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BROMINATED INDOLES FROM LAURENCIA BRONGNIARTII

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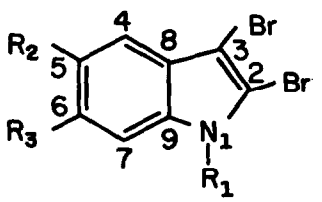
During the Alpha Helix Caribbean Expedition in February-March 1978 (AHCE 1978), we examined numerous samples of Laurencia by GC/MS[1] on shipboard, as part of a comprehensive survey[2] of Caribbean Laurencia species. In the main, these examinations identified bromoterpenes of types previously found by GC/MS of west coast Laurencia species.[3,4] However, Laurencia brongniartii J. Agardh (AHCE 22-III-78-2-101), which belongs to a different section (Planae) of the subgenus Laurencia,[2] produced, instead, a mixture of brominated indoles (1-4) in copious amounts.



	R_1	R_2	R_3
1	CH_3	H	Br
2	CH_3	Br	H
3	H	Br	Br
4	CH_3	Br	Br

Our attention was initially drawn to Laurencia brongniartii by its antimicrobial activity vs. Bacillus subtilis (a gram-positive bacterium) and Saccharomyces cerevisiae (a yeast), which correlates well with prediction from its high organic halogen content.[5] Shipboard GC/MS analysis indicated nearly all the organic halogen to reside in isomeric compounds of molecular weights[6] 365 (Br_3), 429 (Br_4) and 443 (Br_4),[7] the odd molecular weights indicating immediately the compounds' nitrogen content.

To isolate the brominated compounds a sample of the frozen alga (450 g) was homogenized in chloroform, affording 1.5 g of dark green oil, which was subjected to silica gel chromatography. Elution with petroleum ether (30-60°) gave 1 (173 mg), followed by 2 and 4 as a 2:3 mixture (73 mg). Increasing the polarity of the solvent with added chloroform eluted 3, which after additional silica gel chromatography in chloroform yielded 177 mg of pure material. Compounds 2 and 4 were separated by fractional crystallization and by the preferential sublimation of 2. Properties of 1-4 are summarized in Table 1.

The mass spectra of 1 and 2 show molecular ion quartets characteristic of three bromine atoms and their molecular formula was assigned as $C_9H_6Br_3N$ by high resolution mass spectrometry (HRMS). Similarly, the molecular ions of 3 and 4 were quintets for four bromine atoms and

their molecular formulas were assigned as $C_8H_3Br_4N$ and $C_9H_5Br_4N$, respectively, by HRMS. Compounds 1, 2 and 4 all show loss of CH_3 in their mass spectra; 3 does not and 4 could be assumed to be a methyl derivative of 3, as well as a bromo derivative of 1 and 2, which were isomers.

The N-methylindole nucleus of 1 was recognized from the close correspondence of its ^{13}C NMR spectrum ($CDCl_3$) to that of N-methylindole itself.[8] The ^{13}C NMR spectrum of 1 clearly shows[8] resonances due to the N-CH_3 carbon at 32.4, C-3 at 93.1 (non-protonated, therefore bromine-bearing), C-8 at 125.9 and C-9 at 137.0 ppm. The three protonated aromatic carbons appear at 112.7, 120.2 and 134.3 ppm and the other two brominated carbons at 115.7 and 116.8 ppm. The positions of the bromine atoms in 1 were determined from the ^1H NMR spectrum of 1 (Table 1), which has signals for a 1,2,4-trisubstituted benzene (J_o = 8.3 Hz, J_m = 1.6 Hz), thus assigning one bromine to C-5 or C-6 and the other two to C-2 and C-3. (The C-3 bromine was already noted above from the ^{13}C NMR spectrum.) Changing the solvent from deuteriochloroform to hexadeuterioacetone causes a substantial downfield shift (0.31 ppm) of the 1.6-Hz doublet. Previous studies have shown that this type of solvent shift is expected only for H-2 and H-7 in the indole system;[9] thus the resonance at 7.44 ppm ($CDCl_3$, J = 1.6 Hz) was assigned to H-7, and the third bromine must be at C-6.

The tribromo isomer of 1 also shows a 1,2,4-trisubstituted benzene pattern (J_o = 8.7, J_m = 1.7 Hz) and it must then be 1-methyl-2,3,5-tribromoindole (2). In this case the solvent-shift experiment (Table 1) showed the signal due to H-7 (7.14 ppm, d, J = 8.7 Hz, $CDCl_3$) to shift downfield by 0.35 ppm in deuterioacetone.

