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POTASSIUM LESPEDEZATE AND POTASSIUM ISOLESPEDEZATE, BIOACTIVE SUBSTANCES CONCERNED WITH THE CIRCADIAN RHYTHM IN NYCTINASTIC PLANTS

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Summary: In an effort to search for stimulants concerned with the circadian rhythm in several nyctinastic plants, potassium lespedezate and potassium isolespedezate were isolated as bioactive substances from the plant Lespezeza cuneata L. G. Don. These potassium salts have been proved to be quite effective for leaf-opening of the plant Cassia mimosoides L.

The plant Mimosa pudica (Ojigi So in Japanese) and related nyctinastic plants have been well known for their organismus having an internal clock, and great efforts have been made for understanding the mechanism of the thigmonastic and nyctinastic movements of the plant Mimosa pudica.[1] In 1916, Ricca reported that the movements of the plant were controlled by some chemical substances.[2] Since then, a number of scientists have attempted to search for these bioactive compounds. Recently, Schildknecht et al. have isolated the leaf movement factor (KPLM-1) (**1**) from the plant Mimosa pudica and others, and reported that both thigmonastic and nyctinastic movements were controlled by KPLM-1 (**1**).[1,3] In 1987, we have isolated potassium chelidonate (**2**) from the plants Cassia mimosoides L. and Cassia occidentalis as a leaf-closing factor.[4] We report herein isolation of the leaf-opening factors from the nyctinastic plant Lespedeza cuneata L. G. Don. (Medohagi in Japanese).

In order to detect stimulants which control the leaf-opening, we have used the leaves of the plant Cassia mimosoides L. The young leaves to be tested have been immersed in distilled water and allowed to stand at room temperature for several hours, and used for bioassay. All the leaves closed at evening. When the bioactive principles were contained in the test solution, the leaves opened again at night (around 10 p.m.). These Cassia tests are quite reproducible, but must be carried out carefully, because the minimum active concentration varied with the conditions (temperature, humidity and others).

The fresh whole harb of the plant Lespedeza cuneata L. G. Don was extracted with methanol for 1 week, and then carefully separated according to the isolation procedure cited in Fig. 1, affording two bioactive substances (**3** and **4**) as colourless powder having the following spectral data: **3** $[\alpha]^{25}_{D}$ -57.4° (c 0.65, H_2O); FAB-MS (negative) 379 $(M-H)^-$, 341 $(M-K)^-$; UV (H_2O) 268 nm(ε, 13000); ^1H NMR [D_2O, internal reference; dioxane (δ 3.71)] δ 3.41 (1H, dd, J = 7.8, 9.8Hz), 3.46(1H, dd, J = 7.8, 9.3Hz), 3.50(1H, m), 3.54(1H, dd, J = 9.3, 9.8Hz), 3.84(1H, dd, J = 5.9, 12.7Hz), 3.89(1H, dd, J = 2.0, 12.7Hz), 4.88(1H, d, J = 7.8 Hz), 6.12(1H, s), 6.79(2H, d, J = 8.8Hz), 7.18(2H, d, J = 8.8Hz).

