Homocyclotirucallane and Two Dihydrophenanthrenes from *Spiranthes sinensis*

Yun-Lian Lin,*a* Wan-Yi Wang,*a* Yuch-Hsiung Kuo,⁎b and Yi-Hung Liu⁎b

National Research Institute of Chinese Medicine,*a* Taipei 112, Taiwan and Department of Chemistry, National Taiwan University,*a* Taipei 106, Taiwan. Received March 9, 2001; accepted May 10, 2001

A novel homocyclotirucallane, sinetirucallol (1), and two additional new dihydrophenanthrenes, sinensols G (2) and H (3), were isolated from the aerial parts of *Spiranthes sinensis* (Pers.) AMES. Their structures were determined by various spectral analyses, including MS and two-dimensional nuclear magnetic resonance techniques. The structure of compound 1 was further confirmed by single-crystal X-ray analysis. The absolute configuration of 1 was determined by modified Mosher's method.

**Key words** *Spiranthes sinensis*; Orchidaceae; aerial part; homocyclotirucallane; dihydrophenanthrene

In a previous paper,¹ we have described the characterization of six new dihydrophenanthrene derivatives (sinensols A—F) together with five known dihydrophenanthrenes from the aerial parts of *Spiranthes sinensis* (Pers.) AMES. (Orchidaceae), which has been used as a folk drug in Taiwan.²,³ Our continuing chemical studies on this plant has resulted in the isolation of a novel homocyclotirucallane, sinetirucallol (1), and two new dihydrophenanthrene derivatives, sinensols G (2) and H (3), together with spiranthesol,⁴ 2-(3',4'-dihydroxyphenyl)-1,3-benzodioxole-5-aldheyde,⁵ ergosterol per-oxide,⁶ and a series of phenolic compounds, p-hydroxybenzaldehide,⁷ 3,4-dihydroxybenzaldehide,⁷ 3,4-dihydroxybenzyl alcohol,⁹ hydroquinone,⁷ 4-hydroxybenzyl methyl ether,¹⁰ 4-hydroxybenzyl methyl ether,¹¹ and methyl 3-(4-hydroxyphenyl)propanoate.¹² This paper reports the isolation and the structural elucidation of the novel homocyclotirucallane (1) and two new additional dihydrophenanthrene derivatives, sinensol G (2) and sinensol H (3).

**Results and Discussion**

The EtOH extract of the aerial parts of *S. sinensis* (Pers.) AMES was fractionated into EtOAc-soluble, n-BuOH-soluble and H₂O-soluble fractions. The cytotoxic EtOAc-soluble fraction was further subjected to repeated column chromatography to afford a novel homocyclotirucallane (1) and two additional dihydrophenanthrenes, 2 and 3.

Compound 1 was obtained as colorless needles. It gave the molecular formula C₃₁H₅₂O₃ from HR-EI-MS. The EI-MS showed fragment ions at *m/z* 425 [M–CH₃]⁺, 407 [M–CH₃–H₂O], and 313 [M–side chain–2H]⁺. Its IR had hydroxyl (3381, 1097 cm⁻¹) and olefinic (1616, 1590, 1494 cm⁻¹) absorptions. The ¹H-NMR spectrum (see Experimental) revealed two characteristic cyclopropane protons ¹Hₙ 0.67 (C-18). Such data indicated that the compound was a member of the Δ⁸- euphane or Δ⁸-tirucallane series of compounds.¹³,¹⁴ And a strong ion at M⁺–15 also supported the assignment of a double bond to C-9 (11) for an allylic cation resulted from this process. The negative optical rotation ([α]D⁵⁻⁵.5°) revealed that it belongs to the tirucallane rather than euphane series.¹⁴ The side chain and the cyclopropane of 1 were deduced from 1D total correlation spectroscopy (TOCSY1D), heteronuclear multiple bond correlation (HMBC) and nuclear Overhauser enhancement spectroscopy (NOESY) experiments. From TOCSY1D spectrum, the contiguous protons from cyclopropane H-31 are as follows: H-31, H-24, H-22, and H-20. In addition, long-range correlations (HMBC) and NOE correlations observed between the cyclopropane protons and H-26, -27 confirmed the two methyl groups (H₇-26, -27) connecting to the terminal carbon of the cyclopropane. The signal at δ 3.22 [[dd, J = 10.8, 5.5 Hz]] was assigned as H-3 being on α-axial orientation due to the larger coupling constant and having HMBC correlations with C-4, C-28, and C-29. From the above evidence, the structure of 1 was established as 24,31-homocyclotirucall-9(11)-ene-3β-ol, a novel homocyclotirucallane skeleton. The relative configuration of 1 was determined by NOESY technique (see Experimental) and X-ray single-crystal analysis (Fig. 2). Its absolute configuration was determined by the modified Mosher’s method.¹⁵,¹⁶ Treatment of 1 with [(S)- and (R)-2-methoxy-2-trifluoromethyl-2-phenylacetyl chloride (MT-PACI) afforded (S)- and (R)-MTPA esters (1a, 1b, respectively). The ¹H-NMR signals of the two derivatives were assigned by TOCSY1D. The Δδ values (δ_S–δ_R in Hz) of the individual protons of ring A are shown in Fig. 1. The systematic arrangement of positive and negative Δδ values indicated that the configuration of C-3 is R.

