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Unusual Sulfamate Indoles and a Novel Indolo[3,2-*a*]carbazole from *Ancorina* sp.[1]

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Received February 21, 2002

Four new indoles, ancorinolates A−C and bis-ancorinolate B, which contain sulfamate and sulfate groups, were isolated from the aqueous extract of the sponge *Ancorina* sp. In addition, ancorinazole, an indolo[3,2-*a*]carbazole also possessing sulfamate and sulfate groups, was isolated from two separate New Zealand collections of *Ancorina* sp. Ancorinazole is the first indolo[3,2-*a*]carbazole described as a natural product. Ancorinolates A (**1**) and C (**3**) showed weak HIV-inhibitory activity in the XTT-based, anticytopathicity assay.

Introduction

The aqueous extract of the sponge *Ancorina* sp. collected in New Zealand by NCI showed HIV-inhibitory activity in the NCI's primary screen.[2] Since only a tetramic acid glycoside[3] and its magnesium salt[4] had been reported from this genus, we undertook the study of this extract searching for new anti-HIV metabolites. Analysis of the extract at NCI afforded four new indoles and one indolo[3,2-*a*]carbazole,[5] all of them sulfamate derivatives and bearing sulfate groups. In an independent investigation by the Victoria University group, a collection of *Ancorina* sp. from Three Kings Island in New Zealand also yielded ancorinazole from the methanolic extract of the sponge.

There are few examples of natural products bearing a sulfamate functional group, such as the minalemines D−F,[6] the ianthesines C and D,[7] and phaseolotoxin.[8] This is the first report of indole with sulfamate and sulfate functional groups isolated from sponges. Several indole[9] and bis-indoles[10,11] have been described from sponges, which are usually halogenated or bear oxygenated substituents. Sulfated indoles have only been isolated from toads[12] and from the mollusc *Dicathais orbita* Gmelin.[13] Indoles with sulfamate substituents have been identified from the plant *Isatis tinctoria* L.[14] and from the toad *Bufo alvarius* Girard.[15]

Compounds from the indolocarbazole family are known to exhibit a wide range of biological effects such as inhibition of protein kinase C, cytotoxic, antihypertensive, antibacterial, antiedema, antiallergic, and antiinflammatory activities;[16−18] however, no biological activity has been reported for the [3,2-*a*] isomer. Indolo[3,2-*a*]-carbazoles have been synthesized[19−23] but this is the first time one has been isolated from a natural source.

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(1) Number 78 in the series: HIV-Inhibitory Natural Products. For 77 see ref 31.

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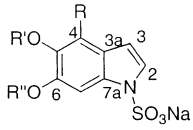
10.1021/jo020120k CCC: $22.00
Published on Web 08/24/2002

TABLE 1. NMR Assignments of Ancorinolates A (1), B (2), and C (3) in DMSO-d_6

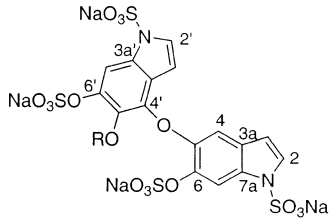
	ancorinolate A (**1**)		ancorinolate B (**2**)		ancorinolate C (**3**)	
position	δ_C mult	δ_H mult (*J* in Hz)	δ_C mult	δ_H mult (*J* in Hz)	δ_C mult	δ_H mult (*J* in Hz)
2	128.8 d	7.41 d (3.0)	127.5 d	7.32 d (3.0)	125.3 d	7.21 d (3.0)
3	99.2 d	6.25 d (3.0)	100.2 d	6.17 d (3.0)	98.3 d	6.13 d (3.0)
3a	125.6 s		126.1 s		120.2 s	
4	110.4 s		105.8 d	6.86 s	109.1 s	
5	141.4 s		144.2 s		137.3 s	
6	139.0 s		137.8 s		143.3 s	
7	108.3 d	7.51 s	108.7 d	7.50 s	98.8 d	7.28 s
7a	128.5 s		128.8 s		128.1 s	
OH-5		8.85 s		8.32 s		8.36 s
OH-6						9.39 s

Results and Discussion

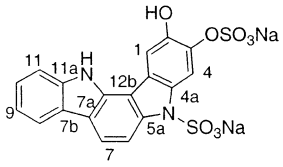
The aqueous extract of the sponge *Ancorina* sp. was fractionated by using vacuum-liquid chromatography with mixtures of MeOH−H_2O. The anti-HIV activity was concentrated in the two most polar fractions. Purification of those fractions afforded five compounds: four indoles, ancorinolates A−C (**1**−**3**) and bis-ancorinolate B (**4**), and an indolo[3,2-*a*]carbazole alkaloid, ancorinazole (**5**). A second collection of the sponge *Ancorina* sp. also yielded **5** from the methanolic extract of this sponge, which was fractionated on polymeric reversed-phase support (PS-DVB) and molecular exclusion Sephadex LH-20.



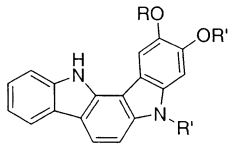
	R	R'	R''
1	Cl	H	SO_3Na
2	H	H	SO_3Na
2b	H	Ac	SO_3Na
3	Cl	H	H



