Ecteinascidins 770 and 786 from the Thai Tunicate *Ecteinascidia thurstoni*

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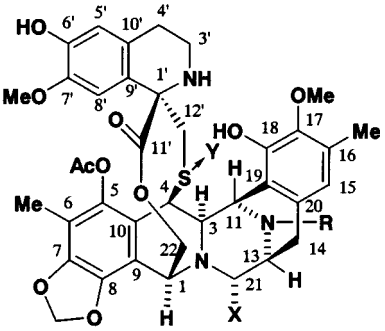
Ecteinascidins 770 (**1b**) and 786 (**3b**) were isolated from the pretreated Thai tunicate *Ecteinascidia thurstoni* with potassium cyanide in buffer solution (pH 7). These structures were fully elucidated by extensive 2D NMR analysis.

Ecteinascidin 743 (**1a**) is a novel marine natural product obtained from the Caribbean tunicate *Ecteinascidia turbinata*, which was discovered along with ecteinascidin 729 (**2**) and related compounds (Figure 1).[1] Among this group, **2** is generally the most cytotoxic compound, and it was initially planned that **2** would advance to clinical trials. The choice was switched to **1a**, however, due to its greater abundance in the tunicate. Now, **1a** is undergoing phase II clinical trials for cancer therapy.[2] The combination of unique structural features and the high degree of functionalization of **1a** presents a formidable challenge to the synthetic chemist. The first elegant total synthesis of **1a** and an improvement of the original synthesis were described by Corey and co-workers.[3] In addition, an efficient process for the preparation of **1a** starting from cyanosafracin B, which is readily prepared from microbial safracin B, was recently described by the Pharma Mar Laboratories group.[4] With an aim to elucidate the structure−activity relationships, Corey, Schreiber, and co-workers discovered phthalascidin, which is easily made from a synthetic intermediate and is considerably more stable in solution than **1a**.[5,6]

As a part of our search for new anticancer metabolites in the isolation and characterization of biologically active compounds from Thai marine animals, we found a Thai tunicate, *Ecteinascidia thurstoni* Herdman 1891, which is the first example of the Asian tunicate contained this class of molecules, growing around Phuket Island. We now report the isolation and structural elucidation of ecteinascidin 770 (**1b**) and ecteinascidin 786 (**3b**) from the KCN-pretreated Thai tunicate. Compound **1b** was discovered in very small amounts from the Caribbean tunicate, and ecteinascidin 786 (**3b**) is a derivative of ecteinascidin 759B (**3a**).[7]

The tunicate *Ecteinascidia thurstoni* was collected by scuba divers at Phuket Island at a depth of 1−5 m during March and July 2000. Initial attempts to extract the alkaloid were performed on 50 g of wet animal using the Rinehart procedure, but there were only trace amounts of alkaloid in the crude fraction. Natural products that have an α-hydroxyamine functionality such as **1a** are relatively unstable during extraction. We attempted to stabilize **1a** by converting it to **1b** by addition of potassium cyanide to the homogenized tunicate during workup.[8] Phosphate buffer solution was added to the homogenized samples of frozen tunicate (38 kg, wet weight) until a pH of 7 was



ecteinascidins
743 (1a): R = Me, X = OH, Y = none
729 (2): R = H, X = OH, Y = none
759B (3a): R = Me, X = OH, Y = O
770 (1b): R = Me, X = CN, Y = none
786 (3b): R = Me, X = CN, Y = O

Figure 1.

achieved, and then potassium cyanide solution was added and the mixture was allowed to stand for 5 h and was further macerated with methanol. The extract was evaporated to an aqueous emulsion, which was partitioned with ethyl acetate. The organic layer was evaporated to dryness and then partitioned again between methanol and hexane. The methanol layer extract was concentrated, and the residue was further separated by chromatography on a Sephadex LH-20 column to afford a pale yellow solid. The pale yellow solid was subjected to chromatography on a silica gel column with 1:2 hexane−ethyl acetate to afford ecteinascidin 770 (**1b**, 225 mg, 6.0×10^{-4}% of wet weight). Further elution with 1:4 hexane−ethyl acetate afforded ecteinascidin 786 (**3b**, 60 mg, 1.6×10^{-4}% of wet weight).

