Microbial Hydroxylation and Reduction of the Diterpene Psiadin

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Z. Naturforsch. **56 c,** 216–222 (2001); received October 4/November 10, 2000

Psiadin, Microbial Bioconversion, 2α-Hydroxydeoxopsiadin

Microbial bioconversion studies conducted on the diterpene psiadin have revealed that it was metabolized by *Aspergillus niger* (NRRL 2295) to give 2α-hydroxydeoxopsiadin, *Cunninghamella blakesleeana* (ATCC 8688a) to give 11β-hydroxypsiadin, and *Cylindrocephalum aureum* (ATCC 12720), *Gongronella butleri* (ATCC 22822), *Kloeckera africana* (ATCC 20111), and *Kluyveromyces marxianus var. lactis* (ATCC 2628) to yield 7α-hydroxypsiadin. Their structures have been established on the basis of spectral data. The structure and relative stereochemistry of 7α-hydroxypsiadin was confirmed by single-crystal X-ray analysis.

Psiadin (**1**) is a diterpene isolated from the aerial parts of *Psiadia arabica* Jaub. et Spach. (Fam. Asteraceae) (Mossa *et al.*, 1992). The leaf decoction of some *Psiadia* species was reported to be used as a folk medicine in Kenya for the treatment of colds and fevers (Kokwaro, 1976), and for the removal of ectoparasites from cattle (Beentje, 1994).

The utilization of microbes as models for mammalian metabolism of xenobiotics has been well established since this concept was first introduced by Smith and Rosazza in the early seventies (Orabi, 2000; Abourashed *et al.*, 1999; Davis, 1987; Rosazza and Smith, 1979, 1975; Rosazza, 1982). This concept depends on the fact that fungi, being eukaryotes, possess metabolizing enzymatic machinery similar to those of mammals. Hence, the outcome of xenobiotic metabolism in both systems is expected to be similar, if not identical, and, thus, fungi can serve as reliable, convenient, predictive models for mammalian metabolism of various xenobiotics.

It is anticipated that the microbial metabolism of **1** would produce significant quantities of metabolites that would be difficult to obtain from either animal systems or chemical synthesis. Moreover, this may provide some novel metabolites that may serve as starting compounds for semisynthesis of other derivatives, or as analytical standards for mammalian metabolic studies.

On the other hand, similarity in the biogenetic/biodegradation pathways in plants and microbes, which might imply the involvement of similar enzymatic machinery, is demonstrated herein this report, as was observed before (Orabi, in press). This report accounts on the microbial bioconversion of **1** and the isolation and characterization of its metabolites.

Results and Discussion

Out of seventy five fungal cultures screened for their ability to metabolize **1**, only six were successful. *Aspergillus niger* (NRRL 2295) and *Cunninghamella blakesleeana* (ATCC 8688a) yielded 2α-hydroxydeoxopsiadin (**2**) and 11β-hydroxypsiadin (**3**), respectively, upon preparative-scale fermentation of **1**. *Cylindrocephalum aureum* (ATCC 12720), *Gongronella butleri* (ATCC 22822), *Kloeckera africana* (ATCC 20111), and *Kluyveromyces marxianus var. lactis* (ATCC 2628) were able to covert **1** into 7α-hydroxypsiadin (**4**), and

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0939–5075/2001/0300–0216 $ 06.00

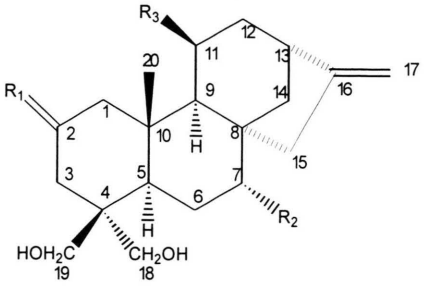
of those *C. aureum* was chosen for preparative-scale fermentation.

