

A New Indolinepeptide from *Paecilomyces* sp. J300

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A new indolinepeptide (**3**) was isolated, together with two known compounds, a cerebroside (**1**) and an alloxazine (**2**), from silkworm larvae infected with *Paecilomyces* sp. J300. On the basis of spectroscopic data, their structures were elucidated as (4*E*, 8*E*, 2*S*, 2'*R*, 3*R*)-*N*-2'-hydroxy-hexadecanoyl-1-*O*- β -D-glucopyranosyl-9-methyl-4, 8-sphingadienine (**1**), 7,8-dimethylalloxazine (**2**) and 3 β ,5-dihydroxy-1-*N*-methyl-indoline-2 β -carbonyl amino-D-alanyl-erythro- β -hydroxyisoleucinyl-glycine (**3**).

Key words: *Paecilomyces* sp. J300, Cerebroside, Alloxazine, Indolinepeptide

INTRODUCTION

Paecilomyces species has been used in the Chinese traditional medicine to treat impotence, spermatorrhea and backache, and as a tonic to nourish lungs and kidneys (Shanghai Science and Technologic Publisher and JungDam Publisher, 1997; Pemberton, 1999). Previous pharmacological study on *Paecilomyces* species reported antitumor (Lee *et al.*, 2001), immunomodulation (Shim *et al.*, 2000), hypoglycemic activity (Shim *et al.*, 2000), antimicrobial (Rossi *et al.*, 1987) and antioxidative activity (Li *et al.*, 2001). Leucinostatin (Rossi *et al.*, 1987), cyclopeptide (Bernardini *et al.*, 1975), sphingofungin (Horn *et al.*, 1992), benzanthracene (Yamashita *et al.*, 1990), ergosterol (Nam *et al.*, 2001) and galactomannan derivatives (Domenech *et al.*, 1999) were reported from fungus *Paecilomyces* species.

Paecilomyces sp. J300 was produced by inoculating *Paecilomyces* sp. with the homogeneous fungi strain, *Paecilomyces* sp. J300, which was developed at the National Institute of Agricultural Science and Technology in Korea. We have previously reported seven cytotoxic ergosterol derivatives in the methanol extract of *Paecilomyces* sp. J300 (Kwon *et al.*, 2002). In the continuing study on this source, a new indolinepeptide (**3**) together with a known cerebroside (**1**) and an alloxazine

(**2**) were isolated from methanol- and 70% acetone soluble portion of water extract. The present paper describes the isolation and structural characterization of these compounds.

MATERIALS AND METHODS

General procedure

Mps: uncorr. NMR: in CDCl₃, Bruker AMX 500 and Varian UNITY INOVA 500. IR: Bruker Vector 22 FT-IR spectrophotometer. UV: Shimadzu UV-1601 UV-Visible Spectrophotometer. Polarimeter : JASCO P-1020. MS: JEOL JMS700 mass spectrometer (Japan). Hewlett-Packard 6890 GC (column : HP-5MS 30m 0.25mm)/Hewlett-Packard 5973 MSD system. Prep. HPLC : JAI LC-908 model with refractive index detector, UV detector and Alltech Econosil Silica 10 μ m column (250 mm \times 22 mm). HPLC for amino acid analysis : Hewlett Packard 1100 Series with UV detector (254 nm), column oven (46 $^{\circ}$ C) and HP. Eclipse XDB-C18 column (4.6 \times 250 mm, 5 μ m), Column Chromatography : Silica gel (Merck, 70-230, 230-400 mesh) and Sephadex LH-20 (Pharmacia). TLC: Merck precoated Si gel F₂₅₄ plates and RP-18 F_{254s} plates. LPLC: Merck Lichroprep Lobar-A Si 60 & Lobar-A RP-18 (240 \times 10 mm).

Material

Paecilomyces sp. J300 was supplied by the National Institute of Agricultural Science and Technology, Suwon, Korea.

