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Assignment of the absolute configuration of hepatoprotective highly oxygenated triterpenoids using X-ray, ECD, NMR *J*-based configurational analysis and HSQC overlay experiments

Xiaojuan Wang^{a,b,1}, Jiabao Liu^{a,1}, Pankaj Pandey^{c,1}, Jiabao Chen^a, Frank R. Fronczek^d, Stuart Parnham^e, Xinzhu Qi^a, Robert J. Doerksen^c, Daneel Ferreira^f, Hua Sun^a, Shuai Li^{a,*}, and Mark T. Hamann^{b,*}

^aState Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

^bDepartment of Drug Discovery and Biomedical Sciences, College of Pharmacy, Medical University of South Carolina, Charleston, SC 29425, United States

^cDepartment of BioMolecular Sciences, Division of Medicinal Chemistry, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, MS 38677-1848, United States

^dDepartment of Chemistry, Louisiana State University, Baton Rouge, LA 70803, United States

^eDepartment of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC 29425, United States

^fDepartment of BioMolecular Sciences, Division of Pharmacognosy, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, MS 38677-1848, United States

Abstract

Background—The plants of the genus *Kadsura* are widely distributed in China, South Korea, and Japan. Their roots and stems are traditionally used to treat blood diseases and pain. The main bioactive constituents of *Kadsura longipedunculata* comprise highly oxygenated triterpenoids. Schiartane-type nortriterpenoids showed anti-HIV, anti-HBV, and cytotoxic bioactivities. For such compounds, the absolute configuration influences the bioactivities, and hence its unambiguous determination is essential. In this work, the absolute configurations of three highly oxygenated

*Corresponding authors. lishuai@imm.ac.cn (S. Li), hamannm@musc.edu (M.T. Hamann).

¹These authors contributed equally to this manuscript.

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Appendix A. Supplementary data

Supplementary data including X-ray crystallographic data, experimental and calculated ECD spectra and HR-ESIMS, and 1D and 2D NMR spectra and DP4+ calculations of **2**, as well as general experimental procedures, plant materials, extraction and isolation, the detailed experimental conditions for hepatoprotective assay, of **2** can be found in the online version. Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.bbagen.2017.09.001>.

schiantane-type nortriterpenoids were unequivocally assigned using X-ray, ECD, and *J*-based configuration analysis and HSQC overlay data.

Methods—The ethanol extract of *Kadsura longipedunculata* Finet et Gagnep was purified by column chromatography using silica, Sephadex LH-20, and ODS as substrates. To help assign the absolute configuration of schiantane-type nortriterpenoids, X-ray diffraction analysis, ECD experiment compared to ab initio computed data, DP4+ analysis, HSQC overlay, NOESY, and *J*-based configuration analysis were carried out. Hetero- and homo-nuclear coupling constants were extracted from HETLOC experiments.

Results—Three new highly oxygenated triterpenoids, micrandilactone I (**1**), micrandilactone J (**2**), and 22,23-diepi-micrandilactone J (**3**) were isolated. Their 2D structures were solved using NMR and HRESIMS data and their absolute configurations were elucidated using X-ray diffraction analysis, ECD experimental results compared to ab initio computed spectra, HSQC overlay, DP4+, NOESY, and *J*-based configuration analysis. Micrandilactone I (**1**) and 22,23-diepi-micrandilactone J (**3**) showed moderate hepatoprotective activity against APAP-induced toxicity in HepG2 cells with cell survival rates of 53.0 and 50.2%, respectively, at 10 μ M (bicyclol, 49.0%), while micrandilactone J (**2**) was inactive.

General significance—This is the first comprehensive stereochemical assignment of a non-crystalline schiantanetype nortriterpenoid like **3**. This general protocol may contribute towards solving the problems hampering the assignment of the absolute configurations of other members of this class of nortriterpenoids.

