Natural and Synthetic Derivatives of Discorhabdin C, a Cytotoxic Pigment from the New Zealand Sponge *Latrunculia* cf. bocagei

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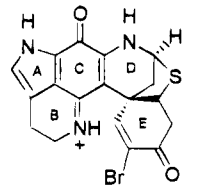
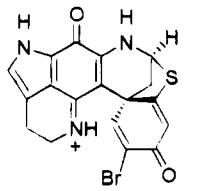
Modification of the spiro-cyclohexadienone skeleton of discorhabdin C (**3**) led to a series of derivatives **6–11**. The biological properties of this series were evaluated in a wide range of screens for selective cytotoxicity, antifungal, and antimicrobial properties. The structure and biological properties of a further discorhabdin, discorhabdin E (**5**), from a New Zealand sponge *Latrunculia* cf. bocagei are also presented.

Introduction

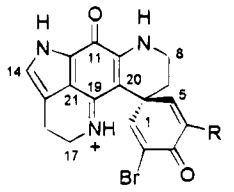
Three species of sponges belonging to the genus *Latrunculia* du Bocage (family Latrunculiidae, order Hadromerida) are commonly found in New Zealand waters over latitudes ranging from the subtropical north to the subantarctic Islands. In the screening of extracts from marine organisms collected from around New Zealand the extracts prepared from sponges of this genus invariably showed activity against a variety of microorganisms and were strongly inhibitory against the P388 leukemia cell line. These biological activities are due to a series of cytotoxic alkaloids, discorhabdins A (**1**), B (**2**),[2] C (**3**),[3] and D (**4**),[4] but of these only discorhabdin D (**4**) had *in vivo* activity against the P388 cell line (T/C 132%). Discorhabdins A and D and other related compounds have also been reported from marine sponges of the genus *Prianos* (family Hymeniacidonidae, order Halichondrida), collected in Japanese waters.[4,5] Discorhabdin A (**1**) and a series of less cyclized pyrroloiminoquinones have recently been isolated from a Fijian sponge of the genus *Zyzzya*.[6] The discorhabdins contain the new pyrrolo[1,7]-phenanthroline ring system with a spiro-cyclohexadienone or cyclohexenone moiety. Several approaches to the synthesis of the discorhabdins have been reported;[7a–f] two groups have now successfully completed the total synthesis of discorhabdin C.[7g–i]



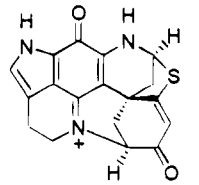
1 Discorhabdin A



2 Discorhabdin B



3 R = Br Discorhabdin C
5 R = H Discorhabdin E



4 Discorhabdin D

We now report studies on the chemistry of discorhabdin C together with a further natural product in this series, discorhabdin E (**5**). Acid-catalyzed rearrangement of discorhabdin C gave another new ring system, with five fused rings including a 2,3-dihydro-1*H*-azepine. The conformational exchange in these ring systems, as detected in their nuclear magnetic resonance (NMR) spectra, is also discussed.

To explore the structural features responsible for the biological activities of the discorhabdins, all the derivatives in this study were tested for toxicity toward leukemia cells and a range of microorganisms. Discorhabdin C and some of these derivatives have also been tested in the U. S. National Cancer Institute (NCI) *in vitro* primary screen.[8] The comprehensive testing by the NCI has shown that members of the discorhabdin family of compounds exhibit *selective* cytotoxicity against certain cancer types.

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Table 1. ^{1}H-NMR Data for Compounds 3, 5–11[a]

H	3[b]	5	6	7	8	9	10	11
1	7.73, s	7.70, d, 2.8	–	–	6.46, s	–	–	–
3	–	–	–	–	4.79, s	7.84, d, *2.0*	–	–
4	–	6.55, d, *9.9*	–	–	–	–	–	–
5	7.73, s	7.19, dd, *2.8*, *9.9*	7.45, s	7.54, s	6.46, s	7.49, d, *2.0*	8.03, s	7.52, s
7	2.12, t, *6*	2.05, m	2.95, ddd, *1.5*, *14*	3.01, ddd, *1.0*, *4.8*, *14.2*	1.99, t, *5.8*	3.04, bdd, *4.0*, *14.2*	3.13, bdd, *4.8*, *14.3*	2.48, dt, *15.9*, *7.5*
			3.24, m	3.30, m		3.30, m	3.35, m	2.58, dt, *15.9*, *3.7*
8	3.73, t, *6*	3.70, t, *5.5*	3.98, ddd, *2.5*, *15*	4.00, ddd, *1.6*, *4.8*, *15.0*	3.59, t, *5.8*	4.03, bdd, *4.0*, *14.6*	4.02, ddd, *1.0*, *5.2*, *14.8*	3.51, m
			3.46, bdd, *10.5*, *15*	3.46, bdd, *11.0*, *15.0*		3.47, bdd, *11.1*, *14.6*	3.51, bdd, *10.8*, *14.8*	
14	7.22, s	7.18, s	7.23, t, *0.8*	7.23, s	7.17, s	7.23, s	7.26, s	7.25, t, *0.9*
16	2.90, t, *7*	2.87, t, *7.5*	2.96–3.02, m	2.99, bdd, *7.0*, *8.7*	2.89, t, *7.4*	2.99,bdd, *6.2*, *8.8*	3.01,bdd, *6.3*, *8.6*	2.99, td, *8.0*, *0.9*
17	3.79, t, *7*	3.77, t, *7.5*	3.73, dt, *14.0*, *8.5*	3.74, dt, *14.2*, *8.7*	3.84, t, *7.4*	3.75, dt, *14.3*, *8.8*	3.77, dt, *14.0*, *8.6*	3.80, dt, *14.4*, *8.0*
			3.90, dt, *14.0*, *6.5*	3.91, dt, *14.2*, *7.0*		3.94, dt, *14.3*, *6.2*	3.95, dt, *14.0*, *6.3*	3.93, dt, *14.4*, *7.3*
22	–	–	–	3.89, s	–	–	–	–