Ecteinascidin 770 (**1b**) was obtained as colorless prisms, mp 216−218 °C (from methanol). High-resolution FABMS of **1b** demonstrated a molecular composition of $C_{40}H_{43}N_4O_{10}S$ $(M + H)^+$ by observation of the peak at a *m*/*z* 771.2693 (Δ −0.7 mmu) and the elemental analysis. The ^1H chemical shifts and proton coupling patterns of this compound were identical with those reported by Rinehart and co-workers. However, the lack of published data for **1b**, particularly ^{13}C NMR data, frustrated our attempts to identify our sample by simple comparison of NMR data.[1b] Therefore, extensive analyses of spectral data were necessary to confirm the structure. The IR spectrum of the KBr disk had characteristic absorption bands such as 3530, 3500, 3350, 2930, 1770, 1740, and 1630 cm^{-1}, but there was only

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a very weak band of the cyano group in the region 2100−2300 cm^{-1}.[9] To confirm the incorporation of a cyano group at the C-21 position including the stereochemistry, HMBC, HMQC, and NOESY experiments of **1b** allowed for the unambiguous assignment of almost all of the quaternary carbons and confirmed the proposed structure. The ^{13}C NMR spectral data of **1b** and **1a** revealed only two major differences. The carbinolamine-containing carbon of **1b** (C-21) shifted from 92.1 to 59.6 ppm, and an additional carbon resonance was present at 118.2 ppm.[10] The optical rotation of **1b** was −58.5 ° (*c* 1.0, $CHCl_3$).[11,12]

The new compound **3b** was obtained as colorless prisms, mp 197−199 °C (from methanol), and exhibited $[\alpha]_D^{24}$ −156.9° (*c* 0.6, $CHCl_3$). Its structure was elucidated predominantly by interpretation of NMR and MS data and comparison to spectral data for **1b**. High-resolution FABMS of **3b** demonstrated the molecular composition of $C_{40}H_{43}N_4O_{11}S$ $(M + H)^+$ by observation of the peak at *m/z* 787.2649 (Δ +0.3 mmu). Therefore, it might be the oxygenated analogue of **1b**. All protons and carbons were assigned by extensive NMR measurements (including COSY, NOESY, HMQC, and HMBC techniques). The major differences in the ^{13}C NMR spectral data of **3b**, in comparison to those of **1b**, were in the downfield shifts of C-4 and a methylene C-12′. Furthermore, the ^1H NMR spectral data of **3b** also revealed the distinct upfield shift of H-4 and downfield shift of H_2-12′, comparable to those of **1b**. These observations suggested that the sulfur atom between C-4 and C-12′ might be oxidized. An NOE between H-14*β* and H-21 revealed the relative stereochemistry at C-21. Thus, the structure of ecteinascidin 786 was deduced to be ecteinascidin 770 *S*-oxide.[7]

Isolation of marine natural products using this procedure has the advantage of increasing the yield of labile ecteinascidin-type compounds. Actually, **1b** was easily transformed to **1a** in high yield with silver nitrate,[13] and the transformation product was identical with authentic data in all respects.

Compounds **1b** and **3b** have potent cytotoxic activity, exhibiting IC_{50} values of 2.5 and 7.6 nM against the breast cancer cells (BC) and of 0.034 and 0.15 *μ*M against nasopharynx carcinoma cells (KB), respectively. Moreover, **1b** and **3b** showed antitubercular activity against *Mycobacterium tuberculosis* H37Ra at MIC of 0.13 and 2.0 *μ*M, respectively. More details of other biological activities of these compounds, evaluation of the antitumor activity, and the isolation and structure elucidation of other minor components are currently in progress.[16]

Experimental Section

General Experimental Procedures. CD was obtained on a JASCO J-720WI. Optical rotations were measured on a Horiba-SEPA. IR spectra were obtained on a Hitachi 260-10. Melting points determined with a Yanagimoto micromelting point apparatus are uncorrected. ^1H and ^{13}C NMR spectra were recorded at 500 and 125.65 MHz, respectively, on a JEOL JNM-LA-500 FT-NMR spectrometer and at 300 and 75 MHz, respectively, on a Bruker Avance DPX-300 FT-NMR spectrometer (ppm, *J* in Hz with TMS as internal standard). Elemental analysis: Perkin-Elmer Model 240B. TLC: precoated Kieselgel 60 F_{254} plates (Merck, 0.25 mm thick). Column chromatography: Kieselgel 60 [Merck, 70−230 (for open chromatography) and 230−400 mesh (for flash chromatography)]. Sephadex LH-20 [Pharmacia Biotech].

