VOL. 56 NO. 2, FEB. 2003 THE JOURNAL OF ANTIBIOTICS pp. 114 — 122

Coniosetin, a Novel Tetramic Acid Antibiotic from *Coniochaeta ellipsoidea* DSM 13856

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(Received for publication August 12, 2002)

The coprophilic ascomycete *Coniochaeta ellipsoidea* DSM 13856 forms the new antibiotic coniosetin (**1**) in surface cultures grown on a medium containing malt extract and oatmeal. The structure of the compound $C_{25}H_{35}NO_4$, MW 413, was determined by 2D-NMR and mass spectrometric studies. Coniosetin belongs to the class of tetramic acids; it consists of a substituted aliphatic bicyclic ring system linked to a tetramic acid subunit through a carbonyl center. The absolute configuration was determined by measuring its circular dichroism spectrum and comparing the data with those of equisetin. Coniosetin has a pronounced antibacterial and antifungal action, inhibiting even multi drug-resistant strains of *Staphylococcus aureus* at a concentration of 0.3 μg/ml, though it is inactive against Gram-negative bacteria.

An obvious consequence of the development of resistance by pathogenic microorganisms[1] is a growing demand for new medicines for the control of infectious diseases. However, a review article by J. J. BRONSON and J. F. BARRETT[2] has shown that the pharmaceutical industry, no doubt as a result of having abandoned the intensity with which it had conducted antibiotic screenings in earlier decades, is now for the most part working on antibacterial products that are merely variants of known classes of antibiotic. In the course of our investigations into new natural products, we isolated an antibiotic that bore no resemblance to antiinfective agents in current use and embarked on an investigation of the new compound (**1**).

Reports in the scientific literature of secondary metabolites of ascomycetes of the genus *Coniochaeta* have up to now been few and far between, the only examples being the benzopyranone derivatives coniochaetone A and B[3], isolated from *C. saccardoi* and *C. tetraspora*, which show not only antifungal, but also monoamine oxidase inhibiting activity[4]. This prompted an investigation of constituents of the coprophilic fungus *Coniochaeta ellipsoidea* Udagawa, DSM 13856, which led to the discovery of a new antibiotic which we named coniosetin (**1**). In this article we describe the microbiological production, isolation, structural characterization, and some biological properties of the compound **1**.

Materials and Methods

General

Quantitative ultraviolet absorption spectra were recorded using a Perkin Elmer Lambda 2 spectrometer; for all other purposes, including the performance of HPLC analyses, Hewlett-Packard series 1100 equipment fitted with diode array detectors was used. Preparative HPLC was performed using Gilson 331 and 332 pumps (Abimed Analysen-Technik GmbH, Langenfeld, Germany), model 87.00 variable-wavelength monitors (Dr. KNAUER, Berlin, Germany), and a Büchi B-684 fraction collector (Büchi Labortechnik GmbH, Flawil, Switzerland). Pharmacia model 2248/2252 HPLC equipment (Uppsala, Sweden) was

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also used.

Circular dichroism (CD) spectra were recorded on a Jasco spectropolarimeter, model J-810.

Fermentation

A starter culture was produced by inoculating 200 ml of nutrient medium (20 g/liter malt extract, 2 g/liter yeast extract, 10 g/liter glucose, 0.5 g/liter $(NH_4)_2HPO_4$, pH 6.0) in a sterile 300 ml conical flask with the strain *Coniochaeta ellipsoidea* Udagawa, DSM 13856, which was then incubated on a rotary shaker for 4 days at 25°C and 140 rpm. This starter culture was then used as the inoculum for the production of the main cultures.

Production of coniosetin was carried out in 100 sterile 22.5×22.5 cm^2 Nunc plates, each charged with 200 ml of nutrient medium A (20 g/liter malt extract, 20 g/liter oatmeal, 0.5 g/liter $CaCO_3$, pH 7.0) as well as 20 g/liter agar. After allowing the nutrient medium to cool, the plates were inoculated with 2 ml of the starter culture and incubated at 25°C. Maximum production of coniosetin was achieved after approx. 670 hours.

