which was used in the subsequent coupling reactions without purification.

Slow addition of **9** (1 equiv over 45 min) to a dichloromethane solution of acetaldehyde (2 equiv) and **1** (0.75 equiv) at 0 °C, followed by workup with 10% (w/v) aqueous sodium tartrate, gave an 8:2:1 mixture of three diastereomers from which the major cross-coupled product (**10**) was isolated by flash chromatography in 41% yield from **8** (Scheme II). An excess of acetaldehyde and **1** has been found to be optimum. Addition of **9** (1 equiv over 45 min) to a solution of **1** (0.5 equiv) and isovaleraldehyde (1.1 equiv) at 25 °C gave a single product (**11**), obtained in 61% yield from **8** (Scheme II). The poor stereoselectivity observed in the acetaldehyde cross-coupling reaction is presumably a consequence of the low steric requirements of the acetaldehyde methyl group.

The dithiolane protecting group in **10** was removed using $Hg(ClO_4)_2(H_2O)_3$[13] in methanol to give a mixture of methyl *N*-Cbz-D-furanosides (**12**) (2:1 at the anomeric carbon) in 95% yield.[14] The overall yield of **12** from *N*-Cbz-L-aspartic acid was 21%. To confirm its relative stereochemistry, compound **12** was transformed into the known *N*-benzoyl-D-3-*epi*-daunosamine (mp 218–220 °C; lit.[9c,15] mp 215–218 °C) in 60% yield (Scheme II). Reaction of **11** with $Hg(ClO_4)_2(H_2O)_3$ in methanol gave methyl *N*-Cbz-D-pyranoside (**13**) in 96% yield (Scheme II).[14,16] The overall yield of **13** from *N*-Cbz-L-aspartic acid was 31%.

The syntheses described above represent a new and stereoselective approach for construction of the 3-amino 1,2-diol unit. The tolerance of **1** toward relatively acidic and reactive functional groups demonstrates that it is a mild but effective reducing agent that will undoubtedly find further applications in cross-coupling reactions involving aldehydes.

Acknowledgment. S.F.P. is grateful to the National Institutes of Health (GM38735), the National Science Foundation for a Presidential Young Investigator Award (Grant No. CHE-8552735), Eli Lilly and Company, and Rohm and Haas Company for financial support.

Supplementary Material Available: A representative cross-coupling procedure, ^1H NMR, ^{13}C NMR, and FAB mass spectra, and elemental analyses data for compounds **2–5** and **8–13** (14 pages). Ordering information is given on any current masthead page.

(13) Fujita, E.; Nagao, Y.; Kaneko, K. *Chem. Pharm. Bull.* **1978**, *26*, 3743.

(14) Assigning furanoside and pyranoside structures to **12** and **13**, respectively, is based on ^1H NMR decoupling experiments on acylated derivatives of these compounds. See the supplementary material for further details.

(15) Fronza, G.; Fuganti, C.; Grasselli, P.; Marinoni, G. *Tetrahedron Lett.* **1979**, 3883.

(16) A crystalline *N*-benzoyl derivative (mp 109–110 °C) of methyl pyranoside **13** was prepared using steps 1 and 2 in the last equation in Scheme II (70% yield).

Antitumor Tetrahydroisoquinoline Alkaloids from the Colonial Ascidian *Ecteinascidia turbinata*

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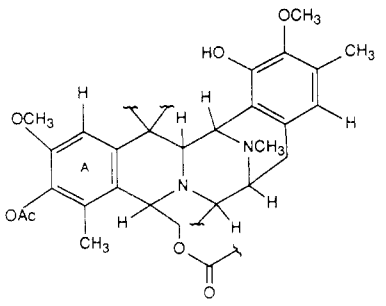
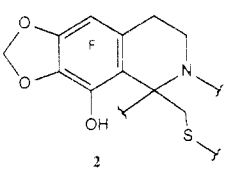
Summary: A bioassay guided approach was used to isolate two antitumor tetrahydroisoquinoline alkaloids, **3** and **4**, from the marine ascidian *Ecteinascidia turbinata*. The structures of **3** and **4** were determined through spectroscopic methods.