	R
4	H
4b	Me



5



5b R, R' = H
5c R = Me, R' = SO_3H

The first isolated compound, ancorinolate A (**1**), was obtained as a white powder. Its FAB mass spectrum showed an isotopic cluster at *m*/*z* 388, 390 with relative intensities approximately 3:1, which suggested the presence of a chlorine atom. Careful analysis of the relative abundances [100% (*m*/*z* 388) and 43% (*m*/*z* 390)] indicated the presence of additional elements which contributed to the intensity of the peak at *m*/*z* 390.[24] Combinations of the natural isotopic abundances of different elements suggested that compound **1** had one chlorine and two sulfur atoms. Its molecular formula was established as $C_8H_4O_8NClS_2Na_2$ by HRFABMS of its pseudomolecular ion $[M + H]^+$ (*m*/*z* 387.8948, calcd 387.8941). The skeleton of compound **1** was easily identified as indole. The ^1H NMR spectrum recorded in DMSO-d_6 (Table 1) showed signals for four protons, two of which appeared as doublets at δ 7.41 (1H, d, $J = 3.0$ Hz) and δ 6.25 (1H, d, $J = 3.0$ Hz), and two as singlets, one of which was the signal for an exchangeable proton at δ 8.85. The ^{13}C NMR spectrum (Table 1) showed signals for all eight carbons between 99 and 142 ppm, suggesting that compound **1** was a fully aromatic system. The chemical shifts of the two doublets and of their corresponding carbon signals (δ_H 7.41, δ_C 128.8 and δ_H 6.25, δ_C 99.2) were consistent with the NMR data for C-2 and C-3, respectively, in indoles. Surprisingly, HMBC correlations between the signal of the exchangeable proton at δ_H 8.85 and C-4 (δ_C 110.4), C-5 (δ_C 141.4), and C-6 (δ_C 139.0) placed it as a hydroxyl group at C-5. As it was the only signal for an exchangeable proton in the ^1H NMR, the nitrogen of the indole had to be substituted. DEPT experiments showed only three protonated carbons, and since two of them were assigned to C-2 and C-3, this indicated that the aromatic ring of the indole had only one position without substitution. All these data indicated that ancorinolate A (**1**) was an indole with four substituents, with one of them attached at the nitrogen of the indole. With the spectral data indicating the presence of a chlorine and a hydroxyl as substituents on the indole ring, the remaining elements from the molecular formula of seven oxygen, two sulfur, and two sodium atoms had to constitute two substituents.

The FAB linked-scan spectrum in negative ion mode of *m*/*z* 364 ($[M - Na]^-$) showed a peak at *m*/*z* 300 corresponding to the loss of SO_2. Initially, the loss of this fragment suggested the presence of a sulfite functional group (−OSO_2), but careful research of the MS literature concerning sulfur substituents indicated that the cleavage of SO_2 could be produced in negative FAB after a rearrangement of a −SO_3 group to sulfite prior to fragmentation.[25] Strong absorbance bands at 1270, 1250, 1129, and 1054 cm^{-1} in the IR spectrum supported the presence of oxygenated sulfur groups. Accounting for three oxygen, one sulfur, and one sodium atom with −SO_3Na, the remaining elements from the molecular formula, SO_4Na, suggested the presence of a sulfate group.

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The position of the substituents was not trivial to assign because ancorinolate A (**1**) contained only four signals in the ^{1}H NMR spectrum. The protonated carbon in the aromatic ring was assigned as C-7 by the HMBC correlations between δ_H 7.51 (H-7) and δ_C 125.6 (C-3a), 141.4 (C-5), 139.0 (C-6), 128.5 (C-7a). The only signal for a quaternary carbon at high field (δ 110.4) was assigned to the carbon bearing the chlorine and was determined to be C-4 by HMBC correlations between this carbon and δ 6.25 (H-3). To determine the position of the sulfate and $-SO_3$ groups, the compound was reacted with 1% TFA yielding a dark brown oil. Purification by reversed-phase HPLC afforded a product whose molecular formula was established as $C_8H_5O_5NClSNa$ by HRFABMS of its pseudomolecular ion $[M - Na]^-$ (*m*/*z* 261.9573, calcd 261.9577), and corresponded to a loss of SO_3Na from ancorinolate A (**1**) plus one H, suggesting that only the sulfate group was removed. The ^{1}H NMR spectrum of the reaction product showed signals for five protons, two doublets at δ 6.13 (1H, d, J = 3.0 Hz, H-3) and 7.21 (1H, d, J = 3.0 Hz, H-2), one singlet at δ 7.28 (H-7), and two broad singlets at δ 8.36 and 9.39 belonging to exchangeable protons. HMBC correlations between the signal of the exchangeable proton at δ_H 8.36 and C-4 (δ_C 109.1) indicated that this signal belonged to the hydroxyl group at C-5. The other exchangeable proton was assigned to a hydroxyl group at C-6 by the HMBC correlations observed between δ_H 9.39 and C-5 (δ_C 137.3), C-6 (δ_C 143.3), and C-7 (δ_C 98.8), and by the NOE enhancement observed at H-7 when OH-6 was irradiated. The presence of a new hydroxyl group at C-6 in the product indicated that the sulfate group was present at C-6. Since the chlorine, the hydroxyl group, and the sulfate had been assigned to C-4, C-5, and C-6, respectively, only the $-SO_3$ group could be attached to the indole nitrogen, forming a sulfamate functional group. Thus, the structure of ancorinolate A (**1**) was determined to be disodium-4-chloro-5-hydroxyindole-1-sulfamate-6-sulfate.

The FABMS spectrum of ancorinolate B (**2**) did not show the typical isotopic pattern for chlorine as seen for ancorinolate A (**1**). The molecular formula was established as $C_8H_5O_8NS_2Na_2$ ($[M - H]^-$, *m*/*z* 351.9193, calcd 351.9174) by HRFABMS in negative mode, confirming the absence of chlorine. The negative FAB linked-scan spectrum at *m*/*z* 330 ($[M - Na]^-$) showed loss of SO_2 suggesting the presence of a sulfamate group as in ancorinolate A (**1**). The NMR data (Table 1) were nearly identical with those for compound **1**, except for the presence of a new protonated carbon (δ_H 6.86, δ_C 105.8) and the absence of a quaternary carbon. The new methine carbon was assigned to C-4 by the HMBC correlations between δ_H 6.86 (H-4) and C-3 (δ_C 100.2), C-3a (δ_C 126.1), C-5 (δ_C 144.2), C-6 (δ_C 137.8), and C-7a (δ_C 128.8). To confirm the presence of a hydroxyl group as in ancorinolate A, 5.4 mg of compound **2** was acetylated. The ^{1}H NMR spectrum of the derivative **2b** (Experimental Section) showed an additional singlet at δ 2.19 and absence of the signal for the exchangeable proton at δ 8.32. The position of the hydroxyl group was identified as C-5, as in ancorinolate A (**1**), by HMBC correlations in the spectrum of ancorinolate B (**2**) between the signal for the exchangeable proton at δ_H 8.32 and C-4 (δ_C 105.8), C-5 (δ_C 144.2), and C-6 (δ_C 137.8). Furthermore, HMBC correlations between δ_H 7.50 and C-3a, C-5, C-6, and C-7a confirmed that C-7 was protonated as in compound **1**. All these data indicated that ancorinolate B (**2**) was the dechlorinated derivative of ancorinolate A (**1**).

