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Thelepamide: An Unprecedented Ketide-Amino Acid from *Thelepus crispus*, a Marine Annelid Worm

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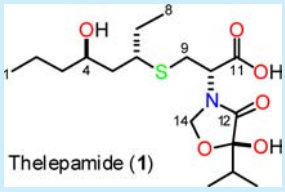
Supporting Information

ABSTRACT: Thelepamide (**1**) was characterized during a program to study cytotoxic substances from an unusual source, the tidal zone-derived annelid *Thelephus crispus*. Its structure contains a tetraketide and a tripeptide subunit and possesses striking atom diversity, consisting of 17 carbons and 8 heteroatoms. The relative configurations at four chiral sites were elucidated via ROESY, *J*-based configurational analysis, and DFT calculations. It was modestly active against leukemia cells (IC_{50} = 5 μg/mL) and inactive against solid tumor cell lines.



Marine invertebrates, especially sponges and tunicates, are recognized as an incredible source of diverse and bioactive secondary metabolites.[1] Strikingly, current awareness scans of the review literature for the biosynthetic products of marine worms such as those from the *Annelida* or *Hemichordata* phyla show few hits.[2] However, there are some important molecular structure dereplication outcomes that arise from such a search. Heading this short list are the bis-steroidal pyrazine cephalostatins, which are exceedingly potent natural cytotoxins from the hemichordate tubeworm *Cephalodiscus gilchristi*.[3] Other metabolites isolated from organisms belonging to these two phyla are: (1) bromophenols,[4] (2) halogenated indoles,[5] (3) chlorinated metabolites,[6] (4) *ortho*-antraquinones,[7] and (5) polypeptides.[8]

Early in our campaign to examine coastal zone-derived worms, we selected one specimen for rigorous study. The methanol extract of *Thelepus crispus* (Phylum *Annelida*, class Polychaeta, family Terebellidae) showed cytotoxic activity against leukemia tumor cells (ED_{50} < 5 μg/mL). Once the molecular formula of the major metabolite was established, it was clear that the compound in hand, named thelepamide (**1**), was different compared to those isolated during previous studies on *T. crispus* which included polybrominated phenols and hemoglobin.[9]

The reasonable yield of a semipure extract fraction (20 mg) which contained almost pure **1** facilitated our investigation. The sample of *T. crispus* (Coll. No. CBMT 93311, 571 g of wet weight) collected off the coast of Friday Harbor, WA was easily taxonomically identified. Our standard workup procedure[10] afforded a cytotoxic active MeOH/H_2O (1:1) fraction, which eventually yielded **1** as an optically active amorphous solid.

The (−)-HRFABMS analysis of compound **1** provided the $[M-H]^-$ pseudomolecular ion at *m/z* 376.1808, appropriate for a molecular formula of $C_{17}H_{31}NO_6S$ (Δ 1.4 mmu). Additional confirming data for the proposed molecular formula were derived from the following: (a) pseudomolecular ions $[M + Na]^+$ at *m/z* 400.1781 (Δ 1.1 mmu) and $[M - H + 2Na]^+$ at *m/z* 422.1594 (Δ 0.5 mmu) by (+)-HRFABMS; (b) *m/z* 416 $[M + K]^+$, (c) *m/z* 438 $[M - H + Na + K]^+$, and (d) *m/z* 454 $[M - H + 2K]^+$ by (+)-LRFABMS. The IR spectrum suggested the presence of hydroxyl (3336 cm^{-1}), carboxylic acid (1697 cm^{-1}), and amide (1613 cm^{-1}) functionalities.

The next step in the structure elucidation involved detection of four isolated proton spin systems outlined in Figure 1. The

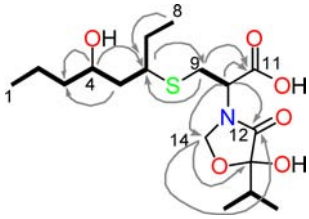


Figure 1. Diagnostic NMR correlations (see also Supporting Information (SI)) including: (a) $^1H-^1H$ COSY (black bonds) and (b) HMBC ($^1H\rightarrow^{13}C$) to support the overall framework proposed for compound **1**.

