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New Guaiane Sesquiterpenes and Furanocoumarins from *Notopterygium incisum*

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Abstract

In addition to twenty-nine known compounds, two new guaiane sesquiterpenes and two new furanocoumarins were isolated from the chloroform extract of the rhizomes of *Notopterygium incisum*. The new structures were elucidated by means of spectroscopic methods including 2D NMR techniques and mass spectrometry to be 8 β -acetoxy-4 α ,6 α -dihydroxy-1 α ,5 α (*H*)-guai-9-ene (incisumdiol A, **1**), 4 α ,6 α -dihydroxy-1 α ,5 α (*H*)-guai-9-ene (incisumdiol B, **2**), 5-[(2*E*,5*Z*)-7-hydroxy-3,7-dimethyl-2,5-octadienoxy]psoralene (**3**) and 5-[(2,5)-epoxy-3-hydroxy-3,7-dimethyl-6-octenoxy]psoralene (**4**).

Keywords

Notopterygium incisum Ting ex H. T. Chang; Apiaceae; structure elucidation; guaiane sesquiterpenes; incisumdiols; furanocoumarins

Introduction

The rhizomes of the plant of *Notopterygium incisum* Ting ex H. T. Chang (Apiaceae), known as “Qianghuo” in Chinese, have been used for centuries as a common traditional Chinese medicine (TCM) to relieve arthritic pain and to alleviate headache from sinus infections [1]. The rhizomes were previously reported to afford furanocoumarins [2], [3], [4]; however, no sesquiterpenoid has been isolated, although oxygenated sesquiterpenes have been recently detected by GC-MS from the essential oil in the rhizomes [5]. In this paper, we report the isolation and structure elucidation of two new guaiane sesquiterpenes (**1**, **2**)

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and two new furanocoumarins (**3**, **4**) from the chloroform extract of the rhizomes of *N. incisum*.

Materials and Methods

General experimental procedures

Melting points were measured on a WRS-1A Digital Melting-Point Apparatus (Shanghai YICE Apparatus & Equipments CO., LTD). Optical rotations were determined by using a Perkin-Elmer 341 polarimeter. IR spectra were measured on a Nicolet NEXUS-670 FT-IR spectrophotometer. UV spectra were recorded on a Shimadzu UV-2550 spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer using TMS as the internal reference. EIMS analyses were performed on a Thermo Trace GC Ultra-DSQ instrument. LR-ESI-MS were carried out on a Bruker Esquire 3000plus instrument. HR-ESI-MS were measured on a Bruker Daltonics micrOTOF mass spectrometer. All the solvents were of analytical grade and were purchased from Shanghai Chemical Reagents Company Ltd. Column chromatography was performed using silica gel (Qingdao Ji-Yi-Da Silysia Chemical Ltd.; 200–300 mesh) or Sephadex LH-20 (Pharmacia Biotech Ltd.).

Plant material

The dry rhizomes of *N. incisum* were purchased from Shanghai Kang-Qiao traditional Chinese medicinal (TCM) materials Co. Ltd, and were originally collected from Sichuan province, China. The plant was identified by Prof. Jian-Wei Chen (College of Pharmacy, Nanjing University of TCM). A voucher specimen (No. 060305) was deposited at the Herbarium of the Shanghai Key Laboratory of Brain Functional Genomics, East China Normal University.