Animal Material. The tunicate was identified by T. Nishikawa of the Nagoya University Museum as *Ecteinascidia thurstoni* Herdman 1981.[14,15]

Extraction and Isolation. The tunicate *Ecteinascidia thurstoni* Herdman 1891 was collected by scuba divers on the east coast of Phuket Island at a depth of 1−5 m during March and July 2000 and frozen until used. Freeze-dried animals (38 kg, wet weight) were homogenized in a blender with methanol. Phosphate buffer solution was added to the resulting homogenized solution (40 L) to a pH of 7. Potassium cyanide (25 g) was added, and the mixture was allowed to stand for 5 h. Afterward the mixture was macerated with methanol (3 × 20 L). The extract was filtered, and the filtrate was concentrated. The aqueous methanol extract (2 L) was partitioned by the addition of ethyl acetate (8 L), and the solvent was removed to obtain a residue (20 g), which was dissolved in methanol (150 mL) and partitioned with hexane (600 mL). The methanol extract was concentrated to give a residue (12 g), which was successively chromatographed three times on Sephadex LH-20 columns using chloroform−methanol (1:1), ethyl acetate, and hexane−ethyl acetate (9:1) as the eluents to obtain a mixture of ecteinascidins 770 and 786. This mixture was further separated on a silica gel column using hexane−ethyl acetate (1:2 to 1:4) as eluent to give ecteinascidins 770 (**1b**, 225 mg) and 786 (**3b**, 60 mg).

Ecteinascidin 770 (1b): colorless prisms from methanol, mp 216−218 °C (dec); $[\alpha]^{24}_D$ −58.5 (*c* 1.0, $CHCl_3$); CD Δϵ nm (*c* 0.13 mmol/L, methanol, 24 °C) 0 (306), −12.0 (289), 0 (270), +12.8 (254), 0 (242), −51.0 (221), 0 (211), +39.4 (295); ^1H NMR ($CDCl_3$) *δ* 6.60 (1H, s, 15-H), 6.46 (1H, s, 5′-H), 6.44 (1H, s, 8′-H), 6.04 (1H, d, *J* = 1.2 Hz, OCHO), 5.97 (1H, d, *J* = 1.2 Hz, OCHO), 5.77 (1H, s, 18-OH), 5.59 (1H, br s, 6′-OH), 5.01 (1H, d, *J* = 11.6 Hz, 22-H*β*), 4.57 (1H, br s, 4-H), 4.32 (1H, br s, 1-H), 4.28 (1H, dd, *J* = 4.9, 1.2 Hz, 11-H), 4.18 (1H, d, *J* = 2.8 Hz, 21-H), 4.12 (1H, dd, *J* = 11.6, 2.0 Hz, 22-Hα), 3.78 (3H, s, 17-OCH_3), 3.60 (3H, s, 7′-OCH_3), 3.51 (1H, d, *J* = 4.9 Hz, 3-H), 3.41 (1H, m, 13-H), 3.11 (1H, br t, 3′-Hα), 2.91 (2H, br d, *J* = 7.6 Hz, 14-H_2), 2.79 (1H, m, 3′-H*β*), 2.60 (1H, m, 4′-H*β*), 2.42 (1H, dt, *J* = 15.9, 3.4 Hz, 4′-Hα), 2.35 (1H, br, 12′-H)*, 2.32 (3H, s, 16-CH_3), 2.26 (3H, s, 5-$OCOCH_3$), 2.19 (3H, s, 12-CH_3), 2.15 (1H, m, 12′-H)*, 2.04 (3H, s, 6-CH_3) (* the signal overlapped with the methyl signal); ^{13}C NMR ($CDCl_3$) *δ* 172.6 (s, 11′-C), 168.2 (s, 5-*O*C*O*CH_3), 147.9 (s, 18-C), 145.3 (s, 7-C), 144.6 (s, 6′-C), 144.4 (s, 7′-C), 143.1 (s, 17-C), 141.4 (s, 5-C), 140.2 (s, 8-C), 130.8 (s, 20-C), 129.4 (s, 16-C), 129.2 (s, 10′-C), 125.8 (s, 9′-C), 121.2 (s, 10-C), 120.7 (d, 15-C), 118.4 (s, 19-C), 118.7 (s, 21-CN), 114.2 (d, 5′-C), 114.1 (s, 9-C), 113.4 (s, 6-C), 109.9 (d, 8′-C), 102.0 (t, OCH_2O), 64.6 (s, 1′-C), 61.2 (d, 1-C), 60.4 (q, 17-OCH_3), 60.1 (t, 22-C), 59.7 (d, 3-C), 59.6 (d, 21-C), 55.2 (q, 7′-OCH_3), 54.8 (d, 11-C), 54.7 (d, 13-C), 42.3 (t, 12′-C), 41.9 (d, 4-C), 41.6 (q, NCH_3), 39.7 (t, 3′-C), 28.8 (t, 4′-C), 24.2 (t, 14-C), 20.4 (q, 5-OCO*C*H_3), 15.8 (q, 16-CH_3), 9.7 (q, 6-CH_3); IR (KBr) 3530, 3500, 2940, 2250w, 1770, 1740, 1630, 1595, 1520, 1478 cm^{-1}; HR-FABMS *m/z* 771.2693 (calcd for $C_{40}H_{43}N_4O_{10}S$ $[M + H]^+$ 771.2700); *anal.* C 62.33%, H 5.49%, N 7.27%, calcd for $C_{40}H_{42}N_4O_{10}S$, C 62.00%, H 5.48%, N 6.90%.

