NEW ALKALOIDS FROM THE ROOT OF *STEPHANIA TETRANDRA* (FEN-FANG-JI)

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Abstract — Four new alkaloids named fenfangjines F, G, H, and I were isolated from the root of *Stephania tetrandra* S. MOORE, the Chinese traditional medicine " Fen-Fang-Ji". The chemical structures of fenfangjines F, G, H, and I were determined to be **1**, **2**, **3**, and **4** by spectral analyses and chemical methods, respectively.

In the preceding paper, we reported the isolation of thirteen known alkaloids, and the structural determination of four new bisbenzylisoquinoline (BBI) alkaloids, fenfangjines A, B, C, and D from the root of *S. tetrandra*.[1] In succession of the chemical examination on constituents of this plant monitoring the inhibitory activity against angiotensin I converting enzyme (ACE), we have isolated four new alkaloids, one phenanthrene alkaloid, one morphinane alkaloid, and two BBI alkaloids, named fenfangjines F, G, H, and I. This paper presents details of the isolation of these

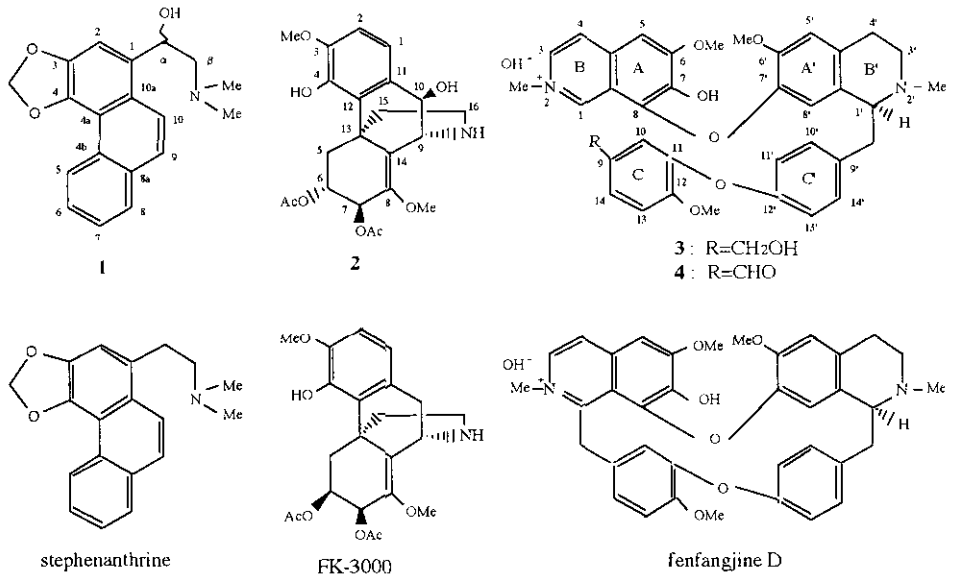

Figure 1. New Alkaloids (**1-4**) from *S. tetrandra* and their Related Alkaloids

alkaloids and the structural determination of fenfangjines F, G, H, and I.

The powdered root was extracted with MeOH. The MeOH extract was partitioned between hexane and 90% MeOH. The 90% MeOH extract was partitioned between $CHCl_3$ and 2% NH_4OH. Repeated chromatographic separation of the $CHCl_3$ extract gave four new alkaloids, fenfangjines F (**1**), G (**2**), H (**3**), and I (**4**). Their yields are 0.0003% (**1**), 0.005% (**2**), 0.0004% (**3**), and 0.005% (**4**).

