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FUNGAL TRANSFORMATION OF OFLOXACIN AND ENROFLOXACIN

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Absrtact– The ability of *Rhizoctonia solani* VKM F-942 to regioselective metabolize the fluoroquinolones ofloxacin and enrofloxacin was investigated. The fungus was grown in sucrose/peptone broth at 28°C, dosed with 100 µg/mL ofloxacin or enrofloxacin, and incubated with shaking for 18 days. The cultures were extracted with ethyl acetate, which was evaporated *in vacuo*. High-performance liquid chromatography showed that both drugs were transformed to single metabolites, which were identified by mass spectrometry and proton nuclear magnetic resonance spectrometry. The product from ofloxacin (40.0% of the total peak area at 280 nm) was ofloxacin *N*-oxide and the product from enrofloxacin (14.4% of the total peak area at 280 nm) was enrofloxacin *N*-oxide.

INTRODUCTION

The fluoroquinolones are synthetic antimicrobial agents that are active against a broad spectrum of pathogenic bacteria. Two of the widely used fluoroquinolones are ofloxacin and enrofloxacin; ofloxacin is used for treatment of various bacterial infections in humans (Monk and Campoli-Richards, 1987) but enrofloxacin is restricted to veterinary use (Brown, 1996). Both drugs are used in Japan to treat respiratory diseases of poultry (Nakamura, 1995).

Ofloxacin is transformed in experimental animals to ofloxacin *O*-glucuronide, *N*-desmethyl-ofloxacin, and ofloxacin *N*-oxide (Sudo *et al.*, 1986). Enrofloxacin is transformed in rats to enrofloxacin *O*-glucuronide and to desethyl-enrofloxacin (= ciprofloxacin) in several mammals (Heitzman, 1995; Mengozzi *et al*., 1996). In poultry, enrofloxacin metabolites are formed by deethylation and *N*-hydroxylation as well as by opening of the piperazine ring (Heitzman, 1995).

Although the transformation of ofloxacin by fungi has not been reported, cultures of wood-decaying fungi have been shown to convert enrofloxacin to CO_2 and several other metabolites (*Martens et al*., 1996; Wetzstein *et al*., 1997). The fungus *Mucor ramannianus* transforms enrofloxacin to enrofloxacin *N*-oxide, desethylene-enrofloxacin, and *N*-acetylciprofloxacin (Parshikov *et al.*, 2000). We now have investigated the transformation of fluoroquinolones by another fungus, *Rhizoctonia solani* VKM F-942.

MATERIALS AND METHODS

Rhizoctonia solani VKM F-942 was from the All-Russian Microorganism Collection and maintained on agar slants (Modyanova *et al.*, 1999; Parshikov *et al.*, 1999; Khasaeva *et al.*, 2016a,b). The mycelium was scraped from the surface of the agar, suspended in 5 mL of sterilized water, and used to inoculate 500 ml flasks. Each flask contained 100 mL of a medium containing (per liter): 30.0 g sucrose, 5.0 g peptone, 3.0 g NaCl, 3.0 g $NaNO_3$, 5.0 g KH_2PO_4, 0.5 g $MgSO_4 7H_2O$, 0.5 g KCl, 0.1 g $FeSO_4$, 1 mg $MnSO_4$, and 1000 mL deionized water. The pH was adjusted to 5.0. Cultures were grown for 2 days on a rotary shaker at 28°C with shaking at 180 rpm.

Ofloxacin and enrofloxacin were purchased from Sigma Chemical Co. These compounds were dissolved (at 10 mg mL^{-1}) in 20 mM aqueous KOH and filter-sterilized; 1.0 mL was added to each flask to make the final concentrations 249 µM ofloxacin and 253 µM enrofloxacin. After dosing, the cultures

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were incubated for an additional 18 days at 28°C with shaking at 180 rpm.

