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NONTOXIC CONSTITUENTS OF THE MARINE MOLLUSK

Stylocheilus longicauda

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN CHEMISTRY

DECEMBER 1975

By

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ACKNOWLEDGEMENTS

I would like to acknowledge the assistance of Dr. Alfred Asato who insisted that a compound with aromatic protons can be a phenol and also for his assistance in interpretation of several pmr spectra. Mr. James Loo operated the Varian XL-100 Spectrometer providing all the Fourier transformed spectra and also taught me to use and maintain the Varian HA-100. Dr. David Brent was extremely patient in his instruction on the operation of the Varian Mat-311 Mass Spectrometer. Many of the mass spectra were determined by Sister Mary Roger Brennan or Mr. Chris Huckins. Dr. Ronald Takata provided much timely assistance with gas chromatography and general laboratory techniques.

Dr. Jon Clardy's laboratory at Iowa State University determined the structure of the only solid product of this work by x-ray diffraction. Dr. William Fenical at Scripps Institute of Oceanography provided the 220MHz PMR spectrum of stylocheilamide.

Special mention must go to Dr. Paul Scheuer, a man for whom I have great respect and admiration. He is a man who cares.

To the golfers, bridge players, slight-of-hand magician, uke players, picnickers and party-goers who helped maintain my sense of reality and human faith, mahalo.
ABSTRACT

From the marine mollusk *Stylocheilus longicauda* we have isolated two structurally related chlorine containing amides. The structures of stylocheilamide (1), C$_{28}$H$_{44}$ClNO$_6$, and deacetylstylocheilamide (2), C$_{26}$H$_{40}$ClNO$_4$, were determined by a combination of chemical degradations and spectroscopic methods.

Lemieux oxidation of 1 or 2 yielded 3-methoxydecanoic acid (3). Lithium aluminum hydride reduction of 1 followed by acetylation and exhaustive ozonolysis, resulted in 2β,4β-diacetoxy-3β-methylcyclohexanone (4) and methyl 2β,4β-diacetoxy-1β-hydroxy-3β-methylcyclohexanecarboxylate (5). Low temperature selective ozonolysis of 1 led to compound (6), which was isolated as the dimethyl acetal.
The structures of all degradation products were determined by detailed spectral analysis. The structure of 3 was confirmed by synthesis and 4, which was the only crystalline compound encountered in this work, was elucidated by single crystal x-ray diffraction in Dr. J. Clardy's laboratory at Iowa State University.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF CHARTS</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>xi</td>
</tr>
<tr>
<td>I.  Introduction</td>
<td>1</td>
</tr>
<tr>
<td>A.  Background</td>
<td>1</td>
</tr>
<tr>
<td>B.  Sea Hare Secondary Metabolites</td>
<td>7</td>
</tr>
<tr>
<td>C.  Algal Secondary Metabolites</td>
<td>10</td>
</tr>
<tr>
<td>D.  Algal Taxonomy</td>
<td>18</td>
</tr>
<tr>
<td>E.  Statement of Research Objectives</td>
<td>20</td>
</tr>
<tr>
<td>II. Experimental</td>
<td>25</td>
</tr>
<tr>
<td>A.  General Information</td>
<td>25</td>
</tr>
<tr>
<td>1.  Structures</td>
<td>25</td>
</tr>
<tr>
<td>2.  Nomenclature</td>
<td>30</td>
</tr>
<tr>
<td>3.  Chromatography</td>
<td>32</td>
</tr>
<tr>
<td>a.  Preparation of Columns</td>
<td>32</td>
</tr>
<tr>
<td>b.  Preparation of Dry-Columns</td>
<td>33</td>
</tr>
<tr>
<td>c.  Preparation of Thin Layer Chromatography Plates</td>
<td>34</td>
</tr>
<tr>
<td>d.  TLC Visualization Methods</td>
<td>34</td>
</tr>
<tr>
<td>e.  Column Chromatography Monitoring</td>
<td>35</td>
</tr>
<tr>
<td>4.  Molecular Weight Determination</td>
<td>36</td>
</tr>
<tr>
<td>5.  Physical Measurements</td>
<td>37</td>
</tr>
<tr>
<td>B.  Collection of Animals</td>
<td>39</td>
</tr>
<tr>
<td>C.  Storage of Specimens</td>
<td>39</td>
</tr>
</tbody>
</table>
D. Isolation

1. Stylocheilamide and Deacetylstylocheilamide
2. Dioctyl Phthalate
3. Tri(n-butoxyethoxy)phosphate
4. Chimyl Alcohol

E. Characterization of Stylocheilamide

1. Classification Tests
2. Spectral Data of \( I \)

F. Characterization of Deacetylstylocheilamide

1. Classification Tests
2. Spectral Data of \( II \)

G. Catalytic Hydrogenation of Stylocheilamide

1. Quantitative Hydrogenation
2. Platinum Hydrogenation
3. Palladium Hydrogenation, Selective

H. Rearrangement of \( I \)

1. Rearrangement of Stylocheilamide with Sodium Acetate
2. Palladium Hydrogenation of \( IX \)
3. Alumina Chromatography of Stylocheilamide

I. Hydride Reduction of \( I \)

1. Lithium Aluminum Hydride
   a. Reduction of Stylocheilamide
   b. Acetylation of 25
   c. Reduction of \( VII \)
   d. Acetylation of 15
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Sodium Borohydride Reduction of Stylocheilamide</td>
<td>57</td>
</tr>
<tr>
<td>3. Red-Al Reduction of Stylocheilamide</td>
<td>58</td>
</tr>
<tr>
<td>4. Lithium Aluminum Deuteride Reduction of Stylocheilamide</td>
<td>59</td>
</tr>
<tr>
<td>J. Oxidations</td>
<td>60</td>
</tr>
<tr>
<td>1. Basic Lemieux of Stylocheilamide</td>
<td>60</td>
</tr>
<tr>
<td>2. Neutral Lemieux of Stylocheilamide</td>
<td>61</td>
</tr>
<tr>
<td>3. Ozonolysis of XII</td>
<td>63</td>
</tr>
<tr>
<td>4. Ozonolysis of Stylocheilamide</td>
<td>65</td>
</tr>
<tr>
<td>K. Catalytic Hydrogenation of Deacetylstylocheilamide (II)</td>
<td>67</td>
</tr>
<tr>
<td>1. Palladium Hydrogenation</td>
<td>67</td>
</tr>
<tr>
<td>L. Ozonolysis of Deacetylstylocheilamide (II)</td>
<td>68</td>
</tr>
<tr>
<td>1. Oxidative Workup</td>
<td>68</td>
</tr>
<tr>
<td>2. Reductive Workup</td>
<td>69</td>
</tr>
<tr>
<td>M. Hydride Reduction of Deacetylstylocheilamide (II)</td>
<td>70</td>
</tr>
<tr>
<td>1. Sodium Borohydride Reduction</td>
<td>70</td>
</tr>
<tr>
<td>a. Esterification of 51</td>
<td>71</td>
</tr>
<tr>
<td>b. Esterification of 49</td>
<td>73</td>
</tr>
<tr>
<td>N. Synthesis</td>
<td>74</td>
</tr>
<tr>
<td>1. Preparation of 5-Decyne (XXVI)</td>
<td>74</td>
</tr>
<tr>
<td>2. Preparation of trans-5-Chlorodec-5-ene (XXVII)</td>
<td>75</td>
</tr>
<tr>
<td>3. Preparation of 3-Hydroxydecanoic Acid (XXVIII)</td>
<td>75</td>
</tr>
<tr>
<td>4. Preparation of Methyl 3-Hydroxydeconate (XXIX)</td>
<td>76</td>
</tr>
</tbody>
</table>
5. Preparation of Methyl 3-Methoxydeconate (XXX) ........................................ 77

6. Preparation of N-Allylhexamide (XXXI) ........................................ 78

O. Proton Magnetic Resonance Spectra ........................................ 80

P. Infrared Spectra ................................................................. 109

Q. Mass Spectra ................................................................. 128

III. Discussion ................................................................. 142

A. Choice of Sea Hare Species .............................................. 142

B. Homogeneity of Stylocheilamide ........................................ 143

C. Characterization of Functional Groups in Stylocheilamide ......... 145

D. PMR and CMR Spectra of Stylocheilamide ............................... 153

E. Degradations of Stylocheilamide ......................................... 159
   1. Hydrogenation ......................................................... 159
   2. Lithium Aluminum Hydride ........................................ 161
   3. Lithium Aluminum Deuteride ......................................................... 166
   4. Lemieux Oxidation ............................................... 167
   5. Ozonolysis of LAH Product (XII) ........................................... 171
   6. Ozonolysis of Stylocheilamide ........................................ 180
   7. Rearrangement of Stylocheilamide ........................................ 188

F. Stereochemistry of Stylocheilamide ................................... 194

G. Deacetylstylocheilamide ................................................. 198
   1. Characterization of α,β-Unsaturated Ketone ........................ 198
   2. Abnormal Hydrogenation: Aromatization .............................. 201
   3. Phenolic Substitution ......................................................... 202
   4. Mechanism of Phenol Formation ........................................ 206

H. Structure of Deacetylstylocheilamide ................................ 207

IV. Conclusion ................................................................. 211
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The Distribution of Photosynthetic Pigments in Algae</td>
<td>19</td>
</tr>
<tr>
<td>2. Distribution of Sterols in Algae</td>
<td>21</td>
</tr>
<tr>
<td>3. Flow Chart for Isolation of Stylocheilamide</td>
<td>42</td>
</tr>
<tr>
<td>4. Correlation of PMR and CMR Resonances of Stylocheilamide by Single Frequency On-Resonance Decoupling</td>
<td>152</td>
</tr>
<tr>
<td>5. Effect of Ketone Infrared Frequency by α-Substitution</td>
<td>174</td>
</tr>
<tr>
<td>6. The Effect of Substituents on the Chemical Shift of Benzene</td>
<td>206</td>
</tr>
</tbody>
</table>

LIST OF CHARTS

<table>
<thead>
<tr>
<th>Chart</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mass Spectral Fragmentation Pattern of VII</td>
<td>160</td>
</tr>
<tr>
<td>2. Mass Spectral Fragmentation Pattern of XII and XIII</td>
<td>163</td>
</tr>
<tr>
<td>3. Mass Spectral Fragmentation Pattern of XVII</td>
<td>168</td>
</tr>
<tr>
<td>4. Mass Spectral Fragmentation Pattern of XVIII</td>
<td>178</td>
</tr>
<tr>
<td>5. Mass Spectral Fragmentation Pattern of XXI</td>
<td>185</td>
</tr>
</tbody>
</table>
LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>The Digestive and Nervous System of Applysiiid</td>
<td>2</td>
</tr>
<tr>
<td>2.</td>
<td>Numbering System for Stylocheilamide</td>
<td>31</td>
</tr>
<tr>
<td>3.</td>
<td>220 MHz PMR (CDCl₃) Spectrum of Stylocheilamide (I)</td>
<td>81</td>
</tr>
<tr>
<td>4.</td>
<td>100 MHz PMR (CDCl₃) Spectrum of Stylocheilamide (I)</td>
<td>82</td>
</tr>
<tr>
<td>5.</td>
<td>100 MHz PMR (acetone-d₆) Spectrum of Stylocheilamide (I)</td>
<td>83</td>
</tr>
<tr>
<td>6.</td>
<td>100 MHz PMR (acetone-d₆-benzene-d₆) Spectrum of Stylocheilamide (I)</td>
<td>84</td>
</tr>
<tr>
<td>7.</td>
<td>100 MHz PMR (CDCl₃) Spectrum of Deacetylstylocheilamide (II)</td>
<td>85</td>
</tr>
<tr>
<td>8.</td>
<td>100 MHz PMR (acetone-d₆) Spectrum of Deacetylstylocheilamide (II)</td>
<td>86</td>
</tr>
<tr>
<td>9.</td>
<td>100 MHz PMR (benzene-d₆) Spectrum of Deacetylstylocheilamide (II)</td>
<td>87</td>
</tr>
<tr>
<td>10.</td>
<td>100 MHz PMR (CDCl₃) Spectrum of Dioctylphthalate (III)</td>
<td>88</td>
</tr>
<tr>
<td>11.</td>
<td>100 MHz PMR (CDCl₃) Spectrum of Tri(n-butoxyethoxy)phosphate (IV)</td>
<td>89</td>
</tr>
<tr>
<td>12.</td>
<td>100 MHz PMR (CDCl₃) Spectrum of Chimyl Alcohol (V)</td>
<td>90</td>
</tr>
<tr>
<td>13.</td>
<td>100 MHz PMR (CDCl₃) Spectrum of VI</td>
<td>91</td>
</tr>
<tr>
<td>14.</td>
<td>100 MHz PMR (CDCl₃) Spectrum of VII</td>
<td>92</td>
</tr>
<tr>
<td>15.</td>
<td>100 MHz PMR (CDCl₃) Spectrum of IX</td>
<td>93</td>
</tr>
<tr>
<td>16.</td>
<td>100 MHz PMR (CDCl₃-benzene-d₆, 2:1) Spectrum of IX</td>
<td>94</td>
</tr>
<tr>
<td>17.</td>
<td>100 MHz PMR (CDCl₃) Spectrum of XI</td>
<td>95</td>
</tr>
<tr>
<td>18.</td>
<td>100 MHz PMR (CDCl₃) Spectrum of XII</td>
<td>96</td>
</tr>
<tr>
<td>19.</td>
<td>100 MHz PMR (CDCl₃) Spectrum of XVI</td>
<td>97</td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>20. 100 MHz PMR (CDCl₃) Spectrum of XVII</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>21. 100 MHz PMR (CDCl₃) Spectrum of XVIII</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>22. 100 MHz PMR (CDCl₃) Spectrum of XIX</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>23. 100 MHz PMR (CDCl₃) Spectrum of XXI</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>24. 100 MHz PMR (CDCl₃) Spectrum of XXII</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>25. 100 MHz PMR (CDCl₃) Spectrum of XXIII</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>26. 100 MHz PMR (CDCl₃) Spectrum of 5-Decyne (XXVI)</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>27. 100 MHz PMR (CDCl₃) Spectrum of trans-5-Chlorodec-5-ene (XXVII)</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>28. 100 MHz PMR (CDCl₃) Spectrum of Methyl 3-Hydroxydeconate (XXIX)</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>29. 100 MHz PMR (CDCl₃) Spectrum of N- Allylhexamide (XXXI)</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>30. 100 MHz PMR (CDCl₃) Spectrum of 37</td>
<td>108</td>
<td></td>
</tr>
<tr>
<td>31. Infrared (film) Spectrum of Stylocheilamide (I)</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>32. Infrared (film) Spectrum of Deacetylstylocheilamide (II)</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>33. Infrared (1 mm cell, CCl₄) Spectrum of Dioctylphthalate (III)</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>34. Infrared (1 mm cell, CCl₄) Spectrum of Chimyl Alcohol (V)</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>35. Infrared (1 mm cell, CCl₄) Spectrum of VI</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>36. Infrared (1 mm cell, CCl₄) Spectrum of VII</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>37. Infrared (1 mm cell, CCl₄) Spectrum of IX</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td>38. Infrared (1 mm cell, CCl₄) Spectrum of XI</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>39. Infrared (1 mm cell, CCl₄) Spectrum of XII</td>
<td>118</td>
<td></td>
</tr>
<tr>
<td>40. Infrared (1 mm cell, CCl₄) Spectrum of XVI</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>41. Infrared (1 mm cell, CCl₄) Spectrum of XVII</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>42. Infrared (1 mm cell, CCl₄) Spectrum of XVIII</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>Figure</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>43. Infrared (1 mm cell, CC14) Spectrum of XIX</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>44. Infrared (0.1 mm cell, CC14) Spectrum of XXI</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>45. Infrared (0.1 mm cell, CC14) Spectrum of XXII</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>46. Infrared (0.1 mm cell, CC14) Spectrum of XXIII</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>47. Infrared (film) Spectrum of N-Allylhexamide (XXXI)</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>48. Infrared (1 mm cell, CC14) Spectrum of 25</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>49. Mass Spectrum (20 ev) of Stylocheilamide (I)</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>50. Mass Spectrum (20 ev) of Deacetylstylocheilamide (II)</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>51. Mass Spectrum (70 ev) of VI</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>52. Mass Spectrum (70 ev) of VII</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>53. Mass Spectrum (70 ev) of X</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td>54. Mass Spectrum (20 ev) of XII</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td>55. Mass Spectrum (20 ev) of XVI</td>
<td>135</td>
<td></td>
</tr>
<tr>
<td>56. Mass Spectrum (20 ev) of XVII</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>57. Mass Spectrum (70 ev) of XVIII</td>
<td>137</td>
<td></td>
</tr>
<tr>
<td>58. Mass Spectrum (20 and 70 ev) of XIX</td>
<td>138</td>
<td></td>
</tr>
<tr>
<td>59. Mass Spectrum (70 ev) of XXI</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>60. Mass Spectrum (70 ev) of XXII</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td>61. Mass Spectrum (70 ev) of XXIII</td>
<td>141</td>
<td></td>
</tr>
<tr>
<td>62. A Computer Generated Perspective Drawing of 2β,4β-Diacetoxy-3β-methylicyclohexanone (XIX)</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>63. Steroview of Steric Hinderance to Hydride Attack</td>
<td>197</td>
<td></td>
</tr>
<tr>
<td>64. LAOCOON-2 Best Fit Stick Plot of the Aromatic Region of the PMR Spectrum of XXIII</td>
<td>204</td>
<td></td>
</tr>
</tbody>
</table>
I. INTRODUCTION

A. Background

The phylum Mollusca contributes a number of conspicuous species to the Hawaiian marine fauna. Members of this phylum differ greatly in appearance and include the snails, clams, squids, octopuses, sea slugs and sea hares. Over 80,000 living and some 35,000 fossil species have been described. The best known feature of mollusks is a shell and it is due in large part to shell collecting that so many species are known. However a spectacular shell is not characteristic of all mollusks; in sea hares, it is small and covered by the mantle and it is absent in nudibranchs.

Sea hares are marine members of the gastropod molluscan subclass Opisthobranchia in the family Aplysiidae. More than twenty species of aplysiids have been described from the Hawaiian Islands\textsuperscript{1,2}. Their trivial name derives from their resemblance to rabbits when they are seen grazing on a stand of seaweed. The animals may be observed in varying numbers on reef flats or in detrital limestone tidepools. The coloring is variable and frequently blends with the surroundings so that they are difficult to see in wind-rippled tidepools.

The digestive and nervous systems of an aplysiid are shown in Figure 1. Thompson and Bebbington\textsuperscript{3} describe the reproductive system of aplysiids.
Figure 1. Digestive and Nervous System of Aplysiid.
Sea hares feed on seaweeds and the crop is lined with chitinous plates. The salivary glands secrete amylase into the crop and the digestive gland secretes protease and lipase into the stomach. *Aplysia kurodai* feeds on brown (Phaeophyta) and green (Chlorophyta) algae but more often on the brown *Udaria pinnatifida*. The animal attacks the soft leafy parts and when these are exhausted, consumes the stipe and will devour 10-15% of its body weight of algae per day. This voracious appetite diminishes when the water temperature is outside a range of 15-24°C. *Aplysia willooxi* feeds on macroscopic red algae with less than 5% of the crop content being brown or green algae. *Stylocheilus longicauda* has been observed feeding on *Acanthophera spicifera* (red) and *Lyngbya majuscula* (blue-green) and is reported to feed on *Spyridia filamentosa* (red) and *Padina* sp (brown).

Susswein and Kupfermann report that the bulk properties of food are important in producing satiation in *Aplysia californica*. When the animal is prefed a partial meal of nonnutritional bulk, it eats significantly less food as compared to control animals. The total mass of material consumed by the animals that were fed bulk, and then fed to satiation with seaweed, did not differ significantly from the mass consumed by animals fed to satiation on seaweed alone. That study also indicates that although sea hares have an algal preference, they will not restrict their diet to a single algal species.

There are few reports in the literature of predation on sea hares, but Bertsch and Smith report that during the
month of July *Navanax inermis* (a flatworm) feeds on *S. longicauda*, which is the most common opisthobranch found in Las Cruces Bay, Mexico. The sea hare frequently escapes by violently jerking its body, turning somersaults or rapidly crawling away. *Aplysia dactylomela* has been reported to avoid contact with the sea cucumber *Actinopygia agassizi* which contains the toxin holothurin and with the jellyfish *Cassiopea xamachanc* which contains toxic nematocysts. The remains of partially digested *Aplysia californica* have been found in the enteric cavity of the great green sea anemone *Anthopleura xanthogrammica* by Windler. Winkler tried to feed the digestive gland of the sea hare, known to contain aplysia toxin, to the anemone. The digestive gland was ingested but was followed by slow regurgitation in 5-10 min. The regurgitated material was covered with an envelope of thick mucus. When the anemone was fed on frog muscle, no regurgitation occurred.

These few reported incidences of predation might make one wonder what role, if any, sea hares have in the marine food chain. MacGinite provides a partial answer. He reports that a sea hare, *Aplysia californica* (synonym *Tethys californicus*), weighing 2.6 kg, laid 478,190,000 ± 5% eggs between October 29, 1933 and March 6, 1934. During 27 egg-laying periods, the animal laid a total of 60,565 cm of egg strings averaging 39 capsules per cm, 188 eggs per capsule. The sea hare laid its string of eggs at an average rate of 5.9 cm or 230 capsules or 41,000 eggs per minute. The egg masses are eaten by sea stars (Astroidea). Practically all
of the egg masses become veliger larvae which escape after about twelve days. The larvae are used as food by plankton feeders but the greatest mortality probably occurs at and just after the time the larvae settle to the ocean floor. The sea hare is herbivorous with its diet restricted to seaweed so that only those larvae which settle near the shore can be expected to survive.

Toevs and Brackenbury\textsuperscript{12} have identified the proteins which initiate egg laying in \textit{A. californica}.

A great deal of biological research has been carried out with sea hares. Invertebrate cell preparations have had a prominent role in the growth of cellular neurophysiology. The functional organization of invertebrate ganglia was reviewed by Kandel and Kupfermann\textsuperscript{13}. The nervous system of \textit{Aplysia californica} has been studied extensively and a neuron designation system devised\textsuperscript{13,14}. Some of the neurons in \textit{A. californica} are often 1,000\,	extmu m in diameter. These large neurons have made it possible to study the biochemical mechanisms for the characteristic physiological function of these nerve cells. Several groups are studying peptide synthesis by identified \textit{Aplysia} neurons\textsuperscript{15,16,17}. The large abdominal ganglion (R2) of \textit{A. californica} allowed Triestman and Schwartz\textsuperscript{18} to inject radioactive material directly into a living axon to study metabolism and transport within a nerve cell. The neurons of \textit{A. californica} have also been used to study cerebral nerve synapse transmission\textsuperscript{19}, the response of an individual neuron to drug application\textsuperscript{20} and
synthesis, storage and metabolism of the transmitter compound liberated at a cerebral nerve terminal\textsuperscript{21}. The structure of the eye of \textit{Aplysia} has been extensively studied\textsuperscript{22}.

Lederhendler, Bell and Tobach\textsuperscript{9} have done a behavioral study of \textit{Aplysia dactylomela} in North Bimini, Bahama Islands.

Several groups working on marine natural products have demonstrated the ability of sea hares to concentrate algal metabolites. Yamamura and Hirata\textsuperscript{23} isolated aplysin, debromoaplysin and aplysinsol from the digestive gland of \textit{Aplysia kurodai} collected at Hokkaido, Japan. Several years later Irie\textsuperscript{24} isolated these three compounds from a red alga, \textit{Laurencia glandulifera}. Faulkner\textsuperscript{25} also demonstrated the presence of these compounds in the midgut (digestive) gland of \textit{Aplysia californica} which feeds upon \textit{Laurencia pacifica} (red), \textit{Plocamium cocineum} (red), and \textit{Gelidium apecies} (red).

Further examination of the digestive gland of \textit{A. californica} by Faulkner\textsuperscript{25} led to the isolation of pacifidiene, pacifenol and johnstonol. Pacifenol and pacifidiene were isolated from a red alga \textit{Laurencia pacifica} by Sims\textsuperscript{26} and johnstonol was also isolated by Sims\textsuperscript{27} from \textit{L. johnstonii}.

Because of the demonstrated correlation between the organic compounds isolated from sea hares and the algal metabolites which they ingest, a summary of known halogenated sea hare constituents and of selected algal secondary metabolites will be presented. An extensive review of marine
natural products is given by Scheuer\textsuperscript{28} and Premuzic\textsuperscript{29}.

Marine toxins have been reviewed by Scheuer\textsuperscript{30}, Halstead\textsuperscript{31} and Russell\textsuperscript{32}. Sterols isolated from mollusks have been reviewed by Idler and Wiseman\textsuperscript{33}. Chang\textsuperscript{34} gives a short review of marine pigments.

B. Sea Hare Secondary Metabolites

1. Aplysioviolin\textsuperscript{35,36}, mp 315°
   Isolated from Aplysia limacina and A. californica.
3. Aplysinol$^{24}$, mp 158-160°
Isolated from A. kurodai, A. californica and the alga Laurencia glandulifera.

