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STEREOCHEMICAL STUDIES ON THE BIOSYNTHESIS OF PROTOBERBERINE, PROTOPINE, AND BENZOPHENANTHRIDINE ALKALOIDS USING PAPAVERACEAE PLANT CELL CULTURES

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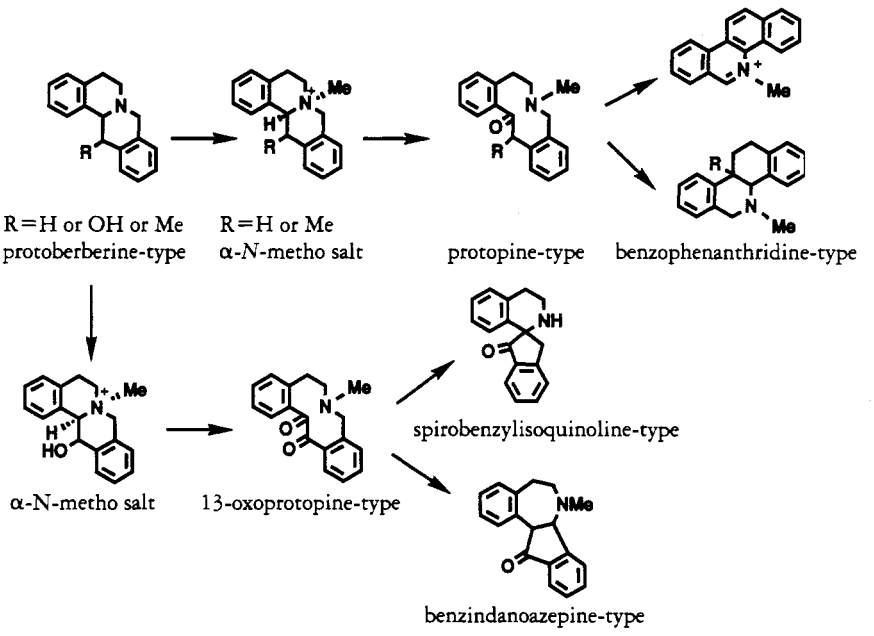
ABSTRACT.—In the cultured cells of *Corydalis incisa*, (−)-enantiomers of the α-*N*-metho salts of the *trans*- and *cis*- 13-methylprotoberberines having trans and cis configurations of the protons at C-13 and C-14, respectively, were stereospecifically bioconverted into the (−)- and (+)-13-methylprotopines [(−)-(13*S*)- and (+)-(13*R*)-enantiomers], respectively. The α-*N*-metho salts of the eight different 13-methylprotoberberines were demonstrated to be the precursors of the 13-methylprotopines, though there is a difference in conversion yields. The α-*N*-metho salts of the *trans*-13-methylprotoberberines bearing a methylenedioxy bridge at C-2 and C-3 are more favorable precursors of the 13-methylprotopines than those having methoxyl groups at the same positions. The α-*N*-metho salts of *cis*-13-methylprotoberberines with either methylenedioxy or methoxy substitution are efficiently converted into 13-methylprotopines. The (+)-(13*R*)-13-methylprotopines were more effective precursors of the benzophenanthridines than the (−)-(13*S*)-methylprotopines. The 13-methylprotopines possessing a methylenedioxy bridge were more preferred precursors of the benzophenanthridines than those having methoxyl groups. (+)-Corynoline [**14**] is more easily oxidized than (−)-**14**, resulting in (+)-corynoloxine [**18**]. (−)-Corynoline is acetylated more easily than its (+) isomer to afford (−)-acetylcorynoline [**19**]. Corynoline analogue **15**, having methoxy groups at C-7 and C-8, must not be as easily oxidized as corynoline, which has a methylenedioxy bridge at C-7 and C-8. (+)-Chelidonine [**27**] was converted into dihydrosanguinarine [**32**] and dehydrochelidonine [**28**]. Biotransformation of the protoberberines into the benzophenanthridines occurred similarly in both cultured cells and live whole plants of *Co. incisa*.

Two different biogenetic pathways (Scheme 1) from the protoberberines have been defined in several *Corydalis* species (*Corydalis incisa, Corydalis ophiocarpa, Corydalis ochotensis* var. *raddeana, Corydalis platycarpa,* and *Corydalis pallida* var. *tenuis*), *Macleaya cordata*, and *Chelidonium majus* of Papaveraceae using whole plants or their cell cultures (1–6). The first pathway involves the sequence protoberberine or 13-methylprotoberberine→α-*N*-metho salt→protopine→benzophenanthridine; while the second includes the conversion 13-hydroxyprotoberberine→α-*N*-metho salt→13-oxoprotopine→spirobenzylisoquinoline or benzindanoazepine (Scheme 1).

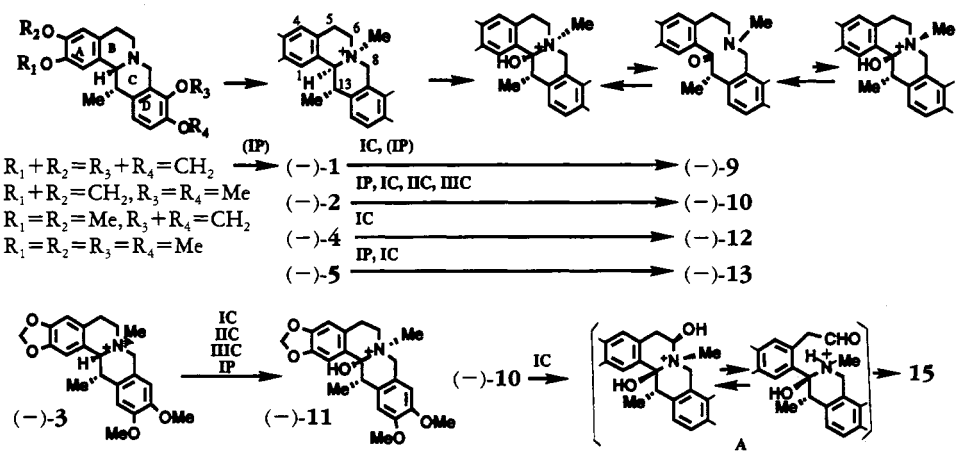
The purpose of this work was to investigate the stereospecificity of the first pathway and to compare the biotransformation of the alkaloids in the cultured cells with that in *Co. incisa* plants. Some related experiments with the cultured cells of *Co. ochotensis* var. *raddeana* and *Co. ophiocarpa* were also covered.

The results of the previous studies on the first pathway are as follows (1–6). The α-*N*-metho salts (e.g., **1**, **2**, **6**, **7**, **23**, and **24**) (Schemes 2, 3, and 4) of (±)-protoberberines and (±)-13-methylprotoberberines having the cis-B/C fused system but not the corresponding trans-fused β-*N*-metho salts, are biotransformed into the corresponding protopines (**9**, **10**, **25**, and **26**). Both α-*N*-metho salts (**1** and **6** or **2** and **7**) of (±)-13-methylprotoberberines, having the trans and cis configurations of the protons at C-13 and C-14, respectively, are bioconverted into the 13-methylprotopines **9** or **10**. The (−)-enantiomer of the α-*N*-metho salts is more preferred as a substrate than the (+)-isomer.