Fenfangjine F (**1**) was obtained as a colorless oil. The HRMS gave the molecular formula as $C_{19}H_{19}NO_2$. The HCl salt of **1**, mp 133-134°C, $[\alpha]_D$ -4.8° ($CHCl_3$), was obtained as colorless needles from EtOH. The IR spectrum (KBr) of the HCl salt showed absorption of a hydroxyl group at 3244 cm^{-1}. The EIMS of **1** exhibited at *m/z* 309 $[M]^+$ and 250. The molecular ion peak at *m/z* 309 of **1** corresponds to plus 16 mass units in comparison with the ion peak at *m/z* 293 of stephenanthrine.[1,2] In the ^{1}H-NMR spectrum of **1**, the signals of two *N*-methyl groups, three aliphatic protons, one methylenedioxy group and seven aromatic protons were observed, and these signals were closely similar to those of stephenanthrine except for the presence of a hydroxymethine proton signal at δ 5.54. In the COLOC of **1**, a cross peak was observed between the proton signal of the hydroxymethine at δ 5.54 and the carbon signal of 2-position at δ 107.1. And a cross peak was also exhibited between the proton signal of the *N*-methyl group at δ 2.43 and the carbon signal of the methylene at δ 66.7 (Figure 2). Therefore the hydroxymethine was confirmed to be α-position. Thus, the structure of fenfangjine F was elucidated to be **1** (Figure 1). But the configuration of the hydroxyl bond at α-position in **1** has not been able to confirm yet, because the derivartives of **1** such as *p*-brombenzoate were not obtained fine crystals for X-Ray diffraction.

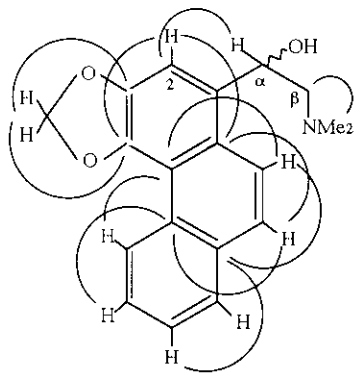

Figure 2. Long-Range Correlation in COLOC of **1**

Fenfangjine G (**2**), mp 203-205°C, $[\alpha]_D$ -63.7°, was obtained as colorless needles from AcOEt. The HRMS gave the molecular formula as $C_{22}H_{27}NO_8$. The EIMS of **2** showed at *m/z* 433 $[M]^+$, 415, 374, 314, 274, 258, and 243. The IR spectrum (KBr) of **2** showed absorptions of hydroxyl groups at 3528-3138 cm^{-1} and acetoxy groups at 1747 cm^{-1}. In the ^{1}H-NMR spectrum of **2**, two acetoxy groups, two methoxy groups, three methylene groups, three methine groups, and two aromatic protons were observed, and these signals were very similar to those of FK-3000[3] except for the proton signals of the hydroxymethine at δ 4.62 (1H, d, *J*=2.0 Hz) and the methine at δ 5.21 (1H, dt, *J*=13.3, 3.4 Hz). The hydroxymethine at δ 4.62 was confirmed to be at

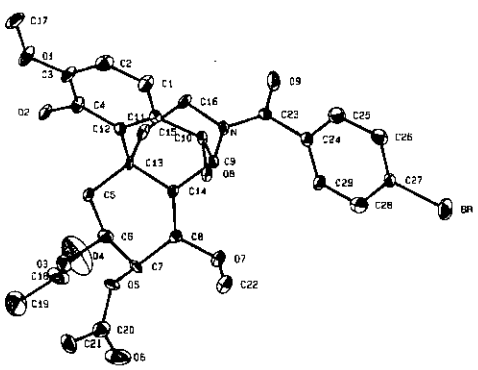

Figure 3. X-Ray Crystal Structure of **2a**

10-position by the COSY, in which the proton signal of the hydroxymethine was observed a cross peak to the methine signal of 9-position at δ 4.34. Thus, **2** was assumed to be the structure having hydroxyl group at 10-position of FK-3000. On treatment with *p*-bromobenzoyl chloride in dry THF, **2** afforded mono *p*-bromobenzoate (**2a**) which was obtained as colorless prisms from EtOH. A crystal of **2a** was analyzed by the X-Ray diffraction method and the absolute stereochemistry were confirmed to be 6*R*, 7*S*, 9*R*, and 10*R* (Figure 3), while those of FK-3000 are to be 6*S*, 7*S* and 9*R*. As the result, the structure of fenfangjine G was proved to be **2**.