4. Aplysin-20$^{36,37}$, mp 146-147°
Isolated from A. kurodai.

5. Dactylyne$^{38}$, mp 62.2-63.0°
Isolated from A. dactylomela.
6. Monoterpane$^{26}$, mp 54°
Isolated from *A. californica* and the red alga *Plocamium pacificum*.

7. Monoterpine alcohol$^{25}$, oil
Isolated from *A. californica* and the red alga *P. pacificum*.

8. Aplysiatoxin$^{39}$, oil
Isolated from *Stylocheilus longicauda*.
C. Algal Secondary Metabolites

1. Caulerpicin\textsuperscript{40}, mp 95°
   Isolated from \textit{Caulerpa lamourouzi} (green).

\[
\text{CH}_2\text{OH} \quad \text{O} \\
\text{CH}_3-(\text{CH}_2)_{13}-\text{CH}-\text{NH}-\text{C}-(\text{CH}_2)_n-\text{CH}_3
\]

\(n = 23, 24, 25\)

2. Caulerpin\textsuperscript{41}, mp 317°
   Isolated from \textit{C. lamourouzi} (green).

3. Laurene\textsuperscript{42}, oil
   Isolated from \textit{Laurencia glandulifera} (red).

4. Laurinterol\textsuperscript{43, 44}, mp 54.0-55.0°
   Isolated from \textit{Laurencia glandulifera} (red).
5. Laurensisol\textsuperscript{45}, oil
Isolated from \textit{L. nipponica} (red).

\[
\begin{array}{c}
\text{HO} \\
\text{CHBr}
\end{array}
\]

6. Spirolaurenone\textsuperscript{46}, oil
Isolated from \textit{L. glandulifera} (red).

\[
\begin{array}{c}
\text{Br} \\
\text{O}
\end{array}
\]

7. Laurefucin\textsuperscript{47}, mp 107-108\textdegree
Isolated from \textit{L. nipponica} (red).

\[
\begin{array}{c}
\text{Br} \\
\text{OH}
\end{array}
\]
8. Chondriol\textsuperscript{48,49}, oil
Isolated from Chondria oppositoclada (red).

\[
\begin{array}{c}
\text{HO} \\
\text{Br} \\
\text{Cl}
\end{array}
\]

9. Laurencin\textsuperscript{50,51}, mp 73-74\degree
Isolated from L. glandulifera (red).

\[
\begin{array}{c}
\text{Br} \\
\text{H} \\
\text{Ac}
\end{array}
\]

10. Laureatin\textsuperscript{52}, mp 82-83\degree
Isolated from L. nipponica (red).
11. Elatol$^{53}$, oil
Isolated from *L. elata* (red).

![Elatol structure](image)

12. Dictyopterene A$^{54}$, oil
Isolated from *Dictyopteris plagiogramma* and *D. australis* (brown).

![Dictyopterene A structure](image)

13. Dictyopterene B$^{55}$, oil
Isolated from *D. plagiogramma* (brown).

![Dictyopterene B structure](image)
14. S-(3-oxoundecyl)-thioacetate\textsuperscript{56}, oil
Isolated from \textit{D. plagiogramma} (brown).

![Structure of S-(3-oxoundecyl)-thioacetate]

15. 3-hexyl-4,5-dithiacycloheptanone\textsuperscript{56}, oil
Isolated from \textit{D. plagiogramma} (brown)

![Structure of 3-hexyl-4,5-dithiacycloheptanone]

16. Violacene\textsuperscript{57}, mp 71.0-71.5°
Isolated from \textit{Placodium violaceum} (red).

![Structure of Violacene]
17. Pacifenol$^{26}$, mp 149.0-150.0°
Isolated from *L. tasmanica*.

18. Prepacifenol$^{58}$, mp 109-126° (rearrangement)
remelts 147°
Isolated from *L. pacifica* and *L. filiformis*.

19. Johnstonol$^{27}$, mp 178°
Isolated from *L. johnstonii*, *L. pacifica* and *L. okamurai* (red).
20. Zonarol\textsuperscript{59}, gum  
Isolated from \textit{D. zanaroides} (brown).

![Chemical structure of Zonarol]

21. Pachydictyol A\textsuperscript{60}, oil  
Isolated from \textit{Pachydictyon coriaceum} (brown).

![Chemical structure of Pachydictyol A]

22. Oppositol\textsuperscript{61}, mp 54-55\textdegree  
Isolated from \textit{L. subopposita} (red).

![Chemical structure of Oppositol]
23. **Rhodophytin**\(^6\)\(_2\), oil
   Isolated from *Chondria oppositicladada* (red).

24. **Chondrochol**\(^6\)\(_3\), oil
   Isolated from *Chondrococcus hornemanni* (red).

25. **Snyderol**\(^6\)\(_4\)
   Isolated from *L. snyderol* (red).
26. Nidifidienol\textsuperscript{65} Isolated from \textit{L. nidifica} (red).

D. Algal Taxonomy

Algae can be broadly classified into 10 divisions:\textsuperscript{66} Cyanophyta (blue-green), Chlorophyta (green), Xanthophyta (green), Chrysophyta, Bacillariophyta (diatoms), Pyrrophyta, Cryptophyta, Euglenophyta, Phaeophyta (brown) and Rhodophyta (red). These primary classifications are based on five main criteria: 1. Photosynthetic pigments, 2. The nature of the food reserves, 3. The nature of cell wall components, 4. The types of flagella, and 5. Certain details of cell structure.

The color of an alga is due to the presence of various photosynthetic pigments and can often be used as a preliminary classification (Table 1). However, the concentration of a pigment too frequently varies with depth, age and reproductive stage of the alga and therefore cannot be the sole taxonomic feature.

Only the Chlorophyta accumulate \(\alpha\)-1,4-polysaccharides as primary carbon dioxide fixation products. However, polysaccharides with \(\beta\)-1,3 linkages are found in the Phaeophyta,
**TABLE I**
The distribution of photosynthetic pigments in algae*

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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* The data in the above table are taken from ref 66.

* a α-carotene is present in the Cystophyceae.

* b γ-carotene, lycopene, and zeaxanthin are present in the Charophyceae.
the Euglenophyta, the Chrystophyta and the Bacillariophyta.

Attempts have been made to classify algae based on their sterol content. The Cyanophyta appear to be lacking sterols. Some of the sterols found in algae are listed in Table 2. However, taxonomic identification by steroid content has proven difficult because the same species collected at different locations and seasons contains fluctuating steroid concentrations and composition.

Shilo\textsuperscript{67} presents a short review of phycotoxins and their action.

E. Statement of Research Objectives

The sea hare \textit{Stylocheilus longicauda} (Quoy and Gaimard, 1824)\textsuperscript{68} is abundant from late May through mid-June in Kaneohe Bay, Oahu, Hawaii. Engle\textsuperscript{69} reports that \textit{S. longicauda} is circumtropical. An adult specimen of \textit{S. longicauda} reaches a maximum length of about 100 mm, on the average about 60 mm. The body and parapodia are brownish-green with longitudinal grey-brown striations. A distinguishing feature of this species is whitish-ringed ocelli with blue ring centers\textsuperscript{2}. The head and tentacles retract into the body; the tail is long and pointed.

This species of sea hare is known to be toxic\textsuperscript{70,71} and the structure of the toxic substance, aplysia toxin, was determined in this laboratory by Kato and Scheuer\textsuperscript{39}.

The initial intent of the present research project was to examine the diethyl ether extractable oil from \textit{S.}
Table 2

Distribution of sterols in algae*

<table>
<thead>
<tr>
<th></th>
<th>Cyanophyta</th>
<th>Chlorophyta</th>
<th>Xanthophyta</th>
<th>Chrysophyta</th>
<th>Bacillariophyta</th>
<th>Pyrrophyta</th>
<th>Cryptophyta</th>
<th>Euglenophyta</th>
<th>Phaeophyta</th>
<th>Rhodophyta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitosterol</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>?c</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fucosterol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sargasterol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>?</td>
<td>-</td>
<td>+</td>
<td>d</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td>?</td>
<td>-</td>
<td>-</td>
<td>+e</td>
</tr>
<tr>
<td>Ergosterol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chondrillasterol</td>
<td>-</td>
<td>_a</td>
<td>-</td>
<td>_b</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* The data in the above table are taken from ref. 66

a Fucosterol has been identified in a species of *Cladophora*
b Chondrillasterol has been found in a species of *Scenedesmus*
c Pyrrophyta and Cryptophyta do not appear to have been examined
d Sargasterol is much less common than fucosterol
e Cholesterol is sometimes replaced by dehydrocholesterol
longicauda for biochemical precursors to aplysiatoxin. One prominent feature of the PMR spectrum of aplysiatoxin is a benzylic methoxy singlet at 3.2 δ. The diethyl ether soluble oil, 2, was chromatographed on a Bio-Sil A column and each 100 ml fraction examined by PMR spectroscopy for methoxy groups, TLC with Dragendorff's spray reagent and Beilstein test for halogens. 

In the course of this screening process, stylocheilamide was detected and the direction of the research project changed. Stylocheilamide bears no structural relationship to aplysiatoxin. The aim of the research project then became the structural elucidation of Sl-54 (Sl for Stylocheilus longicauda, 54 for the notebook page number where the compound was first isolated, albeit impure), later to be called stylocheilamide. Over a 5 yr period, approximately 32 kg of animal was collected yielding nearly 2.5 g of stylocheilamide.

Because of the small size of the animal, no attempt was made to excise parts of the animal which might contain high concentrations of stylocheilamide. It is unknown whether the digestive gland (midgut) contains high stylocheilamide content which would indicate a dietary origin of the compound. At present it is believed that the compound is at least in part synthesized by the sea hare. The predominant alga on the reef where the animal used in this study was collected is Acanthophera spicifera, a red alga. The sea hares were observed devouring this alga and one could observe
the disappearance of the alga as the sea hares moved across the reef flat.

In 1952 the first small fragment of the red alga *Acanthophora spicifera* (Vahl) Boergesen (1910) was found in the Hawaiian Islands at Pearl Harbor. By the middle of 1953 the alga was found abundantly on the leeward side of Oahu and in June 1956 it was found on the windward side of Oahu. At present the alga is ubiquitous on Oahu and can be found on all the Hawaiian Islands.\(^7\)

The means of introduction of this species remains in doubt but Doty\(^7\) proposes that this upstream migration is man-made. A heavily fouled fuel oil barge, the "Yon 146," was towed to Pearl Harbor from Guam in February, 1950, and placed in drydock. This drydock is only 8 km in a straight line from the first collection of *A. spicifera*. This same barge is also reported to have transported several invertebrates which have become established.

There seems a just end to the "Yon 146," for Doty\(^7\) reports, "though we do not know what other biological consternation may be associated with this vessel, it may cause little more for Hawaiian biologists, for it is reported to have been sunk in Subic Bay on October 16, 1955."

*Acanthophora spicifera*, 2.3 kg wet weight, was steeped in acetone, filtered and the filtrate concentrated to an oil. The oil was subjected to the liquid-liquid portion of the extraction scheme used to isolate stylocheilamide from the sea hare (Table 2). The carbon tetrachloride layer gave
a weakly positive Beilstein test for halogens. Tlc analysis of this oil failed to show the presence of stylocheilamide or deacetylstylocheilamide.

Hence tlc analysis excludes stylocheilamide arising from the sea hare ingestion of *A. spicifera* but it is not known what the diet of the sea hare is before it comes onto the reef where it spawns. The contents of the midgut gland were not analyzed to determine the algae present.

*Lyngbya majuscula*, a blue-green alga, is also present where the sea hares are collected but is much less common than *A. spicifera*. While *L. majuscula* is known to contain a compound containing both nitrogen and chlorine, the sea hares were rarely observed on this alga.
II. Experimental Section

A. General Information

1. Structures

\[ \text{I} \]

\[ \text{II} \]

\[ \text{III} \]

\[ \text{IV} \]
\[
\text{CH}_2 \text{O} (\text{CH}_2)_{15} \text{CH}_3 \\
\text{CHOH} \\
\text{CH}_2 \text{OH}
\]

\[v\]

\[\text{VI}\]

\[\text{VII}\]

\[\text{VIII}\]
\[ \text{XXVII} \]

\[ \text{XXVIII} \quad R = R' = H \]

\[ \text{XXIX} \quad R = H; \quad R' = \text{CH}_3 \]

\[ \text{XXX} \quad R = R' = \text{CH}_3 \]

\[ \text{XXXI} \]

\[ L \]
2. Nomenclature

Mixtures will be designated by italicized Arabic numerals. Italicized Roman numerals will be used for homogeneous compounds. Structures corresponding to the Roman numerals can be found in section II-A-1 or in the text. Partial structures will be assigned italicized capital letters while fragments will be identified by lower case italic letters.

\[ \text{Cl} \]

The term "chloromethylidene" is used to mean \(-\text{C}=\), the chlorine-carbon portion of a vinyl chloride group.

The numbering system for stylocheilamide (Figure 2) was adopted for simplicity in the discussion section. The notation "H-10" is used to indicate all the protons attached at the 10 position. When necessary, the protons at a center are further distinguished by lower case letters. The notation "C-4'" indicates the carbon atom at position 4' in the cyclohexane ring. The corrugated line between C-3' and C-4' is used to indicate that the cyclohexanone ring is both \textit{cis} and \textit{trans} to the chlorine. The epoxide is \textit{beta} and the 3'-4' bond is \textit{alpha}.

The experimental section is arranged more by reaction sequence than by reaction type. Hence esterification of a reaction product will be found following a reduction rather than in a separate acylation section. While stylocheilamide and deacetylstylocheilamide are related, it seemed more convenient and less confusing to present the degradation...
Figure 2. Numbering System for Stylocheilamide Skeleton.
experiments and their interpretation separately. The discussion section of deacetylstylocheilamide is much shorter than that of stylocheilamide because most of the structural elements of the compounds are common to both.

Journal abbreviations used in the reference section were adopted by the International Organization for Standardization and published in the "Literature Source Index" of Chemical Abstracts.

3. Chromatography

a. Preparation of columns.

Columns were prepared by carefully pouring a well mixed slurry of the adsorbent in the chromatographic solvent into the column. In the case of gradient elution, the least polar solvent was used. The glass columns were fitted with a fritted glass disk and Teflon stopcock. The glass drip tip was adapted for Teflon tubing so that the column effluent could be drained through an automatic fraction collector.

The usual method of applying material to the column was to drain the solvent to the top of the support, dissolve the compound in a minimum amount of the chromatographic solvent and apply the solution to the top of the column. The compound-liquid level was drained slightly below the top of the support and the column filled with solvent. For small columns (less than 4 cm in diameter) the flow rate did not exceed 3 ml/min.

Another method for preparation of material for column chromatography consisted of adding a solution of the organic
material in a small amount of suitable solvent to the column support and removing of the solvent in \textit{vacuo}. This gave a powdery residue of the organic material coated on the support. The column was prepared as above, the solvent drained to the top of the adsorbent and the coated support deposited onto the column. Solvent was added and fractions were collected in the normal manner.

Adsorbents used were Bio-Rad Laboratories Bio-Sil A (200-325 mesh), Mallinckrodt Chemical Works SilicAR CC-7 Special for Column Chromatography, MCB Silica Gel (60-200 mesh) SX 144-5, Floridin Company Florisil (less than 200 mesh) FB-6 and Brinkmann Instruments, Inc. Silica Gel HF-254+366 for TLC according to Stahl, type 60.

Analytical reagent grade redistilled solvents were used for all chromatographies. Whenever possible solvents were reclaimed, fractionally distilled and reused. No attempt was made to keep the solvents anhydrous.

b. Preparation of Dry-columns\textsuperscript{75}

Woelm silica gel for Dry-Column Chromatography (activity III) was poured into 3 cm diameter polyethylene tubing. Small perforations were made in the bottom of the tubing. The organic material was dissolved in a small amount of solvent and applied to the top of the column. Solvent was added to the column. The usual development time was 60-90 min.

The developed column was cut into sections based on color and Rf values of a parallel TLC system. The silica gel was extracted with acetone or acetone-methanol.
c. Preparation of Thin Layer Chromatography Plates 76,77

The adsorbents used for thin-layer plates were Brinkmann Instruments Silica Gel HF 254+366 type 60, Silica Gel PF-254 containing calcium sulfate (15%) and Floridin Company's Florisil for TLC (# 07228).

Analytical TLC plates (5 x 20 cm) were prepared by wiping the glass panes with hexanes then, by means of a Desaga-Brinkmann spreader, layering an aqueous suspension of the adsorbent onto the plates. The thickness of the wet plates was 0.25 mm and was somewhat less on drying. The air dried plates were activated by drying at 120° for 1 to 1.5 hr. Plates were stored in contact with a very moist atmosphere. The TLC plates were developed in filterpaper lined, glass stoppered 5.5 x 25 cm jars. The sample for analysis was a small spot applied to the adsorbent via a thin glass capillary.

Preparative TLC plates were manufactured by coating an aqueous suspension of the adsorbent onto 20 x 20 cm or 20 x 40 cm glass plates. The thickness of the wet adsorbent was 0.60 mm and dried to approximately 0.5 mm. The air dried plates were activated at 120° for 2 hr and stored in contact with the atmosphere.

d. TLC Visualization Methods 78,79

Most commonly a combination of visualization methods was used. Examination of 254 or 366 nm inorganic phosphor impregnated plates by long and short wavelength UV light was used to detect compounds with chromophores near these frequencies. The plates were then immersed in an iodine-saturated
tank and yellow to brownish spots observed under natural light or dark spots observed on long wavelength UV examination. The plates were sprayed with methanolic sulfuric acid (1:1), air dried, examined under UV light, then heated at 110° until the maximum color developed. Some classes of compounds have characteristic colors: cholesterol is red, then turns brown, while vitamin A and esters are blue turning to grey. Prolonged heating generates brown to dark grey spots.

Dragendorff's Reagent\textsuperscript{78} (Vágújfalvi modification) was used to visualize deacetylstylocheilamide. Subsequent spraying of the plate with 0.05N sulfuric acid produces red spots on a grey background rather than the normal orange spots on a yellowish background. The test is normally used to detect alkaloids and other nitrogen-containing bases but some other non-nitrogen compounds give a positive test\textsuperscript{80}.

Rhodamine 6G, a general spray reagent for lipids, phosphatides and phenols, was also used.

The products of oxidation were examined by spraying with bromocresol green, an indicator for organic acids. When aldehydes or ketones were suspected, the developed plate was sprayed with a 0.04% solution of 2,4-dinitrophenylhydrazine in 2M hydrochloric acid.

e. Column Chromatography Monitoring

Whenever possible, one of two types of automatic fraction collection apparatuses was used. An Isco Model AT fraction collector equipped with a time operated advancement
test tube turntable was used for fraction size up to 25 ml.

An Isco Model UA-4 absorbance monitor and Model 1130 channel alternator were coupled with a Type 5 optical unit so that two UV wavelengths (usually 254 and 280 nm) could be monitored. The fraction collector was an Isco Model 1200 PUP usually operated in the drop advance mode.

Fractions were combined by UV absorbance or TLC equivalence.


The Hitachi Perkin-Elmer 115 Molecular Weight Apparatus was disassembled, cleaned, oven dried (120°) and immediately reassembled. Benzene (Mallinckrodt, analytical reagent dried over sodium) was used as the solvent while benzil was used as the standard for the molality-resistance plot. The instrument was allowed to come to thermal equilibrium overnight. The suboven was at 34.0° (VR-4 set at 590) and the main oven at 40.0° (VR-5 set at 688). Four to six measurements were made for each solution. The thermistor was allowed to equilibrate for 1.5 min prior to recording the resistance reading. A total of thirteen benzil solutions of different molality (0.00276 to 0.04530) were prepared.

Least squares regression analysis of the data on a Wang calculator equipped with a CP-2M card programmer, program 360.47-ST, gave the following equation,

\[ C = 0.00005429 \Delta R - 0.00053710 \]
where \( C \) is the molality of the solution and \( \Delta R \) is the change in the resistance. A "t" test for the fourteen points (thirteen degrees of freedom) gave a high value of 19.23 or better than 99.9% confidence level.

The average molecular weight, \( \bar{M} \), is then determined by,

\[
\bar{M} = \frac{W_1 (1000)}{(W_1 + W_2) C}
\]

where \( W_1 \) is the weight of the solute and \( W_2 \) is the weight of the solvent.

Cholesterol (molecular weight 386), used as a reference, gave a calculated molecular weight of 395 for an error of 2.4%. It was assumed that the 2.4% would represent the uncertainty of other results.

Stylocheilamide (10.72 mg in 2.01313 g of benzene) gave a \( \Delta R \) of 197.5 for a calculated average molecular weight of 520 \( \pm \) 12.

Deacetylstylocheilamide (13.66 mg in 3.20165 g of benzene) gave a \( \Delta R \) of 180.5 for a calculated average molecular weight of 459 \( \pm \) 11.

5. Physical Measurements.

Melting points were taken on a Fisher-Johns block and are uncorrected. Infrared spectra (IR) were recorded on a Beckman IR-10 instrument calibrated to the 1601 cm\(^{-1}\) line of polystyrene. Most samples were recorded in carbon tetrachloride using 0.1 mm liquid cells. Viscous oils were recorded as films on sodium chloride disks. A Varian HA-100
nuclear magnetic resonance (NMR) spectrometer and a Varian XL-100 NMR spectrometer with Fourier transform were used to record the proton magnetic resonance (PMR) spectra. Chemical shifts were calculated relative to internal TMS at $\delta = 0$. Homonuclear decoupling on the Varian HA-100 spectrometer required a Hewlett-Packard Model 200AB audio oscillator. PMR spectra at 220 MHz were obtained at the University of California, San Diego. Natural abundance carbon-13 nuclear magnetic resonance (CMR) spectra were obtained with a Varian XL-100 spectrometer operating at 25.2 MHz coupled to a Digilab 400-2 pulser, 410-H (100 MHz) radiofrequency pulse amplifier and Nova 1200 minicomputer for Fourier transform. CMR chemical shifts were calculated relative to internal TMS and are reported in ppm. Ultraviolet spectra were obtained on a Cary-14 recording spectrometer in fused silica cells of 1 cm path length. Optical rotations were determined on a Bendix-Ericsson model ETL-NPL automatic polarimeter, type 143A. Low resolution mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6E or Varian MAT 311 spectrometer. High resolution mass spectra were obtained on a Varian MAT 311 at 70 ev or on an Associated Electrical Industries LTD MS-9 with dual EI-CI ion source at Battelle Memorial Institute, Mass Spectral Laboratory, Columbus, Ohio. Chemical ionization mass spectra were also obtained on an AEI MS-9 at Battelle Memorial Institute. Elemental analyses were performed at the University of California, Chemical Analysis Services, Berkeley, California. Analytical GLC was performed
on a Beckman GC-5 equipped with flame ionization detectors. Molecular weight determinations were performed on a Hitachi Perkin-Elmer model 115 molecular weight apparatus.

B. Collection of Animals

*Stylocheilus longicauda* (Quoy and Gaimard)<sup>64,68</sup> was plentiful from late May through mid-June on a low, flat, silted reef on the southeast side (east of the Kaneohe Yacht Club) of Kaneohe Bay, Oahu, Hawaii. At low tide the water level on the reef varies from 0-1 m and approximately 6 kg of animals could be collected in a single collection trip. The reef is rather barren; the predominant algae are *Acanthophera spicifera* (red), *Lyngbya majuscula* (blue-green) and *Dictyosphaeria cavernosa* (bubble alga).

*Stylocheilus longicauda* could also be found in sparse numbers on a reef at Kahala Beach Park on the south shore of Oahu, and on a sandy reef at Kaaawa Beach Park on the east shore of Oahu. Recently, I collected the animal in mid-June on the reef fronting Ala Moana Beach Park on the south shore of Oahu.

The animals could be safely and easily captured by picking them from the algae or the sand with bare hands.

C. Storage of Specimens

The animals were collected into plastic bags for transport to the laboratory. The animals were separated from algae and sand, rinsed with tap water, weighed, returned to plastic bags and stored in a freezer (-10°) until needed.
No decrease in the concentration of stylocheilamide was noted in specimens stored for periods of up to three years.

D. Isolation

1. Stylocheilamide and deacetylstylocheilamide

A typical isolation of stylocheilamide and deacetylstylocheilamide involves mild maceration of 8.5 kg of whole, frozen animals with acetone in a Waring Blender. The solvent is decanted from the solid mass, evaporated on an R-20 Büchi Rotary Evaporator and the condensate is used to reextract the animals. The process is repeated three times. The tissue mass is discarded and the resulting concentrated aqueous organic mixture partitioned between diethyl ether (2 l) and water (2 l).

The dark green organic phase is reextracted with water and the diethyl ether removed at reduced pressure to give a viscous, dark green oil, 1. The oil, 1, is then partitioned between aqueous methanol (1:9) and petroleum ether (30-60°). The petroleum ether phase, 2, (39 g, 0.45%) gave a weakly positive Beilstein test for halogen and was not further investigated.

The concentrated aqueous methanolic phase, 3, is then partitioned between carbon tetrachloride (2 l) and aqueous methanol (1:4, 2 l). Thin layer chromatography showed the presence of Dragendorff positive material in the carbon tetrachloride layer. Careful azetropic removal of carbon tetrachloride with methanol showed that the hydrocarbon layer contained halogenated material shown by a positive
Beilstein test. The methanolic phase was not investigated.

