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Whole-Cell Biotransformation of *m*-Ethyltoluene into 1*S*,6*R*-5-Ethyl-1,6-dihydroxycyclohexa-2,4-diene-1-carboxylic Acid as an Approach to the C-Ring of the Binary Indole–Indoline Alkaloid Vinblastine

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The title compound **3**, a potential building block for the construction of analogues of the clinically important anti-cancer agent vinblastine (**1**), has been prepared in an efficient manner through a whole-cell biotransformation of *m*-ethyltoluene (**4**) using the microorganism *Pseudomonas putida* BGXM1 which expresses the enzyme toluate dioxygenase (TADO). Metabolite **3** was readily converted into derivatives **5**–**8** using conventional chemical techniques and the structure, including absolute stereochemistry, of the last of these was established by single-crystal X-ray analysis.

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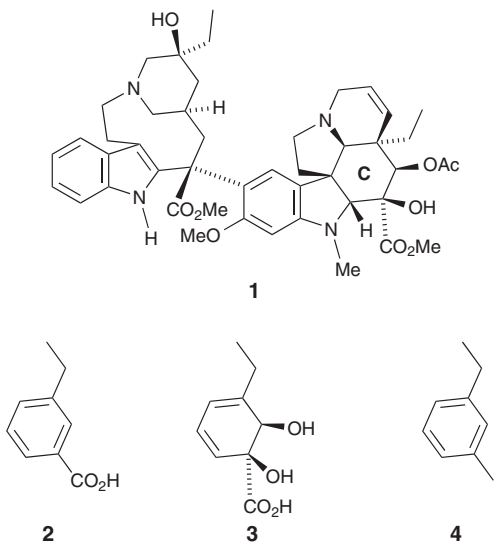
The binary indole–indoline alkaloid vinblastine (**1**, Scheme 1) and several derivatives are currently in clinical use for the treatment of various human cancers including Hodgkin's and non-Hodgkin's lymphoma, testicular cancer, bladder cancer, and lung cancer as well as acute childhood leukaemia.[1] As such, compounds of this class have been the target of many synthetic studies over more than three decades.[1] Notwithstanding important contributions from the laboratories of Kuehne, Kutney, Langlois/Potier, and Magnus,[2] the daunting structural complexity of vinblastine meant that it eluded de novo total synthesis until 2002 when Fukuyama and coworkers first reported their landmark work.[3] For the same reasons, there has been an attendant lack of medicinal-chemistry-type studies so that the minimum structure responsible for the therapeutically useful anti-mitotic properties of compound **1** remains ill-defined.[1,4] We have recently established, using Pd[0]-catalyzed Ullmann cross-coupling chemistries, new methods for the construction of the indole and indoline ring systems,[5,6] each of which is incorporated within the vinblastine framework. Accordingly, we have begun a program directed towards the synthesis of 'stripped down' vinblastine analogues that might retain clinically useful therapeutic properties. During the course of analyzing structure **1** we recognized that enzymatic dihydroxylation of *m*-ethylbenzoic acid (**2**) using the toluate dioxygenase (TADO) enzyme system[7] might be expected[8] to give metabolite **3** embodying key structural features associated with the C-ring of vinblastine. While this conversion has been described previously by Whited et al.,[9] these researchers were unable to assign the absolute stereochemistry of the metabolite and actually displayed (with an appropriate caveat) *ent*-**3** in their paper. As a consequence, this structure (namely *ent*-**3**) has been propagated, without qualification, in a review[10] even though it is at variance with the well-defined actions of this same enzyme system on the parent benzoic acid which delivers the des-ethyl derivative of compound **3**.[8] In order to resolve this seeming stereochemical contradiction and to establish serviceable routes to



Scheme 1.

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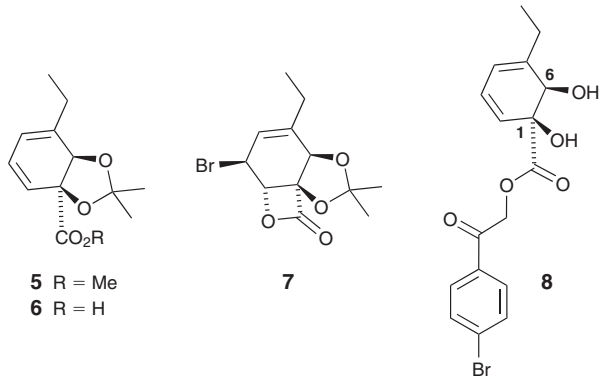

Scheme 2.

