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Novel Guaianoids, Nardoguaianone E–I, from *Nardostachys chinensis* Roots

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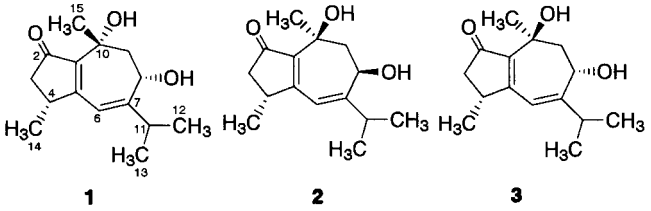
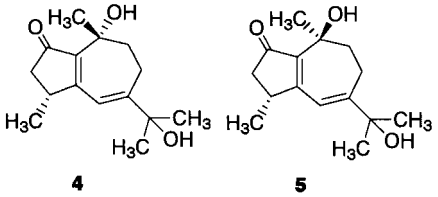
Abstract—Five novel guaianoids, nardoguaianone E–I (**1**–**5**), were isolated from *Nardostachys chinensis* roots. They possessed a dienone moiety in their molecules. The relative structures were elucidated by spectral means and chemical transformation, and the absolute configurations were determined using the modified Mosher's method and by comparison of their CD spectra.

Introduction

The rhizomes and roots of *Nardostachys chinensis* Batalin (Valerianaceae) are used as the crude drug 'Kanshoko' in Oriental medicines. In our previous papers, we have already reported guaiane sesquiterpenes bearing an endoperoxide moiety from the plant, and some of them showed antimalarial activity.[1,2] Further phytochemical investigation on the constituents of the plant afforded five novel guaiane-type sesquiterpenes named nardoguaianone E–I. We describe herein the isolation and structure elucidation of these compounds.



1 **2** **3**



4 **5**

Results and Discussion

Isolation of nardoguaianone E–I (1–5)

Dried rhizome of *N. chinensis* (3.0 kg) was extracted three times with methanol. The extracts (357 g) were concentrated in vacuo and partitioned between ethyl acetate and water to yield ethyl acetate solubles (230 g). The ethyl acetate solubles were fractionated by conventional silica-gel column chromatography with stepwise gradient of *n*-hexane and ethyl acetate (10:0–0:10). The *n*-hexane–ethyl acetate (7:3) fraction of the column chromatography was fractionated repeatedly using silica-gel and reverse-phase ODS column to give three sesquiterpenes, nardoguaianone E (**1**), F (**2**) and G (**3**). Repeated chromatography of the *n*-hexane–ethyl acetate (5:5) fraction gave nardoguaianone H (**4**) and I (**5**).

Planar structures of nardoguaianone E (1), F (2) and G (3)

The molecular formula of nardoguaianone E (**1**) was established to be $C_{15}H_{22}O_3$ from its ^1H and ^{13}C NMR and HREI-MS (*m/z* 250.1570 $[M]^+$ (*m/z* 250.1569 calcd for $C_{15}H_{22}O_3$)). Its ^{13}C NMR spectrum demonstrated the presence of four methyl carbons, two methylene carbons, two methine carbons, one oxymethine carbon, one oxygen-attached quaternary carbon, four olefinic carbons and one carbonyl carbon. The two double bond carbons (δ 141.6 and 169.9) and carbonyl carbon (δ 210.5) showed characteristic chemical shifts for an α,β-unsaturated carbonyl group in a five-membered ring, and the UV absorption band at 293 nm indicated a dienone moiety in the molecule. The ^1H–^1H COSY spectrum suggested three partial structures: (i)

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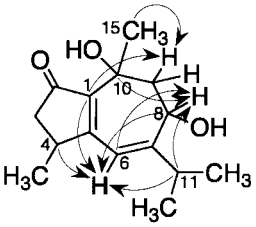

Figure 1. HMBC signals of nardoguaianone E (**1**). The arrows indicate the correlations.

–CH(OH)–CH_2–, (ii) –CH(CH_3)–CH_2– and (iii) –CH(CH_3)$_2$. The partial structures were further combined on the basis of cross peaks in the HMBC spectrum as shown in Fig. 1. As for **1**, cross peaks of C-11/H-6, C-11/H-8, C-10/H-8, C-1/H-6 and C-1/H-9 constructed a seven-membered ring.

