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

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In our continuing search for microbial metabolites that inhibit the activity of 5-lipoxygenase (5-LPO), we had reported carbazomycins1*, epocarbazolins2 as 5-LPO inhibitors. Further search resulted in the isolation of new 5-LPO inhibitors designated nitrosoxacin 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%, CaCO3 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.

Fermentation broth (9 liters)
extracted with BuOH (5 liters) concentrated to dryness
extracted with EtOAc (450 ml) concentrated to dryness

Crude solid of nitrosoxacin complex (2.3 g)
silica gel column chromatography CH\(_2\)Cl\(_2\) - MeOH (8:2)
YMC ODS AM-S50 column chromatography CH\(_3\)CN - phosphate buffer, pH 3.5 (85:15)

Nitrosoxacins A and B
(107 mg)
preparative HPLC on Cosmosil 5C18AR
CH\(_3\)CN - phosphate buffer, pH 3.5 (85:15)

Semi-pure nitrosoxacin C
(41 mg)

Semi-pure nitrosoxacin A
(58 mg)
Sephadex LH-20 chromatography
CH\(_2\)Cl\(_2\) - MeOH (1:1)

Nitrosoxacin A (48 mg)

Semi-pure nitrosoxacin B
(12 mg)
Sephadex LH-20 chromatography
CH\(_2\)Cl\(_2\) - MeOH (1:1)

Nitrosoxacin B (9 mg)

Nitrosoxacin C (26 mg)
**Table 1.** \(^1\)H NMR data of nitrosoxacin A, B and C in CDCl\(_3\).

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

* Disappeared upon D\(_2\)O addition.

**Table 2.** \(^{13}\)C NMR data of nitrosoxacins A, B and C in CDCl\(_3\).

<table>
<thead>
<tr>
<th>Nitrosoxacin A</th>
<th>Nitrosoxacin B</th>
<th>Nitrosoxacin C</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.64 (q)*</td>
<td>14.09 (q)</td>
<td>22.63 (q)</td>
</tr>
<tr>
<td>23.00 (q)</td>
<td>22.68 (t)</td>
<td>22.63 (t)</td>
</tr>
<tr>
<td>26.12 (t)</td>
<td>26.13 (t)</td>
<td>26.12 (t)</td>
</tr>
<tr>
<td>26.60 (t)</td>
<td>26.60 (t)</td>
<td>26.59 (t)</td>
</tr>
<tr>
<td>27.41 (t)</td>
<td>28.82 (t)</td>
<td>27.38 (t)</td>
</tr>
<tr>
<td>27.98 (d)</td>
<td>29.29 (t)</td>
<td>27.95 (d)</td>
</tr>
<tr>
<td>28.82 (t)</td>
<td>29.29 (t)</td>
<td>27.95 (d)</td>
</tr>
<tr>
<td>29.29 (t)</td>
<td>29.45 (t)</td>
<td>29.27 (t)</td>
</tr>
<tr>
<td>29.45 (t)</td>
<td>29.56 (t)</td>
<td>29.45 (t)</td>
</tr>
<tr>
<td>29.56 (t)</td>
<td>29.62 (t)</td>
<td>29.55 (t)</td>
</tr>
<tr>
<td>29.62 (t)</td>
<td>29.65 (t)</td>
<td>29.64 (t)</td>
</tr>
<tr>
<td>29.65 (t)</td>
<td>29.65 (t)</td>
<td>29.89 (t)</td>
</tr>
<tr>
<td>29.70 (t)</td>
<td>29.66 (t)</td>
<td>39.04 (t)</td>
</tr>
<tr>
<td>29.93 (t)</td>
<td>29.66 (t)</td>
<td>61.38 (t)</td>
</tr>
<tr>
<td>39.06 (t)</td>
<td>31.91 (t)</td>
<td>61.40 (t)</td>
</tr>
<tr>
<td>61.40 (t)</td>
<td>61.40 (t)</td>
<td>61.40 (t)</td>
</tr>
</tbody>
</table>

* Multiplicity determined by DEPT spectra.

Nitrosoxacin A: MP 44.0~44.5°C; UV \(\lambda_{\text{max}}\) (0.1 N HCl-MeOH (1:9)) nm (e) 229 (6,300), \(\lambda_{\text{max}}\) (0.1 N NaOH-MeOH (1:9)) 249 (9,800); IR \(v_{\text{max}}\) (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\(_2\)O\(_2\): 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 \(\lambda_{\text{max}}\) (0.1 N HCl-MeOH (1:9)) nm (e) 230 (6,400), \(\lambda_{\text{max}}\) (0.1 N NaOH-MeOH (1:9)) 249 (9,900); IR \(v_{\text{max}}\) (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\(_2\)O\(_2\): C 67.09, H 11.96, N 9.78, Found: C 66.95, H 12.02, N 10.01.

Nitrosoxacin C: MP 35.5~36.0°C; UV \(\lambda_{\text{max}}\) (0.1 N HCl-MeOH (1:9)) nm (e) 230 (6,200), \(\lambda_{\text{max}}\) (0.1 N NaOH-MeOH (1:9)) 249 (9,900); IR \(v_{\text{max}}\) (KBr) cm\(^{-1}\) 2920, 2850, 1465, 1080, 965, 720; FAB-MS (m/z) 259 (M+H)+, 257 (M-H)-; Elemental analysis, Calcd for C\(_{16}\)H\(_{34}\)N\(_2\)O\(_2\): C 67.09, H 11.96, N 9.78, Found: C 66.95, H 12.02, N 10.01.

The molecular formula of nitrosoxacin A was established as C\(_{16}\)H\(_{34}\)N\(_2\)O\(_2\) 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\(_2\)O 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-methylpentadecylamino (Fig. 1).

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-alkylyhydroxylamine is characterized as chelating...
agent). The 5-LPO inhibitory activity of nitroso-
xAxeins is probably due to their chelating activity.
However, cupferron (N-nitroso-N-phenylhydroxyl-
amine ammonium salt) and N-nitroso-N-cyclohexyl-
hydroxylamine showed poor 5-LPO inhibition
(Table 3). The fact that an alkylamine, 14-methyl-
pentadecylamine 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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