In summary, complicated proton NMR spectra of enzyme/inhibitor complexes can be simplified and made interpretable by using 2D NOE difference spectroscopy and deuterium labeled ligands. Analogous to the structural information obtained by isotope-editing procedures,[6] these techniques allow the conformations of bound ligands and their active site environments to be defined. Unlike the isotope-editing techniques, however, this method does not require additional hardware for x-nucleus decoupling, fixed delays in the experiment which decrease the sensitivity, or carbon-13 labeling of ligands which is typically more difficult and expensive than deuterium labeling.

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Isolation and Structure of the Cytostatic Depsipeptide Dolastatin 13 from the Sea Hare *Dolabella auricularia*[1]

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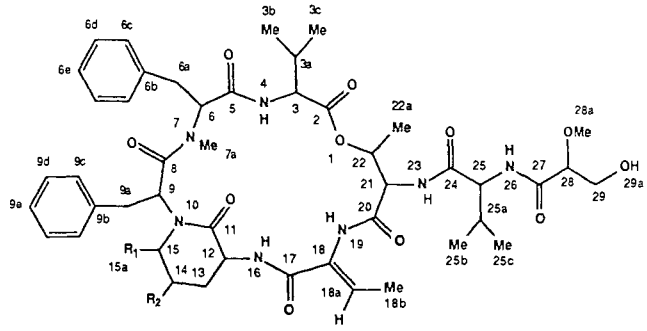
The Indian Ocean shell-less mollusc *Dolabella auricularia* superficially appears to lack predator defenses. Such seemingly defenseless animals are only attacked by certain carnivorous[2a] members of this gastropod subclass. Evidence is now accumulating that Opisthobranchia species have developed very powerful chemical defenses by careful selection and/or biosynthetic manipulation of various dietary sources such as blue-green algae[1a] and sponges[2b,3] (employed by nudibranchs, including possible protection of egg masses[4]). Our early (1968-1972)[5] assumptions that certain species of shell-less molluscs of, e.g., the *Aplysia*[5] and *Dolabella*[6] genera contain potentially useful defensive constituents of the cell growth inhibitory type has been amply realized by isolation of the exceptionally potent antimelanoma pentapeptide dolastatin 10 from *D. auricularia*.[6] We now report the isolation and structural elucidation of a new cell growth inhibitory (P388 lymphocytic leukemia, PS system[7]) constituent of this animal that represents a hitherto unknown type of cyclodepsipeptide.

A small (1.72 g), albeit PS active, fraction prepared as previously summarized[1] from 1600 kg (wet wt) of *D. auricularia* collected (1982) in the Indian Ocean (East Africa) was further separated (PS bioassay) by gradient HPLC (RP8 silica gel, 1:1 methanol-water 100% methanol as mobile phase) to afford dolastatin 13 (**1**) as crystals (from methylene chloride-hexane, 10.6



1: R_1= OH; R_2=H
2: R_1=R_2= $\Delta^{14,15}$

mg, 6 × 10^{-8}% yield): mp 286-289 °C; $[\alpha]_D$ +94° (*c* 0.01, CH_3OH); R_f 0.56 in 90:10:0.8 CH_2Cl_2-CH_3OH-H_2O; see ref 13 for mass spectroscopy; UV (CH_3OH) λ_{max} (log ϵ), 220 (3.04) nm; and IR (NaCl plate), and ν_{max} 3384, 3315, 2960, 2930, 1733, 1677, 1653, 1529, 1205, 750, and 700 cm^{-1}. Dehydrodolastatin 13 (**2**) was obtained as a minor component together with depsipeptide **1** from the same fraction; crystals from methylene chloride-hexane (0.74 mg, 4 × 10^{-9}% yield); mp 127-132 °C; $[\alpha]_D$ +38° (*c* 0.005, CH_3OH); R_f 0.64 (in preceding solvent system); HR FAB MS[M + H]$^+$ obsd 888.4492, calcd 888.4508 for $C_{46}H_{62}N_7O_{11}$; UV_{max} (CH_3OH) λ_{max} (log ϵ), 220 (3.11) nm; and IR (NaCl plate), λ_{max} 3382, 3311, 2960, 2930, 1732, 1678, 1653, 1530, 1467, 1202, 750, and 700 cm^{-1}.

