The occurrence of the reverse PO → PS reaction is of interest in view of its potential utility for the conversion of phosphoryl compounds into corresponding phosphorothioyl analogues. Although such a synthetic aspect was not evaluated in the current study, the optimization of the reaction conditions and carboxylic thioanhydride or thiocarboxylate/anhydride sytem might produce a new mild reagent for the PO → PS transformation.

The stereochemical results of oxidation of 1 and 2 are explained by the racemization of the sulfide, selenide, or oxide due to the multiple occurrence of the reversible processes shown in Scheme III (each occurring with inversion at phosphorus) before the inactivation of the trifluorothioacetate can take place. The relatively higher degree of inversion observed in oxidations of triesters 3 and 4 can be explained by a much lower nucleophilicity of selenium atom in these cases as compared to phosphine selenide 2 (due to electronic effect of oxygen substituents at phosphorus), slowing down the rates of formation of 12 and 13 and lowering the probability of a return to phosphoroselenonates. In fact, the low-temperature $^{31}P$ NMR spectra of *O,O,O*-trimethyl phosphoroselenoate/TFAA did not indicate the formation of such intermediates at concentrations above the detection limit.

## Experimental Section

All substrates were obtained according to known procedures and were characterized by their $^{31}P$ NMR spectra (Table I) and other physical data prior to use. $^{31}P$ Chemical shifts were referenced indirectly to 85% phosphoric acid. Organic solvents were reagent grade and were dried before use by routine methods and were stored in Teflon stopcock sealed ampoules over appropriate dessicants. Trifluoroacetic anhydride was from Merck and was stored in sealed ampoules over $P_2O_5$. NMR samples and reaction mixtures were prepared using vacuum-line technique to avoid TFAA hydrolysis upon contact with moisture and to protect samples against TFA-catalyzed thiono-thiolo rearrangement in cases when phosphylthioic and -selenoic esters were used a substrates.

**General Procedure.** Phosphylthionate or -selenonate (10 mmol) was dissolved in methylene chloride (10 mL) and TFAA (15 mmol) was added. The progress of the reaction was monitored by TLC. After the reaction was complete, the solution was treated with methanol (1 mL) and neutralized by washing with aqueous sodium bicarbonate. Methanol (10 mL) was added, and the precipitate of sulfur or selenium was removed by filtration. The solution was concentrated, and the residue was chromatographed on a silica gel column. Esters 6 and 7 were distilled under vacuum.

8: *m/z* 168 (MI, 5), 139 (BP), 125 (18), 91 (7), 77 (21), 47 (15).

9: *m/z* 184 (MI, 55), 156 (BP), 141 (30), 123 (15), 107 (8), 78 (21), 77 (14), 63 (27).

5: *m/z* 182 (MI, 23), 154 (BP), 139 (95), 125 (55), 109 (6), 91 (65), 77 (35), 51 (18).

1: *m/z* 198 (MI 31), 156 (BP), 141 (25), 123 (12), 109 (7), 107 (4), 91 (10), 78 (20), 63 (18).

**Registry No.** 1, 13153-92-9; 2, 33995-97-0; 3, 33996-01-9; 4, 33996-02-0; 5, 2328-23-6; (*S*)-5, 1515-99-7; 6, 33996-03-1; 7, 33996-04-2; 8, 7309-49-1; 9, 13639-73-1; 10, 129848-65-3; 11, 21690-87-9; 12, 129848-67-5; 13, 129834-42-0; TFAA, 407-25-0.

# Structures of Three New Cyclic Heptapeptide Hepatotoxins Produced by the Cyanobacterium (Blue-Green Alga) *Nostoc* sp. Strain 152[1]

Michio Namikoshi, Kenneth L. Rinehart,* and Ryuichi Sakai

*Roger Adams Laboratory, University of Illinois, Urbana, Illinois 61801*

Kaarina Sivonen

*Department of Microbiology, University of Helsinki, SF-00710 Helsinki, Finland*

Wayne W. Carmichael

*Department of Biological Sciences, Wright State University, Dayton, Ohio 45435*

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Three new hepatotoxic cyclic heptapeptides in the microcystin class were isolated from the cyanobacterium (blue-green alga) *Nostoc* sp. strain 152 and assigned structures based on their high-resolution FABMS, FABMS/MS, $^1H$ and $^{13}C$ NMR spectra, amino acid analysis, and GC on a chiral capillary column. All three toxins (1-3) have 9-acetoxy-3-amino-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid as an unusual structural component (Scheme I) instead of the corresponding 9-methoxyl derivative (Adda) found in the microcystins.

