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# ALKALOIDS OF CALLUS TISSUES AND REDIFFERENTIATED PLANTLETS IN THE PAPAVERACEAE*

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**Key Word Index**—*Papaver*; *Eschscholtzia*, etc. Papaveraceae; callus tissues; alkaloids; chemotaxonomy; differentiation.

**Abstract**—The callus tissues from 11 representative species of the Papaveraceae and the redifferentiated plantlets from four species were successfully derived and maintained. The alkaloids in the callus tissues and redifferentiated plantlets were examined in comparison with those of the original plants. All the callus tissues are similar in their alkaloid chemistry and contain benzophenanthridine, protopine and aporphine type alkaloids. By contrast, the plantlets have a more specific alkaloid pattern, being similar in content to the original plants.

## INTRODUCTION

IN A PREVIOUS paper,[1] it was reported that the new alkaloid norsanguinarine and other known alkaloids were obtained from all the callus tissues derived from seedling root, stalk and capsule of opium poppy *Papaver somniferum* L. (Keshi-Ikkanshu: in Japanese). We have now examined the callus tissues from 11 representative species of the Papaveraceae and the plantlets produced from the above callus tissues. The results of an alkaloid survey of these materials are presented here.

## RESULTS AND DISCUSSION

### *Alkaloids of callus tissues*

Alkaloids in 15 callus tissues (Table 1) derived from 11 species of Papaveraceae, growing in Japan and representing different subfamilies and tribes within the family, were examined. The results are shown in Table 2. The alkaloidal patterns of the various callus tissues were almost identical. Furthermore, there was no variation in alkaloidal pattern caused by differences in origin (stem, root, capsule, seedling) in culture age or in growth conditions. All the callus tissues contain mainly benzophenanthridine, protopine and aporphine type alkaloids, which are distributed widely in the Papaveraceae, and also choline; protoberberine type alkaloids present in the original plants were not detected.

Norsanguinarine, which was isolated first from opium poppy callus tissues, was detected in the alkaloidal fraction of all callus tissues, although it is not present in the original plants. Norsanguinarine, therefore, seems to accumulate as a by-product of the pathway of biosynthesis of benzophenanthridine alkaloids in Papaveraceae callus tissues.[1]

* Part XXIII in the series "Studies in Plant Tissue Cultures". For Part XXII see HIROTANI, M. and FURUYA, T. (1974) *Phytochemistry* **13**, 2135–2142.

[1] FURUY, T., IKUTA, A. and SYŌNO, K. (1972) *Phytochemistry* **11**, 3041.

TABLE 1. DERIVATION OF PAPAVERACEAE CALLUS TISSUES

| Plants | Year | Basic medium | Origin |
|---|---|---|---|
| *Papaver somniferum* | 1966.6 | I 1, K 0·1 | stem |
| | 1967.4 | D 1, K 0·1 | seedling root |
| | 1967.6 | D 1, K 0·1 | capsule |
| | 1971.6 | D 1, K 0·1, CM | capsule |
| *P. setigerum* | 1969.3 | D 1, K 0·1, CM | seedling |
| *P. bracteatum* | 1968.4 | D 1, K 0·1, CM | seedling |
| *P. orientale* | 1969.3 | D 1, K 0·1, CM | seedling |
| *P. rhoeas* | 1969.3 | D 1, K 0·1, CM | seedling |
| *Eschscholtzia californica* | 1969.7 | D 1, K 0·1, CM | root |
| | 1969.7 | D 0·1, K 0·1 | stem |
| *Dicentra peregrina* | 1969.7 | D 0·1, K 0·1 | stem |
| *Macleaya cordata* | 1969.7 | D 1, K 0·1, CM | stem |
| | | D 0·1, K 0·1 | stem |
| *Chelidonium japonicum* | 1970.5 | D 1, K 0·1, CM | hypocotyl |
| *Corydalis incisa* | 1971.5 | D 1, K 0·1 | petiole |
| *C. pallida* | 1971.5 | D 1, K 0·1 | stem |

Key: I = 3-indolylacetic acid; D = 2,4-dichlorophenoxyacetic acid; K = kinetin, CM = coconut milk; basic medium = M & S inorganic solution and vitamin mixture.

