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TWO NEW DIBROMOTYROSINE DERIVATIVES FROM THE CARIBBEAN SPONGE *PSEUDOCERATINA CRASSA*

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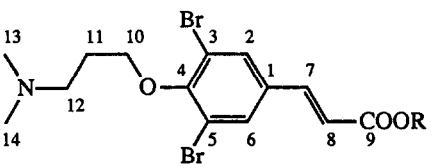
Abstract· Two novel dibromotyrosine derivatives were isolated from the Caribbean sponge *Pseudoceratina crassa* (Hyatt). The structures of ethyl 3,5-dibromo-4-(3'-N,N-dimethylaminopropyloxy)cinnamate (**1**) and 3,5-dibromo-4-(3'-N,N-dimethylaminopropyloxy)cinnamic acid (**2**) were proposed on the basis of spectroscopic evidence and were confirmed by synthesis

Marine invertebrates provide a wealth of secondary metabolites with interesting biological properties,[1] many of which point to potential pharmaceutical applications Our studies of the chemistry of marine sponges are therefore guided by bioassays for antimicrobial activity and cytoxicity The ethanolic extract of a Caribbean specimen of the Verongid sponge *Pseudoceratina crassa* (Hyatt) exhibited significant cytotoxicity, antifungal activity, and antibacterial activity against both Gram positive and Gram negative bacteria The bioactivity was associated with several UV-absorbing compounds. We wish to report the structural elucidation and synthesis of two new dibromotyrosine derivatives (**1**) and (**2**), compounds typical of sponges of the order Verongida [2]



1 R = Et
2 R = H

Partition of the ethanolic extract between water and hexane and subsequently water and ethyl acetate gave two organic extracts which were similar in constitution and were recombined The aqueous phase from the solvent partition was lyophilized and successively triturated with *n*-butanol, chloroform, and methanol. When examined by tlc, these extracts were similar and were also recombined This material is referred to as the aqueous extract

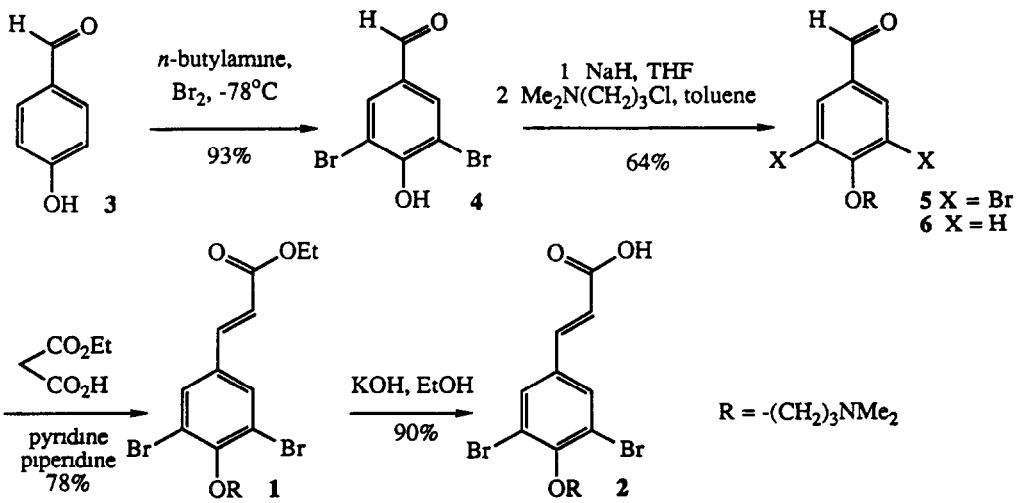
The organic extract contained one UV-absorbing compound that was antimicrobial, and also cytotoxic and genotoxic in the *E coli* K-12 SOS chromotest agar spot test [3] The active compound was isolated by preparative tlc

