THE APLYSIATOXINS

Y. KATO and P. J. SCHEUER

Department of Chemistry, University of Hawaii, Honolulu, HI 96822, USA

ABSTRACT

From the marine gastropod mollusc *Stylocheilus longicauda* we isolated aplysiatoxin and debromoaplysiatoxin (LD_{100}, 0.3 mg/kg) and equal amounts of their non-toxic acetates. The structures of these compounds were deduced by spectral studies and by reductive and hydrolytic degradation.

INTRODUCTION

The attention of many natural product chemists was directed towards marine-oriented research at the 1964 symposium in Kyoto, through R. B. Woodward's lecture on the just then completed structure of tetrodotoxin[1]. During the intervening ten years we have witnessed a tremendous increase in research activity in this area, including such highlights as a total synthesis of tetrodotoxin[2], and structural elucidation of saxitoxin[3] and of surugatoxin[4]. Not surprisingly, all three marine toxins—tetrodotoxin, saxitoxin and surugatoxin—had achieved considerable notoriety as public health hazards long before their molecular structures became of interest to organic chemists, and long before organic chemists had the tools for unravelling such complex structures.

For about five of the ten years since the Kyoto Symposium we have wrestled with the structure of yet another marine toxin, which is the topic of this lecture. We are honoured and grateful that the Organizing Committee has invited us to present this work at the Ottawa Symposium, the first of the natural products symposia to include specifically a section on marine research.

Unlike the marine toxins that were mentioned above, the aplysiatoxins have never, to our knowledge, been a human health problem. This is not to say that the toxicity of sea hares, the animals that harbour the aplysiatoxins, has gone unnoticed by human observers. Yet it appears to us that the literary record in this case is perhaps a bit stranger than our own experience would indicate. Halstead[5] quotes the sixteenth century French poet and physician Grévin as follows.

> So deadly is the force of this poison that it poisons not only those who took it in by mouth, but also those who touched or looked at it, as Pliny reports, and if a pregnant woman sees it or even comes near it, especially if this happens to be a young woman, she immediately feels pains in the belly and nausea, and then she has an abortion.

Our own curiosity in sea hares as a research subject was first aroused by our unsuccessful attempt to isolate the purple defensive secretion, which is a

characteristic of some members of the family; this problem was eventually solved elsewhere[6] and led to the characterization of aplysioviolin. Our interest was again stimulated some years later by Watson's research on the physiological activities of sea hare toxin[7].

Sea hares are members of the marine molluscan family Aplysiidae, and are characterized by having only a small internal shell or no shell at all. Unlike many of the 80000 or so described living species of molluscs (in addition to 35000 fossil species), which are familiar to us because of their magnificent shells, their ability to generate pearls or their delicious taste, sea hares are inconspicuous, rather unattractive and, in contrast to some other molluscs, are not considered a dietary delicacy. In spite of the reputed toxicity of the animals, the first serious attempt to characterize the toxin was made only in 1915 by Flury[8]. Flury isolated the colourless unpleasant-smelling external secretion of *Aplysia depilans* and studied its physiological and chemical properties. Flury concluded that the secretion displayed neuromuscular activity in marine invertebrates, was non-nitrogenous and resembled terpenes. In addition to this colourless external secretion, Flury studied the non-toxic purple fluid, subsequently identified by Rüdiger[6]. A third, internal, secretion had been described by Flury[8] as white and viscous and was first investigated by Winkler *et al.*[9], who isolated a partially purified toxin, which they called aplysin, from the digestive gland of two California species, *Aplysia californica* and *A. vaccaria.* The digestive gland of sea hares, also known as midgut gland, hepatopancreas or liver, is a large organ which occupies about one-third of the body cavity of sea hares. Winkler *et al.*[9] studied the effect of the toxin on tissue preparations and on living animals, and concluded that the pharmacological activity of the toxin resembled that of choline esters.

Watson[7] isolated the toxins from the digestive gland of four sea hares found in Hawaiian waters—*Aplysia pulmonica, Dolabella auricularia, Dolabrifera dolabrifera* and *Stylocheilus longicauda.* Watson[7,10] found that all four species contained two toxins distinct in bioactivity and solubility. The water-soluble toxin exhibited hypotensive and the ether-soluble toxin hypertensive properties[11]. Watson's work, although not dealing with pure toxins, provided sufficient definition of at least the ether-soluble toxin to allow us to initiate research towards structural elucidation of this toxin. Successful completion of this work has been the subject of a preliminary report[12].

