An Antidepressant Principle of *Lobelia inflata* L. (Campanulaceae)

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Abstract □ A crude methanolic extract of the leaves of *Lobelia inflata* exhibited antidepressant activity in mice. The extract was fractionated, monitored by the activity, to give β-amyrin palmitate as an active component.

The leaves of *Lobelia inflata* L. (Campanulaceae), which have been used as a remedy for spasmodic asthma,[1] reportedly contain numerous piperidine-type alkaloids.[2] Among them, lobeline is known as the major and most important alkaloid affecting both the central nervous system[1,3] and the cardiovascular system.[4] Except for these alkaloids, other active constituents from this plant have not been reported. In the course of our survey of pharmacologically active substances in medicinal plants, much attention has been given to the occurrence of natural products possessing antidepressant activity. We have found that methanolic extract of the leaves of *L. inflata* showed antidepressant activity in the forced swimming test with mice. In this paper, we report the isolation and structural elucidation of an antidepressant substance from the plant.

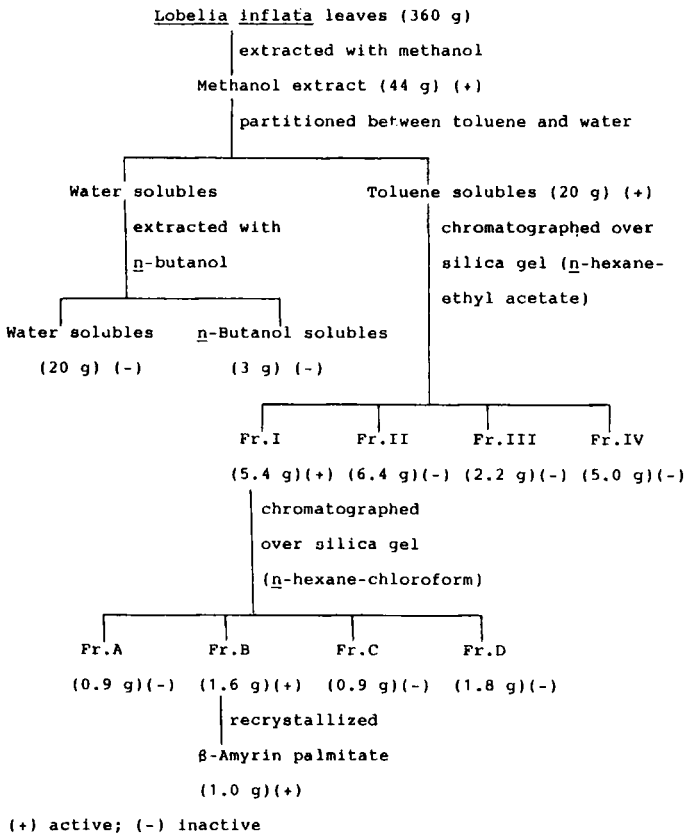
Experimental Section

Instruments used for structural elucidation are as follows: the melting point was determined on a Yanaco micro melting point apparatus and are uncorrected; optical rotation was measured on a JASCO DIP-360 polarimeter; the IR spectrum was recorded on a Shimadzu IR-408 spectrometer; ^1H and ^{13}C NMR spectra were measured on JEOL JNM FX-100 and FX-500 spectrometers, respectively (tetramethylsilane as an internal standard); the field desorption–mass spectrum (FD–MS) was determined with a JEOL-JMS-DX 303 spectrometer; the electron impact–mass spectrum was determined with a Hitachi M-52 spectrometer.

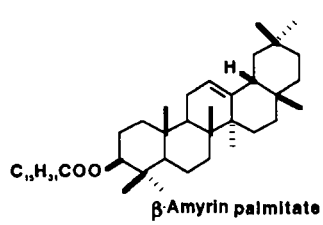
Isolation—Leaves of *L. inflata* were collected in West Java, Indonesia, and dried at room temperature. The active ingredient in the leaves was extracted and isolated (Scheme I) to yield β-amyrin palmitate[5] as colorless crystals: mp 55–57 °C; $[\alpha]_D$ +29.4° (concentration, 1.0 g/mL; $CHCl_3$); IR (Nujol) cm^{-1}: 1735 (carbonyl); FD–MS *m/z*: 665 ($M^+ + 1$), 409, 239, 218, 203; ^1H NMR ($CDCl_3$) δ: 0.81 (3H), 0.85 (12H), 0.86, 0.94, 0.95 (3H each), 1.10 (3H), 2.25 (2H, t, J = 7.5 Hz), 4.48 (1H, dd, J = 6.9, 9.4 Hz), and 5.15 (1H, t, J = 3.8 Hz); ^{13}C NMR ($CDCl_3$) δ: 14.3, 15.7, 17.0, 18.4, 22.9, 23.7, 23.9, 25.4, 26.1, 26.3, 27.1, 28.2, 28.6, 29.4, 29.5, 29.6, 29.9, 31.2, 32.1, 32.8, 33.5, 34.9, 35.0, 37.1, 37.3, 37.9, 38.5, 39.6, 40.0, 41.9, 47.0, 47.4, 47.7, 55.4, 80.7, 121.8, 145.3, and 173.8.

