Absolute configuration of biologically active marine natural products
by David Ernest Barnekow

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry
Montana State University

Abstract:
Investigation of the absolute configuration and biological activity of three nakafuran derivatives isolated from the marine sponge Dysidea etheria resulted in the determination of the absolute configuration of the secondary alcohol 5-hydroxynakafuran-8, 40, by means of a modified Korean's method. This modification was the substitution of 2-phenylbutanoyl chloride for 2-phenylbutanoic anhydride.

The stereochemistry of the other two nakafurans, 5-acetoxy-nakafuran-8, 41, and 5-ketonakafuran-8, 42, was assigned by chemical degradation of transformation to the lead compound 40. All three nakafurans were evaluated for their phytotoxicity, cytotoxicity, insecticidal and antimicrobial activity.

Investigation of the cytotoxic organic extract of the marine green alga Neomeris annulata resulted in the isolation of three novel metabolites, 2-bromo-5-hydroxy-cis-selin-6-ene, 114, 1R-bromo-ent-maaliol, 115, and neomeranol, 116, which possesses a previously unknown regular isoprenoid sesquiterpene carbon skeleton. In the brine shrimp toxicity assay compounds 114 and 115 were twice as toxic as 116, although all three were inactive in the KB in vitro assay. Neomeranol, 116, was the only compound to display phytotoxic activity against johnsongrass. All three compounds were also screened for insecticidal and antimicrobial activity. The absolute configuration of compound 115 was determined by chemical degradation and comparison to the natural product maaliol, 118. The absolute configuration of neomeranol, 116, was determined by the same modified Korean's method that was employed in the nakafuran work.

The breadth of application of the modified Korean's method was evaluated with nine secondary alcohols of varying steric hindrance. These alcohols were esterified with both the anhydride and acid halide. The results demonstrated that sterically hindered secondary alcohols gave higher optical yields when resolved with the acid halide and less hindered secondary alcohols gave better optical yields with the anhydride.

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APPROVAL

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David Barnekow

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

28 Apr 89
Date

Chairperson, Graduate Committee

Approved for the Major Department

April 28, 1989
Date

Edwin H. Abbott
Head, Major Department

Approved for the College of Graduate Studies

May 11, 1989
Date

Henry L. Parsons
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Date April 28, 1989

Sue

my best friend
wife
and the mother of our children

an encourager
a motivator
and a provider

Leah Marie, Jeremy Alan and Baby

for the special meaning of the word

Daddy

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ABSTRACT

Investigation of the absolute configuration and biological activity of three nakafuran derivatives isolated from the marine sponge Dysidea etheria resulted in the determination of the absolute configuration of the secondary alcohol 5-hydroxynakafuran-8, **40**, by means of a modified Horeau's method. This modification was the substitution of 2-phenylbutanoyl chloride for 2-phenylbutanoic anhydride. The stereochemistry of the other two nakafurans, 5-acetoxy-nakafuran-8, **41**, and 5-ketonakafuran-8, **42**, was assigned by chemical degradation of transformation to the lead compound **40**. All three nakafurans were evaluated for their phytotoxicity, cytotoxicity, insecticidal and antimicrobial activity.

Investigation of the cytotoxic organic extract of the marine green alga Neomeris annulata resulted in the isolation of three novel metabolites, 2-bromo-5-hydroxy-cis-selin-6-ene, **114**, 1R-bromo-ent-maaliol, **115**, and neomeranol, **116**, which possesses a previously unknown regular isoprenoid sesquiterpene carbon skeleton. In the brine shrimp toxicity assay compounds **114** and **115** were twice as toxic as **116**, although all three were inactive in the KB in vitro assay. Neomeranol, **116**, was the only compound to display phytotoxic activity against johnsongrass. All three compounds were also screened for insecticidal and antimicrobial activity. The absolute configuration of compound **115** was determined by chemical degradation and comparison to the natural product maaliol, **118**. The absolute configuration of neomeranol, **116**, was determined by the same modified Horeau's method that was employed in the nakafuran work.

The breadth of application of the modified Horeau's method was evaluated with nine secondary alcohols of varying steric hindrance. These alcohols were esterified with both the anhydride and acid halide. The results demonstrated that sterically hindered secondary alcohols gave higher optical yields when resolved with the acid halide and less hindered secondary alcohols gave better optical yields with the anhydride.

INTRODUCTION

The concept of stereoisomerism of organic molecules was first recognized in 1815 by a French physicist, Jean Baptiste Biot[1]. While working with the oils of laurel and lemon and a solution of camphor in alcohol, he observed that these solutions would rotate plane polarized light. Thus the term optically active compound was developed.

