SYNTHESIS, STEREOCHEMISTRY, AND BIOLOGICAL PROPERTIES OF THE DEPIGMENTING AGENTS, MELANOSTATIN, FELDAMYCIN AND ANALOGS

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Syntheses of melanostatin and feldamycin have been completed from l-serine and l-threonine, respectively, and the configuration of unknown asymmetric carbons determined. Feldamycin analogs have also been prepared and the l-tryptophyl analog was the most potent in the depigmentation of Streptomyces bikiniensis and B16 melanoma cells.

In the course of screening for melanin biosynthesis inhibitors using Streptomyces bikiniensis NRRL B-1049, BMY-28565 and BMY-28566 were isolated from Streptomyces calvus No. N924-1 and Streptomyces clavifer No. N924-2, respectively\(^1\). They inhibited melanin synthesis in the growing cells of S. bikiniensis and B16 melanoma, but did not show any inhibitory activity on mushroom tyrosinase.

From structural studies BMY-28565 was identical to feldamycin\(^3,4\), reported as an antibacterial antibiotic, whereas BMY-28566 was revealed to be a new compound and named melanostatin\(^2\), which was deduced to have the pseudotripeptide structure 1. Two of the three asymmetric centers of 1 were determined to have the S-configuration, but the stereochemistry of the remaining carbon was still uncertain. The structure of feldamycin (2) has been reported\(^3,4\) with no description of the stereochemistry of the four asymmetric carbons in the molecule. Our degradation work indicated that the histidine moiety of 2 has the same S-configuration as that of 1. However, the stereochemistry of the other three carbons remained unknown (Fig. 1).

This report describes the total synthesis and stereochemistry of melanostatin and feldamycin, preparation of the analogs and their inhibitory activity on melanin biosynthesis.

Synthesis of Melanostatin (1) and Feldamycin (2)

The total synthesis of melanostatin has been accomplished from l-serine, according to the procedure shown in Scheme 1.
Nakagawa et al.\(^5\) reported that an attempted cyclization of \(N,O\)-ditosyl-\(L\)-serine ethyl ester (3, Et ester) to an optically active \(N\)-tosylaziridine ester (4, Et ester) under basic conditions (triethylamine (Et\(_3\)N) - THF) resulted in elimination of the O-tosyl group to give a dehydroamino ester, although the corresponding \(N\)-trityl ester\(^6\) gave the \(N\)-tritylaziridine derivative. Nakajima et al.\(^7\) applied this observation to the preparation of optically active \(N\)-acylaziridine derivatives which were prepared by cyclization of the \(N\)-trityl derivative followed by detritylation and \(N\)-acylation. We confirmed that the reaction of 3 with Et\(_3\)N in

\[\text{i: MeOH} - \text{SOCl}_2, \text{ii: TsCl} - \text{pyridine, iii: Et}_3\text{N} - \text{MeOH, iv: NaOH} - \text{L-His, v: Na-\text{liq NH}_3, vi: \(N\)-BOC-\(N\)-Me-L-PheOSu - Et}_3\text{N, vii: TFA, viii: \(N^\alpha,N^\alpha\)-di-BOC-\(N^\alpha\)-Me-L-His-OSu - Et}_3\text{N.}\]

\(\text{Nakagawa et al.}^5\) reported that an attempted cyclization of \(N,O\)-ditosyl-\(L\)-serine ethyl ester (3, Et ester) to an optically active \(N\)-tosylaziridine ester (4, Et ester) under basic conditions (triethylamine (Et\(_3\)N) - THF) resulted in elimination of the O-tosyl group to give a dehydroamino ester, although the corresponding \(N\)-trityl ester\(^6\) gave the \(N\)-tritylaziridine derivative. Nakajima et al.\(^7\) applied this observation to the preparation of optically active \(N\)-acylaziridine derivatives which were prepared by cyclization of the \(N\)-trityl derivative followed by detritylation and \(N\)-acylation. We confirmed that the reaction of 3 with Et\(_3\)N in
THF resulted in 1,2-elimination to give a dehydroamino ester as reported in the literature\(^5\), but in the reaction in MeOH, 1,3-elimination of 3 proceeded smoothly to afford the desired N-tosylaziridine ester 4 in good yield, although the reason for this observation could not be explained clearly. The nucleophilic ring opening reaction of 4 with L-histidine gave the \(\alpha,\beta\)-diamino acid derivative 5 with a small amount of a regio-isomer (6). The desired product 5 was isolated and the N-tosyl group removed in the usual manner to give 7. The acylation of 7 with N-methyl-L-phenylalanine active ester followed by deblocking afforded (2S)-2-[(2S)-2-methylamino-3-phenylpropionyl]amino-3-[[(1S)-1-carboxy-2-(1H-imidazol-4-yl)ethyl]amino]propionic acid (1), which was identical with the natural product\(^2\) (IR, \(^1\)H NMR, \([\alpha]_D\) and TLC). This confirmed that the unknown asymmetric carbon of melanostatin was of the \(S\)-configuration.