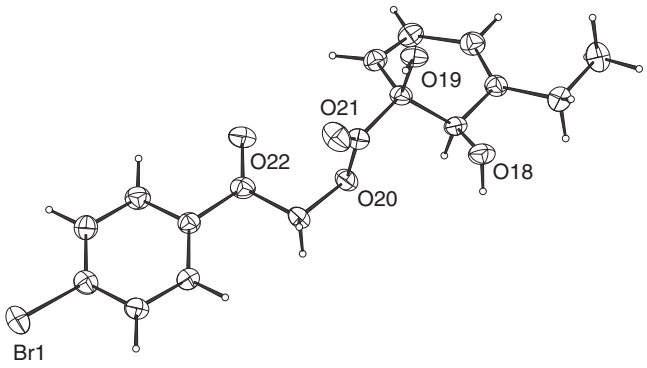

Fig. 1. ADEP derived from a single-crystal X-ray analysis of compound **8**.

compound **3** we have investigated a closely related biotransformation and now detail the outcomes of our studies in this area.

The microorganism used by Whited et al.[9] in carrying out the biotransformation **2** → *ent*-**3**/**3** was the *Pseudomonas putida* (Pp) strain BGXM1 which is an NTG mutant of the wild-type strain PpBG1 containing a TOL plasmid encoding the pathway for the catabolism of toluene. The mutation in strain BGXM1 inactivates the gene encoding the enzyme *cis*-toluate diol dehydrogenase which oxidizes *cis*-toluate diols to the corresponding catechols. As such, the mutant accumulates the *cis*-diols metabolically formed from the corresponding acids. Since the *P. putida* strain BGXM1 also expresses enzymes capable of oxidizing toluenes to benzoic acids, commercially available *m*-ethyltoluene (**4**) was examined as a substrate for this microorganism in the expectation this would be oxidized to acid **2** in situ. If this approach were successful it would avoid the need to prepare *m*-ethylbenzoic acid (**2**), which is not readily accessible.

In the event, presentation of compound **4** to the above-mentioned mutant resulted in the smooth formation (>55% yield) of the targeted metabolite **3** which could be extracted from the fermentation broth by conventional methods. This rather thermally sensitive material was immediately subjected to the derivatization protocols outlined below and detailed in the Experimental section.

Derivatization of compound **3** was undertaken with a view to obtaining a crystalline product, ideally one incorporating a heavy atom since such features should allow for a single-crystal X-ray analysis, and, thereby, determination of the absolute stereochemistry of this metabolite. To such ends the acid was treated with diazomethane in a 10 : 1 v/v mixture of dichloromethane/ethanol and the resulting methyl ester immediately treated with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid (*p*-TsOH). In this manner the acetonide ester **5** (Scheme 2) was obtained as a clear, colourless oil and in 49% overall yield from the starting hydrocarbon used in the biotransformation. Saponification of this material using potassium carbonate in methanol then gave, after careful acidic workup, the free acid **6** (100%), which was also obtained as a clear, colourless oil. A more direct route to this same acid involved treatment of metabolite **3** with 2,2-dimethoxypropane in the presence of *p*-TsOH but product **6** was now contaminated with significant quantities of aromatic products, making this a less efficient process than the three-step protocol just described.