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Extraction and purification

The dried and ground *Paecilomyces* sp. J300 (2.0 kg) were extracted with MeOH five times at room temperature, and then extracted five times at 60°C. The resultant methanol extract (200 g) was suspended in water and then successively partitioned to give hexane (110 g), chloroform (6 g) and butanol (15 g) soluble fractions. After extracting the material with methanol, the residue was extracted with water three times at 100°C. The resultant water soluble portion was extracted with 70% acetone to give acetone fraction (200 g). *N*-Containing compounds were isolated by monitoring with dragendorff and ninhydrin reagent on TLC. The hexane extract (110 g) was subjected to silica gel column chromatography, eluted with a gradient solvent mixture of hexane/ethylacetate to give three fractions (H1~H3). H2 fraction (70 g) was then applied to a silica gel column chromatography using hexane/ethylacetate/methanol (2:5:1) as an eluent to afford three subfractions (H21~H23). H22 fraction (2.8 g) was further subjected to silica gel column chromatography using methylene chloride/methanol/water (60:10:1) as eluent to give three subfractions (H221~H223). H222 subfraction was purified with Sephadex LH-20 column chromatography using methylene chloride/methanol (1:1) and preparative HPLC (methylene chloride/methanol/water 70:10:1) to afford **1** (300 mg).

The acetone extract (200 g) was subjected to HP-20 column chromatography, eluted with a gradient solvent mixture of water/methanol to give three fractions (A1~A3). A2 subfraction (15 g) was then applied to silica gel column chromatography using ethylacetate/methanol/water (9:5:2) as eluent to afford five subfractions (A21~A25). A21 fraction (500 mg) was subjected to Sephadex LH-20 column chromatography using methanol as eluent to give five subfractions (A211~A215). A214 was subjected to C-18 RP column chromatography (50% methanol) to three subfractions (A2141~A2143). A2141 subfraction was purified with RP Lobar-A column chromatography (40% methanol) and silica gel column chromatography using ethylacetate/methanol (6:1) to give **2** (7 mg). A2143 subfraction was purified with silica gel column chromatography using ethylacetate/methanol (20:1) to give **3** (10 mg).

(4*E*, 8*E*, 2*S*, 2'*R*, 3*R*)-*N*-2'-Hydroxyhexadecanoyl-1-*O*- β -D-glucopyranosyl-9-methyl-4,8-sphingadienine (**1**)

White powder, mp. 194°C; $[\alpha]_D^{20} +9.1^\circ$ (c 1.0, 1-PrOH); FAB-MS *m/z* (rel. int.): 750 ($[M+Na]^+$, 100), 728 ($[M+H]^+$, 10), FAB-CID-MS *m/z* (rel. int.): 750.3 ($[M+Na]^+$, 100), 495.3 ($[M-255+Na]^+$, 15); 1H -NMR (500 MHz, pyridine-*d*₅): δ 0.88 (6H, m, CH₃), 1.28 (40H, m, H-11~H-17 and H-3'~H-15'), 1.63 (3H, s, 9-Me), 2.02 (2H, dd, *J*=8.5, 7.5 Hz, H-10), 2.17 (4H, m, H-6 and H-7), 3.89 (1H, m, H-5''), 4.02

(1H, br.t, *J*=7.5 Hz, H-2''), 4.22 (3H, m, H-1, H-3'' and H-4''), 4.34 (1H, dd, *J*=11.9, 5.2 Hz, H-6''), 4.50 (1H, dd, *J*=11.9, 2.1 Hz, H-6''), 4.58 (1H, dd, *J*=7.9, 3.7 Hz, H-2''), 4.71 (1H, dd *J*=10.4, 5.8 Hz, H-1), 4.76 (1H, t, *J*=6.1 Hz, H-3), 4.80 (1H, m, H-2), 4.90 (1H, d, *J*=7.6 Hz, H-1''), 5.69 (1H, br.s, H-8), 5.94 (1H, dt, *J*=15.3, 5.5 Hz, H-5), 6.01 (1H, dd *J*=15.3, 6.1 Hz, H-4), 8.35 (1H, d, *J*=9.0 Hz, NH); ^{13}C -NMR (125 MHz, pyridine-*d*₅): δ 16.26 (16'-CH₃ and 18-CH₃), 18.06 (9-CH₃), 30.15~34.10 (C-3'~C-15' and C-11~C-17), 30.17 (C-7), 35.02 (C-6), 41.95 (C-10), 56.61 (C-2), 64.66 (C-6''), 72.12 (C-1), 73.52 (C-4''), 74.31 (C-3), 74.50 (C-2'), 77.08 (C-2''), 80.44 (C-3''), 80.52 (C-5''), 107.64 (C-1''), 124.21 (C-9), 133.87 (C-4), 134.26 (C-5), 137.84 (C-8), 177.58 (C-1').