The tetrabromoindoles might reasonably be expected by analogy to 1 and 2 to be 2,3,5,6-tetrasubstituted. This is confirmed by the ^1H NMR spectrum ($CDCl_3$) of 4, which shows H-7 (the solvent-shifted proton) as a singlet at 7.58 ppm, near the position of H-7 in 1, and H-4 (which does not shift) at 7.75 ppm, near the position of H-4 in 2. The substitution of bromine at C-3 is confirmed by lack of proton absorption in the region characteristic of indoles H-3.[9] Thus, 4 is 1-methyl-2,3,5,6-tetrabromoindole. The close correspondence of the ^1H NMR spectrum of 3 to that of 4 ($CDCl_3$, Table 1) assigns it as 2,3,5,6-tetrabromindole: a broad resonance at 8.32 ppm assigned to the N-H, a singlet at 7.76 ppm for H-4 (slightly broadened due to small coupling between N-H and H-4),[10] and a very sharp singlet for H-7 at 7.61 ppm, which shifts downfield in deuterioacetone (Table 1).

Of the four purified compounds, only 3 showed antimicrobial activity. At a level of 100 µg per 12.7-mm disc, 3 produced zones of inhibition of 16 mm for Bacillus subtilis and 14 mm for Saccharomyces cerevisiae after 24 h of growth. In addition, 3 shows an ID_{50} of 3.6 µg/ml vs. L1210 tumor cells in tissue culture.

None of the bromoindoles 1-4 has previously been reported as a natural product, although 3[11] and 4[12] have been prepared synthetically and the mp's of the synthetic samples (149-151° and 168-170°) agree reasonably well with those in Table 1.[13] A few, usually complex, halogenated indoles derived from marine animals are known[14] and, quite recently, the first simple halogenated indoles from a marine alga from the South Island of New Zealand, Rhodophyllis membranacea, were reported.[15] Phylogenetically, R. membranacea is not closely related to Laurencia brongniartii, though it belongs to the same subclass (Floridiophycidae) in the Rhodophyta. The haloindoles from R. membranacea differed from the present compounds in that they consisted of difficultly separable

Table 1. Properties of Bromoindoles 1-4

	1				2				3				4			
Mp, °C	90.5-91				120-122				152.5-154 dec				171.5-172			
UV:[a] λ_{max}, nm (ε_{max})	230 (39,000), 288 (10,000), 294 (10,000)				228 (34,000), 282 (7,300), 290 (7,700), 297 (7,700)				230 (47,000), 294 (9,600), 301 (9,500)				233 (49,000), 296 (10,000), 303 (10,000)			
^{1}H NMR			δ[c]				δ[c]			δ[c]				δ[c]		
	m[b]	J,Hz	C	A	m	J, Hz	C	A	m	C	C:A:1:1	A	m	C	T:A:1:1	A
N-CH_3	s		3.75	3.89	s		3.78	3.88					s	3.75	3.88	3.90
H-1[d]									br	8.32						
H-4	d	8.3	7.36	7.39	d	1.7	7.64	7.58	s	7.76	7.74	7.76	s	7.75	7.71	7.74
H-5	dd	8.3,1.6	7.26	7.31												
H-6					dd	1.7,8.7	7.32	7.38								
H-7	d	1.6	7.44	7.75	d	8.7	7.14	7.49	s	7.61	7.72	7.81	s	7.58	7.82	7.96
MS	m/e (% base)				m/e (% base)				m/e (% base)				m/e (% base)			
M	371 (29), 369 (100), 367 (100), 365 (36)[e]				371 (35), 369 (94), 267 (100), 365 (36)[e]				437 (16), 435 (65), 433 (100), 431 (68), 429 (18)[e]				451 (15), 449 (65), 447 (100), 445 (68), 443 (18)[e]			
M - CH_3	350 (6)[e]				350 (6)[e]								428 (2)[e]			
M - Br	286 (5)[e]				286 (7)[e]				350 (10)[e]				364 (4)[e]			
M - CNBrR[f]	245 (4)[e]				245 (4)[e]				323 (1)[e]				323 (1)[e]			

[a]EtOH solution. [b]Multiplicity. [c]Ppm from TMS. Solvent: C = $CDCl_3$, A = CD_3COCD_3, T = CCl_4. [d]N-H signals not observed in acetone solution. [e]Assignment confirmed by HRMS. [f]R = CH_3 for 1, 2, 4; R = H for 3.

mixtures of chlorinated and brominated analogs and the substitution patterns assigned were 2,3,4- and 2,3,7- for the trihalindoles and 2,3,4,7- for the tetrahaloindoles.

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