

A NEW BROMINATED BIINDOLE FROM AN AUSTRALIAN CYANOBACTERIUM, *RIVULARIA FIRMA*

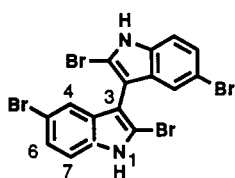
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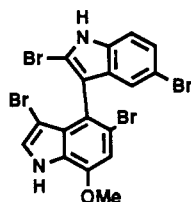
ABSTRACT.—A cyanobacterium, *Rivularia firma*, collected from Flinders on the southern coast of Victoria, Australia, was found to contain the new brominated biindole **7** along with several known examples of this structure class. The structure of **7** was established by spectroscopic analysis.

We were encouraged to undertake a re-investigation of the chemistry of *Rivularia firma* Womersley (Rivulariaceae) [*Rivularia firma* has been proposed to be *Calothrix crustaceae* (Thuret) (1)] because of an observation made in an earlier study. On reporting the isolation and structure elucidation of a variety of novel polybrominated biindoles from a Westernport Bay collection of *R. firma* (2), researchers

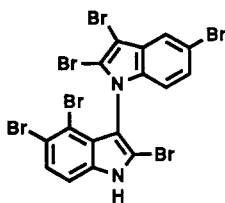
from the Roche Research Institute of Marine Pharmacology commented on the co-occurrence of related minor components that were neither isolated nor identified. Of those biindoles **1–6** that were isolated, all but the first possess an axis of chirality. Furthermore, these chiral biindoles were reported to possess significant anti-inflammatory activity. We were keen to attempt to isolate and iden-



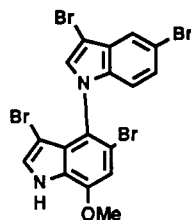
1



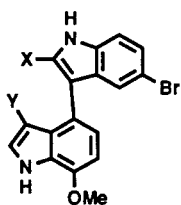
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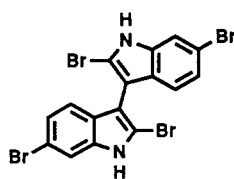


4



5 X = H, Y = Br

6 X = Br, Y = H



7

tify the aforementioned unidentified minor components, to determine if these minor components could shed some light on the biosynthetic origins of the polybrominated biindoles, and to investigate whether they might facilitate a determination of absolute stereochemistry.

Initial attempts to locate an acceptable quantity of *R. firma* from localities described in the earlier study proved unsuccessful. We did however succeed in obtaining a collection from rock platforms exposed at extreme low tide near Flinders in Westernport Bay. Although the collection was made from a low intertidal zone rather than high intertidal, fractionation of the crude EtOH-CH₂Cl₂ (9:1) extract yielded two known biindoles **2** and **3**, together with a new nonchiral biindole **7**, isomeric with **1**.

The ¹³C-nmr spectrum of **7** revealed eight discrete carbon resonances, while the ei mass spectrum confirmed a molecular formula of C₁₆H₈⁷⁹Br₄N₂. This together with an [α]_D of 0° suggested that **7** was a symmetrically substituted bromo biindole isomeric with **1**. In keeping with this assessment, the ¹H-nmr (CDCl₃) spectrum of **7** displayed only three aromatic proton resonances (δ 7.27, dd, *J* = 0.6, 8.0 Hz; 7.33, dd, *J* = 1.9, 8.0 Hz; 7.46, ddd, *J* = 0.6, 0.6, 1.9 Hz) and an NH resonance (δ 8.42, bs). Addition of D₂O succeeded in exchanging the NH, and hence deleting the ¹H-nmr resonance at δ 8.42 and one of the 0.6 Hz couplings to the H-7 resonance at δ 7.46. The resonance at δ 7.46 was confirmed as belonging to H-7; therefore the lack of an ortho coupling to this proton required that C-6 be substituted by bromine. Meta (1.9 Hz) and para (0.6 Hz) couplings confirmed resonances at δ 7.33 and 7.27 as belonging to H-5 and H-4, respectively. Thus the structure assigned to **7** was as shown.

