On the Origin of the Dimeric Aplysinopsin Alkaloids

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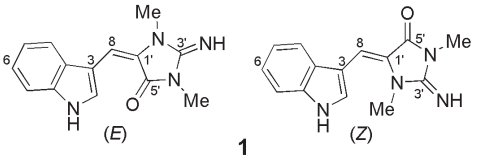
Introduction

The aplysinopsins comprise a group of compounds isolated from marine sources and are unusual in that they contain a series of dimers of unexplained biogenetic origin. These dimers have long been thought to arise from the Diels-Alder cycloaddition between two monomers with one acting as the diene and the other as the dienophile. However, recent findings have evoked a possible second biosynthetic pathway whereby the dimers are formed by the rearrangement of a corresponding dimeric cyclobutane aplysinopsin. A chronological history of this fascinating class of alkaloids is discussed herein.

Monomeric Aplysinopsins

In 1977, Wells and co-workers isolated two novel tryptophan-based secondary metabolites from the sponges *Thorecta* sp. collected from the Australian Great Barrier Reef.[1] These natural products were subsequently named aplysinopsin (**1**) and 3'-deimino-3'-oxoaplysinopsin (**2**) that occur as (*E*)- and (*Z*)-isomers (below and Table 1).[1] It was shown that **1** existed as a mixture of double bond isomers (~ 9:1) of which the major isomer is the (*E*)-isomer.[2] The isolation report also confirmed the structure of **1** by synthesis. Condensation of indole-3-carbaldehyde with a creatinine derivative gave rise to **1** identical in every respect to the natural material.[1]



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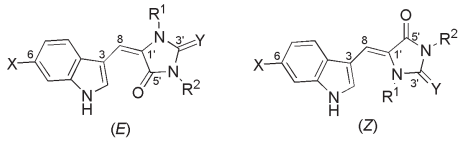
The years following Wells' initial report saw the isolation of several more aplysinopsin-type secondary metabolites (Table 1) and in the latter part of 1977, **1** was isolated from the sponge *Verongia spengelii*.[2] In 1980, an extract of the sponge *Dercitus* sp. from Belize was also shown to contain **1**, along with minor amounts of **3** and **4** (Table 1).[3] In late 1980, analysis of the extracts of the Caribbean sponge *Smenospongia aurea* afforded the novel 6-bromo-

Table 1. The family of monomeric aplysinopsins



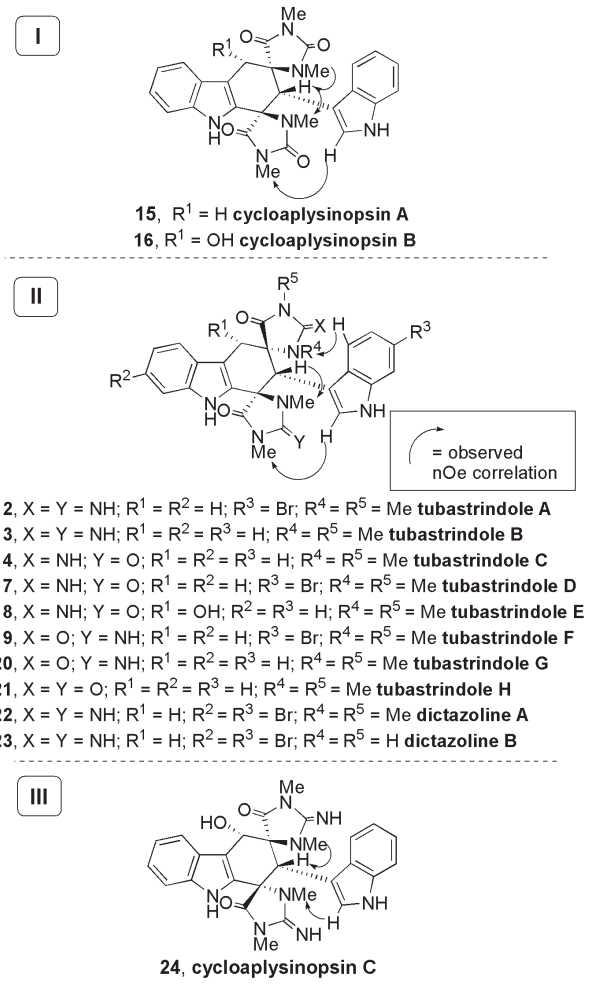
No.	Natural Product	R^1	R^2	X	Y	*E*/*Z* ratio	Ref. *E*/*Z* ratio
1	aplysinopsin	Me	Me	H	NH	> 95:5	10
2	3'-deimino-3'-oxo-aplysinopsin	Me	Me	H	O	> 95:5	10
3	2'-de-*N*-methyl-aplysinopsin	H	Me	H	NH	< 5:95	10
4	6-bromo-2'-de-*N*-methylaplysinopsin	H	Me	Br	NH	< 5:95	10
5	6-bromo-3'-deimino-2',4'-bis(demethyl)-3-oxoaplysinopsin	H	H	Br	O	1:1	9
6	*N*-methylaplysinopsin	Me	Me	H	NMe	High *E*	5
7	6-bromoaplysinopsin	Me	Me	Br	NH	100% *E*	5
8	6-bromo-3'-deimino-3'-oxoaplysinopsin	Me	Me	Br	O	5:2	9
9	3'-deimino-2',4'-bis(de-methyl)-3'-oxoaplysinopsin	H	H	H	O	<5:95	10
10	2'-demethyl-3'-*N*-methyl-aplysinopsin	H	Me	H	NMe	<5:95	10
11	6-bromo-2'-demethyl-3'-*N*-methylaplysinopsin	H	Me	Br	NMe	<5:95	10

