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Studies on the Alkaloids of Papaveraceous Plants. XXV.[1] Biosynthesis of the Alkaloids of *Corydalis incisa* Pers. and *Chelidonium majus* L. Incorporations of Tetrahydroprotoberberines, N-Methosalts of Tetrahydroprotoberberines, and Protopine

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Since *dl*-cheilanthifoline (**2**) was converted in *Corydalis incisa* plants into corynoline (**3**) and protopine (**4**), it was shown that **2** was the intermediate between scoulerine (**15**) and stylopine (**10**).

Corycavine (**9**) was shown to be biosynthesized in *Corydalis incisa* plants from *dl*-mesotetrahydrocorysamine (**7**) and *dl*-tetrahydrocorysamine (**8**) *via* their α-N-methosalts (**7α** and **8α**). Protopine (**4**) is shown to be formed *in vivo* from the α-N-methosalt of *dl*- or *l*-stylopine (**10α**). Incorporation of the β-N-methosalts (**10β** and **8β**) into **4** and **9** might be absent or negligible. These results imply that α-N-methosalts of tetrahydroprotoberberines are stereospecifically converted into protopine-type alkaloids.

Sanguinarine (**13**) and chelidonine (**14**) were shown to be biosynthesized in *Chelidonium majus* plants from α-N-methosalt of *dl*- or *l*-stylopine (**10α**) *via* protopine (**4**). This shows that protopine-type alkaloid is the intermediate between tetrahydroprotoberberine-type and benzo[*c*]phenanthridine-type alkaloids.

The biosynthetic conversions of the tetrahydroprotoberberine skeleton into the benzo[*c*]-phenanthridine and protopine skeletons have been proved by the tracer experiments.[3,4] The biosynthetic pathway has been presented as illustrated in Chart 1.

The present investigation was undertaken in order to study intermediates on the pathway and the biosynthetic relationship between protopine-type alkaloid and benzo[*c*]phenanthridine-type alkaloid. *Chelidonium majus* plants used in the present experiments involve tetrahydroprotoberberine-type, protopine-type, and benzo[*c*]phenanthridine-type alkaloids. *Corydalis incisa* plants mainly contain the same type alkaloids having a C-methyl group at the position corresponding to C-13 of tetrahydroprotoberberine-type alkaloid.

Experiment with (8,13a-T_2)-*dl*-Cheilanthifoline (2a)

As *l*-cheilanthifoline had been isolated[5] from nutrient stage *Corydalis incisa* plants, the position of this alkaloid on the biosynthetic pathway was studied.

Labelled *dl*-cheilanthifoline (**2a**) was prepared by reduction of dehydrocheilanthifoline chloride (**1**) with sodium borotritiide in dimethyl sulfoxide as shown in Chart 2. The labelled sites of **2a** were estimated on the basis of the proton magnetic resonance (PMR) and mass spectra of (8,13a-D_2)-*dl*-cheilanthifoline (**2b**), prepared by reduction of **1** with sodium boro-

1) Part XXIV; T. Tagahara, S. Ko, and M. Kanaguchi, *Shoyakugaku Zasshi*, accepted.
2) Location; *Motoyama-Kitamachi, Higashinada-ku, Kobe.*
3) *a*) A.R. Battersby, R.J. Francis, E.A. Ruveda, and J. Staunton, *J. Chem. Soc. Chem. Commun.*, **1965**, 89; *b*) A.R. Battersby, R.J. Francis, M. Hirst, R. Southgate, and J. Staunton, *ibid.*, **1967**, 602; *c*) A.R. Battersby, P.W. Sheldrake, and J.A. Milner, *Tetrahedron Letters*, **1974**, 3315; *d*) D.H.R. Barton, R.H. Hesse, and G.W. Kirby, *J. Chem. Soc.* (*C*), **1965**, 6379; *e*) C. Tani and K. Tagahara, *Chem. Pharm. Bull.* (Tokyo), **22**, 2457 (1974); *f*) A.R. Battersby, J. Staunton, H.R. Wiltshire, R.J. Francis, and R. Southgate, *J. Chem. Soc.* (*C*), **1975**, 1147.
4) G. Nonaka, Dissertation, Kyushu University, 1974.
5) G. Nonaka and I. Nishioka, *Phytochemistry*, **13**, 2620 (1974).

