Studies on the Constituents of Orchidaceous Plants. VIII.[1,2] Constituents of *Spiranthes sinensis* (PERS.) AMES var. *amoena* (M. BIEBERSON) HARA. (1). Isolation and Structure Elucidation of Spiranthol-A, Spiranthol-B, and Spirasineol-A, New Isopentenyldihydrophenanthrenes

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Constituents of *Spiranthes sinensis* (PERS.) AMES var. *amoena* (M. BIEBERSON) HARA (Japanese name "nezibana") were examined and several new dihydrophenanthrenes were isolated along with orchinol (1), *p*-hydroxybenzaldehyde, *p*-hydroxybenzyl alcohol, hydrocarbons, a sterol mixture, and a ferulate mixture (5). The structures of three new dihydrophenanthrenes, spiranthol-A (2), spiranthol-B (3), and spirasineol-A (4), were determined by spectroscopic methods including two-dimensional nuclear magnetic resonance techniques. These compounds are the first examples of natural dihydrophenanthrenes containing an isopentenyl substituent.

Keywords *Spiranthes sinensis* var. *amoena*; Orchidaceae; spiranthol-A; spiranthol-B; spirasineol-A; orchinol; dihydrophenanthrene; *p*-hydroxybenzaldehyde; *p*-hydroxybenzyl alcohol; ferulic acid ester

The crude drug "Chheng-thian liong-thiau" (青天竜桂) in Taiwan is the dried whole plants of *Spiranthes sinensis* (PERS.) AMES (Orchidaceae), and is used as a folk remedy for treatment of hemoptysis, epistaxis, headache, chronic dysentery, meningitis, and as a tonic.[3] In China, the same plant is called "Pan-long-sheng" (盤竜参)[4] and is used as a crude drug in the treatment of fever, cough, hemoptysis, vertigo, low back pain, and so on.[5] In a recent study, Lin and Namba[6] showed that not only *S. sinensis*, but also *S. sinensis* (PERS.) AMES var. *amoena* (M. BIEBERSON) HARA (Japanese name "nezibana")[7] is used as a source of both of these crude drugs. In the course of our chemical study on Orchidaceous medicinal plants, we examined the constituents of *S. sinensis* var. *amoena* and isolated a known dihydrophenanthrene derivative, orchinol (**1**, DHP-II), and seven new dihydrophenanthrenes (DHP-I and DHP-III to VIII). This paper deals with the isolation and identification of orchinol (**1**) and with the structure elucidation of three new dihydrophenanthrenes named spiranthol-A (**2**, DHP-III), spiranthol-B (**3**, DHP-I), and spirasineol-A (**4**, DHP-VIII).

The plant material, collected at Toyama in June 1987, was divided into the underground part (roots) and the aerial part, and each was cut into small pieces and extracted successively with ether and methanol under reflux to give the ether and methanolic extracts. Among these, the ether extract from the roots showed a mild cytotoxic activity toward HeLa-S_3 cells and thus examination of the constituents of this ether extract was undertaken. As shown in Chart 2, the ether extract was roughly separated by silica gel column chromatography and each fraction was further separated by preparative thin-layer chromatography (TLC) to give *p*-hydroxybenzaldehyde, *p*-hydroxybenzyl alcohol, and eight dihydrophenanthrenes (DHP-I to VIII), along with fractions containing hydrocarbons, fatty acids, sterols, and ferulic acid esters.

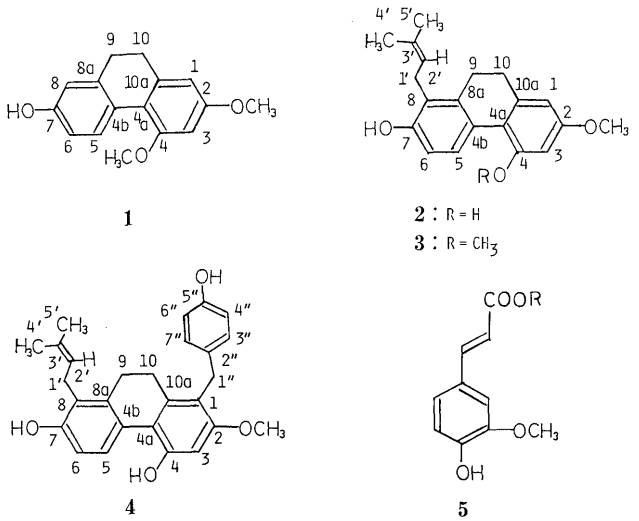

Chart 1

Among these, *p*-hydroxybenzaldehyde and *p*-hydroxybenzyl alcohol were identified by direct comparisons with authentic samples and the hydrocarbons were identified as tricosane, tetracosane, pentacosane, hexacosane, heptacosane, octacosane, and nonacosane in a ratio of 3:3:28:4:36:7:19 by gas chromatographic (GC) and gas chromatography-mass spectroscopic (GC-MS) comparisons with authentic samples. The sterols were also identified as campesterol, β-sitosterol, and stigmasterol (in a ratio of 3:80:14) by GC and GC-MS analyses.