3'-deimino derivative **5**[4] and, in 1981, the *N*-methyl **6** was isolated from *Aplysinopsis recticulata*.[5] Interestingly, various monomeric aplysinopsins have also been found in several scleractinian corals of the family Dendrophylliidae. In 1982, the extracts of *Tubastraea coccinea* revealed the presence of **3** and **4**, as well as the new natural product 6-bromoaplysinopsin (**7**).[6] Aplysinopsins **1** and **7** have been found in *Astroides calycularis,*[7] and **1** also from *Tubastraea aurea*.[8] In 1988, two novel 3'-oxoaplysinopsins were identified from two separate corals.[9] Thus, extracts of *Tubastraea* sp. contained **2** and the novel oxoaplysinopsin **8**, with the extracts of *Leptopsammia pruvoti* containing both the bis(demethyl)-3'-oxoaplysinopsin **5** and the novel bis(demethyl)-3'-oxoaplysinopsin **9**. A year later, two further analogues [2'-demethyl-3'-*N*-methylaplysinopsin (**10**) and its 6-bromo derivative (**11**)] were isolated from *Dendrophyllia* sp. along with **3** and **4**.[10] In a series of important findings, it was subsequently shown that various aplysinopsins undergo a thermally-reversible photoisomerization.[9] An instructive review regarding the synthetic efforts towards this monomeric class of natural products is available.[11]

The Dimeric Aplysinopsins

In 2000, the aplysinopsin story acquired a new dimension when it was reported that an extract of the coral *Tubastraea faulkneri* contained a dimer of **4**. This dimer was identified from the extract using *spectral and melting point analyses*, but the only spectroscopic information reported was the molecular weight.[12] It took a further three years before a fully characterized aplysinopsin dimer appeared.

Thus, the first fully characterized dimeric aplysinopsins were reported in 2003. Extracts of *Tubastraea* sp. contained the cyclohexenyl tubastrindoles A-C (**12-14**; Fig.1), along with aplysinopsin **1**[13] Around the same time, extracts of two corals afforded cycloaplysinopsin A (**15**), smaller amounts of B (**16**) and the known monomers **2** and **8**.[14] Interestingly, detailed 2-D NMR studies conducted by separate groups confirmed that the tubastrindoles A-C (**12-14**) and **15** and **16** possess different relative stereochemistry (Fig. 1). Further investigation of the extracts of *Tubastraea aurea* in 2008 led to the discovery of five further tubastrindoles D-H (**17-21**)[15] that had the same relative stereochemistry as previously established **12-14**.[13] The first dibrominated aplysinopsin dimers appeared when analysis of the extracts of the sponge *Smenospongia cerebriformis* afforded dictazolines A and B (**22** and **23**)[16] along with the previously described tubastrindoles **12** and **13**. Subsequently, dictazolines A and B were shown to have the same relative stereochemistry as all the previously isolated tubastrindoles.[13,15] In 2009 the isolation of a novel aplysinopsin dimer took the total number of distinct diastereomer classes to three when it was shown that the extracts of the sponge *Tubastraea* sp. (collected in Yemen) yielded cycloaplysinopsin C (**24**). Detailed 2-D NMR studies confirmed that **24** possesses different relative stereochemistry from both the previously described cycloaplysinopsins **15** and **16** (Class I) and the tubastrindoles and dictazolines **12-14**, and **17-23** (Class II) (Fig. 1).[17]