The crude aqueous ethanol extracts of the colonial ascidian *Ecteinascidia turbinata* were first reported to possess in vivo antitumor activity by Sigel et al. in 1969.[1] A number of research groups have been working on the isolation of the active constituents of the extract, most notably the researchers at the University of Illinois at Urbana–Champaign led by Professor Kenneth Rinehart.[2] In 1986, they suggested that the active compounds contained three tetrahydroisoquinoline rings.[2a] At a recent meeting[2b] they extended this analysis to partial structures **1** and **2** and suggested that the compounds are related to the safracin class of antitumor antibiotics. As part of our program to discover new antitumor agents, a butanol



1



2

partition of a crude methanol–toluene (3:1) extract of *E. turbinata* (8-V-85-3-9), collected near Ramrod Key in the Florida Keys in May 1985, was found to statistically prolong the life of mice infected with P388 murine leukemia by 45%. The isolation and structure elucidation of the active components was undertaken in 1986, and we now report in this paper our progress toward the structure elucidation of two active constituents of the extract. The compounds were isolated by repeated reversed-phase chromatography. The purification was followed by in vitro bioassay against a P388 murine leukemia tumor cell line. The structures of the compounds were determined through spectroscopic methods and were dependent upon the

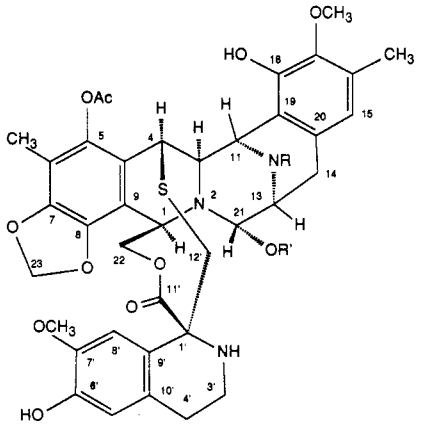
(1) Sigel, M. M.; Wellham, L. L.; Lichter, W.; Dudeck, L. E.; Gargus, J. L.; Lucas, L. H. In *Food-Drugs From the Sea, Proceedings, 1969*; Youngken, H. W., Ed.; Marine Technology Society; Washington DC, 1970; pp 281–295.

(2) (a) Holt, T. G. Ph.D. Dissertation, University of Illinois, Urbana, 1986; *Chem. Abstr.* **1987**, *106*, 193149u. (b) Rinehart, K. L. 30th Ann. Mtg. Am. Soc. Pharmacognosy, San Juan, Puerto Rico, Aug. 6–10, 1989.

0022-3263/90/1955-4508$02.50/0

availability of 2D ^1H detected heteronuclear correlation experiments (HMQC[3] and HMBC[4]), which greatly facilitated the structure elucidation, as many of the critical correlations were not observed in the carbon detected experiments (e.g. HETCOR[5] optimized for 6–10-Hz coupling constants). The structure elucidation was complicated by the low quantities of the compounds present in the ascidian (0.000 16% and 0.000 14% of wet weight, respectively) as well as poor sensitivity in the NMR experiments.

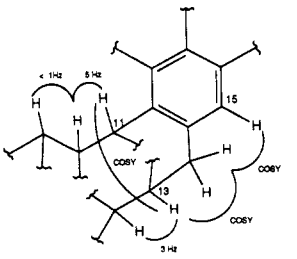
Analysis of the ^1H and ^{13}C NMR and high-resolution positive-ion FABMS data of **3** and **4** suggested the formulas of $C_{38}H_{39}N_3O_{10}S$ (M + H^+ obsd 730.2426, calcd 730.2434) and $C_{39}H_{41}N_3O_{10}S$ (M + H^+ obsd 744.2648, calcd 744.2591) for compounds **3** and **4**, respectively, as previously reported.[2a] The ^1H and ^{13}C NMR spectral data of



	R	R'
3	H	H
4	CH_3	H
13	H	CH_3
14	CH_3	CH_3

3 and **4** (Table I)[6] were very similar, with the major difference being the presence of signals attributable to an *N*-methyl group in **4** (^1H, 2.12, 3 H, s; ^{13}C, 41.3, q). Both compounds have NMR resonances attributable to two aromatic methyls, three isolated aromatic protons, a methylenebis(oxy) group, two methoxy groups, and an acetate.[2b] The molecular formulas suggested by the positive ion FABMS of **3** and **4** require 21 degrees of unsaturation. The presence of resonances attributable to 18 olefinic carbons and 2 carbonyl groups in both **3** and **4** accounts for 11 degrees of unsaturation. As no additional unsaturation was suggested by the NMR spectra, both compounds were presumed to contain 10 rings.