On the other hand, the fraction containing ferulic acid esters (**5**) showed infrared (IR) absorptions at 3550 (OH), 1700 (CO), 1635 (C=C), 1605, 1515, and 1470 cm^{-1} (aromatic ring) and its proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed signals arising from a methoxy methyl (δ 3.93), two olefinic protons [δ 6.29 and 7.61 (each d, J=6 Hz)], and 1,3,4-trisubstituted benzene protons [δ 6.91 (1H, d, J=8 Hz), 7.03 (1H, d, J=2 Hz), and 7.06 (1H, dd, J=8, 2 Hz)], along with signals assignable to alkyl chains. These spectral data were almost identical with the reported data of hexacosyl ferulate obtained from *Rabdosia japonica* (BURM.) HARA (Labiatae)[8] and finally this substance was concluded to be a mixture of octacosyl ferulate, heptacosyl ferulate, hexacosyl ferulate, pentacosyl ferulate, tetracosyl ferulate, tricosyl ferulate, henicosyl ferulate, icosyl ferulate, and nonadecyl ferulate, based on the appearance of the respective molecular ion peaks in the MS.

Dihydrophenanthrene DHP-II (**1**), $C_{16}H_{16}O_3$, showed ultraviolet (UV) absorptions at 215, 280, and 300 nm and IR absorptions at 3600, 3300 (br, OH), 1605, 1573, and 1462 cm^{-1} (aromatic ring). The ^{1}H-NMR spectrum of **1**

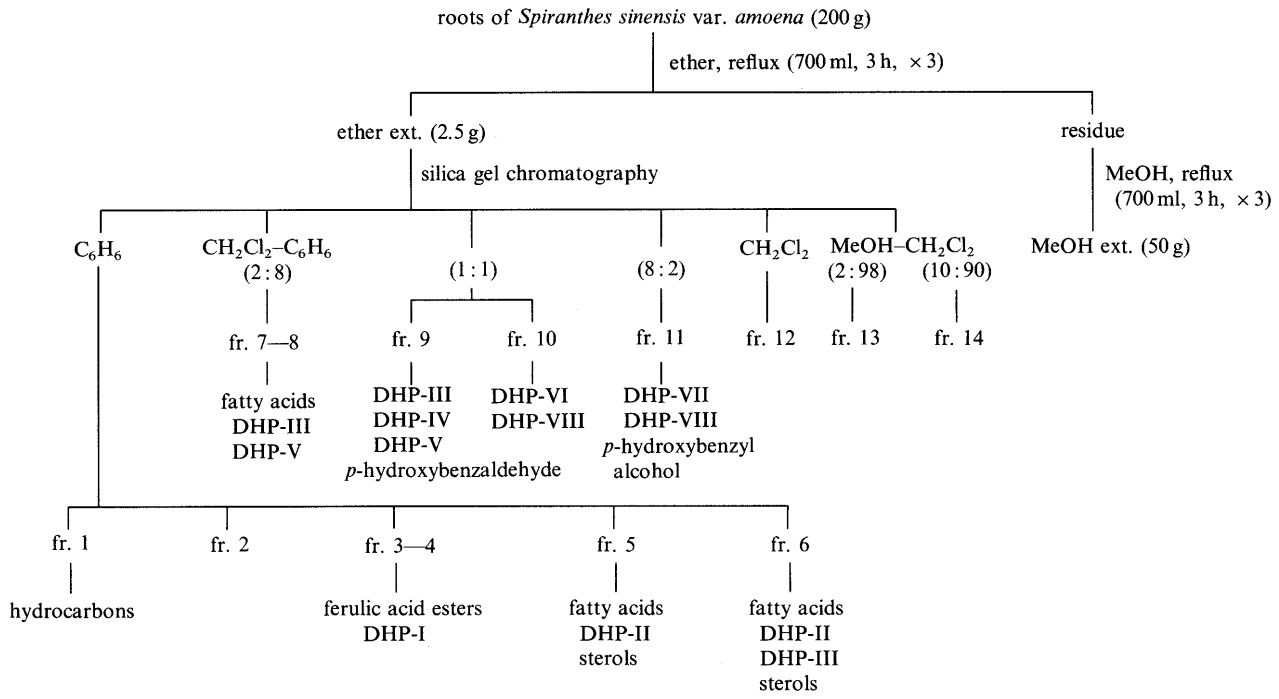

Chart 2

TABLE I. ^{1}H-NMR Data for Dihydrophenanthrenes from *Spiranthes sinensis* var. *amoena* in $CDCl_3$ (*J* in Hz)