Metabolites **2–4** were isolated by solvent extraction, purified by chromatography and subjected to spectral analyses. In addition, the structure and relative stereochemistry of **3** was further confirmed by X-ray analysis. Complete unambiguous assignments of ^{1}H and ^{13}C resonances of psiadin were previously reported (El-Domiaty *et al.*, 1993).

Metabolite **2** was isolated as colorless needles in a 2.6% yield. It was shown to possess the molecular formula $C_{20}H_{32}O_3$ as derived from the presence of an ion peak at *m/z* 321 $[M+1]^+$ and from NMR data (Table I). The absence of any strong absorption around the 1695 cm^{-1} region in the IR spectrum indicated the conversion of the original psiadin carbonyl group into another functionality, as was also shown in the ^{13}C NMR spectrum. Moreover, the NMR data confirmed the identity of **2** to be 2α-hydroxydeoxopsiadin, and identical to that previously isolated from *Psiadia arabica* (El-Domiaty *et al.*, 1993). The similarity in the biogenetic/biodegradation pathways in plants and microbes, which might imply the involvement of similar enzymatic machinery, is evident in this compound.

Metabolite **3** was isolated as colorless prisms in a 4.6% yield. Low Resolution Electron Impact Mass Spectroscopy (LREIMS) of **3** gave a characteristic ion peak at *m/z* 335 $[M+1]^+$, and was consistent with the molecular formula $C_{20}H_{30}O_4$. No significant functional group change was observed as indicated from its IR spectrum. The ^{13}C NMR spectra revealed the oxygenation of one methylene carbon (C-11) (Table I). The HMBC spectra showed strong three-bond correlations between H-17a & b (δ 4.74, 4.88, both as s) and C-13 (δ 45.4, d) and C-15 (δ 50.2, t). C-13, in turn, showed a three-bond correlation with an oxygenated methane proton; H-11 (δ 4.57, ddd, J = 7.9, 7.0, 7.0 Hz). On the other hand, this oxygenated methane proton showed a three-bond correlation with C-13. Furthermore, a two-bond correlation exists between H-9 (δ 1.91, d, J = 7.4 Hz) and C-11 (δ 70.9, d). Other important three-bond correlations were between H-20 (δ 1.59, s) and C-1 (δ 60.7, t), H-1a & b (δ 2.56, 4.27, both as d, J = 15.6 Hz) and C-9 (δ 61.4, d) and C-20 (δ 22.5, q), H-3a & b (δ 3.05, 3.14, both as d, J = 15.2 Hz) and C-18 (δ 69.3, t) and C-19 (δ 66.1, t), and between H-13 (δ 2.66, br s) and C-8 (δ 47.6, s). Other protons and carbons assignments were unambiguously made from the COSY, HMQC and HMBC spectra. The stereochemical disposition of the hydroxyl group on C-11 was concluded to be a *beta*-orientation due to the presence of nuclear Overhauser effects in the NOESY spectra between the oxygenated methane H-11 and H-5α and H-9α. These data unambiguously determined that **3** was 11β-hydroxypsiadin.

Metabolite **4**, isolated as colorless needles in a 70% yield, showed LREIMS spectra similar to those of **3**, and was consistent with the molecular formula $C_{20}H_{30}O_4$, suggesting its possible chemistry as hydroxypsiadin. On the other hand, the ^{13}C NMR spectra showed the oxygenation of one methylene carbon (C-7). The *Co*rrelation Spectroscopy for *Lo*ng-Range *C*ouplings (COLOC) spectra showed only two weak three-bonds correlations between this position; H-7 (δ 3.77, br s) and C-5 (δ 39.5, d) and C-9 (δ 49.2, d). No other significant long-range correlations were shown between either H-7 or C-7 and other carbons or protons, respectively. Single-crystal X-ray analysis for **4** was done to confirm the chemical and stereochemical identity of this metabolite. Other ^{3}J-cross peaks were also observed between H-1a & b (δ 2.11, 2.68, each as d, J = 13.7 Hz) and C-5 and C-20 (δ 19.3, q), and H-3a & b (δ 2.93, 3.08, each as d, J = 14.7 Hz) and C-5, H-5 (δ 3.07, br s) and C-3 (δ 45.4, t) and C-20, H-6a (δ 1.92, dd, J = 13.7, 13.7 Hz) and H-6b (δ 2.23, br d, J = 13.7 Hz) and



