3628 J. Am. Chem. Soc. 1982, 104, 3628–3635

A Series of Chiral Polybrominated Biindoles from the Marine Blue-Green Alga *Rivularia firma*. Application of ^{13}C NMR Spin–Lattice Relaxation Data and ^{13}C–^{1}H Coupling Constants to Structure Elucidation

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Received June 11, 1981

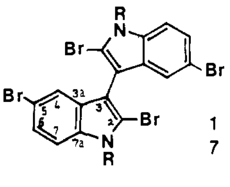
Abstract: Six novel polybrominated biindole derivatives have been isolated from the marine blue-green alga *Rivularia firma*: 2,2′,5,5′-tetrabromo-3,3′-bi-1*H*-indole (**1**), (+)-2,3′,5,5′-tetrabromo-7′-methoxy-3,4′-bi-1*H*-indole (**2**), (+)-3′,5,5′-tribromo-7′-methoxy-3,4′-bi-1*H*-indole (**3**), (+)-2,5,5′-tribromo-7′-methoxy-3,4′-bi-1*H*-indole (**4**), (−)-3,3′,5,5′-tetrabromo-7′-methoxy-1,4′-bi-1*H*-indole (**5**), and (+)-2,2′,3,4′,5,5′-hexabromo-1,3′-bi-1*H*-indole (**6**). Structures were solved by analysis of ^{1}H and ^{13}C chemical shifts, ^{13}C–^{1}H coupling constants, and ^{13}C spin–lattice relaxation data (at 15.04 MHz). The contributions of ^{13}C–^{1}H dipolar interactions to the spin–lattice relaxation times (T_1) of quaternary carbons were determined from measured T_1 and nuclear Overhauser enhancement values and then used to estimate the numbers of protons two bonds removed from each quaternary carbon. This information was used in structure determination as well as for resonance assignments. Spin–lattice relaxation data were also used to identify brominated carbons. The biindoles **2**–**6** are optically active and are therefore new representatives of a very small number of natural products in which chirality is due solely to restricted rotation.

Polyhalogenated indoles have been isolated recently from the marine algae *Rhodophyllis membranacea*[1] and *Laurencia brongniartii*.[2] However, with the exception of 6,6′-dibromoindigotin, the major constituent of the ancient dye Tyrian purple, which has been isolated from a number of marine gastropod mollusks[3] and might be regarded as a derivative of 2,2′-bi-1*H*-indole, biindoles have not been isolated previously from marine organisms. In this paper we describe the isolation and structural elucidation of six novel polybrominated biindoles from the blue-green alga *Rivularia firma*.

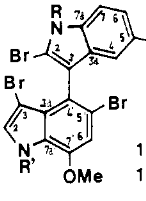
The structures of these metabolites are determined by analysis of ^{1}H and ^{13}C chemical shifts and ^{13}C–^{1}H coupling constants, as well as ^{13}C spin-lattice relaxation data for quaternary carbon resonances. The use of relaxation data for determination of the structures of unknown metabolites represents a novel application of such measurements. The basis of the approach is that the number of hydrogen nuclei two bonds removed from each quaternary carbon atom in a molecule may be estimated from the magnitude of the contribution of ^{13}C–^{1}H dipole–dipole interactions to the spin-lattice relaxation of that carbon. This information is then compared with predicted values for various possible structures that fit other data on the compound, such as ^{1}H NMR and mass spectra. The details of this method, together with some applications, have recently been outlined elsewhere.[4–6]

Results and Discussion

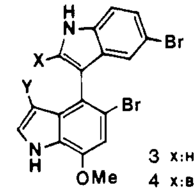
The blue-green alga *Rivularia firma* Womersley (Cyanophycophyta, Rivulariaceae) was collected on two separate occasions at Westernport, Victoria, where the alga occurred as dark green gelatinous hemispherical lumps, about 1 cm in diameter, on rocks in the intertidal zone.



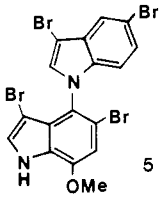
1 R:H
7 R:Ac



2 R:R′:H
11 R:Ac, R′:H
12 R:R′:Ac



3 X:H,Y:Br
4 X:Br,Y:H



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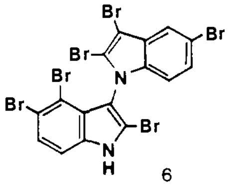

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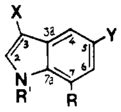

8 X:Br, R:R′:Y:H
9 Y:Br, R:R′:X:H
10 X:Y:Br, R:R′:H
13 Y:OMe, R:R′:X:H
14 Y:OMe, R′:Ac, R:X:H
15 R:OMe, R′:X:Y:H
16 R:OMe, R′:Ac, X:Y:H

Extraction of the dried alga with dichloromethane–methanol followed by trituration of the gummy extract with dichloromethane and chromatography of the dichloromethane soluble material on silica gel gave a mixture of biindoles (0.04% based on wet alga), which were separated by HPLC on silica gel to give 2,2′5,5′-tetrabromo-3,3′-bi-1*H*-indole (**1**) and (+)-2,3′,5,5′-tetrabromo-7′-methoxy-3,4′-bi-1*H*-indole (**2**) as major components, together with minor quantities of (+)-3′,5,5′-tribromo-7′-methoxy-3,4′-bi-1*H*-indole (**3**), (−)-3,3′,5,5′-tetrabromo-7′-methoxy-1,4′-bi-1*H*-indole (**5**), (+)-2,2′,3,4′,5,5′-hexabromo-1,3′-bi-1*H*-indole (**6**), and three other related compounds present in trace quantities only. A second collection of the alga yielded compounds **1** and **2** as the dominant biindoles, together with a small amount of (+)-2,5,5′-tribromo-7′-methoxy-3,4′-bi-1*H*-indole (**4**).

2,2′,5,5′-Tetrabromo-3,3′-bi-1*H*-indole (1). **1** crystallized from chloroform as colorless rosettes; High-resolution mass spectrometry established the formula as $C_{16}H_8Br_4N_2$. The ^{1}H NMR spectrum in acetone-d_6 shows only three aromatic resonances at δ 7.44, 7.40, and 7.24 and the ^{13}C NMR spectrum only eight aromatic carbon resonances, indicating that **1** is symmetrical. The ^{13}C chemical shifts (Table I) suggest that **1** is a biindole.

Treatment of **1** with acetic anhydride–pyridine slowly produces a diacetate (**7**), which crystallizes directly from the reaction mixture. The ^{1}H NMR spectrum of **7** shows resonances at δ 8.11

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Table I. ^{13}C Chemical Shifts and $^{13}C-^{1}H$ Spin-Spin Coupling Constants for Biindoles from *R. firma*[a]

C[b]	1		2		3		4	5		6	
	δ[c]	J_{CH}[d]	δ	J_{CH}	δ	J_{CH}	δ	δ	J_{CH}	δ	J_{CH}
2	112.5	0; NH, 2.4	113.7	0; NH[f]	127.8	d, 183.7; NH, 4.3		131.6	d, 192.9[e]	120.3	0,[e]
3	108.3	d, 2.8; NH, 2.7	113.9	d < 2.4; NH[f]	112.9	f		90.6	t, 3.9[e]	94.2	d, 4.9[e]
3a	130.4	d, 5.2; NH, 5.8	132.0	d, 5.2; NH, 5.5	131.8	t, 6.3; NH, 6.3		129.4	t, 5.9[e]	129.5	d, 5.5[e]
4	125.7	d, 166.6; d, 5.5[e]	125.0	d, 166.2; d, 5.4[e]	124.4	d, 165.6; d, 5.3[e]	125.4	126.7	d, 167.2; d, 5.9[e]	127.4	d, 168.2; d, 5.8[e]
5	113.7	d, 8.7; d, 3.8[e]	113.5	d > 7.3; d;[f] NH[f]	112.7	f		114.3	d, 10; t, 3[e]	115.4	f
6	122.1	d, 165.9; d, 4.7[e]	121.8	d, 165.7; d, 5.2[e]	122.7	d, 164.2; d, 5.2[e]	122.3	121.9	d, 166.5; d, 5.2[e]	121.6	d, 167.8; d, 5.5[e]
7	113.7	d, 164.8[e]	113.3	d, 163.9[e]	114.0	d, 163.0[e]	113.5	113.7	d, 165.4[e]	114.0[g]	d, 166.0[e]
7a	136.1	t, 8.1; NH, 3.0	135.6	t, 7.9; NH, 2.9	135.5	qa, 7.9; NH, 4.0		137.9	qa, 7.4	138.5	d, 9.3; d, 7.5[e]
2'			126.8	d, 189.8; NH, 3.8	126.8	d, 189.8; NH, 4.0	126.2	127.9	d, 190.9; NH, 4	115.4	0,[e]
3'			90.4	d, 4.9; NH, 8.1	90.8	d, 4.9; NH, 7.9	103.2	88.0	d, 5.2; NH, 8.5	112.7	0,[e]
3a'			127.3	d, 5.5; NH[f]	127.4	d, 7.0; NH[f]		125.6	d, 7; NH[h]	125.9	d, 3.1; NH, 2.4
4'			117.8	d, 5.8[e]	120.0	d, 5.5[e]		121.4	d, 6[e]	112.8	d, 5; d, 2; NH[f]
5'			118.4	d, 4.3[e]	118.3	d, 4.3[e]		116.3	d, 5[e]	118.5	d, 5.8; d, 2.1[f]
6'			107.6	d, 164.8[e]	107.7	d, 164.2[e]	107.0	107.5	d, 166.3[e]	128.3	d, 169.7[f]
7'			147.3	qi, 3.7[e]	146.9	qi, 4.1[e]		148.2	qi, 4[e]	113.7[g]	d, 167.2[f]
7a'			126.1	t, 7.8; NH, 3.8	126.2	t, 7.9; NH, 3.1		127.1	e NH[h]	135.6	d, 6.7; d, 4.0; NH[h]
7'-OCH_3			56.2	qa, 144.7[e]	56.2	qa, 144.7[e]	56.2	56.8	qa, 145.6		