Ecteinascidin 786 (3b): colorless prisms from methanol, mp 197−199 °C (dec); $[\alpha]^{24}_D$ −156.9 (*c* 0.6, $CHCl_3$); CD Δϵ nm (*c* 0.13 mmol/L, methanol, 24 °C) 0 (314), −13.0 (291), −2.3 (247), −60.1 (223), 0 (214), +70.6 (207); ^1H NMR ($CDCl_3$) *δ* 6.65 (1H, s, 15-H), 6.49 (1H, s, 5′-H), 6.37 (1H, s, 18-OH), 6.20 (1H, s, 8′-H), 6.06 (1H, d, *J* = 1.2 Hz, OCHO), 6.02 (1H, d, *J* = 1.2 Hz, OCHO), 5.49 (1H, br s, 6′-OH), 4.67 (1H, dd, *J* = 4.3, 1.2 Hz, 11-H), 4.60 (1H, d, *J* = 11.6 Hz, 22-H*β*), 4.31 (1H, d, *J* = 2.1 Hz, 1-H), 4.31 (1H, dd, *J* = 11.6, 2.1 Hz, 22-Hα), 4.18 (1H, br s, 4-H), 4.07 (1H, d, *J* = 2.8 Hz, 21-H), 3.85 (3H, s, 17-OCH_3), 3.74 (1H, dd, *J* = 4.3, 2.8 Hz, 3-H), 3.66 (1H, d, *J* = 14.3 Hz, 12′-H*β*), 3.63 (3H, s, 7′-OCH_3), 3.43 (1H, ddd, *J* = 9.2, 2.8, 1.2 Hz, 13-H), 3.01 (1H, dd, *J* = 17.7, 9.2 Hz, 14-Hα), 2.99 (1H, ddd, *J* = 12.2, 8.2, 4.0 Hz, 3′-Hα), 2.86 (1H, ddd, *J* = 12.2, 5.2, 4.9 Hz, 3′-H*β*), 2.70 (1H, d, *J* = 17.7 Hz, 14-H*β*), 2.62 (1H, ddd, *J* = 15.9, 8.2, 4.9 Hz, 4′-H*β*), 2.46 (1H, ddd, *J* = 15.9, 5.2, 4.0 Hz, 4′-Hα), 2.31 (3H, s, 16-CH_3), 2.27 (3H, s, 5-$OCOCH_3$), 2.24 (3H, s, 12-CH_3), 2.19 (1H, d, *J* = 14.3 Hz, 12′-Hα)*, 2.07 (3H, s, 6-CH_3) (* the signal overlapped with the methyl signal); ^{13}C NMR ($CDCl_3$) *δ* 171.8 (s, 11′-C), 168.9 (s, 5-*O*C*O*CH_3), 148.0 (s, 18-C), 147.0 (s, 17-C), 146.1 (s, 7-C), 145.0 (s, 6′-C), 144.7 (s, 7′-C), 142.2 (s, 5-C), 140.7 (s, 8-C), 130.6 (s, 16-C), 129.5 (s, 10′-C), 129.4 (s, 20-C), 124.5 (s, 9′-C),

