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REDUCTIVE DECHLORINATION OF DYSIDENIN FROM *DYSIDEA HERBACEA*. STRUCTURE OF A NOVEL BINUCLEAR ZINC METALLOCYCLE

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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 5S,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 *iso*dysidenin (2), a diastereomer of 1 that was erroneously reported as 2*R*, 5*S*, 7*R*, 13*R* 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

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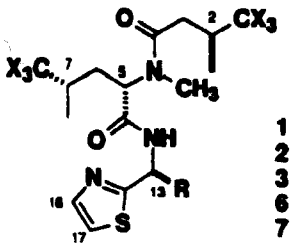
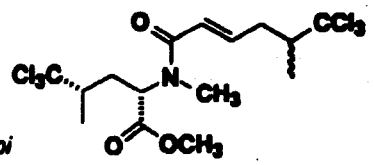
that were made during the synthesis[6] of (+)-demethyldysidenin, the enantiomer of the natural product 3.[7]



1 R = CH_3, X = Cl
2 R = CH_3, X = Cl, C5-*epi*
3 R = H, X = Cl
6 R = CH_3, X = H
7 R = CH_3, X = D



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Aside from chiroptical comparisons[2] there is no independent determination of the *absolute* configuration at the α-carbon (C5) in 1.¶

In the structure elucidation of herbaceamide 4 from *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 AcOH and AcOD.[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 metallocycle (5*S*, 13*S*) 5 and suggests that analogous Zn derivatives may be useful for independent configurational assignments in other *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.[8]

RESULTS

Dysidenin (1)[1] was isolated from *Dysidea herbacea* as described elsewhere[8] and subjected to reductive dechlorination (Zn dust, AcOH, 70°). After *non-aqueous* work-up (dilution of the hot mixture with $CHCl_3$ and removal of insoluble products by filtration), the clear filtrate was concentrated and triturated with $CHCl_3$ to provide a single product 5 upon removal of solvent. ^1H NMR of the residue in $CDCl_3$ showed the appearance of signals due to the newly formed isopropyl group,

¶ 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.[2]

All bond angles and bond lengths appear normal, however, the 20 membered macrocycle was unexpected. The complex is soluble in $CDCl_3$ (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.[9,10] Both methods gave the same result. The Flack parameter is -0.02(7) (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 5*S*, 13*S* for 5. It follows that the absolute configuration of 1 can now be assigned confidently as 2*S*, 5*S*, 7*S*, 13*S* consistent with earlier predictions.[2,5]

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 (NH_4Cl aq. wash) gave d_6-compound 7 ($C_{17}H_{23}D_6N_3O_2S$, FABMS MH^+, *m/z* 346.2432, Δmmu 1.3). The ^1H NMR spectrum of 7 ($CDCl_3$, 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 diastereotopic Me groups at C7 in 6 (prepared by Zn-AcOH reduction,[2] 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[6] 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 5*S*, 13*S*. Combined with high-field ^1H NMR assignments

† 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**[2] and suggested incorporation of Zn.

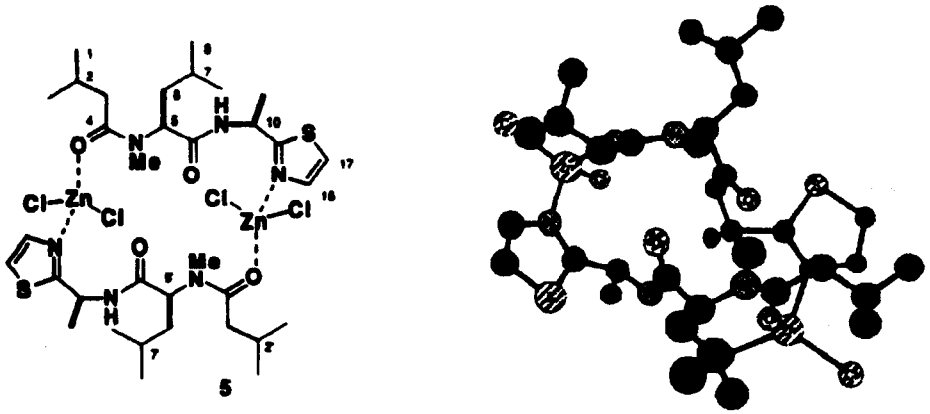

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Figure 1: X-ray structure (Chem3D representation) of dimeric Zn-chelate **5** ($CHCl_3$ omitted)

Although the ^1H NMR spin systems were the same for both **5** and **6**, the two showed slightly different ^1H chemical shifts in $CDCl_3$, particularly those of the thiazole ring H16, 17. When the crude product was washed first with saturated aqueous NH_4Cl, the Zn-free peptide **6** (amorphous) was obtained whose ^1H NMR and MS spectral characteristics were identical with those of literature values.[2]

A $CHCl_3$ 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, $CHCl_3$ removed for clarity) in the monoclinic space group $P2_1$, a = 12.640(5), b = 17.834(7), c = 13.083(3) Å, β = 102.11(3)° (T = 120 K, λ(Cu Kα) = 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 carbonyl 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 thiophilic 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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§ In FABMS, only the monomeric form was observed $C_{17}H_{29}N_3O_2SZnCl$ (m/z 440 MH^+, 100%), with the isotope ratios 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 majuscula*[15] 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 stereochemistries 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 2*S*, 7*S* configuration in all members of the series examined so far.

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