Extraction and isolation

Dry crude material (10 kg) was extracted with chloroform (10×2000 mL, total amount 20 L) at room temperature. After filtration, the extracts were combined and evaporated under vacuum. The residue (ca. 1000 g) was chromatographed on silica gel (7 kg, column: 150×14 cm) with an *n*-hexane-acetone gradient (15:1, 8:1, 4:1, 1:1, 0:1, each 6 L) to yield twelve fractions (Fr.1–Fr.12). Fr.2 (between 0.8 and 2.0 L, 1.8 g) was chromatographed on silica gel (200 g, column: 70×4 cm) with a petroleum ether-chloroform gradient (8:1, 6:1, 4:1, each 600 mL) to yield compounds **9** (between 120 and 800 mL, 60 mg) and **31** (between 1200 and 1800 mL, 19 mg); Fr.3 (between 2.0 and 3.6 L, 1.5 g) was chromatographed on silica gel (180 g, column: 70×4 cm) with a petroleum ether-chloroform gradient (8:1, 6:1, 4:1, each 500 mL) to yield compounds **24** (between 600 and 800 mL, 4 mg) and **25** (between 1000 and 1300 mL, 8 mg); Fr.4 (between 3.6 and 5.0 L, ca. 8 g) was chromatographed on silica gel (1000 g, column: 70×5 cm) with a petroleum ether-ethyl acetate gradient (10:1, 8:1, 6:1, 5:1, each 800 mL) to yield compounds **11** (between 500 and 1200 mL, 38 mg), **12** (between 1200 and 1800 mL, 30 mg), **14** (between 1800 and 2100 mL, 900 mg) and **16** (between 2100 and 2800 mL, 35 mg). Fr.5 (between 5.0 and 8.0 L, ca. 10 g) was chromatographed on silica gel (1000 g, column: 70×5 cm) with a petroleum ether-ethyl acetate gradient (8:1, 6:1, 5:1, 3:1, each 700 mL) to yield compounds **19** (between 1200 and 1300 mL, 15 mg), **20** (between 1800 and 2100 mL, 30 mg), **27** (between 800 and 1000 mL,

90 mg), **28** (between 1300 and 1600 mL, 15 mg), **30** (between 1600 and 1800 mL, 35 mg) and **33** (between 600 and 1100 mL, 30 mg).

Fr.6 (between 8.0 and 14 L, ca. 11 g) was chromatographed on silica gel (1000 g, column: 70×6 cm) using a gradient of chloroform-ethyl acetate (5 :1, 4:1, 2:1, 0:1, each 800 mL) to yield six subfractions (Fr.6–1 to Fr.6–6). Compounds **4** (15 mg) and **13** (38 mg) were obtained from Fr.6–1 (between 0 and 1100 mL, 1.2 g) by gel permeation chromatography on Sephadex LH-20 (column: 110×4 cm) in acetone. Compounds **7** (25 mg) and **32** (15 mg) were obtained from Fr.6–2 (between 1100 and 1800 mL, 1.4 g) on Sephadex LH-20 (column: 120×4 cm) in methanol. Compounds **3** (20 mg), **5** (8 mg) and **6** (11 mg) were isolated from Fr.6–3 (between 1800 and 2100 mL, 1.0 g) by preparative TLC (chloroform-ethyl acetate 5:1, **3**: R_f = 0.2, **5**: R_f = 0.4, **6**: R_f = 0.8) and further purified on Sephadex LH-20 (column: 120×4 cm) in methanol. Compounds **1** (between 200 and 650 mL, 32 mg), **2** (between 0 and 200 mL, 30 mg), **17** (between 1000 and 1200 mL, 32 mg) and **18** (between 1200 and 1800 mL, 30 mg) were isolated from Fr.6–5 (between 2700 and 3100 mL, 1.8 g) by repetitive silica gel column (50×4 cm) chromatography with *n*-hexane-ethyl acetate (10:1, 2000 mL), and were further purified on Sephadex LH-20 (column: 100×3 cm) in methanol. Fr.7 (between 14 and 16.5 L, ca. 8 g) was chromatographed on silica gel (column: 70×4 cm) with a gradient of *n*-hexane-ethyl acetate (10:1, 8:1, 5:1, each 1000 mL) to yield compounds **22** (between 1500 and 1800 mL, 15 mg) and **23** (between 1200 and 1500 mL, 40 mg). Compound **15** (1 g) was obtained from Fr.8 (between 16.5 and 18 L, ca. 12 g) by crystallization from ethyl acetate. Compounds **8** (between 800 and 1000 mL, 15 mg), **10** (between 500 and 800 mL, 40 mg), **21** (between 1200 and 1350 mL, 40 mg) and **26** (between 1500 and 1750 mL, 110 mg) were isolated from Fr.9 (between 18 and 21.5 L, ca. 8 g) by silica gel column (50×4 cm) chromatography with a gradient of *n*-hexane-acetone (4 : 1, 3:1, 2:1, each 800 mL). Compound **29** (300 mg) was obtained from Fr.10 (between 21.5 and 22 L, 2:1, ca. 3 g) by crystallization from ethyl acetate.

