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# FORMATION OF ALKALOIDS IN *CORYDALIS OPHIOCARPA* CALLUS CULTURE

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**Key Word Index**—*Corydalis ophiocarpa*; Papaveraceae; callus tissue; biotransformation; isoquinoline alkaloids.

**Abstract**—*Corydalis ophiocarpa* callus tissue was examined for its isoquinoline alkaloid content. The culture has good biosynthetic capabilities for transformation of exogenous alkaloids.

## INTRODUCTION

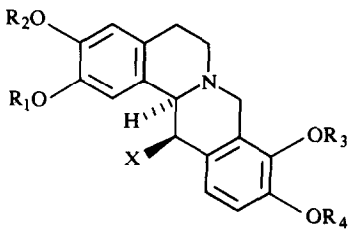
*Corydalis ophiocarpa* Hook et. Thoms. native to Taiwan [1, 2] and species cultivated in Ottawa [3] and North Carolina [4] have previously been shown to contain the protoberberine-type alkaloids, (−)-stylopine (**1**) [1, 2], (−)-canadine (**2**) [1, 3, 4], (−)-cheilanthifoline (**3**) [1], (−)-corypalmine (**4**) [3], (−)-isocorypalmine (**5**) [1], (−)-corycarpine (**6**) [1, 4], (−)-ophiocarpine (**7**) [1–4], coptisine (**8**) [1], berberine (**9**) [1–3] and dehydrocheilanthifoline (**10**) [1]; the protopine-type alkaloids, protopine (**12**) [1–4], α-allocryptopine (**13**) [1–3] and cryptocavine (**14**) [3]; the benzophenanthridine-type alkaloid, sanguinarine (**15**) [1]; the phthalidisoquinoline-type alkaloid, (−)-adlumine (**17**) [3]; and the isoquinoline-type alkaloids, noroxyhydrastinine (**18**) [1], dehydrocorypalline (**19**) [1] and corypalline (**20**) [1].

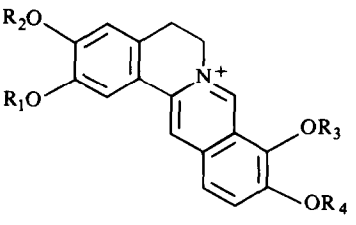
## RESULTS AND DISCUSSION

The alkaloids of *C. ophiocarpa* collected in Japan were investigated. From the whole plants, dehydroisocorypalmine (**11**) and chelerythrine (**16**) were isolated for the first time along with alkaloids (−)-**1**, (−)-**2**, (−)-**5** to (−)-**7**, **8**, **9**, **12**, **13**, **15** and **20**. From the stem callus tissue was derived and studies were made on the alkaloids produced. Sanguinarine (**15**) was identified in the callus (strain A) derived in 1976. Protopine (**12**) as well as sanguinarine (**15**) were present in the callus (strain B) derived in 1978. Base I was also isolated from the callus of strain B and structure **21** was assigned to this new alkaloid.

The capacity of the cell culture to transform administered intermediates was examined. Seven mg of sanguinarine (**15**) was isolated from 630 mg of strain A callus grown on agar containing non-radioactive protopine (**12**) hydrochloride (90 mg) for 20 days. Sanguinarine production in the cells was increased by increasing the period of subculturing. The biosynthetic products, 34 mg of α-allocryptopine (**13**) and 1 mg of chelerythrine (**16**), were obtained from 9.02 g of strain B callus grown on solid medium containing 100 mg of non-radioactive (±)-tetrahydroberberine α-*N*-methochloride for 30 days along with 6 mg of protopine, 2 mg of sanguinarine chloride and 48 mg of (+)-tetrahydroberberine α-*N*-methochloride which is *ca* 50% of the amount administered.