and purified on Sephadex LH-20 to obtain the crystalline ester **1** (4 mg, 0.13% dry weight) The ester **1** has a molecular formula of $C_{16}H_{21}Br_2NO_3$, as revealed by high resolution mass measurement (20 eV) of the molecular ion at m/z = 432 9860. The molecular ion was a cluster of m/z = 437, 435, 433 in the ratio of 1 2 1, due to the presence of two bromine atoms in the compound. A low resolution mass spectrum (70 eV) showed no fragment ions of significant intensity except the base peak at m/z = 58 ($Me_2N{=}CH_2^+$), an ion typical of dimethylamino groups attached to the end of a carbon chain [4] The NMe_2 moiety was also detected in the infrared spectrum. The low wave number of 2758 cm^{-1} for a C-H stretching band is typical of methyl or methylene groups next to nitrogen The infrared spectrum contained bands at 1712 (unsaturated ester) and 1635 (aromatic) cm^{-1} The UV-spectrum showed absorption maxima at 230 nm (ε 11200) and 282 nm (ε 9500) which are appropriate for a cinnamic ester The ^1H NMR spectrum was deceptively simple and implied elements of symmetry Several isolated spin systems were observed The signals at δ 1 30 (t, 3 H, J = 7.1 Hz) and 4 23 (q, 2 H, J = 7 1 Hz) were due to the ethyl ester and those at 7 46 (d, 1 H, J = 16 0 Hz, H-7) and 6 04 (d, 1 H, J = 16 0 Hz, H-8) were assigned to a *trans* cinnamic ester Two triplets at δ 4 06 (2 H, J = 6.2 Hz, H-10) and 2 72 (2 H, J = 7 1 Hz, H-12), both coupled to a multiplet at 2 12 (2 H, H-11), and a singlet at 2.38 (6 H, NMe_2) were typical of a N,N-dimethylaminopropyloxy moiety The remaining signal at δ 7.63 (s, 2 H, H-2,6) was assigned to the aromatic protons on a symmetrically substituted aromatic ring The ^{13}C NMR spectrum contained only 13 signals with three signals at δ 45 4 (q, C-13,14), 118 9 (s, C-3,5) and 132 0 (d, C-2,6) being due to two carbons each The aromatic signals at δ 154 7 (s, C-4), 133 0 (s, C-1), 132 0 (d, 2 C, C-2,6), and 118 9 (s, 2 C, C-3,5) are appropriate for a 3,5-dibromo rather than a 2,6-dibromo substitution when compared with values obtained from incremental estimations [4] The remaining signals were those of the ethyl ester at δ 14 3 (q) and 60 7 (t), and 28 2 (t, C-11), 56 1 (t, C-12), 72 2 (t, C-10), 120 1 (d, C-8), 141 0 (d, C-7), and 166 3 (s, C-9) These data are completely compatible with ethyl 3,5-dibromo-4-(3'-N,N-dimethylaminopropyloxy)cinnamate

The aqueous extract contained several very polar UV-absorbing compounds which could be chromatographed on normal phase silica using dichloromethane methanol ammonia mixtures (typically 74 21 5) The recovery of material, however, was unsatisfactory Repeated chromatography on Sephadex LH-20 followed by reversed phase HPLC with gradient elution allowed the isolation of the acid **2** (6 mg, 0 20% dry weight) The NMR spectra of this compound were almost identical to those of the cinnamic ester **1**, only the signals due to the ethyl ester were absent and the carbonyl signal in the ^{13}C NMR spectrum was at δ 174 0 (unsaturated acid) The expected molecular formula $C_{14}H_{18}Br_2NO_3$ was confirmed by HRFABMS which gave a peak at m/z = 407 9641 $(M+H)^+$ All other spectroscopic data are in agreement with the proposed structure The carbonyl band in the

infrared spectrum was shifted to 1565 cm^{-1} (unsaturated acid) and accompanied by the typical broad OH absorption (3700-2900 cm^{-1}) of an acid The absorption maxima in the UV-spectrum were shifted to lower wavelengths with absorption maxima at 223 nm (ε 13700) and 267 nm (ε 11000)