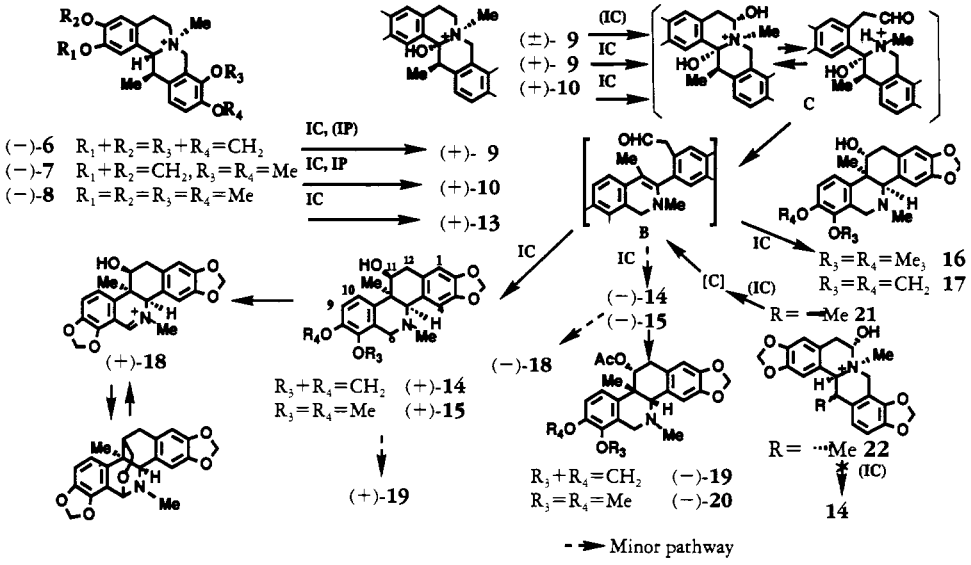


SCHEME 1

Corycavine [**9**] is further biotransformed into corynoline [**14**], corynoloxine [**18**], and acetylcorynoline [**19**]. A stereospecific removal of hydrogen from C-6 of corynoline [**14**] occurs in its bioconversion into corynoloxine [**18**]. The α-*N*-metho salt of 6-hydroxy-13-methylprotoberberine [**21**], having the cis relative configuration of protons at C-13 and C-14, is also converted into **14** and **18**; the corresponding α-*N*-metho salt [**22**], possessing the trans configuration, is not. This is in marked contrast to the situation with the 13-methylprotoberberines **1** and **6**, which are both metabolized to benzophenanthridines via protopines. (±)-Corycavine [**9**] is incorporated 4 times more efficiently into corynoline [**14**] and 6 times more efficiently into corynoloxine [**18**] than is 6-hydroxy-13-methylprotoberberine [**21**]. The 13-methylprotoberberines likely undergo hydroxylation at C-14 prior to hydroxylation at C-6 in the major biosynthetic pathway to



SCHEME 2. *Corydalis incisa* (I), *Corydalis ophiocarpa* (II), *Corydalis ochotensis* var. *raddeana* (III). P: Plant. C: Callus. ( ): Previous feeding experiment.



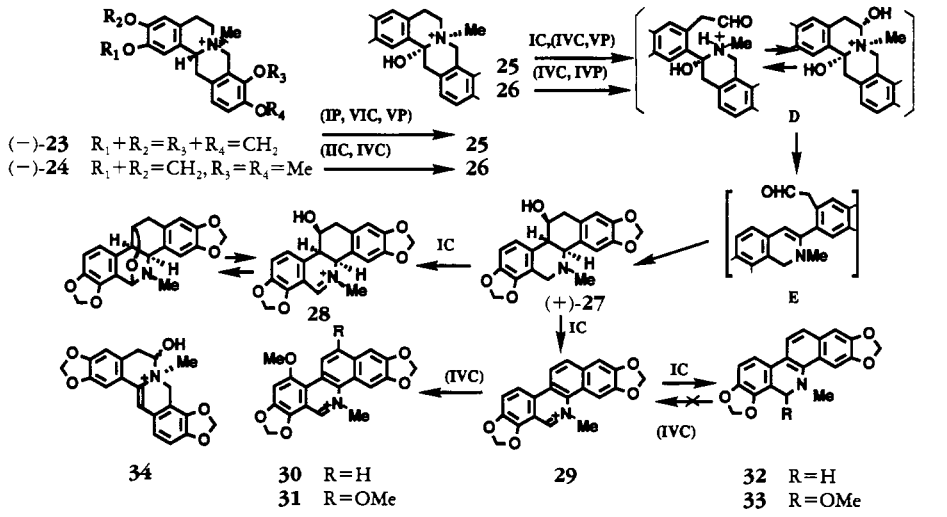
SCHEME 3. *Corydalis incisa* (I). P: Plant. C: Callus. ( ): Previous feeding experiment.

benzophenanthridines such as corynoline [**14**]. Protopine [**25**] is found to be incorporated into chelidonine [**27**] and the fully aromatized quaternary benzophenanthridine sanguinarine [**29**]. Sanguinarine [**29**] is converted via 10-methoxysanguinarine (chelirubine [**30**]) to 10,12-dimethoxysanguinarine (macarpine [**31**]).

RESULTS AND DISCUSSION

FEEDING EXPERIMENTS IN THE CULTURED CELLS.—Feeding experiments were carried out to provide information about hydroxylation at C-14 of the protoberberine α-*N*-metho salts. We also wanted to have information about intermediates involved in the conversion of the protopines into the benzophenanthridines.

α-*N*-Metho salts **1**–**5** were prepared by treating tetraoxygenated *trans*-13-methyl-



SCHEME 4. *Corydalis incisa* (I), *Corydalis ophiocarpa* (II), *Macleaya cordata* (IV), *Chelidonium majus* (V). P: Plant. C: Callus. ( ): Previous feeding experiment.

protoberberines, which have a trans configuration of protons at C-13 and C-14, with ^{13}C-labeled MeI. ^{1}H- and ^{13}C-nmr spectral data for **3**, **4**, and **5**, compounds never before prepared, are summarized in Tables 1 and 2. These ^{13}C-labeled α-*N*-metho salts **1–5** (Scheme 2) were first fed to the cultured cells of *Co. incisa* (Table 3, experiments 1–5). All substrates **1–5** were bioconverted into the corresponding 13-methylprotopines **9–13** (Scheme 2). The ^{1}H- and/or ^{13}C-nmr spectra for **9–13** showed that the ^{13}C enrichment of the *N*-methyl group of each compound is almost the same as that of the corresponding starting substrate of **1–5** (Table 3). The structures of **11**, **12**, and **13** were determined by comparing ^{1}H- and ^{13}C-nmr spectra with those of known compounds **9** and **10** (Tables 1 and 2). The five different α-*N*-metho salts were hydroxylated at C-14 to produce the 13-methylprotopines.