[a] Measured at 300 MHz in CD_3OD; β_H in ppm. followed by multiplicity; J_{HH} values in hertz are italicized. [b] The assignments for discorhabdin C in CD_3OD have been reported previously.[2]

Table 2. ^{13}C-NMR Data for Compounds 3,5–11[a]

C	3[b]	5	6	7	8[c]	9	10	11
1	153.0	152.8	132.8	130.1	136.4	131.7	142.3	153.3
2	125.0	127.0	112.4	119.1	125.8	126.8	117.8	128.5
3	173.1	179.0	152.7	155.7	73.1	136.4	152.9	173.2
4	125.0	129.9	115.7	122.8	125.8	124.0	135.2[d]	121.8
5	153.0	153.2	132.8	133.3	136.4	132.0	124.1	154.5
6	46.5	44.2	138.2	143.0	43.9	148.0	136.6[d]	78.2
7	35.5	35.7	33.8	34.0	36.6	34.6	34.0	44.5
8	39.8	39.9	52.6	52.3	39.8	52.1	52.0	42.3
10	154.1	154.1	153.1	153.2	153.6	153.1	153.6	154.1
11	166.7	166.9	169.0	168.8	167.2	168.7	168.3	167.3
12	125.1	125.1	125.3	125.3	125.1	125.3	125.3	125.4
14	128.1	128.0	127.9	127.9	127.9	128.0	128.2	128.3
15	121.7	121.6	121.3	121.3	121.6	121.3	121.5	121.4
16	19.7	19.8	19.8	19.8	19.9	19.8	19.8	19.7
17	45.6	45.5	44.8	44.8	45.6	44.9	44.9	45.0
19	156.6	156.6	158.1	158.2	156.5	158.0	158.1	156.5
20	93.4	94.0	99.1	98.5	97.9	98.0	99.1	99.1
21	125.4	125.5	124.5	124.4	125.5	124.5	124.4	124.1
22				61.2				

[a] Measured at 75 MHz in CD_3OD; δ_C values in ppm. [b] ^{13}C-NMR data for discorhabdin C has been previously reported for a DMSO-d_6 solution.[2] [c] Major signals observed are presented in the table. Other minor signals were observed at δ_C 135.55, 72.21, 44.62, 39.00, 35.55 ppm. [d] Values may be interchanged.

Discorhabdin E. Discorhabdin C (**3**) was reisolated from the same sponge species, now classified as *Latrunculia* cf. bocagei, and identified by ^{1}H- and ^{13}C-NMR, UV, and IR spectroscopy.[3] The ^{13}C-NMR spectrum, in CD_3OD solution, was fully assigned with the aid of an HMBC 2D-NMR experiment[9] and by comparison with the data previously obtained in DMSO-d_6.[2] While reisolating discorhabdin C, a new, minor component was discovered. Discorhabdin E (**5**), a red solid, was characterized as the trifluoroacetate salt. High resolution FABMS established the formula of MH^+ as $C_{18}H_{15}BrN_3O_2$, while the UV, IR, ^{1}H- and ^{13}C-NMR data (Tables 1 and 2) showed many similarities with those for discorhabdin C (**3**, MH^+ $C_{18}H_{14}Br_2N_3O_2$). The differences in the ^{1}H-NMR spectra centered on those resonances comprising the cyclohexadienone system. The observation of an AMX system in the olefinic region led to the assignment of discorhabdin E (**5**) as the monodebromo derivative of discorhabdin C (**3**). The ^{1}H chemical shifts and proton–proton coupling constants of the dienone system of discorhabdin E (**5**) (Table 1) were comparable with those reported for a monobrominated tyrosine metabolite from a *Verongia* sp. sponge.[10] The signals due to C-1, C-4, C-5, and C-14 were assigned by a heteronuclear correlation experiment, and the other signals by comparison with discorhabdin C (**3**) (Table 2).

