

On the Alkaloids of Genus *Corydalis* of the Papaveraceous Plants

Narao Takao

Kobe Women's College of Pharmacy, Kobe 658, Japan

This review consists of two parts. Part I will be on the chemical structure of the alkaloids, studied in my laboratory. Part II will discuss their biosynthesis.

Part I

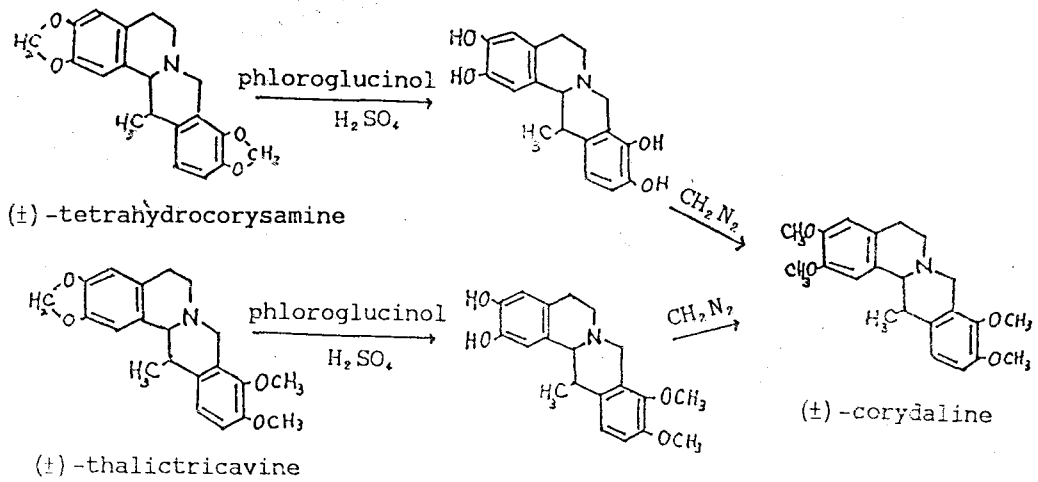
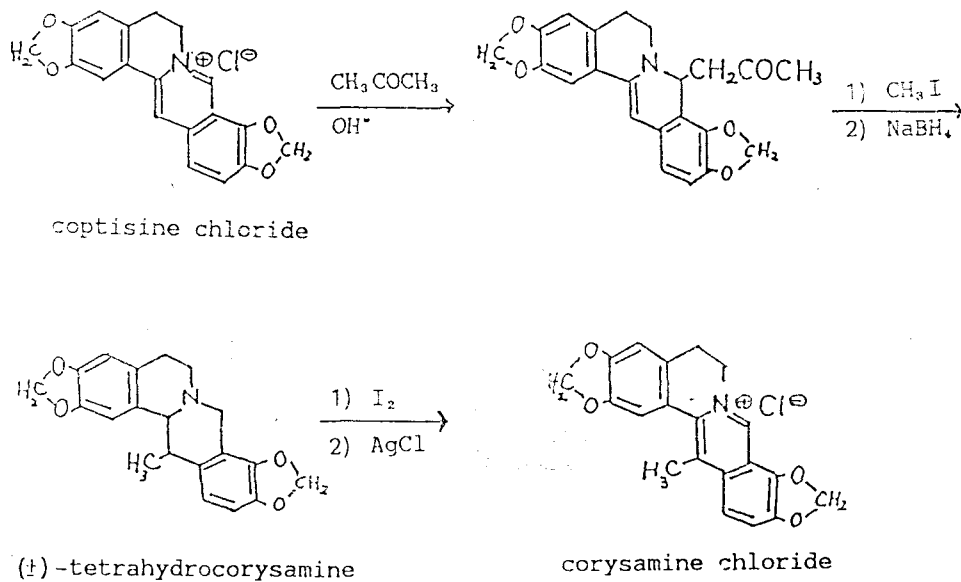
The plants of Genus *Corydalis* are distributed over the temperate zones of Europe, Africa, Asia and North America. At present 200 species¹⁾ have been described. Of these, 64 species²⁾ have been chemically studied, as of 1986. The plant of Genus *Corydalis* are classified into two types. Type one has tubers, and is used as plant-drugs. These plant-drugs are used as analgetics. Type two has no tuber. The plants of type two are mostly useless and regarded as toxic plants. *Corydalis incisa* belongs to type two, and Japanese consider this plant to be toxic and of no use. It grows in bamboo groves in Japan. It has red-violet blossoms from March to May.