1. Introduction

Highly oxygenated triterpenoids isolated from plants of the genera *Schisandra* and *Kadsura* have revealed a variety of important biological activities including potent anti-HIV [1], anti-HBV [2], and cytotoxic properties [3–6]. Their 2D structures and relative configurations have been successfully elucidated by NMR spectroscopic methods. Assignment of their absolute configurations is critical since it influences their bioactivities and is essential for future efforts focused on synthesis and medicinal chemistry applications. For the assignment of the relative configuration, NMR-based techniques including NOE and *J*-based configuration analysis (*J*BCA) in combination with molecular and quantum mechanics calculations including DP4 [7] as well as DP4+ calculations [8] were applied [9]. For the absolute configuration, X-ray diffraction and electric circular dichroism (ECD) methods have often been used. However, even with these advanced techniques, it has proven difficult to determine the absolute configuration for the schiantane-type nortriterpenoids due to the presence of 12 non-contiguous stereogenic centers as well as conformationally flexible side chains. Herein, we report an integrated analysis to comprehensively assign the absolute configurations of schiantane-type nortriterpenoids using a combination of methods.

2. Material and methods

2.1. General procedures

Optical rotations were measured on a JASCO P-2000 polarimeter and UV spectra with a JASCO V-650 spectrophotometer. ECD spectra were measured on a JASCO J-815

spectrometer. The ^1H and ^{13}C NMR spectra were recorded on INOVA-500 and BRUKER AV500-III spectrometers. HETLOC and HSQC with decoupling were measured on a Bruker 850 MHz NMR spectrometer. The standard Bruker pulse sequences were used for each 2D measurement. HRESIMS data were acquired using an Agilent 6520 Accurate-Mass Q-ToF LC/MS mass spectrometer. Analytical reversed-phase HPLC was performed on a COSMOSIL 5C₁₈-PAQ Waters column (4.6 mm I. D. $\times$ 250 mm) eluted with water/methanol (flow rate, 1 mL/min; 220 nm UV detection) at room temperature. Preparative reversed-phase HPLC was performed on a COSMOSIL 5C₁₈-PAQ Waters column (10 mm I. D. $\times$ 250 mm) at room temperature. Column chromatography was performed with silica gel (40–63 μm ; Silicycle), C₁₈ 120 Å reversed-phase silica gel (RP-18; 50 μm ; YMC), and Sephadex LH-20 (GE Healthcare Bio-Science AB). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H₂SO₄ in ethanol.

2.2. Extraction and purification

The plant material of *Kadsura longipedunculata* was collected from the Jiujiang prefecture of Jiangxi province, People's Republic of China, in March 2010. The air-dried and powdered roots (34.0 kg) were percolated with 95% aqueous ethanol (48 L) at room temperature. The combined extract was concentrated in vacuo to yield a residue (2400 g). The residue (2400 g) was subjected to silica gel (200–300 mesh, 2400 g) column chromatography, eluting with a petroleum ether/acetone gradient system (100:1, 50:1, 10:1, 5:1, 3:1, 1:1), acetone, and 80% aqueous ethanol, to give fractions 1–15. Fraction 13 was chromatographed on an ODS (octadecylsilyl) column eluted with a gradient of water/methanol (4:6–1:9) to yield four subfractions A–D. Fraction B was further chromatographed on a silica gel column eluted with a gradient of chloroform/methanol (60:1, 50:1, 30:1, 20:1) and pure methanol to yield five fractions B1–B5. Fraction B4 was purified by semi-preparative HPLC (60% methanol/water), to give micrandilactone I (**1**, 7 mg). Fraction 14 was partitioned between water and ethyl acetate. The ethyl acetate extract (60 g) was chromatographed on an ODS column eluted with a gradient of water/methanol (4:1, 3:2, 2:3, 1:4) and pure methanol to yield five fractions A–E. Fraction D was chromatographed on Sephadex LH-20 (eluted with chloroform/methanol) to give five subfractions D1–D5. Fraction D1 (1.7 g) was subjected to silica gel (200–300 mesh, 3 g) column chromatography, eluting with a chloroform/methanol gradient system (20%–80% methanol) to afford nine subfractions, D1.1–D1.9. Fraction D1.3 (365 mg) was purified by semi-preparative HPLC (46% methanol/water), to give micrandilactone J (**2**, 164 mg) and 22,23-di-epi-micrandilactone J (**3**, 1 mg).