ISOLATION

We compared toxicity and reliable availability of ten species of aplysiids and decided to base our work on *Stylocheilus longicauda* (Quoy and Gaimard, 1824), despite the small size of this species, which averages only about 27 mm in length[13]. Because of the smallness of the animal we did not excise the toxic gland, but usually extracted whole animals in 500 g or 1 kg batches with acetone in a Waring Blendor, followed by a series of purification steps, which are summarized in *Figure 1.* Toxicity was monitored by i.p. injection in mice. In view of the unsavoury reputation of sea hares, it is worth noting that we observed no ill effects from handling the animals. Manipulation of

Stylocheilus longicauda (1 kg, wet)

↓ extraction with acetone

acetone soluble material (40 g)

↓ ether–water partition

ether-soluble material (20 g; LD_{100}, 10 mg/kg mouse)

↓ silicic acid column chromatography

toxic fractions (1.5 g)

↓ dry column chromatography

toxic fractions (0.6 g)

↓ gel filtration

toxic fractions (0.37 g)

↓ t.l.c.

aplysiatoxins (0.25 g; LD_{100}, 0.3 mg/kg mouse; coloured orange)

Figure 1. Isolation and purification of aplysiatoxins

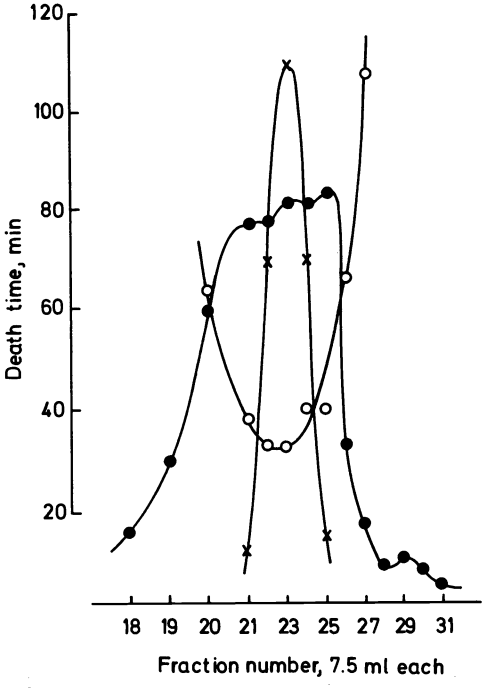


Figure 2. Distribution of toxicity (-○-○-), absorbancy at 446 nm (- × - × -) and weight (-●-●-) of fractions from gel filtration column chromatography. The scales are arbitrary except for the toxicity curve, which indicates the death time of mice in min.

the toxic extracts, on the other hand, caused reddening and swelling of the mucous membranes of eyes and nose. Accidental skin contact with a toxic extract produced redness and pus. Following the gel filtration step, we checked toxicity versus weight of fraction and versus non-volatile organic matter as described by Johnson[14]. The results are shown in *Figure 2*. The weight distribution curve (*Figure 2*) clearly showed that the toxin consisted of several components. However, preparative t.l.c. in numerous systems failed to achieve greater resolution of the toxin, which had the appearance of a viscous orange oil that was stable between pH 7 and pH 4.

CHARACTERIZATION

Ultra-violet spectral characteristics (*Figure 3*) of the unresolved aplysiatoxin were those of an alkylphenol. The i.r. spectrum confirmed the phenolic nature and revealed, in addition, the presence of at least one non-phenolic hydroxy group. Acetylation under mild conditions yielded a resolvable mixture of four non-toxic diacetates. The tenacious companion pigment luckily also reacted and became separable by t.l.c. All four diesters possessed

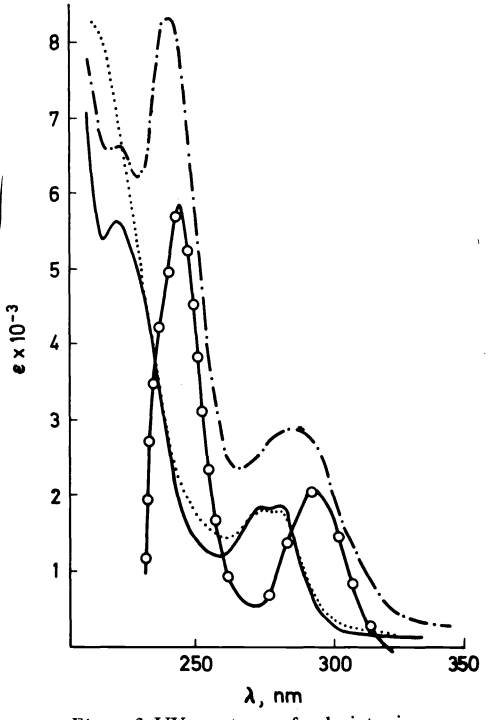


Figure 3. UV spectrum of aplysiatoxin:
———— in MeOH
–·–·– in 0.1 N NaOH/MeOH
– – – – – in 0.1 N H_2SO_4
–○–○– Δε curve: ε in basic–ε in neutral

identical ester functions, one phenolic and one secondary alcoholic ester per component. Each pair of diacetates consisted of an alkylphenol and of an alkylbromophenol derivative. The relationship of the two pairs to each other remained obscure to us for several years.