7,8-Dimethylalloxazine (**2**)

Yellowish gum, UV (MeOH) λ_{max} (log ϵ): 211.5 (4.53), 242 (4.54), 252 sh (4.47), 258 (4.66), 331 (3.93), 377 (3.87); EI-MS *m/z* (rel. int.): 242 (100), 227 (3), 171 (50), 156 (40), 130 (5), 100 (7); 1H -NMR (500 MHz, pyridine-*d*₅): δ 2.01 (3H, s, 7-CH₃), 2.13 (3H, s, 8-CH₃), 7.68 (1H, br.s, H-6), 7.86 (1H, br.s, H-9); ^{13}C -NMR (125 MHz, pyridine-*d*₅): δ 20.25 (CH₃), 20.83 (CH₃), 127.60 (C-9), 130.15 (C-6), 131.46 (C-5a), 139.53 (C-7), 140.27 (C-9a), 143.49 (C-4a), 145.25 (C-8), 148.15 (C-2), 152.22 (C-10a), 162.46 (C-4).

3 β ,5-Dihydroxy-1-methyl-indoline-2 β -carbonylamino-D-alanyl-erythro- β -hydroxyisoleucinyl-glycine (**3**)

Colorless gum, HR-FAB-MS: C₂₁H₃₁N₄O₈, Found: *m/z* 467.2139 $[M+H]^+$ Calcd.: *m/z* 467.2142; 1H -NMR (500 MHz, CD₃OD): δ 0.95 (3H, t, *J*=7.3 Hz, H-17), 1.20 (1H, d, *J*=7.1 Hz, H-12), 1.66 (3H, s, H-18), 1.66 (1H, m, H-16, overlap with the signal of H-18), 2.04 (1H, sext, *J*=7.3 Hz, H-16), 2.67 (3H, s, NCH₃), 3.55 (1H, d, *J*=8.6 Hz, H-2), 3.75 (1H, d, *J*=17.1 Hz, H-20), 3.86 (1H, d, *J*=17.1 Hz, H-20), 4.49 (1H, br.q, *J*=7.1 Hz, H-11), 4.72 (1H, s, H-14), 4.78 (1H, d, *J*=8.6 Hz, H-3), 6.89 (1H, d, *J*=8.3 Hz, H-8), 7.14 (1H, br.d, *J*=8.3 Hz, H-7), 7.30 (1H, br.s, H-5); ^{13}C -NMR (500 MHz, CD₃OD): δ 9.09 (C-17), 16.75 (C-12), 23.30 (C-18), 31.47 (C-16), 33.14 (NCH₃), 44.81 (C-20), 51.07 (C-11), 60.72 (C-14), 70.06 (C-2), 73.44 (C-3), 86.52 (C-15), 119.04 (C-8), 123.57 (C-7), 124.83 (C-5), 132.12 (C-4), 143.90 (C-9), 152.60 (C-6), 168.52 (C-10), 170.78 (C-19), 172.84 (C-13), 176.38 (C-21).

Methanolysis of **1**

Compound **1** (2mg) was heated with 5 % HCl in MeOH at 74°C for 24 h. After addition of H₂O, the reaction mixture was extracted with hexane and dried by adding magnesium sulfate anhydrous. The resultant hexane soluble fraction was concentrated to yield 2-hydroxy-hexadecanoyl methyl ester, which was analyzed by GC-MS.

Enzyme hydrolysis of 1

Compound **1** (2mg) and 10 mg cellulase (ICN) in H₂O was kept at 37°C for 24 h, followed by solvent partitioning with chloroform and water. The water soluble portion was purified with Sephadex LH-20 column chromatography (MeOH) to afford glucose.