The mycelia were harvested by using filter paper in a Büchner funnel and then extracted with three equal volumes of ethyl acetate, which was dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residues were dissolved in methanol : acetonitrile: acetic acid (10:10:2) for analysis. Extracts were analyzed and metabolites were purified by high-performance liquid chromatography (HPLC), using a Phenomenex Prodigy ODS-3 column (10 × 250 mm). The mobile phase components were solvent A (water:methanol, 90:10) and solvent B (water:methanol, 10:90); acetic acid (2 mL L^{-1}) was added to both components and the pH was adjusted to 3.0. The mobile phase (flow rate = 3.0 mL min^{-1}) was a gradient increasing from 10% to 95% solvent B over 20 min (Parshikov *et al.*, 1999). Metabolite concentrations were estimated from the HPLC peak areas at 280 nm.

Liquid chromatography/electrospray ionization mass spectrometry (LC/ESI MS) was performed with a Hewlett-Packard 1090L/M HPLC system and a Hewlett-Packard 5989B quadrupole mass spectrometer operated using positive-ion electrospray ionization (ESI) with in-source collision-induced dissociation (CID). Components were resolved using a Phenomenex Prodigy ODS-3 HPLC column (2.0 × 250 mm). The mobile phase (flow rate = 0.2 mL/min) was a linear 40-min gradient from 5% acetonitrile / 95% water to 95% acetonitrile / 5% water in 20 min with constant 0.1% formic acid.

Proton nuclear magnetic resonance (NMR) spectral analyses were performed at 500 MHz as previously described (Parshikov *et al.*, 1999) except that the compounds were dissolved in deuterated methanol.

RESULTS

HPLC analysis of the ethyl acetate extract from cultures of *Rh. solani* VKM F-942 dosed with enrofloxacin showed that enrofloxacin eluted from the HPLC column at 10.0 min and a metabolite at 11.6 min. After 18 days, as shown by the integrated peak area (A_{280}) for enrofloxacin, 85.6% of the starting material remained.

The enrofloxacin metabolite had a yield of 14.4% of the total A_{280} and a UV absorption spectrum with λ_{max} = 274, 319 and 331 nm. The positive-ion ESI mass spectrum included ions at *m*/*z* 376 $[M+H]^+$, 360 $[M+H-O]^+$, 359 $[M+H-{\cdot}OH]^+$, 358 $[M+H-H_2O]^+$, 344 $[M+H-CH_3OH]^+$, 315 (100) $[M+H-{\cdot}OH-CO_2]^+$, and 300 $[M+H-CH_3OH-CO_2]^+$. The mass spectra are different from those published previously (Parshikov *et al.*, 2000) because positive-ion ESI MS with in-source CID was used instead of regular ESI MS or MS/MS. The ^1H NMR data presented in Table 1 are virtually the same as those previously reported (Parshikov *et al.*, 2000); the metabolite was dissolved in deuterated methanol instead of deuterium oxide. Based on the MS and NMR results, the metabolite (Fig. 1) was identified as enrofloxacin *N*-oxide.

HPLC analysis of the ethyl acetate extract from cultures of *Rh. solani* VKM F-942 dosed with ofloxacin showed that ofloxacin eluted from the HPLC column at 9.4 min and a metabolite at 11.4 min. After 18 days, as shown by the integrated peak area (A_{280}) for ofloxacin, 60.0% of the starting material remained.

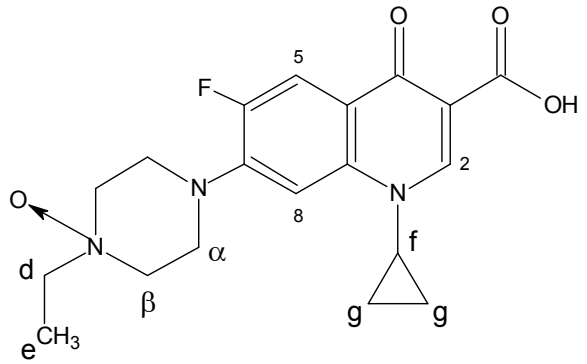
The ofloxacin metabolite had a yield of 40.0% of the total A_{280} and a UV absorption spectrum with λ_{max} = 296 and 330 nm. The positive-ion ESI mass spectrum included ions at *m*/*z* 378 $[M+H]^+$, 361 $[M+H-{\cdot}OH]^+$, 317 (100) $[M+{\cdot}OH-CO_2]^+$, 247, and 246. The proton resonances for H2 and H5 in ^1H NMR spectrum had similar chemical shifts as those in other fluoroquinolones. The resonances for H_a, H_b, and H_c were assigned based on their chemical shifts, multiplicities, integration, and homonuclear