[a] All spectra were recorded with acetone-d_6 as solvent, except for measurements of $^{13}C-^{1}H$ coupling constants in the N-deuterated forms, which were carried out in acetone-d_6 containing a few drops of D_2O. [b] See structure diagram. [c] Chemical shift of NH form in ppm from Me_4Si; estimated accuracy 0.1 ppm. [d] Coupling constant of ND form in Hz; estimated accuracy 1 Hz. d = doublet, t = triplet, qa = quartet, qi = quintet. NH indicates coupling constant to exchangeable proton. [e] Not coupled to exchangeable proton. [f] Coupling constant not measured because of peak overlap. [g] Assignments may be reversed. [h] Coupling to NH observable but not measurable.

Table II. ^{13}C Spin-Lattice Relaxation Times and NOE Values for Biindoles from *R. firma*[a]

C[b]	1								2								6							
	NH				ND				NH				ND				NH				ND			
	$\eta + 1$[c]	T_1[d]	T_1^{CH} [e]	T_1°,[f]	$\eta + 1$	T_1	T_1^{CH}	T_1°	$\eta + 1$	T_1	T_1^{CH}	T_1°	$\eta + 1$	T_1	T_1^{CH}	T_1°	$\eta + 1$	T_1	T_1^{CH}	T_1°	$\eta + 1$	T_1	T_1^{CH}	T_1°
2	1.5	7.7	32	10	1.2	11	132	12	1.9	7.9	17	15	$\lesssim 1.6$[k]	14[k]	$\gtrsim 43$	$\lesssim 21$	1.0	13	>500	14	1.1	12	>200	12
3	1.9	60	133	110	1.8	70	170	115	2.1	$\gtrsim 44$[i]	$\gtrsim 77$	$\gtrsim 102$	$\gtrsim 1.6$[k]	31[k]	$\lesssim 95$	$\gtrsim 46$	1.1	16	>300	16	1.0	14	>2000	14
3a	2.1	30	51	70	2.6	30	37	159	2.5	28	36	125	2.6	24	30	110	2.7	27	32	171	2.7	21	25	134
4	3.0	0.91	0.91		2.9	0.78	0.78		2.8	0.66	0.66		2.9	0.61	0.61		3.0	0.86	0.86		3.1	0.74	0.74	
5	1.9[g]	9.1[h]	19	17	2.2[g]	7.2[h]	12	18	2.4[g]	6.5[h]	9.3	22	2.1[g]	8.3[h]	15	18	2.0[l]	8.9	18	18	o			
6	3.0	0.92	0.92		3.1	0.72	0.72		2.9	0.70	0.70		2.8	0.59	0.59		3.0	0.75	0.75		3.0	0.69	0.69	
7	3.0[g]	1.15[h]	1.15		3.0[g]	0.86[h]	0.86		3.0[g]	0.69[h]	0.69		3.0[g]	0.87[h]	0.87		2.9[l]	0.76	0.76		2.9[l]	0.66	0.66	
7a	2.3	19	29	57	2.4	24	34	87	2.8	15	17	133	2.8	18	21	156	2.2	23	38	61	2.2	23	38	58
2'									3.3	0.77	0.77		3.2	0.70	0.70		1.4[l]	7.8	40	10	m			
3'									1.5	12	46	17	1.6	12	40	16	1.5[l]	30	>100	40	1.4	44	~200	57
3a'									2.1	$\gtrsim 49$[i]	$\gtrsim 92$	$\gtrsim 107$	2.0	53	110	102	1.3	34	~200	41	1.3	36	~200	43
4'									1.7	$\gtrsim 58$[i]	$\gtrsim 162$	$\gtrsim 91$	1.7	54	151	85	1.1[l]	24	>250	26	1.3	14	81	16
5'									1.8	13	31	22	1.8	13	30	22	1.4	10	46	13	1.8	11	30	19
6'									2.9	0.82	0.82		3.0	0.71	0.71		3.0	0.74	0.74		3.0	0.66	0.66	
7'									2.4	22	32	71	2.5	21	28	86	2.9[l]	0.73	0.73		2.9[l]	0.63	0.63	
7a'									2.7	20	23	132	2.1	37	68	79	2.1	13	24	29	2.1	22	40	46
7'-OCH_3									3.0	j			3.0	j										

[a] Samples with hydrogen-bearing indole nitrogen (NH) were run in acetone-d_6; those with deuterated indole nitrogen (ND) in acetone-d_6 containing a few drops of D_2O. The deuterated samples were lyophilized at least once from a acetone-d_6/D_2O mixture beforehand. Sample conditions were as follows: 1, NH 0.2_5 M, ND 0.2_5 M, 31 °C; 2, NH 0.6 M, ND 0.6 M, 31 °C; 6, NH 0.3 M (sample contained 1 drop H_2O), ND 0.2_5 M (sample contained 1.3 mM Na_2EDTA), 27 °C. [b] See structure diagram. [c] Intensity obtained by setting the average of the intensities of the resolved protonated carbon resonances equal to 2.99 ($\eta = 1.99$). Estimated accuracy 0.3 unit. [d] Experimental ^{13}C T_1 (in s). Estimated accuracy 10% for protonated carbons, 15% for quaternary carbons. [e] Contribution (in s) of $^{13}C-^{1}H$ dipolar interactions to observed T_1, obtained from eq 2 as described in text. [f] Contribution (in s) of relaxation mechanisms other than $^{13}C-^{1}H$ dipolar interactions to observed T_1, obtained from eq 4 as described in text. [g] Peaks overlap. The protonated carbon C-7 is assumed to have an intensity of 3.0. [h] Estimated error ~20% because of peak overlap. [i] Obtained with recycle time of 150 s ($< 3.5 \times T_1$). [j] Not measured. [k] Peaks overlap. Intensities of peaks from C-2 and C-3 assumed to be equal for purposes of calculation, although C-3 probably makes the greater contribution. [l] Partial peak overlap. [m] Peaks overlap. Total intensity = 3.2, average T_1 = 11 s.

(d, $J = 9$ Hz), 7.32 (dd, $J = 9, 2$ Hz), and 7.17 (d, $J = 2$ Hz), with the acetyl methyl at δ 2.89. Conversion of an indole to its *N*-acetate is expected to cause significant deshielding of (C-7)H, in which case the coupling constants observed in the ^{1}H NMR of **7** indicate that C-4, C-4′, C-6, C-6′, C-7, and C-7′ of the biindole are protonated. This is confirmed by analysis of ^{13}C–^{1}H spin–spin coupling constants and ^{13}C spin–lattice relaxation measurements, which independently yield the complete structure.

The use of spin–lattice relaxation data from quaternary carbon atoms for elucidation of the structures of natural products has been described previously.[4-6] An outline of the way in which such data are utilized is given here for compound **1**. The same principles are employed later for compounds **2** and **6**. ^{13}C NMR spin–lattice relaxation times and nuclear Overhauser enhancement (NOE) values[7] were measured for all carbons of **1** in its NH and ND forms (see Table II). As the methine carbon resonances experience the maximum NOE ($\eta + 1 = 2.99$), it may be concluded that their relaxation is dominated by ^{13}C–^{1}H dipolar interactions,[8] with a rotational correlation time that satisfies the "extreme narrowing" condition:

$$(\omega_H + \omega_C)^2 \tau_R^2 \ll 1 \qquad (1)$$

where ω_H and ω_C are the resonance frequencies in rad/s of ^{1}H and ^{13}C, respectively. Therefore, the contribution of ^{13}C–^{1}H dipolar interactions to the spin–lattice relaxation time of any carbon (T_1^{CH}) can be obtained from eq 2. Values of T_1^{CH} for

$$T_1^{CH} = (1.99/\eta) T_1 \qquad (2)$$

the quaternary carbons of **1** are given in Table II.