The presence of spin systems **5**–**8** in both **3** and **4** was suggested by interpretation of the spectra obtained from a series of ^1H homonuclear decoupling experiments and 2D COSY spectra. These partial structures were extended to partial structures **9** and **10** based upon chemical shift arguments and the long-range ^1H–^{13}C connectivities which were determined through a series of ^1H detected 2D HMBC experiments. Representative long-range ^1H–^{13}C couplings are given in Table I. No single HMBC experiment allowed us to detect all of the long-range correlations listed in Table I; rather the structures were assembled through a combination of experiments utilizing different solvents.



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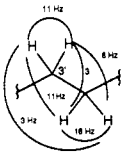

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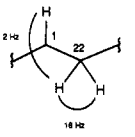

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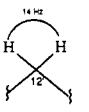

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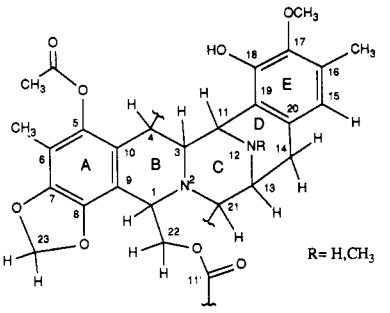
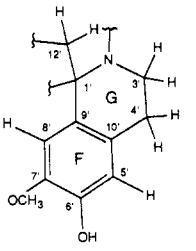


R= H,CH_3

9



10

The aromatic substituents of the E ring were assigned as follows: A methyl group was located on C-16 based upon long-range ^1H–^{13}C correlations observed between H-15 and the 16-CH_3 carbon and between the 16-CH_3 protons and both C-15 and C-16. C-17 was assigned based upon a long-range ^1H–^{13}C correlation observed between the 16-CH_3 protons and C-17. The chemical shift of C-17 suggested oxygen substitution.[7] A methoxy group was attached to C-17 based upon a long-range ^1H–^{13}C correlation observed between the 17-OCH_3 protons and C-17. The position of C-18 was assigned based upon a long-range ^1H–^{13}C correlation observed between H-11 and C-18. Its chemical shift suggested oxygen substitution.[7] The position of C-19 was assigned based upon long-range ^1H–^{13}C correlations observed between C-19 and the following protons: H-3, H-11, H-14ab, and H-15. The position of C-20 was assigned based upon long-range ^1H-^{13}C correlations observed between C-20 and the three protons H-14ab and H-11.

The D ring was closed by insertion of a nitrogen between C-11 and C-13. This was based upon the chemical shifts of C-11 and C-13[7] and the long-range ^1H–^{13}C correlations observed between H-11 and C-13 and H-13 and C-11. In **4**, long-range ^1H–^{13}C correlations were observed between the 12-NCH_3 protons and both C-11 and C-13, which located the *N*-CH_3 group at atom 12.

The A–C rings were elucidated based on the following arguments: The C ring was formed by insertion of a nitrogen between C-3 and C-21 based upon the chemical shifts of C-3 and C-21,[7] as well as the following long-range ^1H–^{13}C correlations: C-21 to H-3, and C-3 to H-21. The position of C-1 with respect to C-21 was deduced from its

(3) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 565–569.
(4) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.
(5) Bax, A.; Morris, G. *J. Magn. Reson.* **1981**, *42*, 501–505.
(6) The chemical shifts reported in Table I are for the methanol-d_4 adducts of **3** and **4** which form immediately upon addition of the NMR solvent.
(7) For examples of ^{13}C NMR chemical shifts in isoquinoline alkaloids, see: Shamma, M.; Hindenlang, D. M. *Carbon-13 NMR Shift Assignments of Amines and Alkaloids*; Plenum Press: New York, 1979; pp 144–185.

Table I. ^1H and ^{13}C NMR Data for 3 and 4[6]

