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Microbial models of soil metabolism: biotransformations of danofloxacin

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Danofloxacin is a new synthetic fluoroquinolone antibacterial agent under development for exclusive use in veterinary medicine. Such use could lead to deposition of low levels of danofloxacin residues in the environment in manure from treated livestock. This study was conducted to evaluate the potential for indigenous soil microorganisms to metabolize danofloxacin. Cultures of 72 soil microorganisms representing a diverse panel of bacteria, fungi and yeast were incubated with danofloxacin mesylate substrate and samples analyzed periodically by high performance liquid chromatography for loss of danofloxacin and formation of metabolites. Some samples were further analyzed by liquid chromatography-mass spectrometry and mass spectrometry to confirm metabolite identification. Twelve organisms, representing eight different genera, biotransformed danofloxacin to metabolites detectable by the chromatographic methods employed. Two *Mycobacterium* species, two *Pseudomonas* species, and isolates of *Nocardia* sp, *Rhizopus arrhizus* and *Streptomyces griseus* all formed *N*-desmethyldanofloxacin. The formation of the 7-amino danofloxacin derivative, 1-cyclopropyl-6-fluoro-7-amino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid by cultures of *Candida lipopytica*, *Pseudomonas fluorescens*, two *Mycobacterium* species and three *Penicillium* species demonstrates the propensities of these cultures to completely degrade the piperazine ring. At least two additional and unidentified metabolite peaks were observed in chromatograms of *Aspergillus nidulans* and *Penicillium* sp cultures. Radiolabled [2-^{14}C]danofloxacin added to cultures of the fungus *Curvularia lunata* was apparently mineralized, with approximately 31% of the radiolabel recovered as volatile metabolites after 24 h of incubation, indicating the susceptibility of the quinolone ring to microbial metabolic degradation.

Keywords: microbial transformations; danofloxacin

Introduction

Danofloxacin (1-cyclopropyl-6-fluoro-7-[(1*S*, 4*S*)-5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid) (**1**, Figure 1) is a new, synthetic antibacterial agent of the fluoroquinolone class for treatment of respiratory diseases of livestock [19,29,30]. Fluoroquinolone antimicrobials target bacterial DNA gyrase (topoisomerase II), binding to the enzyme and interfering with DNA replication, repair and transcription, leading to rapid cell death [12,17,28,32,51]. The potent cidal activity of fluoroquinolones against a broad spectrum of Gram-negative and Gram-positive bacteria and mycoplasmas has led to development of a number of these agents for clinical use [6,32,51]. Danofloxacin, developed exclusively for veterinary use, has excellent activity against bacterial respiratory pathogens of cattle, swine and poultry and demonstrated efficacy in therapeutic treatment of both induced and natural respiratory infections in these animals [8,14,15,19,30,48].

Veterinary use of danofloxacin will result in its deposition into the environment in manure from treated animals. Soil levels will be quite low, and sorption properties insure that bioavailable levels of danofloxacin will be far below levels inhibitory to soil microorganisms. It is important to determine whether these danofloxacin residues will persist in the environment or be degraded. Soils contain many bacterial, yeast, and fungal species that are well known for their individual or collective abilities to catalyze nearly every type of oxidative, reductive, conjugative or degradative reaction sequence with natural or xenobiotic organic compounds [5,9,13,18,21,23,26,36,38–42,50], suggesting that the mechanisms necessary to degrade danofloxacin might be present in some soil microorganisms. Although soil biodegradation studies indicate that danofloxacin will indeed be biotransformed by indigenous soil microorganisms [49], relatively slow rates of transformation under *in vitro* test conditions and the production of a number of minor metabolites make it difficult to extract sufficient quantities of degradation products for identification. The use of selected microorganisms as 'models for xenobiotic metabolism' [37,43–46] has been successfully exploited under similar circumstances in mammalian metabolism studies of diverse compounds to aid in predicting degradative pathways where direct isolation of mammalian metabolites proves difficult [4,7,10,11,22,24,35]. This approach has been recently exploited in a similar manner as a model for soil metabolism of clomazone, providing the basis for predicting degradative pathways for the herbicide in the environment [24]. We decided to exploit such an approach with danofloxacin.

