VOL. 56 NO. 10, OCT. 2003 THE JOURNAL OF ANTIBIOTICS pp. 862 — 865

Isolation and Identification of Cissetin—A Setin-like Antibiotic with a Novel *cis*-Octalin Ring Fusion

CHRISTIE BOROS[†,*], ALISSA DIX[†], BARRY KATZ[††], YELENA VASINA[†††] and CEDRIC PEARCE[†]

MYCOsearch, a subsidiary of OSI Pharmaceuticals, 4905 Pine Cone Drive, Durham, North Carolina, USA 27707

(Received for publication June 9, 2003)

In our search for fungal metabolites with antibiotic activity against methicillin-resistant *Staphylococcus aureus* (MRSA), we have isolated three different setin antibiotics from several different fungi—the previously reported equisetin (**2**)[1] and trichosetin (**3**),[2] and a novel setin-like compound (**1**) from OSI 50185. Like the known setin antibiotics, cissetin (**1**) is active against several Gram-positive organisms,[2~5] but it is most notable for the atypical *cis* ring fusion in the octalin portion of the molecule.

Cissetin (**1**) was isolated from OSI 50185 as a white foamy powder for which positive ion detection HR-FAB-MS suggested a molecular formula of $C_{23}H_{33}NO_4$ [*m/z*, found 388.24893 $(M+H)^+$, calcd 388.24892]. In contrast to reports of the NMR data for the setin class as extremely broad and ill-defined,[1~5] **1** gave crisp, clean ^1H and ^{13}C NMR spectra in $CDCl_3$ at ambient temperature (Table 1).

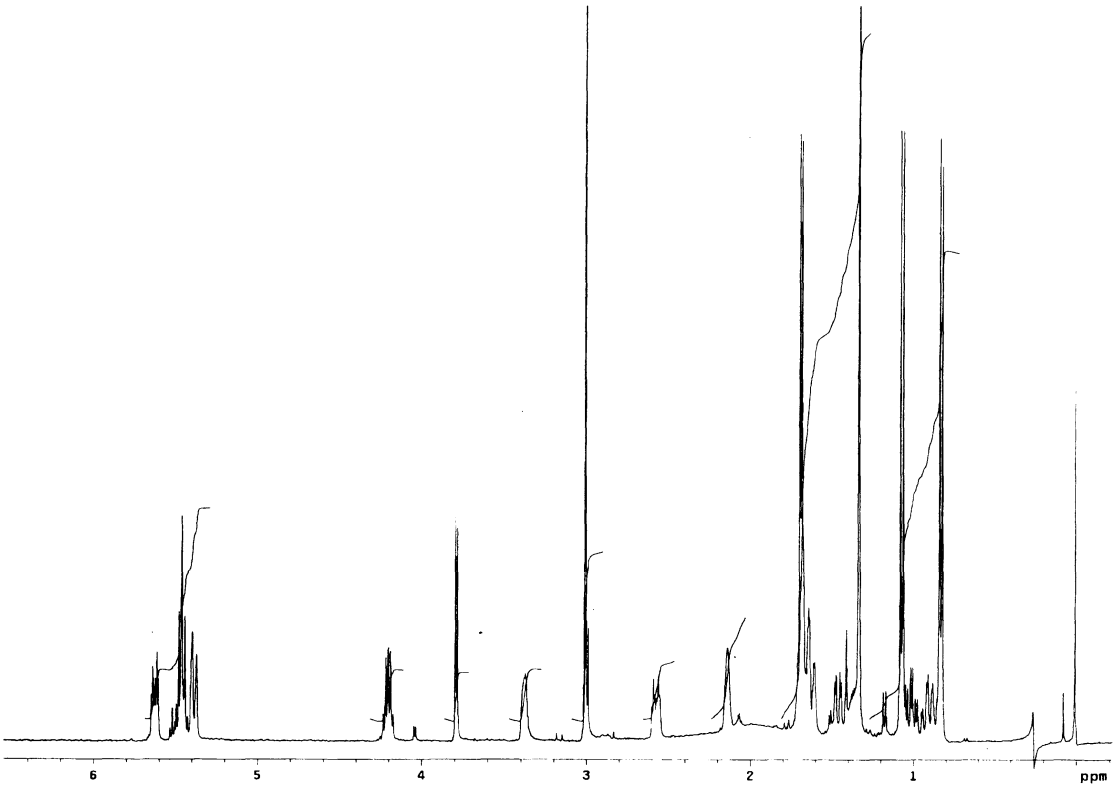
Table 1. ^1H and ^{13}C NMR data of cissetin (**1**) in $CDCl_3$ (T=20°C).