On the basis of results of detailed high field (400 MHz) ^1H and ^{13}C NMR and high resolution FAB MS peak matching experiments, a molecular formula of $C_{46}H_{63}N_7O_{12}$ was deduced for dolastatin 13 (**1**). A combination of ^1H,^1H COSY, ^1H,^{13}C COSY, and ^1H,^1H relayed COSY[8] experiments indicated eight discreet spin coupled systems of which four corresponded to the well-known amino acids threonine (Thr), *N*-methylphenylalanine (MePhe), and valine (Val, two units). Threonine and the two valine units were also detected by amino acid analyses of the products from acid-catalyzed (6 N HCl, 110 °C, 24 h) hydrolysis. Assignment of the fifth and sixth units as an N,N-disubstituted phenylalanine derivative was realized by NMR interpretations. From a series of double and triple relayed coherence transfer experiments (homonuclear relay) and sensitivity enhanced heteronuclear multiple bond correlation experiments (HMBC)[9] the sixth unit was found to be the new cyclic hemiacetal (Ahp, for 3-amino-6-hydroxy-2-piperidone, cf. **1**) presumably derived from a Phe-Glu dipeptide precursor (Glu-γ-carboxyl-γ-aldehyde).[10]

Continuation of the NMR experiments led to assignment of the seventh unit as the rare[11] dehydro amino acid α,β-dehydro-2-aminobutanoic acid (*cis*-Abu),[12] presumably from dehydration of Thr, and the eighth, as 2-*O*-methylglyceric acid (MeGlc). Because of some ambiguity, the Δ-Abu olefin was only tentatively

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(1) In commemoration of Professor Carl Djerassi's 65th birthday. Contribution 174 of the series "Antineoplastic Agent". Consult the following for part 173: Pettit, G. R.; Kamano, Y.; Kizu, H.; Dufresne, C.; Herald, C. L.; Bontems, R.; Schmidt, J. M.; Boettner, F. E.; Nieman, R. A. *Heterocycles*, in press.

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Table I. ^1H and ^{13}C NMR Assignments and Selected NOE's for Dolastatin 13 in CD_2Cl_2 Solution and HMBC Correlations from CD_2Cl_2 (0.031 M) and C_5D_5N (0.015 M) Solutions[a]

	C no.	^{13}C	^1H (mult, J (Hz), intgrtn)	NOE's	HMBC[b]
Val_2	2	174.38			
	3	59.05	4.18 t, 7.5		2, 3a, 3b, 3c, 5
	3a	30.93	2.02 oct, 6.8	4	3, 3b, 3c
	3b	19.26	0.92 d, 6.7, 3 H		3, 3a, 3c
	3c	19.59	0.95 d, 6.9, 3 H		3, 3a, 3b
	4		7.42	3a	5, 6
MePhe	5	171.86			
	6	62.39	5.28		5, 6a, 6b
	6a	34.14	3.85 dd, 14.0, 2.8	6	
			2.81 dd, 14.0, 11.5	6, 6c	6, 6b, 6c
	6b	137.96			
	6c	129.72	7.35		6a, 6d, 6e
	6d	129.31	7.36		6b, 6c
	6e	127.55	7.26		6d
	7a	31.69	2.89 s, 3 H		6, 8
Phe	8	172.49[c]			
	9	51.46	5.07 dd, 11.2, 5.0	9a, 9c, 15	8, 9a, 9b, 11, 15
	9a	35.27	2.93 dd, 14.6, 11.2	9c, 15	8, 9, 9b
			2.09 dd, 14.6, 4.8		9, 9b
	9b	136.28			
	9c	129.55	6.84 dd, 7.7, 2.1	9, 9a, 15	9a, 9d, 9e
	9d	128.69	7.24		9b, 9c
	9e	127.30	7.23		9d
Ahp	11	171.86			
	12	49.52	4.05		11
	13	22.21	2.21 dq, 3.0, 14.6	15, 16	11, 12, 14, 15
			1.68		
	14	29.80	1.85 ddd, 14.0, 5.0, 2.6		12
			1.59		
	15	75.85	5.22	9, 9a, 9c, 12	11, 13
	15a		3.98		
	16		7.12 d, 9.5	19	12, 17
ΔAbu	17	163.78			
	18	129.53			
	18a	134.15	6.76 br q, 7.0		17, 18b
	18b	13.24	1.66 d, 7.0, 3 H	19	17, 18, 18a, 20
	19		7.99 br s	16, 21, 23, 29	20
Thr	20	169.94			
	21	55.40	4.90 d, 10.4	22b	20, 22, 22a, 24
	22	73.96	5.24	3	
	22a	21.06	1.41 d, 6.8, 3 H	21	21, 22
	23		6.72 d, 10.4	19, 26	24
Val_1	24	172.56[c]			
	25	61.93	4.01		24, 25a, 25b
	25a	29.59	2.41 oct, 6.9	23, 26	24, 25, 25b, 25c
	25b	19.42	1.13 d, 6.9, 3 H		25, 25a, 25c
	25c	19.82	1.11 d, 6.9, 3 H		25, 25a, 25c
	26		7.38	25a, 25b	25, 25a, 27
MeGlc	27	172.82			
	28	83.43	3.74 t, 1.8	28b	27, 28a
	28a	58.23	3.38 s, 3 H	28	28
	29	63.09	4.01		
			3.63 ddd, 12.0, 4.7, 1.8	29b	27, 28
	29a		4.42 t, 5.1	19, 29	29