All components of crude aplysiatoxin and its derivatives were recognizable by white fluorescence when an acidified t.l.c. plate was viewed under 360 nm light. After we discovered this property, we noticed in the crude sea hare extracts non-toxic components that fluoresced identically and were less polar than the toxic compounds. These constituents proved to be a group of four alcoholic monoacetates that occurred naturally in approximately equal yield (0.025%) and were otherwise fully parallel to the four toxins.

Demonstration that structural integrity has been preserved during acetylation and that the non-toxic acetates could be reconverted to the toxic phenolic alcohols proved to be difficult because of the known sensitivity of the toxins to acid and base. We eventually achieved a successful transformation, albeit in poor yield, on an alumina column, patterned after the work by Johns and Jerina[15] in the steroid series. One of the bromophenols was recovered in sufficient quantity for bioassay: LD_{100} of this toxin was 0.2 mg/kg, as against 0.3 mg/kg for the toxin mixture.

The phenolic hydroxy group reacted slowly with diazomethane in alcoholic ether. This etherification furnished a mixture of four non-toxic anisoles, which readily formed monoacetates. The anisole-monoacetates were convenient derivatives, since they allowed us to utilize fully the natural alcoholic acetates. Another useful interconversion was debromination of the phenols by palladium-on-charcoal hydrogenolysis in the presence of triethylamine[16].

Determination of the molecular formulas of the toxins and of their derivatives appeared to be straightforward: mass spectrometry and combustion data led to $C_{32}H_{45}BrO_9$ (652) and $C_{32}H_{46}O_9$ (574) for the underivatized toxins and to corresponding formulations for the monoacetates, diacetates and anisole acetates. We seemingly were dealing with two pairs of isomeric toxins, each pair consisting of a phenol and of a bromophenol, and with a parallel set of two pairs of non-toxic monoacetates. As we hinted earlier, the true relationship of the pairs to each other was not revealed until rather late in the structural investigation.

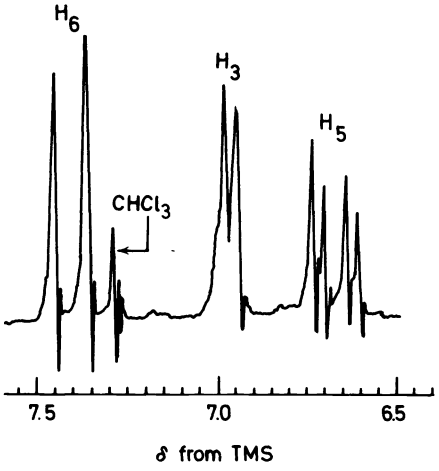
STRUCTURE

The Aromatic Moiety Ultra-violet data (*Figure 3*) clearly indicated the presence of a phenol, and the $\Delta\varepsilon$ curve in *Figure 3* further showed that the phenol lacked extended conjugation[17].

P.m.r. spectra of the bromophenols and their derivatives displayed ABX or AMX spin systems, as seen in *Figure 4* for one of the anisoles, which appears to be a 2-alkyl-4-methoxybromobenzene. Chemical shift values for this substitution pattern may be calculated[18], and these values for three aromatic protons are compared with the observed values in *Figure 4*. Correspondence for H-3 is poor, but choice of this substitution pattern is necessary in order to accommodate chemical shift data for the benzylic proton. The derivative in *Figure 4* displays a one-proton triplet at δ 4.52, while its debromo analogue

has the corresponding signal at δ 4.07, which requires *o*-alkylbromo substitution.

For the more complex four-spin system of the debromo analogue, computer-generated spectra were compared with the observed spectra in CCl_4 and benzene-d_6.



Chemical shift values (δ) in a 2-alkyl-4-methoxybromobenzene

	Observed	Calculated[18]
H-3	6.97	6.56
H-5	6.68	6.53
H-6	7.40	7.34

Figure 4. P.m.r. signals of the aromatic portion of an aplysiatoxin derivative, and comparison of observed and calculated δ-values for the aromatic protons

A three-proton singlet at δ 3.18 had been assigned to a benzylic methoxy group. This structural feature was confirmed by loss of this signal following hydrogenolysis in acetic acid-ethanol. This reaction also eliminated the benzylic proton triplet at δ 4.07. The triplet nature of this signal therefore places a methylene group next to the benzyl carbon. Appropriate prominent mass spectral peaks for substituted tropylium ions were in complete agreement with part structure I, *Figure 5*.