Subjection of the β,γ-unsaturated acid **6** to standard *trans*-selective bromolactonization conditions[11] afforded the expected brominated β-lactone **7** in 92% yield. While it is anticipated that this highly functionalized compound will serve as a useful intermediate in the construction of vinblastine analogues, it was, unfortunately, only ever obtained as an oil. Following work reported by Widdowson et al.,[8a] we eventually achieved access to a solid derivative of compound **3** through its reaction with commercially available α,*p*-dibromoacetophenone in the presence of triethylamine. By such means the ester **8** was obtained in 65% yield and this crystalline material proved amenable to single-crystal X-ray analysis, details of which are provided in Fig. 1 and the Experimental section. This analysis clearly reveals that compound **3** possesses the illustrated absolute configuration, a result in keeping with reports[8] on related biotransformations of benzoic acid and as required for exploitation of the title metabolite in the preparation of vinblastine analogues incorporating C-ring surrogates. The crystal structure reveals that the diene moiety within compound **8** assumes *P* helicity with a torsion angle of +13.6 and that the C1 and C6 OH groups (see structure **8**, Scheme 2) are pseudo-axially and pseudo-equatorially orientated, respectively.

The work detailed herein serves to further highlight the extraordinary utility of dioxygenases in the large-scale preparation of monochiral and highly functionalized building blocks likely to be suitable for use in the synthesis of biologically active natural products and their analogues.[12] Work along such lines and directed towards the synthesis of analogues of vinblastine (**1**) from metabolite **3** is now underway in these laboratories. Results will be reported in due course.

Experimental

Melting points were measured on a Reichert hot-stage microscope apparatus and are uncorrected. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on either a Varian Inova 500 spectrometer, operating at 500 MHz (^{1}H) and 126 MHz (^{13}C), or a Gemini 300 NMR spectrometer, operating at 300 MHz (^{1}H) and 75 MHz (^{13}C). Unless otherwise specified, spectra were acquired at 20°C in $CDCl_3$ that had been filtered through basic alumina immediately before use. Chemical shifts are recorded as δ values in parts per million (ppm). Infrared spectra

(ν_{max}) were recorded on a Perkin–Elmer 1800 FTIR spectrometer and samples were analyzed as KBr disks (for solids) or plates (for oils). Low-resolution mass spectra were recorded on a Micromass-Waters LC-ZMD single quadrupole liquid chromatograph-MS or a VG Quattro II triple quadrupole MS instrument using electron-impact techniques. High-resolution mass spectra were recorded on an AUTOSPEC spectrometer. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at the sodium-D line (589 nm) and at the concentrations (c; g 100 mL^{-1}) indicated using spectroscopic-grade $CHCl_3$ unless otherwise specified. The measurements were carried out between 19 and 21°C in a cell with a path length (l) of 1 dm. Specific rotations $[\alpha]_D$ were calculated using the equation $[\alpha]_D = 100\alpha/(cl)$ and are given in 10^{-1} deg cm^2 g^{-1}. Elemental analyses were performed by the Australian National University's Microanalytical Services Unit based at the Research School of Chemistry. Dichloromethane was distilled from calcium hydride, and THF was distilled, under nitrogen, from sodium benzophenone ketyl. Where necessary, reactions were performed under a nitrogen atmosphere.

Biotransformation and Chemical Derivatization Studies

Whole-Cell Biotransformation of m-*Ethyltoluene into 1*S,*6*R-*5-Ethyl-1,6-dihydroxycyclohexa-2,4-diene-1-carboxylic Acid* **3**

Psuedomonas putida strain BGXM1[9] was used to produce the title compound **3** through biotransformation of *m*-ethyltoluene (**4**) in a 14 L fed batch culture. Frozen cells that had been maintained at −70°C in Lauria broth containing 25% glycerol were used for the biotransformation. An isolated colony of strain BGXM1 grown on Lauria agar at 30°C was used as inoculum for a 500 mL preculture. The preculture was grown in MSB (2) medium supplemented with 0.4% glycerol and 0.05% yeast extract at 30°C on a rotary shaker overnight. A 14 L fermentor was charged with 8.5 L of MSB medium supplemented with 0.4% glycerol and 0.05% yeast extract. The fermentor was inoculated with the preculture and allowed to grow at 30°C until the initial glycerol was depleted. The growth conditions were maintained at 25% dissolved oxygen by automatic adjustment of stirring, the pH was controlled at 7.2 with a 25% solution of ammonium hydroxide, and the air flow was constant at 8 L min^{-1}. After the initial depletion of glycerol (approx. 7 h) the culture was fed at a constant rate of 0.25 g min^{-1} with a 25% solution of glycerol for 10 h after which the rate was increased to 1 g min^{-1}. The culture reached an optical density of approx. 18 at 600 nm about 19 h after inoculation. At this time a slow drip of *m*-ethyltoluene (**4**, ex. Aldrich) was delivered directly to the fermentation broth using an HPLC pump. Metabolite **3** began to accumulate immediately as determined by measuring the spectrum of a sample of the clarified broth and quantifying the absorbance peak at 268 nm. The diol absorbance peak continued to increase proportionally to the addition of hydrocarbon until about 20 g of substrate was delivered.