Amino acid composition analysis of 3

Compound **3** (5 mg) was dissolved in 6 N HCl (1.5 mL) and heated at 110°C for 24 h. The mixture was cooled to room temperature and concentrated *in vacuo*. 1 mg of hydrolysis resultant was treated with an phenylisothiocyanate (PITC). The reaction mixture was applied to HPLC for the analysis of amino acid. HPLC: solvent A-1.4 mM NaAc, 0.1% TEA, 6% acetonitril, pH 6.1; solvent B-60% acetonitril; elution linear gradient of solvent B (0-100%); flow rate 1.0 ml/min. 4 mg of Acid hydrolysis resultant was subjected to Dowex-50 resin and eluted with 2 N NH₄OH. Removal of aqueous ammonia *in vacuo* afforded a amino acid mixture, which was subjected to silica gel column chromatography using ethylacetate/methanol/water (9:5:1) as eluent to give glycine and D-alanine.

D-Alanine : $[\alpha]_{D}^{25}$ 14.4° (c. 0.016, MeOH), ¹H-NMR (500 MHz, CD₃OD) : δ 1.45 (3H, d, $J=7.5$ Hz), 3.57 (1H, q, $J=7.5$ Hz).

Glycine : ¹H-NMR (500 MHz, CD₃OD) : δ 3.60 (2H, s).

RESULTS AND DISCUSSION

A known cerebroside (**1**) was characterized by comparing their ¹H- and ¹³C-NMR data with those reported in the literatures (Toledo *et al.*, 1999). The enzyme hydrolysis, acid hydrolysis and the analysis of FAB-CID MS as well

as 2D-NMR spectra allowed to assign for the structure of **1**. 7,8-Dimethylalloxazine (**2**) was characterized by the analysis of 2D-NMR spectra, as well as ¹H- and ¹³C-NMR data. The NMR spectral data of 7,8-dimethylalloxazine have not been yet assigned in the previous literature (Sikorski *et al.*, 1999).

Compound **1** was obtained as amorphous powder and its quasimolecular ion peak showed at m/z 750 ([M+Na]⁺) in FAB-MS spectrum. Its IR spectrum displayed absorption bands at 3350 and 1650 cm⁻¹, indicating the presence of hydroxyl and amide functionalities. The characteristic signals of 2-amino-1,3-diol of hydrocarbon chain were observed at δ 4.22 (1H, m), 4.71 (1H, dd, $J=10.4, 5.8$ Hz), 4.80 (1H, m) and 4.76 (1H, t, $J=6.1$ Hz) in the ¹H-NMR spectrum and at δ 72.12, 56.61 and 74.31 in the ¹³C-NMR spectrum (Mori and Fumaki, 1985). In addition, the ¹H-NMR spectrum showed the signals corresponding to sugar moiety at δ 4.90 (1H, d, $J=7.6$ Hz), 4.02 (1H, br.t, $J=7.5$ Hz), 4.22 (1H, m, H-3''), 4.22 (1H, m, H-4''), 3.89 (1H, m, H-5''), 4.34 (1H, dd, $J=11.9, 5.2$ Hz) and 4.50 (1H, dd, $J=11.9, 2.1$ Hz), long chain aliphatic hydrocarbons at δ 0.88 (6H, m) and 1.28 (40H, m), three olefinic protons at δ 5.69 (1H, br.s), 5.94 (1H, dt, $J=15.3, 5.5$ Hz) and 6.01 (1H, dd, $J=15.3, 6.1$ Hz), an oxygenated proton at δ 4.58 (1H, dd, $J=7.9, 3.7$ Hz), allylic methyl signal at δ 1.63 (3H, s) and NH signal at δ 8.35 (1H, d, $J=9.0$ Hz). The ¹³C-NMR spectrum showed the signals by sugar moiety at δ 107.64, 77.08, 80.44, 73.52, 80.52 and 64.66, the signals by two terminal methyl groups in aliphatic hydrocarbon chains at δ 16.26, four olefinic carbons at δ 133.87, 134.26, 137.84 and 124.21, an oxygenated carbons at δ 74.50, an allylic methyl carbon at δ 18.06 and an amide carbon at δ 177.58. The position and geometry of double