The carbon tetrachloride phase is separated and concentrated under vacuum to a very dark viscous oil, 4, (35 g, 0.41%). Oil 4 is chromatographed on a large column of Bio-Sil A (200-325 mesh), with a load factor of 20:1. Gradient elution from hexanes through diethyl ether gave Beilstein positive material eluted with 60-80% diethyl ether. Concentration of these fractions gave approximately 6 g (0.07%) of dark oil, 5.

Dark mixture 5 is placed onto a short Florisil (minus 200 mesh) column which is eluted with diethyl ether-hexanes (2:1) to give 5.50 g (0.065%) of clear yellow oil, 6. Thin layer chromatography on silican gel HF 254+366 developed with benzene-5% methanol shows the presence of low Rf material.

Column chromatography on Bio-Sil A (200-325 mesh) of 6 and elution with benzene-5% methanol gives 1.256 g (0.015%) of Beilstein positive oil, 7. Thin layer chromatography of 7 on Sephadex LH-20 using methanol as eluent gives a mixture, 8, of stylocheilamide and deacetylstylocheilamide as the major fraction (1.067 g, 0.013%).

Preparative thin layer chromatography of 8 on silica gel HF 254+366 using multiple development technique with diethyl ether-n-hexane (3:1) gives 300 mg each of stylocheilamide and deacetylstylocheilamide. This represents only a recovery of 60% by weight of a mixture which by tlc was predominantly stylocheilamide.
Table 3
Flow Chart for Isolation of Stylachellamide

Stylachellus tanicaulida

8.5kg
- acetone
- all
diethyl ether/water

- diethyl ether
  - all, 80g
  - aqueous methanol (1:9)/
    petroleum ether
  - petroleum ether
    - all, 25g
    - aqueous methanol (1:4)/
      carbon tetrachloride
  - carbon tetrachloride
    - all, 35g (0.41%)
    - Bio-Sili A
      column
    - 60-300, diethyl ether
      in hexanes
      - all, 6g (0.07%)
      - Florisil
        column
    - diethyl ether
      in hexanes (2:1)
      - all, 5.5g (0.063%)
    - Bio-Sili A
      column
    - benzene:9% methanol
      - all, 1.25g (0.015%)
      - Sephadex LH-20
        methanol
      - oil, 1.05g (0.010%)
      - Bio-Sili A
        column
    - diethyl ether in
      cyclohexane (2:1)
      - stilachellamide
        - all, 0.005g (0.00%)
      - prep. TLC
        silica gel HF 254+R66
        diethyl ether in
        cyclohexane (1:1)
      - stilachellamide
        - all, 0.35g (0.003%)
      - diethyl ether in
        cyclohexane (1:1)
A satisfactory separation was achieved by adsorption chromatography of the mixture 8 on Bio-Sil A (200-325 mesh) using diethyl ether-cyclohexane (2:1). This method gives two major fractions: A mixture (1:3) of stylocheilamide (I) and deacetylstylocheilamide (II) (25 weight percent) and stylocheilamide (55 weight percent). The stylocheilamide thus obtained is homogeneous by tlc: silica gel HF 254+366, diethyl ether-n-hexane (3:1), Rf (I) 0.26, (II) 0.32; benzene-5% methanol, Rf (I) 0.35, (II) 0.37; ethyl acetate-diethyl ether (2:1), Rf (I) 0.68, (II) 0.71; toluene-2-butanone (4:3), Rf (I) 0.49, (II) 0.51; dichloromethane-5% methanol, Rf (II) 0.74, (I) 0.76.

The yield of stylocheilamide from the animal was about 0.007% while deacetylstylocheilamide was isolated in about 0.003%. In total about 32 kg of animal was processed to give nearly 2.5 g of stylocheilamide.

2. Isolation of dioctyl phthalate.

Column chromatography of the carbon tetrachloride soluble oil 4 on Bio-Sil A with gradient elution from hexanes through diethyl ether gave oil 9 eluting with about 5% diethyl ether in hexane. Column chromatography of 9 on SilicAR CC-7 eluting with dichloromethane-1.5% methanol gave dioctyl phthalate, III, in two fractions. Tlc: silica gel HF 254+366, dichloromethane-methanol (40:1), Rf 0.78.

IR(CCl₄): 3080(aromatic), 1730(ester), 1460, 1380, 1275(C-O), 1110, 1070 cm⁻¹. PMR(CCl₄), Figure 10: 7.25(m,
4H), 4.05(m), 1.8-1.1(m, 24H), 0.9(bt, 6H). Mass spectrum at 70 ev: m/e 390 (M+).

3. Isolation of tri(n-butoxyethoxy)phosphate

Column chromatography of the diethyl ether extract, oil 1, of Stylocheilus longicauda on silica gel gave mixture 10 eluted with chloroform-10% acetone.

Trituration of 10 with n-hexane gave a hexane-soluble fraction 11. Column chromatography of 11 on silica gel eluted with benzene-acetone (1:1) gave tri(n-butoxyethoxy)phosphate, IV, as a colorless oil. Tlc: silica gel HF 254+366, benzene-acetone (1:1), Rf 0.67.

IR(film): 2980, 2960, 2850, 1460, 1210, 1150, 1130, 1040, 990 cm⁻¹. PMR(CDC13), Figure 11.

4. Isolation of Chimyl Alcohol

One of the persistent contaminants present in the preliminary purification of stylocheilamide was shown to be chimyl alcohol.

The initial silica gel column chromatography after liquid-liquid extraction gave Beilstein positive material eluted with 60-80% diethyl ether in hexanes, 5. Tlc of this material developed with benzene-5% methanol showed material at Rf 0.27, slightly lower than stylocheilamide. This material was visible as a blue spot under long or short wavelength UV light after spraying with methanolic sulfuric acid (1:1). The spot was light grey after heating at 120° for 30 min or 0.5 hr.

Column chromatography of 5 on Mallinckrodt SilicAR CC-7 silica gel eluting with benzene-3.5% methanol gave material
in fractions 27-34 which upon combination and concentration gave crystals of chimyl alcohol, V. Beilstein test for halogens was negative. Recrystallization of V from methanol gave pure chimyl alcohol, mp 61.0-62.0°. Tlc: silica gel HF 254+366, diethyl ether-cyclohexane (3:1), Rf 0.16.

IR(CCl₄): 3600, 3440 (OH); 1460; 111(C-O) cm⁻¹.
PMR(acetone-d₆): 3.6-3.3(m, 6H), 2.9(bs, 1H, D₂O exchangeable), 1.3(bs, 26H), 0.9(bt, J=6Hz, 3H). Mass spectrum at 70ev: m/e 316 (M⁺). Chemical analysis for C₁₉H₄₀O₃: requires C,72.10; H,12.74; found C,72.03; H,12.12.

E. Characterization of Stylocheilamide (I)
1. Classification tests
   a. Stylocheilamide was dissolved in 95% ethanol, placed onto a spot test plate and 2,4-dinitrophenyl-hydrazine in acidified ethanol was added. The compound failed to give a precipitate.
   b. Stylocheilamide was placed onto a tlc plate and sprayed with silver nitrate-ammonium hydroxide-fluorescein in a test for easily hydrolyzed halogen. The compound failed to give a rose-colored positive test.
   c. Stylocheilamide was dissolved in ethanol and two drops of 5% ethanolic silver nitrate were added. A precipitate of silver chloride did not form even on prolonged boiling.

2. Spectral data of I
   Optical rotation: [α]²⁸° + 10.6° (c 28.2, methanol).
   UV (methanol), end absorption. IR(CCl₄): 2920, 2860, 2820;
1745 (acetate), 1720 (ketone), 1655 (amide); 1450, 1390, 1360, 1230, 1100, 1040, 1000, 960, 880 cm$^{-1}$.

M$^+$ C$_{28}$H$_{44}$ClNO$_6$, measured 525.2856 calculated 525.2856.

Chemical analysis: C$_{28}$H$_{44}$ClNO$_6$ requires C, 63.92; H, 8.43; N, 2.66; Cl, 6.74; found C, 64.74; H, 8.20; N, 2.85. CMR (CDCl$_3$, ppm from TMS): SP$^2$,C=O: 202.8, 202.3 (ketone); 172.8, 172.5 (acetate); 168.9 (amide). SP$^2$,C=C: 132.1 (s), 131.6 (s); 131.0 (d); 126.8 (d); 121.5 (d), 120.2 (d). SP$^3$,C-O,N: 80.5 (d); 76.3 (d); 64.0 (d); 61.2 (s), 60.6 (s); 56.3 (q); 50.8 (t), 48.3 (t); 41.2. SP$^3$,C-C: 36.2; 35.0; 33.8; 33.2; 32.3; 31.8; 29.7; 29.7; 28.0; 25.2; 22.6; 20.8; 14.1; 10.8. PMR (CDCl$_3$, 220MHz spectrum, Figures 3-6.

F. Characterization of Deacetylstrylocheilamide (II)

1. Classification tests

   a. Deacetylstrylocheilamide was dissolved in 95% ethanol, placed onto a spot test plate and 2,4-dinitrophenylhydrazine in acidified ethanol was added. The compound failed to give a precipitate.

   b. Deacetylstrylocheilamide was dissolved in 95% ethanol and two drops of 5% silver nitrate was added. A precipitate of silver chloride did not form even on prolonged boiling of the solution.

   c. Deacetylstrylocheilamide was placed onto an analytical tlc plate and developed with diethyl ether-hexanes (3:1). The air dried tlc plate was sprayed with nitroprusside (sodium)-ferricyanide (FCNP reagent)$^{78}$ reagent to test for aliphatic nitrogen compounds, creatine and creatinine. A
blue spot was formed by creatine while \( II \) gave a negative test forming a yellow spot.

d. Deacetylstylocheilamide was placed onto an analytical tlc plate and developed with diethyl ether-hexanes (3:1). The air dried tlc plate was sprayed with Dragendorff's reagent (Vágnjfalvi modification)\textsuperscript{78} to test for nitrogen and other compounds\textsuperscript{80}. An orange spot which turned red when sprayed with 0.05N sulfuric acid indicated a positive test.

e. Deacetylstylocheilamide was spotted near the bottom of an analytical tlc plate and placed into a jar of iodo-methane vapors. The plate was then developed with diethyl ether-hexanes (3:1). The Rf value of \( II \) exposed to iodo-methane was unaltered from a control tlc plate which had not been exposed to iodomethane. This excludes a basic center in \( II \).

2. Spectral data of \( II \)

Optical rotation: \([\alpha]_D^{25\circ} -11.5^\circ (c15.7, \text{methanol})\).

UV (methanol): \( \lambda_{\text{max}} \) 241 nm (\( e = 6,800 \)). IR (film): 1660 (amide; \( \alpha,\beta \)-unsaturated ketone), 1450, 1400, 1120, 1090, 1060, 960, 880, 830 cm\(^{-1}\). Molecular weight determination (isopiestic method) in benzene, 459 ± 11. \( M^+ \) C\textsubscript{26}H\textsubscript{40}ClNO\textsubscript{4}\', measured 465.2621, requires 465.2646. Chemical analysis; C\textsubscript{26}H\textsubscript{40}ClNO\textsubscript{4} requires C, 67.01; H, 8.65; Cl, 7.61; N, 3.01; found C, 67.07; H, 8.71; Cl, 6.9; N, 3.02. CMR (CDC\textsubscript{13}): SP\textsuperscript{2}, C=O: 191.9, 172.5(amide). SP\textsuperscript{2}, C=C: 137.7(d), 133.4(s), 132.5(s), 130.9(d), 126.9(d), 121.0(d), 119.1(d). SP\textsuperscript{3}, C-O,N: 80.6(d), 60.3(d), 59.9(s), 59.4(s), 56.3(q),
51.0(t), 48.6(t). Sp\(^3\), C-C: 36.3, 35.2, 33.9, 33.3, 32.3, 31.7, 29.6, 27.9, 25.2, 22.5, 16.1, 14.0. PMR (CDCl\(_3\)), Figures 7-9.

G. Catalytic Hydrogenation of \(I\)

1. Quantitative Hydrogenation of Stylocheilamide

A Brown\(^2\) microhydrogenation apparatus was charged with 10\% palladium-on-carbon (5 mg) and 2.5 ml of 2-propanol, then flushed with nitrogen. Hydrogen was introduced via syringe and the catalyst was stirred until no further hydrogen uptake occurred. Stylocheilamide (20.58 mg, 0.0563 mmol) in 0.15 ml 2-propanol was added. Hydrogen uptake (0.077 mmol) was complete in 6 hr. Ratio: 1.4 mol hydrogen per mole stylocheilamide.

2. Platinum Hydrogenation of Stylocheilamide

Into a round-bottomed flask containing reduced platinum oxide (3 mg) in 7 ml ethyl acetate was added stylocheilamide (15 mg) in 1 ml ethyl acetate. After 1.5 hr thin layer analysis indicated the absence of starting material. The reaction was flushed with nitrogen, filtered through Celite filter aid and the solvent was removed \textit{in vacuo} to give a mixture, \(12\), that contained one major component. Tlc: silica gel HF 254+366, diethyl ether-cyclohexane (3:1), Rf 0.16.

Preparative thin layer chromatography of \(12\) on silica gel HF 254+366 developed with benzene-methanol (20:1; Rf 0.48-0.62) gave \(VI\) (10 mg) as a clear oil. Beilstein test for halogens: Negative. IR (CCl\(_4\)): 1745, 1725, 1650 (C=O); 1455, 1400, 1370, 1225, 1090, 1000, 900 cm\(^{-1}\). PMR (CCl\(_4\)):
5.0 (m, 1H), 3.22 (s, 3H), 3.0 (bs, 3H), 1.97δ (s, 3H).

Mass spectrum at 20ev (rel %): 495(M⁺, 5), 470(20), 463(15), 435(10), 396(38), 336(20), 297(40), 268(30), 252(25), 241(45), 164(85), 44(100).

3. Palladium Hydrogenation of Stylocheilamide.

Impure stylocheilamide (50 mg) was dissolved in 5 ml of redistilled ethyl acetate, 5 mg of 10% palladium-on-carbon was added. The system was flushed twice with nitrogen then twice with hydrogen. The reaction was stirred at atmospheric pressure for 8 hr then flushed with nitrogen. Celite filter aid was added to the reaction mixture and the catalyst was removed by filtration through Celite. The filter cake was washed with ethyl acetate and evaporation of the solvent under vacuum gave a pinkish oil 13 (45 mg). Tlc: silica gel HF 254+366, diethyl ether-n-hexane (3:1), Rf: 0.15, 0.20 (major), 0.25, 0.30.

Preparative thin layer chromatography of mixture 13 on silica gel HF 254+366 using diethyl ether-n-hexane (3:1), multiple development (10 and 40 min), allowed the removal of four fractions.

The major fraction, 14 (35 mg), was chromatographed on preparative thin layer plates using benzene-methanol (20:1), multiple development of 5, 10 and 40 min. The major fraction, VII (22.6 mg), could not be visualized by short wavelength ultraviolet light but was an area between two faintly visible bands.
VII. IR (CCl₄): 1745, 1730, 1655 (C=O); 1450, 1400, 1370, 1215, 1090, 900 cm⁻¹. PMR (CDCl₃): 6.16, 6.08 (s, 1H); 5.02 (bs, 2H); 4.00 (bs, 2H); 3.58 (bs, 1H); 3.28 (s, 3H); 3.2–2.9 (m, 2H); 3.0, 2.92 (–CO–NCH₃); 2.5–2.2 (m, 6H); 2.0 (s, 3H); 1.02δ (d, J=7Hz, 3H). Mass spectrum at 70ev (rel %): 527, 529(M⁺, 3), 512(5), 492(M⁺–Cl, 100), 432(28), 428, 430(32), 368, 370(12), 288(18), 252(17), 250(12), 192(20), 174(35), 69(35), 44(32), 43(30). CMR (CDCl₃, ppm from TMS): SP², C=O: 202.5, 173.0, 169.8. SP², C=C: 131.9, 121.4. SP³, C–O, N: 80.7, 75.7, 63.8, 61.2, 56.2, 48.3, 41.2. SP³, C–C: 33.2(2C), 31.7, 29.5(3C), 22.5, 20.8, 14.0, 10.7.

H. Rearrangement of I

1. Rearrangement of Stylocheilamide with Sodium Acetate

To a degassed solution of stylocheilamide (0.3316 g, 0.632 mmol) in 95% ethanol was added sodium acetate trihydrate (0.1030 g, 0.76 mmol). The reaction was stirred at room temperature for 1 hr then refluxed for 7 hr. The ethanol was removed at reduced pressure and the resulting gum was dissolved in diethyl ether and extracted with water. The separated organic phase was dried (Na₂SO₄) and concentrated to a tan oil 21 (0.3165 g).

Preparative thin layer chromatography of the 21 on silica gel HF 254+366 plates, developed with diethyl ether-hexanes (3:1), gave one major short wavelength ultraviolet band (22). This band was extracted from the silica gel with acetone and concentrated to give oil 22 (0.2585 g).
Preparative thin layer chromatography of II on silica gel HF 254+366 by multiple development in ethyl acetate-hexanes (1:1) gave deacetylstylocheilamide (II) (0.0755 g) and IX (0.1538 g). Tlc: silica gel HF 254+366, ethyl acetate-hexanes (1:1), Rf (IX) 0.43. IX: M⁺: C₆H₄NO₄Cl Calculated 465.2645, Found 465.2648. Chemical analysis for C₆H₄NO₄Cl: requires C, 67.10; H, 8.60; Cl, 7.53; N, 3.01. Found: C, 65.37; H, 8.61; Cl, 8.3; N, 3.24. UV: \( \lambda_{\text{max}} \) (methanol) 260 nm (ε = 5,300). IR (CCl₄): 3040 (olefin); 1775 (\( \gamma \)-lactone); 1655 (amide); 1600 (olefin); 1120, 1070 (C-O); 960 cm⁻¹ (trans olefin). PMR (CDCl₃): 6.25 (m, 3H); 5.5 (m, 4H); 4.63 (bd, J=16Hz, 1H); 4.18 (bs, 1H); 3.92 (bd, J=16Hz, 1H); 3.30 (s, 3H); 3.15 (m, 1H); 2.98, 2.90 (-CO-NCH₃); 1.52δ (d, J=6.5Hz, 3H). CMR (CDCl₃, ppm from TMS): \( \text{sp}^2 \), C=O: 172.4 (lactone), 168.7 (amide). \( \text{sp}^2 \), C=C: 144.7(s), 133.5(s), 130.9(d), 130.7(d), 127.4(t), 127.1(d), 125.1(d), 120.6(d), 114.7(d). \( \text{sp}^3 \), C-O,N: 80.7(d), 56.4(q), 49.8(t), 40.2. \( \text{sp}^3 \), C-C: 36.4, 34.9, 33.4(2C), 31.8, 29.7, 29.3, 28.0, 25.3, 22.6, 14.4, 14.1.

2. Palladium Hydrogenation of IX

To a degassed solution of ethyl acetate was added 10% palladium-on-carbon (5 mg), IX (47.7 mg, 0.102 mmol) and hydrogen. Hydrogenation at atmospheric pressure was continued (2.5 hr) until tlc analysis showed the absence of starting material. Celite filter aid was added to the nitrogen-flushed reaction and the catalyst was removed through Celite. The organic solution was extracted with
water, dried (\text{Na}_2\text{SO}_4) and concentrated \textit{in vacuo} to an oil, 23. TLC: silica gel HF 254+366, ethyl acetate-hexanes (1:1), Rf 0.064, 0.35, 0.47, 0.57, 0.70, 0.80.

Preparative thin layer chromatography of 23 on silica gel HF 254+366 using multiple development with ethyl acetate-hexanes (1:1) gave \(X\) (11.0 mg, Rf 0.37-0.50) and \(XI\) (14.1 mg, Rf 0.50-0.56) as oils.

\textbf{Compound X:} \(M^{+}, C_{26}H_{47}N_{04}\): found 437.3512, calculated 437.3505. IR (CCl\(_4\)): 1755 (\(\gamma\)-lactone); 1650 (amide); 1130, 1090 cm\(^{-1}\) (C-O). PMR (CDCl\(_3\)): 5.35 (m, \(<\text{IH}\)); 4.4 (bd, J=16Hz, 1H); 3.8 (bd, J=16Hz, 1H); 3.3 (s, 3H); 2.95, 2.90 (3H, \(-\text{CO-NCH}_3\)); 1.13 (d, J=6Hz, 3H). Mass spectrum at 70ev (rel %): 437 (M\(^+\), 30), 422(6), 409(11), 405(10), 366(30), 338(85), 310(50), 252(50), 241(58), 240(60), 44(100).

\textbf{Compound XI:} \(M^{+}, C_{26}H_{47}N_{04}\): found 437.3513, calculated 437.3505. IR (CCl\(_4\)): 1760 (\(\gamma\)-lactone); 1655 (amide); 1130, 1090, 1070 cm\(^{-1}\) (C-O). PMR (CDCl\(_3\)): 4.42 (d, J=15Hz, 1H); 3.82 (d, J=15Hz, 1H); 3.35 (s, 3H); 2.9, 2.85 (\(-\text{CO-NCH}_3\)); 1.15 (d, J=7Hz, 3H). Mass spectrum at 70ev (rel %): 437 (M\(^+\), 28), 422(5), 409(17), 366(45), 338(60), 310(70), 280(30), 252(30), 241(30), 240(55), 44(100).

3. Alumina Chromatography of Stylocheilamide

Aluminum oxide (Bio-Rad Laboratories neutral alumina AG-7, 100-200 mesh) was washed with water. The bulk of the water was removed by Büchner funnel filtration on the water aspirator. The residual water was removed by oven drying (150°) for 24 hr.
A water-washed aluminum oxide column (45 x 1.5 cm) in diethyl ether-hexanes (3:1) was prepared and loaded with stylocheilamide (0.2369 g). A yellow band 2 cm long formed at the top of the column which could not be eluted with diethyl ether.

The alumina containing the yellow material was removed from the top of the column and extracted with methanol. Most of the yellow color appeared to remain adsorbed on the alumina. Concentration of the methanol gave 41 (5 mg) as an oil. Tlc: silica gel HF 254+366, diethyl ether-hexanes (3:1), Rf 0.04, 0.21, 0.35, 0.49 (major); benzene-5% methanol, Rf 0.15, 0.18, 0.30, 0.57 (major).

Mixture 41 was placed onto a 20 x 20 x 0.05 cm tlc plate coated with silica gel HF 254+366 and developed with benzene-5% methanol. Visualization of the plate under short wavelength UV light allowed the removal of one major band. The band was scraped from the plate and the adsorbent extracted with acetone-dichloromethane. Evaporation of the solvent gave XXII as a slightly yellow oil. Tlc: silica gel HF 254+366, dichloromethane-methanol (15:1), Rf 0.73. Ferric chloride test for phenols was positive.

**XXII: M⁺ C_{26}H_{40}ClNO_{3}:** measured 449.2693, calculated 449.2696. IR (CCl₄): 3400, 3200 (OH); 1640 (amide), 1460, 1410, 1360, 1240, 1090, 760 cm⁻¹. PMR (CDCl₃): 9.2(bs, 1H, D₂O exchangeable), 7.1(dd, J=6.5, 2.5, 1H), 6.8(m, 2H), 4.29(bs, 2H), 3.35(s, 3H), 3.1(bs overlapping m, 4H), 2.26(s overlapping m, 8H), 1.5-1.1(m, 12H), 0.87(bt,
J=5.5Hz, 3H). UV (methanol): $\lambda_{\text{max}}$ 283 nm ($\varepsilon=2,300$), $\lambda_{\text{max}}$ (basic) 290 nm ($\varepsilon=4,100$).

I. Hydride Reductions of I

1. Lithium Aluminum Hydride

   a. Reduction of Stylocheilamide. Lithium aluminum hydride (0.600 g, 65 mmol) was slurried in 50 ml dry diethyl ether under nitrogen, and stylocheilamide (1.2188 g, 2.31 mmol) in 20 ml dry diethyl ether was added dropwise over 20 min. The reaction was stirred at room temperature for 1 hr, then heated to reflux for 3 hr.

   Excess hydride was destroyed by dropwise addition of 2-propanol. Saturated sodium chloride was then added dropwise until a white, granular precipitate formed. The reaction mixture was filtered and the filter cake was washed with diethyl ether. The combined organic phases were extracted with 5% hydrochloric acid. The aqueous acidic phase was basified with solid sodium carbonate to about pH 10 and extracted with diethyl ether. The organic phase was washed with water until neutral, dried (MgSO$_4$) and evaporated to a pale yellow oil, $24$ (0.9761 g). Tlc: silica gel HF 254+366, diethyl ether-hexanes (3:1), Rf 0.08, 0.16, 0.30*(2spots), 0.38*, 0.50, *Dragendorff positive for basic nitrogen.

IR (CCl$_4$): 3400(OH); 1190, 1160, 1125(C-O); 960 cm$^{-1}$ (trans olefin).

