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POLYHALOGENATED INDOLES FROM THE MARINE ALGA RHODOPHYLLIS MEMBRANACEA HARVEY

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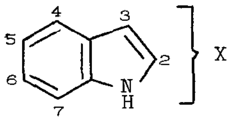
Several halogenated indole derivatives have been isolated from marine molluscs,[1] sponges,[2] and hemichordates.[3] In screening New Zealand marine algae for antimicrobial activity, the crude extract of Rhodophyllis membranacea Harvey was noted to possess strong antifungal activity. This activity is due to the presence of polyhalogenated indoles.

The alga was collected off Seal Reef on the Kaikoura Coast of the South Island of New Zealand. The fresh material was homogenized and extracted with methanol for several days. Partitioning of the concentrated extract between water and ethyl acetate gave an organic extract which was chromatographed on a silica gel column. Several crystalline fractions were obtained: A (10% benzene/hexane); B (10% benzene/hexane); C (20% benzene/hexane); and D (50% benzene/hexane) in yields of 18, 12, 11, and 1.5% respectively from the crude extract. Fractions C and D could be further separated into two fractions each, C_1 and C_2, and D_1 and D_2, using carbon tetrachloride as the eluting solvent. All fractions were crystalline, colorless, and homogeneous on TLC with several solvent systems, and all but D_2 melted sharply or over a narrow range (Table I). Infrared spectra for all fractions showed a sharp NH band (3480 cm^{-1}) and ^1H NMR spectra showed only aromatic hydrogens. Mass spectral analysis revealed each fraction to be a mixture of simple polyhalogenated indoles (Table I).[4]

While separation of the components of the major fractions (A, B, and C_2) was eventually achieved by vapor phase chromatography, spectral data on the unresolved materials allowed the assignment of substitution type. Thus, the ^{13}C NMR spectra indicated that none of the major components of fractions A, B, and C_2 possessed a free β-carbon as there was no nonquaternary absorption above δ 110.[5] The aromatic hydrogens in the 60 MHz ^1H NMR spectrum of fraction A occurred as a series of multiplets between δ 6.8-7.5 while fractions B and C_2 showed sharp singlets (δ 7.20 and 6.90 respectively) in CCl_4. No appreciable difference in the spectra of A and B was observed when the solvent was changed to acetone; however, the C_2 singlet changed to a series of multiplets wherein one hydrogen had shifted to considerably lower field. Since solvent shifts of this type are expected only for protons on the 2- or 7-positions of indoles,[6] these data suggest that fractions A and B have both of these positions substituted while C_2 has at least one of them open. Therefore, the A components are 2,3,7-trihaloindoles (1). The B components, substituted also at the 2-, 3-, and 7-positions, should have the fourth halo sub-

Table I. Haloindoles from R. membranacea Harvey



Fraction	MP	X
A	95°	Br_3, Br_2Cl, $BrCl_2$, Cl_3
B	130°	Br_4, Br_3Cl, Br_2Cl_2, $BrCl_3$, Cl_4
C_1	205°	Br_5, Br_4Cl, Br_3Cl_2, Br_2Cl_3
C_2	105-108°	Cl_3, $BrCl_2$, Br_2Cl
D_1	212-215°	Br_6, Br_5Cl, Br_4Cl_2, Br_3Cl_3
D_2	$\sim$135°+	Br_5, Br_4Cl, Br_3Cl_2, Br_2Cl_3, Br_2Cl_2, Br_4, Br_3Cl, $BrCl_3$

stituent at the 4-position (2) to fit the singlet nature of the proton spectrum.[7]

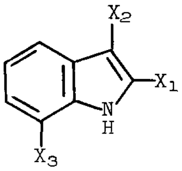
The C_2 fraction has either the 2- or the 7-position open. The presence of a nonquaternary carbon absorption at δ 109.7 in the ^{13}C NMR indicates that it is the C-7 position rather than the C-2 position that is unsubstituted.[5,8] The placement of the third halogen in the C_2 fraction would then be limited to positions 4, 5, or 6. Although the above data did not allow an unambiguous assignment, the appearance of the ^{1}H NMR pattern suggested that the C_2 components are 2,3,4-trisubstituted indoles (3).[6]

Vapor phase chromatography of the A, B, and C_2 fractions on an OV-1 column permitted their resolution in quantities sufficient for 270 MHz FT ^{1}H NMR analysis. These spectra (Table II) confirmed the substitution type assigned to the A and B mixtures. The A components displayed two doublets and a triplet indicative of three adjacent hydrogen atoms on the benzene ring. The B components displayed AB patterns indicative of two adjacent hydrogens in very similiar environments on the benzene ring. The major C_2 component did not give a first order spectrum, in keeping with the assumed 2,3,4-trisubstitution type.[6]