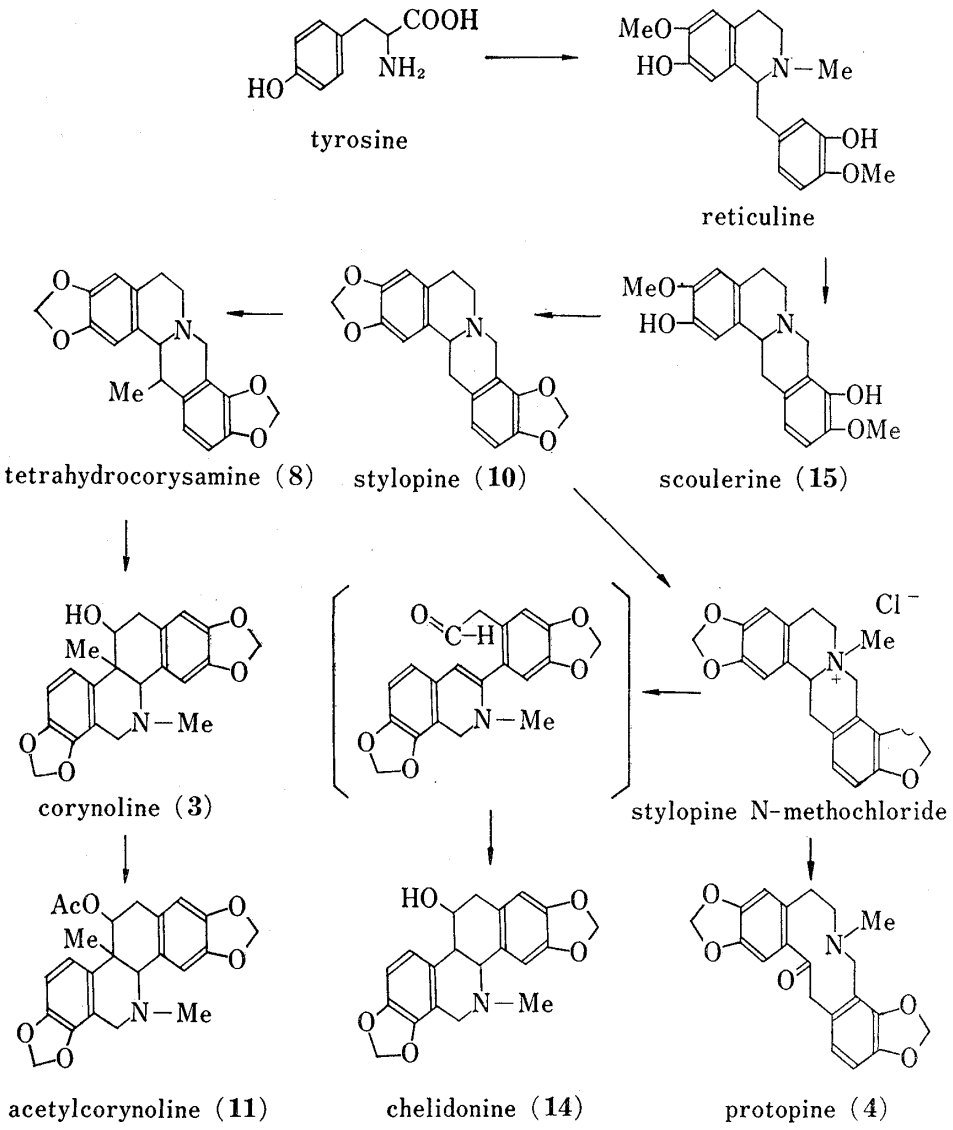

Chart 1

deuteride in a similar method to the above. The C-8 protons in *dl*-cheilanthifoline (2) appeared at δ 3.56 (1H) and δ 4.07 (1H), while those in **2b** at δ 3.52 (0.45H) and δ 4.05 (0.45H). The mass spectrum of **2** showed a molecular ion at *m/e* 325 and fragment ions at *m/e* 176 and 148, while that of **2b** the ions at *m/e* 327 and at *m/e* 177 and 149, respectively. This indicates that the protons on C-8 and C-13a were substituted with deuteriums. Similarly **2a** should be labelled at C-8 and C-13a.

The hydrochloride of **2a** (38.01 mCi/mM) was fed into *Corydalis incisa* plants (Table I-A, experiment 1). The radioactive protopine (**3a**) and corynoline (**4a**) were isolated by the usual way. The obserbed incorporations into protopine (3) and corynoline (4) were 0.043% and 0.200%, respectively.

Oxidation of **3a** with potassium permanganate yielded a mixture of radioinactive 6-oxocorynoline (**5**) and radioactive corynoloxine (**6a**) as shown in Chart 2. After reduction of **6a** with sodium borohydride to **3**, the activity of **3** retained still. These results show that the label of **3** should reside at C-6 and not at C-14. This tritium loss from C-14 which occurred during the biosynthetic conversion of **2** into **3** is in keeping with the intermediate suggested by Battersby, *et al.*[3a] as shown in Chart 1. Analogously, protopine (**4a**) from experiment 1 should be labelled at C-8.

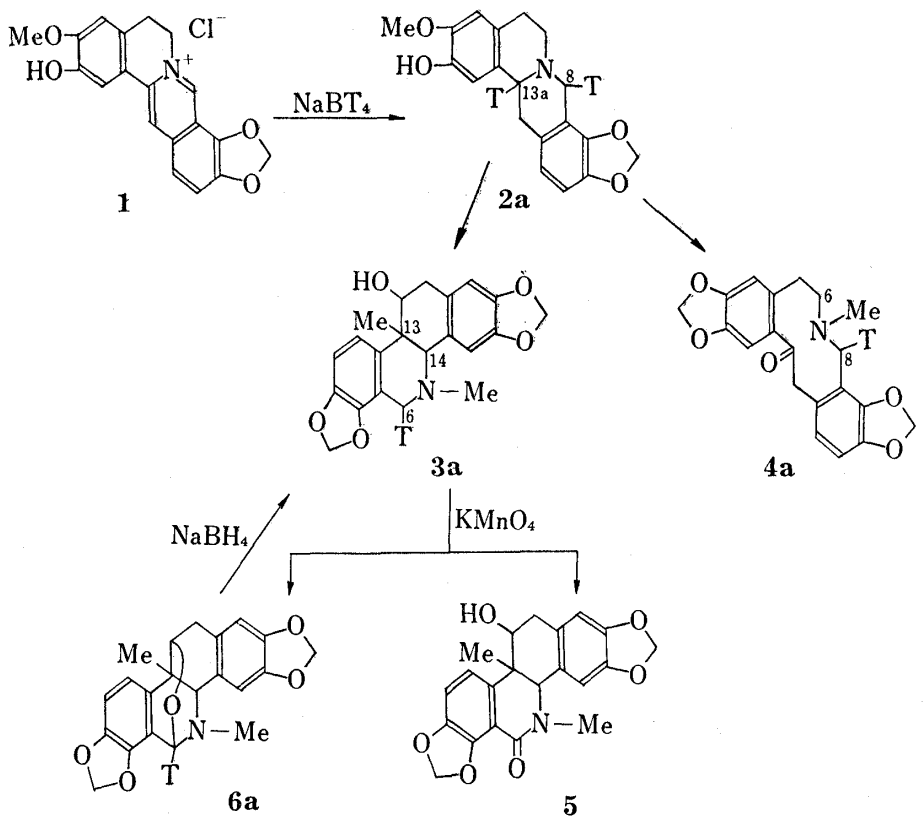

Chart 2

TABLE I. Feeding Experiments with *Corydalis incisa* Plants (X) and *Chelidonium majus* Plants (Y)