The addition of TFA to a solution of compound **2** in MeOH produced complete removal of the sulfate group in 24 h. The ^{1}H NMR spectrum of the product showed shielding of all protons, with the most significant effect observed for H-7. A similar result was obtained with ancorinolate A (**1**). To follow the reaction of ancorinolate B (**2**), 1% TFA was added to a solution of compound **2** in CD_3OD. The reaction was done in a NMR microtube and monitored by ^{1}H NMR. During the first 10 min after the addition of TFA, the ^{1}H NMR spectra showed a time-dependent decrease of the integration of the H-3 signal, and change of the H-2 multiplicity (δ 7.44) from doublet to singlet, suggesting the exchange of H-3 with deuterium under acidic conditions. Similar chemical reactivity was described for the arnoamines A and B, which are pentacyclic pyridoacridine with two rings resembling an indole system.[26] Subsequently, the ^{1}H NMR spectra showed a decrease of the integration of the signals for the three protons [δ 7.44 (H-2), 6.99 (H-4), and 7.86 (H-7)] and the simultaneous appearance of three new singlets [δ 7.28 (H-2), 6.85 (H-4), and 7.38 (H-7)].

The ^{13}C chemical shift assignment of C-5 and C-6 in ancorinolates A (**1**) and B (**2**) without ambiguity was not trivial. H-7 and the proton of the hydroxyl group in both compounds, and H-4 in ancorinolate B (**2**) showed HMBC correlations with C-5 and C-6, making the signals belonging to both carbons undistinguishable. The attempt to compare the ^{13}C NMR spectra in CD_3OD versus that in CD_3OH did not reveal any significant difference between the carbon bearing the hydroxyl group and the carbon bearing the deuterated hydroxyl substituent. However, the HMBC spectrum of the acetylated derivative **2b** showed a four-bond correlation between the protons of the acetate methyl (δ_H 2.19) and δ_C 137.5 (C-5). Although four-bond HMBC correlations are not always considered reliable, there have been several examples in the literature where these have been used in structural elucidation.[27] Support for the assignment of these carbons is provided by the acetylated derivative **2b**. Since acetylation in aromatic systems causes shielding at the ipso carbon and deshielding at the carbons in the ortho position,[28] the assignment of the signal at δ 144.2 to C-5 is based on its upfield shift to δ 137.5 in **2b** and therefore δ 137.8 is assigned to C-6 in ancorinolate B (**2**).

All the spectral data (Table 1 and Experimental Section) for ancorinolate C (**3**) were identical with those obtained for the reaction product of ancorinolate A (**1**), indicating that compound **3** is the desulfated derivative of ancorinolate A (**1**). Since no acidic conditions were employed during the isolation or purification of compound **3**, it is considered unlikely that ancorinolate C (**3**) is an artifact.

The molecular formula of compound **4** was deduced as $C_{16}H_8O_{16}N_2S_4Na_4$ by HRFABMS in negative ion mode (*m*/*z* 680.8472, calcd 680.8451 for $C_{16}H_8O_{16}N_2S_4Na_3$). This

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TABLE 2. NMR Assignments of Bis-ancorinolate B (4) in DMSO-d_6

position	C δ_C mult	H δ_H mult (J in Hz)	HMBC correlations
2	127.3 d	7.26 d (3.0)	H-3
3	100.5 d	6.06 d (3.0)	H-2, H-4
3a	124.7 s		H-2, H-3, H-4, H-7
4	103.8 d	6.42 s	H-3
5	145.9 s		H-4, H-7
6	138.2 s		H-4, H-7
7	108.4 d	7.88 s	
7a	129.3 s		H-2, H-3, H-4, H-7
2′	126.6 d	7.19 d (3.0)	H-3′
3′	98.0 d	6.03 d (3.0)	H-2′
3a′	120.3 s		H-2′, H-3′, H-7′
4′	133.3 s		H-3′
5′	136.7 s		H-7′
6′	139.0 s		H-7′
7′	104.9 d	7.58 s	
7a′	128.1 s		H-2′, H-3′, H-7′
OH		8.18 s	

TABLE 3. NMR Assignments of Ancorinazole (5) in DMSO-d_6

position	C δ_C mult	H δ_H mult (J in Hz)	HMBC correlations
1	108.3 d	8.09 s	OH
2	145.1 s		H-1, H-4, OH
3	139.8 s		H-1, H-4, OH
4	110.6 d	7.92 s	
4a	133.4 s[a]		H-1, H-4
5a	140.1 s		H-6, H-7
6	107.6 d	8.00 d (9.0)	H-7
7	117.9 d	8.02 d (9.0)	
7a	115.9 s		H-6, H-7, H-8, NH
7b	124.0 s		H-9, H-11, NH
8	119.6 d	8.07 d (7.5)	H-10
9	119.4 d	7.16 t (7.5)	H-11
10	124.2 d	7.32 t (7.5)	H-8
11	111.8 d	7.58 d (7.5)	H-9
11a	140.2 s		H-8, H-10, NH
12a	133.6 s[a]		H-7, NH
12b	107.7 s		H-1, H-6
12c	120.0 s		H-4
OH		8.67 s	
NH		11.57 s	

[a] Assignments may be interchanged.