   The basic oil $24$ was chromatographed on silica gel HF 254+366 preparative thin layer plates, multiply developed with diethyl ether-hexanes (3:1). Three bands were removed
as seen by short wavelength ultraviolet light; the center band, 25 (0.5163 g), was the major fraction.

b. Acetylation of 25. Mixture 25 (0.5163 g) was dissolved in dry (KOH) pyridine and redistilled acetic anhydride (0.80 ml, 0.74 g, 7.28 mmol) was added, the reaction mixture was left at room temperature for 5 hr and then heated to 50-55° for 3 hr. The solvent was removed on the vacuum pump and the resulting oil partitioned between water and dichloromethane. The organic phase was extracted with 10% sodium carbonate, washed with water until neutral, dried (MgSO₄) and evaporated to a clear oil 26. Tlc: silica gel HF 254+366, diethyl ether-hexanes (3:1), Rf 0.25, 0.30, 0.34, 0.44, 0.50, 0.54, 0.62.

Column chromatography of acetylated mixture 26 on Bio-Sil A (200-325 mesh) and elution with diethyl ether-cyclohexane (3:1) gave an oil, XII (0.1988 g), homogeneous by tlc. Tlc: silica gel HF 254+366, diethyl ether-cyclohexane (3:1), Rf 0.48. IR (CCl₄): 3100 (OH); 1735 (acetate); 1600 (olefin); 1215, 1085, 1020, 1000 (C-O); 955 cm⁻¹ (trans olefin). PMR (CDCl₃): 6.04 (bs, 1H); 5.44 (bt, J=3.5Hz, 2H); 4.96 (m, 2H); 3.65 (bd, J=14Hz, 1H); 3.30 (s, 3H); 2.4 (bs, 3H); 2.05 (s, 3H); 2.03 (s, 3H). Mass spectrum at 20 ev (rel %): 557, 559 (M⁺, 50); 522(80); 498(20); 482, 484(25); 462(22); 414(M⁺ -143, 20); 346(57); 328, 330(100); 143(45). CMR (CDCl₃, ppm from TMS): SP², C=O: 170.1 (s, 2C); SP², C=C: 137.5 (s), 131.3 (d), 126.9 (d), 118.4 (d). SP³, C-O,N: 80.6 (d), 75.6, 75.1, 71.1, 63.1, 56.2,

c. Reduction of VII. To a nitrogen-flushed 3-necked round-bottomed flask fitted with a gas inlet tube, condenser and dropping funnel, was added lithium aluminum hydride (0.50 g, 52.9 mmol) and 80 ml dry diethyl ether. The solvent was refluxed for 1 hr, then cooled in an ice-water bath to $0^\circ$ and VII (0.8405 g, 1.60 mmol) in 25 ml dry diethyl ether was added to the magnetically stirred mixture. The reaction was refluxed for 2 hr, cooled to room temperature and the excess hydride was destroyed by careful dropwise addition of 2-propanol. Saturated aqueous ammonium chloride was added dropwise until a grey-white precipitate formed.

The diethyl ether phase was decanted; the precipitate was washed with more diethyl ether; and the combined organic phases were washed with water, dried (MgSO$_4$) and evaporated to give mixture 15 (0.6588 g) as an oil. Tlc: silica gel HF 254+366, diethyl ether-hexanes (3:1), Rf 0.05, 0.19, 0.36, 0.45, 0.50, 0.60, 0.73. IR (CCl$_4$): 3450 cm$^{-1}$ (OH), no C=O.

d. Acetylation of 15. Mixture 15 (0.6500 g) was dissolved in 10 ml dry (KOH) pyridine, flushed with nitrogen and acetylated with acetic anhydride (0.515 ml, 0.505 g, 5.56 mmol) at 40$^\circ$ overnight. Excess acetic anhydride was hydrolyzed with water and the solvent was removed in vacuo. The resulting oil was dissolved in diethyl ether and water. The separated organic phase was washed several times with
water then extracted with 5% hydrochloric acid to give three phases.

The aqueous acidic layer was removed, basified with solid potassium hydroxide to pH 10 and extracted with diethyl ether. The organic phase was washed with water, dried (MgSO\(_4\)) and concentrated to a clear oil 16 (0.0315 g). Tlc: silica gel HF 254+366, diethyl ether-cyclohexane (3:1), Rf 0.29, 0.44, 0.52, 0.65.

The interface oil was dissolved in chloroform, washed with water, dried (MgSO\(_4\)) and the solvent removed to give 17 (0.4560 g) as a pinkish oil. Tlc: silica gel HF 254+366, diethyl ether-cyclohexane (3:1), Rf 0.29, 0.44, 0.52, 0.65.

Preparative thin layer chromatography of 17 on silica gel HF 254+366 plates developed with diethyl ether-cyclohexane (3:1) produced two major fractions, 18 (0.1832 g) and 19 (0.1508 g). Tlc: silica gel HF 254+366, diethyl ether-cyclohexane (3:1), Rf (18) 0.42, 0.49, 0.51, 0.61; RF (19) 0.52, 0.61, 0.64, 0.70.

2. Sodium Borohydride Reduction of Stylocheilamide

Stylocheilamide (0.1829 g, 0.349 mmol) was dissolved in 10 ml redistilled 2-propanol, cooled to 0\(^\circ\) in an ice bath and sodium borohydride (14.5 mg, 1.53 mmol) in 50 ml 2-propanol was added dropwise to the magnetically stirred solution. After stirring in the cold for 10 min, saturated aqueous ammonium chloride was added until hydrogen evolution ceased. The solvent was removed at reduced pressure and the resulting gum partitioned between diethyl ether and water.
The separated organic phase was then extracted with 10% sodium carbonate, 5N hydrochloric acid, washed with water, dried (MgSO₄) and evaporated to a clear oil 20 (0.1699 g).

Oil 20 was placed onto a 2.0 x 65 cm Bio-Sil A (200-325 mesh) column eluted with benzene-5% methanol to give the majority of the material eluted in three fractions, which were combined to give compound VIII (0.1777 g). Tlc: silica gel HF 254+366, diethyl ether-cyclohexane (3:1), Rf 0.34. IR (CCl₄): 3600, 3400 (OH); 1750 (lactone); 1650 (amide); 1230, 1210 (C-O); 1090 cm⁻¹. Mass spectrum at 20 ev (rel %): 527, 529 (M⁺, 16); 492 (M⁺-Cl, 11); 385, 387 (M⁺-142, 100); *285 (527+385); 143(23).

3. Red-al Reduction of Stylocheilamide

Red-al (70% solution of sodium bis (2-methoxyethoxy)-aluminum hydride in benzene), 2.5 ml, was dissolved in 50 ml diethyl ether, cooled to 0° in an ice bath and stylocheilamide (0.1078 g) in 10 ml dry diethyl ether was added. The mixture was stirred at room temperature for 2 hr, then refluxed for 3 hr and allowed to stand at room temperature for 11 hr.

2-Propanol was added to the rapidly stirred solution until no hydrogen was evolved. Saturated aqueous sodium chloride was added dropwise until a white precipitate formed. The solution was filtered and the filter cake was washed with diethyl ether. The separated organic phase was washed with water then extracted with 3% hydrochloric acid. Evaporation of the organic solvent gave a trace of neutral oil.
The aqueous acid was basified with solid sodium bicarbonate to about pH 9 and extracted with diethyl ether. The separated organic layer was washed with water, dried (MgSO₄) and evaporated to an oil (0.0403 g), which was a complex mixture. Thin layer chromatography of the mixture gave more than ten spots, all of which gave approximately equal color density following sulfuric acid-heat charring.

4. Lithium Aluminum Deuteride Reduction of Stylocheilamide

Stylocheilamide (0.1309 g, 0.349 mmol) was dissolved in 2 ml sodium-dried diethyl ether and added to a slurry of lithium aluminum deuteride (0.050 g, 4.78 mmol) in 10 ml dry diethyl ether at 5°. After 30 min the reaction was allowed to warm to room temperature and then refluxed for 3 hr. Water work-up gave a clear oil, 27 (0.1133 g).

Beilstein test for halogens was positive. IR (CCl₄): 3700, 3450 (OH), 1470, 1100, 970 cm⁻¹. PMR (CDCl₃): 6.10 (s, 1H), 5.43 (, 3H), 3.31 (s, 3H), 3.32 (d, J=14Hz, 1H), 2.75 (d, J=14Hz, 1H), 2.2 (s, 3H), 1.166 (d, J=7Hz, 3H). CMR (CDCl₃, ppm from TMS): SP², C=C: 140.7, 131.3, 127.0, 119.1. SP³, C-O,N: 80.7, 71.0, 61.5, 56.4, 40.1. SP³, C-C: 36.4, 33.4, 31.8, 30.1, 29.8, 29.2, 25.9, 25.3, 22.6, 15.0, 14.1.

Oil 27 was acetylated with excess acetic anhydride in pyridine (50°, 2 hr) to give a mixture 28 (0.1064 g). Beilstein test for halogens was positive.

Attempted column chromatography of 28 on Bio-Sil A (200-325 mesh) using diethyl ether-hexanes (3:1) failed to
elute anything. Elution of the column with methanol gave approximately 5 mg of oil (XIII). M\(^{+}\): C\(_{30}\)H\(_{48}\)D\(_{4}\)ClNO\(_{6}\) measured 561.3703, calculated 561.3733. M\(^{+}\)-142: C\(_{21}\)H\(_{29}\)D\(_{4}\)ClNO\(_{5}\) measured 418.2290, calculated 418.2297. M\(^{+}\)-211: C\(_{16}\)H\(_{21}\)D\(_{4}\)ClNO\(_{5}\) measured 350.1674, calculated 350.1671.

J. Oxidations

1. Basic Lemieux Oxidation of Stylocheilamide\(^{81}\)

Stock solution: 0.12 g potassium permanganate, 1.00 g potassium carbonate and 4.10 g sodium periodate was slurried in 100 ml distilled water.

To a stirred solution of stylocheilamide/deacetylstylocheilamide (0.2652 g) in 170 ml redistilled t-butanol was added 30 ml of stock solution. The color slowly turned from purple to brown. After 4 hr the reaction was filtered and the solvent was removed under vacuum to yield a tan oil. The oil was dissolved in diethyl ether and extracted with water. The aqueous phase was back-extracted with diethyl ether and the combined diethyl ether phases washed with a small amount of saturated aqueous sodium chloride, dried (MgSO\(_{4}\)) and evaporated to give a tan, neutral oil 29 (0.1093 g).

The neutral oil 29 was chromatographed on a column of tlc grade silica gel HF 254+366, 1.5 x 10 cm, eluted with diethyl ether-acetone (3:1) to give mixture 30 (41.6 mg) as the major fraction. IR (CCl\(_{4}\)): 3450 (OH), 2860 (OCH\(_{3}\)), 1715 (C=O), 1625 (amide, 1080 cm\(^{-1}\) (C=O).
The aqueous basic phase was acidified with concentrated phosphoric acid to pH 2 and extracted twice with dichloromethane. The organic phase was washed with water until neutral, dried (MgSO\(_4\)) and evaporated to give compound XIV.

2. Lemieux Oxidation of Stylocheilamide

Stock solution: sodium periodate (10.70 g) in 50 ml water; potassium permanganate (15.8 g) in 100 ml water.

To impure stylocheilamide (0.500 g) dissolved in 15 ml acetone was added 5.5 ml stock solution of sodium periodate and 0.2 ml of the potassium permanganate solution. The solution was stirred at reflux for 10 min, when a brownish precipitate began to form. The slurry was refluxed for another 4 hr, another 1 ml of the potassium permanganate solution was added and reflux was continued for 4 additional hr. The solvent was removed by distillation. The pot residue was extracted twice with diethyl ether, and the organic phase was successively extracted with 15% sodium carbonate, water and saturated aqueous sodium chloride. The diethyl ether phase was then dried (MgSO\(_4\)) and evaporated to give a yellowish oil 31 (0.18 g) containing neutral and basic compounds. Tlc: silica gel HF 254+366, dichloromethane-methanol 20:1, Rf 0.15, 0.20, 0.22, 0.32, 0.36, 0.50, 0.52, 0.75. IR (CCl\(_4\)): 3500 cm\(^{-1}\) (OH), 1710 (C=O), 1630 (amide), 1080 (C-O).

Oil 31 was chromatographed on a 2.5 x 65 cm Mallinkrodt SilicAR CC-7 column eluted with dichloromethane-methanol (20:1). Fractions 7 and 8 were combined and
subjected to gel permeation chromatography on Sephadex LH-20 eluted with methanol. Two fractions (19 and 20) were again combined and placed back onto a Sephadex LH-20 column to give a single, symmetrical peak. Evaporation of the solvent gave a colorless oil, \textit{XV} (0.072 g). Tlc: silica gel HF 254+366, dichloromethane-methanol (20:1), Rf 0.31. IR (CCl$_4$): 3480 (OH); 1720, 1625 (C=O); 1410; 1090 cm$^{-1}$ (C-O). Mass spectrum at 20ev (rel %): 515, 517(5), 480(28); 448(12); 330(75); 328(100); 246(16); 244(19).

The aqueous basic phase was acidified to pH 2 with concentrated phosphoric acid and extracted with diethyl ether. The separated organic phase was washed with water to neutrality, dried (MgSO$_4$) and evaporated to a slightly yellow oil, \textit{XVI} (0.0672 g). IR (CCl$_4$): 3400-2400 (acid): 1715 (C=O); 1270, 1070 cm$^{-1}$ (C-O). PMR (CDCl$_3$): 10.7 (b, 1H), 3.74 (p, J=6Hz, 1H), 3.38 (s, 3H), 2.51 (dd, J=6, 3Hz, 2H), 2.3 (bs, 12H), 0.96 (t, J=6Hz, 3H). Mass spectrum at 20ev (rel %): 187 (M$^+$-15, 12), 172(4), 170(7), 133(19), 103(100), 69(17), 61(21).

Esterification of \textit{XVI}. Acid \textit{XVI} was dissolved in diethyl ether, cooled to 0° and freshly prepared (from Diazald) diazomethane in diethyl ether was added until the solution was bright yellow. Stirring was continued for 30 min and the solvent was removed at reduced pressure 30mm Hg).

The slightly impure ester, \textit{32}, was placed onto a 1.5 x 13 cm Mallinckrodt SilicAR CC-7 column and eluted with dichloromethane-methanol, 20:1. Column fractions were
monitored by tlc to give pure methyl ester XVII (59 mg) in three fractions. Tlc: silica gel HF 254+366, dichloro-methane-methanol (20:1), Rf 0.72. GLPC: 10 ft x 1/8 in 6% EGSS-X column, 118°, retention time 4.7 min. Chemical analysis for C₁₂H₂₄O₃ requires C: 66.63, H: 11.18. Found C: 66.60, H: 10.98. IR (CCl₄): 2940; 2860 (OCH₃); 1740 (ester); 1260, 1090 cm⁻¹ (C-O). PMR (CDCl₃): 3.68 (s, 3H), 3.65 (m, 1H), 3.35 (s, 3H), 2.47 đ (dd, J=6, 3Hz, 2H). Mass spectrum at 20ev (rel %): 201 (M⁺-15, 15), 186(12), 184(8), 143(33), 127(15), 117(100), 65(80).

3. Ozonolysis of XII. Compound XII (0.1988 g) was dissolved in 20 ml dry methanol, flushed with nitrogen and cooled to -75° in a methanol-dry ice bath. Ozone, generated by a Welsbach model T-23 Ozonator, was bubbled through the solution for 15 min giving a bluish solution. The solution was allowed to stand at -75° for an additional 15 min, then the excess ozone was removed by a nitrogen flush. The solution was warmed to 0° and approximately 20 mg of 10% palladium-on-carbon was added, and the reaction was hydrogenated at atmospheric pressure for 3.5 hr.

Hydrogen was displaced by nitrogen, Celite filter aid was added and the catalyst was removed by filtration through Celite. The solvent was removed at the water aspirator (30mm Hg, room temperature) to give a mixture 33 (0.1864 g). Gas chromatography (6 ft x 1/8 in 3% OV-17 column, oven temperature 70°) of the mixture gave 13 peaks, but two fractions (retention time 90 and 110 min) contained 80% of
the material. Tlc: silica gel HF 254+366, diethyl ether-hexanes (3:1), Rf 0.07, 0.10, 0.20, 0.30*, 0.43*, 0.60*.

*2,4-Dinitrophenylhydrazone positive for aldehydes and ketones.

Preparative thin layer chromatography of 33 on silica gel HF 254+366 developed with diethyl ether-hexane (3:1) gave two mixtures, 34 (Rf 0.25-0.35) and 35 (Rf 0.35-0.45). Oils 34 and 35 were placed under a pump vacuum and more than half of the material sublimed. Thin layer chromatograms of the two mixtures were identical to chromatograms before loss of material on sublimation.

Mixture 34 (tlc: silica gel HF 254+366, diethyl ether, Rf 0.1, 0.16, 0.34, 0.36) was chromatographed on Sephadex LH-20 with methanol as the solvent. The major material, 20 mg, mixture 36, was chromatographed on Bio-Sil A (200-325 mesh) eluting with diethyl ether to give XVIII (12 mg).

XVIII: Glc (6 ft x 1/8 in 3% OV-17, oven temperature 130°) gave a single peak (retention time 36.5 min). Tlc (silica gel HF 254+366, diethyl ether, Rf 0.40) also indicated that the compound was homogeneous. High resolution mass spectroscopy: C_{13}H_{20}O_7, calculated 288.1209, found 288.1232; UV (methanol): λ\text{max} 217 nm (ε=158). IR (CCl_4): 3600, 3500 (OH); 1730 (acetate, ester); 1370, 1250-1380, 1030 cm^{-1} (C-O). PMR (CDCl_3): 5.00 (q, J=3Hz, 1H), 4.82 (d, J=2.5Hz, 1H), 3.74 (s, 3H), 2.45-1.8 (6H), 2.10 (s, 3H), 2.08 (s, 3H), 0.95δ (d, J=7.5Hz, 3H). Mass spectrum at 20ev (rel %): 288 (M^+, 4) 261 (M^+-OH, 2), 246(68), 228(20), 186(100), 169(30), 170(32), 109(30), 84(30). Base peak at 70ev m/e 43.
Mixture 35, a gummy solid, was triturated with petroleum ether (30-60°) to give a white solid, \textit{XIX}. Recrystallization of \textit{XIX} from cyclohexane gave fine rods, mp 89.0-90.0°.

\textit{XIX}: Tlc: silica gel HF 254+366, diethyl ether, Rf 0.55; diethyl ether-cyclohexane (3:1), Rf 0.30. IR (CCl$_4$): 1760 (ketone); 1740 (acetate); 1370, 1210, 1080, 1030 cm$^{-1}$ (C-O). PMR (CDCl$_3$): 5.35 (p, J=5Hz, 1H), 5.3 (d, J=5.5Hz, 1H), 2.8-1.8 (5H), 2.15 (s, 3H), 2.08 (s, 3H), 0.958 (d, J=7Hz). Mass spectrum at 20ev (rel %): 186 (M$^{+}$-CH$_2$=C=O, 20), 168(18), 143(45), 126(100), 98(50), 80(40), 68(18), 43(40). Base peak at 70ev m/e 43.

4. Ozonolysis$^{83}$ of Stylocheilamide

Stylocheilamide (0.5561 g, 1.06 mmol) was dissolved in 50 ml redistilled methanol and placed into a 125 ml side-arm filter flask fitted with a drying tube (CaCl$_2$), flushed with nitrogen and cooled in a dry ice-acetone bath to -75°. Ozone (generated from a Welsbach model T-23 Ozonator) was passed through the solution until the exit tube indicated the presence of ozone (formation of iodine) and the reaction was immediately flushed with nitrogen.

Palladium-on-carbon (10%, 200 mg) was added to the cold (-75°) solution and the mixture was subjected to hydrogenation at atmospheric pressure. After 40 min the reaction was allowed to warm to 0° and hydrogenation was continued for 1 hr. The reaction was flushed with nitrogen and the catalyst was removed by filtration through Celite. The filter cake was washed with methanol and the filtrates were combined.
Approximately 10 ml of the solvent was distilled under nitrogen into an aqueous methanolic dimedon solution. No crystal formation was observed, even on extended standing at 0°.

The pot residue was concentrated under nitrogen and the resulting oil triturated with petroleum ether (30-60°) to give a non-polar fraction, oil 37 (0.2291 g) and a polar fraction, oil 38 (0.3107 g).

Mixture 38 was subjected to gel filtration on Sephadex LH-20 with methanol as solvent. Six fractions (tubes 15-20) were combined (0.2204 g) and chromatographed on preparative silica gel HF 254+366 plates developed with dichloromethane-methanol (20:1). Extraction of a faintly short wavelength ultraviolet visible band (Rf 0.45-0.55) with acetone gave oil 39 (0.1294 g).

Preparative thin layer chromatography of 39 on silica gel HF 254+366 using a multiple development technique with diethyl ether gave oil 40 as the major (53 mg) component.

Oil 40 was purified by thin layer chromatography on silica gel HF 254+366 developed with dichloromethane-methanol (100:9). Two chromatographically homogeneous compounds, XX (16.3 mg) and XXI (30.5 mg), were eluted as oils. Tlc: silica gel HF 254+366, dichloromethane-methanol (10:1), Rf 0.49 (XX) and Rf 0.71 (XXI).

XXI: M+ C19H28N07Cl measured 417.1549, calculated 417.1554. IR (CCl4): 1745 (acetate), 1730 (ketone), 1650 (amide), 1230, 1115, 1070, 1050 (C-O), 900 cm⁻¹ (epoxide).
PMR (CDCl₃): 6.26, 6.12 (t, J=1.5Hz, 1H); 5.1 (m, 1H); 4.40 (t, J=6Hz, 1H); 4.04 (bs, 2H); 3.64 (m, 1H); 3.37 (s, 6H); 2.0 (s, overlapping m, 3H); 1.0δ (d, J=7Hz, 3H).

CMR (CDCl₃, ppm from TMS): SP², C=O: 202.5 (ketone), 172.4 (acetate), 169.9 (amide). SP², C=C: 131.9 (s), 121.8 (d). SP³, C-O,N: 103.7 (d), 75.7 (d), 63.9 (d), 60.9 (s), 53.1 (q), 53.1 (q), 48.3 (t), 41.2. SP³, C-C: 29.6, 27.8, 27.8, 20.8, 10.7.

K. Catalytic Hydrogenation of Deacetylstylocheilamide (II)

1. Palladium Hydrogenation

Deacetylstylocheilamide (0.0857 g, 0.184 mmol) was dissolved in redistilled ethyl acetate (10 ml). Palladium-on-carbon (10%, 8 mg) was added and the system was flushed with nitrogen. Hydrogen was then added via a balloon (atmospheric pressure). After 1 hr tlc analysis indicated the absence of starting material. The hydrogen was removed by a nitrogen flush and the catalyst removed by filtration through Celite. The solution was concentrated to an oil, 42.

Gel filtration of 42 on Sephadex LH-20 eluting with methanol and monitoring the column effluent with a 254 nm UV detector gave material in six fractions. Fractions 4 and 5 were combined to give 43, 36.9 mg. Tlc (43): silica gel HF 254+366, diethyl ether-hexanes (3:1), Rf 0.225*, 0.295* (major), 0.40. *Visible under short wavelength UV light.

Preparative thin layer chromatography of 43 on silica gel HF 254+366 developed with diethyl ether-hexanes (3:1)
showed the presence of three short wavelength UV light visible bands. The bands were scraped from the glass plate and the silica gel extracted with acetone-methanol (3:1).

Removal of the solvent from the central band gave oil \(XXIII\) (29.2 mg) as the major fraction. The oils from the two other bands were not further investigated. \(XXIII\) gave a positive Beilstein test for halogens and positive ferric chloride test for phenols\(^{84}\). Tlc (\(XXIII\)): silica gel HF 254+366, diethyl ether-hexanes (3:1), \(R_f\) 0.27. \(M^+\cdot C_{26}H_{42}ClNO_3\): measured 451.2835, calculated 451.2854. IR\((CCl_4)\): 3250(OH), 1640 (amide)', 1460, 1420, 1340, 1280, 1240, 1200, 1090 cm\(^{-1}\).

PMR (\(CDCl_3\)): 8.1(bs, 1H, \(D_2O\) exchangeable), 7.1(dd, \(J=6.5Hz, 3Hz, 1H\)), 6.85(m, 2H), 6.20(t, \(J=1.5Hz, 1H\)), 4.05(bs, 2H), 3.32(s, 3H), 3.1(bs overlapping m, 4H), 2.26(s overlapping multiplet, 5H), 1.7-1.0(approximately 23H), 0.85(bt, \(J=5Hz, 3H\)). UV: \(\lambda_{max}\) methanol 283 nm (\(\varepsilon = 2,100\)).

L. Ozonolysis of Deacetylstylocheilamide (II)

1. Oxidative Workup\(^{85}\)

A 25 ml filter flask with a side arm and fitted with a drying tube (\(CaCl_2\)) and gas inlet tube was charged with deacetylstylocheilamide (0.1376 g, 0.296 mmol) in 15 ml dry methanol. The solution was flushed with nitrogen, cooled to \(-78^\circ\) (dry ice-acetone) and ozone (generated by a Welsbach Model T-23 Ozonator) bubbled through the solution until the pale blue color remained. The solution was allowed to stand \((-78^\circ)\) for 15 min, then excess ozone was removed by a nitrogen flush. Hydrogen peroxide (30%, 9 ml) and potassium
carbonate (75 mg) were added and the solution stirred at -78° for 0.5 hr, warmed to room temperature and stirred overnight.