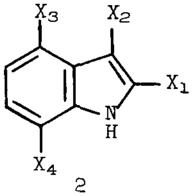
The structures (Figure I) for the A components and for the C_2 trichloro component were proven by independent synthesis of these as well as of all the other possible mixed halo isomers for the A components.[9] The B components have not yet been synthesized, but the 270 MHz ^{1}H NMR spectra allow some structural assignments to be made. The tetrabromide must have structure 2e. The chemical shifts of the two aromatic hydrogens in 2c and 2d are very similiar to those of 2e. Two of the bromines in these can then be assigned to the 4- and 7-positions. In 2b, where there is only one bromine, the difference in chemical shift between the two aromatic hydrogens has increased substantially indicating that the halogens on the 4- and 7-positions are no longer identical. We are unable at this point to determine whether the single bromine is at the 4- or 7-position in 2b and whether the single chlorine is at the 2- or 3-position in 2d. Compound 2a was not present in sufficient concentration for collection, and its structure is assigned on the basis of analogy to the other components of fraction B.

From vapor phase chromatography an estimation of the ratios of components in the A, B, and C_2 fractions is possible and is given in Figure I. The small quantities of the C_1, D_1, and D_2 fractions have precluded further study of these materials apart from their mass spectral data which established their compositions as shown in Table I.

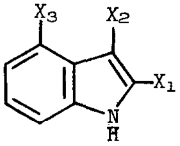
Figure I



1



2



3

1a $X_1 = X_2 = X_3 = Cl$ (4%)
1b $X_1 = X_2 = Cl$, $X_3 = Br$ (60%)
1c $X_1 = X_3 = Br$, $X_2 = Cl$ (31%)
1d $X_1 = X_2 = X_3 = Br$ (4%)

2a $X_1 = X_2 = X_3 = X_4 = Cl$
2b $X_1 = X_2 = Cl$, $X_3 = Br(Cl)$, $X_4 = Cl(Br)$ (24%)
2c $X_1 = X_2 = Cl$, $X_3 = X_4 = Br$ (57%)
2d $X_3 = X_4 = Br$, $X_1 = Br(Cl)$, $X_2 = Cl(Br)$ (17%)
2e $X_1 = X_2 = X_3 = X_4 = Br$ (2%)

3a $X_1 = X_2 = X_3 = Cl$ (83%)[10]

Table II. 270 MHz ^1H NMR Spectra of Haloindoles (CD_3COCD_3)

Compound	H-4	H-5	H-6	H-7	J (Hz)
1a	7.48 (d)	7.21 (t)	7.32 (d)	-	$J_{4,5} = J_{5,6} = 7.8$
1b	7.52 (d)	7.15 (t)	7.47 (d)	-	
1c	7.53 (d)	7.14 (t)	7.45 (d)	-	
1d	7.46 (d)	7.14 (t)	7.45 (d)	-	
2b*	-	7.13 (d)	7.42 (d)	-	$J_{5,6} = 8.1$
2c*	-	7.30 (d)	7.36 (d)	-	
2d*	-	7.29 (d)	7.34 (d)	-	
2e*	-	7.31 (d)	7.36 (d)	-	
3a	-		7.1-7.4 (m)		

*Assignments may be reversed in these compounds.

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References and Notes

1. J. T. Baker and C. C. Duke, *Tetrahedron Letters*, 1233 (1976) and references cited therein.
2. G. E. Van Lear, G. O. Morton, and W. Fulmor, *ibid.*, 299 (1973); W. D. Raverty, R. H. Thomson, and T. J. King, *J. Chem. Soc. Perkin I*, 1204 (1977).
3. T. Higa and P. J. Scheuer, *Naturwissenschaften*, **62**, 395 (1975); T. Higa and P. J. Scheuer, *Heterocycles*, 227 (1976).
4. The occurrence of halogenated indoles as apparently homogeneous, sharp-melting, inseparable mixtures is not without precedent: B. E. Leggetter and R. K. Brown, *Can. J. Chem.*, **38**, 1467 (1960); H. Ishii, Y. Murakami, K. Hosoya, H. Takeda, Y. Suzuki, and N. Ikeda, *Chem. Pharm. Bull.*, **21**, 1481 (1973).
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6. S. P. Hiremath and R. S. Hosmane in "Advances in Heterocyclic Chemistry," A. R. Katritzky and A. J. Boulton, Eds., Academic Press, New York, N.Y., 1973, Vol. 15, p. 277.
7. The 5- and 6-hydrogens of indole are very close in chemical shift.[6] With identical or very similiar substituents at the 4- and 7-positions, the superposition of these two protons would be expected.
8. The position of this carbon is quite invariant in 2,3-dihaloindoles: M. R. Brennan and K. L. Erickson, Unpublished observations.
9. The synthesis and properties of these materials will be described elsewhere.
10. The other major component, $C_8H_4BrCl_2$, comprised 17% of the C_2 fraction.