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Isolation of Potassium Chelidonate as a Bioactive Substance Concerning with Circadian Rhythm in Nyctinastic Plants

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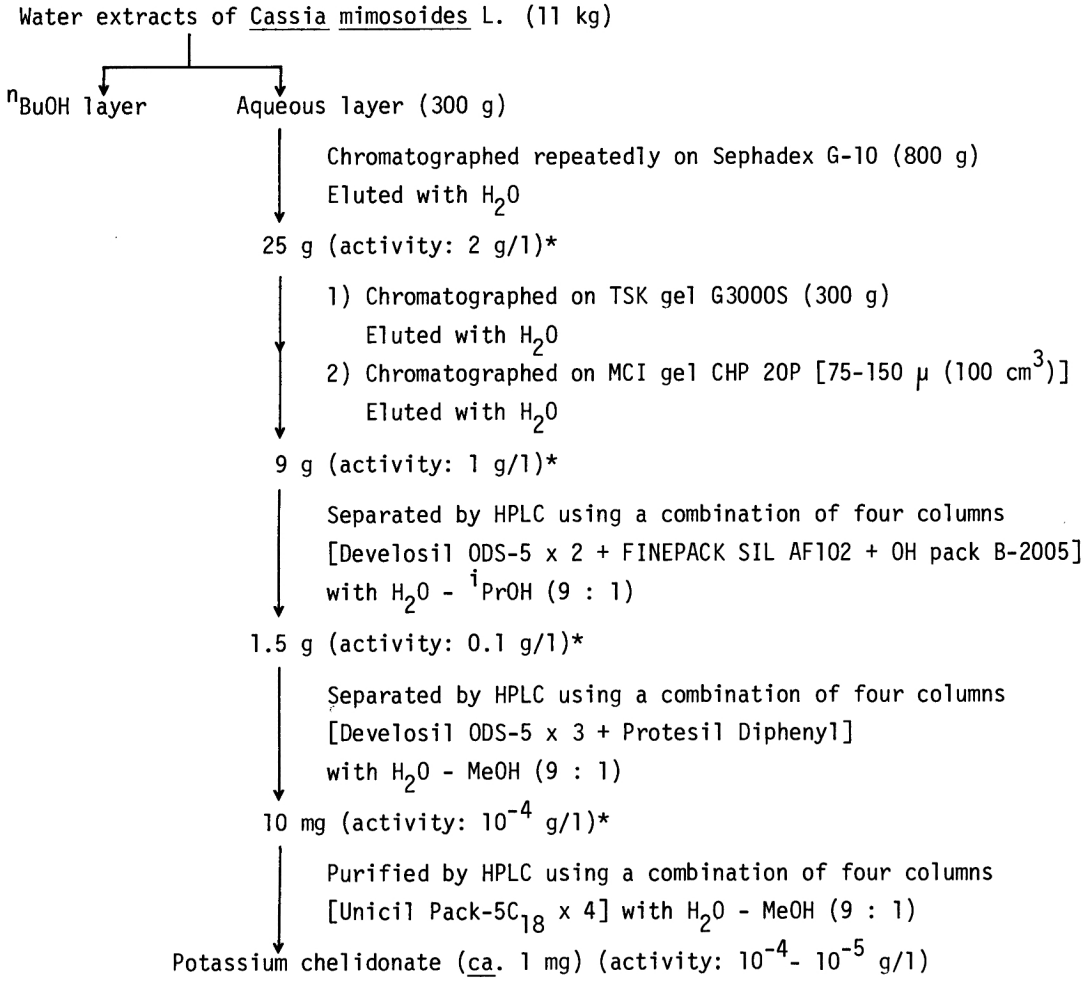
In an effort to search for stimulants concerning with circadian rhythm in several nyctinastic plants, potassium chelidonate was isolated as a bioactive substance from the plant Cassia mimosoides L. and C. occidentalis L. This potassium salt has been proved to be quite effective for leaf-closing of the plants Mimosa pudica, Cassia mimosoides L. and others.

The plant Mimosa pudica (Ojigi So in Japanese) and related nyctinastic plants have been well known for their organismus having internal clocks, 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 his ingeneous experiments which strongly suggested that the movements of the plant must be controlled by some bioactive substances.[2] Since then, a number of scientists have attempted to search for these bioactive compounds. Recently, Schildknecht et al. have isolated some turgolins from the plants Mimosa pudica, Acacia karroo, Albizia julibrissin, Oxalis strica, and others.[1,3] In particular, they have reported that the leaf movement factor (K-PLMF) (1) is regarded as the truly bioactive substance which controls both thigmonastic and nyctinastic movements.[1,3] We report herein isolation of another type of turgolin from the nyctinastic plants Cassia mimosoides L. (Kawara Ketsumei in Japanese) and C. occidentalis L. (Habu So in Japanese). In addition, some new information on the bioactive substances of the plant Mimosa pudica is also presented herein.

In order to detect stimulants of the nyctinastic plants, we have adopted two different methods using the leaves of the plants Mimosa pudica and Cassia mimosoides L. Our Mimosa test is quite similar to Fittig-Hesse-Schildknecht test using the leaves of the plant,[1,3] which shows a rapid responce, but must be carefully carried out because it is quite sensitive to H^+ ion [the leaves were closed on addition of dil. H_2SO_4 (10^{-2} g/l)], temperature, humidity, and others. On the other hand, Cassia test is not so sensitive as Mimosa test but quite reproducible. The leaves of the plant to be tested have been immersed in distilled water and allowed to stand at room temperature overnight. The leaves, which open again in the next morning, are used for bioassay, wherein the reaction

time depends on the concentration of active substances, the minimum amount of which is judged by leaf-closing in one hour.