Some genera of fresh and brackish cyanobacteria (blue-green algae) produce potent hepatotoxic cyclic peptides.[2,3] Microcystins,[4] cyclic heptapeptides illustrated by microcystin-LR (4),[3] are the most common of these cyanobacterial hepatotoxins, and nine chemically defined microcystins have been reported (4 and 6-13, Scheme II).[5] Nodularin, whose structure we recently reported,[3] is thus far the only cyclic pentapeptide in this class of hepatotoxins. These compounds all have a unique $C_{20}$ amino acid (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid (Adda), a remarkable structural feature.[6] Adda seems to be important for their

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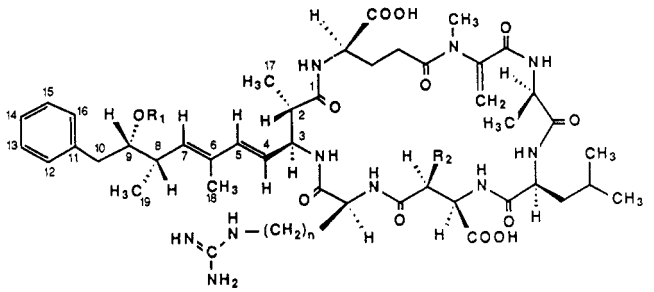
**Scheme I**



1: $R_1$ = $COCH_3$, $R_2$ = $CH_3$, n = 3

2: $R_1$ = $COCH_3$, $R_2$ = $CH_3$, n = 4

3: $R_1$ = $COCH_3$, $R_2$ = H, n = 3

**Table I. High-Resolution FABMS Data for 1, 2, and 3**

| compd | calcd for (M + H) | observed | Δ (mmu) |
|---|---|---|---|
| 1 | $C_{50}H_{75}N_{10}O_{13}$, 1023.5547 | 1023.5531 | 1.6 |
| 2 | $C_{51}H_{77}N_{10}O_{13}$, 1037.5702 | 1037.5687 | 1.5 |
| 3 | $C_{49}H_{73}N_{10}O_{13}$, 1009.5359 | 1009.5365 | 0.6 |

hepatotoxicity since hydrogenation or ozonolysis of the diene system gives an inactive compound[7] and stereoisomers at the $\Delta^6$ double bond are inactive.[8] We recently deduced the stereochemistry[3] and reported the total synthesis[6] of Adda. The microcystins primarily differ in the two L-amino acids and secondarily involve the absence of the methyl groups on D-*erythro*-β-methylaspartic acid (D-MeAsp) and/or *N*-methyldehydroalanine (Mdha) (Scheme II).[5]

We are interested in the structure–activity relationships of these hepatotoxins and have recently reported the isolation and cultivation of *Nostoc* sp. strain 152, which produces several hepatotoxic peptides.[1] We describe here the structure assignment of three new hepatotoxic cyclic heptapeptides, isolated from this strain, which possess an interesting modification in their Adda residue.

**Molecular Weights and Molecular Formulas.** Low-resolution fast atom bombardment (FAB) mass spectra in a matrix of dithiothreitol/dithioerythritol ("magic bullet")[9] showed $(M + H)^+$ ions at *m/z* 1023, 1037, and 1009 for **1**, **2**, and **3**, respectively. The spectra were reproduced in the previous paper, and compounds **1**, **2**, and **3** in the present report correspond to P14, P15, and P16, respectively, in that paper.[1] High-resolution (HR) FAB mass spectral data on the $(M + H)^+$ ions agreed with the molecular formulas $C_{50}H_{74}N_{10}O_{13}$ for **1**, $C_{51}H_{76}N_{10}O_{13}$ for **2**, and $C_{49}H_{72}N_{10}O_{13}$ for **3** (Table I).

**Amino Acid Components and Their Stereochemistry.** The amino acid components of the toxins (Table II) were first analyzed with a Waters Pico Tag HPLC system, and the chromatograms were shown in the previous paper.[1] The difference of 14 mu ($CH_2$) between **1** and **2** is ascribable to the replacement of arginine (Arg) in **1** with homoarginine (Har) in **2**, and the difference between **1** and **3** is due to replacement of MeAsp in **1** by Asp in **3**. In order to determine the stereochemistry, GC analysis on a chiral capillary column was carried out. The toxins were hydrolyzed with 6 N hydrochloric acid (HCl) in solution, and the hydrolysates were derivatized with 4 N HCl–2-propanol followed by trifluoroacetic anhydride (TFAA) to give *N*-trifluoroacetyl isopropyl esters. For the analysis of Arg and Har, the hydrolysates were treated first with acetylacetone to afford 4,6-dimethylpyrimidine derivatives of the guanido group[10] prior to esterification and acylation. The authentic amino acids were derivatized in the same manner.