There is a big difference in the conversion yield between one group of α-*N*-metho salts, **1**, **2**, and **3**, and the others, group **4** and **5**. The former, containing a methylenedioxy bridge at C-2 and C-3, are more favorable precursors of the 13-methylprotopine-type alkaloids than are **4** and **5**, with methoxy groups at the same positions. This is consistent with the fact that no 13-methylprotopine possessing methoxyl groups on the A ring has been isolated in nature. The amount of **10** is smaller than that of **9** or **11** because **10** is further converted into the benzophenanthridine alkaloids such as **15** (Table 3).

The 13-methylprotopines **10–12** were levorotatory and were obtained in states of relatively high configurational purity, as can be seen by comparing their $[\alpha]_D$ values with that of (−)-corycavine [**9**] (8) (Table 3). These (−)-13-methylprotopines must have a 13*S* configuration, since (−)-corycavine [**9**] has been determined by X-ray analysis to have a 13*S* configuration (8). All recovered substrates had dextrorotation. Therefore, the (−)-enantiomers of the α-*N*-metho salts **1-5**, rather than the (+)-isomers, were also the preferred precursors of the 13-methylprotopine bases **9–13** in agreement with previous results (2–6) (Scheme 2).

Similar feeding experiments of the substrates **2–5** were carried out using the cultured cells of *Co. ochotensis* var. *raddeana* and *Co. ophiocarpa*. α-*N*-Metho salts **2** and **3** were converted in low yields into the corresponding 13-methylprotopines, **10** and **11**, respectively. However, no 13-methylprotopine was detected in the feeding experiments of **4** and **5**. α-*N*-Metho salts **2** and **3** are better precursors of the 13-methylprotopines than **4** or **5** in cultured cells of *Co. ochotensis* and *Co. ophiocarpa* as well as those of *Co. incisa*.

Next, incorporation experiments (Table 4, experiments 6–11) of the ^{13}C-labeled *cis*-13-methylprotoberberine α-*N*-metho salts **6**, **7**, and **8** (Scheme 3), having cis protons at C-13 and C-14, were undertaken for comparison with **1**, **2**, and **5**, bearing the trans protons. Substrates **6–8** were incorporated into the corresponding 13-methylprotopines, (+)-**9**, (+)-**10**, and (+)-**13**, which were dextrorotatory and of high optical purity. The recovered substrates **6–8** had dextrorotation. The (−)-enantiomers of the α-*N*-metho salts, rather than the (+)-isomers, were therefore more favorable precursors of the 13-methylprotopines. Of the *cis*-13-methylprotoberberine α-*N*-metho salts **6–8**, compound **8**, having methoxyl groups on the A ring, is the preferred precursor of the 13-methylprotopines. This is in contrast to the behavior displayed by *trans*-13-methylprotoberberine α-*N*-metho salt [**5**], which is methoxylated on ring A and is a poor substrate. (−)-Enantiomers of the α-*N*-metho salts **6–8** were converted into (+)-13-methylprotopines **9**, **10**, and **13** (Scheme 3).

Corynoline analogue **15** and 11-epicorynoline analogue **16**, having dextrorotation, and acetylcorynoline analogue **20** were found after feeding compound **7** (Table 4). The structures of **15**, **16**, and **20** were confirmed by comparison of ^{1}H-nmr and mass spectra wtih those of the known corynoline [**14**], 11-epicorynoline [**17**], and acetylcorynoline [**19**] (Tables 5 and 6). In the mass spectra (Table 6), the difference between corynoline

TABLE 1. ^{1}H-nmr Data[a] for the α-*N*-Metho Salts of Protoberberines[b] and 13-Methylprotopines.[c]

Proton	Compound								
	3	**4**	**5**	**8**	**9**	**10**	**11**	**12**	**13**
C-Me	1.50 3H d (6.8)	1.49 3H d (6.8)	1.49 3H d (6.8)	1.99 3H d (7.7)	1.35 3H d (7.3)	1.34 3H d (7.0)	1.39 3H d (7.2)	1.35 3H d (7.0)	1.29 3H d (7.2)
N-Me	3.15 0.26H s	3.16 0.30H s	3.19 0.26H s	3.26 0.12H s	1.81 0.18H s	1.76 0.30 H s	1.78 0.36H s	1.81 0.36, s	1.74 0.30H s
N-13 Me	2.74H d (144)	2.70H d (144)	2.74H d (144)	2.88H d (145)	2.82H d (135)	2.70H d (140)	2.64H d (135)	2.64H d (140)	2.70H d (136)
H-13	overlap	overlap	overlap	overlap	4.27 1H q (7.2)	4.20 1H m	4.25 1H q (7.2)	4.27 1H m	4.18 1H q (7.2)
H-14	4.41 1H d (10)	4.44 1H d (10)	4.40 1H d (10)	4.85 (overlap)					
H-8	4.81 2H ABq d (15)	4.85 2H brs	4.91 2H ABq d (16)	4.95 2H ABq d (15)	3.50, 3.85 each 1H m	3.80, 2H overlap	3.06 1H dd (13.5 3.5[d]) 4.16 1H dd (13.5 8.0[d])	3.50, 3.87 overlap	3.82, 2H overlap
OMe	3.84, 3.85 each 3H s	3.86, 3.88 each 3H s	3.86, 3.88, 3.89, 3.91 each 3H s	3.86, 3.89, 3.87, 3.92 each 3H s		3.80, 3.88 each 3H s	3.88, 3.93 each 3H s	3.89, 3.91 each 3H s	3.76, 3.84, 3.85, 3.88 each 3H s
OCH_2O	6.03 2H s	6.07 2H, ABq (1.0)			5.96 2H s 6.01 2H, ABq (1.3)	5.96 2H, ABq (1.3)	5.96 2H, ABq (1.5)	5.95 2H s	
Ar-H	6.83, 6.84, 6.85, 6.94 each 1H s	6.89, 6.94 each 1H s 6.93 2H s	6.90, 6.94 each 1H s 7.15 2H s	6.88, 6.93 each 1H s 7.15 2H s	6.66, 6.98 each 1H s 6.81, 6.82 each 1H d (8.5)	6.66, 7.02 each 1H s 6.90, 7.09 each 1H d (8.3)	6.65, 6.68, 6.93, 7.00 each 1H s	6.67, 7.03 each 1H s 6.81, 6.86 each 1H d (8.3)	6.66, 7.04 each 1H s 6.88, 7.08 each 1H d (8.4)

[a]Chemical shift (δ), coupling constant (Hz, in parentheses).
[b] 200 MHz, CD_3OD.
[c] **9** and **13**, 200 MHz; **10**, **11**, and **12**, 500 MHz, in $CDCl_3$.
[d] ^{13}C Coupling constant between H-8 and N-^{13}Me group.

TABLE 2. ^{13}C-nmr Data[a] for the α-*N*-Metho Salts of Protoberberines and 13-Methylprotopines.

