Sesquiterpenes and Alkaloids from *Lindera chunii* and Their Inhibitory Activities against HIV-1 Integrase

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Three new eudesmane type sesquiterpenoid lindenanolides E (1), F (2) and G (3), and two new aporphine alkaloid lindechunines A (18) and B (20) were isolated from roots of *Lindera chunii* MERR., together with seven known sesquiterpenes including a new naturally-occurring lindenanolide H (4) and eight known aporphine alkaloids. The structures of these compounds were determined by spectroscopic means. Of the isolated compounds, hernandonine (14), laurolistine (16), 7-oxohernangerine (17) and lindechunine A (18) showed significant anti-human immunodeficiency virus type 1 (HIV-1) integrase activity with IC_{50} values of 16.3, 7.7, 18.2 and 21.1 μM, respectively. The major alkaloids presented in the roots of *L. chunii* were quantitatively analyzed by an HPLC method.

Key words sesquiterpene; aporphine alkaloid; *Lindera chunii*; HIV-1 integrase; Lauraceae

The root of *Lindera chunii* MERR. is used as a Chinese folk medicine, is of the *Lindera* genus (Lauraceae) and is distributed in the provinces of Guangtong and Guangxi of China. The root of *L. chunii* is frequently used as a substitute for *L. aggregata* (SIMS) KOSTERM, which is said to have the power to improve the wind-cold-dampness arthralgia syndrome in traditional Chinese medicine.[1] Isoquinoline alkaloids, especially aporphine alkaloids, were reported as the characteristic constituents of the genus *Lindera*.[2] In a previous paper, we reported the isolation of constituents, including new sesquiterpenoids and aporphine alkaloids, from the root of *L. aggregata* and the pharmacological activities of these compounds.[3,4] Phytochemical studies on *L. chunii* have not yet been conducted, however, which encouraged us to investigate the chemical constituents of this root and their biological activity.

In this paper, we report the isolation and structural elucidation of compounds from the root of *L. chunii* and their inhibitory effects on human immunodeficiency virus type 1 integrase (HIV-1 IN), as well as the quantitative analysis of inhibitory substances present in an alkaloid fraction of this plant extract.

Results and Discussion

From the root of *L. chunii* we isolated 20 compounds, including three new compounds called lindenanolides E (**1**), F (**2**) and G (**3**), new naturally occurring lindenanolide H (**4**),[5] two new aporphine alkaloids called lindechunines A (**18**) and B (**20**), six known sesquiterpenes: pseudoneolinderane (**5**),[6] lindeneol (**6**),[7] lindeneyl acetate (**7**),[7] lindera lactone (**8**),[7] strychinstenolide 6-*O*-acetates A (**9**) and B (**10**),[8] and eight known aporphine alkaloids: hernandine (**11**),[9] hernangerine (**12**),[9] ocokryptine (*N*-methylhernandine) (**13**),[10] hernandonine (**14**),[11] *N*-methylhernangerine (**15**),[12] laurolistine (norboldine) (**16**),[13] 7-oxohernangerine (**17**)[14] and 7-oxohernagine (**19**)[14] (Chart 1). The structures of known compounds were determined by comparing their spectral data with those of authentic samples or previously reported data. Of these, compounds **1**—**4**, **17**, **18** and **20** were isolated for the first time from this plant family. Structures of the new compounds were determined as follows:

Lindenanolide E (**1**) was obtained as colorless needles, mp 151—153 °C. The molecular formula was assigned to be $C_{15}H_{16}O_4$ on the basis of the molecular ion peak at *m/z* 260 $[M]^+$ in the electron impact mass (EI-MS) spectrum and elemental analysis. The ^1H–^1H correlation spectroscopy (COSY) experiment showed the presence of a cyclopropane ring (H-1, H_2-2, H-3). The presence of three carbonyl carbons, including an aldehyde group, was suggested on the basis of the ^{13}C-NMR signals at δ 164.5, 167.9 and 186.0, and the IR absorption bands at ν_{max} 1768 and 1653 cm^{-1}. Extensive analysis of the ^1H-detected multiple quantum coherence (HMQC) and heteronuclear multiple-bond correlation (HMBC) spectra of **1** established the structure (Fig. 1A), *i.e.*, correlations of methylene proton signals (H_2-9) at δ 2.70 (1H, d, J=13.2 Hz) and 2.97 (1H, d, J=13.2 Hz) with three carbon signals at δ 49.1 (C-10), 140.1 (C-8) and 164.5 (C-7) indicated that the carbon at δ 140.1 (C-8) and the carbon at δ 32.9 (C-9) were linked together. Furthermore, long-range correlations were also observed among a methyl proton signal at δ 1.42 (H_3-14) and two quarternary carbon signals at δ 49.1 (C-10) and 134.1 (C-5); between a proton signal at δ 9.81 (H-6) and a quarternary carbon signal at δ 134.1 (C-5); among a methyl proton signal at δ 2.21 (H_3-15) and two carbon signals at δ 167.9 (C-4) and 134.5 (C-5), suggesting that an aldehyde group is connected at C-5. In the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) experiment, NOE correlations were observed among the following proton signals (Fig. 1B): H-2α to H-1/H-3; H-2β to H_3-14; H-1 to H-3/H-9α; H_3-14 to H-9β, which confirmed the stereostructure, as shown in Fig. 1. This is the first example of a seco-eudesmanolide.