2.2.1. Micrandilactone I (1**)**—Micrandilactone I (**1**): white crystals (methanol); $[\alpha]_{\text{D}}^{20}$ -3.4 (c 0.1, methanol); ECD (c 5.0×10^{-5} M, methanol) $[\theta]$ (nm) $+1.4 \times 10^3$ (200), $+0.32 \times 10^3$ (215), λ_{max} (e) 200 (+6.86), HRESIMS m/z: 539.2994 $[\text{M} + \text{Na}]^+$ (calcd 539.2979 for C₃₀H₄₄NaO₇).

2.2.2. Micrandilactone J (2**)**—Micrandilactone J (**2**): white crystals (methanol); $[\alpha]_{\text{D}}^{20}$ -24 (c 0.25, methanol); ECD (c 3.0×10^{-5} M, methanol) $[\theta]$ (nm) -12.6×10^3 (217), λ_{max} (e) 217 (−21.0); HRESIMS m/z: 557.2740 $[\text{M} + \text{Na}]^+$ (calcd 557.2721 for C₂₉H₄₂NaO₉).

2.2.3. 22,23-di-epi-micrandilactone J (3)—22,23-Di-epi-micrandilactone J (**3**): white amorphous powder; $[\alpha]_D^{20} +50$ (c 0.3, methanol); ECD (c 9.0×10^{-5} M, methanol) ECD (methanol) $[\theta]$ (nm) $+2.8 \times 10^3$ (216), $\lambda_{\max}$ (ϵ) 217 (+14.1). HRESIMS m/z : 557.2722 $[M + Na]^+$ (calcd 557.2721 for $C_{29}H_{42}NaO_9$).

3. Results and discussion

Micrandilactone I (**1**) was obtained as white crystals. Its molecular formula was established as $C_{30}H_{44}O_7$ by the HRESIMS ion at m/z 539.2994 $[M + Na]^+$ (calcd 539.2979), suggesting nine indices of hydrogen deficiency. The 1H , ^{13}C , and HSQC NMR data of micrandilactone I (**1**) indicated the presence of two ester carbonyls, six methyl, eight methylene, eight methine (one olefinic and three oxygenated), and six non-protonated carbons (one olefinic and three oxygenated). The 1H NMR spectra (Table 1) revealed three resonances at δ_H 4.27, 2.98, and 2.75, representing the ABX spin system of H-1, H-2 α , H-2 β , respectively, one olefinic proton (δ_H 6.43), three oxygenated methines (δ_H 4.50, 4.31, and 4.27), one methyl doublet (δ_H 0.97), five methyl singlets (δ_H 1.90, 1.56, 1.26, 1.10, and 0.93), and several methylene protons between δ_H 1.30 and 2.85. The 1D NMR data and indices of hydrogen deficiency of micrandilactone I (**1**) suggested that it was a highly oxygenated 3, 4: 9, 10-disecocycloartane containing six rings [10]. Additionally, HMBC cross-peaks of H-27 (δ_H 1.90) with C-24, C-25, and C-26 at δ_C 140.0, 128.0 and 166.2, respectively, and of H-24 at δ_H 6.43 with C-22, C-23, C-26 and C-27 at δ_C 80.4, 23.7, 166.2, and 17.2, respectively, revealed the presence of an α -methyl- α , β -unsaturated- γ -lactone moiety. These 1D and 2D NMR data indicated that micrandilactone I (**1**) had a structure closely similar to that of kadcocilactone A [11], but with a hydroxy group located at C-15, instead of at C-17. X-ray analysis of kadcocilactone A was reported but it only afforded the relative configuration [11]. The absolute configuration of micrandilactone I (**1**) was determined as (1*R*, 5*S*, 8*S*, 9*S*, 10*R*, 13*R*, 14*S*, 15*S*, 17*R*, 20*S*, 22*R*) via X-ray crystallographic analysis (Fig. 2). Accordingly, the structure of micrandilactone I (**1**) was established as shown in Fig. 1.

