Notes

NITROSOXACINS A, B AND C, NEW 5-LIPOXYGENASE INHIBITORS

MAKI NISHIO, MASAMI HASEGAWA, KIYOSHI SUZUKI, YOSUKE SAWADA, DEREK J. HOOK† and TOSHIKAZU OKI

Bristol-Myers Squibb Research Institute, 2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan
†Bristol-Myers Squibb Pharmaceutical Research Institute, Department of Microbiology Biochemistry, 5 Research Parkway, Wallingford, CT 06492-7660, U.S.A.

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In our continuing search for microbial metabolites that inhibit the activity of 5-lipoxygenase (5-LPO), we had reported carbazomycins[1], epocarbazolins[2] as 5-LPO inhibitors. Further search resulted in the isolation of new 5-LPO inhibitors designated nitrosoxacins A, B and C from strain AA4091. In this paper, we describe the production, isolation and structural studies of these new inhibitors.

The producing strain was isolated from a soil sample collected in Kisarazu City, Chiba Prefecture, Japan, and taxonomic studies indicated that the strain belonged to the genus *Streptomyces*. This strain was fermented in 500-ml Erlenmeyer flasks in 100 ml of medium (soluble starch 2.5%, glucose 1%, Pharmamedia 1%, Brewer's yeast extract 0.3%, $CaCO_3$ 0.1% and Allophane 0.5%. The pH of the medium was adjusted to pH 7.0 before autoclaving.). The 5-LPO inhibitory activity was determined as described previously[1].

The active substances were isolated according to the procedures shown in Scheme 1. Nitrosoxacins A, B and C complex were extracted from the fermentation broth with 1-butanol and each component was separated by reversed phase silica gel column chromatography followed by Sephadex LH-20 chromatography. They were obtained as white amorphous powders and were soluble in dimethyl sulfoxide and chloroform, slightly soluble in methanol and hexane, but insoluble in water.

Scheme 1. Isolation procedure of nitrosoxacins A, B and C.

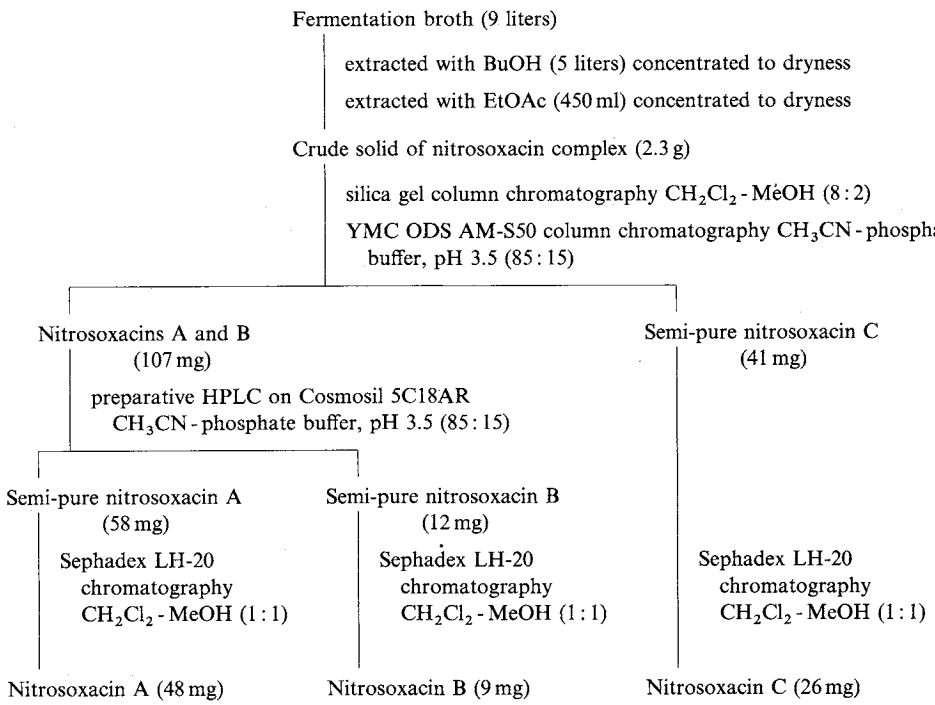

Table 1. ^{1}H NMR data of nitsoxacin A, B and C in $CDCl_3$.

Nitrosoxacin A	Nitrosoxacin B	Nitrosoxacin C
0.86 (6H, d, J=6.84 Hz)	0.88 (3H, t, J=6.84 Hz)	0.86 (6H, d, J=6.84 Hz)
1.13~1.33 (22H, m)	1.25~1.34 (26H, m)	1.13~1.32 (18H, m)
1.51 (1H, m)	1.94 (2H, qui, J=7.26 Hz)	1.51 (1H, m)
1.97 (2H, qui, J=7.26 Hz)	4.13 (2H, t, J=7.27 Hz)	1.94 (2H, qui, J=7.26 Hz)
4.13 (2H, t, J=7.26 Hz)	11.63 (1H, s)*	4.13 (2H, t, J=7.26 Hz)
11.53 (1H, s)*		11.52 (1H, s)*

* Disappeared upon D_2O addition.

Table 2. ^{13}C NMR data of nitrosoxacins A, B and C in $CDCl_3$.

