Pironetin, a Novel Plant Growth Regulator
Produced by Streptomyces sp. NK10958

III. Biosynthesis

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In our screening of microbial secondary metabolites
for plant growth regulators, we found a new compound
from the culture broth of Streptomyces sp. NK10958
and named it pironetin\(^\text{1}\). The structure of pironetin
was determined to be (5jR,6i?)-5-ethyl-5,6-dihydro-6-((,E)
\((2R,3S,5R,5S)-2\)-hydroxy-4-methoxy-3,5-dimethyl-7-
nonenyl\()-2\)-H-pyran-2-one\(^\text{2}\). In this report, we describe
incorporation experiments with \(^{13}\)C-labeled precursors
and the biosynthetic origin of the carbon atoms in
pironetin. The usefulness of pironetin in agriculture
will be reported separately\(^\text{3}\).

Materials and Methods

Labeled Compounds

- Sodium \([l-^{13}\text{C}]\)acetate (99% \(^{13}\)C enriched), sodium
- \([2-^{13}\text{C}]\)acetate (99%), sodium \([1,2-^{13}\text{C}_2]\)acetate (99%),
- sodium \([l-^{13}\text{C}]\)propionate (99%), sodium \([l-^{13}\text{C}]\)butyrate
- (99%) and \(L-[methyl-^{13}\text{C}]\)methionine (99%) were
- purchased from Sigma Chemical Co., U.S.A.

Fermentation

A loopfull spores of the strain Streptomyces sp.
NK10958 was inoculated into 100 ml of a seed medium
consisted of glycerin 2%, soy bean meal 2% and NaCl
0.3% (pH 7.0 before sterilization) in a 500-ml Erlen-
meyer flask, and cultured at 27°C for 2days on a rotary
shaker (220 rpm). One milliliter of this seed culture was
transferred to 1 00 ml of the production medium consisted
of glycerin 4%, soy bean meal 2% and NaCl.0.3% (pH
7.0 before sterilization) in a 500-ml Erlenmeyer flask and
cultured at 27°C on a rotary shaker (220rpm). One
milliliter of each \(^{13}\)C-labeled compound solution at a
concentration of 30mg/ml in water was added into a
flask on 48 hours after inoculation. The fermentation
was continued 48 hours after the feed of \(^{13}\)C-labeled
compound.

Isolation

Each fermentation broth (100 ml \(\times 10\) flasks) was
combined and extracted with 1.0 liter of EtOH. The
residual mycelia were filtered and the filtrate was con-
centrated \textit{in vacuo} to an aqueous solutions. The aqueous
solution was extracted three times with 500 ml of ethyl
acetate. After washing with 200 ml of saturated NaCl
solution, the extract was concentrated to dryness \textit{in vacuo}
The extract was chromatographed on a silica gel
(Merck, type 60) and eluted with the mixture of \(n\)-hexane
and acetone (10:1). The pironetin containing fractions
were collected and concentrated \textit{in vacuo}. The residue
was re-chromatographed on a silica gel (Fuji-Davison,
Japan, BW-700) and eluted with the mixture of \(n\)-hexane
and acetone (10:1). The pironetin containing fractions
were collected and concentrated \textit{in vacuo} to give colorless
crystals of pironetin.

NMR

\(^{13}\)C NMR spectra were recorded on a Bruker AC 300
plus spectrometer. Each \(^{13}\)C-enriched pironetin was dis-
solved in CDC\(_3\) at the concentration of about 20 mg in
a NMR tube. The increment of signal intensities caused
by \(^{13}\)C-enrichment were determined from each signal
intensity of \(^{13}\)C-enriched pironetins by comparison with
the signal intensity of natural pironetin.

Results and Discussion

Relative enrichments and \(^{13}\)C-\(^{13}\)C coupling constants
based on the \(^{13}\)C NMR spectra of pironetin derived from
\(^{13}\)C-labeled precursors are listed in Table 1. Relative
enrichments were normalized to peak intensities for the
C-19 signals on \([l-^{13}\text{C}]\)acetate, \([2-^{13}\text{C}]\)acetate, \([l-
^{13}\text{C}]\)propionate and \([l-^{13}\text{C}]\)butyrate-labeled pironetins
and for the C-7 signal on \(L-[methyl-^{13}\text{C}]\)methionine-
labeled pironetin. In the \(^{13}\)C NMR spectrum of \([l-
^{13}\text{C}]\)acetate-labeled pironetin, high level of enrichments
were observed for C-1, C-3, C-5, C-13 and C-15.
These enrichments correspond to high level of enrich-
ment for C-2, C-4, C-6, C-12, C-14 and C-16 in the
\(^{13}\)C NMR spectrum of \([2-^{13}\text{C}]\)acetate-labeled pironetin
respectively. The \(^{13}\)C-\(^{13}\)C coupling of intact doubly-
labeled acetate units were observed at these positions.
These results indicate the incorporation of six acetates
into pironetin.