Carbon	Compound						
	3	**4**	**5**	**9**	**10**	**11**	**13**
13-Me	20.21	20.32	19.85	16.46	16.39	15.98	16.37
C-5	24.70	24.28	23.13	33.27	33.06	32.88	32.87
C-13	40.16	39.71	38.39	46.11	45.69	45.61	45.85
C-6	52.26	52.77	50.07	57.39	57.52	57.86	57.73
N-Me	51.75	51.96	50.75	40.93	41.12	40.69	41.46
C-8	66.13	60.80	60.24	49.56	49.63	57.01	49.33
C-14	74.05	73.90	70.75	196.68	195.50	[d]	[d]
OMe	57.01	56.99	55.81, 56.01		55.63	55.66	55.81, 56.10
	57.01	57.22	56.30, 61.42		60.68	55.81	56.13, 60.83
OCH_2O	103.49	104.05		100.66	101.24	100.89	
				100.97			
C-1, C-4	110.18, 110.60	109.45, 110.69	111.53, 111.93	106.64, 110.18	110.24, 110.41	109.96, 111.13	111.13, 113.77
C-11, C-12[b]	111.35, 112.16	114.02, 122.22	113.30, 122.43	111.15, 120.35	111.77, 123.04	111.43, 112.56	115.07, 123.19
C-1a, C-4a	119.05, 124.11	122.86, 124.76	119.45, 119.96	116.46, 132.17	128.47, 132.23	126.92, 132.00	128.66, 130.99
C-8a, C-12a	125.80, 129.12	124.76, 130.07	123.61, 127.18	133.72, 134.96	132.43, 132.91	132.18, 134.66	133.29, 133.96
C-2a, C-3a	148.50, 150.69	146.05, 148.31	145.51, 147.99	145.44, 145.44	146.43, 147.11	145.97, 146.58	147.45, 147.75
C-9a, C-10a[c]	150.69, 151.84	149.75, 152.09	149.81, 151.15	146.02, 147.88	148.23, 151.19	147.83, 147.85	149.66, 151.35

[a] **3**, **4**, and **5**, 126 MHz in CD_3OD; **9** and **10**, 50 MHz in $CDCl_3$; **11** and **13** 126 MHz in $CDCl_3$; **13** measured at 50°.

[b]C-9, C-12 for **11**.

[c]C-10a, C-11a for **11**.

[d]The carbonyl absorption is absent because of the tetracyclic form.

TABLE 3. Feeding Experiments of the α-*N*-Metho Salts of *trans*-13-Methylprotoberberines in Cell Static Cultures of *Corydalis incisa*.

Experiment	Substrate[a] (mg) (^{13}C enrichment, %)[b]	Medium (liters)	Incubation time (weeks)	Wt of dry cells (g)	Product, yield %, (^{13}C enrichment, %)[b]		
					Corycavine [**9**] or its analogue	Corynoline analogue **15**	Recovered material
1	**1** 200 (99)	1.6	4.5	5.63	**9**[c] 41 (94) [−112] [−124][d]		57 [+124] [+76]
2	**2** 200 (90)	1.6	4.5	4.32	**10** 16 (90) [−152] [−152]	**15** 0.25 (83)	62 [+90] [+11]
3	**3** 100 (91)	0.8	4.5	2.82	**11** 36 (88) [−139] [−145]		34 [+85] [+23]
4	**4** 100 (90)	0.8	4.5	2.21	**12** 1.5 (88) [−107][e]		55 [+47] [+5.9]
5	**5** 150 (91)	0.8	4.5	2.47	**13** 0.9 (90)		41 [+11] [+2.2]

[a]Compounds **1**–**5** are racemate.
[b]These values were determined by ^{1}H nmr spectra.
[c](−)-**9**: $[\alpha]_D = -170°$ (c=1.0, $CHCl_3$).
[d]Values in brackets are $[\alpha]_D$: left, callus; right, medium.
[e]There is an error resulting from c=0.14.

TABLE 4. Feeding Experiments of the α-*N*-Metho Salts of *cis*-13-Methylprotoberberines, Benzophenanthridines, and Protopines in Cell Static Cultures of *Corydalis incisa*.

Experiment	Substrate[a] (mg) (^{13}C enrichment, %)[b]	Medium (liters)	Incubation time (weeks)	Wt of dry cells (g)	Product, yield %, (^{13}C enrichment, %)[b]		
					13-Methylprotopines	Benzophenanthridines	Recovered material
6	**6** 192 (88)	1.6	4.5	6.91	**9** 7.5 (77) [+138] [+87][d]	**14**[c] 2.5 (87) [+37][e] **18**[c] 4.6 (81) [+92][e] **19** 0.7 (79)	58 [+80] [+30][f]
7	**7** 86 (85)	0.8	4.5	2.80	**10** 6.9 (82) [+149][e]	**15** 5.9 (83) [+40][d,e] **16** 1.7 (79) [+32][d,e] **20** 1.3 (84)	57 [+86] [+18]
8	**8** 190 (96)	0.8	4.5	5.0	**13** 27 (92) [+159][e]		62 [+26]
9	**14** 150	1.2	4.5	5.43		**14**[c] 9.4 [−71] [−54] **18**[c] 6.6 [+62][d] [+103] **19**[c] 34 [−73] [−61]	
10	**25** 200	1.2	4.5	6.5		**33** 25	
11	**27** 200	1.2	4.5	8.5		**28** 1.7 **32** 47	

[a]Compounds **6–8**, **14**, and **25** are racemate and **27** is (+)-enantiomer.
[b]These values were determined by ^{1}H-nmr spectra.
[c][α]D ($CHCl_3$) (+)−**14** +116° (*c*=1.7), (−)-**14** -116° (*c*=2.5), (+)-**18** +150°, (+)-**19** +75° (*c*=2.3).
[d]There is an error resulting from *c*=0.15–0.1.
[e]Only one [α]D value, from material derived from callus and medium.
[f]Values in brackets are [α]D: left, callus; right, medium.

[**14**] and its analogue **15** or 11-epicorynoline [**17**] and its analogue **16** or acetylcorynoline [**19**] and its analogue **20** is either 17 or 16 mass units. Whether it is 17 or 16 depends on the existence of ^{13}C plus CH_4 or only CH_4. Corynoline [**14**] (32% optical purity) and corynoloxine [**18**] (61% optical purity), which are a partial racemate containing an excess of the (+)-form, and acetylcorynoline [**19**] were obtained after feeding compound **6** (Table 4). No benzophenanthridine alkaloid was detected after administering compound **8**.

Corynoloxine [**18**] was formed from **6**, but no corynoloxine-type alkaloid was obtained from **7**. ^{13}C enrichments of benzophenanthridine products **14–16** and **18–20** are shown in Table 4. The varieties of the *N*-methyl enrichments among the precursors and metabolites do not result from making H-D exchange during the course of the biosynthetic experiments, but they seem to arise from the error in the measurement of nmr spectra.