CH containing substructures could be drawn based on analysis of NMR data observed in CD_3OD (^1H, ^{13}C, DEPT, COSY, HMQC, and HMBC) of **1** accompanied by correlating all of the ^1H and ^{13}C NMR chemical shifts with specific atoms.[11]

The first proton spin system C1−C8 was easily identified by the $^1H-^1H$ COSY correlations starting from a multiplet at δ_H 3.65 (H4). The presence of a cysteine moiety bearing the

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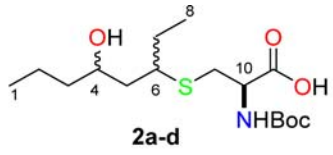
A)



2a-d

δ_C Pos.	1	2a-d
1	14.5	14.5
2	19.9	19.7, 19.8, 20.0
3	40.9	40.6, 40.9, 41.0
4	69.5	69.1, 69.3, 69.8, 69.9
5	43.6	44.1, 43.7, 43.4, 43.1
6	44.7	45.8, 45.2, 45.1, 44.6
7	27.5	30.4, 29.8, 28.0, 27.8
8	11.2	11.4, 11.1, 11.0
9	30.9	34.2, 34.7, 34.8
10	56.8	56.9, 56.7

B)

3a R_1 = Bn, R_2 = Me
3b R_1 = Me, R_2 = Ph

	3a[13a]		**3b**[13b]		**1**	
Pos.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
13	99.5		99.5		104.7	
14	77.1	5.00 d 4.75 d *J*= 3.3 Hz	79.3	5.05 d 5.20 d *J*= 3.2 Hz	77.3	5.26 d 4.94 d *J*= 2.5 Hz

Figure 2. Comparison of the NMR spectral data of compound **1** to those of synthetic analogs **2a–d** (500 MHz in CD_3OD) and 5-hydroxyoxazolidin-4-ones **3a,b** (in $CDCl_3$).

second spin system was suggested by the ^{1}H NMR signals, connected to their corresponding carbons in the HMQC experiment, at δ_H 3.28 (dd, J = 13.0 and 3.5 Hz, H9l) and 2.70 (t, J = 13.0 Hz, H9h)/δ_C 30.9 (C9), δ_H 4.69 (dd, J = 13.0 and 3.5 Hz, H10)/δ_C 56.8 (C10). These two substructures could be linked through HMBC correlations from H6 (δ_H 2.86) to C9 and from H9 (δ_H 3.28 and 2.70) to C6 (see Figure 1).

The presence of an isolated isopropyl moiety was deduced from the ^{1}H–^{1}H COSY correlations between two diastereotopic methyl groups at δ_H 0.98 (d, J = 6.6 Hz, Me16) and δ_H 1.10 (d, J = 6.6 Hz, Me17) and methine H15 at δ_H 2.18 (sept., J = 6.6 Hz). Moreover, two diastereotopic methylene protons at δ_H 5.26 and 4.94 (H14) with a small geminal coupling (J = 2.5 Hz) were observed as the last isolated spin system. The carbon chemical shift of this methylene group at δ_C 77.3 suggested that it must be linked to two heteroatoms. Key HMBC correlations from methylene protons H14 to carbons C12 and C13 along with H15/C13 supported the presence of an oxazolidienone ring. Further correlations between methine H10 and carbons C14 and C12 enabled connection of these remaining two substructures thereby completing the planar structure of **1**.