This study was undertaken to examine danofloxacin

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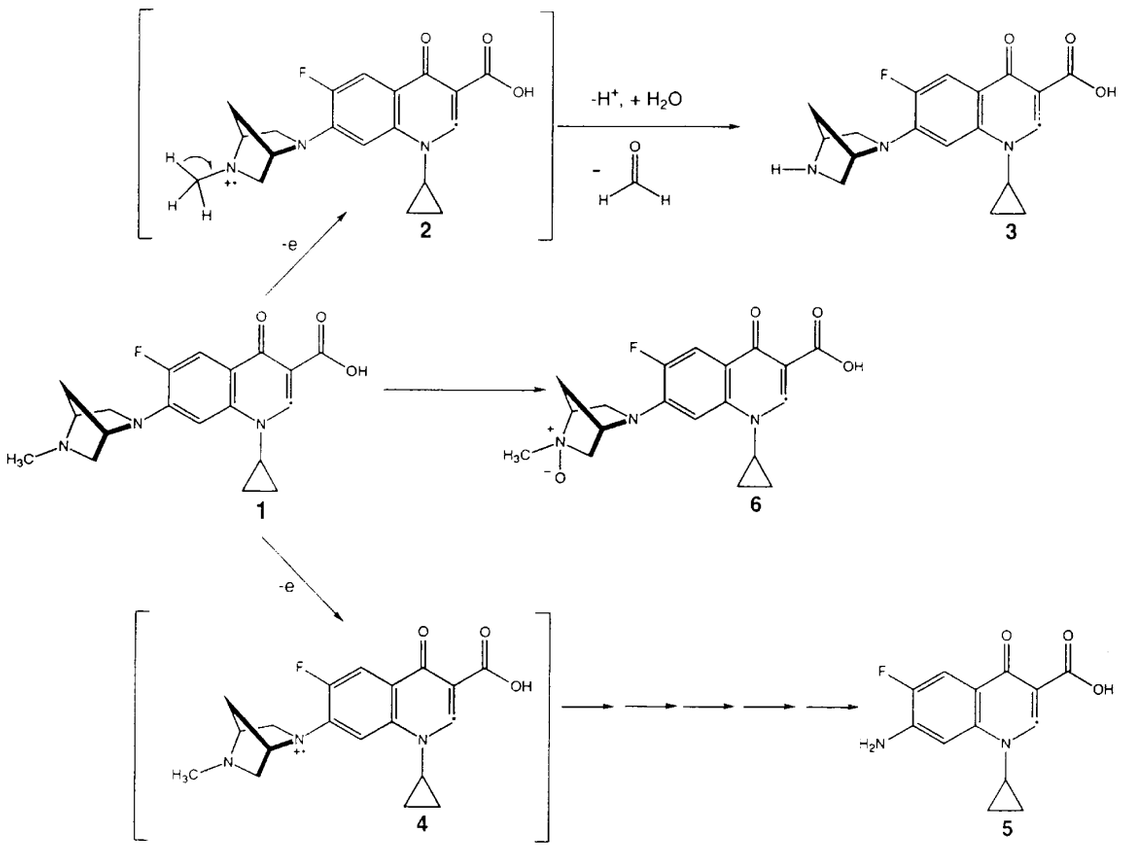

Figure 1 Structures of danofloxacin (**1**), *N*-desmethyl danofloxacin (**3**), the 7-amino danofloxacin derivative (**5**), danofloxacin *N*-oxide (**6**) and possible metabolic intermediates in the production of **3** and **5** from danofloxacin. The (*) indicates the position of radiolabel in [2-^{14}C]danofloxacin.

degradation by pure cultures of microbes, representative of those commonly found in soil. We wished to assess the potential for diverse organisms to degrade this xenobiotic and to identify predominant metabolites produced *in vitro* as an indication of those that might be produced *in situ*. We report the identification of two metabolites that indicate the propensities for these representative soil microorganisms to catalyze oxidations of the piperazine ring and demonstrate that one of the organisms studied readily metabolized [2-^{14}C]-danofloxacin to volatile products, indicating susceptibility of the quinolone ring to microbial metabolism.