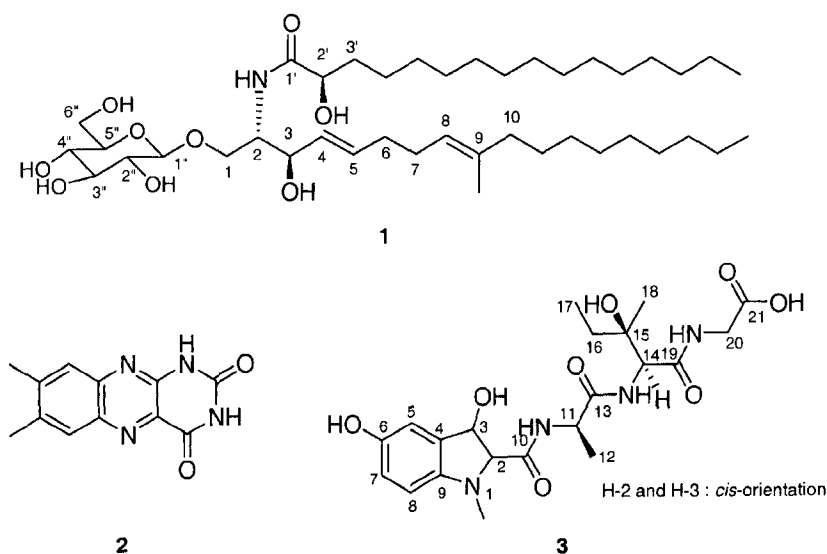


Fig. 1. Structures of compounds 1–3 from *Paecilomyces* sp. J300

bonds were confirmed by the analysis of ^1H - ^1H COSY, NOESY and coupling constants data. The coupling constants of the anomeric proton (7.6 Hz) in sugar group indicated it to be β -configuration. Analysis of the HMBC spectrum (Fig. 2) allowed for the position of fatty acid chain, sugar group and allylic methyl group. Methanolysis with HCl in MeOH yielded 2-hydroxyhexadecanoic acid methyl ester, which was identified by GC-MS analysis (Higuchi *et al.*, 1996), and the major fragment ion at m/z 495 $[\text{M}-255+\text{Na}]^+$ by CID (Collision-induced dissociation) spectrum of $[\text{M}+\text{Na}]^+$ ion in FAB-MS of **1** indicated the presence of C_{18} amino alcohol glycoside and 2-hydroxy fatty acid (Isobe *et al.*, 1997). The enzyme hydrolysis of **1** with cellulase yielded glucose, which was identified by cellulose TLC with authentic glucose. The absolute configuration of C-2, 3 and 2' of cerebrosides could be determined by the comparison of ^{13}C -NMR (Kang *et al.*, 1999). The chemical shift of H-2 (δ 4.80) in ^1H -NMR spectrum and the chemical shift of C-1 (δ 72.12), C-2 (δ 56.61), C-3 (δ 74.31), C-1' (δ 177.58) and C-2' (δ 74.50) were very similar to those of (4*E*, 2*S*, 2'*R*, 3*R*,)-*N*-2'-hydroxy fatty acid-1- O - β -D-glucopyranosyl-4,8-sphingadienine (Inagaki *et al.*, 1998). The optical rotation of **1** (9.1°) was also in good agreement with that of (4*E*, 8*E* 2*S*, 3*R*, 2'*R*)-*N*-2'-hydroxy hexadecanoyl-1- O - β -D-glucopyranosyl-4,8-sphingadienine (Inagaki *et al.*, 1998). These evidences showed that the absolute configuration at C-2, C-3 and C-2' in **1** was 2*S*, 3*R* and 2'*R*, respectively. Accordingly, the structure of **1** was determined to (4*E*, 8*E*, 2*S*, 3*R*, 2'*R*)-*N*-2'-hydroxyhexadecanoyl-1- O - β -D-glucopyranosyl-9-methyl-4,8-sphingadienine (Toledo *et al.*, 1999).