1: R_1 = O, R_2 = R_3 = H

2: R_1 = a-OH, b-H, R_2 = R_3 = H

3: R_1 = O, R_2 = H, R_3 = OH

4: R_1 = O, R_2 = OH, R_3 = H

Table I. NMR data of compounds **3** and **4**.[a]

Position	**3**		**4**	
	δ_H (m, *J* [Hz])	δ_C	δ_H (m, *J* [Hz])	δ_C
1	4.27 (d, 15.6) 2.56 (d, 15.6)	60.7, t[b]	2.68 (d, 13.7) 2.11 (d, 13.7)	56.0, t
2	–	213.9, s	–	212.0, s
3	3.14 (d, 15.2) 3.05 (d, 15.2)	46.7, t	3.08 (d, 14.7) 2.93 (d, 14.7)	45.4, t
4	–	48.4, s	–	47.0, s
5	2.22 (d, 11.6)	50.4, d	3.07 (br s)	39.5, d
6	2.0 (m) 1.53 (m)	22.9, t	2.23 (br d, 13.7) 1.92 (dd, 13.7, 13.7)	28.8, t
7	1.43 (m) 1.46 (m)	43.4, t	3.77 (br s)	75.7, d
8	–	47.6, s	–	48.8, s
9	1.91 (d, 7.4)	61.4, d	1.99 (br d, 6.5	49.2, d
10	–	45.4, s	–	43.1, s
11	4.57 (ddd, 7.0, 7.0, 7.0)	70.9, d	1.58 (m) 1.42 (m)	28.8, t
12	2.02 (m) 2.01 (m)	44.6, t	1.74 (br d, 11.4) 1.13 (br d, 11.4)	38.2, t
13	2.66 (br s)	45.4, d	2.62 (br s)	43.9, d
14	0.99 (dd, 11.5, 4.5) 2.06 (m)	41.2, t	1.44 (m) 1.58 (m)	18.2, t
15	2.11 (d, 11.0) 1.94 (d, 11.0)	50.2, t	2.55 (s) 2.55 (s)	45.9, t
16	–	155.6, s	–	155.6, s
17	4.88 (s) 4.74 (s)	105.4, t	4.89 (s) 4.86 (s)	103.5, t
18	4.13 (d, 10.6) [c] 3.94 (d, 10.6) [d]	69.3, t[c]	3.92 (dd, 11.1, 3.2) [c] 4.09 (dd, 11.1, 2.6) [d]	67.5, t[c]
19	4.03 (d, 10.3) [c] 4.10 (d, 10.3) [d]	66.1, t[c]	4.01 (d, 10.4) [c] 4.12 (d, 10.4) [d]	63.9, t[c]
20	1.59 (s)	22.5, q	1.17 (s)	19.3, q
OH	6.55 (br s)	–	5.77 (br s)	–
OH	6.20 (br s)	–	6.18 (br s)	–
OH	6.14 (br s)	–	6.19 (br s)	–

[a] Spectra recorded in pyridine-d_5. [b] ^{13}C multiplicities were determined by DEPT 135°. [c,d] Assignments may be interchanged within the same column.

C-8 (δ 48.8, s) and C-10 (δ 43.1, s), H-15 (δ 2.55, s) and C-9 and C-17 (δ 103.5, t), H-17a & b (δ 4.86, 4.89, each as s) and C-15 (δ 45.9, t) and between H-20 (d 1.37, s) and C-1 (d 56.0, t), C-5 and C-9. In addition, ^2J-cross peaks exist between H-3a & b and C-4 (d 47.0, s), H-5 and C-6 (d 28.8, t), H-13 (d 2.62, br s) and C-14 (d 18.2, t), and between H-20 and C-10.