The synthesis of feldamycin (2) from L-threonine was achieved by a similar sequence. The N-tosylaziridine \(^8\) was also obtained by direct cyclization of the corresponding N,O-ditosyl ester with Et\(_3\)N in MeOH and converted to the intermediate \(^9\) which retained the configuration of the C-3 asymmetric carbon of L-threonine by a double inversion mechanism\(^10\). Protected N-methyl-L-histidine\(^11\) was coupled with 9 and the acylated product was deblocked to give the pseudotripeptide (2), which was then converted to the corresponding hydrochloride (2-HCl). The \([\alpha]_D\) values of the synthetic product (−7.1°) and its HCl salt (+11.4°) were nearly identical to those reported for feldamycin (−6.6° and +12°)\(^3\), respectively. Thus, the structure of feldamycin has been established as (2S,3R)-2-[(2S)-2-methylamino-3-(1H-imidazol-4-yl)propionyl]amino-3-[[1S]-1-carboxy-2-(1H-imidazol-4-yl)ethyl]aminobutyric acid (2).

**Feldamycin Analogs and Their Inhibition of Melanin Biosynthesis**

A variety of feldamycin analogs (10~30) were prepared to characterize the relationship between structure and depigmenting activity. The coupling of 9 with various amino acids was carried out by the active ester method. The products were purified by column chromatography on silica gel and ion exchange resin. The derivatives prepared by this procedure are listed in Table 1 with their depigmenting activity, which was determined by in vitro assays using \(S.\) *bikiniensis* B-1049 and B16 melanoma cells\(^12\). The l-asparaginyl derivative (22) has been reported\(^6,9\) as the immunomodulating agent FR900490, but its depigmenting activity has never been described.

As shown in Table 1, the inhibitory activity on melanin synthesis was not affected to a large extent by the third amino acid residue (R−CO in Table 1). Little correlation was observed between the activities measured by the two assay methods. The stereochemistry of the amino acid residue (10 vs. 11, 16 vs. 17, 19 vs. 20 and 22 vs. 23) had little effect on the activity of the diastereoisomers. The L-tryptophan analog (15) showed the best activity in both assay systems and was followed by the L- and D-phenylglycine (16 and 17), L- and D-asparagine (22 and 23), and L-tyrosine (13) derivatives.

**Experimental**

IR spectra were measured on an Analect FX-6160 EV spectrometer. \(^1\)H NMR spectra were recorded on a Varian FT-80A (80 MHz) or Jeol GX-400 (400 MHz) spectrometer using TMS or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard. \([\alpha]_D\) values were recorded on a Jasco DIP-140 digital polarimeter. Mass spectra were obtained with a Jeol JMS-AX 505H mass spectrometer.

(2S)-1-Tosyl-2-methoxycarbonylaziridine (4)

A mixture of \(N,O\)-bis(tosyl)-L-serine methyl ester (3)\(^3\) (12.82 g, 30 mmol) and Et\(_3\)N (4.15 ml, 30.5 mmol)
Table 1. Inhibition of melanin biosynthesis by feldamycin analogs (10~30).

<table>
<thead>
<tr>
<th>Amino acid residue (R—CO)</th>
<th>Inhibition of melanin synthesis</th>
<th>Amino acid residue (R—CO)</th>
<th>Inhibition of melanin synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Streptomyces bikiniensis</td>
<td></td>
<td>Streptomyces bikiniensis</td>
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<tr>
<td></td>
<td>B16 melanoma</td>
<td></td>
<td>B16 melanoma</td>
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<tr>
<td></td>
<td>IC₅₀⁺</td>
<td>MEC⁺</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>MEC⁺</td>
</tr>
<tr>
<td>Aromatic amino acid:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 L-Phe</td>
<td>0.37</td>
<td>100</td>
<td>22 l-Asn</td>
</tr>
<tr>
<td>11 D-Phe</td>
<td>0.84</td>
<td>100</td>
<td>23 d-Asn</td>
</tr>
<tr>
<td>12 L-Me-L-Phe</td>
<td>0.23</td>
<td>25~50</td>
<td>24 l-Gln</td>
</tr>
<tr>
<td>13 l-Tyr</td>
<td>0.35</td>
<td>12.5</td>
<td>Acidic or basic substitution;</td>
</tr>
<tr>
<td>14 L-His</td>
<td>0.75</td>
<td>6.3~12.5</td>
<td>25 l-Asp</td>
</tr>
<tr>
<td>15 l-Try</td>
<td>0.13</td>
<td>6.3</td>
<td>26 l-Lys</td>
</tr>
<tr>
<td>16 l-(Phenyl)Gly</td>
<td>0.21</td>
<td>50</td>
<td>27 l-Arg</td>
</tr>
<tr>
<td>17 D-(Phenyl)Gly</td>
<td>0.28</td>
<td>6.3~12.5</td>
<td>Glycyl derivatives:</td>
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<tr>
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<td></td>
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<td>28 Gly</td>
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<td></td>
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<td></td>
<td>30 l-Asn-Gly</td>
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<tr>
<td>Non-aromatic amino acid:</td>
<td></td>
<td></td>
<td>Natural product:</td>
</tr>
<tr>
<td>18 l-Pro</td>
<td>0.72</td>
<td>50</td>
<td>Melanostatin (1)</td>
</tr>
<tr>
<td>19 l-Ala</td>
<td>0.54</td>
<td>12.5~50</td>
<td>0.76</td>
</tr>
<tr>
<td>20 D-Ala</td>
<td>0.96</td>
<td>50</td>
<td>Feldamycin (2)</td>
</tr>
<tr>
<td>21 l-Thr</td>
<td>0.55</td>
<td>12.5~25</td>
<td>1.29</td>
</tr>
</tbody>
</table>

* µg/ml.