Micrandilactone J (**2**) is an isomer of micrandilactone C, based on comparison of their 1D NMR and MS data [6]. The absolute configuration of micrandilactone J (**2**) was determined via X-ray crystal diffraction analysis by using the anomalous dispersion of Cu K α radiation (at 1.54184 Å) combined with ECD data. Crystals of X-ray diffraction quality of micrandilactone J (**2**) were obtained from methanol/water. The resulting data unambiguously permitted the assignment of its relative configuration (Fig. 3). The negative Cotton effect around 220 nm in the ECD spectrum indicated the (2*S*) absolute configuration of the 3-methylfuran-2(5*H*)-one moiety based on comparison with the calculated ECD spectrum (Fig. 4) as well as reported data [12]. Details of the computational methods and results are presented in the Supplementary Information, Section 1.3. Fig. 4 depicts overlays of the experimental spectrum of compound **2**, the average calculated spectrum of (2*S*)-**2** and the Boltzmann-weighted ECD spectrum of the major conformer **2a** (60.17% Boltzmann population) using B3LYP/6-311++G(2d,p). The calculated ECD spectrum of compound (2*S*)-**2** shows Cotton effects characteristic of $\pi \rightarrow \pi^*$ electronic transition at 221.26 nm region (Fig. 4). The molecular orbitals (MOs) involved in key transitions for the ECD of (2*S*)-**2** (conformer **2a**) are shown in Supplementary Information Fig. S2. The electronic

transitions from MO139, MO140 and MO141 (H-3) to MO145 (LUMO) involving a $\pi \rightarrow \pi^*$ transition of the enoate chromophore (Supplementary Information Fig. S2) show negative rotatory strengths at 221.26 nm in (2*S*)-**2** (conformer **2a**), which is consistent with the strong negative Cotton effect at 217 nm in the experimental ECD spectrum of **2**. These results match well with the experimental ECD curve, and support the assignment of the absolute configuration of compound **2** as (1*R*, 5*S*, 8*R*, 9*S*, 10*R*, 13*R*, 14*S*, 15*S*, 17*R*, 20*S*, 22*S*, 23*S*). In addition, ¹H and ¹³C NMR chemical shift calculations were performed to assign the relative configurations of compounds **2** (1*R*, 5*S*, 8*R*, 9*S*, 10*R*, 13*R*, 14*S*, 15*S*, 17*R*, 20*S*, 22*S*, 23*S*) and **2'** (1*S*, 5*R*, 8*S*, 9*R*, 10*S*, 13*S*, 14*R*, 15*R*, 17*S*, 20*R*, 22*R*, 23*S*) considering (2*S*) absolute configuration based on ECD. We used the gauge including atomic orbital (GIAO) method at the PCM/B3LYP/ 6–311+G(d,p) level of theory for DP4+ calculations. The computed chemical shifts of **2** and **2'** (Supplementary Information Fig. S3) were compared with the experimental values utilizing DP4+ probability analyses. The latter yielded the structure of **2** with 100% probability, leading to unequivocal assignment of (2*S*) absolute configurations for compound **2** (Supplementary Information Fig. S4 and Tables S6–S8). Combining the relative configuration assigned via the X-ray diffraction data, the comparison of experimental and ab initio computed ECD spectra, and the DP4+ analysis, the absolute configuration of micrandilactone **J** (**2**) was unequivocally defined as (1*R*, 5*S*, 8*R*, 9*S*, 10*R*, 13*R*, 14*S*, 15*S*, 17*R*, 20*S*, 22*S*, 23*S*).