If **1** behaved as an isotropic rigid rotor, the T_1 values for its methine carbons would all be the same. The data in Table II indicate that this is not true for **1** (NH or ND form). In this case, we must expect some variation among T_1^{CH} values for quaternary carbons, even if they have the same number of hydrogens two bonds removed. Therefore, the quantitative approach used previously[5,6] to solve the structures of a series of polybrominated oxydiphenol derivatives from the sponge *Dysidea herbacea* cannot be employed here. Nevertheless, we may still use quaternary carbon T_1^{CH} values in a qualitative sense to identify those carbons with no hydrogens two bonds removed and to determine which carbons are two bonds removed from readily exchangeable hydrogens. Even with this limitation, useful structural information is obtained.

We begin by comparing T_1^{CH} values for the quaternary carbons of **1** in its NH and ND forms. Before doing so, it is necessary to correct for the slower overall motion of the ND form,[6,9] which is reflected in the smaller methine T_1 values for this form (Table II). Considering only the two well-resolved methine carbon resonances, the average T_1 is 0.75 s for the ND form and 0.91_5 s for the NH. The contribution of the exchangeable hydrogen to the relaxation of each quaternary carbon may then be obtained from eq 3.

$$(\Delta T_1)^{-1} = (T_1^{CH}[NH])^{-1} - [(0.91_5/0.75) T_1^{CH}[ND]]^{-1} \qquad (3)$$

Values obtained in this way for peaks at 108.3, 112.5, 113.7, 130.4, and 136.1 ppm (Table I) are approximately 400, 40, ∞, ∞, and 96 s, respectively. These compare with a value of 51 s, which is the calculated contribution[6,9] of a proton 2.1_5 Å away from a quaternary carbon in an isotropic rigid rotor having $\tau_R = 0.054$ ns (corresponding to $T_1 = 0.91_5$ s for a methine carbon with $r_{CH} = 1.10$ Å). In **1**, the presence of anisotropic overall motion or unequal nonbonded C–H distances (or both) leads to a divergence from the ideal case, but the data can still be used to conclude that the peaks at 112.5 and 136.1 ppm correspond to C-2 and C-7a. Specific assignments can be made from the T_1^{CH} values in the deuterated form. The carbon giving rise to the peak at 112.5 ppm has no hydrogens two bonds away in the ND form, while the carbon at 136.1 ppm has at least one (Table II). As ^{13}C–^{1}H and ^{1}H–^{1}H coupling constants indicate that the heterocyclic ring is fully substituted, specific assignments of these two resonances to C-2 and C-7a, respectively, follow readily, as does the information that C-7 is protonated. Of the remaining three quaternary carbons, the two at 108.3 and 130.4 ppm are coupled to an exchangeable hydrogen, but not significantly relaxed by it. The carbon at 108.3 ppm has no hydrogens two bonds removed and is therefore assigned to C-3. The carbon at 130.4 ppm has at least one hydrogen two bonds removed and must be assigned to C-3a (this also confirms that C-4 bears a hydrogen).

In order to determine the locations of the two bromine substituents, it is necessary to assign the brominated carbon resonances. Previous work[10-12] has shown that resonances from brominated quaternary carbons are readily identified from spin–lattice relaxation data because of the strong contributions of carbon–bromine scalar and dipolar interactions[8] to their relaxation.

The contribution of relaxation mechanisms other than ^{13}C–^{1}H dipolar interactions to the observed T_1 values may be estimated from eq 4, which is valid provided eq 1 is satisfied. These contributions are given in Table II.

$$T_1^0 = [1.99/(1.99 - \eta)] T_1 \qquad (4)$$

In the NH form of **1** the quaternary carbons at 112.5 and 113.7 ppm have T_1^0 values of 10 and 17 s, respectively, whereas the other three have T_1^0 values of 57, 70, and 110 s. Corresponding values in the ND form are 12, 18, 87, 159 and 115 s, respectively. The peak at 112.5 ppm has already been assigned to C-2 on the basis of relaxation data (see above), while the peak at 113.7 ppm must arise from C-5 or C-6, as it is the only quaternary carbon not coupled to an exchangeable proton (Table I). Because these carbons are far apart in the molecule, it is unlikely that they are relaxed by a specifically bound paramagnetic impurity. As **1** contains two bromine atoms, we assign these peaks to the two brominated carbons, their short T_1 values being due mainly to carbon–bromine scalar and dipolar interactions.[13] Since C-2 and C-5 (or C-6) are brominated, C-3 must represent the point of linkage between the two rings.

It now remains to establish whether the second bromine is substituted at C-5 or C-6. This follows from the long-range ^{13}C–^{1}H coupling constants for C-3a and C-7a, the resonances of which have been assigned from relaxation data (see above). In substituted benzenes, ortho ($^2J_{CH}$) coupling constants are usually smaller than 2 Hz (except for carbons substituted with highly electronegative atoms, in which case they can be as large as 5 Hz in magnitude), meta ($^3J_{CH}$) coupling constants are large (7–10 Hz), and para ($^4J_{CH}$) coupling constants are less than 2 Hz.[14,15] Therefore, the observation that C-7a in the ND form is a triplet with $J_{CH} = 8$ Hz (Table I) requires that C-5 be substituted (we have already established that C-7 is protonated), as does the doublet splitting of C-3a ($J_{CH} = 5$ Hz, Table I). The structure of **1** is as shown in the diagram. Having established the structure, the three protonated carbon resonances can be assigned from

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[7] NOE is defined here as the increase in the integrated intensity of a ^{13}C resonance under conditions of complete proton decoupling over its intensity in the absence of proton decoupling.

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[13] Other possible sources of spin–lattice relaxation can be eliminated. ^{13}C–^{14}N dipolar interactions (Norton, R. S.; Allerhand, A. *J. Am. Chem. Soc.* **1976**, *98*, 1007–1014. Oldfield, E.; Norton, R. S.; Allerhand, A. *J. Biol. Chem.* **1975**, *250*, 6368–6380) to C-2 from the directly bonded nitrogen 1.35 Å away would contribute about 200 s to T_1 in the ND form of **1**. Spin rotation[8] can be ignored for these molecules at ambient temperature, and chemical shift anisotropy[8] would make a negligible contribution at the low magnetic field strength employed here (Norton, R. S.; Clouse, A. O.; Addleman, R.; Allerhand, A. *J. Am. Chem. Soc.* **1977**, *99*, 79–83).

[14] Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972.

[15] Gottlieb, H. E. *Isr. J. Chem.* **1977**, *16*, 57–67.

Table III. ^{13}C Chemical Shifts and ^{13}C–^{1}H Spin–Spin Coupling Constants for Brominated Indoles[a]

	8		9		10	
C[b]	δ[c]	J_{CH}[d]	δ	J_{CH}	δ	J_{CH}
2	124.5	d, 188.6; NH, 4.3	126.5	d, 183.3; d, 8.4; NH, 3.9	126.1	d, 189.8; NH, 4.6
3	89.9	m; NH[e]	101.3	d, 173.8; d, 7.8; d, 3.1; NH[e]	89.0	m; NH[e]
3a	126.9	m; NH[e]	130.1	m; NH[e]	128.6	m; NH[e]
4	118.5[f] 120.2[f] 122.6[f]		123.9	d, 165.7; d, 5.5[g]	125.4	d, 167; d, 6[g]
5			112.1	d, 7.8; d, 4.1[g]	113.1	d, 8.2; d, 3.7[g]
6			122.7	d, 164.5; d, 4.0[g]	120.8	d, 166.5; d, 4.7[g]
7	112.1	m; NH[e]	113.2	d, 164.2[g]	114.0	d, 166[g]
7a	136.0	m; NH[e]	135.1	m; NH[e]	134.6	m; NH[e]

[a] All spectra were recorded with solute concentrations of 0.5–1.5 M in acetone-d_6, except for measurements of ^{13}C–^{1}H coupling constants in the N-deuterated forms, which were carried our in acetone-d_6 containing a few drops of D_2O. Peak assignments are based on chemical shifts of methylated indoles[16] and known substituent effects.[14] Where necessary, ^{13}C spin-lattice relaxation measurements were used to assign brominated carbon resonances (see text). Assignments of C-4 and C-6 in the 5-bromoindoles were supported by the observation that the C-6 resonances were broader than the C-4 resonances in coupled spectra, presumably because of weak coupling to H-7. [b] See structure diagram. [c] Chemical shift of NH form in ppm from Me_4Si; estimated accuracy 0.1 ppm. [d] Coupling constant of ND form in Hz; estimated accuracy 1 Hz; d = doublet, t = triplet, qa = quartet, qi = quintet, m = multiplet. NH indicates coupling constant to exchangeable proton. [e] Coupling constant not measured because of peak overlap. [f] Peaks not assigned individually. Coupling constants for peaks at 118.5, 120.2, and 122.6 ppm are respectively d, 161.7/d, 6.7; d, 159.6/d, 7.0; d, 158.7/d, 7.9. Each peak shows additional coupling with $J \simeq 1$ Hz. [g] Not coupled to exchangeable proton.

literature data on methylated indoles[16] and known substituent effects[14] (see Table I).

