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# Antimicrobial and Antineoplastic Tyrosine Metabolites from a Marine Sponge, *Aplysina fistularis*

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**Abstract** □ Examination of the constituents of a marine sponge, *Aplysina fistularis*, indicated that brominated tyrosine metabolites were mainly responsible for antimicrobial and antineoplastic activities. Halogenated tyrosine metabolites, 2, 6-dibromo-(**2**), 2-bromo-3-chloro-(**3**) and 2, 6-dichloro-(**4**) 4-hydroxy-2, 5-cyclohexadienone-4-acetamides, as well as the lactam derivatives, 5-amino-2, 6-dibromo-(**5**), 5-amino-2-bromo-6-chloro-(**6**) and 5-amino-2, 6-dichloro-(**7**) 4-hydroxy-2-cyclohexenone-4-acetic acid lactams were identified as the major antineoplastic and antimicrobial principles. Many other brominated tyrosine metabolites were also confirmed, but they did not show antimicrobial and antineoplastic activities.

**Keywords** □ Marine Natural Products, Marine Sponge, *Aplysina fistularis*, Antimicrobial Compounds, Antineoplastic compounds, Halogenated Tyrosine Metabolites.

Nature is the most abundant source for new medicines useful for treatment of human disease. During last a couple of decades terrestrial plants and microorganisms were extensively screened to develop new antineoplastic and antiviral agents without much success. Although the medicinal use of marine organisms was a very long time ago in China, the extensive search for biologially active substances from marine sources was started only recently. It has been proposed that, because of differences between the environments of marine and terrestrial organisms, the former may produce structurally very different compounds, some of which might be useful as new drugs for human disease control.

Antimicrobial cmpounds isolated from marine organisms include sesquiterpenes, laurinterol[1,2] and its analogs[3,4] isolated from marine algae, and other terpenoids having an isonitrile group,[5,6] or having a furane ring,[7,8] in their structures. Several species of marine organisms, such as algae, sea cucumbers,[9] gorgonians,[10] anemones,[11] tunicates,[12] and microorganisms[13] were reported to produce compounds having antimicrobial, antineoplastic or antiviral activities[14,15] In 1952, Nigrelli first reported the antimicrobial activity of some sponges which were tested by placing fresh sponge fragments on a seeded Petri dish.[16] It was shown that about 35% of sponges tested exhibited inhibitory activity against Gram positive organisms, 15% against Gram negative and 10% against fungi.[17] Also, some sponges such as *Verongia fistularis*, *Ianthella ardis* and *Specciospongia vesparia* have been indicated to have strong antitumor activity.[18] Chemical work on extracts of marine sponges was started by Bergmann, who in 1955 isolated 1-(β-D-arabinofuranosyl) uracil (spongouridine) from the West Indian sponge, *Cryptotheya crypta*,[19] which is known to have antiviral and antitumour activity. Recently many terpenoids were isolated from marine sponges and some of them were reported to have antimicrobial acti-

vity. These include axisonitrile,[6] nitenin,[7] furospongin,[8] fasciculatin,[20] ircinin,[21] variabillin,[23] etc. Up to now brominated tyrosinemetabolites were isolated from two families of sponges, Verongidae and Disideidae in the order of Dictioceratia. These species showed strong antimicrobial activity as well as antineoplastic activity,[23-25] and many brominated tyrosine metabolites isolated from these organisms were reported to have antimicrobial activity, but actually it was found that the antimicrobial activity was limited in a couple of compounds only[26] and still many compounds are remained to be identified. In this report,a marine sponge, *Aplysina fistularis*, belong to the family of Verongidae, was extracted and studied for antimicrobial and antineoplastic principles.

## EXPERIMENTAL METHODS

### *Collection of the Organism and Screening of the Bioactivity*

The marine sponge was collected from Baja California (near Bahia Los Angeles, latitude, 29°N, 40ft) and it was identified as *Aplysina fistularis* by Dr. Backus in Sourthern California University. About 20 kg of the sponge was collected and stored in ethanol. 2 g of the collected sponge was blended with toluene-methanol (3:1, 10 ml) for 1 min in Virtis Blender and centrifuged. The liquid layer was separated and added with 10 ml of 1 *N* sodium nitrate. The toluene layer was separated and tested for antimicrobial activity. Also the extract was evaporated and dried under vacuum to give a residue for test of inhibition of KB 11 cell growth.

