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CONSTITUENTS OF MORPHOLOGICALLY SIMILAR SPONGES

APLYSINA AND *SMENOSPONGIA* SPECIES[1,2]

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Abstract—Compounds from the marine sponge *Smenospongia aurea* have been isolated and characterized as 5-bromo- and 5,6-dibromo-N,N-dimethyltryptamines (**1** and **2**), aureol (**3**), and two new aplysinopsin derivatives (6-bromoaplysinopsin and 6-bromo-4'-N-demethylaplysinopsin, **4** and **5**, respectively). Morphologically similar species from the Caribbean have been surveyed and found to contain either mixtures of these metabolites or bromotyrosine-derived compounds. ^{1}H NMR spectra of bromoindole-containing metabolites are discussed.

The presence of antimicrobial compounds in marine sponges has been reported as a general phenomenon[3] and has been suggested to reflect the defensive strategy of these sedentary, filter-feeding animals.[4] A survey of Caribbean marine organisms during the 1978 Alpha Helix Caribbean Expedition (AHCE 1978) revealed a high incidence of antimicrobial activity in extracts of Porifera,[5] including extracts of the genus *Aplysina*. Analysis of several *Aplysina* (formerly *Verongia*)[2] extracts by gas chromatography/mass spectrometry (GC/MS), both on the *R/V Alpha Helix* and in Urbana, showed them to contain brominated phenolic metabolites of tyrosine, many of which had been previously identified, synthesized and tested for antimicrobial activity in our laboratory.[6-8] Indeed, these bromophenols have been proposed as being of chemotaxonomic significance for the genus *Aplysina*.[9]

In contrast to these typical *Aplysina* extracts, eight specimens tentatively classified on shipboard (under difficult taxonomic conditions) as *Aplysina spongelii* or *A. lacunosa* and collected in Belizean, Colombian, Honduran and Mexican waters posed a distinct problem (Table 1). Some (AHCE #141, 180, 259, 286, 360) gave GC/MS data indicative of bromophenols (e.g. *m/z* 338, presumably aeroplysinin I).[6] On careful reexamination, the first three of these have been classified as *A. lacunosa*, noting that *A. spongelii* has recently been reclassified as *A. lacunosa*,[2] and the last two (AHCE #286 and 360) have been classified as *A. archeri*. Again, one notes that all five samples contain the typical *Aplysina* bromophenols. However, the remaining three specimens (AHCE #295, 462 and 583) were found by GC/MS to contain volatile metabolites different from the typical bromophenols, though they demonstrated varying degrees of antibacterial activity (Table 1). A large sample (#583, AHCE 18-III-78-3-1) of one of the three anomalous specimens originally identified as *A. lacunosa* was studied further in an attempt to identify the bioactive compounds.

In contrast to the complex mixtures in typical *Aplysina* species, extracts of sponge AHCE #583 gave only two peaks by GC (Fig. 1), although the broad peak with the longer retention time in the chromatogram was resolved into two components by GC/chemical ionization (CI)MS (Fig. 1) with selective ion monitoring. GC/high resolution (HR)MS indicated that the first and second peaks contained related amines (mono- and dibrominated, respectively) and that a non-halogenated compound coeluted with the less volatile dibromoamine. Extraction with acid or silica gel column chromatography readily separated the amines from the non-halogenated component in the second peak. The brominated analogs were then separated from each other by preparative GC and crystallized from methanol, while the neutral constituent was purified by crystallization from hexane.

The amines were identified as mono- and dibromo derivatives of N,N-dimethyltryptamine by their characteristic fragments and HR electron ionization (EI)MS molecular formulas (Scheme 1). Solvent shift studies[10,11] (Table 2) of the ^{1}H NMR resonances for **1**, **2** and the related compound **7**, as well as for indoles **8–10**, indicated that the halogen was at C-5 of the indole nucleus in **1** and allowed the assignment of **1** and **2** as the basic antimicrobial components of AHCE #583. Furthermore, the dimethyltryptamine prepared from commercial 5-bromoindole (Scheme 2) was found to be identical to the natural product **1**.

It is of some interest that the dibromotryptamine **2** has significantly greater antimicrobial activity than the monobromo analog **1** (Table 3). Synthesis and testing of the 6-bromo isomer of **1** should shed further light on the structure-activity dependence of these antimicrobial tryptamines.

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Table 1. Antimicrobial properties and constituents of extracts of specimens classified on shipboard as *Aplysina lacunosa* (= *A. spongelii*)[a]

	AHCE Sample[b]							
	#141[c]	#180[c]	#259[c]	#286[d]	#360[d]	#295[e]	#462[e]	#583[e]
Activity[f]								
Bacillus subtilis	24	20	17	16	21	16	14	15
Escherichia coli	21	17	15	13	18	-	-	-
Saccharomyces cerevisiae	-	-	15	-	-	-	-	-
Penicillium atrovenetum	-	-	-	-	-	-	-	-
Constituent								
1[g]	-	-	-	-	-	+	trace	+
2[g]	-	-	-	-	-	+	-	+
3[g]	-	-	-	-	+	+	+	+
4, 5[h]	-	-	-	-	-	+	-	+
m/z 338[g]	+	+	+	+	+	-	-	-

[a]Shipboard identification; see c-e for reclassification. [b]See Experimental Section for collection sites and depths. [c]Reclassified as Aplysina lacunosa (Pallas, 1766). [d]Aplysina archeri (Higgin, 1875). [e]Smenospongia aurea (Hyatt, 1875). [f]Activity of crude extracts measured as zone of inhibition (mm) of growth of a microbial lawn. [g]Detection by GC/MS and FDMS of crude extracts (+ present, - not detected); see Scheme I for structures of 1 and 2. [h]Detection by TLC (silica, $CHCl_3$:MeOH : 4:1)with UV visualization, see Scheme 3 for structures.