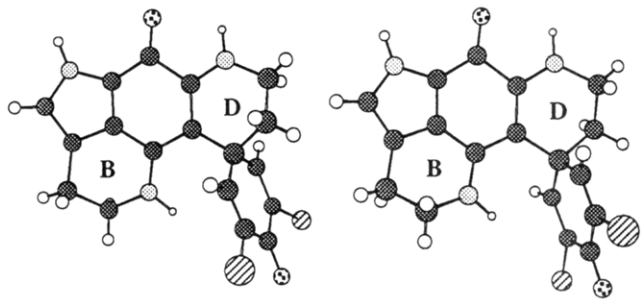

Figure 1. Enantiomeric conformations of discorhabdin C in solid state. Redrawn from X-ray data.[3]

A feature of the ^{1}H-NMR spectrum of discorhabdin C (**3**) was the equivalence of H-1/H-5 and the appearance of 2H-7, 2H-8, 2H-16, and 2H-17 as triplets (Table 1). This demonstrated that the enantiomeric conformations found in the solid state (Figure 1)[3] exchange rapidly in solution by inversion of the two half-chair rings B and D, leading to averaging of the 2H-7 to 2H-8 and 2H-16

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to 2H-17 coupling constants. The conformational inversions of rings B and D are not necessarily linked, as the diastereotopic H-17 protons in discorhabdin A (**1**), which has the conformation of ring D locked by a sulfur bridge, still showed averaged coupling constants to the 2H-16 protons.[2,5] Of the known discorhabdin derivatives, only D (**4**) has ring B locked into a single, half-chair conformation due to bonding between rings B and E.[4]

In contrast to discorhabdin C (**3**), there is a chiral center at C-6 in discorhabdin E (**5**), so all the methylene protons are diastereotopic and 2H-7 did not give a triplet signal in the ^{1}H-NMR spectrum (Table 1). However, rapid conformational inversion of rings B and D was still occurring, since the methylene protons at a greater distance from the chiral center were observed as triplets. Like many of the chiral brominated tyrosine derivatives from sponges of the order Verongida,[10,11] discorhabdin E (**5**) was found to be racemic.

Chemical Modifications of Discorhabdin C. While the pyrrolo[1,7]phenanthroline ring system of the discorhabdins (**1**–**5**) is unique, the spiro-cyclohexadienone function is well represented among plant products. For example, there are the proaporphine phenolic alkaloids[12] and more recently, the eupodienones, neolignans from an Australian plant.[13] The classic reaction of spirocyclohexadienones is the acid-catalyzed dienone–phenol rearrangement.[14] Such a rearrangement in the case of discorhabdin C (**3**) would lead to a new, ring-expanded system, by either alkyl (C-7) or alkenyl (C-20) migration. Aryl migration was observed for the proaporphines[12] and in the prohomoaporphine system,[15] whereas the eupodienones rearranged by alkyl migration.[16]

When discorhabdin C (**3**) was dissolved in concentrated sulfuric acid, a single product **6** was recovered. The ^{1}H-NMR spectrum (Table 1) confirmed that a dienone–phenol rearrangement had occurred, as evidenced by two one-proton signals, each due to olefinic or aromatic protons. One signal was assigned to the pyrrole proton H-14, while the other signal (at 7.45 ppm) showed a nuclear Overhauser effect (NOE) interaction with one of the H-7 signals (2.95 ppm) (Figure 2). This confirmed that the rearrangement had occurred by an alkenyl (C-20) shift to give the phenol **6** containing the 2,3-dihydro-1*H*-azepine system. The ^{13}C-NMR spectrum of the phenol **6** (Table 2) was fully assigned with the aid of an HMBC 2D-NMR experiment and was consistent with assignments for discorhabdin C (**3**) and with values of similarly substituted aromatic carbons.[17,18] The same dienone–phenol rearrangement by alkenyl shift also occurred in the model compounds used in synthetic approaches to the discorhabdins.[7a,h]

In the 2,3-dihydro-1*H*-azepine ring system of the phenol **6** there is no longer fast exchange between equivalent positions for the geminal protons on C-7 and C-8 since the signals are resolved and show distinct vicinal couplings (Table 1). A model of the conformation of this ring consistent with the coupling constants and the observed NOE interaction is shown in Figure 2. This slow conformational exchange of the 2,3-dihydro-1*H*-azepine leads to the two H-17 protons being diastereotopic (the plane of the chromophore is no longer averaged to a plane of symmetry). However, rapid conformational exchange of ring B was still occurring in solution, since the two H-17 protons showed averaged couplings to the H-16 protons (Table 1).