### *Bioactivity Test*

Antimicrobial activity test was performed by two different methods; a disk diffusion method and bioautography. The disk diffusion method was carried out by the usual method using a paper disk (12.5 mm in diameter) absorbed with 100 $\mu$l of a sample solution at a fixed concentration. The paper disk was then dried at room temperature for 10~20 min, and put onto an agar plate seeded with a test organism. The agar plate was then incubated at 29°C and examined for inhibition of the growth of the microorganism. *B. subtilis* was purchased from Difco and *E. coli*, *P. atrovenetum* or *Saccharomyces cerevisiae* was from laboratory of Dr. Hager (Department of Biochemistry, University of Illinois) or Dr. Shaw (Department of Plant Pathology, University of Illinois). The media and broths used for antimicrobial activity test and for maintainance of the microorganisms are those suggested by Dr. Shaw and described previously.[27]

Bioautography of a sample was carried out by developing the sample on a TLC plate, then placing it on a seeded agar plate. The TLC plate was allowed to contact the agar for 10~20 min in a refregerator. After the TLC plate was removed, the seeded agar plate was incubated. The result was read after one day with an inhibition zone being observed around any spot which had an antimicrobial component.

### *General*

Column chromatography was performed with a glass column slurry packed with silica gel (100-200 mesh, BIO-RAD Lab., or Brinkmann) and usually fractional collection was carried out in tubes of about 10 ml copacity. The fractions were devided after examination of the components in tubes by TLC. TLC was carried out with commercially precoated glass plates (Brinkmann or Analtech) of 0.25 mm in thickness after cutting the plate at an appropriate size. $^1$H NMR spectra were recorded with EM-390 or HR-220 (Fourier Transform) spectrrometer

and the chemical shifts were reported as ppm from the TMS peak as an internal standard.IR spectra were recorded on on Perkin-Elmer 521 or Beckmann IR-12 spectrometer and EI mass spectrometric analysis were obtained from Varian CH-5 mass spectrometer. Melting point was obtained from Hoover Capillary Melting Point Apparatus and reported without correction.

*Extraction and Preparation of V-60-B*

The marine sponge (200 g; wet weight) stored in ethanol was homogenized in Waring Blender with 500 ml of chloroform-methanol (1:1). The homogenized organism was filtered and the solid residue was refluxed with 1,000ml of the same solvent for one day and filtered. All the organic solvent used in this extract (ethanol and chloroform-methanol) was combined together and evaporated to give a residue. This procedure was repeated to get 340g of total extract from 1950 g of the sponge (wet weight). The extract was then dissolved in chloroform-methanol (1:1) and loaded on the top of a column packed with silica gel (10kg) and eluted with chloroform-methanol (1:1) to give V-60-B (110.5g) and with methanol to give V-60-A (200.9g). About 20 l of chloroform-methanol (1:1) and about 30 l of methanol was used to collect these residue.

*Sephadex LH-20 Column Chromatography*

Preliminary examination of V-60-B on distri bution of the antimicrobial components by size separation on Sephadex LH-20 column was done by dissolving the sample (223.7mg) in 1ml of chloroform-methanol (1:1), by loading the solution on the top of the column (1′×26′) and by elution with the same solvent.The Sephadex LH-20 used in this work was purified by refluxing in a Soxhlet apparatus for 2 days with methanol. The column chromatography gave 11 fractions; V-61-1 (3.9mg), V-61-2 (8.7mg), V-61-3 (13.6mg), V-61-4 (20.3mg), V-61-5 (8.7mg), V-61-6 (22.2mg), V-61-7 (20.8mg), V-61-8 (45.6mg), V-61-9 (42.2mg), V-61-10 (18.3mg) and V-61-11 (14.0mg). A large scale fractionation was carried out with a column packed with Sephadex LH-20 (1.75′×29′) by loading 1.12g of V-60-B dissolved in chloroform-methanol (1:1, 5ml). The column was developed with the same solvent to give V-64-A (62mg), V-64-B (352mg), V-64-C (552.2mg), V-64-D (598.8mg), V-64-E(1.562 g) and V-64-F (1.1253g).

*Sephadex LH-20 Column Chromatography of V-64-F)*

V-64-F (1.1253g) was chromatographed on the same column used in the above experiment in a large scale separation of V-60-B by elution with the same solvent to give V-69-B (114mg), V-69-C (264mg), V-69-D (169mg), V-69-E (553mg), V-69-F (93.2mg) and V-69-G (2.1 mg).