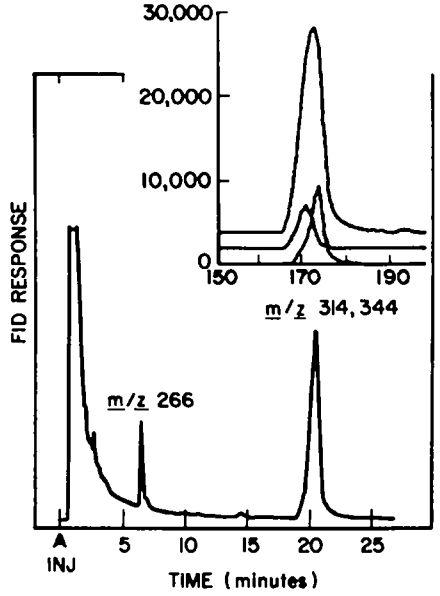
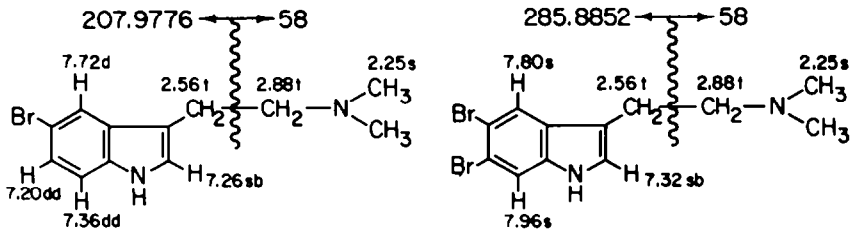


Fig. 1. GC trace of volatile components of sponge AHCE #583. Conditions: 6 ft × 1/8 in column, 3% OV-17 on GCQ, 250° isothermal, helium flow 30 mL/min; injector, 270°; detector, 300°. Inset: selected ion traces of ions at *m/z* 344 (dibromodimethyltryptamine, 2, middle) and 314 (aureol, 3, bottom), as well as a total ion trace of the mixture (top).

After we had completed our assignments of structures 1 and 2, the same bromotryptamines were reported by Djura *et al.* from two *Smenospongia* spp. sponges.[12] In that case, one sample of the sponge *Smenospongia aurea* was reported to contain 5-bromo-N,N-dimethyltryptamine, while two samples of *Smenospongia echina* were reported to contain the dibromo analog.[12] Careful comparison of AHCE #583 with *S. aurea* confirmed their identity. Other bromotryptamines (5,6-dibromotryptamine and 5,6-dibromo-N-methyltryptamine) have been reported by Van Lear *et al.* from *Polyfibrospongia maynardii*,[13] but that sponge is now believed to have also been a representative of the *Smenospongia* genus.[14]

Physical data obtained for the neutral component, which had modest *Bacillus subtilis* activity (Table 3), gave evidence for a sesquiterpene hydroquinone structure. In particular, our examination of its ^{13}C NMR spectrum indicated a close resemblance to that of zonarol (11), a natural product previously isolated from the brown alga *Dictyopteris zonarioides* (=*D. undulata*).[15,16] At that point in our studies, the X-ray structure of aureol (3) obtained from *Smenospongia aurea* appeared[12] and a comparison of physical properties showed the volatile terpene from sponge AHCE #583 to be identical to aureol.

Although AHCE #583 is the first sponge reported to contain all three metabolites, mixtures of 1 and 3 or of 2 and 3 were reported in different *Smenospongia* species.[12] As noted above, the observation of these compounds in *Smenospongia* samples prompted us to

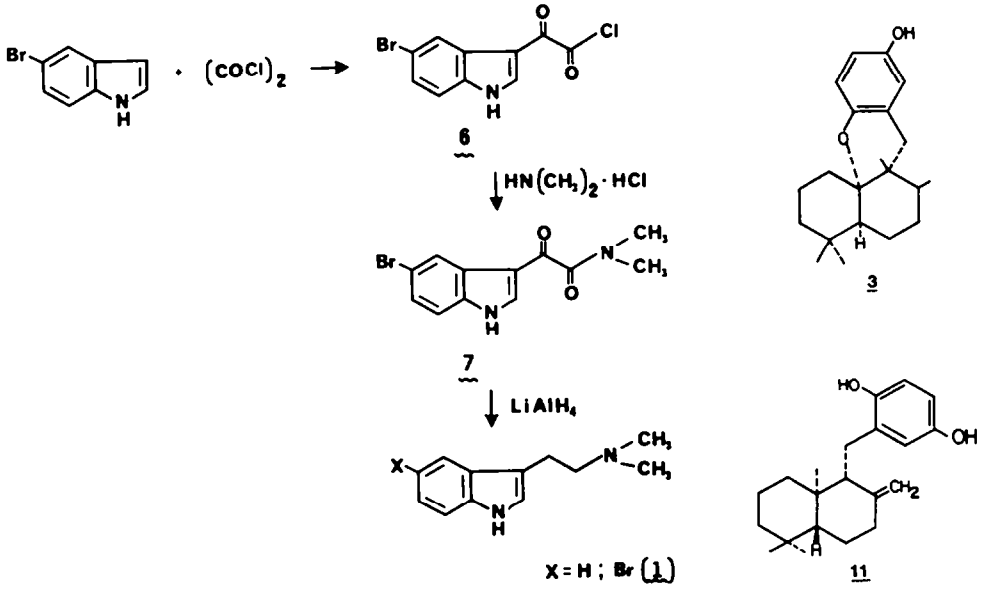


Scheme 1. Mass spectral and ^1H NMR (CD_3COCD_3) assignments for bromotryptamines 1 and 2.

