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# ANTINEOPLASTIC AGENTS 370. ISOLATION AND STRUCTURE OF DOLASTATIN 18[1]

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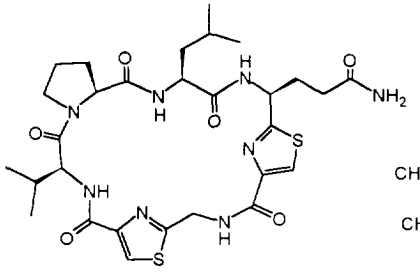
Abstract: Bioassay-guided separation of cancer cell growth inhibitory fractions derived from the sea hare *Dolabella auricularia* obtained in Papua New Guinea led to isolation (1.51 x $10^{-7}$% yield) of the new thiazole-containing peptide, dolastatin 18 (4). Structural determination was completed by employment of results from high-field (500 MHz) 2-D NMR experiments and tandem MS/MS mass spectral sequence analyses. Dolastatin 18 (4) was found to inhibit a selection of cancer cell lines among which $GI_{50}$ 0.39 μg/mL was found for the nonsmall cell lung cancer NCI-H460. © 1997 Elsevier Science Ltd.

Biosynthetic pathways in certain superficially defenseless marine organisms and/or their dietary sources occasionally favor production of cancer cell growth inhibitory substances with thiazole- and thiazolidine-type ring systems. Illustrative are the shell-less mollusc *Dolabella auricularia* constituents dolastatins 3[2] (1), 10[3] (2), and dolabellin[4a] (3) as well as the tunicate genus *Lissoclinum* components that include the patellamides,[5a] ulithiacyclamides,[5b] cyclodidemnamide,[5c] and lissoclinamides.[5d] In addition, the thiazolidine-ring-containing latrunculins A and B have been found in a nudibranch.[5] Since 1972 we have been exploring the antineoplastic potential of selected constituents from *D. auricularia* collected in the Indian Ocean (Mauritius), and in 1983 this investigation was extended to specimens collected in Papua New Guinea (PNG). More recently, Yamada and coworkers[4] have isolated an interesting series of biologically active constituents that include dolabellin (3),[4a] doliculide,[4a] and dolastatin H[4b] from *D. auricularia* obtained in Japanese ocean areas. We now report the isolation and structural elucidation of a new cancer cell growth inhibitory peptide designated dolastatin 18 (4) from PNG specimens of *D. auricularia*.

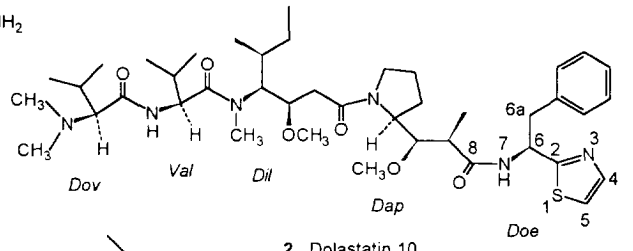
By employment of the murine P388 lymphocytic leukemia and selected human cancer cell lines (e.g., the nonsmall cell lung cancer NCI-H460), the active dichloromethane-soluble

†Dedicated to the memory of Dr. Matthew Suffness, deceased June, 1995

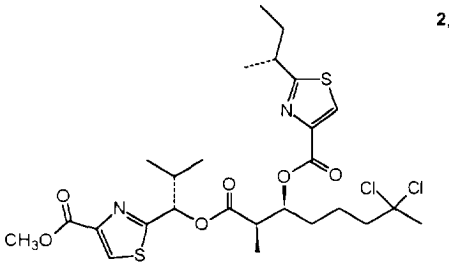
fraction prepared from 1000 kg (wet wt.) of the sea hare was separated by a series of size exclusion chromatographic procedures on Sephadex LH-20 combined with high-speed countercurrent distribution. Final separation and purification was performed by reverse-phase (C8) HPLC ($CH_3CN:H_2O$, 1:1) to give 1.51 mg (1.51 x $10^{-7}$% yield) of pure dolastatin 18 (4) as a colorless powder, $[\alpha]_D$ -2.3° (*c* 0.094, $CH_3OH$).