*Silica Gel Column Chromatography of V-69-D*

A column was prepared by a slurry packing with silica gel (BIO-SIL A, 100-200 mesh, BIO-RAD Lab.) dissolved in chloroform-methanol (1:1). V-69-D (0.1693g) was dissolved in chlorform-methanol (1:1), mixed with 2g of silica gel, dried under vacuum and loaded on the column. The column was developed with the same solvent to give V-71-B (48.1mg). Combining the rest of fractions and washing of the column with methanol gave V-71-A (52.2 mg) and V-71-C (91.5mg), respectively.

*Silica Gel Column Chromatography of V-69-E*

V-69-E (552.2mg) was dissolved in 1ml of chloroform-methanol (1:1) and absorbed in 2g of silica gel. The silica gel was dried and loaded on the top of the column packed with silica gel. The column was developed with benzene-chloroform-methanol (5:5:1) to give V-

74-A (15.9mg), V-74-B (370.3mg) and V-74-C (44.6mg).

*Silica Gel Column Chromatoraphy of V-74-B*

V-74-B (470.3mg) was dissolved in toluene-acetonitrile (1:1) and loaded on the top of a silica gel column and the column was developed with the same solvent to give V-77-A (126.5 mg), V-77-B (72.7mg) and V-77-C (40.1 mg).

*Silica Gel Column Chromatography of V-69-F and Isolation of V-80-A*

V-69-F (93.2mg) was dissolved in 2ml of chloroform-methanol (1:1) and mixed with 2g of silica gel. After evaporation of the solvent, the silica gel was placed on the top of a column packed with silica gel. The column was developed with toluene-acetonitrile (1:1) to give V-79-A (30.3mg) and V-79-B (247.4mg). When the fraction, V-79-A was dissolved in toluene-acetonitrile (1:1) and standed at room temperature, V-80-A was isolated as a crystalline compund, m.p. 188~190°C.

## RESULTS AND DISCUSSION

*Collection and Extraction of the Sponge*

*Aplysina fistularis*[28] was collected from the coastal waters of Baja California during March, 1977 and extracted with toluene-methanol (3:1) for test of antimicrobial activity against *Bacillus subtilis, Esherichia coli, Penicilium atrovenetum* and *Saccharomyces cerevisiae*. The extract showed strong antimicrobial activities against *B. subtilis* and *E. coli*. Separate test for antineoplastic activity of this extract also showed atrong growth inhibitory against KB 11 cells cultured.[29] About 20kg of the sponge was collected and stored in ethanol (95%). The organism stored in ethanol was extracted by homogenation and by reflux with chlorform-methanol (1:1). The

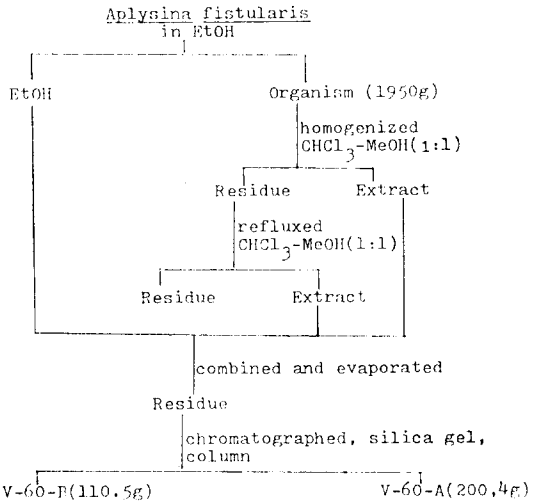



**Fig. 1**: Extraction and preparation of sample V-60-B

extract combined was dissolved in chloroform-methanol (1:1), filtered and evaporated to give totally 17.4% of the weight of the wet organism extractactable. To remove inorganic salts and other unnecessary material in the extract, it was filtered through a silica gel column by eluting with chloroform-methanol (1:1) to give fraction V-60-B (32.4% of the extract was recovered in this fraction). Washing of the column with methanol made addlitional material (V-60-A, 58.8% of the extract was colected) recovered. Fraction V-60-B retained most of the antimicrobial activity against *B.subtililis* and *E. coli* but did not show activity against *P. atrovenetum*. The extraction procedure is diagramed in Figure 1 and all the antimicrobial activity test results included in this report is given in Table I.