Table 2. Solvent effects on bromoindole protons' chemical shifts

	Chemical shift, δ[a]											
	4		5		6[b]		8[c]		9[c]		10[c]	
	C	A	C	A	C	A	C	A	C	A	C	A
Indole Proton												
H-2	7.04 (sb)	7.25 (sb)	7.04 (sb)	7.29 (sb)	7.75 (d,3.0)	8.14 (s)	Br	Br	Br	Br	Br	Br
H-4	7.71 (sb)	7.72 (d,1.7)	7.85 (s)	7.78[d] (s)	8.49 (d,1.6)	8.43 (d,1.7)	7.64 (d,1.7)	7.58 (d,1.7)	7.36 (d,8.3)	7.39 (d,8.3)	7.75 (s)	7.74 (s)
H-5	Br	Br	Br	Br	Br	Br	Br	Br	7.26 (dd,8.3, 1.6)	7.31 (dd,8.3 1.6)	Br	Br
H-6	7.24	7.19 (dd,8.7, 1.7)	Br	Br	7.36 (dd,8.6, 1.7)	7.44 (dd,8.6, 1.7)	7.32 (dd,8.7 1.7)	7.38 (dd,8.7 1.7)	Br	Br	Br	Br
H-7	7.21	7.34 (d,8.7)	7.63 (s)	7.93[d] (s)	7.22 (d,8.6)	7.55 (d,8.6)	7.14 (d,8.7)	7.49 (d,8.7)	7.44 (d,1.6)	7.75 (d,1.6)	7.58 (s)	7.96 (s)

[a] Solvent: C = $CDCl_3$, A = CD_3COCD_3. δ in ppm from TMS (multiplicity, J in Hz). Multiplicities: s = singlet, d = doublet, b = broad. [b] See Scheme 2 for structure 6. [c] See G. T. Carter, K. L. Rinehart, Jr., L. H. Li, S. L. Kuentzel, J. L. Connor, Tetrahedron Lett. 4479–4482 (1978); 8, 1-methyl-2,3,5-tribromoindole; 9, 1-methyl-2,3,6-tribromoindole; 10 1-methyl-2,3,5,6-tetrabromoindole. [d] Assignments may be reversed.



Scheme 2. Synthesis of 5-bromo-N,N-dimethyltryptamine (1).

Table 3. Antimicrobial properties of AHCE #583 constituents

Component	Antimicrobial Activity[a]			
	B. subtilis	E. coli	S. cerevisiae	P. atrovenetum
1	trace[b]	0	15	trace
2	24	22	18	18
3	15	0	0	0
4 and 5	14	0	0	0

[a]Zone of inhibition (mm) for 100 μg/ 12.5-mm disk. [b]Clear zone not observed.

reinvestigate the taxonomy of our "*Aplysina lacunosa*" sample AHCE # 583, causing its reassignment as *S. aurea*. From the chemotaxonomic point of view, sample #295 was like #583 in containing **1**, **2** and **3**, while sample #462 contained only a trace of **1** plus **3**. This systematic study demonstrated that when halogenated tryptamines were abundant in a sponge (#295, 583), they were always detected by GC/MS as a monobromo–dibromo pair (Table 1). This contrasts with the report of Djura *et al.*[12] who found **1** or **2**, but not both, in single *Smenospongia* samples and demonstrates the value of GC or GC/MS examination of extracts. The earlier identification of **1** and **2** was by isolation rather than GC and one of the components could easily have been overlooked.

During a large scale isolation of the bromotryptamines from *S. aurea* (#583), a yellow solid precipitated from the more polar silica gel column eluents. The solid was purified by recrystallization from methanol–water and was found to have no significant antimicrobial activity (Table 3). HREIMS indicated that the solid was a mixture of two monobromo compounds with molecular formulas $C_{13}H_{11}BrN_4O$ and $C_{14}H_{13}BrN_4O$. UV absorption and ^1H NMR data (Table 4) on the mixture indicated that these homologs were bromo and bromodimethyl derivatives (**4** and **5**) of the previously isolated[17,18] sponge metabolite aplysinopsin (**12**), which shows antineoplastic activity.[17] The acetate salt of **5** was prepared by precipitation.

Aplysinopsin itself (**12**) was reportedly isolated from a Florida *A. spongelii* sample[17] and also from an Australian *Thorecta* species.[18] Our identification of aplysinopsin derivatives in *S. aurea* but not in *A. lacunosa* (≡*A. spongelii*) suggests a reexamination of the Florida sponge.[17] Derivatives and analogs of **12** are also known (Table 4 and Scheme 3),[13–18] and imino-N-methylaplysinopsin, isolated from an *Aplysinopsis* species, is a potent antidepressant.[19,20] Wells[21] first detected a monobromoaplysinopsin in a *Thorecta* sp. sponge but was unable to isolate or assign a structure to the trace metabolite. Two other aplysinopsin analogs (**14** and **15**), both 2′-N-demethylaplysinopsins from a *Dercitus* species, were later identified[22] by ^1H NMR comparisons between the natural products and synthetically prepared analogs such as **16** and **17**. Since the aplysinopsin analog **15** contains a 6-bromoindole unit, bromoaplysinopsin itself might be expected to have structure **4**. To confirm this, the 5-bromo isomer was prepared from the readily available 5-bromoindole-3-carboxaldehyde (Scheme 4) and its ^1H NMR spectrum was shown to be different.

The bromo-demethyl analog of aplysinopsin isolated from *Smenospongia aurea* (AHCE #583) might have been expected to be either **15** or the isomeric **5**. The compound isolated clearly differed from **15** in its ^1H NMR spectrum (Table 4). Here again, in order to aid the ^1H NMR interpretation, an authentic sample of the related **18** was prepared by an unequivocal synthesis[16] from 5-bromoindole-3-carboxaldehyde (Scheme 4) and shown to be different from **5** by ^1H NMR (Table 4). The 6-bromo assignment is further supported by a recent report[12] of a closely related sponge-derived indole (**19**).