In 1950, R.H.F. Manske³⁾ reported, that from *Corydalis incisa*, cultivated in Ottawa, Canada, he had isolated four alkaloids: protopine, adlumidine, *l*-corypalmine and a new one F-62. Our results were surprisingly different. Of the items isolated, only protopine was the same in both plants⁴⁾. We were unable to explain the great differences.

We could isolate a new quaternary alkaloid from *Corydalis incisa*. We named it corysamine⁵⁾. Corysamine chloride has a melting point of 240° and its formula is $C_{20}H_{16}O_4NCl \cdot 1/2H_2O$. Its

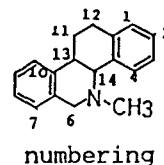
functional groups are as follows, two methylenedioxy groups and one C-methyl group. The reduction of this chloride gives tetrahydrocorysamine, a tertiary base, mp 203° ($C_{20}H_{19}O_4N$). Later we were able to isolate (+)-tetrahydrocorysamine from this plant. Tetrahydrocorysamine was treated with phloroglucinol-sulfuric acid, and the resulting product was methylated with diazomethane. In such a way, we obtained (±)-corydaline. On the other hand, from (±)-thalictricavine, the same (±)-corydaline was obtained. So we could determine the structure of (±)-tetrahydrocorysamine (Scheme I).⁷⁾ Corysamine-chloride was synthesized from coptisine-chloride via acetonecoptisine. Methylation and reduction gave tetrahydrocorysamine. Tetrahydrocorysamine was oxidized with iodine. This iodide was converted with AgCl to chloride (Scheme I).^{6,7)} Thus the structure of corysamine was determined.

From the non-phenolic tertiary fraction we could isolate a new alkaloid. We named it (±)-corynoline. It has the formula $C_{21}H_{21}O_5N$ and its melting point is 217°, $[\alpha]_D=0^\circ$. Functional groups are as follows: two methylenedioxy groups, one alcoholic hydroxyl group, one N-methyl group and one C-methyl group. This belongs to the hydrobenzo(c)-phenanthridine type alkaloids, as concluded from the following

Structure of (±)-tetrahydrocorysamineSynthesis of corysamine chloride

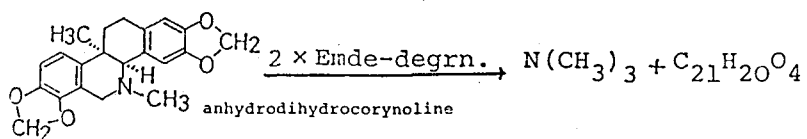
Scheme I

(±)-Corynoline

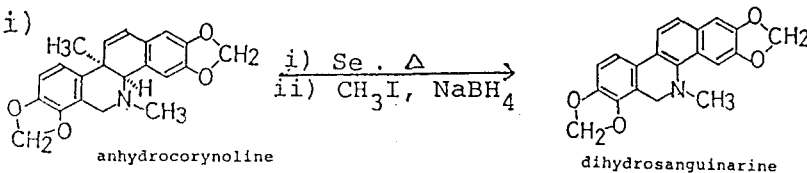


A) This belongs to hydrobenzo(c)-phenanthridine-alkaloid.

i)

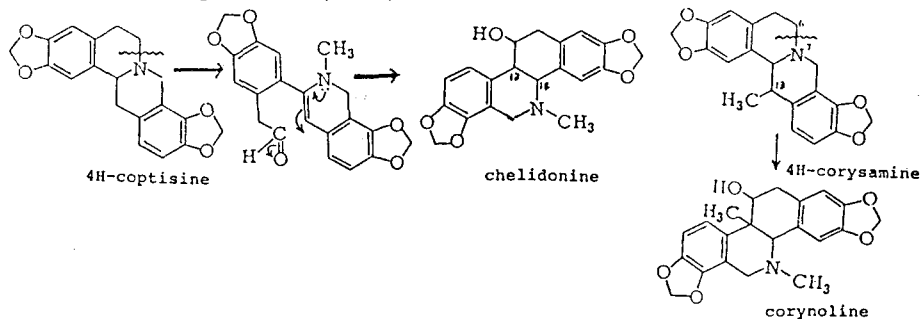


ii)