Table 1. ^1H NMR parameters for the metabolites[a] of ofloxacin and enrofloxacin produced by *Rh. solani* VKM F-942

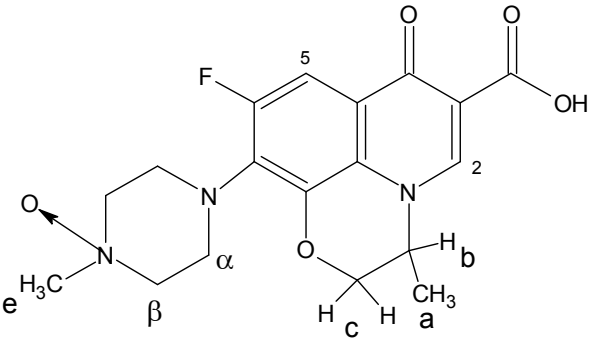
Proton	Ofloxacin *N*-oxide[b]	Enrofloxacin *N*-oxide[c]
H2	8.78	8.84
H5	7.74	8.03
H8	7.71	
Piperazine a	3.35, 4.00	
Piperazine b	3.32, 3.63	
Piperazine a+ b		3.33, 3.62-3.78
H_a	1.53	
H_b	4.72	
H_c	4.40, 4.56	
H_d		3.45
H_e	3.26	1.43
H_f		3.77
H_g		1.22, 1.43

[a]Samples were dissolved in deuterated methanol.
[b]Coupling constants were $J_{5,F}$ = 12.0; $J_{a,b}$=6.9; $J_{b,c}$ = 11.6; $J_{a,c}$ = 2.2.
[c]Coupling constants were $J_{5,F}$ = 12.9; $J_{8,F}$ = 6.9; $J_{f,g}$ = 7.1.

decoupling experiments. The piperazine α and β resonances were assigned based on homonuclear decoupling and NOE experiments. The large downfield shifts of the piperazine β resonances indicated the formation of an *N*-oxide The ^{1}H NMR data are shown in Table 1. The metabolite (Fig. 1) was identified ‘as ofloxacin *N*-oxide.



Enrofloxacin *N*-oxide



Ofloxacin *N*-oxide

Fig. 1. Structures of the enrofloxacin *N*-oxide and ofloxacin *N*-oxide metabolites formed from ofloxacin and enrofloxacin by *Rh. solani* VKM F-942.

DISCUSSION

The biotransformation of fluoroquinolones that have an *N*-alkyl substituent on the piperazine ring, such as ofloxacin and enrofloxacin, may proceed by different pathways in different organisms. In animals, such fluoroquinolones are metabolized at either the *N*-alkylpiperazine ring or the carboxyl group (Borner *et al*., 1990). For instance, ofloxacin, amifloxacin, difloxacin, fleroxacin, and pefloxacin are modified in mammals by *N*-oxidation of the alkylpiperazine rings as well as by other reactions (Sudo *et al*., 1986; Carlucci, 1998).

Several pathways are also used in fungal bioconversions of fluoroquinolones. Fungi metabolize the piperazine ring by *N*-oxidation, dealkylation, *N*-acetylation, removal of two carbons, or total breakdown of the ring (Wetzstein *et al*., 1997; Parshikov, 2016; Parshikov, 2017). We have studied the transformation of fluoroquinolones by different strains of fungi, such as *M. ramannianus* R-56 (Parshikov *et al*., 2000) and *Rh. solani* VKM F-942. The transformation of ofloxacin and enrofloxacin by *Rh. solani* VKM F-942 involves a regioselective *N*-oxidation of the terminal nitrogen of the piperazine ring, as reported previously for the metabolism of enrofloxacin by *M. ramannianus* (Parshikov *et al*., 2000). Because the antibacterial activity of enrofloxacin *N*-oxide is much less than that of enrofloxacin (Wetzstein *et al*., 2000), fungal *N*-oxidation may reduce the antibacterial activity of fluoroquinolone residues in the environment.