The ^{1}H and ^{13}C NMR spectra of nardoguaianone F (**2**) and G (**3**), bearing the molecular formula $C_{15}H_{22}O_3$, resembled those of **1**. In addition, HMBC spectra of **2** and **3** also exhibited the same cross peaks as those of **1**. These spectral features unambiguously indicated the compounds **1**–**3** to be stereoisomers.

Stereochemistries of nardoguaianone F (2), E (1) and G (3)

The compound **1** bears three asymmetric centers at C-4, C-8 and C-10 in its molecule, and NOE experiments revealed no information on the relative stereochemistry because C-4 is isolated from the other two chiral carbons, C-8 and C-10, by the planar dienone moiety. Thus, we tried to introduce a 'mediator' to the dienone moiety. Osmium tetroxide oxidation of **1** using microencapsulated OsO_4 (MC-OsO_4)[3] gave a 6,7-*cis*-dihydroxylated product (**6**). As shown in Fig. 2, NOE's of **6** revealed that all the hydroxyl groups are situated in the same side of the molecule. The methyl group at C-4 also must be in the same side of the hydroxyl groups because of the NOE between H-4 and H-6. The absolute configuration at C-8 was established by the modified Mosher's method.[4] The $\Delta\delta$ values obtained for the MTPA esters at C-8 of **1** indicated the absolute configuration of C-8 to be *S* (Fig. 3).

The relative stereochemistry of **2** was investigated in the same manner as that for **1**. Treatment of **2** with MC-OsO_4 yielded an oxidized product (**7**). NOE experiments in **7**, together with the evidence that the chemical shifts of H-8, H-9 and the methyl hydrogens at C-10 of **2** were very similar to those of **1**, indicated that all the hydroxyl groups of **7** were in the same plane of the molecule. The δ value of H-4 shifted more by the osmium oxidation than that of H-14, and the NOE between H-11 and H-14 was observed in **7**. From these findings, the relative stereochemistry of the hydroxyl groups leads to determination of the β-configuration of the C-4 methyl group. The $\Delta\delta$ values obtained for the MTPA esters at C-8 of **2** demonstrated the absolute configuration of **1** to be 4*R*,8*R*,10*S*.

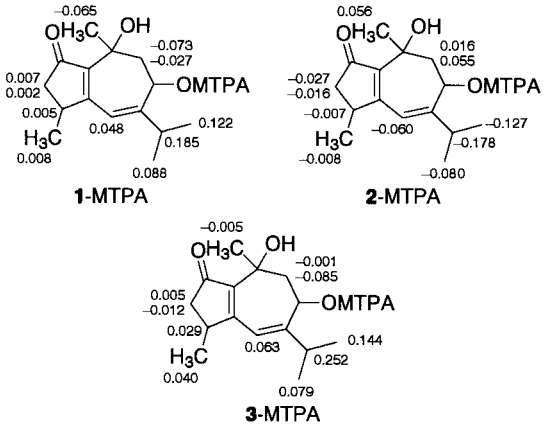

Figure 3. $\Delta\delta$ values obtained for the MTPA ester of nardoguaianone E (**1**), F (**2**) and G (**3**).

Because osmium tetroxide oxidation of **3** was unsuccessful and gave no oxidized product, its NOE experiments could not be examined. We obtained information that the absolute configuration of C-8 is *S* from the result of the modified Mosher's method. CD spectra of **1**–**3** was evaluated to know the absolute configuration of C-4 and C-10 in **3**. The CD spectra of **2** and **3** are very similar, and they are almost antipodal to that of **1** (Fig. 4), indicating that the signs of the Cotton effects of the compounds are thought to depend on the absolute stereochemistry at C-10. From this point of view, the methyl group at C-10 of **3** was presumed to be α, and this was substantiated by the ^{1}H NMR data. The chemical shifts of the H-9 signals of **3** are distinctive (δ_H 2.01 and 2.47), whereas those of **1** and **2**, where the hydroxyl groups at C-8 and C-10 are oriented to the same side, appear almost in the same region (δ_H~2.1). The chemical shifts of the H-9 signals may be affected by the conformational change around C-9. One of the H-9 signals is closer to the adjacent hydroxyl group than another in **3**, while, in **1** and **2**, neither are close to the adjacent hydroxyl groups and they may be similarly affected by the hydroxyl groups and π-electron of the double bond (Fig. 5).