22,23-di-epi-micrandilactone **J** (**3**) had a molecular formula, C₂₉H₄₂O₉, based on HRESIMS analysis ([M + Na]⁺ *m/z* 557.2722) and its ¹³C NMR spectrum, indicative of it being an isomer of micrandilactone **J** (**2**). The 1D NMR data and index of hydrogen deficiency also suggested a highly oxygenated schiartane-type nortriterpenoid possessing six rings [6]. Analysis of the 2D NMR data (Fig. 6B and C) showed an identical 2D structure compared to micrandilactone **J** (**2**). The overlaid HSQC spectra of **2** and **3** showed highly coincidental crosspeaks of the fused 5/5/7/6/5 ring systems (Fig. 7), which indicated that micrandilactone **J** (**2**) and 22,23-di-epi-micrandilactone **J** (**3**) had identical pentacyclic core structures. The misalignment of cross-peaks combined with the opposite Cotton effect near 220 nm in the ECD spectra indicated that **3** and **2** had opposite absolute configurations at some of the stereocenters. The absolute configuration of C-23 was assigned as *R* based on the positive Cotton effects at 217 nm in the ECD spectrum [11]. The absolute configurations of the remaining stereocenters of compound **3** were established via *J*-based configuration analysis.

¹JBCA data were utilized in an attempt to determine the relative configurations at C-23, C-22, and C-20 [9]. This method was applied to analyze the relative configurations of polyhydroxylated acyclic molecules by detailed analysis of the ³*J*_{H, H} and ², ³*J*_{C, H} values [9]. The “small” values (0 Hz) of ³*J*_{H-23, H-22} and ³*J*_{H-22, H-20} of micrandilactone **J** (**2**) indicated a structure without significant conformational changes, thus giving reliable ¹JBCA data [9]. The gauche orientations of H-22/H-23, H-22/H-20, H-23/C-20, H-22/C-24 and H-20/C-23 were evidence of the small ³*J*_{H-22, H-23}, ³*J*_{H-22, H-20}, ³*J*_{H-23, C-20}, ³*J*_{H-22, C-24}, and ³*J*_{H-20, C-23}, respectively, and the anti H-22/C-17 orientations gave large ³*J*_{H-22, C-17} values. These interactions established the relative configurations of C-23–C-20 as depicted in Fig. 5. These data matched the results of the X-ray diffraction (Fig. 3) and NOESY studies (Fig. 6).

The absolute configurations of the stereogenic centers of the side chain of 22,23-di-epi-micrandilactone **3** were deduced from the *J*BCA, combined HSQC overlay with **2**, NOESY, as well as ECD data. The positive Cotton effect around 220 nm in the ECD spectrum indicates its (23*R*) absolute configuration [13]. *J*BCA data were utilized to determine the relative configurations at C-23, C-22, C-20 as was described for micrandilactone **2** [9]. The gauche orientations of H-22/H-23, H-22/H-20, H-22/C-17, and H-20/C-23 were suggested by the small $^3J_{\text{H-22, H-23}}$, $^3J_{\text{H-22, H-20}}$, $^3J_{\text{H-22, C-17}}$, and $^3J_{\text{H-20, C-23}}$ values, respectively, and the anti-orientation of H-22/C-24 by a large $^3J_{\text{H-22, C-24}}$ value, and thus the relative configurations of C-22 and C-20 were as depicted in Fig. 6C. However, due to the absence of key $^3J_{\text{C-20, H-23}}$ and $^2J_{\text{C-22, H-23}}$ couplings, the relative configurations of C-23 and C-22 as shown in Fig. 5A and B could not be differentiated. Also, because of the complex splitting pattern of H-17, the key $^3J_{\text{H, H}}$ values were unavailable. Such an absence of coupling constant data presented a significant challenge for assignment of the absolute configuration of C-22, C-20, and C-17, because NOE data could not be applied to associate the stereogenic carbons with a proton attached to the rigid ring system. Thus, the HSQC method combined with *J*BCA and NOESY data of a degassed sample were applied to define the absolute configuration of the remaining stereogenic carbons.