^{13}C spin–lattice relaxation data have been used in a number of ways in solving the structure of **1**. Analysis of ^{13}C–^{1}H dipolar contributions to quaternary carbon relaxation has been used to assign resonances (e.g., those from carbons adjacent to the exchangeable hydrogen) and to identify those carbons with no hydrogens two bonds removed (e.g., C-2 in the ND form, C-3 in either form). Contributions from other relaxation mechanisms have been used to identify the brominated carbons and then to aid in positioning the bromines in the structure. The arguments used for **1** will also be used for **2** and **6**.

Before proceeding, it is useful to consider in greater detail the long-range ^{13}C–^{1}H coupling constants for **1** (Table I), as they are relevant to subsequent structures. Coupling to the exchangeable NH is finite but cannot be used to distinguish C-2 and C-7a from C-3 and C-3a. C-2 shows no other coupling under our experimental conditions, although C-3 is weakly coupled to a single proton, presumably (C-4)H. Except for C-5, none of the carbons in the six-membered ring is coupled to protons in the ortho position. The brominated carbon, C-5, is coupled to only one of its two ortho protons. We have confirmed that this pattern also occurs in model compounds.

In Table III ^{13}C chemical shifts and ^{13}C–^{1}H coupling constants are given for some brominated indoles. The coupling constants for C-5 of **9** (5-bromoindole) and **10** (3,5-dibromoindole) show the same pattern as C-5 of **1**, viz., strong coupling to the meta proton and weaker coupling to only one of the ortho protons. The coupling constants for the protonated carbons in the six-membered rings are similar in **1**, **9**, and **10**.

(+)-2,3′,5,5′-Tetrabromo-7′-methoxy-3,4′-bi-1*H*-indole (2). This was the major biindole isolated from both collections of *R. firma*. The molecular formula $C_{17}H_{10}Br_4N_2O$ was established by high-resolution mass spectrometry. The ^{1}H NMR spectrum in $CDCl_3$ shows two NH resonances at δ 8.48 and 8.30, a complex series of signals between δ 7.4 and 6.9 due to five aromatic protons, and a methoxyl group at δ 3.88. Although **2** is optically active ($[\alpha]^{20}_{\mathrm{D}}$ +71°), there are no resonances attributable to asymmetric carbon atoms in either the ^{1}H or ^{13}C NMR spectra, and, therefore, asymmetry must be due to restricted rotation in **2**.

Treatment of **2** with acetic anhydride–pyridine at room temperature overnight gave a mixture of mono- and diacetates **11** and **12** which were separated by HPLC on silica gel. The ^{1}H NMR spectrum of the monoacetate **11** shows resonances at δ 8.76 (1 H, br s, NH), 8.26 (1 H, d, J = 9 Hz), 7.37 (1 H, dd, J = 9, 2 Hz), 7.16 (1 H, d, J = 3 Hz, becoming a singlet on exchange of NH with D_2O), 7.12 (1 H, d, J = 2 Hz), 6.98 (1 H, s), 3.96 (3 H, s), and 2.90 (3 H, s). By analogy with **1**, it appears that the indole ring that is acetylated first is substituted at C-5. Furthermore, the unacetylated ring must have a proton at C-2. The ^{1}H NMR spectrum of the diacetate **12** supports these assignments, showing one low-field ortho-coupled proton, one ortho-meta-coupled proton, one-meta-coupled proton, two aromatic proton singlets, a methoxyl signal, and two *N*-acetyl groups at δ 2.75 and 2.90.

The ^{13}C spectrum of **2** contains 17 resonances, 7 of which have chemical shifts and ^{13}C–^{1}H coupling constants (Table I) very similar to those of C-2 and C-3a to C-7a of **1**. This indicates that one indole ring in **2** has the same substitution pattern as the rings of **1**. This is confirmed by analysis of the ^{13}C spin-lattice relaxation data for these carbons in **2** (Table II) and is consistent with ^{1}H NMR data. The peak at 113.9 ppm is assigned to C-3 based on its long-range coupling (Table I) and relaxation behavior (Table II), the 5.6 ppm downfield shift relative to C-3 of **1** indicating a different mode of attachment to the second ring.

In order to establish the substituent pattern in the second ring, we must employ ^{13}C spin-lattice relaxation data in conjunction with ^{13}C chemical shifts and ^{13}C–^{1}H coupling constants. The observation of a resonance with $^{1}J_{\mathrm{CH}}$ = 190 Hz (Table I) indicates[14] that the second ring has a hydrogen at C-2′, consistent with the ^{1}H NMR data. The quaternary carbon resonance at 90.4 ppm is assigned to a brominated C-3′ by comparison with chemical shifts in model compounds (**8** and **10** in Table III). This is confirmed by its ^{13}C spin-lattice relaxation behavior (Table II), which shows it to be a brominated carbon adjacent to at least one nonexchangeable proton,[17] in this case (C-2′)H. The resonances from C-3a′ and C-7a′ in this ring are identified as those at 126.1 and 127.3 ppm (although not in this order) due to their coupling to an exchangeable proton. Specific assignments follow from analysis of the ^{13}C–^{1}H dipolar contributions to their ^{13}C relaxation behavior (Table II). The carbon giving rise to the peak at 126.1 ppm is adjacent to at least one proton in the NH form, but none in the ND form, showing that it must be assigned to C-7a′. The carbon yielding the peak at 127.3 ppm has no protons two bonds removed from it in either form, consistent with its assignment to C-3a′. These relaxation data also indicate that C-4′ and C-7′ are quaternary.

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(16) Parker, R. G.; Roberts, J. D. *J. Org. Chem.* **1970**, *35*, 996–999. The assignments of C-5 and C-6 given in this paper should be reversed; see: Bradbury, J. H.; Norton, R. S. *Biochim. Biophys. Acta* **1973**, *328*, 10–19.

(17) Average T_1 value for resolved methine carbon resonances of the NH form of **2** is 0.74 s, corresponding to τ_R = 0.066 ns (r_{CH} = 1.10 Å); therefore, a nonbonded ^{1}H 2.15 Å away would contribute 41 s to ^{13}C relaxation. Corresponding values for the ND form are 0.65 s, 0.076 ns, and 36 s, respectively.

Table IV. ^{13}C Chemical Shifts of Methoxyindoles[a]

C[b]	13	14[c]	15	16[c]
2	126.0	127.6	125.0	128.6
3	102.2	108.9	102.7	108.0
3a	132.3[d]	132.4[d]	130.5	134.9
4	112.4[e]	113.8[e]	113.9	114.5
5	154.9	157.2	120.4	124.9
6	102.7[e]	104.1[e]	102.1	107.7
7	112.6	117.6	147.3	149.0
7a	129.4[d]	131.0[d]	127.4	125.3
$-OCH_3$	55.8	55.7	55.5	56.0

[a] 0.3–1.3 M solutions in acetone-d_6. [b] See structure diagram. [c] Chemical shifts of methyl and carbonyl carbons, respectively, of acetyl moiety are as follows: 14, 23.6, 169.1 ppm; 16, 25.9, 170.0 ppm. [d] Assignments may be reversed in same column. [e] Assignments may be reversed in same column.

Having assigned these resonances from their relaxation behavior, we use their long-range couplings to obtain further structural information. C-7a′ is coupled to (C-2′) H and one other proton with J_{CH} = 8 Hz. The magnitude of this coupling indicates that the second proton is meta, and, since we have shown that C-4′ is quaternary, C-6′ must be protonated.[18]

It remains to determine the locations in the six-membered ring of the bromine, the methoxyl group, and the other indole ring. The peak at 118.4 ppm is assigned to the brominated carbon on the basis of its relaxation behavior (strong non-CH dipolar contribution; see Table II), the peak at 147.3 ppm is assigned to the methoxyl-substituted carbon on the basis of its chemical shift[14] and its coupling to four protons (Table I), and the remaining peak at 117.8 ppm must by elimination arise from the carbon to which the other indole ring is attached. The relaxation data in Table II show clearly that both the brominated carbon and the methoxyl-substituted carbon are adjacent to at least one proton, whereas the carbon giving rise to the peak at 117.8 ppm has no proton two bonds removed. As we have established that C-6′ bears a proton, the bromine and methoxyl substituents must occur at C-5′ and C-7′ (not necessarily in that order) while the other indole ring must be attached to C-4′.

In order to resolve the uncertainty regarding the positions of the bromine and methoxy substituents, we consider the effects of acetylation at N-1′ on the ^{13}C chemical shifts of the methoxyl carbon and the indole carbon to which the methoxyl group is attached. Acetylation of **11** to yield **12** produces downfield shifts of 1.6_3 and 0.4_1 ppm, respectively, for $C-OCH_3$ and $-OCH_3$. The corresponding shifts caused by acetylation of 5-methoxyindole (**13**) are 2.3_5 and -0.0_8 ppm, respectively, and for 7-methoxyindole (**15**) 1.6_5 and 0.5_2 ppm, respectively (Table IV). The close correspondence between the effects on **11** and **15** indicates that the methoxyl group in **2** is located at C-7′ and the bromine at C-5′. This structure has since been confirmed by X-ray crystallography,[19] which also established the absolute configuration of the compound.