in MeOH (60 ml) was heated at reflux for 30 minutes and cooled to room temperature. The resulting mixture was diluted with EtOAc (600 ml) and washed with 10% citric acid, 1 M NaHCO₃, and brine. The organic extract was dried and concentrated. The residual oil (8.0 g) was passed through an alumina column (160 g). The eluate with n-hexane-EtOAc (4: 1~1: 1) was concentrated to obtain 3.3 g of 4 as an oily product. Yield 43%. IR v_max (film) cm⁻¹ 1745, 1590, 1330, 1230, 1160; ¹H NMR (CDCl₃) δ 2.5 (3H, s), 2.6 (2H, m), 3.3 (1H, dd, J = 5 and 7 Hz), 3.7 (3H, s), 7.3 (2H, d, J = 7 Hz), 7.8 (2H, d, J = 7 Hz); [α]D²⁶ −63° (c 2.1, MeOH); HRFAB-MS: Calcd for C₁₁H₁₄NO₄S (M+1), m/z 256.0644; Found m/z 256.0648.

(2S)-3-[(1S)-1-Carboxy-2-(1H-imidazol-4-yl)ethyl]amino-2-tosylaminopropionic Acid (5) and Its Regio Isomer (6)

A suspension of 4 (3.13 g, 12.3 mmol) in MeOH (12.5 ml) and 1 N NaOH (12.5 ml) was stirred under cooling in an ice bath for 1 hour and to the resulting solution were added l-histidine monohydrochloride hydrate (7.74 g, 36.9 mmol) and NaOH (2.0 g, 50 mmol). The mixture was heated to reflux for 1 hour and then concentrated to dryness. The residual oil was diluted with water (15 ml) and the solution was adjusted to pH 7.0 by adding 2 N H₂SO₄. The resulting crystalline precipitate was filtered off (1.27 g of histidine was recovered) and the filtrate was acidified to pH 4. The acidified solution was adsorbed onto a column of high porous resin Diaion HP-20 (400 ml) which was washed with water and eluted with 50% aq MeOH. The desired fractions were combined and concentrated to obtain 4.7 g of yellow solid, which was subjected to column chromatography (Kieselgel 60, 120 g). Elution with CHCl₃-MeOH-conc NH₄OH (5:3:1) was monitored by TLC. The desired fractions were combined and concentrated to afford 2.26 g of 5. Yield 46%. MP 195~198°C (dec); IR v_max (KBr) cm⁻¹ 1640~1610, 1560, 1410, 1370, 1350, 1320, 1160, 1090,
80; ¹H NMR (D₂O) δ 2.40 (3H, s), 2.7-2.9 (4H, m), 3.36 (1H, t, J=6.7 Hz), 3.74 (1H, dd, J=4.4 and 8.7 Hz), 6.92 (1H, s), 7.40 (2H, d, J=7.9 Hz), 7.73 (2H, d, J=7.9 Hz), 7.78 (1H, s); ¹³C NMR (D₂O) δ 21.5 (q), 29.9 (t), 49.8 (t) 58.1 (d), 63.7 (d), 118.6 (d), 127.7 (Ph), 130.8 (Ph), 136.3 (d). HRFAB-MS: Calcd for C₁₆H₂₁N₄O₆S (M+ l), m/z 397.1182; Found m/z 397.1180.

The slower moving fractions gave 669 mg of the regio isomer (6). Yield 14%. MP 232-239°C (dec); IR vs max (KBr) cm⁻¹ 1630, 1505, 1455, 1395, 1355, 1315, 1155, 1090, 835, 810; ¹H NMR (D₂O) δ 2.39 (3H, s), 2.85 (1H, dd, J=6.8 and 15.1 Hz), 2.92 (1H, dd, J=5.1 and 15.1 Hz), 3.09 (2H, d, J=5.1 Hz), 3.21 (1H, t, J=5.1 Hz), 3.38 (1H, dd, J=6.8 and 5.7 Hz), 6.96 (1H, s), 7.40 (2H, d, J=8.1 Hz), 7.68 (2H, d, J=8.4 Hz), 7.78 (1H, s); ¹³C NMR (D₂O) δ 21.5 (q), 29.95 (t), 44.9 (t), 61.5 (d), 62.4 (d), 118.9 (d), 127.6 (Ph), 130.9 (Ph), 136.25 (d). HRFAB-MS: Calcd for C₁₆H₂₁N₄O₆S (M+ l), m/z 397.1182; Found m/z 397.1170.

(S)-2-Amino-3-[(1S)-1-carboxy-2-(1H-imidazol-4-yl)ethyl]aminopropionic Acid (7)

To a stirred mixture of 5 (1.83 g, 4.62 mmol) in liq ammonia (20 ml) was added Na metal (940 mg, 40.9 mmol atom) in small pieces. The resulting deep blue mixture was stirred for 1 hour at −78°C and then quenched by the addition of NH₄Cl (2.55 g, 48 mmol). Ammonia was removed on standing under atmospheric pressure for 2 hours. The residual solid was dissolved in water (20 ml), adjusted to pH 4 by the addition of 1 N HCl and was passed through an Amberlite IR-120 column (H⁺ type, 100 ml). After being washed with water, the column was eluted with 2.8% NH₄OH. The desired fractions were combined, concentrated to dryness, and the residue was lyophilized to afford 1.01 g of amorphous powder, which was crystallized from aq MeOH to give 654 mg of white powder 7. Yield 58%. MP 232-235°C (dec); IR vs max (KBr) cm⁻¹ 1633-1614, 1582, 1440, 1400, 1367, 840; ¹H NMR (D₂O+NaOD) δ 2.55 (1H, dd, J=8.8 and 12.1 Hz), 2.71 (1H, dd, J=4.4 and 11.7 Hz), 2.86 (1H, d, J=6.6 Hz), 2.87 (1H, d, J=7.3 Hz), 3.31 (1H, t, J=6.6 Hz), 3.31 (1H, dd, J=4.4 and 8.4 Hz), 6.88 (1H, s), 7.64 (1H, s).