Water and 5% hydrochloric acid were added until approximately pH 1. The reaction mixture was extracted with chloroform (3X) and the combined organic extracts were washed with a little water, dried (MgSO₄) and evaporated to give 44 (0.0294 g) as a clear oil. IR (CCl₄): 3350 (OH), 1740 (C=O, weak), 1650 (C=O, weak), 1450, 1370, 1100 cm⁻¹. PMR shows no -COOH.

2. Reductive Workup

To a 25 ml filter flask with a side arm and fitted with a drying tube (CaSO₄) and a gas bubbler tube was added deacetylstylocheilamide (0.1322 g, 0.285 mmol) in dry methanol (15 ml). The reaction was flushed with nitrogen, cooled in a dry ice-acetone bath (-78°) and ozone (generated by a Welsbach Model T-23 Ozonator) bubbled through the solution until the exit tube showed the presence (KI + I₂) of ozone. The reaction was allowed to stand for 10 min, flushed with nitrogen and dimethyl sulfide 5 ml, 68 mmol) was added. The reaction was allowed to warm to room temperature over 1 hr and more dimethyl sulfide was added (5 ml, 68 mmol). The reaction stood for 2 hr, then the solvent was removed on the water aspirator. PMR showed aldehyde signals at 9.80. The oil was dissolved in diethyl ether and extracted (2X) with water, dried (Na₂SO₄) and evaporated to oil 45, 0.1366 g.

Trituration of the 45 with n-hexane gave a hexane-soluble fraction 46 (0.0422 g) and a hexane-insoluble fraction 47
(0.941 g). Tlc: silica gel HF 254+366, diethyl ether-hexanes (2:1), Rf (46) 0.58, 0.66(major); diethyl ether-acetone (1:1), Rf (47) 6 spots. 46: IR (CCl₄): 2820, 2710 (CHO); 1730(s), 1700(m), C=O; 1460, 1370, 1180, 1090 cm⁻¹. PMR (CDCl₃): 9.8(t, J=2.5Hz, 1H), 3.7(q, J=6Hz, 2H), 3.33 (s, 3H), 2.57 (t, J=2.5Hz, 1H), 2.52(m, 1H), 1.2-1.6 (14H), 0.88(dt, J=6Hz, 3H). Double irradiation: Irradiation of the 9.8 signal collapses 2.57t to a δ and 2.52m to a bs; Irradiation of the 3.75 signal collapses 2.57t and 2.52m to a bs at 5.5δ. 47: IR (CCl₄): 3450, 3300 (OH); 2820, 2720 (CHO); 1720, 1650 (C=O); 1400, 1220, 1110, 1060 cm⁻¹. PMR (CDCl₃): 9.8(s), no (CH₂)ₓ envelope.

M. Hydride Reduction of Deacetylstylocheilamide (II)
1. Sodium Borohydride Reduction.

A nitrogen flushed 250 ml round-bottomed flask equipped with a magnetic stirrer and pressure equalizing dropping funnel was charged with sodium borohydride (0.020 g, 2.12 mmol) in 90 ml redistilled (CaCl₂) 2-propanol. Deacetylstylocheilamide (0.5560 g, 0.12 mmol) in 10 ml 2-propanol was added to the stirred solution over 50 min. The reaction was allowed to stir at room temperature for 10 hr. Excess hydride was destroyed by the dropwise addition of saturated aqueous ammonium chloride. The solvent was removed in vacuo and the resulting oil partitioned between hexane and water. The separated organic layer was washed with water, dried (Na₂SO₄) and filtered through Celite. Evaporation of the solvent gave 48 (0.4534 g) as an unstable oil. Tlc analysis showed more than ten components.
Mixture 48 (0.4530 g) was placed onto a Sephadex LH-20 column and eluted with methanol. Beilstein positive material was removed in eight fractions which were combined (similar tlc) to give oils 49 (0.1207 g) and 50 (0.3086 g). Tlc: silica gel HF 253+366, ethylacetate-hexanes (1:1), Rf (49) 0.30, 0.37, 0.39; Rf (50) 0.05, 0.09, 0.19, 0.22, 0.26, 0.37.

49: IR (CCl₄): 3400 (OH), 2940, 2880, 2820, 1730, 1645, 1460, 1400, 1380, 1260, 1180, 1100, 960 cm⁻¹. PMR (CDCl₃): 5.98(bs, 1H), 5.5(bs, 2H), 4.46(Ld, J=15Hz, 1H), 3.68(bd, J=15Hz, 1H), 3.32(s, 3H).

50: IR (CCl₄): 3400(OH), 2940, 2880, 2820, 1730(weak), 1645, 1450, 1400, 1090, 960 cm⁻¹. PMR (CDCl₃): 6.0(m, 1H), 5.5(bs, 2H), 3.3(s, 3H), 3.1(m, 1H), 2.9(d, -CO-NCH₃).

Mixture 50 was subjected to gel filtration on Sephadex LH-20 and eluted with methanol. Beilstein positive and short wavelength UV absorbing material was removed in six tubes. The tubes were combined and concentrated to give 51 (0.1730 g) as an unstable oil. Tlc: silica gel HF 254+366, ethyl acetate-hexanes (1:1), Rf 0.05, 0.20, 0.26, 0.35, 0.41.

a. Esterification of 51. To a 10 ml round-bottomed flask, equipped with magnetic stirring, was added 51 (0.1730 g), pyridine and p-bromobenzoyl chloride (0.15 g). The reaction flask was then flushed with nitrogen and stirred at room temperature overnight. The solvent was removed at reduced pressure and the resulting semisolid partitioned between diethyl ether and water. The organic phase was
extracted with dilute sodium carbonate, water, dried (Na\textsubscript{2}SO\textsubscript{4}) and evaporated to a tan oil \(\delta 2\) (0.2050 g). Tlc: silica gel HF 254+366, diethyl ether-hexanes (3:1), Rf 0.19, 0.22*, 0.42*, 0.44, 0.52. *dark to short UV light.

Adsorption column chromatography of mixture \(\delta 2\) on BioSil A eluted with diethyl ether-hexanes (3:1) gave Beilstein positive material eluted in seven tubes. Tlc analysis indicated tubes 1 and 2 to be identical and containing one major and two minor components. The tubes were combined to give \(\delta 2\), 0.0281 g. Tlc: Silica gel HF 254+366, diethyl ether-hexanes (3:1), Rf 0.38, 0.40*, 0.51. *major component.

Preparative thin layer chromatography of \(\delta 2\) on silica gel HF 254+366 developed with diethyl ether-hexanes (3:1) showed three bands when the plate was viewed under short wavelength UV light. The central band (Rf 0.40-0.55) was scraped from the plate and extracted with acetone-methanol (1:1). Evaporation of the solvent gave \(XXIV\) (20.3 mg) as a pale yellow oil. \(XXIV\): \(M^+\) C\textsubscript{33}H\textsubscript{45}BrCIN\textsubscript{5}: measured 649.2173, calculated 649.2168. IR (CC\textsubscript{1}4): 1725, 1655 \(\text{cm}^{-1}\); 1255, 1170, 1090, 1005, 960 \(\text{cm}^{-1}\). PMR (CDCl\textsubscript{3}): 7.92 (d, \(J=8\text{Hz}\), 2H), 7.55 (d, \(J=8\text{Hz}\), 2H), 5.95 (bm, 2H), 5.45 (bm, 3H), 4.05 (m, 2H), 3.30 (s, 3H), 1.7 (bs, 3H). Mass spectrum at 70 ev (rel %): 649(M\textsubscript{+}, 2), 650(2), 651(5), 652(3); 596(M\textsubscript{+}−Cl, 10), 597(3), 598(10), 599(3); 193, 195(100).
b. Esterification of 49. Mixture 49 (0.1207 g) was placed into a 10 ml round-bottomed flask fitted with a magnetic stirrer. The flask was flushed with nitrogen and pyridine (5 ml) and p-bromobenzoyl chloride (Aldrich Chemical Co. B5920, 0.15 g) were added. The reaction was stirred at room temperature for 3 hr. The excess acid chloride was decomposed by the addition of water.

The solvent was removed in vacuo to yield a semisolid which on trituration with hexane gave a precipitate of p-bromobenzoic acid. The solid acid was removed by filtration and the filtrate concentrated to a tan oil 53 (0.1226 g). Tlc: silica gel HF 254+366, diethyl ether-hexanes (3:1), Rf 0.21, 0.22, 0.41.

Mixture 53 was placed onto a 20 x 20 x 0.05 cm preparative silica gel HF 254+366 tlc plate. Multiple development with diethyl ether-hexanes (9:5) and visualization of the air dried plates under short wavelength UV light indicated two major bands. The bands were scraped from the plate and separately extracted with dichloromethane-methanol (3:1). Filtration and evaporation of the solvent gave oils 54 (0.0432 g) and XXV (0.0540 g). Tlc: silica gel HF 254+366, ethyl acetate-hexanes (1:1), Rf (54) 0.37, 0.38, 0.42; Rf (XXV) 0.46. XXV: IR (CCl₄): 3080, 2940, 2860, 1730, 1655, 1460, 1400, 1260, 1170, 1100, 1000, 960 cm⁻¹. PMR (CDCl₃): 7.9(d, J=9Hz, 2H), 7.55(d, J=9Hz, 2H), 5.95(m, 2H), 5.45(m, 2H), 4.96(p, J=6.5Hz, 1H), 4.15(m, 1H), 4.02 (m, 1H), 3.32(s), 3.15(m, 1H), 2.85(m, 4H), 2.25(bm, 6-8H),
1.3 (bs, 8-10H), 1.2 (d, J=7Hz, 3H?), 0.90 (bt, J=6Hz, 3H).

Mass spectrum at 70 ev (rel %): 709, 710, 711, 712, 713, 714, 715; 694, 695, 696, 697, 698, 699; 674, 675, 676, 677, 687, 769, 680; 666, 667, 668, 669, 670, 671, 672, 673, 674; 509, 510, 511, 512, 513; 366(100), 371(30); 315(25); 280(80); 143(95).

N. Synthesis

1. Preparation of 5-Decyne (XXVI)87

To an oven-dried three-necked 250 ml round-bottomed flask fitted with a Hershberg stirrer, dry ice condenser and dropping funnel, was added approximately 175 ml liquid ammonia. Sodium (2.20 g 0.096 mol) in small cubes was added, followed by dropwise addition of 1-hexyne (Farchan Research Laboratories AA600) (11.3 ml, 8.21 g, 0.10 mol). After this addition the colorless solution was refluxed for 0.5 hr and freshly distilled n-butyl bromide (10.8 ml, 13.7 g, 0.10 mol) added dropwise over 0.5 hr to the stirred solution. The solution was then stirred at reflux for 5.5 hr and the ammonia was allowed to boil off (trapped by a water scrubber).

The resulting oily solid was dissolved in diethyl ether and washed repeatedly with water until neutral. The organic phase was dried (MgSO4) and concentrated to a clear oil 54. Distillation of 54 gave 5-decyne, XXVI, as a colorless oil, bp 170-174° (760mm Hg) (lit. bp 177 at 751mm Hg). GPLC: 6ft x 1/8in 3% OV-17, oven 75°, retention time 3.10 min.
2. Preparation of trans-5-Chlorodec-5-ene (XXVII)

5-Decyne (2.06 g, 0.015 mol) was dissolved in 15 ml dried glacial acetic acid. Tetraethylammonium chloride (1.66 g, 0.10 mol) was added followed by the addition of acetyl chloride (1.42 ml, 1.56 g, 0.02 mol). Water (0.27 ml, 0.27 g, 0.015 mol) was added slowly to the rapidly stirred solution. The homogeneous reaction mixture was stirred at room temperature for 8 d.

The reaction mixture was poured into hexane and the organic phase was extracted repeatedly with water until neutral. The yellowish hexane solution was dried (Na₂SO₄) and evaporated to a yellow oil 55 (1.94 g). Beilstein test for halogens was positive. GPLC (6ft x 1/8in 3% OV-17, oven 75°) showed two peaks of equal area.

Column chromatography of 55 on Bio-Sil A (200-325 mesh) and elution with n-hexane gave 0.813 g (31.5%) of trans-5-chlorodec-5-ene, XXVII. PMR (neat): 5.38(tt, J=7, 1.5 Hz, 1H), 4.2(p, J=7Hz, 4H), 1.36(m, 8H), 0.906(t, J=7Hz, 6H).
CMR (CDCl₃, ppm from TMS): SP²: 134.5, 125.1. SP³: 39.1, 30.8, 29.6, 28.2, 22.2, 21.6, 13.7, 13.7.

3. Preparation of 3-Hydroxydecanoic Acid (XXVIII)

Octanal (Aldrich Chemical Co., 12.0 g, 0.094 mol) and ethyl bromoacetate (Aldrich Chemical Co., 43.5 g, 0.284 mol) were dissolved in 70 ml dry benzene and added dropwise to
activated zinc (16.2 g, 0.25 mol, acid washed, methanol rinse) and covered with dry benzene. Iodine was required to catalyze the reaction mixture, which slowly turned grey. When nearly all zinc had reacted, the reaction was refluxed for 2.5 hr. The cooled reaction was poured into cold, dilute sulfuric acid and extracted with diethyl ether. The orange organic layer was separated, washed with water (2X), dried (MgSO$_4$) and concentrated to an oil, 56.

Oil 56 was dissolved in methanol and 6N potassium hydroxide was added to about pH 10. The solution was stirred at room temperature for one hour, then refluxed for 1 hr. The solvent was removed at reduced pressure and the resulting basic aqueous solution was extracted with diethyl ether. The separated basic solution was acidified with concentrated phosphoric acid to about pH 2 and extracted with dichloromethane. The organic phase was separated, washed with water, dried (MgSO$_4$) and evaporated to a semisolid 57. Recrystallization of 57 from cyclohexane gave 3-hydroxydecanoic acid XXVIII as a white solid (10.2 g, 58%), mp 55.0-55.5°.

IR (CCl$_4$): 3600-2400 (acid tailing), 1715 (C=O), 1420, 1290, 1220 cm$^{-1}$. PMR (CDCl$_3$): 7.35(bs, 2H, D$_2$O exchangeable); 4.02(m, 1H); 2.52(s, 1H), 2.45(d, J=5Hz, 1H); 2.6-2.2 (12H); 0.88δ(bt, J=6Hz, 3H). CMR (CDCl$_3$, ppm from TMS): SP$^2$, C=O: 177.0. SP$^3$: 68.2(d), 41.2, 36.4, 31.8, 29.6, 29.5, 25.5, 22.6, 14.0.

4. Preparation of Methyl 3-Hydroxydecanoate (XXIX)

3-Hydroxydecanoic acid, XXVIII, (1.50 g, 8.0 mmol) was dissolved in 25 ml diethyl ether, cooled to 0° and ethereal
diazomethane (freshly prepared from Diazald) added dropwise until the bright yellow color remained. After stirring at 0° for 1.5 hr, the solution was extracted with dilute sodium bicarbonate, water and dried (Na₂SO₄). Evaporation of the solvent gave the methyl ester, XXIX, as a clear oil (1.62 g, 100%). IR (neat): 3550 (OH); 1740 (C=O); 1370, 1250, 1170 cm⁻¹ (C-O). PMR (CDCl₃): 4.00 (m, 1H), 3.68 (s, 3H), 3.25 (bs, 1H, D₂O exchangeable), 4.48 (s, 1H), 4.42 (d, J=3Hz, 1H), 4.48 and 4.42 signals collapse to a bs at 4.46 upon irradiation at 4.00δ), 1.7–1.2 (12H), 0.89δ (bt, J=6Hz, 3H). CMR (CDCl₃, ppm from TMS): SF², C=O: 173.0. SF³: 68.1 (d), 51.5 (q), 41.9, 37.1, 32.1, 29.9, 29.7, 25.8, 22.9, 14.2.

5. Preparation of Methyl 3-Methoxydecanoate (XXX)

Attempted 0-methylation of the hydrogen bonded hydroxy ester with iodomethane and potassium carbonate in methanol or diazomethane and fluoroboric acid in dichloromethane or diazomethane and boron trifluoride in diethyl ether failed to give the desired methyl ether.

Methyl 3-hydroxydecanoate, XXIX, (0.420 g, 0.210 mmol) was dissolved in 10 ml chloroform, dried over magnesium sulfate and filtered into a dry round-bottomed flask. The solution was flushed with nitrogen and silver oxide (0.35 g) catalyst added. Three portions of iodomethane (0.5 ml each, 1.5 ml, 24.2 mmol) were added at 15 min intervals. The reaction was stirred at room temperature for 2 d and the catalyst removed by filtration through Celite. Evaporation of the solvent gave a greenish oil 58 (0.428 g, 95%).
3-methoxydecanoate \( l740(ester) \), sp\(^2\), c=o:

Oil \( S8 \) was chromatographed on Bio-Sil A (200-325 mesh) eluted with diethyl ether-hexanes (s:l) to give methyl 3-methoxydecanoate \( XXX \) as an oil (0.4011 g, 89\%). IR (CCl\(_4\)): 1740(ester); 1270, 1160 cm\(^{-1}\) (C-O). CMR (CDCl\(_3\), ppm from TMS): sp\(^2\), C=O: 173.0. sp\(^3\): 77.7, 56.8, 51.4, 41.4, 36.5, 31.7, 29.2, 29.1, 25.4, 22.5, 13.9. PMR (CDCl\(_3\)):

6. Preparation of N-allylhexamide (\( XXXI \))

Hexanoic acid (Aldrich Chemical Co., 5.80 g, 0.05 mol) was placed into a 100 ml 3-necked round-bottomed flask fitted with a condenser topped with a drying tube (Drierite), addition funnel and magnetic stirrer. The apparatus was flushed with dry nitrogen and thionyl chloride (4.35 ml, 7.08 g, 0.06 mol) was added dropwise. The reaction was stirred at room temperature for 3 hr, then refluxed for an additional 30 min. The solution was cooled in an ice-water bath and thoroughly flushed with nitrogen.

Allylamine (Aldrich Chemical Co., 12.6 mol, 11.6 g, 0.10 mol) was added dropwise via the dropping funnel into the rapidly stirred solution. The solution turned dark brown and when approximately half of the amine had been added, the reaction became gummy and had to be stirred by shaking and swirling the apparatus. After addition of the amine, the reaction mixture was allowed to stand at room
temperature for 15 min. Water was added, followed by diethyl ether. The dark organic phase was extracted with water (8X) until the pH was neutral then extracted with dilute hydrochloric acid. The separated dark organic phase was extracted sequentially with water, 5% aqueous potassium hydroxide and water. The dark brown organic layer was dried (MgSO₄) and concentrated to an amber oil, XXXI, 7.44 g (0.488 mol, 97%).

XXXI: IR (CCl₄): 3470, 3400(NH); 3080(=CH); 1650(amide); 1525, 1460, 1420, 1250, 980, 910 (RCH=CH₂). PMR (CDCl₃): 7.95(bt, 1H), 5.7(m, 1H), 5.1(m, 2H), 3.78(t, J=5Hz, 2H), 2.2(t, J=6Hz, 2H), 1.8-1.1(m, 6H), 0.88(t, J=5.5Hz, 3H).

CMR (CDCl₃): SP², C=O: 173.3. SP², C=C: 134.3(d), 115.2(t). SP³, C-C: 41.6(t), 36.1(t), 31.3(t), 25.2(t), 22.2(t), 13.6(q). Mass spectrum at 70 ev: m/e 155 (M⁺, 10%).
O. Proton Magnetic Resonance Spectra
Figure 3. 220MHz PMR (CDCl₃) Spectrum of Stylocheilamide (I)
Figure 4. 100 MHz PMR (CDCl₃) Spectrum of Stylocheilamide (I)
Figure 5. 100 MHz PMR (acetone-$d_6$) of Stylocheilamide (I)
Figure 6. 100 MHz PMR (acetone-$d_6$-benzene-$d_6$, 1:2) Spectrum of Stylocheilamide (I)
Figure 7. 100 MHz PMR (CDCl$_3$) Spectrum of Deacetylstylocheilamide (II)
Figure 8. 100 MHz PMR (acetone-$d_6$) of Deacetyl$s_3$tylocheilamide (II)
Figure 9. 100 MHz PMR (benzene-d₆) Spectrum of Deacetylstylocheilamide (II)
Figure 10. 100 MHz PMR (CDCl₃) Spectrum of Dioctylphthalate (III)
Figure 11. 100 MHz PMR (CDCl₃) Spectrum of tri(n-butoxyethoxy)phosphate (IV)
Figure 12. 100 MHz PMR (CDCl₃) Spectrum of Chimyl alcohol (V)
Figure 13. 100 MHz NMR (CDCl3) of VI.
Figure 16. 100 MHz PMR (CDCl$_3$-benzene-$_d_6$, 2:1) Spectrum of IX
Figure 17. 100 MHz PMR (CDCl₃) Spectrum of XI
Figure 21. 100 MHz PMR (CDCl₃) Spectrum of XVIII
Figure 22. 100 MHz PMR (CDCl₃) Spectrum of XIX
Figure 27. 100 MHz PMR (CDCl₃) Spectrum of trans-5-Chlorodec-5-ene (XXVII)
Figure 28. 100 MHz PMR (CDCl₃) Spectrum of Methyl 3-Hydroxydeconate (XXIX)
Figure 29. 100 MHz PMR (CDCl₃) Spectrum of N-allylhexamide (XXXI)
P. Infrared Spectra
Figure 31. Infrared (film) Spectrum of Stylocheilamide (I)
Figure 32. Infrared (film) Spectrum of Deacetylstylocheilamide (II)
Figure 33. Infrared (1 mm cell, CC\textsubscript{4}) Spectrum of Dioctylphthalate (III)
Figure 34. Infrared (1 mm cell, CCl₄) Spectrum of Chimyl Alcohol (V)
Figure 35. Infrared (1 mm cell, CCl₄) Spectrum of VI
Figure 36. Infrared (1 mm cell, CCl₄) Spectrum of VII
Figure 37. Infrared (1 mm cell, CCl₄) Spectrum of IX
Figure 38. Infrared (1 mm cell, CCl₄) Spectrum of XI
Figure 39. Infrared (0.1 mm cell, CCl₄) Spectrum of XII
Figure 40. Infrared (1 mm cell, CH₂Cl₂) Spectrum of XVI
Figure 41. Infrared (1 mm cell, CCl₄) Spectrum of XVII
Figure 42. Infrared (1 mm cell, CCl₄) Spectrum of XVIII
Figure 43. Infrared (1 mm cell, CCl₄) Spectrum of XIX
Figure 44. Infrared (0.1 mm cell, CCl₄) Spectrum of XXI
Figure 45. Infrared (0.1 mm cell, CCl₄) Spectrum of XXII
Figure 46. Infrared (0.1 mm cell, CCl₄) Spectrum of XXIII
Figure 47. Infrared (film) Spectrum of N-Allylhexamide (XXXI)
Figure 48. Infrared (1 mm cell, CCl₄) Spectrum of 25
Q. Mass Spectra
Figure 49. Mass Spectrum (21 ev) of Stylocheilamide (I)
Figure 50. Mass Spectrum (20 ev) of Deacetylstylocheilamide (II)
Figure 52. Mass Spectrum (70 ev) of VII
Figure 56. Mass Spectrum (20 ev) of XVII
Figure 57. Mass Spectrum (70 ev) of XVIII
Figure 58. Mass Spectrum (20 and 70 ev) of XIX
Figure 59. Mass Spectrum (70 ev) of XXI
III. Discussion

A. Choice of Sea Hare Species

Sea hares are conspicuous members of the Hawaiian fauna. _Stylocheilus longicauda_ was chosen for the present study for two reasons: 1. previous work in this laboratory on the structure of aplysia toxin provided an initial supply of diethyl ether soluble oil isolated from _S. longicauda_ and 2. the animal was plentiful, especially in June when it moved onto a shallow, flat reef to spawn. Although large supplies (usually 15 kg/yr) were available in Kaneohe Bay for part of the year, the concentration of stylocheilamide did not decrease if the animals were stored in the freezer (-10°). The yield of stylocheilamide was the same whether recently collected or animals frozen for two years were extracted.

_S. longicauda_ was collected on Oahu at Kahala Beach Park and off Ala Moana Beach Park on the south shore and at Kaaawa Beach Park and Kaneohe Bay near the Kaneohe Bay Yacht Club on the east shore. Stylocheilamide was present in the sea hares collected from all locations.

Preliminary stability studies indicated that stylocheilamide was stable in refluxing ethanol (bp 78°) for 0.5 hr. Thin layer chromatography studies showed the compound to be unstable to acid or base. Stylocheilamide was adsorbed onto silica gel, a small amount of ethanol added and the solvent refluxed for 0.5 hr. Thin layer chromatographic examination of the solution after reflux showed that stylocheilamide was
unaltered. Silica gel chromatography did not affect the structure of the compound. Column chromatography on neutral alumina caused a rearrangement of stylocheilamide and was not used in the purification procedure.

Most of the animals used in the present study were collected in Kaneohe Bay, east of the Kaneohe Bay Yacht Club. The structure of stylocheilamide required approximately 32 kg of S. longicauda which yielded nearly 2.5 g of the natural product.