	1	2	3	4
1-H	6.41 d (2.5)	6.42 d (2.4)	6.42 d (2.4)	—
3-H	6.45 d (2.5)	6.36 d (2.4)	6.44 d (2.4)	6.45 s
5-H	8.11 d (8.5)	7.75 d (8.5)	7.98 d (8.6)	7.66 d (8.2)
6-H	6.71 dd (8.5, 2.5)	6.74 d (8.5)	6.70 d (8.6)	6.75 d (8.2)
8-H	6.68 d (2.5)	—	—	—
9-H_2	2.74 m	2.73 m	2.74 m	2.60 br s
10-H_2	2.72 m	2.68 m	2.70 m	
2-OCH_3	3.83 s	3.79 s	3.83 s	3.79 s
4-OCH_3	3.86 s	—	3.85 s	—
4-OH	—	5.34[a] s	—	5.50[a] s
7-OH	—	4.99[a] s	4.89 br s	4.81[a] br s
1′-H_2	—	3.44 d (6.7)	3.43 d (6.7)	3.42 d (7.0)
2′-H	—	5.15 tqq (6.7, 1.2, 1.2)	5.15 tqq (6.7, 1.2, 1.2)	5.12 tqq (7.0, 1.5, 1.5)
4′-H_3	—	1.82 br s	1.81 br s	1.79 br s
5′-H_3	—	1.72 d (1.2)	1.71 d (1.2)	1.71 d (1.5)
1″-H_2	—	—	—	3.98 s
3″,7″-H	—	—	—	6.98 d (8.5)
4″,6″-H	—	—	—	6.68 d (8.5)
5″-OH	—	—	—	5.11[a] s

a) Assignments may be interchanged in each column.

TABLE II ^{13}C-NMR Data for Dihydrophenanthrenes from *Spiranthes sineensis* var. *amoena* in $CDCl_3$

	1	2	3	4
C-1	105.0 d	106.0 d	104.4 d	118.5 s
C-2	158.7 s	158.6 s	158.6 s	157.2 s
C-3	97.7 d	100.8 d	97.6 d	98.3 d
C-4	157.7 s	153.4 s	157.6 s	151.7 s
C-4a	116.6 s	115.4 s	117.2 s	115.2 s
C-4b	125.8 s	125.6 s	125.9 s	125.9 s
C-5	129.2 d	124.6 d	127.1 d	124.1 d
C-6	112.8 d	113.3 d	112.8 d	113.4 d
C-7	153.5 s	152.5 s	152.3 s	152.6 s
C-8	114.3 d	125.3 s	123.9 s	125.3 s
C-8a	139.8 s	138.4 s	137.9 s	138.7 s
C-9	30.0 t	25.6 t	25.5 t	25.6[a] t
C-10	29.7 t	30.5 t	30.7 t	26.4[a] t
C-10a	140.7 s	140.9 s	140.6 s	139.7 s
2-OCH_3	55.3 q	55.3 q	55.3 q	55.7 q
4-OCH_3	55.6 q	—	55.5 q	—
C-1′	—	25.5 t	25.5 t	25.5 t
C-2′	—	122.1 d	122.3 d	122.0 d
C-3′	—	133.4 s	133.2 s	133.6[b] s
C-4′	—	18.0 q	18.0 q	18.0 q
C-5′	—	25.7 q	25.8 q	25.7 q
C-1″	—	—	—	30.1 t
C-2″	—	—	—	133.6[b] s
C-3″,7″	—	—	—	129.1 s
C-4″,6″	—	—	—	115.1 d
C-5″	—	—	—	153.4 s

a) Assignments may be interchanged. *b*) At a higher concentration (125 mg/ml), these signals appeared at δ 133.5 (C-3′) and 133.1 (C-2″).

showed signals due to two methoxyl methyls at δ 3.83 and 3.86 and five aromatic protons at δ 6.41 (d, J=2.5 Hz), 6.45 (d, J=2.5 Hz), 6.68 (d, J=2.5 Hz), 6.71 (dd, J=8.5, 2.5 Hz), and 8.11 (d, J=8.5 Hz), together with signals corresponding to four protons at around δ 2.7, which are characteristic of the 9- and 10-protons of dihydrophenanthrenes.[9]

From these spectral data, DHP-II (**1**) was considered to be orchinol (2,4-dimethoxy-7-hydroxy-9,10-dihydrophenanthrene), which was reported as a phytoalexin of *Orchis militalis* L.[10] This was confirmed by comparison of its carbon-13 NMR (^{13}C-NMR) spectrum with the published data[11] (Table II).

