pure (*EE*) dienic sulfone **13** in 40% yield (entry 14).

Other electrophiles such as aldehydes, acid chlorides, and alkyl halides did not react in satisfactory yields with the alkenylcopper reagents **2b** due to their moderate reactivity.[19]

In summary, the facile insertion of zinc into the carbon–halogen bond of alkenyl halides bearing an electron-withdrawing substituent led to a very general preparation of new vinylogous *unmasked* acyl anion equivalents. Their reaction in the presence of Pd(0) or CuCN·2LiCl with various classes of electrophiles led to highly functionalized molecules. Extensions of this methodology are currently underway in our laboratories.

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Supplementary Material Available: Typical experimental procedure and spectral data for all new compounds (8 pages). Ordering information is given on any current masthead page.

(19) Alkenylcopper reagents are less reactive than alkylcopper derivatives limiting somewhat the range of electrophiles reacting with **2b**. See: Normant, J. F. In *Modern Synthetic Methods*; Scheffold, R., Ed.; Salle and Sauerländer: Frankfurt, **1983**, Vol. 3, p 139.

Wakayin: A Novel Cytotoxic Pyrroloiminoquinone Alkaloid from the Ascidian *Clavelina* Species

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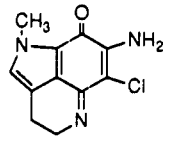
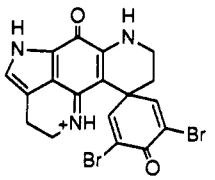
Summary: Wakayin (**3**) is the first reported example of a pyrroloiminoquinone alkaloid to be isolated from an ascidian.

Ascidians have been the recent source of many iminoquinone-bearing polycyclic aromatic alkaloids.[2] These structurally related compounds typically exhibit a wide range of biological activities, including murine cell-line cytotoxicity,[2] topoisomerase II,[2e] and microbe/fungus[2d] inhibition. The related pyrroloiminoquinone alkaloids, isobatzellines (e.g., isobatzelline C (**1**)), and discorhabdins/prianosins (e.g., discorhabdin C (**2**)) are, however, known only from phylum Porifera.[3]



1



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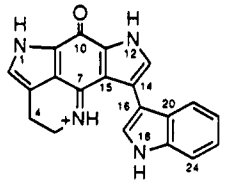
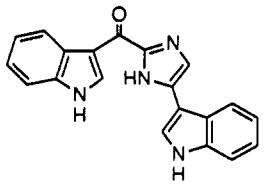


3



4

In continuation of our search for biologically active secondary metabolites from ascidians, we now report the structure of a new pyrroloiminoquinone derivative, wakayin (**3**), isolated from the ascidian *Clavelina* sp. Wakayin exhibited in vitro cytotoxicity against the human colon tumor cell line (HCT116 IC_{50} 0.5 μg/mL). Inhibition of topoisomerase II enzyme (250 μM) and the observation of a 3-fold differential toxicity toward the CHO cell line EM9[4] (sensitive to DNA-damaging genotoxic agents) versus BR1[5] (resistant to BCNU) provided preliminary evidence that wakayin exhibits its cytotoxicity by interfering with or damaging DNA. Antimicrobial activity against *Bacillus subtilis* (MIC $\approx$ 0.3 μg/mL) was also observed.

The methanol–chloroform extract of the ascidian[6] was crudely partitioned by reversed-phase flash chromatography using methanol–aqueous trifluoroacetic acid solvent mixtures.[7] Biologically active fractions were combined and purified by repeated elution through Sephadex LH-20,

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affording wakayin (3) as the dark blue trifluoroacetate salt[8] (15 mg, 0.005% wet weight): HRFABMS, MH^+ 327.1262, $C_{20}H_{15}N_4O$ requires 327.1246; UV (MeOH) λ_{max} 222.2 (ϵ 33500), 243.0 (ϵ 28200), 311.4 nm (ϵ 15000); (MeOH/KOH) λ_{max} 226.4 (ϵ 24600), 298.9 (ϵ 14000), 412.5 (ϵ 5100), 473.5 nm (ϵ 4600); FTIR (TFA salt, KBr) ν_{max} 3668.0–3130.1, 1792.4, 1715.3, 1661.9, 1651.8, 1446.6, 1206.5, 1174.1, 1150.5, 846.4, 804.1, 726.2 cm^{-1}; (free base, KBr) ν_{max} 3668.0–3126.6, 1806.0, 1713.5, 1693.8, 1681.7, 1633.6, 1446.3, 1333.9, 1209.9, 1140.7, 846.7, 804.4, 726.2 cm^{-1}.

Several features of the ^1H and ^{13}C NMR spectra of (3) were reminiscent of the discorhabdins and isobatzellines, indicating the presence of a pyrroloiminoquinone moiety. The spin system comprising δ 13.04 (br s, NH1), 7.11 (d, J = 2.5 Hz, H2), 2.93 (t, J = 8.0 Hz, H_24), 3.78 (br t, J = 8.0 Hz, H_25), and 10.32 (br s, NH6) was established by DQCOSY[9] and 1-D ^1H difference NOE[10] NMR experiments. HMQC[11] and HMBC[12] NMR experiments confirmed that this spin system was part of a pyrrolodihydropyridine ring system. An iminoquinone ring was evident from IR bands at 1662 and 1447 cm^{-1} and ^{13}C NMR signals at δ 166.28 (C10) and 156.72 (C7). Long-range ^1H–^{13}C NMR correlations observed for H2 (δ 120.74, C8; 128.16, C9), H_24 (δ 120.74, C8), and H_25 (δ 156.72, C7) established connectivities between the pyrrolodihydropyridine system and C7, C8, and C9 of the iminoquinone ring.