(+)-3′,5,5′-Tribromo-7′-methoxy-3,4′-bi-1*H*-indole (3). This compound separated from chloroform as colorless prisms, and the molecular formula $C_{17}H_{11}Br_3N_2O$, was obtained by high-resolution mass matching. The similarity of **3** with the tetrabromo compound **2** is apparent by comparison of the ^{1}H NMR spectra, in which the only variation is the appearance of an extra single-proton aromatic resonance in **3**.

The ^{13}C spectrum of **3** contains 7 resonances with chemical shifts and ^{13}C–^{1}H coupling constants (Table I) almost identical with those of C-2′ to C-3a′ and C-5 to C-7a′ of **2**, indicating that the 3-brominated indole ring in **2** conserved in **3**. The C-4′ resonance is shifted downfield by 2.2 ppm, suggesting a change in the five-membered ring of the other indole nucleus in **3**. This is readily accounted for by the observation of a resonance at 127.8 ppm with $^1J_{CH}$ = 184 Hz and 4 Hz coupling to an exchangeable proton, which must be assigned to a methine carbon at position 2. The long-range coupling data for C-3a and C-7a further confirm that C-2 is protonated in **3**, while the ^{13}C chemical shifts and ^{13}C–^{1}H coupling constants for C-4 to C-7 (Table I) indicate that the substitution pattern in the six-membered ring is conserved. Thus, **3** is identical with **2** except for debromination of the latter at C-2.

(+)-2,5,5′-Tribromo-7′-methoxy-3,4′-bi-1*H*-indole (4). High-resolution mass spectrometry established the molecular formula as $C_{17}H_{11}Br_3N_2O$. A significant feature of the ^{1}H NMR spectrum is the presence of a one-proton doublet resonance at δ 5.98 (J = 3 Hz) attributable to an indole H-3 coupled to H-2. Because of the limited quantity of material available, only seven protonated carbon resonances were observed in the ^{13}C NMR spectrum. Six of these are nearly superimposable on the six protonated carbon resonances in the spectrum of **2** (Table I), while the new resonance at 103.2 ppm has a chemical shift characteristic of C-3 of an indole.[14,16] Thus, the available NMR data are consistent with a structure identical with **2** except for debromination at C-3′.

(−)-3,3′,5,5′-Tetrabromo-7′-methoxy-1,4′-bi-1*H*-indole (5). **5** separated from chloroform–hexane as a poorly crystalline colorless solid, for which high-resolution mass matching established the molecular formula as $C_{17}H_{10}Br_4N_2O$. The ^{1}H NMR spectrum shows resonances at δ 7.70 (1 H, d, J = 2 Hz), 7.48 (1 H, s), 7.44 (1 H, s), 7.30 (1 H, dd, J = 9, 2 Hz), 7.16 (1 H, s), 6.98 (1 H, d, J = 9 Hz), and 4.18 (3 H, s).

These data suggest that, as with other biindoles in this series, one of the indole rings is substituted at C-5. However, the C-7 ortho-coupled proton at δ 6.98 is at considerably higher field than in compounds **1**–**4**. Furthermore, consideration of the molecular formula and ^{1}H NMR data indicates that **5** can have only a single NH group. This could be explained if the indole ring protonated at C-4, C-6, and C-7 was attached to the second indole ring via the nitrogen.

The ^{13}C NMR spectrum (Table I) contains two resonances at 88.0 and 90.6 ppm, with chemical shifts attributable to C-3 (Br) and two resonances at 127.9 ($^1J_{CH}$ = 191 Hz) and 131.6 ppm ($^1J_{CH}$ = 193 Hz), with ^{13}C–^{1}H coupling constants which require assignment to C-2 (H). The peaks at 88.0 and 127.9 ppm are each coupled to an exchangeable hydrogen. Resonances from the other two carbons coupled to this hydrogen are at 125.6 and 127.1 ppm. The chemical shifts of these latter carbons and their coupling to nonexchangeable protons are similar to those of C-3a′ and C-7a′ of **2**, suggesting that this six-membered ring of **5** has the same substitution pattern as **2**. This is supported by the presence of peaks at 107.5, 116.3, and 148.2 ppm in the spectrum of **5**, which have chemical shifts and ^{13}C–^{1}H coupling constants very similar to those of C-6′, C-5′, and C-7′, respectively, of **2**. The peak at 121.4 ppm is assigned to C-4′ of this ring on the basis of its ^{13}C–^{1}H coupling constant. Its downfield shift of 3.6 ppm relative to C-4′ of **2** is consistent with a change in the point of linkage to the other ring from C-3 in **2** to N-1 in **5**. The peaks at 90.6 and 131.6 ppm are readily assigned to C-3 and C-2, respectively, of the ring linked through N-1, as neither is coupled to an exchangeable proton. Because the C-3 resonance is a triplet in the ^{1}H-coupled spectrum, C-4 must be protonated. Taken in conjunction with the ^{1}H NMR data, which indicate that three aromatic protons are substituted in an ortho/meta/para pattern, this suggests that this ring is brominated at C-5. This is confirmed by the presence of ^{13}C resonances at 126.7, 114.3, 121.9, and 113.7 ppm, which have chemical shifts and ^{13}C–^{1}H coupling constants consistent with their assignment to C-4, C-5, C-6, and C-7, respectively, of a 5-brominated indole ring (Table I). The remaining resonances at 129.4 and 137.9 ppm are assigned to C-3a and C-7a, respectively, on the basis of chemical shifts and long-range ^{13}C–^{1}H coupling constants (Table I).

(+)-2,2′,3,4′,5,5′-Hexabromo-1,3′-bi-1*H*-indole (6). **6** crystallized from acetonitrile and gave a molecular formula of $C_{16}H_6Br_6N_2$ by high-resolution mass matching. The ^{1}H NMR spectrum shows resonances at δ 7.68 (1 H, d, J = 2 Hz), 7.52 (2 H, s), 7.30 (1 H, dd, J = 9, 2 Hz), and 6.92 (1 H, d, J = 9

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(18) This is confirmed by the long-range coupling to C-3a′. This carbon is coupled only to (C-2′)H, indicating that the proton in the six-membered ring cannot be meta to C-3a′ and must be attached to C-4′ or C-6′.

(19) Blount, J. F.; Wells, R. J. *Aust. J. Chem.*, submitted for publication.

Hz). The three single-proton resonances have chemical shifts almost identical with those of (C-4)H, (C-6)H and (C-7)H, respectively, of **5**. In conjunction with the fact that only one NH is present, this suggest that a 5-brominated ring is linked through N-1, as in **5**.

In the ^{13}C NMR spectrum, resonances from C-2, C-3, C-3a, and C-7a of the ring with (N-1)H would normally be coupled to this proton (Table I). However, as discussed below, C-2 and C-3 of this ring are not coupled to (N-1)H. Therefore, we have used ^{13}C spin–lattice relaxation data to solve this structure.

Comparison of T_1^{CH} values for quaternary carbons of the NH and ND forms of **6** (Table II) shows that the carbon giving rise to a peak at 135.6 ppm is relaxed by the exchangeable proton and at least one nonexchangeable proton.[20] This could come from C-7a′ (with C-7′ protonated) or C-2′ (with C-3′ protonated). We may reject the second alternative because there is no methine carbon resonance in the spectrum with the characteristic chemical shift of an unsubstituted C-3′ (see above). However, the C-7a′ resonance is the only *well-resolved* quaternary carbon resonance relaxed by an exchangeable proton. As there is no ^{13}C resonance with properties attributable to a C-2′ methine (Table I), C-2′ must be quaternary and its resonance must be one component of the two-carbon peak at 115.4 ppm. The two components of this peak are barely resolved in the NH form, but the relaxation data clearly indicate that both carbons are brominated (Table II). In the ND form the two peaks are not resolved, but if the relaxation of one component in the ND form is assumed to be the same as in the NH form, the data show that the T_1^{CH} value for the second component increases significantly in the ND form. Only one component of the 115.4 ppm peak displays ^{13}C–^{1}H coupling, and neither is coupled to (N-1′)H. As C-3′ must be quaternary (see above), the singlet component of the peak at 115.4 ppm is assigned to C-2′.

The resonance at 135.6 ppm, assigned to C-7a′ on the basis of its relaxation behavior, is coupled to two nonexchangeable protons (Table I). Because C-2′ and C-3′ are quaternary, these protons must be located in the six-membered ring, one meta to C-7a′ and the other probably ortho. In order to verify the positions of these protons, we must also assign the C-3a′ resonance. On the basis of its long-range coupling to (N-1′)H, the peak at 125.9 ppm is assigned to C-3a′. The relaxation data for this peak (Table II) show that C-3a′ has no protons two bonds removed, indicating that C-3′ and C-4′ are quaternary. Therefore, the long-range couplings to C-7a′ must come from protons ortho (4 Hz) and meta (7 Hz) to this carbon. Thus, C-6′ and C-7′ are protonated. Of the five protonated carbon resonances, only the two at 113.7 and 114.0 ppm have chemical shifts characteristic of a proton-bearing C-7 (see Table I). Regardless of which is assigned to C-7′, the fact that neither displays *any* long-range coupling indicates that the carbon meta to each is quaternary. Therefore, C-5 and C-5′ are quaternary.