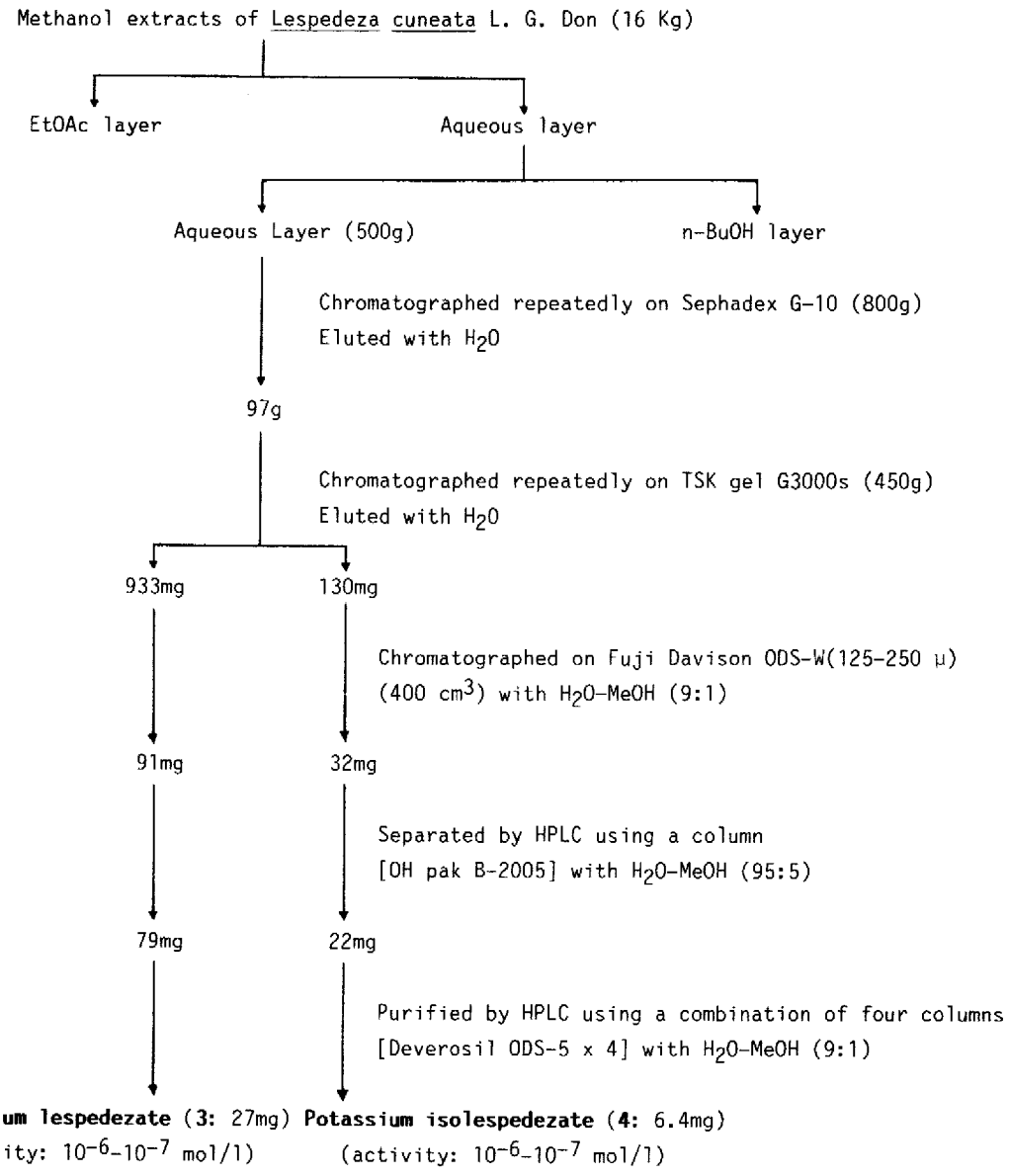
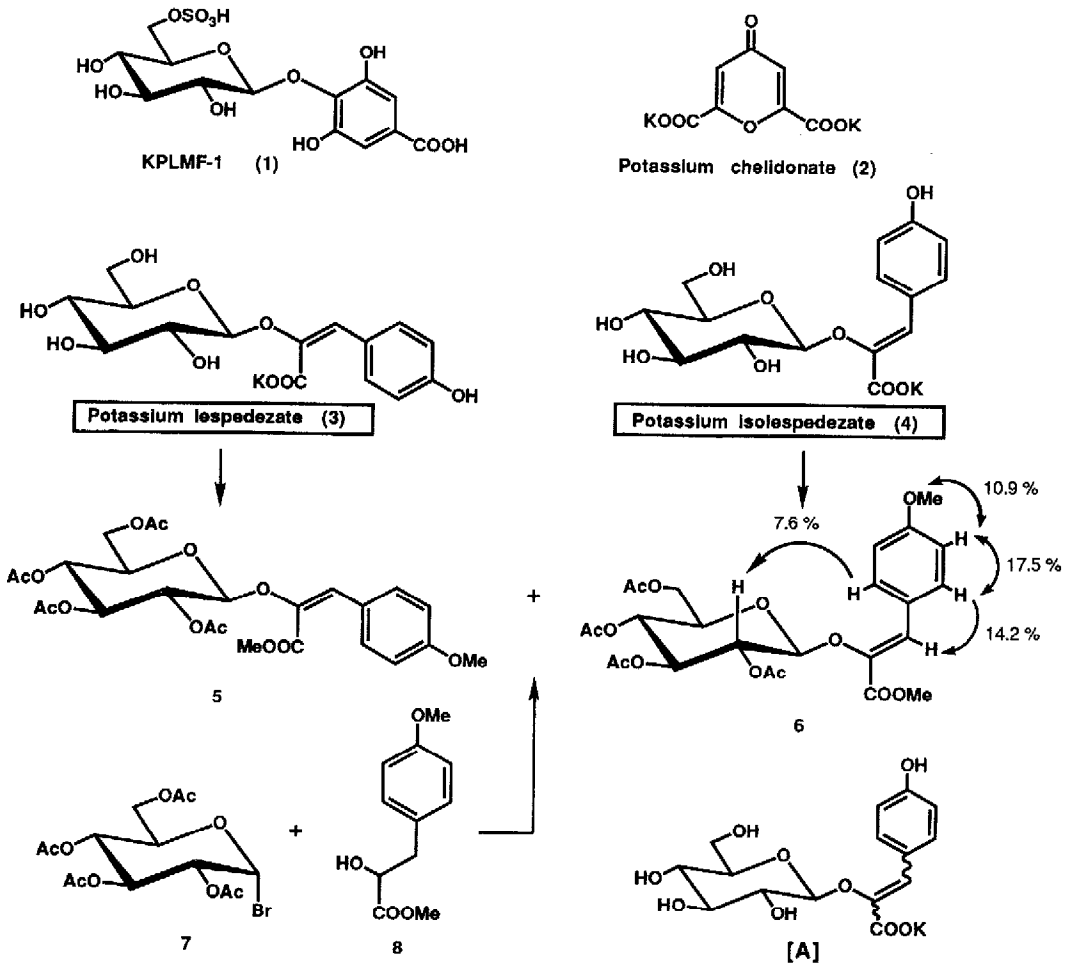