The French scientist Louis Pasteur[2] was the first to recognize that the optical activity was caused by an asymmetric grouping of atoms in the molecule (chiral molecules). Pasteur was famous for his work with (+)-tartaric acid, but it was not until 1951 that the Dutch scientist J.M. Bijvoet determined the absolute configuration of (+)-tartaric acid *via* a special x-ray technique.[3]

With the rapid evolution of this new field, stereochemistry, in organic chemistry, a general method for designating the absolute structure of a stereoisomer was needed. The *R* - *S* convention for defining the absolute configuration of an asymmetric carbon was developed by two English scientists, R.S. Cahn and C.K. Ingold, and a Swiss scientist, V. Prelog.[4]

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Although enantiomers have the same physical properties, their biological activities are often very different. R and S carvone were shown to have different odors; R-carvone had a spearmint odor while S-carvone was the source of a caraway odor.[2] In an undergraduate organic text[5], the importance of knowing which enantiomer displayed a particular biological activity was emphasized by the following examples. When grown on a diet of racemic tartaric acid, the mold Penicillium glaucum was shown to consume only the (+)-tartaric acid while leaving the (-)-tartaric acid. The hormone (-)-adrenaline was demonstrated to be many times greater in activity than the enantiomer (+)-adrenaline. Only one of the stereoisomers of chloromycetin shows antibiotic activity. In the case of (-)-ephedrine, which was used as a drug, there was actually interference with its activity by the other enantiomer, (+)-ephedrine. Among amino acids only one of the stereoisomers of asparagine and leucine was sweet, and only one of the stereoisomers of glutamic acid enhanced the flavor of foods.

There are many examples of biochemicals, agrochemicals, and pharmaceuticals in which only one of the isomers displays a particular activity. Knowing which enantiomer of a compound displayed the desired activity would be crucial to the commercial development of that

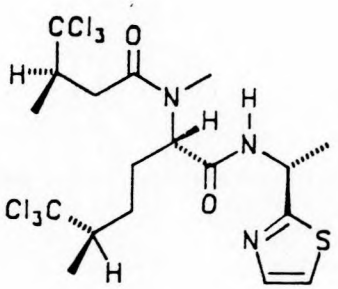
compound as a drug or agrochemical. Therefore, the complete characterization of a new biologically active, chiral natural product would conclude with the determination of the absolute configuration possessed by that compound.

The goals of the research presented herein were to isolate, fully characterize and then evaluate novel marine natural products with various bioassays to determine their biological activities. The complete characterization would involve the determination of the gross structure and relative stereochemistry *via* spectral techniques and the absolute configuration by chemical methods. Screening of biological activities of these marine natural products would utilize the following in house assays: brine shrimp toxicity, cut-leaf assay (phytotoxicity), tobacco hornworm and grasshopper assays (insecticidal) and differential DNA repair. The KB assay for cytotoxicity was performed at the National Cancer Institute.

KNOWN CHEMISTRY OF THE SPONGE GENUS Dysidea

The sponge Dysidea has been one the most chemically studied genera of sponges. The study of this genus has led to the isolation of a wide varity of secondary metabolites representing totally unrelated structural classes. These classes were represented by the following structural types; polyhalogenated nitrogenous metabolites, diphenyl ethers, phenols, and terpenoids.

Hexachloro-amino acid derivatives like isodysidenin 1, were isolated from Dysidea herbacea.[6,7,8,9,10,11] The absolute configuration for isodysidenin was established by x-ray diffraction[8]. D. herbacea also produces the diketopiperazine 2.[12] The isolation of a large number of closely related amino acid metabolites from blue-green algae, as well as the discovery of the presence of blue-



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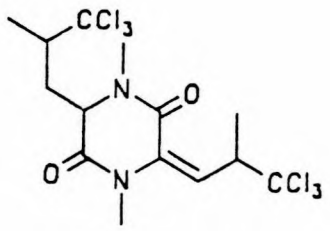

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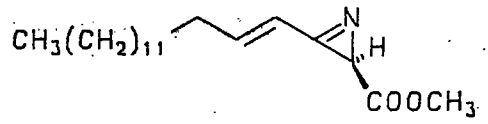
green algae in the sponge tissue, which at times represents half the cellular weight of the collection, has led to the belief that these metabolites were from the blue-green algae.[13]

From D. fragilis, an interesting cytotoxic and antimicrobial active azacyclopropene, dysidazirine, **3**, was isolated.[14] The absolute configuration of **3** was determined after hydrolysis of the ester by circular dichroic spectral analysis the of p-bromobenzamide derivative.