The ^{1}H NMR spectrum contains three resonances arising from protons substituted ortho/meta/para in the same ring, in addition to a two-proton singlet. The latter resonance must arise from (C-6′)H and (C-7′)H, the lack of coupling being due to their coincident chemical shifts. Attempts to resolve these resonances in other solvents failed because of solubility problems.

Thus, we have established that C-2′ is brominated and that C-6′ and C-7′ are protonated, and we have assigned the resonances from C-3a′ and C-7a′. It is now necessary to determine which of the remaining quaternary carbons in this ring, C-3′, C-4′, and C-5′, is linked to N-1 of the other ring. There are eight unassigned quaternary carbon resonances in the spectrum, five corresponding to brominated carbons. Only one of these (94.2 ppm) exhibits the shielding characteristic of a brominated C-3 (Table I). Relaxation data (Table II) confirm that this resonance corresponds to a brominated carbon. This carbon is coupled to a single proton (J_{CH} = 5 Hz), which, because both C-2 and C-2′ are quaternary (see above), must be H-4 or H-4′ (compare with C-3 of **5**, Table I). As we have established that C-4′ is quaternary, the peak at 94.2 ppm cannot be assigned to C-3′, and must correspond instead to C-3. If C-3′ is not brominated, it must represent the point of attachment to the other indole ring. To assign the resonance from C-3′, we note that, of the three unassigned quaternary carbon resonances which arise from nonbrominated carbons, the relaxation data in Table II show that only one, at 112.7 ppm, corresponds to a carbon with no hydrogens two bonds removed. This must be C-3′. It follows by elimination that C-4′ and C-5′ are brominated, although we cannot assign their resonances at this stage.

Turning to the other ring, we have established that N-1 is linked to C-3′ and C-3 is brominated. As C-2 is quaternary, it must also be brominated. There are two remaining resonances from nonbrominated quaternary carbons, at 129.5 and 138.5 ppm, which must arise from the bridgehead carbons. They are assigned to C-3a and C-7a, respectively, on the basis of their chemical shifts. The relaxation data (Table II) show that each carbon is ortho to at least one hydrogen and their long-range coupling constants (Table I) are consistent only with the presence of protons at C-4, C-6, and C-7. This agrees with the ^{1}H NMR data. The substituent at C-5 must, by elimination, be bromine.

The assignment of resonances from the brominated carbons C-2, C-5, C-4′, and C-5′ follows from their relaxation behavior (Table II). Two of the unassigned brominated carbon resonances, at 115.4 and 118.5 ppm, come from carbons having hydrogens ortho to them, with the former having a T_1^{CH} value roughly half that of the latter. Therefore, the peak at 115.4 ppm is assigned to C-5, and that at 118.5 ppm to C-5′. This assignment could not have been made from coupling constant data. The remaining two brominated carbon resonances correspond to carbons having no ortho protons. The peak at 112.8 ppm is assigned to C-4′ because it is coupled to two protons, (C-6′)H and (C-7′)H, while the peak at 120.3 ppm is assigned to C-2 due to its lack of ^{13}C-^{1}H coupling. Therefore, the structure of **5** and its ^{13}C resonance assignments are complete. The structure has been confirmed by X-ray crystallography.[19]

A number of features of the coupling constants in the ring linked via C-3′ are noteworthy: (i) Neither C-2′ nor C-3′ is coupled to (N-1)H, even though C-3a′ and C-7a′ are. (ii) The meta coupling between C-3a′ and (C-7′)H is small ($^3J_{\mathrm{CH}}$ = 3 Hz). (iii) C-7a′ is strongly coupled to its ortho proton ($^2J_{\mathrm{CH}}$ = 4 Hz). (iv) C-4′ is coupled (J_{CH} = 2 Hz) to the para proton (C-7′)H or, via through-space interactions, to one of the protons on the other ring.

Concluding Remarks

The structures presented in this paper have been solved by analysis of ^{1}H and ^{13}C NMR chemical shifts, ^{13}C–^{1}H coupling constants, and ^{13}C spin–lattice relaxation data. The use of ^{13}C relaxation data for this purpose represents a novel application of such measurements. Previously, we have reported that ^{13}C spin–lattice relaxation measurements can be used to identify brominated carbons,[11] an application useful in structural analysis,[12] and have shown how quantitative analysis of T_1^{CH} values for quaternary carbons could be used to solve the structure of polybrominated oxydiphenol derivatives from the sponge *D. herbacea*.[5,6] The present work extends these initial studies by demonstrating that the method is useful even when it can be applied only semiquantitatively (because of anisotropic reorientation, probable variation in distances to hydrogens two bonds removed, or peak overlap). We believe that this approach will be useful in a number of instances because, although relaxation time measurements require more instrumental time than routine spectra, they provide information on ortho protons, which, as the data for the compounds described here show quite clearly, are often not detectable by analysis of long-range ^{13}C–^{1}H coupling constants.

Although the symmetrical biindole (**1**) shows no optical activity, the biindoles **2**–**6** are all optically active, with the chirality in each case being due to restricted rotation only. Therefore, these biindoles are new representatives of a very small class of natural products, exemplified by the bianthraquinone pigments of the

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[20] Average T_1 value for methine carbon resonances of the NH form of **6** is 0.77 s, corresponding to τ_R = 0.064 ns (r_{CH} = 1.10 Å); therefore, a nonbonded ^{1}H 2.15 Å away would contribute 43 s to ^{13}C relaxation. Corresponding values for the ND form are 0.68 s, 0.072 ns, and 38 s, respectively.

skyrin class,[21] which are chiral only because of restricted rotation within the molecule.

Experimental Section

All solvents used were analytical grade. Optical rotations were measured on a Jasco DIP-4 polarimeter. UV spectra were recorded on a Varian Superscan-3 UV-visible spectrophotometer and IR spectra were measured on a Hitachi 285 spectrophotometer. Mass spectra were recorded on a V.G. Micromass 70/70 F system and molecular formulas were obtained by mass matching of at least two bromine isotope peaks. All high-performance liquid chromatography (HPLC) was performed on a Whatman Magnum 9 10/50 partisil semipreparative column. The eluting solvent used for each separation is given in parentheses in the appropriate section. Injection quantities for each separation varied from 10 to 50 mg, depending on difficulty of separation, and were monitored by refractive index with a Waters R403 monitor. PLC and TLC separations were performed on Merck Si60 silica gel plates (Cat. No. 5717 and 5715).

^{1}H NMR spectra were measured in the continuous wave mode on a JEOL JNM-MH-100 spectrometer operating at 100 MHz. ^{13}C NMR spectra were obtained at 15.04 MHz on a JEOL JNM-FX-60 spectrometer incorporating a 4000-Hz bandpass crystal filter (JEOL) and operating in the pulsed-Fourier transform mode. On-resonance, noise-modulated proton decoupling was used unless otherwise indicated. Ten-millimeter o.d. spinning sample tubes were used, and the probe temperature was 26–31 °C, as indicated in the text. The sweep width was 2500 Hz in all cases. Spectra were accumulated in 8192 time-domain addresses (giving a digital resolution of 0.61 Hz after Fourier transformation), except for some protonated carbon T_1 measurements where 4096 time-domain addresses were used. Spectra were processed with 1.0-Hz exponential broadening. Chemical shifts were measured digitally and are reported in parts per million downfield from internal Me_4Si. The solvent CD_3 resonance (at 29.8 ppm) was employed as primary standard. Estimated accuracy is ±0.1 ppm. ^{13}C–^{1}H spin–spin coupling constants were measured from spectra accumulated with the use of gated proton decoupling[22] (^{1}H decoupling off during data acquisition).

Spin-lattice relaxation times (T_1) were determined by the inversion–recovery method,[23] in which a $(180°–\tau–90°–t)_n$ pulse sequence is employed. The delay time, t, between each 90° radiofrequency pulse and the following 180° radiofrequency pulse was greater than 3.5 times the longest T_1 being measured unless otherwise noted. The width of a 90° pulse was 18 μs. T_1 values were determined from a least-squares fit of the exponential magnetization recovery curve M_τ vs. τ, where M_τ is the initial value of the magnetization following a 90° pulse at time τ after the 180° pulse. Satisfactory fits to a single exponential function were obtained in all cases. This was to be expected for the methine carbons, where cross-correlation effects[24] do not have to be considered. For quaternary carbons relaxed by more than one proton, any effects of cross-correlation on recovery were within experimental error. Only values of $\tau \lesssim T_1$ were employed in calculating the T_1 values reported. Separate sets of inversion–recovery sequences were used for measuring protonated and quaternary carbon T_1 values in each case. Estimated accuracy of T_1 values is 10% for protonated carbons and 15% for quaternary carbons.