Although plants of the genus *Papaver* normally contain alkaloids of the morphinane type, these compounds are not produced in the derived callus tissues. Thus, the alkaloidal components of callus tissues are simpler than those of the original plants and are mainly of the widely distributed benzophenanthridine, protopine and aporphine types.[2]

In our callus tissues of *Macleaya cordata* (Takenigusa: in Japanese) and *Chelidonium japonicum* (Yamabukiso: in Japanese) plants, magnoflorine was not detected, and also

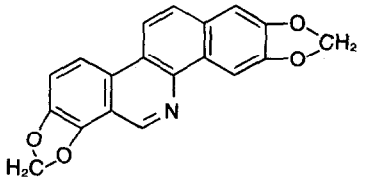
TABLE 2. THE ALKALOIDS OF PAPAVERACEAE CALLUS TISSUES

| Subfamily tribe | Original plants of callus tissues | Type of alkaloids: Benzophenanthrizine 1 | 2 | 3 | 4 | 5 | Protopine 6 | Aporphine 7 | Unknown CS | QS |
|---|---|---|---|---|---|---|---|---|---|---|
| Papaveroideae | | | | | | | | | | |
| Eschscholtzea | *Eschscholtzia californica* | + | + | + | + | + | + | + | + | – |
| Chelidonieae | *Chelidonium japonicum* | + | + | + | – | – | + | – | + | – |
| | *Macleaya cordata* | + | + | + | + | + | + | – | + | – |
| Papavereae | *Papaver somniferum* | + | + | + | + | – | + | + | + | + |
| | *P. setigerum* | + | + | + | + | – | + | + | + | |
| | *P. bracteatum* | + | + | + | + | + | + | + | + | + |
| | *P. orientale* | + | + | + | + | – | + | + | + | + |
| | *P. rhoeas* | + | + | + | + | – | + | + | + | + |
| Fumarioideae | | | | | | | | | | |
| Corydaleae | *Dicentra peregrina* | + | + | + | + | – | + + | + | + | + |
| | *Corydalis incisa* | + | + | + | + | – | + | + | + | – |
| | *C. pallida* | + | + | + | + | – | + | + | + | + |
| Hypecoideae | *Pteridophyllum racemosum** | + | + | + | – | – | + + | + | | |

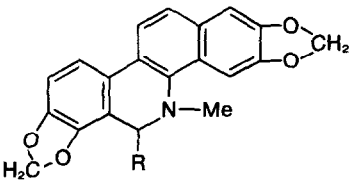
Key: – absent; + present; + + present (large amount); * original plants; CS had $R_f$ 0·31 (yellow fluorescence) in $CHCl_3$–MeOH (3:1); QS had $R_f$ 0·3 (yellow fluorescence) in MeOH–$H_2O$–$NH_4OH$ (8:1:1).

[2] ŠANTAVÝ, F. (1970) *The Alkaloids* Vol. XII, pp. 429. Academic Press, New York.

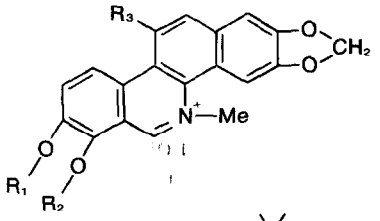
aporphine type alkaloids were not present in the original plants. This is of chemotaxonomic interest. We have failed to derive callus tissues from *Pteridophyllum racemosum** (Osabagusa: in Japanese) which is the only species of this genus in Japan and therefore we could not investigate the alkaloids of its callus tissues.