Further confirmation of the presence of C1–C8 and C9–C11 fragments in the proposed structure of **1** was sought. This involved the preparation of analogs **2a–d** using a non-stereoselective synthesis (see SI). Thus, 4-octyne was converted to (*E*)-oct-5-en-4-one which was reacted with *N*-Boc-cysteine through a Michael type addition[12] and finally reduced with $NaBH_4$ to yield the mixture of analogs **2a–d**. Comparison of the NMR data between **2a–d** and the corresponding atoms in **1** clearly showed very similar chemical shifts (see Figure 2A). Another set of benchmark oxazolidinone (**3a** and **3b**) isomers possessing varying ring substituents were prepared by different compounds from two known synthetic methods.[13] Again, the NMR spectral data for **3a** and **3b** displayed a similar set of chemical shifts (see Figure 2B) which were also similar in comparison to the corresponding atoms in **1**. These data further justified the connectivities proposed for the heterocyclic ring.

The relative stereochemistry of **1** was addressed next. Heteronuclear *J*-based configurational analysis (JBCA) was used to determine the relative stereochemistry of chiral centers at C4 and C6 in fragment C1–C8. Although there are many examples in the literature for studies of methine 1,2 or 1,3 chiral centers containing oxygen, nitrogen, or chlorine atoms,[14] *this is the first example* of applying the JBCA methodology to a sulfur-containing fragment. We assumed that there were not too many differences between oxygen and sulfur atoms due to their similar electronegativity values. The HETLOC, HECADE, and *J*-HMBC experiments were performed to measure key heteronuclear coupling constants (boxed *J* values in the Figure 3). Taking into account the six possible rotamers **I–VI** for the

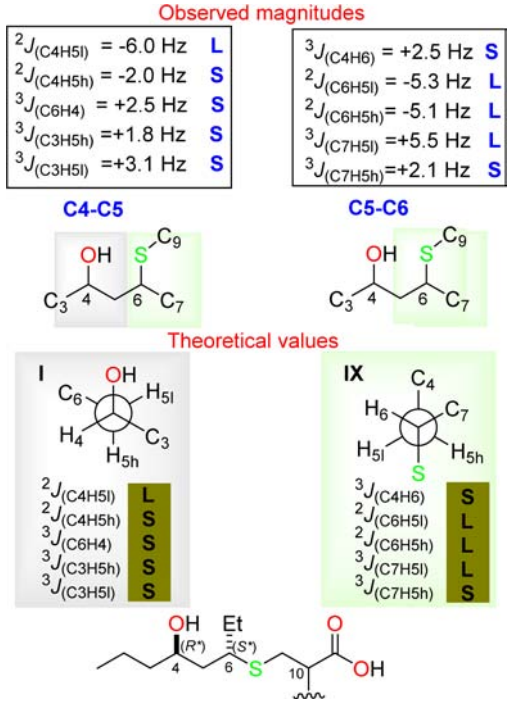


Figure 3. Rotamers with *J* values consistent with the measured heteronuclear coupling constants and the resulting 4*R**, 6*S** relative stereochemistry for **1**. Note: **L** (large) and **S** (small) refer diagnostic J_{CH} values discussed in ref 15 (see the complete analysis in SI).

erythro and *threo* conformers around the C4–C5 bond (see SI), following Murata's methodology,[15] conformer **I** was the only one which agrees with the experimental heteronuclear $^2J_{(C4H5l)}$, $^2J_{(C4H5h)}$, $^3J_{(C6H4)}$, $^3J_{(C3H5h)}$, and $^3J_{(C3H5l)}$ values observed (Figure 3). Using the same approximation for the six rotamers of the C5–C6 bond consisting of *erythro* and *threo* conformers **VII–XII** (see SI), we found that only the conformation **IX**

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corresponds to the rotamer with $^{3}J_{(C4H6)}$, $^{2}J_{(C6H5l)}$, $^{2}J_{(C6H5h)}$, $^{3}J_{(C7H5l)}$, and $^{3}J_{(C7H5h)}$ values consistent with the measured heteronuclear coupling constants (Figure 3). On the basis of these data, the overall relative stereochemistry for these two centers was concluded to be 4*R**, 6*S**.