The α-*N*-metho salts of the (−)-*trans*-13-methylprotoberberines **1–5** were transformed into (−)-13-methylprotopines **9–13** [(−)-(13*S*)-enantiomers], from which the formation of the benzophenanthridines was detected only with **10** (Scheme 2). On the other hand, α-*N*-metho salts of the (−)-*cis*-13-methylprotoberberines **6–8** were biotransformed into (+)-13-methylprotopines **9**, **10**, and **13** [(+)-(13*S*)-enantiomers] (Scheme 3). Products **9** and **10** were further converted into benzophenanthridines. Therefore, the (+)-enantiomer of the 13-methylprotopine **9** or **10**, having a methylenedioxy bridge on the A ring, is bioconverted more efficiently into benzophenanthridines than is the (−) isomer. The (+)-enantiomer as well as the (−)-enantiomer of the 13-methylprotopines bearing methoxyl groups on the A ring is not significant as a precursor of the benzophenanthridines. Formation of (+)-corynoline [**14**], having low configurational purity (32%), from (+)-corycavine [**9**] with high configurational purity (81%) supports the intermediacy of an achiral enamino aldehyde (Scheme 3, B).

Next, the biotransformations of benzophenanthridines, such as corynoline [**14**] and chelidonine [**27**], were studied. Unlabeled (±)-corynoline hydrochloride was fed into the cultured cells of *Co. incisa* (Table 4, experiment 9). (±)-Corynoline [**14**] was converted into (−)-acetylcorynoline [**19**] of 81% optical purity (9) and (+)-corynoloxine [**18**] of 69% optical purity (10). (−)-Corynoline of 47% optical purity (9) was recovered. (+)-Corynoline [**14**] is more easily oxidized than (−)-**14**, resulting in (+)-corynoloxine [**18**]. (−)-Corynoline is acetylated more easily than the (+) isomer to afford (−)-acetylcorynoline [**19**] (Scheme 3). Corynoline analogue **15**, having methoxy groups at C-7 and C-8, must not be as easily oxidized as corynoline with a methylenedioxy bridge, because corynoloxine-type alkaloid was not obtained from **15**.

Chelidonine [**27**] has been shown to be biosynthesized from the α-*N*-metho salt of (−)-stylopine [**23**] via protopine [**25**] (Scheme 4) (1,6,7). The next step was to establish the relationship between chelidonine [**27**] and the more highly oxidized sanguinarine [**29**]. Hydrochlorides of unlabeled protopine [**25**] and (+)-chelidonine [**27**] were administered to cultured cells of *Co. incisa* (Table 4, experiments 10 and 11). Protopine [**25**] was found to be converted into sanguinarine [**29**] by the isolation of 6-methoxysanguinarine [**33**] in *Co. incisa* as well as in *M. cordata* (6) and *Ch. majus* (1). (+)-Chelidonine [**27**] was converted into dihydrosanguinarine [**32**] and a small amount of dehydrochelidonine [**28**]. The structure of **28** was determined by comparing its tlc and ^1H-nmr spectrum with those of corynoloxine [**18**] (Table 5). Acetylchelidonine was not detected. This indicated that (+)-chelidonine just as (+)-corynoline are not easily acetylated. It has been demonstrated that dihydrosanguinarine [**32**] is not effective as a precursor of sanguinarine [**29**] in the cultured cells of *M. cordata* (6). Pathways (+)-

TABLE 5. ^{1}H-nmr Data[a] for the Authentic Benzophenanthridines **14**, **17**, **19**, and **18** and Their Analogues **15**, **16**, **20**, and **28**, Obtained from Feeding Experiments.

Proton	Compounds							
	14	**15**	**16**	**17**	**19**	**20**	**18**	**28**
Ac					1.87 3H s	1.79 3H s		
C-Me	1.13 3H s	1.14 3H s	1.10 3H s	1.10 3H s	1.27 3H s	1.26 3H s		
N-Me	2.22 3H s	2.24 0.52H s 2.48H d (135)	2.17 0.48H s 2.52H d (134)	2.17 3H s	2.49 3H s	2.40 0.64H s 2.36H d (134)	2.15 3H s	2.16 3H s
N-^{13}Me								
H-12	3.07 1H dd (17.7, 4.5) 3.16 1H d (17.7)	3.11 1H dd (18.0, 4.5) 3.16 1H d (18.0)	2.62 1H dd (18.0, 10.0) 3.20 1H dd (18.0, 7.0)	2.62 1H dd (17.5, 9.5) 3.18 1H dd (17.5, 7.0)	2.91 1H dd (16.0, 7.5) 2.96 1H dd (16.0, 8.0)	2.87 1H dd (16.0, 6.5) 2.96 1H dd (16.0, 8.0)	2.98 1H dd (18.0, 3.5) 3.12 1H dd (18.0, 1.1)	3.0 1H dd (18.0, 4.0) 3.18 1H dd (18.0, 6.0)
H-11	3.96 1H m	3.98 1H m	4.77 1H t like	4.55 1H dd (9.5, 7.0)	5.25 1H dd (8.0, 7.5)	5.22 1H t like	3.65 1H m	3.94 1H m
H-14	3.32 1H d (1.7)	328 1H brs	3.14 1H brs	3.18 1H s	3.56 1H s	3.42 1H brs	2.86 1H d (1.7)	3.10 1H d (1.0)
H-6	3.51 2H, ABq (16.0)	3.60 2H, ABq (15.5)	3.89 2H, ABq (17.0)	3.73 2H, ABq (16.0)	3.78 2H, ABq (16.0)	3.74 2H, ABq (16.5)	5.32 1H s	5.34 1H s
OMe		3.86, 3.88 each 3H s	3.86, 3.88 each 3H s			3.81, 3.85 each 3H s		
OCH_2O	5.94 4H m	5.96 2H, ABq (1.5)	5.95 2H, ABq (1.4)	5.94 4H m	5.94 4H m	5.93 2H s	5.97 6.03 each 2H, ABq (1.4)	5.95 6.10 each 2H, ABq (1.3)
Ar-H	6.69, 6.71 each 1H s 6.83, 6.86 each 1H d (8.5)	6.65, 6.66 each 1H s 6.91, 7.20 each 1H d (8.5)	6.64, 6.66 each 1H s 6.86, 7.14 each 1H d (8.6)	6.63, 6.64 each 1H s 6.74, 6.88 each 1H d (8.5)	6.57, 6.92 each 1H s 6.76, 6.98 each 1H d (8.5)	6.54, 6.86 each 1H s 6.78, 7.15 each 1H d (8.5)	6.64, 6.72 each 1H s 6.86 2H s	6.66, 6.71 each 1H s 6.81 2H s

[a]**14**, **15**, and **20**, 500 MHz, in $CDCl_3$; **16**, **17**, **18**, **19**, and **28**, 200 MHz, in $CDCl_3$.

TABLE 6. Comparison of Mass Spectra (*m/z*) of Corynoline [**14**], 11-Epicorynoline [**17**], and Acetylcorynoline [**19**] with Those of their Analogues **15**, **16**, and **20**.

