mation constant for other metal ion–carbonic anhydrase complexes,[7] combined with a narrower range of formation rate constants,[8,13,22,23] indicates that this may be a general rule for metallo derivatives of this particular enzyme.

Acknowledgment. This work was supported by a grant from the National Science Foundation (GP-36783). We are grateful to Drs. Pat Harrington and Alan Van Heuvelen for their help in determining manganese in concentrations by epr.

Communications to the Editor

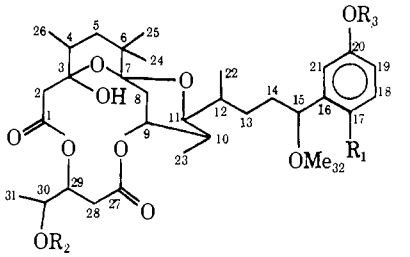
Aplysiatoxin and Debromoaplysiatoxin, Constituents of the Marine Mollusk *Stylocheilus longicauda* (Quoy and Gaimard, 1824)

Sir:

Accounts of toxicity of sea hares, which are gastropod mollusks without shells, date to Roman times,[1] but attempts to characterize the toxin, initiated by Flury in 1915,[2] have been few[3–6] and have shed no light on its chemical nature. In contrast, recent investigations into secondary metabolites of sea hares, irrespective of physiological activity, have yielded a rich harvest of mono-, sesqui-, and diterpenoids, most of them halogenated.[7–10] The early literature[1,2] mentions several toxic sea hare secretions, but Winkler, *et al.*,[4] and Watson[5] linked the animal's toxicity to its digestive or midgut gland. Watson[5] distinguished two toxic entities by solubility and bioactivity, one soluble in water and one in ether.

We now wish to report that we have isolated from *Stylocheilus longicauda* Watson's ether-soluble toxin (LD_{100} 0.3 mg/kg, ip mouse; 0.025% of wet whole animal) as a difficultly separable oily mixture of aplysiatoxin (**1**) and debromoaplysiatoxin (**2**) and that we have determined their structures. The homogeneity of all compounds was verified by tlc, mass, and nmr spectrometry. Two parallel noncrystalline nontoxic acetates, **3** and **4**, accompany the toxins. The phenolic hydroxyl and the tertiary hydroxyl at C-3 render the toxins labile above pH 7 and below pH 4. Diazomethane treatment furnished stable anisoles **5–8**, but the C-3 hydroxyl was readily eliminated under many experimental conditions and by active adsorbents. The ensuing artifacts, four C-3,4 olefins corresponding to **1–4**, doubled the components of the natural mixture.

Chemical ionization mass spectrometry on **8** with ammonia as the carrier gas[11] led to an unambiguous



1, $R_1 = Br$; $R_2 = R_3 = H$
2, $R_1 = R_2 = R_3 = H$
3, $R_1 = Br$; $R_2 = Ac$; $R_3 = H$
4, $R_1 = H$; $R_2 = Ac$; $R_3 = H$
5, $R_1 = Br$; $R_2 = H$; $R_3 = Me$
6, $R_1 = R_2 = H$; $R_3 = Me$
7, $R_1 = Br$; $R_2 = Ac$; $R_3 = Me$
8, $R_1 = H$; $R_2 = Ac$; $R_3 = Me$

molecular weight of 648, while mass spectrometry under all other conditions had furnished only M − 18. Composition was determined by high resolution mass spectroscopy[11] on the anhydrophenyl acetate of **4**, 658.3377 (calcd for $C_{36}H_{50}O_{11}$ 658.3353).

The molecular architecture of the aplysiatoxins (**1**, **2**), which are bislactones of 3,4-dihydroxyvaleric acid and of 4,6,6,10,12-pentamethyl-3,7,9,11,15-tetraoxy-15-phenylpentadecanoic acid, encompassing a symmetrical trioxacyclododecane was revealed by the following major degradations and spectral data.

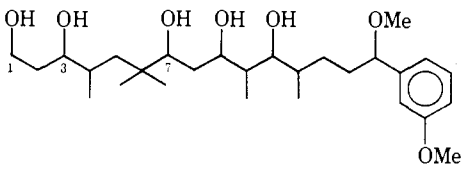
Principal evidence for the aromatic portion of the toxins includes phenolic uv in MeOH at 283 nm (ε 1950), shifted to 290 nm (ε 3000) in 0.1 *N* NaOH–MeOH, and typical mass spectral fragments resulting from benzylic cleavage. Nmr chemical shifts (δ 6.28, 6.97, 7.40) and coupling constants (<1.0, 3.1, 8.0 Hz) of **1** are those of a 1,2,4-trisubstituted benzene, mass spectral data indicate that the three substituents are Br, OH, and the aliphatic moiety, and the chemical shifts of **5** calculated from aromatic substituent effects[12] matched those of a 1-Br-2-R-4-$OMeC_6H_3$. The more complex four-spin system of **2** was confirmed by matching nmr spectra in $CDCl_3$ and in benzene-d_6 with computer generated spectra.