Materials and methods

Materials

Danofloxacin (**1**), *N*-desmethyl danofloxacin (**3**), 1-cyclopropyl-6-fluoro-7-amino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**5**), danofloxacin *N*-oxide (**6**) and [2-^{14}C]danofloxacin (specific activity 47.1 μCi mmol^{-1}, radiochemical purity greater than 98% as determined by HPLC) were provided by Pfizer Inc (Groton, CT, USA). All of these compounds were characterized by ^{1}H-NMR and mass spectrometry prior to use. Tetrabutyl-ammonium hydroxide (TBAH) was purchased from Sigma Chemical Co (St Louis, MO, USA).

Analytical methods

Thin-layer chromatography (TLC) was performed on 0.25-mm thick layers prepared by slurrying 25 g of $SiO_2 \cdot GF_{254}$ (Merck, EM Science, Gibbstown, NJ, USA) in 50 ml of H_2O and spreading it with a Quickfit Industries (London, UK) spreader on glass plates. Plates were oven-activated at 120°C for 30 min before use. Plates were developed with EtOAC-$CH_3COCH_2CH_3$-HCO_2H-H_2O (3 : 3 : 2 : 1, v/v/v/v) and visualized under both 254-nm and 365-nm UV lights. With this system, danofloxacin (**1**), *N*-desmethyl danofloxacin (**3**) and the 7-amino derivative of danofloxacin (**5**) display brilliant blue-green fluorescent spots with R_f values of 0.4, 0.5 and 0.8, respectively.

High performance liquid chromatography (HPLC) (Method 1) was routinely performed using a Shimadzu LC-6A pump, a CR 501 Integrator/Recorder, and a variable wavelength UV detector set at 278 nm (Shimadzu Sci. Instruments, Columbia, MD, USA). In general, 5 μl fermentation samples were resolved on a PRP-1 column (10 μm, 7.0 × 305 mm, i.d.) preceded by a 0.45 × 1 cm guard column of the same stationary phase, using asolvent system of CH_3CN-$(CH_3CH_2)_3N$-HOAc-H_2O (10 : 1.4 : 0.2 : 88.4, v/v/v/v) pumped isocratically at 2 ml min^{-1}. The retention volumes (R_v) of danofloxacin (**1**), *N*-desmethyl danofloxacin (**3**), and the 7-amino derivative of danofloxacin (**5**) (Figure 1) were 18.6, 13.5, and 9.4 ml, respectively, in this system.

A second HPLC analytical system (Method 2) was employed to confirm the identities of eluting metabolites. This second system consisted of three LDC solvent delivery pumps (2 model CM 3200 and 1 CM 3500 with controller and high pressure mixer), a Shimadzu RF-10A spectrofluorometric detector with excitation and emission wavelengths of 280 and 440 nm, respectively, an ISS 200 autosampler, and a Spectra Physics model SP4200 computing integrator. For this system, samples of culture supernatants were diluted 400-fold in 0.01 M NH_4HCO_2 adjusted to pH 10 with aqueous NH_4OH. Samples of 50 μl of diluted supernatants were injected for analysis on a 4.6 × 150 mm, PLRP-S (Polymer Labs, Amherst, MA, USA) 5-μm column preceded by a PL-GCT (Polymer Labs) 5 × 30 mm guard cartridge. Samples were eluted at a flow rate of 1 ml min^{-1} unless otherwise noted using different mixtures of (A) 0.01 M NH_4HCO_2 (Aldrich, Milwaukee, WI, USA, 97%) adjusted to pH 10 with NH_4OH and (B) CH_3CN with the following gradient: 0–5 min, 94%A, 6%B; 5–10 min, ramp to 89%A, 11%B and hold for 10–30 min; 30–32 min, ramp to 94%A, 6%B and hold for 32–47 min with 1.5 ml flow rate. Under these conditions, retention volumes of **1**, **3**, **5** and **6** were 22.4 ml, 15.2 ml, 6.0 ml and 4.0 ml, respectively.

