ANTAGONISM BETWEEN PHENYLALANINE AND VARIOUS ISOMERS OF PHENYLSERINE IN TWO LACTOBACILLI*

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β-Phenylserine has been used as a phenylalanine antagonist in studies of growth (1-7). In the course of investigation of aromatic amino acid biosynthesis in this laboratory (8), it became evident that phenylserine, as usually prepared by the Erlenmeyer methods (9, 10), was of uncertain identity and purity (11). Impetus was thus added to attempts to separate conveniently phenylserine (the threo form) and allophenylserine (12) (the erythro form) for antibacterial studies. It became of interest to study also two resolved forms which have become available more recently (13).

Materials and Methods

The phenyl-L-serine and allophenyl-L-serine were prepared by methods previously described (12). Phenyl-L-serine and phenyl-D-serine were gifts of Dr. William S. Fones of the Organic Chemistry Unit of the Laboratory of Biochemistry of the National Institutes of Health. L-Phenylalanine was from a bottle of U. S. P. reference standard.

The organisms tested were Lactobacillus arabinosus 17-5 and Lactobacillus brevis, with medium described in a recent paper (14). Concentrations of phenylalanine and of phenylserine were varied as shown in Table I. Because of the limited amounts of phenyl-D- and L-serine available, the total amount of finally diluted culture medium was 1.00 ml. in each tube. After 48 hours of incubation, the cultures were titrated with 0.05 N sodium hydroxide from a 5 ml. burette bearing a micro tip. Each figure in Table I is the average of duplicates.

Results

The effects of the isomers of phenylserine in the presence of various amounts of phenylalanine in the medium for L. arabinosus and L. brevis are presented in Table I. These results confirm, with quantitative figures, all of the trends observed in an earlier set of results which covers part of the same range and in another set covering the same range. For each of the two more complete experiments, the level of growth was different, but the inhibition ratios were closely similar.

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120 INHIBITION BY PHENYLSERINE ISOMERS

With *L. arabinosus*, phenyl-DL-serine exhibits substantial inhibition at all ratios of phenyl-DL-serine to phenyl-L-alanine, whereas allophenyl-DL-serine exhibits virtually no inhibition. At a ratio of 100:1, the phenyl-L-serine and phenyl-n-serine effect high degrees of inhibition. At a ratio of 100:1, 20:1, or 4:1 the D isomer is the considerably stronger antagonist of the two.

With *L. brevis*, phenyl-DL-serine and allophenyl-DL-serine each causes strong inhibition at a 2000:1 ratio to phenyl-L-alanine. At 200:1 the phenyl-DL-serine is somewhat inhibitory, whereas the allophenyl-DL-serine is strongly so. At lower ratios the phenyl-DL-serine is slightly stimulatory. Inhibitory effects of the phenyl-DL-serine at the various ratios are similar to those of the L isomer alone.

These results indicate that phenylserine and allophenylserine may function differently as phenylalanine antagonists. Furthermore, the pattern of response to each varies with the organism. In antimetabolite studies which employ phenylserine, the mode of preparation and identity and purity of product are thus of significance. The results also demonstrate an antipodal dependence in the inhibition by phenylserine. Here too, the D form is more inhibitory for one organism, whereas the L isomer is the stronger inhibitor for another.

Comparison with inhibition of rat growth by phenylserine and by allo-

### TABLE I

*Titration Values of Cultures of L. arabinosus 17-5 and L. brevis in Presence of Various Ratios of Phenylalanine and Phenylserine Isomers*

All values are in ml. of 0.05 N sodium hydroxide solution.

<table>
<thead>
<tr>
<th>Phenylserine isomer</th>
<th>L-Phenylalanine, γ per tube</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td><em>L. arabinosus</em></td>
<td></td>
</tr>
<tr>
<td>400 γ phenyl-DL-serine</td>
<td>0.285</td>
</tr>
<tr>
<td>400 &quot; allophenyl-DL-serine</td>
<td>0.375</td>
</tr>
<tr>
<td>200 &quot; phenyl-L-serine</td>
<td>0.310</td>
</tr>
<tr>
<td>200 &quot; phenyl-n-serine</td>
<td>0.340</td>
</tr>
<tr>
<td>None</td>
<td>0.335</td>
</tr>
<tr>
<td><em>L. brevis</em></td>
<td></td>
</tr>
<tr>
<td>400 γ phenyl-DL-serine</td>
<td>0.325</td>
</tr>
<tr>
<td>400 &quot; allophenyl-DL-serine</td>
<td>0.355</td>
</tr>
<tr>
<td>200 &quot; phenyl-L-serine</td>
<td>0.353</td>
</tr>
<tr>
<td>200 &quot; phenyl-n-serine</td>
<td>0.469</td>
</tr>
<tr>
<td>None</td>
<td>0.335</td>
</tr>
</tbody>
</table>

is strongly so. At lower ratios the phenyl-DL-serine is slightly stimulatory. Inhibitory effects of the phenyl-DL-serine at the various ratios are similar to those of the L isomer alone.

These results indicate that phenylserine and allophenylserine may function differently as phenylalanine antagonists. Furthermore, the pattern of response to each varies with the organism. In antimetabolite studies which employ phenylserine, the mode of preparation and identity and purity of product are thus of significance. The results also demonstrate an antipodal dependence in the inhibition by phenylserine. Here too, the D form is more inhibitory for one organism, whereas the L isomer is the stronger inhibitor for another.

Comparison with inhibition of rat growth by phenylserine and by allo-
phenylserine (15) indicates a greater similarity to the pattern with *L. arabinosus* than to the results with *L. brevis*.

**SUMMARY**

Antagonism between phenylserine and phenylalanine is shown to vary with the phenylserine isomer, normal (threo) or allo (erythro), L-threo or D-threo, and with the microorganism studied.

**BIBLIOGRAPHY**

ANTAGONISM BETWEEN PHENYLALANINE AND VARIOUS ISOMERS OF PHENYLSEERINE IN TWO LACTOBACILLI
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