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A Novel Chlorinated Ketide Amino Acid, Herbamide A, from the Marine Sponge *Dysidea herbacea*[1]

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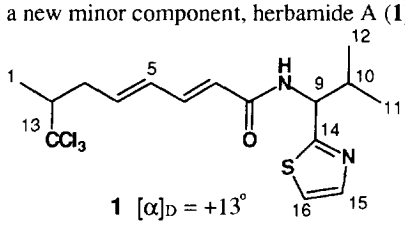
Abstract: A collection of the marine sponge *Dysidea herbacea*, containing cyanobacterial symbionts, from Papua New Guinea yielded a new ketide amino acid, herbamide A (**1**), along with the previously reported polychlorinated tetrapeptide, dysidenin (**2**). The structure of **1** was determined by spectroscopic methods.

The chemistry[2] and biology[3] of *Dysidea herbacea*, a common Indo-Pacific sponge, have been studied extensively. The morphology as well as the pigmentation of this sponge varies considerably; some specimens are heavily laden with symbionts that include cyanobacteria and/or rod-shaped bacteria, while others appear to be devoid of prokaryotic microorganisms altogether. Samples without cyanobacteria have been a good source of sesquiterpenoids[4] which are sometimes isolated as antipodal compounds.[4,5] "Green" forms of this sponge are laced with the cyanobacteria *Oscillatoria spongeliae*[6] and are rich in polychlorinated peptides[7] or polybrominated phenols[8]. Recently, the sesquiterpenoids and chlorinated polypeptides have been shown to be concentrated in sponge or cyanobacteria cells, respectively.[9] We have been conducting a survey of various forms of *D. herbacea* from Papua New Guinea and report below on the chemistry of one sample which contains new peptide constituents.

A collection of *D. herbacea* (coll. no. 93153) presumed to be rich in cyanobacteria because of the rich presence of chlorophyll identified by TLC was investigated further. Its methanol extract was processed according to our standard procedure,[1] and the dichloromethane solvent partition fraction was further explored because its $^{1}H/^{13}C$ NMR spectrum showed lowfield resonances characteristic of polypeptides. Subsequent purification of this fraction by HPLC (ODS, MeOH:THF:MeCN:H_2O, 54:9:13:24) afforded two compounds; a new minor component, herbamide A (**1**)[10], and a known major component, dysidenin (**2**)[11].



1 $[\alpha]_D = +13^\circ$

The structural elucidation work on herbamide A (**1**) proceeded efficiently once it was recognized that this compound was highly chlorinated. Evidence for this was shown in the LRFABMS (positive mode) where the molecular ion peak exhibited an isotope distribution characteristic of three chlorine atoms. A molecular formula of $C_{16}H_{21}Cl_3N_2OS$ was established by a HRFABMS $[MH]^+$ peak at *m/z* 394.0442. The presence of an NH was evident by comparison of the MS data to the ^{13}C NMR APT formula of $C_{16}H_{20}$ along with the assignment of the δ 165.6 resonance to an amide moiety. Seven ^{13}C resonances (Table 1) were identified as being characteristic of a thiazole ring and two disubstituted double bonds which accounted for the remaining elements of unsaturation. The UV (λ_{max} 258) and IR (ν 1672, 1602 cm^{-1}) spectra were consistent with extended conjugation represented by a dieneamide functionality. Analysis

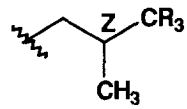
Table 1. ^{1}H (300 MHz), ^{13}C (75.5 MHz) and HMBC (500 MHz) NMR Data ($CDCl_3$) of **1**.