Instrumentation

Liquid chromatography/mass spectrometry (LC/MS) analyses were performed using a Finnigan (San Jose, CA, USA) TSQ-700 spectrometer. The samples were ionized using an Atmospheric Pressure Chemical Ionization (APCI) source. Typically, data collection was over a mass range of 200–450 Da. When scanned in MS/MS (daughter ion scan) mode, argon was admitted to the second quadrupole at a pressure sufficient to attenuate the primary parent ion beam by 50%, and data were acquired over a mass range of 50–400 Da.

Scintillation counting was accomplished using a Beckman LS 3801 Liquid Scintillation System, where samples were automatically counted for 20 min.

Incubation protocol

All microorganisms used in this study are maintained in the University of Iowa, College of Pharmacy culture collection. In general, microorganisms are stored on solid media at 5°C in a refrigerator prior to use. The standard protocol for screening used a two-stage fermentation procedure [3]. The medium used for growing microorganisms was composed of glucose 20 g, NaCl 5 g, K_2HPO_4 5 g, soybean meal 5 g, yeast extract 5 g, distilled H_2O 1 L, and the pH of the final mixture was adjusted to 7.0 with 6 N HCl. Stainless steel-capped DeLong flasks (125 ml), holding one fifth volumes (25 ml) of culture medium were sterilized for 15 min. Fresh one-week old slants were flooded with sterile medium and agitated to suspend spores or vegetative growth to inoculate Stage I cultures. The Stage I cultures were incubated for 72 h at 27°C with shaking at 250 rpm in a gyratory shaker (Model G-25; New Brunswick Scientific Co, Edison, NJ, USA) before being used as inoculum (10% volume) for Stage II cultures. Stage II cultures were incubated as described for 24 h, then danofloxacin mesylate substrate dissolved in 0.2 ml of distilled water was added to each flask. Substrate-containing cultures were incubated as before, and samples (cells plus medium) were withdrawn for analysis at 24, 72 and 168 h. Controls consisted of microbial cultures containing no substrate, and incubations of substrate alone in buffers held at different pH values (pH 3, 7, 9) to insure that transformations observed are enzyme mediated, and not simply artifacts formed by incubation conditions.

In a variation of the incubation protocol [16], Stage I cultures were incubated for only 24 h before being used as inoculum (10% volume) for Stage II cultures. Stage II cultures were incubated for 48 h and substrate dissolved in 0.2 ml of distilled water was then added to each flask. All other procedures of sampling and analysis were the same as described for the standard protocol.

For TLC analysis, each sample was adjusted to pH 9 with tetrabutylammonium hydroxide (TBAH) and extracted with 1 ml of CH_2Cl_2. The organic layers (30 μl) were spotted onto TLC plates. Distribution coefficients of **1**, **3** and **5** were 1.1, 1.3, and 1.7 respectively, using this extraction procedure.

For HPLC analysis, microbial transformation samples were centrifuged at 3000 × *g* for 5 min in a desk-top centrifuge. A 0.5-ml sample of the clear supernatant was transferred to a microtube which was centrifuged at 15 850 × *g* for 5 min in a Beckman Microfuge E. The resulting supernatant phase was then used for HPLC analysis. This procedure enabled a direct analysis of substrate and metabolites by HPLC and eliminated the potential for inefficient recovery of danofloxacin and danofloxacin metabolites by solvent extraction.

Incubation of ^{14}C-labeled danofloxacin with Curvularia lunata

Incubations with [2-^{14}C]danofloxacin were conducted using the normal fermentation protocol in 125-ml DeLong flasks holding 25 ml of medium. Unlabeled danofloxacin (2.5 mg) and ^{14}C-labeled danofloxacin (1 μCi) were each dissolved in 0.2 ml distilled water and added to duplicate, 24 h-old Stage II cultures of *C. lunata* NRRL 2178. The flasks were connected by vacuum to an aspirated KOH-trap to retain any $^{14}CO_2$ produced by the cultures. Control flasks consisted of fermentations containing only unlabeled danofloxacin. After 24 h of incubation the control flask was sampled for HPLC analysis to verify that all the danofloxacin substrate had been removed from the culture medium.