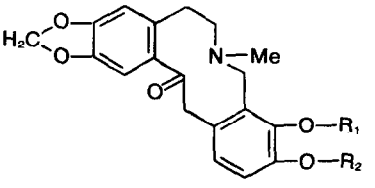
**(1)** Norsanguinarine



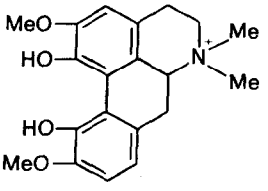
**(2)** Oxysanguinarine, R = O
**(3)** Dihydrorosanguinarine, R = $H_2$



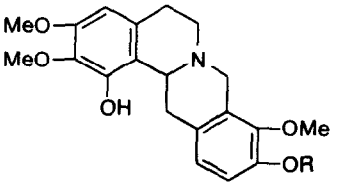
**(4)** Sanguinarine, $R_1 = R_2 = \overset{\diagdown\diagup}{CH_2}$; $R_3$ = H
**(5)** Chelirubine, $R_1 = R_2 = \overset{\diagdown\diagup}{CH_2}$; $R_3$ = OMe
**(8)** Chelerythrine, $R_1 = R_2$ = Me; $R_3$ = H



**(6)** Protopine, $R_1 = R_2 = \overset{\diagdown\diagup}{CH_2}$
**(11)** Allocryptopine, $R_1 = R_2$ = Me



**(7)** Magnoflorine



**(9)** Capauridine, R = Me
**(10)** Capaurimine, R = H

*Alkaloids of redifferentiated plantlets*

*Macleaya cordata.* Both morphology and alkaloid pattern of the redifferentiated plantlets and original plants were found to be almost the same. Norsanguinarine is the main alkaloid in the callus tissues, but only a trace is present in plantlets. On the other hand, the benzophenanthridine type alkaloid chelerythrine was not found in the callus tissues, but occurred in the redifferentiated plantlets and original plants. The amount of protopine and allocryptopine in the callus tissues was lower than that in the redifferentiated plantlets.

* We have detected norsanguinarine, dihydrosanguinarine, oxysanguinarine, magnoflorine and unknown spots from these plants, which have not been reported yet, except protopine type alkaloids.[3,4]

[3] KOJIMA, K. and ANDO, Y. (1951) *J. Pharm. Soc. Jap.* **71**, 625.
[4] HAGINIWA, J. and HARADA, M. (1961) *J. Pharm. Soc. Jap.* **80**, 1231.

*Corydalis pallida.* The plantlets accompanying the callus tissues of *C. pallida* (Miyamakikeman: in Japanese) were observed to contain all alkaloids which are present in the callus tissues, except one or two new unknown constituents. Norsanguinarine was mainly found in the callus tissues cultured on a medium containing 2,4-D, and also in a trace amount in both the plantlets accompanying with callus tissues and original plants. Furthermore, protoberberine type alkaloids, capauridine and capaurimine which were isolated from *C. pallida*,[5] were detected in plantlets with no callus tissues, but capaurimine only was present in a small amount in callus tissues with no plantlets; both alkaloids were absent from the callus tissues cultured on a medium containing 2,4-D.

*Papaver bracteatum.* In *P. bracteatum* (Botangeshi: in Japanese), the buds accompanying the callus tissues were not developed more than 1–1·5 cm in height and did not form shoot and root on medium containing IAA under light. The buds accompanying the callus tissues subcultured on medium containing IAA under light, the callus tissues subcultured on medium containing IAA under dark and also the callus tissues subcultured on medium containing 2,4-D had similar patterns of alkaloids. Thebaine, produced mainly in original plants, was not detected in the buds accompanying the callus tissues subcultured on medium containing IAA, but alkaloids were observed to be present in a larger amounts in the buds accompanying with callus tissues subcultured on medium containing IAA than in the callus tissues subcultured on medium containing 2,4-D.

*Papaver somniferum.* The scattering bud formation was observed in albino type[1] callus tissues subcultured on medium containing IAA under light and the buds redifferentiated from albino type callus tissues had not developed more than 0·5–1·0 cm in height.