	3[a]		4[c]		
atom no.	^{13}C δ_{mult}[f]	^1H δ (J in Hz)	^{13}C δ_{mult}[f]	^1H δ (J in Hz)	correlations from C no.
1	57.2 d	4.74 bs	56.6 d	4.86 bs	H21,[bcd] H22a[acd]
3	58.8 d	3.64 m	59.6 d	3.66 bd (5)	H21,[e] H11[cd]
4	43.3 d	4.56 bs	43.7 d	4.57 bs	H3,[c] H12′a[b]
5	142.6 s		142.7 s		6-CH_3[e]
6	113.9 s		113.9 s		6-CH_3[e]
7	147.0 s*		146.8 s		6-CH_3,[e] H23[e]
8	141.9 s		141.9 s		H1,[ad] H23a,[bcd] H23b[abd]
9	116.5 s		116.8 s		H1,[e] H22a[e]
10	122.9 s		122.9 s		H1,[abd] H3,[c] H4[d]
11	48.3 d	4.47 d (4)	55.8 d	4.25 dd (5, 1)	H13,[a] H3,[cd] NCH_3[cd]
13	47.2 d	3.72 m	53.9 d	3.40 m	H11,[acd] H14ab,[e] N-CH_3[cd]
14	28.3 t	3.06 m 2 H	24.7 t	2.83 m 2H	H15[e]
15	121.4 d	6.55 s	121.0 d	6.51 s	H14ab,[e] 16-CH_3[e]
16	131.0 s		131.0 s		16-CH_3[e]
17	144.6 s		145.1 s		H15,[e] 16-CH_3,[e] 17-OCH_3[e]
18	148.3 s		150.2 s		H11[e]
19	124.9 s		119.9 s		H3,[acd] H11,[e] H14ab,[e] H15[e]
20	132.9 s		132.4 s		H11,[e] H14ab[e]
21	91.5 d	4.17 d (3)	93.1 d	4.20 d (3)	H1,[abd] H3,[c] H14ab[e]
22	63.1 t	a 5.17 d (11)	61.5 t	a 5.16 d (11)	
		b 4.09 dd (11, 2)		b 4.09 dd (11, 2)	
23	103.4 t	a 6.10 s	103.4 t	a 6.08 s	
		b 6.01 s		b 5.98 s	
1′	65.6 s		65.6 s		H3′a,[abd] H8′,[e] H12′a[a]
3′	40.7 t	a 3.15 ddd (11, 11, 4)	40.7 t	a 3.14 ddd (11, 11, 3)	H4′ab[acd]
		b 2.76 ddd (11, 6, 3)		b 2.78 ddd (11, 6, 3)	
4′	28.9 t	a 2.58 ddd (16, 11, 3)	28.8 t	a 2.61 ddd (16, 11, 6)	H3′ab,[a] H5′[ac]
		b 2.43 m		b 2.43 m	
5′	115.9 d	6.40 s	115.9 d	6.38 s	
6′	146.8 s*		146.8 s		H8′[e]
7′	146.7 s*		146.8 s		H5′,[e] 7′-OCH_3[e]
8′	111.7 d	6.45 s	111.7 d	6.44 s	
9′	126.0 s		126.0 s		H4′ab,[acd] H5′,[e] H8′[cd]
10′	129.3 s		129.3 s		H3′ab,[a] H4′ab,[e] H8′[e]
11′	173.6 s		173.5 s		H22ab,[e] H12′b[abd]
12′	43.1 t	a 2.44 m	43.2 t	a 2.37 d (14)	
		b 2.04 m		b 2.02 d (14)	
5-OAc	170.2 s		170.2 s		5-OAc methyl[e]
	20.5 q	2.30 s 3 H	20.5 q	2.29 s 3 H	
6-CH_3	9.8 q	2.04 s 3 H	9.7 q	2.02 s 3 H	
16-CH_3	16.1 q	2.28 s 3 H	16.0 q	2.28 s 3 H	H15[e]
17-OCH_3	58.8 q	3.73 s 3 H	60.3 q	3.73 s 3 H	
7′-OCH_3	55.8 q	3.58 s 3 H	55.9 q	3.57 s 3 H	
12-NCH_3			41.3 q	2.12 s 3 H	H11,[cd] H13[cd]

*Assignments may be interchanged; [a] 3 in methanol-d_4. [b] 3 in methanol-d_4–TFA (5:1). [c] 4 in methanol-d_4. [d] 4 in methanol-d_4–CD_2Cl_2 (1:3). [e] Correlation observed under all conditions run. [f] Carbon multiplicities were measured using the DEPT sequence.