Experiment No.		Substrate, No. (α-form:β-form)	Feeding[a] weight (mg)	Numbers of stems	Dried weight of plants (g)	Green weight (g)
A	1	**2a**	71	X —	23.3	
	2-1	**7a**	80	X 61	34	
	2-2	**8a**	80	X 76	36	
	3-1	**10αa** (4.3:1)	150	X 63	75	
	3-2	**10βa** (1:30)	210	X 92	116	
	4	**7αa** (only α)	56	X 34	55	
	5-1	**8αa** (1:3.8)	60	X 53	9.5	
	5-2	**8βa** (only β)	70	X 53	9.5	
	6-1	**10αa** (3:1)	40	Y 19	37.5	
	6-2	**10βa** (only β)	23	Y 9	34.5	
B	7-1	**10αa** (3:1)	30			Y 40
	7-2	**10βa** (only β)	30			Y 40
	8	**4a**	28			Y 30

a) concentration 5—10 mM

Thus, the incorporation of *dl*-cheilanthifoline (**2**) into corynoline (**3**) and protopine (**4**) was established (Chart 3).

Experiments with [C(13)-$^{13}CH_3$, (8,13,13a-D_3)]-*dl*-Mesotetrahydrocorysamine and -Tetrahydrocorysamine (7a and 8a)

l-Tetrahydrocorysamine,[6] which possesses a C-methyl group at C-13, occurs in *Corydalis incisa* plants together with corynoline, *d*-14-epicorynoline,[7] *etc.* (benzo[*c*]phenanthridine-type

6) G. Nonaka and H. Okabe, I. Nishioka, and N. Takao, *Yakugaku Zasshi*, **93**, 87 (1973).
7) N. Takao, H.W. Bersch, and S. Takao, *Chem. Pharm. Bull.* (Tokyo), **21**, 1096 (1973).

alkaloids), and corycavine (protopine-type alkaloid), which have a C-methyl group similarly. The incorporations of *dl*-tetrahydrocorysamine and the diastereomer, *dl*-mesotetrahydrocorysamine, into benzo[*c*]phenanthridine-type and protopine-type alkaloids were examined.

Labelled compounds (**7a** and **8a**) were prepared from acetonecoptisine[8] by $^{13}CH_3I$ (89% enrich) treatment followed by reduction with zinc dust and deuterio chloride. The hydrochlorides of **7a** and **8a** were administered to *Corydalis incisa* plants (Table I-A, experiment 2-1 and 2-2). The isolated alkaloids were used for PMR and carbon magnetic resonance (CMR) spectral studies. By CMR spectra, it was found that the C-methyl group of each corycavine (**9a**) from experiments 2-1 and 2-2 was enriched with ^{13}C (Table II-B). The approximate enrichment of each **9a** was determined by PMR spectroscopy (Table III).

Table II. Enhancement of the Signal Intensities of Labelled Alkaloids determined by CMR Spectra

Experiment, No.	Product	Peak height of carbon atom			
		C-8	C-6	N-CH_3	C-CH_3
A	**4**	1	1.01	0.84	
3-1	**4a**	1	0.87	88.0	
3-2	**4a**	1	1.05	5.10	
6-1	**4a**	1	1.06	10.18	
B	**9**	1	0.81	0.76	0.73
2-1	**9a**	—[a]	1	—[a]	3.58
2-2	**9a**	1	—[a]	—[a]	5.12
4	**9a**	1	0.72	41.06	—[a]
5-1	**9a**	1	0.75	—[a]	68.67
C	**12**		1	0.54	
6-1	**12a**		1	1.58	

a) Uncertainty of signal prevented estimation of these values.

Table III. PMR Data for Labelled Alkaloids and Approximate Enrichment

Experiment No.	Product	Chemical shift (ppm)		$J_{^{13}C-H}$ (Hz)	Approximate[a] Enrichment (%)
		N-CH_3	C-CH_3		
2-1	**9a**	—[b]	1.34	2×64.0[c]	5.0
2-2	**9a**	—[b]	—[d]	—[d]	9.0
3-1	**4a**	1.93	—[b]	134.5[e]	55.5
3-2	**4a**	—[d]	—[b]	—[d]	8.0
4	**9a**	1.82	—[b]	134.5[e]	38.8
5-1	**9a**	—[b]	1.34	2×64.0[c]	30
6-1	**4a**	1.93	—[b]	134.5[e]	9.9
	12a	2.60	—[b]	135.0[e]	5.5
7-1	**4a**	1.93	—[b]	67.0[c]	38.0
	12a	2.60	—[b]	2×67.0[c]	31.0
	14a	2.27	—[b]	—[d]	29.0
8	**12a**	2.60	—[b]	—[d]	42.0
	14a	2.27	—[b]	—[d]	38.0

a) ratio of integrals, labelled peak/unlabelled and labelled peaks $\times100$
b) unlabelled signal
c) determined by 90 MHz PMR spectra
d) same value
e) determined by 60 MHz PMR spectra

8) T. Tani, N. Takao, and S. Takao, *Yakugaku Zasshi*, **82**, 748 (1962).