122.6 (d, 15-C), 120.3 (s, 10-C), 120.2 (s, 19-C), 117.6 (s, 21-CN), 114.5 (d, 5′-C), 114.1 (s, 6-C), 111.6 (s, 9-C), 109.4 (d, 8′-C), 102.3 (t, OCH_2O), 71.0 (d, 4-C), 67.7 (t, 12′-C), 61.8 (t, 22-C), 61.6 (s, 1′-C), 60.7 (d, 1-C), 60.6 (d, 21-C), 60.4 (q, 17-OCH_3), 60.0 (d, 3-C), 55.2 (q, 7′-OCH_3), 54.9 (d, 11-C), 54.6 (d, 13-C), 41.8 (q, NCH_3), 39.9 (t, 3′-C), 29.1 (t, 4′-C), 24.9 (t, 14-C), 20.8 (q, 5-$OCOCH_3$), 16.0 (q, 16-CH_3), 10.1 (q, 6-CH_3); IR (KBr) 3430, 2930, 2250w, 1760, 1730, 1620, 1590, 1510, 1460 cm^{-1}; HR-FABMS *m*/*z* 787.2652 (calcd for $C_{40}H_{43}N_4O_{11}S$ $[M + H]^+$ 787.2649).

Transformation of 1b to 1a. Ecteinascidin 770 (**1b**, 7.7 mg, 0.01 mmol) was dissolved in a mixture of acetonitrile and water [3:2 (v/v), 2.5 mL], and silver nitrate (34 mg, 0.3 mmol, 30 equiv) was added to this solution. The suspension was stirred at room temperature for 11 h. A mixture of saturated aqueous sodium chloride solution and saturated aqueous sodium bicarbonate solution [1:1 (v/v), 6.25 mL] was added, and the mixture was stirred vigorously at room temperature for 15 min. The reaction mixture was extracted with dichloromethane (3 × 20 mL), and the combined organic layers were dried with sodium sulfate and filtered through a pad of cellulose powder. The filtrate was concentrated to give ecteinascidin 743 (**1a**, 7.2 mg, 96.9%) as a colorless solid, which was identical in all respects with that of the authentic sample.

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Supporting Information Available: Color pictures of *Ecteinascidia thurstoni* Herdman 1891 are available free of charge via the Internet at http://pubs.acs.org.

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(8) In our previous research on the extraction and isolation of saframycin antibiotics, there was an inherent difficulty in the development of these satellite antibiotics for chemotherapeutic agents because of their extremely low production in culture. A satisfactory way of circumventing the low production level, however, was found quite unexpectedly with the observation that cyanide was directly incorporated in the cyano residue of saframycin A; see: Arai, T.; Takahashi, K.; Nakahara, S.; Kubo, A. *Experientia* **1980**, *36*, 1025−1027; Arai, T.; Takahashi, K.; Ishiguro, K.; Yazawa, K. *J. Antibiot*. **1980**, *33*, 951−960. And also see quinocarcin: Saito, H.; Hirata, T. *Tetrahedron Lett*. **1987**, 35, 4065−4068. Cyanocycline A: Zmijewski, Jr. M. J.; Goebel, M. *J. Antibiot*. **1982**, *35*, 524−526. Gould, S. J.; He, W.; Cone, M. C. *J. Nat. Prod*. **1993**, *56*, 1239−1245.

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(10) These spectral changes are consistent with those reported for saframycins A and S, and naphthyridinomycin and cyanocycline A.[8]

(11) The CD spectra of **1b** (from the Thai tunicate) and **1a** (from the Caribbean tunicate) displayed almost superimposable curves. Therefore, these compounds have the same absolute confirguration.

(12) Rinehart reported the optical rotation of **1b** (from the Caribbean tunicate), $[\alpha]^{25}_D$ +52° (*c* 0.1, methanol), in contrast with that of **1b** (synthetic compound), $[\alpha]^{20}_D$ −35° (*c* 0.1, CH_2Cl_2).

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(16) T. Fukuyama of the University of Tokyo has just finished the total synthesis of (−)-ecteinascidin 770, and the synthetic sample has been identified with our natural one in all respects; see: Endo, A.; Yanagisawa, A.; Abe, M.; Tohma, S.; Kan, T.; Fukuyama, T. *J. Am. Chem. Soc.*, in press. We thank T. Fukuyama for communicating the results prior to publication.

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