Spiranthol-A (**2**, DHP-III) was obtained as a slightly

colored amorphous powder and its molecular formula was determined to be $C_{20}H_{22}O_3$ (M^+ m/z 310) from the high-resolution mass spectrum (HR-MS). It showed UV absorptions at 219, 280, 290 sh, and 311 sh nm and IR absorptions at 3600, 3400 (br, OH), 1615, and 1470 cm^{-1} (aromatic ring). In the ^{1}H-NMR spectrum (Table I), **2** showed signals of two methylene groups ascribable to the 9- and 10-H_2 of a dihydrophenanthrene nucleus, four aromatic protons, two hydroxyl protons, and a methoxy methyl group. Further, the ^{1}H-NMR spectrum showed signals due to two vinyl methyls at δ 1.72 and 1.82 (each d, J=1.2 Hz), a methylene at δ 3.44 (d, J=6.7 Hz), and an olefinic proton at δ 5.15 (tqq, J=6.7, 1.2, 1.2 Hz), suggesting that **2** may be a dihydrophenanthrene having an isopentenyl, a methoxyl, and two hydroxyl groups. The locations of these four substituents were suggested to be C-2, -4, -7, and -8 by the coupling pattern of the aromatic protons and by the fact that there was only one proton signal at around δ 8, which is characteristic of 4- or 5-H of the dihydrophenanthrene nucleus.[12]

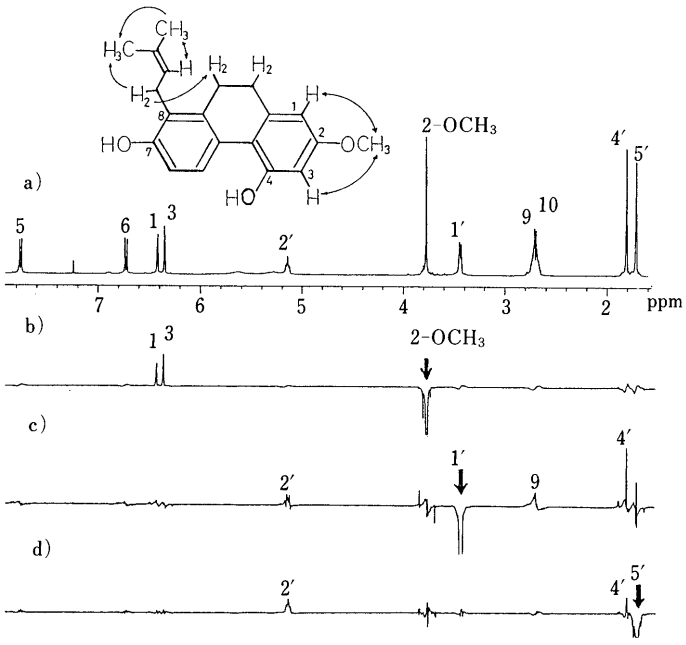

Fig. 1. NOE Difference Spectra of Spiranthol-A (**2**)
a) Normal spectrum. b, c, d) Difference spectra.

Then, the nuclear Overhauser effect (NOE) difference spectra were measured in order to determine the position of each substituent. As shown in Fig. 1, irradiation of the methoxyl and methylene protons enhanced the intensity of the signals at δ 6.36 (3-H) and 6.42 (1-H) and the signals at δ 1.82 (4′-H_3) and 2.73 (9-H_2), respectively. Therefore, the methoxyl group must be attached to C-2 and the isopentenyl group to C-8, and hence the two hydroxyl groups to C-4 and C-7. This was confirmed by ^{1}H–^{13}C shift correlation spectroscopy (^{1}H–^{13}C COSY) and long-range ^{1}H–^{13}C COSY experiments (Fig. 2).

On the basis of the above findings, spiranthol-A was determined to be 4,7-dihydroxy-8-isopentenyl-2-methoxy-9,10-dihydrophenanthrene (**2**), which is the first example of a dihydrophenanthrene containing an isopentenyl group. This compound (**2**) exhibited a mild cytotoxic activity toward HeLa-S_3 cells (IC_{50} 10.8 μg/ml).

Spiranthol-B (**3**, DHP-I) is a minor component obtained as a slightly colored amorphous solid and its molecular formula was determined to be $C_{21}H_{24}O_3$ (M^+ m/z 324) by MS and HR-MS. It showed UV and IR absorptions similar to those of **2** (see Experimental section). The ^{1}H- and ^{13}C-NMR spectra (Tables I and II) were also similar to those of **2** except for the presence of two methoxyl groups (δ_H 3.83 and 3.85; δ_C 55.3 and 55.5). Difference NOE experiments irradiating the methoxyl protons at δ 3.83 and 3.85 showed enhancement of the signal intensities of 1- and 3-H and 3- and 5-H, respectively, suggesting the positions of the methoxyl groups to be C-2 and C-4.

From these spectral data, spiranthol-B was determined to be 2,4-dimethoxy-7-hydroxy-8-isopentenyl-9,10-dihydrophenanthrene (**3**).