Both *dl*-mesotetrahydrocorysamine (**7**) and *dl*-tetrahydrocorysamine (**8**) were thus proved to be incorporated into corycavine (**9**) (Chart 3). The incorporation of **8** into **3** has been confirmed[4] by tracer experiment with tritium-labelled compound (Chart 1), but the enrichment of **3** from experiments 2-1 and 2-2 could not be detected by PMR and CMR spectroscopies. It is thought that a low incorporation might arise from experimental conditions, *e.g.* the procedure and season of the administration.

The occurrence[9] and biosynthetic incorporation studies[10,3e] of N-quaternary tetrahydroprotoberberines in papaveraceous plants have been reported. Labelled compounds were prepared by N- or C-methylation of tetrahydroprotoberberines with $^{13}CH_3I$ (89% enrich). The α-form and β-form of N-methosalts[11] could be differentiated by the chemical shifts of N-methyl group in PMR and CMR spectra (Table V). In some case, the separation of α-methosalt from β-methosalt was difficult. Labelled compounds were fed as an aqueous solution into *Corydalis incisa* and *Chelidonium majus* plants either by introducing through cotton wicks directly or by cutting stems off. Each sample was given to the plants grown under the same environment in the conditions as same as possible.

Experiments with α- and β-(N-$^{13}CH_3$)-*l*-Stylopine Methochlorides (10αa** and 10β**a**)**

Labelled compounds (**10α**a and **10β**a) were administered to *Corydalis incisa* plants (Table I-A, experiments 3-1 and 3-2). The yields of acetylcorynoline (**11**) from experiments 3-1, 3-2, and 4 were 0.026%, 0.030%, and 0.025%, respectively and those of **3** from experiments 3-1, 3-2, and 4 0.075%, 0.066%, and 0.084%, respectively. While the yields of **4** from experiments 3-1 and 3-2 were 0.055% and 0.010%, respectively. These facts suggest the positive incorporation of the quaternary salts into **4**. CMR spectra showed that the N-methyl group of each protopine (**4a**) in experiments 3-1 and 3-2 was enriched with ^{13}C (Table II-A). The approximate enrichment of each **4a** was determined by PMR spectroscopy (Table III). The data on the mass spectra of **4** and **4a** are listed in Table IV-A. By mass spectral analysis, the approximate enrichments of **4** from experiments 3-1 and 3-2 were determined to be 54.2% and 10.1%, respectively, which agreed with the values determined by PMR spectroscopy. The apparent poor enrichment in experiment 3-2 as shown in Table III may be explained

Table IV. Mass Data of Protopine (**4**), Corycavine (**9**), Labelled Protopine (**4a**) from Experiments 3-1 and 3-2, and Labelled Corycavine (**9a**) from Experiment 4

	Peak (*m/e*)	Intensities[a] (%)				
		4	**4a** from Ex. 3-1	**4a** from Ex. 3-2	**9**	**9a** from Ex. 4
A	354	25.2	100	32.8		
	353(M^+)	100	70.8	100		
	191	15.0	100	22.5		
	190	100	83.8	100		
B	368				26.3	85.7
	367(M^+)				100	100
	205				18.6	77.8
	204				100	100

a) relative to most intense peak (=100) in this group

9) a) K. Haisová and J. Slavík, *Coll. Czech. Chem. Commum.*, **38**, 2307 (1973); b) J. Haisová, V.J. Slavík, and L. Dulejš, *ibid.*, **38**, 3312 (1973); c) V. Haisová, V. Šimárnek, L. Dulejš, O. Gašič, A. Nemečková, and F. Šantavý, *ibid.*, **38**, 3662 (1973).

10) H. Rönsch, *Eur. J. Biochem.*, **28**, 123 (1972).

11) Fujii, *et al.* has assigned B/C-*cis* fused structure to the α-methosalt and B/C-*trans* fused structure to the β-methosalt [*Chem. Pharm. Bull.* (Tokyo), **23**, 144 (1975)].

by the possibility of the incorporation of α-form involved slightly in the quaternary salt fed in the experiment.

Thus, it is shown that the stereospecific incorporation of the α-form of *l*-stylopine methochlorides (**10α**) into protopine (**4**) is most highly probable (Chart 3).

Experiment with α-(N-$^{13}CH_3$)-*dl*-Mesotetrahydrocorysamine Methochloride (7αa)

Labelled substrate (**7αa**) was fed into *Corydalis incisa* plants (Table I-A, experiment 4). As described above, the differences in the yields of **4** and **11** from experiments 3-1, 3-2, and 4 were small. The yields of **9** from experiments 3-1 and 3-2 were 0.010% and 0.009%, respectively, while that of **9** from experiment 4 was 0.022%. These results suggest the positive incorporation of the quaternary salt into **9**. By CMR spectrum, it was found that the N-methyl group of corycavine (**9a**) from experiment 4 was enriched with ^{13}C (Table II-B). The approximate enrichment of **9a** is given in Table III. The data on the mass spectra of **9** and **9a** are collected in Table IV-B. By mass spectral analysis, the approximate enrichment of **9** from experiment 4 was determined to be 39.0%, which was consistent with the value determined by PMR spectroscopy.

Thus, the conversion of the α- form of *dl*-mesotetrahydrocorysamine methochloride (**7α**) into corycavine (**9**) was proved (Chart 3).

Experiments with α- and β-N-Methochlorides of [C(13)-$^{13}CH_3$, (8,13,13a-D_3)]-*dl*-Tetrahydrocorysamine (8αa and 8βa)

Labelled compounds (**8αa** and **8βa**) were administered to *Corydalis incisa* plants (Table I-A, experiments 5-1 and 5-2). CMR spectrum showed that the C-methyl group of corycavine (**9a**) from experiment 5-1 is enriched with ^{13}C (Table II-B). The approximate enrichment of **9a** is listed in Table III. The enrichment of **3** from experiment 5-1 and **3** and **9** from experiment 5-2, in which only the β-form of *dl*-tetrahydrocorysamine methochlorides was administered, could not be detected by CMR and PMR spectroscopies.