15, R^1 = H **cycloaplysinopsin A**
16, R^1 = OH **cycloaplysinopsin B**

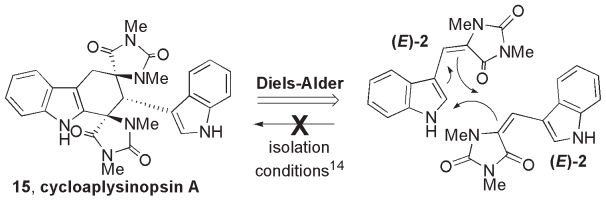
12, X = Y = NH; R^1 = R^2 = H; R^3 = Br; R^4 = R^5 = Me **tubastrindole A**
13, X = Y = NH; R^1 = R^2 = R^3 = H; R^4 = R^5 = Me **tubastrindole B**
14, X = NH; Y = O; R^1 = R^2 = R^3 = H; R^4 = R^5 = Me **tubastrindole C**
17, X = NH; Y = O; R^1 = R^2 = H; R^3 = Br; R^4 = R^5 = Me **tubastrindole D**
18, X = NH; Y = O; R^1 = OH; R^2 = R^3 = H; R^4 = R^5 = Me **tubastrindole E**
19, X = O; Y = NH; R^1 = R^2 = H; R^3 = Br; R^4 = R^5 = Me **tubastrindole F**
20, X = O; Y = NH; R^1 = R^2 = R^3 = H; R^4 = R^5 = Me **tubastrindole G**
21, X = Y = O; R^1 = R^2 = R^3 = H; R^4 = R^5 = Me **tubastrindole H**
22, X = Y = NH; R^1 = H; R^2 = R^3 = Br; R^4 = R^5 = Me **dictazoline A**
23, X = Y = NH; R^1 = H; R^2 = R^3 = Br; R^4 = R^5 = H **dictazoline B**

24, **cycloaplysinopsin C**

Fig. 1. Dimeric aplysinopsins and key nOe correlations.

Dimeric Aplysinopsins: Diels-Alder Cycloadducts?

When considering the biosynthetic origin of the cyclohexenyl aplysinopsin dimers, their structural similarity to the monomeric aplysinopsins is striking. Alas, in the vast majority of cases, the dimeric aplysinopsin is co-isolated from the same host organism along with its respective monomer, suggesting that the monomeric aplysinopsins are related to the dimers.[13,14] To investigate this possible relationship, Mancini and co-workers instigated an investigation involving **2** and its dimeric partner, cycloaplysinopsin A (**15**), two natural products isolated from the same coral. This group showed that subjecting synthetic **2** to the exact conditions used during the extraction process did not result in the formation of **15**. This suggests that cycloaplysinopsin A (**15**) is not an artefact of the isolation procedure and is indeed a natural product in its own right (Scheme 1).



15, **cycloaplysinopsin A**

Scheme 1. Diels-Alder cycloaddition with two molecules of (*E*)-**2**.

The observations regarding the biogenesis of the dimeric aplysinopsins present a fascinating possibility that they may, in fact, be cycloadducts of their respective aplysinopsin monomers. Diels-Alder cycloaddition is a credible pathway for this dimerization, whereby the monomeric aplysinopsin is acting both as the diene and the dienophile in the [4+2] process (Scheme 1).[13,14] After dimerization, further modifications occur in some cases, *i.e.* oxidation at C8 in the case of **16**, **18** and **24**.

What Promotes the Dimerization?