In order to confirm the structures of the ester **1** and the acid **2** and to provide sufficient amounts of material for thorough biotesting we have synthesized both compounds 4-Hydroxybenzaldehyde (**3**) was brominated using bromine in toluene containing *n*-butylamine at -78°C [5] The alkylation of the 3,5-dibromoaldehyde **4** was successful only after refinement of the reaction conditions The phenolic hydroxy group of **4** was highly acidic and could be deprotonated even with concentrated aqueous KOH The phenolate, however, was sterically crowded and alkylated only under aprotic conditions The aldehyde **4** was therefore dissolved in anhydrous THF and deprotonated with sodium hydride Excess alkylating agent was added in toluene solution, which allowed the reaction to run at a higher temperature The desired aldehyde **5** was obtained in 64% yield (based on conversion of starting material) The maximum turnover of the reaction was 62% with remaining starting material being readily recovered It was not possible to improve the overall yield of the reaction sequence by brominating 4-(3'-N,N-dimethylaminopropyloxy)benzaldehyde (**6**) under a variety of conditions, because **6** was either initially brominated at the nitrogen or did not react at all A Perkin reaction[6] of the 4-alkoxy-3,5-dibromobenzaldehyde **5** with monoethyl malonate gave the desired cinnamic ester **1** in 78% yield The ester **1** was converted to the free acid **2** in 90% yield by treatment with excess 0 1 M ethanolic KOH Both compounds were identical with the natural products in all respects



Experimental section

Collection, Extraction and Purification

The specimen of *Pseudoceratina crassa* (Hyatt), a dictyoceratid sponge, was collected by hand using SCUBA (-15 m) near Albert Town, Bahamas, in July, 1989. The entire sample (1 016 g dry weight) was stored in 70% ethanol/water and kept at 5°C for several months. The ethanolic extract was filtered and the organic solvent removed under reduced pressure The resulting aqueous suspension was diluted to 100 ml and extracted subsequently with hexane and EtOAc (3x 100 ml each). Each extract was dried ($MgSO_4$), filtered, and evaporated *in vacuo* to yield extract 1 (13 mg) and extract 2 (73 mg). The remaining aqueous layer was freeze dried and the lyophilisate (2.93 g) triturated with 250 ml of *n*-butanol (680 mg, extract 3), chloroform (180 mg, extract 4), and methanol (70 mg, extract 5). After examining the ^1H NMR spectra and thin layer chromatograms, extracts 1 and 2 and extracts 3 - 5 were combined and called organic and aqueous extract.

A single spot appeared in the tlc of the organic extract when examined under UV light (254 nm). This material was obtained by preparative tlc (CH_2Cl_2.MeOH 4:1), and then rechromatographed on Sephadex LH-20 to remove residual fat to yield **1** (4 mg, 0.13% dry weight). The compound inhibited the growth of *Bacillus subtilis* and *Staphylococcus aureus* at 100 μg/disk and *Candida albicans* at 50 μg/disk It was also cytotoxic and genotoxic in the *Escherichia coli* K-12 SOS chromotest agar spot test[3] at the same minimal concentration

The aqueous extract was separated in batches to give a total of 6 mg of **2** (0 20% dry weight) The purification sequence usually consisted of three column runs on Sephadex LH-20 using successively MeOH, MeOH H_2O 4 1, CH_2Cl_2 MeOH 1 1 as eluents and HPLC on reversed phase silica using a linear gradient elution profile from 30 70 MeOH H_2O to pure methanol as a final step The acid **2** had no bioactivity