Incisumdiol A (1)—Yellow gum; $[\alpha]_D^{23}$: -30.6 (c 2.45, CHCl_3); IR (CHCl_3) ν_{max} = 3451, 2954, 2928, 2867, 1734, 1670, 1459, 1383, 1374, 1248, 1183, 1150, 1025 cm^{-1} ; $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data, see Table 1; ESI-MS: m/z = 319 $[\text{M}+\text{Na}]^+$, 615 $[2\text{M}+\text{Na}]^+$; HR-ESI-MS: m/z = 319.1881 $[\text{M}+\text{Na}]^+$ (calcd. for $\text{C}_{17}\text{H}_{28}\text{O}_4\text{Na}$: 319.1885).

Incisumdiol B (2)—Yellow gum; $[\alpha]_D^{23}$: $+62.1$ (c 3.25, CHCl_3); IR (CHCl_3): ν_{max} = 3434, 2927, 2868, 1637, 1460, 1385, 1374, 1137 cm^{-1} ; $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data, see Table 1; ESI-MS: m/z = 261 $[\text{M} + \text{Na}]^+$, 499 $[2\text{M} + \text{Na}]^+$.

5-[(2E,5Z)-7-Hydroxy-3,7-dimethyl-2,5-octadienoxy]psoralene (3)—Yellow powder; m.p. 80–81 °C; $[\alpha]_D^{23}$: $=0$ (c 0.43, CHCl_3); UV (MeOH): λ_{max} = 221 (2.94), 248 (2.99), 258 (2.92), 268 (2.90), 311 (2.78) nm; IR (KBr): ν_{max} = 3476, 3125, 2974, 2925, 2853, 1721, 1626, 1608, 1579, 1544, 1455, 1400, 1203, 1166, 1093, 1072 cm^{-1} ; $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data, see Table 2; ESI-MS: m/z = 377 $[\text{M} + \text{Na}]^+$, 731 $[2\text{M} + \text{Na}]^+$; HR-ESI-MS: m/z = 377.1359 $[\text{M} + \text{Na}]^+$ (calcd. for $\text{C}_{21}\text{H}_{22}\text{O}_5\text{Na}$: 377.1364).

5-[(2,5)-Epoxy-3-hydroxy-3,7-dimethyl-6-octenoxy]psoralene (4)—Yellow gum;

$[\alpha]_{\text{D}}^{23}$: +1.5 (*c* 0.62, CHCl₃); UV (MeOH): λ_{max} = 220 (3.11), 249 (3.08), 258 (2.97), 267 (3.04), 308 (3.12) nm; IR (KBr): ν_{max} = 3429, 3119, 2973, 2924, 2853, 1721, 1624, 1578, 1546, 1400, 1205, 1155, 1069 cm⁻¹; ¹H-NMR and ¹³C-NMR data, see Table 2; ESI-MS: *m/z* = 371 [M+ H]⁺, 741 [2 M+ H]⁺, 763 [2 M+ Na]⁺, 785 [2 M+ HCOO]⁻.

Supporting Information

Structures of the isolates (**1–33**) (Fig. 1S), ¹H- and ¹³C-NMR spectra of compounds **1** (Fig. 2S–Fig. 5S), **2** (Fig. 6S–Fig. 8S), **3** (Fig. 9S and Fig. 10S), and **4** (Fig. 11S and Fig. 12S) are available as Supporting Information.