Compound **2** was obtained as yellowish gum and EIMS spectrum showed a molecular ion peak at m/z 242. From the EIMS and DEPT ($\text{C}\times 8$, $\text{CH}\times 2$, $\text{CH}_3\times 2$) spectral data, the molecular formula was deduced to be $\text{C}_{12}\text{H}_{10}\text{N}_4\text{O}_2$. The ^1H -NMR spectrum showed two methyl signals at δ 2.01 (3H, s) and 2.13 (3H, s), and two aromatic proton signals at δ 7.68 (1H, br.s) and 7.86 (1H, br.s), indicated the presence of heteroaromatic ring. The ^{13}C -NMR spectrum and DEPT spectra showed 12 carbon signals which were composed of two methyl signals at δ 20.25 and 20.83,

carbon signals in heteroaromatic ring δ 127.60, 130.15, 131.46, 139.53, 140.27, 143.49, 145.25, and 152.22 and two amide carbon group at δ 148.2, and 162.46. The HMBC spectrum showed cross-peaks by long-range correlation of 7- CH_3 (δ 2.01)/C-6 (δ 130.15) and C-7 (δ 139.53), 8- CH_3 (δ 2.13)/C-8 (δ 145.25) and C-9 (δ 127.60), H-6 (δ 7.68) /C-5a (131.46), and H-9 (δ 7.86)/C-9a (δ 140.27). On the basis of these spectroscopic evidences, the structure of **2** was identified to 7,8-dimethylalloxazine (Sikorski *et al.*, 1999).

Compound **3** was obtained as colorless gum and its molecular formula was determined to be $\text{C}_{21}\text{H}_{30}\text{N}_4\text{O}_8$ by HR-FAB-MS ($[\text{M}+\text{H}]^+$, m/z 467.2139). Its IR spectrum displayed absorption bands at 3350 and 1650 cm^{-1} , indicating the presence of hydroxyl and amide functionalities. The analysis of amino acid composition after HCl hydrolysis revealed the presence of a glycine, an alanine and two nonprotein amino acids. Signals by the glycine unit showed at δ 3.75 (1H, d, $J=17.1$ Hz) and 3.86 (1H, d, $J=17.1$ Hz) in the ^1H -NMR spectrum and at δ 44.81 and 176.38 in the ^{13}C -NMR spectrum (Sikorski *et al.*, 1999). Typical signals by the alanine unit showed at δ 1.20 (1H, d, $J=7.1$ Hz) and 4.49 (1H, br.q, $J=7.1$ Hz) in ^1H -NMR spectrum and at δ 16.75, 51.07 and 172.84 in ^{13}C -NMR spectrum (Jegorov *et al.*, 1994). In addition, the ^1H -NMR spectrum showed the signals corresponding to protons in β -hydroxyisoleucine unit at δ 0.95 (3H, t, $J=7.4$ Hz), 1.66 (3H, s), 1.66 (1H, m), 2.04 (1H, sext, $J=7.3$ Hz) and 4.72 (1H, s) (Dobson and Vining, 1968), and 3,5-dihydroxy-1-*N*-methyl-indoline-2-carboxylic acid amide unit at δ 2.67 (3H, s, NCH_3), 3.55 (1H, d, $J=8.6$ Hz), 4.78 (1H, d, $J=8.6$ Hz), 6.89 (1H, d, $J=8.3$ Hz), 7.14 (1H, br.d, $J=8.3$ Hz) and 7.30 (1H, br.s). ^{13}C -NMR spectrum showed signals by the β -hydroxyisoleucine unit at δ 9.09, 23.30, 31.47, 60.72, 86.52 and 170.78, and 3,5-dihydroxy-1-*N*-methyl-indoline-2-carboxylic acid amide unit at δ 33.14, 70.06, 73.44, 119.04, 123.57, 124.83, 132.12, 143.90, 152.60 and 168.52. The analysis of ^1H - ^1H COSY, HMQC and HMBC spectra was allowed to assign for all signals in ^1H - and ^{13}C -NMR spectrum and the connectivities between amino acid unit. The HMBC spectrum showed cross-peaks due to long-