Anal Calcd for C₉H₁₄N₄O₄·H₂O: C 43.97, H 5.90, N 22.79. Found: C 43.66, H 5.92, N 23.17.

(2S,3S)-1-Tosyl-2-methoxycarbonyl-3-methylaziridine (8)

A mixture of N,O-ditosyl-L-threonine methyl ester (93 g, 0.21 mol) and Et₃N (29.3 ml, 0.21 mmol) in MeOH (450 ml) was refluxed for 1.5 hours and then evaporated to dryness. The residue was diluted with EtOAc and washed with water. The organic extract was dried, concentrated, and the residual oil was diluted with isopropl ether. The crystalline precipitate was collected by filtration to obtain 37.2 g of 8,
as colorless crystals. Yield 66%. MP 59~60°C; IR \nu_{\text{max}} (\text{KBr}) \text{ cm}^{-1} 2880, 1735; ^1\text{H NMR (CDCl}_3\text{)} \delta 1.33 (3\text{H}, d, J=5.5\text{ Hz}), 2.47 (3\text{H}, s), 3.20 (2\text{H}, m), 3.70 (3\text{H}, s), 7.4~8.1 (4\text{H}, m). [\alpha]_{D}^{25} = -41.0^\circ (c 2.1, \text{MeOH}) (\text{literature}^5) [\alpha]_{D}^{23} = -40.2^\circ (c 2.0, \text{MeOH}).

(2R,3R)-2-Amino-3-[(1S)-1-carboxy-2-(1H-imidazol-4-yl)ethyl]aminobutyric Acid (9)

A suspension of 8 (51.15 g, 0.19 mol) in MeOH (200 ml) and 1 \text{ n NaOH (190 ml)} was stirred at room temperature for 30 minutes and to the resulting solution were added L-histidine monohydrochloride hydrate (145 g, 0.69 mol) and NaOH (64.75 g, 1.62 mol) in MeOH (36.5 ml) and water (50 ml). The mixture was heated to reflux for 2 hours and was concentrated to dryness. The residual oil was diluted with water, and pH of the solution was adjusted to 5.0 by adding 2\text{ n H}_2\text{SO}_4. The resulting crystalline precipitate was collected by filtration, and washed with water and MeOH to obtain 35.0 g of the ring-opened product (yield 45%). MP 187~190°C (the regio isomer was isolated from the mother liquor).

To a stirred mixture of the above intermediate (34.6 g, 84 mmol) in liq NH\textsubscript{3} (350 ml) was added sodium metal (15.9 g, 0.69 mol atom) in small pieces, and the resulting deep blue mixture was stirred for 40 minutes at -78°C. The reaction was quenched by the addition of NH\textsubscript{4}Cl, and NH\textsubscript{3} was removed on standing under atmospheric pressure. The residual solid was dissolved in water, adjusted to pH 4 by the addition of 2\text{ n H}_2\text{SO}_4 and passed through an Amberlite IR-120 column. After being washed with water, the column was eluted with 2.8% NH\textsubscript{4}OH. The desired fractions were combined and concentrated to dryness and the residue was crystallized from MeOH to afford 6.71 g of 9 as a colorless powder. Yield 31%. MP 220~225°C. IR \nu_{\text{max}} (\text{KBr}) \text{ cm}^{-1} 2995, 2850, 1620, 1580. ^1\text{H NMR (D}_2\text{O+NaOD)} \delta 1.13 (3\text{H}, d, J=7\text{ Hz}), 2.6~4.3 (5\text{H}, m), 7.06 (1\text{H}, s), 7.81 (1\text{H}, d, J=1\text{ Hz}).

(2S,3R)-2-[(2S)-2-Methylamino-3-(1^-imidazol-4-yl)propionyl]amino-3-[(1S)-1-carboxy-2-(1H-imidazol-4-yl)ethyl]aminobutyric Acid (Feldamycin) (2)