Results and Discussion

In a reinvestigation of the chemical components of *N. incisum*, the chloroform extract from the plant's rhizomes was subjected to column chromatography (CC) over silica gel and Sephadex LH-20 to furnish two new *cis*-fused ⁹-guaienes (**1**, **2**) and two new furanocoumarins (**3**, **4**) (Fig. 1), along with eight known guaiane sesquiterpenes (**5–12**) and fourteen known coumarins (**13–26**) as well as seven miscellaneous constituents (**27–33**). Comparing their MS and NMR data as well as the optical activity with the literature, the known compounds were identified as 4 β ,6 β -dihydroxy-1 α ,5 β (*H*)-guai-9-ene (**5**) [6], teucladiol (**6**) [7], chrysathol (**7**) [8], teuclatriol (**8**) [7], hanamyol (**9**) [9], 1,5,11-trihydroxyguaiane (**10**) [10], grilactone (**11**) [11], 3,7(11),10(14)-guaiatrien-1 β ,5 β (*H*)-12,6 β -olide (**12**) [12], notoitol (**13**) [4], isoimperatorin (**14**) [3], [13], notoitol (**15**) [2], [4], bergamottin (**16**) [14], anhydronotoitol (**17**) [4], bergapten (**18**) [3], [13], 6-methoxy-7-geranyloxy coumarin (**19**) [15], ostruthin (**20**) [16], scopoletin (**21**) [17], isofraxidin (**22**) [18], 7-isopentenyl-6-methoxy coumarin (**23**) [19], 5-geranyloxy-7-methoxy coumarin (**24**) [20], 5-isopentenyl-7-methoxy coumarin (**25**) [21], marmesin (**26**) [15], [22], phenethyl ferulate (**27**) [23], [24], pterostilbene (**28**) [25], pregnenolone (**29**) [26], falcariindiol (**30**) [2], falcariinol (**31**) [27], (2*E*,9*Z*)-heptadecadiene-4,6-diyn-1-ol (**32**) [28] and 8-acetoxy-(1,9*Z*)-heptadecadiene-4,6-diyn-3-ol (**33**) [29]. Among them, compounds **5–12**, **20–25** and **31–33** were reported from *N. incisum* for the first time.

The molecular weight of compound **1** and its chemical formula of C₁₇H₂₈O₄ were deduced from the positive mode high-resolution electrospray ionization mass spectrum (HR-ESI-MS), which resulted in an [M + Na]⁺ ion peak at *m/z* = 319.1881 (C₁₇H₂₈O₄Na requires 319.1885). The IR spectrum of **1** showed absorptions (ν_{max}) at 3451 (hydroxy groups), 1734 and 1025 (ester carbonyl), 1670 and 1459 (double bond), 1383 and 1374 (isopropyl group) cm⁻¹. In the high field region of the ¹H-NMR spectrum (CDCl₃, 298 K), five methyl signals were observed: two secondary methyls for an isopropyl group (δ = 1.07 and 0.93), one tertiary methyl (δ = 1.13), one olefinic methyl (δ = 1.75), and one acetyl methyl (δ = 2.09). Based on these spectroscopic data and on the degrees of unsaturation, the backbone structure of **1** was elucidated to be a guaiane-type skeleton that was also found for the known compounds **5–12** [6], [7], [8], [9], [10], [11], [12].

In the ^1H -NMR spectrum (CDCl_3 , 298 K), the signals for H-1, H-5, and H-8 appeared very broad. Similarly, in the ^{13}C -NMR spectrum recorded in CDCl_3 at 310 K, extremely weak and broad signals were observed for C-1, C-2, C-3, C-7, C-8, C-9, C-10, and C-14. These broadened signals suggested that **1** exists as a mixture of conformations that interconvert at a moderate rate on the NMR time scale. To test this hypothesis, we re-acquired the NMR spectra at a higher temperature (343 K, $\text{DMSO}-d_6$). Indeed, at higher temperatures, the signals for H-1, H-5 and H-8 coalesce to sharp, averaged multiplets at $\delta = 2.47$, 2.57, and 5.16, respectively (Table 1). In the ^{13}C -NMR spectrum acquired under these conditions, the intensity of the eight carbon signals of C-1, C-2, C-3, C-7, C-8, C-9, C-10, and C-14 was significantly increased, although they were still less intense than the other carbon signals (Table 1).