The same alkaloidal pattern was found in these buds. Norsanguinarine is the main askaloid in the buds and other alkaloids were minor.[1] The alkaloids of all callus tissues were the same, with norsanguinarine as the main component. But in both the redifferentiated plants from these callus tissues of *M. cordata* and buds from these callus tissues of *C. pallida* and their original plants, norsanguinarine was absent or only detected in a trace amount. Norsanguinarine must be exceptionally accumulated as secondary metabolite in the callus tissues. In *M. cordata*, chelerythrine having the benzophenanthridine skeleton was detected in the redifferentiated plants and original plants, but not in the callus tissues. The alkaloidal pattern of the rootless aerial plants accompanying the callus tissues in *C. pallida* is different from that of callus tissues and the original plants. On the other hand, the alkaloidal pattern of the buds accompanying the callus tissues in *P. bracteatum* and *P. somniferum* was demonstrated to be the same as the callus tissues. In *Coptis japonica* (Seribawōren: in Japanese) callus tissues, the hair root formation was observed and the alkaloid pattern of the callus tissues with hair root was identical with that of callus tissues. The rhizomes of redifferentiated whole plants were demonstrated to be different from the callus tissues in alkaloid pattern, but to be similar to the crude drug "wōren".[6] In *Scopolia parviflora* callus tissues, the alkaloidal content was also reported to be significantly increased through the development of roots.[7] These results seem to indicate the lack of biosynthetic versatility in these callus tissues, and suggest that more favorable redifferentiation conditions are required to obtain the same components as those contained in the original plants.

[5] KANEKO, H. and NARUTO, S. (1971) *Yakugaku Zasshi*, **91**, 101.
[6] IKUTA, A., SYŌNO, K. and FURUYA, T. (1974) *Phytochemistry*. In press.
[7] TABATA, M., YAMAMOTO, H., HIRAOKA, N. and KONOSHIMA, M. (1972) *Phytochemistry* **11**, 949.

## EXPERIMENTAL

*Plant materials. Eschscholtzia californica* (Hanabishiso: in Japanese), *Chelidonium japonicum, Macleaya cordata, Corydalis incisa* (Murasakikeman: in Japanese), *C. pallida* and *Pteridophyllum racemosum* were collected near Tokyo and *Dicentra peregrina* (Komakusa: in Japanese) was cultivated in Rokko Alpine Botanical Garden of Japan. The seeds of *Papaver* were obtained from Kasukabe Experiment Station of Medicinal Plants, National Hygienic Laboratory.

*Derivation and culture of callus tissues.* Each callus tissue, derived as indicated earlier,[1] was subcultured every 4 weeks onto fr. Murashige and Skoog (M & S) medium (minus glycine) containing 2,4-D (1 mg/l), kinetin (0·1 mg/l) and coconut milk (CM) (7%) at 26° in the dark for 3–4 yr.

*Preparation of alkaloidals.* Each fresh callus tissue (500–1000 g fr. wt) was homogenized in cold MeOH in a Waring blender, and was refluxed with MeOH and benzene–MeOH. The combined solution was conc. and acidified with HCl. The acidic solution was extracted with benzene to remove the neutral and acidic fraction. The aqueous solution was made alkaline to pH 8–9 with conc. $NH_4OH$ and extracted repeatedly with $CHCl_3$ to yield the alkaloidal fraction and also the quaternary base was obtained by the same method as described in a previous paper.[1] The alkaloidal fraction of the redifferentiated plantlets and original plants collected were prepared by the same method as described above.