**Table II. Amino Acid Components of 1, 2, and 3**

| compd | amino acid[a] |
|---|---|
| 1 | D-Ala L-Leu D-MeAsp[b] D-Glu L-Arg ($MeNH_2$)[c] |
| 2 | D-Ala L-Leu D-MeAsp D-Glu L-Har[d] ($MeNH_2$) |
| 3 | D-Ala L-Leu D-Asp D-Glu L-Arg ($MeNH_2$) |

[a] Based on amino acid analysis (Waters Pico Tag) and GC analysis on a chiral capillary column. [b] D-*erythro*-β-Methylaspartic acid. [c] Methylamine from *N*-methyldehydroalanine. [d] Homoarginine.

Since commercially available Har is the L isomer,[11] several conditions for epimerization were examined using L-Arg as a model compound; heating L-Arg with 0.5 equiv of 4-(dimethylamino)pyridine (DMAP)[12] in pyridine–water solution was suitable for this purpose, giving a ca 9:1 L:D mixture under these conditions in 24 h. L-Har gave after 36 h treatment a ca 4:1 L:D mixture, which was used for the determination of stereochemistry.

The GC conditions used for analysis are given in the Experimental Section, and the results are summarized in Table II. Compound **1** gave the same profile of five amino acid components as **4**, and **3** has the same five amino acids as [D-Asp[3]]microcystin-LR (**11**).[8,13]

The peak due to methylamine in the HPLC chromatograms for amino acid analysis[1] suggested the presence of an *N*-methyl dehydro amino acid as a sixth component. The $^1H$ NMR spectra of **1** and **2** (Table III; the spectra of **1**, **2**, and **4** were reproduced in the previous paper[1]) revealed a three-proton singlet at δ 3.33 and two one-proton singlets at δ 5.43 and 5.89, which are ascribable to the *N*-methyl group and two olefinic protons of Mdha. $^{13}C$ NMR data for **1** and **2** showed further evidence for the existence of Mdha (Table IV); thus, Mdha was identified as the sixth amino acid component of **1**, **2**, and **3**.

The total masses of the six amino acid residues are 681 ($C_{29}H_{48}N_9O_{10}$) for **1**, 695 ($C_{30}H_{50}N_9O_{10}$) for **2**, and 677 ($C_{28}H_{46}N_9O_{10}$) for **3**. Subtraction of these values from their respective molecular weights (formulas) gave 341 mu ($C_{21}H_{27}NO_3$), which is not identical with the usual Adda residue (313, $C_{20}H_{27}NO_2$) found in microcystins and nodularin. The difference of 28 mu (CO) suggests a structural variation from this amino acid. Careful assignment of $^1H$ NMR data for **1** and **2** reveals the presence of an amino acid closely related to Adda (Table III). The obvious differences between the $^1H$ NMR spectra of **1** (or **2**) and **4** are as follows: a three-proton singlet due to the methoxyl group, at δ 3.24 in the spectrum of **4**, is not observed in spectra of **1**, **2**, and **3**; instead, a methyl singlet ascribable