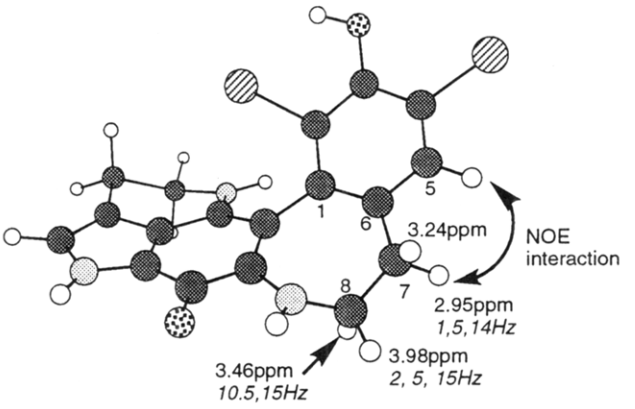
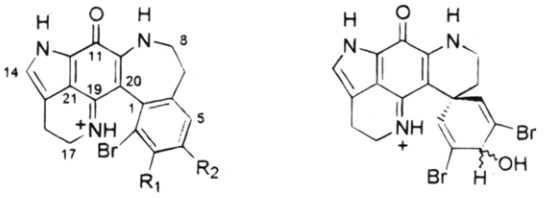


Figure 2. Model of one solution conformation of **6** produced by molecular mechanics calculations.[33]

From the phenol **6**, the *O*-methyl derivative **7** was prepared by methylation with CH_2N_2. The ^{1}H-(Table 1) and ^{13}C-NMR (Table 2) spectra of **7** showed the presence of signals characteristic of an aromatic methoxyl moiety (C-22, 61.17 ppm; 3H-22, 3.89 ppm) as well as changes in the C-1 to C-6 signals consistent with *O*-methylation of a phenol.[18] No N-13 methylation of phenol **6** was detected, which is in contrast to the reported reaction of prianosin A (discorhabdin A) (**1**).[5]



8 dienol

6	R_1 = OH	R_2 = Br	phenol
7	R_1 = OCH_3	R_2 = Br	methylated phenol
9	R_1 = H	R_2 = Br	benzene
10	R_1 = OH	R_2 = NO_2	nitrophenol

The dienol **8** was prepared for two reasons. Firstly, to gain a measure of the importance of the ring E ketone to the biological activity of the discorhabdin series, and secondly, as a further entry point into the 2,3-dihydro-1*H*-azepine system *via* the dienol/benzene rearrangement. To this end discorhabdin C (**3**) was reduced with $NaBH_4$ in methanol. The initial red color of the solution changed to yellow and then back to red upon swirling the solution in air. This was presumed to be due to the reduction of the iminoquinone chromophore followed by aerial reoxidation. This solution yielded a red solid which appeared to be a single compound by reverse-phase liquid chromatography, silica gel thin-layer chromatography and its ^{1}H-NMR spectra in three different solvents. Comparison of the ^{1}H-NMR data for a CD_3OD solution with those for discorhabdin C (**3**) (Table 1) showed a 1.3

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ppm upfield shift of the H-1/H-5 signal and a new singlet signal (H-3) at 4.79 ppm, consistent with this compound being one diastereoisomer of dienol **8**.[13] However, the ^{13}C-NMR spectrum of this compound in CD_3OD showed that all the more intense signals had satellite signals, resolved by up to 1 ppm, at about 30% intensity (Table 2). This could be due to either the presence of both C-3 epimers or to slow exchange between different conformations of the 1,4-cyclohexadiene ring.[19] No couplings or NOE interactions were observed between H-3 and the other protons, so there was no evidence to distinguish between the two possibilities. Rapid conformational inversion of rings B and D was occurring, since the relevant protons showed averaged coupling constants (Table 1).

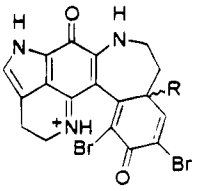
The reaction of dienol **8** with sulfuric acid gave a single product in high yield. The signals of two meta-coupled aromatic protons in the ^{1}H-NMR spectrum confirmed that the reaction had given the desired benzene derivative **9**. Alkenyl (C-20) migration was demonstrated by appropriate difference NOE experiments, as for **6** above. The ^{1}H-NMR (Table 1) and ^{13}C-NMR (Table 2) assignments of derivative **9** were based upon the phenol **6** and the methylated phenol derivative **7**. The 2,3-dihydro-1*H*-azepine ring of **9** was not undergoing rapid conformational exchange, but ring B was (see discussion for **6** above).

Since the phenol **6** was stable in strongly acidic solutions, the introduction of a nitro group into this skeleton was a possibility. This would provide further derivatives for structure–activity studies. The phenol **6** was nitrated with fuming nitric acid in glacial acetic acid at 0 °C.[20] Purification of the reaction mixture yielded two products, in 10 and 70% yields (starting material **6** was also present).