To a stirred solution of 9 (319 mg, 1.24 mmol) and Et\textsubscript{3}N (455 mg, 4.5 mmol) in water (4.5 ml) was added a solution of N-hydroxysuccinimide ester of AU-bis-BOC-N-methyl-L-histidine (1.0 g, 2.14 mmol) in dioxane (9 ml). The mixture was stirred at room temperature overnight and then concentrated to dryness. The residue was dissolved in a small volume of BuOH-H\textsubscript{2}O-AcOH (4:1:2) and purified on a column of Kieselgel 60 (30 g) eluted with the same solvent (500 ml). The eluate was fractionated and desired fractions (Nos. 4~10) combined and concentrated to afford 1.35 g of oily residue, which was diluted with TFA (4 ml). The resulting solution was left at room temperature for 1 hour and then concentrated to dryness. The residue was dissolved with a small volume of water, concentrated again, and diluted with water (20 ml). The solution was passed through a column of Amberlite IR-120 (H\textsuperscript{+} type, 5 ml), and after washing with water, the column was eluted with 1\text{ n NH}_4\text{OH} and the eluate was fractionated. The desired fractions (Nos. 5 and 6) were combined and concentrated to obtain 325 mg of amorphous powder, which was dissolved with BuOH-AcOH-H\textsubscript{2}O (4:1:2), and was subjected to column chromatography on Kieselgel 60 (20 g). The column was successively eluted with BuOH-AcOH-H\textsubscript{2}O (4:1:2, 150 ml; 3:1:2, 200 ml; and 2:1:2, 150 ml), and collected in 15 ml fractions. The desired fractions (Nos. 11~16) were combined and concentrated. The residue was dissolved in a small amount of water and passed through a column of Amberlite IR-120 (H\textsuperscript{+} type, 5 ml). After washing with water, the column was eluted with 1\text{ n NH}_4\text{OH}. The desired fraction (No. 6) was concentrated and lyophilized to give 118 mg of 2, as an amorphous powder. Yield 23%. MP 160~162°C; IR \nu_{\text{max}} (\text{KBr}) \text{ cm}^{-1} 1623, 1389; [\alpha]_{D}^{24} = -7.1^\circ (c 1.0, \text{H}_2\text{O}), +24^\circ (c 1.08, 0.1\text{ n HCl}) (\text{literature}^3) [\alpha]_{D}^{25} = -6.6^\circ (c 1, \text{H}_2\text{O})); ^1\text{H NMR (D}_2\text{O)} \delta 1.10 (3\text{H}, d, J=7\text{ Hz}, d=7\text{ Hz}), 2.11 (3\text{H}, s), 3.17 (2\text{H}, m), 3.35 (1\text{H}, dq, J=5 and 7\text{Hz}), 3.90 (1\text{H}, dd, J=5 and 7\text{ Hz}), 3.99 (1\text{H}, t-like, J=6\text{Hz}), 4.30 (1\text{H}, d, J=5\text{Hz}), 7.05 (2\text{H}, d, J=1\text{Hz}), 7.88 (2\text{H}, d, J=1\text{Hz}). \text{HRFAB-MS: Calcd for } \text{C}_{17}\text{H}_{26}\text{N}_{7}\text{O}_{5} (M+1), \text{m/z 408.1995; Found, m/z 408.1989.}

Preparation of Feldamycin Hydrochloride (2-HCl)

A solution of 2 (30 mg, 0.074 mmol) in 50% MeOH (0.1 ml) was diluted with 4\text{ n HCl in MeOH (0.2 ml, 0.8 mmol)}. The solution was added dropwise to stirred acetone (20 ml) and the precipitate was collected by filtration, washed with acetone and dried to obtain 31 mg of 2 hydrochloride as a hygroscopic white powder. IR \nu_{\text{max}} (\text{KBr}) \text{ cm}^{-1} 1687, 1656, 1623, 1556; [\alpha]_{D}^{25} = +11.4^\circ (c 1.0, \text{H}_2\text{O}),(\text{literature}^3) [\alpha]_{D}^{23} = +12^\circ (c 1, \text{H}_2\text{O}).
Preparation of Feldamycin Analogs (10~30)

Procedure for preparation of 10~30 in Table 1 is fundamentally the same as that described for feldamycin above. Analytical data of the compounds are shown below. The yield is indicated as isolated yield from 8.

**L-Phenylalanyl Analog (10)**

Yield 33%. MP 180°C; IR ν_max (KBr) cm⁻¹ 1623, 1386; ¹H NMR (D₂O + NaOD) δ 1.02 (3H, d, J = 6.5 Hz), 2.9 (5H, m), 3.48 (1H, t-like, J = 7 Hz), 3.63 (1H, t-like, J = 6 Hz), 3.93 (1H, d, J = 8 Hz), 6.90 (1H, s), 7.35 (5H, m), 7.65 (1H, s); HRFAB-MS: Calcd for C₁₉H₂₆N₅O₅ (M+1), m/z 404.1934; Found, m/z 404.1926.

**D-Phenylalanyl Analog (11)**

Yield 43%. MP 174~176°C (dec); IR ν_max (KBr) cm⁻¹ 1623, 1384; ¹H NMR (D₂O) δ 0.75 (3H, d, J = 7 Hz), 2.8~3.5 (5H, m), 3.94 (1H, dd, J = 5 and 9 Hz), 4.26 (1H, dd, J = 7 and 10 Hz), 4.53 (1H, d, J = 4 Hz), 7.21 (1H, s), 7.43 (5H, m), 8.01 (1H, d, J = 1 Hz); HRFAB-MS: Calcd for C₁₉H₂₆N₅O₅ (M+1), m/z 404.1947; Found, m/z 404.1947.

**N-Methyl-L-phenylalanyl Analog (12)**

Yield 56%. MP 178~183°C; IR ν_max (KBr) cm⁻¹ 1630, 1390; ¹H NMR (D₂O + NaOD) δ 0.75 (3H, d, J = 7 Hz), 2.40 (3H, s), 2.9 (5H, m), 3.48 (1H, t-like, J = 7 Hz), 3.63 (1H, t-like, J = 6 Hz), 7.03 (1H, s), 7.75 (1H, s); HRFAB-MS: Calcd for C₂₀H₂₈N₅O₅ (M+1), m/z 418.2091; Found, m/z 418.2097.