Compound					
14	**15**[a]	**17**	**16**[a]	**19**	**20**[a]
$[M]^+$ 367 (78.0)	$[M]^+$ 384 (62.8)	$[M]^+$ 367 (100)	$[M]^+$ 384 (100)	$[M]^+$ 409 (2.8)	$[M]^+$ 426 (4.6)
349 (100)	366 (87.8)	336 (36.5)	352 (57.1)	408 (4.8)	425 (7.5)
334 (70.4)	351 (62.4)	321 (38.4)	337 (36.3)	349 (100)	366 (100)
318 (56.0)	334 (37.6)	307 (15.5)	323 (36.4)	334 (51.0)	351 (43.5)
308 (26.7)	324 (27.1)	202 (24.7)	219 (33.6)	318 (42.9)	334 (36.0)
202 (60.2)	219 (100)	190 (22.7)	207 (22.6)	202 (32.3)	219 (54.0)
190 (54.8)	207 (45.6)	176 (34.2)	192 (25.2)	190 (22.0)	207 (14.4)
176 (42.4)	192 (27.0)				
162 (51.2)	178 (29.3)				

[a] (N-^{13}Me)-labeled product.

chelidonine [**27**]→sanguinarine [**29**]→dihydrosanguinarine [**32**] and (+)-chelidonine [**27**]→dehydrochelidonine [**28**] were defined in the cultured cells of *Co. incisa*.

FEEDING EXPERIMENTS IN WHOLE PLANTS OF *CORYDALIS INCISA*.—Four final studies were carried out to compare the biotransformation of protoberberine alkaloids in whole plants with that in cultured cells. Feeding experiments of **2**, **3**, **5**, and **7** were undertaken using *Co. incisa* plants (Table 7, experiments 12–17). The (−)-enantiomers of labeled precursors **2**, **3**, and **7** were incorporated into the corresponding 13-methylprotopines (−)-**10**, (−)-**11**, and (+)-**10**, respectively, which are partial racemates. 13-Methylprotopine-type alkaloid was not detected in the feeding experiment of **5**. In plant as well as in cultured cells, the α-*N*-metho salts of the protoberberines with a methylenedioxy bridge on the A ring are more preferred as substrates than alkaloids having methoxyl groups on ring A. The biochemical processes in the conversion of the protoberberines into the benzophenanthridines occur similarly in intact plants and in callus tissues.

The values for optical purity vary among the 13-methylprotopines **10** and **11** obtained. This indicates racemization of corycavine-type alkaloids in plants. The optical purities of corycavine [**9**] isolated from feeding experiments (experiments 12–15) and parallel controls (experiments 16 and 17) also vary greatly. In contrast, the optical purities of other metabolites such as corynoline [**14**] and corynoloxine [**18**] obtained from *Co. incisa* plants do not vary largely.

Benzophenanthridine alkaloids are biosynthesized from protoberberine alkaloids via oxidation at C-14 and then again at C-6. This is followed by C-6–N bond fission and the elimination of H_2O leading to an enamino aldehyde and then intramolecular condensation between the positions corresponding to C-6 and C-13 of the protoberberine skeleton. Some detailed routes to the benzophenanthridines from the protoberberines, based on the present results and earlier works (1–6), are summarized as follows (Schemes 2–4). The (−)-(13*S*,14*S*)-,(−)-(13*R*,14*S*)- and (−)-(14*S*)-protoberberines are *N*-methylated to afford the α-*N*-metho derivatives (e.g., **1**, **6**, **23**). The *S* hydrogen at C-14 of the α-*N*-metho salts is replaced by a hydroxyl group. Battersby *et al.* (7) have suggested that complete loss of tritium from C-14 of scoulerine as well as chelidonine, during the transformation from scoulerine into chelidonine, is in keeping with the suggested dihydroisoquinoline intermediate **34** (Scheme 4). The (−)-(13*S*,14*R*)-, (+)-(13*R*,14*R*), and (+)-(14*R*)-protopines [e.g., (−)-**9**, (+)-**9**, and (+)-**25**] result from the α-*N*-metho salts of (−)-(13*S*,14*S*), (−)-(13*R*,14*S*), and (−)-(14*S*) protoberberines, respectively. (−)-(13*S*,14*R*)- and (+)-(13*R*,14*R*)-protopines are in equilibrium through the 10-membered ring compound with (−)-(13*S*,14*S*)- and (+)-(13*R*,14*S*)-protopines, respectively. Since the stereospecific loss of the pro-*S* hydrogen atom at C-6 of the protoberberine skeleton has been determined (11), the same hydrogen of the (+)-(13*R*,14*R*)- and (+)-

TABLE 7. Feeding Experiments of the α-*N*-Metho Salts of *trans*- and *cis*- 13-Methylprotoberberines in Intact Plants of *Corydalis incisa*.

Experiment	Substrate[a] (mg)	Period of cultivation (weeks)	Wt of dry plants (g)	Product, mg (^{13}C enrichment, %)[b]				
				Corycavine analogue	Corycavine [9]	Corynoline [14]	Corynoloxine [18]	Recovered material
12	**2** 90	1	2.68	**10** 7.0[c] (88) [−167][d,e]	9 [+114]	20 [+9.9]	33 [+100]	57[c] [+8.0]
13	**3** 90	1	3.97	**11** 6.2[c] (86) [−33]	12 [+33]	31 [+4.2]	19 [+75]	71[c] [+12]
14	**5** 90	1	2.49		6 [+91]	16 [+5.2]	28 [+116]	71[c]
15	**7** 97	1	3.85	**10** 7.7[c] (80) [+110]	10 [+37]	32 [+14]	27 [+99]	73[c] [+14]
16[f]		1	2.85		9 [+99]	15 [+2.6]	38 [+111]	
17[f]		1	2.87		5 [+19]	trace	31 [+95]	

[a]Compounds, **2**, **3**, **5**, and **7** are racemates.
[b]This value was determined by ^{1}H nmr spectra.
[c]These values are %.
[d]There is an error resulting from c=0.03.
[e]Values in brackets are [α]D.
[f]Parallel control experiment.

(14*R*)- protopines could be replaced by a hydroxyl group. This would lead, respectively, to 6,14-dihydroxy intermediates C and D (Schemes 3 and 4). The (−)-(13*S*,14*R*)-protopines may be converted via a 10-membered ring (5) into (−)-(13*S*,14*S*)-protopines, of which the pro-*R* hydrogen at C-6 would be substituted by a hydroxyl group to afford a 6,14-dihydroxy intermediate (Scheme 2, A).

The low conversion yields of (−)-(13*S*,14*R*)-protopines into benzophenanthridines might result from their required epimerization to (−)-(13*S*,14*S*)-isomers before transformation into benzophenanthridines. An enamino aldehyde intermediate (Scheme 3, B) could then be generated by the elimination of H_2O from the 6,14-dihydroxyprotoberberine (Schemes 2–4, A, C, or D). The aldehyde B (Scheme 3) evidently yields much more (+)-corynoline than (−)-isomer and 11-epicorynoline analogue [**16**] in *Co. incisa.* (+)-Corynoline is oxidized to give (+)-corynoloxine, and (−)-corynoline is acetylated to afford (−)-acetylcorynoline.