After all of the hydrocarbon substrate had been delivered the pH of the fermentation medium (now approx. 10 L in volume) was adjusted to 8.0 with KOH (1 M aqueous solution) and the cells were then removed by centrifugation. The 8.5 L of clarified supernatant thus obtained was concentrated to 1.6 L under reduced pressure using a water bath temperature of approximately 55°C. This concentrated material was centrifuged again, to remove residual cells and precipitated salts, then shipped from San Francisco to Canberra. The yield of diol in this concentrate was estimated, by UV-vis spectroscopy and using the molar extinction coefficient of 3000 (at 268 nm) reported[9] for the corresponding *cis*-diol derived from *p*-toluic acid, to be approximately 16.9 g (about 55% yield). Appropriate portions of this material were cooled to 0°C and treated with HCl (10 M aqueous solution) until the pH was between 2 and 3. The resulting orange-brown solution was extracted three times with ethyl acetate and the combined extracts were then dried ($MgSO_4$), filtered, and concentrated under reduced pressure on a rotary evaporator (with the associated bath temperature maintained below 37°C) to afford samples of compound **3**[9] contaminated with ethyl acetate. δ_H (300 MHz) 6.25 (2H, br s), 6.10 (1H, dd, J 5.5 and 9.3), 5.69 (2H, m), 4.82 (1H, s), 2.24 (2H, q, J 7.5), 1.05 (3H, t, J 7.5), signal due to CO_2H not observed. δ_C (75 MHz) 171.5 (C), 144.6 (C), 127.7 (CH), 122.5 (CH), 116.3 (CH), 75.0 (C), 72.7 (CH), 25.0 (CH_2), 12.0 (CH_3). This material was used in all the derivatization reactions detailed below.

*Methyl (3a*S,*7a*R*)-7-ethyl-2,2-dimethyl-1,3-benzodioxole-3a(7a*H*)-carboxylate* **5**

A solution of compound **3** (300 mg of crude material obtained by ethyl acetate extraction of the processed fermentation broth—see above) in dichloromethane/ethanol (4 mL of a 10 : 1 v/v mixture) was treated with diazomethane (8 mL of a 0.24 M solution in ether) and the resulting mixture was left to stand at 18°C for 0.5 h. Excess diazomethane was then blown off by passing a stream of nitrogen gas through the solution. After 10 min the residual solvent was removed under reduced pressure to yield a pale orange residue, that was then dissolved in 2,2-dimethoxypropane (2 mL) and treated with *p*-toluenesulfonic acid (5 mg). The ensuing mixture was stirred at 18°C for 16 h then concentrated under reduced pressure. The resulting light yellow oil was subject to column chromatography (silica; 5 : 95 v/v ethyl acetate/hexane elution) and concentration of the relevant fractions (R_F 0.3) afforded the *title compound* **5** (190 mg, 49% from compound **4**) as a clear, colourless oil, $[\alpha]_D$ −226 (c 0.9 in $CHCl_3$) [Found: $(M - H^{\bullet})^+$ 237.1133. $C_{13}H_{18}O_4$ requires 237.1127]. δ_H (300 MHz) 6.07 (1H, dd, J 5.7 and 9.3), 5.80 (1H, dm, J 5.7), 5.69 (1H, dm, J 9.3), 4.81 (1H, s), 3.77 (3H, s), 2.31 (2H, m), 1.43 (3H, s), 1.36 (3H, s), 1.11 (3H, app. t, J 7.2). δ_C (75 MHz) 172.4 (C), 139.7 (C), 125.0 (CH), 122.0 (CH), 117.3 (CH), 107.3 (C), 80.5 (C), 76.0 (CH), 52.8 (CH_3), 26.9 (CH_3), 26.7 (CH_2), 25.5 (CH_3), 11.4 (CH_3). ν_{max} (neat)/cm^{-1} 2968, 1736, 1454, 1435, 1378, 1371, 1256, 1176, 1031, 892, 798, 720. *m/z* (EI, 70 eV) 237 [$(M - H^{\bullet})^+$, 2%], 207 (17), 191 (70), 173 (57), 148 (52), 146 (100), 145 (81), 120 (57), 77 (36).