Compound 2 was isolated as a colorless amorphous powder, and gave the molecular formula C₂₀H₂₂O₃ from HR-EI-MS. The IR spectrum indicated hydroxyl (3417, 1158 cm⁻¹), and aromatic ring (1616, 1590, 1494 cm⁻¹) absorptions. Its ¹H-NMR showed signals (see Experimental) due to four protons [δ 2.71 (4H, m)] typical H-9 and H-10 signals of 9,10-dihydrophenanthrenes,¹⁷ an isopentenyl group [δ 5.38 (1H, t, J = 7.0 Hz), 3.40 (2H, d, d = 7.0 Hz), 1.79 and 1.81 (3H each, s)], a methoxyl [δ 3.81 (3H, s), two meta-phenyl protons [δ 6.37, 6.43 (d, d = 2.0 Hz)] and two para-phenyl protons [δ
6.72, 7.78 (s, 1H each)]. The relatively lower field at δ 7.78 was assigned as H-5 due to the deshielding by the C-4 hydroxyl group on the adjacent aromatic ring. The 13C-NMR indicated three oxygenated aromatic carbons (δ C 152.8, 153.5, 158.9). The HMBC correlations, CH₃O (δ 3.81)/C-2; H-1 (δ 6.98)/C-5, -6, -7, -2, and -3; H-9 (δ 7.11)/C-1, -4a, -4b, -8, -8a, and -10a; H-1/C-2, -3, and -4a, and H-3/C-1, -2, and -4, determined the situation of the methoxyl, isopentenyl, and hydroxyl groups on the dihydrophen-anthrene. From these spectral data, 2 was identified as 2-methoxy-4,7-dihydroxy-6-isopentenyl-9,10-dihydrophenanthrene. This assignment was further confirmed by the NOE correlations as follows: CH₃O (δ 3.81)/H-1, -3; H-1 (δ 3.40)/H-5 (δ 7.78).

Table 1. Crystal Data and Structure Refinement for IC7156

- Identification code: ic7156
- Empirical formula: C₃₃H₅₈O₆
- Formula weight: 470.79
- Temperature: 150(2) K
- Wavelength: 0.71073 Å
- Crystal system: Monoclinic
- Space group: C2
- Unit cell dimensions:
  - a = 44.2182 (5) Å, alpha = 90°
  - b = 9.04440 (10) Å, beta = 97.7020 (10)°
  - c = 7.44880 (10) Å, gamma = 90°
- Volume, Z: 2952.10 (6) Å³, 4
- Density (Calculated): 1.059 mg/m³
- Absorption coefficient: 0.061 mm⁻¹
- F(000): 1056
- Crystal size: 0.40 x 0.30 x 0.20 mm
- θ range for data collection: 0.93 to 27.50°
- Limiting indices: h00, k00, l00
- Reflections collected: 14545
- Independent reflections: 6758 (R(int) = 0.0235)
- Absorption correction: Used SADABS
- Max. and min. transmission: 0.830 and 0.781
- Refinement method: Full-matrix least-squares on F²
- Data/restraints/parameters: 6463/1/308
- Goodness-of-fit on F²: 1.045
- R indices [I>2σ(I)]:
  - R1 = 0.0922, wR2 = 0.2400
  - R indices (all data):
  - R1 = 0.1136, wR2 = 0.2653
- Absolute structure parameter: 0 (5)
- Extinction coefficient: 0.0008 (8)
- Largest diff. peak and hole:
  - 0.866 and -0.340 e Å⁻³

