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# Kottamide E, the first example of a natural product bearing the amino acid 4-amino-1,2-dithiolane-4-carboxylic acid (Adt)

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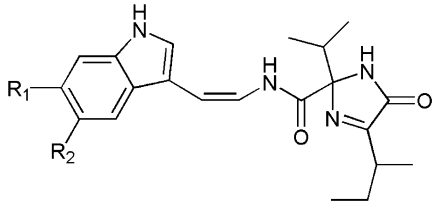
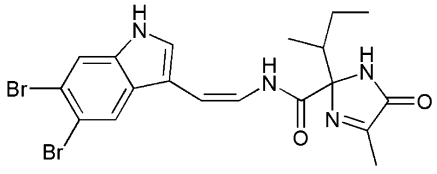
**Abstract**—Kottamide E, a novel alkaloid containing dibrominated indole enamide, oxalic acid diamide and 4-amino-1,2-dithiolane-4-carboxamide moieties, has been isolated from the New Zealand ascidian *Pycnoclavella kottae*. Characterisation was achieved by interpretation of spectroscopic data.


As part of our ongoing search for novel biologically active metabolites from New Zealand marine organisms[1] we recently reported kottamides A–D (**1**–**4**), isolated from the endemic ascidian *Pycnoclavella kottae* (Millar, 1960) (Order Aplousobranchia, Family Pycnoclavellidae) collected at the Three Kings Islands, New Zealand.[2] Alkaloids **1**–**4** exhibited a range of biological activities including anti-inflammatory, antitumour and anti-metabolic properties. While kottamides A–D represented the major components of the crude extract, HPLC, mass spectrometry and NMR indicated the presence of additional minor structurally related compounds.

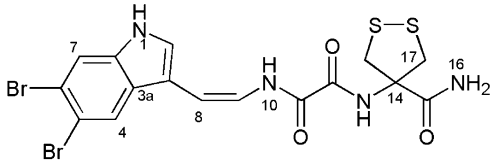
Fractionation of a portion of the $CH_2Cl_2$–MeOH extract of the organism (32 g dry wt) using repeated reversed-phase $C_{18}$ flash column chromatography (MeOH:$H_2O$) followed by HPLC ($C_{18}$; MeCN:$H_2O$ (80:20); 5 mL/min), as previously described,[2] afforded semi-purified samples of kottamides A–D. The semi-pure mixture of kottamides B (**2**) and C (**3**) was subjected to aminopropyl-derivatised silica chromatography in a Luer lock cartridge (500 mg). Elution with $CH_2Cl_2$ yielded a clean mixture of **2** and **3**, while subsequent elution with MeOH yielded kottamide E (**5**) as an optically inactive white amorphous solid (1.2 mg, 0.008% dry weight).[3,4]



**1** $R_1 = R_2 = Br$
**2** $R_1 = Br$, $R_2 = H$
**3** $R_1 = H$, $R_2 = Br$



**4**



**5**

A molecular formula of $C_{16}H_{14}Br_2N_4O_3S_2$ for **5** was established by HRFAB mass spectrometry [*m*/*z* 531.8830/533.8838/535.8835 (M), Δ +4.4 mmu] with the observed isotope pattern supporting the presence of two bromine atoms, as seen for kottamides A (**1**) and D (**4**).[4] UV absorptions at 239 (log ε 4.41) and 305 nm (3.92) suggested the presence of an extended aromatic

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**Table 1.** $^{1}H$, $^{13}C$ and $^{15}N$ NMR data ($d_6$-dmso) for kottamide E (**5**)

| Atom no. | $\delta_H$ (mult, $J$ (Hz)) | $\delta_C$ | $\delta_N$[a] | HMBC ($^{1}H\rightarrow^{13}C$ and $^{1}H\rightarrow^{15}N$) |
|---|---|---|---|---|
| N-1 | 11.67 (br s) | – | 136.2 | 3, 3a |
| 2 | 7.63 (br s) | 125.9 | – | 3, 3a, 7a |
| 3 | – | 109.2 | – | – |
| 3a | – | 127.3 | – | – |
| 4 | 8.05 (s) | 123.0 | – | 3, 5, 6, 7a |
| 5 | – | 113.7 | – | – |
| 6 | – | 116.0 | – | – |
| 7 | 7.84 (s) | 116.4 | – | 3a, 5, 6 |
| 7a | – | 135.4 | – | – |
| 8 | 6.23 (d, 9.1) | 105.4 | – | 2, 3a, 9, N-10 |
| 9 | 6.70 (dd, 10.9, 9.2) | 118.0 | – | 3, 8 |
| N-10 | 9.45 (d, 10.8) | – | 126.0 | – |
| 11 | – | 159.4[b] | – | – |
| 12 | – | 156.5[b] | – | – |
| N-13 | 8.99 (br s) | – | 122.7 | 11, 14, 15, 17 |
| 14 | – | 71.6 | – | – |
| 15 | – | 170.5 | – | – |
| N-16 | 7.54 (br s) | – | 101.7 | 15 |
| | 7.33 (br s) | – | | 14 |
| 17 | 3.76 (d, 12.1) | 47.7 | – | N-13, 14, 15, 17 |
| | 3.54 (d, 12.2) | | – | N-13, 14, 15, 17 |