The small $^3J_{\text{H-22, H-23}}$, $^3J_{\text{H-22, C-17}}$, $^3J_{\text{H-20, C-23}}$, and $^3J_{\text{H-20, H-22}}$ values (Fig. 5) of 22,23-di-epi-micrandilactone **3** indicated that rotation of the C-17–C-23 moiety is restricted. Thus, NOESY data of a degassed sample could be applied to determine the relative configuration of the H-17–H-23 stereocenters. The correlation of H-23/H-22, H-22/H-20, and H-21/H-17 indicated (17*R*, 20*S*, 22*R*) absolute configuration. The NOESY data for C-20 and C-17 were also supported by the *J*BCA data. H-N HSQC overlay is frequently applied to monitor the binding and conformational changes of proteins based on the peak shift [14]. Herein, H-C HSQC overlay was used to compare the configurations of **2** and **3**. In the overlaid HSQC spectrum of **2** and **3**, the C-1, C-5, C-8, and C-17 resonances of the two isomers are completely overlapped, indicating that they have the same relative configurations (Fig. 7A). For the non-protonated stereogenic carbons, the signals of the adjacent carbons could be used to determine their relative configurations. The cross-peaks of C-1, C-8, C-15, and C-17 are completely overlapped, suggesting that C-10, C-9, C-14, and C-13 of **2** and **3** had the same relative configurations (Fig. 7A). This is consistent with the results of the NOESY experiment (Fig. 5). Combined with the (17*R*) absolute configuration, the absolute configuration of 22, 23-di-epi-micrandilactone **3** was assigned as (1*R*, 5*S*, 8*R*, 9*S*, 10*R*, 13*R*, 14*S*, 15*S*, 17*R*, 20*S*, 22*R*, 23*R*) (Fig. 1). The peak shifts of C-23 on the HSQC overlay spectra of the two isomers indicated different configurations, matching the opposite Cotton effects in the ECD spectra of the two compounds. However, the different C-23 configuration of the two compounds will influence the HSQC overlay results of C-20 and C-22 (Fig. 6). Thus, the absolute configurations of C-20 and C-22 could not be elucidated from the developed HSQC method.

Compounds **1–3** were evaluated for their in vitro hepatoprotective activities against *N*-acetyl-*p*-aminophenol (APAP)-induced toxicity in HepG2 cells by means of the MTT method [12]. Compounds **1** and **3** showed moderate hepatoprotective activity by integrated

analysis against APAP-induced toxicity in HepG2 cells with cell survival rate of 53.0 and 50.2% at 10 μ M, respectively (positive control, bicyclol, 49.00%), while compound **2** was inactive.

4. Conclusion

Our study of *K. longipedunculata* exemplifies a combination approach to determine the absolute configuration for the schiartane-type nortriterpenoids by use of ECD (including comparison of experimental and calculated spectra), DP4+ analysis, X-ray and NMR spectroscopic data. Two of the compounds demonstrated moderate hepatoprotective activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Structures of micrandilactone I (**1**), micrandilactone J (**2**), and 22,23-di-epi-micrandilactone J (**3**).

Fig. 2.
ORTEP drawing of micrandilactone I (1).

Fig. 3.
ORTEP drawing of micrandilactone J (2).

Fig. 4.

Experimental ECD spectrum of compound **2** (black), calculated averaged Boltzmann weighted ECD spectra of conformers (**2a–2c** and **2e**) (green), and ECD spectrum of conformer **2a** (major conformer) in MeOH (red). σ -value (artificial line broadening) was set to 0.16 eV.