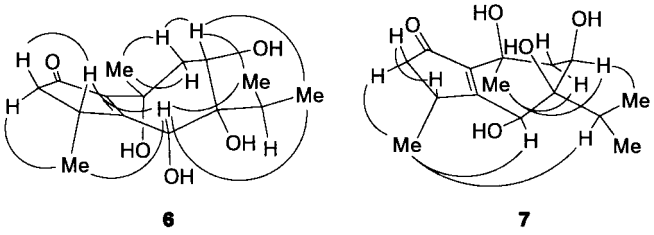

Figure 2. NOEs of oxidized nardoguaianone E (**6**) and F (**7**).

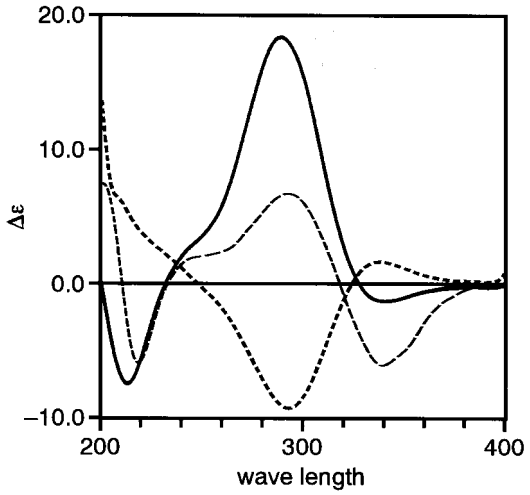

Figure 4. CD spectra of nardoguaianone E (**1**), F (**2**), and G (**3**). (- - -) **1**, (—) **2**, (– – –) **3**.

Biogenetic consideration that all the guaianoids isolated from the plant bear the α-configuration implies that **3** also has the same configuration.

Structure of nardoguaianone H (4) and I (5)

The molecular formulas of nardoguaianone H (**4**) and I (**5**) were also given as $C_{15}H_{22}O_3$ from the same molecular ion peaks as nardoguaianones E–G (**1**–**3**) at *m/z* 250 in their EI-MS, along with ^1H and ^{13}C NMR data and their HREI-MS. Their NMR spectra quite resembled those of **1**–**3** except for the following findings. In the spectra of **4** and **5**, two hydrogens and one carbon of a methylene, two singlet methyl hydrogens and one oxygen-attached quaternary carbon were observed instead of the H-8 oxymethine hydrogen and the two methyl and one methine hydrogens of the isopropyl group in the NMR spectra of **1**–**3**, respectively. From these results, **4** and **5** were assumed to be structural congeners of **1**–**3** which possess a hydroxyl group at C-11 instead of C-8. 2D NMR spectra of **4** and **5** elucidated the planar structures as shown in Fig. 6.

As **4** and **5** have a dienone moiety in their molecules, NOE experiments were not helpful to confirm the absolute configurations of these compounds. On the other hand, as shown in Fig. 7, CD spectra of **4** and **5** resembled those of **1** and **2**, respectively. On the basis of the spectral analysis mentioned above, the absolute configurations of **4** and **5** were confirmed to be 4*R*,10*R* and 4*R*,10*S*, respectively.

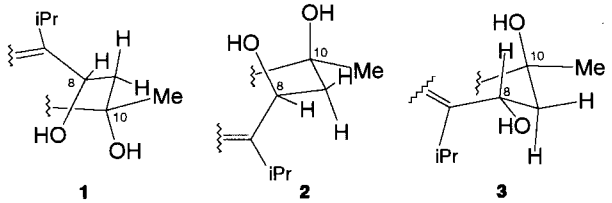

Figure 5. Conformation around C-9 of nardoguaianone E (**1**), F (**2**) and G (**3**).

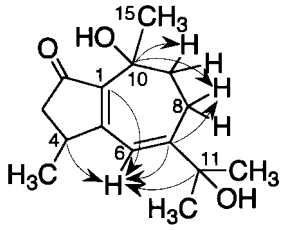

Figure 6. HMBC signals of nardoguaianone H (**4**). The arrows indicate the correlations.