formula contains twice the elements of the formula obtained for ancorinolate B (**2**) minus two H, suggesting a dimer. The IR spectrum showed absorbance bands at 1265, 1242, 1159, and 1054 cm^{-1}, indicating the presence of sulfate and sulfamate groups. The ^1H NMR spectrum (Table 2) showed four doublets with a coupling constant of 3.0 Hz (δ 6.03, 6.06, 7.19, and 7.26) supporting the presence of two indole groups. The spectrum also showed four singlets, one of which was an exchangeable proton, indicating that only one of the indoles was substituted with a hydroxyl group. Also, as for the ancorinolates A−C (**1**−**3**), the spectrum of **4** did not show signals for NH, suggesting that the nitrogens of both indoles were substituted. The ^{13}C NMR spectrum (Table 2) showed signals for all 16 carbons between 98 and 146 ppm, suggesting that compound **4** was a bis-indole. The COSY spectrum showed correlations between δ 7.26 (H-2) and δ 6.06 (H-3). HMBC correlations between δ_C 124.7 (C-3a) and δ_C 129.3 (C-7a) and δ_H 7.26 (H-2), δ_H 6.06 (H-3), δ_H 6.42 (H-4), and δ_H 7.88 (H-7) indicated that one of the indole monomers was substituted at C-5 and C-6. The NMR data of this monomer (A) were similar to those of ancorinolate B (**2**), except for the absence of the signal for an exchangeable proton (OH-5) belonging to a hydroxyl group. Similar spectroscopic analysis provided evidence to identify the other indole group (B). HMBC correlations between δ_C 120.3 (C-3a′) and δ_C 128.1 (C-7a′) and δ_H 7.19 (H-2′), δ_H 6.03 (H-3′), and δ_H 7.58 (H-7′) indicated that the indole B was substituted at C-4′, C-5′, and C-6′. To determine which fragment had the hydroxyl group, compound **4** was methylated with CH_2N_2. The ^1H NMR spectrum of the product (**4b**) (Experimental Section) showed an additional singlet (δ 3.70) that integrated for three protons and was assigned as a methoxyl group. HMBC correlations between δ_C 139.6 (C-5′) and δ_H 3.70 (OMe) and δ_H 7.81 (H-7′) suggested that the pentasubstituted aromatic ring was bearing the methoxyl group, meaning that the hydroxyl group in compound **4** was in fragment B. The two indoles were shown to be linked between C-5 and C-4′ by the NOE enhancement observed at H-3′ when H-4 was irradiated. The low-field chemical shift of C-5 (δ 145.9) and C-4′ (δ 133.3) suggested that both monomers were linked by an oxygen atom through an ether linkage. The indole dimer accounted for $C_{16}H_8O_2$ of the molecular formula. The remaining four sulfur atoms together with fourteen oxygen and four sodium atoms suggested the presence of two sulfate and two sulfamate groups. Furthermore, since C-5, C-4′, and C-5′ had been previously shown to bear the ether bond and the hydroxyl group, respectively, the sulfate groups were placed at C-6 and C-6′, and the sulfamate groups were attached to both of the indole nitrogens. The very close similarity of the NMR data of compound **4** with those obtained for the ancorinolates A−C (**1**−**3**) supported this proposed structure.

The molecular formula of compound **5** was deduced as $C_{18}H_{10}O_8N_2S_2Na_2$ by HRFABMS of its pseudomolecular ion $[M - Na]^-$ (m/z 468.9785, calcd 468.9777). The FABMS (negative mode) peak at m/z 427 suggested loss of SO_2 from the $[M - H]^-$ ion, and the FABMS (positive mode) peak at m/z 413 suggested loss of SO_3 from the $[M + H]^+$ ion. The IR spectrum showed absorbance bands at 1262, 1232, and 1053 cm^{-1}, supporting the presence of sulfate and sulfamate groups. Additional support for the sulfate functionality was provided by the formation of a white precipitate ($BaSO_4$) under mild acid hydrolysis in the presence of $BaCl_2$. The UV spectrum of **5** was complex and showed absorption maxima at λ = 242, 280, 308, 341, and 357 nm, indicating a polycyclic aromatic compound.

The ^1H NMR spectrum (Table 3) showed three singlets (δ 7.92, 8.09, and 8.67) one of which was the signal for an exchangeable proton, similar to the signals for the aromatic protons and the hydroxyl group in ancorinolate B (**2**). However, no signals for the two characteristic doublets at H-2 and H-3 were observed, suggesting that these positions were substituted. The three singlets observed in the ^1H NMR spectrum (δ 7.92, 8.09, and 8.67) were confirmed to be signals for the protons in para positions and a hydroxyl group (OH-2) on the aromatic ring of an indole fragment by HMBC correlations between δ 139.8 (C-3) and 145.1 (C-2) and H-1, H-4, and OH-2. The ^1H NMR spectrum also contained signals for an ortho-disubstitued aromatic ring [δ 8.07 (1H, d, J = 7.5 Hz), 7.58 (1H, d, J = 7.5 Hz), 7.32 (1H, t, J = 7.5 Hz), and 7.16 (1H, t, J = 7.5 Hz)], an AB system [δ 8.02

(1H, d, J = 9.0 Hz) and 8.00 (1H, d, J = 9.0 Hz)], and another exchangeable proton (δ 11.57). A series of 1D TOCSY experiments confirmed the disubstituted phenyl ring fragment. In particular, selective excitation of the proton resonance of H-8 (δ_H 8.07, δ_C 119.6) in a series of one-dimensional TOCSY experiments with increasing mixing times sequentially revealed protons assigned to carbons C-9 (δ_H 7.16, δ_C 119.4), C-10 (δ_H 7.32, δ_C 124.2), and C-11 (δ_H 7.58, δ_C 111.8). Similarly, selective irradiation of H-11 sequentially revealed the protons H-10, H-9, and H-8, clearly establishing the linear sequence of this spin system. These signals were assigned to a carbazole system by HMBC correlations between the signal for the exchangeable proton (δ_H 11.57, NH-12) and C-7a, C-7b, C-11a, and C-12a, and between some of these carbons with protons of the disubstituted aromatic ring, and with those of the AB system (between C-7a and H-6, H-7, and H-8, between C-7b and H-9 and H-11, between C-11a and H-8 and H-10, and between C-12a and H-7).