Fenfangjine H (**3**), $[C_{37}H_{39}N_2O_7]^+$ OH^-, $[\alpha]_D$ -89.5° (MeOH), was obtained as an orange amorphous powder. In the FABMS (positive), **3** gave the [M-OH]$^+$ ion peak at *m/z* 623, and the HRMS in the same mode revealed the formula to be $C_{37}H_{39}N_2O_7$ moiety. The IR spectrum of **3** showed absorption of hydroxyl groups at 3408 cm^{-1}. In the ^{1}H-NMR spectrum of **3**, the signals were similar to those of fenfangjine D[1] except for five signals at δ 4.42, 4.49 (each 1H, d, *J*=12.9 Hz), 8.59 (1H, s) and 6.39, 6.64 (each 2H, d, *J*=8.5 Hz). The signals at δ 4.42 and 4.49 were assignable to the hydroxymethylene at α-position by the COLOC, for their signals were exhibited cross peaks to the carbon signals at 10 (δ 119.2) and 14-position (δ 122.6) on C-ring (Figure 4). And the signal at δ 8.59 was ascribable to be the proton at 1-position on B-ring, because its signal was observed cross peaks to two carbon signals of *N*-methyl group at 2-position (δ 43.1) and at 3-position (δ 126.8). It is known that four protons on C'-ring of BBI alkaloids having two ether-linkages generally exhibit four individual ABX-type double-doublet signals for rigid structures.[4] But those of **3** showed two AA'BB'-type doublet signals at δ 6.39 and 6.64 (each 2H, d, *J*=8.5 Hz). The observations of these proton signals and the presence of the proton at 1-position and the hydroxymethylene at α-position in **3** were supposed to be the cleaved structure between 1 and α-position of fenfangjine D. As the result, the structure of fenfangjine H was determined as **3**.

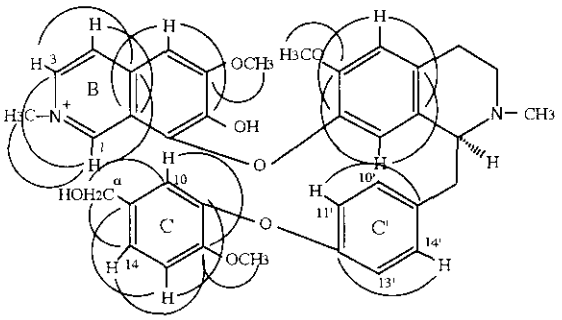

Figure 4. Long-Range Correlation in COLOC of **3**

Fenfangjine I (**4**), $[C_{37}H_{37}N_2O_7]^+$ OH^-, $[\alpha]_D$ -46.5° (MeOH), was obtained as an orange amorphous powder. The IR spectrum of **4** showed absorptions of a hydroxyl group at 3412 cm^{-1} and a carbonyl group at 1686 cm^{-1}. In the FABMS (positive), **4** gave the [M-OH]$^+$ ion peak at *m/z* 621, and the HRMS in the same mode revealed the formula to be $C_{37}H_{37}N_2O_7$ moiety. The [M-OH]$^+$ ion peak at *m/z* 621 of **4** corresponds to minus 2 mass units in comparison with the ion peak at *m/z* 623 of **3**. In the ^{1}H and ^{13}C-NMR spectra of **4**, the signals were also similar to those of **3** except for the signals of α-position. The hydroxymethylene signals of α-position at δ_H 4.42, 4.49, δ_C 63.6 observed in the ^{1}H and ^{13}C-NMR spectra of **3** disappeared, while the proton and carbon signals of the formyl group exhibited at δ_H 9.77, δ_C 190.3 in **4**. Thus, the structure of fenfangjine I was proved to be **4**.

ACE activity of these four compounds were measured according to the method of Morota *et al.*[5] Three alkaloids (**1**, **3**, and **4**) inhibited ACE by 54.5%, 36.0%, and 55.4% at a concentration of 1 mM, respectively. Compound (**2**) had no inhibitory activity at the same concentration.

