Synthesis of Antimicrobial Agents. II. Syntheses and Antibacterial Activities of Optically Active 7-(3-Hydroxypyrrolidin-1-yl)quinolones

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Two optically active isomers of 1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-hydroxypyrrolidin-1-yl)-4-oxoquinoline-3-carboxylic acid (**10**) were prepared. One of the isomer, 7-[(3*S*)-hydroxypyrrolidin-1-yl] derivative **8**, was about 4 times more potent *in vitro* than the other, 7-[(3*R*)-hydroxypyrrolidin-1-yl] derivative **4**, and approximately two times more active than the racemate, 7-[(3*RS*)-hydroxypyrrolidine-1-yl] derivative **10**.

Optical active **8** was the most active in *in vivo*, followed by **10**, and **4** was the least active compound. But, they were more potent than **CI-934** **12** and norfloxacin.

From the results, (3*S*)-hydroxypyrrolidinyl group was found to be one of the beneficial group for PCA-antibacterial agent.

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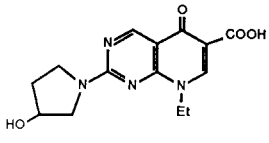
Since 8-ethyl-5,8-dihydro-2-(3-hydroxypyrrolidin-1-yl)-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid (**1**) was found to be the active metabolite of piromidic acid (**PA, 2**) when administered to animals and to humans orally [1-2], many compounds containing a pyridone carboxylic acid moiety (PCA-antibacterials) which have 3-hydroxypyrrolidinyl group have been synthesized [2-6].

Though these compounds have an asymmetric center at the 3-position in the pyrrolidine ring, there have been no reports on the synthesis of the two optical isomers.

On the other hand, Hironaka *et al.* [7] reported that **PA** was converted to **1** microbiologically using the strain of *Streptomyces endus,* although the configuration of the metabolite **1** has not been mentioned.



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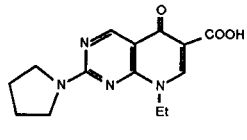

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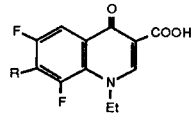
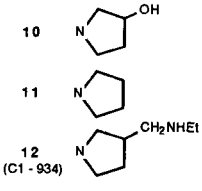




10

11

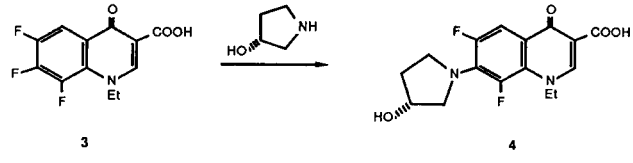
12
(CI - 934)

In the course of our study to search for novel antibacterial agents, 1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-hydroxypyrrolidin-1-yl)-4-oxoquinoline-3-carboxylic acid (**10**) was found to be more effective than **CI-934** [8] which was reported to show the best gram positive activity among the current derivatives, such as norfloxacine (**NFLX**), enoxacin (**ENX**), ciprofloxacin (**CPFX**) and ofloxacin (**OFLX**), when administered orally to mice. This compound **10** also contains 3-hydroxypyrrolidine moiety, our interest was directed to the syntheses of the two optical isomers and their antibacterial activities.

In this paper, we report the syntheses and evaluation of optically active 1-ethyl-6,8-difluoro-1,4-dihydro-7-[(3*R*)-hydroxypyrrolidin-1-yl]-4-oxoquinoline-3-carboxylic acid (**4**) and 1-ethyl-6,8-difluoro-1,4-dihydro-7-[(3*S*)-hydroxypyrrolidin-1-yl]-4-oxoquinoline-3-carboxylic acid (**8**).

7-[(3*R*)-Hydroxypyrrolidin-1-yl] derivative **4** was prepared by the condensation of 6,7,8-trifluoroquinolone **3** [9] with (3*R*)-hydroxypyrrolidine which was obtained by the method according to the literature [10], as shown in Scheme I. The specific rotation in 0.1*N* sodium hydroxy solution of **4** was $[\alpha]_D^{20}$ +125° (c = 1.0).