The α-form of *dl*-tetrahydrocorysamine methochlorides (**8α**) was thus proved to be stereospecifically incorporated into corycavine (**9**) (Chart 3).

Experiments with α- and β-(N-$^{13}CH_3$)-*dl*-Stylopine Methochlorides (10αa and 10βa)

Labelled substrates (**10αa** and **10βa**) were fed into *Chelidonium majus* plants (Table I-A, experiments 6-1 and 6-2). Sanguinarine (**13**) was reduced with sodium borohydride to dihydrosanguinarine (**12**). By CMR spectra, it was found that the N-methyl groups of **4a** and **12a** from experiment 6-1 were enriched with ^{13}C (Table II-A, C). The approximate enrichments of **4a** and **12a** are collected in Table III. By CMR and PMR spectroscopies, the enrichment could not be detected in **4** and **12** from experiment 6-2, in which only the β-form of *dl*-stylopine methochlorides was made use. In spite of the low enrichment, the stereospecific conversions of the α-form of *dl*-stylopine methochlorides (**10α**) into protopine (**4**) and sanguinarine (**13**) were proved (Chart 3).

Experiments with α- and β-(N-$^{13}CH_3$)-*dl*-Stylopine Methochlorides (8αa and 8βa)

Labelled compounds (**8αa** and **8βa**) were administered to *Chelidonium majus* plants (Table I-B, experiments 7-1 and 7-2). PMR spectra showed that the N-methyl groups of **4a**, **12a**, and chelidonine (**14a**) from experiment 7-1 were enriched with ^{13}C (Table III). By PMR spectroscopy, the enrichment could not be detected in **4**, **12**, and **14** from experiment 7-2 with the β-form of *l*-stylopine methochlorides.

Thus, the stereospecific conversions of the α-form of *l*-stylopine methochlorides (**10α**) into protopine (**4**), sanguinarine (**13**), and further chelidonine (**14**) were established (Chart 3).

The difference in enrichment between experiment 6-1 and experiment 7-1 may be arisen from the far greater efficiency for the biosynthesis of the alkaloids of *l*-form of **10α** than that of *d*- form of **10α**.

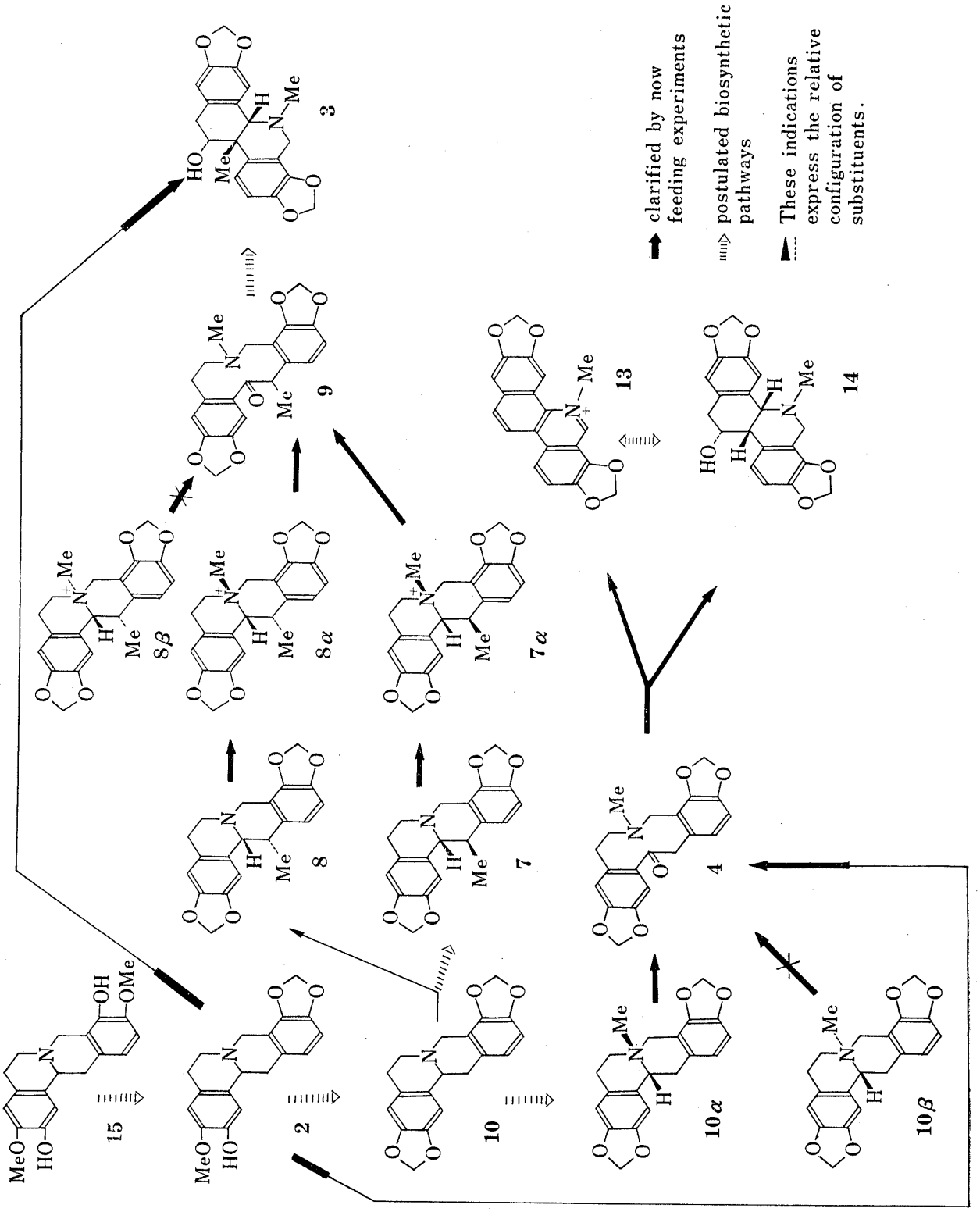

Chart 3

Experiment with (N-$^{13}CH_3$)-Protopine (4a)