Nitrosoxacin A	Nitrosoxacin B	Nitrosoxacin C
22.64 (q)*	14.09 (q)	22.63 (q)
22.64 (q)	22.68 (t)	22.63 (q)
26.12 (t)	26.13 (t)	26.12 (t)
26.59 (t)	26.60 (t)	26.59 (t)
27.41 (t)	28.82 (t)	27.38 (t)
27.96 (d)	29.29 (t)	27.95 (d)
28.82 (t)	29.35 (t)	28.81 (t)
29.29 (t)	29.45 (t)	29.27 (t)
29.45 (t)	29.56 (t)	29.45 (t)
29.56 (t)	29.62 (t)	29.55 (t)
29.62 (t)	29.65 (t)	29.64 (t)
29.65 (t)	29.65 (t)	29.89 (t)
29.70 (t)	29.66 (t)	39.04 (t)
29.93 (t)	29.66 (t)	61.38 (t)
39.06 (t)	31.91 (t)	
61.40 (t)	61.40 (t)	

* Multiplicity determined by DEPT spectra.

Nitrosoxacin A: MP 44.0~44.5°C; UV λ_{max} (0.1 N HCl-MeOH (1:9)) nm (ε) 229 (6,300), λ_{max} (0.1 N NaOH-MeOH (1:9)) 249 (9,800); IR ν_{max} (KBr) cm^{-1} 2920, 2850, 1470, 1060, 965, 720; FAB-MS (m/z) 287 $(M+H)^+$, 285 $(M-H)^-$; Elemental analysis, Calcd for $C_{16}H_{34}N_2O_2$: C 67.09, H 11.96, N 9.78, Found: C 66.95, H 12.02, N. 10.01.

Nitrosoxacin B: MP 38.5~39.5°C; UV λ_{max} (0.1 N HCl-MeOH (1:9)) nm (ε) 230 (6,400), λ_{max} (0.1 N NaOH-MeOH (1:9)) 249 (9,900); IR ν_{max} (KBr) cm^{-1} 2920, 2850, 1465, 1080, 965, 720; FAB-MS (m/z) 287 $(M+H)^+$, 285 $(M-H)^-$.

Nitrosoxacin C: MP 35.5~36.0°C; UV λ_{max} (0.1 N HCl-MeOH (1:9)) mm (ε) 230 (6,200), λ_{max} (0.1 N NaOH-MeOH (1:9)) 249 (9,900); IR ν_{max} (KBr) cm^{-1} 2930, 2850, 1465, 1060, 965, 720; FAB-MS (m/z) 259 $(M+H)^+$, 257 $(M-H)^-$.

The molecular formula of nitrosoxacin A was established as $C_{16}H_{34}N_2O_2$ by the FAB-MS and the elemental analysis. The ^{1}H NMR and the ^{13}C NMR data (Tables 1 and 2) clearly showed the presence of 14-methylpentadecyl group. The remaining N_2O_2 group was assigned to *N*-nitrosohydroxylamino group based on the UV and IR spectra. The UV absorption maximum at 229 nm in acidic methanol and 249 nm in alkaline methanol and the characteristic absorption around 1470 cm^{-1} in the IR spectrum were consistent with the *N*-nitrosohydroxylamino group of synthesized sample[3]. Upon hydrogenolysis over platinum dioxide, nitrosoxacin A afforded an amine whose structure was determined to be 14-methylpentadecylamine by spectral data. Thus, the structure of nitrosoxacin A has been determined to be *N*-nitroso-*N*-14-methylpentadecylhydroxylamine (Fig. 1).

Fig. 1. Structures of nitrosoxacins A, B and C.

Nitrosoxacin A $R=(CH_3)_2CH(CH_2)_{13}-$
Nitrosoxacin B $R=CH_3(CH_2)_{15}-$
Nitrosoxacin C $R=(CH_3)_2CH(CH_2)_{11}-$

Table 3. Inhibition of 5-LPO.

Compounds	IC_{50} (μM)
Nitrosoxacin A	1.7
Nitrosoxacin B	1.3
Nitrosoxacin C	2.1
Cupferron	49
N-Nitroso-*N*-cyclohexylhydroxylamine	55
14-Methylpentadecylamine	>100

The UV and IR spectra of nitrosoxacins B and C indicated that they shared the same chromophore as nitrosoxacin A. The ^{1}H and ^{13}C NMR data showed the presence of a hexadecyl group for nitrosoxacin B and a 12-methyltridecyl group for nitrosoxacin C. From these data, the structures of nitrosoxacins B and C have been assigned to *N*-nitroso-*N*-hexadecylhydroxylamine and *N*-nitroso-*N*-12-methyltridecylhydroxylamine, respectively (Fig. 1).

The 5-LPO inhibitory activities of nitrosoxacins A, B and C are shown in Table 3. *N*-Nitroso-*N*-alkylhydroxylamine is characterized as chelating

agent[4]. The 5-LPO inhibitory activity of nitrosoxacins is probably due to their chelating activity. However, cupferron (*N*-nitroso-*N*-phenylhydroxylamine ammonium salt) and *N*-nitroso-*N*-cyclohexylhydroxylamine showed poor 5-LPO inhibition (Table 3). The fact that an alkylamine, 14-methylpentadecylamine did not show 5-LPO inhibition indicates that the combination of a certain alkyl chain with a *N*-nitroso-*N*-hydroxylamine group has an important role to express 5-LPO inhibitory activity.

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