From 1 *N* KOH (EtOH) or $NaBH_4$ (EtOH) treatment of 3,4-anhydro-**8** we isolated after acetylation 4-acetoxy-*trans*-2-pentenoic acid (ir 3400–2500, 1720–1700 cm^{-1}; nmr ($CDCl_3$, δ) H-28 5.96 (1 H, dd, 16.0, J = 1.5 Hz), H-29 6.95 (1 H, dd, 16.0, J = 5.0 Hz), H-30 5.45 (1 H, m), H-31 (3 H, d, J = 6.5 Hz), $MeCO_2$– 2.10 (3 H, s)), thus describing the five carbons of the valeric acid moiety.

The principal carbon chain of the aplysiatoxins, comprising C-1 to C-26 and C-32, was revealed after pro-

(1) B. W. Halstead, "Poisonous and Venomous Marine Animals of the World," Vol. 1, U. S. Government Printing Office, Washington D. C., 1965, p 709.
(2) F. Flury, *Arch. Exp. Pathol. Pharmakol.*, **79**, 250 (1915).
(3) Y. Ando, *Kagaku (Tokyo)*, **22**, 87 (1952); *Chem. Abstr.*, **46**, 6277g (1952).
(4) L. R. Winkler, B. E. Tilton, and M. G. Hardinge, *Arch. Int. Pharmacodyn. Ther.*, **137**, 76 (1962).
(5) M. Watson, *Toxicon*, **11**, 259 (1973).
(6) M. Watson and M. D. Rayner, *Toxicon*, **11**, 269 (1973).
(7) S. Yamamura and Y. Hirata, *Tetrahedron*, **19**, 1485 (1963); K. Yamada, H. Yazawa, M. Toda, and Y. Hirata, *Chem. Commun.*, 1432 (1968).
(8) M. Matsuda, Y. Tomiie, S. Yamamura, and Y. Hirata, *Chem. Commun.*, 898 (1969); S. Yamamura and Y. Hirata, *Bull. Chem. Soc. Jap.*, **44**, 2560 (1971).
(9) D. J. Faulkner, M. O. Stallard, J. Fayos, and J. C. Clardy, *J. Amer. Chem. Soc.*, **95**, 3413 (1973).
(10) D. J. Faulkner and M. O. Stallard, *Tetrahedron Lett.*, 1171 (1973).
(11) We are indebted to Dr. R. L. Foltz, Battelle Columbus Laboratories, for this determination.
(12) L. M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed, Pergamon Press, Oxford, 1969, p 202.

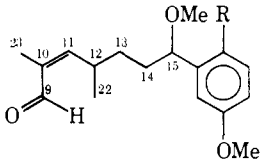
longed LAH treatment of **8**. The resulting pentaol **9**



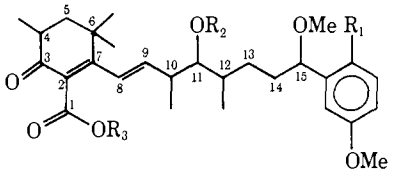
9

(pentaacetate, *m/e* 708), $C_{28}H_{50}O_7$, failed to react with periodate, but formed a diacetonide (*m/e* 578), which in turn was converted to a monoformate (*m/e* 606). LAD reduction of **8** yielded a tetradeuteriopentaol, consistent with deuterium incorporation at C-1 (D_2), C-3, and C-7.

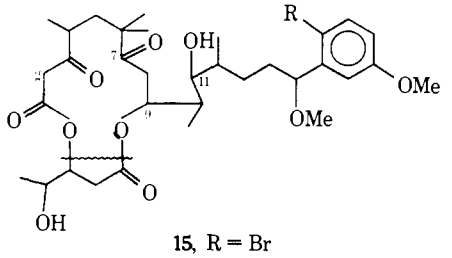
The manner in which the polyoxy chain of **9** is fashioned into the bicyclic hemiketal–spiroketal function of the aplysiatoxins (**1**, **2**) became manifest by treating mixed **7** and **8** with 0.5 *N* KOH in aqueous MeOH for 5 hr, resulting in two aldehydes **10** and **11**



