

Notes

New Lignan Isolated from *Phyllanthus niruri* Linn. Structure Elucidation by NMR Spectroscopy

Wan-Xing Wei, Xing-guo Gong,[†] Omar Ishrud, and Yuan-Jiang Pan*

Department of Chemistry, Zhejiang University, Hangzhou 310027, P.R. China

[†]College of Life Sciences, Zhejiang University, Hangzhou 310027, P.R. China

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Phyllanthus niruri linn was a well-known medicinal plant that grows in many countries, and was used as a medicine for the treatment of hepatitis disease in India.¹ Many other studies on this plant showed that it has many bioactivities.²⁻¹² Many works had been done on the chemical compositions of this plant.¹³⁻¹⁶ The interesting biological activities of this plant prompted us to work on this specie. We report here the isolation and structure of a new compound by a combination of NMR techniques, including ¹H-¹H COSY, ¹³C-¹H COSY, DEPT, HMBC and NOESY.

Neonirtetralin (**1**) has a molecular formula as C₂₄H₃₀O₇ that was determined by EI-MS and elemental analysis. The ¹H, ¹³C, and DEPT NMR spectra of **1** (see Table 1) showed the signals for five methyl groups, four methylene groups, seven methine groups (four aromatic methine groups among

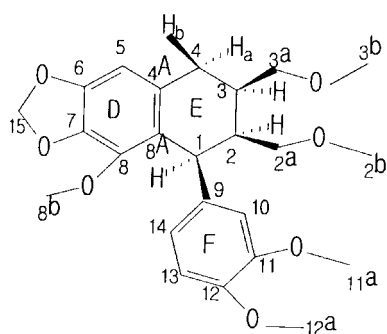


Figure 1. Configuration of compound (**1**).

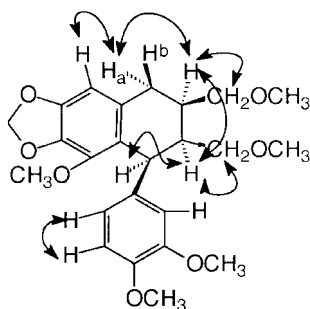


Figure 2. Principal NOESY correlations in **1**.

Table 1. ¹H, ¹³C chemical shift and principal HMBC correlations of (**1**) in CDCl₃

Atom	¹ H (Multiplicity)	¹³ C	HMBC (Carbon)
1	4.26(1H)	41.64	3, 2, 2a, 10, 14, 8A, 4a, 9, 8
2	1.98(1H)	45.56	4, 3, 1, 3a, 2a, 9, 8a
3	1.81(1H)	37.16	—
4a	2.69(1H)	33.61	3, 2, 3a, 5, 8a, 4A, 6, 8, 7
4b	2.55(1H)	33.61	3, 2, 3a, 5, 8a, 4A, 6, 8,
5	6.37(1H)	103.12	4, 8a, 6, 8, 7
6	—	135.79	4, 8a, 6, 8, 7
7	—	147.64	—
8	—	142.08	—
2a	3.24(2H)	73.64	2, 4
3a	3.24(2H)	76.24	3
2b	3.31(3H)	59.14	2a
3b	3.26(3H)	59.10	3a
4A	—	132.14	—
8A	—	125.02	—
8b	3.45(3H)	59.20	8
15	5.82(2H)	101.35	6, 7
9	—	139.95	—
10	6.68(1H)	112.10	1, 14, 9, 11, 12
11	—	148.64	—
11a	3.79(3H)	56.11	11
12a	3.79(3H)	56.03	12
12	—	147.02	—
13	6.68(1H)	110.88	1, 14, 9, 11, 12
14	6.53(1H)	120.10	1, 13, 10, 11, 12

Multiplicity was provided by DEPT (90° and 135°) spectra

these seven methine groups) and eight quaternary carbons.

Compound (**1**) is a stereo-isomer of nirtetralin.¹³ Their NMR data and other properties are different. When sprayed with 5% sulfuric acid in methanol on a silica gel plate and then heated, compound (**1**) had a blue black spot and nirtetralin formed a violet spot.¹³