Alkaline Hydrolysis—A solution of β-amyrin palmitate (10 mg) in 10 mL of 5% KOH in ethanol–chloroform was heated at 70 °C for 3 days. Usual workup of the reaction mixture afforded palmitic acid and β-amyrin (5 mg) as colorless needles: electron impact–mass spectrometry *m/z*: 426 (M^+), 218, 203; ^1H NMR ($CDCl_3$) δ: 0.80, 0.84 (3H each), 0.88 (6H), 0.94, 0.96, 0.98, 1.12 (3H each) (all s, CH_3), 3.20 (1H, dd, J = 6.9, 9.4 Hz), and 5.16 (1H, t, J = 3.8 Hz).

Pharmacological Test—Male standard ddY (Funabashi Farm Company, Chiba, Japan) mice weighing 24–27 g were used. They were housed under standard laboratory conditions for at least 4 days before the experiment. The samples were either dissolved in saline solution or dispersed in a suspension of polysorbate 80 (0.5%, w/v; 0.9% NaCl). Control mice were given the vehicle only. Measurement of immobility in mice was carried out as described by Porsolt et al.[6] Mice were individually placed for 5 min in vertical glass cylinders (height, 20 cm; diameter, 10 cm) containing 8 cm of 25 °C water. They were removed and allowed to dry for ~15 min in a 30 °C drying room. On the next day, 1 h after intraperitoneal injection of the samples, the animals were placed in the cylinder and the total duration of immobility was measured every 5 min during a 15-min test. The mice were judged to be immobile whenever they remained floating passively in the water in a slightly hunched but upright position with their head above the surface.



Scheme I—Procedure for isolation of β-amyrin palmitate.



β-Amyrin palmitate

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Table I—Effects of β-Amyrin Palmitate and Imipramine on the Duration of Immobility of Mice

Drug	Dose, mg/kg[a]	Duration of Immobility[b]		
		0–5 min	5–10 min	10–15 min
Control	—[c]	100	100	100
β-Amyrin palmitate	5.0	78.0 ± 4.0[d]	97.0 ± 5.2	111.2 ± 4.2
	10.0	67.4 ± 6.4[e]	93.6 ± 4.3	106.4 ± 2.3
	20.0	62.4 ± 7.4[e]	95.3 ± 3.1	106.4 ± 4.2
Imipramine	5.0	76.7 ± 9.3	89.0 ± 4.6	96.7 ± 2.6
	10.0	64.5 ± 6.1[e]	89.3 ± 6.5	99.8 ± 6.1
	20.0	54.4 ± 9.4[e]	99.3 ± 6.8	95.7 ± 6.1

[a] *10 mice/dose.* [b] *Expressed as mean percent of control ± standard error; the durations of immobility during the 0–5, 5–10, and 10–15 min intervals in the mice administered polysorbate 80 were 139.3 ± 10.3, 235.6 ±6.5, and 241.4 ± 13.0 s, respectively.* [c] Not applicable. [d] Statistically significantly different from control according to Dunnette's test ($p < 0.05$). [e] Statistically significantly different from control according to Dunnette's test ($p < 0.01$).

Results and Discussion

The methanol extract of *L. inflata* leaves reduced the duration of immobility of mice in the forced swimming test. The methanol extract was further partitioned into fractions that are soluble in toluene, *n*-butyl alcohol, and water. The toluene-soluble fraction that showed the activity was subjected to activity-directed fractionation to yield β-amyrin palmitate as an active component (Scheme I).

β-Amyrin palmitate ($C_{46}H_{80}O_2$) has an IR absorption band at 1735 cm^{-1} due to a carbonyl group. Structural information for this compound was obtained from its ^1H NMR spectrum, which indicates signals for nine tertiary methyls at δ 0.81–1.10, an oxymethine hydrogen at δ 4.48 (1H, dd, J = 6.9 and 9.4 Hz), and an olefinic hydrogen at δ 5.15 (1H, t, J = 3.8 Hz). The mass spectrum exhibited ion peaks at *m/z* 218 and 203, peaks that are characteristic of the oleanane-type triterpenoid. The accumulated spectral evidence, along with the ^{13}C NMR data, indicates the compound to be β-amyrin palmitate. This conclusion was confirmed by alkaline hydrolysis of the compound to β-amyrin and palmitic acid.

The efficacy of clinically effective antidepressant drugs such as imipramine and mianserine in the forced swimming test is closely related to the clinical data.[7] β-Amyrin palmitate (5–20 mg/kg, intraperitoneally), like imipramine (Table I) and mianserine,[7] significantly decreased the duration of immobility of mice during the first 5 min of a 15-min test, whereas the duration of immobility after 5 min was not changed. These observations suggest that β-amyrin palmitate has antidepressant properties.

References and Notes

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