Lindenanolide F (**2**) was assigned the molecular formula $C_{30}H_{34}O_6$ on the basis of the quasi-molecular ion peak at *m/z* 513.2233 $[M+Na]^+$ and 473.2314 $[M+H-H_2O]^+$ in the positive high resolution (HR)-FAB-MS spectrum. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra showed 15 signals of two methyl, three

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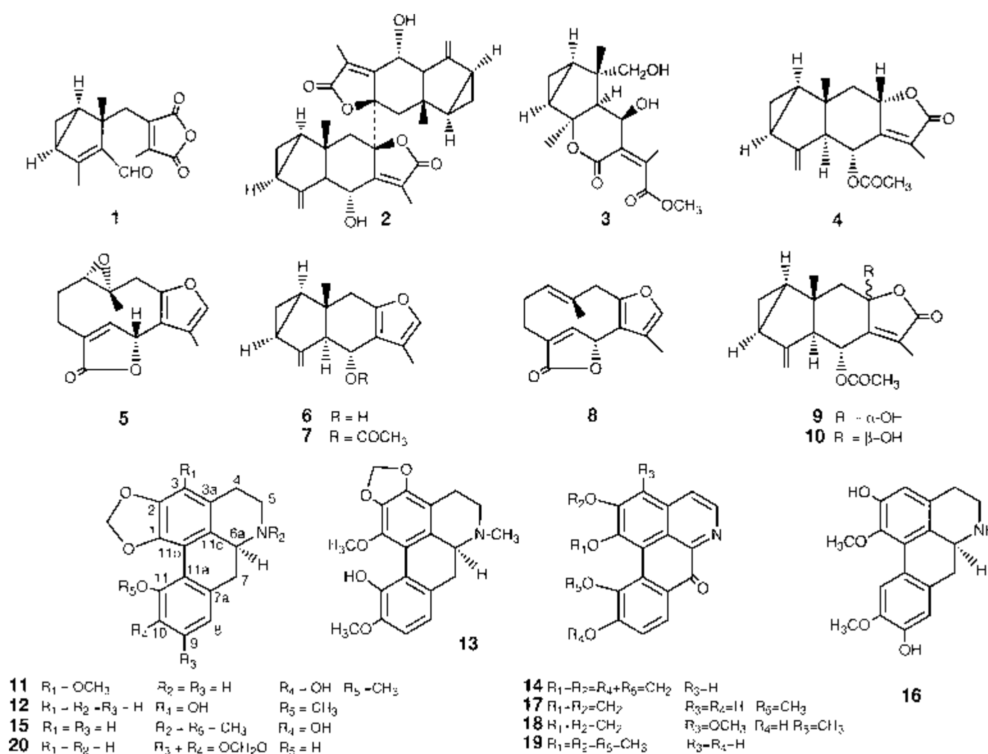

Chart 1. Structures of Compounds Isolated from the Roots of *L. chunii*

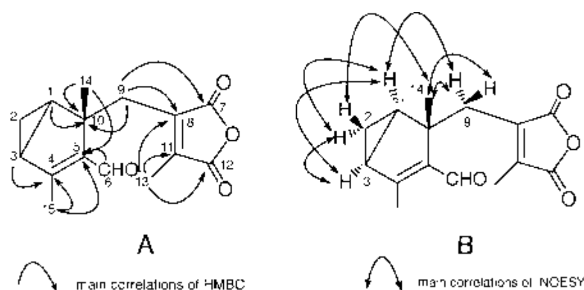

Fig. 1. Correlations in the HMBC and NOESY Spectra of Compound **1**

methylene, four methine, six quarternary carbons, corresponding to a half in number of the total carbon atoms which are estimated from the molecular formula, suggesting that compound **2** may consist of two identical units. The structure of this unit was elucidated by various spectral data as below: in the IR spectrum, compound **2** showed an absorption at $1745\,cm^{-1}$ assignable to the α,β-unsaturated five-membered lactone, which was supported by the presence of a carbon signal at δ 172.7 in the ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum showed signals of one *exo*-olefine at δ 5.06 and 5.03. The ^{13}C-NMR spectrum also indicated carbon signals of a terminal double bond at δ 106.9 and 149.6 and a tetrasubstituted double bond at δ 158.0 and 133.6. The degree of unsaturation in the molecule was 14, giving seven per unit. Two of these were assigned to two double bonds, one to an ester carbonyl group, and four to a tetracyclic structure.

The ^{1}H–^{1}H COSY spectral data confirmed the presence of a cyclopropane ring (H-1, H_2-2, H-3). The HMBC spectral data assembled the carbon skeleton. In particular, the multiple correlations to the signal at δ 38.8 and to the signal at δ 64.4 can be used for the elucidation of the ring system. Correlations of a proton signal at δ 1.87 (H_3-13) with carbon signals at δ 158.0 (C-7) and 172.7 (C-12), and proton signals at δ 2.30 and 2.65 (H_2-9) with a carbon signal at δ 92.3 (C-8) indicated that an α,β-unsaturated lactone was located at C-7 and C-8, and a methyl group was attached at C-11. A correlation of a signal at δ 0.42 (H_3-14) and a signal at δ 38.8 (C-10) suggested that a methyl group (H_3-14) was located at C-10. The structure of **2** was unambiguously assigned by detailed analysis of the two-dimensional (2D)-NMR [^{1}H–^{1}H COSY, HMQC and HMBC (see Fig. 2)] spectra and by comparison of the ^{13}C-NMR spectral data with those of strychnistenolides A (**9**) and B (**10**),[8] as well as lindenanolide H (**4**) (Table 1). Compound **2** was consequently deduced to be a bis-sesquiterpene linked together at C-8.