In ¹H-¹H COSY spectrum, the proton signal at δ 4.26 ppm (H-1) and the proton signal at δ 1.98 ppm (H-2), H-2 and the proton signal at δ 1.81 ppm (H-3), H-3 and the proton signal

at δ 2.69 ppm (H-4a) and δ 2.55 ppm (H-4b) had cross-peaks. Meanwhile H-2 and the proton signal at δ 3.24 ppm (H-2a), H-3 and the proton signal at δ 3.24 ppm (H-3a), the proton signal at δ 6.68 ppm (H-13) and the proton signal at δ 6.53 ppm (H-14) had cross-peaks. Those cross-peaks revealed the facts that H-1 was proximate to H-2, H-2 was proximate to H-3, H-3 was proximate to H-4, and H-2 and H-3 were proximate to H-2a and H-3a, respectively. H-13 was proximate to H-14. The HMBC data supported the structure elucidation of compound (1). The proton signal at δ 4.26 ppm (d, $J = 6.28$) and carbon signal at δ 41.64 ppm were assigned to H-1 and C-1, respectively. The carbon signal at δ 37.16 ppm correlated with the proton signal at δ 4.26 ppm (d, $J = 6.28$ Hz) which showed cross-peaks with the carbon signals at δ 45.56 ppm (C-2), δ 73.64 ppm (C-2a), δ 112.10 ppm (C-10), δ 120.10 ppm (C-14), δ 125.02 ppm (C-8a), δ 132.14 ppm (C-4A), δ 139.95 ppm (C-9) and δ 142.08 ppm (C-8). These indicated that ring F was located at the C-1 position. The carbon signal at δ 135.79 ppm (C-6) correlated with the proton signal at δ 5.82 ppm (H-15) which showed cross-peak with the carbon signal at δ 147.64 ppm (C-7). Proton signal at δ 6.37 ppm (H-5) had cross-peaks with carbon signals at δ 33.61 ppm (C-4), δ 135.79 ppm (C-6), δ 147.64 ppm (C-7), and δ 125.02 ppm (C-8A), δ 142.08 ppm (C-8). These evidences indicated that the methenedioxy group was connected to C-6 and C-7. Meanwhile the HMBC correlations from the carbon signal at δ 73.64 ppm (C-2a) to the proton signal at δ 1.98 ppm (1H, m, $J = 4.80$ Hz, H-2), the carbon signal at δ 76.24 ppm (C-3a) to the proton signal at δ 1.81 ppm (1H, m, $J = 4.45$ Hz, H-3), the carbon signal at δ 59.10 ppm (C-3b) to the proton signal at 3.24 ppm (2H, m, H-3a), the carbon signal at δ 59.14 ppm (C-2b) to the proton signal at δ 3.24 ppm (2H, m, H-2a), and their DEPT spectrum indicated that C-2a and C-3a were methylene group respectively and these two carbons connected to methoxy group respectively. HMBC spectrum also showed that proton signal at δ 3.79 ppm (3H, s, H-11a) correlated to the carbon signal at δ 148.64 ppm (C-11), the proton signal at δ 3.79 ppm (3H, s, H-12a) correlated to the carbon signal at δ 147.0 ppm (C-12), and the proton signal at δ 3.45 ppm (3H, s, H-8b) correlated to the carbon signal at δ 142.0 ppm (C-8). These evidences and DEPT spectrum revealed that C-11, C-12 and C-8 connected with methoxy group respectively. The HMBC correlations of proton signal at δ 6.53 ppm (1H, m, $J = 6.95, 1.35$ Hz, H-14) to the carbon signals at δ 41.64 ppm (C-1), δ 110.88 ppm (C-13), δ 112.10 ppm (C-10) and the proton signal at δ 6.68 ppm (1H, d, $J = 1.35$ Hz, H-10) to δ 41.46 ppm (C-1), δ 139.95 ppm (C-9), δ 148.64 ppm (C-11), δ 147.02 ppm (C-12), δ 120.10 ppm (C-14), and ^1H - ^1H COSY of H-14, H-13 showed that H-13 and H-14 located at *ortho* position, H-14 and H-10 at *meta* position in ring F. The coupling constants of H-13 and H-14 ($J = 6.95$ Hz) and of H-14 and H-10 ($J = 1.35$ Hz) support the proton locations at the ring F. The correlation of proton signal at δ 6.37 ppm (H-5) with carbon signal at δ 33.61 ppm (C-4) in HMBC, and the NOESY evidences of protons signals at δ 6.37 ppm (H-5) and δ 2.69 ppm (4a) confirmed

that the carbon signal at δ 33.61 ppm connected to C-5 at ring C. Meanwhile the correlations of the proton signal at δ 4.26 ppm (H-1) with the carbon signal at δ 142.08 ppm (C-8), δ 125.02 ppm (C-8A), and the proton signal at δ 3.45 ppm (H-8) with the carbon signal at δ 142.08 ppm (C-8) in HMBC indicated that the carbon signal at δ 41.64 ppm (C-1), which connected with the proton (H-1), connected to the aromatic carbon signal at δ 125 ppm (C-8A).