It has been suggested[10] that protopine-type alkaloid is a possible intermediate in the biosynthesis of rhoeadine-type alkaloid. Recently this proposal was supported[12] from the observation that tritium-labelled protopine is incorporated into rhoeadine. Labelled protopine

12) K. Tagahara, *et al.* presented this work at Meeting of Kinki Branch Pharmaceutical Society of Japan, Osaka, November 1974.

(**4a**) was administered to *Chelidonium majus* plants (Table I-B, experiment 8). PMR spectra showed that the N-methyl groups of **13a** and **14a** from experiment 8 were enriched with ^{13}C (Table III). Protopine (**4**) was thus proved to be incorporated into sanguinarine (**13**) and chelidonine (**14**) (Chart 3). This conversion could be explained by the contribution of the quaternary form[13] of protopine-type alkaloid.

The following conclusion can be made about experiments 1—8: (a) Since the conversion of *dl*-cheilanthifoline (**2**) into corynoline (**3**) and protopine (**4**) were confirmed in experiment 1, it is probable that *d*- or *l*-cheilanthifoline is the true intermediate between scoulerine (**15**) and stylopine (**10**). There is one possible intermediate, nandinine, between **15** and **10** besides **2**, but it has been reported[3f] by Battersby, *et al.* that nandinine is not effective as a precursor of chelidonine, stylopine or protopine during the preparation of this article. (b) Experiments 2-1, 2-2, 5-1, and 5-2 showed that 13-methyltetrahydroprotoberberine-type alkaloid was converted *via* the α-N-methosalt into protopine-type alkaloid regardless of the configuration of the hydrogens at C-13 and C-13a. Pursuing further this point might be important. (c) Experiments 3—7 showed that the α-N-methosalts of tetrahydroprotoberberines were stereospecifically converted to protopine-type and benzo[*c*]phenanthridine-type alkaloids. (d) Experiment 8 showed that benzo[*c*]phenanthridine-type alkaloids were biosynthesized from protopine-type alkaloids. Battersby, *et al.*[3f] have also proved the conversions of stylopine methochloride into protopine and chelidonine, independently with us employing ^{14}C- and ^{3}H-doubly labelled compounds, but the experiment has been undertaken without consideration of the different contributions of α-form and β-form in quaternary N-methosalts. They have also shown that stylopine methochloride lies on the pathway to both protopine and chelidonine (Chart 1). By the present experiments, it was confirmed that the α-form of stylopine methochlorides is stereospecifically converted *via* protopine (**4**) into sanguinarine (**13**) and chelidonine (**14**). Further studies will be necessary to clarify the interconversion of sanguinarine and chelidonine, the conversion of corycavine into corynoline, *etc.*, and the detailed mechanism of the formation of these alkaloids in the biosynthetic pathway.

Experimental

Radioactivity was assayed Ten Liquid Scintillation Counter Model G_{SL}-260. Samples were dissolved in a solution consisting of dioxane (50 ml), naphthalene (5 g), PPO (200 mg), and POPOP (3 mg).

PMR spectra were determined for solution in $CDCl_3$ and CD_3OD with a Varian A-60D instrument and NV-21 spectrometer at 90 MHz (tetramethylsilane as internal reference). CMR spectra were determined for solution in $CDCl_3$ and CD_3OD with NV-21 spectrometer at 22.6 MHz by using tetramethylsilane as an internal standard. Mass spectra were recorded on a JEOL-OIS spectrometer at an ionizing potential of 75 eV and ionizing current of approximately 200 μA. Samples were introduced through an all-glass inlet system.

Preparation of (8,13a-D_2)-*dl*-Cheilanthifoline (2b)——$NaBD_4$ (5 mg) was added to a solution dehydrocheilanthifoline chloride (**1**) (100 mg) in Me_2SO (2 ml) and the mixture was kept under stirring for 2.5 hr at room temperature. After addition of $NaBD_4$ (5 mg), the mixture was stirred for 2 hr and kept overnight at room temperature. To the reaction mixture H_2O was added, and the solution was extracted with ether. The dried ether solution was evaporated and the crystalline residue was recrystallized from MeOH–$CHCl_3$ to give **2b** (22 mg), mp 150—158°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570 (OH), 2040 (CD). Mass Spectrum *m/e*: 327 (M^+), 177, 149. PMR ($CDCl_3$): 3.84 (3H, s, OCH_3), 5.93 (2H, m, OCH_2O), 6.61 (1H s, Ar–H), 6.65 (2H, s, Ar–H×2), 6.80 (1H, s, Ar–H).

Preparation of (8,13a-T_2)-*dl*-Cheilanthifoline (2a)——$NaBT_4$ (25 mCi, 1 mg) was added to a solution of dehydrocheilanthifoline chloride (154 mg) in Me_2SO (2 ml) and after standing for 40 hr at room temperature, the mixture was stirred for 4 hr. After addition of $NaBH_4$ (160 mg), the mixture was kept for 1 hr at room temperature, then to the mixture H_2O was added and the solution was extracted with ether. The dried ether solution was evaporated and the crystalline residue was recrystallized from MeOH–ether to give **2a** (42 mg). The crystals were dissolved in the minimum of methanolic hydrogen chloride and the deposited hydrochloride was collected and recrystallized from MeOH–acetone. 40 mg, 38.01 mCi/mM.