At that time, radiolabeled danofloxacin-containing cultures were centrifuged at 10 000 × *g* for 10 min, and the supernatants were reserved for radioactivity determination. The cell pellets were washed with 10 ml distilled H_2O, and the wash was combined with the original supernatant. Flasks were further washed with 10 ml methanol to extract possible residual and labeled danofloxacin adhering to the culture flask.

The cells were extracted with 20 ml acetone, and the suspension was filtered through sintered glass to obtain the dried pellet and an acetone fraction. The pellet was then extracted with a mixture of 15 ml ethanol and 5 ml N HCl, and filtered through sintered glass. Finally, the pellet was boiled with 6 N HCl for 1 h and filtered through sintered glass to give an HCl extract. Each of the cell extracts, the

fermentation supernatant, methanol flask extract and KOH trap contents were subjected to radioactivity determinations.

Radioactivity was determined by dissolving 0.1 ml of each sample in 10 ml of Budget-Solve (Research Products International Corp, Mount Prospect, IL, USA) cocktail in 15 ml scintillation vials. Scintillation vials were placed into a Beckman LS 3801 Liquid Scintillation System (Beckman Instruments, Palo Alto, CA, USA), and samples were automatically counted for 20 min.

Samples of 1 ml of each of the extracts were brought to dryness under a stream of N_2, redissolved in 0.1 ml MeOH, and 5-μl volumes were injected for HPLC analysis using HPLC Method 1. Samples which yielded each chromatographic peak were collected, dissolved in scintillation cocktail and counted as described above.

Results and discussion

Danofloxacin possesses several functional groups that could be subject to microbial transformation and metabolism. Based on previous work, microbe-mediated reactions expected to occur with **1** include *N*-demethylation, further piperazine ring degradation, oxidation of the *N*-cyclopropyl group, and quinolone ring and carboxylic acid reduction [2,36,38].

TLC was used as a preliminary method to assess the abilities of microorganisms to transform danofloxacin to metabolites. TLC offers advantages of speed and ease of handling numerous samples for analysis compared to HPLC. Danofloxacin (**1**) and suspected metabolite standards **3** and **5** could be readily isolated from aqueous fermentation medium by ion pair extraction using TBAH and CH_2Cl_2 as the extraction solvent. A relatively favorable distribution coefficient, and the brilliant fluorescence of **1**, **3**, and **5** spots on TLC plates exposed to short and long UV light rendered this technique suitably sensitive. A possible shortcoming was the possibility that biotransformation reactions could result in metabolites that lacked fluorescence, and would not be amenable to isolation from fermentation broths by the TBAH/CH_2Cl_2 extraction protocol.

A total of 69 cultures representing 23 genera of soil bacteria, fungi, yeast and actinomycetes were screened for their abilities to transform danofloxacin using the TLC analytical method. Substrate levels in these incubations were at 1 mg ml^{-1} of culture medium. These represented the following genera (number of species): *Aspergillus* (18), *Absidia* (1), *Arthrobotrys* (1), *Bacillus* (3), *Caldariomyces* (1), *Candida* (2), *Chaetonium* (1), *Corynebacterium* (1), *Cunninghamella* (3), *Curvularia* (1), *Cylindrocarpon* (1), *Gliocladium* (1), *Microsporum* (1), *Mucor* (3), *Mycobacterium* (3), *Nocardia* (4), *Penicillium* (9), *Pseudomonas* (5), *Rhodotorula* (1), *Rhizopus* (3), *Stemphylium* (1), *Streptomyces* (4), and *Stysanus* (1). Although initial results from TLC suggested that some 15 cultures had biotransformed danofloxacin into possible metabolites, this could not be clearly verified by the methods used. Interestingly, one culture, *Curvularia lunata* NRRL 2178, appeared to completely consume danofloxacin but without accumulating metabolites detected by the TLC method.

