L-THREO-β-HYDROXYASPARTIC ACID
AS AN ANTIBIOTIC AMINO ACID

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In the course of our screening for new
antibiotics, Arthrinium phaeospermum T-53 and
Streptomyces sp. 7540-MC1 were found to
produce an antibiotic substance. This paper
describes the production, isolation, chemical
and biological properties of the antibiotic.

Organisms and Fermentation
A strain of A. phaeospermum1,2) T-53 was
isolated from a fruiting body of Psathyrella
obtusata (mushroom) and maintained on agar
slants containing glucose 2 %, peptone 0.2 %,
MgSO4 0.05 %, KH2PO4 0.06 %, K2HPO4 0.1 %
and agar 1.5 %. The fungus was cultured on
a reciprocal shaker at 28°C in a vegetative
medium containing the following ingredients;
soybean meal 1.5 %, dry yeast 0.2 soluble
starch 2.5 %, CaCO3 0.4 % and NaCl 0.5 %.
Two ml of 2 day old culture was transferred
into 100 ml of the same medium in a 500-m1
Erlenmeyer flask and was cultivated for 3 days
on a rotary shaker at 28°C.
Bacillus subtilis was used as a test organism
in the medium of peptone 0.5 % and agar
1.2 %, pH 7.0. Streptomyces sp. 7540-MC1
was isolated from a soil sample collected at
Nagano Prefecture, Japan and was cultivated
for 3 days in the same manner as described
above for A. phaeospermum.

Isolation Procedure
The broth filtrate was passed through col-
umns of activated carbon and Amberlite
IRC-50 (H+ type), successively. The effluent
was charged onto an anion-exchange column
(Chromatography 51×8 or IRA 410 (OH− type)) and the
column was washed with water and developed
with 0.04 N HCl. The active eluate was
concentrated in vacuo to a small volume and
ethanol was added to yield crude precipitates.
These were further purified through a column
of Amberlite XAD-2 using water as a solvent
to give active crystals. As an alternate of this
step, the IRC-50 effluent was adsorbed on
Amberlite IR-120 (H+ type) and eluted with
0.3 N NH3·H2O. The crystals thus obtained
were recrystallized from water-ethanol.

Physicochemical Properties
The active component was colorless crystals
of mp. ~210°C (dec.). It was soluble in hot
water and conc. HCl, slightly soluble in water
and almost insoluble in most organic solvents.
It gave a positive color reaction with ninhydrin
reagent but negative with FeCl3, 2,4-dinitro-
phenyl hydrazine and phenol red reagents, and
negative Molisch, Fehling, Biuret and Sa-
Kaguchi reactions. The Rf values on paper-
chromatograms were as follows; BuOH - AcOH-
H2O (2 : 2 : 1) 0.41, BuOH - AcOH - H2O (2:
1 : 1) 0.24 and BuOH - EtOH - H2O (5 : 2 : 3)
0.24.

In the UV region it showed no characteristic
absorption and in the NMR spectrum (100 MC,
DCl) it exhibited two methine protons at δ 4.71
and 5.08 with small coupling constant (~ 2 Hz).
The IR spectrum is shown in Fig. 1. Elemental
analysis suggests C4H7O5N for its empirical
formula.

Calcd. for C4H7O5N: C 32.22, H 4.73,
O 53.66, N 9.40
(MW 154)
Found: C 32.52, H 4.65,
O 53.68, N 9.01
(MW 149.1, Titration)

Methylation
Methylation of this material in boiling 3 n
HCl-MeOH for 3 hours gave several products
on a paper chromatogram (BuOH - AcOH - H2O,
2 : 1 : 1). Preparative precipitate and repeated
crystallizations from hot water gave a mono-
methyl ester as a main product of C5H9O5N.
mp. 143~145°C, IR 1725, 1765 cm−1, NMR
(DCl); 3.90 (3H, s), 4.72 (1H, d) and 5.05
(1H, d) ppm.

Identification with L-threo-β-Hydroxyaspartic
acid
These physicochemical properties suggest

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Fig. 1. IR Spectra of \( \beta \)-hydroxyaspartic acids (KBr)

A: \( \text{l-erythro-} \beta \)-Hydroxy-aspartic acid
B: \( \text{dl-threo-} \beta \)-Hydroxy-aspartic acid
C: \( \text{l-threo-} \beta \)-Hydroxy-aspartic acid
D: The antibiotic substance

Table 1. Antimicrobial spectrum of \( \text{l-threo-} \beta \)-hydroxyaspartic acid

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Concentration (mcg/ml)</th>
<th>Inhibition zone (Diameter, mm)*</th>
<th>Medium**</th>
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<tbody>
<tr>
<td>* Assays were performed with 8 mm filter paper discs.</td>
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<tr>
<td>** A Peptone 0.5%, agar 1.2%, pH 7.0</td>
<td>B Glucose bouillon, pH 7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C Mukow Watanabe pH 7.0</td>
<td>D Glycerin Czapek, pH 7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E Sabouraud, pH 7.0</td>
<td>F Yeast ext. 0.2%, starch 1.2%, agar 1.2%, pH 7.0</td>
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</table>
\(\beta\)-hydroxyaspartic acid for the antibiotic substance. The comparison of this material with \(\text{erythro}\) and \(\text{threo}\)-\(\beta\)-hydroxyaspartic acid* in IR spectra (Fig. 1) revealed its identity with the \(\text{threo}\) isomer.3) On high voltage paper electrophoresis (3,500 V, 20 min.) using a buffer of formic acid - acetic acid - water (25 : 75 : 900, v/v), its mobility (2.9 cm), was the same as that of the \(\text{threo}\) isomer (2.9 cm), but different from that of the \(\text{erythro}\) isomer (4.1 cm).

Optical rotations of this antibiotic and \(\text{L-threo}\)-hydroxyaspartic acid were both \([\alpha]_{D}^{256}=+46^\circ\) (c 0.5, 1 N HCl), thus, the antibiotic was identified as \(\text{L-threo}\)-\(\beta\)-hydroxyaspartic acid. It is worth to note that an analogous amino acid \(\text{L-threo}\)-\(\alpha\)-amino-\(\beta\),\(\gamma\)-dihydroxybutyric acid has been isolated by Westley et al.7) from the fermentation broth of an unidentified \(\text{Sreptomyces}\).

**Biological Properties**

This amino acid has been isolated from several organisms,4,5) but its antimicrobial activity is not described as yet. It shows inhibitory activity against \textit{Bacillus subtilis}, \textit{Xanthomonas oryzae}, \textit{Mycobacterium phlei} and \textit{Botrytis cinerea} as summarized in Table 1. It did not show any toxicity at 250 mg/kg (i.v., mice). So far as tested, the biological activity of this antibiotic was not reversed by the addition of known amino acid such serine, threonine, varine and aspartic acid.

**Summary**

\(\text{L-threo}\)-\(\beta\)-Hydroxyaspartic acid was isolated from cultured broths of \textit{Arthrinium phaeospermum} sp. T-53 and \textit{Streptomyces} sp. 7540-MC1. The amino acid exhibited broad antimicrobial activity.

**Acknowledgement**

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**References**


* Authentic \(\text{l-erythro}\) and \(\text{threo}\)-\(\beta\)-hydroxyaspartic acids were kindly presented by Prof. T. Shiba.