The Lactone Moiety Infra-red spectra of the aplysiatoxins and their functional derivatives have carbonyl absorption at 1730 cm^{-1}, which we interpreted as originating from one or two ester and/or lactone functions. Two ester functions were particularly attractive, since only three (ArOH, ROH, ArCHOMe) of the nine oxygen atoms had been assigned. We were able to demonstrate the bis-lactone nature of the aplysiatoxins by mild

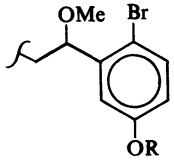


Figure 5. Part structure I

base treatment (1 N KOH/EtOH or $NaBH_4$/EtOH) of the debromoanisole acetates at room temperature. The potassium hydroxide reaction was relatively clean. It produced a major product, a hydroxy acid, that could be isolated and characterized after methylation and acetylation. The stoichiometry of the reaction indicated that a seven-carbon fragment had been lost from the starting material, but we were unable to isolate the small compound. Sodium borohydride reaction, on the other hand, resulted in a complex mixture which was subjected to gel filtration. From one group of fractions we isolated, after acetylation, 4-acetoxy-*trans*-2-pentenoic acid (II), which was identified by spectral analysis. The over-all reaction and p.m.r. data for II are shown in *Figure 6*. Since we had no evidence in the aplysiatoxins of a disubstituted *trans* olefin or of an α,β-unsaturated ester/lactone, we concluded that this structural feature had been generated by base treatment. Since the large fragment resulting from the base reaction was a hydroxy acid, the bis-lactone nature of the aplysiatoxins was plausible, as was generation of a new double bond by base hydrolysis-elimination.

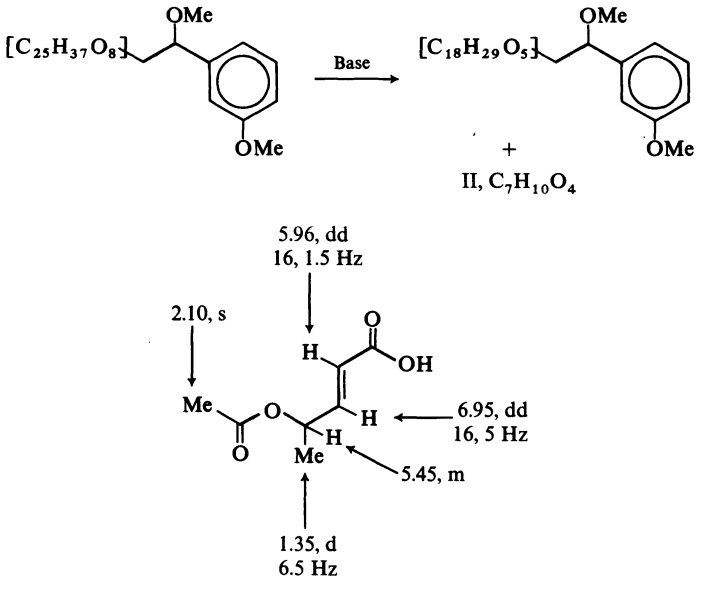


Figure 6. Base treatment of an aplysiatoxin derivative leading to 4-acetoxy-*trans*-2-pentenoic acid (II).

Figure 7 shows the bis-lactone moiety (part structure III) of the intact toxin acetates with appropriate p.m.r. data. Irradiation at δ 4.97 caused the signal at 5.40 to collapse to a broad doublet with J = 10.5 Hz, and the signal at δ 1.23 to a singlet. Irradiation at δ 5.40, on the other hand, collapsed the 4.97 signal to a quartet, J = 6.8 Hz, and simplified the δ 2.7 multiplet. It may be noted here that the aplysiatoxins prior to acetylation lack the proton signal at δ 4.97. Instead a proton signal may be found near δ 3.8. These data clearly show that this is the site of the aliphatic alcohol and that the small part of the aplysiatoxins is 3,4-dihydroxyvaleric acid, which is doubly esterified through the carboxy group and through its 3-hydroxy function to the rest of the molecule.

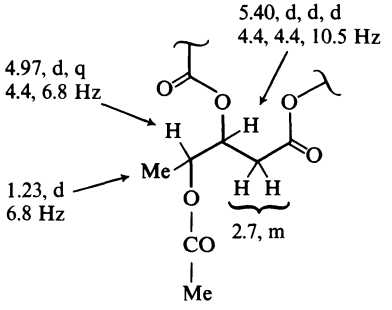


Figure 7. P.m.r. data of the lactone portion of aplysiatoxin acetates, part structure III.

The Principal Carbon Skeleton. Metal Hydride Reduction We mentioned earlier that base treatment of the aplysiatoxin methyl ether acetates led to isolation and characterization of two hydroxy acids, which had lost the valeric acid fragment. Reductive cleavage with lithium aluminium hydride or with Vitride under a variety of conditions not only eliminated the valeric acid moiety but also produced several major degradation products that became vital to the solution of the structural problem.