Atom #	^{13}C (δ)	^{1}H (δ, mult., J = Hz)	HMBC (C → H)
1	16.2	1.29, d, 6.0	–
2	54.6	2.58, m	H1, H3
3	36.6	2.96, m; 2.19, m	H5
4	138.8	6.02, ddd, 14.7, 8.4, 2.4	H3, H3', H6
5	131.1	6.25, dd, 14.7, 11.0	H7
6	141.2	7.24, dd, 14.7, 11.0	H4, H5,
7	122.8	5.90, d, 14.7	H5
8	165.6	–	H6, H7, NH
9	56.0	5.33, dd, 8.7, 6.3	H11, H12
10	34.1	2.33, m	H9, H11, H12
11	18.2	0.97, d, 6.9	H9, H12
12	19.2	0.93, d, 6.9	H9, H11
13	105.7	–	–
14	170.0	–	H9, H15, H16
15	142.3	7.74, d, 3.0	H16
16	118.8	7.26, d, 3.0	H15
NH	–	6.46, bd, 8.4	–

of ^{1}H-^{1}H COSY NMR data confirmed the presence of the thiazole ring, and suggested the presence of two acyclic chains consisting of respectively an *N*H-CH(CH_3)$_2$ and C1 through C7 as shown in **1**. Evidence for attaching the Cl_3C residue to C2, rather than to C7 or C9, came from a ^{13}C NMR chemical shift analysis (Table 2),[12] as agreement between the shift of CZ in the substructure shown and of C2 (δ 54.6) in **1** was achieved for the case R = Cl_3 where the calculated shift is δ 53. This analysis was important because no HMBC correlations were observed to C13 (δ 105.7). Establishing the final atom connectivities to C7 and C9, as shown in the final structure **1**, was guided by a variety of HMBC correlations (Table 1). The most important of these being from the thiazole ring carbon C14 (δ 170.0) to the methine proton H9 (δ 5.33), from the amide *N*H(δ 6.46) to carbonyl C8 (δ 165.6), and from the vinyl proton H7 (δ 5.90) to C8.

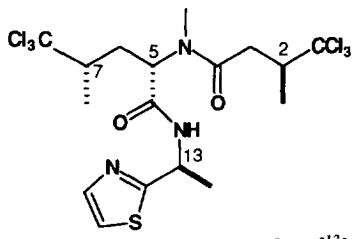
Table 2. ^{13}C NMR Comparison Data of Trichloroisopropyl Group.

R_3	Z(δ)	CH_3(δ)	Ref.
H_3	28.2	22.3	12a
H_2Cl	34.5	17.2	12a
HCl_2	42.2	15.1	12b
Cl_3	53.2	16.1	12c

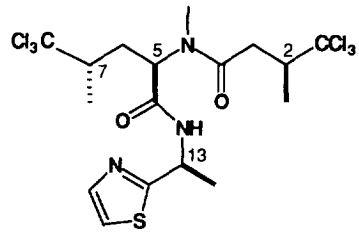


The gross structure of the major component was rapidly deduced by comparing our NMR and mass spectral data[11] to that reported in the literature for dysidenin.[13,14] Less straightforward was the assignment of the absolute stereochemistry at C2, C7 and C13. We measured an $[\alpha]_D = -68°$ which was close to the $[\alpha]_D = -98°$ reported for (–)-dysidenin (**2**)[13a], 2*S*, 5*S*, 7*S*, 13*S*, but distinctly different compared to the $[\alpha]_D = +47°$ for (+)-isodysidenin (**3**)[13b], 2*S*, 5*R*, 7*S*, 13*S*. The differences in the ^{1}H NMR chemical shifts at H2, $H_2$3, H5, $H_2$6 and H7 reported in the literature for (–)-dysidenin (**2**) and (+)-isodysidenin (**3**) diastereomers reflects the

stereochemical difference at C5 between these two compounds. The shifts measured for these hydrogens in our material[11] were nearly identical to those reported for (–)-dysidenin (**2**)[13] suggesting that the stereochemistry at C2, C5, and C7 is identical for this pair of compounds. Unfortunately, the reference data for the protons attached to C13 in the dysidenin framework are incomplete, because no chemical shift assignments have been made for the CH13 of the *R* isomer.[15] Also, comparison of the $[\alpha]_D$ between (–)-dysidenin (–98°)[13a] and (+)-13-demethyldysidenin (+96°)[14] shows that this parameter is probably insensitive to epimeric changes at C13. Combining all of the above considerations with the added assumption that there must be a chemotaxonomic analogy between this and the past work on *D. herbacea* prompts the provisional conclusion that our compound is identical to (–)-dysidenin (**2**).