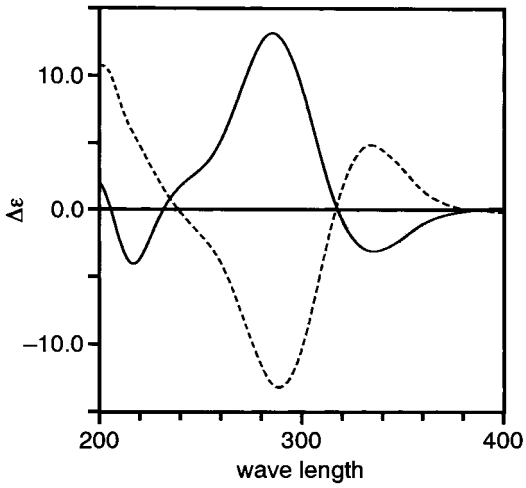

Figure 7. CD spectra of nardoguaianone H (**4**) and I (**5**). (- - -) **4**, (—) **5**.

Conclusion

In this paper,isolation and structure elucidation of five novel guaiane-type sesquiterpenes, nardoguaianone E–I (**1**–**5**), from *N. chinensis* were described. Since they possess a dienone moiety in their molecules, NOE experiments were not practical to get information on the stereochemistries of the compounds. This problem was overcome by osmium tetroxide oxidation, the modified Mosher's method, and comparison of the CD spectra. The biological activity of the compounds may be interesting, but they are unstable to air and light, and care has to be taken in their treatment.

Experimental

General

UV and IR spectra were recorded on Hitachi U-3200 and JASCO FT/IR-5399 spectrometers, respectively. Optical rotations and CD spectra were measured with a JASCO DIP-370 polarimeter and a JASCO J-720 circular dichroism spectrometer. ^1H and ^{13}C NMR spectra were recorded on JEOL LAMBDA-600 (^1H: 600 MHz and ^{13}C: 150 MHz) and JEOL JNM GSX-500 (^1H: 500 MHz and ^{13}C: 125 MHz) spectrometers. All NMR spectra were recorded in $CDCl_3$. Chemical shifts for ^1H and ^{13}C NMR are given in parts per million (δ) relative to tetramethylsilane (δ_H 0.00) and $CDCl_3$ (δ_C 77.1) as internal standards, respectively. LR and HR EI-MS were obtained with JEOL JMS DX-303 and AX-500 mass spectrometers. Analytical TLC was

Table 1. ^{1}H NMR Data for nardoguaianone E (**1**)–I (**5**)

Positions	1	2	3	4	5
3α	2.03 (dd, 18.8, 1.8)	2.03 (dd, 18.9, 1.7)	2.07 (dd, 18.8, 1.7)	2.021 (dd, 18.9,1.8)	2.06 (dd, 18.7, 1.8)
3β	2.62 (dd, 18.8, 6.4)	2.66 (dd, 18.9, 6.7)	2.68 (dd, 18.8, 6.8)	2.65 (dd, 18.9, 6.4)	2.67 (dd, 18.7, 7.0)
4	2.75 (ddq, 6.4, 1.8, 7.0)	2.88 (ddq, 6.7, 1.7, 7.1)	2.85 (ddq, 6.8, 1.7, 7.0)	2.80 (ddq, 6.4, 1.8, 7.0)	2.84 (ddq, 7.0, 1.8, 7.3)
6	5.87 (s)	5.75 (s)	5.83 (s)	6.33 (s)	6.29 (s)
8	4.52 (br.t, 5.2)	4.51 (br.d, 9.0)	4.44 (br.d, 8.4)	2.43 (br.dd,16.8, 8.8)[a] 2.33 (ddd,16.8,11.0,1.2)[a]	2.45 (br.dd, 17.2, 7.0)[a] 2.36 (ddd, 17.2, 10.3, 1.8)[a]
9	2.10 (2H, br.d, 5.2)	2.06 (br.dd, 13.0, 9.0) 2.11 (br.d, 13.0)	2.01 (dd, 14.6, 1.8) 2.47 (dd, 14.6, 8.4)	1.75 (ddd,13.7,8.8,1.2)[a] 2.019 (ddd,13.7,11.0,0.9)[a]	1.74 (br.dd, 13.9, 7.0)[a] 2.04 (ddd, 13.9, 10.3, 1.5)[a]
11	3.02 (sept, 6.7)	3.02 (sept, 6.8)	2.64 (sept, 6.8)		
12 (CH_3)	1.18 (d, 6.7)	1.18 (d, 6.8)	1.15 (d, 6.8)	1.41 (s)[b]	1.42 (s)[b]
13 (CH_3)	1.14 (d, 6.7)	1.15 (d, 6.8)	1.13 (d, 6.8)	1.44 (s)[b]	1.43 (s)[b]
14 (CH_3)	1.26 (d, 7.0)	1.13 (d, 7.1)	1.21 (d, 7.0)	1.23 (d, 7.0)	1.20 (d, 7.0)
15 (CH_3)	1.46 (s)	1.44 (s)	1.49 (s)	1.45 (s)[b]	1.44 (s)[b]