Single-crystal X-ray analysis established the complete structure and relative stereochemistry of **4** unequivocally. The absolute stereochemistry follows from that of psiadin (El-Domiaty *et al.*, 1993). A view of the solid-state conformation is presented in Fig. 1. Bonded distances in **4** are not significantly different from the corresponding values in psiadin, and all lie close to expected values (Allen *et al.*, 1987). Endocyclic torsion angles (ω_{ij}, σ 0.3–0.5°) about the bonds between atoms *i* and *j* in **4**, with corresponding values for psiadin (σ 0.4–0.6°) in parentheses, follow: $\omega_{1,2}$ −54.5(−59.8), $\omega_{2,3}$ 48.1(54.0), $\omega_{3,4}$ −42.3(−43.6), $\omega_{4,5}$ 47.5(45.5), $\omega_{5,10}$ −54.1(−51.0), $\omega_{10,1}$ 54.6(54.5°) in cyclohexanone ring A; $\omega_{5,6}$ −62.6(−63.5), $\omega_{6,7}$ 57.0(56.1), $\omega_{7,8}$ −47.4(−46.5), $\omega_{8,9}$ 45.9(46.2), $\omega_{9,10}$ −50.6 (−51.2), $\omega_{10,5}$ 57.6(58.7°), in cyclohexane ring B; $\omega_{8,9}$ 51.2(51.4), $\omega_{9,11}$ −35.7(−34.9), $\omega_{11,12}$ 42.2(39.8), $\omega_{12,13}$ −61.7(−60.4), $\omega_{13,14}$ 73.8(72.9), $\omega_{14,8}$ −70.0(−70.6°) in cyclohexane ring C; $\omega_{8,14}$ 45.6(44.8), $\omega_{14,13}$ −39.1(−44.0), $\omega_{13,16}$ 16.5(25.2), $\omega_{16,15}$ 12.5(3.5), $\omega_{15,8}$ −36.0(−29.9°) in ring D. The

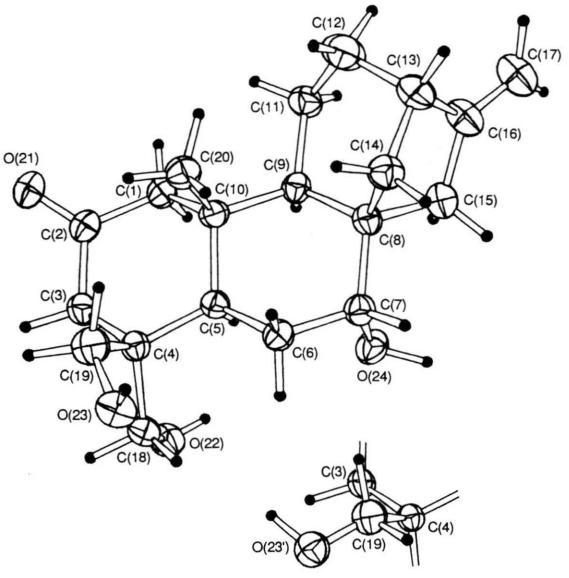


Fig. 1. ORTEP diagram (40% probability ellipsoids) showing the crystallographic atom numbering scheme and solid-state conformation of compound **4**; the hydroxy group at C-19 is disordered *ca.* 5:1 over two orientations O-23 and O-23′, respectively. Small filled circles represent hydrogen atoms.

conformations of rings A and B (flattened chairs) and ring C (distorted chair) are thus very similar to those in psiadin whereas ring D lies closer to a half-chair form in **4** than to the envelope form found in psiadin. In crystals of **4**, one of the hydroxy groups is disordered *ca.* 5:1 over two positions [O(23) and O(23')]. A like situation was encountered in crystals of psiadin where the hydroxy groups of the axial hydroxymethyl in two of the three molecules in the asymmetric unit were found to be disordered approximately equally over two orientations. All of the hydroxy groups in **4** are involved in intermolecular hydrogen bonds: O(22)...O(23) (at x, y, $1 + z$) = 2.963(3)Å, O(23)...O(24) (at x, y, $-1 + z$) = 2.711(3) Å, O(23')...O(21) (at $-1/2 - x$, $-y$, $-1/2 + z$) = 3.016(12) Å, O(24)...O(22) (at $1/2 + x$, $1/2 - y$, $1 - z$) = 2.895(3) Å.