2 $[\alpha]_D$ = -68°/-98°[13a]



3 $[\alpha]_D$ = +47°[13b]

Herbamide A (**1**) has an interesting structure that extends a theme represented in the *Dysidea* chloropeptides which are isolated when the cyanobacterial symbiont is present. We contend that the trichlorovaline subunit present in **2** and **3** is also represented in **1** and this conclusion is based on the following analysis. The nine carbon side chain of **1** is reminiscent of the ten carbon side chain present in the bengamide family of sponge metabolites. A parallel biogenesis can be imagined for these substructural features as both can be dissected into condensation products of valine (for herbamide) or leucine (for bengamide) plus a diketide.[16] This presents a slightly different analysis compared to that advanced recently for the dysidenin skeleton.[3a] Finally, the thiazole portion of **1** is analogous to the terminal unit of dolastatin 10.[17] In contrast to the potent cytotoxicity exhibited by the dolastatins, compound **1** was inactive in the NCI disease oriented cytotoxicity screen.

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REFERENCES AND NOTES

1. Part 17 in the series Novel Sponge Derived Amino Acids. For part 16 see: Jaspars, M.; Rali, T.; Laney, M.; Schatzman, R. C.; Diaz, M. C.; Schmitz, F. J.; Pordesimo, E. O.; Crews, P. *Tetrahedron* **1994**, *50*, 7367-7373.
2. Krebs, H. Chr. *Progress in the Chemistry of Organic Natural Products*, **1986**, *49*, 152-363.

