Littoralisone, a Novel Neuritogenic Iridolactone Having an Unprecedented Heptacyclic Skeleton Including Four- and Nine-Membered Rings Consisting of Glucose from *Verbena littoralis*

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Verbena littoralis H. B. K. (Verbenaceae) has been widely used as a traditional folk medicine for diarrhea, typhoid fever and tonsillitis in South America.[1] Phytochemical studies on *V. brasiliensis*, *V. officinalis*, and *V. hastata* have been extensively done and many compounds have been isolated.[2,3] However, ethnobotanical and phytochemical information on *V. littoralis* have been published in only one literature[1] and only two constituents have been reported.[1] In the course of our investigations of biologically active substances from medicinal plants,[4−6] we have devoted our attention to the occurrence of natural products having new skeletons as well as the neuritogenic activity since these compounds may not only be useful for the basic study and medical treatment of dementia, but also be attractive synthetic target compounds of chemists. Recently, we isolated nardosinone and picrosides from *Nardostachys chinensis* and *Picrorhiza scrophulariflora* as enhancers of the action of nerve growth factor (NGF), respectively.[4,5] More recently, the crude extract of *V. littoralis* has been shown to potentiate NGF-induced neurite outgrowth from PC12D cells. This extract was chromatographed by monitoring the potentiation of NGF-action to give littoralisone (**1**, Figure 1). In this paper, we present the first report on the successful isolation and structure elucidation of littoralisone (**1**) having a novel skeleton, a heptacyclic iridolactone glucoside from *V. littoralis*.

The EtOAc-soluble materials of the MeOH extract of aerial parts of *V. littoralis* collected in Paraguay were subjected to silica gel columns (EtOAc/MeOH) and Sephadex LH-20 column (MeOH) followed by reversed-phase

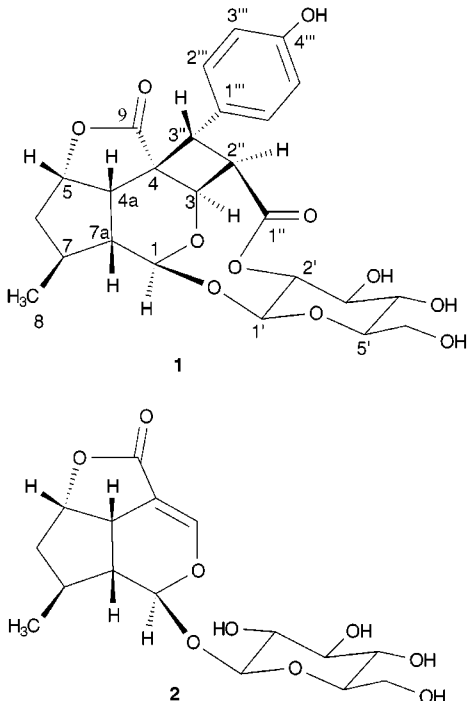

Figure 1. Chemical structures of littoralisone (**1**) and brasoside (**2**).

semipreparative HPLC on a YMC-NH_2 column (90% MeCN in H_2O) to yield littoralisone (**1**, 13 mg) as colorless amorphous powder.

Littoralisone showed a molecular ion peak at *m*/*z* 504 in EIMS spectrum, and its molecular formula was determined to be $C_{25}H_{28}O_{11}$ by HREIMS (*m*/*z* 504.1617, Δ −1.4 mmu). The IR spectrum indicated the presence of hydroxyl (3600−3300 cm^{-1}) and lactone (1745 cm^{-1}) moieties. ^1H and ^{13}C NMR data (Table 1) disclosed the presence of two ester carbonyl, one sp^3, and two sp^2 quaternary carbons, 14 sp^3 methines (seven of which were oxygen-bearing), four sp^2 methines, two sp^3 methylenes (one of which was oxygen-bearing), and one methyl groups. The initial analysis of the NMR spectral data indicated that the molecule consisted of a monoterpene iridoid and a sugar moiety (Figure 1). In the ^{13}C NMR spectrum, six carbons appeared at δ_C 100.37 (C-1′), 80.49 (C-2′), 74.59 (C-3′), 71.93 (C-4′), 79.54 (C-5′), and 62.63 (C-6′), suggesting the presence of a glucopyranosyl group which was confirmed by the detailed analysis of correlations in the $^1H-^1H$ COSY (Figure 2), HMBC (Table 1), and NOESY spectra (Figure 3).