A: C-23~C-22 of micrandilactone **1** (**2**)

	Measured data	Reported data
$^3J_{H-23, H-22}$	Gauche: 0 (small)	Gauche 0-3 (small) Anti 7-10 (large)
$^3J_{H-23, C-20}$	Gauche: 3.1 (small)	Gauche 1-3 (small) Anti 5-7 (large)
$^3J_{H-22, C-24}$	Gauche: 2.6 (small)	Gauche 1-3 (small) Anti 5-7 (large)
$^2J_{H-23, C-22}$	Anti: 2.0 (small)	Gauche -4 to -6 (large) Anti 2-0 (small)
$^2J_{H-22, C-23}$	Anti: 1.9 (small)	Gauche -4 to -6 (large)
		Anti 2-0 (small)

C: C-23~C-22 of 22, 23-di-epi-micrandilactone **1** (**3**)

	Measured data	Reported data
$^3J_{H-23, H-22}$	Gauche: 0 (small)	Gauche 0-3 (small) Anti 7-10 (large)
$^3J_{H-23, C-20}$	Not observed	Gauche 1-3 (small) Anti 5-7 (large)
$^3J_{H-22, C-24}$	Anti: 6.0 (large)	Gauche 1-3 (small) Anti 5-7 (large)
$^2J_{H-23, C-22}$	Not observed	Gauche -4 to -6 (large) Anti 2-0 (small)
$^2J_{H-22, C-23}$	Gauche: -4.2 (large)	Gauche -4 to -6 (large)
		Anti 2-0 (small)

B: C-22~C-20 of micrandilactone **1** (**2**)

	Measured data	Reported data
$^3J_{H-22, H-20}$	Gauche: 0 (small)	Gauche 1-4 (small) Anti 8-11 (large)
$^3J_{H-22, C-17}$	Anti: 6.4 (large)	Gauche 1-3 (small) Anti 6-8 (large)
$^3J_{H-20, C-23}$	Gauche: 3.1 (small)	Gauche 1-3 (small) Anti 6-8 (large)
$^2J_{H-20, C-22}$	Anti: 2.5 (small)	Gauche -5 to -7 (large) Anti 1-2 (small)
$^2J_{H-22, C-20}$	Anti: 2.7 (small)	Gauche -5 to -7 (large) Anti 1-2 (small)

D: C-22~C-20 of 22, 23-di-epi-micrandilactone **1** (**3**)

	Measured data	Reported data
$^3J_{H-22, H-20}$	Gauche: 0 (small)	Gauche 1-4 (small) Anti 8-11 (large)
$^3J_{H-22, C-17}$	Gauche: 3.4 (small)	Gauche 1-3 (small) Anti 6-8 (large)
$^3J_{H-20, C-23}$	Gauche: 3.4 (small)	Gauche 1-3 (small) Anti 6-8 (large)
$^2J_{H-20, C-22}$	Anti: -2.6 (small)	Gauche -5 to -7 (large) Anti 1-2 (small)
$^2J_{H-22, C-20}$	Not observed	Gauche -5 to -7 (large) Anti 1-2 (small)

Fig. 5.

Rotamers and coupling constants for C-23~C-22(A), C-22~C-20 (B) of **1**, C-23~C-22(C), C-22~C-20 (D) of **2**. Key NOESY correlations of **1** (E), (G), and **2** (F), (H).

Fig. 6. Key HMBC (A) correlations of micrandilactone I (**1**); HMBC (B) COSY (C), and NOESY (D) correlations of 22,23-di-epi-micrandilactone J (**3**); and NOESY (E) correlations of micrandilactone J (**2**).

Fig. 7.

(A) Entire HSQC overlay. The black parts of the structures are identical and the blue parts are different based on the HSQC data. Key non-overlaid HSQC crosspeaks of micrandilactone J (**2**) and 22,23-di-epi-micrandilactone J (**3**) are shown.