EXPERIMENTAL

Melting points were determined on a Yanaco MP-J3 micro melting apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 polarimeter. IR spectra were taken with a Hitachi 270-30 spectrophotometer. ^{1}H and ^{13}C-NMR spectra were measured on JEOL JNM-FX200 and Bruker AM 500 spectrometers using TMS as an internal standard. MS and HRMS were obtained with a JEOL DX-300 and Shimadzu KRATOS CONCEPT 32 IH and 32 IS spectrometers. TLC was conducted on precoated Kieselgel 60 F_{254} plates (Merck) and spots were visualized by spraying Dragendorff's reagent. Plant material was purchased from Raw Medical Trading Co., Ltd. (Tokyo, Japan).

Extraction and Separation

The dried root (15 kg) of *S. tetrandra* was milled and extracted twice with hot MeOH (50 L) at 2 h. The MeOH extract (790 g) was partitioned between hexane and 90% MeOH. 2% NH_4OH (2 L) was added to the 90% MeOH extract (459 g) and the NH_4OH solution was extracted with $CHCl_3$ (2 L× 3). The $CHCl_3$ layer was concentrated *in vacuo* to yield 331 g of the $CHCl_3$ extract. This extract was separated on alumina column (2 kg) by elution with $CHCl_3$, followed with $CHCl_3$-MeOH (10:1) to give two fractions; Fr. A-I (255 g) and Fr. A-II (34 g). Chromatography of Fr. A-I on silica gel by elution with $CHCl_3$-MeOH (40:1) yielded two alkaloids, tetrandrine (118 g) and fangchinoline (56 g).[1] Fr. A-II was partitioned between $CHCl_3$ and 5% acetic acid. The $CHCl_3$ layer was washed with 2% NH_4OH and then evaporated to yield 15.8 g of non-phenolic alkaloidal portion. This portion extract was chromatographed on Sephadex LH20 column by using MeOH as an eluent to furnish crude **1** (74.8 mg). 5% HCl-MeOH was added to crude **1** and the HCl salt was crystallized from EtOH as colorless needles (46 mg, 0.0003%). The 5% acetic acid layer, after basification with 25% NH_4OH, was extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated and a residue (17.9 g) was chromatographed on alumina column with a gradient of $CHCl_3$-MeOH (50:1 → 10:1) to give three fractions; Fr. A-II-2-1 (11.9 g), Fr. A-II-2-2 (1.9 g), and Fr. A-II-2-3 (2.5 g). Repeated chromatography of Fr. A-II-2-1 on silica gel with $CHCl_3$-MeOH (30:1) yielded crude **2** (953 mg). Crude **2** was crystallized from AcOEt as colorless needles (493 mg, 0.003%). Fr. A-II-2-2 was chromato- graphed on silica gel column by using $CHCl_3$-MeOH-25% NH_4OH (60:10:1) to furnish **3** (66 mg, 0.0004%) and **4** (71 mg, 0.0005%). Fr. A-II-2-3 yielded crude fenfangjine D.[1]

Fenfangjine F (1) : Colorless oil. EIMS *m/z* : 309 $[M]^+$, 250, HRMS: Calcd for $C_{19}H_{19}NO_3$ $[M]^+$ 309.1365. Found 309.1361. IR (KBr) cm^{-1}: 3244, 2980, 2916, 2824, 2788, 1596, 1502, 1446, 1390, 1278, 810. ^{1}H-NMR ($CDCl_3$) δ : 2.43 (6H, s, NCH_3), 2.53 (1H, dd, *J*=12.6, 10.4 Hz, H-ß), 2,62 (1H, dd, *J*=12.6, 3.0 Hz, H-ß), 5.54 (1H, dd, *J*=10.4, 3.0 Hz, H-α), 6.19 (1H, dd, *J*=6.5, 1.7 Hz, OCH_2O), 7.53 (1H, d, *J*=9.4 Hz, H-9), 7.54-7.60 (2H, m, H-6, 7), 7.61 (1H, s, H-2), 7.73 (1H,