Detailed analysis of NMR spectral data, in particular $^1H-^1H$ COSY, HMQC, and HMBC data, revealed the structure for the aglycon moiety. The ^1H NMR spectrum showed a singlet methine proton signal at δ 5.23 (H-1), which was connected to C-1 (δ_C 96.45) by the HMQC spectrum. Correlations of H-1/C-3 (δ_C 66.84), H-1/C-4a (δ_C 43.87), and H-1/C-1′ (δ_C 100.37) were observed in the HMBC spectrum. The other two oxygenated methine protons at δ 5.06 (H-3) and 5.16 (H-5) were connected to C-3 (δ_C 66.84) and C-5 (δ_C 83.47) according to the HMQC spectrum, respectively. H-3 correlated to C-1 (δ_C 96.45), C-4 (δ_C 48.13), C-4a (δ_C 43.87), C-9 (δ_C 176.94), C-1″ (δ_C 175.13), and C-2″ (δ_C 50.14), and H-5 correlated to C-7

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Table 1. NMR Spectral Data of Littoralisone (CD_3OD, ^1H NMR 500 MHz, ^{13}C NMR 125 MHz)

position	δ_H[a]	*J* (in Hz)	δ_C[a]	HMBC correlation
1	5.23	s	96.45 (d)	C-3, C-4a, C-7, C-1′
3	5.06	d, 11.1	66.84 (d)	C-1, C-4, C-4a, C-9, C-2″, C-1″
4			48.13 (s)	
4a	3.35	dd, 9.2, 4.6	43.87 (d)	C-1, C-4, C-7, C-7a, C-3″
5	5.16	dd, 5.0, 4.6	83.47 (d)	C-7, C-7a, C-9
6	2.03 (H-6α)	dd, 12.6, 3.8	43.23 (t)	C-4a, C-5, C-7, C-7a
	1.42 (H-6*β*)	dd, 12.6, 5.0		C-7, C-8
7	1.49	m	33.21 (d)	C-6
7a	1.70	dd, 11.8, 9.2	46.98 (d)	C-4, C-4a, C-7, C-8
8	1.03	d, 5.7	16.58 (q)	C-6, C-7, C-7a
9			176.94 (s)	
1′	4.83	d, 8.4	100.37 (d)	C-1, C-2′, C-3′, C-5′
2′	4.73	dd, 9.9, 8.4	80.49 (d)	C-1′, C-1″, C-3′
3′	3.72	dd, 9.9, 8.4	74.59 (d)	C-2′, C-4′
4′	3.37	dd, 9.9, 8.4	71.93 (d)	C-3′, C-5′, C-6′
5′	3.41	ddd, 9.9, 5.3, 2.3	79.54 (d)	C-6′
6′	3.68 (H-6′a)	dd, 11.8, 5.3	62.63 (t)	C-5′
	3.90 (H-6′b)	dd, 11.8, 2.3		C-4′
1″			175.13 (s)	
2″	3.94	dd, 11.1, 4.6	50.14 (d)	C-3, C-4, C-1″, C-3″, C-1‴
3″	4.15	d, 4.6	49.65 (d)	C-3, C-4, C-4a, C-9, C-1″, C-2″, C-1‴, C-2‴
1‴			128.37 (s)	
2‴	7.06	dd, 6.5, 1.9	130.32 (d)	C-3″, C-4‴, C-6‴
3‴	6.72	dd, 6.5, 1.9	116.19 (d)	C-1‴, C-4‴, C-5‴
4‴			157.79 (s)	
5‴	6.72	dd, 6.5, 1.9	116.19 (d)	C-1‴, C-3‴, C-4‴
6‴	7.06	dd, 6.5, 1.9	130.32 (d)	C-2‴, C-4‴, C-5‴

[a] 1D NMR signals were assigned by $^1H-^1H$ COSY, long range $^1H-^1H$ COSY, DEPT, HMQC, and HMBC experiments.

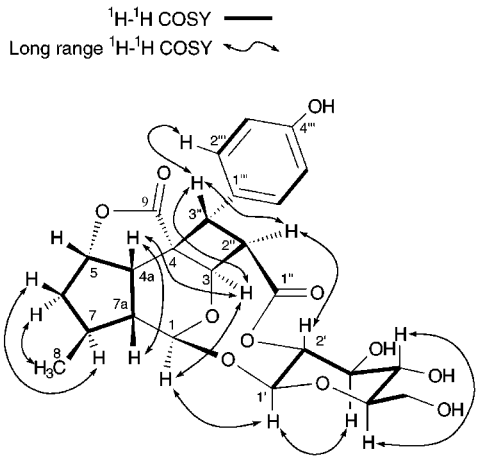

Figure 2. Selected cross-peaks of $^1H-^1H$ COSY and long-range $^1H-^1H$ COSY spectra of littoralisone.