Comparison of the ¹H-nmr spectroscopic data for **7** with those reported for **1** confirmed that they were indeed different. Furthermore, although acetylation

of **1** provided valuable supportive evidence used in assigning its structure (**2**), repeated attempts to acetylate **7** using the same (and more vigorous) conditions proved unsuccessful. While ¹³C-nmr spin-lattice relaxation measurements and ¹H-¹³C-nmr coupling constants were key techniques used in assigning structure **1-6** (**2**), at least in the case of **5** and **6** the bromine substitution pattern was determined by a comparison of ¹³C-nmr shifts with **2**. As the ¹³C-nmr spectra of the 5-bromo isomer **1** and the 6-bromo isomer **7** were almost superimposable, this raises some doubt as to whether such a comparison could differentiate between alternative substitution patterns.

Several other species of *Rivularia* are common to southern Victorian waters, and although they are difficult to collect in quantity the findings outlined above suggest that an investigation of their chemistry could prove rewarding.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—General experimental procedures were as reported by Butler *et al.* (3).

COLLECTION, EXTRACTION, AND ISOLATION.—A collection of a *R. firma* (120 g dry wt less extractables) was made during an extreme low tide, off rock platforms in the intertidal zone near Flinders, Westernport Bay, Victoria, in March 1990. A type sample has been lodged with the University of Melbourne, Department of Botany, Herbarium, Accession number MELU A-38635. The freshly collected alga was stored in a plastic bag, packed in ice, and transported to the laboratory where it was transferred to a polyethylene sample bottle and steeped in EtOH/CH₂Cl₂ (800 ml) at 0°. The decanted extract was concentrated under reduced pressure and partitioned sequentially with 400 ml aliquots of hexane (82 mg), CH₂Cl₂ (282 mg), and MeOH (250 mg). The combined hexane and CH₂Cl₂ fractions were subjected to rapid silica filtration (10% stepwise elution from hexane to EtOAc) followed by normal phase hplc (Phenomenex Spherex 5μ 250 × 10 mm column, elution at 2.0 ml/min with 20% EtOAc/hexane) to yield, in increasing order of polarity, **2** (15 mg, 0.012%), **3** (4 mg, 0.003%), and **7** (8 mg, 0.006%).

(+)-2,3',5,5'-Tetrabromo-7'-methoxy-3,4'-bi-1H-indole [**2**].—A stable viscous oil with [α]_D,

^1H -nmr and ^{13}C -nmr data as reported previously (2).

(+)-2,2',3,4',5,5'-Hexabromo-1,3'-bi-1H-indole [3].—A stable viscous oil with $[\alpha]_D$, ^1H -nmr, and ^{13}C -nmr data as reported previously (2).

2,2',6,6'-Tetrabromo-3,3'-bi-1H-indole [7].—A stable viscous oil: ν max (film) 3415, 1643 cm^{-1} ; λ max (MeOH) 302 (ϵ 11000), 293 (12140), 286 nm (10800); ^1H nmr (CDCl_3) δ 7.27 (dd, $J=0.6, 8.0$ Hz, H-4), 7.33 (dd, $J=1.9, 8.0$ Hz, H-5), 7.46 (ddd, $J=0.6, 0.6, 1.9$ Hz, H-7), 8.42 (bs, NH); ^1H nmr ($\text{Me}_2\text{CO}-d_6$) δ 7.45 (dd, 0.8, 8.8 Hz, H-4), 7.31 (dd, $J=2.0, 8.8$ Hz, H-5), 7.41 (bd, $J=2.0$ Hz, H-7), 11.3 (bs, NH); ^{13}C nmr (CDCl_3) (^{13}C nmr assignments were tentatively made on the basis of comparisons with model compounds) 108.2 (C-3), 111.1 (C-6), 112.0 (C-2), 114.0 (C-7), 122.0 (C-5), 125.7 (C-4), 129.6 (C-3a), 134.7 ppm (C-7a); ^{13}C nmr ($\text{Me}_2\text{CO}-d_6$) sample too dilute to measure (C-3), 112.6 (C-2), 113.9 (C-6 and C-7), 122.1 (C-5), 125.7 (C-4), 130.5 (C-3a), 136.3 (C-7a); eims m/z (%) 544, 546, 548, 550, $[\text{M}]^+$ 552 (1), 149 (20), 135 (28), 111 (28), 97

(38), 83 (43), 71 (82), 57 (100); hreims m/z 543.7418 ($\text{C}_{16}\text{H}_8^{79}\text{Br}_4\text{N}_2$ requires 543.7420).

ACKNOWLEDGMENTS

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