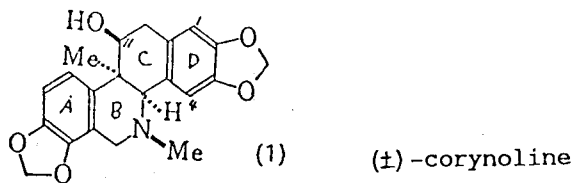
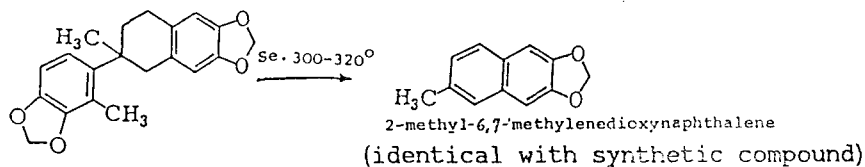


B) The position of angular methylgroup (C-13 or C-14)

i) Robinson's biogenesis (1955)



ii) Selenium dehydrogenation of anhydrodihydro-des-N-product



Scheme II

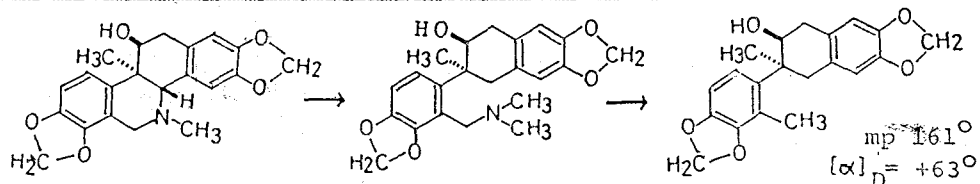
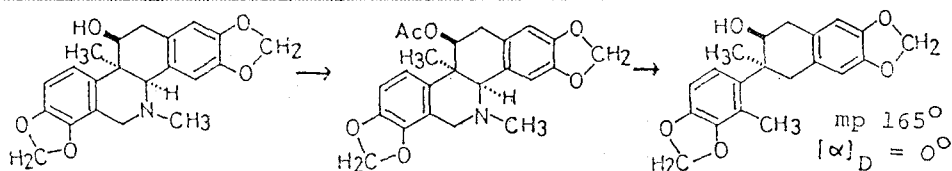
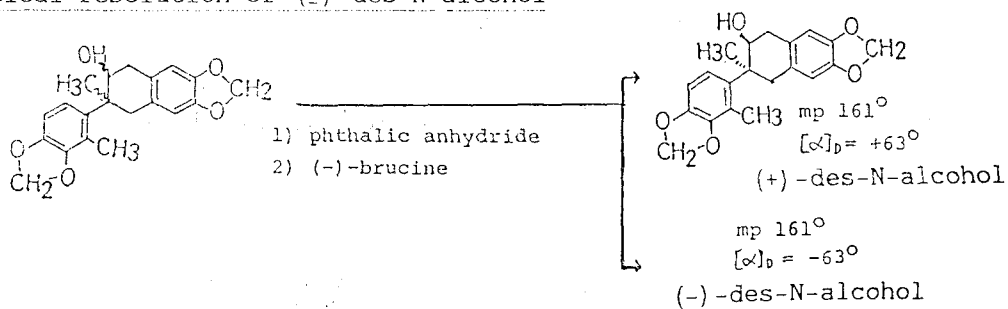
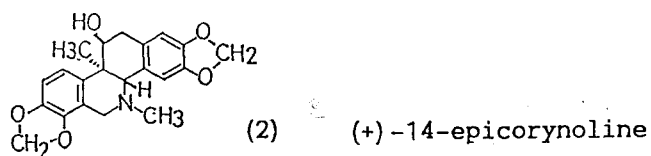
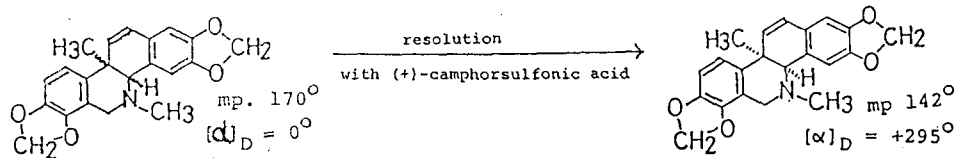
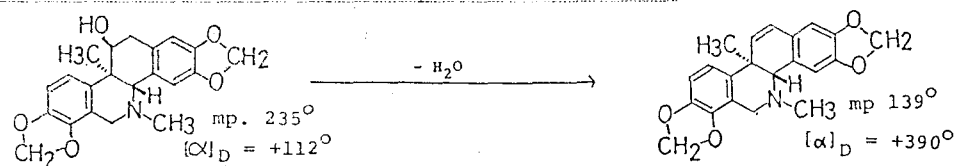
two facts (Scheme II).^{8,9)} 1) Anhydrocorynoline gave trimethylamine and des-N-product by the repetition of Emde degradation. This shows, that this alkaloid does not belong to the protoberberine type alkaloids. 2) After selenium pyrolysis of anhydrocorynoline, N-methylation and reduction give dihydrosanguinarine. These results show that (\pm)-corynoline belongs to the hydrobenzo(c)-phenanthridine type alkaloids. Regarding the position of the alcoholic hydroxy group, 1) Oppenauer oxidation gives a ketone. This means that the hydroxyl group is a secondary and stands at C-11 or C-12. 2) Anhydrocorynoline has a conjugated double bond, which means the alcoholic hydroxyl group is located at C-11 or C-12. 3) The intra hydrogen bond appears in the IR spectrum. This indicates that hydrogen bond is between the hydroxyl group and nitrogen atom. Thus the secondary alcoholic hydroxyl group must be located at C-11. At the same time, this means that the B/C ring juncture is *cis*.