*Fractionation and Isolation*

Preliminary examination of V-60-B by LH-20 Sephadex column chromatography indicated that the antimicrobial components are eluted from the column following a green band in which mainly steroids were found. The fractions

**Table I:** Bioactivities of the fractions obtained from the extract of *Aplysina fistularis*.

| Sample | Wieght | Zone of Inhibition, mm* | | | | | |
|---|---|---|---|---|---|---|---|
| | | 50mcg | | | 200mcg | | |
| | | *B. sub.* | *E. coli* | *P. atro.* | *B. sub.* | *E. coli* | *P. atro.* |
| V-60-A | 200.9g | 13.0 | 13.0 | —** | 17.0 | 17.0 | — |
| V-60-B | 110.0g | 18.0 | 18.0 | — | 27.0 | 28.0 | — |
| V-61-1 | 3.9mg | — | — | — | — | — | — |
| V-61-2 | 4.7 | — | — | — | — | — | — |
| V-61-3 | 13.6 | — | — | — | — | — | — |
| V-61-4 | 20.3 | — | — | — | — | — | — |
| V-61-5 | 8.7 | — | — | — | — | — | — |
| V-61-6 | 22.2 | — | — | — | — | — | — |
| V-61-7 | 20.3 | — | — | — | 13.5 | 13.0 | — |
| V-61-8 | 45.6 | — | — | — | — | — | — |
| V-61-9 | 42.2 | 14.5 | 14.0 | — | 37.0 | 31.0 | — |
| V-61-10 | 28.3 | 15.5 | 15.5 | — | 37.0 | 31.0 | — |
| V-61-11 | 14.0 | 15.5 | 13.5 | — | 36.0 | 36.0 | — |
| V-64-A | 62.0 | — | — | — | — | — | — |
| V-64-B | 351.0 | — | — | — | — | — | — |
| V-64-C | 552.2 | — | — | — | — | — | — |
| V-64-D | 598.8 | — | — | — | — | — | — |
| V-64-E | 1562.1 | — | — | — | — | — | — |
| V-64-F | 1125.3 | 19.0 | 19.0 | — | 33.0 | 38.0 | — |
| V-69-B | 114.0 | — | — | — | — | — | — |
| V-69-C | 264.2 | 25.0 | 26.0 | — | 40.0 | 43.0 | — |
| V-69-D | 169.3 | 27.0 | 21.0 | — | 42.0 | 38.0 | — |
| V-69-E | 552.2 | 23.0 | 21.0 | — | 38.0 | 37.0 | — |
| V-69-F | 93.2 | 20.0 | 19.0 | — | 34.0 | 30.0 | — |
| V-71-A | 52.2 | 16.0 | 31.0 | — | 21.0 | 32.0 | — |
| V-71-B | 48.1 | — | — | — | — | — | — |
| V-71-C | 91.5 | — | — | — | — | — | — |
| V-74-A | 15.9 | 26.0 | 26.0 | — | 34.0 | 34.0 | — |
| V-74-B | 370.3 | 29.0 | 27.0 | — | 48.0 | 47.0 | — |
| V-74-C | 44.6 | 18.0 | 20.0 | — | 29.0 | 32.0 | — |
| V-77-A | 126.5 | 34.0 | 34.0 | — | 43.0 | 46.0 | — |
| V-77-B | 72.7 | 27.0 | 22.0 | — | 37.0 | 84.0 | — |
| V-77-C | 40.1 | 27.0 | 25.0 | — | 38.0 | 36.0 | — |
| V-79-A | 30.3 | 33.0 | 36.0 | — | 41.0 | 40.0 | — |
| V-79-B | 47.4 | 15.0 | 13.0 | — | 19.0 | 23.0 | — |

*: antimicrobial activity results were obtained by a disk diffusion method using 12.5mm disks (in diameter) against *B. sub.* (*Bacillus subtilis*), *E. coli* and *P. atro* (*Penicilium atrovenetum*) and were reported by the diameter of the inhibition zone.