The ^{13}C -NMR and DEPT spectra recorded in $\text{DMSO}-d_6$ at 343 K confirmed that compound **1** contained seventeen carbons classified as three quaternary carbons at $\delta = 170.3$, 131.1 and 58.8, seven methines at $\delta = 128.2$, 71.1, 69.8, 68.3, 48.8, 42.4 and 25.3, two methylenes at $\delta = 37.6$ and 23.2, and five methyls at $\delta = 23.0$, 20.8, 20.7, 19.3 and 16.0 (Table 1). In the COSY spectrum ($\text{DMSO}-d_6$), a clear proton spin system was observed between H-5 at $\delta = 2.57$ and H-6 at $\delta = 3.40$, between H-6 and H-7 at $\delta = 1.30$, as well as between H-7 and H-8 at $\delta = 5.16$. The correlation between H-8 and its vicinal olefinic proton H-9 is ambiguous due to the dihedral angle (ca. 90°) between H-8 and H-9. The chemical shifts of the corresponding methine carbons were determined through the HSQC NMR experiment. The unusual lowfield chemical shift (68.3 ppm) of C-5, a non-oxygenated methine, was also found in chrysanthol (**7**) and its derivatives [8]. In the HMBC spectrum (Fig. 2), the correlations (nJ , $n = 2$ or 3) between H-15 and C-3, C-4 and C-5, as well as between H-6 and C-1, C-5, C-7, C-8 and C-11 indicated that the two hydroxy groups were linked to C-4 and C-6, respectively. The correlation between H-8 at $\delta = 5.16$ and the carbonyl carbon at $\delta = 170.3$ showed that the acetoxy group is located at C-8. The double bond at C-9 was also confirmed by the HMBC correlations (nJ , $n = 2$ to 4) between H-14 and C-1, C-8, C-9 and C-10 (Fig. 2).

The relative configuration of **1** was established by the coupling constants (Table 1) in the ^1H -NMR and the correlations in the NOESY spectrum ($\text{DMSO}-d_6$, 343 K). When H-5 was assumed to be in the α -orientation, H-1 should be also α , and both H-6 and H-7 should be β due to the coupling constants between H-5 and H-1 ($J_{1,5} = \text{ca. } 0 \text{ Hz}$), between H-5 and H-6 ($J_{5,6} = 7.4 \text{ Hz}$), and between H-6 and H-7 ($J_{6,7} = \text{ca. } 0 \text{ Hz}$). Moreover, the large coupling constant (11.5 Hz) between H-7 and H-8 requires that these two protons be *trans*-diaxial. Clear NOE correlations (Fig. 2) were observed between H-1 and H-8, between H-5 and H-8, between H-6 and H-7, and between H-6 and H-15. Thus, the structure of **1** was established to be 8β -acetoxy- $4\alpha,6\alpha$ -dihydroxy- $1\alpha,5\alpha$ -(*H*)-guai-9-ene.

The molecular formula $\text{C}_{15}\text{H}_{26}\text{O}_2$ of compound **2** was determined by the ESI-mass spectrum with an $[\text{M} + \text{Na}]^+$ peak at $m/z = 261$, together with its ^{13}C - and ^1H -NMR data (Table 1). Its ^1H -NMR spectrum recorded in CDCl_3 showed general features similar to those of **1**, indicating that **2** also has a *cis*-fused 9 -guaiene skeleton. Compound **2** also showed broadened signals at lower acquisition temperatures as described for **1**. The most obvious difference between these two compounds is that the proton signal at $\delta = 5.07$ (H-8) and the

acetyl methyl at $\delta = 2.09$ in **1** are absent in **2**. This was confirmed by the ^{13}C -NMR (CDCl_3 , 310 K) spectrum of **2** (Table 1), which only showed fifteen carbon signals. All the above data indicated that **2** is an 8-deacetoxy derivative of **1**. This is further supported by its IR spectrum, which did not show any absorption for the ester carbonyl. Therefore, compound **2** was deduced to be 4*a*,6*a*-dihydroxy-1*a*,5*a*-(*H*)-guai-9-ene. Detailed 2 D NMR (COSY, HMQC, HMBC and NOESY) spectra of **2** were in full agreement with the proposed structure.

The molecular formula $\text{C}_{21}\text{H}_{22}\text{O}_5$ of compound **3** was deduced from the positive mode HR-ESI-MS, with an $[\text{M} + \text{Na}]^+$ ion peak at $m/z = 377.1359$ ($\text{C}_{21}\text{H}_{22}\text{O}_5\text{Na}$ requires 377.1364). The IR spectrum of **3** showed absorptions (ν_{max}) at 3476 (hydroxy group), 1721 (ester carbonyl), and 1626, 1608, 1544 (aromatic ring) cm^{-1} . Its UV spectrum exhibited absorption maxima at 221, 248, 258, 268 and 311 nm, which is very similar to those of the furanocoumarins notoptol (**13**) [4] and notopterol (**15**) [2], [4]. In the low-field region of the ^1H -NMR spectrum (500 MHz, CDCl_3), the proton signals of the coumarin part were observed at $\delta = 6.27$, 7.15 and 8.14, and the furan part at $\delta = 6.95$ and 7.60. Both compounds **3** and **13** have a similar monoterpenyloxy side chain at the C-5 position. The smaller coupling constant in **3** ($J_{\text{H-18,H-19}} = 11.7$ Hz) compared with that in **13** (16.0 Hz) [4] indicated that **3** is an isomer of **13** with a *cis*-double bond at C-18 rather than a *trans*-double bond as in **13**. Furthermore, in its ^{13}C -NMR spectrum, the chemical shifts of C-13 to C-16 of **3** are close to those of **13**, but these of C-17 through C-22 are quite different (Table 2). Therefore, both compounds **3** and **13** contain the same geometric configuration of the double bond at C-14, but possess a different geometric configuration of the double bond at C-18. Thus, compound **3** was deduced to be 5-[(2*E*,5*Z*)-7-hydroxy-3,7-dimethyl-2,5-octadienoxy]psoralene.