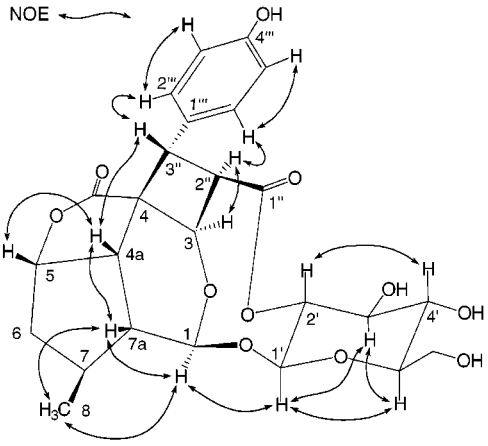

Figure 3. Selected correlations of the NOESY spectrum of littoralisone.

(δ_C 33.21), C-7a (δ_C 46.98), and C-9 in the HMBC spectrum. The five-membered ring was determined by the analysis of $^1H-^1H$ COSY, long-range $^1H-^1H$ COSY (Figure 2), and HMBC spectral data. One doublet signal at δ 1.03 (3H, J = 5.7 Hz, H-8) was connected to the methyl carbon C-8 (δ_C 16.58). The cross-peak between H-8 (δ 1.03) and H-7 (δ 1.49) was observed in the $^1H-^1H$ COSY spectrum, and the correlations of C-8/H-6*β*, C-8/H-7a, H-8/C-6 (δ_C 43.23), H-8/C-7, and H-8/C-7a were given in the HMBC spectrum. The presence of a five-membered lactone ring was deduced by the analysis of the downfield chemical shift signals of C-5 (δ_C 83.47) and H-5 (δ 5.16), which revealed that the methine (C-5) was linked to an acyloxy group, and this was confirmed by the correlations of C-9/H-3, C-9/H-5, and C-9/H-3″ (δ 4.15) in the HMBC spectrum. The iridoid substructure was demonstrated as shown in Figure 1 by the above analysis.

A pair of two-proton double doublets (J = 6.5, 1.9 Hz) at δ 7.06 (H-2‴(6‴)) and 6.72 (H-3‴(5‴)) implied the presence of a *p*-hydroxyphenyl group, and this was supported by the bathochromic shift of the UV absorption at 279 nm under alkaline condition. The corresponding carbon signals of the *p*-hydroxyphenyl group were assigned by the HMQC and HMBC spectra (Table 1). The remaining two methine signals at δ 3.94 (1H, dd, J = 11.1, 4.6 Hz, H-2″) and 4.15 (1H, d, J = 4.6 Hz, H-3″) were connected to the C-2″ (δ_C 50.14) and C-3″ (δ_C 49.65) positions of the four-membered ring, because cross-peaks of H-3″/H-2″ and H-2″/H-3 were observed in the $^1H-^1H$ COSY spectrum. The characteristic four-membered ring structure was further confirmed by the correlations of C-2″/H-3, C-2″/H-3″, C-3″/H-4a, C-3″/H-2″, C-3″/H-2‴(6‴), H-2″/ C-3, H-2″/C-4, H-2″/C-1″, H-2″/C-1‴, H-3″/C-3, H-3″/C-4/C-4a, H-3″/C-9, H-3″/C-1″, H-3″/C-1‴, and H-3″/C-2‴(6‴) in the HMBC spectrum. Furthermore, the carbonyl carbon at δ_C 175.13 (C-1″) was connected to C-2″ by the correlations of C-1″/H-2″, C-1″/H-3″, and C-1″/H-3. The *p*-hydroxyphenyl group was

connected to C-3″ position by the correlations of C-1‴/H-3″, C-1‴/H-2″, and C-3″/H-2‴ (6‴) in the HMBC spectrum.

The NOESY spectrum of **1** (Figure 3) allowed many of the stereochemical features of littoralisone to be assigned. The presence of cross-peaks of H-1/H-7a, H-1/7-CH_3, H-7a/H-4a, H-7a/7-CH_3, and H-4a/H-5 provided that H-1, H-4a, H-5, H-7a, and 7-CH_3 oriented on the same side of the cyclopentane−pyran in the β-position. The correlations of H-3/H-2″ and H-3″/H-4a as well as the absence of NOE between H-2″ and H-3″ confirmed the location of a four-membered ring, the α-position of H-3 and H-2″, and the β-position of H-3″. The analysis of NOESY correlations of H-2‴ (6‴)/H-2″ and H-2‴ (6‴)/H-3″ indicated the α-position of the *p*-hydroxyphenyl group. The configulation at C-1 had the oxygen in the β-position, with the proton α, like other iridoids including brasoside (**2**)[2,7] from the detailed analysis of the molecular model of **1** on the basis of following observations; 1) there were NOEs between H-1 and H-7a and between H-1 and H-1′, but not between H-1 and H-4a, 2) H-1 appeared as a singlet, so the angle between H-1 and H-7a should be near 90°.