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REFERENCES

Borner, K., Borner, E., Hartwig, H. and Lode, H. 1990. HPLC of recent quinolone antimicrobials, p. 131-144 In: E. Reid, and I.D. Wilson, (Eds.) *Analysis for Drugs and Metabolites, Including Anti-Infective Agents*. Royal Society of Chemistry. Cambridge, UK

Brown, S.A. 1996. Fluoroquinolones in animal health. *J. Vet. Pharmacol. Ther.* 19 : 1-14.

Carlucci, C. 1998. Analysis of fluoroquinolones in biological fluids by high-performance liquid chromatography. *J. Chromatogr. A.* 812: 343-367.

Heitzman, R.J. 1995. Enrofloxacin, p. 31-44 In: *Residues of Some Veterinary Drugs in Animals and Foods, monographs prepared by the 43rd meeting of the Joint FAO-WHO Expert Committee on Food Additives*. Food and Agriculture Organization of the United Nations. Rome, Italy

Khasaeva, F.M., Parshikov, I.A. and Zaraisky, E.I. 2016a. Degradation of 2,6-Dimethylpyridine by *Arthrobacter crystallopoietes*. *Ecology, Environment and Conservation.* 18: 1673-1676.

Khasaeva, F.M., Parshikov, I.A. and Zaraisky, E.I. 2016b. Biodegradation of 4-methylpyridine by *Àrthrobacter* sp. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences.* 22 : 75-77.

Martens, R., Wetzstein, H.G., Zadrazil, F., Capelari, M.,

Hoffmann, P. and Schmeer, N. 1996. Degradation of the fluoroquinolone enrofloxacin by wood-rotting fungi. *Appl. Environ. Microbiol.* 62: 4206-4209.

Mengozzi, G., Intorre, L., Bertini, S. and Soldani G. 1996. Pharmacokinetics of enrofloxacin and its metabolite ciprofloxacin after intravenous and intramuscular administrations in sheep. *Am. J. Vet. Res.* 57: 1040-1043.

Modyanova, L. V., Duduchava, M.R., Piskunkova, N.F., Grishina, G.V., Terentyev, P.B. and Parshikov, I.A. 1999. Microbial transformations of piperideine and pyridine derivatives [in Russian]. *Khim. Geterotsikl. Soed.* 35 : 649-655.

Monk, J.P. and Campoli-Richards, D.M. 1987. Ofloxacin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic use. *Drugs.* 33 : 346-391.

Nakamura, S. 1995. Veterinary use of new quinolones in Japan. *Drugs* 49 : 152-158.

Parshikov, I.A., Freeman, J.P., Lay, J.O., Beger, R.D., Williams, A.J. and Sutherland, J.B. 1999. Regioselective transformation of ciprofloxacin to *N*-acetylciprofloxacin by the fungus *Mucor ramannianus*. *FEMS Microbiol. Lett.* 177 : 131-135.

Parshikov, I.A., Freeman, J.P., Lay, J.O., Beger, R.D., Williams, A.J. and Sutherland, J.B. 2000. Microbiological transformation of enrofloxacin by the fungus *Mucor ramannianus*. *Appl. Environ. Microbiol.* 66 : 2264-2267.

Parshikov, I.A. 2016. Microbial Transformation of Nitrogen Containing Heterocycles. Primedia E-launch LLC, Dallas. 130 p.

Parshikov, I.A. 2017. Microorganisms in Chemistry of Nitrogenous Heterocycles. Primedia E-launch LLC, Dallas. 130 p.

Sudo, K., Okazaki, O., Tsumura, M. and Tachizawa, H. 1986. Isolation and identification of metabolites of ofloxacin in rats, dogs and monkeys. *Xenobiotica* 16: 725-732.

Wetzstein, H. G., Karl, W., Hallenbach, W., Himmler, T. and Petersen, U. 2000. Residual antibacterial activity of metabolites derived from the veterinary fluoroquinolone enrofloxacin, p. 2 In: *Abstr. 100th Gen. Meet. Am. Soc. Microbiol.* Los Angeles, USA

Wetzstein, H.G., Schmeer, N. and Karl, W. 1997. Degradation of the fluoroquinolone enrofloxacin by the brown rot fungus *Gloeophyllum striatum*: Identification of metabolites. *Appl. Environ. Microbiol.* 63: 4272-4281.