13) F.R. Stermitz, R.M. Coomes, and D.R. Harris, *Tetrahedron Letters*, **1968**, 3915.

Preparation of [C(13)-$^{13}CH_3$, (8,13,13a-D_3)]-*dl*-Mesotetrahydrocorysamine and -Tetrahydrocorysamine (7a and 8a)——Acetonecoptisine (680 mg) and $^{13}CH_3I$ (900 mg) in a sealed tube were heated at 100° for 7 hr. The separated crystals were dissolved in MeOH and converted to the chloride by AgCl. The crude chloride (520 mg) was dissolved in MeOD (50 ml) and conc. DCl (10 ml) and after addition of zinc dust (1 g), the mixture was heated under reflux for 4.5 hr at 70°. The zinc dust was filtered off. The filtrate was diluted with H_2O and concentrated to a small volume under a reduced pressure. After cooling the solution was made alkaline with NH_4OH and extracted with $CHCl_3$. The dried $CHCl_3$ solution was evaporated and the residue was chromatographed on silica gel with benzene–ether. The benzene–ether (19: 1) elute was recrystallized from $CHCl_3$–MeOH to give **8a** (90 mg) mp 206—208°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2020, 2140 (CD). Mass Spectrum *m/e*: 341 (M^+), 175, 165. The benzene–ethre (4: 1) elute was recrystallized from $CHCl_3$–MeOH to give (8,13,13a-D_3)-*dl*-stylopine (90 mg) mp 225—228°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2030, 2150, 2200 (CD). Mass Spectrum *m/e*: 326 or 327 (M^+), 175, 151, 150. The benzene–ether (1: 1) elute was purified by preparative TLC and recrystallized from MeOH–ether to give **7a** (90 mg), mp 132—134°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2140 (CD). Mass Spectrum *m/e*: 341 (M^+), 175, 165.

Preparation of (N-$^{13}CH_3$)-*l*-Stylopine Methochlorides (10αa and 10βa)——*l*-Stylopine (580 mg) in acetone (4 ml) and $^{13}CH_3I$ (500 mg) in acetone (5 ml) were admitted into a glass-stoppered bottle. It was heated for 1.5 hr at 78° in a water bath. The precipitated crystalline was separated by decantation. The methiodide was dissolved in MeOH and converted to the chloride by AgCl. The crude chloride (390 mg) was recrystallized from acetone–MeOH to afford **10βa** (320 mg), mp 270—277° (decomp.). The mixture of the mother liquor of the methiodide and additional $^{13}CH_3I$ (300 mg) was heated 1.5 hr under similar conditions as above. The separated crystalline was converted to the chloride and it was recrystallized from acetone–MeOH to afford **10αa** (170 mg), mp 248—252° (decomp.). The chemical shifts of the N-methyl groups were collected in Table V.

Preparation of α-(N-$^{13}CH_3$)-*dl*-Mesotetrahydrocorysamine Methochloride (7αa)——To a solution of *dl*-mesotetrahydrocorysamine (57 mg) in acetone (1 ml) $^{13}CH_3I$ (100 mg) in acetone (1 ml) was added. After standing at room temperature for 2.5 hr, additional $^{13}CH_3I$ (100 mg) was added. After standing 30 minutes, the separated crystalline was filtered, colorless needles 79 mg, mp 298° (decomp.). The methiodide was dissolved in MeOH and converted to the chloride by AgCl. The crude chloride was recrystallized from acetone to afford **7αa** (50 mg), colorless needles, mp 278° (decomp.). The chemical shift of the N-methyl group was listed in Table V.

Preparation of α- and β-N-Methochlorides of [C(13)-$^{13}CH_3$, (8,13,13a-D_3)]-*dl*-Tetrahydrocorysamine (8αa and 8βa)——[C(13)-$^{13}CH_3$, (8,13,13a-D_3)]-*dl*-Tetrahydrocorysamine (85 mg) in acetone and CH_3I (5 ml) were admitted into a glass-stoppered bottle. It was heated at 90° for 1.5 hr. After cooling separated crystal was dissolved in MeOH and converted into chloride by AgCl. The chloride recrystallized from MeOH–acetone was warmed in acetone. The crystal insolved in hot acetone was recrystallized from MeOH–acetone to give **8βa** (60 mg). From the fraction dissolved in acetone, **8αa** (*ca.* 70 mg, oil) was obtained. The chemical shifts of the N-methyl groups were collected in Table V.

Table V. The Chemical Shift (ppm) of N-Methyl Group in α- and β-N-Methochlorides of Tetrahydroprotoberberines

Substrate No.	In RMR (60 MHz)		In CMR Chemical shifts
	Chemical shift	$J_{^{13}C-H}$ (Hz)	
10αa	3.31	145	51.2
10βa	3.00	144	40.5
8αa	3.33	—a)	53.3
8βa	3.11	—a)	44.2
7αa	3.19	145	51.9

a) no observation for unlabelled N-methyl group

Preparation of (N-$^{13}CH_3$)-*dl*-Stylopine Methochlorides (10αa and 10βa)——These compounds were prepared by the method described for *l*-stylopine methochlorides.

Substrate, **4a**, was obtained by the feeding experiment of α-(N-$^{13}CH_3$)-*l*-stylopine methochloride (**10αa**) in *Corydalis incisa* plants.