Fig. 1. Isolation procedure of the bioactive substances from the plant Lespedeza cuneata L. G. Don

4: $[\alpha]^{25}_{D}$ +53.1° (c 1.0, H_2O); FAB-MS (positive) 381$(M+H)^+$, 343$(M-K+H_2)^+$; UV (H_2O) 286 nm(ε, 20000); ^1H NMR [D_2O, internal reference; dioxane (δ3.71)] δ 3.33(1H, m), 3.42(1H, t, J = 9.3Hz), 3.52(1H, t, J = 9.3Hz), 3.55(1H, dd, J = 7.3, 9.3Hz), 3.64(1H, dd, J = 5.1, 12.5Hz), 3.75(1H, dd, J = 1.7, 12.5Hz), 5.01(1H, d, J = 7.3Hz), 6.73(1H, s), 6.87(2H, d, J = 8.8Hz),

7.69(2H, d, J = 8.8Hz); ^{13}C NMR [D_2O, internal reference; dioxane (δ67.6)] 61.5(t), 70.4(d), 74.8(d), 76.9(d), 77.4(d), 102.2(d), 116.5(d x 2), 121.4(d), 127.0(s), 132.8(d x 2), 146.2 (br.s), 156.9(s), 172.6(br.s).



The ^1H and ^{13}C NMR spectra of **4** indicate the presence of β-D-glucopyranoside(δ 3.33, 3.42, 3.52, 3.55, 3.64, 5.01, 61.5, 70.4, 74.8, 76.9, 77.4 and 102.2), 1,4-disubstituted benzene ring(δ 6.87, 7.69, 116.5, 127.0, 132.8, and 146.2), a trisubstituted double bond (δ 6.73, 121.4 and 156.9) and a carboxylate group(δ 172.6). The isomer (**3**) also has the corresponding signals. Furthermore, both potassium lespedezate (**3**) and potassium isolespedezate (**4**), having the same molecular formula ($C_{15}H_{17}O_9K$), afforded the mixture of these two compounds on standing at room temperature, suggesting that **3** and **4** were isomeric to each other. Thus, the structures of both potassium salts are represented by [**A**]. Finally, from the comparison of chemical shifts of olefinic protons (**3**, δ 6.12; **4**, δ 6.73) and aromatic protons (**3**, δ 6.79 and 7.18; **4**, δ 6.73 and 7.69) it is obvious that the