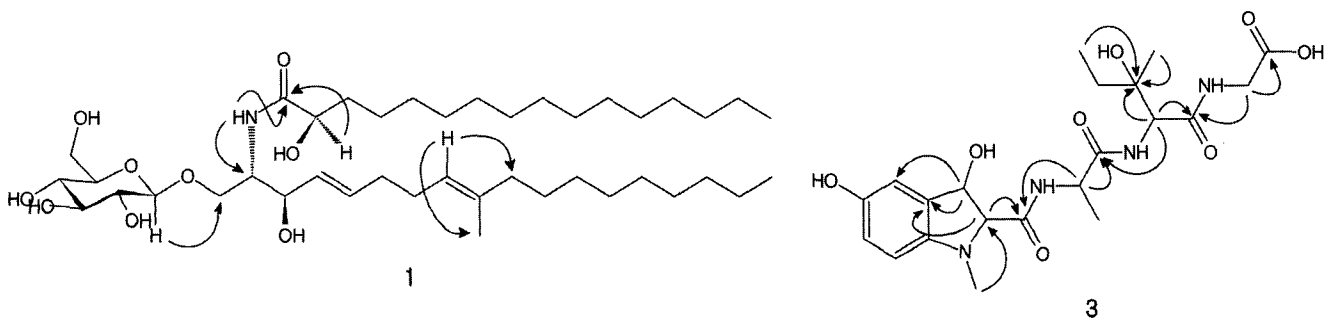


Fig. 2. Major long range correlations in HMBC spectra of compounds **1** and **3**

range correlations of NCH₃ (δ 2.67)/C-2 (δ 70.06), H-2 (δ 3.55) and H-11 (δ 4.49)/C-10 (δ 168.52), H-11 (δ 4.49) and H-14 (δ 4.72)/C-13 (δ 172.84), H-14 (δ 4.72), H-17 (δ 0.95) and H-18 (δ 1.66)/C-15 (δ 86.52), H-14 (δ 4.72) and H-20 (δ 3.86)/C-19 (δ 170.78), H-20 (δ 3.86)/C-21 (δ 176.38), and H-2 (δ 3.55) and H-3 (δ 4.78)/C-4 (δ 132.12), respectively. (Fig. 2.) The proton-carbon long-range correlation of the indoline unit in HMBC spectrum of **3** was in good agreement with those of indoline unit in benzastatins A (Kim *et al.*, 1997). On the basis of above evidences, the gross structure of **3** was suggested to be 3,5-dihydroxy-1-*N*-methyl-indoline-2-carboxylamino-alanyl- β -hydroxyisoleucinyglycine. The optical rotation value of alanine obtained by the HCl hydrolysis of **3** was -14.4° (c. 0.016, MeOH), indicated it was D-alanine, which was also identified by the comparison with optical rotation value of authentic sample (Aldrich 16,265-5). In β -hydroxyisoleucine unit of **3**, $J_{16,17}$ value (7.3 Hz) and chemical shift of H-16 (δ 1.66 and 2.04) and H-18 (δ 1.66) suggested it was erythro- β -hydroxyisoleucine (Dobson and Vining, 1968). The H-2 (δ 3.55) gave strong NOESY correlation with both H-3 (δ 4.78) and NCH₃ (δ 2.67). Thus, the stereochemistry of H-2, H-3, and NCH₃ are supposed to be *cis*-form. In addition, the coupling constant ($J_{2,3}$ =8.6 Hz) was in good agreement with the *cis*-vicinal coupling constant ($J_{2,3}$) in *cis*-3-(carboxymethyl)indoline-2-carboxylic acid (*cis* $J_{2,3}$ = 8.9 Hz; *trans* $J_{2,3}$ = 5.5 Hz) (Collot *et al.*, 1999). But, the relative configuration of H-2 and H-3 could not be determined by spectroscopic data. The structure of **3**, therefore, was determined to 3,5-dihydroxy-1-*N*-methyl-*cis*-indoline-2-carboxylamino-D-alanyl-erythro- β -hydroxyisoleucinyglycine, which was first reported from natural sources.

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