Integrated intensities were obtained digitally from fully relaxed spectra recorded with 90° radiofrequency pulse excitation. The protonated carbon resonances were shown to have the full NOE by comparing intensities in spectra recorded with complete proton decoupling with those in spectra recorded with gated proton decoupling[25] (^{1}H decoupling on only during data acquistion). The experimental intensities reported in Table I (fully decoupled spectra) were adjusted to give a value of 2.99 for the average intensity of the resolved protonated carbon resonances. Estimated accuracy of integrated intensities is ±0.3.

Isolation of Biindoles (1–6) from *Rivularia firma*. The alga[26] (10 kg) was cooled to −60 °C, ground, and extracted twice with water. The residue was freeze-dried and percolated with dichloromethane (10 L), methanol (10 L), dichloromethane (10 L), and, finally, methanol (10 L). Concentration of the combined extracts in vacuo gave 80 g (0.8% based on algal wet weight) of a green-black gum. A 40-g sample of this extract was triturated with dichloromethane (4 × 100 mL), with decantation of the dichloromethane extracts between each treatment. Concentration of the combined dichloromethane extracts gave 12.0 g of a dark gum, which was dissolved in dichloromethane and applied to a bed of silica gel (300 g, Merck Kieselgel, Type H) drypacked into a Büchner funnel and the column run by application of vacuum to the receiving flask. The first yellow carotenoid band which was eluted was rejected, and the remainder of the dichloromethane eluate was concentrated to give 2.6 g of a mixture which contained ~75% of the biindoles **1–3**, **5**, and **6**.

This crude mixture, dissolved in an equivolume mixture of dichloromethane–hexane (100 mL), was applied to a short column of silica gel (200 g, Merck Kieselgel, Type H) drypacked in a Büchner funnel and run by application of vacuum. The column was eluted with dichloromethane–hexane (1:1, 700 mL collected in 100 mL fractions, A–F) and dichloromethane (fraction G). Fractions A–C were devoid of biindoles. Compound **6** (70 mg) crystallized directly from fraction D upon concentration, and compound **5** (90 mg) was separated from two minor indoles of unknown structure by HPLC (diisopropyl ether–hexane, 1:1) of fraction E. Fractions F and G were combined, and the biindoles **1–3** separated by HPLC (diisopropyl ether) to give, in order of elution, 1 (600 mg), **2** (1.2 g), and **3** (100 mg).

A second collection[26] of *R. firma* (2 kg, wet) was freeze-dried and extracted as above to give a mixture of biindoles which was separated by HPLC (diisopropyl ether) to yield 1 (160 mg), **2** (300 mg), and **4** (35 mg). Trace amounts of other biindoles were not characterized.

2,2′,5,5′-Tetrabromo-3,3′-bi-1*H*-indole (1) crystallized from chloroform as colorless rosettes: mp 239–240 °C; IR ν_{max} (KBr disk) 3450, 3420, 1450, 1430, 1400, 1340, 1330, 1100, 1045, 795 cm^{-1}; UV λ_{max} (acetonitrile) 297 (ϵ 15 500), 289 (ϵ 17 600), 228 (ϵ 57 400), 209 (ϵ 61 400) nm; ^{1}H NMR (acetone-d_6) δ 7.44 (1 H, br s), 7.40 (1 H, d, J = 9 Hz), 7.24 (1 H, dd, J = 9, 2 Hz); high-resolution mass measurements, found 545.7389, $C_{16}H_8N_2{}^{79}Br_3{}^{81}Br$ requires 545.7396; found 547.7359, $C_{16}H_8N_2{}^{79}Br_2{}^{81}Br_2$ requires 547.7378.

(+)-2,3′,5,5′-Tetrabromo-7′-methoxy-3,4′-bi-1*H*-indole (2) separated as colorless prisms: mp 178–179 °C dec on slow evaporation of a dichloromethane–hexane solution; $[\alpha]^{20}{}_D$ +71° (c 1, $CHCl_3$); IR ν_{max} (KBr disk) 3425, 1620, 1560, 1410, 1280, 1100, 1000, 780 cm^{-1}; UV λ_{max} (acetonitrile) 299 (ϵ 14 000), 290 (ϵ 17 000), 280 (16 000), 227 (ϵ 66 000) nm; ^{1}H NMR ($CDCl_3$) δ 8.48 (1 H, br s, D_2O exch), 8.30 (1 H, br s, D_2O exch), 7.4–6.9 (5 H, m), 3.88 (3 H, s); high-resolution mass measurements, found 575.7522, $C_{17}H_{10}N_2O^{79}Br_3{}^{81}Br$ requires 575.7503; found 577.7445, $C_{17}H_{10}N_2O^{79}Br_2{}^{81}Br_2$ requires 577.7482; found 579.7459, $C_{17}H_{10}N_2O^{79}Br^{81}Br_3$ requires 579.7462.

(+)-3,5,5′-Tribromo-7′-methoxy-3,4′-bi-1*H*-indole (3) separated from chloroform as colorless prisms: mp 220–223 °C, $[\alpha]^{20}{}_D$ +8.5° (c 1, $CHCl_3$); IR (KBr disk), ν_{max} 3340, 1630, 1570, 1480, 1440, 1430, 1400, 1380, 1280, 1265, 1100, 1000, 765 cm^{-1}; UV λ_{max} (acetonitrile) 297 (ϵ 11 500), 288 (ϵ 13 000), 283 (ϵ 12 700), 225 (ϵ 73 000) nm; ^{1}H NMR (acetone-d_6) δ 7.5–7.2 (5 H, m), 7.00 (1 H, s), 3.96 (3 H, s); high-resolution mass measurements, found 497.8397, $C_{17}H_{11}N_2O^{79}Br_2{}^{81}Br$ requires 497.8399; found 499.8382, $C_{17}H_{11}N_2O^{79}Br^{81}Br_2$ requires 499.8378.

(+)-2,5,5′-Tribromo-7′-methoxy-3,4′-bi-1*H*-indole (4) was obtained as a foam: $[\alpha]^{20}{}_D$ +11.3 (c 1, $CHCl_3$); IR (Nujol) ν_{max} 3325, 1630, 1570, 1485, 1435, 1390, 1380, 1280, 1100, 1000, 770 cm^{-1}; UV λ_{max} (acetontrile) 298 (ϵ 13 000), 289 (ϵ 16 000), 279 (ϵ 17 000), 227 (ϵ 69 000) nm; ^{1}H NMR (acetone-d_6) δ 7.38 (1 H, d, J = 9 Hz), 7.3–7.15 (3 H, m), 7.00 (1 H, s), 5.98 (1 H, br d, J = 3 Hz), 3.92 (3 H, s); high-resolution mass measurements, found 497.8390, $C_{17}H_{11}N_2O^{79}Br_2{}^{81}Br$ requires 497.8399.

(−)-3,3′,5,5′-Tetrabromo-7′-methoxy-1,4′-bi-1*H*-indole (5) separated from chloroform–hexane as a poorly crystalline colorless solid: mp 196–200 °C, $[\alpha]^{20}{}_D$ −6.0° (c 1, acetonitrile); IR (KBr disk) ν_{max} 3450, 1560, 1506, 1501, 1490, 1445, 1340, 1295, 1280, 1104, 1000, 780 cm^{-1}; UV λ_{max} (acetonitrile) 296 (ϵ 13 600), 287 (ϵ 15 100), 226 (ϵ 66 600) nm; ^{1}H NMR (acetone-d_6) δ 7.72 (1 H, d, J = 2 Hz), 7.50 (1 H, s), 7.45 (1 H, s), 7.31 (1 H, dd, J = 9, 2 Hz), 7.14 (1 H, s), 6.80 (1 H, d, J = 9 Hz), 4.04 (3 H, s); high-resolution mass measurements, found 575.7511, $C_{17}H_{10}N_2O^{79}Br_3{}^{81}Br$ requires 575.7503; found 577.7479, $C_{17}H_{10}N_2O^{79}Br_2{}^{81}Br_2$ requires 577.7482; found 579.7465, $C_{17}H_{10}N_2O^{79}Br^{81}Br_3$ requires 579.7462.

(+)-2,2′,3,4′,5,5′-Hexabromo-1,3′-bi-1*H*-indole (6) crystallized from acetonitrile as prisms: mp 263–264 °C, $[\alpha]^{20}{}_D$ +18.7° (c 1, acetonitrile); IR ν_{max} ($CHCl_3$) 3450, 1700, 1600, 1555, 1435, 1400, 1360, 1300, 1200, 1175, 1150 cm^{-1}; UV λ_{max} (acetonitrile), 287 (ϵ 17 000), 226 (ϵ 63 000) nm; 1H NMR (acetone-d_6) δ 10.8 (1 H, br s) 7.68 (1 H, d, J = 2 Hz), 7.52 (2 H, s), 7.30 (1 H, dd, J = 9, 2 Hz), 6.92 (1 H, d, J = 9 Hz);

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[26] Collections of *Rivularia firma* were taken from Westernport Bay, Victoria, Australia. Compounds **1–3**, **5**, and **6** were obtained from a collection made by M. Mackay in April 1979, RRIMP specimen FN 1907/000/02, and compound **4** was obtained, together with **1** and **2**, from a collection made in February 1978, by K. Harada and R. Berthold, RRIMP specimen FN1907/000/01.