B. Homogeneity of Stylocheilamide

The homogeneity of oils is always difficult to ascertain. One or two dimensional thin layer chromatography of stylocheilamide in a number of solvent systems failed to produce additional spots visible by ultraviolet light, iodine staining, Dragendorff complexing or sulfuric acid–heat charring. The molecular weight of stylocheilamide was measured as 525±11 on a Hitachi Perkin-Elmer 115 molecular weight apparatus. Use of this isopiestic method gives an average molecular weight, hence stylocheilamide cannot be a mixture containing compounds differing by more than eleven mass units, but does not exclude the possibility of isomers.

The molecular composition was determined by high resolution mass spectroscopy on a Perkin-Elmer MS-9 and a Varian Mat-311 instrument. Chemical ionization mass spectroscopy confirmed that the ion at m/e 525 is indeed the molecular ion and also showed the absence of significant contaminant compounds at other molecular weights. Chemical analysis
confirmed the presence of nitrogen and chlorine and the absence of phosphorus and sulfur. Thus the elemental composition of $\text{C}_{28}\text{H}_{44}\text{ClNO}_6$ for stylocheilamide was secure.

However, the problem of isomers remained. The proton magnetic resonance spectrum lacks the sharp signal definition and resolution associated with "pure" compounds. More significantly, two signals which appear at 6.26 and 6.14\(\delta\) (H-3') integrate for one proton. Double irradiation studies show these signals to be long range coupled to a slightly broadened singlet at 4.02\(\delta\) (H-1'). Likewise, there are two singlets at 2.90 and 2.98\(\delta\) (H-10') which integrate for a total of three protons. If the pmr spectrum is determined in dimethyl sulfoxide-d$_6$ at 100°, the signals at 2.90 and 2.98\(\delta\) collapse to a single line at 2.95\(\delta\). Thus it appears that stylocheilamide is a mixture of isomers.

The nature of the isomerism remains in doubt. A mixture of conformers would present no difficulty to chemical degradation, because irrespective of conformational differences, identical compounds are produced. Ozonolysis of a mixture of cis- and trans-hex-2-ene generates only acetaldehyde and butanal. If the conformational relationship is not destroyed in the degradative process, then one is in no worse position than initially. If, on the other hand, the isomerism is positional, then chemical degradation will always lead to mixtures, the complexity of which depends on the nature of positional isomerism.

Sephadex LH-20 gel filtration separates compounds on the basis of molecular size. Chromatography of stylocheilamide
by this technique shows little spreading, an indication that the sample is homogeneous in size. This tends to indicate that no drastic positional isomerism exists.

The carbon magnetic resonance spectrum determined in deuterochloroform or hexadeuterobenzene shows doubletting with diminished intensity of several signals, an indication of conformational isomerism. The ketone and acetate signals (202.8, 202.3 and 172.8, 172.5 ppm, respectively) are doubletted, probably as a result of conformational (boat-chair ?) isomerization. Two olefinic signals, H-2' and H-3', (132.1, 131.6 and 121.5, 120.2 ppm) likewise appear as doublets and could indicate cis and trans forms about this bond. Two other signals (61.2, 60.6 and 50.8, 48.3 ppm) are obvious doublets. If these signals are counted as single carbon atoms, the cmr carbon count agrees with the elemental formula determined by high resolution mass spectrometry. The absence of obvious doubletting in the sp³ carbon region supports the hypothesis of conformational isomerism.

C. Characterization of Functional Groups in Stylocheilamide

When added to silver ion, labile chlorine atoms will give a precipitate of silver chloride. The failure of stylocheilamide to give a silver chloride precipitate, even after refluxing with ethanolic silver nitrate, indicates that the chlorine is aromatic, vinylic, beta to an ether linkage, at the bridgehead of a small ring system, or alpha to a carbonyl. There are no aromatic signals in the pmr
or cmr spectra and no indication of aromaticity in the infrared spectrum. The unsaturation number does not allow for a tricyclic system.

In 1-chloropropene the cmr chemical shift of C-1 is 126.2 ppm for cis (methyl and chlorine) and 128.9 ppm for the trans form while that of the β-carbon is 119.6 for cis and 117.2 ppm for the trans compound\(^9\). To determine the carbon chemical shifts of vinyl halogens in larger molecules, \(\text{trans-5-chlorodec-5-ene}\) was prepared. The chemical shift of the chlorine-bearing carbon (C-5) is 134.5 ppm while the shift of C-6 is 125.1 ppm. Stylocheilamide has olefinic carbon absorptions at 132.3, 131.6 (singlets) for the vinyl chloride carbon (C-2'), and 121.5, 120.2 ppm (doublets) for the α-olefinic carbon (C-3'). Likewise, the proton chemical shift of the vinyl hydrogen in \(\text{trans-5-chlorodec-5-ene}\) is at 5.38 (tt) with long range coupling of 1.5Hz to the methylene. In stylocheilamide the proton chemical shift of H-3', the vinyl hydrogen, is 6.3 with long range methylene coupling of 1.5Hz.

Additional chemical support of a vinyl chloride comes from lithium aluminum hydride reduction of stylocheilamide. The basic product of the reaction contains chlorine. However, the chlorine atom is lost upon platinum-catalyzed hydrogenation.

The infrared spectrum of stylocheilamide shows a band at 1720 cm\(^{-1}\) assignable to a ketone\(^9\). The cmr spectrum also shows ketone functionality by a signal at 202 ppm. The
failure of the compound to form a 2,4-dinitrophenylhydrazone derivative indicates that the ketone is hindered. Chemical evidence for ketone assignment comes from sodium borohydride reduction of the natural product. The infrared spectrum of the product of this reaction shows strong -OH stretching and no 1720 cm\(^{-1}\) carbonyl band. The position of the stretching frequency dictates that the ketone is saturated and either aliphatic or in a ring larger than five carbons.

The infrared spectrum of stylocheilamide also shows carbonyl absorption at 1745 cm\(^{-1}\) and strong C-O stretching vibrations at 1230 cm\(^{-1}\) consistent with an acetate. The pmr spectrum shows a three-proton singlet at 2.00δ while the cmr spectrum has a carbonyl signal at 172 ppm, both consistent with acetate assignment. Attempted hydrolysis of the acetate in acid or base leads to rearrangement of the carbon skeleton and loss of the acetate functionality.

Support for an amide carbonyl comes from the infrared spectrum. The spectrum of stylocheilamide does not show NH or OH absorptions. The strong stretching vibration at 1655 cm\(^{-1}\) is assigned to a tertiary amide\(^{97}\). Consistent with this assignment is a cmr signal at 169 ppm\(^{98}\). Chemical support comes from the fact that stylocheilamide is a neutral, water-insoluble compound, while the product of lithium aluminum hydride reduction is basic.

The pmr spectrum contains a three-proton singlet at 3.30δ assignable to a methyl ether. The stretching frequency range of aliphatic methyl ethers\(^ {99}\) is 2830-2815 cm\(^{-1}\).
and stylocheilamide has an infrared absorption at 2820 cm\(^{-1}\). The mass spectrum also shows an M\(^{+}\)-OCH\(_3\) at m/e 494.

The cmr spectrum also shows two additional olefinic carbons at 131(d) and 126(d) ppm. The pmr spectrum shows a two-proton broad triplet at 5.45\(\delta\) while the infrared spectrum shows a \textit{trans} olefinic C-H out-of-plane deformation at 960 cm\(^{-1}\).

The chemical formula for stylocheilamide allows for seven sites of unsaturation. Quantitative platinum hydrogenation, cmr and pmr spectra support the presence of only two olefinic linkages. Three additional sites of unsaturation and four oxygen atoms are accounted for by an amide, a ketone and an acetate. This accounts for all but two unsaturation sites, hence the compound contains two rings.

![XXXIV](image)

In cuauhtemone\(^{101}\) \textit{XXXIV} the epoxide carbons resonate at 59.6(d) and 59.9(s) ppm. Substitution on one of the epoxide carbons affects both carbon chemical shifts, but tertiary carbons are normally deshielded more than secondary carbons. Substitution of an electronegative group at C-3 of propylene oxide causes C-2 to resonate at lower field but
C-1 is little changed\textsuperscript{102}. For example, in propylene oxide C-1 and C-2 resonate at 47.7 and 48.1 ppm, while in epichlorohydrin these carbons resonate at 47.0 and 51.6 ppm. In a series of indene diepoxides, Anet\textsuperscript{102} reports a chemical shift range for the epoxide carbons to be 43.0 to 74.6 ppm.

The high resolution mass spectrum of stylocheilamide shows a large peak at m/e 143 of composition C\textsubscript{9}H\textsubscript{19}O. The unsaturation number of this fragment is 0.5 indicating a saturated ion. A large peak at m/e 383 and a strong metastable ion at m/e 279.5 support the one-step loss of a C\textsubscript{9}H\textsubscript{18}O radical directly from the molecular ion. The pmr spectrum shows a large methylene envelope at 1.25, indicative of a long chain aliphatic fragment. This indicates that stylocheilamide contains an aliphatic chain consistent with part structure A.

![Structure A](image)
Thus, all the unsaturation sites and heteroatoms are identified. Stylocheilamide contains the following structural elements.

![Chemical structure of Stylocheilamide]

Stylocheilamide contains six oxygen atoms. Five of the oxygens are contained in the ketone, amide, acetate and methoxy groups. The environment of one oxygen atom was in doubt for some time. The infrared spectrum does not show OH stretching and the pmr spectrum contains no exchangeable protons. Three carbonyl bands seen in the infrared and cmr spectra are assigned. The infrared spectrum also excludes such oxygen-containing functional groups as isocyanate, nitro, nitroso, nitrate ester and N-oxide. Thus, by elimination, the remaining oxygen atom is an ether.

There are seven carbon atoms which by cmr are shown to be bonded to oxygen or nitrogen. The methyl ether, C-15, (56.3 ppm) is a quartet in the off-resonance spectrum. The
carbon atom (triplet) at 50.8, 48.3 ppm was shown by on-
resonance $^{13}$C-proton decoupling to be correlated to the pmr
broadened singlet at 4.02δ and is assigned as a methylene
adjacent to an olefin and the amide nitrogen, C-1'. The
signal at 41.2 ppm is assigned to a methyl, C-10', on a
nitrogen atom. On-resonance cmr studies demonstrated that
the signal at 76.3 ppm (doublet) correlates with the pmr
one-proton signal at 5.05δ and was assigned to an acetate
methine at C-7'. The acetate signals are absent in
deaacetylstylocheilamide. These firm assignments account
for 4 of the 7 C-N,O carbons.

Thus the number of unidentified hetero-bonded carbons
is reduced to three. The cmr resonance at 80.5 ppm (doublet)
was assigned as a methine bearing the methoxy group. Methyl
3-methoxydecanoate was prepared and the methine bearing the
methoxy group in this molecule absorbs at 77.7 ppm, in
reasonable chemical shift agreement for the above assignment.
Roberts et al.\textsuperscript{103} indicate that the carbon chemical shift
for the C-2 carbon in exo-2-hydroxynorbornane is 74.5 ppm.
Upon methylation of the hydroxy group the carbon would be
expected to shift downfield by about 5 ppm\textsuperscript{104}.

Therefore, only two oxygen bonded carbons remain to be
assigned; a doublet at 64.0 ppm and a singlet at 61.2 ppm.
Lukas et al.\textsuperscript{105} report that the range for epoxide carbons
in a six-membered saturated A-ring of steroids is 50-60
ppm.
Table 4

Correlation of PMR and CMR Resonances of Stylocheilamide by Single Frequency On Resonance Decoupling

<table>
<thead>
<tr>
<th>PMR, ppm</th>
<th>CMR, ppm</th>
<th>Number of Protons</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.28</td>
<td>121.5 d</td>
<td>1</td>
</tr>
<tr>
<td>6.17</td>
<td>120.2 d</td>
<td></td>
</tr>
<tr>
<td>5.50</td>
<td>131.0 d</td>
<td>1</td>
</tr>
<tr>
<td>5.50</td>
<td>126.8 d</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>131.6 s</td>
<td></td>
</tr>
<tr>
<td></td>
<td>132.1 s</td>
<td></td>
</tr>
<tr>
<td>5.15</td>
<td>76.3 d</td>
<td>1</td>
</tr>
<tr>
<td>4.05</td>
<td>50.8 t</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>48.3 t</td>
<td></td>
</tr>
<tr>
<td>3.62</td>
<td>64.0 d</td>
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</tr>
<tr>
<td>3.32</td>
<td>56.3 q</td>
<td>3</td>
</tr>
<tr>
<td>3.15</td>
<td>80.5 d</td>
<td>1</td>
</tr>
<tr>
<td>2.93</td>
<td>41.2 d</td>
<td>1</td>
</tr>
</tbody>
</table>
D. PMR and CMR Spectra of Stylocheilamide

The CMR spectrum and quantitative hydrogenation of stylocheilamide indicate the presence of only two double bonds. The PMR signal at 5.5δ is a broad triplet which integrates for two protons and is similar in shape to olefinic absorptions in unsaturated fatty acids\textsuperscript{106}. This symmetry indicates the similarity of the magnetic environment on both sides of the olefinic bond. CMR heteronuclear decoupling of the proton signal at 5.5δ causes the C-13 signal at 131.0 and 126.8 ppm (doublets in the off-resonance) to collapse to singlets.

The proton magnetic resonance (PMR) of stylocheilamide, Figure 3 shows a two proton broad triplet at 5.5δ (H-4,5). Irradiation of these protons causes a broad triplet (J=7Hz) at 2.18δ, H-6, to collapse to a doublet (J=6Hz) pointing downfield. Irradiation of H-6 sharpens the 5.5δ signal but also sharpens a quartet (J=6Hz) at 3.1δ (H-7). Likewise, decoupling of H-7 collapses H-6 to a broad singlet. This indicates part structure B.

\[-\text{C=CH-CH}_{2}\text{-CH-O,N} \quad B\]

Single frequency on-resonance decoupling (SFORD) of the protons at 3.1δ and observing the CMR spectrum shows that signals at 82 and 41 ppm are collapsed to singlets. Thus the proton signal at 3.1δ contains two protons of coincidental chemical shift.
The CMR signal at 80.5 ppm (doublet in the off-resonance) is assigned to a methine bearing a methoxyl group (H-7). In ovalicin\(^\text{140}\), \(XXXV\), the methoxyl methine is reported at 85.9 ppm while in 3-methoxy-1-butanol this carbon resonates at 75.3 ppm.

The PMR chemical shift of H-7 (3.1\(\delta\)) does not allow it to contain additional electron withdrawing groups and must be attached to carbon, hence the part structure \(B\) can be expanded to \(C\).

\[
\begin{align*}
&\begin{array}{c}
\text{-C-CH}_2\text{-CH=CH-CH}_2\text{-CH-OCCH}_3
\end{array} \\
&\text{C}
\end{align*}
\]

The PMR signal at 5.15\(\delta\) is not olefinic as shown by enhancement of a signal in the CMR spectrum at 76.3 ppm (off-resonance doublet) upon SFORD. This signal is assigned to an acetoxy methine, H-7'. Homonuclear proton decoupling of H-7' results in sharpening of the multiplet at 3.1\(\delta\). Likewise, decoupling of the 3.1\(\delta\) signal not only causes the H-7' (5.15\(\delta\)) signal to sharpen but collapses a three proton methyl doublet (H-11') at 0.95\(\delta\) to a singlet. Irradiation of the H-11' methyl group results in collapse of the 3.1\(\delta\) multiplet to a broad singlet.
SFORD has shown the correspondence between the PMR 3.10 signal and the CMR 41.2 ppm resonance. These chemical shifts exclude the direct attachment of a hetero atom at this center. The proton chemical shift allows for this methine to be adjacent to a double bond or carbonyl group. If the methine is adjacent to an olefin it must be attached to a fully substituted center. The only such center is the vinyl chloride carbon, part structure D.

\[
\text{AcO} \quad \text{CH}_3 \quad \text{Cl} \\
\quad \text{-CH} \quad \text{CH} \quad \text{C} = \text{CH} \quad \text{D}
\]

The other possibility is to have the methine adjacent to a carbonyl as in part structure E.

\[
\text{AcO} \quad \text{CH}_3 \quad \text{O} \\
\quad \text{-CH} \quad \text{CH} \quad \text{C} \quad \text{E}
\]

The proton signal at 6.26 and 6.14\(\delta\) integrates for one hydrogen and shows long range (1.5Hz) coupling to a broadened methylene signal at 4.02\(\delta\) (H-1'). The only change in the PMR spectrum upon irradiation of H-1' is sharpening of the 6.26 and 6.14\(\delta\) signals. This implies part structure F.

\[
\text{-CH} = \text{CCl} \quad \text{-CH}_2 \quad \text{X} \quad \text{F}
\]

The chemical shift of the H-1' (4.02\(\delta\)) requires that it be bonded to an electronegative group. Correlation of the PMR and CMR spectra shows the relationship between H-1' (4.02\(\delta\)) and the CMR signals at 50.8 and 48.3 (t) ppm. The CMR chemical shift is a little too high field for X=oxygen in
part structure $F$. In farnesol$^{107}$, which has a methylene (C-12) equivalent of $X=\text{oxygen}$, part structure $F$, the CMR chemical shift of C-12 is at 58.8 ppm.

A chemical shift of 6.26 or 6.14$\delta$ for an olefinic proton vicinal to a chlorine seems a bit low-field. Pascual, Meier and Simon$^{108}$ have empirically derived a formula for determining the chemical shifts of olefinic signals by use of a substituent shielding coefficient, $Z$. Their correlation is based on a total of 1070 proton signals and 99.6% of the experimental values were within 0.45$\delta$ (74% within 0.15$\delta$) of the calculated value. Using their equation and $Z$-factors, the calculated chemical shift of the olefinic proton in part structure $F$ should be 5.73$\delta$ for $\text{cis}$ hydrogen and chlorine, 5.49$\delta$ for $\text{trans}$ hydrogen and chlorine.

The difference between observed and calculated values could come from the use of an incorrect substituent: $-\text{N-R}_1\text{R}_2$ may not be equivalent to $-\text{N-CO-}$, which is not on the substituent list. For example, the $Z$-value for a $\text{cis}$ alkoxy moiety is -1.06 while the $Z$-value for a similar acetoxy is -0.40, a difference of 0.6 ppm.

Shoolery$^{109}$ has devised a set of empirical rules for determining the chemical shift of methylene protons in compounds of the type $Y-\text{CH}_2-X$. The shielding constant for an acetoxy ($-\text{OCOCH}_3$) group is 3.13, for hydroxy ($-\text{OH}$) 2.56 and for alkoxy ($-\text{OR}$) 2.36. Again, amide moieties are not listed, but if the magnitude of the shielding constant between a disubstituted amine ($-\text{N-R}_1\text{R}_2$) and an amide ($-\text{NCO-}$) is as large as the difference between alkoxy and acetoxy
substituents, considerable variation between the calculated and experimental shift values would be expected. The calculated chemical shift of the methylene group in part structure $F$ using Shoolery's rules ($X=-NR_2$) is $3.12\delta$. However, there is a downfield shift of $0.77\delta$ going from an alkoxy to an acetoxy group. The same downfield shift might be expected for the change from amine to amide, and the adjusted chemical shift value for the methylene would then be $3.98\delta$, as compared with an experimental value of $4.02\delta$.

This allows part structure $F$ to be expanded to $G$.

\[
-\text{CH}=\text{CCl}-\text{CH}_2-\text{N}-\overset{\text{O}}{\text{C}}- 
\]

In order to support the calculated methylene chemical shifts of $3.98\delta$ in $G$, N-allylhexamide ($XXXI$) was synthesized. In $XXXI$ the allylic methylene PMR chemical shift is $3.78\delta$ while the CMR chemical shift is $41.6$ ppm. The CMR chemical shift of $41.6$ ppm seems a bit high-field considering that the chemical shift of the allylic methylene in allylamine ($\text{CH}_2=\text{CH}-\text{CH}_2-\text{NH}_2$) is $43.6$ ppm. However, SFORD experiments clearly show the correlation of the PMR $3.78\delta$ and CMR $41.6$ ppm signals.

The discrepancy of $0.24\delta$ between the experimental and calculated proton chemical shift values for the methylene group in part structure $G$ can be explained by including the deshielding effect of the chlorine atom. Because of the higher electronegativity of Cl (3.15) versus H (2.20), it is anticipated that a halogenated olefin would be more
deshielding to an adjacent methylene than would an olefinic linkage without a halogen. Electronegative atom deshielding effects fall off rapidly as the number of intervening bonds increase and lends support to the chlorine being alpha rather than beta to the methylene as in G.

Levy and Nelson\textsuperscript{110} report that the CMR spectrum of N,N-di-n-butylformamide shows four resolved pairs of aliphatic resonances. The pairing is attributed to the carbons being cisoid and transoid to the formyl hydrogen. Consistent with this interpretation is the fact that the difference between cisoid and transoid aliphatic carbon chemical shifts gets smaller farther away from the carbonyl group. The restricted motion about the amide C-N bond results in an upfield steric compression shift for the carbons cisoid to the carbonyl. However, in XXXI single resonances are observed for each aliphatic carbon indicating that C-N bond rotation (or nitrogen inversion) in secondary amides is rapid or that only one conformer was present. The CMR spectrum of XXXI remained unchanged at -25°.

Part structures D and G have the vinyl chloride linkage in common and cannot both be correct. Part structure D was eliminated by examination of the PMR spectrum of compound \textit{VI}, the platinum hydrogenation product of stylocheilamide. Hydrogenation of the double bond and hydrogenolysis of the chlorine will cause the methyl group in D to shift to higher field in the PMR spectrum. The chemical shift of the methyl group, H-11', in \textit{VI} is not shifted from the natural product.
Hence, part structure $G$ is the correct choice in stylocheilamide.

Part structure $D$ and $E$ represent two possible environments for a methine proton chemical shift of 3.1$\delta$. Based on methyl group PMR chemical shifts required by olefin hydrogenation, part structure $D$ was eliminated in stylocheilamide, indicating that alternative part structure $E$ is part of the natural product.

E. Degradations of Stylocheilamide

1. Hydrogenation

The rate of hydrogenation of the two double bonds in stylocheilamide is sufficiently different that it was possible to hydrogenate selectively the least hindered bond. Thus, mild atmospheric hydrogenation of the natural product in ethyl acetate using palladium-on-carbon gave dihydro-stylocheilamide, $VII$. The PMR spectrum (Figure 14) indicates the disappearance of the two proton signal at 5.5$\delta$ and the peak shape of the signal from 2.1 to 2.6$\delta$ has changed and diminished in area. Likewise, the width of the long chain methylene envelope has increased extending downfield to 1.9$\delta$ and shows a definite shoulder (1.4$\delta$). These results are consistent with reduction of a double bond in a long chain.

The mass spectrum, Figure 52, of $VII$ indicates a molecular ion at m/e 527, 529 (3%) corresponding to a formula of $C_{28}H_{46}ClNO_6$. The mass spectrum also shows peaks at m/e 512, 514 ($M^+\text{-CH}_3$), 492 ($M^+\text{-Cl}$). The probable fragmentation of $VII$ is shown in Chart 1.
Chart 1. Mass Spectral Fragmentation Pattern of VII

m/e 428

m/e 527

m/e 492

m/e 432
Platinum hydrogenation of I gave VI as an oil which had no low-field olefinic signals. The mass spectrum exhibits a molecular ion at m/e 495 (5%) for C_{28}H_{49}NO_{6}. Expected losses of methyl (M^+ -15), methanol (M^+ -32) and acetic acid (M^+ -60) are observed. No carbons are lost in the reaction, thereby excluding the possibility of allylic ethers.

The infrared absorptions of the three carbonyls in stylocheilamide (1745, 1720 and 1655 cm\(^{-1}\)) remain unchanged in VI. This demonstrates lack of conjugation with a carbonyl group. The infrared spectrum of VI does not show NH or OH absorption (3200-3600 cm\(^{-1}\)) indicating that the sole nitrogen atom in stylocheilamide is not part of an imine, nitrile, nitroso or nitro; it also excludes a peroxide linkage. Ketone reduction normally requires more vigorous conditions (3-4 atm)\(^{111}\).

2. Lithium Aluminum Hydride Reduction

Lithium aluminum hydride (LAH) reduction of I frequently led to mixtures. On one occasion the product isolated after workup had a strong carbonyl absorption at 1775 cm\(^{-1}\). The least complex mixture was obtained by using anhydrous diethyl ether (stored over sodium) and adding a large excess (20 fold) of LAH. It was necessary to reflux the solvent under a dry, inert atmosphere (nitrogen) for a minimum of 1 hr to ensure exclusion of moisture. The compound dissolved in dry diethyl ether was added slowly to the cold, stirred hydride suspension.
Use of a strong reducing agent, lithium aluminum hydride, resulted in the formation of an amine, mixture 25. The infrared spectrum of 25, Figure 48, shows no carbonyl absorption (1750-1650 cm\(^{-1}\)) but shows the expected strong OH stretching frequency near 3400 cm\(^{-1}\).