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**Scheme II**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| [ADMAdda$^5$]microcystin-LR (**1**): | cyclo(-D-Ala- | -L-Leu- | -D-MeAsp- | -L-Arg- | ADMAdda- | D-Glu- | -Mdha-) |
| [ADMAdda$^5$]microcystin-LHar (**2**): | cyclo(-D-Ala- | -L-Leu- | -D-MeAsp- | -L-Har- | ADMAdda- | D-Glu- | -Mdha-) |
| [D-Asp$^3$,ADMAdda$^5$]microcystin-LR (**3**): | cyclo(-D-Ala- | -L-Leu- | -D-Asp- | -L-Arg- | ADMAdda- | D-Glu- | -Mdha-) |
| microcystin-LR (**4**): | cyclo(-D-Ala- | -L-Leu- | -D-MeAsp- | -L-Arg- | Adda- | D-Glu- | -Mdha-) |
| microcystin-LA (**6**): | cyclo(-D-Ala- | -L-Leu- | -D-MeAsp- | -L-Ala- | Adda- | D-Glu- | -Mdha-) |
| microcystin-YA (**7**): | cyclo(-D-Ala- | -L-Tyr- | -D-MeAsp- | -L-Ala- | Adda- | D-Glu- | -Mdha-) |
| microcystin-YR (**8**): | cyclo(-D-Ala- | -L-Tyr- | -D-MeAsp- | -L-Arg- | Adda- | D-Glu- | -Mdha-) |
| microcystin-YM (**9**): | cyclo(-D-Ala- | -L-Tyr- | -D-MeAsp- | -L-Met- | Adda- | D-Glu- | -Mdha-) |
| microcystin-RR (**10**): | cyclo(-D-Ala- | -L-Arg- | -D-MeAsp- | -L-Arg- | Adda- | D-Glu- | -Mdha-) |
| [D-Asp$^3$]microcystin-LR (**11**): | cyclo(-D-Ala- | -L-Leu- | -D-Asp- | -L-Arg- | Adda- | D-Glu- | -Mdha-) |
| [D-Asp$^3$]microcystin-RR (**12**): | cyclo(-D-Ala- | -L-Arg- | -D-Asp- | -L-Arg- | Adda- | D-Glu- | -Mdha-) |
| [D-Asp$^3$,Dha$^7$]microcystin-RR (**13**): | cyclo(-D-Ala- | -L-Arg- | -D-Asp- | -L-Arg- | Adda- | D-Glu- | -Dha-) |

MeAsp = *erythro*-β-Methylaspartic acid. ADMAdda = *O*-Acetyl-*O*-demethylAdda.
Mdha = *N*-Methyldehydroalanine. Har = Homoarginine. Dha = Dehydroalanine.

**Table III. $^1$H NMR Data for 1, 2, and 4**[a]

| proton (mult)[b] | δ (*J* in Hz) 1 | 2 | 4 | proton | 1 | 2 | 4 |
|---|---|---|---|---|---|---|---|
| [Adda][c] | | | | | | | |
| H-2 (m) | 3.05 (10.5, 7.0) | 3.04 (10.5, 7.0) | 3.05 (10.5, 7.0) | | | | |
| H-3 (dd) | 4.57 (10.5, 9.0) | 4.58 (10.5, 9.0) | 4.56 (10.5, 9.0) | | | | |
| H-4 (dd) | 5.53 (15.5, 9.0) | 5.53 (15.5, 9.0) | 5.48 (15.5, 9.0) | | | | |
| H-5 (d) | 6.26 (15.5) | 6.26 (15.5) | 6.24 (15.5) | [Glu] | | | |
| H-7 (d) | 5.39 (9.5) | 5.39 (9.5) | 5.42 (9.5) | H-2 (dd) | 4.20 (7.5, 7.5) | 4.20 (7.5, 7.5) | 4.20 (7.5, 7.5) |
| H-8 (m) | 2.74 (9.5, 7.5, 7.0) | 2.74 (9.5, 7.5, 7.0) | 2.58 (9.5, 7.0, 7.0) | H-3 (m) | 1.93 | 1.93 | 1.94 |
| H-9 (m) | 4.96 (8.5, 7.5, 4.0) | 4.96 (8.5, 7.5, 4.0) | 3.27 (7.5, 7.0, 3.5) | (m) | 2.11 | 2.11 | 2.10 |
| H-10 (dd) | 2.71 (14.0, 8.5) | 2.72 (14.0, 8.5) | 2.68 (13.5, 7.5) | H-4 (m) | 2.54 | 2.53 | 2.54 |
| (dd) | 2.90 (14.0, 4.0) | 2.90 (14.0, 4.0) | 2.81 (13.5, 3.5) | (m) | 2.65 | 2.65 | 2.64 |
| H-12, 16 (d) | 7.16 | 7.16 | 7.18 | [Leu] | | | |
| H-13, 15 (t) | 7.24 | 7.24 | 7.24 | H-2 (dd) | 4.26 (11.5, 3.5) | 4.26 (11.5, 3.5) | 4.26 (11.5, 3.5) |
| H-14 (t) | 7.17 | 7.17 | 7.19 | H-3 (m) | 1.54 | 1.54 | 1.54 |
| $H_3$-17 (d) | 1.04 (7.0) | 1.04 (7.0) | 1.03 (7.0) | (m) | 2.02 | 2.02 | 2.02 |
| $H_3$-18 (s) | 1.69 | 1.69 | 1.61 | H-4 (m) | 1.78 | 1.78 | 1.78 |
| $H_3$-19 (d) | 0.98 (7.0) | 0.98 (7.0) | 1.00 (7.0) | $H_3$-5 (d) | 0.87 (7.0) | 0.87 (7.0) | 0.87 (7.0) |
| $H_3$-20′ (s) | | | 3.24 | $H_3$-6 (d) | 0.89 (7.0) | 0.89 (7.0) | 0.89 (7.0) |
| $H_3$-21 (s) | 1.92 | 1.92 | | [MeAsp] | | | |
| [Ala] | | | | H-2 (d) | 4.46 (3.5) | 4.46 (3.5) | 4.46 (3.5) |
| H-2 (q) | 4.55 (7.0) | 4.56 (7.0) | 4.55 (7.0) | H-3 (m) | 3.15 (7.0, 3.5) | 3.16 (7.0, 3.5) | 3.15 (7.0, 3.5) |
| $H_3$-3 (d) | 1.33 (7.0) | 1.33 (7.0) | 1.33 (7.0) | $H_3$-5 (d) | 1.04 (7.0) | 1.04 (7.0) | 1.05 (7.0) |
| [Arg][d] | | | | [Mdha][e] | | | |
| H-2 (dd) | 4.34 (9.0, 3.5) | 4.31 (10.5, 3.0) | 4.34 (9.0, 3.5) | H-3 (s) | 5.43 | 5.43 | 5.43 |
| H-3 (m) | 1.58 | 1.58 | 1.57 | (s) | 5.89 | 5.89 | 5.89 |
| (m) | 2.02 | 2.02 | 2.02 | *N*-$CH_3$ (s) | 3.33 | 3.33 | 3.33 |
| $H_2$-4 (m) | 1.52 | 1.34 | 1.52 | | | | |
| $H_2$-5 (m) | 3.13 | 1.55 | 3.13 | | | | |
| [$H_2$-6 (t)] | | 3.11 (7.0) | | | | | |

