Melophlins P, Q, R, and S: Four New Tetramic Acid Derivatives, from Two Palauan Marine Sponges of the Genus Melophlus

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Four new tetramic acid derivatives, named melophlins P, Q, R, and S (1—4), were isolated from two marine sponges of the genus Melophlus collected at Palau, together with seven known melophlins A, D, E, G, H, I, and O. The structures of the new compounds were elucidated on the basis of their spectral data. The absolute stereochemistries at the tetramic acid moieties of the new compounds were determined as 1:1 mixtures (racemic) by ESI-LC/MS analysis of derivatives obtained by oxidation and hydrolysis of the respective parent compounds. Melophlins P—S (1—4) showed cytotoxicity against the murine leukemia cell line L1210 with IC50 values of 20.0, 10.5, 0.85, and 5.13 μM, respectively.

Key words Melophlus cf. sarasinorum; Melophlus sp.; melophlin; tetramic acid derivative; marine sponge; cytotoxicity

Melophlins, tetramic acid derivatives possessing a long alkyl chain, have been isolated from the marine sponge Melophlus sarasinorum collected in Indonesia.2–4) Tetramic acid derivatives were also reported from other species of marine sponges.5—11)

During our continuing study on biologically active metabolites from marine organisms, we found four new melophlins, named melophlins P, Q, R, and S (1—4, Chart 1), from the ethanol extract of a marine sponge Melophlus cf. sarasinorum collected in Palau, together with seven known melophlins A (5), D (6), E (7), G (8), H (9), I (10), and O (11). Three of the new melophlins Q—S (2—4) and seven known compounds (5—11) were also detected in the ethanol extract of a Palauan Melophlus sp., which had a different appearance from that of the above species.

We describe herein the isolation, structures, and growth inhibitory activity against the murine leukemia cell line L1210 of four new melophlins.

Ethanol extract of M. cf. sarasinorum inhibited growth of L1210 cells. Bioassay-guided isolation from the extract by repeated column chromatographies and HPLC gave four new melophlins P—S (1—4, Chart 1) and five previously known melophlins D (6), G (8), H (9), I (10), and O (11). Melophlins A (5) and E (7) were also detected in a fraction obtained by silica gel column chromatography. Ethanol extract of the other Melophlus sp. showed cytotoxicity against L1210 cells, and seven known compounds 5—11 were isolated from the extract. Three new compounds 2—4 were contained in a fraction separated by a silica gel column, but melophlin P (1) was not detected in any fractions. The structures of 5—11 were assigned on the basis of their spectral data and confirmed by comparing the data with reported values.2,3)

The 1H- (Table 1) and 13C-NMR signals (Table 2) of four new melophlins 1—4 suggested that these compounds were also tetramic acid derivatives.2,3,8,12) The 1H- and 13C-NMR spectra of melophlin P (1) were assigned on the basis of their spectral data and confirmed by comparison of the NMR data for B, C, J, and L—O. The 1H- and 13C-NMR spectra of 1—4 showed that these compounds were mixtures of two tautomers (exo A and exo B forms)13—15) in the ratio of 9:1 at the tetramic acid moiety as similar to the other melophlins.2,3)

Melophlin P (1) was isolated as a yellowish oil. The ESI-MS of 1 showed an (M+H) + ion at m/z 366. The molecular formula (C22H39NO3) was determined from high-resolution (HR) FAB-MS and NMR data. In the 1H-NMR spectrum of 1, one methyl-triplet (δ 0.84, J=6.8 Hz) was detected besides

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two methyl-signals due to N-CH$_3$ and 5-CH$_3$. Subtraction of the tetracetic acid moiety from the molecular formula gave C$_{11}$H$_{11}$, which was assigned as a (CH$_2$)$_2$H$_2$ unit (C-7 to C-21) from the NMR data (Tables 1, 2). This C$_{11}$-alkyl chain was the same as that in melophlin A (5).$^{2,3}$ $^{13}$C-NMR data for I from C-7 to C-21 were identical with those for melophlin A (5). Therefore the structure of melophlin P (1) was the 5-methyl derivative of melophlin A as shown in Chart 1. Compound 1 has an asymmetric carbon at the 5 position but was not found optically active ($\alpha$-$\alpha_0$=ca. 0°). The absolute stereochemistry at the 5 position was determined utilizing the advanced Marfey’s method.$^{2,3,16–19}$ Compound 1 was treated with NaIO$_4$ and KMnO$_4$ followed by aqueous HCl to yield ESI-MS. The molecular formula was deduced as C$_{21}$H$_{37}$NO$_3$. The 1H- and 13C-NMR spectra of I were analyzed by HR-FAB-MS and NMR data. The 1H-NMR spectrum of I showed a methyl-triplet due to H$_3$-19 ($\delta$ 0.84, 6H, d, $J$=6.8 Hz) and a methyl-doublet ascribed to the branched methyl group ($\delta$ 0.82, $J$=6.4 Hz), besides N-CH$_3$ and 5-CH$_3$ signals. The 1H-1H COSY and HMBC spectra of 3 revealed the presence of an anteiso-type moiety, and the alkyl chain of 3 was the same as that of melophlin I (10),$^3$ whose NMR data due to C-6 to C-19 and 17-CH$_3$ were identical to those for 3. Thus the structure of melophlin R (3) was assigned as the 5-methyl derivative of melophlin I (10) as shown in Chart 1. The stereochemistry at the 5 position was also identified by the advanced Marfey’s method as described above and demonstrated a 1:1 mixture of S and R-configurations.