3. (a) Garson, M. J. In *Sponges in Time and Space*, Van Soest, R. W. M.; Van Kampen, Th. M. G.; Braekman, J. C. Eds.; Balkema: Rotterdam,1994; pp. 427-440. (b) Bergquist, P. R. *New Zeal. J. Zoo.* **1980**, *7*, 443-503. (c) Dunlop, R. W.; Kazlauskas, R.; March, G.; Murphy, P. T.; Wells, R. J. *Aust. J. Chem.* **1982**, *35*, 95-103.
4. Horton, P.; Inman, W. D.; Crews, P. *J. Nat. Prod.* **1990**, *53*, 143-151.
5. Searle, P. A.; Jamal, N. M., Lee, G. M.; Molinski, T. F. *Tetrahedron* **1994**, *50*, 3879-3888.
6. (a) Berthold, R. J.; Borowitzka, M. A.; Mackay, M. A. *Phycologia* **1982**, *21*, 327-335. (b) Larkum, A. W. D.; Cox, G. C.; Hiller, R. G.; Parry, D. L.; Dibbayawan, T. P. *Mar. Biol.* **1987**, *95*, 1-13.
7. (a) Hofheinz, W.; Oberhansli, W. E. *Helv. Chim. Acta.* **1977**, *60*, 660-669. (b) Erickson, K. L.; Wells, R. J. *Aust. J. Chem.* **1982**, *35*, 31-38.
8. Norton, R. S.; Croft, K. D.; Wells, R. J. *Tetrahedron* **1981**, *37*, 2341-2349.
9. (a) Faulkner, D. J.; He, H. Y.; Unson, M. D.; Bewley, C. A. *Gazz. Chim. Ital.* **1993**, *123*, 301-308. (b) Unson, M. D.; Faulkner, D. J. *Experientia* **1993**, *49*, 349-353. (c) Unson, M. D., Rose, C. B.; Faulkner, D. J.; Brinen, L. S.; Steiner, J. R.; Clardy, J. *J. Org. Chem.* **1993**, *58*, 6336-6343.
10. *Herbamide A* (**1**): $C_{16}H_{21}Cl_3N_2OS$, oil, $[\alpha]_D$ = +13° (*c* 0.013, $CHCl_3$); UV ($CHCl_3$) λ_{max} 258; IR ($CHCl_3$) ν, 3422, 1672, 1602, cm^{-1}; HRFABMS (m/z 394.0442, MH^+, Δ 0.3 mmu of calcd.).
11. *Dysidenin* (**2**): $C_{17}H_{23}Cl_6N_3O_2S$, oil, $[\alpha]_D$ = −68° (*c* 0.034, $CHCl_3$); UV ($CHCl_3$) λ_{max} 248; IR ($CHCl_3$) ν, 1678, 1643 cm^{-1}; LRFABMS (m/z 546, MH^+); ^1H NMR (250 MHz, $CDCl_3$) δ 7.71 d *J* = 3.3 Hz (H16), 7.28 d *J* = 3.3 Hz (H17), 6.84 bd *J* = 7.0 Hz (NH), 5.37 dd *J* = 10.5, 2.5 Hz (H5), 5.36 q *J* = 6.8 Hz (H13), 3.38 ddq *J* = 10.0, 6.5, 2.5 Hz (H2), 3.13 dd *J* = 16.3, 2.5 Hz (H3), 3.04 s (H10), 2.62 dd *J* = 14.8, 10.5 Hz (H6), 2.49 dd *J* = 16.3, 10.0 Hz (H3'), 2.22 ddq *J* = 10.5, 6.5, 2.5 Hz (H7), 1.92 ddd *J* = 14.8, 10.5, 2.5 Hz (H6'), 1.59 d *J* = 6.8 Hz (H14), 1.39 d *J* = 6.5 Hz (H1), 1.36 d *J* = 6.5 Hz (H8); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.1 s (C4), 172.0 s (C15), 168.9 s (C12), 142.5 d (C16), 119.2 d (C17), 105.6 s (C9), 105.2 s (C11), 54.1 d (C5), 51.9 d (C7), 51.5 d (C2), 47.3 d (C13), 37.5 t (C3), 31.0 t (C6), 30.9 s (C10), 21.9 q (C14), 17.4 q (C1), 16.3 q (C8).
12. (a) Pretsch, E; Clerc, T; Seibl, J; Simon, W *Spectral Data for Structure Determination of Organic Compounds*; Fresenius, W; Huber, J. F. K.; Pungor, E; Rechnitz, G. A.; Simon, W; West, Th. S. Eds.; Springer-Verlag: Berlin, 1989, 2nd ed.; pp. C10-C25. (b) Isaacs, S.; Berman, R.; Kashman, Y. *J. Nat. Prod.* **1991**, *54*, 83-91. (c) Lee, G. M.; Molinski, T. F. *Tetrahedron Lett.* **1992**, *33*, 7671-7674.
13. (a) Kazlauskas, R.; Lidgard, R. O.; Wells, R. J. *Tetrahedron Lett.* **1977**, *18*, 3183-3186. (b) Charles, C.; Braekman, J. C.; Daloze, D.; Tursch, B.; Karlsson, R. *Tetrahedron Lett.* **1978**, *19*, 1519-1520.
14. For example see data in De Laszlo, S. E.; Williard, P. G. *J. Am. Chem. Soc.* **1985**, *107*, 199-203.
15. Biskupiak, J. E.; Ireland, C. M. *Tetrahedron Lett.* **1984**, *25*, 2935-2936.
16. (a) Adamczeski, M.; Quiñoá, E.; Crews, P. *J. Am. Chem. Soc.* **1989**, *111*, 647-654. (b) Adamczeski, M.; Quiñoá, E.; Crews, P. *J. Org. Chem.* **1990**, *55*, 240-242.
17. Pettit, G. R.; Singh, S. B.; Hogan, F.; Lloyd-Williams, P.; Herald, D. L.; Burkett, D. D.; Clewlow, P. J. *J. Am. Chem. Soc.* **1989**, *111*, 5463-5465.

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