The number of bonds between the NH group and the carbons identified in the HMBC experiment was established from a ^{13}C NMR spectrum that was run in the presence of a mixture of H_2O/D_2O 1:1 (DMSO-d_6). This experiment results in an apparent splitting or broadening of carbons two and three bonds away from an exchangeable proton due to the deuterium isotope effect on the chemical shift of the ^{13}C resonance.[29] Carbon C-11a and the carbon resonance δ_C 133.6 (C-12a) showed large apparent splittings of 0.15 (Δ ppm), indicating a short-range (two bond) deuterium isotope effect, establishing the attachment of the NH-12 group to C-11a and C-12a. Additional long-range (three bond) deuterium isotope effects were observed for carbons C-7b and C-11, and two carbon resonances δ_C 115.9 (C-7a) and δ_C 107.7 (C-12b), with small apparent splittings of 0.04, 0.03, 0.05, and 0.04, respectively. These isotope shift effects confirmed the connection of C-7b and C-11 to the carbon C-11a and required that the substituted carbons C-7a and C-12b be directly connected to C-12a.

All these data suggested that compound **5** contained indole and carbazole fragments. The presence of only 18 carbons in the molecular formula indicated the systems were fused. The fragments were fused between C-5a and C-12b based on HMBC correlations between C-12b and H-1 and H-6. The [3,2-*a*] fusion geometry of the indolocarbazole was deduced by the NOE enhancement observed in H-1 (δ_H 8.09) when the NH signal (δ_H 11.57) was irradiated, as only this geometrical isomer has proximity between these two protons. These data also suggested the placement of a sulfate group at C-3 and a sulfonate at N-5 to form a sulfamate group, analogous to the substitutions found for ancorinolate B (**2**).

Treatment of **5** with mild acid (pTsOH·H_2O) then quenching with H_2O afforded the hydrolysis product **5b**. The HRESMS in positive ion mode of the product (**5b**) gave an $[M + H]^+$ ion at m/z 289.0977 (Δ 0.3 ppm) corresponding to the molecular formula $C_{18}H_{12}N_2O_2$ and confirmed the loss of two $-SO_3Na$ groups from the molecular formula of ancorinazole (**5**). All 18 carbons and 12 protons were observed in the ^1H and ^{13}C NMR spectra (Experimental Section). The ^1H NMR spectrum of **5b** (acetone-d_6) was similar to that of **5** except that it now contained four exchangeable proton resonances (δ_H 10.76, 10.19, 8.16, and 7.40) that showed no correlations to carbon in the HSQC spectrum.

The presence of hydroxyl groups at C-2 and C-3 in **5b** was determined from HMBC correlations from two of the new exchangeable resonances at δ_H 7.40 and δ_H 8.16. The resonance at δ_H 7.40 (OH-2) showed HMBC correlations to C-1 (δ_C 107.5), and carbon resonances attributed to C-2 and C-3 (δ_C 140.8, δ_C 145.2, respectively). Similarly, correlations from δ_H 8.16 (OH-3) to C-2, C-3, and C-4 (δ_C 97.8) confirmed the connection of hydroxyl groups to C-2 and C-3. Additional correlations were observed from a new exchangeable resonance at δ_H 10.19 (NH-5). The exchangeable resonance showed correlations to carbons assigned to C-5a (δ_C 140.4)and C-12b (δ_C 108.5) and the carbon resonance δ_C 115.3 (C-12c) assigned to carbon C-5a of the carbazole ring and carbon C-4a (δ_C 135.0) of the phenyl ring. The presence of additional split carbon resonances in the H_2O/D_2O ^{13}C NMR experiment for carbons C-5a and the carbon resonance δ_C 135.0 (Δ 0.19 and 0.14 ppm, respectively), respectively, confirmed the connection of the NH group to C-5a and C-4a (δ_C 135.0). Long-range deuterium isotope effects were also observed for the carbon resonances assigned to C-4 (δ_C 97.8), C-6 (δ_C 104.5), C-12b (δ_C 108.5), and C-12c (δ_C 115.3), confirming the placement of the NH group, and establishing the structure of **5b** as a 2,3-dihydroxyindolo[3,2-*a*]carbazole.

Finally, the location of the hydroxyl group at C-2 was confirmed by the reaction of **5** with CH_2N_2 resulting in the formation of a highly unstable trimethylated compound that rapidly decomposed to give a monomethylated product (**5c**). Compound **5c** showed an $[M - H]^-$ ion at m/z 461.0113 in the HRESMS corresponding to the molecular formula $C_{19}H_{14}N_2O_8S_2$, one carbon and two hydrogens greater than ancorinazole (**5**). The mass spectrum also showed significant fragment ions at m/z 381 and 300 corresponding to the facile loss of two $-SO_3$ groups from the molecular ion similar to fragment ions observed for **5**. The ^1H NMR spectrum of **5c** contained a methoxyl resonance at δ_H 3.96 (δ_C 60.0), which showed an HMBC correlation to an oxygenated carbon at δ_C 147.1 assigned to C-2. The position of the methoxyl group was confirmed from an NOE enhancement observed in a 1D GOESY experiment. Selective irradiation of δ_H 8.10 (H-1) revealed OMe-2 (δ 3.96) and NH-12 (δ 11.67). Similarly, irradiation of δ_H 3.96 (OMe-2) revealed a proton resonance at δ_H 8.13 (H-1). These correlations established the attachment of methoxyl to C-2 (δ 147.1) and supported the placement of the sulfate at C-3.

All the isolated compounds were tested in the XTT-tetrazolium based anti-HIV screen.[2] Only ancorinolates A (**1**) and C (**3**) showed weak activity (EC_{50} = 10.7 and 10.6 μg/mL, respectively) with 70−80% maximum protection. Ancorinolates A (**1**) and B (**2**) were also tested in a HIV-1 integrase in vitro assay,[30] but both showed only very weak inhibition with IC_{50} higher than 100 μM

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(ancorinolate A (**1**), IC_{50} = 156 μM; ancorinolate B (**2**), IC_{50} = 116 μM).