Administration of Labelled Compound to *Corydalis incisa* Pers. and *Chelidonium majus* L.——Labelled compounds in experiments 1, 2-1, and 2-2 were fed as an aqueous solution of the hydrochloride into the stems of generative stage *Corydalis incisa* plants through cotton wicks. The cotton wicks passed through numbers of stems. The ends were allowed to dip into an aqueous solution of the substrate in small containers. Distilled H_2O was repeatedly fed after the solution of the substrate had been absorbed.

Labelled compounds in experiments 3—5 were fed as an aqueous solution into the stems of generative stage *Corydalis incisa* plants by cotton wick method.

Labelled compounds in experiments 6-1 and 6-2 were fed as an aqueous solution into the stems of generative stage *Chelidonium majus* plants by cotton wick method.

Labelled compounds in experiments 7-1 and 7-2 were fed as an aqueous solution into the cuttings of nutrient stage *Chelidonium majus* plants according to the following procedure. Cuttings of *Chelidonium majus* (*ca.* 20 cm long) were dipped into an aqueous solution of the substrate in small tubes, which was repeatedly refilled with distilled H_2O after the original solution had been absorbed. Then the stems were transferred to the container of distilled H_2O.

Labelled Compound in experiment 8 was fed as an aqueous solution of the hydrochloride into the cuttings of nutrient stage *Chelidonium majus* plants according to the procedure described for experiments 7-1 and 7-2.

After one week, the plants from the individual feeding experiments were dried.

Extraction and Isolation of Alkaloids from the Plants——The alkaloids were extracted by the usual way. The tertiary non phenolic alkaloids were isolated by the column chromatography. The alkaloids isolated from experiment 1 were repeatedly recrystallized from $CHCl_3$–MeOH to give the pure corynoline (**3**), 13.6 mg, 0.087 mCi/mM and protopine (**4**), 2.5 mg, 0.212 mCi/mM. The alkaloids from experiments 2—8 were purified by preparative TLC (DC-Fertig-platten Kieselgel 60 F_{254}, solvent, ether/benzene=1/9—1/1 or MeOH) and recrystallization from $CHCl_3$-MeOH. The individual alkaloids were identified with the authentic sample by comparison of mp, TLC, and PMR spectra. Corynoline (**3**) from experiment 3-1, 56 mg, $[\alpha]_D^{23}$ +58°[14] (2.40 in $CHCl_3$), mp 216—217° (*dl*-form); protopine (**4**) from experiment 3-1, 41 mg, mp 208—211°; corycavine (**9**) from experiment 3-1, 8 mg, $[\alpha]_D^{23}$ +139°[14] (0.65 in $CHCl_3$), mp 220—223° (*dl*-form); acetylcorynoline (**11**) from experiment 3-1, 20 mg, $[\alpha]_D^{23}$ +50°[14] (1.08 in $CHCl_3$), mp 153—155° (*dl*-form); **3** from experiment 3-2, 77 mg, $[\alpha]_D^{23}$ +75° (3.20 in $CHCl_3$); **4** from experiment 3-2, 12 mg; **9** from experiment 3-2, 10 mg, $[\alpha]_D^{23}$ +123° (0.75 in $CHCl_3$); **11** from experiment 3-2, 35 mg; **3** from experiment 4, 46 mg, $[\alpha]_D^{23}$ +62° (1.85 in $CHCl_3$); **9** from experiment 4, 12 mg, $[\alpha]_D^{23}$ +40° (0.20 in $CHCl_3$), mp 185—195° (mixture of *dl*- and *d*-forms), mp 220—221° (*dl*-form); **11** from experiment 4, 17 mg; **4** from experiment 6-1, 37 mg; dihydrosanguinarine (**12**) from experiment 6-1, 15 mg, mp 188—189°; chelidonine (**14**) from experiment 6-1, 80 mg, mp 135—136°; **4** from experiment 6-2, 31 mg; **12** from experiment 6-2, 20 mg; 14 from experiment 6-2, 75 mg. The corresponding alkaloids from experiments 2-1, 2-2, 5-1, and 5-2 and from experiments 7-1, 7-2, and 8 were identified as above. PMR spectral data were collected in Table III.

Oxidation of Labelled Corynoline (3a)——The **3a** (1 mg) and the unlabelled material (50 mg) were dissolved in acetone (100 ml). A stirred solution was treated dropwise with potassium permanganate (150 mg) in acetone and the mixture was stirred for 1 hr. An excess of $KMnO_4$ was decomposed by adding MeOH and MnO_2 was filtered off. The filtrate was evaporated *in vacuo* and the residue was dissolved in AcOEt. The AcOEt solution was extracted with 5% HCl solution and then 5% NH_4OH solution. The dried AcOEt solution was evaporated and the residue was recrystallized from $CHCl_3$–MeOH to give radioinactive 6-oxocorynoline (**5**), 7 mg. The foregoing HCl layer was made alkaline with NH_4OH solution and extracted with $CHCl_3$. The dried $CHCl_3$ solution was evaporated and the residue was purified by silica gel chromatography and recrystallization from $CHCl_3$-MeOH to give corynoloxine (**6a**), 1.8 mg, 1.16×10^{-3} mCi/mM, corresponding to 67% of the activity in **3a**.

Reduction of Labelled Corynoloxine (6a)——The **6a** (0.8 mg) and inactive material (10 mg) were dissolved in 5% HCl solution. $NaBH_4$ (27 mg) was added to the solution. When the mixture was made alkaline with 10% NaOH solution, the color was rapidly discharged. To the mixture H_2O was added and the solution was extracted with ether. The dried ether solution was evaporated and the residue was recrystallized from $CHCl_3$–MeOH to give corynoline (**3a**), 5.6 mg, 0.82×10^{-4} mCi/mM, corresponding to 77% of the activity in **6a**.

14) The partial racemate with the *d*-form were isolated from *Corydalis incisa* PERS. for the first time.