SOME NEW BIOSYNTHETIC PENICILLINS

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In our laboratories some 200 compounds, including phenylacetic acid derivatives, have been studied as possible precursors for new penicillins, and several of these appeared to give rise to new penicillins. In this report, five new penicillins, four of which were obtained in crystalline form, are described and their production, isolation, and characterization discussed. The methods employed in our studies were substantially the same as those already reported (1–3).

Some comparative blood level data have been obtained on these and other penicillins and will be published elsewhere.

EXPERIMENTAL

Fermentation—In the study of penicillin precursors, the initial test on the compound was carried out in shaken culture to determine the toxicity of the material and the effect on the fermentation as indicated by the yield of the penicillin obtained. Three levels of precursor were tested in a synthetic (Table II) medium. The control cultures contained the same medium without any precursor. Quantities of medium (100 ml.) in 500 ml. Erlenmeyer flasks were inoculated with Penicillium chrysogenum X-1612 or Q-176 and incubated at 24° on a reciprocating shaker. Samples were taken for analysis after 3, 4, 5, 6, and 7 days incubation. The results obtained with five different precursors are shown in Table I. The figures represent maximum yields in units per ml. of culture broth as determined by our standard assay procedure with Staphylococcus aureus as the test organism.

Since the results obtained in shaken flasks suggested the possibility that penicillins incorporating the precursor were formed, sufficient quantities of the penicillins were produced to permit isolation and characterization.

Small fermenters containing 12 liters of medium were inoculated with vegetative culture of P. chrysogenum X-1612 or Q-176. The design and operation of our fermenters were similar to the fermenters described by Rivett et al. (8). Vegifat-Y was added as needed to control foaming. Quantities of culture varying from 80 to 120 liters were prepared with each precursor. A synthetic medium shown in Table II was used, since
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the regular corn steep medium ordinarily employed for penicillin production contains a number of substances which can act as precursors for various penicillins. In the case of the phenylmercaptoacetic acid, P. chrysogenum X-1612 was employed. In all other instances P. chrysogenum Q-176 was used. The level of precursor was determined on the basis of the shaken flask results, the level giving the highest yield being used. The results obtained in the small fermenters are shown in Table III.

TABLE I
Penicillin Yields in Shaken Culture

The values are given in units per ml. of culture broth.

<table>
<thead>
<tr>
<th>Precursor</th>
<th>No. of precursor</th>
<th>Concentration of precursor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>concentrate</td>
<td>100 mg. per liter</td>
</tr>
<tr>
<td>Phenylmercaptoacetic acid (4)</td>
<td>102</td>
<td>6th</td>
</tr>
<tr>
<td>p-Bromophenylmercaptoacetic acid (5, 7)</td>
<td>164</td>
<td>7th</td>
</tr>
<tr>
<td>Benzylmercaptoacetic acid (6)</td>
<td>114</td>
<td>6th</td>
</tr>
<tr>
<td>Phenethylmercaptoacetic acid (4)</td>
<td>14</td>
<td>7th</td>
</tr>
<tr>
<td>p-Hydroxyphenylmercaptoacetic acid (7)</td>
<td>89</td>
<td>6th</td>
</tr>
</tbody>
</table>

The figures in parentheses represent bibliographic references. * Day on which maximum yield was obtained.

TABLE II
Synthetic Medium

| Lactose | 30 | FeSO₄·7H₂O | 0.2 |
| Glucose | 5 | ZnSO₄·7H₂O | 0.02 |
| Glacial acetic acid | 6 | CuSO₄·5H₂O | 0.005 |
| NH₄NO₃ | 6 | ml. |
| KH₂PO₄ | 2 | Water | 1000 |
| MgSO₄·7H₂O | 0.5 | KOH to pH 6.2 |

Isolation and Characterization—The technique used in the isolation of these penicillins has already been described (1, 2). The filtered beer was extracted with 0.4 volume of amyl acetate, and this in turn with potassium phosphate buffer at pH 7.5. An ether extract (at pH 2.0) of this buffer extract was then chromatographed on a silica gel-buffer column, pH 6.2, and the principal active fraction was transferred to chloroform and subjected to further chromatography on a buffer-silica gel column. The most active bands from this chromatogram were converted to sodium salts and lyophilized, and crystallization attempts were made. Table IV shows the penicillins which were isolated and the activities in units per mg. versus S. aureus and Bacillus subtilis.
Phenylmercaptomethylpenicillin—80 liters of broth contained 15.8 million units. The principal band of the first chromatogram occupied a position comparable to that of benzylpenicillin when run in a similar manner and was equivalent to 66 per cent of the units applied to the column. Chromatography of this fraction in chloroform and subsequent conversion to sodium salt gave 2.57 gm. of amorphous material having an activity of 2700 units per mg. This was crystallized from acetone and then from 90 per cent acetone-water; yield, 1.525 gm.

