Reducive Dechlorination of Dysidenin from Dysidea Herbacea. Structure of a Novel Binuclear Zinc Metallocycle

Eugene K. Trousdale, Steven W. Taylor, Sean Parkin, Hakon Hope, Tadeusz F. Molinski*

Department of Chemistry
University of California, Davis, CA 95616

(Received 5th February 1998)

Abstract:
The structure of a novel 20-membered macrocyclic Zn chelate, prepared by reductive dechlorination of dysidenin, was solved by single crystal X-ray diffraction and confirms the SS,13S configuration.

Keywords: Dysidea herbacea, trichloroleucine, peptide, X-ray analysis

Introduction:

Dysidenin (1)\(^1\) belongs to a family of about a dozen modified peptides isolated from the marine sponge Dysidea herbacea that contain the remarkable chlorinated groups N-methyl-5,5,5-trichloroleucine and 4,4,4-trichloroisovaleramide. In our study of the biosynthesis of trichloroleucine marine natural products we required reliable assignments of configuration for 1, however, the absolute configuration in 1 has been a matter of some controversy.

A determination of absolute configuration for 1 was published\(^2\) based on comparison with isodyidenin (2), a diastereomer of 1 that was erroneously reported as 2R, 5S, 7R, 13R from an X-ray crystal structure determination.\(^3\) The configuration of 2 has since been revised,\(^4\) but determination of configuration in 1 and other members of the family by X-ray crystallography has been problematic.\(^5\) The relative configuration at C2 and C4 (amino acid numbering) of the N-methyl-5,5,5-trichloroleucine residue in 1 was not in doubt because of correlations.

* Author for correspondence: tel. (530) 752 6358, FAX, (530) 752 8995, tfmolinski@ucdavis.edu
that were made during the synthesis\textsuperscript{6} of \((\pm\text{-})\text{-demethyldysidenin}, the enantiomer of the natural product 3.\textsuperscript{7}

Aside from chiroptical comparisons\textsuperscript{2} there is no independent determination of the absolute configuration at the \(\alpha\)-carbon (C5) in 1.\textsuperscript{9}

In the structure elucidation of herbaceamide 4 from \textit{D. herbacea} we developed a spectroscopic method for assignment of the relative stereochemistry at the remote C7 center with respect to C5 in N-methyl 5,5,5-trichloroleucyl residues based on reductive dechlorination in the presence of \(\text{AcOH} \) and \(\text{AcOD}\).\textsuperscript{8} However, when Zn-promoted dechlorination 1 was carried out an unusual metal chelate 5 was isolated which provided the opportunity for independent confirmation of C5 and C13 configurations. This communication describes the X-ray crystal structure and absolute configuration of a novel 20-membered, dimeric Zn metallolcycle (55, 135) 5 and suggests that analogous Zn derivatives may be useful for independent configurational assignments in other \textit{Dysidea} peptides. From this result and the known relative configuration of 1, it can be deduced that C2 and C7 configurations are both S - the same as other members of the dysidenin family - which supports a conservative biogenesis of the trichloroisopropyl group.\textsuperscript{8}

\textbf{RESULTS}

Dysidenin (1)\textsuperscript{1} was isolated from \textit{Dysidea herbacea} as described elsewhere\textsuperscript{8} and subjected to reductive dechlorination (Zn dust, \(\text{AcOH}, 70^\circ\)). After non-aqueous work-up (dilution of the hot mixture with \(\text{CHCl}_3\) and removal of insoluble products by filtration), the clear filtrate was concentrated and triturated with \(\text{CHCl}_3\) to provide a single product 5 upon removal of solvent. \(^1\text{H} \) NMR of the residue in \(\text{CDCl}_3\) showed the appearance of signals due to the newly formed isopropyl group.

\textsuperscript{1} The correlation of 1 and 2 by hydrolysis and derivatization was compromised by the fact that epimerization occurred in both 1 and 2 at C5.\textsuperscript{2}
All bond angles and bond lengths appear normal, however, the 20
membered macrocycle was unexpected. The complex is soluble in
CDCl₃ (99.7% d) but it is not clear from the ¹H NMR data whether the
dimeric structure persists or whether a monomeric species is preferred
in solution.

The absolute configuration for 5 was determined from the X-ray
data. Two computational methods were used: determination of the sign
of f°, and calculation of the Flack x parameter. Both methods gave
the same result. The Flack parameter is -0.027 (expected values are 0,
within 3 esd's, for correct and +1 for inverted absolute structure). The
absolute structure determination is now simplified because of the
number of different, significant anomalous scatterers (Zn, Cl, S) and
leads to 5S, 13S for 5. It follows that the absolute configuration of 1 can
now be assigned confidently as 2S, 5S, 7S, 13S consistent with earlier
predictions.