An HPLC method (Method 1) which cleanly resolves **1** and available standards **3** and **5** (Figure 2a) was used in rescreening 45 cultures. The standard fermentation protocol was used in this experiment with danofloxacin substrate at a level of 0.1 mg ml^{-1} of culture medium. Fermentation samples were clarified by centrifugation, and clear supernatants were directly injected for HPLC. Screening in this manner identified eight cultures which appeared to be producing metabolites from danofloxacin (Table 1). Presumptive identification of the metabolites was made by co-injections of standard compounds and samples. Peaks eluting with an R_v of 9.4 ml (HPLC Method 1) are consistent with the 7-amino derivative of danofloxacin (**5**) in which the piperazine ring has been completely degraded. This 7-amino compound was detected in cultures of *Candida*, two *Mycobacterium* strains, and one *Pseudomonas*. *N*-Desmethyldanofloxacin (**3**) which elutes at R_v of 13.5 ml using this HPLC method was confirmed by co-injection as a metabolite in supernatants of both *Mycobacterium* cultures, *Nocardia* sp, two *Pseudomonas* species, *Rhizopus* and *Streptomyces griseus*. An HPLC chromatogram of the 72-h sample of *Mycobacterium smegmatis* showing peaks for both **3** and **5** is shown in Figure 2b as an example. HPLC analysis using Method 2 confirmed the presence of **3** and **5** as metabolites of danofloxacin in these cultures with the 7-amino metabolite (**5**) also apparent from cultures of *Nocardia* sp, *Rhizopus* and *S. griseus* using this system; representative chromatograms are presented in Figure 3. Yields of metabolites were generally not high with any cul-

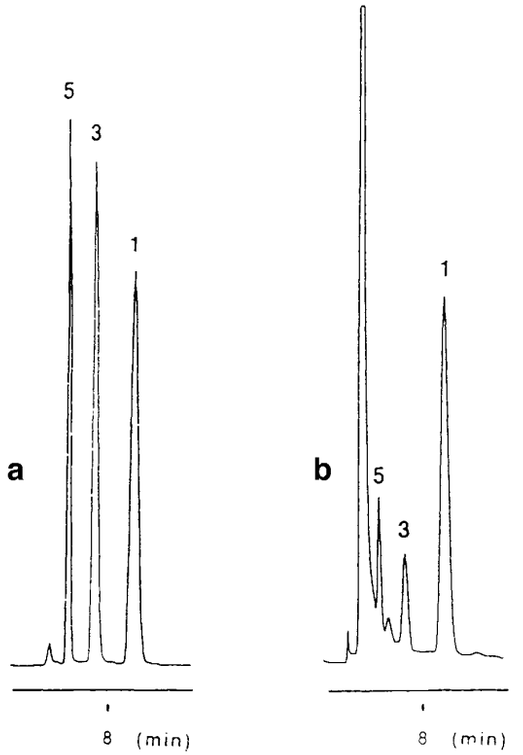

Figure 2 Representative HPLC chromatograms (Method 1, see Materials and Methods). (a) Standards: 7-amino-danofloxacin derivative (**5**); *N*-desmethldanofloxacin (**3**); and danofloxacin (**1**). (b) Chromatogram showing **1**, **3**, and **5** in the culture supernatant of *Mycobacterium smegmatis* after 72 h of incubation; additional peaks were also present in chromatograms of control cultures and do not represent danofloxacin metabolites.

Table 1 Cultures metabolizing danofloxacin (**1**)

Culture number	Organism	Metabolite		
		3 (R_v 13.5 ml)[a]	**5** (R_v 9.4 ml)	Other
Standard incubation protocol[b]:				
NRRL 5699	*Candida lipolytica*	–	+	–
UI-AM-463	*Mycobacterium bisrymcum*	+	+	–
UI-AM-563	*Mycobacterium smegmatis*	+	+	–
NRRL 5646	*Nocardia* sp	+	–	–
UI 60690	*Pseudomonas aeruginosa* var	+	–	–
UI AM-670	*Pseudomonas fluorescens*	+	+	–
ATCC 11145	*Rhizopus arrhizus*	+	–	–
ATCC 10137	*Streptomyces griseus*	+	–	–
Modified incubation protocol[b]:				
ATCC 24528	*Aspergillus nidulans*	–	–	5.1 ml[a]
UI-X-251	*Penicillium chrysogenum*	–	+	–
UI-MR-70	*Penicillium frequentans*	–	+	–
ATCC 12556	*Penicillium* sp	–	+	6.2 ml[a]

[a]Retention volumes were obtained by HPLC analysis (Method 1).
[b]See Materials and Methods.