The configurations of H-5 and H_3-14 were assigned by the NOESY spectrum; appreciable correlations were observed between signals at δ 0.42 (H_3-14) and at 2.65 (H-9β)/4.38 (H-6), but not with a signal at δ 3.48 (H-5). The coupling constant of signals at δ 3.48 (H-5) and 4.38 (H-6) was 10.5 Hz, showing a *trans diaxial* relationship of these protons. Furthermore, NOE correlations were observed among H-9α/H-1, H-9β/H_3-14 and H-2β/H_3-14 (Fig. 2), suggesting that a cyclopropane ring is oriented to be close with a 14-methyl group.

Recently, a bissesquiterpene having the above type of the structure was isolated from *L. aggregata*,[15] which is a derivative of **2** and has two double bonds at C-1 and C-2, as well as at C-1′ and C-2′. However, the stereochemistry at C-8 in **2** seemed to be different from this compound, because a signal of H-9α in **2** appeared exclusively in the low field of δ 2.30, due to the presence of the neighboring ether-oxygen of the γ-lactone moiety. This can be supported by comparison of the spectral data with those of strychnistenolide A, in which a

Table 1. ^{13}C-NMR Spectral Data of Compounds **1**, **2**, **3** and **4** in CD_3Cl (100 MHz)

No.	**1**	**2**	**3**	**4**
1	26.0	29.4	27.2	26.9
2	14.2	16.3	5.1	16.3
3	25.3	23.8	27.1	23.6
4	167.9	149.6	92.8	147.5
5	134.1	64.4	53.5	66.3
6	185.9	63.9	65.3	69.2
7	164.4	58.0	134.5	158.3
8	140.1	92.3	167.1	79.5
9	32.9	45.2	73.4	43.2
10	49.1	38.8	19.3	38.5
11	140.5	133.6	133.5	121.2
12	164.1	172.7	170.0	170.4
13	8.3	8.9	15.9	8.6
14	20.8	19.9	18.3	18.3
15	12.3	106.9	33.0	108.9
1′		29.4		
2′		16.3		
3′		23.8		
4′		149.6		
5′		64.4		
6′		63.9		
7′		158.0		
8′		92.3		
9′		45.2		
10′		38.8		
11′		133.6		
12′		172.7		
13′		8.9		
14′		19.9		
15′		106.9		
OCH_3			52.8	
$\underline{C}OCH_3$				173.7
$CO\underline{CH_3}$				20.7

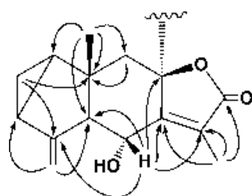

Fig. 2. Correlations in the HMBC Spectrum of Compound **2**

hydroxy group at C-8 is oriented in the α-configuration. A methyl signal (H_3-14) at δ 0.42 in **2** was rather similar to that of strychnistenolide A (**9**) (δ 0.61), but greatly different from that of strychnistenolide B (**10**) (δ 1.23). Such an arrangement may be possible when the counterpart is oriented in the α-configuration at C-8. An appreciable NOE correlation was observed between H-6 and H_3-13 in the NOESY spectrum of **2** (Fig. 3), which can be seen in the spectrum of strychnistenolide A, but not in that of strychnistenolide B. Therefore, compound **2** was assigned as a bissesquiterpene, in which the identical sesquiterpene units are linked together at C-8 or C-8′, as shown in Fig. 3.

Lindenanolide G (**3**) showed a quasi-molecular ion peak at m/z 311 $[M+H]^+$ in the positive ion FAB-MS spectrum and the ^{13}C-NMR spectrum showed 16 signals assignable to four methyl, two methylene, four methine and six quarternary carbons. These data, together with a positive HR-FAB-MS spectral datum m/z 311.1509 (Calcd for $C_{16}H_{23}O_6$, 311.1548) established the molecular formula $C_{16}H_{22}O_6$. The ^{1}H-NMR spectrum of **3** showed a methoxyl signal at δ 3.75 (OCH_3) and three tertiary methyl signals at δ 1.35 (H_3-14), 1.65 (H_3-15), and 2.05 (H_3-13). The presence of a cyclopropane ring (H-1, H_2-2, H-3) system was confirmed by the ^{1}H–^{1}H COSY experiment.

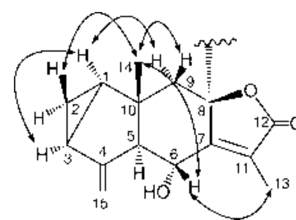

Fig. 3. NOE Correlations of Compound **2**

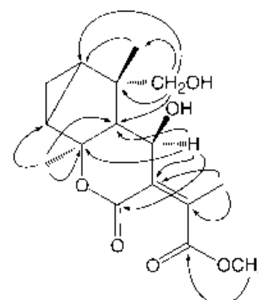

Fig. 4. Correlations in the HMBC Spectrum of Compound **3**

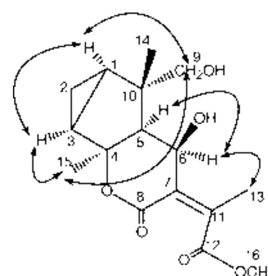

Fig. 5. NOE Correlations of Compound **3**

The presence of ester and lactone groups was supported by ^{13}C-NMR signals at δ 167.1 and 170.0 and the IR absorptions at 1718 and 1644 cm^{-1}. The ^{1}H- and ^{13}C-NMR spectral data of **3** were quite similar to those of strychnilactone,[8] except that an ethyl group in strychnilactone is replaced by a methyl group in **3**. Extensive analysis of the 2D-NMR [^{1}H–^{1}H COSY, HMQC and HMBC (see Fig. 4)] spectra of **3** established the structure. On the basis of the NOE correlations in the NOESY spectrum, the relative stereostructure of **3** is shown in Fig. 5, which represented the second example of a secoeudesmanolide, in which a C-8, C-9 bond of the eudesmanolide may be subjected to cleavage to form a C-8, C-4 δ-lactone.[8]