Cultures of *Coniochaeta ellipsoidea* Udagawa, DSM 13856, were also grown on the following media:

Nutrient medium A: 20 g/liter malt extract, 20 g/liter oatmeal, 0.5 g/liter $CaCO_3$, pH 7.0.

Nutrient medium B: 30 g/liter sucrose, 3 g/liter $NaNO_3$, 1 g/liter K_2HPO_4, 0.5 g/liter $MgSO_4 \cdot 7H_2O$, 0.01 g/liter $FeSO_4 \cdot 7H_2O$, 0.5 g/liter KCl, pH 7.3.

Nutrient medium C: 5 g/liter cornsteep liquor, 40 g/liter tomato paste, 10 g/liter oatmeal, and 10 g/liter of trace element solution, adjusted to pH 6.8. The trace element solution contained: 1 g/liter $FeSO_4 \cdot 7H_2O$, 1 g/liter $MgSO_4 \cdot 7H_2O$, 0.025 g/liter $CuCl_2 \cdot 2H_2O$, 0.1 g/liter $CaCl_2 \cdot 2H_2O$, 0.056 g/liter H_3BO_3, 0.019 g/liter $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 0.2 g/liter $ZnSO_4 \cdot 7H_2O$.

Nutrient medium D: 30 g/liter oatmeal, 0.5 g/liter K_2HPO_4, 0.2 g/liter KNO_3, 0.2 g/liter $MgSO_4 \cdot 7H_2O$.

Nutrient medium E: 20 g/liter malt extract, adjusted to pH 6.0.

For the shake cultures, 100 ml portions of the respective sterile nutrient media A~E in 300 ml conical flasks were inoculated with the starter culture of *Coniochaeta ellipsoidea* Udagawa, DSM 13856, and incubated for varying lengths of time on a shaking machine at 25°C and 100, 140, or 180 rpm. Shake flasks were removed after 5, 10, 17, 20, 23, and 28 days and the cultures were freeze dried, extracted with methanol, and their contents investigated by HPLC.

For the preparation of surface cultures, 20 g/liter agar was added to media A~E. The mixtures were then sterilized and 30 ml portions were poured into petri dishes. After cooling, the plates were each inoculated with 0.3 ml of the *Coniochaeta ellipsoidea* Udagawa, DSM 13856, starter culture and incubated at 25°C, without agitation, for varying lengths of time. Agar cultures were removed after 7, 9, 12, 14, 16, 18, 21, and 28 days and then freeze-dried, extracted with methanol, and their coniosetin content determined by HPLC.

Isolation

The colonized agar of the surface cultures grown on nutrient medium A was removed from the plates, freeze-dried (499 g), and extracted with 8 liters of methanol. The clear liquid phase was concentrated to 500 ml under reduced pressure (solid content: 79.8 g), diluted with water, and loaded onto a column (volume: 580 ml, dimensions 5×30 cm) packed with the adsorption resin MCI Gel® CHP20P (Mitsubishi Chemical Industries, Tokyo, Japan). The column was eluted with a solvent gradient of 5 to 90% acetonitrile in water and the eluate was collected at a rate of 40 ml/minute in fractions of 120 ml. The coniosetin-containing fractions 30~32 (checked by HPLC analyses) were pooled, concentrated under reduced pressure, and freeze-dried (0.98 g). The enriched coniosetin antibiotic was purified on a LiChrospher® 100 RP-18e HPLC column (dimensions: 2.5×25 cm), which was eluted at a flow rate of 30 ml/minute with a gradient of 75 to 100% acetonitrile in 0.05% acetic acid, with the eluate collected in 60 ml fractions. Fractions 6~8 (checked by HPLC analyses) were pooled on the basis of their coniosetin content, concentrated under reduced pressure, and freeze-dried, yielding 503 mg of coniosetin in 98% purity. ESI-MS: m/z=414.2640 amu, calculated for $C_{25}H_{36}NO_4$ (MH)= 414.2639; UV λ_{max}^{MeOH} nm (ε) 232 (19000), 287 (5500); IR (KBr): ν_{max}=3396, 2868, 1676, 1567, 1454, 1380 cm^{-1}; $[\alpha]_D^{23}=-319°$ (c=0.1, MeOH). The CD spectrum was measured as a 0.12 mM solution in methanol: $\Delta\varepsilon_{max}=-12.0$ at 232 nm, $\Delta\varepsilon_{min}=-1.4$ at 251 nm, and $\Delta\varepsilon_{max}=-11.7$ at 283 nm. NMR data are shown in Table 1.