**L-Tyrosyl Analog (13)**

Yield 17%. MP 216~218°C; IR ν_max (KBr) cm⁻¹ 1653, 1623, 1617, 1516, 1399, 1250; ¹H NMR (D₂O) δ 1.04 (3H, d, J = 7 Hz), 2.9~3.4 (5H, m), 3.7~4.05 (2H, m), 4.28 (1H, d, J = 7 Hz), 6.90 (2H, m) 7.13 (1H, s), 7.18 (2H, m), 7.86 (1H, d, J = 1 Hz); HRFAB-MS: Calcd for C₁₉H₂₆N₅O₅Na (M+1), m/z 420.1883; Found, m/z 420.1893; Calcd for C₁₉H₂₆N₅O₅Na (M+Na), m/z 442.1705; Found, m/z 442.1705.

**L-Histidyl Analog (14)**

Yield 30%. MP >175°C; IR ν_max (KBr) cm⁻¹ 1623, 1386; ¹H NMR (D₂O) δ 1.10 (3H, d, J = 7 Hz), 3.15 (2H, m), 3.43 (1H, dq, J = 5 and 7 Hz), 3.91 (1H, dd, J = 6 and 8 Hz), 4.12 (1H, t, J = 7 Hz), 4.33 (1H, d, J = 5 Hz), 7.10 (2H, s), 7.95 (2H, s); HRFAB-MS: Calcd for C₁₆H₂₄N₇O₅ (M+1), m/z 394.1839; Found, m/z 394.1839.

**L-Tryptophyl Analog (15)**

Yield 31%. MP >184°C (dec); IR ν_max (KBr) cm⁻¹ 1628, 1384; ¹H NMR (D₂O) δ 1.05 (3H, d, J = 7 Hz), 3.25 (2H, m), 3.50 (3H, m), 3.96 (1H, dd, J = 6 and 8 Hz), 4.31 (1H, d, J = 5 Hz), 4.37 (1H, t-like, J = 8 Hz), 7.20 (1H, s), 7.2~7.8 (5H, m), 8.00 (1H, d, J = 1 Hz); HRFAB-MS: Calcd for C₂₁H₂₇N₆O₅ (M+1), m/z 443.2043; Found, m/z 443.2031.

**Anal** Calcd for C₁₉H₂₆N₅O₅·H₂CO₃·H₂O: C 52.8, H 5.94, N 16.66.

**Found:**

C 51.87, H 6.10, N 16.87.
L-Phenylglycyl Analog (16)
Yield 58%. MP 182~185°C; IR \( \nu_{\text{max}} \) (KBr) cm\(^{-1}\) 1628, 1389; \(^1\)H NMR (D\(_2\)O+NaOD) \( \delta \) 1.05 (3H, d, \( J = 6 \) Hz), 2.9 (3H, m), 3.5 (1H, t, \( J = 6.5 \) Hz), 4.0 (1H, d, \( J = 8.5 \) Hz), 4.65 (1H, s), 6.9 (1H, d, \( J = < 1 \) Hz), 7.45 (5H, s), 7.65 (1H, d, \( J = < 1 \) Hz).

**Anal Calcd for C\(_{18}\)H\(_{23}\)N\(_5\)O\(_5\)-2H\(_2\)O:** C 49.76, H 6.50, N 16.12.

**Found:** C 49.53, H 6.10, N 16.51.

D-Phenylglycyl Analog (17)
Yield 51%. MP 198~200°C; IR \( \nu_{\text{max}} \) (KBr) cm\(^{-1}\) 1672, 1633, 1499, 1394; \(^1\)H NMR (D\(_2\)O+NaOD) \( \delta \) 0.95 (3H, d, \( J = 8 \) Hz), 3.0~3.5 (3H, m), 3.95 (1H, dd, \( J = 6 \) and 9 Hz), 4.6 (1H, d, \( J = 5.5 \) Hz), 5.25 (1H, s), 7.18 (1H, s), 7.6 (5H, s), 8.00 (1H, s).

**Anal Calcd for C\(_{18}\)H\(_{23}\)N\(_5\)O\(_5\)-2H\(_2\)O:** C 50.81, H 6.40, N 16.46.

**Found:** C 50.59, H 6.01, N 16.75.

L-Prolyl Analog (18)
Yield 36%. MP 173~177°C; IR \( \nu_{\text{max}} \) (KBr) cm\(^{-1}\) 3397, 3233, 2883, 1627, 1489, 1385, 1262; \(^1\)H NMR (D\(_2\)O) \( \delta \) 1.15 (3H, d, \( J = 7 \) Hz), 1.5~2.5 (4H, m), 3.17~3.43 (5H, m), 3.85 (1H, t, \( J = 7 \) Hz), 4.30 (1H, d, \( J = 7 \) Hz), 4.35 (1H, m), 7.1 (1H, s), 7.9 (1H, s).

**Anal Calcd for C\(_{15}\)H\(_{23}\)N\(_5\)O\(_5\)
-\( \frac{1}{2} \)H\(_2\)O:** C 47.36, H 6.98, N 18.41.

**Found:** C 47.18, H 6.90, N 18.70.

L-Alanyl Analog (19)
Yield 76%. MP 182~188°C; IR \( \nu_{\text{max}} \) (KBr) cm\(^{-1}\) 1630, 1385; \(^1\)H NMR (D\(_2\)O+NaOD) \( \delta \) 1.32 (3H, d, \( J = 8 \) Hz), 2.9 (3H, d, \( J = 8 \) Hz), 2.95 (1H, s), 3.53 (2H, m), 3.98 (1H, d, \( J = 8 \) Hz), 6.92 (1H, s), 7.69 (1H, s); HRFAB-MS: Calcd for C\(_{13}\)H\(_{22}\)N\(_5\)O\(_5\) (M+1), m/z 328.1621; Found, m/z 328.1637.