Note: Solvent: C₂H₆
typical H-9, H-10 of dihydrophenanthrene protons [δ 3.35 (2H, m), δ 7.21 (2H, m)], and a methoxyl group [δ 3.78 (3H, s)]. Its NMR spectrum showed six oxygenated carbons (δc 155.9, 156.0, 156.1, 156.1, 156.4) and five exchangeable phenolic protons [δ 4.59 (br s), exchangeable]. The key HMBC correlations, H-1/C-7 (δc 155.9), -8, -8a; H-1/C-1, -2 (δc 158.4), -10a; H-3/C-1, -2, -4 (δc 156.1), -4a, and OCH3/C-2, suggested the connection of two 4-hydroxybenzyl and methoxyl groups at C-1, C-8, and C-2, respectively, while two hydroxylated carbons were located at C-4 and C-7. This assignment was further confirmed from the NOE correlations of H-1'/H-9, H-1'/H-10, OCH3/H-3, H-3'(7')/H-9, and H-3'(7'')/H-10 in its NOSY spectrum.

Acetylation of 3 with Ac2O/pyridine yielded triacetate (3a) (δ 2.17, 2.28, 2.28). The IR spectrum of 3a showed hydroxyl (3459 cm⁻¹), ester (1763, 1202 cm⁻¹), and aromatic ring (1600, 1515 cm⁻¹) absorptions. Two phenolic hydroxy groups [δ 4.67 (br s), exchangeable] resisted acetylation due to the steric effect from the benzyl and phenyl groups. The chemical shift of the two oxygenated carbons at δc 155.9 (C-7) and 156.0 (C-5) excluded a catechol-type composition. Therefore, the structure of 3 can be assigned as 4,5,7-trihydroxy-1,8-bis(4-hydroxybenzyl)-3-methoxy-6-phenyl-9,10-dihydrophenanthrene.

Experimental

General Experimental Procedures Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP-370 polarimeter. IR spectra were recorded on a Nicolet avatar 320 FT-IR spectrophotometer. UV spectra were measured on a Hitachi U-3200 spectrophotometer. NMR were run on a Bruker AC-300 and a Varian unity INOVA-500 spectrometer. Mass spectra (EI-MS and HR-ESI-MS) were taken on a JEOL JMS-HX110 and a JEOL SX-102A instrument, respectively.

Plant Material

The aerial parts of Sprantasis sinensis (Pers.) Ames. were purchased from a local herbal medicine store, Taipei, Taiwan, in April, 1999, and identified by comparison with the voucher specimens already deposited at the Herbarium of the Department of Botany, National Taiwan University, Taipei, Taiwan (no. TAIE 218182, collected on April 12, 1934).

Extraction and Isolation

The aerial parts of Sprantasis sinensis (Pers.) Ames. (3 kg) were extracted with EtOH (50 l) at 50 °C three times (8 h each time). The EtOH extract was evaporated under reduced pressure. The concentrate was taken up in H2O, and partitioned into EtOAc-soluble, nBuOH-soluble and H2O fractions. The EtOAc-soluble fraction (35 g) was subjected to column chromatography over silica gel using an n-hexane-EtOAc-methanol gradient. The fractions of 25—33% ethyl acetate were further separated on a Si gel column to yield 1 (645 mg), phytosterol (465 mg), and ergosterol peroxide (48 mg). The fraction of 5—10% methanol/ethyl acetate yielding three fractions were further purified by Sephadex LH-20 (Baker, ME). MeOH/H2O:1 11:9 (1.5 l, A, methyl), 60, 7 µm, using n-hexane-EtOAC=2:1 or 1:1 column chromatography, yielded 2 (32 mg), 3 (18 mg), and a series of phenolic compounds, 4-hydroxybenzaldehyde (112 mg), 3,4-dihydroxybenzaldehyde (345 mg), 3,4-dihydroxybenzyl alcohol (48 mg), hydroquinone (65 mg), 4-hydroxybenzyl ethyl ether (59 mg), 4-hydroxybenzyl methyl ether (98 mg), methyl 3-(4-hydroxyphenyl)propanoate (83 mg).