Analytical HPLC of coniosetin was carried out on a Superspher 100 RP-18e® 250-4 column (E. Merck, Darmstadt, Germany). The column was eluted with a mobile phase consisting of 75% acetonitrile in 0.1% phosphoric acid at a flow rate of 1 ml/minute, with UV detection at 210 nm. The retention time of coniosetin under these conditions was 13.6 minutes.

NMR Spectroscopy

NMR samples of coniosetin were prepared by dissolving 4.5 mg of compound in 0.5 ml of methanol-d_4 or 6.5 mg in

Table 1. ^{1}H and ^{13}C NMR chemical shift assignments for coniosetin in DMSO-d_6 and methanol-d_4 at 300 K.

	DMSO-d_6		Methanol-d_4	
Position	^{13}C δ (ppm)	^{1}H δ (ppm)	^{13}C δ (ppm)	^{1}H δ (ppm)
1	48.94	-	51.18	-
1-Me	13.34	1.33 s	14.31	1.42 s, br
2	48.45	3.19	50.59	3.28 br
3	130.99	-	133.15	-
3-Me	22.04	1.53 t	22.67	1.58 t
4	125.88	5.19 s, br	127.31	5.20 s
5	38.61	1.80	40.68	1.86 m
6	42.06	1.78, 0.82	44.10	1.83 d, br, 0.87 m
7	32.90	1.49	35.00	1.52 m, br
7-Me	22.40	0.89 d	23.07	0.94 d
8	35.44	1.72, 1.01	37.20	1.77 d, br, 1.10 m
9	27.59	1.94 d, 1.00	29.47	2.01 d, br, 1.06 m
10	39.28	1.57	41.39	1.66 m
11	130.42	5.18 m	132.05	5.19
12	131.96	5.72 t	134.03	5.78 t
13	131.31	5.91 t	132.71	5.90 t
13a	127.83	5.52 m	129.12	5.51 m
13b	17.73	1.65 d	18.23	1.67 d
14	198.23	-	201.30	-
14-OH	-	17.49 s, br	-	-
15	99.46	-	101.50	-
16	179.52	-	181.53	-
17	-	9.22 s, br	-	-
18	66.57	3.62	68.19	3.62 br
19	191.09	-	193.51	-
20	65.66	3.91	68.11	4.06 br
20-OH	-	4.76 d	-	-
21	20.67	1.17 d	20.65	1.29 d, br

0.5 ml of DMSO-d_6. Wilmad NMR tubes (grade 528) with a diameter of 5 mm were used.

All spectra were recorded on a Bruker DRX600 spectrometer operating at 600.13 MHz and 150.92 MHz for ^{1}H and ^{13}C respectively or on a Bruker DRX500 operating at 500.13 MHz (^{1}H) and 125.76 MHz (^{13}C). The temperature was set at 300 K for all samples. All experiments were performed in phase-sensitive mode using standard pulse sequences employing time-proportional phase incrementation (TPPI) for quadrature detection in F_1[5].

For the homonuclear experiments (double quantum-filtered [DQF]-COSY[6~8], NOESY[9,10], ROESY[11]) the spectral width was set to 7 ppm (methanol-d_4) or 19 ppm (DMSO-d_6) in both dimensions, F_1 and F_2. For all experiments 512 increments in t_1 were recorded with 2048 complex data points in t_2. Either 8, 24, or 32 transients were averaged for each t_1 value. The ROESY spectrum was recorded with a mixing time of 150 ms and a B_1 field strength of 3 kHz. In the NOESY experiment the mixing delay was set to 600 ms.