carboxylate group and the benzene ring are located in cis position in **3**, and trans one in **4**. To confirm the structures, both potassium lespedezate (**3**) and potassium isolespedezate (**4**) were converted into the acetates (**5**)[5] and (**6**)[5], respectively (1, Amberlite IR-120B/H_2O; 2, CH_2N_2/MeOH; 3, Ac_2O/Pyr.). The stereostructure of **4** was confirmed by NOE experiments of compound (**6**). The acetates (**5**) and (**6**) were synthesized starting from D-acetobromoglucose (**7**) and methyl 3-p-methoxyphenyl-2-hydroxy-propionate (**8**) (1, AgOTf - Molecular Sieves 4A / CH_2Cl_2; 2, DDQ / toluene at refluxing temperature). The physical properties of the synthetic acetates (**5**) and (**6**) were completely identical with those of the compounds obtained from natural bioactive salts in all respects of spectral data including optical rotation.

Two natural samples of potassium lespedezate and potassium isolespedezate opened the leaves of Cassia mimosoides L. at the same concentration(10^{-6} - 10^{-7} mol/l). Synthetic studies on potassium lespedezate(**3**), potassium isolespedezate(**4**) and their analogues as well as biological activities of these compounds are further in progress.

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References and notes

1) H. Schildknecht, Angew. Chem. Int. Ed. Engl., **22**, 695 (1983) and many references cited therein.

2) U. Ricca, Nuov G. Bot. Ital. (Nuova Seria), **23**, 51 (1916).

3) H. Schildknecht and K. Schumacher, Pure Appl. Chem., **54**, 2501 (1982) and many references cited therein.

4) E. Miyoshi, Y. Shizuri and S. Yamamura, Chem. Lett., **1987**, 511.

5) **5** as a colourless oil: $C_{25}H_{30}O_{13}$[m/z 538.1673(M^+)]; IR (film) 1755, 1720, 1640, 1605, 1570, and 1515 cm^{-1}; δ (C_6D_6) 1.65(3H, s), 1.68(3H, s), 1.71(3H, s), 1.91(3H, s), 3.13 (1H, ddd, J = 2.2, 5.4, 9.3Hz), 3.23(3H, s), 3.28(3H, s), 4.01(1H, dd, J = 2.2, 12.2Hz), 4.17(1H, dd, J = 5.4, 12.2Hz), 4.80(1H, d, J = 7.8Hz), 5.25(1H, dd, J = 9.3, 9.8Hz), 5.45 (1H, t, J = 9.3Hz), 5.55(1H, dd, J = 7.8, 9.8Hz), 6.69(2H, d, J = 8.8Hz), 6.88 (1H, s), and 7.30(2H, d, J = 8.8Hz); $[\alpha]^{25}_D$ -35.1° (c 0.70, $CHCl_3$).
6 as a colourless oil: $C_{25}H_{30}O_{13}$[m/z 538.1666(M^+)]; IR (film) 1760, 1720, 1640, 1605, 1575, and 1515 cm^{-1}; δ (C_6D_6) 1.59(3H, s), 1.62(3H, s), 1.70(3H, s), 1.80(3H, s), 3.08 (1H, ddd, J = 2.5, 4.4, 9.8Hz), 3.22(3H, s), 3.45(3H, s), 3.82(1H, dd, J = 2.5, 12.2Hz), 4.04(1H, dd, J = 4.4, 12.2Hz), 5.29(1H, t, J = 9.8Hz), 5.48(1H, t, J = 9.8Hz), 5.59 (1H, d, J = 7.8Hz), 5.69(1H, dd, J = 7.8, 9.8Hz), 6.75(2H, d, J = 9.3Hz), 7.20(1H, s), and 7.79(2H, d, J = 9.3Hz); ^{13}C NMR (δ, $CDCl_3$) 20.5(q), 20.6(q), 20.6(q), 20.7(q), 52.2 (q), 55.3(q), 61.6(t), 68.4(d), 71.6(d), 71.9(d), 72.8(d), 99.3(d), 113.8(d x 2), 125.3 (s), 126.9(d), 132.6(d x 2), 138.1(s) 160.6(s), 164.2(s), 169.5(s), 170.0(s), 170.2(s), and 170.5(s); $[\alpha]^{26}_D$ -10.0° (c 1.0, $CHCl_3$).

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