*Detection of alkaloids.* Norsanguinarine, sanguinarine, oxysanguinarine, dihydrosanguinarine, chelirubine, protopine and magnoflorine were detected using the same nine TLC solvent systems as described earlier.[1] Capauridine (capaurine) (**1**) and capaurimine (**2**) were detected using TLC on silica gel G (Merk) in (a) $CHCl_3$–MeOH (9:1), $R_f$ (**1**) 0·77, (**2**) 0·46, (b) xylene–MeCOEt–MeOH–$Et_2NH$ (20:20:3:1), $R_f$ (**1**) 0·88, (**2**) 0·58, (c) BuOH–AcOH–$H_2O$ (4:1:5 upper), $R_f$ (**1**) 0·45, (**2**) 0·5.

*Differentiation from callus tissues: Macleaya cordata.* The plantlets were initiated on subcultured callus tissues on M & S medium containing 2,4-D (1 mg/l), kinetin (0·1 mg/l) and CM (7%). The plantlets were transferred to M & S medium containing IAA (1·0 mg/l) and kinetin (0·1 mg/l) and cultures were maintained in growth cabinet with 12/12 hr. light/dark cycles under light (4500–9000 lx) at 25 ± 1°. Whole plants induced on this condition were transferred to pots with soil and was cultivated for about 2 yr from 1971 to Aug 1973 and the root was harvested in Aug 1973 and original plants was collected at the same time for investigation of alkaloids and morphological comparison. The leaves were alternate, and divided narrowly into several lobes. The lower surface of them was white and covered with short hairs. The roots were gross and orange.

*Corydalis pallida.* The buds were initiated on subcultured callus tissues of *C. pallida* on M & S medium containing 2,4-D (1 mg/l) and kinetin (0·1 mg/l). The buds were transferred to M & S medium containing IAA (0·1 mg/l) and kinetin (0·1 mg/l) and cultures were maintained in the growth cabinet with 12/12 hr. light/dark cycles under light (4500–9000 lx) at 25 ± 1° and aerial parts were initiated, but the root formation was not induced. Leaflets of the regenerated shoot had less deeply toothed serration than those of the original plants. The rootless plantlets accompanying with callus tissues were subcultured onto fr. M & S medium every 5–6 wks. These aerial parts accompanying the callus tissues harvested from cultured flasks were prepared for alkaloid investigation and the callus tissues subcultured on medium containing 2,4-D and the original plants were comparatively studied for alkaloids. One of the rootless plantlets induced roots on medium containing IAA (1 mg/l) and kinetin (0·1 mg/l), and was transferred to pots with soil and grew 10–15 cm in height, but its plants were not available for alkaloid investigation because they were too small.

*P. bracteatum.* The buds were initiated in about 3 yr on subcultured callus tissues of *P. bracteatum* on M & S medium containing IAA (1 mg/l), kinetin (0·1 mg/l) and CM (7%). The buds were transferred to the growth cabinet with 12/12 hr. light/dark cycles under light (4500–9000 lx) at 25 ± 1°, and were subcultured onto fr. M & S medium containing IAA (1 mg/l), kinetin (0·1 mg/l) and CM (7%) for 5–6 wks growth interval. The callus tissues were also subcultured onto the same medium under dark; a few buds formed without chlorophyll. The buds and the callus tissues collected were prepared for alkaloid study.

*P. somniferum* L. The buds were initiated on subcultured callus tissues of albino type on M & S medium containing 2,4-D (0·1 mg/l) and kinetin (0·1 mg/l). The buds obtained were transferred to M & S medium containing IAA (0·1 mg/l) and kinetin (0·1 mg/l) and the culture were maintained in the growth cabinet with 12/12 hr. light/dark cycles under light (4500–9000 lx) at 25 ± 1°.

*Alkaloid concentrations.* These were measured approximately, as the amount in the crude $CHCl_3$ extract, and are expressed here as the % total alkaloid/fr. wt. Results were: *Macleaya cordata* original plant (8 g fr. wt) 0·61, redifferentiated plantlets (18 g) 0·50, callus tissue (462 g) 0·009; *Corydalis pallida* plantlets (227 g) 0·17, callus tissue (492 g) 0·01; *Papaver bracteatum* buds (600 g) 0·028, callus tissue (245 g) 0·024.

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