No.	^{13}C[a]	Mult[b]	^1H (m, *J* in Hz)[c]	HMBC
1	201.8	s	-	
2	49.6	s	-	
3	42.2	d	3.38 (br)	
4	130.3	d	5.63 (ddd, 10.0, 3.6, 3.5)	49.6, 42.2, 34.9
5	129.3	d	5.39 (dd, 10.0, 1.2)	42.2, 40.8, 37.7
6	34.9	d	2.12 (br)	
7	40.8	t	1.63 (m, obsc)[d]	
			1.01(app dt, 12.6, 5.0)[e] ax	129.3, 34.9, 28.4, 22.6
8	28.4	d	1.37 (br m)	
9	35.4	t	1.68 (m, obsc)[d]	
			0.90 (app dq, 12.4, 3.0)[e] ax	22.9
10	22.9	t	1.66 (m, obsc)[d]	
			1.46 (app dq, 12.7, 3.7)[e] ax	49.6, 37.7, 35.4, 18.4
11	37.7	d	2.58 (ddd, 12.1, 3.9, 3.4)	201.8, 129.3, 49.6, 42.2, 35.4, 22.9
12	18.4	q	1.33 (s)	201.8, 49.6, 42.2, 37.7
13	131.1	d	5.45 (dd, 15.5, 8.6)	42.2
14	127.6	d	5.47 (dq, 15.5, 5.1)	42.2, 18.1
15	18.1	q	1.68 (d, 5.1)	131.1, 127.6
16	22.6	q	0.84 (d, 6.5)	40.8, 35.4, 28.4
2'	177.6	s	-	
3'	98.1	s	-	
4'	191.4	s	-	
5'	67.3	d	3.80 (d, 4.4)	191.4, 177.6, 66.4, 17.3
6'	66.4	d	4.20 (dq, 6.4, 4.4)	191.4
7'	17.1	q	1.07 (d, 6.4)	67.3, 66.4
8'	27.5	q	3.00 (s)	177.6, 67.3
enol -OH	-	-	17.75 (s)	

[a] 100 MHz, $CDCl_3$ ref. 77.0 ppm. [b] Determined by DEPT. [c] 400 MHz, $CDCl_3$ ref. 7.27 ppm. [d] Obsc: obscured by overlapping resonances. [e] App: apparent.

Current addresses: [†] Mycosynthetix, Inc., 4905 Pine Cone Drive, Suite 5, Durham, NC 27707 USA.
[††] 5801 Cascade Drive, Chapel Hill, NC 27514 USA.
[†††] Paradigm Genetics, Inc., P.O. Box 14528, Research Triangle Park, NC 27709 USA.
* Corresponding author: cboros@mycosynthetix.com

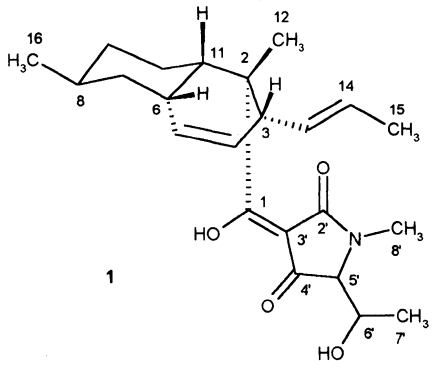
Fig. 1. ^{1}H NMR spectrum of cissetin (**1**) in $CDCl_3$ (T=20°C).



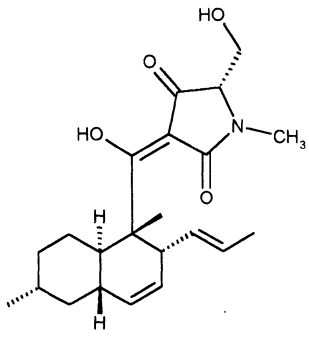
Absorbance maxima at 235 and 291 nm supported the presence of the tetramic acid moiety;[3] and a sharp, one-proton singlet at 17.75 ppm in the ^{1}H NMR spectrum was attributed to the enolic proton in the same system. Furthermore, the ^{13}C NMR spectrum displayed a pattern of resonances reminiscent of that noted for the setins; namely, three quaternary carbons of the keto-enol system (δ 201.8, 191.4, and 98.1 ppm), an amide carbonyl (δ 177.6 ppm), and four sp^2 methine carbons (δ 131.1, 130.3, 129.3, 127.6). The atypical clarity and resolution of the NMR resonances allowed unambiguous homo- and heteronuclear correlations by COSY, HMQC, and HMBC which permitted the complete assignment of all resonances in both of the 1D NMR spectra (Table 1).