**Table III**

*Precursor Data*

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Concentration</th>
<th>Maximum yield</th>
<th>Day of maximum yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylmercaptoacetic acid</td>
<td>250 mg. per l.</td>
<td>446 unit per ml.</td>
<td>4th</td>
</tr>
<tr>
<td>p-Bromophenylmercaptoacetic acid</td>
<td>500 mg. per l.</td>
<td>366 unit per ml.</td>
<td>4th</td>
</tr>
<tr>
<td>Benzylmercaptoacetic acid</td>
<td>100 mg. per l.</td>
<td>240 unit per ml.</td>
<td>4th</td>
</tr>
<tr>
<td>Phenethylmercaptoacetic acid</td>
<td>100 mg. per l.</td>
<td>195 unit per ml.</td>
<td>3rd</td>
</tr>
<tr>
<td>p-Hydroxyphenylmercaptoacetic acid</td>
<td>500 mg. per l.</td>
<td>242 unit per ml.</td>
<td>4th</td>
</tr>
</tbody>
</table>

**Table IV**

*New Penicillins*

<table>
<thead>
<tr>
<th>Penicillin isolated</th>
<th>Units per mg. vs. <em>S. aureus</em></th>
<th>Units per mg. vs. <em>B. subtilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylmercaptomethylpenicillin</td>
<td>3400</td>
<td></td>
</tr>
<tr>
<td>p-Bromophenylmercaptomethylpenicillin</td>
<td>2100</td>
<td>1700</td>
</tr>
<tr>
<td>Benzylmercaptomethylpenicillin</td>
<td>1900</td>
<td>1400</td>
</tr>
<tr>
<td>Phenethylmercaptomethylpenicillin</td>
<td>2050</td>
<td>1500</td>
</tr>
<tr>
<td>p-Hydroxyphenylmercaptomethylpenicillin</td>
<td>600</td>
<td>800</td>
</tr>
</tbody>
</table>

* This penicillin was not isolated in crystalline form. The assay was carried out on an amorphous preparation.

Analysis—C₁₅H₁₁O₅N₅S₅Na. Calculated. C 49.48, H 4.38, S 16.49, N 7.21

Found. C 49.13, H 4.28, S 16.87, N 7.28

*p-Bromophenylmercaptomethylpenicillin*—The harvest amounted to 120 liters and contained 25 million units. 68 per cent of the activity recovered from the ether chromatogram was found in the filtrates in a position comparable to the position occupied by n-heptylpenicillin in similar isolations. The chloroform chromatogram was run at pH 6.7, and 60 per cent of the activity was in the top half of the column. Crystallization of the 324 mg. of amorphous sodium salt from acetone resulted in 200 mg. of analytically pure crystals.
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**Analysis**—C₁₂H₁₄O₄N₂S₂BrNa. Calculated. C 41.12, H 3.45

**Found.** " 41.44, " 3.52

In this fermentation, apparently only a very small amount of the desired penicillin was produced. The positions of the bands on the column indicate that mostly n-heptylpenicillin, 2-pentenylpenicillin, and other aliphatic penicillins were formed virtually to the exclusion of benzylpenicillin.

**Benzy1mercaptomethylpenicillin**—The harvest (120 liters) contained 15.5 million units. 75 per cent of the activity recovered from the ether chromatogram was found in the filtrates. The subsequent chloroform chromatogram was therefore run at pH 6.7, and 60 per cent of the applied activity was in the top half of the column. The resulting 2.22 gm. of sodium salt assaying 1780 units per mg. were crystallized from acetone; yield, 677 mg. of analytically pure product.

**Analysis**—C₁₅H₁₅O₄N₂S₂Na. Calculated. C 50.73, H 4.75

**Found.** " 50.63, " 4.58

**Phenethylmercaptomethylpenicillin**—15 million units were contained in 116 liters of harvest. 75 per cent of the activity from the ether chromatogram was in the filtrates. The subsequent chloroform chromatogram at pH 6.7 yielded 66 per cent of the activity in the filtrates, and as a result another chloroform chromatogram was run at pH 7.2. 85 per cent of this applied activity was in the top half of the column and yielded 2.055 gm. of amorphous sodium salt. Crystallization from acetone and three recrystallizations from acetone-water yielded 438 mg. of analytically pure crystals.

**Analysis**—C₁₃H₁₅O₄N₂S₂Na. Calculated. C 51.90, H 5.08

**Found.** " 51.66, " 4.70

**p-Hydroxyphenylmercaptomethylpenicillin**—The harvest (119 liters) contained 11.6 million units. 30 per cent of the activity applied to an ether chromatogram was recovered from the very top of the column in a position comparable to that occupied by p-hydroxybenzylpenicillin. A subsequent chloroform chromatogram, pH 6.0, was run and resulted in very little, if any, separation of activity and pigmented impurities from the top of the column. This was followed by an ether chromatogram at pH 5.6 which brought about some resolution of the activity into a 25 cm. band, 8 cm. from the top of a 40 cm. high column.

All attempts to obtain crystals from the resulting amorphous sodium salt (1.474 gm.) failed. Although no clear cut evidence was obtained to demonstrate the formation of this p-hydroxyphenylmercaptomethylpenicillin, it appears probable that the precursor was utilized and the peni-
cillin formed, since in no other similar fermentations has there been any significant amount of this type of comparatively higher acidic activity.

The authors wish to express their gratitude to W. J. White for assistance in carrying out the fermentations, and to E. F. Shelberg and L. F. Reed who performed the microanalyses.

SUMMARY

The production, isolation, and characterization of five new biosynthetic penicillins have been described. Four of these were obtained in crystalline form and varied in potency from 1900 to 3400 units per mg. The other, p-hydroxyphenylmercaptomethylpenicillin, was isolated as an amorphous sodium salt having a potency of 600 units per mg. versus Staphylococcus aureus.

BIBLIOGRAPHY

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