D-Alanyl Analog (20)
Yield 41%. MP > 181°C (dec); IR \( \nu_{\text{max}} \) (KBr) cm\(^{-1}\) 1629, 1389; \(^1\)H NMR (D\(_2\)O) \( \delta \) 1.26 (3H, d, \( J = 7 \) Hz), 1.62 (3H, d, \( J = 7 \) Hz), 3.2 (2H, m), 3.53 (1H, dq, \( J = 5 \) and 7 Hz), 4.05 (1H, dd, \( J = 5.5 \) and 8 Hz), 4.22 (1H, q, \( J = 7 \) Hz), 4.56 (1H, d, \( J = 5 \) Hz), 7.21 (1H, s), 8.02 (1H, s); HRFAB-MS: Calcd for C\(_{13}\)H\(_{22}\)N\(_5\)O\(_5\) (M+1), m/z 328.1621; Found, m/z 328.1621.

**Anal Calcd for C\(_{13}\)H\(_{22}\)N\(_5\)O\(_5\) \( \cdot \frac{1}{2} \)H\(_2\)O:** C 47.36, H 6.40, N 18.61.

**Found:** C 47.02, H 6.61, N 18.61.

L-Threonyl Analog (21)
Yield 23%. MP 165~170°C; IR \( \nu_{\text{max}} \) (KBr) cm\(^{-1}\) 3400, 1628, 1452; \(^1\)H NMR (D\(_2\)O) \( \delta \) 1.35 (3H, d, \( J = 8 \) Hz), 3.2 (2H, m), 3.5~4.0 (1H, m), 4.0~4.5 (2H, m), 4.6 (1H, d, \( J = 8 \) Hz), 7.2 (1H, d, \( J = < 1 \) Hz), 8.19 (1H, d, \( J = < 1 \) Hz). HRFAB-MS: Calcd for C\(_{14}\)H\(_{24}\)N\(_5\)O\(_6\) (M+1), m/z 358.1727; Found, m/z 358.1712.

L-Asparaginyl Analog (22)
Yield 83%. HRFAB-MS: Calcd for C\(_{14}\)H\(_{24}\)N\(_6\)O\(_6\) (M+1), m/z 371.1679; Found, m/z 371.1669. The IR spectrum was identical to that of the authentic sample kindly provided by Dr. Kohsaka of Fujisawa Pharmaceutical Co., Ltd. The \(^1\)H NMR spectrum was also identical with the reported one\(^{12}\).

D-Asparaginyl Analog (23)
Yield 42%. MP 185~190°C; IR \( \nu_{\text{max}} \) cm\(^{-1}\) 3366, 1623, 1396; \(^1\)H NMR (D\(_2\)O) \( \delta \) 1.15 (3H, d, \( J = 6.5 \) Hz), 2.65 (2H, m), 3.0~3.5 (3H, m), 3.5~4.0 (2H, m), 4.25 (1H, d, \( J = 6.5 \) Hz), 7.1 (1H, s), 7.85 (1H, s). HRFAB-MS: Calcd for C\(_{14}\)H\(_{24}\)N\(_6\)O\(_6\) (M+1), m/z 358.1727; Found, m/z 358.1712.

L-Glutaminyl Analog (24)
Yield 80%. MP 182~186°C; IR \( \nu_{\text{max}} \) (KBr) cm\(^{-1}\) 1675, 1625; \(^1\)H NMR (D\(_2\)O+NaOD) \( \delta \) 1.10 (3H, d, \( J = 6 \) Hz), 1.7~2.6 (4H, m), 2.93 (3H, m), 3.50 (2H, m), 3.95 (1H, d, \( J = 8 \) Hz), 6.93 (1H, s), 7.68 (1H, s); HRFAB-MS: Calcd for C\(_{15}\)H\(_{25}\)N\(_6\)O\(_6\) (M+1), m/z 385.1836; Found, m/z 385.1839.


**Determination of The Inhibition of Melanin Synthesis**

(1) Inhibition of melanin synthesis in *S. bikiniensis* B-1049 is indicated in terms of IC₅₀ value, determined as follows: Suspended cell spores of *S. bikiniensis* B-1049 with the vegetative medium (ISP-7 supplemented with 0.2% yeast extract, inoculum size of 1.4 x 10⁶ cfu/ml) were cultured in the presence of 0.4% KN0₃ and varying concentrations of the test compound in a total volume of 1 ml at 28°C for 18 hours. The percent inhibition was estimated by reading the OD at 450 nm of the supernatant of the cultures, and the IC₅₀ value was calculated from the % inhibition (of at least 8 serial concentrations of an inhibitor). Data are a mean of duplicate runs.

(2) Inhibitory activity of melanin synthesis in B-16 melanoma is indicated in terms of minimum effective concentration (MEC) value, which was determined as follows: Test compound solution (0.4 ml) and B-16 melanoma cells (3 x 10⁴ cells/ml) in Eagle’s minimum essential medium supplemented

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**L-α-Aspartyl Analog (25)**

Yield 23%. MP 180~185°C; IR νmax (KBr) cm⁻¹ 1661, 1651, 1646, 1615, 1558, 1504, 1394; ¹H NMR (D₂O) δ 1.32 (3H, d, J = 7 Hz), 2.75~3.20 (2H, m), 3.25~3.50 (2H, m), 3.55~3.90 (1H, m), 4.0~4.50 (1H, m), 4.60 (1H, d, J = 5 Hz), 7.38 (1H, s), 8.44 (1H, s); HRFAB-MS: Calcd for C₁₄H₂₂N₅O₇ (M+1), m/z 372.1519; Found, m/z 372.1515.