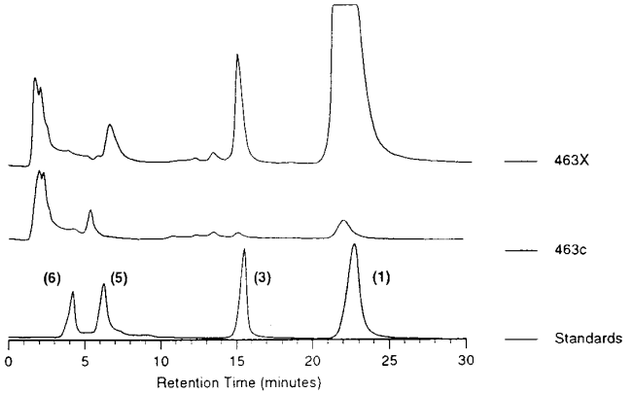

Figure 3 A representative HPLC chromatogram (Method 2) of danofloxacin metabolites found in a 72-h culture extract of *Mycobacterium bisrymcum*, strain UI-AM-463. X, danofloxacin culture extract; c, control. Standards in order of elution: *N*-oxide (**6**), 7-amino derivative (**5**), desmethyldanofloxacin (**3**), danofloxacin (**1**).

ture, and were estimated at no greater than 5–10% of the amount of danofloxacin substrate used during the 168-h sampling period.

A third screening experiment was conducted using 18 *Aspergillus* and 9 *Penicillium* strains selected on the basis of a previous report of successful microbial transformations of the related compound nalidixic acid by members of these genera [16]. The modified incubation protocol was used for this experiment with a danofloxacin substrate level of 0.2 mg ml^{-1} of culture medium. In this case, analysis using HPLC Method 1 showed that the 7-amino metabolite (**5**) was again produced by three *Penicillium* species (Table 1), and two additional and unidentified peaks with R_v of 5.1 ml and 6.2 ml were formed by *Aspergillus nidulans* and *Penicillium* sp respectively. Further evaluation of culture broths from these latter two strains revealed a number of polar components which may include the *N*-oxide danofloxacin metabolite (**6**) and a presumed piperazine ring metabolite also observed in cattle bile during mammalian metabolism studies [25].

Extracts obtained from *R. arrhizus*, *Pseudomonas fluorescens*, and *Penicillium* sp (ATCC 12556) were examined by LC/MS and LC/MS/MS in order to confirm the identities of the metabolites detected by HPLC and to determine whether additional polar eluting peaks were danofloxacin metabolites. These culture extracts were also compared by HPLC (Method 2) to extracts from the corresponding control cultures containing no danofloxacin substrate.

The presence of the 7-amino metabolite (**5**) and the *N*-desmethyl metabolite (**3**) were confirmed by the elution of peaks at common retention volumes which produced MH^+ ions at m/z 263 and 344, respectively. The MS/MS daughter ion spectra from these MH^+ ions showed excellent agreement with those produced by synthetic standards. The *Rhizopus* and *Pseudomonas* cultures produced low yields (less than 1% yield) of polar metabolites which gave MH^+ ions at m/z 374, the expected mass of the *N*-oxide metabolite (**6**). The low metabolite yield in both cultures was insufficient to obtain additional structural information by MS/MS. A common peak at retention volume 8.4 ml which produced an MH^+ ion at m/z 360 was observed in all three cultures. MS/MS analysis of the peak showed daughter ions at m/z 342 ($MH^+ - H_2O$) and 318 (MH^+ – cyclopropane), which are structurally characteristic of danofloxacin metabolites. However, it is difficult to speculate on the structure of this unknown compound without producing sufficient quantities for complete NMR analysis.