Regarding the position of the angular methyl group (C-13 or C-14), in 1955, Robert Robinson published the book, *The Structural Relation of Natural Products*¹⁰⁾. He proposed in this book a biogenetic pathway from protoberberine to the hydrobenzo(c)-phenanthridine alkaloids (Scheme II). This scheme shows that stylophine is converted to (+)-chelidone. Based on Robinson's biogenesis, the position will be C-13, because we have already found (+)-tetrahydrocorysamine in this plant. Selenium pyrolysis of anhydrodihydrocorynoline-des-N-product gives 2-methyl-6,7-methylenedioxy-naphthalene, which proves chemically that the position of the angular methyl group is at C-13. From these reactions, we obtained the following results, 1) (\pm)-corynoline belongs to the hydrobenzo(c)-phenanthridine type alkaloids, 2) this is the first example of this type, to have an angular methyl group, and 3) the B/C ring juncture is

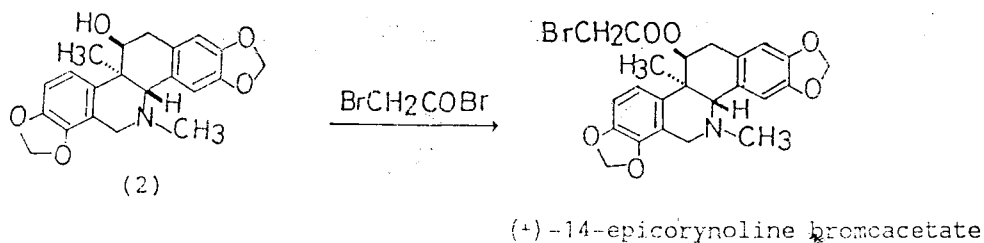
cis. Thus the relative structure of (\pm)-corynoline can be shown as 1 (Scheme II).