Having established the relative configurations between the C4 and C6 stereocenters, attention was turned to assignment of the C10 and C13 chiral centers which proved to be more challenging. The four possible diastereoisomers around C10 and C13 chiral centers consisted of possibilities **1a**–**d** (Figure 4A). These were distinguished on the basis of NOESY

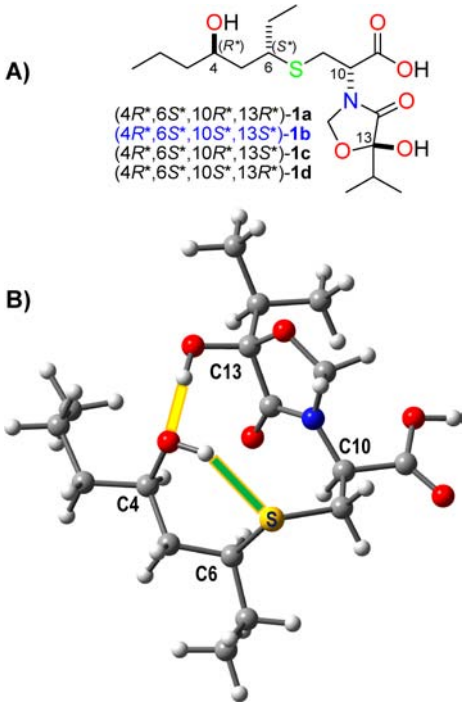

Figure 4. (A) Structures of the four possible diastereomers **1a**–**d** around C10 and C13. (B) Energy-minimized conformer for **1b** (4*R**,6*S**,10*S**,13*S**). The yellow and green bars denote hydrogen bonds.

experiments and by DFT molecular modeling calculations. Particularly diagnostic NOEs were observed among H4, H6, and H10, suggesting that these protons must be placed in a 2–5 Å proximal environment. In view of these restrictions, we carried out conformational searches (50 000 steps) using the GMMX module of PCMODEL for each of the four possible diastereoisomers.

Energy-minimized structures in a 2.5 kcal/mol window were obtained first for each of the four diastereoisomers **1a**–**d**. Next each was then optimized at the B3LYP/6-31G(d) level of theory in order to evaluate its DFT energy. Only conformers of **1b**, with the 4*R**, 6*S**, 10*S**, 13*S** relative stereochemistry (Figure 4B), appear wholly consistent with the NMR experimental data (*J*-values and NOE restrictions). Interestingly, the 17 conformers corresponding to diastereoisomer **1b** showed the presence of intramolecular hydrogen bonds between (a) the OH at C4 and S (green bar) and (b) the HO at C13 and O at C4 (yellow bar), suggesting the existence of a more rigid structure. This fact agrees with the large difference in chemical shifts of the H9*l* and H9*h* diastereotopic protons and their different proton–proton coupling constants to H10 ($^{3}J_{H9h\text{-}H10}$ = 13.0 Hz vs $^{3}J_{H9l\text{-}H10}$ = 3.5 Hz).

A final step was taken to explore the relative configurations proposed above for thelepamide (**1**). This involved the use of DFT chemical shift calculations. Therefore, *ab initio* NMR shielding constants were calculated for each structure using the DFT Gauge Independent Atomic Orbital (GIAO) method in the gas phase. This analysis included representing all the available configurational and conformational space for **1a**, **1b**, **1c**, and **1d**. It involved the MP1MPW91 functional tool in conjunction to the 6-31G(d,p) basis set. The output of ^{13}C and ^{1}H chemical shifts was calculated using the TMS at the same level of calculation as the reference and taking in account the Maxwell–Boltzmann population averaged on the basis of the SCF energy differences. The mean absolute error (MAE), R^2 of $\delta_{calcd}/\delta_{expt}$, by the linear regression of calculated (δ_{scaled})[16] was considered for the four possible diastereoisomers **1a**–**d**. The best fit was found in all cases for **1b** (see SI). Finally the computed chemical shifts for each diastereoisomer were introduced in the JAVA web applet provided by Goodman's group[17] in order to calculate the DP4 probability for carbon chemical shifts. These calculations predicted a single diastereoisomer **1b** (4*R**,6*S**,10*S**,13*S**) with >99% probability in having the correct relative configuration. This conclusion proved to be in accord with the proposed configurations determined by our NMR analysis (**1b**, Figure 4).