[a] $^{15}N$ chemical shifts were determined indirectly from $^{1}H$–$^{15}N$ HSQC (optimised for 87 Hz) and $^{1}H$–$^{15}N$ HMBC (optimised for 6.0 Hz) NMR experiments. Data were referenced to liq. $NH_3$ using urea as an external standard.
[b] Assignments may be interchanged.

chromophore while the IR spectrum indicated the presence of amide carbonyl and NH functional groups.[3] Comparison of the $^{1}H$, $^{13}C$ and $^{15}N$ NMR data observed for **5** with those previously reported for **1** and **4**, established the presence of a 5,6-dibromo-indole-3*Z*-enamide moiety in **5** (Table 1). The remaining atoms, $C_6H_7N_2O_3S_2$, requiring four degrees of unsaturation, comprised three carbonyl ($\delta$ 159.5, 159.4 and 170.5), a quaternary $sp^3$ ($\delta$ 71.6) and two chemically equivalent $sp^3$ methylene ($\delta$ 47.7) $^{13}C$ resonances in addition to three broad singlets ($\delta$ 7.33, 7.54 and 8.99) and an alkyl AB quartet ($\delta$ 3.54 (2H, d, $J=12.2$ Hz) and 3.76 (2H, d, $J=12.1$ Hz)) observed in the $^{1}H$ NMR spectrum. Full assignment of this oxalic acid diamide-1,2-dithiolane-4-carboxamide fragment was achieved by interpretation of $^{1}H$–$^{13}C$ HMBC, $^{1}H$–$^{15}N$ HSQC and $^{1}H$–$^{15}N$ HMBC NMR data. $^{1}H$–$^{15}N$ HSQC correlations defined the presence of two amide groups; one primary ($\delta$ $^{15}N$ 101.7, $\delta$ H 7.33 (br s), 7.54 (br s)) and one secondary ($\delta$ $^{15}N$ 122.7, $\delta$ H 8.99 (br s)).[5] $^{1}H$–$^{13}C$ HMBC correlations from the primary amide proton resonances to C-15 ($\delta$ 170.5) and C-14 ($\delta$ 71.6) combined with correlations from the secondary amide proton H-13 to C-11 ($\delta$ 159.4), C-14 and C-15 defined the backbone structure from C-11/C-12 to N-16. Placement of the remaining atoms required by the molecular formula ($C_2H_4S_2$) at C-14 was necessitated by the observation of $^{1}H$–$^{13}C$ HMBC correlations from the diastereotopic methylene protons H-17 to C-14, C-15 and C-17 and by correlations observed between NH-13 and C-17. Chemical shift considerations (C-17 $\delta$ 47.7) combined with evidence for symmetry ($^{1}H$–$^{13}C$ HMBC correlations from H-17 to C-17) suggested the presence of a 4,4-disubstituted-1,2-dithiolane ring at C-14. Excellent agreement between the observed chemical shifts of **5** to those reported for both 4-*N*-Boc-1,2-dithiolane-4-carboxymethyl ester[6] and igzamide, a brominated-indole-3*Z*-enamide-oxalic acid diamide alkaloid isolated from the Pacific sponge *Plocamissa igzo*,[7] further supported the spectroscopic assignments and the structure of kottamide E.

To the best of our knowledge, this is the first report of the presence of a 4-amino-1,2-dithiolane-4-carboxylic acid (Adt) residue in a natural product. Adt is of current interest as a conformationally restricted analogue of cysteine.[8,9]

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3. UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 202 (4.36), 239 (4.41), 305 (3.92) nm; IR (neat) $\nu_{max}$ 3345, 2923, 1673, 1606, 1523, 1486, 1118, 1022 $cm^{-1}$.
4. EIMS: 301/303/305 (15%). FABMS 532/534/536 (M+, 0.55%), 533/535/537 (MH+, 0.35%). While the presence of two bromine atoms was easily deduced by inspection of the observed isotope pattern, the presence of sulphur was masked by the overlapping ion clusters of M+ and MH+ observed in the FAB mass spectrum.
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