d, *J*=9.4 Hz, H-10), 7.77 (1H, m, H-8), 9.06 (1H, m, H-5). ^{13}C-NMR($CDCl_3$) δ : 45.3 (2C, q, NCH_3), 66.2 (d, C-α), 66.7 (t, C-β), 101.1 (d, OCH_2O), 107.1 (d, C-2), 116.6 (s, C-4a), 121.4 (d, C-10), 124.6 (s, C-10a), 125.2 (d, C-9), 126.4 (d, C-6), 126.7 (d, C-7), 127.3 (d, C-5), 127.6 (d, C-8), 128.7 (s, C-4b), 131.7 (s, C-8a), 132.8 (s,C-1), 142.7 (s, C-4), 145.5 (s, C-3). The HCl salt of **1** was obtained as colorless needles from EtOH. mp 133-134°C, $[\alpha]_D^{27}$ -4.8°(*c*=0.67, $CHCl_3$).

Fenfangjine G (2) : Colorless needles (from AcOEt), mp 203-205°C, $[\alpha]_D^{24}$ -63.7° (*c*=0.85, $CHCl_3$). EIMS *m/z* : 433 $[M]^+$, 415, 374, 314, 274, 258, 243. HRMS : Calcd for $C_{22}H_{27}NO_8$ $[M]^+$ 433.1736. Found 433.1732. IR (KBr) cm^{-1} : 3528, 3310, 3288, 3138, 2940, 1747, 1688, 1605. ^{1}H-NMR ($CDCl_3$) δ : 1.91 (2H, m, H-15), 2.01, 2.03 (each 3H, s, $OCOCH_3$), 2.36 (1H, t, *J*=13 Hz, H-5), 2.45 (1H, dt, *J*=13.3, 3.8 Hz, H-16), 2.68 (1H, m, H-16), 2.86 (1H, dd, *J*=13.3, 3.2 Hz, H-5), 3.55 (3H, s, 8-OCH_3), 3.88 (3H, s, 3-OCH_3), 4.34 (1H, d, *J*=2.0 Hz, H-9), 4.62 (1H, d, *J*=2.0 Hz, H-10), 5.21 (1H, dt, *J*=13.3, 3.4 Hz, H-6), 5.90 (1H, dd, *J*=3.4, 0.9 Hz, H-7), 6.82 (1H, d, *J*=8.4 Hz, H-2), 6.95 (1H, d, *J*=8.4 Hz, H-1). ^{13}C-NMR ($CDCl_3$) δ : 20.85, 20.88 (each q, $OCOCH_3$), 32.9 (t, C-5), 37.8 (t, C-15), 38.8 (s, C-13), 39.9 (t, C-16), 52.2 (d, C-9), 56.1 (q, 3-OCH_3), 57.2 (q, 8-OCH_3), 64.6 (d, C-7), 68.2 (d, C-6), 73.0 (d, C-10), 109.3 (d, C-2), 120.8 (d, C-1), 125.3 (s, C-14), 127.4 (s, C-12), 132.7 (s, C-11), 142.7 (s,C-8), 143.0 (s, C-4), 146.8 (s, C-3), 170.2, 170.4 (each s, $OCOCH_3$).