**: — means negative antimicrobial activity.

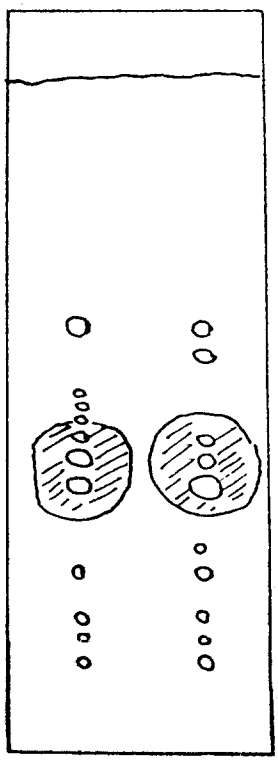


**Fig. 2**: Bioautography of Samples V-61-10 and V-61-11 (Benzne-chloroform-Methanol=5:5:1). Inhibition areas are given by shade.

after the green band (V-61-9, V-61-10 and V-61-11; Table I) were composed of about 38% of the material loaded on the column. When these fractions were studied by bioautography, the plate (Figure 2) showed that the three components found in the middle region of the plate were mainly responsible for the bioactivity.

Larege scale fractionation on a Sephadex LH-20 column gave the same result and in this experiment about 25.2% of the loaded material was collected in fraction V-64-F as the only antimicrobial fraction. Further fracionation of V-64-F on the same column divided the antimicobial activity into four fractions. Except the first one collected, all the rest of the fractions (V-69-C, V-69-D, V-69-E and V-69-F) showed antimicrobial activity. Examination of V-69-D by thin layer chromatography (TLC) showed two major components. Column chromatography (silica gel) of this fraction gave a pure compound, V-71-B. This compound showed a $R_f$ value at 0.4 (benzenechloroform-methanol =5:5:1) and 28.4% of V-69-D was recovered in this fraction. But this compound did not show antimicrobial activity. Rest of the fractions obtained from this column chromatography were combined together to give V-71-A, which retained the bioactivity. Washing of the column with methanol gave V-71-C but this fraction did not show the bioactivity.

Fraction V-69-E contained the TLC spots showing antimicrobial activity in bioautography. Silica gel column chromatography of V-69-E by elution with chloroform-benzene-methanol (5:5:1) gave three fractions (V-74-A, V-74-B and V-74-C). The TLC spots mainly responsible for antimicrobial activity in bioautography were found in fraction V-74-B and it showed two bioactive spots by TLC analysis. The two spot showed $R_f$ values at 0.17 and 0.21 when a silica gel TLC plate was developed with benzene-chloroform-methanol (5:5:1). About 67.1% of V-69-E was recovered in fraction V-74-B. Examination of this fraction by TLC (silica gel) with toluene-acetonitrile (1:1) as the developing solvent showed the two spots at 0.48 and 0.36 of the $R_f$ values. Thus silica gel column chromatography of the fraction with this solvent system gave V-77-A (34.2% of V-74-B was recovered in this fraction) and V-77-B (19% of V-74-B was recovered) in which the compounds having $R_f$ values at 0.48 and 0.36 were collected, respectively. Both compcunds retained antimicrobial activities.

TLC analysis of V-69-F showed one major

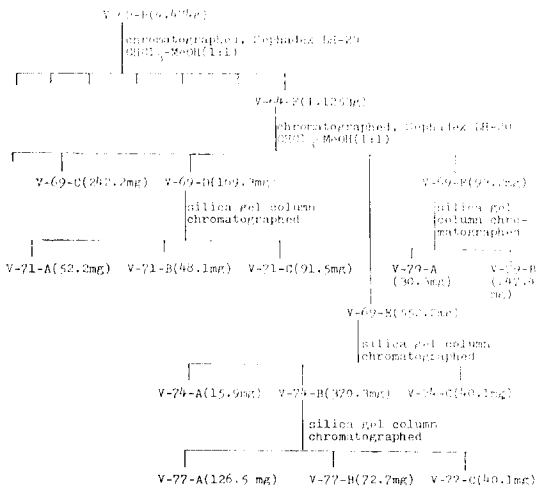


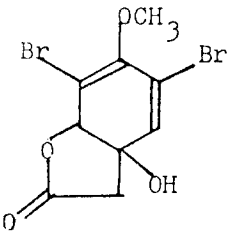

**Fig. 3**: Fractionation of sample V-60-B and isolation of pure compounds.

component ($R_f$=0.52; toluene-acetonitrile=1:1). Silica gel column chromatography of V-69-F gave a fraction V-79-A (32.5% of V-69-F) having the spot ($R_f$=0.52) as the major component. Standing V-79-A solution of toluene-acetonitrile(1 : 1) gave V-80-A as a crystalline compound. Although V-79-A showed antimicrobial activity, V-80-A was completely devoid of the biological activity.