[a] Where no multiplicity is noted, it could not be determined due to overlapping signals. [b] Underlined numbers are carbonyl carbons. [c] Chemical shift values are interchangeable.

assigned the Z-configuration shown. Confirmation for the Δ-Abu and MeGlc assignments was obtained from results of the HMBC experiment. Initial attempts at sequencing dolastatin 13 using NOE data proved unsuccessful presumably due to a folded solution conformation and the small magnitude of the observed NOEs. The correct sequence of units was achieved by using HMBC and by combining the results from experiments in two different solvents (Table I). With dichloromethane-d_2 as solvent, several segments of dolastatin 13 were established, but due to overlapping signals in the carbonyl region it was necessary to use data obtained in pyridine-d_5, where only two carbonyl chemical shifts overlapped. The only assumption made to complete the overall structure was that the carbonyl group at 174.38 ppm corresponded to an ester and must therefore be attached to the Thr oxygen. The resultant structure **1** was confirmed by a sequence determination[13] using FAB combined with tandem mass spectrometry.[14]

Upon standing in chloroform solution dolastatin 13 was found to selectively lose 1 mol equiv of water to yield dehydrodolastatin 13 (**2**). The same ready dehydration of hemiacetal **1** was also

(13) Exact mass, deviation in ppm, formula: 906.4590, −2.4, $C_{46}H_{64}N_7O_{12}$ for $[M + H]^+$; 888.4492, −1.6, $C_{46}H_{62}N_7O_{11}$; 789.3807, −2.2, $C_{41}H_{53}N_6O_{10}$; 687.3528, 3.2, $C_{37}H_{47}N_6O_7$; 646.3426, −3.8, $C_{32}H_{48}N_5O_9$; 628.2977, −0.8, $C_{31}H_{42}N_5O_9$; 487.2344, 0.5, $C_{28}H_{31}N_4O_4$; 404.1981, 1.8, $C_{24}H_{26}N_3O_6$; 368.1817, −1.2, $C_{17}H_{26}N_3O_6$; 326.1495, −2.9, $C_{18}H_{20}N_3O_3$; 292.1344, 2.2, $C_{19}H_{18}NO_2$; 279.1703, −2.0, $C_{15}H_{23}N_2O_3$; 202.1075, −2.1, $C_9H_{16}NO_4$; 174.1129, 0.7, $C_8H_{16}NO_3$; 75.0444, −2.8, $C_3H_7O_2$.

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observed in the mass spectral results. Once the structure of dolastatin 13 (**1**) was in hand, it was clear from the combined NMR and mass spectral studies that dehydrodolastatin 13 was the Ahp dehydration product of depsipeptide **1**, thereby corresponding to structure **2**. The ^{1}H and ^{13}C NMR spectra of depsipeptide **1** and **2** appeared almost identical, with exception of the Ahp signals, and the amide proton of Val-2 at δ 7.42 (H-4) which upon dehydration shifts upfield by 1 ppm. Perhaps the latter shift indicates hydrogen bonding between H-4 and O-15 in dolastatin 13.

Interestingly, dolastatin 13 appears only remotely related to the cyclodepsipeptides dolastatins 11 and 12[1] and not at all to the previous cytostatic diterpenes[5] and peptides[6] we isolated from *D. auricularia*. Hence, this sea hare's facility for selecting and concentrating cytostatic and antineoplastic constituents and/or synthesizing such substances is a truly virtuoso performance. Presently we are pursuing experiments directed at determining the asymmetric centers of dolastatin 13 and evaluating various biological properties. Stereochemical assignments completed to date for other cytostatic and/or antineoplastic peptides of the remarkable dolastatin series[1,6] suggests that dolastatins **1** and **2** are most likely derived from *S*-amino acids. So far dolastatin 13 has been found to strongly inhibit growth of the PS cell line exhibiting an ED_{50} of 0.013 μg/mL, whereas dehydrodolastatin 13 proved to be marginally inactive in this system and revealed the first structure/activity insight.

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Additions and Corrections

Molecular and Electronic Structure of Electron-Transfer Active Main-Group Organometallics [*J. Am. Chem. Soc.* **1989**, *111*, 2126–2131]. JENS BAUMGARTEN, CHRISTIAN BESSENBACHER, WOLFGANG KAIM,* and THOMAS STAHL

The designations "cage" and "escape" in formula 1 should be exchanged. The arrow between both words should be deleted.