Examination of the ^1H NMR data of these several aplysinopsin derivatives (Table 4) provides a number of correlations. A striking one is that H-2 in those imidazolidones containing a 2′-N-Me substituent (**12**, **13**, **4**, **5**, **17**, **18**) occurs further downfield than in those lacking the 2′-N-Me (**14–16**), thus arguing for the structure assignment of **5** as shown. Similarly, the 2′-methyl is always found downfield of the 4′-methyl. Other correlations can be found for the benzenoid protons, and it may be appropriate at this point to discuss briefly the benzenoid ^1H NMR signals of bromo-substituted indoles in general, since some of these signals have occasionally been misassigned.[22]

The proton spectrum of indole itself in the aromatic region is a jumble at 60 MHz,[10] but at 360 MHz it is well resolved (Fig. 2). Among these protons, H-3 is farthest upfield at 6.50 ppm ($CDCl_3$) and thus readily recognized, and is shown by homonuclear decoupling to be coupled to the signal at 6.98 ppm, establishing it as H-2. Similarly, homonuclear decoupling shows the signals at 7.62, 7.10, 7.17 and 7.22 ppm to belong to H-4, H-5, H-6 and H-7, respectively (or, alternatively, to H-7, H-6, H-5 and H-4). That the former is the correct assignment is demonstrated by the downfield shift of the signal at 7.22 ppm (→7.42) in acetone solvent ((CD_3COCD_3)).[10,11] A similar solvent-related downfield shift is observed for H-2 (6.98 → 7.28 ppm). Small upfield shifts are observed for H-6 (7.17 → 7.10 ppm) and H-5 (7.10 → 7.02). The 360-MHz spectrum in deuterioacetone agrees almost exactly with that reported previously at 100 MHz.[23] The spectra of benzo-unsubstituted indoles in the aplysinopsin series (**12**, **14**, **17**) show signals at positions similar to those of indole itself.

The substitution of a single bromine in the benzene ring of indole shifts the proton signals for adjacent (*ortho*) hydrogens downfield by 0.1–0.5 ppm (compare **12** to **13** and **4**, **17** to **18** and **5** in Table 4), with a smaller effect (≤0.05 ppm) on *meta* protons. The substitution

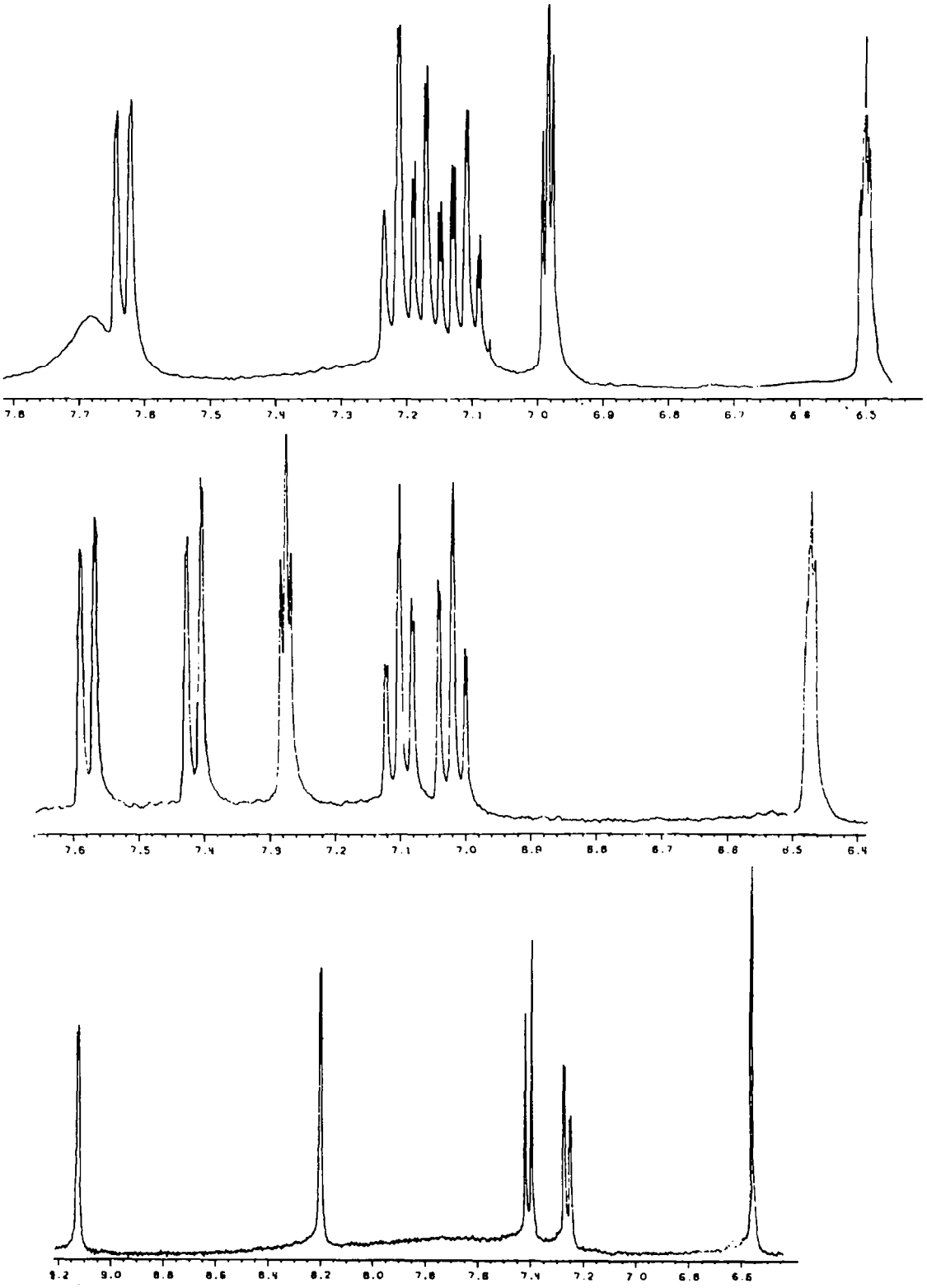


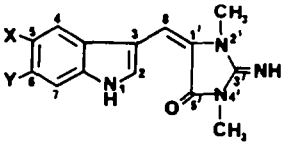
Fig. 2. ^{1}H NMR spectra (360 MHz) of indoles: top and middle, indole, $CDCl_3$ and CD_3COCD_3, respectively; bottom, **18**, DMSO-d_6.