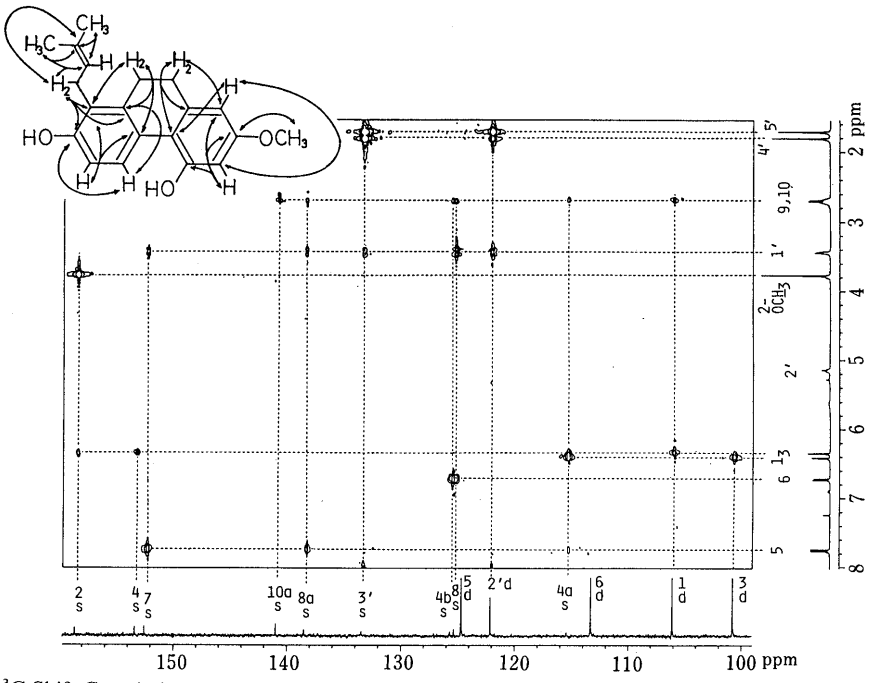

Fig. 2. Long-Range ^{1}H–^{13}C Shift Correlation Spectrum of Spiranthol-A (**2**) in $CDCl_3$

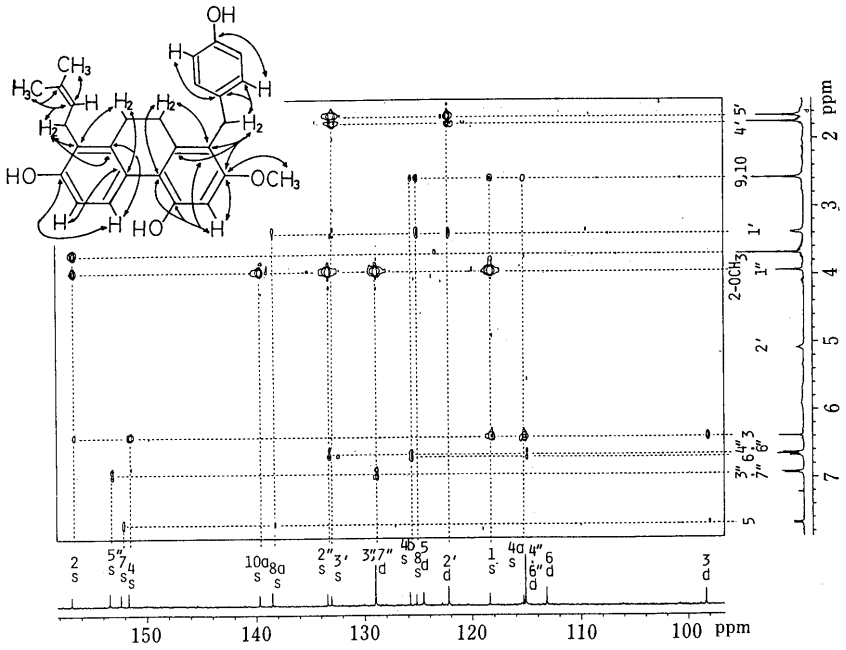

Fig. 3. Long-Range ^1H–^{13}C Shift Correlation Spectrum of Spirasineol (**4**) in $CDCl_3$

Spirasineol-A (**4**, DHP-VIII) was obtained as slightly colored needles (ether), mp 165—167.5 °C, and its molecular formula was determined to be $C_{27}H_{28}O_4$ (M^+ *m/z* 416) by MS, HR-MS, and elemental analyses. It showed UV absorptions at 216, 280, 299 sh, and 311 sh nm and IR absorptions at 3600, 3350 (OH), 1600, 1510, and 1463 cm^{-1} (aromatic ring). In the ^1H-NMR spectrum (Table I), **4** showed signals ascribable to 9- and 10-H_2 of dihydrophenanthrene, an isolated aromatic proton (δ 6.45), and a pair of *ortho*-coupled aromatic protons (δ 6.75 and 7.66, J= 8.2 Hz) along with signals of a methoxyl, an isopentenyl group, and three hydroxy protons. In addition, it showed two signals of *ortho*-coupled aromatic protons at δ 6.68 and 6.98 (each d, J=8.5 Hz), each corresponding to two protons, and a signal due to methylene protons at δ 3.98 (s). In the MS it showed a fragment ion at *m/z* 107 ($C_7H_7O^+$) which was assignable to a hydroxytropylium cation. These spectral data led us to deduce that spirasineol might be a derivative of **2**, having a *p*-hydroxybenzyl substituent at the C-1 or C-3 position.