¹H and ¹³C NMR data for micrandilactone I (1), micrandilactone J (2) and 22,23-di-*epi*-micrandilactone J (3) in pyridine-*d*₅ using an INOVA-500 MHz spectrometer.

Table 1

No.	1	2		3	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{C}
1	4.27 (d, 5.0)	81.8	4.28 (d, 4.5)	82.4	4.30 (d, 4.5) 82.4
2 α	2.75 (d, 17.5)	36.5	2.71 (d, 17.5)	37.3	2.73 (m) 37.3
2 β	2.98 (dd, 17.5, 5.0)		2.95 (dd, 17.5, 4.5)		2.94 (dd, 18.0, 4.5)
3		175.2		176.0	176.0
4		84.8		85.3	85.4
5	2.55 (dd, 13.5, 4.0)	60.0	2.70 (dd, 13.5, 3.5)	59.0	2.70 (overlapped) 59.0
6 α	1.63 (m)	28.8	1.75 (overlapped)	28.9	1.74 (m) 29.0
6 β	1.32 (br t, 13.5)		1.29 (overlapped)		1.29 (overlapped)
7 α	2.82 (dd, 14.0, 9.0)	24.6	2.69 (overlapped)	24.7	2.71 (overlapped) 24.8
7 β	2.08 (overlapped)		2.19 (overlapped)		2.19 (m)
8	1.84 (overlapped)	53.9	2.00 (br d, 11.0)	57.0	2.02 (br d, 11.0) 56.9
9		73.8		72.4	72.3
10		100.3		100.0	100.0
11 α	2.11 (overlapped)	39.5 ^a	1.88 (m)	38.6	1.90 (m) 38.7
11 β	1.70 (br d, 13.0)		1.63 (br d, 14.0)		1.65 (overlapped)
12 α	2.22 (overlapped)	30.6	2.79 (m)	39.3	2.80 (t, 12.5) 39.3
12 β	1.46 (br d, 13.0)		1.71 (br d, 14.0)		1.67 (overlapped)
13		46.9		46.1	46.1
14		50.9		87.0	86.8
15	4.30 (dd, 10.0, 5.0)	76.7	4.48 (s)	77.2	4.49 (s) 77.4
16 α	1.99 (overlapped)	39.4 ^a	2.53 (overlapped)	36.5	2.53 (m) 35.9
16 β	1.81 (overlapped)		2.18 (overlapped)		2.25 (m)
17	1.79 (overlapped)	44.8	2.53 (overlapped)	54.6	2.53 (overlapped) 54.9
18	0.93 (s)	15.5	1.48 (s)	18.6	1.48 (s) 18.7
19 α	2.03 (d, 5.0)	48.0	2.05 (d, 15.5)	47.7	2.07 (d, 15.0) 47.8

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No.	1		2		3	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
19 β	2.05 (d, 5.0)		2.13 (d, 15.5)		2.14 (d, 15.0)	
20	2.05 (overlapped)	39.3 ^a	2.57 (m)	38.1	2.38 (m)	37.3
21	0.97 (d, 6.5)	13.7	1.32 (d, 6.0)	18.8	1.22 (d, 7.0)	18.9
22	4.50 (dt, 13.0, 3.0)	80.4	4.06 (br s)	73.9	4.31 (overlapped)	73.8
23 α	2.18 (overlapped)	23.7	5.26 (s)	83.0	5.24 (s)	83.5
23 β	1.97 (overlapped)					
24	6.46 (d, 6.0)	140.0	7.19 (overlapped)	148.8	7.39 (br s)	147.8
25		128.0		130.4		131.0
26		166.2		175.1		174.7
27	1.90 (s)	17.2	1.81 (s)	10.9	1.85 (s)	11.0
28	1.56 (s)	13.0				
29	1.10 (s)	22.9	1.29 (s)	30.3	1.29 (s)	30.3
30	1.26 (s)	29.4	1.08 (s)	23.9	1.08 (s)	23.9

^aInterchangeable.