The molecular formula $\text{C}_{21}\text{H}_{22}\text{O}_6$ of compound **4** was assigned by ESI-MS with a clear $[\text{M} + \text{H}]^+$ peak at $m/z = 371$, together with its ^{13}C - and ^1H -NMR data (Table 2). The IR and UV spectra of **4** were very similar to those of **3**, indicating the presence of hydroxy and furanocoumarin moieties in **4**. The ^1H - and ^{13}C -NMR data (Table 2) showed that both compounds **4** and **3** have the same mono-substituted furanocoumarin skeleton with a different monoterpenyloxy side chain at C-5. The structure of the side chain in **4** was determined by 1 D and 2 D NMR experiments. In the COSY spectrum, two proton spin systems were observed for the side chain: H_2 -13 and H-14 comprise one spin system, while H_2 -17, H-18, H-19, H-21, and H-22 comprise the other. The proton signal of the tertiary Me-16 in **4** exhibited a sharp singlet at $\delta = 1.45$, rather than the broad singlet at $\delta = 1.73$ in **3** (Table 2). The ^{13}C -NMR data indicated that C-15 is an oxygenated quaternary carbon ($\delta = 78.6$). Based on these data and the molecular formula, there should be a ring system in the side chain; we therefore deduced that an ether bond is formed between C-14 ($\delta_{\text{C}} = 83.4$) and C-18 ($\delta_{\text{C}} = 74.3$). This tetrahydrofuran ring structure was confirmed by an HMBC correlation across the oxygen (Fig. 3). Clear HMBC correlations were observed between H-14 and C-13, C-15, C-16, C-17 and C-18, respectively. Therefore, compound **4** was elucidated to be 5-[(2,5)-epoxy-3-hydroxy-3,7-dimethyl-6-octenoxy]psoralene. The relative configuration of the chiral centers at C-14, C-15 and C-18 in the tetrahydrofuran ring were determined by the NOESY spectrum, which showed clear correlations between H-14*a* and

H-17 α , between H-16 β and H-17 β , between H-16 β and H-13/H-13', between H-16 β and H-18 β , and between H-17 β and H-18 β . Moreover, the olefinic proton of H-19 at $\delta = 5.39$ was also detected to show NOE correlation with H-14 α , H-17 α , and H-21, respectively (Fig. 3).

Supplementary Material

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Acknowledgments

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Fig. 1.
Structures of guaiane sesquiterpenes (**1**, **2**) and furanocoumarins (**3**, **4**).

Fig. 2.
Key HMBC and NOE correlations of **1**.

Fig. 3.
Key HMBC and NOE correlations of **4**.