In the ^{1}H NMR spectrum it was noted that the octalin ring junction signals H-6 (δ 2.12 ppm) and H-11 (δ 2.58 ppm) were deshielded by 0.3 and 0.95 ppm, respectively, relative to those of all previously reported setins.[1~5] Additionally, the small coupling of ~3.5 Hz between these protons indicated that they were oriented equatorially and could not be disposed *trans* to one another at the ring junction. Thus, *cis* relative stereochemistry at the octalin ring junction—previously unobserved in the setin antibiotics—was postulated. Support for this assignment was garnered by comparing the ^{1}H NMR spectra of muurola-4,11-diene (**4**, *cis* ring junction) and cadina-4,11-diene (**5**, *trans* ring junction).[6] The ring junction protons of **4** (δ 2.06 and 2.28~2.34 ppm) are significantly deshielded compared to those of **5** for which no non-vinylic signals appear above 1.99 ppm.[7]

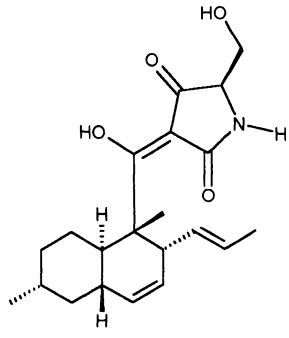
As in the previously reported setins, the saturated cyclohexane ring adopts a chair conformation, and the axial H-7β, H-8α, H-9β, H-10α, and H-11β protons were assigned based both on coupling constants ($J_{Hax\text{-}Hax}$ ~12 Hz; see Table 1) and on observed NOEs of 3% each for the axial protons H-7β (δ 1.01 ppm) and H-9β (δ 0.90 ppm) upon irradiation of H-11 (δ 2.58 ppm). To accommodate *cis* ring junction stereochemistry, the cyclohexene ring assumes a slightly twisted boat conformation. NOEs observed between H-3 (δ 3.38 ppm) and H_3-12 (δ 1.33 ppm, 2%) and between H-11 and H_3-12 (3%) suggested that the C-12 methyl group was on the same side of the molecule as both of those protons. ROESY correlations between H_3-12 and H-3, H-6, and H-11



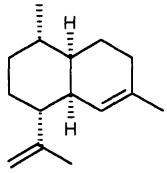
1



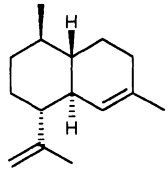
2



3



4



5

confirmed a pseudo-axial β placement of the methyl on C-2 as well as further supporting the *cis* orientation between H-6 and H-11. Additional ROESY correlations between the axial H-10α (δ 1.46 ppm) and the vinylic H-13/H-14 protons of the propenyl side chain (δ 5.45 and 5.47 ppm) fixed H-3 as β and consequently pseudo-equatorial. The β placement of the methyl on C-8 was made by considering the coupling constants of H-7β (δ 1.01 ppm) and H-9β (δ 0.90 ppm). H-7β manifested only a single axial coupling (J=12.6 Hz), and this was attributed to H-8 (δ 1.37 ppm). H-9β, on the other hand, experiences two axial couplings (J~12.5 Hz), one attributable to H-10α (δ 1.46 ppm) and the other thereby assigned to an axial H-8α. Thus, CH_3-16 is placed in the equatorial β position on C-8.

The placement of the hydroxyethyl group on the heterocyclic ring was straightforwardly made by observation of tidy, isolated interactions in both the hetero- and homonuclear correlation experiments (Table 1). Furthermore, NOEs were observed between the N-Me H_3-8′ (δ 3.00 ppm) and both H-5′ (δ 3.80 ppm, 2.1%) and H-6′ (δ 4.20 ppm, 4.1%). Because epimerization of C-5′ has been observed,[1,3] the relative stereochemistries of the chiral centers of the tetramic acid moiety were not determined.

The biological activity of cissetin (**1**) was assessed relative to that of equisetin (**2**) and trichosetin (**3**). All compounds were similarly active against MRSA (ATCC 33591, MIC 2~4 μg/ml), MSSA (ATCC 25923, MIC

2~4 μg/ml), vancomycin-resistant *Enterococcus faecium* (ATCC 51559, MIC 4~8 μg/ml), *E. faecalis* (ATCC 29212, MIC 4~8 μg/ml), and inactive against *Escherichia coli* (ATCC 25922, MIC >128 μg/ml). Cissetin (**1**) was 4~8 times more active than **2** and **3** against penicillin-resistant *Streptococcus pneumoniae* (ATCC 35088, MIC 2 μg/ml); however, while **2** and **3** were marginally active against *Candida albicans* (ATCC 90028, MIC 64 μg/ml and 32 μg/ml, respectively), **1** was completely inactive (MIC >128 μg/ml). Because the shapes of the *cis*- and *trans*-fused octalin ring systems are quite different, the similarity of antibiotic activities suggests, not surprisingly,[8] that it is the tetramic acid moiety which confers the antibiotic qualities of the setins.