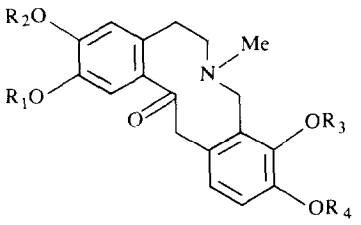
Thus, dehydroisocorypalmine (**11**) and cheleryth-



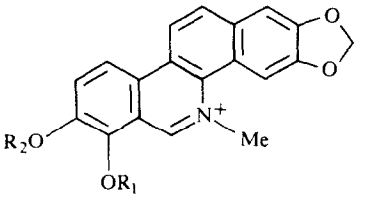




**1** $R_1 + R_2 = R_3 + R_4 = CH_2$, X = H
**2** $R_1 + R_2 = CH_2$, $R_3 = R_4$ = Me, X = H
**3** $R_1$ = H, $R_2$ = Me, $R_3 + R_4 = CH_2$, X = H
**4** $R_1 = R_3 = R_4$ = Me, $R_2$ = H, X = H
**5** $R_1$ = H, $R_2 = R_3 = R_4$ = Me, X = H
**6** $R_1 + R_2 = R_3 + R_4 = CH_2$, X = OH
**7** $R_1 + R_2 = CH_2$, $R_3 = R_4$ = Me, X = OH

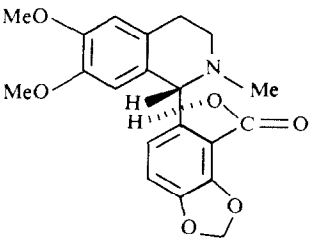
**8** $R_1 + R_2 = R_3 + R_4 = CH_2$
**9** $R_1 + R_2 = CH_2$, $R_3 = R_4$ = Me
**10** $R_1$ = H, $R_2$ = Me, $R_3 + R_4 = CH_2$
**11** $R_1$ = H, $R_2 = R_3 = R_4$ = Me



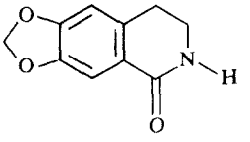
**12** $R_1 + R_2 = R_3 + R_4 = CH_2$
**13** $R_1 + R_2 = CH_2$, $R_3 = R_4 = Me$
**14** $R_1 = R_2 = Me$, $R_3 + R_4 = CH_2$



**15** $R_1 + R_2 = CH_2$
**16** $R_1 = R_2 = Me$



**17**



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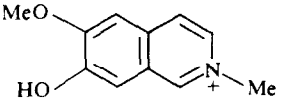

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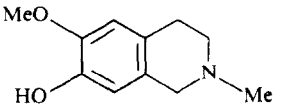

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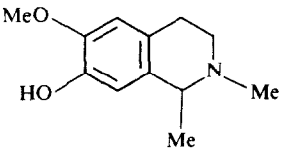


**21**

rine (**16**) were isolated from intact plants. Sanguinarine (**15**), protopine (**12**) and Base 1 (**21**) were produced in the callus cells. The cultured cells are able to convert protopine into sanguinarine and (−)-tetrahydroberberine $\alpha$-*N*-methochloride bearing a *cis* fused quinolizidine ring via $\alpha$-allocryptopine into chelerythrine (Scheme 1). That is, there is the capacity to convert tetrahydroprotoberberine type compounds via protopine type compounds into benzophenanthridine type compounds in the cell culture similar to other *Papaver* plants [5]. We have also demonstrated that the (+)-form of tetrahydroberberine $\alpha$-*N*-methochloride is not transformed by the cultured cells of *C. ophiocarpa*. It is now possible to use the cell culture for biosynthetic studies since *C. ophiocarpa* tissue culture has the ability to perform biogenetic conversion of the alkaloids.

## EXPERIMENTAL

Mps are uncorr. Si gel $60F_{254}$ plates (Merck) were used for prep. TLC. $^1H$ NMR spectra were taken in $CDCl_3$ using TMS as internal standard at 60 MHz or 90 MHz. EIMS was determined at 75 eV.