Lindechunine A (**18**) was obtained as a yellow powder, positive to Dragendorff's test, $[\alpha]_D^{25.6}$ 0° (c=0.1, MeOH). The molecular formula $C_{19}H_{13}O_6N$ was assigned by HR-EI-MS measurement (m/z 351.0734 $[M]^+$, Calcd 351.0765). The 7-oxoaporphine skeleton was deduced by its UV spectrum (absorption maxima at λ 220, 250, 285, 365, 425 nm), which was similar to that of oxoduocine.[10,19] A bathochromic shift

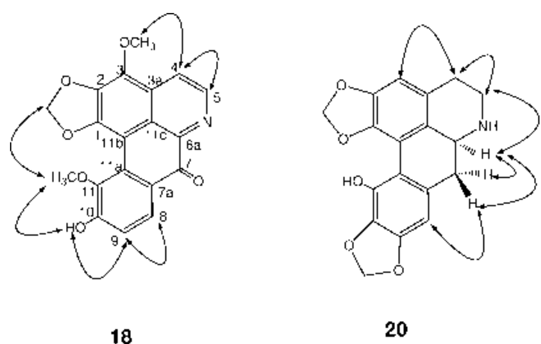

Fig. 6. NOE Correlations of Compounds **18** and **20**

at 325 and 450 nm in the UV spectrum by the addition of alkali, as well as an absorption at 3461 cm^{-1} in the IR spectrum, suggested the presence of a phenolic functional group. The ^{1}H-NMR spectrum showed signals at δ 4.22 and 3.47 corresponding to two methoxyl groups, along with four doublet signals at δ 8.77 and 8.16 (J=5.2 Hz, each one proton), 7.96 and 7.10 (J=8.9 Hz, each one proton). These data suggested the structure of 1,2,3,10,11-oxygenated oxoaporphine alkaloid. The complete proton assignments of **18** were made by the COSY and NOESY (Fig. 6) experiments. A signal at δ 8.16 (H-4) showed significant correlations with signals at δ 4.22 (CH_3O-3) and δ 8.77 (H-5), and a signal at δ 3.47 (CH_3O-11) displayed correlations with those at δ 6.38 (OCH_2O) and δ 10.51 (HO-10) in the NOESY spectrum. In the ^{13}C-NMR spectrum, fifteen aromatic carbon signals were observed in a range of δ 180.2 and 99.5, two methoxyl carbon signals at δ 60.2 and 60.0, and a methylene carbon signal at δ 102.4, and the structure of **18** was further confirmed by extensive analysis of the HMBC spectrum. Thus, the structure of **18** was determined as 10-hydroxy-3,11-dimethoxy-1,2-methylenedioxydibenzo[de,g]quinolin-7-one.

Lindechunine B (**20**), a greyish amorphous powder, $[\alpha]_D^{26.5}$ +43.0° (c=0.01, MeOH), was positive to Dragendorff's test. The molecular formula $C_{18}H_{15}O_5N$ was assigned by HR-EI-MS (m/z 325.0949 $[M]^+$, Calcd 325.0925). Two methylenedioxy groups were shown by the presence of ^{13}C-NMR signals at δ 100.1 and 100.3, and ^{1}H-NMR signals at 5.87 and 5.99 (each 1H, d, J=0.8 Hz) and 5.92 and 6.01 (each 1H, d, J=0.8 Hz). The ^{1}H-NMR spectrum also showed two singlet signals at δ 6.73 (aromatic, H-3 and H-8) and seven ones (aliphatic) at δ 3.51 (1H, dd, J=4.4, 13.4 Hz), 2.50—3.17 (4H, m), 2.34 (1H, br t, J=13.4 Hz) and 2.75 (1H, dd, J=4.4, 10.2 Hz), which are similar to those of hernangerine (**12**).[9] The ^{13}C-NMR spectrum showed 12 aromatic carbon signals in a range of δ 146.4 and 103.6, two methylenedioxy carbon signals as mentioned above, three methylene carbon signals at δ 42.2, 36.6 and 23.6 and a methine carbon signal at δ 53.8. By extensive analysis of HMQC and HMBC spectral data, aromatic protons at δ 6.73 were assigned to H-3 and H-8. In the NOESY (Fig. 6) experiment, significant correlations were observed among H-3/H_2-4/H_2-5 and H-6a/H-7/H-8. Thus, the structure of **20** was determined to be 11-hydroxy-1,2:9,10-bis(methylenedioxy)noraporphine.

The isolated compounds were tested for their inhibitory activities on HIV-1 IN (Table 2). Hernandonine (**14**), laurolistine (**16**), 7-oxohernangerine (**17**) and lindechunine A (**18**) exhibited appreciable inhibitory activities against HIV-1 IN with IC_{50} values of 16.3, 7.7, 18.2, and 21.1 μM, respectively, while the others showed no significant activities at 100 μM. Of the isolated 7H-dibenzo[de,g]quinolin-7-ones, compounds **14**, **17** and **18**, bearing a methylenedioxy group linked at C-1 and C-2), showed appreciable inhibitory activity, while compound **19**, having no methylenedioxy group, was inactive at 100 μM. This suggests that the 1,2-methylenedioxy group in the 7-oxoaporphine alkaloids may play an important role in the inhibition against HIV-1 IN. Of the all alkaloids isolated, compound **16** (a noraporphine alkaloid) exhibited the most potent inhibitory activity against HIV-1 IN. This compound has an additional hydroxyl group compared to the other alkaloids.