Scheme I



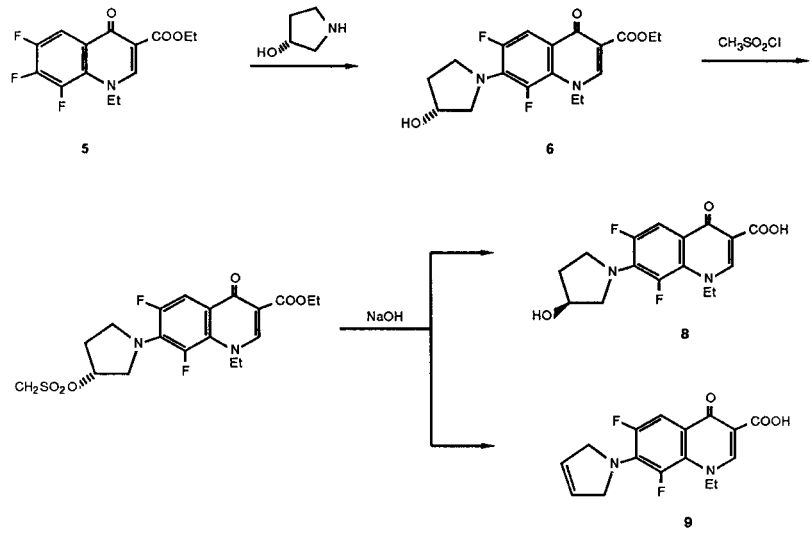
3

4

On the other hand, 7-[(3*S*)-hydroxypyrrolidin-1-yl] derivative **8** was prepared by the chemical conversion of the ethyl ester **6** which was obtained by the condensation of 6,7,8-trifluoroquinolone ethyl ester **5** [9] with (3*R*)-hydroxypyrrolidine, as shown in Scheme II.

Treatment of **6** with methanesulfonyl chloride in the presence of triethylamine to yield 7-[(3*R*)-hydroxypyrrolidin-1-yl]quinolone ethyl ester **7**. Alkaline hydrolysis of **7** gave a mixture of desired **8** and 7-(3-pyrrolin-1-yl) derivative **9**. These compounds were isolated by the column chromatography on silica gel. The configuration at the 3-position in the pyrrolidine ring of **8** was assigned

Scheme II



from the fact that **8** showed the same physical properties (ir, nmr and ms spectrum data) as those of **4** except specific rotation $[\alpha]_D^{20}$ -123° (c = 1.0, 0.1*N* sodium hydroxide). On the other hand, the structure of **9** was identified as 7-(3-pyrrolin-1-yl)quinolone by the comparison with authentic sample which was prepared by condensation of **3** with 3-pyrroline.

Racemate, 7-[(3*RS*)-hydroxypyrrolidin-1-yl]quinolone **10**, was prepared according to the synthetic method of **4** using (3*RS*)-hydroxypyrrolidine, and whose value of specific rotation was $[\alpha]_D^{20}$ 0° (c = 1.0, 0.1*N* sodium hydroxide).

In Table I are summarized the *in vitro* antibacterial activities of the optical isomers (**4** and **8**) and racemate **10** against 8 organisms. The results of 7-(pyrrolidin-1-yl) derivative **11** [8] **CI-934** and **NFLX** [11] are also included for comparison.

Table I

In vitro Antibacterial Activity of Optically Active 7-(3-Hydroxypyrrolidin-1-yl) Derivatives

Compound	Organism, Minimum Inhibitory Concentration (MIC) [a] (μg/ml)							
	Sa	Se	Sf	Bs	Ec	Kp	Pv	Pa
4	0.20	1.56	3.13	0.05	1.56	0.05	0.025	6.25
8	0.10	0.39	0.78	0.0125	0.20	0.0125	0.006	1.56
10	0.10	0.78	1.56	0.025	0.39	0.0125	0.0125	3.13
11	0.20	1.56	0.78	0.025	0.78	0.20	0.10	3.13
CI-934	0.10	0.39	0.39	0.05	0.39	0.025	0.05	12.5
NFLX	0.20	3.13	1.56	0.20	0.20	0.05	0.10	3.13

[a] The MICs were determined by the twofold agar dilution on sensitivity test agar. Organisms selected for inclusion in the table: Sa, *Staphloccus aureus* FDA 209P JC-1; Se, *Staphylococcus epidermidis* IAM 1296; Sf, *Streptococcus faecalis* IID 682; Bs, *Bacillus subtilis* ATCC 6633; Ec, *Escherichia coli* NIHJ JC-2; Kp, *Klebsiella pneumoniae* PCI-602; Pr, *Proteus vulgasris* OX-19; Pa, *Pseudomonas aeruginosa* IFO 3445.