Experimental Section

General. NMR spectra were acquired at 500 MHz for ^1H and 125 MHz for ^{13}C in DMSO-d_6, acetone-d_6, CD_3OD, and CD_3OH as solvents. NMR experiments include 1D NOESY, $^1H-^1H$ COSY, 1D-TOCSY, 1D GOESY, HSQC, and HMBC. Low- and high-resolution mass spectra were obtained in FAB in positive and negative mode, with magic bullet (dithiothreitol−dithioerytritol, 1:1) as matrix. UV spectra were run in MeOH. IR spectra were obtained as a film on NaCl. HPLC separations were performed in reversed phase with a C_{18} column (1 × 25 cm, MeOH−H_2O 68:32 or gradient 0:100 to 100:0, flow rate 1.0 mL/min, UV detection at 230 nm) followed by normal phase HPLC with a diol column (1 × 25 cm, gradient 100:0 to 50:50 CH_2Cl_2−MeOH, flow rate 1 mL/min, UV detection at 254 nm).

Collection, Extraction, and Isolation. The NCI collection of *Ancorina* sp. Schmidt (Ancorinidae) was collected in East Side Port Hutt, Chatham Island, New Zealand, in October 1988, by J. W. Blunt, under contract with the National Cancer Institute. A voucher specimen has been deposited at the Smithsonian Institution. The taxonomy was determined by Chris Battershill.

A portion (5.07 g) of aqueous extract of *Ancorina* was fractionated by vacuum-liquid chromatography (C_4, 40 μm) with mixtures of MeOH−H_2O to give 6 fractions (A−F). The anti-HIV activity was concentrated in the two most polar fractions (A and B). A portion of the A fraction (810 mg) was subjected to successive gel permeation on Sephadex LH-20 (MeOH−H_2O, 1:1 and 7:3) to give ancorinolate B (**2**) (8.9 mg, 0.75% of the extract), ancorinolate C (**3**) (1.3 mg, 0.08% of the extract), and bis-ancorinolate B (**4**) (7.6 mg, 0.47% of the extract), and an impure fraction (46.3 mg), which was purified by reversed phase HPLC [MeOH−H_2O (gradient 0:100 to 100:0)], affording ancorinolate A (**1**) (7.1 mg, 0.44% of the extract) and ancorinolate B (**2**) (3.2 mg). Permeation on Sephadex LH-20 (MeOH−H_2O, 1:1) of the B fraction (879 mg) afforded ancorinazole (**5**) (7.3 mg, 0.14% of the extract). Final purification of **1**−**5** was accomplished by normal phase HPLC (diol, CH_2Cl_2−MeOH gradient).

The Victoria University collection of *Ancorina* sp. (1.1 kg, NIWA no. MNP 2055) was collected from the Three Kings Islands, North Island, New Zealand. The sponge was extracted with MeOH (2 × 2 L) for 24 h. The second and then the first extract were passed through a column of HP-20 (7 × 15 cm) preequilibrated with MeOH. The eluents were combined and passed through the column. The eluent was diluted with H_2O (20 L) and passed through the column. The column was eluted with 1-L fractions of (1) H_2O, (2) 20% Me_2CO/H_2O, (3) 40% Me_2CO/H_2O, (4) 60% Me_2CO/H_2O, (5) 75% Me_2CO/H_2O, and (6) Me_2CO. Fraction 3 was concentrated under vacuum to give a black oil (653 mg). The oil was fractionated by Sephadex LH-20, gel permeation chromatography (90% MeOH/H_2O). The late eluting fractions, displaying a long-wavelength UV absorbing spot on silica gel TLC (15% MeOH/CH_2Cl_2), were concentrated under vacuum to give ancorinazole (**5**) (86.6 mg).

Ancorinolate A (1): white powder; HRFABMS *m/z* 387.8948 (calcd for $C_8H_5O_8NClS_2Na_2$, 387.8941); FABMS (positive mode) *m/z* 410 $[M + Na]^+$ (12), 388 $[M + H]^+$ (14), 329 $[M + Na - SO_3H]^+$ (25); FABMS (negative mode) *m/z* 386 $[M - H]^-$ (30), 364 $[M - Na]^-$ (98), 262 $[M - SO_3Na - Na + H]^-$ (13); linked-scan spectrum (negative ion mode) at *m/z* 364, *m/z* 328 $[M - Na - ClH]^-$ (8), 300 $[M - SO_2Na]^-$ (1), 284 $[M - SO_3Na]^-$ (3); UV λ_{max} (log ϵ) 300.0 (3.56), 271.0 (3.86), 220.0 nm (4.31); IR ν_{max} 3496, 1651, 1645, 1634, 1446, 1270, 1250, 1129, 1054 cm^{-1}; ^1H and ^{13}C NMR (DMSO-d_6), see Table 1.

Conversion of 1 to 3: Ancorinolate A (**1**) (4 mg) was dissolved in 4 mL of MeOH and TFA (40 μL) was added. The solution was stirred at room temperature for 5 h. The solution was evaporated under reduced pressure, then the residue was purified by HPLC (C_{18}, MeOH−H_2O 68:32) to give 2.0 mg of ancorinolate C (**3**).

Ancorinolate B (2): white powder; HRFABMS *m/z* 351.9193 (calcd for $C_8H_4O_8NS_2Na_2$, 351.9174); FABMS (positive mode) *m/z* 376 $[M + Na]^+$ (11), 354 $[M + H]^+$ (23), 274 $[M - SO_3 + H]^+$ (12); FABMS (negative mode) *m/z* 352 $[M - H]^-$ (7), 330 $[M - Na]^-$ (31), 227 $[M - SO_3Na - Na]^-$ (4); linked-scan spectrum (negative ion mode) at *m/z* 330, *m/z* 266 $[M - SO_2Na]^-$ (12), 250 $[M - SO_3Na]^-$ (3); UV λ_{max} (log ϵ) 296.0 (3.39), 265.0 (3.61), 222.0 nm (4.08); IR (NaCl) ν_{max} 3486, 2924, 2851, 1628, 1457, 1234, 1114, 1054 cm^{-1}; ^1H and ^{13}C NMR (DMSO-d_6), see Table 1; ^1H NMR (CD_3OD) δ 7.86 (1H, s, H-7), 7.44 (1H, d, J = 3.5 Hz, H-2), 6.99 (1H, s, H-4), 6.30 (1H, d, J = 3.5 Hz, H-3).