Experimental Section

General experimental procedures

Melting points were determined in open capillary tubes, using a Mettler 9100 electrothermal melting point apparatus and were uncorrected. The IR spectra were recorded in KBr disk using an ATI Mattson Genesis Series FTIR spectrophotometer. UV spectra were measured in methanol using a UV-160 IPC UV-visible dual-beam spectrophotometer. Optical rotations were taken with a Perkin-Elmer 241 MC polarimeter. The ^{1}H and ^{13}C NMR were obtained on a Bruker DRX-500 spectrometer operating at 500 and 125 MHz, respectively. Both ^{1}H and ^{13}C NMR spectra were recorded in pyridine-d_5, and the chemical shift values were expressed in δ (ppm) relative to the internal standard TMS. For the ^{13}C NMR spectra, the number of attached protons was determined by DEPT 135°. 2D NMR data were obtained using the standard pulse sequence of the Bruker DRX-500 for COSY, HETCOR, HMQC, COLOC, HMBC and NOESY. Low resolution EIMS were obtained using a Shimadzu QP5000 Gas Chromotography/Mass Spectrometer.

Cultures and fermentation screening procedure

The microbial cultures were originally obtained from the American Type Culture Collection (ATCC), Rockville, Maryland, or from the USDA Northern Regional Research Laboratories (NRRL), Peoria, Illinois, and are maintained in King Saud University, Department of Pharmacognosy Culture Collection. *Helicostylum piriforme* QM 6945 was obtained from Quartermaster Culture Collection, Quartermaster Research and Engineering Command, United States Army Natick Laboratories, Natick, Massachusetts, USA. *Cunninghamella bainieri* (UI 3605) and *Mucor mucedo* (UI 4605) were obtained from Dr. John P. Rosazza, Division of Medicinal Chemistry and Natural Products, University of Iowa, Iowa City, Iowa, USA. *Mucor ramannianus* 1839 (Sih) was obtained from Dr. Charles J. Sih, Department of Pharmaceutical Biochemistry, University of Wisconsin, Madison, Wisconsin, USA. Stock Cultures were maintained on agar slants of media recommended by the ATCC and were stored at 4 °C.

All the preliminary screening and preparative-scale experiments were carried out as reported before (Orabi *et al.*, 1999) and according to a standard two-stage protocol (Orabi, 2000; Rosazza and Smith, 1975). *Aspergillus niger* NRRL 2295, *Cunninghamella blakesleeana* ATCC 8688a, *Cylindrocephalum aureum* ATCC 12720, *Gongronella but-*

leri ATCC 22822, *Kloeckera africana* ATCC 20111 and *Kluyveromyces marxianus var. lactis* ATCC 2628 showed metabolizing capabilities of **1**, and, thus, *A. niger, C. blakesleeana* and *C. aureum* were selected for preparative-scale fermentation to produce metabolites **2**, **3** and **4**, respectively. Substrate **1** was prepared as a 15% solution in *N,N*-dimethylformamide (DMF) and added to the 24-h-old stage II culture medium of the microorganism at a concentration 0.3 mg/ml of medium. Substrate controls were composed of sterile medium to which the substrate (4 mg/100 µl DMF) was added and incubated without microorganisms. Culture controls consisted of fermentation blanks in which the microorganisms were grown under identical conditions but without the substrate addition. After two weeks of incubation, each control was harvested and analyzed by TLC.