Table 2. Inhibitory Activities of Compounds from *L. chunii* against HIV-1 IN

Compound	IC_{50} (μM)
Lindenanolide E (**1**)	>100
Lindenanolide F (**2**)	>100
Lindenanolide G (**3**)	>100
Lindenanolide H (**4**)	>100
Pseudoneolinderane (**5**)	>100
Lindeneol (**6**)	>100
Lindeneyl acetate (**7**)	>100
Linderalactone (**8**)	>100
Strychinstenolide 6-*O*-acetates A and B (**9** and **10**)	>100
Hernandine (**11**)	>100
Hernangerine (**12**)	>100
Ocokriptine (**13**)	>100
Hernandonine (**14**)	16.3
N-Methyl hernangerine (**15**)	>100
Laurolistine (**16**)	7.7
7-Oxohernangerine (**17**)	18.2
Lindechunine A (**18**)	21.1
7-Oxohernagine (**19**)	>100
Lindechunine B (**20**)	>100
Suramin (positive control)	2.4

Burke *et al.* reported that phenolic moieties in compounds, such as flavones, caffeic acid phenyl esters and curcumins, were required for HIV-1 IN inhibitory activity.[17] A few kinds of alkaloids have been reported as HIV-1 IN inhibitors: ascidian alkaloids from marine natural products were selective inhibitors on HIV-1 IN,[18] and styrylquinoline alkaloids showed potent HIV-1 IN inhibitory effects, followed by blocking of the replication of HIV-1 in cell culture.[19] Pommier *et al.* found that three classes of compounds, DNA-binding molecules, polyhydroxylated aromatic compounds and various nucleotides, effectively inhibited HIV-1 IN activity.[20]

Since the main constituents of the root of *L. chunii* were alkaloids and some isolated alkaloids exhibited HIV-1 IN inhibitory activity, an HPLC method was developed to determine the contents of each alkaloids. The method utilized a C_{18} reversed phase analytical column with MeOH and CH_3COONH_4 buffer as a mobile phase (see Experimental), monitoring the elution profile at 275 nm (Fig. 7). Using the standard curves prepared with authentic sample, the contents (mean±S.D.) of the respective compounds in the crude alkaloid fraction were determined as follows: hernandine (**11**) 17.1±2.4% (w/w), hernangerine (**12**) 15.9±1.0%, hernandonin (**14**) 4.6±1.1%, laurolistine (**16**) 1.5±3.5%, 7-oxohernangerine (**17**) 11.1±3.3%, lindechunine A (**18**) 12.4±1.2%,

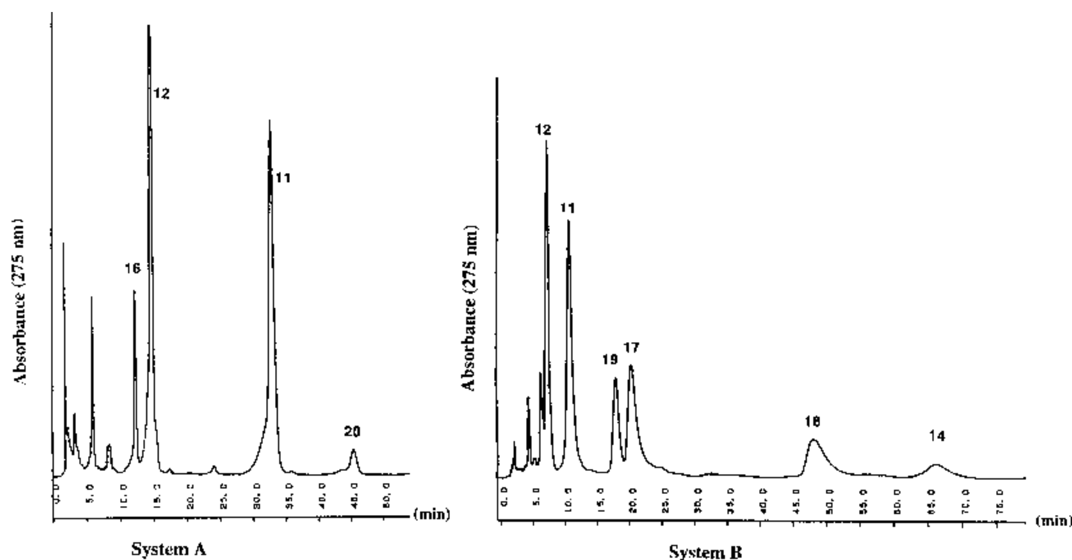

Fig. 7. Elution Profile of a Crude Alkaloid Fraction from the Root of *L. chunii*

Chromatographic conditions: column, PR-18 (4.6×150 mm); flow rate, 1.0 ml/min; detector, 275 nm; elution, MeOH–0.05 M CH_3COONH_4/2% CH_3COOH (20 : 80, system A) and MeOH–0.01 M CH_3COONH_4/0.01% NH_4OH (pH 8.0) (41 : 59, system B). **11**, hernandine; **12**, hernangerine; **14**, hernandonine; **16**, laurolistine; **17**, 7-oxohernangerine; **18**, lindechunine A; **19**, 7-oxohernagine; **20**, lindechunine B.

7-oxohernagine (**19**) 10.6±4.0%, lindechunine B (**20**) 1.5±4.8%. This indicates that hernandonin (**14**), laurolistine (**16**), 7-oxohernangerine (**17**) and lindechunine A (**18**) are the major inhibitory constituents of the root of *L. chunii* against HIV-1 IN.

Although aporphine alkaloids are reported to have vasorelaxing and antioxidant activities,[21] it is worth investigating other biological activities of these aporphine alkaloids, such as antirheumatic and hemostatic activities, in the light of the traditional usage of the root of *L. chunii*.