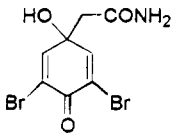
The minor, less polar product had very similar ^{1}H- and ^{13}C-NMR spectra to the starting material **6**, except for the signals associated with the phenol ring (Tables 1 and 2). The mass spectrum showed the presence of only one bromine atom, supported by the HR mass data, so this compound was the product of a nitrodebromination reaction. The nitro-substituent was located at C-4, adjacent to the aromatic proton, by comparison with the ^{1}H- and ^{13}C-NMR data reported for the model compound 2-bromo-4-methyl-6-nitrophenol,[21] to give structure **10** for the nitrophenol.

The major, more polar product was shown to be the 4-hydroxy-dienone **11**. The structural elucidation of the 4-hydroxy-dienone **11** relied on NMR spectroscopy (Tables 1 and 2) and was supported by HRFAB mass spectrometry. In the ^{13}C-NMR spectrum the signal at 173.18 ppm was appropriate for a cyclohexadienone carbonyl signal (see discorhabdin C (**3**), Table 2) and this was correlated with a one-proton singlet (H-5) at 7.52 ppm in an HMBC experiment. The carbon signal at 78.16 ppm was appropriate for a hydroxy-bearing quaternary carbon[17] and was correlated with the two-proton multiplets 2H-7 and 2H-8 in the HMBC experiment.

The formation of the 4-hydroxy dienone **11** most likely proceeded *via* the initial formation of the 4-nitro dienone **12** followed by a radical dissociation-recombination pathway leading to the 4-nitrito dienone **13**, which on hydrolysis would yield the 4-hydroxy-dienone **11**.[22] The 2-nitrophenol **10** may also have arisen from the 4-nitro dienone **12** as there have been repeated findings that 4-nitro dienones can rearrange in a wide variety of solvents to give 2-nitrophenols.[20,23,24] Alternatively, the 2-nitrophenol **10** could have been formed directly by a nitrodebromination reaction.[25] The mechanisms by which the 4-hydroxydienone **11** and the 2-nitrophenol **10** arose were not explored.



11 R = OH hydroxydienone
12 R = NO_2
13 R = ONO



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The ^{1}H-NMR data (Table 1) showed that the 2,3-dihydro-1*H*-azepine ring D of the nitrophenol **10** was not undergoing rapid conformational exchange. However, the vicinal coupling constants of 2H-7 and 2H-8 of the hydroxy dienone **11** are averaged (Table 1), so conformational exchange in the seven-membered ring D of this compound is most probably rapid.

Biological Activities of the Discorhabdin Derivatives. Discorhabdin C (**3**) and the derivatives **5**–**11** were assayed in-house for cytotoxicity against the BSC monkey kidney and the P388 murine leukemia cell lines and for antimicrobial activity against gram positive and gram negative bacteria and a fungus (Table 3). This selection of discorhabdin derivatives showed the induced changes in biological activities caused by varying selected structural features.

Cytotoxicities and activities against *Escherichia coli* seem to correlate with the presence of the α-bromo-α,β-unsaturated ketone moiety. This moiety is present in a number of marine natural products, notably the brominated tyrosine derivatives found in the sponge families Aplysinidae and Aplysinellidae (order Verongida).[11] The most studied example is the 2,6-dibromocyclohexadienone **14**, which was active against *Bacillus subtilis*, *E. coli*, and *Pseudomonas aeruginosa* (but not *Candida albicans*)[26] and was cytotoxic at an unspecified level.[11] The cytotoxic action of compound **14** could be due to the inhibition of enzymatic Na^+–K^+-ATPase activity[27] or by the Michael-type addition of enzymatic thiol-bearing groupings to the α-bromo-α,β-unsaturated ketone moiety, as demonstrated in quinone methide and α-methylene lactone enzyme inhibitors.[28]

The 1**H**-azepine derivatives **6**, **7**, **9**, and **10**, which do not contain an α-bromo enone moiety, are much less cytotoxic than the α-bromo enones **3**, **5**, and **11**. However, they are still sufficiently cytotoxic ($IC_{50} \leq 10$ μg/mL) to warrant further testing.[11] This cytotoxicity could be due

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Table 3. *In Vitro* Biological Activities of Compounds 3, 5–9

	cytotoxicity[a]			antimicrobial[c]			
compound	μg/disc	activity	P388: IC_{50}[b]	Ec	Bs	Pa	Ca
3	0.2	3+	40 ± 10	2	8	0	0
	0.05	2+					
5	0.2	2+	206 ± 25	6	4	0	1
6	20.0	3+	3050 ± 600	0	9	0	2
	5.0	1+					
7	5.0	4+	4000 ± 500	0	8	0	0
	2.0	2+					
8	20.0	4+	530 ± 15	4	5	0	0
	5.0	2+					
9	2.0	4+	1700 ± 50	1	9	0	2
	0.5	1+					
10	20.0	3+	8500 ± 400	0	0	0	0
	5.0	1+					
11	2.0	2+	385 ± 10	2	3	0	0
	0.5	1+					