The NOESY of compound (1) confirmed the structure (see Fig. 1). Cross-peaks of proton signal at δ 4.26 ppm (H-1) with the proton signal at δ 1.98 ppm (H-2), the proton signal at δ 1.98 ppm (H-2) with the proton signal at δ 1.81 ppm (H-3), the proton signal at δ 1.81 ppm (H-3) with the proton signals at δ 2.69 ppm (H-4a) and the proton signals at δ 2.69 ppm (H-4a) with the proton signals at δ 6.37 ppm (H-5) appeared in the NOESY spectrum. Those NOESY spectrum evidences showed that H-1 and H-2, H-2 and H-3, H-3 and H-4a, H-4a and H-5 were proximate in space. H-5 was in the plane of benzene ring, and it had NOE with H-4a. It means that H-4a was at the plane, which connected to the benzene ring. The protons at 1, 2, 3 positions were linked to relative carbons in equator orientation. If one of H-1, H-2, H-3 was at the axial orientation just as in nirtetralin, the three hydrogen would not have NOE each other. It was deduced that H-1, H-2, H-3, H-4 linked to correlative carbons respectively at the equator orientation. The NOESY evidence of H-4 and the proton signal at δ 6.37 ppm (H-5) revealed the fact that the another only one proton had to locate at 5 position in ring C. The coupling constants of these protons supported the fact. The coupling constant between H-1 and H-2 was 6.18 Hz, the coupling constants between H-2 and H-3, H-3 and H-4 were 4.25 Hz and 4.51 Hz, respectively. On the basis of above evidences, the three protons at C-1, C-2, C-3 were assigned to be in the β, β, β orientation. Therefore, neonirtetralin (1) was elucidated to be (1 β ,2 β ,3 β)-2,3-dimethoxymethyl-6,7-methenedioxy-8-methoxy-1-(11,12-dimethoxyphenyl) naphthalene. Structure of neonirtetralin, and its configuration was showed in Figure 1.

Compound (2) was elucidated as which spectral data was in good agreement with those of previous reported phyllanthin¹⁴ and compound (3) was elucidated as hypophyllanthin on the basis of those of reported compound from literature.^{15,16}

Experimental Section

General methods. ^1H -NMR (500 MHz) and ^{13}C -NMR (125 MHz) were recorded on a Bruker AM-500 spectrometer. Sample dissolved in CDCl_3 for NMR studies. Chemical shifts were reported in ppm. Coupling constants (J -Values) were given in Hertz (Hz). EIMS spectrum was recorded using HP5989B MASS spectrometer. Elementary analysis was performed on a PE2400 CHN elemental analyzer.

Plant material: The plant material was collected from Longan county, Guangxi province at August 2001 and was identified as *Phyllanthus niruri* L. A voucher specimen was

kept in the botany Department of Zhejiang University.

Isolation and purification. The air dried *P. niruri* (3.5 kg) was extracted with ethanol, with constant mechanical stirring; this procedure was repeated until no further extract bud was obtained. The combined extracts were filtered while warm through glass wool, concentrated. The residue (120 g) was extracted successively with petroleum ether and ethyl acetate. The mixture of petroleum ether extracts and ethyl acetate extracts was centrifuged, and concentrated to dryness and got 15 g residue. Chromatography of the 15 g residue was applied in silica gel (40-60 μm) column using the solvent system of petroleum ether and ethyl acetate as eluent. The chromatography different polarities systems demonstrated after 15×500 mL eluent was applied. The fraction obtained at a ratio of 20 : 3 of petroleum ether and ethyl acetate (volume ratio) gave 250 mg liquid residue and, was purified by vacuum liquid chromatography and preparative TLC to give 78 mg of pure compound (**1**). The fraction obtained at a ratio of 20 : 4 of petroleum ether and ethyl acetate gave a solid residue (450 mg). This solid residue was crystallized repeatedly in methanol to give 250 mg compound (**2**). The fraction obtained at a ratio of 3 : 1 of petroleum ether and ethyl acetate gave solid residue (1.35 g). This 1.35 g residue was crystallized in methanol repeatedly to give 660 mg compound (**3**).

Neonirtetralin: colorless oil. EIMS m/z : 430, and elemental analysis C67.0% H7.0% (calcd. 430.49, 66.96C%, H7.02%) for $\text{C}_{24}\text{H}_{30}\text{O}_7$. ^1H , ^{13}C , DEPT NMR spectral data and principal HMBC are listed in Table 1. Most of NOESY correlations are in Figure 2.

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