A new alkaloid was obtained from the non-phenolic tertiary base fraction. This alkaloid could be isolated easily from the other alkaloids by shaking the hydrochloric acidic solution with chloroform. This alkaloid has the same formula and functional groups as (\pm)-corynoline. But it is optically active, $[\alpha]_D^{25} = +112^{\circ}$, showing a diastereomer of corynoline. This alkaloid has no hydrogen bond and so quaternarization proceeds easily. After twice doing the Emde degradation, we obtained the optically active des-N-alcohol (Scheme III).¹²⁾ On the other hand, the quaternarization of (\pm)-corynoline does not proceed because of the hydrogen bond. So we had to do esterification. This O-acetate provides, after doing the Emde degradation twice and saponification, (\pm)-des-N-alcohol (Scheme III).^{ibid.)} The (\pm)-des-N-alcohol obtained was resolved to optically active des-N-alcohol. The alcohol was esterified with phthalic anhydride to a monoester. The monoester was led to diastereomeric salt with (-)-brucine. Thus we could obtain optically active des-N-alcohol (mp 161°, $[\alpha]_D^{25} = +63^{\circ}$) (Scheme III).^{ibid.)} This optically active des-N-alcohol is identical with the des-N-alcohol from the new alkaloid. This means the configurations are the same at C-11 and C-13. This left only the problem of the configuration of C-14 remaining to be solved.

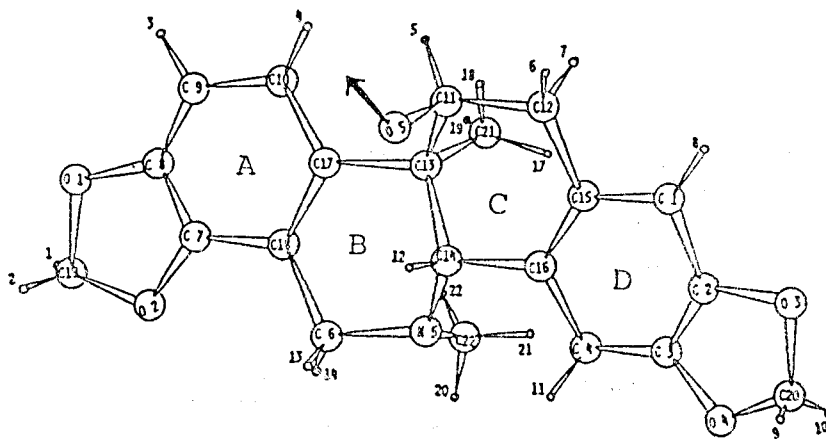
This new alkaloid was dehydrated to the optically active anhydro-product (mp 139°, $[\alpha]_D^{25} = +390^{\circ}$). On the other hand, (\pm)-anhydrocorynoline could be optically resolved, via the diastereomeric salt of (+)-camphorsulfonic acid. (+)-Anhydrocorynoline (mp 142°, $[\alpha]_D^{25} = +295^{\circ}$) differs from the optically active anhydro-product. Thus the B/C ring juncture of this alkaloid is *trans*, namely this new alkaloid has the structure of (+)-14-epicorynoline (2) (Scheme III).^{ibid.)} It is the first example in hydrobe-

(+)-14-Epicorynoline a diastereomer of (+)-corynolineFormation of des-N-alcohol of (+)-14-epicorynolineFormation of des-N-alcohol of (±)-corynolineOptical resolution of (±)-des-N-alcoholTrans B/C ring juncture of (+)-14-epicorynoline

Scheme III

Crystal structure (stereostructure) of (+)-14-epicorynoline bromoacetate**Table.** Crystallographic data

$\text{C}_{23}\text{H}_{22}\text{O}_6\text{NBr}$, M.G. 488.34, mp 188~189°
colourless, transparent needle, monocline
$a=10.056(6)$, $b=7.400(6)$, $c=13.959(6)\text{Å}$
$\beta=94.09^\circ(3)$
$V=1036.1\text{Å}^3$, $Z=2$, $F(000)=500$
$\mu(\text{for Cu-K}\alpha)=32.58\text{cm}^{-1}$
$D_{\text{obsd.}}=1.551\text{g/cm}^3$, $D_{\text{calcd.}}=1.565\text{g/cm}^3$
space group $P2_1$

Stereostructure of (+)-14-epicorynoline**(+)-14-epicorynoline****Scheme IV**

nzo(c)-phenanthridine type alkaloids, that the B/C ring juncture is *trans*.