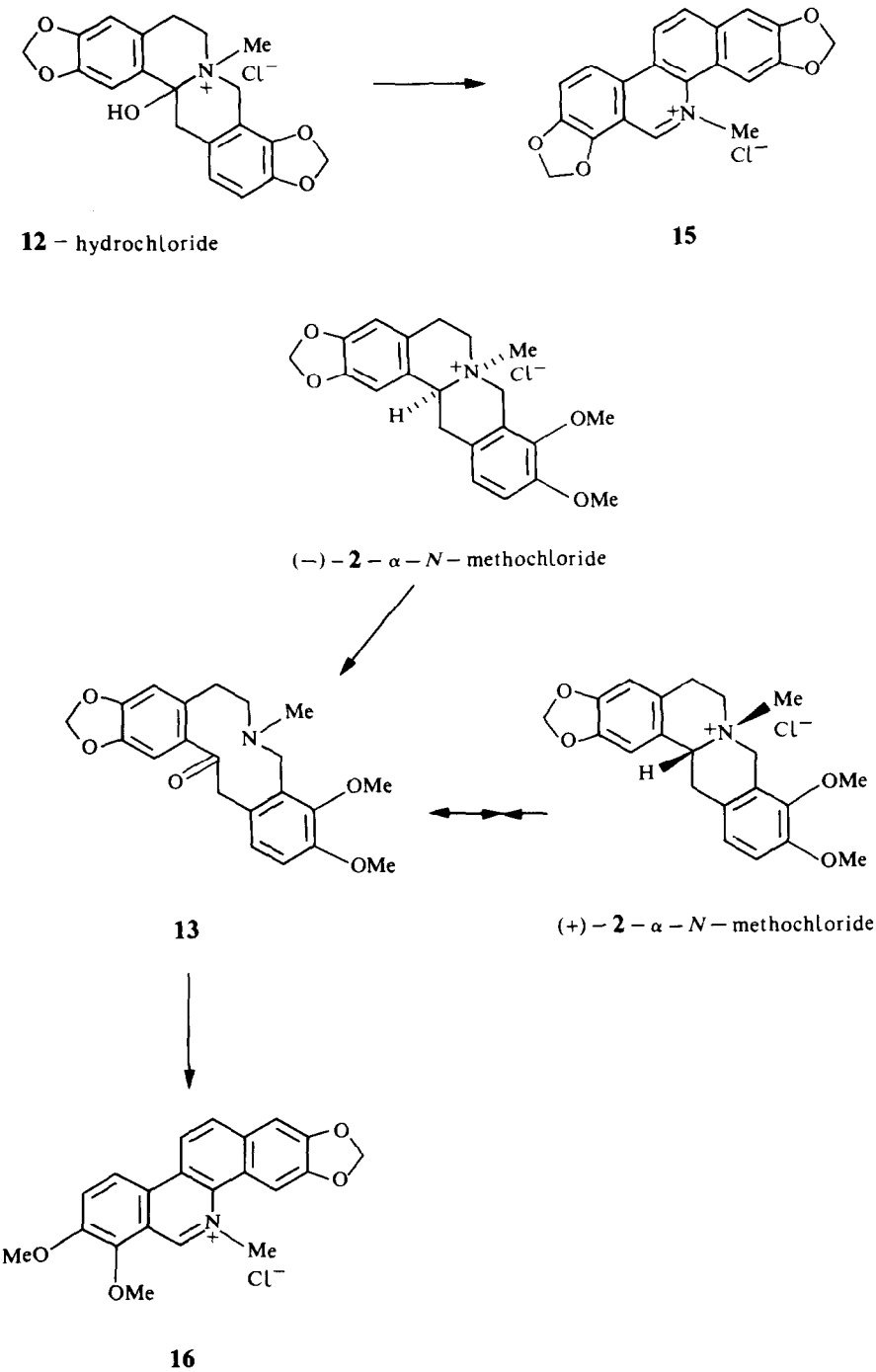
*Extraction and isolation of alkaloids.* After drying and cutting, the whole plant (36.2 g collected in 1976) was extracted with MeOH. The MeOH extract was evapd under red. pres. and the residue re-extracted with 3% aq. tartaric acid. The acidic soln was washed with $Et_2O$, made basic with $NH_4OH$ and extracted with $Et_2O$. The alkaline $Et_2O$ extract (373 mg) was separated by prep. Si gel TLC in $C_6H_6$–$Et_2O$ (7:3) and nine bands were extracted with $CHCl_3$–MeOH (3:1) to give bases A–I. Bases A–E were recrystallized from $CHCl_3$–MeOH to afford 6 mg of (−)-stylopine (**1**), mp 202–203° $[\alpha]_D$ −291° ($CHCl_3$; *c* 0.25), 10 mg of (−)-canadine (**2**), mp 126–127° $[\alpha]_D$ −262° ($CHCl_3$; *c* 0.31), a trace of (−)-isocorypalmine (**5**), 25 mg of (−)-corycarpine (**6**), mp 223–224° $[\alpha]_D$ −277° ($CHCl_3$; *c* 0.47) and 103 mg of (−)-ophiocarpine (**7**) mp 190–191° $[\alpha]_D$ −295° ($CHCl_3$; *c* 0.40). Bases F and G had characteristic orange and yellow colours, respectively and were reduced with $NaBH_4$ in MeOH to afford their dihydroderivatives, dihydrosanguinarine and dihydrochelerythrine. Bases H and I were prepared by prep. TLC in MeOH to give 15 mg of protopine (**12**), mp 210–211° and 5 mg of $\alpha$-allocryptopine (**13**), mp 157–158°. The quaternary base fraction was obtained from the remaining aq. layer by acidification with HCl, addition of a soln of KI and repeated extraction with $CHCl_3$. The $CHCl_3$ extract was reduced and fractionated by prep. TLC as above to afford 2 mg of (±)-stylopine (**1**), mp 218–219° and 20 mg of (±)-canadine (**2**), mp 167–168°.