To record heteronuclear multiple quantum correlation (HMQC) spectra[12], 512 increments with 16 (DMSO-d_6) or 64 scans (methanol-d_4) and 2048 complex data points in t_2

were collected. Sweep widths of 7 ppm in the proton dimension and 160 ppm in the carbon dimension were used. A bilinear rotation decoupling (BIRD) pulse was applied to suppress magnetization of protons bound to ^{12}C. A delay of 3.45 ms, corresponding to 145 Hz, was used for the evolution of ^{1}H-^{13}C one-bond couplings. The heteronuclear multiple bond correlation (HMBC) spectra[13] were acquired with a sweep width of 7 ppm (methanol-d_4) or 19 ppm (DMSO-d_6) in the proton dimension and 220 ppm in the carbon dimension. 512 increments in t_1 and 2048 complex data points in t_2 were recorded. The delay for the evolution of long-range correlations was set to correspond to $^{n}J_{CH}$ coupling constant values of 7 Hz. 32 (DMSO-d_6) or 64 (methanol-d_4) scans per increment were collected.

Before undergoing Fourier transformation, all 2D time domain data were subjected to apodization using adjusted sine and squared sine bell window functions. The software packages XWINNMR and AURELIA (Bruker, Rheinstetten, Germany) were used for data processing.

Mass Spectrometry

Mass spectra were recorded by infusing a MeCN/H_2O solution (50/50) of the sample into the external ESI source of a Bruker Apex III FTICR-MS (7T) instrument at a flow rate of 4 μl/minute. MS/MS spectra were obtained by IRMPD. The elemental compositions of MS/MS fragment ions were verified based on a mass accuracy <10 ppm.

Antibacterial Activity

Minimal inhibitory concentrations (MIC) were measured by a two-fold microdilution method in Mueller-Hilton broth medium (pH 7.4; Diagnostic Pasteur, France). A standard inoculum of 10^5 cfu/ml was used. Plates were incubated at 37°C for 24 hours. MIC was defined as the lowest concentration at which no visible growth could be detected.

Results

The organism *Coniochaeta ellipsoidea* Udagawa, DSM 13856, was isolated from a Japanese soil sample. The strain forms oblong ascospores arranged in packets of eight in yellow to brown perithecia. At 20~25×10~11.5 μm, they are larger than many ascospores of other species[14].

The strain *Coniochaeta ellipsoidea* Udagawa, DSM 13856, grew well on all the nutrient media investigated, with the exception of the synthetic nutrient medium B. Both the shake cultures and the surface cultures formed abundant mycelia, good growth being observed even in submerged cultures in 30 liter fermenters at 28°C and 170 rpm over a period of 500 hours. However, the antibiotic coniosetin was only formed by the strain *Coniochaeta ellipsoidea* Udagawa, DSM 13856, in stationary cultures on nutrient medium A (malt extract/oatmeal), on which the product was obtained in reproducibly good yield. HPLC analysis of individual plates showed that the biosynthesis of coniosetin begins after 7 days, increasing sharply in the period between day 16 and day 27, but with only moderate additional product formation beyond this point up to day 35. The coniosetin was harvested from agar plates cultured for 28 days.

Isolation and Characterization

The microbiological yield on the malt/yeast medium was more than 5 mg coniosetin[15] per 22.5×22.5 cm plate. The antibiotic was isolated by freeze-drying the agar surface cultures, extraction of the antibiotic with methanol, and chromatography of the alcoholic extract on adsorption resin CHP20P to remove the dissolved agar. Repeated preparative HPLC gave 0.5 g of coniosetin in 98% purity. Physicochemical data are listed in Table 2. Coniosetin is a colorless substance soluble in polar organic solvents and in water at pH 9. The UV spectrum [maximum at 232 nm (ε=19000)] is consistent with a diene structure and [287 nm (ε=5500)] with the tetramic acid moiety proposed below. The characteristic bands in the IR spectrum at 1676 and 1567 cm^{-1} likewise correspond to those reported for other tetramic acids.