[a] 500 MHz; $CD_3OD$ (δ 3.30). [b] s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet, q = quartet. [c] Adda for **4**, *O*-acetyl-*O*-demethylAdda for **1** and **2**. [d] Arg for **1** and **4**, Har for **2**. [e] *N*-Methyldehydroalanine.

to an acetyl group appears at δ 1.92 in the spectra of **1**, **2**, and **3**; the H-9 proton signal is observed at lower field (δ 4.96) for **1** and **2** than for **4** (δ 3.27). These data show that the methoxyl group at the C-9 position of Adda in **4** is replaced with an acetoxyl group in **1**, **2**, and **3**; that is, they contain *O*-acetyl-*O*-demethylAdda (ADMAdda). Analysis of $^{13}$C NMR data for **1**, **2**, and **4** supports this assignment, in that the difference in the chemical shifts between **1** (or **2**) and **4** can be explained by substitution effects.[14]

$^1$H and $^{13}$C NMR data for **1** and **2** also allow assignment of the stereochemistry of ADMAdda. The chemical shifts and coupling constants for C-1 to C-8 and C-10 to C-19 and their attached protons in **1**, **2**, and **4** show great similarity, and the differences in chemical shift for C-9 and H-9 (and the small β and γ effects) agree with the difference of the substituent, while their $^1$H coupling constants resemble each other. The stereochemistry of ADMAdda in these toxins should, therefore, be identical with that of Adda in microcystins and nodularin.[3]

Thus, the structures of the seven amino acid constituents of **1**, **2**, and **3**, including stereochemistry, were assigned.

**Tandem Mass Spectrometric Analysis.** The $(M + H)^+$ ions of **1**, **2**, **3**, and **4** obtained by the FAB method were subjected to collisionally induced tandem MS (MS/MS, B/E scan)[15] to give the daughter ion peaks summarized in Table V. Significant fragment ions were observed, including some with Mdha. The peaks at *m/z* 859, 155,

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(15) Argon was used as the collision gas.

Table IV. $^{13}C$ NMR Data for 1, 2, and 4[a]