[a] The test compound was applied to a 6 mm paper disc and incubated with the BSC cell line growing in continuous culture in a 16 mm well for 24 h at 36 °C in an atmosphere containing 5% CO_2. Zones of cytotoxicity were measured microscopically as excess radii from the disc and indicated by −, none detectable; +, 1–2 mm; 2+, 2–3.5 mm; 3+, 3.5–4.5 mm; 4+, greater than 4.5 mm. [b] IC_{50} against the P388 D1 murine leukemia cell line; concentrations in ng/mL. [c] Zone of microbial inhibition against *E. coli*, *B. subtilis*, *P. aeruginosa*, and *C. albicans* for 30 μg of test compound on a 6 mm paper disc. Incubation for 18 h at 35 °C. Zones measured as excess radii in millimeters.

to the iminoquinone moiety since other iminoquinone-bearing compounds have been found to be cytotoxic.[29,30] The need for further testing of compounds **7-11** is reinforced by the *in vivo* antitumor activity of discorhabdin D (**4**), which also lacks the α-bromo enone moiety, and is the least cytotoxic of discorhabdins A to D in *in vitro* screening.[4] A recent report has shown that simpler pyrroloiminoquinones are inhibitors of topoisomerase II.[6] However, discorhabdin A (**1**) was not active in this assay, so it is likely that the discorhabdins with the spiro-center are acting by an alternative mode of action.[6]

The biological properties of discorhabdin C (**3**) and derivatives **6**, **8**, and **9** were further evaluated by the NCI in their *in vitro* disease-oriented primary antitumor screen. The NCI testing provides observations on the mean response parameters (GI_{50}, TGI_{50}, LC_{50}), differential cellular sensitivity and subpanel-specific patterns of sensitivity.[8] In these primary assays discorhabdin C (**3**) was found to be selective for the colon and leukemia subpanels, while the dienol **8** was selective for the small cell lung and colon subpanels (see Experimental Section for details). Following successful testing against the recently instituted prostate cancer screen, discorhabdin C has also been referred to the Biological Evaluation Committee for Prostate Cancer for further evaluation.

This discovery of selective cytotoxicity for some discorhabdins against certain human-tumor cell lines is particularly encouraging and adds focus to the considerable synthetic and semisynthetic interest[7] in this new class of alkaloid.

Experimental Section

General Methods. Details of instrumental methods and general experimental procedures have been reported previously.[2]

Discorhabdin E 5. Specimens of *Latrunculia* cf. bocagei sponge were collected by SCUBA diving off the Auckland Islands in May, 1986. A voucher specimen, 86AKI06-02, is held at the Department of Chemistry, University of Canterbury, Christchurch, New Zealand. *Latrunculia* cf. bocagei is distinct from the other two species of *Latrunculia* found in New Zealand waters. These are *L. brevis*[31] and *L.* sp. 2, which remains undescribed. *Latrunculia* cf. bocagei is characterized by having a knobbly appearance and is colored brown, unlike the more abundant *L. brevis* which is green. Spicules are also distinctive, in particular the discorhabds are stout with three central whorls.

The sponge (550 g) was blended and extracted with CH_3OH to give, after removal of solvent, a red solid (44 g). This was partitioned on a C18-reverse phase column[32] to give a number of fractions containing both discorhabdin C (**3**) and discorhabdin E (**5**). Discorhabdin E was purified by a further three stages of semipreparative HPLC to yield a red solid (7 mg) which was characterized as the trifluoroacetate salt. HRFABMS MH^+ 384.0341, $C_{18}H_{15}N_3O_2{}^{79}Br$ requires 384.0348; λ_{max} (CH_3OH) 201 (ε 14 500), 244 (ε 18 000), 360 (ε 7300), 551 nm (ε 1000); λ_{max} (CH_3OH/KOH) 213 (ε 15 700), 337 nm (ε 7500); υ_{max} (smear) 3235, 1675, 1585, 1535, 1410, 1325, 1015 cm^{-1}; [α] = 0° (*c* 0.003, CH_3OH, λ = 250–700 nm); ^{1}H-NMR (Table 1); ^{13}C-NMR (Table 2).