**1**, Dolastatin 3




**2**, Dolastatin 10



**3**, Dolabellin

Dolastatin 18 (4) exhibited a FAB-MS quasimolecular ion peak at *m/z* 619 ($[M+H]^+$), corresponding to a molecular formula of $C_{35}H_{46}N_4O_4S$, which was consistent with the carbon and hydrogen content estimated from the NMR spectra. That dolastatin 18 was a peptide was evident from its $^1H$ and $^{13}C$ NMR spectra, which exhibited two amide NH, one amide $NCH_3$, and four carbon signals between δ 169 and 175 ppm. A ketone carbonyl (δ 210.60) was also apparent. Interpretation of the $^1H$-$^1H$ COSY, TOCSY, HMQC, and HMBC spectra (500 MHz) taken in three solvents ($CDCl_3$, $CD_2Cl_2$, and $CD_3CN$) revealed the structure of peptide 4 to be derived from two α-amino acids (Leu and MePhe), a dolaphenine (Doe) unit, and the new carboxylic acid 2,2-dimethyl-3-oxohexanoic acid (herein named dolahexanoic acid, Dhex). Interestingly, Dhex appears to be biosynthetically related to the β-oxo-2,2-dimethyl amino acid unit of dolastatin 11.[7]

Table 1: The High-Field (500 MHZ) $^{1}H$- and $^{13}C$-NMR Spectral Assignments for Dolastatin 18 (4) in $CDCl_3$.

| *Position No.* | $^{13}C$ (ppm) | $^{1}H$ (ppm) | J (Hz) | *HMBC* ($^{1}H$ to $^{13}C$) | *Position No.* | $^{13}C$ (ppm) | $^{1}H$ (ppm) | J (Hz) | *HMBC* ($^{1}H$ to $^{13}C$) |
|---|---|---|---|---|---|---|---|---|---|
| 2 | 174.13 s | | | | 9e | 126.75 d | 7.18 m | | |
| 4 | 139.41 d | 7.50 d | 3.5 | | 10 | 31.36 q | 2.89 s | | 9,11 |
| 5 | 119.96 d | 7.38 d | 3.5 | | 11 | 174.04 s | | | |
| 6 | 52.40 d | 5.63 m | | 2 | 12 | 48.42 d | 4.55 m | | 11,12a |
| 6a | 40.87 t | 3.47 dd | 8.0,19 | 2,6,9b,9c | 12a | 41.11 t | 1.05 m | | |
| | | 3.37 dd | 7.5,19 | | | | 0.88 m | | |
| 6b | 135.80 s | | | | 12b | 24.39 d | 1.07 m | | 17 |
| 6c | 129.33 d | 7.23 m | | 6a | 12c | 21.97 q | 0.73 d | 7.5 | 12a,12b,12d |
| 6d | 128.73 d | 7.26 m | | | 12d | 22.97 q | 0.69 d | 8.0 | 12a,12b,12c |
| 6e | 127.23 d | 7.24 m | | | 13 | | 6.29 d | 8.0 | 14 |
| 7 | | 7.81 d | 3.5 | | 14 | 172.80 s | | | |
| 8 | 169.86 s | | | | 15a | 55.47 s | | | |
| 9 | 57.46 d | 5.10 dd | 6.5,7.0 | 8,10 | 15b | 22.48 q | 1.30 s | | 14,15 |
| 9a | 33.53 t | 3.30 m | | 9 | 15c | 22.55 q | 1.32 s | | 15,16 |
| | | 2.92 m | | 9 | 16 | 210.20 s | | | |
| 9b | 136.41 s | | | | 17 | 40.06 t | 2.42 dt | 4.0,9.0 | 16,18,19 |
| 9c | 128.66 d | 7.28 m | | 9a | 18 | 17.10 t | 1.52 m | | 16 |
| 9d | 128.50 d | 7.22 m | | | 19 | 13.59 q | 0.83 t | 8.0 | |

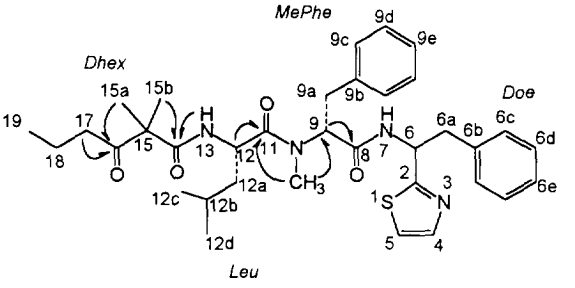



Figure 1: Dolastatin 18 (**4**) with selected HMBC correlations (↷).