In the NOE experiments, irradiation at δ 3.98 (1″-H) and at δ 3.42 (1′-H_2) enhanced the intensities of the signals at δ 2.60 (9- and 10-H_2) and 6.98 (3″- and 7″-H) and at δ 2.60 (9- and 10-H_2), 5.12 (2′-H), and 1.71 (5′-H_3), respectively. In turn, irradiation of the methylene protons at δ 2.60 (9- and 10-H_2) enhanced the intensities of the signals at δ 5.12 (2′-H), 6.98 (3″- and 7″-H), 3.42 (1′-H), and 3.98 (1″-H_2), while irradiation of the methoxyl protons (δ 3.79) enhanced the intensity of the isolated aromatic proton (δ 6.45), suggesting that the *p*-hydroxybenzyl group is located at C-1, the isopentenyl group at C-8, and possibly the methoxyl group at C-2.

Finally, the location of each substituent was clearly determined by a long-range ^1H–^{13}C COSY experiment. As shown in Fig. 3, the carbon at δ 157.2 (C-2) showed long-range correlations with the protons at δ 3.79 (OCH_3), 3.98 (1″-H_2), and 6.45 (3-H), indicating the location of the methoxyl group at C-2. The carbons at δ 118.5 (C-1) and 125.3 (C-8) showed long-range correlations with the protons at δ 2.60 (10-H_2), 3.98 (1″-H_2), and 6.45 (3-H) and the protons at δ 2.60 (9-H_2) and 3.42 (1′-H_2), respectively. Thus, the *p*-hydroxybenzyl group must be attached to C-1 and the isopentenyl group to C-8. Also, other long-range correlations observed are shown by arrows in the formula in Fig. 3.

On the basis of the above spectral evidence, spirasineol-A was concluded to be 4,7-dihydroxy-1-*p*-hydroxybenzyl-8-isopentenyl-2-methoxy-9,10-dihydrophenanthrene (**4**). It should be noted that a dihydrophenanthrene having a *p*-hydroxybenzyl substituent has been isolated from *Bletilla striata* (Orchidaceae),[13] but **4** is the first example of a dihydrophenanthrene containing both isopentenyl and *p*-hydroxybenzyl substituents.

Experimental

Melting points were determined on a Kofler-type apparatus and are uncorrected. UV spectra were taken with a Shimadzu 202 UV spectrometer in EtOH solutions and IR spectra with a JASCO IRA-2 spectrometer in $CHCl_3$ solutions. ^1H- and ^{13}C-NMR spectra were measured on a JEOL GX-400 spectrometer in $CDCl_3$ solutions using tetramethylsilane as an internal standard; chemical shifts are recorded in δ values and coupling constants in hertz (Hz). Multiplicities of ^{13}C-NMR spectra were determined by means of the distortionless enhancement by polarization transfer (DEPT) method. 2-D NMR spectra (^1H–^{13}C COSY and long-range ^1H–^{13}C COSY) were measured by the use of JEOL standard pulse sequences (^1H–^{13}C COSY: VBDCHSHF, J=140 Hz and long-range ^1H–^{13}C COSY: VCHSHF, J=10 Hz) and collected data were treated by JEOL standard software. NOE difference spectra were obtained by the use of the JEOL standard sequence (DIFNOE2) with irradiation for 5 s. MS and HR-MS were obtained with a JEOL JMS-D 300 spectrometer (ionization voltage, 70 eV; accelerating voltage, 3 kV) using a direct inlet system or a GC injection system [2% OV-17 2 m × 2 mm i.d. glass column; column temperature, 180—280 °C (5 °C/min) for hydrocarbons and 260 °C for sterols; injection temperature, 295 °C; carrier gas, He]. GC was done on a Shimadzu GC-6AM instrument with a 2% OV-17 column [2 m × 3 mm i.d. glass tube; injection temperature, 295 °C; column temperature, 180—280 °C (5 °C/min) for hydrocarbons and 260 °C for sterols; carrier gas, N_2] and the peak area ratio was obtained with a Shimadzu Chromatopak E1A calculator. Column chromatography was done with a mixture of Mallinkrodt silica gel and Merck Kieselgel (Art. 7734) (1 : 1). Preparative TLC was carried out on precoated Merck Kieselgel GF_{254} plates (0.25 or 0.5 mm) and the plates were examined under UV light. Extraction of substances from silica gel was done with MeOH–CH_2Cl_2 (1 : 9) and

solutions were concentrated *in vacuo*.

Authentic samples of nonacosane, heptacosane, hexacosane, pentacosane, tetracosane, tricosane, and *p*-hydroxybenzaldehyde were purchased from Tokyo Kasei Kogyo Co., Ltd. and octacosane was from Wako Pure Chemical Industries, Ltd.