[a] Assignments of the stereochemistry for these signals were uncertain.
[b] Data can be interchanged within the group.

performed on silica gel 60 F254 (Merck). Column chromatography was carried out on silica gel 60 (70–230 mesh, Merck) and Cosmosil 75C18-OPN (Nacalai Tesque, Kyoto). (*R*)-(−)-α Methoxy-α-(trifluoromethyl)phenylacetyl chloride ((*R*)-MTPA-Cl), (*S*)-(+)-α-methoxy-α (trifluoromethyl)phenylacetyl chloride ((*S*)-MTPA-Cl) and 4-methylmorpholine *N*-oxide (NMO) were purchased from Aldrich. Microencapsulated osmium tetroxide (MC-OsO_4) was purchased from Wako Pure Chemicals (Osaka).

Extraction and isolation

Methanol extract of dried rhizome (3 kg) of *N. chinensis* and ethyl acetate solubles were obtained as described in Ref. 2. Ethyl acetate solubles were chromatographed over silica gel, and the column was eluted with *n*-hexane–ethyl acetate mixtures by increasing polarity (10:0–0:10). The resulted *n*-hexane–ethyl acetate (7:3) eluate (29.8 g) was further fractionated with repeated chromatography using silica gel with a mixed solvent of *n*-hexane–acetone, followed by *n*-hexane–isopropanol as eluants. Fractionation of the *n*-hexane–isopropanol (95:5) eluate (333 mg) using silica gel column (chloroform–methanol), ODS columns (35% acetonitrile–H_2O) and preparative HPLC with ODS column (35% acetonitrile–H_2O) afforded nardoguaianone E (**1**) (1.8 mg), nardoguaianone F (**2**) (6.9 mg). The *n*-hexane–isopropanol (93:7) eluate (879 mg) was fractionated using silica gel column (chloroform) and ODS column (30% acetonitrile–H_2O and 30% acetone–H_2O) to give nardoguaianone G (**3**) (41 mg). Nardoguaianone H (**4**) (5.5 mg) and nardoguaianone I (**5**) (2.8 mg) were obtained from *n*-hexane–ethyl acetate (5:5) eluate (18.5 g) by fractionation with silica gel (chloroform–methanol and benzene–acetone) and preparative HPLC with ODS columns (25% acetonitrile–H_2O).

Nardoguaianone E (1). $[\alpha]_D^{25}=-238.4°$ (*c* 0.18, MeOH); a yellowish amorphous solid; UV λ_{max} (MeOH) (nm (log ϵ)) 293 (4.04); CD λ (MeOH) nm ($\Delta\epsilon$) 338.0 (+1.52), 292.8 (−9.25); IR ν_{max} ($CHCl_3$) 1671, 1589 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data are shown in Tables 1 and 2; HREI-MS: *m/z* 250.1570 $[M]^+$ (250.1569 calculated for $C_{15}H_{22}O_3$).

Nardoguaianone F (2). $[\alpha]_D^{25}=+541.7°$ (*c* 0.62, MeOH); a yellowish amorphous solid; UV λ_{max} (MeOH) (nm (log ϵ)) 292 (4.14); CD λ (MeOH) nm ($\Delta\epsilon$) 341.4 (−1.06), 289.6 (+18.0), 213.6 (−7.06); IR ν_{max} ($CHCl_3$) 3443, 1672, 1595 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data are shown in Tables 1 and 2; HREI-MS: *m/z* 250.1572 $[M]^+$ (250.1569 calculated for $C_{15}H_{22}O_3$).