Acetylation (pyridine and acetic anhydride) of 25 gave a diacetate, compound XII, which gave a strongly positive Beilstein test for halogens. The infrared spectrum, Figure 39, shows very low frequency hydroxyl stretching near 3100 cm\(^{-1}\), strong acetate carbonyl absorption at 1735 cm\(^{-1}\) and olefinic absorption at 1600 and 955 cm\(^{-1}\). The absence of a strong band near 1650 cm\(^{-1}\) assignable to an acetamide dictates that the amide in stylocheilamide is tertiary.

The mass spectrum of XII shows a molecular ion at m/e 557, 559 (50% corresponding to C\(_{30}\)H\(_{52}\)ClNO\(_6\) with the anticipated losses of methyl, chloride, acetate and acetic acid. Also seen is a loss of 143 mass units corresponding to C\(_9\)H\(_{19}\)O. There is also a small peak at m/e 599 (1.5%) for a triacetate which loses methyl (m/e 584), chloride (m/e 564) and ketene (m/e 557). There is no peak at m/e 539 for the loss of acetic acid from the triacetate. Loss of ketene from the molecular ion is usually observed from vinyl acetates, vicinal diacetates or acetoacetates. Chart 2 shows a probable fragmentation of XII.

The mass spectrum of XII does not show loss of water from the molecular ion or from high mass fragment ions. One might expect a tertiary alcohol to lose water easily by a
Chart 2. Mass Spectral Fragmentation of XII and XIII

\[ \text{m/e 559 } \text{C}_{30}\text{H}_{52}\text{ClNO}_6, \text{XII} \]
\[ \text{m/e 561 } \text{C}_{30}\text{H}_{48}\text{D}_4\text{ClNO}_6, \text{XIII} \]
Most thermal dehydrations are 1,2-eliminations; however, deuterium labeling experiments indicate that dehydration from molecular ions occurs predominantly by 1,4-elimination through a six-membered intermediate. The primary route for most cyclanols is α-cleavage of the C-O bond with the charge residing on oxygen.

The PMR spectrum, Figure 18, of XII shows two acetate methyl signals at 2.03 and 2.06δ, an aliphatic methoxy singlet at 3.30δ and a broad N-methyl singlet at 2.2δ. The broadened one-proton singlet at 6.04δ is the vinyl halogen absorption, while the broad two-proton triplet at 5.45δ is the disubstituted olefin in the long chain. The signal at 5.95δ is a two-proton resonance assigned to the two acetate methines.

The PMR spectrum of the natural product has an acetate methine at 5.05δ (75.6 ppm in the CMR) which is shifted upfield by 0.1 ppm after reduction and reacetylation, indicating that the acetate methine of I is in the magnetic field of a group that was reduced. The methine is not more than three bonds removed from the ketone or amide carbonyls. The acetate cannot be α to the ketone or a large (0.5δ) downfield shift would have been expected. The acetate is probably β to the ketone carbonyl although an α-position to the amide carbonyl cannot be eliminated and part structure H is proposed for the natural product, thereby lending support to part structure E.
The CMR spectrum of XII shows two signals assignable to carbons bearing acetates at 75.6 (d) and 75.1 (d) ppm. The acetyl carbonyls both resonate at 170.1 ppm while the acetate methyls absorb at 20.8 and 20.6 ppm. Four olefinic signals are seen at 137.5(s), 131.3(d), 126.9(d) and 118.4(d). The C-2' olefinic carbon in XII is shifted downfield by 5.5 ppm from stylocheilamide.

Minor changes in the electron density about a nucleus create variations in the repulsions between electrons. The result is an alteration of the effective nuclear charge by increasing the negative charge about the atom. This is equivalent to electron expansion and results in an upfield shift of the chemical resonance. For proton-proton compression shifts, Grant and Cheney\textsuperscript{113} have derived an equation to predict the magnitude of the induced shift based on proton-proton distance and on the angle between the proton-proton axis and the perturbed HC\textsuperscript{13} bond. Even though the bond in question is Cl-\textsuperscript{13}C, the direction of the shift should be the same and the downfield shift of this resonance reflects a relief of steric compression.

The chemical formula for XII, C\textsubscript{30}H\textsubscript{52}ClNO\textsubscript{6}, has an unsaturation number of five. Two sites of unsaturation and four oxygen atoms are accounted for by the acetates. Two olefinic linkages reduce the unsaturation number to one. The remaining oxygens are accounted for by an alcohol and a
methyl ether. The remaining unsaturation number dictates a ring which is either carbocyclic or a nitrogen heterocycle.

The two acetates must be derived from an acetate (hydrolysis and acetylation of the hydroxy) and a ketone (reduction and acetylation of the hydroxy) while the tertiary hydroxyl most likely results from oxirane or oxetane ring opening. It is doubtful that the hydroxyl was introduced during the reaction because the hydride reduction was carried out under nitrogen. Stylocheilamide is a monoacetate of a secondary alcohol, thus excluding acetylation at the tertiary center.

3. Lithium Aluminum Deuteride Reduction

Reduction of stylocheilamide with lithium aluminum deuteride in diethyl ether under nitrogen and acetylation of the product with acetic anhydride in pyridine yielded a diacetyl amine, compound XIII. High resolution mass spectrometry gave a formula of $C_{30}H_{48}D_4ClN_6$. Incorporation of four deuterium atoms is consistent with amide reduction (2-D), ketone reduction (1-D) and ring opening (1-D). Analysis of fragment ions, Chart 2, indicates that the deuterium atoms are all contained in the polar portion of the molecule.

A common fragmentation route of amines is cleavage of a carbon-carbon bond $\alpha$-phà to the amine with loss of the largest alkyl group. This type of fragmentation will not increase the unsaturation number. An even-electron fragment at m/e 346, $C_{16}H_{25}ClN_5O$, (m/e 350, $C_{16}H_{21}D_4ClN_5O$), has an unsaturation number of 4.5, hence only one site of
unsaturation is present in the expelled radical. No ion is observed at m/e 270 (C\textsubscript{17}H\textsubscript{34}NO) corresponding to cleavage of the \textit{alpha} carbon-carbon bond on the other side of the nitrogen, indicating the formation of an unstable cation or radical.

4. Lemieux Oxidation

Lemieux oxidation of stylocheilamide under basic conditions gave a complex mixture and low weight recovery. However, under neutral conditions a single acidic product, compound \textit{XVI}, was isolated. The mass spectrum of \textit{XVI} did not exhibit a molecular ion; the highest mass peak was seen at m/e 187 (M\textsuperscript{+}-15). A probable fragmentation route of \textit{XVII} is given in Chart 3.

The infrared spectrum of \textit{XVI}, Figure 40, shows carbonyl stretching at 1715 cm\textsuperscript{-1} and acid tailing 3400-2400 cm\textsuperscript{-1}. The strong C=O stretching frequency at 1070 cm\textsuperscript{-1} suggests an ether\textsuperscript{115}.

The PMR spectrum of \textit{XVI}, Figure 19, shows a broad one-proton signal at 10.7\textdelta assign able to the carboxylic acid hydrogen. The three-proton singlet at 3.38\textdelta is assigned as a methyl ether supported by the IR. The one-proton signal at 3.74\textdelta indicates the direct attachment of oxygen, but the resonance is too high-field for an ester methine and is therefore assigned to an ether methine. Irradiation of the 3.74\textdelta pentet collapses a doublet of doublets at 2.51\textdelta to a broad singlet. Irradiation of the 2.51\textdelta resonance collapses the 3.74\textdelta signal to a triplet, suggesting part structure \textit{I}.
Chart 3. Mass Spectral Fragmentation Pattern of XVII

- m/e 127
- m/e 184
- m/e 186

no M$^+$ (m/e 216)

- m/e 59
- m/e 143
- m/e 201

- m/e 117
The chemical shift of the 2.51δ methylene doublet of doublets is in accord with an adjacent carbonyl group. The multiplicity of the signal argues for hindered rotation about the methylene-methine bond. Part structure I can be extended to J.

The methylene envelope at 2.3δ integrates for about 12 protons supporting structure XVI.

Treatment of acid XVI with diazomethane in diethyl ether yielded a methyl ester, XVII. The infrared spectrum of XVII, Figure 41, lacked the acid tailing of XVI, but showed a strong carbonyl stretching frequency at 1740 cm⁻¹. Chemical analysis of XVII was in excellent agreement for C₁₂H₂₄O₃, but again the mass spectrum failed to show a molecular ion at m/e 216. The highest mass peak was at m/e 201 (15%), indicating loss of methyl from the molecular ion.

The PMR spectrum, Figure 20, is consistent with structure XVII indicating the methyl ester singlet at 3.68δ. which partially overlaps the methoxyl methine resonance. Irradiation at 3.65δ collapses a methylene doublet of doublets at 2.47δ to a singlet.

In order to prove the structure of XVII, a synthesis of racemic methyl 3-methoxydecanoate was undertaken. The
Reformatsky reaction\textsuperscript{89} was used to condense octanal and ethyl bromoacetate. The hydroxy ester was not isolated; instead the ester was hydrolyzed with basic aqueous methanol. The hydroxy acid was isolated as a semisolid which was recrystallized from cyclohexane to give a 58\% yield of 3-hydroxydecanoic acid. The acid was quantitatively methylated with diazomethane in diethyl ether to yield racemic methyl 3-hydroxydecanoate. The PMR spectrum, Figure shows the nonequivalence of the methylene protons adjacent to the carbonyl.

Attempted O-methylation of the hydrogen bonded methyl 3-hydroxydecanoate with iodomethane and potassium carbonate in methanol\textsuperscript{91} or diazomethane and fluoboric acid in dichloromethane\textsuperscript{92} or diazomethane and boron trifluoride in diethyl ether\textsuperscript{93} failed to give the desired methyl ether.

Methyl 3-methoxydecanoate was successfully prepared by treatment of methyl 3-hydroxydecanoate in dry chloroform with iodomethane and silver oxide as catalyst\textsuperscript{90}. The reaction was slow but an 89\% yield of racemic methyl 3-methoxydecanoate (XXX) was obtained. The PMR spectrum of XXX was nearly identical to XVII, the ester from the natural product. In XVII the methylene adjacent to the carbonyl is a doublet of doublets (J=6, 3Hz) while in XXX this signal is a doublet (J=6Hz).

Thus, Lemieux oxidation demonstrates the existence of part structure $K$ in stylocheilamide.
The neutral fraction from the Lemieux oxidation did not show an aldehyde resonance in the PMR near 9.5δ. The complex mixture did not contain a major component and no meaningful data was obtained by further investigation of this material.

5. Ozonolysis of LAH Product (XII)

Ozonolysis of stylocheilamide in dichloromethane or hexane gave complex mixtures. When the ozonolysis was carried out in methanol, the product was much less complex. Reductive workup with either methyl sulfide or palladium catalyzed hydrogenation gave the same products. Hydrogenation was the preferred workup, because it gave higher yields of aldehyde in a model system.

Trituration of the stylocheilamide ozonolysis oil with petroleum ether gave 37. The PMR spectrum of 37, Figure 30, contaminated by the corresponding dimethyl acetal, clearly shows the presence of L. Irradiation of the aldehyde triplet at 9.6δ collapsed the 2.4δ multiplet to a doublet while irradiation of the 2.4δ methylene multiplet collapsed the aldehyde signal to a singlet. This demonstrates the presence of a CHO-CH₂-CH unit in L. Irradiation of the methoxyl methine multiplet (3.65δ) caused the 2.4δ multiplet to collapse to a broad singlet.

Isolation of the aldehyde from the ozonolysis of stylocheilamide lends support to part structure K in the natural product.
The structure of the petroleum ether insoluble oil, 38, will be discussed below.

Ozonolysis of XII in methanol using reductive workup gave two compounds XVIII and XIX. The structural similarity of the two compounds was shown by comparing their PMR spectra, Figures 21 and 22.

Compound XIX crystallized from cyclohexane to give small, fine rods. The infrared spectrum, Figure 37, shows no -OH or -NH stretching and only weak C-H stretching at 2980 cm\(^{-1}\). Two carbonyl stretching frequencies of approximately equal intensity are present at 1760 and 1740 cm\(^{-1}\). The strong C-O stretching vibration at 1210 cm\(^{-1}\), although at the lower end of the frequency range, is assigned to an acetate C-O\(^{116}\). Aliphatic ethers which absorb in the frequency range 1150-1070 cm\(^{-1}\) are excluded.

It would be odd to ozonize an olefinic compound and not generate a carbonyl. There are reports in the literature of the products of ozonolysis that underwent further reaction either thermally or on workup\(^{117}\). For example, patchouline, XXXVI, gave XXXVII on workup.

\[ XXXVI \rightarrow XXXVII \]
The infrared absorption at 1740 cm\(^{-1}\) in \(XIX\) was therefore suspected to represent two carbonyls, one an acetate and the other a ketone. The normal range for five-membered-ring ketones\(^9\) is 1750-1740 cm\(^{-1}\) but this is difficult to rationalize in terms of the PMR spectrum. It was thought that a six-membered-ring ketone fits the data better.

The infrared frequency of alpha halogen substituted ketones is shifted to higher wavenumber by about 20 cm\(^{-1}\), and it was thought that a similar shift might be noted with alpha acetoxy substitution. There appears to be no systematic study of the effect of alpha-hydroxy or alpha-acetoxy substitution on ketone infrared absorption. The infrared spectral data available on substituted steroids is extensive and several examples, Table 5, of alpha-hydroxy and alpha-acetoxyketone carbonyl shifts are given to support a shift of +5 to 10 cm\(^{-1}\) for alpha-hydroxy and +15-30 cm\(^{-1}\) for alpha-acetoxy substitution.

Thus the 1740 cm\(^{-1}\) carbonyl band in \(XIX\) is assigned to an acetate and an alpha-acetoxy ketone in a six-membered ring.

The PMR spectrum, Figure 22, of \(XIX\) shows a three proton doublet at 0.95\(\delta\). Irradiation of a multiplet at 2.74\(\delta\) collapsed the acetoxy methine signal at 5.35\(\delta\) to a broad singlet suggesting part structure \(L\).

\[
\begin{array}{c}
\text{AcO} \\
\text{CH}_3 \\
\text{OAc}
\end{array}
- \text{CH} - \text{CH} - \text{CH} - \quad L
\]
Table 5
Effect of Ketone Infrared Frequency by α-Substitution

<table>
<thead>
<tr>
<th>Ketone (cm⁻¹)</th>
<th>Acetoxy (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1700 (β)</td>
<td>-</td>
</tr>
<tr>
<td>1705 (α)</td>
<td>-</td>
</tr>
<tr>
<td>1705</td>
<td>-</td>
</tr>
<tr>
<td>1730</td>
<td>1755</td>
</tr>
<tr>
<td>1705</td>
<td>-</td>
</tr>
<tr>
<td>1715</td>
<td>1740</td>
</tr>
</tbody>
</table>
The acetoxy methine signals overlap but look like a doublet \((J=5.5\text{Hz})\) and a pentet \((J=5\text{Hz})\), thus allowing expansion of the part structure to \(M\).

\[
\begin{array}{cccc}
O & OAc & CH_3 & OAc \\
\text{-} & \text{C} & \text{-CH} & \text{-CH} & \text{-CH} & \text{-CH}_2 & \text{M}
\end{array}
\]

Irradiation of the acetoxy methines sharpened the multiplet at 2.74\(^\delta\) to a broadened quartet and affected the region near 1.8\(^\delta\). The two acetate methyls resonate at 2.15 and 2.08\(^\delta\).

The highest mass peak in the mass spectrum of \(XIX\) is at \text{m/e} 186 indicating a formula of \(C_8H_{10}O_5\) (unsaturation number 4) or \(C_7H_{22}O_5\) (unsaturation number 3). Part structure \(M\) contains ten carbon atoms, hence the ion at \text{m/e} 186 is not the molecular ion.

An x-ray crystal structure determination\(^{118}\) demonstrated the structure as \(XIX\).

The magnetic equivalence of the C-2 and C-4 acetoxy methine protons of \(XIX\) is somewhat unexpected. The low field resonance of the C-4 methine probably results from the
anisotropy of the ketone group and places the methine in the deshielding region of the carbonyl.

Compound \( XVIII \), isolated from the ozonolysis of \( XII \), was more polar on a tlc plate than \( XIX \) and was insoluble in petroleum ether. The ultraviolet maximum of \( XVIII \) is at 217 nm with a low extinction coefficient of 158, suggesting \( n^+\pi^* \) transition of an ester carbonyl. The infrared spectrum, Figure 42, supports this hypothesis with a strong, broadened carbonyl stretching absorption at 1730 cm\(^{-1}\).

The complexity and broadness of the infrared signal in the region 1300-1200 cm\(^{-1}\) also suggests several types of C-O-C stretching frequencies of esters. The high frequency value for the maximum absorbance (1230 cm\(^{-1}\)) is in good agreement for C-O-C asymmetric stretching of acetates. The infrared spectrum also shows -OH stretching at 3600 and 3500 cm\(^{-1}\). The medium strength bands at 1160 and 1370 cm\(^{-1}\) can be assigned to C-O stretching of a tertiary alcohol\(^{119} \).

The PMR spectrum of \( XVIII \), Figure 21, shows three three-proton singlets at 3.74, 2.10 and 2.08\( \delta \) assignable to carbomethoxy and two acetates, respectively. The methyl doublet at 0.85\( \delta \) collapses to a singlet upon irradiation of the broad multiplet centered at 2.37\( \delta \). This same irradiation collapses the 4.82\( \delta \) acetoxy methine to a singlet and the 5.00\( \delta \) acetoxy methine is collapsed to a broad singlet suggesting part structure \( L \).

Irradiation of a multiplet at 1.88\( \delta \) collapses the 5.00\( \delta \) methine to a doublet allowing placement of a methylene
adjacent to one of the acetoxy methines and a full substituted carbon on the other, allowing expansion of part structure L to N.

\[
\text{OAc} \quad \text{CH}_3 \quad \text{OAc} \quad \text{- C - CH - CH - CH - CH}_2 - \quad \text{N}
\]

Based on $XIX$, the structure is a cyclohexane skeleton so that $N$ can be cyclized to $O$.

A hydroxyl and carbomethoxy group must still be attached.

High resolution mass spectrometry of $XVIII$ assigned the highest mass peak at m/e 288 to the formula $C_{13}H_{20}O_7$ (unsaturated number 4). Part structure $O$ has a composition of $C_{11}H_{16}O_4$ so that the unplaced hydroxyl and carbomethoxyl groups must be attached to the same carbon atom to give $XVIII$. If the hydroxyl is attached to another than a tertiary position, a PMR signal near 48 would be expected for a hydroxymethine (-CH-OH), but no such signal is observed.

The mass spectral fragmentation route of $XVIII$ is given in Chart 4.
Chart 4. Mass Spectral Fragmentation Pattern of XVIII

XVIII

\[
\begin{array}{c}
\text{AcO} \quad \text{OH} \\
\text{AcO} \quad \text{OH} \\
\text{m/e 288}
\end{array}
\]

\[
\begin{array}{c}
\text{COOMe}^+ \\
\text{COOMe}^+ \\
\text{m/e 228}
\end{array}
\]

\[
\begin{array}{c}
\text{HO} \quad \text{OH} \\
\text{OH} \\
\text{m/e 186}
\end{array}
\]

\[
\begin{array}{c}
\text{HO} \\
\text{OH} \\
\text{m/e 127}
\end{array}
\]

\[
\begin{array}{c}
\text{OH} \\
\text{OH} \\
\text{m/e 109}
\end{array}
\]

\[
\begin{array}{c}
\text{AcO} \quad \text{OH} \\
\text{AcO} \quad \text{OH} \\
\text{m/e 228}
\end{array}
\]

\[
\begin{array}{c}
\text{AcO} \quad \text{OAc} \\
\text{m/e 186}
\end{array}
\]

\[
\begin{array}{c}
\text{AcO} \quad \text{OAc} \\
\text{m/e 108}
\end{array}
\]

\[
\begin{array}{c}
\text{AcO} \quad \text{OAc} \\
\text{m/e 168}
\end{array}
\]

\[
\begin{array}{c}
\text{AcO} \quad \text{OAc} \\
\text{m/e 108}
\end{array}
\]
The isolation of a methyl ester from the ozonolysis of a vinyl chloride in methanol fixes the position of halogen attachment\textsuperscript{120} and suggests part structure $P$ in stylocheilamide,

![Chemical Structure](image)

where one of the oxygens is a ketone and the other attached to an acetyl group.

However, part structure $Q$, obtained by PMR homonuclear spin decoupling, argues for attachment of the chlorine at the adjacent olefinic carbon atom to give part structure $Q$. 
The isolation of a cyclohexanone (compound XIX) and a methyl cyclohexylcarboxylate (compound XVIII) resulting from the ozonolysis of a chloromethylidene (-ClC=) bond seems strange at first. It was anticipated that ozonolysis of the chloromethylidene portion of XII would generate only a methyl ester. Support for the formation of both XVIII and XIX from the ozonolysis of this bond comes from the ozonolysis of 1-vinyl cyclohexanol, XXXVIII, which does not give the anticipated aldehyde; the reaction products are cyclohexanone, formic acid and formaldehyde.\textsuperscript{121}

\[
\text{XXXVIII} \quad \xrightarrow{\text{O}} \quad \text{O} + \text{CHO} + \text{HCOOH}
\]

6. Ozonolysis of Stylocheilamide

Ozone will oxidize amines to N-oxides, resulting in the loss of the nitrogen-containing fragment either during the workup due to water solubility, or by irreversible binding to the adsorbent during chromatography. Amides, however, are unreactive to ozone. Hydrogenation studies of stylocheilamide demonstrated a significant rate difference for the reduction of the hindered olefin and the olefin in the aliphatic chain. It was anticipated that low temperature selective ozonolysis of the less hindered alkyl olefin would be possible.
Low temperature ozonolysis of stylocheilamide in dry methanol yielded an ozonide which was decomposed by palladium-catalyzed hydrogenation. Ozone was bubbled into the solution until the exit tube indicated the presence of ozone so that more than one molar equivalent of ozone was present. Distillation of a portion of the methanol into an aqueous methanolic dimedon solution failed to give a precipitate, demonstrating the absence of small aldehydes. Evaporation of the remaining methanol gave an oil, the IR spectrum of which showed the presence of aldehyde(s) and amide.

Trituration of the oil with low boiling petroleum ether gave a soluble oil 37 and an insoluble oil 38. The PMR spectrum of 37 clearly showed the presence of 3-methoxy-decanal (L) contaminated by a small amount of 3-methoxy-decanal dimethyl acetal. The infrared and PMR spectra of 38 indicated the presence of an amide and the absence of an aldehyde.

Mixture 38 was purified by gel filtration on Sephadex LH-20 to give an oil which was subjected to preparative silica gel tlc in dichloromethane-methanol (20:1) to give 39. Silica gel preparative tlc of 39 developed with diethyl ether yielded 40. Preparative tlc of 40 with dichloromethane-methanol (100:9) gave XXI as a homogeneous oil.

The infrared spectrum of XXI, Figure 44, shows the absence of -OH or -NH but indicates three carbonyls at 1745 (acetate), 1730 (ketone) and 1650 cm\(^{-1}\) (amide). The strong
C-O stretching frequency, unshifted from the natural product, is due to the acetate and the epoxide. Three strong bands at 1115, 1075 and 1055 cm⁻¹ are indicative of acetal C-O-C-O-C stretching vibrations. The 1115 cm⁻¹ band is due to a C-H deformation vibration perturbed by the neighboring C-O group and is characteristic of acetals. Thus, the newly formed aldehyde is masked as an acetal.

Excess ozonolysis of stylocheilamide results in cleavage of the vinyl chloride to generate an acid chloride. Reaction of the acid chloride with methanol generates a methyl ester and hydrochloric acid. In dry alcoholic media, acids catalyze acetal and ketal formation. The failure of the ketone to form a dimethyl ketal under the reaction conditions reflects the hindered nature of the carbonyl and the difference in the rate of formation of acetals over ketals.

The CMR spectrum of XXI clearly shows the acetal carbon resonance at 103.7 ppm, a doublet in the off-resonance. The carbonyls which resonate at 202.5 (ketone), 172.4 (acetate) and 169.9 ppm (amide) are only slightly shifted from the natural product. The olefinic signal at 131.9 ppm (s) is the chloromethylidene carbon (-CCl=) while the signal at 121.8 ppm (d) is the alpha-carbon (-CCl=CH-). The acetoxy carbon, 75.7 ppm (d) and epoxide carbons, 63.9 (d) and 60.9 ppm (s), are shifted less than 0.7 ppm from their position in stylocheilamide.

The one-proton signal centered at 6.1δ shows coupling to a two-proton broadened singlet at 4.04δ. The chemical
shift of the methylene signal requires an electron withdrawing group and part structure $R$ or $S$.

$$\text{HClC} = \text{C-CH}_2 \cdot \text{X} \quad R \quad \text{CH} = \text{CCl-CH}_2 \cdot \text{X} \quad S$$

The CMR signal at 53.1 ppm (q) integrates for more than one carbon and is assigned as the two acetal methyl groups. The PMR spectrum of $XXI$, Figure 23, shows a six proton singlet at 3.35$\delta$ for the dimethyl acetal group. The acetal methine proton is a triplet at 4.40$\delta$ which on irradiation collapses a multiplet near 1.8$\delta$. Irradiation at 1.85$\delta$ collapses the dimethyl acetal methine to a singlet. The chemical shift of 1.85$\delta$ for a methylene suggests its attachment to carbon and part structure $T$.

$$\text{C} - \text{CH}_2 - \text{CH} \cdot \text{OCH}_3)_2 \quad T$$

Irradiation of a methine multiplet near 3.2$\delta$ causes the methyl resonance at 1.0$\delta$ to collapse to a broadened singlet and perturbs the signal near 2.4$\delta$. The chemical shift of 3.2$\delta$ for a methine suggests an adjacent carbonyl. This methine is collapsed to a broadened quartet upon irradiation of the acetoxy methyl methine at 5.13$\delta$, consistent with part structure $E$ in $XXI$.