In the above experiments, *C. lunata* NRRL 2178 consistently consumed all the danofloxacin substrate within 24 h, but did not produce metabolites detected by the methods used. The possibility that *C. lunata* NRRL 2178 transformed danofloxacin to unknown metabolites was examined using ring-labeled, [2-^{14}C]danofloxacin as substrate. Control flasks containing unlabeled danofloxacin were analyzed by HPLC 24 h after substrate addition to show that all substrate had been consumed by the culture. Analysis of [2-

^{14}C]danofloxacin-containing cultures indicated that there was no residual radioactivity in the culture medium at this time. However, each of the fractions obtained when cells were extracted with acetone, acidified ethanol, and 6 N HCl, were radioactive. HPLC analysis (Method 1) of these extracts revealed a peak with a retention volume corresponding to danofloxacin with no other apparent metabolites. To verify that no other metabolites were present in the extracts, every chromatographic peak was collected as it eluted, and its radioactivity was determined. Peaks corresponding to danofloxacin were the only ones containing any radioactivity, and accounted for over 68% of the radioactivity originally added to the cultures (Table 2). In addition, 31% of the radiolabel was recovered in the KOH traps suggesting that a significant portion of the danofloxacin substrate was degraded to CO_2 or other volatile components by *C. lunata*. The total recovery of radioactivity from culture medium, cell extracts and the KOH trap was 99.6% (Table 2).

Pathways for the formation of danofloxacin metabolites are uncertain. However, *N*-demethylation reactions are microbial transformations commonly observed with alkaloids [36]. Such reactions may be catalyzed by peroxidases, or by cytochrome P450 enzyme systems commonly found in microorganisms. *N*-Demethylation of danofloxacin and of other *N*-methylated fluoroquinolones has been observed in mammalian metabolism studies as well [20,31,33,47] and is consistent with the microbial mediated activity observed here. These types of biotransformations usually involve initial electron abstraction from nitrogen, loss of an adjacent hydrogen atom, hydroxylation to an unstable carbinolamine, and subsequent elimination of the methyl group as formaldehyde (Figure 1) [36]. The degradation of the piperazine ring is more complicated, and would have to proceed through initial oxidation of the nitrogen atom attached to the aromatic ring as suggested in Figure 1.

The apparent degradation of danofloxacin by *C. lunata* likely involves conversion of the ^{14}C-label in the quinolone ring into carbon dioxide. Little literature exists concerning such ring degradations except for the recent work of Martens *et al*, who demonstrated the evolution of $^{14}CO_2$ from a related fluoroquinolone, enrofloxacin by wood rotting fungi [27]. Precedence for a degradation pathway may be evident in the work of Otten and Rosazza [34] who have reported the only example of the fungal cleavage of a quinone ring system. With lapachol and dichloroallyllawsone as substrates, quinone cleavage occurs by oxidative ring fission involving a putative epoxide intermediate. Through a similar epoxidation of the quinolone ring and subsequent epoxide hydrolysis, we believe that the labeled carbon atom can be eliminated as $^{14}CO_2$.

Table 2 Radioactivity from *Curvularia lunata* NRRL 2178 cell extracts and KOH trap

Fraction	d.p.m.	Radioactivity (%)[a]
KOH-Trap	678 378	31.2
Acetone	267 600	12.6
EtOH-HCl	622 600	29.3
HCl	556 600	26.5

[a]Approximately 1 μCi (an average of 2 185 600 d.p.m.) of [2-^{14}C]danofloxacin (**1**) was added to each of two culture flasks. The values shown are the average levels of radioactivity found in each extract or fraction.

The results presented here demonstrate the potential for danofloxacin to be transformed or degraded by microorganisms. This intrinsic degradability of danofloxacin is consistent with results obtained in an *in vitro* aerobic soil biodegradation study using three different soil types: transformation of danofloxacin to minor metabolites was observed in all three soils, with estimated times to 50% transformation ranging from 87–143 days [49]. The organisms shown in the present study to be capable of metabolizing danofloxacin represent a variety of Gram-negative and Gram-positive eubacteria, actinomycetes, fungi and yeasts that are common to the terrestrial environment [1]. Demonstration of danofloxacin metabolism by such a diversity of organisms suggests that the compound will be susceptible to degradation under a variety of environmental conditions, where fluxes in physical and chemical parameters can alter the dominant microbial populations. Moreover, the apparent metabolism of the quinolone ring by one fungal organism in the panel tested establishes the inherent susceptibility of danofloxacin to ultimate degradation or mineralization by soil microbes.

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