Sinetirucallic (1): Colorless needles, mp: 96—97 °C, [α]D25 +66° (c=0.5, CHCl3). IR νmax (KBr cm⁻¹): 3381, 3045, 2940, 2869, 1625, 1515, 1097. 1H-NMR (500 MHz, CDCl3): δ 10.8 (1H, br s, H-1), 6.37 (1H, d, J=5.5 Hz, H-5), 5.38 (1H, d, J=4.6 Hz, H-31), 3.67 (2H, d, J=4.0 Hz, H-31), 0.33 (dd, J=10.8, 5.5 Hz, H-3), 26.0 (q, C-4), 29.6 (t, C-9), 30.2 (t, C-1), 36.0 (d, C-20), 36.7 (t, C-16), 37.5 (s, C-10), 37.8 (t, C-12), 39.2 (s, C-11), and aromatic ring (1600, 1515 cm⁻¹) absorptions. 13C-NMR (125 MHz, CDCl3): δc 149.9 (C-15), 135.1 (s, C-3); 128.4 (2C, C-4); 128.0 (2C, C-7); 124.3 (2C, C-8); 122.3 (3C, C-10 or 11); 121.0 (2C, C-12 or 13); 117.8 (2C, C-22 or 23); 116.5 (2C, C-14); 114.6 (2C, C-17); 112.2 (2C, C-19 or 21); 109.1 (1C, C-1); 101.2 (1C, C-5); 81.6 (3C, C-18, C-26, C-27); 69.9 (2C, C-24, C-25); 51.5 (2C, C-13); 42.9 (2C, C-28, C-29); 35.2 (2C, C-15 or 16); 33.3 (2C, C-19 or 20); 31.5 (2C, C-30 or 31); 28.1 (1C, C-28, C-29); 26.6 (3C, C-32); 25.4 (3C, C-24, C-25); 24.6 (C, C-23); 22.9 (2C, C-14 or 21); 18.4 (2C, C-19); 18.1 (C, C-18); 14.9 (2C, C-12); 13.9 (2C, C-11); 13.8 (2C, C-10); 13.9 (2C, C-9), 13.9 (C, C-4), 39.3 (C, C-3), 40.5 (C, C-8); 44.0 (C, C-13); 44.5 (C, C-5); 46.6 (C, C-14); 50.8 (C, C-27), 79.1 (C, C-3), 116.9 (C, C-5), 150.4 (C, C-9), HMBC correlations: H-3/C-1, -2, -4, -5, -28, -29; H-18/C-12; -13, -14, -17; H-11/C-8, -9, -10, -12, -13; H-19/C-5, -5, -9, -10; H-24/C-22, -23, -25, -26, -27, -31; H-31/C-23, -24, -25, -26, -27. NOE correlations: H-3/C-1, -2, -4, -5, -28, -29; H-8/C-11, -12, -19, -20/18-17, -21; -22, -24/C-23, -31; H-31/C-24, -25, -26, -27. EI-MS (20eV) m/z (rel. int.): 440 [M⁺] 58, 425 (100), 313 (45), 259 (9), 220 (31), 123 (12), 95 (28), 83 (10), 69 (15). HR-ESI-MS m/z 440.4013 (Calcd for C35H38O2: 440.4018).
4), 158.9 (s, C-2), HMBC correlations: H-1/C-2, -3, -4a, -10a, -10; H-3/C-1, -2, -4a, -15/C-4a, -4b, -6, -7a; H-8/4b, -6, -7, H-1/C-5, -6, -7, -2', -3', H-9(10)/C-1, -4a, -8a, -8; H-4'(5)'C-2', -3'. NOE correlations: OCH3/H-1, -3, H-9/H-8; H-10/H-1, H-1/H-5. El-MS (20 eV) m/z 310 ([M]+), 254 (100), 243 (62), 241 (15). HR-El-MS m/z 310.1569 (Caled for C35H30O6: 310.1569).

Sinensol H: Pale yellow amorphous powder. [ε]20° (c=0.5, MeOH), IR νmax (KBr) cm⁻¹: 3585, 3028, 1615, 1599, 1497, 1103, 910-920 cm⁻¹.