For the sugar moiety, the NOESY correlations among the protons of the glucopyranosyl moiety (Figure 3) and the coupling constants (see Table 1, in particular, $J_{\text{H-1}',\text{H-2}'} = 8.4$ Hz) indicated a $^1C_4$ chair conformation of β-glucopyranose moiety. The C-1′ and C-2′ carbon atoms of β-glucopyranosyl group were attached to the C-1 and C-1″ position of the aglycon through oxygen atoms by the obvious correlations from H-1′ to C-1 and from H-2′ to C-1″ in the HMBC spectrum, respectively. The absolute stereostructure of sugar was determined as the D-form by the HPLC analysis of a thiazolidine derivative which were obtained by condensation of the aqueous acidic hydrolysate of **1** with L-cysteine methyl ester hydrochloride.[8] The iridoid moiety of **1** is related to **2** and its dihydro derivative and has the absolute configuration depicted in the Figure 1.

Although there are many research reports on the iridoid compounds,[9−15] littoralisone is the first heptacyclic iridolactone bearing four-, five-, six-, and nine-membered rings. For the biosynthetic point of view, the four-membered ring may be formed by the intramolecular [2 + 2] cycloaddition of *trans*-cinnamate moiety on the convex side of iridolactone. Unfortunately, precursory side-products of littoralisone failed to be isolated from the crude extract. Littoralisone (100 μM) showed an enhancing activity (30%) of NGF-mediated neurite outgrowth from PC12D cells. Detailed pharmacological properties of this compound will be reported elsewhere.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded at 500 or 600 MHz for protons and at 125 or 150 MHz for carbons in CD_3OD. Chemical shifts refer to the MeOH-d_4 multiplet (^{1}H, 3.30 ppm; ^{13}C, 49.0 ppm). Infrared spectra were determined as thin films. Melting points were uncorrected. Enhancing activity of NGF-mediated neurite outgrowth from PC12D cells was examined by the same method as described previously.[4]

Materials. The aerial parts of *V. littoralis* were provided by Seiwa Yakuhin Co., Ltd. (Ibaragi, Japan). The botanical identification was made by Mr. Tetsuo Nakasumi (Instituto de Pesquisas de Plantas Medicinais do Brasil).

Extraction and Isolation. The aerial parts of *V. littoralis* (1 kg) were extracted with MeOH (3 L) three times, and the MeOH extract (48 g) was partitioned between EtOAc and H_2O (1:1). The EtOAc-soluble fraction (18 g) was subjected to silica gel column chromatography (EtOAc/MeOH, 5:1) and Sephadex LH-20 column chromatography (MeOH) followed by reversed-phase semipreparative HPLC (YMC-Pack NH_2, 5 μm, 300 Å∼10 mm; eluent, MeCN/H_2O, 90:10; flow rate, 1 mL/min; RI detector) to afford littoralisone (**1**, 13 mg).

Littoralisone (1): colorless amorphous powder; mp 159−161 °C; $[\alpha]^{26}_D$ −49.5° (*c* 0.43, MeOH); UV (MeOH) λ_{max} 204 (log ϵ, 0.51), 229 (0.30) and 279 (0.05) nm; UV (MeOH + NaOH) λ_{max} 206 (log ϵ, 1.08), 244 (0.30) and 295 (sh.) nm; IR (neat) ν_{max} 3450, 1745, 1635, 1520 and 1450 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* 504 $[M]^+$ (1.4), 476 (1.1), 396 (1.9), 309 (25.8), 164 (10.2), 147 (100), 119 (17.0), 107 (11.5); HREIMS *m/z* 504.1617 $[M]^+$ (calcd for $C_{25}H_{28}O_{11}$, 504.1631).

HPLC Analysis of Sugar Component in 1. A solution of **1** (1.5 mg) in 4 N aqueous HCl (2.0 mL) was heated under reflux for 7 h. The reaction mixture was neutralized with 1 N NaOH and concentrated under reduced pressure. The residue was dissolved in pyridine (1 mL) and treated with L-cysteine methyl ester hydrochloride (4.5 mg) at 60 °C for 1 h. The resulting thiazolidine derivatives were analyzed by HPLC. HPLC conditions: detection, UV (220 nm) and RI; column, Hibar LiChrosorb NH_2 (4.6 × 250 mm); mobile phase, 95% MeOH (in H_2O); flow rate 1 mL/min. t_R: methyl 2- (D-*gluco*-pentahydroxypentyl)-thiazolidine-4*R*-carboxylate = 19.5 and 26.5 min. Each t_R was identical with that of authentic sample.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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