Under mild conditions (LAH, ether, $-8°$, 2.5 h) aplysiatoxin methyl ether acetates (C_{35} compounds) produced C_{28} diols which could be selectively acetylated to mono- and diacetates. Mild lithium aluminium deuteride reduction produced a dideuteriodiol, thereby indicating that only the lactone linkages had been affected by the reaction. The hindered secondary alcoholic group furnished an additional structural detail. Slow acetylation (3 d for complete diacetylation) frequently points to an axial C—O bond at a conformationally rigid cyclohexane system[19]. The p.m.r. signal for the proton of this secondary acetate is a sharp quartet at δ 4.80, J = 2.7 Hz. This would indicate that this is an equatorial hydrogen that is coupled equally to three vicinal protons. Of further importance is the fact that this characteristic one-proton quartet may be seen in the spectra of the original toxins.

When the aplysiatoxin methyl ether acetates were refluxed with LAH in ether for 6 h, one major product resulted, which could be purified on a silicic acid column. The resulting homogeneous polyol did not show a

molecular ion, but it slowly formed a peracetate which had a molecular ion at *m/e* 708. Spectral data of the polyol and its acetate showed that the product of drastic LAH reduction was a pentaol of composition $C_{28}H_{50}O_7$ (498). The pentaol failed to react with periodate but formed a diacetonide, which could be converted to a monoformate. LAD reduction under identical conditions led to the incorporation of four deuterium atoms. The p.m.r. spectra of the pentaol and its derivatives were too complex to allow full structural assignments without information not yet discussed.

However, this crucial transformation resulted in a pentahydroxy-alkylbenzene derivative which had lost only the valeric acid moiety and which had lost all unsaturation and all rings with the exception of the familiar aromatic part of the molecule. More importantly yet, this transformation posed a dilemma which after its resolution contributed fundamentally towards untying of the structural knot.

Aplysiatoxins, $C_{32}O_9$		Pentaol, $C_{28}O_7$	
phenol	1	anisole	1
benzylmethyl ether	1	benzylmethyl ether	1
sec-alcohol	1	(hydroxyvaleric acid	3)
bis-lactone	4	hydroxy groups	5
unknown functions	2		
	9		10

Figure 8. Inventory of oxygen functions in aplysiatoxins and in pentaol

We should recall that mass spectral and combustion data had established the aplysiatoxins to be isomeric pairs of $C_{32}O_9$ compounds that were derivatives either of *m*-cresol or of 4-bromo-3-alkylphenols. An inventory of the demonstrated oxygen functions is shown in *Figure 8*. Two oxygen atoms are securely assigned to the benzyl part of the molecule and remain intact during the LAH reduction. Three oxygen atoms are lost with the hydroxyvaleric acid fragment (*Figure 7*). The expected LAH reduction product, therefore, should have four oxygen atoms in addition to the two in the aromatic moiety. But we know that it has five. This bit of arithmetic led us to the inescapable conclusion that the native aplysiatoxins must possess an additional hitherto undetected oxygen atom. Chemical ionization mass spectrometry with ammonia did indeed reveal that all of our earlier electron impact data had resulted in M^+-18. These data not only provided correct molecular formulas, $C_{32}H_{47}BrO_{10}$ and $C_{32}H_{48}O_{10}$, for the aplysiatoxins, but also solved the puzzling 'isomeric pairs'. Correctly, the natural toxins are two compounds, aplysiatoxin and debromoaplysiatoxin. Both compounds readily lose one molecule of water, invariably and completely during electron impact mass spectrometry and partially during purification on silica gel. The apparent isomers, in reality anhydro derivatives, are creatures of the mass spectrometer and of active adsorbents.

Base Degradation When a mixture of aplysiatoxin and debromoaplysiatoxin methyl ether acetates was refluxed for 5 h with 0.5 N aqueous methanolic KOH and the ensuing mixture treated with diazomethane,

t.l.c. analysis revealed six major products, which were separated and purified by Sephadex LH-20 chromatography and preparative t.l.c.

Two compounds, IV and V, were C_{17}-aldehydes, the structures of which are shown in *Figure 9*. Composition of $C_{17}H_{23}BrO_3$ and $C_{17}H_{24}O_3$ was seen in the mass spectra, as was the retention of the aromatic moiety. In addition to the aromatic u.v. (280 nm, ε 1920) the compounds had a second u.v. maximum at 225 nm (18 700). Corresponding i.r. bands at 2710 and 1670 cm^{-1} pointed to an α,β-unsaturated aldehyde structure. Pertinent p.m.r. data are shown in *Figure 9*. Irradiation experiments verified the n.m.r. assignments.

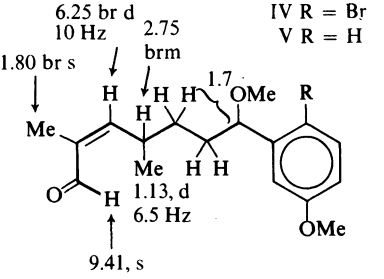


Figure 9. Structure and p.m.r. assignments of aldehydes IV and V

Corresponding α,β-unsaturated acids arose by autoxidation during work-up or could be produced from the aldehydes by oxidation, followed by diazomethane esterification and isolation.