The fresh whole herb of the plant Cassia mimosoides L. was extracted with boiling water for ca. 15 min, and then carefully separated according to the isolation procedure cited in Fig. 1, affording a bioactive substance (2) as white powder having the following spectral data: UV (H_2O) 270 (ε, 7200) and

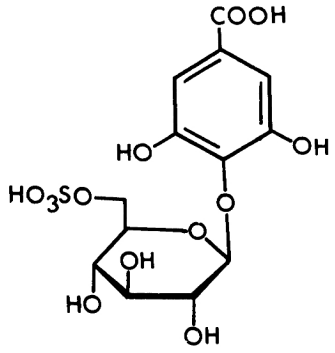


Size of columns: Develosil ODS-5 and Protesil Diphenyl: ϕ 5 mm x 250 mm; FINEPACK SIL AF102: ϕ 8 mm x 500 mm; OH pack B-2005: ϕ 10 mm x 500 mm; Unicil pack-$5C_{18}$: ϕ 6 mm x 250 mm.

* For the plant Mimosa pudica.

Fig. 1. Isolation procedure of the bioactive substances from the plant Cassia mimosoides L.

222 nm (9500); IR (Nujol) 1650 (sh) and 1635 cm^{-1}; ^1H NMR (D_2O) δ7.08 (s); ^{13}C NMR (D_2O) δ 119.0 (d), 162.4 (s), 167.8 (s), and 187.7 (s). An aqueous solution of 2 was passed through a short column of Amberlite IR-120B to afford quantitatively the corresponding acid [$C_7H_4O_6$(M^+, m/z 184.0020)], which was completely identical with the synthetic sample of chelidonic acid[4] in all respects of HPLC, IR and ^1H NMR spectra. From these results together with ion chromatogram of the original substance indicating that it contains two K^+ ions in one molecule, the bioactive substance must be potassium chelidonate, which has been confirmed by synthesis. According to essentially the same procedure, potassium chelidonate (2) was also obtained from the plant Cassia occidentalis L. in 0.00025% yield based on the weight of the fresh whole herb of the plant.



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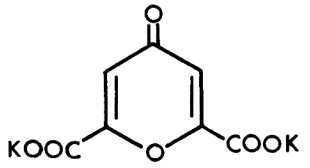

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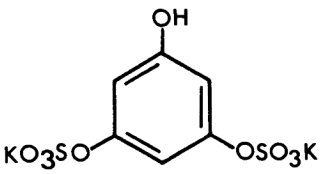

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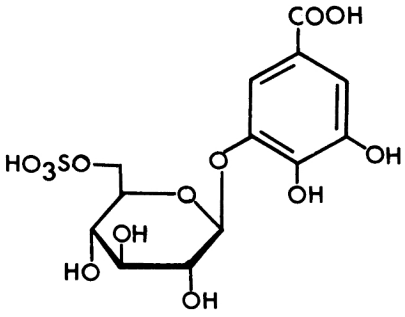

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The natural and synthetic samples of potassium chelidonate (2) closed the leaves of both Mimosa pudica and Cassia mimosoides L., at 10^{-4} - 10^{-5} g/l and 10^{-2} - 10^{-3} g/l, respectively. Furthermore, the leaves of the nyctinastic plants Cassia occidentalis L. and Lespedeza cuneata G. Don. (Medohagi in Japanese) were also closed on addition of potassium chelidonate in the same order of concentration (10^{-2} - 10^{-3} g/l) in the day-time. Interestingly, however, chelidonic acid did not show any activity for leaf-closing of the plants Cassia mimosoides L., C. occidentalis L., and Lespedeza cuneata G. Don. These results prompted us to examine effects of K^+ ion on leaf-closing of the plant Cassia mimosoides L. using potassium acetate, potassium oxalate, and potassium phloroglucinol disulfate (3).[5] However, all of them did not show any activity for the plant. According to essentially the same procedure as described by Shildknecht et al., we synthesized K-PLMF 1 (1),[1,3] its potassium salt, and an isomer (4).[6] In the case of the plant Mimosa pudica, our synthetic sample of K-PLMF 1 (1) showed almost the same activity (ca. 10^{-4} g/l) as reported by Schildknecht et al.[1,3] The isomer (4) also showed the activity at 10^{-2} g/l. However, K-PLMF 1 (1) and its isomer (4) did not show any activity for the plant Cassia mimosoides L. The potassium salt of 1 was effective on leaf-closing of the plant Mimosa pudica,

although its activity (ca. 10^{-1} g/l) was quite weak, while any activity was not observed for the plant Cassia mimosoides L.

The present study has indicated that potassium chelidonate (2) as a turgolin plays an important role in leaf movements of the nyctinastic plants. From a structural point of view, potassium chelidonate (2) is quite different from K-PLMF 1 (1), indicating that 1 and related compounds are not necessarily regarded as common stimulants in the nyctinastic plants.[1,3] In connection with K-PLMF 1 (1), furthermore, it should be noted that our recent study on bioactive substances of the plant Mimosa pudica has led to the isolation of less than 20 μg of stimulants which are quite effective on leaf-closing of the plant at 10^{-8} - 10^{-9} g/l, although they have not yet been obtained as completely pure state.[7] Further study on this point is in progress.

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References

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. R. Riegel and F. Zwilgmeyer, Org. Synth., Coll. Vol. II, 126 (1950).
5) M. A. Ragan, Can. J. Chem., 56, 268 (1978).
6) The synthetic procedure of this compound (4) will be published elsewhere.
7) Y. Shizuri, E. Miyoshi, S. Nishiyama, H. Toshima, K. Nagasawa, and S. Yamamura, 27th Symposium on the Chemistry of Natural Products, Hiroshima, October 1985, Abstract Papers pp. 664 - 671.

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