Fractionation and separation of individual compounds are diagramed in Figure 3 and their biological activity test results are given in Table I.

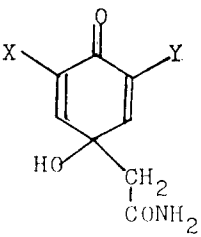
*Structures of the Compounds Isolated*

V-71-B isolated as an oily compound and it showed a molecular ion at m/z 338 ($Br_2$) and fragmented ions at m/z 320 ($Br_2$) and 279 ($Br_2$) by electron impact mass spectrometry (EIMS) analysis. Its $^1$H NMR spectrum showed peaks at 2.77 (s, 2-H), 3.63(s, 3-H), 4.97 (s, 1-H), 6.23 (s, 1-H) and 7.09 ppm(s, 1-H) and its IR spectrum showed a band at 1,750cm$^{-1}$ which implied a $\beta$-or $\gamma$-lactone. From these data, compound **1** was suggested. This compound was




1

isolated from other marine sponges and showed the same physical data reported.[30,31] The compound, V-77-A showed strong antimicrobial activity against Gram positive and Gram negative organisms. Its $^1$H NMR spectrum showed peaks at 2.79 (s, 2-H), 2.85 (s, 2-H), 5.93 (s, 1-H) and 7.60ppm (s, 2-H). The compound showed a molecular ion at m/z 323($Br_2$) and fragmented ions at m/z 306 ($Br_2$) in its EI mass spectrum. For the compound in V-77-A, 2, 6-dibromo-4-hydroxy-2, 5-cyclohexadienone-4-acetamide (**2**) was assigned by comparison of the physical data with those reported.[23]



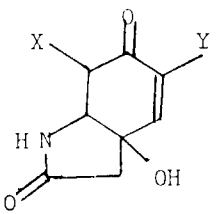

X=Y=Br 2

X=Br Y=Cl 3

X=Y=Cl 4

Compound V-77-B also showed strong antimicrobial activity against G(+) and G(−) organisms and it showed a molecular ion at the same mass ions (at m/z, 323, $Br_2$) with the same high resolution mass spectral data as V-77-A. But it showed more complex peaks in its $^1$H NMR spectrum which are completely different from those observed for V-77-A. It was impossible to assign its structure without other chemical data. Thus a larger scale fractionation was carried out to isolate more compounds of V-71-B, V-77-A and V-77-B. Examination of these isolated materials more carefully, V-77-A was composed of three components, 2,

6-dibromo-(**2**), 2-bromo-6-chloro-(**3**), and 2,6-dichloro-(**4**) 4-hydroxy-2,5-cyclohexadienone-4-acetamides,[31] and V-77-B was found to be constituted by lactams, 5-amino-2,6-dibromo-(**5**), 5-amino-2-bromo-6-chloro-(**6**) and 5-amino-2,6-dichloro-(**7**) 4-hydroxy-2-cyclohexenoneacetic acid lactams.[32] These compounds were confirmed by high resolution electron impact (HREI) mass spectral, field desorption mass spectral, $^1$H NMR and $^{13}$C NMR spectral data with other chemical data. Isolation and structural determination of these compounds are reported separately in papers submitted for publication

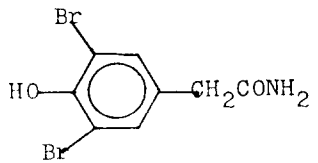


X=Y=Br **5**

X=Cl Y=Br **6**

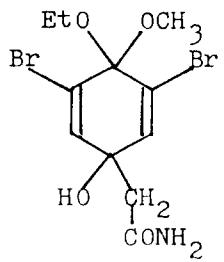
X=Y=Cl **7**

recently.[32,33] The crystalline compound, V-80-A showed m.p. at 188~190°C (190~191°C for 3,5-dibromo-4-hydroxyphenylacetamid,[34] **8**) and a molecular ion at m/z 307 ($Br_2$) in EIMS analysis and was assigned by the compound, **8**.



**8**

During further fractionation of the extract, compound **9**[35] was also isolated as a pure compound but it did not show antimicrobial activity. Compounds, **8** and **9** were claimed to have antimicrobial activity.