Experimental

General

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra and 2-D COSY and ROESY NMR contour plots were recorded in $CDCl_3$ at room temperature (20°C) on a Varian Mercury NMR spectrometer using 5 mm Varian inverse-detection pulse field gradient or broad band probes tuned to the nucleus of interest; $CDCl_3$ solvent signals were used as references (δ 7.27 ppm and δ 77.0 ppm, respectively). The absorbance spectrum was obtained with photodiode array detection of eluent during HPLC with a Gilson 215 using Zorbax XDB-C18 columns and aq MeOH gradient (1 minute at 30% MeOH, 37 minute ramp to 100% MeOH, 10 minutes elution with 100% MeOH). Low- and high-resolution FAB mass spectrometric measurements were made on a modified Finnegan MAT-212 high-resolution, double-focusing spectrometer.

Producing Microorganism and Fermentation

OSI 50185 is a sterile, hyaline fungus and was isolated from decomposed aerial plant litter gathered on Rio Pacaya near Bretaña, Peru. A voucher specimen of OSI 50185 is preserved at Mycosynthetix, Inc. (Durham, NC). OSI 50185 was cultured and fermented using a reported procedure standard in the MYCOsearch laboratories[9] except that the scale-up medium comprised casein (2%), dextrose (2%), and yeast extract (1%).

Isolation of Cissetin (1)

Crude MeOH extract of 3 liters of the total freeze-dried fermentation was dissolved in aq MeOH and was then partitioned sequentially between hexanes and $CHCl_3$. The $CHCl_3$ portion was concentrated to a light brown residue and was chromatographed by reversed phase HPLC. Cissetin (**1**) eluted at 100% MeOH and was concentrated to a white, foamy powder: UV λ_{max} (aq MeOH) nm: 200, 235, 291; FAB-MS *m/z* 432 $[M-H+2Na]^+$, 410 $[M+Na]^+$, 388 $[M+H]^+$; ^{1}H and ^{13}C NMR see Table 1; yield ~170 mg/liter.

Antibacterial Assay

Antibacterial assays for determination of the IC_{50} values for **1**, **2**, and **3** were performed according to the National Committee for Clinical Laboratory Standards' "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically." Vancomycin and ciprofloxacin were used as standards.

References

1) PHILLIPS, N. J.; J. T. GOODWIN, A. FRAIMAN, R. J. COLE & D. G. LYNN: Characterization of the *Fusarium* toxin equisetin: The use of phenylboronates in structure assignment. J. Am. Chem. Soc. 111: 8223~8231, 1989
2) MARFORI, E. C.; S. KAJIYAMA, E. FUKUSAKI & A. KOBAYASHI: Trichosetin, a novel tetramic acid antibiotic produced in dual culture of *Trichoderma harzianum* and *Catharanthus roseus* callus. Z. Naturforsch. 57c: 465~470, 2002
3) SINGH, S. B.; D. L. ZINK, M. A. GOETZ, A. W. DOMBROWSKI, J. D. POLISHOOK & D. J. HAZUDA: Equisetin and a novel opposite stereochemical homolog phomasetin, two fungal metabolites as inhibitors of HIV-1 integrase. Tetrahedron Lett. 39: 2243~2246, 1998
4) SUGIE, Y.; K. A. DEKKER, T. INAGAKI, Y.-J. KIM, T. SAKAKIBARA, S. SAKEMI, A. SUGIURA, L. BRENNAN, J. DUIGNAN, J. A. SUTCLIFFE & Y. KOJIMA: A novel antibiotic CJ-17,572 from a fungus, *Pezicula* sp. J. Antibiotics 55: 19~24, 2002
5) SUGIE, Y.; S. INAGAKI, Y. KATO, H. NISHIDA, C.-H. PANG, T. SAITO, S. SAKEMI, F. DIB-HAJJ, J. P. MUELLER, J. SUTCLIFFE & Y. KOJIMA: CJ-21,058, a new SecA inhibitor isolated from a fungus. J. Antibiotics 55: 25~29, 2002
6) SARITAS, Y.: Isolierung, strukturaufklärung und stereochemische untersuchungen von sesquiterpenoiden inhaltsstoffen aus ätherischen ölen von Bryophyta und höheren pflanzen. Ph.D. Thesis, Universität Hamburg, 2000.
7) NMR shifts in reference 6 are reported in d_6-benzene. Compound **1** gave similar shifts for H-6 (δ 2.35 ppm) and H-11 (δ 2.88 ppm) when recorded in d_6-benzene.
8) ROYLES, B. J. L.: Naturally occurring tetramic acids: structure, isolation, and synthesis. Chem. Rev. 95: 1981~2001, 1995
9) BOROS, C.; B. KATZ, S. MITCHELL, C. PEARCE, K. SWINBANK & D. TAYLOR: Emmyguyacins A and B: unusual glycolipids from a sterile fungus species that inhibit the low-pH conformational change of hemagglutinin A during replication of influenza virus. J. Nat. Prod. 65: 108~114, 2002 and Supporting Information available on the Internet at http://pubs.acs.org