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LINDERNIOSIDES A AND B, OLEANANE SAPONINS FROM *LINDERNIA PYXIDARIA*

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Key Word Index—*Lindernia pyxidaria*; Scrophulariaceae; lindernioside; oleanane saponin; medicagenic acid; 30-normedicagenic acid.

Abstract—Two new oleanane-type triterpene saponins, named linderniosides A and B, were isolated from the whole plants of *Lindernia pyxidaria*, together with a known triterpene glycoside, medicagenic acid 3-*O*-glucuronide. The structures of these compounds were determined on the basis of spectral and chemical evidence.

INTRODUCTION

In connection with a study on the saponin of some plants of the Scrophulariaceae [1–6], we have investigated *Lindernia pyxidaria* L., which is indigenous to Asia and Europe. This paper reports the isolation and the structure elucidation of two new saponins and a known glycoside from the whole plants.

RESULTS AND DISCUSSION

The water extract of the whole plants was passed through a porous polymer gel Mitsubishi Diaion HP-20 column and the methanol eluate was chromatographed on a silica gel column to give fractions A and B. Fraction B was methylated with diazomethane after treatment with cation exchanger and separated into three fractions (frs 1–3). From fractions 1 and 2, compounds **1**–**3** were isolated by preparative HPLC as methyl ester (**1a**–**3a**). Compound **1a** was identified as medicagenic acid 3-*O*-β-D-glucuronide trimethyl ester [7].

Lindernioside A methyl ester (**2a**), obtained as an amorphous powder, exhibited an $[M + Na]^+$ ion peak at *m/z* 905 in the FAB-mass spectrum. The ^{1}H NMR spectrum exhibited the presence of six singlet methyl signals at δ0.85, 0.90, 0.93, 1.22, 1.50 and 1.87, an olefinic proton signal at δ5.37, three carbomethoxyl signals at δ3.63, 3.70 and 3.82 and two anomeric proton signals at δ4.86 (1H, *d*, *J* = 8 Hz) and 5.41 (1H, *d*, *J* = 8 Hz). The ^{13}C NMR spectrum was almost superimposable on that of compound **1a** in the aglycone unit (Table 1 [8]). Methanolysis of **2a** with acetylchloride-methanol gave medicagenic acid dimethyl ester. Acid hydrolysis of **2a** after reduction with $NaBH_4$ gave glucose. These data suggested that **2a** was composed of medicagenic acid dimethyl ester as an aglycone and one glucose and one glucuronic acid methyl ester [9]. We employed the difference NOE experiment to decide the sugar sequence after assignment of most proton signals by homonuclear Hartmann–Hahn (HOHAHA) spectrum. When the signals at δ4.86 for the H-1 of the glucosyl unit and at δ5.41 for the H-1 of the glucuronosyl methyl ester unit were irradiated, NOEs were observed at the signals at δ4.45 (1H, *d*, *J* = 4 Hz) due to the H-3 of the aglycone unit and at δ4.17 (1H, *t*, *J* = 8 Hz) due to the H-3 of the glucosyl unit, respectively. In the heteronuclear multiple bond connectivity (HMBC) spectrum, long-range ^{1}H–^{13}C correlations were observed between the H-1 of the glucosyl unit and the C-3 of the aglycone unit and between the H-1 of the glucuronosyl methyl ester unit and the C-3 of the glucosyl unit. From the above evidence, the structure of **2a** was concluded to be 3-*O*-β-D-glucuronopyranosyl-(1 → 3)-β-D-glucopyranosyl medicagenic acid trimethyl ester.

Lindernioside B methyl ester (**3a**), obtained as an amorphous powder, exhibited an $[M + Na]^+$ ion peak at *m/z* 917 in the FAB-mass spectrum. The ^{1}H NMR spectrum exhibited the presence of four singlet methyl signals at δ0.82, 1.20, 1.51 and 1.89, an olefinic proton signal at δ5.37, two *exo*-methylene proton signals at δ4.73 (1H, *br s*) and 4.77 (1H, *br s*), four carbomethoxyl signals at δ3.60, 3.65, 3.69 and 3.84 and two anomeric proton signals at δ4.94 (1H, *d*, *J* = 8 Hz) and 5.41 (1H, *d*, *J* = 8 Hz). Acid hydrolysis after reduction with $NaBH_4$ gave glucose as a sugar moiety [9]. In the difference NOE spectrum, NOEs were observed at δ4.46 (1H, *d*, *J* = 4 Hz) owing to the H-3 of the aglycone unit and δ4.22 (1H, *t*, *J* = 9 Hz) owing to the H-3 of the inner glucuronosyl unit on irradiation at δ4.94 (1H, *d*,