In addition to the pairs of C_{17} aldehydes and acids, base degradation furnished two C_{28} hydroxy acids, which were isolated as the methyl ester acetates and were assigned structures VI and VII (*Figure 10*). The formulation of VII (by mass spectrometry) as $C_{31}H_{44}O_7$ indicated retention of all aplysiatoxin carbons with the exception of the hydroxy-valeric acid fragment. Infra-red bands at 1730 and 1675 cm^{-1} and a u.v. maximum at 268 nm (29 500) indicated the presence in the molecule of an unsaturated carbonyl

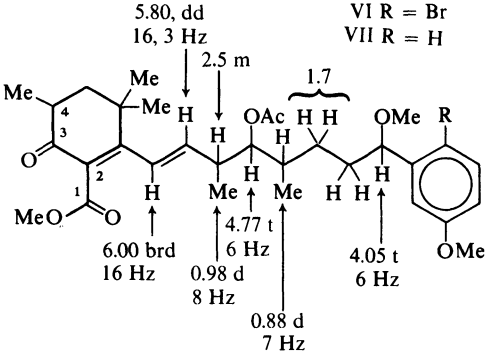


Figure 10. Structure and p.m.r. assignments of base degradation products VI and VII

function in addition to the known methyl ester and acetate groups. The p.m.r. assignments which could be made unambiguously and which were interrelated by double resonance experiments are shown in *Figure 10*. Lack of additional olefinic protons coupled with i.r. and u.v. absorption make it plausible to extend the olefin to a dienone. P.m.r. signals for the remaining three methyl groups (near δ 1.2) could not be assigned, perhaps partly because compound VII probably contained both epimers with respect to C-4.

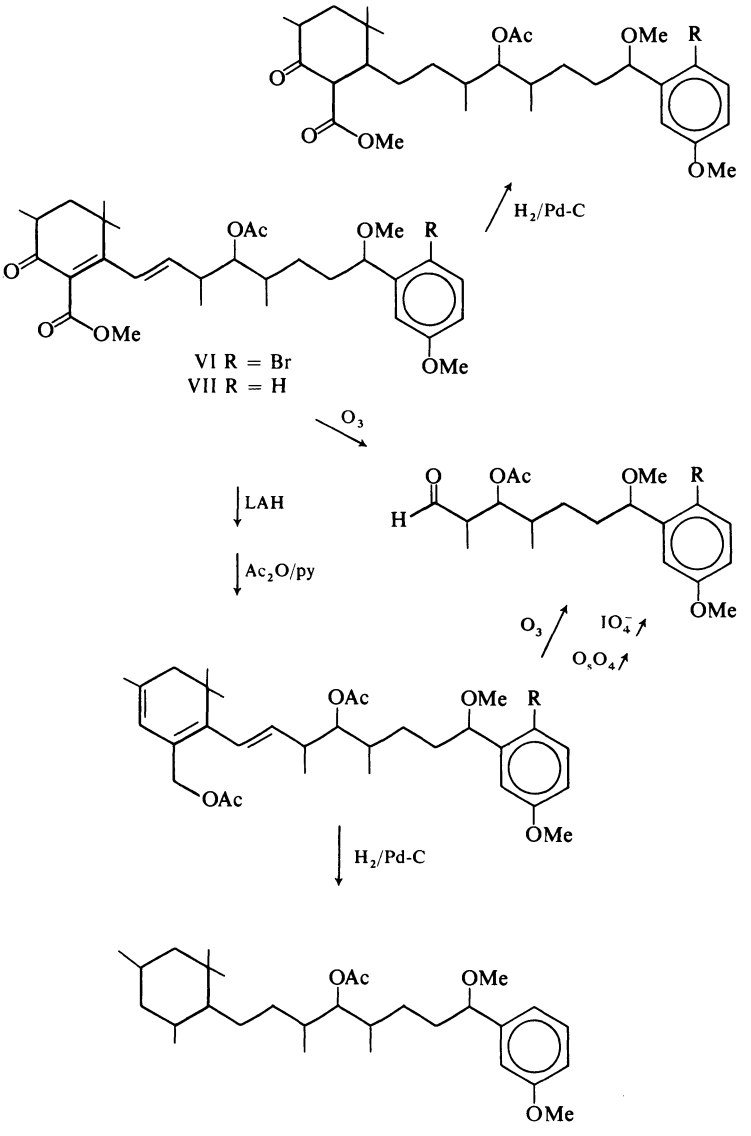


Figure 11. Transformations of compound VII

The postulated structure of VII was further corroborated by a series of reactions which are outlined in *Figure 11*. Only the LAH reduction product of VII was accumulated in sufficient quantity for detailed p.m.r. studies.