It is well established that cycloaplysinopsin **15** is not formed when subjecting the corresponding monomer, **2**, to the extraction conditions. This implies that an external entity associated with the host organism is aiding the dimerization in some way. Cycloaplysinopsins A-B (**15** and **16**) both possess a slight excess of one enantiomer (~30% ee), suggesting that, if a Diels-Alderase is responsible, it is enantio-defective.[14] However, Mancini and co-workers concluded that owing to the ongoing controversy over enzymes that catalyze concerted cycloaddition processes, it is more likely that the host organism (or associated symbiont) contains an adventitious Diels-Alder catalyst that aids the dimerization. Moreover, the chiral environment (steroids and other common metabolites) appears responsible for the slight asymmetric induction.[14] Presumably this postulate can be applied to other dimeric aplysinopsins, as Iwigawa and co-workers also concluded that because of the small optical rotation values seen in the tubastrindoles, it is highly likely that they exist as a mixture of both enantiomers.[15] However, further investigation into this proposal is hindered by the fact that many of the isolation reports do not disclose the enantiomeric excess of the dimeric aplysinopsins. Nonetheless, the Diels-Alder cycloaddition proposal offers potential insight into the different relative stereochemistry observed in the various dimeric compounds. It is possible that both (*E*)- and (*Z*)-aplysinopsins are viable substrates for the cycloaddition process and, as the vast majority of the monomeric aplysinopsins essentially exist as a single regioisomer (Table 1), it is conceivable that photoisomerization[9] is pivotal for the Diels-Alder reaction to proceed in some cases. Accordingly, the dimers can be grouped into three classes and used to rationalize the relative stereochemistry observed: Class I: (*E*)-diene and (*E*)-dienophile **15** and **16**; Class II: (*Z*)-diene and (*E*)-dienophile **12-14** and **17-23**; Class III (*E*)-diene and (*Z*)-dienophile **24** (Fig. 2).

As shown in Fig. 2, it is assumed that during the cycloaddition process, the diene and the dienophile are orientated as depicted. This is based on the orientation being the most favourable from MM calculations for (*E*)-**2** diene and (*E*)-**2** dienophile to give **15**.[14] However, it is also conceivable that **15** may be constructed from an *anti* approach of a (*Z*)-**2** diene and an (*E*)-**2** dienophile. Alas, it is equally feasible that the three distinct classes of diastereomers could arise through the dimerization of (*E*)- and (*Z*)-regioisomers in both *syn* and *anti* orientations.

Dictazoles and the Vinylcyclobutane Rearrangement

In early 2010, the proposed biosynthetic origins of the

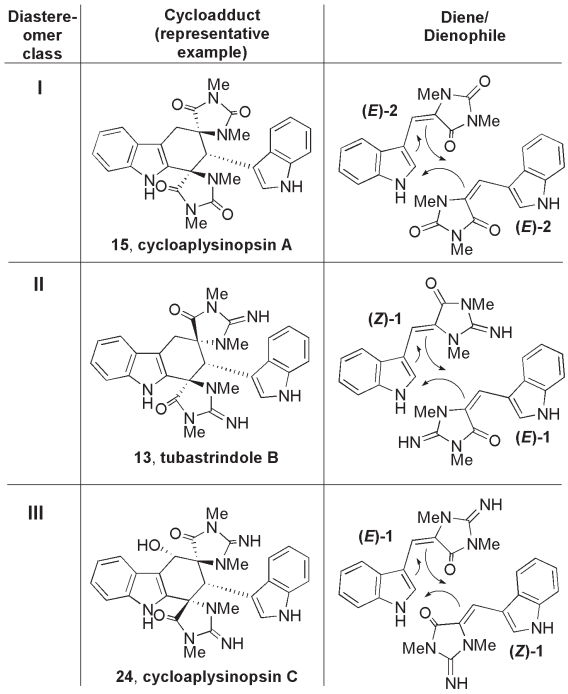


Fig. 2. Three diastereomer classes could arise from different cycloaddition pairs.

aplysinopsin dimers took another twist. Williams and co-workers reported the isolation of the three new dictazolines C-E (**25-27**), along with the structurally unique dictazoles A and B, **28** and **29**, respectively,[18] from the same extract of *S. cerebriformis* that had earlier afforded dictazolines A and B (**22** and **23**)[16] (Fig. 3). Interestingly, the authors did not report the isolation of any monomeric aplysinopsins.

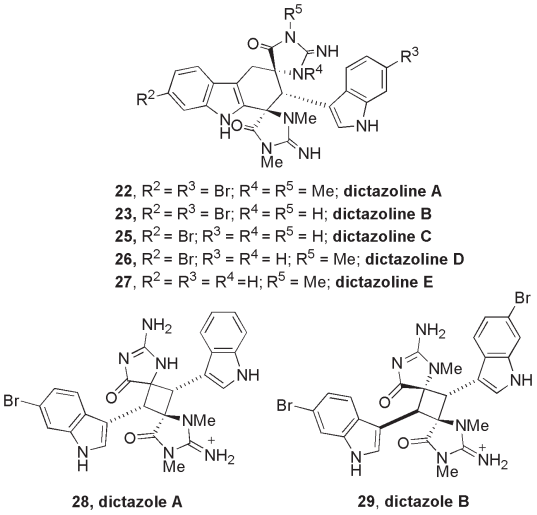
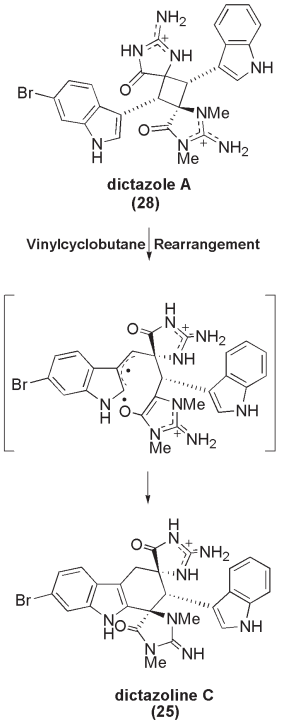


