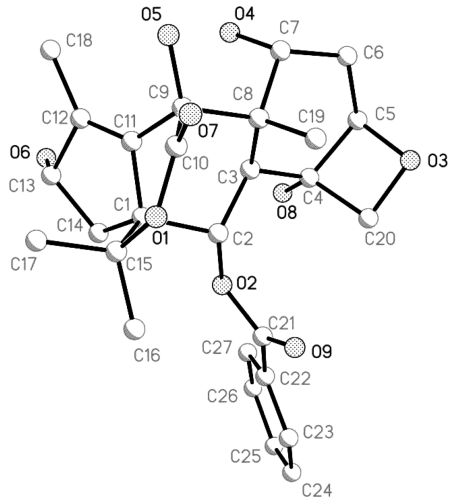


Figure 1. ORTEP drawing of compound **4** showing the atom-numbering scheme.

In the crystalline form, the molecules are stabilized by a three-dimensional network of hydrogen bonds, involving O-4, O-5, and O-6 (O−H---O hydrogen bonds) and O-1, O-3, and O-7 (C−H···O short intermolecular contacts). The water molecules act as either hydrogen bond donors or acceptors. Several intramolecular hydrogen bonds are also observed. Finally, comparison of the NMR data of **5** with those of compound **4** (Tables 1, 2, and 3) indicates that the two compounds share the same ring system, the only difference being the presence in **5** of a C-4 *O*-acetyl instead of an OH group.

Perusal of the literature showed that compound **5** is identical with wallifoliol, a taxoid isolated from the needles of Himalayan *Taxus wallichiana*.[8] Its structure was elucidated solely on the basis of NMR data.[8] Compound **5** was again isolated from *Taxus canadensis*[9] and recently from leaves and twigs of *Taxus sumatrana* together with other taxane derivatives, the tasumatrols.[10] Natural *abeo*-taxanes with ring A contracted to a five-membered ring 11(15→1) have been found in a few yew species, e.g. the *abeo*-taxane taxumairol and 13-*O*-acetyl-wallifoliol in *T. sumatrana*.[10] Compound **5** possesses most of the structural features of the taxoid brevifoliol, which is supposed to arise from a benzylic acid-type rearrangement of a precursor with carbonyl groups at both C-9 and C-10. In fact, the first product isolated by us from the biotransformation of 10-DAB using *C. lunata* as a bioagent was compound **6** (Scheme 2).[5]

The retro-aldol epimerization of the 7-hydroxy group in compound **6**, from a β-equatorial to an α-axial orientation, is characteristic of baccatin III derivatives. The 7α-epimer is stabilized by an intramolecular hydrogen bond between the 7-hydroxy and the 4-acetate carbonyl group.[11] Scheme 2 shows a possible biogenetic pathway from compound **6** to wallifoliol **5**. This scenario requires a subsequent epimerization at C-7.

Wallifoliol, a 11(15→1), 11(10→9) bis-*abeo*taxane, is formally derived from a tandem Wagner−Meerwein benzyl−benzylic-acid type rearrangement of an α-dioxo system such as in 10-oxo-10-DAB.[12] The same authors mimicked this complex reaction and proved that the dioxo system of this taxoid reacted with trichloroacetic acid to give a bis-*abeo*taxane like wallifoliol except for the simultaneous opening of the oxetane ring.[12]

We assume that a C-4 deacetylase is responsible for the transformation of compound **5** to **4**. More specifically, an enzymatic C-4 deacetylation of 10-DAB (**1**) has been observed using a strain of *Rhodococcus* spp.[13]

In conclusion, we have reported for the first time an enzymatic transformation of 10-DAB to the *abeo*-taxane wallifoliol (**5**). Although low-yielding, this microbial rearrangement provides useful experimental proof for the biogenesis of these compounds in *Taxus* spp.

Some fungi are able to biotransform taxanes to compounds featuring a variety of different skeletons common to several natural substances. Therefore, considering that symbioses between *Taxus* sp. and fungi are frequent, we cannot rule out the possibility that some fungal enzymes might be involved in the biosynthesis of these compounds.[14]

Compounds **4**, **5**, and **6** showed weak cytotoxicity on the tumor cell line H 460 with respect to 10-DAB (**1**).

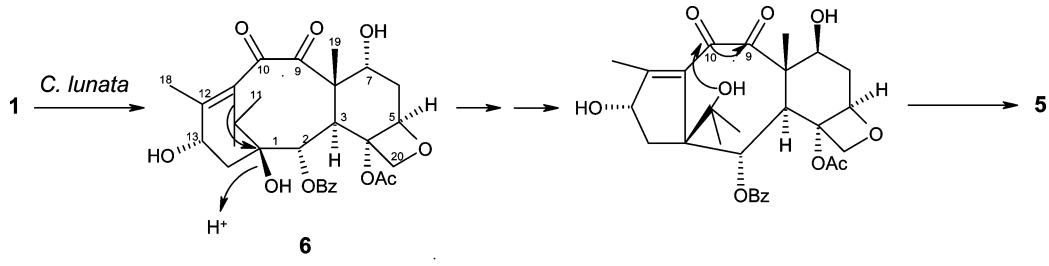
Experimental Section

General Experimental Procedures. Mass spectra were obtained with a Bruker Esquire 3000 and, for HRMS, with a Bruker APEX-QZT ICR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DMX-500 instrument in the temperature range 296−305 K in acetone-d_6 and methanol-d_4. The assignment of the proton signals is based on COSY, while carbon signals were assigned using HSQC and HMBC coupling constants. The nuclear Overhauser effects were determined by monodimensional NOE difference spectra or by NOESY experiments using a mixing time of 700−900 ms.