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Table 1. ^{13}C NMR spectral data of compounds **1a**, **2a**, **3a**, and amaranthus-saponin III [8] (in pyridine-d_5 at 35)*

	1a	2a	3a	Amaranthus saponin III
Aglycone unit				
1	44.3	44.1	44.4	44.2
2	70.7	70.3	70.7	69.8
3	86.4	86.5	86.7	89.6
4	53.4	53.3	53.5	39.0
5	52.9	52.8	52.9	56.0
6	21.1	21.1	21.1	18.6
7	32.8	32.8	32.8	33.1
8	40.1	40.1	40.1	40.0
9	48.6	48.6	48.5	48.3
10	36.9	36.9	36.8	37.0
11	23.4	23.4	23.5	23.5
12	122.8	122.8	†	122.9
13	144.2	144.2	143.5	143.4
14	42.1	42.1	42.0	42.2
15	28.0	28.0	28.0	28.1
16	23.9	23.9	23.8	23.8
17	46.9	46.9	47.3	47.3
18	41.8	41.8	47.7	47.6
19	46.0	46.1	41.6	41.6
20	30.8	30.8	148.5	148.5
21	34.0	34.0	30.1	30.1
22	32.8	32.8	37.9	37.6
23	178.5	178.4	178.4	29.4
24	13.7	13.8	13.6	18.5
25	16.8	16.8	16.8	16.4
26	17.1	17.1	17.0	17.5
27	26.2	26.2	26.2	26.1
28	178.0	178.0	177.2	175.7
29	33.1	33.1	107.4	107.3
30	23.7	23.7		
COOMe	51.6	51.6	51.6	
	52.2	52.2	52.2	
Sugar unit				
Inner				
1	106.6	105.5	106.4	
2	74.5	73.8	73.5	
3	77.6	87.6	86.3	
4	73.0	69.6	71.1	
5	77.1	78.0	76.5	
6	170.6	62.4	170.0	
COOMe	52.0		52.2	
terminal				
1		105.8	105.9	
2		75.4	75.5	
3		77.6	77.5	
4		73.1	73.2	
5		77.1	77.2	
6		170.4	170.4	
COOMe		52.0	52.0	

*Assigned by heteronuclear single quantum coherence spectrum.

†Overlapped with solvent signals.

J = 8 Hz) owing to the H-1 of the inner glucuronosyl unit and at δ5.41 (1H, d, J = 8 Hz) owing to the H-1 of the terminal glucuronosyl unit, respectively. In the HMBC spectrum, the long-range ^{1}H ^{13}C correlations were observed between the H-1 of the inner glucuronosyl unit and the C-3 of the aglycone unit and between the H-1 of the terminal glucuronosyl unit and the C-3 of the inner glucuronosyl unit. Two *exo*-methylene proton signals at δ4.73 and 4.77 were correlated with the C-19 and C-21. The ^{13}C NMR data of the A ring carbons were superimposable on those of compound **1a** and the data of the B, C, D and E ring carbons were superimposable on those of 2β,3β-dihydroxy-30-norolean-12,20(29)-dien-28-oic acid glycoside, amaranthus-saponin III [8] (Table 1). These data led us to conclude the structure of **3a** to be 3-*O*-β-D-glucuronopyranosyl-(1 → 3)-β-D-glucuronopyranosyl 30-normedicagenic acid tetramethyl ester.

Lindernioside B is an interesting compound having two glucuronic acid units as the sugar moiety which has been noted previously only for glycyrrhizin isolated from *Glycyrrhiza* species (Leguminosae).

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR: Jeol α-400 FT NMR spectrometer, with TMS as an int. standard; FAB–MS; Jeol JMS-SX102 MS; optical rotations; Jasco DIP-360 digital polarimeter; GC; Hitachi G 3000 gas chromatograph.

Plant material. Lindernia pyxidaria L. was collected in Shizuoka, Japan in September 1994 and the voucher specimen is deposited in the herbarium, School of Pharmaceutical Sciences (University of Shizuoka, Japan).

