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HERBACEAMIDE, A CHLORINATED *N*-ACYL AMINO ESTER FROM THE MARINE SPONGE, *DYSIDEA HERBACEA*

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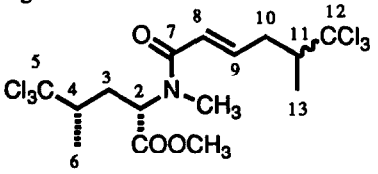
Abstract: *Herbaceamide (1) was isolated from* Dysidea herbacea. *The structure and partial stereochemistry were assigned on the basis of spectroscopic comparision with dysidenin (2) and synthetic analogs.*

Dysidea herbacea is the source of several families of secondary metabolites including polybrominated diphenyl ethers,[1a] rearranged sesquiterpenes[1b] and the chlorinated metabolites, such as dysidenin (2).[1c] Perhaps the most intriguing of these are the chlorine containing peptides which are formally related to each other by the presence of the same modified amino acid, 5,5,5-trichloroleucine. 5,5,5-Trichloroleucine, a lipophilic amino acid unknown from terrestrial sources, is present in **2** as the (2*S*,4*S*) isomer. In the course of investigations into the biosynthesis of this most unusual amino acid we have isolated, herbaceamide (**1**), an acylated trichloroleucine methyl ester and assigned the configurations at C2 and C4 by comparision with synthetic analogs.



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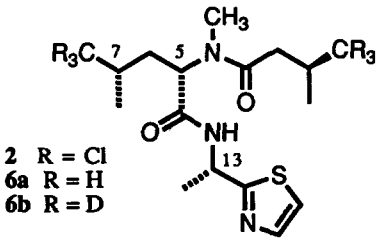

2 R = Cl
6a R = H
6b R = D

The methanol extract from lyophilized *D. herbacea* (collected Great Barrier Reef, May 1990), was partitioned into hexane soluble material and more polar extracts. Purification of the hexane fraction by silica and Florisil flash chromatography followed by HPLC (Dynamax, 20x250 mm, ODS, 87:13 MeOH-H_2O) gave herbaceamide (**1**).[2] The high resolution FAB mass measurement of **1** provided the formula $C_{15}H_{21}Cl_6NO_3$ (m/z 473.9711, MH^+, Δ mmu -2) indicating three unsaturation equivalents, while the UV (λ_{max} 212 nm; $\log_{10}\varepsilon$ 4.20) and IR spectra (ν 1736, 1660, 1616 cm^{-1}) revealed an α,β-unsaturated amide. Interpretation of the ^1H NMR and ^{13}C NMR spectra of **1**, including DEPT, 2D COSY, HETCOR and COLOC experiments defined an

N-methyltrichloroleucine and a 6,6,6-trichloro-2-isoheptenoyl moiety and provided complete spin assignments. The three bond heteronuclear couplings ($^3J_{CH}$) observed from the *N*-CH_3 signal (3.04,s; 31.35, q) to the carboxamide carbon C7 (167.08, s) and C2 (53.17, d) along with the two bond coupling between H8 (6.42, dt, 1H) and C7 (167.08, s), allowed connection of the substructures to give **1**.

Structural confirmation of **1** and the configuration of C2 was obtained by synthesis of (2*S*)-**3** (Fig 1). Thus, isovaleraldehyde was condensed with methyl triethylphosponoacetate (NaH, THF, 92 %) to give methyl 2-isoheptenoate which was saponified (KOH, 1 equiv., aq. MeOH) to the potassium salt (**4**). (S)-*N,O*-Dimethylleucine trifluoroacetate (**5**) was prepared by BOC protection (di-*t*-butyl carbonate, Et_3N, THF, quant.) of (S)-leucine methyl ester (Sigma) followed by methylation (MeI, NaH, DMF, 60°, 74%, some racemization), and deprotection (aq. TFA, quant.). Coupling of **4** and **5** (DCC, *N*-hydroxybenzotriazole, THF, 25°, 10h, 56%) gave **3**[3] after purification by HPLC (Dynamax 10x250 mm, silica, 1:5 EtOAc-hexane). This material was identical by ^1H NMR, ^{13}C NMR and MS with the compound obtained by reductive dechlorination of **1** (Zn dust, CH_3COOH, 60°;[4] aq. NH_4Cl workup, quant.). The circular dichroism spectra of the two samples of **3** showed Cotton effects,[4] identical in sign and magnitude, indicating that the configuration at C2 in **1** was *S*.