UV λmax (MeOH) nm (log ε): 288 (4.12), 283 (4.10). ¹H-NMR (500 MHz, CDCl₃): δ 2.25 and 2.71 (2H each, m, H-2, H-9), 3.78 (3H, s, H-5), 6.63 and 6.68 [2H each, d, J = 8.5 Hz, H-4' (6'), -3' (7')], 6.65 and 6.93 [2H each, d, J = 8.5 Hz, H-4'' (6''), -3'' (7'')], 6.98 (2H, d, J = 8.5 Hz, H-2'' (6''), -7''), 7.11 (1H, dd, J = 8.5, 9.0 Hz, H-4''), 7.21 (2H, dd, J = 8.5, 9.0 Hz, H-3'' (5''), -5''). ¹³C-NMR (125 MHz, CDCl₃): δ 31.1 (t, C-1'a), 31.2 (t, C-1), 33.4 (t, C-10), 37.7 (t, C-9), 55.9 (OCH3), 98.0 (d, C-3), 115.8 (s, C-6), 115.8 (d, C-4', -6''), 115.9 (d, C-4', -6''), 119.5 (s, C-4a), 119.5 (s, C-1), 120.3 (s, C-8), 126.8 (d, C-4, -5a), 129.1 (s, C-4b), 129.2 (d, C-3'', -5''), 129.2 (d, C-2'', -6''), 129.9 (d, C-3', -7'), 130.1 (d, C-3', -7'), 134.3 (s, C-2'), 142.7 (s, C-17), 142.7 (s, C-10a), 143.6 (s, C-8a), 155.9 (s, C-7), 156.0 (s, C-5), 156.1 (s, C-4), 156.1 (s, C-5'), 158.4 (s, C-2). Key HMBC correlations: H-3/C-1, -2, -4a, H-1/C-1', -7, -8a, -2', -3' (7'); H-1'/C-1', -2, 10a, -2', -3' (7'); H-1'/C-1, -2, 10a, -2', -3' (7'); H-2''(6'')C-1, -2, -3'' (7''); H-2''(6'')C-1, -2, -3'' (7''). Key NOE correlations: H-1'/H-9, -3' (7'); H-1'/H-10, -OCH3, -3' (7'). H-3/OCH3. El-MS (20 eV) m/z (rel. int.): 546 ([M]+), 5, 440 (100), 334 (65), 255 (72), 243 (40), 107 (85), 77 (15). HR-El-MS m/z 546.2441 (Calcd for C35H30O6): 546.24034.

(5)- and (R)-MTPA Ester of 3 Compounds (3 mg) were acetylated with Ac₂O (0.5 ml) in pyridine (0.5 ml) at 25 °C. The solvent and excess reagent were then removed under reduced pressure. Purification with Si gel column gave triacetate (3a, 4 mg): IR νmax (KBr) cm⁻¹: 3459, 3050, 1763, 1600, 1515, 1506, 1459, 1202, 1167, 1104, 1044, 1018, 904. ¹H-NMR (500 MHz, CDCl₃): δ 2.17 (3H, s), 2.28 (6H, s), 2.50 and 2.83 (2H each, m), 3.78 (3H, s, OCH3), 3.94 (2H, s), 4.10 (2H, s), 6.63 (1H, s), 6.93 and 7.07 (2H each, d, J=8.0 Hz), 6.97 and 7.09 (2H each, d, J=8.0 Hz), 7.00 (2H, d, J=8.5 Hz), 7.18 (1H, t, J=8.5 Hz), 7.22 (2H, t, J=8.5 Hz).

X-Ray Crystal Structural Analysis of 1 A colorless crystal of 1 with a = 0.40 x 0.30 x 0.20 mm was selected for X-ray analysis. Structure determination was performed by using the SHELXTL program on PC. Data were collected over a hemisphere of reciprocal space, by a combination of three sets of exposures. The compound crystallized in the monoclinic space group C2, a=4.2182 (5) Å, b=9.04440 (10) Å, c=7.44880 (10) Å, β=97.7020 (10)°, V=2925.10 (6) Å³, Z=4, D_cal=1.059 mg·m⁻³, λ=0.71073 Å, μ(MoKα) = 0.061 mm⁻¹, F(000)=1056, and T=150 (2) K. The SMART program was used to make data correction. A total of 15454 reflections, collected in the range 9.3°<θ<27.5°, yielded 6758 unique reflections. The structure was solved using direct methods and refined by full-matrix least-squares on F² values for 6463 reflections with I>2σ(I). Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were R=0.0922, R_w=0.136 with goodness-of-fit=1.045. Scattering factors were taken from the International Tables for X-ray Crystallography.

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References