*(3a*S,*7a*R*)-7-Ethyl-2,2-dimethyl-1,3-benzodioxole-3a(7a*H*)-carboxylic Acid* **6**

Method A: A magnetically stirred solution of ester **5** (40 mg, 0.168 mmol) in methanol (3 mL) was treated with K_2CO_3 (1.5 mL of a 1 M aqueous solution) and stirred at 18°C for 4 h after which time TLC analysis indicated the complete consumption of starting material. As a consequence, the reaction mixture was acidified to a pH of 6 with HCl (1 M aqueous solution) and extracted with diethyl ether (3 × 15 mL). The combined organic fractions were then dried ($MgSO_4$), filtered, and concentrated under reduced pressure to afford the *title acid* **6** (38 mg, 100%) as a pale yellow oil, $[\alpha]_D$ −246 (c 0.6 in $CHCl_3$) (Found: $M^{+\bullet}$ 224.1049. $C_{12}H_{16}O_4$ requires 224.1049). δ_H (300 MHz) 6.11 (1H, dd, J 6.0 and 9.6), 5.90 (1H, br s), 5.82 (1H, dm, J 6.0), 5.70 (1H, dm, J 9.6), 4.82 (1H, s), 2.29 (2H, m), 1.47 (3H, s), 1.14 (3H, s), 1.11 (3H, t, J 6.9), signal due to CO_2H not observed. δ_C (75 MHz) 175.9, 139.6, 125.5, 121.3, 117.3, 107.9, 80.3, 76.1, 26.8, 26.6, 25.5, 11.4. ν_{max} (neat)/cm^{-1} 3184, 2988, 2938, 1732, 1458, 1435, 1382, 1372, 1247, 1215, 1163, 1074, 1054, 1035, 892, 741. *m/z* (EI, 70 eV) 224 [$M^{+\bullet}$, 2%], 166 (40), 149 (36), 148 (39), 121 (100), 107 (51), 77 (43), 43 (47).

Method B: A magnetically stirred solution of diol **3** (300 mg of crude material obtained by ethyl acetate extraction of the processed fermentation broth—see above) in 2,2-dimethoxypropane (3 mL) and acetone (0.5 mL) was treated with *p*-TsOH (5 mg) and the resulting mixture allowed to stir at 18°C for 16 h. Concentration of the reaction mixture afforded the desired acid **6** (as judged by ^{1}H NMR spectroscopic comparisons with the material obtained by method A) albeit contaminated with significant quantities of aromatized material.

*(3a*R,*6*S,*6a*S,*8a*R*)-6-Bromo-4-ethyl-6,6a-dihydro-2,2-dimethyl-8-oxo-3a*H,*8*H*-oxeto[3,2-*d*]-1,3-benzodioxole* **7**

Following protocols developed by Ganem and coworkers,[11] a magnetically stirred solution of acid **6** (50 mg, 0.22 mmol) in $NaHCO_3$ (1.5 mL of a saturated aqueous solution) was cooled to 0°C then treated, dropwise, with a solution of molecular bromine (36 mg, 0.22 mmol) in dichloromethane (1 mL). After 20 min the organic layer was separated and the aqueous phase extracted with dichloromethane (2 × 10 mL). The combined organic fractions were washed with brine (1 × 20 mL) then dried ($MgSO_4$), filtered, and concentrated under reduced pressure to