After the quaternary bases were isolated as their iodides, the aq. layer was made alkaline with aq. NaOH, reduced and extracted with $Et_2O$. The $Et_2O$ extract was fractionated by prep. TLC in MeOH and then in $C_6H_6$–$Et_2O$ (1:1) to afford 7 mg of corypalline (**20**), mp 163°. Extraction and isolation were carried out on the plants (70 g) collected in 1978, as well as described above, to give (−)-**1** (22 mg), (−)-**2**

(30 mg), (−)-**5** (1.5 mg) (−)-**6** (16 mg), (−)-**7** (62 mg), having mp 217–218°, (±)-**1**(19 mg), (±)-**2** (68 mg), (±)-**5** (3 mg) having mp 214–216°, **12** (29 mg), **13** (8 mg) and trace amounts of **15** and **16**. These known isoquinoline bases were identified by mp, TLC, IR, $^1$H NMR and MS.

*Callus culture.* The tissues from *C. ophiocarpa* stem were derived in May 1976 (strain A) and in Sept. 1978 (strain B), respectively. Murashige and Skoog's medium containing 2,4-D and kinetin as plant growth regulators was used for induction of callus tissues. They have been continuously subcultured every 4–6 weeks on the same medium containing 2,4-D (1 mg/l.) and kinetin (0.1 mg/l.).

*Extraction and isolation of alkaloids from callus.* (a) The callus (650 mg and 990 mg dry wt) of strain A cultured at 25° for 20 and 40 days on agar medium, respectively, were extracted in MeOH. Further treatment of the combined MeOH extracts followed the procedures described for intact plants. The alkaline $Et_2O$ extract was evapd and analysed by prep. TLC. The orange band observed on TLC was isolated and found to be identical by TLC and $^1$H NMR with a




Scheme 1.