There are some additional properties of thelepamide (**1**) that merit discussion. First, **1** showed a selective but modest cytotoxicity against leukemia cells CCRF-CE (IC_{50} = 5 μg/mL). It was inactive (IC_{50} > 50 μg/mL) against solid tumors including colon cancer HCT-116 cells or breast cancer MCF-7 cells. To the best of our knowledge, the skeleton of thelepamide (**1**) has no counterparts and represents exciting new chemical space. Furthermore there is no precedent in the literature for any other natural product containing an oxazolidinone ring with substituents as in thelepamide (**1**). Biosynthetically, the C1–C8 fragment of **1** appears to be derived from a tetraketide, while the C9–C18 fragment could come from three different aminoacids: cysteine, isoleucine, and glycine (see SI).

■ ASSOCIATED CONTENT

Supporting Information

Full characterization, ^{1}H NMR, ^{13}C NMR, DEPT-135, HMQC, HMBC, ^{1}H–^{1}H COSY, HETOC, *J*-HMBC, and LRFABMS spectra for thelepamide (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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DEDICATION

[‖]Dedicated to Pr. Ricardo Riguera for his 65th birthday.

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(11) Thelepamide (**1**): $[\alpha]_D$ = −10.4 (*c* 0.016 MeOH); IR (neat) 3336, 2962, 1697, 1613, 1466, 1393, 1091, 1059, 962 cm^{-1}; ^1H NMR (500 MHz, CD_3OD): δ 5.26 (d, *J* = 2.5 Hz, 1H, H14*l*), 4.94 (d, *J* = 2.5 Hz, 1H, H14*h*), 4.69 (dd, *J* = 13.0 and 3.5 Hz, 1H, H10), 3.65 (m, 1H, H4), 3.28 (dd, *J* = 3.5 and 13.0 Hz, 1H, H9*l*); 2.86 (m, 1H; H6), 2.70 (t, *J* = 13.0 Hz, 1H, H9*h*), 2.18 (sept, *J* = 6.6 Hz, 1H, H15), 1.77 (m, 1H, H5*l*), 1.69 (m, 1H; H7*l*), 1.60 (m, 1H, H5*l*), 1.47 (m, 1H, H7*h*), 1.45 (m, 1H; H2*l*), 1.38 (m, 1H, H3*l*), 1.36 (m, 1H, H3*h*), 1.33 (m, 1H, H2*h*), 1.10 (d, *J* = 6.6 Hz, 3H, H17), 0.98 (d, *J* = 6.6 Hz, 3H, H16), 0.94 (t, *J* = 6.4 Hz, 3H, H1), 0.89 (t, *J* = 6.4 Hz, 3H, H8). ^{13}C and DEPT-135 NMR (63 MHz, CD_3OD): 174.3 (C, C11), 171.4 (C, C12), 104.7 (C, C13), 77.3 (CH_2, C14), 69.3 (CH, C4), 56.8 (CH, C10), 44.7 (CH, C6), 43.6 (CH_2, C5), 40.9 (CH_2, C3), 34.9 (CH, C15), 30.9 (CH_2, C9), 27.5 (CH_2, C7), 19.9 (CH_2, C2), 17.7 (CH_3, C16), 15.5 (CH_3, C17), 14.5 (CH_3, C1), 11.2 (CH_3, C8).

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