yield an orange oil. Subjection of this material to flash chromatography (silica; 1 : 9 v/v ethyl acetate/hexane elution) gave, after concentration of the appropriate fractions (R_F 0.3), the *title bromolactone* **7** (58 mg, 92%) as a light yellow oil, $[\alpha]_D$ −27 (*c* 1.0 in $CHCl_3$) [Found: $(M - H_3C^{\bullet})^+$ 286.9922. $C_{12}H_{15}{}^{79}BrO_4$ requires 286.9919]. δ_H (300 MHz) 5.85 (1H, m), 4.92 (1H, dd, *J* 5.1 and 6.0), 4.58 (1H, d, *J* 5.1), 4.55 (1H, m), 2.34 (2H, m), 1.71 (3H, s), 1.58 (3H, s), 1.15 (3H, t, *J* 7.5). δ_C (75 MHz) 170.6, 147.1, 119.1, 114.7, 81.0, 76.3, 74.0, 45.6, 27.0, 25.7, 25.3, 11.1. ν_{max} (neat)/cm^{-1} 2986, 2938, 1805, 1384, 1335, 1302, 1247, 1217, 1157, 1109, 1075, 1055, 939, 874, 787. *m/z* (EI, 70 eV) 289 and 287 [$(M - H_3C^{\bullet})^+$, both 2%], 179 (35), 121 (100), 109 (27), 93 (32), 69 (40).

*2-(4-Bromophenyl)-2-oxoethyl (1*S*,6*R*)-5-Ethyl-1,6-dihydroxycyclohexa-2,4-diene-1-carboxylate* ***8***

A magnetically stirred solution of diol **3** (300 mg of crude material obtained by ethyl acetate extraction of the processed fermentation broth—see above) in acetone (5 mL) maintained at 18°C was treated with triethylamine (184 mg, 1.83 mmol) then α,*p*-dibromoacetophenone (507 mg, 1.83 mmol, ex. Aldrich). After 4 h, at which point a precipitate had formed, the reaction mixture was partitioned between ethyl acetate (30 mL) and water (10 mL). The separated organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to provide a cream-coloured solid that was recrystallized (ethanol/water) to afford the *title diol* **8** (407 mg, 65%) as a white, crystalline solid, mp 124–130°C, $[\alpha]_D$ +11 (*c* 0.9 in $CHCl_3$) (Found: C 53.4, H 4.3, Br 21.1; $M^{+\bullet}$ 380.0266. $C_{17}H_{17}{}^{81}BrO_5$ requires C 53.6, H 4.5, Br 21.0%; $M^{+\bullet}$ 380.0259). δ_H (300 MHz) 7.76 (2H, dm, *J* 8.7), 7.66 (2H, dm, *J* 8.7), 6.17 (1H, dd, *J* 5.7 and 9.0), 5.87 (1H, d, *J* 9.0), 5.71 (1H, m), 5.51 (1H, d, *J* 18), 5.43 (1H, d, *J* 18), 4.95 (1H, m), 3.28 (2H, br s), 2.32 (2H, m), 1.10 (3H, t, *J* 7.2). δ_C (75 MHz) 191.4, 174.7, 144.6, 132.4, 132.1, 129.8, 129.3, 128.0, 122.1, 116.2, 75.5, 73.3, 67.0, 25.1, 12.1. ν_{max} (neat)/cm^{-1} 3493, 3413, 2963, 1739, 1698, 1587, 1428, 1399, 1284, 1224, 1121, 1069, 995, 961, 815, 747, 563. *m/z* (EI, 70 eV) 382 and 380 [$M^{+\bullet}$, both 12%], 364 and 362 [$(M - H_2O)^{+\bullet}$, both 22%], 200 and 198 (both 26), 185 and 183 (both 100), 149 (57), 148 (97), 138 (54), 120 (57), 95 (25), 77 (35).

Crystal Data for Ester ***8***

$C_{17}H_{17}BrO_5$, *M* 381.227, *T* 200 K, orthorhombic, space group $P2_12_12_1$, *Z* 4, *a* 5.3466(1), *b* 15.8093(3), *c* 18.8706(4) Å, *V* 1595.06(5) $Å^3$, D_x 1.588 Mg m^{-3}, 3646 unique data ($2\theta_{max}$ 54.96°), 2546 with $I > 3\sigma(I)$; *R* 0.022, *wR* 0.026, *S* 1.072.