Phenol Derivative 6. Discorhabdin C (**3**) (19.0 mg) was dissolved in concd H_2SO_4 (3 mL) and left at room temperature for 5 min. The reaction mixture was neutralized by the addition of solid $NaHCO_3$. The green solution was then added directly to a C18 reverse phase flash chromatography column (50 mm x 15 mm, 20g) and the column flushed with six column volumes of H_2O (90 mL). The retained material was eluted with CH_3OH to give a green solid which was further purified by semipreparative HPLC to yield the noncrystalline phenol **6** (17 mg). HREIMS M^+ 462.9380, $C_{18}H_{13}{}^{79}Br^{81}BrN_3O_2$ requires 462.9355; $M^+ - Br$ 382.0188, $C_{18}H_{13}{}^{79}BrN_3O_2$ requires 382.0192. DCI/NH_3 MH^+ 462/464/466; λ_{max} (CH_3OH) 208 (ε 25 700), 251 (ε 16 700), 311 (ε 11 500), 562 nm (ε 1000); λ_{max} (CH_3OH/KOH) 332 nm (ε 10 700); υ_{max} (KBr disc) 3700–2500, 1680, 1630, 1600, 1550, 1340, 1095 cm^{-1}; ^{1}H-NMR (Table 1); ^{13}C-NMR (Table 2).

Methylated Phenol Derivative 7. A sample of the phenol **6** (15 mg) was dissolved in CH_3OH (2 mL) and an ethereal solution of CH_2N_2 added. The reaction was allowed to stand for 10 min and then taken to dryness to yield **7** as a green solid. This was further purified by semipreparative HPLC to yield the noncrystalline trifluoroacetate salt (12 mg). HRFABMS MH^+ 477.9574, $C_{19}H_{16}{}^{79}Br^{81}BrN_3O_2$ requires 477.9590; λ_{max} (CH_3OH) 209 (ε 38 900), 249 (ε 20 000), 310 (ε 12 600), 562 nm (ε 1200); λ_{max} (CH_3OH/KOH) 214 (ε 33 790), 332 nm (ε 11 870); υ_{max} (smear) 1675, 1550, 1440, 1340, 1200, 1140, 800, 730 cm^{-1}; ^{1}H-NMR (Table 1); ^{13}C-NMR (Table 2).

Dienol Derivative 8. A sample of discorhabdin C (**3**) (4.7 mg) was dissolved in CH_3OH (2 mL) and excess $NaBH_4$ added. The red solution became yellow, but after swirling in air for 5 min the color had reverted to red. The sample was dried and the red solid taken up in 2 mL of 1% CF_3COOH in water. This was applied to a 20 g C18 reverse phase flash chromatography column (50 mm x 15 mm) and flushed with six column volumes of water (90 mL) to remove salt residues from the sample. The retained material was eluted with CH_3OH to yield **8** (4 mg) as a red solid which was further purified by semipreparative HPLC and characterized as the noncrystalline trifluoroacetate salt. HRFABMS MH^+ 465.9582, $C_{18}H_{16}{}^{79}Br^{81}BrN_3O_2$ requires 465.9589; λ_{max} (CH_3OH) 239 nm (ε 23 700), 351 (6030), 551 (740); λ_{max} (CH_3OH–KOH) 350 nm (ε 6300); υ_{max} (smear) 3225,

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1670, 1585, 1540,1325, 1200, 1130 cm^{-1}; ^1H-NMR (Table 1); ^{13}C-NMR (Table 2).

Benzene Derivative 9. A sample of the dienol **8** (23.0 mg) was dissolved in concd H_2SO_4 (3 mL) and left at room temperature for 5 min. The reaction mixture was neutralized by the addition of solid $NaHCO_3$. The green solution was then added directly to a C18 reverse phase flash chromatography column (50 mm x 15 mm, 20 g) and the column flushed with six column volumes of H_2O (90 mL). The retained material was eluted with CH_3OH to give a green solid which was further purified by semipreparative HPLC to yield **9** as the noncrystalline trifluoroacetate salt (23 mg). HRFABMS MH^+ 447.9467, $C_{18}H_{14}{}^{79}Br^{81}BrN_3O_2$ requires 447.9485; λ_{max} (CH_3OH) 207 nm (ϵ 26 500), 248 (11 500), 302 (7900), 565 (740); λ_{max} (CH_3OH-KOH) 212 nm (ϵ 11 900), 333 (6100); υ_{max} (smear) 1680, 1550, 1440, 1340, 1200, 1140 cm^{-1}; ^1H-NMR (Table 1); ^{13}C-NMR (Table 2).

Nitration of the Phenol Derivative 6. A sample of phenol **6** (10 mg) was dissolved in CH_3COOH (5 mL) and stirred at 0 °C. Fuming nitric acid (1 mL) was added dropwise over 30 s and the solution stirred at 0 °C for 5 min. The solution was then neutralized with solid $NaHCO_3$. Semipreparative HPLC of the resulting red solution gave two products.