Table 4. ^{1}H NMR Data for aplysinopsin derivatives and analogs

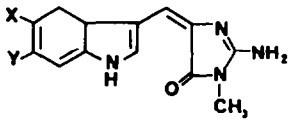
Compound	Chemical Shift, δ (ppm, DMSO-d_6)										
	12[a]	13	4 + 5	14[a]	15[a]	16[a,b]	17[a]	17[c]	18[c]	5[c]	19[d]
Proton											
H-2	8.72	8.96	9.07	8.25	8.09	8.31, 8.36	9.09	9.09	9.11	9.07	8.16
H-4	7.89	8.37	7.89	7.93	7.88	8.09, 8.13	7.86	7.89	8.19	7.88	7.77
H-5	7.15	Br	7.27	7.11	7.13	Br	7.04	7.10[e]	Br	7.24	7.26
H-6	7.15	7.36	Br	7.11	Br	7.27	7.04	7.15[e]	7.27	Br	Br
H-7	7.45	7.47	7.64	7.43	7.54	7.43	7.36	7.42	7.41	7.62	7.63
H-8	6.46	7.24	6.61, 6.50	6.81	6.65	7.02, 7.09	6.52	6.53	6.57	6.50	6.72
2'-CH_3	3.26	3.45	3.29	–	–	–	3.22	3.28	3.30	3.27	–
4'-CH_3	3.07	3.19	3.10	3.06	3.00	3.12	–	–	–	–	–

[a]Data taken from Reference 22. [b]Data reported for a mixture of *E* and *Z* isomers (1:1). [c]Data for the monoacetate salt. [d]See Reference 12. [e]Assignments may be reversed.

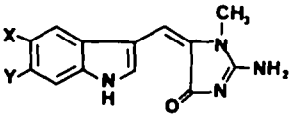
of a second bromine has similar effects, especially on the signal of the *ortho* proton. The structures assigned to the monobromo compounds appear unequivocal, based on syntheses of authentic materials and the distinction between *ortho* coupled protons (e.g. H-6 and H-7 in **18**) is based on small (*ca* 1 Hz) *meta* coupling



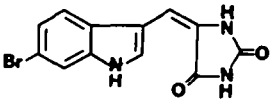
	X	Y
12	H	H
13	Br	H
4	H	Br



	X	Y
14	H	H
15	H	Br
16	Br	H



	X	Y
17	H	H
18	Br	H
5	H	Br



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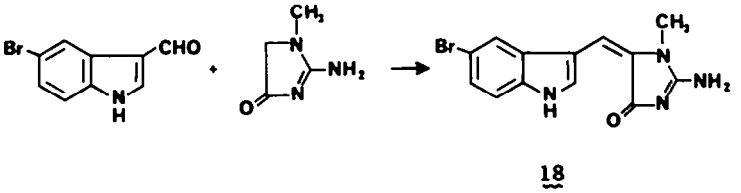
Scheme 3. Aplysinopsin derivatives and analogs.

constants (see, for example, the 360-MHz spectrum of **18** in Fig. 2). To decide in any unknown pair which compound is 5- or 6-bromo substituted (Tables 2 and 4), one notes that in the 5-bromo compound the benzenoid singlet (H-4) is well downfield (usually at least 0.5 ppm) of the *ortho* coupled pair, while in the 6-bromo compound one of the *ortho*-coupled protons (H-4) in the deuteriochloroform or deuteriodimethylsulfoxide spectrum is found near the benzenoid singlet (H-7) or downfield from it.

Mass spectral fragments (Scheme 5) observed for the natural product **5** (and for **4**) and for the synthetic analog **18** support the 2'-methyl assignments for the new bromoindoles. Although equilibration of *E* and *Z* double bond isomers of **12** has been observed in solution,[21] the geometrical isomers depicted are presumed for the natural products **4** and **5** by analogy to the X-ray structure determined for aplysinopsin (**12**).[21] The imidazolidone portions of **14**–**18** as well as **5** are shown as the preferred endocyclic imino tautomers.[24]

Although taxonomic assignments of the sponges in Table 1 are not final, some conclusions may be drawn from the survey of metabolites. First, the sponges listed in Table 1, all originally thought to be *A. lacunosa*, can be divided into two groups, those containing bromotryptamine (*m/z* 266, 344) and those containing bromotyrosine metabolites (*m/z* 306, 320, 338); no sponge contains both groups of compounds. The strongest antimicrobial activity is shown by the sponge extracts containing bromotyrosine metabolites. Secondly, aplysinopsin analogs (**4**, **5**) were found only in sponges containing bromotryptamines. The bromotyrosine derivatives have, to-date, remained uniquely characteristic of the Order Verongida sponges (Scheme 6).[14] The bromotryptamine and aplysinopsin natural products may then be a similar fingerprint of the Dictyoceratida Order sponges, as previously suggested for the Thorectidae family.[14] This would require reclassification of the *A. spongelii*[17] and *Dercitus* sp.[22] samples noted earlier. Further chemotaxonomic studies are clearly warranted. Thirdly, the bromoindoles **1**, **2**, **4**, and **5** appear to be biosynthetically related as alternative metabolites of tryptophan. However, the regiospecific bromination patterns appear to preclude a common bromoindole precursor and suggest divergent



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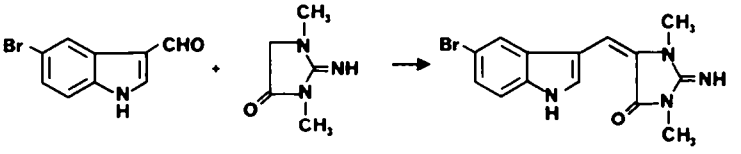

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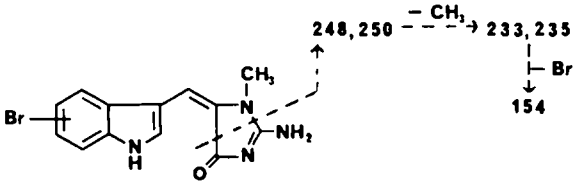
Scheme 4. Synthesis of aplysinopsin analogs.