Sequence assignment of the four units was established by the HMBC correlations shown in Figure 1 and Table 1. Although no HMBC relationships were observed from the two olefin protons at δ 7.50 d (H-4) and δ 7.38 d (H-5) to the carbon (C-2) at δ 174.13 (s), the coupling constants ($J$ = 3.5 Hz) of the proton doublets indicated a *cis* orientation in a five-membered ring. The chemical shifts of the proton and carbon signals led to identification of the thiazole ring. Although it proved difficult to find HMBC correlations from NH-7, H-6, and H-6a to C-2 (δ 174.13 s), the presence of the Doe unit was deduced when it was found that its NMR data (in $CD_2Cl_2$) nearly coincided with those of the dolastatin 10 Doe unit (Table 2).

Table 2: Comparison of the Doe Unit NMR (500 MHz) Data for Dolastatin 18 (4) and Dolastatin 10 (2) in $CD_2Cl_2$.

| *Position* | *Doe Unit of Dolastatin 18* | | *Doe Unit of Dolastatin 10* | |
|---|---|---|---|---|
| *No.* | *Carbon* | *Proton* | *Carbon* | *Proton* |
| 2 | 172.20 | | 170.51 | |
| 4 | 146.65 | 7.78 | 147.77 | 7.72 |
| 5 | 119.27 | 7.26 | 118.76 | 7.25 |
| 6 | 53.03 | 5.52 | 53.02 | 5.52 |
| 7 | | 7.28 | | 7.28 |
| 6a | 41.40 | 3.18 | 41.48 | 3.26 |
| | | 3.40 | | 3.40 |

Two important HMBC correlations involving NH-Leu/CO-Dhex and $CH_3$N-Phe/CO-Leu established the Dhex-Leu-MePhe bonding where Doe and Dhex were assigned the *C*- and *N*-terminal positions of the sequence, respectively. Although no HMBC correlation was found from NH-Doe to CO-MePhe, the sequence of dolastatin 18 (4) was deduced to be Dhex-Leu-MePhe-Doe. The structure (4) elucidated by these NMR and chemical considerations was supported by tandem MS/MS sequential analyses.

The chiral centers of Leu and MePhe were found to be *S* and *R*, respectively, by employment of a 6 N HCl hydrolysis-chiral HPLC analysis (CHIREX phase 3126) sequence.[8] Based on our total synthesis of natural dolastatin 10 and the X-ray crystal structure determination of the chiral isomer 6(*R*)-dolastatin 10,[9] the Doe unit of dolastatin 18 was presumed to have the 6(*S*)-configuration.

Dolastatin 18 (4) was found to significantly inhibit growth of a selection of human

cancer cell lines among which activity against the lung cancer NCI-H460 proved typical at $GI_{50}$ 0.39 μg/mL. A more detailed evaluation of dolastatin 18 will be conducted when we complete a scale-up total synthesis now in progress.

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8. Conditions for the chiral HPLC examination: CHIREX phase 3126 column (4.6 x 50 mm), Phenomenex; solvents, 2 mM $CuSO_4$ $H_2O$:$CH_3CN$ (9:1); detection at 230.4 and 550 nm. The retention times (min) of the authentic amino acids were L-Leu (10.39), D-Leu (11.77), L-MePhe (20.16) and D-MePhe (25.68). By comparison with those retention times, the absolute stereochemistry of the two components in the acid hydrolysate of dolastatin 18 (4) were assigned as L-Leu (10.40) and D-MePhe (25.72).

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