The mass spectrum of $XXI$, Figure 59, shows a small molecular ion at m/e 417(2%) with peaks at m/e 382(M$^+$-Cl, 70), 131(60) and 99(28). High resolution mass spectrometry assigned the formula $C_6H_{11}O_3$, unsaturation number 1.5, to the ion at m/e 131. This fragment can arise by alpha
cleavage of the amide N-CO- bond with charge retention on the carbonyl to give the acylium ion fragment $a$.

\[ ^+\text{O=CC-CH}_2-\text{CH}_2-\text{CH(OCCH}_3)\text{_2} \]

Additional support for fragment $a$ is found in further fragmentation to give ions at m/e 99, $C_5H_7O_2^+$, for loss of methanol from $a$ and a McLafferty rearrangement of the acylium ion $a$ to give protonated ketene at m/e 43.

The loss of chlorine from a vinyl chloride should not be a significant fragmentation route. However, in $XXI$ the large peak (70%) at m/e 382 results from the loss of chlorine probably accompanied by a Claisen rearrangement to fragment $c$, Chart 5. The resulting carbonium ion is allylic and resonance-stabilized by the oxygen accounting for the paucity of peaks in the mass range 200-330. The ion at m/e 350 results from loss of methanol from fragment $c$.

High resolution mass spectrometry assigned the formula $C_{19}H_{28}ClNO_7$ to $XXI$. The formula and the data presented above demonstrate that the long chain aliphatic portion of the natural product had undergone oxidative cleavage. The CMR chemical shifts of the ketone, acetate, amide and olefinic carbons in $XXI$ are unshifted from the natural product. The carbonyl bands in the infrared spectrum are also unshifted from stylocheilamide indicating that the compound isolated had not undergone structural rearrangement.

The substituted cyclohexane ring in stylocheilamide has been established as part structure $P$ or $Q$. Addition of part
Chart 5. Mass Spectral Fragmentation Pattern of XXI

\[
\text{m/e 382, } \text{c} \\
(C_{19}H_{28}NO_7)
\]

\[
\text{m/e 350} \\
(C_{19}H_{28}ClNO_7)
\]

\[
\text{m/e 252} \\
(C_{13}H_{18}NO_4)
\]

\[
\text{m/e 131, } \alpha \\
(C_6H_{11}O_3)
\]

\[
\text{m/e 99} \\
\text{m/e 71}
\]
structure $S$ where $X=N$ expands the part structures to $U$ or $V$.

The acylium ion fragment $\alpha$ can be attached to the nitrogen to form the amide. Addition of a methyl group to the nitrogen forms the tertiary amide, expanding the part structures to $W$ or $X$. 
One of the oxygen atoms is a ketone and the other part of an acetoxy group giving four possible structures ($XXI-XXI-c$) for the ozonolysis product.

$$XXI \ R = 1 \quad XXI-b \ R = 1$$

$$XXI-a \ R = 2 \quad XXI-c \ R = 2$$

PMR data support structures $XXI$ or $XXI-a$ because the olefinic signal ($H_a$: 6.25, 6.16$\delta$) is a triplet with a small coupling constant of 2Hz. The value of the coupling constant is consistent with allylic coupling$^{124}$. However, chemical evidence supports structures $XXI-b$ or $XXI-c$.

Excess ozonolysis yielded a methyl ester attached to the cyclohexane moiety, not to the amide as expected from $XXI$ or $XXI-a$.

Aromatization of the cyclohexane ring of deacetylstylocheilamide ($vide infra$) yields a phenol which fixes the
position of the ketone and supports \(XXI\) or \(XXI-b\) as the correct structure for the ozonolysis product.

7. Rearrangement of Stylocheilamide

Stylocheilamide underwent a base-catalyzed rearrangement to give deacetylstylocheilamide and an isomeric compound \(IX\). The rearrangement occurred under very mild conditions and, in one instance, occurred when lithium aluminum hydride was inadvertently suspended in moist diethyl ether. Compound \(IX\) was also isolated in varying yields from the reaction of stylocheilamide with sodium borohydride in 2-propanol. Reaction of stylocheilamide with sodium acetate in refluxing 95% ethanol gave a 52% yield of \(IX\). The other product was deacetylstylocheilamide.

High resolution mass spectrometry assigns the chemical formula of \(\text{C}_{26}\text{H}_{40}\text{ClNO}_4\) to \(IX\), unsaturation number 7, differing from the natural product by the elements of acetic acid. The infrared spectrum of \(IX\) in carbon tetrachloride, Figure 37, shows the two carbonyls at 1775 (\(\gamma\)-lactone ?) and 1655 cm\(^{-1}\) (amide), the ketone and acetate functional groups of the natural product being expelled or transformed in the reaction. Support for the \(\gamma\)-lactone is found in the CMR which shows a resonance at 172.4 ppm.

The PMR spectra of \(IX\), Figures 15 and 16, contain a three-proton overlapping multiplet at 6.25\(\delta\) and a four-proton broad absorbance at 5.5\(\delta\). Conformation of seven olefinic protons is found in the CMR spectrum which exhibits off-resonance doublets at 130.9, 130.7, 125.1, 120.6 and
114.7 ppm and an off-resonance triplet at 127.1 ppm. Two olefinic resonances which occur at 144.7 and 133.5 ppm are singlets.

Hydrogenation of IX at atmospheric pressure with palladium-on-carbon in ethyl acetate gave a mixture from which was isolated X and XI as isomeric $C_{26}H_{47}NO_4$ compounds. The unsaturation number is 3. The formula difference between IX and XI indicates hydrogenolysis of the chlorine and uptake of three moles of hydrogen. The PMR spectrum of XI, Figure 17, shows the absence of olefinic signals while the infrared spectrum, Figure 38, shows the lactone shifted to lower wavenumber at 1760 cm$^{-1}$.

It thus appears that IX contains only three double bonds and that the excessive CMR olefinic signals represent isomers. The lactone and amide account for two additional sites of unsaturation and indicate that IX contains two rings.

The rearrangement, under mild conditions, of 4,5-epoxy-ketones to cyclopropanes has been documented by Gaoni$^{125,126}$. The mechanism is intramolecular carbanion opening of the
epoxide ring followed by protonation of the alcoholate.

A Favorskii type rearrangement of the epoxylacetone $XXXIX$ in aqueous sodium hydroxide was reported by Chang and Pelletier$^{127}$. The initially formed hydroxy acid lactonizes and the product contains an aldehyde.

House and Gilmore$^{128}$ have made an extensive study of the base-catalyzed rearrangement of piperitone oxide, $XL$. Rearrangement of $XL$ in methanolic potassium hydroxide gave
seven products, however 71% of the yield is hydroxy cyclopentanoic acid derivatives. The cyclopentanoic acids are

![Chemical structure](image)

\[ XL \]

thought to result from acid hydrolysis during workup of an initially formed cyclopropanone.

However, in the steroid series, Rausch and LeMahieu\textsuperscript{129} report that 4α,5-epoxy-cholestan-3-one (XL\textsubscript{I}) failed to give

\[ XL\textsubscript{II} \]

\[ XL\textsubscript{III} \]
the expected Favorskii ring contracted product, yielding instead the vinyl methyl ether \( \text{XLII} \).

Under acidic conditions, Meinwald and Cardoff\(^ {130} \) report that 3,4-epoxyketones can rearrange to lactones (\( \text{XLIII} + \text{XLIV} \), the cleavage being mechanistically similar to the 1,3-diol cleavage.

\[
\begin{align*}
\text{XLIII} & \quad \text{H}^+ \\
\text{XLIV} & 
\end{align*}
\]

The isolation of compound \( \text{XVIII} \) as a degradation product of stylocheilamide infers part structure \( Y \) or \( Z \) to the natural product. It was anticipated that the base-catalyzed
rearrangement product would be identified and the mechanism of its formation could be used to exclude either part structure Y or Z. This turned out not to be the case. Compound IX does not contain cyclopropyl protons in the PMR, thereby excluding products of the type isolated by Gaoni.\textsuperscript{125}

A Favorskii type mechanism such as that of House and Gilmore\textsuperscript{128} seems at first sight reasonable with part structure Y rearranging to part structure AA.

The PMR spectrum of IX excludes AA because the methyl group is a doublet (J=6.5Hz) at 1.52δ and the signal at 4.18δ (bs, 1H) is too high-field for H\textsubscript{a}.

The literature does not contain references to base-catalyzed rearrangements of 3,4-epoxycyclohexanones. The inability to assign a structure to IX based on the known rearrangement mechanisms of 2,3-epoxycyclohexanones lends some support to the assignment of Z as a part structure of stylocheilamide. On the negative side, anionic rearrangements based on Z also failed to account for IX.

The determination of the structure of XXI defines the number and position of atoms between the double bonds and
requires part structure $BB$ of stylocheilamide.

![Structure BB](image)

Addition of part structure $K$ completes the structure, without sterochemistry, of stylocheilamide.

F. Sterochemistry of Stylocheilamide

Some of the sterochemistry in stylocheilamide can be determined. An x-ray crystal structure determination of $XIX$, Figure 62, demonstrates that the acetoxy and methyl groups on the cyclohexanone ring are $cis$. The reaction series which generated $XIX$, lithium aluminum hydride reduction, acetylation and ozonolysis, would not be expected to alter the $cis$ orientation of the two group. The acetoxy and methyl groups are $cis$ in stylocheilamide.

Dauben, Fonker and Noyce\textsuperscript{131} have studied the sterochemistry of the addition of hydride to substituted cyclohexanones. The use of sodium borohydride resulted in steric approach control of the product distribution; the major product isolated resulted from hydride attack from the least hindered side of the molecule. Lithium aluminum hydride resulted in greater product development control of
Figure 62. A Computer Generated Perspective Drawing of 2β,4β-Diacetoxy-3β-methylcyclohexanone (XIX).
the product distribution; the major product isolated depended on the relative stabilities of the products. Thus, reduction of 2-methylcyclohexanone with lithium aluminum hydride gave the more stable trans alcohol in 82% yield while reduction with bulkier sodium borohydride gave the trans alcohol in 70% yield. Thus lithium aluminum hydride reduction of the ketone group in stylocheilamide should produce the more stable trans-diequitorial alcohol. The crystal structure of $XIX$ shows that the alcohol is cis to the methyl group and results from steric approach control.

The ketone in stylocheilamide is known to be hindered. The trans alcohol resulting from lithium aluminum hydride reduction of 2-methylcyclohexanone demonstrates that one methyl group alpha to the carbonyl is not sufficiently bulky to compel steric control approach of the reaction. This implies that in stylocheilamide the epoxide ring is on the same side of the cyclohexanone ring as the methyl group. Examination of stylocheilamide with Dreiding-Stereomodels confirms the hypothesis of a hindered side and a relatively clear side of the molecule (Figure 63). Hydride attack on the ketone from the unhindered face will generate the less stable all cis configuration.

The epoxide, with its backside on the unhindered side of the cyclohexanone ring, will be attacked by hydride to generate an axial tertiary alcohol$^{132}$. Compound $XII$ has the all cis geometry on the cyclohexanone ring.
Figure 63. Steroview of Steric Hinderance to Hydride Attack.
The trisubstituted olefin, C-2' and C-3', of stylocheilamide exists with the cyclohexanone moiety and the chlorine in both the cis and trans configurations. Evidence for this assignment comes from the CMR spectrum which shows doubling of C-2' and C-3' peaks and from the PMR spectrum which shows a chemical shift difference of 12 ppm for H-3' cis and trans to the chlorine. The disubstituted olefin in the alkyl chain is probably trans.

The structure of stylocheilamide with some stereochemical assignments is

\[ I \]

G. Deacetylstylocheilamide

1. Characterization of \(\alpha,\beta\)-Unsaturated Ketone

A second chlorine containing amide was isolated from \textit{Stylocheilus longicauda}, which differs from stylocheilamide \((\text{C}_{28}\text{H}_{44}\text{ClNO}_6)\) by the elements of acetic acid and is called deacetylstylocheilamide, \(\text{C}_{26}\text{H}_{40}\text{ClNO}_4\). The CMR spectrum of deacetylstylocheilamide shows two carbonyl carbons at 191.9 and 172.5 ppm, assignable to an \(\alpha,\beta\)-unsaturated ketone and an amide\(^98\). The infrared spectrum shows a single broad
carbonyl absorption at 1660 cm\(^{-1}\) consistent with these assignments. Thus, it appears that one mole of acetic acid has been lost from stylocheilamide to yield deacetylstylocheilamide, probably as an artifact.

The ultraviolet spectrum of deacetylstylocheilamide in methanol exhibits a maximum at 241 nm with a low extinction coefficient of 6,300. The absorption maximum of \(\alpha,\beta\)-unsaturated ketone systems can be calculated using Woodward's rules\(^{133}\). By analogy with stylocheilamide, a methyl cyclohexenone is expected. However, the calculated values for 2-methylcyclohex-2-enone (XLV) and 3-methylcyclohex-2-enone (XLVI) are too close to allow an unambiguous distinction to be made.

The \(beta\) proton in an \(\alpha,\beta\)-unsaturated ketone would be expected to have a PMR chemical shift of 6.7-7.2\(\delta\). The lowest field signal in the PMR spectrum of deacetylstylocheilamide, Figure 7-9, is at 6.35\(\delta\), thus indicating substitution at the \(beta\) position. The PMR chemical shift of a methyl group in the \(beta\) position of \(\alpha,\beta\)-unsaturated ketones is about 2\(\delta\), while similar \(alpha\) substitution results in a shift of the resonance to higher field by about 0.2\(\delta\). The
methyl group in deacetylstylocheilamide appears at 1.80\(\delta\) in the PMR spectrum. Spin decoupling experiments demonstrated long range coupling (\(J=1.5\)Hz) of the 1.80\(\delta\) methyl signal and the olefinic multiplet at 6.35\(\delta\). The chemical shift of the methyl group suggests it to be \(\alpha\) to the ketone.

The CMR spectrum of deacetylstylocheilamide shows doubleting of peaks as does the spectrum of stylocheilamide. The lowest field signal in the olefinic region is at 137.6 ppm, a doublet in the off-resonance. This signal is not present in stylocheilamide. Addition of a carbonyl to an olefinic group deshields both \(\alpha\)- and \(\beta\)-olefinic carbons with a larger downfield shift for the \(\beta\)-carbon. The highest field signal in stylocheilamide is at 10.8 ppm assigned to a methyl group \(\alpha\) to, and strongly shielded by, a ketone group. This signal is absent in deacetylstylocheilamide. The CMR data supports a 2-methylcyclohex-2-enone type part structure for deacetylstylocheilamide.

Attempts to form a 2,4-dinitrophenylhydrazone derivative of deacetylstylocheilamide failed to yield isolable products, probably because of the strongly acidic (15% sulfuric acid) nature of the reagent. The ketone was not sufficiently reactive to form an oxime with hydroxylamine hydrochloride and pyridine. No complexation and precipitation occurred with picric acid dissolved in diethyl ether.

It was hoped that the \(\alpha,\beta\)-unsaturated ketone could be reduced to the alcohol and a solid alcohol derivative could be formed. Sodium borohydride reduction of deacetylstylo-
cheilamide yielded a complex mixture. The infrared spectrum of the mixture showed strong hydroxyl stretching but also indicated the appearance of a new carbonyl group at 1730 cm\(^{-1}\). Attempts to separate the mixture into its components caused the mixture to decompose and/or rearrange into a more complex mixture. The allylic alcohol (?) was reacted with p-bromobenzoyl chloride and pyridine to give a mixture from which an oily p-bromobenzoate derivative was isolated. All attempts to crystallize the p-bromobenzoates or purify the mixtures by chromatography failed.

2. Abnormal Hydrogenation: Aromatization

Atmospheric pressure palladium-on-carbon catalyzed hydrogenation of deacetylstylocheilamide yielded one major product, \(XXIII\). Tlc analysis of the product indicated that it was more polar than the starting material and was also visible under short wavelength UV light, indicating a UV chromophore near 254 nm.

The infrared spectrum of \(XXIII\), Figure 46, showed strong hydroxyl stretching at 3250 cm\(^{-1}\) and amide carbonyl stretching at 1640 cm\(^{-1}\).

The aromatic region of the PMR spectrum of \(XXIII\), Figure 25, was identical to the aromatic pattern of \(XXII\), Figure 24, the alumina chromatography product of stylocheilamide. Compound \(XXII\) gave a positive ferric chloride test for phenols\(^8\) and the UV maximum of 283 nm \((ε\ 2,300)\) shifted to 290 nm \((ε\ 4,100)\) in base are in accord with the phenolic assignment. Scott\(^13\) reports that estrone has a UV maximum
at 280 nm ($\epsilon$ 2,300) which shifts by approximately +20 nm in alkaline solution. This shift is often diagnostic for the presence of a monohydric phenol chromophore. At the time of the isolation of XXIII, it was not recognized as a phenol and an alkaline UV spectrum was not recorded. However, the positive ferric chloride spray reagent test and PMR spectral pattern identity with XXII confirm XXIII as being a phenol.

3. Phenolic Substitution.

The PMR spectrum of XXIII exhibits a one proton, rapidly exchangeable signal at 8.10 ppm. The signal is unshifted by varying the concentration, a feature diagnostic of intramolecularly hydrogen-bonded phenols\textsuperscript{136}. The PMR spectrum also shows a one proton doublet of doublets centered near 7.10 ppm and a multiplet which integrates for two protons from 6.6-6.96 ppm. The pattern is similar to an ABC system in which the chemical shift of $H_a-H_b \approx 20$ ppm and the chemical shift of $H_a-H_c = 25$ ppm\textsuperscript{137}.

A computer simulated stick plot spectrum was obtained using a LA01P program and a Digital PDP-11 computer. A reasonable fit was obtained for the chemical shifts $H_a=7.1$, $H_b=6.8$, and $H_c=6.78$ and coupling constants $J_{a-b}=J_{b-c}=8\text{Hz}$, $J_{a-c}=2\text{Hz}$. Approximate solutions with negative coupling constants were not tried because the relative sign of the coupling constants in benzenoid systems are all the same and presumed to be positive\textsuperscript{138}. The LA01P approximate fit program assigned line order numbers to the spectral lines. A LAOCOON-2 program was used in the iterative mode to refine
the chemical shifts and coupling constants to obtain a best fit of the experimental data, Figure 64. The best fit values, obtained after two iterations, were: Chemical shifts; $H_a = 7.103$, $H_b = 6.836$, $H_c = 6.741 \delta$ Coupling constants; $J_{a-b} = 1.3$, $J_{a-c} = 8.1$ and $J_{b-c} = 7.8 \text{Hz}$. Protons $H_a - H_c$ and $H_b - H_c$ are ortho to one another, while $H_a - H_b$ are meta, indicating a 1,2,3-trisubstituted benzene system.

A good deal of work has been carried out to predict the magnitude and direction of PMR chemical shifts in aromatic systems caused by a substituent$^{139}$. In 1,3- or 1,4-disubstituted benzene systems, the substituent effects are approximately additive and agreement of ±0.1 ppm between experimental and calculated chemical shifts are possible. If, however, the substituents are ortho, there will often be considerable difference in the magnitude of the calculated chemical shifts. However, the direction of the shift will be correctly predicted.

There are three ways to arrange the three substituents on a 1,2,3-substituted benzene. Jackman and Sternhell$^{139}$ list substituent constants for a number of aromatic substituents. An olefin is not on the substituent list, but
Figure 64. LAOCOON-2 Best Fit Stick Plot of the Aromatic Region of the PMR Spectrum of XXIII
qualitatively a phenyl substituent should be a reasonable replacement. Using the substituent constants for methyl, phenyl and hydroxyl, Table 6, the calculated proton shifts for the three possible model phenols, XLVII, XLVIII and XLIX, and the sample part structure CC are

The calculated chemical shifts for model compound XLIX are in much better agreement with the experimental values, part structure CC, than are the calculated chemical shifts for model compounds XLVII or XLVIII.

This data indicates part structure DD in XXIII.
However, DD is a hydroxylated styrene derivative which should contain a main band UV absorption near 248 nm. The intensity and position of the 248 nm band are susceptible to loss of coplanarity. The absence of a definite maximum indicates that the double bond in XXIII is not conjugated with the aromatic ring.

Table 6
The Effect of Substituents on the Chemical Shift of Benzene.
Negative Sign Denotes Downfield Shift

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Shift Relative to Benzene (7.27 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ortho</td>
</tr>
<tr>
<td>Ph-</td>
<td>-0.18</td>
</tr>
<tr>
<td>CH$_3$-</td>
<td>0.17</td>
</tr>
<tr>
<td>HO-</td>
<td>0.50</td>
</tr>
</tbody>
</table>

4. Mechanism of Phenol Formation

Compound XXIII was isolated from the partial hydrogenation of deacetylstylocheilamide but is the result of aromatization of the cyclohexane ring. In deacetylstylocheilamide, the cyclohexane ring contains an $\alpha,\beta$-unsaturated ketone, a methyl group, an epoxide ring and a vinyl group. Compound XXIII can be formed by hydrogenolysis of the epoxide ring to yield an alcohol. Elimination of water and enolization of the ketone yields a substituted phenol. This sequence requires that the phenolic oxygen in XXIII is the ketone in deacetylstylocheilamide and this information expands the part structure to EE.
High resolution mass spectrometry assigned the chemical formula \( C_{26}H_{42}ClNO_3 \), unsaturation number 6, to \( \text{XXIII} \). A large ion at \( m/e \) 352 indicates the loss of a \( C_7H_{15} \) (99 mass units) radical and the presence of the long chain. The loss of 143 mass units (\( C_9H_{19}O \)) is absent, indicating hydrogenation of the long chain olefin.

H. Structure of Deacetylstylocheilamide

The mass spectral fragmentation pattern is consistent with structure \( \text{XXIII} \) and shows loss of chlorine and an ion at \( m/e \) 145, \( C_{10}H_{9}O \). This fragment ion can arise by cleavage \( \alpha \) to the nitrogen to form stable ion \( b \).
Inclusion of fragment ion b to part structure EE expands the part structure in deacetyl stylocheilamide to FF.

\[ FF \]

The infrared and CMR spectra require a tertiary amide in deacetyl stylocheilamide. By analogy with stylocheilamide, the amide is N-methylated and FF can be expanded to GG.

\[ GG \]

Lemieux oxidation of a mixture of stylocheilamide and deacetyl stylocheilamide gave a single isolable acid, XVI. The isolation of one acidic product requires that part structure K is common to stylocheilamide and deacetyl stylocheilamide. Support for K in deacetyl stylocheilamide is found in a long-chain methylene envelope in the PMR spectrum and the similarity of CMR chemical shifts in the 10-30 ppm region in stylocheilamide and deacetyl stylocheilamide. The mass spectral fragmentation of deacetyl stylocheilamide shows
a large ion at m/e 143, C$_9$H$_{19}$O, corresponding to cleavage alpha to the oxygen in $\kappa$ and formation of fragment ion $c$.

$$\text{CH}_3\overset{\hat{o}}{=\text{CH} - (\text{CH}_2)_6 - \text{CH}_3}$$

This data allows expansion of part structure $GG$ to $HH$.

Only three carbons and five hydrogen atoms remain to be assigned. The PMR spectrum of deacetylstylocheilamide does not show vinyl methyl absorption excluding the possibility of a CH$_3$-C=C- group in the compound. The PMR spectrum does show a two proton broad, symmetrical triplet at 5.5$\delta$ indicative of disubstituted olefins in long chain hydrocarbons. The PMR data suggest the best arrangement of the C$_3$H$_5$ fragment is -CH$_2$-CH$_2$-CH=. The other possibility, -CH(CH$_3$)-CH=, is excluded by the PMR spectrum which does not show a doublet near 1.1$\delta$ as required for the allylic methyl group and by analogy with $XXI$, the ozonolysis product of stylocheilamide.
The structure of deacetylstylocheilamide (II) is
IV. CONCLUSION

The chemical and spectral evidence presented strongly supports the postulated structures of stylocheilamide and deacetylstylocheilamide. The configuration about the vinyl chloride linkage is a mixture of cis and trans isomers. The stereochemistry at C-7 was not determined although it is felt that this center is optically active.

Stylocheilamide is the first reported halogenated amide from a marine source. The biosynthesis of the compound is probably from linear fatty acids but positive biogenesis is always an intriguing problem. Likewise, the biological significance of this compound would be interesting. Stylocheilamide was not toxic to mice and was inactive against Staphylococcus aureus, Escherichia coli, Candida albicans or Mycobacterium smegmatis inoculated agar plates.

The final proof of structure lies in synthesis. The cyclohexanone ring is sufficiently complex to present a reasonable challenge. Studies of the rearrangement of 2,3-epoxycyclohexanone derivatives is also warranted.
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