Acetylation of 2: A 5.4-mg sample of ancorinolate B (**2**) was dissolved in 1 mL of pyridine and 2 mL of acetic anhydride was added. The solution, protected from the light, was stirred for 1 h, and then dried under a stream of N_2, affording **2b**.

Acetyl-ancorinolate B (2b): ^1H NMR (DMSO-d_6) δ 7.87 (1H, s, H-7), 7.39 (1H, d, J = 3.0 Hz, H-2), 7.11 (1H, s, H-4), 6.26 (1H, d, J = 3.0 Hz, H-3), 2.19 (3H, s, OAc); ^{13}C NMR (DMSO-d_6, δ deduced from HSQC and HMBC experiments) δ 169.4 (s, OAc), 140.4 (s, C-6), 137.5 (s, C-5), 132.0 (s, C-7a), 127.6 (d, C-2), 124.2 (s, C-3a), 112.2 (d, C-4), 107.5 (d, C-7), 100.4 (d, C-3), 20.6 (q, OAc).

Reaction of 2 with TFA in MeOH: A 3.2-mg sample of ancorinolate B (**2**) was subjected to acid-catalyzed desulfonation (1% TFA in MeOH, 5 h). ^1H NMR (CD_3OD) of the hydrolysis product: δ 7.38 (1H, s, H-7), 7.28 (1H, d, J = 3.0 Hz, H-2), 6.85 (1H, s, H-4), 6.21 (1H, d, J = 3.0 Hz, H-3).

Reaction of 2 with TFA in CD_3OD: A 1.0-mg sample of ancorinolate B (**2**) was dissolved in 160 μL of CD_3OD and the ^1H NMR was recorded and considered time zero. Subsequently, TFA (16 μL) was added and the reaction was followed by ^1H NMR.

Ancorinolate C (3): white powder; HRFABMS *m/z* 261.9573 (calcd for $C_8H_5O_5NClS$ 261.9577); FABMS (negative mode) *m/z* 262 $[M - Na]^-$ (100); linked-scan spectrum (negative ion mode) at *m/z* 262, *m/z* 226 $[M - ClH - Na]^-$ (3), 182 $[M - SO_3Na]^-$ (12); UV λ_{max} (log ϵ) 302.5 (3.64), 272.0 (3.78), 216.0 nm (4.14); IR (NaCl) ν_{max} 3407, 2928, 2860, 1597, 1473, 1259, 1116, 1053 cm^{-1}; ^1H and ^{13}C NMR (DMSO-d_6), see Table 1.

Bis-ancorinolate B (4): white powder; HRFABMS *m/z* 680.8472 (calcd for $C_{16}H_8O_{16}N_2S_4Na_3$, 680.8451); FABMS (positive mode) *m/z* 727 $[M + Na]^+$ (1), 705 $[M + H]^+$ (1), 625 $[M + H - SO_3]^+$ (4), 603 $[M + 2\,H - SO_3Na]^+$ (2), 523 $[M + 2\,H - 2\,SO_3 - Na]^+$ (3); FABMS (negative mode) *m/z* 703 $[M - H]^-$ (5), 681 $[M - Na]^-$ (14), 601 $[M - SO_3Na]^-$ (2), 579 $[M + H - SO_3Na - Na]^-$ (5), 498 $[M - 2\,SO_3Na]^-$ (1); UV λ_{max} (log ϵ) 301.0 (3.82), 268.0 (4.07), 221.5 nm (4.53); IR (NaCl) ν_{max} 3484, 1648, 1461, 1265, 1242, 1159, 1054 cm^{-1}; ^1H and ^{13}C NMR (DMSO-d_6), see Table 2.

Methylation of Bis-ancorinolate B (4): Bis-ancorinolate B (**4**) (3.1 mg) was dissolved in MeOH (1 mL), and a solution of CH_2N_2 in Et_2O (1 mL) was added. The mixture was kept for 1 h in the dark, and was then dried under nitrogen, to give 5′-methyl-bis-ancorinolate B (**4b**).

5′-Methyl-bis-ancorinolate B (4b): ^1H NMR (DMSO-d_6) δ 8.00 (1H, s, H-7), 7.81 (1H, s, H-7′), 7.24 (1H, d, J = 3.0 Hz, H-2), 7.12 (1H, d, J = 3.0 Hz, H-2′), 6.40 (1H, s, H-4), 6.03 (1H, d, J = 3.0 Hz, H-3), 5.95 (1H, d, J = 3.0 Hz, H-3′), 3.70 (3H, s, OMe); ^{13}C NMR (DMSO-d_6, δ deduced from HSQC and HMBC experiments) δ 146.4 (s, C-5), 139.6 (s, C-5′), 139.7 (s, C-6), 143.5 (s, C-6′), 134.8 (s, C-4′), 131.6 (s, C-7a′), 130.1 (s, C-7a), 127.7 (d, C-2), 127.0 (d, C-2′), 124.8 (s, C-3a), 119.5 (s, C-3a′), 108.0 (d, C-7), 104.5 (d, C-7′), 105.1 (d, C-4), 101.0 (d, C-3), 99.4 (d, C-3′), 61.6 (q, OMe).

Ancorinazole (5): white powder; HRFABMS *m/z* 468.9785 (calcd for $C_{18}H_{10}O_8N_2S_2Na$, 468.9777); FABMS (positive mode) *m/z* 515 $[M + Na]^+$ (18), 493 $[M + H]^+$ (19), 413 $[M + H - SO_3]^+$ (16); FABMS (negative mode) *m/z* 491 $[M - H]^-$ (7), 469

$[M - Na]^-$ (32), 427 $[M - SO_2H]^-$ (3); UV λ_{max} (log ϵ) 356.5 (3.95), 340.5 (3.78), 308.0 (4.00), 296.0 (3.97) (sh), 280.0 (4.23), 242.5 nm (4.28); IR (NaCl) ν_{max} 3462, 1634, 1262, 1232, 1053 cm^{-1}; ^1H and ^{13}C NMR (DMSO-d_6), see Table 3.