Flash CC was performed on Merck silica gel; TLC and PLC were performed with Merck HF_{254} silica gel. The purity of products was checked by TLC, NMR, and MS and was deemed sufficient for the purpose of structural determination. 10-DAB (**1**) was kindly provided by Indena S.p.a., Milano.

Microorganisms, Fermentation, and Extraction Procedures. The microorganisms were two strains of the fungus *C. lunata* deposited in the collection of our Institute (CNR-ICRM) as 176-ICRM and 198-ICRM. Both fungi were maintained at 4 °C as a stock culture on agar slants containing PDA (potato dextrose agar) medium, where they grow forming white (176-ICRM) or black (198-ICRM) colonies. The white strain is a morphogenetic variation of the original black strain formed during subsequently transfers in pure culture and has lost the capability of spore formation.[6] The cultures were grown, fermented, and extracted according to a previously described protocol.[5,6] After 24−48 h of growth the substrate 10-DAB (**1**, 1.2 g), dissolved in DMSO (12 mL), was evenly distributed among the 60 flasks containing stage II cultures (final concentration 0.2 g/L). The fermentations were sampled periodically by TLC analysis, with pure samples as reference; after two weeks, the cultures were filtered, the filtrate was extracted with EtOAc/MeOH (100:1), and the extract was evaporated to dryness. Metabolites **2** and **4** were reproducibly produced by fermentations of both strains 176-ICRM and 198-ICRM; metabolite **3** was produced by cultures of the black strain (198-ICRM),[6] while metabolite **5** was produced only from cultures of the white strain (176-ICRM).

Scheme 2. Possible Biogenetic Relationship between 7-*epi*-10-Oxo-10-DAB (**6**) and Wallifoliol (**5**)



Isolation of 4 and 5 from Cultures of White 176-ICRM and Black 198-ICRM Strains. The crude residues (0.9 g 176-ICRM; 0.85 g 198-ICRM) were purified by column chromatography on silica gel with a stepwise elution with CH_2Cl_2/i-PrOH from 30:1 to 1:1. The fractions obtained were further purified on preparative plates (PLC) in reverse phase (RP-18): metabolite **4** was isolated from both strains of fungus *C. lunata*, using H_2O/MeCN (2:1) as eluent to give 3 mg (0.3%) from white cultures (176-ICRM) and 4 mg (0.4%) from black cultures (198-ICRM). Compound **5** (13 mg, 1.4%) was obtained only from cultures of the white strain using H_2O/MeCN (1:1) as eluent; unreacted 10-DAB (**1**) was recovered (260 mg, 22%).

Compound 4: white solid, mp 190 °C; $[\alpha]_D$ −36 (*c* 0.2, MeOH); UV (EtOH) λ_{max} 203, 230, and 273sh (ε 15.300, 12.400, and 1200); ESIMS *m/z* 523 $[M + Na]^+$, 501 $[M + H]^+$, and 482 $[M + H - 18]^+$; FABMS *m/z* 501 $[M + H]^+$; HREIMS 500.2038 (calcd for $C_{27}H_{32}O_9$, 500.2046); ^1H and ^{13}C NMR data are in Tables 1 and 2.

Compound 5: white solid, mp 165 °C; $[\alpha]_D$ −30 (*c* 0.1, MeOH); ESIMS *m/z* 565 $[M + Na]^+$, 543 $[M + H]^+$, and 524 $[M + H - 18]^+$; ESIMS, negative ion *m/z* 541 $[M - H]^+$; HREIMS 542.2127 (calcd for $C_{29}H_{34}O_{10}$, 542.2148); ^1H and ^{13}C NMR data are in Tables 1 and 2.

Cytotoxicity Bioassay. Compounds **4**, **5**, **6**, and 10-Dab (**1**) were tested for their cytotoxicity against the non-small-cell lung tumor cell line H 460 [IC_{50} (μM): 102.8, 68, 60, and 3.1, respectively].

Crystal Structure Determination of 4. The compound crystallizes in the orthorhombic system, space group $P2_12_12_1$, with unit cell parameters $a = 8.362(1)$ Å, $b = 10.869(1)$ Å, $c = 27.818(2)$ Å,, $V = 2528.3(2)$ Å^3, $Z = 4$, $D_c = 1.362$ g cm^{-3}, $F(000) = 1104$. A crystal suitable for X-ray analysis, with dimensions of 0.4× 0.2 × 0.15 mm, was obtained upon slow recrystallization from acetone/water.