The C4 configuration in herbaceamide (**1**) was determined by comparison of the dechloro-derivatives **3** and **6**. We took advantage of two useful properties i) the diastereotopic methyl ^1H NMR signals of the reduced *N*-methyl leucyl compounds in $CDCl_3$ were sufficiently dispersed at high field, and ii) preparation of stereospecifically deuteriated **6** from **2** provides the assignment of the pro-*R* and pro-*S* methyl groups.[5,1c]

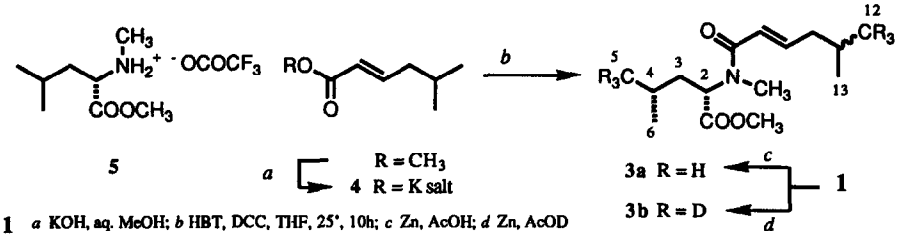

Fig. 1 *a* KOH, aq. MeOH; *b* HBT, DCC, THF, 25°, 10h; *c* Zn, AcOH; *d* Zn, AcOD

Dysidenin (**2**) was dechlorinated with Zn and CH_3COOH or CH_3COOD to give **6a** and **6b**,[1c] respectively. Leucine methyl assignments in **6a** were made at 500 MHz by COSY and HMQC (0.865, d, J = 6.6Hz; 0.921, d, J= 6.6Hz). Since the downfield leucyl methyl signal of **6a** was missing in **6b** and the absolute configuration of dysidenin (**2**) is 7*S*,[6] the pro-*R* and pro-*S* methyl signals of leucine in **6a** are 0.921 and 0.865 ppm, respectively.[7] Likewise, herbaceamide (**1**) was reduced to give **3a** and **3b**. Examination of the leucyl methyl signals in **3a** revealed similar chemical shift differences to those in **6a** and the absence of the downfield methyl

signal (0.954, d) in the hexadeuterio compound, **3b**. Consequently, the configuration of the *N*-methyl 5,5,5-trichloroleucyl residue in **1** must be (2*S*, 4*S*).[8]

The 5,5,5-trichloro-2-isoheptenoate found in **1** probably arises from condensation of an acetate equivalent with 5,5,5-trichloroisovalerate, a putative metabolic intermediate found in **2** which also finds analogy in the catabolism of leucine.[9] The stereochemistry at C11 is presently unassigned, but it is probably also *S*.

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References and notes.

1 For example, (a) Norton, R.; Croft, K.D.; Wells, R.J. *Tetrahedron* **1981**, *37*, 2341-2349; (b) furodysin and furodysinin, Kazlauskus, R.; Murphy, P.T.; Wells, R.J.*Tetrahedron Lett.* **1978**, (*49*), 4951-4954; (c) dysidenin, Kazlauskus, R.; Lidgard, R.O.; Wells, R.J. *Tetrahedron Lett.* **1977**, (*36*), 3183-3186; (d) isodysidenin, Charles, C.; Braekman, J.C.; Daloze, D.; Tursch, B. *Tetrahedron Lett.* **1978**, (*17*), 1519-1520