chemical shift and the following long-range ^1H–^{13}C correlations: C-1 to H-21, and C-21 to H-1. H-1 also had long-range ^1H–^{13}C correlations to C-8, C-9, and C-10, defining it as adjacent to an aromatic ring. C-4 was located benzylic to the A ring based upon the long-range ^1H–^{13}C correlations observed between C-10 and both H-3 and H-4, thus completing the B ring. The substituents of the A ring were assigned as follows: The chemical shifts of C-7 and C-8 both argue for oxygen substitution.[7] Long-range ^1H–^{13}C correlations were observed between the methylenebis(oxy) protons, H-23ab, and the carbons C-8 and C-7, which located the methylenebis(oxy) functionality on ring A. A methyl group has been located at C-6 due to a long-range ^1H–^{13}C correlation observed between the 6-CH_3 protons and C-7.[8] The 6-CH_3 protons also have long-range ^1H–^{13}C correlations to C-6 and C-5 which suggested the positions of these two carbons. The chemical shift of C-5 suggests oxygen substitution.[7] The acetate was assigned to C-5 based upon the observation of an NOE enhancement of the acetate methyl protons when H-4 is irradiated. The position of C-9 was assigned based upon long-range ^1H–^{13}C correlations observed from C-9 to both H-22a and H-1. The position of C-10 was assigned based upon a long-range correlation observed between C-10 and H-1. Long-range ^1H–^{13}C correlations observed between H-22ab and C-11′ suggested the position of the remaining ester carbon, C-11′. This completes the assignment of all atoms in partial structure **9**.

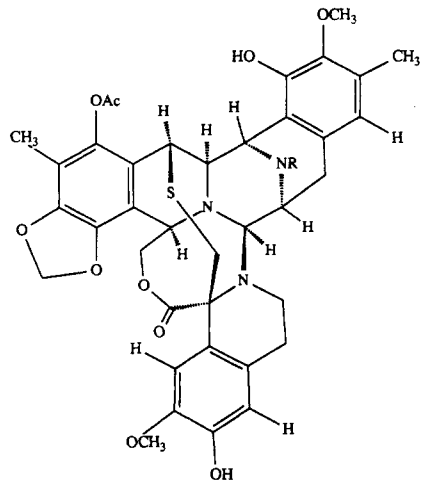
The third isoquinoline ring system, **10**, was put together as follows: C-4′ was assigned as benzylic due to the following long range ^1H–^{13}C correlations: H-3′ab to C-10′; H-4′ab to both C-9′ and C-10′. C-5′ was located based upon a long-range ^1H–^{13}C correlation observed between H-5′ and the carbons C-4′ and C-9′. The position of H-8′ was assigned based upon long-range correlations observed between H-8′ and the carbons C-9′ and C-10′. The position of C-6′ was assigned based upon a long-range ^1H–^{13}C correlation observed between C-6′ and H-8′. The chemical shift of C-6′ suggests oxygen substitution.[7] The position of C-7′ was assigned based upon a long-range ^1H–^{13}C correlation observed between C-7′ and H-5′. A long-range correlation between the 7′-OCH_3 protons and C-7′ located a methoxy group at this position. This was confirmed by

(8) The chemical shifts of the methyl carbons observed at ≈9.7 ppm suggest that they are sterically shielded due to diortho oxygen substitution. See, for example: Levy, G. C. *Carbon-13 Nuclear Magnetic Resonance Spectroscopy*; John Wiley and Sons: New York, 1980; p 107.

observation of an NOE between the 7′-OCH_3 and H-8′. The position of C-1′ was assigned based upon its chemical shift[7] and long-range ^1H–^{13}C correlations observed between C-1′ and both H-3′a and H-8′. A nitrogen was inserted between C-3′ and C-1′, closing the G ring based upon chemical shift arguments[7] and the C-1′ to H-3a′ long-range correlation. A bond was drawn between C-12′ and C-1′ based upon a long-range ^1H–^{13}C correlation observed between H-12′a and C-1′.

Only connections between atoms C-4, C-21, C-1′, C-11′, C-12′, N-2′, and sulfur remained to complete the structures. Long-range correlations were observed between H-12′a and both C-4 and C-1′ which suggested that the methylene group must bridge these two carbons. No coupling was observed between the methylene protons, H-12′ab, and the methine proton, H-4, in either ^1H–^1H decoupling or homonuclear COSY experiments, which suggested that C-4 and C-12′ are not vicinal. Insertion of sulfur between the two carbons accounted for both the chemical shifts[9] of C-4 and C-12′ as well as the long-range correlation observed between H-12′a and C-4. A bond has been drawn between C-1′ and C-11′ based upon a long range ^1H–^{13}C correlation observed between H-12′b and C-11′.