Table 1

¹H- (500 MHz) and ¹³C-NMR (125 MHz) data of **1** and **2**^a

1			2		
No.	δ_{H} (mult, J in Hz) ^b	δ_{C} ^b	δ_{H} (mult, J in Hz) ^c	δ_{C} ^d	δ_{C} ^d
1	2.47 (1H, brd, 10.5)	42.4 ^e	2.58 (1H, brs)	42.0 ^f	39.1 ^f
2	2.22 (2H, m)	23.2 ^e	2.24 (2H, m)	24.3 ^f	23.1 ^f
3	1.18 (1H, m), 1.97 (1H, m)	37.6 ^e	1.20 (1H, m), 2.09 (1H, overlapped)	37.8 ^f	37.3 ^f
4		58.8		59.7	60.5
5	2.57 (1H, brd, 7.4)	68.3	2.76 (1H, brs)	68.4	68.7
6	3.40 (1H, brd, 7.4)	69.8	3.47 (1H, brd, 8.2)	71.0	71.8
7	1.30 (1H, brdd, 11.5, 6.3)	48.8 ^e	1.32 (1H, m)	50.1 ^f	47.0 ^f
8	5.16 (1H, brd, 11.5)	71.1 ^e	5.07 (1H, brs)	73.1 ^f	25.8 ^f
9	5.30 (1H, brs)	128.2 ^e	5.27 (1H, brs)	128.1 ^f	122.8 ^f
10		131.1 ^e		129.8 ^f	137.3 ^f
11	2.01 (1H, m)	25.3	2.09 (1H, overlapped)	26.1	31.7
12	1.07 (3H, d, 6.5)	23.0	1.07 (3H, d, 6.4)	23.4	20.9
13	0.91 (3H, d, 6.5)	20.8	0.93 (3H, d, 6.4)	21.3	21.4
14	1.71 (3H, brs)	19.3 ^e	1.75 (3H, brs)	20.4 ^f	18.3 ^f
15	1.09 (3H, s)	16.0	1.13 (3H, s)	16.1	16.9
OAc	1.99 (3H, s)	170.3, 20.7	2.09 (3H, s)	172.3, 21.1	

^aAssignments were based on 1 D and 2 D NMR experiments (COSY, HSQC and HMBC).

^bIn DMSO-*d*₆ at 343K.

^cIn CDCl₃ at 298K.

^dIn CDCl₃ at 310K.

^eSignals with medium intensity.

^fSignals with weaker intensity and broad.

Table 2¹H- (500 MHz) and ¹³C-NMR (125 MHz) data of **3** and **4** (in CDCl₃, 298 K)^a

No.	3		4	
	¹ H (J values in Hz)	¹³ C ^b	¹ H (J values in Hz)	¹³ C
2		161.2 (161.1)		161.2
3	6.27 (1H, d, 9.5)	112.6 (112.4)	6.26 (1H, d, 9.6)	112.7
4	8.14 (1H, d, 9.5)	139.5 (139.5)	8.21 (1H, d, 9.6)	139.5
5		148.9 (148.8)		148.6
6		114.1 (114.3)		114.1
7		158.1 (158.0)		158.0
8	7.15 (1H, s)	94.2 (94.0)	7.16 (1H, s)	94.6
9		152.6 (152.5)		152.5
10		107.5 (107.4)		107.3
11	6.95 (1H, d, 2.2)	105.0 (105.0)	6.99 (1H, d, 2.0)	104.8
12	7.60 (1H, d, 2.2)	144.9 (144.9)	7.59 (1H, d, 2.0)	145.1
13	4.96 (2H, d, 6.7)	69.7 (69.6)	4.44 (1 h, dd, 9.9, 6.3)	72.8
			4.47 (1H, dd, 9.9, 4.7)	
14	5.59 (1H, brd, 6.7)	119.1 (119.6)	4.23 (1H, dd, 6.3, 4.7)	83.4
15		142.6 (141.7)		78.6
16	1.73 (3H, brs)	17.1 (16.7)	1.45 (3H, s)	22.2
17	3.15 (2H, brd, 7.7)	37.4 (42.0)	1.98 (1H, dd, 12.7, 7.8)	47.9
			2.25 (1H, dd, 12.7, 7.2)	
18	5.34 (1H, dt, 11.7, 7.7)	126.9 (123.5)	4.85 (1H, ddd, 8.5, 7.8, 7.2)	74.3
19	5.61 (1H, d, brd, 11.7)	138.7 (140.6)	5.39 (1H, brd, 8.5)	126.1
20		71.8 (70.5)		136.4
21	1.36 (3H, s)	31.2 (29.8)	1.75 (3H, brs)	25.8
22	1.36 (3H, s)	31.2 (29.8)	1.68 (3H, brs)	18.1

^aAssignments were based on 1 D and 2 D NMR experiments (COSY, HSQC and HMBC).^bThe ¹³C-NMR chemical shifts of compound **13** in the parentheses are consistent with the reported data for notoptol [4].