Acid hydrolysis of ancorinazole (5): To a solution of **5** (7 mg) in Me_2CO (1 mL) was added excess *p*TsOH·H_2O. After 0.5 h the reaction was quenched with H_2O (200 μL) and passed through an Amberchrom column (0.5 × 1 cm). The eluent was diluted with H_2O (4 mL) and passed through the column. The column was eluted with increasing concentrations of Me_2CO in H_2O (0−100%). The 30−40% fractions were concentrated to dryness to give the acid hydroysis product (**5b**) (5 mg).

Acid hydrolysis product (5b): colorless oil; HRESMS, $[M + H]^+$ obsd *m*/*z* 289.0977, calcd for $C_{18}H_{13}N_2O_2$ 289.0974; UV (MeOH) λ_{max} (log ϵ) 360 (2.84), 344 (3.68), 310 (3.92), 286 (4.04), 268 (4.19), 256 (4.20), 210 nm (4.13); IR (KBr) ν_{max} 3425, 1632, 1459, 1262, 1022, 799, 744 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.53 (1H, bs, NH-12), 10.95 (1H, bs, NH-5), 8.02 (1H, d, J = 7.6 Hz, H-8), 7.98 (1H, s, H-1), 7.91 (1H, d, J = 8.6 Hz, H-7), 7.56 (1H, d, J = 7.9 Hz, H-11), 7.28 (1H, t, J = 7.2 Hz, H-10), 7.19 (1H, d, J = 8.6 Hz, H-6), 7.13 (1H, t, J = 7.2 Hz, H-9), 6.94 (1H, s, H-4); ^{13}C NMR (DMSO-d_6) δ 145.0 (s, C-3), 140.2 (s, C-2), 139.4 (s, C-11a), 139.1 (s, C-5a), 133.7 (s, C-4a), 133.5 (s, C-12a), 124.8 (s, C-7b), 123.2 (d, C-10), 118.8 (d, C-9), 118.8 (d, C-8), 116.2 (d, C-7), 114.0 (s, C-7a), 113.7 (s, C-12c), 111.1 (d, C-11), 107.6 (d, C-1), 107.1 (s, C-12b), 103.6 (d, C-6), 97.1 (d, C-4); ^1H NMR (acetone-d_6) δ 10.76 (1H, s, NH-12), 10.19 (1H, s, NH-5), 8.16 (1H, s, OH-3), 8.07 (1H, d, J = 7.6 Hz, H-8), 7.99 (1H, d, J = 8.3 Hz, H-7), 7.95 (1H, s, H-1), 7.57 (1H, d, J = 7.8 Hz, H-11), 7.40 (1H, s, OH-2), 7.29 (1H, d, J = 8.5 Hz, H-6), 7.29 (1H, t, J = 8.3 Hz, H-10), 7.16 (1H, t, J = 7.8 Hz, H-9), 7.09 (1H, s, H-4); ^{13}C NMR (acetone-d_6) δ 145.2 (s, C-3), 140.8 (s, C-2), 140.4 (s, C-5a; s, C-11a), 135.0 (s, C-4a), 134.8 (s, C-12a), 125.3 (s, C-7b), 124.0 (d, C-10), 119.8 (d, C-9), 119.5 (d, C-8), 117.0 (d, C-7), 115.8 (s, C-7a), 115.3 (s, C-12c), 111.8 (d, C-11), 108.5 (s, C-12b), 107.5 (d, C-1), 104.5 (d, C-6), 97.8 (d, C-4).

Methylation of ancorinazole (5): A solution of **5** (10 mg) in ethanol (2 mL) was treated with an excess of CH_2N_2 in Et_2O for 12 h. Removal of the solvent under vacuum yielded an unstable trimethylated product (3 mg) and a DMSO-d_6 soluble monomethylated product (**5c**) (6 mg).

Monomethylated product (5c): a colorless oil; HRESMS, $[M - H]^-$ obsd *m*/*z* 461.0113, calcd 461.0113 for $C_{19}H_{13}N_2O_8S_2$; UV (MeOH) λ_{max} (log ϵ) 356 (4.12), 340 (4.18), 298 (4.04), 280 (4.38), 244 (4.44), 212 nm (4.30); IR (KBr) ν_{max} 3438, 2924, 1615, 1487, 1244, 1051, 653, 617 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.67 (1H, s, NH-12), 8.36 (1H, s, H-4), 8.10 (1H, s, H-1), 8.09 (1H, d, J = 7.6 Hz, H-8), 8.04 (1H, d, J = 8.9 Hz, H-6), 7.99 (1H, d, J = 8.9 Hz, H-7), 7.64 (1H, d, J = 7.9 Hz, H-11), 7.34 (1H, t, J = 8.0 Hz, H-10), 7.17 (1H, t, J = 7.8 Hz, H-9), 3.96 (3H, s, OMe-2); ^{13}C NMR (DMSO-d_6) δ 147.1 (s, C-2), 141.4 (s, C-3), 139.7 (s, C-11a), 139.2 (s, C-5a), 133.2 (s, C-4a), 132.9 (s, C-12a), 123.8 (d, C-10), 123.7 (s, C-7b), 119.2 (d, C-8), 119.0 (d, C-9), 117.4 (s, C-12c), 116.9 (d, C-7), 115.4 (s, C-7a), 111.2 (d, C-11), 108.8 (d, C-4), 107.7 (s, C-12b), 107.3 (d, C-6), 104.4 (d, C-1), 60.0 (q, OMe-2).

Acknowledgment. The NCI group would like to thank Ken Snader and David Newman (Natural Products Branch) for coordinating collections, John Blunt (University of Canterbury) for the collection of the sponge, Chris Battershill (Australian Institute of Marine Sciences) for the taxonomic identification, Tom McCloud (SAIC-Frederick) for extractions, Roberta Gardella and Jennifer Wilson for anti-HIV assays, and Christophe Marchand and Yves Pommier (Laboratory of Molecular Pharmacology, CCR, NCI) for the HIV-1 integrase inhibition assays. The Victoria University group would like to thank Chris Battershill and Michael Page (National Institute of Water and Atmosphere) for assisting with collections.

Supporting Information Available: MS, ^1H, and ^{13}C NMR spectra of compounds **1**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO020120K