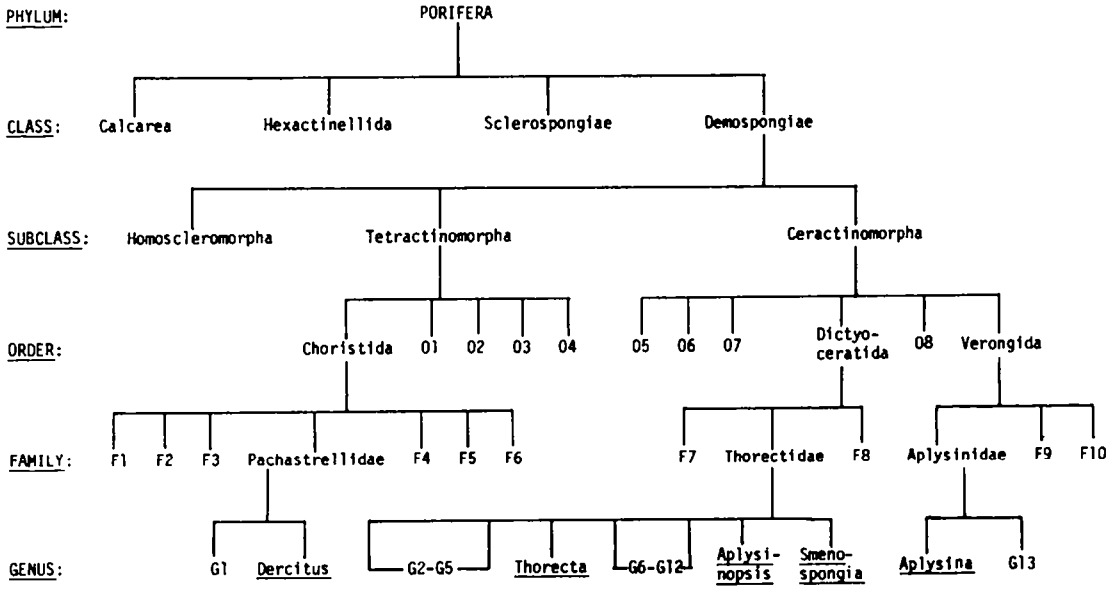


Scheme 5. Mass spectral fragmentations of 5 and 18.

biosynthetic pathways for the tryptamines and aplysinopsin derivatives. Finally, the presence of aureol (3) in a sample did not correlate with either the tentative taxonomic classification or the nature of the brominated metabolites in a sponge. Although it was found in all sponges containing bromotryptamines (AHCE #295, 583 and 462), it was also found in one sponge containing bromotyrosine metabolites (AHCE #360). Thus, algal detritus containing sesquiterpenes such as zonarol (11) is suspected as a dietary source for aureol production.

EXPERIMENTAL

General. M.p.s were determined on a Reichert micro hot stage and are uncorrected. UV and IR spectra were obtained on Perkin–Elmer spectrophotometers, Model Lambda 3 and grating Model 237, respectively. Optical rotations were recorded on a Carl Zeiss polarimeter, 0.01° model. Mass spectrometers used were Finnigan MAT Models 311A and CH-5 and VG Analytical Model 7070. GC was performed using Varian instruments, Model 1700 for analytical and Model 1520 for



Scheme 6. Porifera classification. Orders: O1 = Spirophorida; O2 = Lithistida; O3 = Hadromerida; O4 = Axinellida; O5 = Halichondrida; O6 = Poecilosclerida; O7 = Haplosclerida; O8 = Dendroceratida. Families: F1 = Stelletidae; F2 = Geodiidae; F3 = Calthropellidae; F4 = Theneidae; F5 = Thrombidae; F6 = Jaspidae; F7 = Spongiidae; F8 = Dysideidae; F9 = Aplysinellidae; F10 = Ianthellidae. Genera; G1 = *Pachastrella*; G2 = *Cacospongia*; G3 = *Hyrtios*; G4 = *Ircinia*; G5 = *Sarcotragus*; G6 = *Thorectandra*; G7 = *Luffariella*; G8 = *Psammocinia*; G9 = *Fenestraspongia*; G10 = *Fascaplysinopsis*; G11 = *Taonura*; G12 = *Fasciospongia*; G13 = *Verongula*.

preparative work. NMR spectra were measured on a Nicolet, Model NT-360, spectrometer.

Sponge collection. Sponges were obtained by SCUBA techniques during an expedition (AHCE 1978) on board the *R/V Alpha Helix*[3] and vouchers are stored in the Allan Hancock Foundation Collections, University of Southern California. Collection sites were as follows:

AHCE #141 (27-II-78-1-10, collected at −6 to 12 m at Rada el Cove, Isla San Andrés, Colombia, 12°31'46" N, 81°44'05" W),

AHCE #180 (28-II-78-4-3, collected at −10 to 15 m at Burn Cay, Cayo Media Luna, Honduras, 15°10' N, 82°25' W),

AHCE #259 (3-III-78-1-12, collected at −3 to 27 m at Isla Roatán, Honduras, 16°26' N, 86°22' W),

AHCE #286 and 295 (6-III-78-1-2 and 6-III-78-1-11, respectively, collected at −10 to 27 m at Turneffe Island, Belize, 17°10'48" N, 87°55'18" W),

AHCE #360 (8-III-78-1-2, collected at −5 to 7 m at Lighthouse Reef, Belize, 17°12'48" N, 87°35'48" W),

AHCE #462 (12-III-78-2-12, collected at −7 m at Punta Molas (Palancar Reef), Cozumel, Mexico, 20°37'42" N, 86°44'30" W), and

AHCE #583 (18-III-78-3-1, collected at −3 to 7 m at Turneffe Island, 17°11'18" N, 87°55'36" W).

The animals were either frozen or stored in cans in isopropyl alcohol on shipboard and then were stored at −20° in Urbana, where biological field testing was repeated.