The data for the first three entries (**4**, **8** and **10**) indicates that optically active **8** was found to be the most potent compound whose potency was about 2 times higher than that of racemate **10** and about 4 times higher than that of optical isomer **4**. Above results indicate that the configuration of the hydroxyl group at the 3-position of pyrrolidine ring has significant effect on the antibacterial activity.

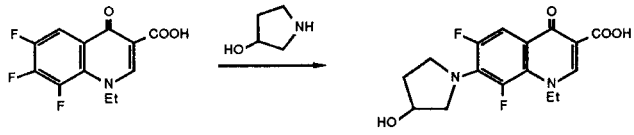
Hitherto, the PCA-antibacterial agents were found to inhibit the DNA-gyrase of bacteria [12-13], and the potency was believed to depend mainly on this inhibition. Therefore, we think that the stereochemistry at this position plays an important role to inhibit DNA-gyrase.

Successively, we investigated *in vivo* antibacterial activities of **4**, **8** and **10** against experimentally induced infection of mice after oral administration. The data are shown in Table II, together with the *in vitro* activity of the infecting strain.

Against gram positive *Staphylococcus aureus* **IID-803**, compound **8** was the most potent compound, followed by **10**, and **4** was the least active compound as expected from the *in vitro* activity. But the potency of **4** was similar to that of **CI-934**, and **11** and **NFLX** were essentially inactive. On the other hand, against gram negative *Escherichia coli* KC-14, **8** and **10** showed similar activity whose potencies were about 2 times higher than that of **4**. **CI-934** and **NFLX** showed lower activities than **4**, and **11** was almost inactive.

The newer derivatives, such as **NFLX, ENX, CPFX** and **OFLX** possess excellent activities against gram negative organisms but have week activities against gram positive organisms. Hence, it is desirable to find other compound having superior activity against gram positive

Scheme III



3 10

Table II

In Vivo Antibacterial Activities of Optically Active 7-(3-Hydroxypyrrolidin-1-yl) Derivatives

Compound	*S. aureus* **IID-803** MIC [a] (μg/ml)	ED_{50} [b]	(mg/kg)	*E. coli* **KC-14** MIC (μg/ml)	ED_{50}	(mg/kg)
4	0.39	10.1	(7.68-13.4) [c]	0.78	7.19	(5.57-9.30)
8	0.10	8.09	(4.74-13.8)	0.20	4.29	(3.09-5.96)
10	0.20	8.82	(5.96-13.1)	0.20	4.29	(3.09-5.96)
11	0.05	>100		0.39	53.6	(38.2-75.3)
CI-934	0.10	10.1	(7.68-13.4)	0.39	10.1	(7.68-13.4)
NFLX	1.56		>100	0.10	8.09	(6.52-10.1)

[a,b] See the Experimental. [c] 95% Confidence limits.

organisms [8].

The above results suggest that the compound **8** is a preferable agent and 3-hydroxypyrrolidinyl group, in particular, 3*S*-hydroxypyrrolidinyl group is one of the beneficial group for the PCA-antibacterial agent.

EXPERIMENTAL

Melting points were determined on a Yanagimoto micro melting point apparatus, and all melting points are uncorrected. Proton nuclear magnetic resonance (^{1}H-nmr) spectra were determined at 100 MHz on a Nihon Denshi PS-100 NMR spectrometer using tetramethylsilane as an internal standard. Mass spectra (ms) were measured with a Hitachi Seisakusyo M-60.

In Vitro Antibacterial Activity.

All the carboxylic acids prepared in this work were tested for antibacterial activity *in vitro* by the serial dilution method [14].

In Vivo Antibacterial Activity.

The *in vivo* antibacterial activity of the test compounds was determined in ddY-strain male mice (20-25 g body weight, five per group). Suspension of the test compounds were made by dispersing in distilled water or in 5% sodium carboxymethylcellulose solution (5% CMC), and diluted distilled water or 5% CMC to the desired concentration.

S. aureus **IID-803** and *E. coli* **KC-14** were incubated in Tripticase-Soy broth at 37° for 18 hours. Cultures (0.5 ml), dilution with phosphate buffered saline were injected intraperitoneally into mice. The LD_{50} for the test organism was calculated from the cumulative mortalities on the seventh day by using the Weil method [15].