The presence of another 2,3,4-trisubstituted pyrrole ring was suggested by ^1H NMR [δ 13.41 (br s), 7.28 (d, J = 2.5 Hz)] and ^{13}C NMR [δ 134.25 (s), 120.44 (s), 114.15 (s), 125.14 (d, $^1J_{CH}$ = 190 Hz)]. Long-range ^1H–^{13}C NMR correlations observed for NH12 [δ 114.15 (C14), 120.44 (C15)] and H13 [δ 120.44 (C15), 134.25 (C11)] confirmed this and also established that the pyrrole ring was bound to C11 and C15 of the iminoquinone ring, as shown in 3.

^1H and ^{13}C NMR also suggested the presence of a 3-substituted indole moiety [δ 11.54 (br d, J = 2.0 Hz), 7.73 (d, J = 2.0 Hz), 7.52 (d, J = 8.0 Hz), 7.49 (d, J = 8.0 Hz), 7.19 (dd, J = 8.0, 8.0 Hz), 7.09 (dd, J = 8.0, 8.0 Hz); ^{13}C [δ 105.90 (s), 112.07 (d, J = 161 Hz), 118.86 (d, J = 161 Hz), 119.66 (d, J = 161 Hz), 121.85 (d, J = 161 Hz), 124.94 (d, J = 183 Hz), 125.49 (s), 136.76 (s)]. This was confirmed by HMQC and HMBC NMR experiments and by comparison with the ^{13}C NMR data reported for the indole–imidazole moiety of topsentin (4).[13]

Long-range ^1H–^{13}C correlations (HMBC and selective INEPT[14] NMR experiments) between H13 and C16 established connectivity between the bipyrroloiminoquinone moiety and the 3-substituted indole.[15] This confirmed the presence of the C14–C16 bond and allowed the assignment of structure 3 to wakayin. Further confirmation for the presence of the C14–C16 bond was established by the observation of ^1H NOE difference enhancements between H13 and H21 (6%) in the free-base form of 3.

^1H NOE difference experiments on the TFA salt of 3 indicated magnetization transfer from H17 to NH6 (2%), NH18 (4%), and H13 (2%), implying rotation about the C14–C16 bond was occurring. Irradiation of H17 in the free-base form of 3, however, only showed enhancement of NH18 (5%), implying restricted rotation about C14–C16, probably due to hydrogen-bond formation between H17 and N6. Although hydrogen bonding between a carbon-bound hydrogen and a nitrogen is unusual, the differences observed between the NMR spectra of the free base and TFA salt forms of 3 [H17 Δ –0.9 ppm, $^1J_{C17-H17}$ Δ –9 Hz] supported the presence of such an electronic interaction in the free-base form of 3.

Biosynthetically, the isobatzellines may be derived from a tryptamine precursor, in which case the discorhabdins and wakayin represent examples of the further incorporation of tyrosine and tryptamine subunits, respectively, to this basic frame.

This represents the first reported isolation of a pyrroloiminoquinone alkaloid from an ascidian. As other sources of this class of compound have been exclusively from phylum Porifera, the question must be raised as to whether these compounds originate from the marine specimen alone or from associated microbes.

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Supplementary Material Available: ^1H, ^{13}C, coupled HMQC, selective INEPT (H13), and NOE difference spectra (H17, TFA salt, and free base) (6 pages). Ordering information is given on any current masthead page.

(8) ^1H NMR (TFA salt, 500 MHz, DMSO-d_6) δ 2.93 (t, J = 8.0 Hz, H_24), 3.78 (t, J = 8.0 Hz, H_25), 7.09 (dd, J = 8.0, 8.0 Hz, H22), 7.11 (d, J = 2.5 Hz, H2), 7.19 (dd, J = 8.0, 8.0 Hz, H23), 7.28 (d, J = 2.5 Hz, H13), 7.49 (d, J = 8.0 Hz, H24), 7.52 (d, J = 8.0 Hz, H21), 7.73 (d, J = 2.0 Hz, H17), 10.32 (br s, NH6), 11.54 (br d, J = 2.0 Hz, NH18), 13.04 (br s, NH1), 13.41 (br s, NH12); ^{13}C NMR (TFA salt, 125.7 MHz, DMSO-d_6) δ 17.88 (t, J = 132 Hz, C4), 44.70 (t, J = 147 Hz, C5), 105.90 (s, C16), 112.07 (d, J = 161 Hz, C24), 114.15 (br s, C14), 118.86 (d, J = 161 Hz, C21), 119.05 (s, C3), 119.66 (d, J = 161 Hz, C21), 120.44 (s, C15), 120.74 (s, C8), 121.85 (d, J = 161 Hz, C23), 123.13 (d, J = 191 Hz, C2), 124.94 (d, J = 183 Hz, C17), 125.14 (d, J = 190 Hz, C13), 125.49 (s, C20), 128.16 (s, C9), 134.25 (s, C11), 136.76 (s, C19), 156.72 (s, C7), 166.28 (s, C10).

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(15) No three-bond correlations between H17 and C14 were detected by either HMBC or INAPT NMR experiments. This may have been due to the broadened nature of the C14 resonance (half-height line width of 9.9 Hz).