Experimental

General Melting points were measured on a Yanagimoto micro hot-stage melting point apparatus. Optical rotations were measured with a DIP-360 automatic polarimeter (Jasco Co., Tokyo), UV spectra with a SHIMADZU UV-2200 recording spectrophotometer (Shimadzu Co., Kyoto), and IR spectra with a FT/IR-230 infrared spectrometer (Jasco Co.). ^{1}H- and ^{13}C-NMR spectra were measured with a JNMLA 400WB Lambda NMR (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer (JEOL Co., Tokyo), the chemical shifts being represented as δ (ppm) with tetramethylsilane (TMS) as an internal standard. EI-MS were measured with a JMS-GC mate mass spectrometer at an ionization voltage of 70 eV (JEOL Co.). FAB-MS spectra were obtained with a JEOL JMS-DX 300L spectrometer using glycerol as a matrix. HR-FAB-MS was measured with a JMS 700T mass spectrometer (JEOL, Co.) with a resolution of 5000 in the presence of glycerol containing a faint amount of NaCl. Column chromatography was carried out on silica gel BW-820MH, ODS (Fuji Silysia Co., Nagoya) and Sephadex LH-20 (Pharmacia Co., Tokyo). Medium pressure liquid chromatography (MPLC) was carried out on LiChroprep Si 60 and LiChroprep RP-18 (Merck Co., Darmstadt, Germany). Preparative HPLC was carried out on a Gilson instrument with a 231XL injector, a 119 UV/VIS detector at 275 nm and a TSK-gel ODS-80T_M column (21.5×300 mm, Tosoh Co., Tokyo). Analytical HPLC was carried out on a Tosoh CCPM system equipped with a Tosoh UV-8020 spectrometer and a TSK-gel ODS-80Ts column [4.6 (i.d.)×150 mm, Tosoh Co.]. The flow rate was kept at 1.0 ml/min and the elution profile of compounds was monitored at 275 nm using MeOH–0.05 M CH_3COONH_4/2% CH_3COOH (20 : 80, system A) and MeOH–0.01 M CH_3COONH_4/0.01% NH_4OH (pH 8.0) (41 : 59, system B) as a mobile phase. Thin-layer chromatography (TLC) was carried out on pre-coated silica-gel 60 F_{254}s plates (0.25 mm, Merck Co.) and RP-18 F_{254} plates (0.25 mm), and spots were detected under a UV light or after spraying anisaldehyde–5% H_2SO_4 followed by heating.

Plant Material The roots of *L. chunii* were collected on Mt. Dinghu in China, in September, 2000. A voucher specimen (2000-09-051) was identified by Q. S. and deposited in the Department of Medicinal Plants of Shenyang Pharmaceutical University, Liaoning, China.

Isolation Procedure The dried roots (1.5 kg) of *L. chunii* were extracted three times with 85% EtOH under reflux to give 105.1 g of an extract. The 85% EtOH extract (100 g) was suspended in 3% HCl solution and extracted three times with $CHCl_3$ to give a $CHCl_3$-soluble fraction (15.3 g). The residual suspension was basified with NH_4OH to pH 8.0 and filtered to give a crude alkaloid fraction (48 g). The $CHCl_3$ fraction (15 g) was applied to a column of silica gel. Elution was started with hexane–acetone (98 : 2, 95 : 5, 90 : 10, 50 : 50) followed by acetone–methanol (5 : 1) to give four fractions (Fr. A—D: 5 g, 3.2 g, 0.9 g, 1.2 g, respectively). Repeated column chromatography of Fr. A on silica gel (hexane–acetone, 50 : 1, 25 : 1, 10 : 1), followed by MPLC on silica gel (hexane–acetone, 5 : 1) afforded lideneol (**6**, 22.6 mg) and lindeneyl acetate (**7**, 5.8 mg). Repeated column chromatography of Fr. B on silica gel (hexane–acetone, 25 : 1, 10 : 1, 5 : 1) afforded linderalactone (**8**, 21.5 mg), lindenanolide E (**1**, 6.5 mg), and lindenanolide H (**4**, 1.4 mg). Fraction C was chromatographed on Sephadex LH-20 with $CHCl_3$–MeOH (1 : 1) and then on an ODS column with 60% MeOH to yield pseudoneolinderane (**5**, 18.6 mg) and strychnistenolide 6-*O*-acetate A and B (**9** and **10**, 25.3 mg). Column chromatography of Fr. D was carried out on an ODS column with 50% MeOH, followed by HPLC on a RP-18 column with 70% MeOH to obtain lindenanoloide F (**2**, 3.2 mg) and lindenanolide G (**3**, 1.6 mg).

The brownish sediment (26 g) was chromatographed over silica gel with $CHCl_3$–MeOH (98 : 2, 95 : 5, 90 : 10, 50 : 50) to give 5 fractions (Fr. E—I). Fraction G was rechromatographed on silica gel ($CHCl_3$: MeOH, 20 : 1) to afford hernandine (**11**, 1.2 g). Fraction F was chromatographed on silica gel with EtOAc–MeOH to give five fractions (Fr. F-1 to Fr. F-5); Fr. F-2 was further chromatographed on an ODS column eluting with 80% MeOH to give *N*-methylhernandine (**13**, 5.5 mg); Fr. F-3 was subjected to column chromatography on silica gel and MPLC with $CHCl_3$–MeOH (15 : 1) to give *N*-methylhernangerine (**15**, 3.5 mg) and hernangerine (**12**, 521.1 mg). Fraction F-4 was further chromatographed on silica gel eluting with EtOAC–MeOH (8 : 1) to yield hernandonine (**14**, 8.9 mg), 7-oxohernangerine (**17**, 45.1 mg), 7-oxohernagine (**19**, 23.0 mg) and lindechunine A (**18**, 35.2 mg). Fraction F-5 was chromatographed on silica gel followed by MPLC eluting with $CHCl_3$–MeOH (10 : 1) to obtain laurolistine (**16**, 12.6 mg). Fraction H was repeatedly chromatographed on silica gel eluting with $CHCl_3$–MeOH (5 : 1) and an ODS column with 80% MeOH to obtain lindechunine B (**20**, 14.1 mg).