Mono *p*-Bromobenzoylation (2a) of Fenfangjine G (2) : To a solution of **2** (51 mg, 0.118 mmol) *in dry THF (10 mL) was added p-bromobenzoyl chloride* (30 mg, 0.137 mmol) and the mixture was stirred at rt for 16 h. The reaction mixture, after addition of H_2O and 25% NH_4OH, was extracted with $CHCl_3$. The $CHCl_3$ extract was separated on silica gel chromatography by elution with $CHCl_3$-MeOH (50:1) to furnish the crude mono *p*-bromobenzoate (**2a**) of **2**, which was crystallized from EtOH as colorless prisms (38 mg, 52%). mp169-171°C, $[\alpha]_D^{24}$ -9.8° (*c=0.54, CHCl*$_3$), IR (KBr) cm^{-1} : 3224, 2936, 2884, 1744, 1678, 1614, 1430, 1364,1276, 1238. FDMS *m/z* : 617 $[M]^+$, 615. ^{1}H-NMR (DMSO-d_6, 120°C) δ : 1.94, 2.00 (each 3H, s, $OCOCH_3$), 3.36 (3H, s , 8-OCH_3), 3.82 (3H, s, 3-OCH_3), 4.49 (1H, s-like, H-10), 5.12 (1H, dt, *J*=13.3, 3.4 Hz, H-6), 5.52 (1H, br. s, H-9), 5.73 (1H, d, *J*=3.4 Hz, H-7), 6.84, 6.93 (each 1H, d, *J*=8.3 Hz, H-1, 2), 7.30, 7.58 (each 2H, d, *J*=8.5 Hz, benzene-H).

X-Ray Chrystallographic Analysis of 2a : The crystal size of **2a** was 0.03× 0.1× 0.22 mm. The unit cell dimension was obtained by least-squares refinement using 25 centered reflections for which 10°< 2 θ < 25° (graphite monochromatized CuKα, λ=1.54184 Å). Intensity data ware collected at ω/2 θ scans on an Enraf-Nonius CAD-4 with three check reflection at intervals of 100 reflections. Other crystal data were: $C_{29}H_{30}NO_9Br$, orthorhombic, space group $P2_12_12_1$, Z=4, a=15.155 (1) Å, b=27.164 (2) Å, c=7.612 (2) Å, V=3113.7 (8) Å^3, Dcalcd=1.307 gcm^{-3} and (CuKα) with 22.0 cm^{-1}. Intensities were measured for 3411 reflections in the range 2°< 2 θ < 140° with 1761 considered as observed by the criteria I > 3 σ (I). The data were corrected for Lorentz and polarization effects. No absorption correction was applied. The structure was solved by the direct-method program Multan and was refined by full-matrix leastsquares, using the Enraf-Nonius SDP programs. All the non-hydrogen atoms were refined anisotropically. Hydrogen atoms were

located from difference maps. The last difference Fourier map was essentially featueless with no peaks greater than 0.619 e/Å^3. The final discrepancy index was R=0.074.

Fenfangjine H (3) : Orange amorphous powder, $[\alpha]_D^{27}$ -89.5° (*c*=0.40, MeOH). Positive-mode FABMS *m/z* : 623 $[M-OH]^+$. HRFABMS : Calcd for $C_{37}H_{39}N_2O_7$ $[M-OH]^+$ 623.2757. Found 623.2758. IR (KBr) cm^{-1} : 3408, 2932, 2836, 1614, 1548, 1504, 1484. ^{1}H-NMR($CDCl_3$) δ : 2.39 (3H, s, 2'-NCH_3), 3.37 (3H, s, 6'-OCH_3), 3.78 (3H, s, 12-OCH_3), 3.87 (3H, s, 6-OCH_3), 4.09 (3H, s, 2-NCH_3), 4.42, 4.49 (each 1H, d, *J*=12.9 Hz, α-CH_2OH), 6.12 (1H, s, H-8'), 6.39 (2H, d, *J*=8.5 Hz, H-11', 13'), 6.45 (1H, s, H-5'), 6.64 (2H, d, *J*=8.5 Hz, H-10',14'), 6.81 (1H, s, H-5), 6.86 (1H, d, *J*=1.8 Hz, H-10), 6.88 (1H, d, *J*=8.4 Hz, H-13), 7.01 (1H, dd, *J*=8.4, 1.8 Hz, H-14), 7.40 (1H, d, *J*=6.4 Hz, H-4), 7.42 (1H, d, *J*=6.4 Hz, H-3), 8.59 (1H, s, H-1). ^{13}C-NMR ($CDCl_3$) δ : 26.9 (t, C-4'), 39.1 (t, C-α'), 43.1 (q, 2'-NCH_3), 47.4 (q, 2-NCH_3), 49.5 (t, C-3'), 55.0 (q, 6'-OCH_3), 56.0 (q, 12-OCH_3), 56.1 (q, 6-OCH_3), 63.6 (t, C-α), 64.5 (d, C-1'), 100.8 (d, C-5), 111.4 (d, C-5'), 112.5 (d, C-13), 112.6 (d, C-8'), 116.7 (2C, d, C-11', 13'), 119.2 (d, C-10), 121.8 (d, C-4), 122.1 (s, C-8a), 122.6 (d, C-14), 126.8 (d, C-3), 126.8 (s, C-4a), 127.3 (s, C-4'a), 129.8 (s, C-8'a), 130.5 (2C, d, C-10', 14'), 132.4 (s, C-8), 134.0 (s, C-9'), 134.7 (d, C-1), 135.6 (s, C-9), 145.4 (s, C-11), 145.6 (s, C-7'), 146.7 (s, C-6'), 150.0 (s, C-12), 155.3 (s, C-12'), 158.6 (s, C-7), 165.6 (s, C-6).