| carbon | 1 | 2 | 4 | carbon | 1 | 2 | 4 | carbon | 1 | 2 | 4 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| [Adda][b] | | | | [Ala] | | | | [Leu] | | | |
| C-1[c] | 176.7 | 176.8 | 176.7 | C-1 | 175.3 | 175.2 | 175.2 | C-1 | 175.4 | 175.4 | 175.4 |
| C-2 | 45.1 | 45.2 | 45.1 | C-2 | 50.1 | 50.0 | 50.1 | C-2 | 55.3 | 55.3 | 55.3 |
| C-3 | 56.7 | 56.6 | 56.8 | C-3 | 17.2 | 17.3 | 17.2 | C-3 | 40.8 | 40.8 | 40.8 |
| C-4 | 127.2 | 127.2 | 126.6 | [Arg][d] | | | | C-4 | 25.8 | 25.8 | 25.8 |
| C-5 | 138.6 | 138.4 | 139.0 | C-1 | 172.0 | 172.4 | 172.0 | C-5 | 21.2 | 21.2 | 21.3 |
| C-6 | 135.0 | 135.0 | 133.9 | C-2 | 52.8 | 53.0 | 52.9 | C-6 | 23.7 | 23.7 | 23.7 |
| C-7 | 135.1 | 135.1 | 137.0 | C-3 | 29.3 | 31.6 | 29.4 | [MeAsp] | | | |
| C-8 | 37.6 | 37.6 | 37.6 | C-4 | 26.7 | 24.2 | 26.7 | C-1[c] | 176.8 | 176.8 | 176.9 |
| C-9 | 79.7 | 79.7 | 88.3 | C-5 | 41.9 | 29.3 | 41.9 | C-2 | 57.2 | 57.1 | 56.9 |
| C-10 | 39.4 | 39.4 | 38.9 | C-6 | 158.5 | 42.1 | 158.5 | C-3 | 42.2 | 42.1 | 42.1 |
| C-11 | 139.2 | 139.2 | 140.5 | (C-7) | | 158.5 | | C-4 | 179.2 | 179.2 | 179.2 |
| C-12, 16 | 130.4 | 130.4 | 130.5 | [Glu] | | | | C-5 | 15.5 | 15.5 | 15.5 |
| C-13, 15 | 129.3 | 129.3 | 129.2 | C-1[c] | 177.1 | 177.2 | 177.0 | [Mdha] | | | |
| C-14 | 127.4 | 127.4 | 127.1 | C-2 | 55.2 | 55.2 | 55.2 | C-1 | 166.0 | 166.0 | 166.0 |
| C-17 | 15.9 | 16.0 | 15.9 | C-3 | 28.4 | 28.4 | 28.3 | C-2 | 146.4 | 146.3 | 146.4 |
| C-18 | 13.1 | 13.1 | 12.9 | C-4 | 33.3 | 33.3 | 33.3 | C-3 | 114.5 | 114.6 | 114.5 |
| C-19 | 17.0 | 17.0 | 16.5 | C-5[c] | 177.0 | 176.9 | 177.0 | $N$-$CH_3$ | 38.4 | 38.5 | 38.4 |
| C-20′ | | | 58.7 | | | | | | | | |
| C-20 | 172.4 | 172.4 | | | | | | | | | |
| C-21 | 20.9 | 20.9 | | | | | | | | | |

[a] 125 MHz; $CD_3OD$ ($\delta$ 49.00). [b] Adda for 4, *O*-acetyl-*O*-demethylAdda for 1 and 2. [c] These assignments may be interchanged. [d] Arg for 1 and 4, Har for 2.

Table V. FABMS/MS Data for 1, 2, 3, 4, and 5

| | fragment ion, $m/z$ (relative intensity)[a] | | | | |
|---|---|---|---|---|---|
| sequence | 1 | 2[b] | 3 | 4 | 5[c] |
| M + H | 1023 | 1037 | 1009 | 995 | 997 |
| M - AcOH | 963 (12) | 977 (11) | 949 (13) | | |
| M - 163[d] | 859 (6) | 873 (9) | 845 (8) | 859 (7, M - 135)[e] | 861 (8, M - 135)[e] |
| CO-Glu-Mdha-Ala - 2H | 309 (wk) | 309 (4) | 309 (4) | 309 (wk) | 311 (wk) |
| Arg-MeAsp + H | 286 (6) | 300[f] (4) | 272[g] (7) | 286 (2) | 286 (wk) |
| Glu-Mdha-Ala - H | 282 (5) | 282 (10) | 282 (7) | 282 (2) | 284 (wk) |
| CO-Glu-Mdha - H | 239 (100) | 239 (82) | 239 (100) | 239 (8) | 241 (7) |
| Glu-Mdha + H | 213 (40) | 213 (32) | 213 (39) | 213 (14) | 215 (9) |
| Mdha-Ala - H | 155 (88) | 155 (64) | 155 (83) | 155 (21) | 157 (10) |
| | | | | 135[e] (100) | 135[e] (100) |