Images were measured on a Nonius Kappa CCD diffractometer ($Mo_{K\alpha}$, graphite monochromator, λ 0.71073 Å) and the data extracted using the *DENZO* package.[13] Structure solution was by direct methods (*SIR97*)[14] and refinement was by full-matrix least-squares on *F* using the *CRYSTALS* program package.[15] The final Flack parameter of 0.012(8) demonstrates that the compound is of the absolute stereochemistry reported here. CCDC 246605 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk.

Acknowledgments

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References

[1] *Antitumor Bisindole Alkaloids from* Catharanthus roseus *(L.)*, in *The Alkaloids, Vol. 37* (Eds A. Brossi, M. Suffness) **1990** (Academic Press: San Diego, CA).

[2] For an up to date minireview on synthetic studies in the area, see: C. Schneider, *Angew. Chem. Int. Ed.* **2002**, *41*, 4217. doi:10.1002/1521-3773(20021115)41:22<4217::AID-ANIE4217>3.0.CO;2-U

[3] (*a*) S. Yokoshima, T. Ueda, S. Kobayashi, A. Sato, T. Kuboyama, H. Tokuyama, T. Fukuyama, *J. Am. Chem. Soc.* **2002**, *124*, 2137. doi:10.1021/JA0177049
(*b*) S. Yokoshima, T. Ueda, S. Kobayashi, A. Sato, T. Kuboyama, H. Tokuyama, T. Fukuyama, *Pure Appl. Chem.* **2003**, *75*, 29.

[4] For a recent and important contribution in this area, see: M. E. Kuehne, W. G. Bornmann, I. Markó, Y. Qin, K. L. LeBoulluec, D. A. Frasier, F. Xu, T. Mulamba, C. L. Ensinger, L. S. Borman, A. E. Huot, C. Exon, F. T. Bizzarro, J. B. Cheung, S. L. Bane, *Org. Biomol. Chem.* **2003**, *1*, 2120. doi:10.1039/B209990J

[5] M. G. Banwell, B. D. Kelly, O. J. Kokas, D. W. Lupton, *Org. Lett.* **2003**, *5*, 2497. doi:10.1021/OL034745W

[6] M. G. Banwell, D. W. Lupton, unpublished.

[7] P. A. Williams, K. Murray, *J. Bacteriol.* **1974**, *120*, 416.

[8] (*a*) G. N. Jenkins, D. W. Ribbons, D. A. Widdowson, A. M. Z. Slawin, D. J. Williams, *J. Chem. Soc., Perkin Trans. 1* **1995**, 2647. doi:10.1039/P19950002647
(*b*) A. G. Myers, D. R. Siegel, D. J. Buzard, M. G. Charest, *Org. Lett.* **2001**, *3*, 2923. doi:10.1021/OL010151M

[9] G. M. Whited, W. R. McCombie, L. D. Kwart, D. T. Gibson, *J. Bacteriol.* **1986**, *166*, 1028.

[10] T. Hudlicky, D. Gonzalez, D. T. Gibson, *Aldrichimica Acta* **1999**, *32*, 35.

[11] B. Ganem, G. W. Holbert, L. B. Weiss, K. Ishizumi, *J. Am. Chem. Soc.* **1978**, *100*, 6483.

[12] M. G. Banwell, A. J. Edwards, G. J. Harfoot, K. A. Jolliffe, M. D. McLeod, K. J. McRae, S. G. Stewart, M. Vögtle, *Pure Appl. Chem.* **2003**, *75*, 223.

[13] Z. Otwinowski, W. Minor, in *Methods Enzymol., Vol. 276: Macromolecular Crystallography, Part A* (Eds C. W. Carter, Jr, R. M. Sweets) **1997**, pp. 307–326 (Academic Press: New York, NY).

[14] A. Altomare, M. C. Burla, M. Camalli, G. L. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori, R. Spagna, *SIR97: J. Appl. Crystallogr.* **1999**, *32*, 115.

[15] D. J. Watkin, C. K. Prout, J. R. Carruthers, P. W. Betteridge, R. I. Cooper, *CRYSTALS Issue 11* **2001** (Chemical Crystallography Laboratory: Oxford).