Antimicrobial testing. Frozen sponge (2 g) was homogenized with MeOH–toluene (3:1, 25 mL) to give a crude extract, as described earlier.[25] Pure compounds were dissolved in MeOH (1 mg/mL) for bioassay. The disk diffusion method was used to test for growth inhibition (100 μL/disk) on agar lawns of *Bacillus subtilis, Escherichia coli, Saccharomyces cerevisiae* and *Penicillium atrovenetum.*

GC/MS of sponge extract AHCE #583. Sponge tissue (10 g) was ground in a mortar and pestle with water (10 mL) and the remaining solid was extracted with diethyl ether (350 mL) in a Soxhlet apparatus for 2 days. The ether extract was dried with $MgSO_4$, filtered and evaporated. MeOH-soluble material was chromatographed at 270° on a glass column (1/8 in × 12 ft) packed with OV-17 (3% on Gas-Chrom Q). The GC effluent was interfaced with a Watson–Biemann two-stage separator to a Finnigan MAT 311A mass spectrometer for GC/CIMS and GC/EIMS. A Varian MAT SS100C data system provided reconstructed chromatograms (ion current vs scan number); difference spectra (scan subtraction) were used to resolve two coeluting components (Fig. 1) by GC/CIMS (isobutane). Some mass spectra were processed with a VG Multispec data system.

Isolation of bromotryptamines **1** *and* **2.** The diethyl ether Soxhlet extract of sponge AHCE #583 was washed with 1 N HCl, 1 N NaOH and brine solns. The acid washes were neutralized and back-extracted with diethyl ether. The organic bases were then separated by preparative GC using an aluminum column (1/4 in × 6 ft) packed with OV-17 (3% on GCQ). The more volatile amine **1** crystallized with MeOH: m.p. 90–92° (lit.[12] 98–99); UV λ_{max}^{MeOH} (ε) 227 (24,000), 282 nm (5500) [lit.[12] 225 (39,000), 285 (4400), 305 (2700)]; IR ($CHCl_3$) $\tilde{\nu}$ 3400, 3300–3100, 1480–1430 cm^{-1} (lit.[12] 3500–3200, 1475); ^1H NMR ($CDCl_3$) δ 2.39 (s, 6H), 2.70 (t, 2H), 2.93 (t, 2H), 7.04 (bs, 1H), 7.20 (m, 2H), 7.72 (s, 1H), 8.24 ppm (1H, D_2O exch.) (see also Table 2) [lit.[12] (CD_3COCD_3) 2.34 (s, 6H), 2.59 (t, 2H), 2.86 (t, 2H), 6.97 (bs, 1H), 7.13 (d, 1H), 7.25(d, 1H), 7.70 (s, 1H)]; GC/HREIMS *m/z* 266.0416 (Calc for $C_{12}H_{15}BrN_2$ 266.0418), 220.9855 ($C_{10}H_8BrN$ 220.9840), 207.9776 (C_9H_7BrN 207.9762), 129.0585 (C_9H_7N 129.0579). The dibromotryptamine **2** also crystallized from MeOH: m.p. 113–115° (lit.[12] 113–115°); UV λ_{max}^{MeOH} (ε) 207 (9600), 232 (30,800), 291 nm (6300) [lit.[12] 230 (40,000), 285 (4900), 300 (3300)]; IR ($CHCl_3$) $\tilde{\nu}$ 3400, 3300–3100, 1480–1430 cm^{-1} (lit.[12] 3500–3100, 1450); ^1H NMR ($CDCl_3$) δ 2.38 (s, 6H), 2.67 (t, 2H), 2.90 (t, 2H), 8.16 ppm (1H, D_2O exch.) (see also Table 2) [lit.[12] (CD_3COCD_3) 2.27 (s, 6H), 2.59 (t, 2H), 2.86 (t, 2H), 7.18 (bs, 1H), 7.70 (s, 1H), 7.86 (s, 1H)]; HREIMS *m/z* 343.9513 (Calc for $C_{12}H_{14}Br_2N_2$ 343.9523).

Synthesis of 5-bromo-N,N-dimethyltryptamine **(1).** Oxalyl chloride (1.1 mL) was added slowly to an ethereal soln (15 mL) of commercial 5-bromoindole (1.96 g, 10 mmol) at 0°. Yellow crystals of **6**, which formed immediately, were filtered off and then added to 70 mL of aqueous (40%) Me_2NH with vigorous stirring for 30 min. The soln was concentrated to give off-white crystals of **7** (39% overall yield from 5-bromoindole): m.p. 201–203°; UV λ_{max}^{MeOH} (ε) 214 (53,000), 250 (24,000), 266 (20,000), 303 nm (22,000); IR (Nujol) $\tilde{\nu}$ 3050, 1630, 1610, 1520, 1450, 970, 885, 805, 795, 780, 750, 740 cm^{-1}; ^1H NMR (CD_3COCD_3) δ 3.04 (s, 6H) (see also Table 2). (Found: C, 48.77; H, 3.85; Br, 27.91; N, 9.33. Calc for $C_{12}H_{11}BrN_2O_2$: C, 48.81; H, 3.73; Br, 27.12; N, 9.49%.)

The amide **7** (0.44 g, 2 mmol) was reduced for 3 hr in dry refluxing dioxane (35 mL) using LAH (0.5 g, 20 mL dioxane). Water quenching and work-up yielded a 1:1 mixture (GC/MS) of **1** and the debrominated N,N-dimethyltryptamine. Pure synthetic **1** was obtained by preparative GC (see above) and found to be identical to the natural product (TLC, GC, MS, NMR).