Nardoguaianone G (3). $[\alpha]_D^{25}=-14.5°$ (*c* 0.84, MeOH); a yellowish amorphous solid; UV λ_{max} (MeOH) (nm (log ϵ)) 296 (3.96); CD λ (MeOH) nm ($\Delta\epsilon$) 340.0 (−3.85), 293.0 (+4.79), 218.6 (−3.72); IR ν_{max} ($CHCl_3$) 3453, 1672, 1607 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data are shown in Tables 1 and 2; HREI-MS: *m/z* 250.1546 $[M]^+$ (250.1569 calculated for $C_{15}H_{22}O_3$).

(*S*)- and (*R*)-MTPA Esterification of nardoguaianone E–G (1–3). Nardoguaianone E (**1**) (0.49 mg) was dissolved to a 100 μL of dry pyridine, and was added (*R*)-MTPA-Cl (30 μL) at 0°C. The reaction mixture was soon warmed to room temperature, and stirred for 1 h. The reaction solution was poured into water (0.5 mL) and extracted twice with ethyl acetate(0.5 mL). The organic layer was combined, washed with water and brine, dried over anhydrous

Table 2. ^{13}C NMR Data of nardoguaianone E–I (**1**–**5**) (abbreviations of multiplicity: q, primary carbon; t, secondary carbon; d, tertiary carbon; s, quaternary carbon)

Positions	1	2	3	4	5
1	141.6 (s)	141.8 (s)	140.3 (s)	142.0 (s)	142.1 (s)
2	210.5 (s)	210.6 (s)	209.9 (s)	210.4 (s)	210.4 (s)
3	43.0 (t)	43.3 (t)	43.3 (t)	43.3 (t)	43.4 (t)
4	36.7 (d)	36.8 (d)	36.8 (d)	37.1 (d)	37.0 (d)
5	169.9 (s)	169.3 (s)	167.1 (s)	167.3 (s)	167.1 (s)
6	113.1 (d)	114.0 (d)	117.4 (d)	116.2 (d)	116.5 (d)
7	167.9 (s)	167.9 (s)	166.4 (s)	164.6 (s)	164.4 (s)
8	69.0 (d)	69.0 (d)	68.8 (d)	25.9 (t)	26.1 (t)
9	54.2 (t)	53.9 (t)	45.8 (t)	37.1 (t)	39.7 (t)
10	71.2 (s)	70.9 (s)	71.5 (s)	[a] (s)	72.0 (s)
11	30.3 (d)	30.1 (d)	35.7 (d)	77.3 (s)	74.2 (s)
12	22.2 (q)	22.3 (q)	21.4 (q)	28.9 (q)[b]	29.1 (q)[b]
13	22.3 (q)	22.5 (q)	21.1 (q)	28.8 (q)[b]	28.7 (q)[b]
14	20.4 (q)	20.0 (q)	19.9 (q)	20.6 (q)	20.2 (q)
15	28.2 (q)	28.0 (q)	28.2 (q)	28.7 (q)[b]	28.2 (q)[b]

[a] The signal was overlapped with the solvent peak.
[b] Data can be interchanged within the group.

magnesium sulfate, and evaporated. The residue was separated by SiO_2 column chromatography to give (*S*)-MTPA ester of **1**. In the same manner, (*S*)-MTPA esters of **2** and **3** and (*R*)-MTPA esters of **1**, **2** and **3** were prepared using (*R*)-MTPA-Cl and (*S*)-MTPA-Cl, respectively.

*(S)-MTPA ester of **1**:* ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.97 (1H, s; H-6), 5.79 (1H, br.d, *J*=8.6 Hz; H-8), 2.79 (1H, ddq, *J*=6.4, 1.8, 7.0 Hz; H-4), 2.68 (1H, dd, *J*=18.9, 6.4 Hz; H-3), 2.52 (1H, sept, *J*=6.7 Hz; H-11), 2.17 (1H, dd, *J*=14.3, 1.5 Hz; H-9), 2.09 (1H, dd, *J*=14.3, 8.6 Hz; H-9), 2.06 (1H, dd, *J*=8.9 Hz; H-8), 1.35 (3H, s; H-15), 1.25 (3H, d, *J*=7.0 Hz; H-14), 1.073 (3H, d, *J*=6.7 Hz; H-12), 1.065 (1H, d, *J*=6.7 Hz; H-13).