Corynoline [**14**] was obtained from **21** but not from compound **22** (5). An intermediate (C) obtained from compound **21** may eliminate H_2O to give an aldehyde (B), which is converted to corynoline [**14**]. It seems reasonable to think that compound **22** could be hydroxylated at C-14, since (−)-mesotetrahydrocorysamine, which has the same configuration at C-13 and C-14 as **22**, is hydroxylated at C-14 to give (−)-corycavine [**9**]. However, it may not be possible to eliminate H_2O from the resulting 6,14-dihydroxy intermediate to afford an aldehyde (B). Similarly, an enamino aldehyde intermediate (Scheme 4, E) could be converted to (+)-chelidonine [**27**] because of high order of stereoselectivity of the enzymes in *Ch. majus.* (+)-Chelidonine is converted into dehydrochelidonine [**28**] and sanguinarine [**29**], which is further bioconverted into dihydrosanguinarine [**32**] and chelirubine [**30**]. Chelirubine [**30**] is then metabolized to macarpine [**31**]. Further studies are necessary to elucidate the process of the elimination of H_2O during formation of the enamino aldehyde intermediate.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were obtained on a Varian XL-200 200 MHz and VXR-500S 500 MHz spectrometers in $CDCl_3$ solvent, except where noted. Ir spectra were recorded on an EPI-G2 (Hitachi) spectrophotometer. Mass spectra were determined on a Hitachi M80 instrument at 75 eV. The cims were obtained by using isobutane as the ionizing gas. Optical rotations were measured using a DIP-SL (Jasco) polarimeter. Microanalyses were performed by the Microanalytical laboratory in Kobe Women's College of Pharmacy. Preparative tlc was performed on Si gel 60F-254 glass plates.

PREPARATION OF THE α-*N*-METHO SALTS OF PROTOBERBERINES.—*α-(N-$^{13}CH_3$)-(±)-Mesotetrahydrocorysamine methochloride* [**1**].—This compound was prepared according to the method of Takao *et al.* (1).

α-(N-$^{13}CH_3$)-(±)-Mesothalictricavine methochloride [**2**].—This compound was prepared according to the method of Iwasa *et al.* (12).

α-(N-$^{13}CH_3$)-(±)-trans-*10,11-Dimethoxy-13-methyl-2,3-methylenedioxy-7,8,13,14-tetrahydroprotoberberine methochloride* [**3**].—$^{13}CH_3I$ (1 g) in Me_2CO (5 ml) was added to a solution of (±)-*trans*-10,11-dimethoxy-13-methyl-2,3-methylenedioxy-7,8,13,14-tetrahydroprotoberberine (13) (400 mg) in Me_2CO (10 ml). After the mixture was allowed to stand at room temperature overnight, the solution was evaporated and Me_2CO was added. The resulting crystals were filtered to give the α-*N*-methyl iodide (500 mg), mp 261–262° (dec), which was treated with AgCl in MeOH to convert it to the α-*N*-methyl chloride **3** (364 mg), mp 237–239° (dec). For ^{1}H and ^{13}C nmr see Tables 1 and 2. *Anal.* calcd for $C_{21}{}^{13}C_1H_{26}NO_4Cl\cdot 1.5\ H_2O$: C 61.40, H 6.77, N, 3.24. Found C 61.24, H 6.83, N 3.17.

α-(N-$^{13}CH_3$)-(±)-Thalictrifoline methochloride [**4**].—$^{13}CH_3I$ (1 g) in Me_2CO (10 ml) was added to a solution of (±)-thalictrifoline (14) (385 mg) in Me_2CO (50 ml). After standing at room temperature overnight, the resulting crystals were filtered to give the α-*N*-methyl iodide (490 mg), mp 259–260° (dec), which was converted to the α-*N*-methyl chloride [**4**] (402 mg): mp 241–243° (dec); ^{1}H and ^{13}C nmr see Tables 1 and 2. *Anal* calcd for $C_{21}{}^{13}C_1H_{26}NO_4\cdot 2H_2O$: C 60.15, H 6.86, N 3.17. Found C 60.70, H 6.81, N 3.29.

α-*(N-$^{13}CH_3$)-(±)-Mesocorydaline methochloride* [**5**].—(±)-Mesocorydaline was prepared according to the method applied to synthesis of (±)-mesotetrahydrocorysamine (1). $^{13}CH_3I$ (1g) in Me_2CO (10 ml) was added to a solution of (±)-mesocorydaline (730 mg) in a mixture of $CHCl_3$ (3 ml) and Me_2CO (30 ml). After standing at room temperature overnight, the solution was evaporated and Me_2CO was added. The resulting crystals were filtered to yield the α-*N*-methyl iodide (1.04 g), mp 206–218° (dec), which was converted to the α-*N*-methyl chloride [**5**] (787 mg): mp 246–250° (dec); ^{1}H and ^{13}C nmr see Tables 1 and 2. *Anal.* calcd for $C_{22}{}^{13}C_1H_{30}NO_4Cl\cdot0.5\ H_2O$: C 64.48; H 7.27, N 3.26. Found C 64.29, H 7.29, N 3.17.

α-N-*(N-$^{13}CH_3$)-(±)-Tetrahydrocorysamine methochloride* [**6**].—This compound was prepared according to the method of Takao *et al.* (1).

α-N-*(N-$^{13}CH_3$)-(±)-Thalictricavine methochloride* [**7**].—This compound was prepared according to Iwasa *et al.* (12).

α-N-*(N-$^{13}CH_3$)-(±)-Corydaline methochloride* [**8**].—(±)-Corydaline was prepared according to the procedure applied to synthesis of (±)-tetrahydrocorysamine (1). A mixture of (±)-corydaline (510 mg) and $^{13}CH_3I$ (1 g) in Me_2CO (15 ml) was allowed to stand at room temperature for 4 days. The resulting crystals were filtered to produce a mixture of the α- and β-*N*-methyl iodides (680 mg), which were converted to a mixture of the α- and β-*N*-methyl chlorides. The crystals were recrystallized from Me_2CO/MeOH to afford the α-*N*-methyl chloride [**8**] (122 mg): mp 255–261° (dec); ^{1}H nmr see Table 1. *Anal.* calcd for $C_{22}{}^{13}C_1H_{30}NO_4Cl\cdot0.5\ H_2O$: C 64.48, H 7.27, N 3.26. Found C 64.69 , H 7.24, N 3.10.

FEEDING EXPERIMENTS WITH CULTURED CELLS.—Each callus was subcultured on Murachige and Skoog's (M-S) agar medium fortified with 2,4-dichlorophenoxyacetic acid (1 mg/liter), kinetin (0.1 mg/liter), and yeast extract (0.1%). Labeled compounds were dissolved in H_2O and introduced through a sterile bacterial filter into 100-ml Erlenmeyer flasks containing ca. 40 ml of the autoclaved M-S medium. The callus (3–5 g) was transferred to the medium containing each substrate and incubated at 25° in the dark for the appropriate time (Tables 3 and 4).