Isolation of aureol **(3).** The neutral portion of the AHCE #583 Soxhlet extract was dried with $MgSO_4$, decolorized with charcoal and evaporated. The solid which formed in the freezer was recrystallized from hexane to give **3**: m.p. 144–146° (lit.[12] 144–144.5°); $[\alpha]_D^{25}$ + 62° (*c* 2.0%, CCl_4) (lit.[12] + 65°); UV λ_{max}^{EtOH} (ε) 225 (4200), 295 nm (2170) [lit.[12] (MeOH) 299 (3100), 216 (4600)]; IR ($CHCl_3$) $\tilde{\nu}$ 3500, 3400–3200, 1480, 1430, 1250, 1230 cm^{-1} [lit.[12] (CCl_4) 3300, 1490, 1450, 950]; ^1H NMR ($CDCl_3$) δ 0.78 (s, 3H), 0.92 (s, 3H), 1.06 (s, 3H), 1.11 (d, 3H), 1.90, 3.39, 6.54, 6.64 ppm [lit.[12] 0.78 (s, 3H), 0.92 (s, 3H), 1.06 (s, 3H), 1.11 (d, 3H, *J* = 7 Hz), 1.96 (d, 1H), 3.38 (d, 1H, *J* = 16 Hz), 6.50 (bs, 1H), 6.62 ppm (m, 3H)]; ^{13}C NMR ($CDCl_3$) δ 148.5, 145.7, 122.2, 117.3, 115.1, 114.1, 82.4, 44.1, 39.4, 38.1, 37.4, 33.9, 32.0, 29.9, 29.4, 27.9, 22.3, 20.2, 18.4, 17.3 ppm [lit.[12] 148.2 (s), 145.8 (s), 122.2 (s), 117.2 (d), 115.2 (d), 114.2 (d), 82.4 (s), 44.0 (d), 39.3 (d), 38.1 (s), 37.4 (t), 33.9 (t, s), 31.9 (q), 29.8 (q), 29.3 (t), 27.9 (t), 22.2 (t), 20.2 (q), 18.4 (t), 17.3 (q)]; HREIMS *m/z* 314.2243 (Calc for $C_{21}H_{30}O_2$ 314.2245).

Chemotaxonomic survey of crude sponge extracts (Table 1). Specimens (2 g) were extracted as for the antimicrobial assays. The crude organic extracts were partitioned into aqueous and toluene layers by the addition of 1 M sodium nitrate (5 mL).[25] Aureol was identified in the toluene fractions by FDMS and GC coinjection with authentic **3** and GC/MS analysis. The tryptamines were prepared for detection (GC, GC/MS and FDMS) by extraction of the aqueous layers with diethyl ether.

Isolation of aplysinopsin derivatives **4** *and* **5.** Sponge AHCE #583 (400 g) was macerated and extracted with toluene–MeOH (1:3) as described above. The aqueous soln remaining after $NaNO_3$ partitioning was extracted exhaustively with EtOAc. The residue after evaporation of the solvent (1.5 g) was chromatographed on a silica gel column (2.1 × 36 cm), providing a separation of **3** (35.5 mg, C_6H_{14}–Et_2O 90:10), tryptamine derivatives (37.5 mg, EtOAc), and the aplysinopsin derivatives **4** and **5** (90.7 mg, EtOAc–MeOH 80:20). The mixture of **4** and **5** crystallized from MeOH–water as a yellow solid: m.p. >250°; UV λ_{max}^{MeOH} (ε) 230 (35,000), 284 (15,000), 364 nm (26,000); IR (Nujol) $\tilde{\nu}$ 3500–3000, 1690, 1660, 1630 cm^{-1}; ^1H NMR (CD_3SOCD_3) (see Table 4); EIMS *m/z* (rel intensity) 332 (8.7), 318 (100), 248 (7.0), 247 (6.7), 233 (15.3) all Br; HREIMS *m/z* 332.0251, 318.0113 (Calc for $C_{14}H_{13}BrN_4O$ 332.0273, $C_{13}H_{11}BrN_4O$ 318.0116). The mixture of **4** and **5** (1.6 mg) was heated to boiling with NaOAc (0.6 mg) and acetic acid (10 μL) in EtOH. A solid acetate salt of **5** precipitated upon cooling and was purified by trituration with cold abs EtOH: m.p. >290°; ^1H NMR (CD_3SOCD_3) (see Table 4).

Synthesis of aplysinopsin derivatives **13, 17** *and* **18.** 5-Bromoindole (0.98 g, 5 mmol) was formylated[26] using $POCl_3$ (0.5 mL, 5 mmol) and dimethylformamide (17 mL, 22 mmol) to

give 5-bromoindole-3-carboxaldehyde (1.1 g, 100% yield, m.p., EIMS, Anal C, H, Br, N).

A mixture of 5-bromoindole-3-carboxaldehyde (0.6 g, 2.7 mmol), creatinine (0.5 g, 3.5 mmol) and NaOAc (0.3 g, 3.7 mmol) suspended in AcOH (1 mL) was heated to boiling then diluted with EtOH (95%, 5 mL). The yellowish solid which formed was filtered and dried to give **18** (0.6 g, 57% yield): m.p. >290 (d)°; IR (Nujol) $\tilde{\nu}$ 3300–3200, 1690, 1630, 1550 cm^{-1}; ^1H NMR (see Table 4 and Fig. 2); EIMS *m/z* (rel intensity) 318 (100), 247 (13), 233 (80). (Found: mol wt, 318.0120 (HREIMS). Calc for $C_{13}H_{11}BrN_4O$: mol wt, 318.0116.)

4-Methylcreatinine hydriodide (0.45 g, 1.76 mmol), prepared according to a known procedure,[24] was condensed with 5-bromoindole-3-carboxaldehyde (0.35 g, 1.56 mmol) as described above to afford a yellow solid (**13**, 0.26 g, 51% yield): m.p. >380°; IR (Nujol) $\tilde{\nu}$ 3600–3200, 1680, 1650, 1550, 1105, 1015, 820 cm^{-1}; ^1H NMR (CD_3SOCD_3) (see Table 4); EIMS *m/z* (rel intensity) 332 (100), 248 (7.5), 247 (10.4), 233 (34.0). (Found: mol wt, 332.0273 (HREIMS). Calc for $C_{14}H_{13}BrN_4O$: mol wt, 332.0272.)

Creatinine (1.2 g, 10.6 mmol) and indole-3-carboxaldehyde (1.5 g, 10.3 mmol) reacted as described above to yield orange **17** acetate (0.94 g, 30%), m.p. 275–285° (dec) [lit.[22] 240–285° (dec)]; MS *m/z* 240 (M); ^1H NMR, see Table 4. (Found: C, 59.44; H, 5.12; N, 19.50. Calc for $C_{13}H_{12}N_4O \cdot C_2H_4O_2$: C, 59.99; H, 5.37; N, 18.66%.)

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