The culture of the above was diluted in 5% (w/v) mucin, and 0.5 ml was injected intraperitoneally into mice. The mice were treated orally (po) with a specific amount of the test compound to be administered at 1 hour after infection. A group of five animals each for at least four dose levels was thus treated, and the deaths were recorded daily for 7 days. Five mice were left untreated as infection control. ED_{50} values were calculated from the cumulative mortalities on the seventh day after infection by using the trimmed version of the Weil method.

1-Ethyl-6,8-difluoro-1,4-dihydro-7-[(3*R*)-hydroxypyrrolidin-1-yl]-4-oxoquinoline-3-carboxylic Acid (**4**).

A mixture of 6,7,8-trifluoroquinolone (**3**, synthesized in accordance with the lit [9], 542 mg), (3*R*)-hydroxypyrrolidine hydrochloride [10] (312 mg), triethylamine (1.1 ml) and DMSO (3 ml) was heated at 100° for 30 minutes with stirring. After cooling, poured into ice water, the mixture was neutralized with 1*N* hydrochloric acid to yield yellow solid. The solid was collected, washed with water, dried and recrystallization from DMF-ethanol to give **4** (472 mg) as pale yellow needles; ^{1}H-nmr (DMSO-d_6): δ 1.44 (3H, br t, J = 7.5 Hz, NCH_2CH_3), 1.7-2.1 and 3.2-4.8 (9H, m, NCH_2CH_3 and pyrrolidine H), 5.02 (1H, d, J = 4.0 Hz, OH), 7.68 (1H, dd, J = 2.0 Hz and J = 12.0 Hz, C_5-H), 8.74 (1H, s, C_2-H), 14.85 (1H, br s, COOH); ms: (m/e) 338 (M^+); $[\alpha]_D^{20}$ +125° (c = 1.0, 0.1*N* sodium hydroxide).

Anal. Calcd. for $C_{16}H_{16}F_2N_2O_4$: C, 56.80; H, 4.77; N, 8.28. Found: C, 56.66; H, 5.01; N, 8.38.

1-Ethyl-6,8-difluoro-1,4-dihydro-7-[(3*R*)-hydroxypyrrolidin-1-yl]-4-oxoquinoline-3-carboxylic Acid Ethyl Ester (**6**).

A mixture of 6,7,8-trifluoroquinolone ethyl ester (**5**, 7.5 g) [9], (3*R*)-hydroxypyrrolidine (5.3 g), triethylamine (12.2 ml) and DMSO (45 ml) was heated at 77-82° for 4 hours. After cooling, the reaction mixture was poured into ice water to yield white solid which was collected and recrystallized from ethanol to give **6** (6.5 g) as colorless needles, mp 212-217°; ^{1}H-nmr (deuteriochloroform): δ 1.42 (3H, t, J = 8.0 Hz, NCH_2CH_3), 1.9-2.3 and 3.4-4.5 (7H, m, pyrrolidine H), 4.42 (2H, q, J = 8.0 Hz, CH_2CH_3), 4.62 (1H, br s, OH), 7.80 (1H, dd, J = 2.0 Hz and J = 12.0 Hz, C_5-H), 8.26 (1H, s, C_2-H); ms: (m/e) 366 (M^+).

Anal. Calcd. for $c_{18}H_{20}F_2N_2O_4$: C, 59.01; H, 5.50; N, 7.65. Found: C, 58.88; H, 5.70; N, 7.65.

1-Ethyl-6,8-difluoro-1,4-dihydro-7-[(3*R*)-methanesulfonyloxypyrrolidin-1-yl]-4-oxoquinoline-3-carboxylic Acid (**7**).

To a solution of **6** (5.49 g) and triethylamine (21 ml) in dichloromethane (150 ml), methanesulfonyl chloride (11.6 ml) was added dropwise with stirring at 0-8°. After the reaction mixture was stirred for 40

minutes at 0-8° and poured into ice water, then the mixture was neutralized with aqueous sodium hydrogen carbonate. The organic layer obtained was washed with water and dried over anhydrous sodium sulfate. The solvent was removed to give brown residue, which was purified by column chromatography on silica gel (chloroform:methanol = 40:1) and recrystallized from ethanol to yield **7** (4.1 g) as pale yellow needles, mp 152-156°; ^{1}H-nmr (deuteriochloroform): δ 1.44 (3H, t, J = 7.5 Hz, NCH_2CH_3), 1.54 (3H, t, J = 7.0 Hz, $COOCH_2CH_3$), 2.10-2.52 and 3.50-4.70 (10H, m), 3.13 (3H, s, SO_2CH_3), 5.44 (1H, m, C_3-H of pyrrolidine ring), 7.96 (1H, dd, J = 2.0 Hz and J = 12.0 Hz, C_5-H), 8.32 (1H, s, C_2-H); ms: (m/e) 444 (M^+).