Nitrophenol Derivative 10. The less-polar product isolated from the mixture was the nitrophenol **10**, which was characterized as the trifluoroacetate salt (1 mg). HRFABMS: MH^+ 429.01959, $C_{18}H_{14}{}^{79}Br^{81}BrN_4O_4$ requires 429.01988; λ_{max} (CH_3OH) 205 nm (ϵ 18 500), 248 (10 100), 374 (7900), 551 (530); λ_{max} (CH_3OH–KOH) 212 nm (ϵ 16 800), 237 (12 800), 359 (6200); υ_{max} 3150, 1675, 1600, 1550, 1400, 1200, 1140, 840, 800, 725 cm^{-1}; ^1H-NMR (Table 1); ^{13}C-NMR (Table 2).

Hydroxy Dienone Derivative 11. The more polar product was found to be the hydroxy dienone derivative **11**. This was characterized as the trifluoroacetate salt (7 mg). HRFABMS: MH^+ 479.9377, $C_{18}H_{14}{}^{79}Br^{81}BrN_3O_3$ requires 479.9381. Mp >360 °C; λ_{max} (CH_3OH) 206 nm (ϵ 34 000), 247 (18 800), 356 (9300), 551 (820); λ_{max} (CH_3OH–KOH) 212 nm (ϵ 28 400), 244 (15 300), 351 (8700); υ_{max} 3200, 1680, 1600, 1540, 1440, 1420, 1340, 1200, 1140, 1080, 800, 720, 695 cm^{-1}; ^1H-NMR (Table 1); ^{13}C-NMR (Table 2).

NCI Biological Testing. Discorhabdin C (**3**), discorhabdin C dienol (**8**), and derivatives **6** and **9** were tested in the NCI's human tumor, disease-oriented *in vitro* screen.[8] Of these only discorhabdin C (**3**) and discorhabdin C dienol (**8**) met the NCI's criteria for further testing. The $\log_{10}$ GI_{50} values for discorhabdin C (**3**) and discorhabdin C dienol (**8**) are listed below (cell name $\log_{10}$ GI_{50} (**3**), $\log_{10}$ GI_{50} (**8**)): CCRF-CEM −7.71, −5.68; HL-60 TB −7.81,-5.74; K562 −7.36, −5.49; MOLT-4 −6.96, -5.68; RPMI-8226 −7.47, −5.76. Non-small cell lung: A-549 −5.99, −5.47; HOP-18 -5.55, −4.67; HOP-62 −5.75, −4.49; NCI-H226 −5.86, −5.22; NCI-H23 −6.81, −5.70; NCI-H322 −5.87, −5.80; NCI-H460 −6.34, −5.77. Small cell lung: DMS-114 −7.01, -6.51; DMS-273 −6.65, −5.67. Colon cancer: COLO-205 −7.68, −6.45; DLD-1 −7.68, -5.78; HCT-116 −7.45, −5.91; HCT-15 −7.11, −5.69; HT-29 −7.19, −6.36; KM-12 -6.07, −5.49; KM-20L2 −7.00, −6.34; SW-620 −7.51, −6.06. CNS: SF-268 −6.48, -5.63; SF-295 −5.96, −5.48; SF-539 −6.79, −5.67; SNB-19 −5.74, −4.41; SNB-75 −5.93, -4.76; SNB-78 −6.53, −5.45; U-251 −6.36, −5.61; XF-498L −6.79, −4.52. Melanoma: LOX-IMVI −6.85, −5.66; M19-MEL −7.12, −5.88; SK-MEL-2 −6.80, −5.49; SK-MEL-28 −6.71, −4.85; SK-MEL-5 −6.83, −5.77; UACC-257 −6.88, −5.81; UACC-62 -6.88, −5.67. Ovarian: IGROV-1 −6.75, −5.69; OVCAR-3 −6.93, −4.51; OVCAR-4 -6.85, −5.80; OVCAR-5 −5.82, −5.55; OVCAR-8 −7.39, −5.82;SK-OV-3 −5.81, −4.88. Renal: A498 −5.71, −4.98; CAKI-1 −6.75, −5.36; RXF-393L −6.79, −5.30; SN-12C -6.79, −5.68; SN12K1 −6.73, −5.40; UO31 −6.36, −4.71. Miscellaneous: MCF-7 -7.39, −6.36; MCF-7/ADR −6.85, −5.38; P388 −7.67, −5.39; P388/ADR −7.58, −5.59. Mean: −6.76, −5.51. Delta: 1.06, 0.99. Range: 2.10, 2.10. Discorhabdin C (**3**) was also tested against the *in vitro* prostate cancer cell strains and again met the criteria for further testing by the NCI's Biological Evaluation Committee for Prostate Cancer. The test data (cell name, GI50) were RB, 5.61E-09; FC, 1.06E-08; WAE, 2.28E-08.

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Supplementary Material Available: ^1H-NMR spectra for compounds **5**–**11** (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.