Found: C 45.28, H 6.46, N 21.12.

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**L-Lysyl Analog (26)**

Yield 57%. MP 170~175°C; IR νmax (KBr) cm⁻¹ 1654, 1636, 1577, 1540, 1458, 1389; ¹H NMR (D₂O) δ 1.10 (3H, d, J = 7 Hz), 1.3~2.0 (6H, m), 2.9~3.4 (5H, m), 3.50~4.0 (2H, m), 4.26 (1H, d, J = 7 Hz), 7.10 (1H, d, J = 1 Hz), 7.83 (1H, d, J = 1 Hz).

Analytical Calcd for C₁₆H₂₈N₆O₅.H₂O: C 47.75, H 7.51, N 20.88.

Found: C 47.36, H 7.81, N 20.87.

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**L-Arginyl Analog (27)**

Yield 43%. MP 206~209°C; IR νmax (KBr) cm⁻¹ 1739, 1667, 1654, 1623, 1558, 1404; ¹H NMR (D₂O) δ 1.46 (3H, d, J = 7 Hz), 1.6~2.2 (4H, m), 3.33 (2H, t, J = 8 Hz), 3.55 (2H, d, J = 8 Hz), 3.65~3.90 (1H, m), 4.15~4.5 (2H, m), 4.98 (1H, d, J = 5 Hz), 7.51 (1H, d, J = 1.5 Hz), 8.76 (1H, d, J = 1.5 Hz); HRFAB-MS: Calcd for C₁₆H₂₉N₈O₅ (M+1), m/z 413.2261; Found, m/z 413.2244.

**Glycyl Analog (28)**

Yield 71%. MP >182°C (dec); IR νmax (KBr) cm⁻¹ 1628, 1386; ¹H NMR (D₂O) δ 1.25 (3H, d, J = 7 Hz), 3.3 (2H, m), 3.58 (1H, dq, J = 5 and 7 Hz), 3.98 (2H, s), 4.03 (1H, m), 4.58 (1H, d, J = 5 Hz), 7.21 (1H, m), 8.02 (1H, s); HRFAB-MS: Calcd for C₁₂H₂₀N₅O₅ (M+1), m/z 314.1465; Found, m/z 314.1470.

**Glycylglycyl Analog (29)**

Yield 72%. MP >171°C (dec); IR νmax (KBr) cm⁻¹ 1628, 1394; ¹H NMR (D₂O) δ 1.26 (3H, d, J = 7 Hz), 3.3 (2H, m), 3.59 (1H, dq, J = 5 and 7 Hz), 4.01 (2H, s), 4.11 (1H, m), 4.16 (2H, m), 4.53 (1H, d, J = 5 Hz), 7.22 (1H, s), 8.04 (1H, s); HRFAB-MS: Calcd for C₁₄H₂₃N₆O₆ (M+1), m/z 371.1679; Found, m/z 371.1659.

**L-Asparaginylglycyl Analog (30)**

Yield 51%. MP >166°C (dec); IR νmax (KBr) cm⁻¹ 1628, 1396; ¹H NMR (D₂O) δ 1.26 (3H, d, J = 7 Hz), 3.0 (2H, m), 3.33 (2H, m), 3.62 (1H, m), 4.13 (3H, m), 4.45 (1H, d, J = 6 Hz), 4.53 (1H, d, J = 5 Hz), 7.25 (1H, s), 8.14 (1H, s); HRFAB-MS: Calcd for C₁₆H₂₆N₇O₇ (M+1), m/z 428.1894; Found, m/z 428.1909.

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**Determination of The Inhibition of Melanin Synthesis**

(1) Inhibition of melanin synthesis in *S. bikiniensis* B-1049 is indicated in terms of IC₅₀ value, determined as follows: Suspended cell spores of *S. bikiniensis* B-1049 with the vegetative medium (ISP-7 supplemented with 0.2% yeast extract, inoculum size of 1.4 x 10⁶ cfu/ml) were cultured in the presence of 0.4% KN0₃ and varying concentrations of the test compound in a total volume of 1 ml at 28°C for 18 hours. The percent inhibition was estimated by reading the OD at 450 nm of the supernatant of the cultures, and the IC₅₀ value was calculated from the % inhibition (of at least 8 serial concentrations of an inhibitor). Data are a mean of duplicate runs.

(2) Inhibitory activity of melanin synthesis in B-16 melanoma is indicated in terms of minimum effective concentration (MEC) value, which was determined as follows: Test compound solution (0.4 ml) and B-16 melanoma cells (3 x 10⁴ cells/ml, 3.6 ml) in EAGLE’s minimum essential medium supplemented
with 10% fetal calf serum were incubated for 6 days at 37°C. During the incubation, the culture medium was once renewed with fresh medium containing the same compound solution. After incubation, cells were counted and solubilized with a solution of 1 n NaOH and 10% DMSO (1:1). The amount of melanin synthesized was colorimetrically measured at 470 nm, and the inhibition of melanin synthesis was determined from these OD values compared to those of control runs (the vehicle group). The MEC values are expressed as the concentration showing 50% inhibition.

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References