The diasterotopic methyl groups in each isopropyl group of 6
exhibit different ¹H NMR (500 MHz) chemical shifts. The pro R and
pro S assignments for each pair of methyl groups were made as follows.
Reduction of 1 with Zn-AcOD, followed by work-up (NH₄Cl aq. wash)
gave d₆-compound 7 (C₁₇H₂₃D₆N₃O₂S, FABMS MH⁺, m/z 346.2432,
Δamu 1.3). The ¹H NMR spectrum of 7 (CDCl₃, 0.06 M, δ 0.953, d, J = 6.6
Hz, C2 Me; 0.865, J = 6.6 Hz, C7 Me; 1.67, d, J = 6.6 Hz, C13 Me) showed
only methyl groups derived from non-chlorinated methyls in 1.
Comparison of the pairs of ¹H NMR signals for diasterotopic Me
groups at C7 in 6 (prepared by Zn-AcOH reduction, δ 0.921, 0.865 ppm)
with the C7 Me group in 7 (δ 0.865 ppm) identified the latter as the C7
pro-S methyl group (relative stereochemistry). Because the relative
configuration of 1 is known and the absolute configuration at C5 was
shown to be S from X-ray of 5, it follows that the upfield Me group at
C7 in 6 is pro-S and the downfield Me group is pro-R.

DISCUSSION

X-ray analysis of Zn metallocyclic derivatives has provided an
alternative for configurational assignment of α-carbons in peptides of
the dysidenin family without reliance on anomalous scattering in the
natural product by Cl alone. Specifically, reductive removal of Cl from
1 afforded the heavy atom derivative 5 that allowed unambiguous
assignment of 5S, 13S. Combined with high-field ¹H NMR assignments

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† All ¹H NMR assignments in 1 and derived compounds were made from interpretation
of COSY spectra, decoupling and matching of coupling constants (J).
however, the mass recovery was unexpectedly high. FABMS of 5 showed a parent ion containing Zn instead of the expected peptide 6 and suggested incorporation of Zn.

![Chem3D representation of dimeric Zn-chelate 5](image)

**Figure 1:** X-ray structure (Chem3D representation) of dimeric Zn-chelate 5 (CHCl₃ omitted)

Although the ¹H NMR spin systems were the same for both 5 and 6, the two showed slightly different ¹H chemical shifts in CDCl₃, particularly those of the thiazole ring H16, 17. When the crude product was washed first with saturated aqueous NH₄Cl, the Zn-free peptide 6 (amorphous) was obtained whose ¹H NMR and MS spectral characteristics were identical with those of literature values.

A CHCl₃ solution of 5 was allowed to evaporate slowly upon which colorless plates were deposited (mp ~168° dec). Single-crystal X-ray diffraction measurements showed that compound 5 crystallizes with one molecule of solvent (Figure 1, CHCl₃ removed for clarity) in the monoclinic space group P2₁, a = 12.640(5), b = 17.834(7), c = 13.083(3) Å, β = 102.11(3)° (T = 120 K, λ(Cu Ka) = 1.54178 Å). There are two dimeric molecules in the unit cell. Based on intensity data, the dimeric structure 5 was determined. The two Zn atoms show slightly distorted tetrahedral geometry with coordination to the C4 carboxyl oxygen, the thiazole N and two Cl's. The bond angles Cl-Zn-Cl / O-Zn-N at each metal center are 117°/101.4° and 118.3°/103.1°, respectively. Surprisingly, the thiolphilic Zn is not coordinated to the available lone pair of the thiazole sulfur but is bound to the thiazole N of one peptide unit and the carbonyl of the isovaleroyl group in the other peptide unit.

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6 In FABMS, only the monomeric form was observed C₁₇H₂₀N₅O₂S₂ZnCl (m/z 440 MH⁺, 100%), with the isotope ratio for MH⁺+1 and MH⁺+2 expected for a molecular ion containing one each of Zn, Cl and S.
of pro R and S Me groups in 6 and 7, the assignment of relative configuration of trichloroleucyl groups in related metabolites can also be made after Zn-promoted dechlorination.

The detailed biosynthesis of 1 is unknown. Reports of compounds containing homologated derivatives of 4,4,4-trichloroisovalerate from D. herbacea.8,11-14 and the blue-green alga Lyngbya majuscula15 raise the intriguing possibility that the latter is derived from catabolism of 5,5,5-trichloroleucine and exploited as a starter unit for ketide synthesis.8,16 If Cl is introduced by enzyme-mediated oxidation after incorporation of leucine, it would be of interest to determine if the C4 configuration is retained or inverted. In any case, it would appear, from comparison of the C7 stereocenters in 1 and 4 with those of related metabolites,5 that the extraordinary biosynthetic transformation that carries out Cl substitution in the unactivated methyl groups of Dysidea herbacea leucine-derived peptides conserves the 2S, 7S configuration in all members of the series examined so far.

ACKNOWLEDGMENTS:

This work was partially funded by grants to TFM from the National Sea Grant College Program (R/MP-57A) and the Committee on Research, UC Davis. We thank Dan Jones, Facility of Advanced Instrumentation, for FABMS data and Jeff DeRopp for 500 MHz NMR spectra. The 500 MHz NMR spectrometer was partially funded through NIH ISIO-RR04795 and NSFBB88-04739.

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