sample of sanguinarine (**15**). (b) The callus (5 g dry wt) of strain B cultured at 25° for 5 weeks on agar medium was extracted in MeOH and 5% HCl and the combined extracts concd. The aq. soln was washed with $Et_2O$ and applied to a 3 × 7 cm Dowex 50W × 8 column. The column was washed with $H_2O$ until the eluate was colorless. The column was then eluted as follows: fraction 1, 5% HCl and mixed soln of 5% HCl and MeOH (9:1–1:9); fraction 2, 4 N HCl and mixed soln of 4 N HCl and MeOH (9:1–1:9). The column resin was shaken with $CHCl_3$ and dil. $NH_4OH$ soln and the organic extract dried and evapd (fraction 3). Fractions 1 and 2 were concd, made alkaline and extracted with $Et_2O$, then with $CHCl_3$. The organic extract was then evapd. Prep. TLC of fraction 1 using MeOH afforded Base I, MS, *m/z*; 207 ($M^+$), 192 (base peak), 177; IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3560 (OH); $^1H$ NMR (90 MHz): $\delta$ 1.45 (3H, *d* $J$ = 6.7 Hz, CH–C$\underline{H}_3$), 2.90 (3H, *s*, N–Me), 2.74–3.29 (4H, *m*) 3.84 (3H, *s*, OMe), 4.07 (1H, *q*, $J$ = 6.7 Hz, C$\underline{H}$–Me) and protopine (**12**) identical by IR comparison with authentic sample. Fractions 2 and 3 were separated by prep. TLC using $C_6H_6$–$Et_2O$ (1:1) to give sanguinarine chloride (6 mg) identical by IR comparison with authentic sample.

*Feeding experiments with unlabelled protopine.* (i) Callus from strain A was incubated at 25° for 20 days on agar (210 ml) containing protopine (**12**) HCl (90 mg). After incubation, the calluses (630 mg, dry wt) were extracted using method (a) described above. Prep. TLC of the $Et_2O$ extract using $C_6H_6$–$Et_2O$ (1:1–7:3) was done to separate bands I and II. Band I was reduced with $NaBH_4$ in MeOH and purified by prep. TLC to give dihydrosanguinarine (7 mg) identical by IR comparison with authentic sample. Protopine (7 mg) was recovered after purification by prep. TLC of band II using MeOH. (ii) The callus from strain A was incubated at 25° for 40 days on agar (430 ml) containing protopine (**12**) HCl (180 mg). After incubation, the calluses (2.34 g, dry wt) were worked up as described above to afford dihydrosanguinarine (24 mg) and protopine (7 mg).

*Feeding experiment with unlabelled (±)-tetrahydroberberine α-N-methochloride.* The callus from strain B was incubated at 25° for 30 days on agar (1600 ml) containing (±)-tetrahydroberberine *α-N*-methochloride (100 mg). After incubation, the calluses (9.02 g, dry wt) were worked up as described above in (b). Prep. TLC of the $Et_2O$ extract from fraction 2 using $CHCl_3$–MeOH (1:1) afforded α-allocryptopine (34 mg) and protopine (6 mg). Prep. TLC of the $CHCl_3$ extract of fraction 2 using $CHCl_3$–MeOH (4:1) gave (+)-tetrahydroberberine *α-N*-methochloride (48 mg), mp 242–247°, $[\alpha]_D$ + 168° ($CHCl_3$; *c* 0.53) identical by IR comparison with an authentic sample. Prep. TLC of the organic extract of fraction 1 using $CHCl_3$–MeOH (1:1) afforded Base I. Prep. TLC of the organic extract of fraction 3 using $C_6H_6$–$Et_2O$ (1:4) gave sanguinarine chloride (2 mg) and chelerythrine chloride (1 mg) identical by IR comparison with authentic samples.

## REFERENCES

1. Tani, C., Nagakura, N. and Kuriyama, C. (1978) *Yakugaku Zasshi* **98**, 1243 and 1658.
2. Lu, S.-T., Wang, S.-J. and Su, T.-L. (1971) *Yakugaku Zasshi* **91**, 778.
3. Manske, R. H. F. (1939) *Can. J. Res. Sect. B* **17**, 51.
4. Jeffs, P. W. and Scharver, J. D. (1975) *J. Org. Chem.* **40**, 644.
5. Takao, N., Iwasa, K., Kamigauchi, M. and Sugiura, M. (1976) *Chem. Pharm. Bull.* **24**, 2859.