Fermentation sampling and chromatographic conditions

The fermentations were sampled by withdrawing 5 mL of culture and extracting it with 5 mL of $CHCl_3$. The concentrated organic phase was analyzed by TLC for the presence of metabolites. TLC analyses were performed on precoated silica gel 60 F_{254} (Merck) plates using 10% MeOH in $CHCl_3$ as solvent system. Visualization was accomplished by spraying with *p*-anisaldehyde spray reagent. The adsorbent used for column chromatography was Si gel 60/230–400 mesh (EM Science, Darmstadt). Psiadin (**1**), the substrate used in this project, was isolated from *P. arabica* as previously reported (El-Domiaty *et al.*, 1993). All solvents used for chromatographic purposes were reagent grade.

Preparative scale fermentation of **1** *by Aspergillus niger*

A. niger NRRL 2295 was grown in thirty 250-ml culture flasks each containing 50 ml of medium α. A total of 300 mg of **1** (in 2.0 ml DMF) was evenly distributed among the 24-h-old stage II culture. After six days, the incubation mixtures were checked by TLC to reveal that most of **1** was transformed into one major metabolite.

The incubation mixtures were combined and filtered to remove the mycelia, and the filtrate was extracted with $CHCl_3$ (1.5 l × 4). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo* at 40 °C to afford a brownish residue (200 mg), which was purified by column chromatography over silica gel (34 g, 2.7 × 15 cm) using MeOH- $CHCl_3$ (3:17 v/v) as the eluting solvent system. Fractions of 20 ml each were collected and pooled on the basis of TLC analyses. Fractions 42–50 yielded pure 2α-hydroxydeoxopsiadin (**2**) (8 mg, 2.6% yield), with R_f = 0.72.

2α-hydroxydeoxopsiadin (**2**): Colorless needles (MeOH-EtOAc): mp 237–238 °C. Other spectral data were indistinguishable from those previously reported (El-Domiaty *et al.*, 1993).

Preparative scale fermentation of **1** *by Cunninghamella blakesleeana*

A total of 680 mg of **1** was dissolved in 4.5 ml of DMF and evenly distributed among sixty eight 250-ml culture flasks each containing 50 ml of 24-h-old *C. blakesleeana* ATCC 8688a stage II culture. After six days, the incubation mixtures were checked by TLC to reveal that most of **1** was transformed into one major (**3**) and two minor metabolites.

The incubation mixtures were combined and filtered to remove the mycelia, and the filtrate was extracted with $CHCl_3$ (3.5 l × 4). The combined extracts were dried over anhydrous Na_2SO_4 and evaporated to dryness *in vacuo* at 40 °C to afford a yellowish residue (700 mg). Part of this residue (400 mg) was purified by column chromatography over silica gel (45 g, 3.3 × 15 cm) using ethyl acetate-n-hexane (3:17 v/v) as the eluting solvent system. Fractions 15–22 yielded pure 11β-hydroxypsiadin (**3**) (33 mg, 4.6% yield), with R_f = 0.20.

11β-hydroxypsiadin (**3**): colorless prisms (EtOAc): mp 99–100 °C; $[a]_D^{25}$ −86.3° (*c* 0.06, MeOH); IR (KBr) ν_{max} 3300 (OH), 2910 (saturated C-H), 1690 (C=O) cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) see Table I; EIMS (70 eV) *m/z* 335 $[M+1]^+$ (29).

Preparative Scale Fermentation of **1** *by Cylindrocephalum aureum*

Using the same procedure, biomass, amount of substrate, and time frame used to scale-up *Aspergillus niger*, *C. aureum* ATCC 12720 culture was

scaled up to yield a yellowish residue (440 mg) which was purified by column chromatography over silica gel (45 g, 2.0 × 44 cm) using MeOH-$CHCl_3$ (1:19 v/v) as the eluting solvent system. Fractions of 20 ml each were collected ad pooled on the basis of TLC analyses. Fractions 58–112 yielded pure 7α-hydroxypsiadin (**4**) (220 mg, 70% yield), with $R_f = 0.17$.