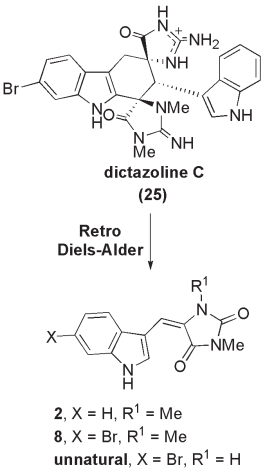
Fig. 3. Dictazolines A-E and dictazoles A and B.

Using Baran's pioneering biomimetic total synthesis of ageliferin from the cyclobutane sceptrin as a guide,[19] the authors suggested that the dictazoles are possible precursors to the corresponding dictazolines. Specifically, dictazole A (**28**) can be converted to dictazoline C (**25**) via the vinylcyclobutane rearrangement, as outlined in Scheme 2.[18] Circumstantial evidence supporting this proposed pathway is the relative abundance of these isolated compounds; the cyclobutane derivative **28** is isolated in significantly higher quantities than the cyclohexenyl analogue **25**.



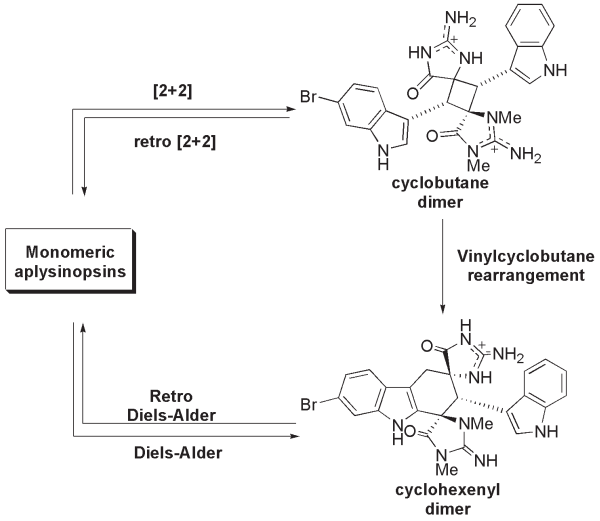
Scheme 2. Vincylcyclobutane rearrangement

An attempt to effect this rearrangement by heating an aqueous solution of pure dictazole A (**28**) to 200 °C in a microwave reactor led to fascinating results. A significant amount of dictazoline C (**25**) was detected by LC-MS along with three monomeric aplysinopsins, presumably arising from a retro-Diels-Alder reaction of **25** (Scheme 3). Disappointingly, owing to a scarcity of natural material, no products could be characterized by NMR nor could the experiments be repeated.[18]



Scheme 3. Retro Diels-Alder fragmentation of dictazoline C

The findings of Williams and co-workers have led to even more unanswered questions. Could the monomeric aplysinopsins undergo conversion to the corresponding cyclobutane dimer in a [2+2]-process? Does the proposed vinylcyclobutane rearrangement to the cyclohexenyl dimer render the Diels-Alder proposal obsolete, or is there a biosynthetic cycle involved that incorporates more than one defined pathway (Scheme 4)? The results from testing the viability of these propositions with labelling studies and chemical syntheses are eagerly awaited.



Scheme 4. Possible biosynthetic cycle.

Concluding Remarks

The fascinating history of the aplysinopsin natural products, from its inception with the isolation of aplysinopsin in 1977 up until the appearance of the dictazoles in early 2010 has been summarized. Upon considering the biosynthetic origin of the cyclohexenyl aplysinopsin dimers, both the Diels-Alder cycloaddition and the vinylcyclobutane rearrangement proposals appear equally feasible. An examination of both compelling biosynthetic pathways through chemical synthesis is in progress in our laboratory and the results will be reported in due course.

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