*(R)-MTPA ester of **1**:* ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.92 (1H, s; H-6), 5.75 (1H, br.d, *J*=9.2 Hz; H-8), 2.79 (1H, ddq, *J*=6.4, 1.8, 7.0 Hz; H-4), 2.67 (1H, dd, *J*=18.9, 6.4 Hz; H-3), 2.34 (1H, sept, *J*=6.7 Hz; H-11), 2.25 (1H, dd, *J*=14.0, 0.9 Hz; H-9), 2.12 (1H, dd, *J*=14.0, 9.2 Hz; H-9), 2.06 (1H, dd, *J*=18.9, 1.8 Hz; H-3), 1.41 (3H, s; H-15), 1.25 (3H, d, *J*=7.0 Hz; H-14), 0.99 (3H, d, *J*=6.7 Hz; H-12), 0.94 (3H, d, *J*=6.7 Hz; H-13).

*(S)-MTPA ester of **2**:* ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.82 (1H, s; H-6), 5.77 (1H, br.d, *J*=9.2 Hz; H-8), 2.88 (1H, d,quint, *J*=1.8, 7.0 Hz; H-4), 2.69 (1H, dd, *J*=18.9, 7.0 Hz; H-3), 2.33 (1H, sept, *J*=6.7 Hz; H-11), 2.26 (1H, dd, *J*=14.0, 1.2 Hz; H-9), 2.11 (1H, dd, *J*=14.0, 9.2 Hz; H-9), 2.08 (1H, dd, *J*=18.9, 1.8 Hz; H-3), 1.39 (3H, s; H-15), 1.16 (3H, d, *J*=7.0 Hz; H-14), 1.00 (3H, d, *J*=6.7 Hz; H-12), 0.94 (3H, d, 6.7 Hz; H-13).

*(R)-MTPA ester of **2**:* ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.88 (1H, s; H-6), 5.81 (1H, br.d, *J*=8.9 Hz; H-8), 2.89 (1H, ddq, *J*=6.7, 1.8, 7.0 Hz; H-4), 2.71 (1H, dd, *J*=19.2, 6.7 Hz; H-3), 2.51 (1H, sept, *J*=6.7 Hz; H-11), 2.21 (1H, dd, *J*=14.3, 1.8 Hz; H-9), 2.11 (1H, dd, *J*=19.2, 1.8 Hz; H-3), 2.09 (1H, dd, *J*=14.3, 8.9 Hz; H-9), 1.33 (3H, s; H-15), 1.17 (3H, d, *J*=7.0 Hz; H-14), 1.08 (3H, d, *J*=6.7 Hz; H-12), 1.07 (3H, d, *J*=6.7 Hz; H-13).

*(S)-MTPA ester of **3**:* ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.95 (1H, s, H-6), 5.75 (1H, dd, *J*=10.1, 2.4 Hz; H-8), 2.80 (1H, ddq, *J*=1.8, 6.7, 7.3 Hz; H-4), 2.67 (1H, dd, *J*=18.9, 6.7 Hz; H-3), 2.527 (1H, dd, *J*=13.7, 10.1 Hz; H-9), 2.526 (1H, sept, *J*=6.9 Hz; H-11), 2.06 (1H, dd, *J*=13.7, 2.4 Hz; H-9), 2.05 (1H, dd, *J*=18.9, 1.8 Hz; H-3), 1.50 (3H, s; H-15), 1.18 (3H, d, *J*=7.3 Hz; H-14), 1.06 (3H, d, *J*=6.9 Hz; H-12), 1.01 (3H, d, *J*=6.9 Hz; H-13).

*(R)-MTPA ester of **3**:* ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.88 (1H, s; H-6), 5.78 (1H, dd, *J*=10.1, 2.4 Hz; H-8), 2.78 (1H, ddq, *J*=1.8, 6.4, 7.0 Hz; H-4), 2.66 (1H, dd, *J*=18.9, 6.4 Hz; H-3), 2.53 (1H, dd, *J*=13.4, 10.1 Hz; H-9), 2.27 (1H, sept, *J*=6.7 Hz; H-11), 2.14 (1H, dd, *J*=13.4, 2.4 Hz; H-9), 2.06 (1H, dd, *J*=18.9, 1.8 Hz; H-3), 1.50 (3H, s; H-15), 1.14 (3H, d, *J*=7.0 Hz; H-14), 0.93 (3H, d, *J*=6.7 Hz; H-12), 0.92 (3H, d, *J*=6.7 Hz; H-13).