The remaining connection between N-2′ and C-21 was made to satisfy the molecular formula determined by positive ion FABMS. The chemical shift of C-21 (91.5 and 93.1 in methanol-d_4 for **3** and **4**, respectively[10]) argues for diheteroatom substitution. The best fit for the chemical shift of C-21 is achieved by substitution with both oxygen and nitrogen rather than two nitrogens, but all of the oxygens required by the molecular formula have been accounted for, leaving only N-2′ to be attached to C-21. Therefore, C-21 should be dinitrogen substituted and vicinal to N-2′, yielding structures **11** and **12**.[11] A similar chemical shift (91.6 ppm) is observed for an sp^3 carbon bearing two nitrogen substituents in flustramine b, isolated from the bryozoan *Flustra foliacea*.[12] Unfortunately, no confirmatory long-range carbon–proton couplings between H-21 and the carbons of the G ring or between H-3′ab and C-21 were observed. Structures **11** and **12** were proposed by us at a recent conference on marine natural products.



11 R = H
12 R = CH_3

While this paper was under review, it came to our attention that the Illinois group observed an ion at m/z 760 for ecteinascidin 743 under negative ion FABMS conditions suggesting that the "molecular ion" observed in the positive ion mass spectrum is for the $M^+ - H_2O$ ion rather than the true molecular ion.[13] Subsequently, the Illinois group has reassigned the substituents on rings A and F in their partial structures **1** and **2**, and made the additional atom connections to form analogues of our structures **11** and **12**. They have placed the additional oxygen provided by the negative ion FABMS onto C-21, which is consistent with the previously observed chemical shifts of safracin b and saframycin s which have hydroxyl functionality at C-21.[14] They also report that the compounds react rapidly with methanol to form the methoxy derivatives **13** and **14** with a subsequent shift of C-21 from 83 to 91.8 ppm for ecteinascidin 743. This value is consistent with our ^{13}C NMR data for C-21, which were all run in the presence of methanol-d_4 except in one instance where acetone-d_6 was used as the solvent. Negative ion mass spectral analysis of **3** and **4** isolated by our group showed that they contain an additional molecule of methanol (when solubilized for mass spectral analysis in methanol). When dried under vacuum followed by addition of matrix, **4** yields a cluster of ions at m/z 760/761/762 consistant with $C_{39}H_{42}N_3O_{11}S$. Under all conditions employed, only the methanol displacement product of **3** was observed as a cluster of ions at m/z 760/761/762. In order to confirm the proposed displacement of the hydroxy by methanol at C-21, we have carried out a number of 2D HMQC and HMBC correlation experiments in $CDCl_3$ or CD_2Cl_2 containing 50–100 μL of protonated methanol. New methoxy carbons appear at 55.0 and 55.3 ppm in the carbon spectra of **3** and **4**, respectively, but no correlations were observed which would define the position of the new methoxy group. To try and improve signal to noise, **3** was analyzed in $CDCl_3$ containing 100 μL of [^{13}C]methanol. In this instance, we observed rapid incorporation of [^{13}C]methanol (^{13}C: 56.9 ppm, q), but no long-range correlations between H-21 and the new methyl were observed. A selective inept experi-

(9) (a) Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. *Tables of Spectral Data for Structure Determination of Organic Compounds*; Springer-Verlag: Berlin, 1983; p C10; (b) p C125.

(10) This carbon is observed at 82.3 ppm in the DEPT spectrum of **4** when run in acetone-d_6 (in which it is sparingly soluble (≈1 mg/mL)).