Structure Elucidation by NMR

The structure of coniosetin was determined by NMR studies using a combination of 1D and 2D NMR techniques including ^{1}H, ^{13}C, DQF-COSY, ROESY, NOESY, ^{1}H-^{13}C HMQC, and ^{1}H-^{13}C HMBC spectra. Proton and carbon resonances were assigned using solutions in methanol-d_4 and DMSO-d_6 (Table 2).

Coniosetin (Fig. 1) consists of three structural subunits: a decalin ring system, a tetramic acid moiety and an olefinic side chain. It is a novel isomer of the HIV integrase inhibitor phomasetin[16] with different activity.

Comparison of the ^{1}H and ^{13}C chemical shifts of coniosetin with those published for phomasetin indicated that the structures of the decalin ring moiety and olefinic side chain are the same in both compounds. This result was further substantiated by the ^{1}H-^{13}C long range correlations observed in the HMBC spectrum of the coniosetin sample (Fig. 2). In particular, the connection of the olefinic side chain to position 2 of the decalin ring is proved by the

Table 2. Physicochemical properties of coniosetin.

Appearance	White powder
Molecular weight	413.5
Molecular formula	$C_{25}H_{35}NO_4$
HRFAB-MS (*m/z*)	
Found:	414.2645 $(MH)^+$
Calcd:	414.2644
$[\alpha]_D^{23}$	-319° (*c* = 0.1, MeOH)
UV (MeOH) λ_{max} nm (ε)	232 (19 000), 287 (5 500)
(MeOH + 0.02 N HCl)	232 (21 700), 287 (6 200)
(MeOH + 0.02 N NaOH)	208 (45 100), 236 (21 800), 288 (8 000)
CD (MeOH) λ_{ext} nm (Δε)	330 (0), 283 (-11.7), 251 (-1.4), 232 (-12.0), 227 (-11.4), 211 (0)
IR (KBr) ν_{max}:	3396, 2868, 1676, 1567, 1454, 1380 cm^{-1}

Fig. 1. Structure of coniosetin.

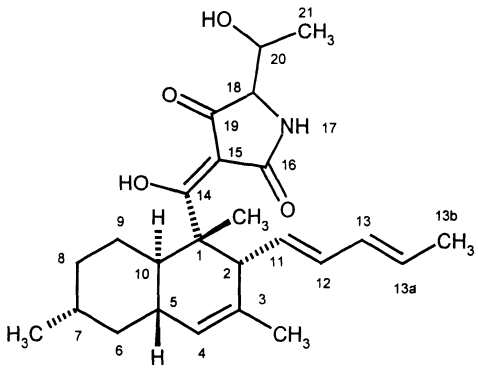
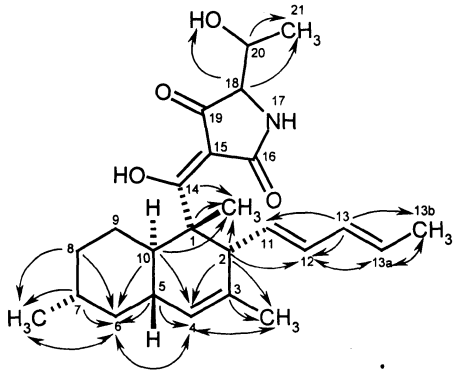


Fig. 2. ^1H-^{13}C long range correlations observed in the HMBC spectrum of coniosetin.

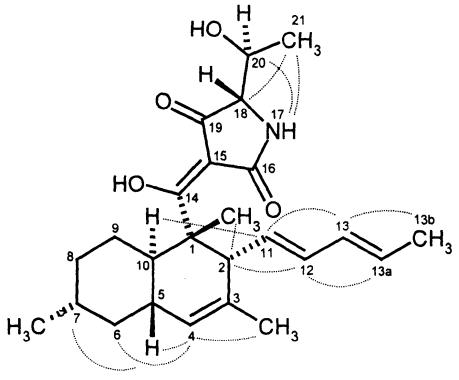


Arrows pointing from carbon atoms to correlated protons indicate correlations detected. Double-headed arrows indicate the observation of both possible correlations.