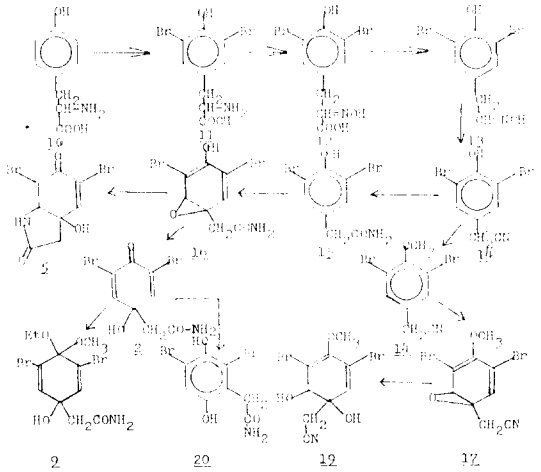
**9**

*Biogenesis of the Isolated Compounds*

The compounds isolated from *Aplysina fistularis* and reported in this paper were supposed to be biosynthesized from tyrosine by the pathway given in Scheme I. Most of the intermediates and metabolites given in this scheme were isolated or confirmed in extracts of *Verongia* or *Aplysina* species[36-38] except the arene oxides, **16** and **18**, and the oxime analogs, **12** and **13**. Among these compounds, compounds **5**, **2** and **19** are presently the only ones confirmed to have strong antimicrobial and antineoplastic activity.

*Bioactivities of the Isolated Compounds*

V-77-A and V-77-B showed strong antineoplastic activity against L1210 cell tube dilution assay at the level of 0.14$\mu$g/ml and 1.2$\mu$g/ml for $ID_{50}$ and 0.41$\mu$g/ml and 3.6$\mu$g/ml for $ID_{90}$, respectively. They also showed strong antimicrobial inhibitory activity against *Staphylococcus aureus*, *B. subtilis*, *Sarcina lutea*, *Klebsiella pneumoniae*, *E. coli*, *Proteus vulgaris*, *Mycobacterium avium*, *Bacteroides fragilis* and *Clostridium perfringens*. Their antimicrobial activity is



**Scheme I:** Biogenesis of the isolated compounds.

comparable to those antibiotics under clinical use. But they did not show any inhibitory activity against *Pseudomonas aureus. Saccharomyces pastorianus, Penicilium oxalicum* and *Candida albicans.*

## CONCLUSION

Extraction and examination of Aplysina fistularis enabled identification of several brominated tyrosine metabolites. From these metabolites, presently 6 compounds were identified to be the major antineoplastic and antimicrobial principles. However, as Table I implies still several other compounds should be existing in the extract which are not identified yet.