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high-resolution mass measurements, found 705.5098, $C_{16}H_6N_2{}^{79}Br_3{}^{81}Br_3$ requires 705.5106; found 707.5090, $C_{16}H_6N_2{}^{79}Br_2{}^{81}Br_4$ requires 707.5086.

1,1′-Diacetyl-2,2′,5,5′-tetrabromo-3,3′-bi-1*H*-indole (7). A solution of 1 (30 mg) in an equivolume mixture of acetic anhydride and pyridine (3 mL) was allowed to stand at 20 °C for 7 days, after which a single crystal of **7** (29 mg) was obtained by decantation of excess liquid followed by washing with diethyl ether: ^{1}H NMR ($CDCl_3$) δ 8.11 (1 H, d, J = 9 Hz), 7.32 (1 H, dd, J = 9, 2 Hz), 7.17 (1 H, d, J = 2 Hz), 2.89 (3 H, s); mass spectrum, M^+ 628; $C_{20}H_{12}N_2O_2{}^{79}Br_4$ requires M^+ 628.

(+)-1-Acetyl-2,3′,5,5′-tetrabromo-7′-methoxy-3,4′-bi-1*H*-indole (11) and (+)-1,1′-Diacetyl-2,3′,5,5′-tetrabromo-7′-methoxy-3,4′-bi-1*H*-indole (12). A solution of **2** (120 mg) in an equivolume mixture of pyridine and acetic anhydride (10 mL) was allowed to stand at 20 °C for 2 days. Water (10 mL) was added to the mixture, which was stirred for 2 h and partitioned between chloroform (40 mL) and water (20 mL). The chloroform layer was washed successively with 1 M HCl (2 × 10 mL), 5% aqueous sodium carbonate (2 × 10 mL), and water (10 mL). HPLC (diisopropyl ether) of dried ($MgSO_4$) chloroform-soluble material gave **11** (51 mg), **12** (57 mg), and unreacted **2** (4 mg).

Monoacetate 11 crystallized from dichloromethane–hexane as colorless prisms: mp 109–111 °C, $[\alpha]^{20}{}_D$ +99° (c 1, $CHCl_3$); IR ν_{max} (KBr disk) 3425, 1710, 1560, 1435, 1360, 1280 cm^{-1}; UV λ_{max} (acetonitrile) 300 (ϵ 15 000), 280 (ϵ 21 000), 247 sh (ϵ 28 000), 227 (ϵ 61 000), 207 (ϵ 55 000) nm; ^{1}H NMR ($CDCl_3$) δ 8.76 (1 H, br s, D_2O exch), 8.26 (1 H, d, J = 9 Hz), 7.37 (1 H, dd, J = 9, 2 Hz), 7.16 (1 H, d, J = 3 Hz, becomes s on D_2O exch), 7.12 (1 H, d, J = 2 Hz), 6.98 (1 H, s), 3.96 (3 H, s), 2.90 (3 H, s); high-resolution mass measurements, found 617.7613, $C_{19}H_{12}N_2O_2{}^{79}Br_3{}^{81}Br$ requires 617.7609, found 619.7607, $C_{19}H_{12}N_2O{}^{79}Br_2{}^{81}Br_2$ requires 619.7589.

Diacetate 12 separated from dichloromethane–hexane as colorless prisms: mp 96–98 °C, $[\alpha]^{20}{}_D$ +71° (c 0.75, $CHCl_3$); IR ν_{max} (KBr disk) 1720, 1540, 1440, 1365, 1260, 1200, 1030 cm^{-1}; UV λ_{max} (acetonitrile) 307 (ϵ 15 000), 276 (ϵ 20 000), 243 (ϵ 39 000); ^{1}H NMR ($CDCl_3$) δ 8.22 (1 H, d, J = 9 Hz), 7.58 (1 H, s), 7.30 (1 H, dd, J = 9, 2 Hz), 7.18 (1 H, s), 7.10 (1 H, d, J = 2 Hz), 4.00 (3 H, s), 2.90 (3 H, s), 2.75 (3 H, s); high-resolution mass measurements, found 661.7717, $C_{21}H_{14}N_2O_3{}^{79}Br_2{}^{81}Br_2$ requires 661.7693, found 663.7660, $C_{21}H_{14}N_2O_3{}^{79}Br{}^{81}Br_3$ requires 663.7674.

Bromination of Indole and 5-Bromoindole. Solid pyridinium bromide perbromide (1.05 mol equiv) was added batchwise over 10 min to a stirred solution of the indole (150 mg) and sodium acetate (1 mol equiv) in methanol (20 mL) cooled in an ice bath. The mixture was concentrated to dryness below 20 °C in vacuo, the solid residue triturated with dichloromethane (20 mL), and the dichloromethane soluble material filtered through a bed of silica gel (Merck, Kieselgel H, 15 g) under vacuum. The silica gel was washed with dichloromethane (50 mL) and the combined dichloromethane filtrate taken to dryness below 20 °C. The resulting foam was used directly for ^{13}C NMR measurements, which, in each case, showed that the product was >95% of the required 3-bromoindole derivative.

Acetylation of 5-Methoxyindole and 7-Methoxyindole. *N*-Butyllithium (2 M in hexane, 3 mL) was added to a stirred solution of the methoxyindole (200 mg) in dry THF (10 mL) at −20 °C and the mixture allowed to stand for 5 min. Acetic anhydride (1 mL) was added dropwise and the mixture allowed to stand for 15 min and then poured onto ice (50 g). The mixture was extracted with pentane (40 mL), the pentane layer washed with water (2 × 20 mL), and the dried ($MgSO_4$) pentane solution concentrated. The acetylated indole was separated from ~5% starting material by HPLC (dichloromethane).

Acknowledgment. We thank Ray Lidgard for mass spectra and high-resolution mass measurements, Gerald Viset for technical assistance, David Edwards for writing the computer program for determining T_1 values, and the collectors already mentioned for supplying alga.

Registry No. 1, 81387-82-8; (+)-**2**, 81387-83-9; (+)-**3**, 81387-84-0; (+)-**4**, 81387-85-1; (−)-**5**, 81387-86-2; (+)-**6**, 81387-87-3; **7**, 81387-88-4; **8**, 1484-27-1; **9**, 10075-50-0; **10**, 81387-89-5; (+)-**11**, 81387-90-8; (+)-**12**, 81387-91-9; **13**, 1006-94-6; **14**, 58246-80-3; **15**, 3189-22-8; **16**, 81387-92-0.

Conformational Analysis. 42. Monosubstituted Tetrahydropyrans[1]

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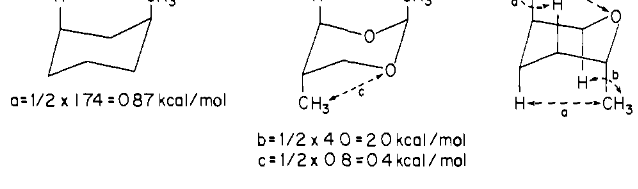
Abstract: The conformational energies of 2-, 3-, and 4-methyl, 2-vinyl, 2-ethinyl, 2-ethyl, 2-carbomethoxy, and 2-hydroxymethyl substituents in tetrahydropyran have been determined (in kcal/mol): 2-Me, 2.86; 3-Me, 1.43; 4-Me, 1.95; 2-CH_2=CH, 2.27; 2-CH≡C, 0.34; 2-Et, 2.62; 2-CO_2Me, 1.38; 2-CH_2OH, 2.89. Some of these values differ appreciably from earlier reported ones, and the 2-CO_2Me value does not support the hypothesis of a reverse anomeric effect.

The tetrahydropyran (oxane, oxacyclohexane) ring system is of wide occurrence in nature, e.g., in the pyranose sugars, in polyether antibiotics, and in thromboxanes. However, conformational information in simple monosubstituted tetrahyodrpyrans is quite limited. The parent compound has been shown by microwave spectroscopy[2] to exist in the chair form, and while it was thought on the basis of the Lambert *R* value[3] that the chair was slightly flattened relative to cyclohexane, this is in fact probably not the case.[4] The inversion barrier in tetrahydropyran (10.3 kcal/mol[5]) is the same as that in cyclohexane. Existing conformational information has been reviewed in various places,[6-8] and the known conformational energies of various polar substituents in the 2-, 3-, and 4-positions of the tetrahydropyran ring in nonpolar solvents are summarized in Table I.[9] Of greatest interest

Scheme I



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