(11) Other possible structures which were considered had both oxygen and nitrogen substitution on C-21 as found in safracin b, saframycin s, and renieramycin e. C-21 in safracin b and saframycin s was observed at 83.3 and 81.6 ppm, respectively,[14] which is similar to the chemical shift we observed in acetone-d_6 for this carbon. It was reported that renieramycin e was too labile to record its ^{13}C NMR spectrum.[15] Given the molecular formulas of **3** and **4** determined by positive ion FABMS, if C-21 is to be substituted with oxygen, one of the oxygens attached to an aromatic carbon in structures **11** and **13** must be used and therefore the carbon would have to be substituted with nitrogen or sulfur rather than oxygen. The HMBC data clearly shows that C-17 and C-7′ bear methoxy groups while C-7 and C-8 bear the methylenebis(oxy) functionality. C-6′ was demonstrated to bear oxygen as follows: Reaction of **3** with diazomethane in methanol yielded a derivative in which two new methoxy signals were observed in its proton NMR spectrum. One of the new methoxys shows an NOE enhancement when H-5′ is irradiated, which demonstrates that C-6′ in **3** is phenolic. The two remaining oxygens are attached to C-18 (observed at 150-148 ppm) and C-5 (observed at 142 ppm). Of these, the chemical shift of C-5 is closer to that expected for an aromatic carbon bearing a nitrogen or sulfur.[9b] NOEs observed between the protons of the acetate and both H-4 and the 6-CH_3 protons suggest that C-5 bears an acetate lending support to structures **11** and **12**. Attempts to determine the lowest energy conformation of a compound in which N-2′ was attached to C-5 while the acetate is attached to C-21 using PCMODEL 3.2 (Serena Software, Box 3076, Bloomington, IN) failed to yield reasonable bond lengths and free energies for such a compound. An alternate structure in which N-2′ is attached to C-4 and the sulfur of the methylenethio bridge is attached at C-5 was ruled out based upon the long-range correlations observed between H-12′a and C-4 and the chemical shift of C-5.

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ment carried out on 4, in $CDCl_3$ containing 30 μL of [^{13}C]methanol, revealed a long-range coupling between H-21 and the new [^{13}C]methoxy carbon, observed at 53.3 ppm, confirming substitution of methoxy at C-21. Pure 4 was supplied to the Illinois group for direct comparison with ecteinascidin 743. The proton NMR, positive ion FABMS, TLC, and HPLC data strongly suggest that the compounds are identical. The major difference is the relative solubility of the compounds. Our compounds exhibit only limited solubility in $CDCl_3$ (≈2–3 mg/mL) requiring addition of methanol for solubilization while the Illinois group's material appears to be very soluble in $CDCl_3$. In conclusion, the data suggests that the reassignment of our proposed structures 11 and 12 to 3 and 4, respectively, by Rinehart et al.[13] is consistent with the observed spectral data and that 3 and 4 are ecteinascidins 729 and 743 or salts thereof.

The relative stereochemistry of rings A–E was found to be the same as that reported for the structurally related renieramycins[15] and saframycins[14c] through a series of ^{1}H–^{1}H NOE experiments. A strong nuclear Overhauser enhancement of H-12′a was observed upon irradiation of H-8′. Models of other possible structures did not account for this enhancement. We are currently attempting to grow crystals suitable for X-ray diffraction studies to confirm the proposed structures of 3 and 4.

Compounds 3 and 4 are structurally related to the safracins, saframycins, and renieramycins. Both the safracins and saframycins are microbial fermentation products. Experiments to locate a microbial source for the ecteinascidins are in progress. Compounds 3 and 4 are potent in vitro inhibitors of P388 murine leukemia with IC_{50}'s of 0.93 and 1.3 ng/mL, respectively. A mixture of the two compounds (≈1:1) was found to be active in vivo against B-16 murine melanoma (T/C = 188% 0.1 mg/kg QD 1-9[16]) and colon carcinoma 26 (T/C = 147 0.1 mg/kg QD 1-9).

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Supplementary Material Available: Experimental procedure for isolation of compounds 3 and 4, proton and carbon NMR data for 3 and 4, and proton and carbon NMR spectra for 3 and 4 (14 pages). Ordering information is given on any current masthead page.

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Ecteinascidins 729, 743, 745, 759A, 759B, and 770: Potent Antitumor Agents from the Caribbean Tunicate *Ecteinascidia turbinata*[1]

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Summary: Ecteinascidins 729, 743, 745, 759A, 759B, and 770, tris(tetrahydroisoquinolines) with potent in vivo antitumor activity, have been isolated from the colonial tunicate *Ecteinascidia turbinata*, and their structures have been assigned.

Reports of the potent in vivo efficacy of extracts of the Caribbean tunicate *Ecteinascidia turbinata* date back to 1969, when it was reported that such extracts gave T/C to 272 vs P388 murine leukemia, with four of six cures in one experiment.[5] The extracts were also powerful immunomodulators, but repeated attempts to isolate the compounds responsible for either activity were unsuccessful.[6] Our own concerted efforts to identify the compounds began in 1981, shortly after the Alpha Helix Caribbean Expedition 1978,[7] where a sample of *E. turbinata* showed cytotoxicity in shipboard assays. These efforts culminated in the isolation by 1986 of six compounds—ecteinascidins (Et's) 729, 743, 745, 759A,

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