Melophlin S (4) was also obtained as a yellowish oil and showed an (M+H)$^+$ ion at $m/z$ 352 in its ESI-MS. The molecular formula (C$_{21}$H$_{37}$NO$_3$) was determined from HR-FAB-MS and NMR data. A methyl-doublet was detected at $\delta$ 0.81 (6H, d, $J$=6.8 Hz) in the 1H-NMR spectrum of 4, together with the terminal methyl signal ($\delta$ 0.86, 3H, t, $J$=6.8 Hz), N-methyl singlet ($\delta$ 2.95, and 5-CH$_3$ signal. The 13C-NMR spectrum of 4 showed a set of signals in a similar ratio of t- and $\beta$-isomers. Therefore melophlin P (1) was a racemic mixture of the 5(S) and 5(R)-enantiomers.

Melophlin Q (2) showed the (M+H)$^+$ ion at $m/z$ 352 in its ESI-MS. The molecular formula was deduced as C$_{21}$H$_{37}$NO$_3$ by HR-FAB-MS and NMR data. The 1H- and 13C-NMR spectra of 2 revealed the presence of an isopropyl-branched terminus by the signals ascribable to H$_2$-19 and 18-CH$_3$ ($\delta$ 0.84, 6H, d, $J$=6.8 Hz; $\delta$ 22.8) and H-18 ($\delta$ 1.49, 1H, m; $\delta$ 29.7). The alkyl chain of 2 assigned by NMR data was identical to that of melophlin H (9),$^3$ and the 1H- and 13C-NMR spectra of 2 and 9 due to C-6 to C-17 and 18-CH$_3$ were superimposable with each other. Therefore 2 was determined as the 5-methyl derivative of melophlin H (9) as shown in Chart 1. The absolute stereochemistry at C-5 in compound 2 was also determined by the advanced Marfey’s method as described above. The result from ESI-MS analysis revealed that 2 was also a racemic mixture of the 5(S) and 5(R)-enantiomers.

Melophlin R (3) was revealed as an isomer of 2 from HR-FAB-MS and NMR data, which established the molecular formula of C$_{21}$H$_{37}$NO$_3$ for 3. The 1H-NMR spectrum of 3 showed a methyl-triplet due to H$_3$-19 ($\delta$ 0.83, $J$=7.2 Hz) and a methyl-doublet ascribed to the branched methyl group ($\delta$ 0.82, $J$=6.4 Hz), besides N-CH$_3$ and 5-CH$_3$ signals. The 1H-1H COSY and HMBC spectra of 3 revealed the presence of an anteiso-type moiety, and the alkyl chain of 3 was the same as that of melophlin I (10),$^3$ whose NMR data due to C-6 to C-19 and 17-CH$_3$ were identical to those for 3. Thus the structure of melophlin R (3) was assigned as the 5-methyl derivative of melophlin I (10) as shown in Chart 1. The stereochemistry at the 5 position was also identified by the advanced Marfey’s method as described above and demonstrated a 1:1 mixture of S and R-configurations.

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Experimental

**General Procedures** NMR spectra were measured by a JEOL AL-400 NMR spectrometer (H, 400 MHz; 13C, 100 MHz) in CDCl3. Mass spectra were obtained by a JEOL HX-110 mass spectrometer (FAB mode) or a Finnigan TSQ 700 triple quadrupole mass spectrometer (ESI mode). UV and IR spectra were recorded on a HITACHI U-3000 and on a JASCO FT/IR 470, respectively. Optical rotations were recorded by a JASCO DIP-1000 digital polarimeter.

**Extraction and Isolation** The marine sponge *M. cf. sarasinorum* (Sponge I) was collected in the Rock Islands at Urakihapel in Palau on February 5, 2000. The other species of *Melophlus* (Sponge II) was taken in the Rock Islands at Eil Malk on the same day. This sponge had the same appearance in the natural habitat as those of Sponge I, but the identification of the species was different from the ordinary *Melophlus* species. Sponge I was transported to Japan by ship on February 5, 2000. The other species of *Melophlus* (Sponge II) was collected at 00-02-05 to 00-02-05 = 2:20, respectively. The sponges were frozen in a storeroom (−50 °C) of the training vessel Shinyo-maru and transported to Japan by ship.

The freeze-dried Sponge I (80 g) was extracted three times with EtOH. The extract, after evaporation of EtOH, was dissolved in CHCl3 and separated by Sephadex LH-20 column with MeOH−CHCl3 (1 : 1) into four fractions. The third fraction (580 mg) was chromatographed on a silica gel with CHCl3−MeOH (gradient elution) to give four fractions, and the 2−5% (v/v) fraction was subjected to preparative TLC with CHCl3−EtOAc−H2O (14:80:20, 0.05% TFA) to give one fraction, obtained by two silica gel column separations after solvent partition, showed the presence of compounds 2, 3, 11, and 12.

**ESI-LC/MS Analysis of Hydrolysate** Tohoku Pharmaceutical University, Komatsushima, Sendai, Japan.

**References and Notes**

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4) The scientific name of this sponge (*Melophlus sarasinorum* THIELE) was accidentally misspelled in literatures 2 and 3.