observation of a cross peak between C2 and H12 in the DMSO-d_6 and methanol-d_4 sample and by a weak four-bond correlation between C11 and 3-CH_3 (seen only in methanol-d_4). The position of the 3-methyl group was determined from the correlations between C2, C3, C4 and the methyl protons in the HMBC spectrum. 1-CH_3 is connected to C1 by ^1H-^{13}C *J*-coupling cross peaks from the methyl protons to C1, C2, C10, and C14. In addition to the ^1H-^{13}C correlations shown, the observed cross peaks for the proton spin systems in the DQF-COSY spectrum allowed the assignment of the strech C5-C10 and of the olefinic side chain.

As was evident from the NMR spectra, the structural difference between coniosetin and phomasetin is located in the tetramic acid moiety. Whereas in phomasetin the amide nitrogen (N-17) is methylated and position 18 bears a hydroxymethyl group, in coniosetin N-17 carries a hydrogen and C18 a 1-hydroxyethyl side chain. The amide proton was observed in the ^1H NMR spectrum of coniosetin as a broad singlet at 9.22 ppm. Signals at 3.91 (^{13}C: 65.66 ppm) and 1.17 ppm (^{13}C: 20.67 ppm) are attributable to the hydroxyethyl side chain. In addition, strong NOE correlations are observed between the amide proton and 21-

Fig. 3. NOE correlations and derived relative stereochemistry for coniosetin.



Observed NOE are indicated by dotted lines. For reasons of clarity, only those correlations of relevance to the relative stereochemistry are shown. Some of the expected correlations could not be detected because of signal overlap.

CH_3 and H20.

The relative stereochemistry of coniosetin was determined from the observed NOE correlations. Strong NOEs between H11/H13, H12/H13a, and H13/H13b pointed to an *E* configuration for both double bonds of the olefinic side chain. The 5,10-*trans* ring fusion of the decalin ring is indicated by absence of a cross peak between H5 and H10 in both the NOESY and ROESY experiments. A strong 1,3-diaxial cross peak between H5 and H7 is evidence for a *cis* orientation for these two protons. The strong NOE correlations between protons H10/H11 and H2/1-CH_3 revealed the stereochemistry of carbons C1 and C2. The relative stereochemistry determined for coniosetin is identical to the published configuration of phomasetin. This is further confirmed by the almost identical chemical shifts observed for the decalin and olefin moieties of the two compounds. The relative configuration of the remaining two stereocenters (C18 and C20) could not be determined unambiguously from the NMR data. However, the presence of a strong NOE between the amide proton H17 and the methyl group 21-CH_3, together with the absence of a NOE between the amide proton and the 20-OH hydroxyl proton indicated hindered rotation of bond C18~C20, most probably attributable to a hydrogen bond between 20-OH and the C19 carbonyl oxygen. Based on this assumption, the missing NOE between H18 and H20 in the NOESY and ROESY experiments would suggest an anti relationship for the two protons and thus point to a *R,R* or *S,S* configuration.