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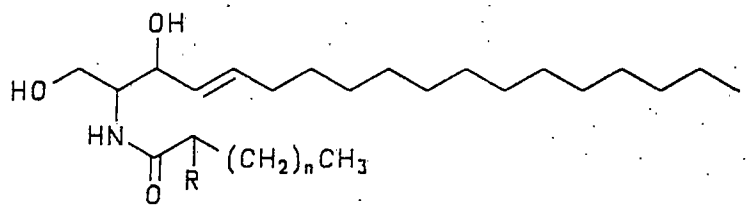
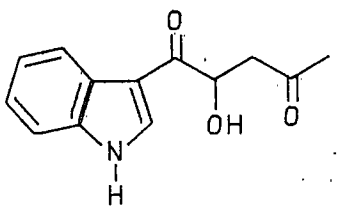
Sphingosine derivatives **4** and **5** were isolated from D. etheria,[15] with predominantly C_{22} or larger fatty acids comprising the amides. The fully saturated acids in **4**



4 R = H, n=12,13,14,15,19,20,21
5 R = OH, n=19,20,21

displayed only weak antimicrobial activity by inhibiting Corynebacterium michiganense.[16] Absolute configuration was determined by comparison of optical rotations for the methyl ester derivatives of the fatty acids to literature values for the corresponding fatty acid derivatives, and comparison of the spectral properties of the sphingosine triacetate to those reported in the literature.

The first plant growth regulator from a sponge, 4-hydroxy-5-(indole-3-yl)-5-oxo-pentan-2-one (**6**), was isolated from D. etheria.[17] This novel indole induced root length extensions of 15% over controls at a maximum effective concentration of 10^{-8} M.[16,18]



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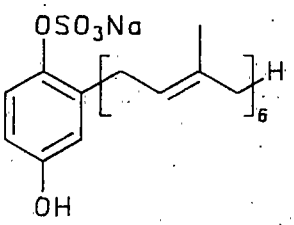
From an unidentified species of Dysidea[19] was isolated the hexaprenylhydroquinone sulfate **7**, was shown to inhibit H,K-ATPase with an IC_{50} of 4.6 x 10-6 M and phospholipase A_2 with an IC_{50} of 1.8 x 10-6 M. Another series of quinones, derived from avarol, **8**, was isolated from D. avara.[20,21,22] These compounds are represented by the sesquiterpenoid amino-quinone **9** and were shown to inhibit

cell cleavage of the fertilized eggs from the sea urchin Sphaerechinus granularis. The absolute configuration of avarol was determined by spectroscopic and chemical methods.



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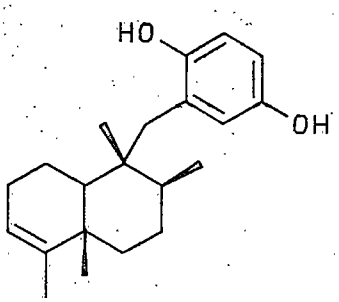

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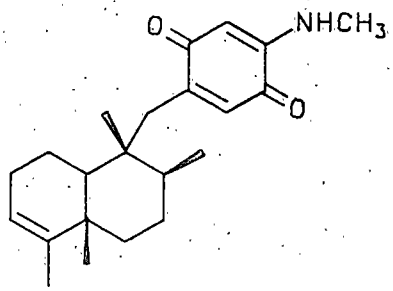

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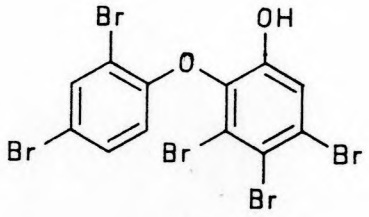
From D. herbacea[23,24,25] ten polybrominated diphenyl ethers have been isolated. These compounds can be placed into two classes- pentabrominated 2-phenoxyphenols such as **10**, and tetrabrominated 2-phenoxyphenols such as **11**; they inhibit the growth of both Gram-negative and Gram-positive microorganisms.

This genus of sponge makes a great number of

biosynthetically unrelated terpenoid compounds. These



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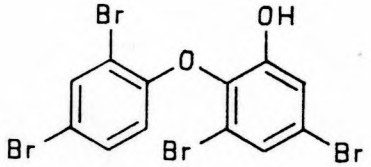

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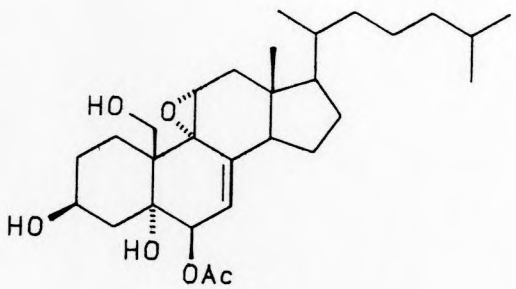
terpenoids include polyhydroxylated sterols[26,27,28,29,30], exemplified by the compound first reported, 9α,11α-epoxycholest-7-ene-3ß,5α,6ß,19-tetrol, **12**.[26] This compound was found to be slightly cytotoxic, ED_{50} in PS[31] of 4.9 µg/mL.[26] Other polyhydroxylated sterols isolated from Dysidea have displayed the following activities; ichthyotoxicity[27], antimicrobial activity[27,29], and brine shrimp toxicity.[29]



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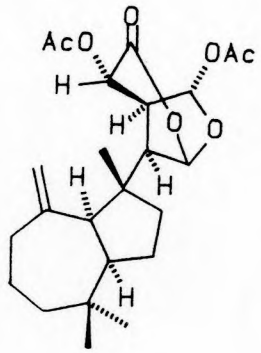

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