NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.
PROCUREMENT AND ISOLATION OF
BIOLOGICALLY ACTIVE SUBSTANCES
DERIVED FROM NATURAL SOURCES
Contact No. DA-18-108-CML-7031
Final Report
28 February, 1963
Division of Natural Products
LOMA LINDA UNIVERSITY
Loma Linda, California
Loma Linda University
Loma Linda, California
PROCUREMENT AND ISOLATION OF
BIOLOGICALLY ACTIVE SUBSTANCES
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G. J. Nelson
Fourth Report, Final, 15 Feb., 1963
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Reports the isolation of toxin
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CML-5390
Title: PROCUREMENT AND ISOLATION OF BIOLOGICALLY ACTIVE SUBSTANCES DERIVED FROM NATURAL SOURCES

Prepared By

George J. Nelson, Ph.D., Project Director

Date: 28 February 1963

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INTRODUCTION

This report is a summary of work done under Contract Number DA-18-108-CML-7031. This was a continuation of the work started under Contract Number DA-18-108-CML-5390 and continued under Grant Number DA-CML-18-108-61-G-17 for the procurement of necessary starting materials and the isolation of the purified toxin from the liver and viscera of the puffer fish, Sphoeroides rubripes and related species.

PROCEDURE

The methods of operation were those developed in the earlier phases of this work and reported in the Final Report\(^{(1)}\) submitted at the termination of Contract Number DA-18-108-CML-5390. No significant changes in the procedures or equipment were developed at this time.

The puffer viscera for this contract was ordered from Dr. Tokiharu Abe, of the University of Tokyo, Japan, early in November, 1961, with instructions for shipping forty or fifty boxes as soon as practical, and the remainder to be shipped when the whole order could be completed. Because the viscera was given a classification as a Class B Poison according to the interpretation of the U.S. Coast Guard rules by the Army Transport Office in Yokohama, the shipment was delayed until a minimum shipment of 9,000 pounds was collected. The first installment arrived in San Bernardino, California on 23 March, 1962. The final shipment of 7,800 pounds was received on 12 June, 1962. This made a total of 16,800 pounds, gross weight, which contained a net weight of 11,200 pounds of viscera.

\(^{(1)}\) George J. Nelson, Screening of Natural Products and Isolation of Their Active Components, Final Report, 1 June, 1961, Project No. 4-08-03-001, Contract DA-18-108-CML-5390.
SUMMARY FOR THE FOURTH PERIOD

During the fourth period of this contract 2,800 pounds of viscera were ground and extracted. Three hundred and fifty-five gallons of methanol extract were obtained and this was precipitated with acetone and the resulting material was put through the process. One thousand seven hundred and seventy-five gallons of acetone were recovered through the solvent recovery still. Four batches of the purified toxin with a total weight of 2,270.6 milligrams were produced and sent to the Army Chemical Center.

SUMMARY FOR THE CONTRACT PERIOD

<p>| | |</p>
<table>
<thead>
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<tbody>
<tr>
<td>Viscera processed</td>
<td>5,834 pounds</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>710 gallons</td>
</tr>
<tr>
<td>Acetone recovered</td>
<td>3,575 gallons</td>
</tr>
<tr>
<td>Toxin produced:</td>
<td></td>
</tr>
<tr>
<td>LLU-5-62-A</td>
<td>506.5 mg.</td>
</tr>
<tr>
<td>LLU-5-62-B</td>
<td>580.3 mg.</td>
</tr>
<tr>
<td>LLU-6-62</td>
<td>447.8 mg.</td>
</tr>
<tr>
<td>LLU-7-62</td>
<td>219.3 mg.</td>
</tr>
<tr>
<td>LLU-8-62</td>
<td>306.0 mg.</td>
</tr>
<tr>
<td>LLU-9-62</td>
<td>497.8 mg.</td>
</tr>
<tr>
<td>LLU-10-62</td>
<td>502.8 mg.</td>
</tr>
<tr>
<td>LLU-11-62-A</td>
<td>629.5 mg.</td>
</tr>
<tr>
<td>LLU-11-62-B</td>
<td>503.1 mg.</td>
</tr>
<tr>
<td>LLU-12-62-A</td>
<td>601.6 mg.</td>
</tr>
<tr>
<td>LLU-12-62-B</td>
<td>536.4 mg.</td>
</tr>
<tr>
<td>Total</td>
<td>5,331.1 mg.</td>
</tr>
</tbody>
</table>

An infra-red spectrogram was supplied with each batch of material submitted.
ACKNOWLEDGMENT

The Contractor wishes to express appreciation for the invaluable assistance given by the following persons:

To Dr. Tokiharu Abe, Tokyo University, for supervising the collection and preparation of the viscera for shipping.

To Major William B. Strough, Deputy for Chemical Ord/CML, U.S. Army Japan, for expediting the shipment from the Port of Yokohama.

To Captain W. L. Morrison, Chief of Operations, U. S. Coast Guard, Long Beach, California, for assistance in getting proper classification of the material for shipment.
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