Mass Spectrometry

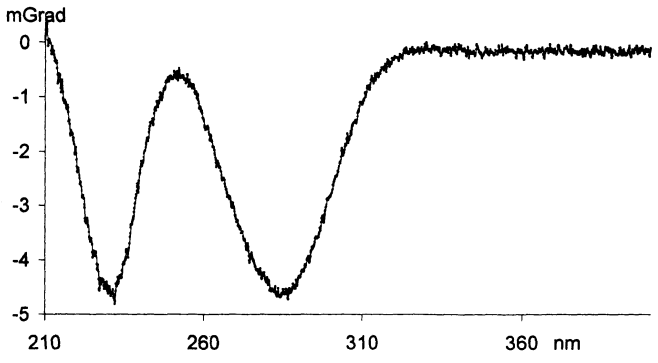
Coniosetin is readily ionized in both ESI^+ and ESI^- modes, in accordance with the presence of both basic and acidic functional groups. In addition to deriving the elemental composition of coniosetin from a high-resolution mass measurement, a structural characterization was carried out using MS/MS measurements. Upon laser excitation, the deprotonated molecule (412 amu) fragments mainly to ions at *m/z* 368 ($C_{24}H_{34}NO_2^-$), 368 ($C_{23}H_{30}NO_3$), 350 ($C_{23}H_{28}NO_2^-$), 324 ($C_{22}H_{30}NO^-$), 268 ($C_{18}H_{22}NO^-$) 229 ($C_{16}H_{21}O^-$), and 138 amu ($C_7H_8NO_2^-$). In the ESI^+ mode, the dominant fragments of the excited protonated molecular ion (414 amu) are at *m/z* 396 ($C_{25}H_{34}NO_3$), 386 ($C_{24}H_{36}NO_3$), 370 ($C_{23}H_{32}NO_3^+$), 346 ($C_{20}H_{28}NO_4^+$), 328 ($C_{20}H_{26}NO_3^+$), 278 ($C_{15}H_{20}NO_4^+$), 271 ($C_{19}H_{27}O^+$), 253 ($C_{19}H_{25}^+$), 243 ($C_{18}H_{27}^+$), 215 ($C_{16}H_{23}^+$), and 196 amu ($C_{10}H_{14}NO_3^+$). Since the molecule contains few labile cleavage sites, several fragmentations are preceded by extensive rearrangements. For example, the loss of CO_2 in the ESI^- mode (324 amu) requires a succession of C–X (X=N, O) bond cleavage/formation steps. However, the following three structure-specific fragments were of diagnostic value: In both modes, the 1-hydroxyethyl side chain is easily cleaved by a retro-aldol reaction; this reaction clearly distinguishes coniosetin from phomasetin or equisetin. In the positive mode, diagnostic fragmentations include loss of the olefinic side chain of the decalin moiety (346 and 328 amu) and cleavage of the tetramic acid moiety (271 and 243 amu).

Whereas the relative configuration of coniosetin thus largely corresponds to that of phomasetin, the specific rotation of the new compound $[\alpha]_D^{23}=-319°$ ($c=0.1$, MeOH) is of the same magnitude and sign as that of equisetin ($[\alpha]_D^{22}=-278°$; $c=0.77$, $CHCl_3$)[16]. Equisetin is a quasi-mirror image stereochemical homolog of phomasetin, which has a reported rotation of $[\alpha]_D^{22}=+93.9°$ ($c=1.05$, $CHCl_3$)[16]. Coniosetin can thus be assigned the absolute configuration of equisetin and not the steric configuration of phomasetin.

To confirm the stereochemistry of coniosetin (Fig. 1), its CD spectrum was recorded. Fig. 4 shows the Cotton effect measured in methanolic solution, with $\Delta\varepsilon_{max}=-12.0$ at 232 nm, $\Delta\varepsilon_{max}=-1.4$ at 251 nm, and $\Delta\varepsilon_{max}=-11.7$ at 283 nm. The reported literature values for equisetin are $\Delta\varepsilon_{max}=-5.5$ at 227 nm, $\Delta\varepsilon_{max}=-7.5$ at 235 nm, $\Delta\varepsilon_{max}=-3$ at 260 nm, and $\Delta\varepsilon_{max}=-8.9$ at 290 nm, and are

consistent with the absolute configuration postulated for coniosetin.

Fig. 4. Circular dichroism spectrum of a 0.12 mM solution of coniosetin in methanol.



Biological Activities

As can be seen from Table 3, coniosetin (**1**) shows strong antimicrobial activity against various Gram-positive bacteria[15] such as *Staphylococcus aureus*, *Enterococcus faecalis*, and *Streptococcus pneumoniae*, but none against Gram-negative bacteria. Coniosetin was effective against various streptococci, including erythromycin-resistant and penicillin-resistant strains and clinically isolated species. Remarkably, **1** also showed potent antimicrobial activities against clinically isolated multiresistant *Staphylococcus aureus* and erythromycin-resistant enterococci.

In addition, coniosetin inhibits the yeast *Candida albicans* at a concentration of 3.1 μg/ml, though it is somewhat less active against *Aspergillus niger*. A cytotoxic

Table 3. Antimicrobial activity of coniosetin against various bacteria, expressed as minimal inhibitory concentrations (MIC).