7α-hydroxypsiadin (**4**): colorless needles ($CHCl_3$-MeOH): mp 211–212 °C; $[a]_D^{25}$ −31.5° (*c* 0.12, MeOH); IR (KBr) ν_{max} 3420 (OH), 2940 (saturated C-H), 1710 (C=O), 1660 (C=C) cm^{-1}; ^1H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz) see Table I; EIMS (70 eV) *m/z* 335 $[M+1]^+$ (25).

X-ray crystal structure analysis of compound **4**

A crystal of dimensions *ca.* 0.15 × 0.18 × 0.50 mm was mounted on the end of a thin glass capillary and oriented on an Enraf-Nonius CAD-4 diffractometer (Cu-Kα radiation, graphite monochromator, λ = 1.5418 Å). Crystal data: $C_{20}H_{30}O_4$, $M_r = 334.46$, orthorhombic, space group $P2_12_12_1(D_2^4)$, $a = 11.003(1)$ Å, $b = 24.027(2)$ Å, $c = 6.265(1)$ Å, $V = 1656.3(6)$ Å^3, $Z = 4$, $D_c = 1.341$ g·cm^{-3}, μ (Cu-Kα radiation) = 6.9 cm^{-1}. One octant of intensity data ($\theta_{max} = 75°$, 1985 reflections) was recorded at 298 K by ω-2θ scans [scanwidth $(0.80 + 0.14\tan\theta)°$]. Four reference reflections, remeasured at 2 h intervals throughout the data collection, showed no significant variation (<1%). The space group was established uniquely by the Laue symmetry and systematic absences ($h00$ when $h \neq 2n$, $0k0$ when $k \neq 2n$, $00l$ when $l \neq 2n$). Refined unit-cell parameters were calculated from the diffractometer setting angles for 25 reflections ($36° < \theta < 40°$) widely separated in reciprocal space.

The crystal structure was solved by direct methods. Initial non-hydrogen atom coordinates were obtained from an *E*-map. The enantiomer was chosen to conform with the known absolute stereochemistry of psiadin (El-Domiaty *et al.*, 1993). Atomic positional and thermal parameters (first isotropic and then anisotropic) were adjusted by means of several rounds of full-matrix least-squares calculations during which $\Sigma w\Delta^2[w = 1/\sigma^2(|F_o|), \Delta = (|F_o| - |F_c|)]$ was minimized; hydrogen atoms were incorporated at their calculated positions during the later cycles. A difference Fourier synthesis, evaluated following convergence, revealed a small but significant maximum within bonding distance of C-19 and in a position compatible with fractional occupancy of an alternative location (*ca.* 18%) by its bonded hydroxy group. Hydrogen atom positional and isotropic thermal parameters were refined during the subsequent least-squares iterations in addition to the non-hydrogen atom parameters; hydrogen atoms associated with the disorder were incorporated at calculated positions. An extinction correction was included as a variable during the later iterations which converged at $R = 0.041$, $R_w = 0.054$, GOF = 1.77 $\{R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|Fo|^2]^{1/2}$, GOF = $[\Sigma w\Delta^2/(N_{observations} - N_{parameters})]^{1/2}\}$ over 1629 reflections with $I > 2.0\sigma(I)$. A final difference Fourier synthesis contained no unusual features [Δ_ρ(e/ Å^3) = 0.27 (max.), −0.16 (min.)].

Crystallographic calculations were performed by use of the Enraf-Nonius Structure Determination Package (SDP 3.0) (Frenz and Associates, 1985). For all structure-factor calculations, neutral atom scattering factors and their anomalous dispersion corrections were taken from International Tables for X-Ray Crystallography (International Tables for X-Ray Crystallography IV, Kynoch Press, Birmingham, U. K., 1974). Final atomic positional and thermal parameters, bond lengths and angles, and torsion angles have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U. K. This material is available from the author (A. T. McPhail) upon request.

Acknowledgements

The authors are grateful to Dr. Charles D. Hufford, School of Pharmacy, University of Mississippi for collecting the NMR data.

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