## LITERATURRE CITED


1) Fenical, W.: *J. Phycol.*, **11**, 245 (1975)
2) Irie, T,, Suzuki, M., and Masamune, T.: *Terahedron Lett.*, 1837 (1965)
3) Sims, J.J., Donnel, M.S., Leary, J.V.,and Lacy, G.H.: *Antimicrob. Ag. Chemother.*, 7, 320(1975)
4) Siuda, J.F., VanBlaricom, G.R., Shaw, P.D., Johnson, R.D,, White, R.H., Hager, L.P., and Rinehart, K.L., Jr.,: *J. Am. Chem. Soc.*, **97**, 937 (1975)
5) Burreson, B.J., Christophersen, C., and Scheuer, J.P.,: *Tetrahedron*, **31**, 2015 (1975)
6) Cafiere, F. C., Fatturosso, E., Magno, S., Santacroce, C., and Sica, D., *Tetrahedron* **30**, 4259 (1973)
7) Fattorusso, E., Minale, L., Sodano, G., and Trievellone, E.,: *Tetrahedron*, **27**, 3909 (1971)
8) Cimino, G., De, Stefano, S., Minale L., and Fattorusso, E.,: *Tetrahedron* **27**, 4673 (1971)
9) Schuer, P.J.,: *Chemistry and Marine Natural Products*, Acadmic Press, New York,N.Y. (1973)
10) Weinheimer, A.J., and Matson, J.A.,: *Lloydia*, **38**, 378 (1975)
11) Sharma, G.M., Michaels, L., and Burkholder, P. R.: *J. Antibiot.*, **21**, 659 (1968)
12) Cheng, M . and Rinehart, K.L., Jr.,: *J. Am. Chem. Soc.*, **100**, 7409 (1978)
13) Okazaki, T., Kitahara, T., and Okami, Y.,: *J. Antibiot.*, **28**, 176 (1975)
14) Rinehart, K.L., Jr., Kobayashi, J., Harbour, G. C., Hughes, R.G., Jr., Mizsak, S.A., and Scahill, T.A.,: *J. Am. Chem. Soc.*, **106**, 1524 (1984)
15) Kobayashi, J., Harbour, G.C., Gilmore, J., and Rinehart, K.L., Jr.,: *J. Am. Chem. Soc.*, **106**, 1526 (1984)
16) Nigrelli, R.F.,: *Zoologica*, **37**, 89 (1952)
17) Rinehart, K.L. Jr., Shaw, P.D., Shield, L.S., Gloer, J.B., Harbour, G.C., Koker, M.E.S., Samain, D., Schwartz, R.E., Tymiak, A.A., Weller, D.L., Carter, G.T., Munro, M.H.G., Hughes, R.G., Jr., Renis, H.E., Swynenberg, E.B., Stringfellow, D.A., Vavra, J.J., Coats, J.H., Zurenko, G.E., Kuentzel, S.L., Li, L.H., Bakus, G.J., Brusca, R.C., Craft, L.L., Young, D.N., Connor, J.L.: *Pure. Appl. Chem.*, **53**, 795 (1981)
18) Burkholder,, P.R., and Sharma G.M. *Lloydia*, **32**, 466 (1969)
19) Bergmann, W., and Burke, D.C., *J. Org.Chem.*, **20**, 2501 (1955)
20) Cafieri, C., Fattorusso, E., Santacroce, C. and Minale, L.,: *Tetrahedron*, **28**, 1579 (1972)
21) Cimino, G. De Stefano, S., and Minale, L.,: *Tetrahedron* **28**, 5983 (1972)
22) Fenical, W., and Sims, J.J. *Tetrahedron Lett.*, 1137 (1974)
23) Sharma, G.M., and Burkholder, P.R.,: *Tetrahedron Lett.*, 4147 (1967)
24) Fulmor, W., Van Lear, G.E., Morton, G.O. and Mills, R.O.,: *Tetrahedron Lett.*, 4551 (1970)
25) Fattorusso, E., Minale, L. and Sodano, G.: *Chem. Commun.*, 751 (1970)
26) Goo, Y.M.: Thesis, Ph. D., University of Illinois, Urbana, Illinois (1980)
27) Goo, Y.M.: *Antibiotics, Research and Development of Penicillins and Cephalosporins*, Seoul National University Press, Seoul, Korea (1983), pp. 212-214

28) *A. fistularis* was originally identified as *Verongia aurea* by Dr. Backus, Sourthern California University.

29) Nemanich, J.W., Senior Thesis, University of Illinois Urbana Illinois (1977)

30) Anderson, R.J. and Faulkner, D.J., *in* "Food-Drugs from the Sea Proceedings", Worthen, L.R., Mar. Technol., Soc., Washington, D.C., (1973)

31) Minale, L., Sodano, G., Chan, W.R., and Chan, A.M.: *J. Chem. Soc., Chem. Commun.*, 674(1972)

32) Goo, Y.M., and Rinehart, Jr., K.L., Jr.: *Tetrahedron Lett.*, submitted

33) Goo, Y.M., and Rinehart, K.L., Jr.: *J.Am. Chem. Soc.*, submitted

34) Stempien, M.F., Jr., Chib, J.S., Nigrelleli,R.F., and Mierzwa, R.A., *in* "Food-Drugs from the Sea Proceedings, 1972" Worthen, L.R., Ed., Mar. Technol. Soc., Washington D.C. (1973), p. 105

35) Anderson, R.J. and Faulkner, D.J., *Tetrahedron Lett.*, 1175 (1973)

36) Goo, Y.M. and Rinehart, K.L., Jr.: *in* Drugs and Food from the Sea, Kaul, P.N. and Sindermann, C.J., Eds., Oklahama University Press (1978), pp. 107-115

37) McMillan, J.A., Paul, I.C., Goo, Y.M., and Rinehart, K.L., Jr.,: *Tetrahedron Lett.*, 22 (1981)

38) Goo, Y.M., and Rinehart, K.L., Jr.,: *J. Org. Chem.*, submitted