10, R = Br
11, R = H



12, R_1 = Br; R_2 = R_3 = H
13, R_1 = R_2 = R_3 = H
14, R_1 = H; R_2 = Ac; R_3 = Me



15, R = Br
16, R = H

and two oxyacids **12** and **13**. If **1** and **2** are rewritten as the chain tautomer (**15**, **16**) of the hemiketal–spiroketal system, degradation products **12** and **13** involve loss of valeric acid (wiggly line) by β-elimination of the resulting C-9 hydroxyl and base catalyzed ring closure from C-2 to C-7. If the C-9 hydroxyl participates in a retroaldol reaction facilitated by the C-7 carbonyl with concomitant β-elimination of the C-11 hydroxyl, aldehydes **10** and **11** are the products. Evidence for **10** includes: *m/e* 356 (13%, $M^+ + 2$), 354 (13% M^+), 229 (100%, $Bz(MeO)_2Br$); uv 225 (15700), 280 (1920) nm; ir 2710, 1685 cm^{-1}; nmr ($CDCl_3$, δ) H-9 9.41 s, H-11 6.25 (br d, J = 10 Hz), H-12 2.75 (br m), H-13, H-14 1.7 (4 H br), H-15 4.50 (br t), H_3-22 1.13 (d, J = 6.5 Hz), H_3-23 1.80 (br s). All assignments were confirmed by appropriate double resonance experiments. Aldehyde **10** autoxidized on silica gel tlc plates to the corresponding acid, characterized as its methyl ester.

Oxyacids **12** and **13** were isolated as their methyl ester acetates, of which **14** was rigorously characterized. The *m/e* 528 was evidence for composition $C_{31}H_{44}O_7$ and a prominent peak at *m/e* 151 confirmed the intact benzyl methyl ether: uv(EtOH) 268 nm (29,500); ir 1730, 1675 cm^{-1}; nmr ($CDCl_3$, δ) H-4 ~2.5, H_2-5 ~1.7, H-8 6.00 (br d, J = 16.0 Hz), H-9 5.80 (dd, 16.0, 3.0), H-10 2.5 (m), H-11 4.77 (t, J = 6.0 Hz), H-12, H_2-13, H_2-14 1.7 (m), H-15 4.05 (t, 6.0), H_3-22 0.88 (d, J = 7.0 Hz), H_3-23 0.98 (d, J = 7.0 Hz), H_9-24,25,26 1.2. Assignments were corroborated by double resonance experiments, and **14** was fully confirmed by catalytic hydrogenation, ozonation, and $LiAlH_4$ reduction followed by acetylation and hydrogenation. All resulting transformation products were isolated and characterized.

Acknowledgment. We are grateful to Hoffmann-La Roche, Inc., and to PHS Grant 15198 for financial support.

(13) From the Ph.D. Thesis of Y. K., University of Hawaii, 1973.

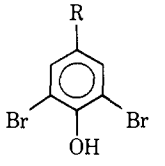
Yoshinori Kato,[13] Paul J. Scheuer*
Department of Chemistry, University of Hawaii
Honolulu, Hawaii 96822
Received December 14, 1973

Thelepin, a New Metabolite from the Marine Annelid *Thelepus setosus*

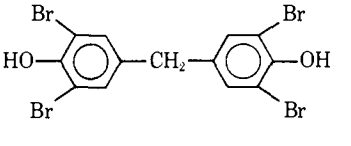
Sir:

Marine annelids (segmented worms) are unfamiliar invertebrates, particularly the sedentary polychaetes which live in self-constructed tubes or permanent burrows. Unsurprisingly, the chemical constituents of these animals have remained unexplored with few exceptions, notably nereistoxin,[1] hallachrome,[2] and arenicochrome.[3]

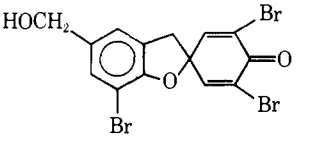
We wish to report the isolation and structural elucidation of five brominated metabolites (**1–5**) from the tube-



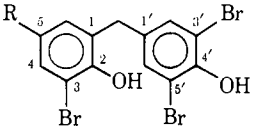
1, R = CH_2OH
2, R = CHO



3



5



4, R = CH_2OH
10, R = CHO

dwelling polychaete *Thelepus setosus* (Quatrefages, 1865), Family Terebellidae, of which spirodienone **5**, which we have named thelepin, bears a striking structural resemblance to the antimycotic agent griseofulvin (**6**), first isolated from the microorganism *Penicillium*

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(3) I. Morimoto, M. I. N. Shaikh, R. H. Thomson, and D. G. Williamson, *Chem. Commun.*, 550 (1970).