ISOLATION, CHARACTERIZATION AND ANTIMICROBIAL ASSAY OF STEROIDS FROM THE CHLOROFORM EXTRACT OF SARCOPHYTON ELEGANS

BRIVER

A Masteral Thesis

Presented to

The Faculty of the Department of Chemistry

College of Science

De La Salle University

In Partial Fulfillment of the Requirement for the Degree Master of Science in Chemistry

> Lorna Milly Avila-Navaja September 1991



ABSTRACT

Three sterols from the chloroform extract of the Philippine soft coral, Sarcophyton elegans were isolated by chromatographic methods.

The structure of one of the sterol (SE3) was partially elucidated based on data from the nuclear magnetic resonance (nmr), infrared (ir) and by comparison with literature data. Comparison with literature data confirmed the presence of a double bond between C-5 and C-6 and the presence of an -OH functional groups attached to C-3 of the sterol. Its molecular formula $C_{28}H_{48}O$ was derived from ¹³C nmr data. Its skeletal system was found to be that of the perhydrocyclopentanophenantrene-type. The structure of the aliphatic side chain was not fully established but was found to contain one quaternary, one methine, three methylene and four methyl carbon atoms.

$$R = C_{9}H_{19} \begin{cases} 1 - C - 1 \text{ CH} \\ 1 \text{ CH} \\ 3 \text{ CH}_{2} \\ 4 \text{ CH}_{3} \end{cases}$$



The skeletal structure of the other two steroids, SE5 and SE9, were also found to be of the perhydrocyclopentanophenantrenetype. Other details of its structure could not be determined due to insufficiency of other pertinent data.

The three isolates showed potential antimicrobial properties. SE5 showed the greatest activity after 24 hours of incubation by inhibiting the growth of six out of seven microorganisms. Isolate SE3 was able to inhibit the growth of all the seven microorganisms after 48 hours.



TABLE OF CONTENTS

| | PAGE |
|--|------|
| ACKNOWLEDGEMENT | i |
| ABSTRACT | iv |
| LIST OF FIGURES | ix |
| | × |
| LIST OF TABLES 1. INTRODUCTION | 1 |
| 1.1 General Introduction | 1 |
| 1.2 Taxonomy of Sarcophyton elegans | 8 |
| 1.3 Steroid Chemistry of Sarcophytons | 11 |
| 1.4 Steroid from Genus Sarcophytons | 18 |
| Sarcophyton sp. | 18 |
| Sarcophyton Tortuosum | 23 |
| Sarcophyton glaucum | 24 |
| Sarcophyton elegans | 32 |
| 1.5 Scope, Limitations and Objectives of the Research | 34 |
| 2. ISOLATION, CHARACTERIZATION AND ANTIMICROBIAL ASSAY | 35 |
| 2.1 Isolation | 35 |
| 2.2 Characterization of Compound SE3 | 37 |
| 2.2.1 Isolation of SE3 | 37 |
| | |



TABLE OF CONTENTS (cont'd.)

| | | | · PAGE |
|-----|-------|--------------------------------|--------|
| | 2.2.2 | Physical Properties | 38 |
| | 2.2.3 | Infrared Spectrum | 38 |
| | 2.2.4 | ¹ H nmr Spectrum | 40 |
| | 2.2.5 | 13C nmr and 13C DEPT Spectra | 43 |
| 23 | | terization of Compound SE5 | 51 |
| | | Isolation of SE5 | 51 |
| | | Physical Properties | 52 |
| | | Infrared Spectrum | 52 |
| | | ¹ H nmr Spectrum | 54 |
| 2.4 | | eterization of Compound SE9 | 56 |
| | | Isolation of SE9 | 58 |
| | | Physical Properties | 57 |
| | | Infrared Spectrum | . 57 |
| | | ¹ H nmr Spectrum | 59 |
| | _ | 13 C nmr and 13 C DEPT Spectra | 59 |
| 2.5 | | icrobial Assay | 65 |
| | | Antimicrobial Assay of SE3 | 65 |
| | | Antimicrobial Assay of SE5 | 67 |
| | | Antimicrobial Assay of SE9 | , 88 |



viii

TABLE OF CONTENTS (cont'd.)

| | | | PAGE |
|----------|-------|--------------------------------|------|
| | 2.6 | Conclusion and Recommendations | .69 |
| | | 2.6.1 Conclusion | 69 |
| | | 2.6.2 Recommendations | 70 |
| 3. | EXPE | RIMENTAL METHODS | 72 |
| . | | General Notes | 72 |
| | | Sampling | 73 |
| | | Extraction | 73 |
| | | Isolation | 74 |
| | | Antimicrobial Assay | 77 |
| ΔPP | ENDIX | | 80 |
| | | | 84 |
| BIB | LIOGR | APHY // // | |



LIST OF FIGURES

| FIGURE | | PAGE |
|--------|--|------|
| 1 | Soft Coral Morphology | 2 |
| 2 | Sarcophyton A Common Soft Coral | 8 |
| 3 | Sarcophyton elegans Moser | 10 |
| 4 | Sarcophyton elegans Sample | 35 |
| 5 | Schematic Diagram for the Isolation of the Steroid Components of Sarcophyton elegans | 36 |
| 6 | IR Spectrum of SE3 | 39 |
| 7 | 300 MHz ¹ H nmr Spectrum of SE3 in Chloroform-d | 41 |
| 8 | ¹ H nmr Spectrum of Gorgosterol(1) | 43 |
| 9 | ¹⁹ C DEPT Spectrum of SE3 in Chloroform-d | 44 |
| 10 | 13C DEPT Spectrum of SE3 in Chloroform-d | 46 |
| 11 | IR Spectrum of SE5 | 53 |
| 12 | 300 MHz ¹ H nmr Spectrum of SE5 in Chloroform-d | 55 |
| 13 | IR Spectrum of SE9 | 58 |
| 14 | 300 MHz ¹ H nmr Spectrum of SE9 in Chloroform-d | 60 |
| 15 | 13C nmr Spectrum of SE9 in Chloroform-d | 61 |
| 16 | ¹³ C DEPT Spectrum of SE9 in Chloroform-d | 63 |
| 17 | Arrangement of Sample Discs in Antimicrobial Assay | 75 |



LIST OF TABLES

| TABLE | | PAGE |
|-------|--|------|
| 1 | Activities Noted with Marine Species | 4 |
| 2 | Biologically Active Metabolites Derived from Soft Corals | 5 |
| 3 | The Main Skeleton of Steroids | 13 |
| 4 | IR Absorptions of SE3 | 40 |
| 5 | Comparison of the 13 C nmr Spectral Data of SE3 with Literature Values | 48 |
| 6 | 13C nmr Signals and their Functionalities | 50 |
| 7 | IR Absorptions of SE5 | 54 |
| 8 | IR Absorptions of SE9 | 57 |
| 9 | Comparison of the 13 C nmr Spectral Data of C-8 with that of C-3 | 64 |
| 10 | Antimicrobial Screening on Compound SE3 | 66 |
| 11 | Antimicrobial Screening on Compound SE5 | 67 |
| 12 | Antimicrobial Screening on Compound SE9 | 68 |
| 13 | Test Organisms and their Incubation Periods | 78 |