OsO_4 Oxidation of nardoguaianone E (1) and F (2)

Nardoguaianone E (**1**) (0.64 mg) was dissolved to a 500 μL of mixed solvent of acetone–acetonitrile–water (1:1:1), and was added 2 mg of MC-OsO_4 and NMO (1 mg). The reaction mixture was stirred at room temperature for 7 days, and then added the same amount of microencapusulated osmium tetraoxide. After stirring for more 4 days, the reaction mixture was then filtered, and the resulted filtrate was evaporated and purified by SiO_2 column chromatography to give oxidized nardoguaianone E (**6**) (0.2 mg). In the same manner, nardoguaianone F (**2**) (0.43 mg) was oxidized within 10 h to give **7** (0.44 mg).

Oxidized nardoguaianone E (6). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.48 (1H, br.d, *J*=2.4 Hz; C-10-O*H*), 4.38 (1H, d, *J*=1.5 Hz; H-6), 4.09 (1H, ddd, *J*=10.7, 4.6, 1.2 Hz; H-8), 3.58 (1H, s; C-7-O*H*), 3.54 (1H, d, *J*=1.5 Hz; C-6-O*H*), 2.93 (1H, ddd, *J*=14.3, 10.7, 2.4 Hz; H-9α), 2.86 (1H, ddq, *J*=7.3, 6.7, 1.5 Hz; H-4), 2.66 (1H, dd, *J*=19.2, 6.7 Hz; H-3β), 2.09 (1H, dd, *J*=19.2, 1.5 Hz; H-3α), 1.96 (1H, d, *J*=4.6 Hz; C-8-O*H*), 1.87 (1H, sept, *J*=6.7 Hz; H-11), 1.63 (1H, br.d, *J*=14.3 Hz; H-9β), 1.45 (3H, s; H-15), 1.31 (3H, d, *J*=7.3 Hz; H-14), 0.99 (3H, d, *J*=6.7 Hz; H-12), 0.89 (3H, d, *J*=6.7 Hz; H-13); EI-MS: *m/z* 284 $[M]^+$.

Oxidized nardoguaianone F (7). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.67 (1H, br.s; C-10-O*H*), 4.51 (1H, br.s; H-6), 4.17 (1H, br.d, *J*=9.0 Hz; H-8) 3.72 (1H, br.s; C-7-O*H*), 3.48 (1H, br.s; C-6-O*H*), 3.09 (1H, ddq, *J*=7.0, 6.6, 2.1 Hz; H-4), 2.73 (1H, dd, *J*=19.4, 6.6 Hz; H-3β), 2.70 (1H, dd, *J*=14.7, 9.0 Hz; H-9α), 2.17 (1H, br.s; C-8-O*H*), 2.10 (1H, dd, *J*=19.4, 2.1 Hz; H-3α), 1.83 (1H, dd, *J*=14.7, 1.1 Hz; H-9β), 1.72 (1H, sept, *J*=6.7 Hz; H-11), 1.51 (3H, s; H-15), 1.26 (3H, d, *J*=7.0 Hz; H-14), 1.02 (3H, d, *J*=6.7 Hz; H-12), 0.99 (3H, d, *J*=6.7 Hz; H-13); EI-MS: *m/z* 284 $[M]^+$.

Nardoguaianone H (4). $[\alpha]_D^{25}=-80.2°$ (*c* 0.48, MeOH); a yellowish oil; UV λ_{max} (MeOH) (nm (log ϵ)) 292 (2.73); CD λ (MeOH) nm ($\Delta\epsilon$) 334.2 (+5.04), 288.4 (−13.2); ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data are shown in Tables 1 and 2; HREI-MS: *m/z* 250.1570 $[M]^+$ (250.1569 calculated for $C_{15}H_{22}O_3$).

Nardoguaianone I (3). $[\alpha]_D^{25}=+135.1°$ (*c* 0.27, MeOH); a yellowish amorphous solid; UV λ_{max} (MeOH) (nm (log ϵ)) 290 (3.20); CD λ (MeOH) nm ($\Delta\epsilon$) 336.8 (−3.23), 285.8 (+13.6), 217.0 (−4.24); ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data are shown in Tables 1 and 2; HREI-MS: *m/z* 250.1565 $[M]^+$ (250.1569 calculated for $C_{15}H_{22}O_3$).

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