In order to rationalize production of aldehyde V and oxyacid VII from a common precursor, we postulate reaction intermediate VIII, shown in *Figure 12*. Production of VII from VIII by Knoevenagel condensation and dehydration presents no difficulty. It is also plausible that VIII, which is a β-ketol, will undergo a retro-aldol reaction and be transformed to IX. Compound IX is an aldol and might be expected to undergo a second retro-aldol reaction. We did not isolate such a product. Instead we isolated α,β-unsaturated aldehyde V. Precursor VIII, incidentally, also serves as a suitable parent for the LAH-produced pentaol which, it will be recalled, gives rise to a bis-acetonide and which incorporates four deuterium atoms (2 at C-1, 1 each at C-3 and C-7) on LAD treatment.

Finally, how can compound VIII be derived from the aplysiatoxins which are bis-lactones of part structure III (*Figure 7*)? One locus of lactonization must be at C-1 and the other is likely to be at C-9. If we combine part structures VIII and III in this fashion, we arrive at a new aplysiatoxin precursor, X (*Figure 13*). We had noted earlier that on the basis of p.m.r. data of the diol produced by mild LAH reduction the carboxyl terminus of the valeric acid must be connected to the rest of the molecule by an axial C—O bond at C-9 and that this C-9 must be part of a structurally rigid 6-ring. Since the

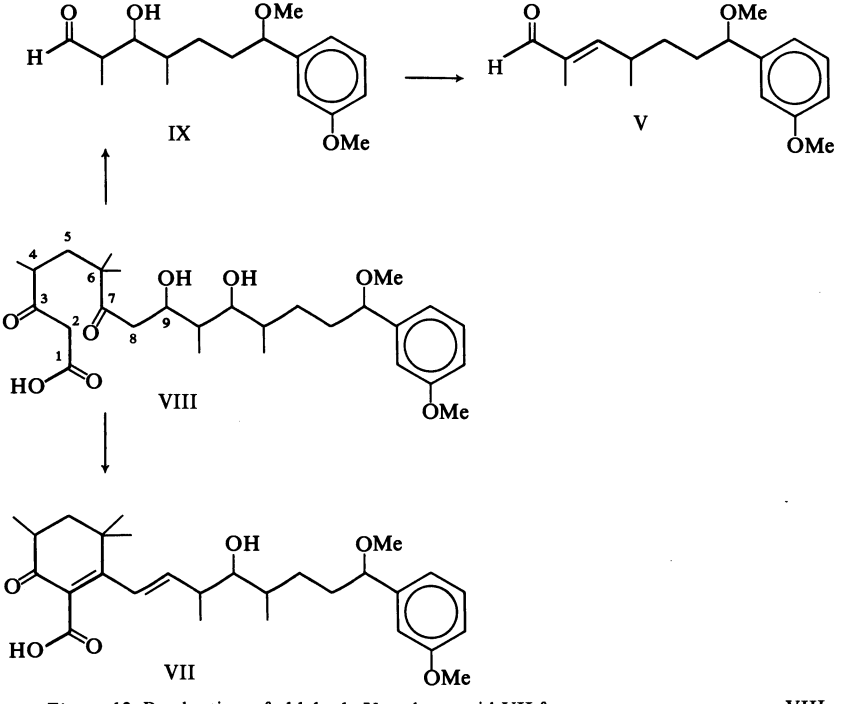


Figure 12. Production of aldehyde V and oxyacid VII from a common precursor VIII

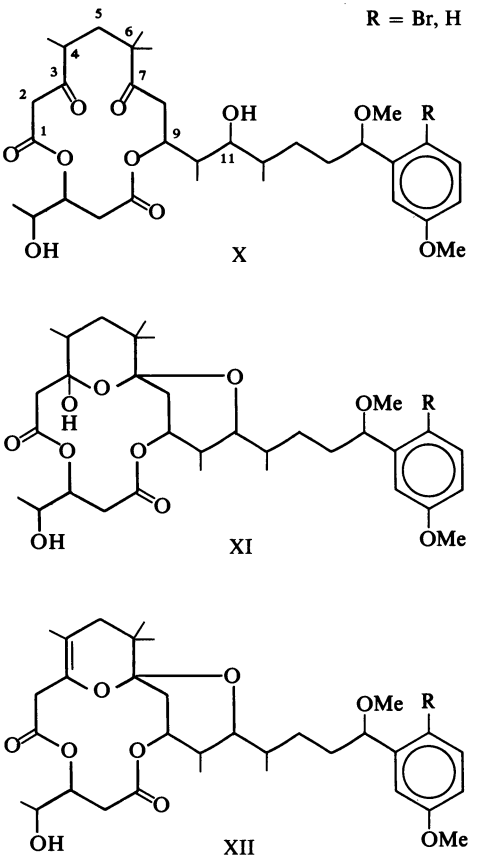


Figure 13. Aplysiatoxins (XI), anhydroaplysiatoxins (XII) and hypothetical precursor (X)

aplysiatoxins have only a secondary alcohol function in the valeric acid appendix and since the aplysiatoxins possess no keto carbonyls, we can combine these structural requirements by forming two six-membered tetrahydropyran rings: a ketal from C-11 to C-7 and a hemiketal from C-7 to C-3. These ring closures result in structure XI, the correct structure of the aplysiatoxins. The facile dehydration of the tertiary alcohol which is *trans* to a convenient proton at C-4 now has a ready explanation.