Extraction and Cytotoxic Activity toward HeLa-S_3 Cells Air-dried plant material (*Spiranthes sinensis* var. *amoena*), collected at Toyama in late June, 1987, was divided into the underground part (roots, 200 g) and the aerial part (540 g). The underground part was cut into small pieces, and extracted successively with ether (700 ml, 3 h, × 3) and MeOH (700 ml, 3 h, × 3) under reflux to give the ether extract (2.5 g) and the MeOH extract (50 g). The aerial part was also extracted with ether and MeOH (each 3.5 l) in the same manner to give the ether and MeOH extracts (14 and 130 g, respectively). Cytotoxic activities of the above extracts toward HeLa-S_3 cells (inhibition rate, %) were as follows: ether extract from the aerial part, 100 μg/ml, 32.7, 10 μg/ml, 0.1, 1 μg/ml, 2.0; MeOH extract from the aerial part, 100 μg/ml, 9.5, 10 μg/ml, 0, 1 μg/ml, 2.7; ether extract from the roots, 100 μg/ml, 100, 10 μg/ml, 5.6, 1 μg/ml, 0; MeOH extract from the roots, 100 μg/ml, 2.6, 10 μg/ml, 2.4, 1 μg/ml, 0.4.

Separation of the Ether Extract from the Roots The ether extract from the roots (2.5 g) was subjected to chromatography on a silica gel (40 g) column and eluted successively with benzene (500 ml, frs. 1—6), CH_2Cl_2–benzene (2 : 8, 1000 ml, frs. 7—8; 1 : 1, 1000 ml, frs. 9—10; 8 : 2, 1000 ml, fr. 11), CH_2Cl_2 (400 ml, fr. 12), and MeOH–CH_2Cl_2 (2 : 98, 1000 ml, fr. 13; 10 : 90, 500 ml, fr. 14).

Fraction 1 (230 mg) was analyzed by GC and GC-MS in comparison with authentic samples and the following normal hydrocarbons were identified: tricosane (t_R 5.3 min, M^+ *m/z* 324, 3.3%), tetracosane (t_R 6.6 min, M^+ *m/z* 338, 2.8%), pentacosane (t_R 8.4 min, M^+ *m/z* 352, 27.6%), hexacosane (t_R 9.5 min, M^+ *m/z* 366, 4.0%), heptacosane (t_R 11.4 min, M^+ *m/z* 380, 36.2%), octacosane (t_R 12.4 min, M^+ *m/z* 394, 7.0%), and nonacosane (t_R 14.2 min, M^+ *m/z* 408, 19.1%).

Fractions 3 and 4 (95 mg) were further separated by preparative TLC with hexane–ether (1 : 2) to give a mixture of ferulates (20 mg) from the less polar fraction and crude DHP-I (spiranthol-B, 14 mg) from the more polar fraction.

Fractions 5 (135 mg) and 6 (260 mg) were also subjected repeatedly to preparative TLC with hexane–ether (1 : 1) and separated into four fractions. The most mobile fraction gave a mixture of fatty acids (140 mg) and the next most mobile fraction gave DHP-II (orchinol, 32 mg). The third most mobile fraction gave a mixture of sterols (60 mg), while the least mobile fraction gave DHP-III (spiranthol-A, 30 mg).

Fractions 7 (180 mg) and 8 (305 mg) were further separated by repeated preparative TLC with hexane–ether (3 : 2 and 1 : 1) into three fractions. The least polar fraction gave an additional crop of fatty acid mixture (33 mg) and the next least polar fraction, DHP-III (spiranthol-A, 240 mg). The third fraction gave crude DHP-V (5.2 mg).

Fraction 9 (100 mg) was separated by preparative TLC with hexane–ether (1 : 1), giving *p*-hydroxybenzaldehyde (9 mg), DHP-III (spiranthol-A, 36 mg), DHP-IV (4 mg), and an additional crop of crude DHP-V (5.4 mg).

Fraction 10 (360 mg) was separated by preparative TLC with MeOH–$CHCl_3$ (2 : 98) to give DHP-VI (5 mg) and DHP-VIII (spirasineol-A, 90 mg).

Fraction 11, on concentration, gave a white precipitate, which was collected by filtration and recrystallized from ether to give *p*-hydroxybenzyl alcohol (39 mg), mp 116—118 °C. The filtrate was concentrated *in vacuo* and the residue (310 mg) was separated by repeated preparative TLC with hexane–ether (2 : 1) and with MeOH–$CHCl_3$ (5 : 95) to give additional crops of *p*-hydroxybenzyl alcohol (6 mg) and DHP-VIII (spirasineol-A, 32 mg) along with DHP-VII (15 mg).

Identification of Sterols Sterol mixture obtained from fractions 5 and 6 was analyzed by GC and GC-MS to show the presence of campesterol (t_R 6.8 min, M^+ *m/z* 400, 2.6%), β-sitosterol (t_R 7.4 min, M^+ *m/z* 414, 80.0%), and stigmasterol (t_R 8.4 min, M^+ *m/z* 412, 13.8%).