Anal. Calcd. for $C_{19}H_{22}F_2N_2O_6S$: C, 51.35; H, 4.99; N, 6.30. Found: C, 51.34; H, 5.05; N, 6.31.

Alkaline Hydrolysis of **7**.

A mixture of **7** (445 mg) and 0.5*N* sodium hydroxide (8 ml) was heated at 100° for 1 hour with stirring. After cooling, water was added and the mixture was adjusted to *p*H 5 with acetic acid to give yellow precipitate which was a mixture of two kinds of products. Column chromatographic separation was carried out on silica gel to give 1-ethyl-6,8-difluoro-1,4-dihydro-7-[(3*S*)-hydroxypyrrolidin-1-yl]-4-oxoquinoline-3-carboxylic acid **8** (74.5 mg) and 1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-pyrrolin-1-yl)-4-oxoquinoline-3-carboxylic acid **9** (38.5 mg).

Compound **8** was recrystallized from ethanol to yield pale yellow needles, $[\alpha]_D^{20}$ -123° (c = 1.0, 0.1*N* sodium hydroxide). Other physical data (ir, nmr and ms) of **8** was compatible with that of **4**.

Anal. Calcd. for $C_{16}H_{16}F_2N_2O_4$: C, 56.80; H, 4.77; N, 8.28. Found: C, 56.60; H, 4.71; N, 8.46.

Compound **9** was recrystallized from a mixture of chloroform-DMF to yield pale yellow needles, mp 293-296° dec; ^{1}H-nmr (deuteriochloroform-perdeuteriomethanol): δ 1.57 (3H, br t, J = 6.5 Hz, NCH_2CH_3), 4.50 (2H, m, NCH_2CH_3), 4.71 (4H, t, J = 3.0 Hz, C_2 and C_5-H of pyrroline ring), 5.94 (2H, s, C_3 and C_4-H of pyrroline ring), 7.85 (1H, dd, J = 2.0 Hz and J = 15.0 Hz, C_5-H), 8.59 (1H, s, C_2-H); ms: (m/e) 320 (M^+).

1-Ethyl-6,8-difluoro-1,4-dihydro-7-(3-pyrrolin-1-yl)-4-oxoquinoline-3-carboxylic Acid (**9**).

A mixture of **3** (3.52 g), 3-pyrroline (1.0 g), triethylamine (7.2 ml) and DMSO (20 ml) was heated at 100° for 1 hour. After cooling, the reaction mixture was poured into ice water, and the mixture was neutralized with 3*N* hydrochloric acid to give yellow solid. The solid was collected and recrystallized from a mixture of chloroform and DMF to give **9** (3.52 g) as pale yellow needles. The physical data (ir, nmr and ms) was compatible with that of **9** which was obtained by alkaline hydrolysis of **7**.

Anal. Calcd. for $C_{16}H_{16}F_2N_2O_4$: C, 60.00; H, 4.41; N, 8.74. Found: C, 60.04; H, 4.51; N, 8.84.

1-Ethyl-6,8-difluoro-1,4-dihydro-7-[(3*RS*)-hydroxypyrrolidin-1-yl]-4-oxoquinoline-3-carboxylic Acid (**10**).

A mixture of **3** (8.13 g) (3*RS*)-hydroxypyrrolidine (Aldrich Chemical Company, 3.2 g), triethylamine (15 ml) and DMSO (47 ml) was heated at 100° for 40 minutes. Post-treatment and purification were carried out according to the synthetic method of **4** to give **10** (7.6 g) as pale yellow needles, $[\alpha]_D^{20}$ 0° (c = 1.0, 0.1 *N* sodium hydroxide). Other physical data (ir, nmr and ms) compatible with those of **4**.

Anal. Calcd. for $C_{16}H_{16}F_2N_2O_4$: C, 56.80; H, 4.77; N, 8.28. Found: C, 56.57; H, 4.80; N, 8.46.

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