Ethyl 3,5-Dibromo-4-(3'-N,N-dimethylaminopropyloxy)cinnamate (1) colorless flakes, mp 67°C, **IR** (neat, NaCl) 2920, 2840, 2805, 2758, 1712, 1635, 1195, 950 cm^{-1}, **UV** (MeOH) λ_{max} 230 nm (ε 11200), 282 (9500), **^1H NMR** ($CDCl_3$) δ 7 63 (s, 2 H, H-2,6), 7 46 (d, 1 H, *J* = 16 0 Hz, H-7), 6 04 (d, 1 H, *J* = 16.0 Hz, H-8), 4 23 (q, 2 H, *J* = 7 1 Hz), 4.06 (t, 2 H, *J* = 6 2 Hz, H-10), 2 72 (t, 2 H, *J* = 7 1 Hz, H-12), 2 38 (s, 6 H, NMe_2), 2 12 (tt, 2 H, *J* = 7 1, 6 2 Hz, H-11), 1 30 (t, 3 H, *J* = 7 1 Hz), **^{13}C NMR** ($CDCl_3$) δ 166 3 (s), 154 (s), 141 0 (d), 133 0 (s), 132 0 (d, 2C), 120 1 (d), 118 9 (s, 2C), 72 2 (t), 60 7 (t), 56 1 (t), 45 4 (q, 2C), 28 2 (t), 14 3 (q), **EIMS** (70 eV) *m/z* 437 (0 7), 435 (1 4), 356 (1), 354 (1), 167 (1), 149 (1), 86 (8), 84 (2), 73 (3), 71 (3), 58 (100), **HREIMS** (20 eV), obsd *m/z* 432 9860 (M^+), $C_{16}H_{21}{}^{79}Br_2NO_3$ requires 432 9888

3,5-Dibromo-4-(3'-N,N-dimethylaminopropyloxy)cinnamic acid (2) white crystals, mp 193-194°C (dec), **IR** (neat, NaCl) 3700-2900, 2940, 2870, 1635, 1565, 930 cm^{-1}, **UV** (MeOH) λ_{max} 223 nm (ε 13700), 267 (11000), **^1H NMR** (MeOH-d_4) δ 7 70 (s, 2 H, H-2,6), 7 21 (d, 1 H, *J* = 15 9 Hz, H-7), 6 44 (d, 1 H, *J* = 15 9 Hz, H-8), 4 10 (t, 2 H, *J* = 5 6 Hz, H-10), 3 40 (t, 2 H, *J* = 7 8 Hz, H-12), 2 83 (s, 6 H, NMe_2), 2 25 (tt, 2 H, *J* = 7 8, 5 6 Hz, H-11), **^{13}C NMR** (MeOH-d_4) δ 174 0 (s), 154 0 (s), 137 8 (d), 136 6 (s), 132 7 (d, 2 C), 128 1 (d), 119 4 (s, 2 C), 71 4 (t), 56 8 (t), 43 8 (q, 2 C), 26 7 (t); **FABMS** *m/z* 410 (46),

408 (94), 406 (61), 325 (11), 323 (24), 321 (14), 307 (8), 305 (19), 303 (10), 244 (12), 242 (12), 58 (100), **HRFABMS**, obsd *m/z* 407 9641 ($M+H^+$), $C_{14}H_{18}{}^{79}Br^{81}BrNO_3$ requires 407 9649.

Synthesis

3,5-Dibromo-4-hydroxybenzaldehyde[7] **(4)** 4-Hydroxybenzaldehyde (**3**) was brominated in a modified Pearson procedure.[5] A solution of *n*-butylamine (9 9 ml, 7.32 g, 100 mmol) in dry toluene (125 ml) in a 250 ml three necked flask with two addition funnels and a low temperature thermometer was cooled in an isopropanol/dry ice bath. While maintaining the temperature below -30°C, bromine (2 57 ml, 7 99 g, 50 mmol) was added with stirring and the mixture was further cooled to -78°C At this temperature a 2 M solution of 4-hydroxybenzaldehyde (**3**) (3 05 g, 25 mmol) in CH_2Cl_2 MeOH (10 1) was added dropwise The reaction mixture was allowed to warm to room temperature, then quenched with water (100 ml), and diluted with EtOAc (50 ml). The phases were separated and the aqueous layer discarded Phenolic compounds were extracted from the organic layer with 10% (w/v) NaOH solution (3x 100) The combined alkaline extract was acidified with cold 4 N hydrochloric acid (62 5 ml), and the desired product extracted with ethyl acetate (3x 150 ml) The combined organic extracts were washed with 5% (w/v) $NaHCO_3$ solution, and brine (100 ml each), dried over $MgSO_4$, filtered and evaporated *in vacuo* to yield 3,5-dibromobenzaldehyde (**4**) (6 50 g, 93%) as pale cream crystals **^{1}H NMR** ($CDCl_3$) δ 9 61 (s, 1H), 7 92 (s, 2H)