Fenfangjine I (4) : Orange amorphous powder, $[\alpha]_D^{27}$ -46.5° (*c*=0.27, MeOH). Positive-mode FABMS *m/z* : 621 $[M-OH]^+$. HRFABMS : Calcd for $C_{37}H_{37}N_2O_7$ $[M-OH]^+$ 621.2602. Found 621.2601. IR (KBr) cm^{-1} : 3412, 2928, 2836, 1686, 1600, 1548, 1504, 1484. ^{1}H-NMR ($CDCl_3$) δ : 2.39 (3H, s, 2'-NCH_3), 3.62 (3H, s, 6'-OCH_3), 3.96 (3H, s, 12-OCH_3), 3.99 (3H, s, 6-OCH_3), 4.23 (3H, s, 2-NCH_3), 6.18 (1H, s, H-8'), 6.50 (2H, d, *J*=8.3 Hz, H-11', 13'), 6.56 (1H, s, H-5'), 6.82 (2H, d, *J*=8.3 Hz, H-10', 14'), 6.85 (1H, s, H-5), 7.08 (1H, d, *J*=8.3 Hz, H-13), 7.24 (1H, d, *J*=1.8 Hz, H-10), 7.36 (1H, d, *J*=6.4 Hz, H-4), 7.43 (1H, d, *J*=6.4 Hz, H-3), 7.60 (1H, dd, *J*=8.3, 1.8 Hz, H-14), 8.66 (1H, s, H-1), 9.77 (1H, s, α-CHO). ^{13}C-NMR ($CDCl_3$) δ : 25.9 (t, C-4'), 40.5 (t, C-α'), 42.8 (q, 2'-NCH_3), 47.4 (q, 2-NCH_3), 48.2 (t, C-3'), 55.4 (q, 6'-OCH_3), 56.0 (q, 12-OCH_3), 56.3 (q, 6-OCH_3), 64.0 (d, C-1'), 100.7 (d, C-5), 111.6 (d, C-5'), 112.0 (d, C-13), 113.2 (d, C-8'), 117.6 (2C,d, C-11', 13'), 118.3 (d, C-10), 121.5 (d, C-4), 122.3 (s, C-8a), 125.8 (d, C-14), 126.3 (s, C-4a), 127.3 (s, C-4'a), 127.6 (d, C-3), 129.9 (s, C-8'a), 130.1 (s, C-9), 130.8 (2C, d, C-10', 14'), 132.5 (s, C-8), 134.3 (d, C-1), 135.4 (s, C-9'), 145.5 (s, C-7'), 146.9 (s, C-11), 147.1 (s, C-6'), 154.0 (s, C-12), 156.0 (s, C-12'), 159.3 (s, C-7), 166.0 (s, C-6), 190.3 (d, C-α-CHO).

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