Mixture of Ferulic Acid Esters (5) IR ν_{max} cm^{-1}: 3550 (OH), 1700 (CO), 1635, 1605, 1515, 1470, 1270, 1180. ^{1}H-NMR: 0.88 (3H, t, J=7 Hz, CH_2CH_3), 1.25 (br s, alkyl chain), 3.93 (3H, s, OCH_3), 4.19 (2H, t, J=7 Hz, $CO_2CH_2CH_2$), 5.83 (1H, br s, OH), 6.29 (1H, d, J=16 Hz, Ar-CH=CH–CO), 6.91 (1H, d, J=8 Hz, H-5), 7.03 (1H, d, J=2 Hz, H-2), 7.06 (1H, dd, J=8, 2 Hz, H-6), 7.61 (1H, d, J=16 Hz, Ar-CH=CH–CO). MS *m/z* (%): 586 (36, M^+ of octacosyl ferulate), 572 (11, M^+ of heptacosyl ferulate), 558 (72, M^+ of hexacosyl ferulate), 544 (31, M^+ of pentacosyl ferulate), 530 (52, M^+ of tetracosyl ferulate), 516 (10, M^+ of tricosyl ferulate), 502 (66, M^+ of henicosanyl ferulate), 488 (21, M^+ of icosanyl ferulate), 474 (62, M^+ of nonadecyl ferulate), 194 (100, ferulic acid cation), 177 (89), 150 (26), 137 (46).

DHP-II (Orchinol, 1) Slightly colored amorphous solid. UV λ_{max} nm (log ε): 215 (4.25), 280 (3.95), 300 (3.72). IR ν_{max} cm^{-1}: 3600, 3300 (br, OH), 1605, 1573, 1462 (aromatic ring), 1438, 1270, 1158, 1120, 1082, 1060, 905, 828. ^{1}H- and ^{13}C-NMR: see, Tables I and II. MS *m/z* (%): 257 (M^++H, 30), 256 (M^+, 100), 255 (M^+−H, 9), 241 (M^+−CH_3, 12), 213 (M^+−CH_3−CO, 13), 128 (12). HR-MS: Found 256.1123, Calcd for $C_{16}H_{16}O_3$ (M^+), 256.1100. Found 241.0838, Calcd for $C_{15}H_{13}O_3$ (M^+−CH_3), 241.0863. ^{13}C-NMR data of this compound (**1**) were identical with those reported.[13]

DHP-III (Spiranthol-A, 2) Slightly colored amorphous powder. UV λ_{max} nm (log ε): 219 (4.61), 280 (4.47), 290sh (4.37), 311sh (4.11). IR ν_{max} cm^{-1}: 3600, 3400 (br, OH), 1615, 1470 (aromatic ring), 1348, 1275, 1198, 1160, 1150, 1043, 1015, 960, 910, 830. ^{1}H- and ^{13}C-NMR: see, Tables I and II. MS *m/z* (%): 311 (22, M^++H), 310 (100, M^+), 255 (37), 254 (73), 211 (10). HR-MS: Found 310.1540, Calcd for $C_{20}H_{22}O_3$ (M^+), 310.1568. This compound showed a weak cytotoxic activity toward HeLa-S_3 cells [inhibition rates (%): 100 μg/ml, 97.54; 25 μg/ml, 98.77; 6.25 μg/ml, 7.03; IC_{50} 10.8 μg/ml].

DHP-I (Spiranthol-B, 3) Slightly colored amorphous solid. UV λ_{max} nm (log ε): 216 (4.69), 281 (4.48), 294sh (4.38), 307sh (4.19). IR ν_{max} cm^{-1}: 3600, 3340 (br, OH), 1605, 1460 (aromatic ring), 1273, 1228, 1201, 1158, 1109, 1050, 952, 909, 832. ^{1}H- and ^{13}C-NMR: see, Tables I and II. MS *m/z* (%): 324 (M^+, 100), 307 (M^+−OH, 9), 269 (23), 268 (45). HR-MS: Found 324.1677, Calcd for $C_{21}H_{24}O_3$ (M^+), 324.1725.

DHP-VIII (Spirasineol-A, 4) Colorless crystals (ether), mp 165—167.5 °C ($CHCl_3$). *Anal.* Calcd for $C_{27}H_{28}O_4 \cdot 1/2H_2O$: C, 76.21; H, 6.87. Found: C, 76.40; H, 6.70. UV λ_{max} nm (log ε): 216 (4.25), 280 (4.09), 299 (3.83), 311sh (3.77). IR ν_{max} cm^{-1}: 3600, 3350 (br, OH), 1600, 1510, 1463 (aromatic ring), 1275, 1170, 1138, 1102, 1052, 1038, 970, 908, 823. ^{1}H- and ^{13}C-NMR: see, Tables I and II. MS *m/z* (%): 417 (44, M^++H), 416 (100, M^+), 361 (13), 360 (22), 310 (10), 266 (7), 254 (10), 107 (17, hydroxytropylium cation). HR-MS: Found 416.2008, Calcd for $C_{27}H_{28}O_4$ (M^+), 416.1988; Found 107.0479, Calcd for C_7H_7O (hydroxytropylium cation), 107.0496.

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