Extraction and isolation. Dried whole plants (1 kg) was extracted with hot H_2O. The extract was passed through a porous polymer gel Mitsubishi Diaion HP-20 column. After washing the column with H_2O, the adsorbed materials were eluted with 50% MeOH aq. (yield 30 g) and MeOH (yield 20 g), successively. The MeOH eluate was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O–AcOH (62:32:5:1) to give fractions A (10.4 g) and B (7.7 g). Fraction B was dissolved in H_2O–MeOH (1:1) and the soln was passed through an Amberlyst 15 column and the column was washed with MeOH. The eluate was methylated with diazomethane in the usual manner. The methylated fraction was chromatographed on a silica gel column with $CHCl_3$–MeOH (9:1) to give fractions 1 (yield 790 mg), 2 (yield 120 mg) and 3 (yield 956 mg). Fraction 1 was subjected to prep. HPLC (Develosil Lop-ODS, 50 mm × 50 cm × 2, 65 → 84% MeOH linear gradient) to afford **1a** (420 mg) and **2a** (43 mg). Fraction 2 was subjected to semi prep. HPLC (Develosil PhA-7, 20 mm × 25 cm, 75% MeOH) to afford **3a** (30 mg).

Lindernioside A methyl ester (**2a**). Amorphous powder, $[\alpha]_D^{20}$ + 22.9° (MeOH; c 3.17). FAB-MS m/z: 905 $[M + Na]^+$. (Found: C, 59.07; H, 8.08. $C_{45}H_{70}O_{17} \cdot 2H_2O$ requires: C, 58.81; H, 8.12%) ^{1}H and ^{13}C NMR: Tables 1 and 2.

Lindernioside B methyl ester (**3a**). Amorphous powder, $[\alpha]_D^{20}$ + 51.1° (MeOH; c 1.80). FAB-MS m/z: 917 $[M + Na]^+$. (Found: C, 59.37; H, 7.64. $C_{45}H_{66}O_{18} \cdot H_2O$

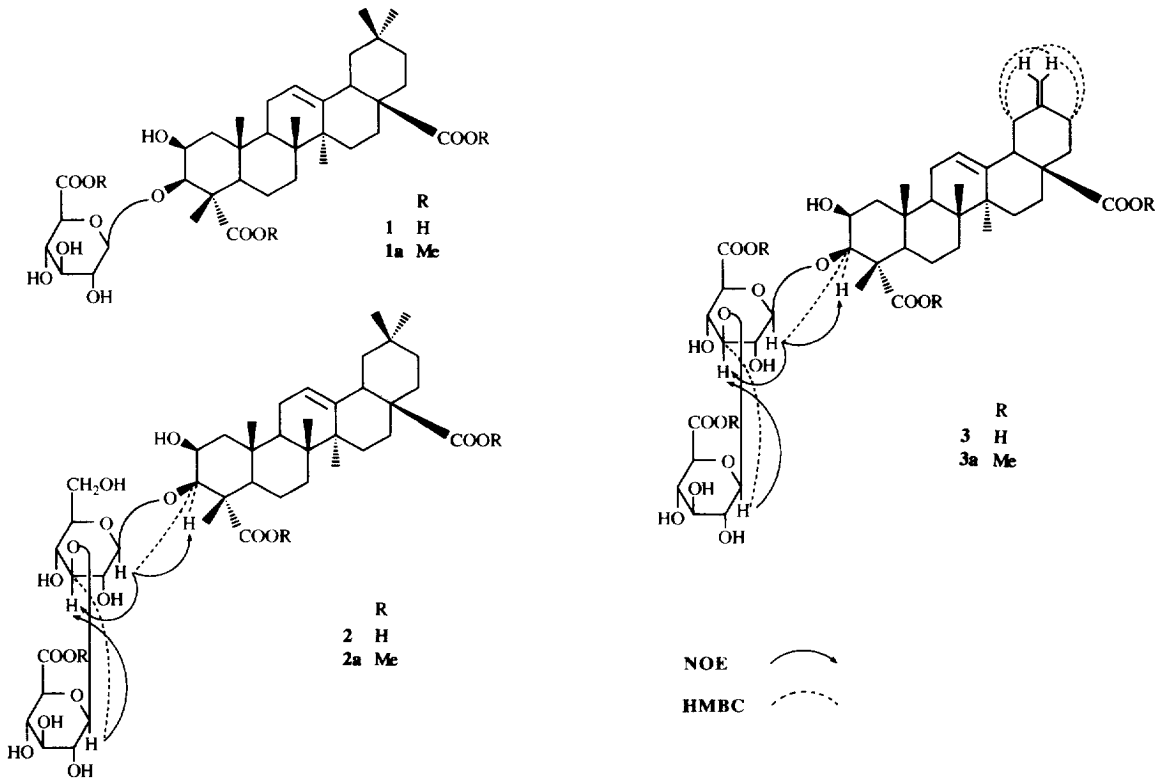

Table 2. ^{1}H NMR spectral data of compounds **2a** and **3a** (in pyridine-d_5 at 35°)