In *Co. ochotensis* var. *raddeana,* compounds **3–5** (each 100 mg) were fed and incubated for 4 weeks. Products **10** and **11** were obtained in yields of 0.5% from **2** and **3**, respectively. In *Co. ophiocarpa* compound **2** (200 mg) and compounds **3–5** (each 100 mg) were fed and incubated for 5 weeks. Products **10** and **11** were obtained in yields of 0.9 and 0.5% from **2** and **3**, respectively. Extraction was carried out as reported in a previous paper (2).

The tertiary-alkaloid fraction soluble in Et_2O and $CHCl_3$ was subjected to preparative tlc. The ^{13}C-labeled products (**14**, **15**, **16**, **18**, **19**, and **20**) and compounds **32** and **33** were purified by preparative tlc with C_6H_6-Et_2O (4:1). The ^{13}C-labeled products **9–13** and compound **28** were obtained by a further preparative tlc (MeOH) of an extract of the low R_f band in a preparative tlc done with C_6H_6-Et_2O (4:1).

LABELED 13-METHYLPROTOPINES **9–13**.—*(−)-Corycavine (−)*-[**9**].—^{1}H nmr δ (200 MHz) 1.82 (2.82 H, d, J=135 Hz, N-$^{13}CH_3$), 1.82 (0.18 H, s, N-Me).

(+)-Corycavin (+)-[**9**].—^{1}H nmr δ (200 MHz) 1.81 (2.31 H, d, J=135 Hz, N-$^{13}CH_3$), 1.81 (0.69 H, s, N-Me).

(−)-13-Methylallocryptopine (−)-[**10**].—^{1}H nmr δ (200 MHz) 1.77 (2.70 H, d, J=135 Hz, N-$^{13}CH_3$), 1.77 (0.30 H, s, N-Me). The product **10** obtained from experiment 6 or 10 was identified by the comparison of ^{1}H nmr and mass spectra: cims *m/z* [MH]$^+$ 385; hrms *m/z* found [M]$^+$ 384.1762 ($C_{21}{}^{13}C_1H_{25}NO_5$ requires 384.1764), 178.1000 ($C_{11}H_{14}O_2$ requires 178.1007).

(+)-13-Methylallocryptopine (+)-[**10**].—^{1}H nmr δ (200 MHz) 1.79 (2.46 H, d, J=135 Hz, N-$^{13}CH_3$), 1.79 (0.54 H, s, N-Me).

(−)-10,11-Dimethoxy-13-methyl-2,3-methylenedioxyprotopine (−)-[**11**].—Ir ν max ($CHCl_3$) cm^{-1} 1650 (C=O): ^{1}H and ^{13}C nmr see Tables 1 and 2; hrms *m/z* [M]$^+$ 384.1758 ($C_{21}{}^{13}C_1H_{25}NO_5$ requires 384.1764), 178.0999 ($C_{11}H_{14}O_2$ requires 178.1007); eims *m/z* (% rel. int.) [M]$^+$ 384 (3.2), 383 (0.5), 297 (5.7), 221 (3.5), 178 (100), 163 (6.1). The product **11** obtained from experiment 7 or 11 was identified by ^{1}H-nmr and mass spectra.

(−)-2,3-Dimethoxy-13-methyl-9,10-methylenedioxyprotopine (−)-[**12**].—Ir ν max ($CHCl_3$) cm^{-1}, 1650 (C=O); ^{1}H nmr see Table 1; hrms *m/z* [M]$^+$ 384.1794 ($C_{21}{}^{13}C_1H_{25}NO_5$ requires 384.1764), 162.0677 ($C_{10}H_{10}O_2$ requires 162.0679); eims *m/z* (% rel. int.) [M]$^+$ 384 (5.3), 383 (0.4), 297 (2.9), 223 (4.3), 205 (4.6), 179 (11.8), 162 (100).

(−)-13-Methylmuramine (−)-[**13**].—Hrms *m/z* [M]$^+$ 400.2067 ($C_{22}{}^{13}C_1H_{29}NO_5$ requires 400.2076), 178.0982 ($C_{11}H_{14}O_2$ requires 178.0993); cims *m/z* [MH]$^+$ 401; eims *m/z* (% rel. int.) [M]$^+$ 400 (4.1), 221 (7.6), 178 (100), 163 (13.6).

(+)-13-Methylmuramine (+)-[**13**].—Hrms *m/z* [M]$^+$ 400.2082 ($C_{22}{}^{13}C_1H_{29}NO_5$ requires 400.2076) 178.0987 ($C_{11}H_{14}O_2$ requires 178.0993); cims *m/z* [MH]$^+$ 401; eims *m/z* (% rel. int.) [M]$^+$ 400 (5.7), 221 (6.8), 178 (100), 163 (7.8).

LABELED BENZOPHENANTHRIDINES **14–20** AND UNLABELED PRODUCTS **28**, **32**, AND **3**.—*Corynoline* [**14**].—^{1}H nmr δ 2.24 (2.61 H, d, J=135 Hz, N-$^{13}CH_3$), 2.24 (0.39 H, s, N-Me).

Corynoline analogue [**15**].—^{1}H nmr and mass spectra see Tables 5 and 6; hrms *m/z* [M]$^+$, 384.1762 ($C_{21}{}^{13}C_1H_{25}NO_5$ requires 384.1764), 366.1633 ($C_{21}{}^{13}C_1H_{23}NO_4$ requires 366.1658), 219.1186 ($C_{12}{}^{13}C_1H_{16}NO_2$ requires 219.1213).

11-Epicorynoline analogue [**16**].—^{1}H nmr and mass spectra see Tables 5 and 6.

Corynoloxine [**18**].—^{1}H nmr δ 2.15 (2.43 H, d, J=135 Hz, N-$^{13}CH_3$), 2.15 (0.57 H, s, N-Me).

Acetylcorynoline [**19**].—^{1}H nmr δ 2.48 (2.37 H, d, J=134 Hz, N-$^{13}CH_3$), 2.48 (0.63 H, s, N-Me).

Acetylcorynoline analogue [**20**].—^{1}H nmr and ms see Tables 5 and 6. The products **14**, **18**, and **19** from experiments 6 and 9 were identified by comparison with authentic samples.

Dehydrochelidonine [**28**].—^{1}H nmr see Table 5. Compounds **32** and **33** from experiments 11 and 10, respectively, were identified by comparison with authentic samples.

FEEDING EXPERIMENTS WITH INTACT PLANTS.—Each ^{13}C-labeled substrate was fed as an aqueous solution into *Co. incisa*, and post-treatment was undertaken according to the procedure described in previous papers (15,16). The tertiary-alkaloid fractions soluble in Et_2O and $CHCl_3$ were subjected to preparative tlc with C_6H_6-Et_2O (1:1) to separate the compounds **9**, **14**, and **18**. The labeled 13-methylprotopine-type alkaloids, **10** and **11**, were purified by a further preparative tlc (MeOH) of an extract of the low R_f band in a preparative tlc done with C_6H_6/Et_2O. The identification of **9**, **14**, or **18** was carried out by comparison with the authentic materials. The products **10** and **11** were identical with those obtained from experiments 2 and 3.

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