Lindenanolide E (**1**): Colorless needles (hexane–acetone). mp 151—153 °C. $[\alpha]_D^{26}$ −70.1° (c=1.01, $CHCl_3$). UV λ_{max} (MeOH): 265 nm. IR (KBr) ν_{max}: 1821, 1768, 1653, 1604 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.20 (1H, m, H_α-2), 1.04 (1H, m, H_β-2), 1.42 (3H, m, H_3-14), 1.78 (1H, m, H-1), 1.83 (1H, m, H-3), 1.90 (3H, s, H_3-13), 2.21 (3H, s, H_3-15), 2.70 (1H, d, J=13.2 Hz, H-9α), 2.97 (1H, d, J=13.2 Hz, H-9β), 9.81 (1H, s, H-6). ^{13}C-

NMR: Table 1. EI-MS *m/z*: 260 $[M]^+$. *Anal.* C 69.23%, H 6.15% (Calcd for $C_{15}H_{16}O_4$,: C 69.09%, H 6.19%).

Lindenanolide F (**2**): An amorphous powder. $[\alpha]_D^{26}$ −303.5° (*c*=0.21, $CHCl_3$). IR (KBr) ν_{max}: 2942, 1745, 1056 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.42 (3H, s, H_3-14), 0.74 (1H, m, H-2α), 0.84 (1H, m, H-2β), 1.40 (1H, m, H-1), 1.87 (3H, s, H_3-13), 2.07 (1H, m, H-3), 2.30 (1H, d, *J*=14.4 Hz, H-9α), 2.65 (1H, d, *J*=14.4 Hz, H-9β), 3.48 (1H, d, *J*=10.5 Hz, H-5), 4.38 (1H, d, *J*=10.5 Hz, H-6), 5.03 (1H, s, H_a-15), 5.06 (1H, s, H_b-15). ^{13}C-NMR: Table 1. FAB-MS *m/z*: 513 $[M+Na]^+$. Positive ion HR-FAB-MS *m/z*: 513.2233 $[M+Na]^+$ (Calcd for $C_{30}H_{34}O_6Na$: 513.2256).

Lindenanolide G (**3**): Oil. $[\alpha]_D^{26}$ −250° (*c*=0.2, MeOH). IR (KBr) ν_{max}: 1718, 1644 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.51 (1H, dt, *J*=5.5, 8.4 Hz, H_α-2), 1.25 (1H, m, H-1), 1.35 (3H, s, H_3-14), 1.49 (1H, ddd, *J*=3.7, 6.1, 8.4 Hz, H-3), 1.58 (1H, m, H_β-2), 1.65 (3H, s, H_3-15), 2.05 (3H, s, H_3-13), 2.10 (1H, d, *J*=4.1 Hz, H-5), 3.50 (1H, br s, H-9), 3.75 (3H, s, $OC\underline{H}_3$), 4.68 (1H, d, *J*=4.1 Hz, H-6). ^{13}C-NMR: Table 1. EI-MS *m/z*: 311 $[M+H]^+$. Positive ion HR-FAB-MS *m/z*: 311.1509 $[M+H]^+$ (Calcd for $C_{16}H_{23}O_6$: 311.1548).

Lindechunine A (**18**): Yellow powder. $[\alpha]_D^{26.5}$ 0° (*c*=0.1, MeOH). UV λ_{max} (MeOH): 220, 265, 365, 425 nm. IR (KBr) ν_{max}: 3461, 1643, 1022, 973 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 3.74 (3H, s, $C\underline{H}_3O$-11), 4.22 (3H, s, $C\underline{H}_3O$-3), 6.38 (2H, s, H_2-1, 2), 7.10 (1H, d, *J*=8.9 Hz, H-9), 7.96 (1H, d, *J*=8.9 Hz, H-8), 8.16 (1H, d, *J*=5.2 Hz, H-4), 8.77 (1H, d, *J*=5.2 Hz, H-5). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 60.0 ($\underline{C}H_3O$-10), 60.2 ($\underline{C}H_3O$-3), 99.5 (C-11c), 102.4 ($O\underline{C}H_2O$), 116.7 (C-9), 117.8 (C-8), 117.8 (C-4), 122.4 (C-3a), 124.8 (C-11b), 126.4 (C-11a), 129.9 (C-6a), 135.1 (C-3), 137.8 (C-2), 143.6 (C-5), 144.1 (C-10), 144.3 (C-11), 149.6 (C-1), 156.9 (C-7a), 180.2 (C-7). EI-MS *m/z*: 351 $[M]^+$. HR-EI-MS *m/z*: 351.0734 $[M]^+$ (Calcd for $C_{19}H_{13}O_6N$: 351.0765).