[a] The intensity is relative to the highest fragment peak (100); wk = weak. [b] $m/z$ 84 (100). [c] Contains *N*-methylalanine instead of Mdha; corresponding peaks are shifted 2 mu higher than those of 4. [d] 163 = Ph-$CH_2$-CH($OCOCH_3$). [e] 135 = Ph-$CH_2$-CH($OCH_3$). [f] Har instead of Arg. [g] Asp instead of MeAsp.

and 135 were detected in the low-resolution FAB mass spectra of 4, and the ion formulas were confirmed by HRMS. Further evidence for these fragment ions was obtained from MS/MS data for the dihydro derivative (5),[16] which was easily made from 4 by reduction with sodium borohydride.[17] Appropriate peaks of 5 were observed two mu higher than those of 4, and the composition of the peak at $m/z$ 215 was confirmed by HRFABMS.

The $(M - AcOH)^+$ and $[M - PhCH_2CH(OCOCH_3)]^+$ ions in the spectra of 1, 2, and 3 confirmed the presence of the acetate at C-9 on the Adda residue. Fragment ions observed at $m/z$ 309, 282, 239, and 213 in the spectrum of 3 revealed that these fragments contain Glu but not MeAsp because 3 has Asp instead of MeAsp. The sequences of Arg-MeAsp for 1, Har-MeAsp for 2, and Arg-Asp for 3 were suggested by peaks at $m/z$ 286, 300, and 272, respectively. Although sequential assignments for Adda, Arg (Har), MeAsp (Asp), and Leu were not clearly made, the sequence -Glu-Mdha-Ala- is defined by the MS/MS spectra. The remaining sequences are presumed by analogy to those of other microcystins, and confirmed by the $^1H$ and $^{13}C$ NMR spectra of 1–4 discussed below.

**Structure of 1.** Compound 1, $[\alpha]^{26}_D$ -79.2° (*c* 0.28, MeOH), contains the same amino acids as microcystin-LR (4), and the sequence proposed for 1 is identical with that of 4, except for the modified Adda unit. The structure of 1 was confirmed by comparison of $^1H$ and $^{13}C$ NMR data for 1 with those of 4 (Tables III and IV). The chemical shifts and coupling constants due to C-1 to C-5 and their attached protons in Adda and the six other amino acids in the spectra of 1 are very similar to those for 4. The $^{13}C$ signals due to the constituents of the ring system in 1 and 4 are almost superimposable. These data argue that 1 has the same sequence as 4 and suggest that the conformations of 1 and 4 are very similar. Compound 1 is, therefore, assigned the structure shown in Scheme I and named [ADMAdda[5]]microcystin-LR.

**Structure of 2.** Compound 2, $[\alpha]^{26}_D$ -75.5° (*c* 0.44, MeOH), is presumed to be an L-Har variant of 1 at the L-Arg residue. The chemical shifts and coupling constants in the $^1H$ and $^{13}C$ NMR spectra of 2 due to C-1 to C-3 and their attached protons of L-Har and six other amino acids closely resembled those of 1 (Tables III and IV), confirming the presumed structure of 2, [ADMAdda[5]]microcystin-LHar, as shown in Scheme I.

**Structure of 3.** Compound 3, a minor component of this strain, was isolated in a very small amount[1] and, accordingly, did not give a good $^1H$ NMR spectrum. The spectrum of 3 was generally similar to that of 1, but a difference was observed in the region of methyl doublets, which would correspond to the replacement of D-MeAsp in 1 by D-Asp in 3. Thus, compound 3 is assigned as the demethyl analogue of 1 at the MeAsp residue and is rep-

(16) Botes, D. P.; Viljoen, C. C.; Kruger, H.; Wessels, P. L.; Williams, D. H. *Toxicon* **1982**, *20*, 1037–1042.
(17) Liesch, J. M.; Millington, D. S.; Pandey, R. C.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1976**, *98*, 8237–8249.

resented as shown in Scheme I, namely as [D-Asp$^3$, ADMAdda$^5$]microcystin-LR.

**Conclusion.** The structures of three new cyclic heptapeptide hepatotoxins produced by *Nostoc* sp. strain 152 were assigned using HRFABMS, FABMS/MS, and $^1$H and $^{13}$C NMR spectra. These compounds have ADMAdda instead of Adda, which was previously the common component of this class of hepatotoxins. The toxicity of **1** is the same as that of **4**;[1] that is, the C-9 methoxyl group on Adda is comparable to the acetoxyl group. These toxins provide interesting information about the structure–activity relationships of the toxins and their derivatives. There are several minor components, which are not identical with known toxins, in the extract of this strain,[1] and the isolation of sufficient amounts of these toxins is now in progress.