Strain		Phenotype of resistance	MIC (μg/ml)
Staphylococcus aureus	011UC4	ery S	0.3
S. aureus	011B18c	oxa S ery R	0.3
S. aureus	011GR12c	oxa R ery R	0.3
S. aureus	011GO25i	oxa S ery R	0.6
S. epidermidis	012GO11i	oxa S ery R	0.3
S. aureus	011CB20c	oxa R ery R tet R	0.3
S. aureus	011GO64	ofl R oxa R ery R tet R	0.6
S. epidermidis	012GO40c	oxa R ery R	1.2
S. pyogenes	02A1UC1	ery S	1.2
Enterococcus faecalis	02D2UC1	ery S	2.5
Enterococcus faecalis	02D2DU15	ery R	2.5
Enterococcus faecium	02D3HT12	tei R van R ery R tet R	1.2
Enterococcus faecium	02D3IP2	tei R van R ery R tet R	1.2
Streptococcus gr.G	02GOGR5	ery S	1.2
S. mitis	02MitCB1	ery S	2.5
S. pyogenes	02A1SJ1c	ery R	1.2
S. agalactiae	02B1SJ1c	ery R	2.5
Streptococcus gr.G	02GOGR4	ery R	0.6
S. sanguis	02SgGr10i		1.2
S. mitis	02MitGR16i		2.5
S. pneumoniae	032UC1	ery R	0.6
S. pneumoniae	030GR20	ery R	1.2
S. pneumoniae	030SJ5i	ery R	0.6
S. pneumoniae	030CR18c	ery R pen R	0.6
S. pneumoniae	030PW23c	ery R pen R	0.6
S. pneumoniae	030RO1i	ery R	0.6
S. pneumoniae	030SJ1c	ery R	0.6
Escherichia coli	250 UC5		> 40

S = sensitive, R = resistant, ery = erythromycin, ofl = ofloxacin, oxa = oxacillin, pen = penicillin, tei = teicoplanin, van = vancomycin, tet = tetracyclin.

effect was observed for coniosetin at concentrations as low as 2 μg/ml, which means that the new compound **1** is two to three times more toxic than amphothericin B under the same conditions.

Discussion

Although the past ten years has seen a downturn in classical antibiotic screening reported in the scientific literature, in the same period there has been an astonishingly high number of publications describing naturally occurring tetramic acids with antibiotic activity. Current understanding of these natural 2,4-pyrrolidinediones was the subject of a 1995 review by B. J. L. ROYLES[17]. Others since reported include vancoresmycin[18], ascosalipyrrolidinone A[19], reutericyclin[20], F-10778[21], rubrosides[22], cryptocin[23], talaroconvolutins[24], xanthobaccin A[25], CJ-17,572[26], and CJ-21,058[27]. This list does not include the new tetramic acids for which effects other than antibiotic activity have been reported. The 2,4-pyrrolidinedione subunit is thus clearly a common structure type in natural products of microbial or marine origin.

The antibiotic coniosetin described here possesses a bicyclic nonpolar ring system and a polar tetramic acid moiety. Its structure resembles that of phomasetin, apart from the unsubstituted ring nitrogen atom in coniosetin. The relative configuration of the two antibiotics is also the same, though their absolute configurations are mirror images of one another, *i.e.* coniosetin and phomasetin represent a rare example of a pair of complex natural products with mirror-image stereochemistry.

Coniosetin shows strong antibiotic activity against Gram-positive bacteria, though its additional inhibitory activity against fungi points to a cytotoxicity similar to that observed for its structural analog equisetin[28]. The equally high activity of coniosetin toward sensitive and resistant microbial pathogens alike demonstrates that nature is still capable of providing us with highly potent active substances that could overcome current problems with resistance in the treatment of bacterial infections. The very specific conditions under which the new antibiotic coniosetin is formed by the common fungus *Coniochaeta ellipsoidea* shows, moreover, that microbial cultures represent a source of active agents that is still not remotely exhausted.

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