Correct location of the secondary methyl group at C-4 and of the two tertiary methyl groups at C-6 was difficult. The C-4 methyl group resonates about δ 0.8, but cannot be clearly observed because of overlapping signals with the other methyl groups. However, in the anhydro series this methyl group resonates at δ 1.60 as a broad singlet. The signal is broadened by long-range coupling with the protons at C-2. This relatively high field resonance is undoubtedly influenced by the oxygen atom of the dihydropyran ring. The signals for the C-2 protons (3.04, br d, J = 16 Hz and 3.23, br d, 16 Hz) are also broad because of long-range coupling with protons of the C-4

methyl group and those at C-5. The p.m.r. signals of the two methyl groups at C-6 could be seen clearly in only two derivatives, one at 300 MHz and the other at 100 MHz with the aid of a shift reagent. One of these derivatives in the anhydro series, which is not discussed here, has signals for the C-5 protons as two broad doublets at δ 1.30 and 2.18 with a coupling constant of 16.5 Hz. Since these are the signals of isolated geminal protons, C-6 must be tetrasubstituted. Furthermore, C-6 is assigned a chemical shift of 38.168 p.p.m. in its c.m.r. spectrum, thereby indicating that C-6 has only carbon neighbours. Chemical shifts for the geminal dimethyls were δ 0.83 and 0.93.

Further degradations, oxidative and under acidic conditions, confirmed all structural assignments[20].

It is worth noting that none of the toxins crystallized nor did any of the numerous derivatives. *In toto*, we degraded about 12 g of toxin, which was extracted from 50 kg of *Stylocheilus longicauda*, about 5000 animals.

ACKNOWLEDGEMENT

We are grateful to Hoffmann-La Roche, Inc., and to the US Public Health Service (Grant 15198) for financial support.

REFERENCES

[1] R. B. Woodward, *Pure Appl. Chem.* **9**, 49 (1964).
[2] Y. Kishi, M. Aratani, T. Fukuyama, F. Nakatsubo, T. Goto, S. Inoue, H. Tanino, S. Sugiura and H. Kakoi, *J. Amer. Chem. Soc.* **94**, 9217, 9219 (1972).
[3] J. L. Wong, R. Oesterlin and H. Rapoport, *J. Amer. Chem. Soc.* **93**, 7344 (1971).
[4] T. Kosuge, H. Zenda, A. Ochiai, N. Masaki, M. Noguchi, S. Kimura and H. Narita, *Tetrahedron Letters*, 2545 (1972).
[5] B. W. Halstead, *Poisonous and Venomous Marine Animals of the World*, Vol. I, p 709. US Government Printing Office: Washington, DC (1965).
[6] W. Rüdiger, *Hoppe-Seyler's Z. Physiol. Chem.* **348**, 129, 1554 (1967).
[7] M. Watson, 'Some aspects of the pharmacology, chemistry and biology of the midgut gland toxins of some Hawaiian sea hares, especially *Dolabella auricularia* and *Aplysia pulmonica*' *PhD Dissertation*, University of Hawaii (1969).
[8] F. Flury, *Arch. Exp. Pathol. Pharmakol.* **79**, 250 (1915).
[9] L. R. Winkler, B. E. Tilton and M. G. Hardinge, *Arch. Int. Pharmacodyn. Ther.* **137**, 76 (1962).
[10] M. Watson, *Toxicon*, **11**, 259 (1973).
[11] M. Watson and M. D. Rayner, *Toxicon*, **11**, 269 (1973).
[12] Y. Kato and P. J. Scheuer, *J. Amer. Chem. Soc.* **96**, 2245 (1974).
[13] E. A. Kay, *Proc. Malacol. Soc. London*, **36**, 173 (1964).
[14] M. J. Johnson, *J. Biol. Chem.* **181**, 707 (1949).
[15] W. F. Johns and D. M. Jerina, *J. Org. Chem.* **28**, 2922 (1963).
[16] R. Augustine, *Catalytic Hydrogenation*, p 125. Dekker: New York (1965).
[17] G. Aulin-Erdtman, *Chem. & Ind. (London)*, 581 (1955).
[18] L. M. Jackman and S. Sternhell, *Application of NMR Spectroscopy in Organic Chemistry*, 2nd ed., p 202. Pergamon: Oxford (1969).
[19] F. Sallmann and Ch. Tamm, *Helv. Chim. Acta*, **39**, 1340 (1956).
[20] Y. Kato, 'Toxic constituents of the marine mollusk *Stylocheilus longicauda*', *PhD Dissertation*, University of Hawaii (1973).