## Experimental Section

**General.** NMR spectra were recorded using deuteriomethanol as solvent and an internal standard; $^1$H and $^{13}$C NMR data are listed in Tables III and IV, and $^1$H NMR spectra of **1**, **2**, and **4** were presented in the previous paper.[1] The $^1$H and $^{13}$C signals were assigned based on the analyses of $^1$H–$^1$H and $^{13}$C–$^1$H COSY spectra and single-frequency decoupling experiments. The data for **4** were compared with the values reported in the literature.[8a] FAB mass spectra were run using xenon atoms and a matrix of "magic bullet".[9] MS/MS spectra in the FAB mode were obtained using argon as a collision gas. HRFABMS and MS/MS data are summarized in Tables I and V, respectively.

**Isolation and Toxicity of Compounds 1, 2, and 3.** The details were reported in the previous paper,[1] in which compounds P14, P15, and P16 correspond to **1**, **2**, and **3**, respectively.

**Gas Chromatography.** Capillary GC analyses were carried out using a Chiralsil Val III column (0.32 mm × 25 m) and helium as a carrier gas (flow rate, 37 mL/min; split ratio, 18:1). The program rate for the analyses of Ala, Leu, Asp, MeAsp, and Glu was 100 °C (2 min) to 180 °C at 8 °C/min. Isothermal conditions (210 °C) were used for the detection of Arg and Har.

**Derivatization of Amino Acids for GC Analysis.** The amino acid (100 μg) was treated with 300 μL of 4 N HCl in 2-propanol at 110 °C for 15 min in a screw-capped reaction vial. The reaction mixture was evaporated to dryness by a nitrogen stream ($N_2$), TFAA (200 μL) was added, and the mixture was allowed to stand at 110 °C for 5 min and evaporated by $N_2$. The residue was dissolved in $CH_2Cl_2$ for GC analysis.

Arg and Har required the following step prior to the above treatment. The amino acid (100 μg) was heated at 110 °C for 4 h with water (25 μL), ethanol (50 μL), $Et_3N$ (25 μL), and acetylacetone (50 μL).[10] The reaction mixture was evaporated to dryness by $N_2$.

**Hydrolysis and Derivatization of Toxins.** Compound **1** (60 μg), **2** (60 μg), or **3** (40 μg) was hydrolyzed with 6 N HCl (120 μL) at 110 °C for 21 h or at 140 °C for 40 min. The reaction mixture was cooled to room temperature and divided into two equal portions. Each portion was evaporated to dryness at 110 °C by $N_2$. One portion was treated with 4 N HCl–2-propanol (200 μL) followed by TFAA (100 μL) as above. Another portion was first treated with the mixture of acetylacetone (30 μL), water (15 μL), ethanol (30 μL), and $Et_3N$ (15 μL) followed by esterification and acylation reactions as above. The residue was dissolved in $CH_2Cl_2$ for GC analysis.

**Epimerization of L-Arginine and L-Homoarginine.** A mixture of L-Arg (21.4 mg, 0.1 mmol) and DMAP (6.1 mg, 0.05 mmol) in pyridine–water (9:1, 1 mL) was heated at 110 °C for 24 h. The solvents were evaporated, and the residue was derivatized as described above.

L-Har[11] (11.3 mg, 0.05 mmol) was treated similarly with DMAP (3.1 mg, 0.025 mmol) in pyridine–water (4:1, 1 mL) for 36 h and then derivatized as above.

**Dihydromicrocystin-LR (5).** A solution of **4** (0.2 mg) and $NaBH_4$ (2 mg) in water (0.15 mL) was stirred for 26 h at room temperature and then acidified with 10% acetic acid and evaporated to dryness. The residue was dissolved in water (0.2 mL) and passed through a Sep-Pak cartridge. The cartridge was washed with water (10 mL), and the product was retrieved with methanol (2 mL). The methanol eluate was evaporated to dryness and analyzed by FABMS followed by FABMS/MS.

Anal. Calcd for $C_{49}H_{77}N_{10}O_{12}$ (M + H) 997.5722, found 997.5740 (HRFABMS).

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**Registry No.** **1**, 129707-51-3; **2**, 129707-52-4; **3**, 129678-93-9; **4**, 101043-37-2; **5**, 129678-94-0; H-Arg-OH, 74-79-3; H-Har-OH, 156-86-5.

**Supplementary Material Available:** $^1$H and $^{13}$C NMR spectra of **1** and **2** (4 pages). Ordering information is given on any current masthead page.