3,5-Dibromo-4-(3'-N,N-dimethylaminopropyloxy)benzaldehyde (5) 3-N,N-Dimethylaminopropyl-chloride was liberated from its commercially available hydrochloride according to Wheatley [8] 3-N,N-dimethylaminopropylchloride hydrochloride (3 20 g, 20 mmol) was stirred with 10 M KOH (3 ml) at 0°C for 30 min and the free base continuously extracted with toluene (5 ml) The layers were then separated and the aqueous phase was extracted with toluene (2x 5 ml) The combined organic extract was dried over $MgSO_4$, filtered and immediately used for the alkylation reaction Powdered sodium hydride (0 24 g, 100 mmol) was suspended in dry THF (5 ml) To this mixture was added a solution of 3,5-dibromobenzaldehyde (**4**) (4 20 g, 15 mmol) in dry THF After the evolution of hydrogen gas had ceased the freshly prepared 3-N,N-dimethylaminopropylchloride solution was added dropwise and the reaction mixture was refluxed for 2 5 h After cooling the mixture to room temperature, ice cold water (50 ml) was added and the phases separated The organic layer was extracted with 10% (w/v) sodium hyroxide solution (3x 50 ml) The desired product **5** remained in the organic layer, which in turn was washed with brine, dried over $MgSO_4$, filtered, and evaporated *in vacuo* to yield 3,5-dibromo-4-(3'-N,N-dimethylaminopropyloxy)benzaldehyde (**5**) (2 20 g, 64%, based on turnover) as yellow oil After acidification with ice cold 4 N HCl remaining starting material could be reextracted from the aqueous phase with EtOAc (1 57 g **4**, 38%) **^{1}H NMR** ($CDCl_3$) δ 9 85 (s, 1 H), 8 03 (s, 2 H), 4 15 (t, 2 H, J = 6 5 Hz), 2 55 (t, 2 H, J = 7 7 Hz), 2 28 (s, 6 H), 2 08 (t, 2H, J = 6 5 Hz), **HRFABMS**, obsd *m/z* 367.9511 $(M+H)^+$, $C_{12}H_{16}{}^{81}Br_2NO_2$ requires 367 9507

Ethyl 3,5-Dibromo-4-(3'-N,N-dimethylaminopropyloxy)cinnamate (1) 3,5-Dibromo-4-(3'-N,N-dimethylaminopropyloxy)benzaldehyde (**5**) (1 88 g, 5 mmol) and monoethyl malonate[9] (1 45 g, 11 mmol) were allowed to react overnight in pyridine (2 50 ml) with piperidine (0.06 ml) as a catalyst and then heated to 60°C

for 6 h until the evolution of CO_2 had stopped [6] The reaction mixture was poured into ice cold 2 N HCl (20 ml) and extracted with CH_2Cl_2 (3x 20 ml). The combined organic layers were washed with 2 N HCl (10 ml), saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The resulting solid was recrystallized twice from ethanol.water (3 2), the final yield of ethyl 3,5-dibromo-4-(3'-N,N-dimethylamino-propyloxy)cinnamate (**1**) was (1 70 g, 78%) The product was identical with the natural product in all spectroscopical features

3,5-Dibromo-4-(3'-N,N-dimethylaminopropyloxy)cinnamic acid (2) The acid **2** was obtained from the ester **1** (43.5 mg, 0 1 mmol) by treatment with 0 1 mM KOH (3 ml) in EtOH overnight at room temperature The reaction mixture was acidified with the calculated amount of ice cold 0 1 M hydrochloric acid After removal of the EtOH under reduced pressure the product could be precipitated in thin flakes from the aqueous solution by adding brine It was filtered with suction, washed with water, and recollected in MeOH Evaporation of the methanolic solution gave 3,5-dibromo-4-(3'-N,N-dimethylaminopropyloxy)cinnamic acid (**2**) (36 mg, 90%) as a white powder which was spectroscopically pure and identical with the natural product.

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