	2a	**3a**
Aglycone unit		
2	4.76 (1H, *m*)	4.71 (1H, *m*)
3	4.45 (1H, *d*, *J* = 4 Hz)	4.46 (1H, *d*, *J* = 4 Hz)
12	5.37 (1H, *t*-like)	5.37 (1H, *t*-like)
18	3.08 (1H, *dd*, *J* = 14, 4 Hz)	2.99 (1H, *dd*, *J* = 13.5, 4.5 Hz)
19α		2.55 (1H, *br t*, *J* = 13.5 Hz)
24	1.87 (3H, *s*)	1.89 (3H, *s*)
25	1.50 (3H, *s*)	1.51 (3H, *s*)
26	0.85 (3H, *s*)	0.82 (3H, *s*)
27	1.22 (3H, *s*)	1.20 (3H, *s*)
29	0.90 (3H, *s*)	4.73 (1H, *br s*)
		4.77 (1H, *br s*)
30	0.93 (3H, *s*)	
COOMe (23)	3.82 (3H, *s*)	3.84 (3H, *s*)
COOMe (28)	3.70 (3H, *s*)	3.65 (3H, *s*)
Sugar unit		
Inner	(glucosyl)	(glucuronosyl)
1	4.86 (1H, *d*, *J* = 8 Hz)	4.94 (1H, *d*, *J* = 8 Hz)
2	3.88 (1H, *t*, *J* = 8 Hz)	3.86 (1H, *dd*, *J* = 8, 9 Hz)
3	4.17 (1H, *t*, *J* = 8 Hz)	4.22 (1H, *t*, *J* = 9 Hz)
4	4.02 (1H, *t*, *J* = 8 Hz)	4.26 (1H, *t*, *J* = 9 Hz)
5	3.85 (1H, *m*)	4.46 (1H, *d*, *J* = 9 Hz)
6	4.19*	
	4.40 (1H, *dd*, *J* = 11, 2 Hz)	
COOMe terminal		3.69 (3H, *s*)
	(glucuronosyl)	(glucuronosyl)
1	5.41 (1H, *d*, *J* = 8 Hz)	5.41 (1H, *d*, *J* = 8 Hz)
2	4.02 (1H, *t*, *J* = 8 Hz)	4.00 (1H, *dd*, *J* = 8, 9 Hz)
3	4.21 (1H, *dd*, *J* = 8, 9 Hz)	4.20 (1H, *t*, *J* = 9 Hz)
4	4.37 (1H, *dd*, *J* = 9, 9.5 Hz)	4.37 (1H, *dd*, *J* = 9, 9.5 Hz)
5	4.51 (1H, *d*, *J* = 9.5 Hz)	4.50 (1H, *d*, *J* = 9.5 Hz)
COOMe	3.63 (3H, *s*)	3.60 (3H, *s*)

*Overlapping with other signals.

requires: C, 59.20; H, 7.51%) ^{1}H and ^{13}C NMR: Tables 1 and 2.

Methanolysis of **2a**. Compound **2a** (8 mg) was refluxed with AcCl–MeOH (1:9) (2 ml) for 7 hr. The reagents were evaporated off and the residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (44:1) to give medicagenic acid dimethyl ester (2.3 mg). This was identified by comparison of ^{1}H and ^{13}C NMR data [10].

Acid hydrolysis of **2a** *and* **3a** [9]. Each compound (1 mg) was reduced with $NaBH_4$ (1 mg) in MeOH (0.5 ml) at room temp. overnight. The reaction mixture was diluted with H_2O and the solution was passed through a Diaion HP-20 column, the column was washed with H_2O and MeOH, successively. The MeOH eluate was concd and the residue was heated at 100° with 5% H_2SO_4–dioxane (1:1) (0.1 ml) for 1.5 hr. The reaction mixture was passed through an Amberlite IRA-60E column and the eluate was reduced with $NaBH_4$ (1 mg) at room temp. for 1 hr and passed through an Amberlite IR-120B column. The eluate was concd and then the reaction mixture was heated at 100° with Ac_2O (0.05 ml) and pyridine (0.05 ml) for 1 hr. The acetylated mixture was subjected to GC, which revealed only one peak for glucitol acetate. GC conditions: column, Supelco SP-2380 capillary column (0.25 mm × 30 m); column temp. 250°; carrier gas, N_2; t_R, 8.8 min.

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