Lindechunine B (**20**): Grayish amorphous powder. $[\alpha]_D^{26.5}$ +43.0° (*c*=0.01, MeOH). UV λ_{max} (MeOH): 222, 280 nm. IR (KBr) ν_{max}: 3461, 1085, 1043, 937 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 2.34 (1H, dd, *J*=13.4, 10.2 Hz, H_{ax}-7), 2.50 (2H, m, H_2-4), 2.75 (1H, dd, *J*=4.4, 10.2 Hz, H_{eq}-7), 2.68—3.17 (2H, m, H_2-5), 3.51 (1H, dd, *J*=4.4, 13.4 Hz, H-6a), 5.87 and 5.99 (each 1H, d, *J*=0.8 Hz, $OC\underline{H}_2O$-1, 2), 5.92 and 6.01 (each 1H, d, *J*=0.8 Hz, $OC\underline{H}_2O$-9, 10), 6.73 (2H, s, H-3 and H-8). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ 23.6 (C-4), 36.6 (C-7), 42.2 (C-5), 53.8 (C-6a), 100.1 ($O\underline{C}H_2O$-1, 2) 100.3 ($O\underline{C}H_2O$-9, 10) 103.6 (C-11b), 106.1 (C-3), 113.6 (C-11a), 116.9 (C-3a), 119.9 (C-8), 129.3 (C-11c), 132.9 (C-10), 138.1 (C-11), 142.9 (C-1 and C-9), 146.4 (C-2). EI-MS *m/z*: 325 $[M]^+$. HR-EI-MS *m/z*: 325.0949 $[M]^+$ (Calcd for $C_{18}H_{15}O_5N$: 325.0925).

Chemicals and Enzyme The isolated compounds were dissolved in 50% DMSO before investigation of HIV-1 IN inhibitory activity. HIV-1 IN protein was expressed in *Escherichia coli* and the purified enzyme was stored at −80 °C. Substrate DNA and oligo-nucleotides of long terminal repeat (LTR) donor DNA were purchased from Japan Bioservice Co. (Asaka City, Japan) and stored at −25 °C. The sequences of biotinylated LTR donor DNA and its unlabelled complement were 5′-biotin-ACCCTTTTAGTCAGTGTGGAAAATCTCAGCAGT-3′ and 3′-GAAAATCAGTCACACCTTTTAGAGATCGTCA-5′, respectively. Digoxigenin labelled target DNA consisted of 5′-TGACCAAGGGCTAATTCACT-digoxigenin and 3′-digoxigenin-ACTGGTTCCCGATTAAGTGA-5′.

Multiplate Integration Assay (MIA) Procedure[22—24] A 96 well plate was coated with 50 μl of a streptavidin solution (40 μg/ml streptatividin, 90 mM Na_2CO_3 and 10 mM (pH 8.0)), and 1 mM NaCl and 40 fmol/μl of LTR donor DNA were added to each well. The plate was gently shaken at room temperature for 30 min, then the wells were washed four times with phosphate-buffered saline (PBS, pH 7.3). A mixture (45 μl) composed of 12 μl of IN buffer [150 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS, pH 7.2), 7.5 mM $MnCl_2$, 5 mM dithiothreitol (DTT), 25% glycerol and 500 μg/ml bovine serum albumin], 1 μl of 5 pmol/μl digoxigenin-labelled target DNA and 32 μl of sterilized water were added. Subsequently, 6 μl of a sample solution and 9 μl of 1/10 dilution of HIV-1 IN enzyme were added to each well and the plate was incubated at 37 °C for 80 min. The wells were washed with PBS and 100 μl of 500 mU/ml alkaline phosphatase (AP) labelled anti-digoxigenin antibody was added. After incubation at 37 °C for 1 h, the wells were washed with washing buffer (0.05% Tween 20 in PBS, 4 times) and PBS 4 times. Then, 150 μl of AP buffer (100 mM Tris–HCl (pH 9.5), 100 mM NaCl, 5 mM $MgCl_2$ and 10 mM *p*-nitrophenyl phosphate) were added to each well and incubated at 37 °C for 1 h. The visible absorbance of each well was measured with a microplate reader (mode 3550 UV, BIORAD) at a wavelength of 405 nm. The positive control group was composed of a reaction mixture, 50% DMSO and HIV-1 IN enzyme. The negative control group was buffer E [20 mM MOPS (pH 7.2), 400 mM potassium glutamate, 1 mM ethylenediaminetetraacetate disodium salt (EDTA 2Na), 0.1% Nonidet P-40 (NP-40), 20% glycerol, 1 mM DTT and 4 M urea] without HIV-I IN enzyme. Suramin was used as a positive control, which inhibited HIV-1 IN activity with an IC_{50} value of 2.4 μM under the above conditions.

Quantitative Analysis of Major Aporphine Alkaloids by HPLC The separation of the respective alkaloids was carried out by HPLC and their contents in the alkaloid fraction were determined using standard lines prepared with authentic samples. The linear lines were obtained for the following compounds in a range of added amounts: hernandine (**11**) at 4.0—20.1 μg, hernangerine (**12**) at 1.2—10.0 μg, hernandonine (**14**) at 0.8—2.5, laurolistine (**16**) at 0.4—2.0 μg, 7-oxohernangerine (**17**) at 1.8—7.5 μg, lindechunine A (**18**) at 1.8—7.5 μg, 7-oxohernagine (**19**) at 2.4—7.5 μg, and lindechunine B (**20**) at 0.6—2.0 μg.

Hernandine (**11**), hernangerine (**12**), laurolistine (**16**) and lindechunine B (**20**) were separated with solvent system A, their contents in the alkaloid fraction and retention times being 17.1±2.6% (mean±S.D. in w/w) (32.8 min), 15.9±1.0% (14.7 min), 1.5±3.5% (12.3 min) and 1.5±4.8% (45.4 min), respectively. The contents and retention times of hernandonine (**14**), 7-oxohernangerine (**17**), lindechunine A (**18**) and 7-oxohernagine (**19**) were 4.6±1.1% of the alkaloid fraction (66.1 min), 11.1±3.3% (20.2 min), 12.4±1.2% (48.1 min) and 10.6±4.0% (17.8 min), respectively, when the elution was carried out with solvent system B.

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