Intensity data were collected, at room temperature, on a Bruker AXS SMART Apex single-crystal diffractometer with a 5 cm crystal-to-detector distance and graphite-monochromatized Mo Kα radiation ($\lambda = 0.71069$ Å) at 50 kV and 30 mA. Unit-cell dimensions were calculated from least-squares refinement of 5017 reflections in the 2θ range 4.8−45.3°. A total of 47 782 reflections was collected in the θ range 2.4−27.0°, corresponding to 5522 unique reflections ($R_{int} = 0.033$). Raw intensity data were corrected for absorption using the SADABS v. 2.10 program.[15]

The structure was solved by the direct method using the SIR97 program,[16] which revealed the position of all non H-atoms. The refinement was carried out on F^2 by a full-matrix least-squares procedure with SHELXL97 for 384 parameters, with anisotropic temperature factors for non-H atoms.[17] The final stage converged to $R = 0.0467$ ($R_w = 0.1192$) for 5080 observed reflections (with $I \geq 2\sigma(I)$), and $R = 0.0510$ for all reflections. The hydroxy and the tertiary H atoms were freely and isotropically refined; all other H atoms were placed in geometrically calculated positions and refined in a riding model.

Full data (excluding structure factors) of the crystal structure have been deposited as supplementary publication No. CCDC 755642. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-(0)12233336033 or e-mail: deposit@ccdc.cam.ac.uk).

Acknowledgment. We are indebted to Prof. G. Appendino for helpful discussions, Dr. F. Zunino (Istituto Nazionale dei Tumori, Milano) for the cytotoxicity tests, and Indena Spa for financial support.

Supporting Information Available: NOEs (Table 4) and ^1H and ^{13}C NMR spectra for the new compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Kingston, D. G. I. *Phytochemistry* **2007**, *68*, 1844–1854.
(2) Dai, J.; Qu, R.; Zou, J.-H.; Chen, X. *Tetrahedron* **2008**, *64*, 8102–8116.
(3) Sun, D.-A.; Nikolakakis, A.; Sauriol, F.; Mamer, O.; Zamir, L. O. *Bioorg. Med. Chem.* **2001**, *9*, 1985–1992.
(4) Jennewein, S.; Croteau, R. *Appl. Microbiol. Biotechnol.* **2001**, *57*, 13–19.
(5) Arnone, A.; Bava, A.; Alemani, S.; Nasini, G.; Bombardelli, E.; Fontana, G. *J. Mol. Catal. B: Enzym.* **2006**, *42*, 95–98.
(6) Arnone, A.; Bava, A.; Fronza, G.; Nasini, G. *J. Nat. Prod.* **2009**, *72*, 2000–2004.
(7) This adduct crystallizes in the orthorhombic system, space group $P2_12_12_1$, with cell parameters $a = 8.383(1)$ Å, $b = 10.846(1)$ Å, $c = 31.148(2)$ Å, $V = 2805.9(2)$ Å^3, $Z = 4$, $D_c = 1.320$ g cm^{-3}, $F(000) = 1188$. In the crystal the molecules are kept together, forming zig-zag chains, elongated parallel to the *c* axis, by C5−H···O4 hydrogen bonding. Full data (excluding structure factors) of this crystal structure have been deposited as supplementary publication No. CCDC 769838.
(8) Vander Velde, D. G.; Georg, G. I.; Gollapudi, S. R.; Jampani, H. B.; Liang, X.-Z.; Mitscher, L. A.; Ye, Q.-M. *J. Nat. Prod.* **1994**, *57*, 862–867.
(9) Zamir, L. O.; Balachandran, S.; Zheng, Y. F.; Nedea, M. E.; Caron, G.; Nikolakakis, A.; Vishwakarma, R. A.; Sauriol, F.; Mamer, O. *Tetrahedron* **1997**, *47*, 15991–16008.
(10) Shen, Y.-C.; Wang, S.-S.; Pan, Y.-L.; Lo, K.-L.; Chakraborty, R.; Chien, C.-T.; Kuo, Y. H.; Lin, Y.-C. *J. Nat. Prod.* **2002**, *65*, 1848–1852.
(11) Appendino, G.; Noncovich, A.; Bettoni, P.; Dambruoso, P.; Sterner, O.; Fontana, G.; Bombardelli, E. *Eur. J. Org. Chem.* **2003**, 4422–4431.
(12) Appendino, G.; Jakupovic, J.; Varese, M.; Bombardelli, E. *Tetrahedron Lett.* **1996**, *37*, 727–730.
(13) Pattel, R. N. *Food Technol. Biotechnol.* **2004**, *42*, 305–325.
(14) Li, Y.-C.; Tao, W.-Y. *Cell Biol. Int.* **2009**, *33*, 106–112.
(15) Sheldrick, G. M. *SADABS*; University of Göttingen, Germany, 1996.
(16) Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, G.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G. P.; Spagna, *J. Appl. Crystallogr.* **1999**, *32*, 115–119.
(17) Sheldrick, G. M. *SHELXL-97, Program for the Refinement of Crystal Structures*; University of Göttingen: Germany, 1997.

NP900765Y