2 *Herbaceamide* (**1**): $C_{15}H_{21}Cl_6NO_3$, oil, $[\alpha]_D$ -35.1 (c 0.55, MeOH); UV (MeOH) 212 nm (ε $\log_{10}$ 4.20); CD (MeOH, 23°) $\Delta\varepsilon$ +4 (210nm), -6.1 (237); IR ($CHCl_3$) ν 1736, 1660, 1616 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.94, ddd, 1H, *J* = 15.4, 8.5, 6.5 Hz (H9); 6.42, dt, 1H, *J*= 15.4,1.3 Hz (H8); 5.56, dd, 1H, *J*= 12.7,3.5 Hz, H2; 3.74, s, 3H, OCH_3; 3.04, s, 3H, NCH_3; 3.05, m, 1H, H10a; 2.68, m, 1H, H11; 2.51, dd, 1H, *J*= 13.2,13.2 Hz, H3a; 2.32, m, 1H, H10b; 2.27, m, 1H, H4; 2.04, m, 1H, H3b; 1.40, d, 3H, *J*= 6.3 Hz, H6; 1.35, d, 3H, *J*= 6.5 Hz, H13; ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 171.07, s, C1; 167.08, s, C7; 144.18, d, C9; 122.25, d, C8; 105.58, s, C5; 105.16, s, C12; 54.16, d, C11; 53.17, d, C2; 52.46, q, OCH_3; 51.77, d, C4; 36.15, t, C10; 31.95, t, C3; 31.35, q, NCH_3; 16.37, q, C6; 16.09, q, C13) FABMS: m/z 473.9711, MH^+, Calculated for $C_{15}H_{22}Cl_6NO_3$ 473.9731.

3 *Hexadechloroherbaceamide* (**3**), $C_{15}H_{27}NO_3$: oil, $[\alpha]_D$ -59° (c 0.18, MeOH); UV(MeOH) 215 (ε $\log_{10}$ 4.13); CD (MeOH) 214 ($\Delta\varepsilon$ +3.5), 237 (-7.9). ^1H NMR ($CDCl_3$, 6:1 mixture of rotamers) 6.95, dt, 1H, *J*= 15.0, 7.5 Hz, 1H; 6.26, dt, *J*= 15.0, 1.4 Hz, 1H); 5.41, dd, *J*= 9.9, 5.9 Hz, 1H; 3.70, s, OCH_3; 2.99, s, NCH_3; 2.15, td, *J*= 7.2, 1.4 Hz, 2H; 1.7, m; 1.52, m; 0.954, d, *J* = 6.7, H5; 0.940, d, *J* = 6.6 Hz, 6H, H12 & H13; 0.940, d, *J*= 6.7, H6; CIMS 270 (MH^+, 9%), 210 (23), 100(100).

4 Corrected for partial racemization of synthetic **3**.

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5 Dysidenin was isolated from another sample of *D. herbacea*, collected on the Great Barrier Reef in 1991. All high field assignments, at 300 or 500 MHz, were made in $CDCl_3$ using 0.06M sample solutions.

6 The has been some confusion in the literature regarding the configurations of dysidenin and isodysidenin. For discussion and resolution of this problem, see de Laszlo, S.E.; Williard, P.G. *J. Am. Chem. Soc.* **1985**, *107*, 199-203 and footnote in Carmely, S.; Gebreyesus, T.; Kashman, Y.; Skelton, B.W.; White, A.H.; Yosief, T. *Aust. J. Chem.* **1990**, *43*, 1881-8. We have confirmed the *(5S , 13S)* configuration in **2** from anomalous scattering in a single crystal X-ray diffraction study of a dysidenin derivative (Molinski, T.F.*et al* , unpublished data).

7 In (*S*)-leucyl peptides the pro-*R* methyl is expected to occur downfield due to a 1,5-pentane interaction of the polar amido group.

8 We have since assigned the C4 configuration in other 5,5,5-trichloroleucyl peptides using the same method. These will be published, together with experimental details, in due course.

9 Lehninger, A.L., *Biochemistry*, 2nd ed., Worth, New York, 1975, p. 570.

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