Low level of enrichments were observed for C-7 and
C-9 in \([1-^{13}\text{C}]\)acetate-labeled pironetin, and C-7, C-8,
C-9, C-10, C-17 and C-18 in \([2-^{13}\text{C}]\)acetate-labeled
pironetin. The \(^{13}\)C-\(^{13}\)C coupling of intact doubly-labeled
acetate units were observed at these positions. These
results indicate the incorporation of six acetates
into pironetin.

The incorporation of methionine showed the methyl carbons
C-17 and C-18 are not derived from methionine. These
results indicate that two propionates were incorporated
Table 1. Incorporation of $^{13}$C-labeled precursors into pironetin.

<table>
<thead>
<tr>
<th>Carbon</th>
<th>Relative enrichment</th>
<th>Jcc (Hz)</th>
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<tr>
<td>NO</td>
<td>$^{13}$C</td>
<td>$^{13}$C</td>
</tr>
<tr>
<td>(ppm)</td>
<td>CH$_3$COONa</td>
<td>CH$_3$COONa</td>
</tr>
<tr>
<td>1</td>
<td>164.6</td>
<td>2.5**</td>
</tr>
<tr>
<td>2</td>
<td>120.8</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>105.6</td>
<td>2.1**</td>
</tr>
<tr>
<td>4</td>
<td>39.1</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>77.8</td>
<td>2.7**</td>
</tr>
<tr>
<td>6</td>
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</tr>
<tr>
<td>7</td>
<td>67.4</td>
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<td>8</td>
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</tr>
<tr>
<td>9</td>
<td>91.0</td>
<td>1.5*</td>
</tr>
<tr>
<td>10</td>
<td>36.1</td>
<td>0.9</td>
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<tr>
<td>11</td>
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<td>2.5**</td>
</tr>
<tr>
<td>12</td>
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<td>13</td>
<td>126.8</td>
<td>2.9**</td>
</tr>
<tr>
<td>14</td>
<td>17.9</td>
<td>0.9</td>
</tr>
<tr>
<td>15</td>
<td>20.7</td>
<td>2.1**</td>
</tr>
<tr>
<td>16</td>
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</tr>
<tr>
<td>19</td>
<td>61.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* Low level of enrichment was observed.
** High level of enrichment was observed.

Relative enrichments were normalized to peak intensities for the C-19 signals on sodium $[1-^{13}$C$]$acetate, sodium $[2-^{13}$C$]$acetate, $[1-^{13}$C$]$propionate and sodium $[1-^{13}$C$]$butyrate-labeled pironetins and for the C-7 signal on $[\text{me}^{13}$C$]$methionine-labeled pironetin.

Due to the $^{13}$C-$^{13}$C coupling, the correct values could not be obtained.

The incorporation experiment of $[\text{me}^{13}$C$]$methionine also showed clearly the methoxyl carbon (C-19) are derived from methyl carbon of methionine. The results mentioned above suggest that two propionate units and one methyl unit of methionine are incorporated into pironetin.

OUMURA et al.4,5) reported that the C-ethyl group of the aglycon of the 16-membered macrolide antibiotics Leucomycin A$_3$ and Tylosin is not directly derived from acetate but it is derived from butyrate in their experiments of incorporation of $^{13}$C-labeled precursors. SETO et al.6) also reported that the C-ethyl group is derived from butyric acid using the incorporation experiment of $[1-^{13}$C$]$butyrate in their carbon assignment work of a polyether antibiotic Lasalocid. In our experiments the relative enrichments of C-3 and C-4 were weaker than those of C-1 and C-2 or C-5 and C-6 in the $[1-^{13}$C$]$- and $[2-^{13}$C$]$-acetates incorporation experiments respectively. C-15 and C-16 also showed weaker relative enrichments. This suggested that the acetate units of C-3 to C-4 and C-15 to C-16 are not directly derived from acetate but derived from butyrate.

In order to confirm this hypothesis, the incorporation experiment of sodium $[1-^{13}$C$]$butyrate was investigated. Relative enrichments of pironetin derived from sodium $[1-^{13}$C$]$-butyrate are also shown in Table 1. The strong enrichment peak was observed for C-3 as expected. This confirmed that the four carbons (C-3, C-4, C-15 and C-16) of pironetin are derived from butyrate directly. In $[1-^{13}$C$]$-butyrate incorporation experiment weak enrichments were observed for C-7 and C-9 and very weak enrichments were observed for C-1, C-11 and C-13. This suggested that butyrate is metabolized to propionate and acetate. Butyrate and propionate are incorporated directly to pironetin in the biosynthesis. Considering our $^{13}$C-labeled acetates, propionate and butyrate incorporation experiments, we concluded that pironetin is derived from four acetate units, two propionate units, one butyrate unit and one methyl unit of methionine. The origin of the all carbon atoms of pironetin are summarized in Fig. 1.

Fig. 1. Incorporation of $^{13}$C-labeled precursors into pironetin.

CH$_3$COONa

CH$_3$CH$_2$COONa

methyl of methionine

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References