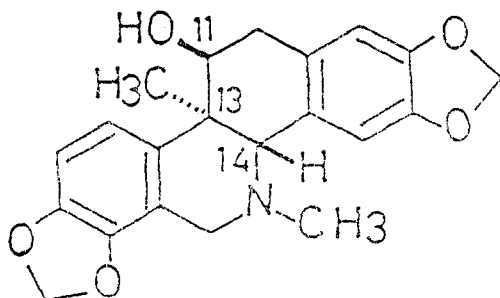
To determine the stereostructure and the absolute configuration of (+)-14-epicorynoline by X-ray analysis, we synthesized (+)-14-epicory-

noline bromoacetate. We obtained crystals of bromoacetate (mp 188°). The Table in Scheme IV shows the crystallographic data of (+)-14-epicorynoline bromoacetate. The structure was determined by the usual heavy atom method.

Absolute configuration of (+)-14-epicorynoline

Table. Comparison of intensities $I(h, k, l)$ with structure factors of Bijvoet-pair

Index	$I(hkl) < I(\bar{h}\bar{k}\bar{l})$	$ Fc(hkl) $	$ Fc(\bar{h}\bar{k}\bar{l}) $
1 1 1	>	187	160
1 1 4	<	182	194
2 1 5	>	159	140
5 1 0	<	138	149
1 1 $\bar{3}$	<	256	270
3 1 $\bar{2}$	>	190	173
4 1 $\bar{7}$	>	66	60
2 2 1	<	191	203
3 2 2	<	182	191
5 2 2	>	119	111
1 2 $\bar{3}$	>	139	130
1 2 $\bar{7}$	>	98	89
1 2 $\bar{8}$	<	63	73
4 2 $\bar{2}$	<	159	168
5 2 $\bar{6}$	<	72	78

(+)–14-epicorynoline 11*S*, 13*R*, 14*S***Scheme V**

The stereostructure was expressed as shown in Scheme IV. We can see, 1) the B/C ring junction is *trans*, 2) the alcoholic hydroxyl group at C-11 is *axial* and 3) the N-methyl group and C-methyl group are 1,3-*diaxial*. The chemically determined structure was confirmed by X-ray structure analysis (Scheme IV).^{13,14)}

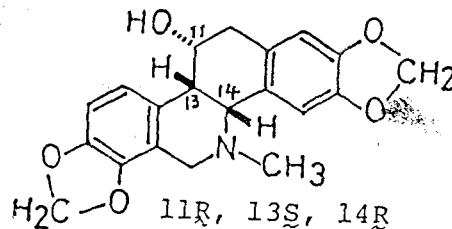
The absolute configuration of (+)-14-epicorynoline bromoacetate was determined using anomalous dispersion effect of the bromine atom. The Table in Scheme V shows a comparison of the intensities with the structure factors of Bij-

voet-pairs. The absolute configuration of (+)-14-epicorynoline was determined as 11*S*, 13*R* and 14*S* (Scheme V).^{ibid.)}

Corynoline, the main alkaloid of *Corydalis incisa*, is racemic. The absolute configuration must be studied with optically active corynoline, so we have tried to resolve (\pm)-corynoline. We tried the route using the diastereomeric ester (Scheme VI).¹⁵⁾ We esterified the (\pm)-corynoline with (–)-menthoxyacetylchloride. The diastereomeric esters were separated by preparative thin layer chromatography (the upper zone (+)-(–)-ester, the lower zone (–)-(–)-ester). (+)-Corynoline was obtained from the upper zone. It has a melting point of 178°, $[\alpha]_D^{25} = +116^\circ$ (Scheme VI).¹⁵⁾

First of all, (+)-corynoline was O-acetylated with acetic anhydride. Without acetylation, the quarternarization does not proceed, because of hydrogen bonding. Doing the Emde degradation twice gives trimethylamine and des-N-alcohol. This des-N-alcohol is identical with the des-N-alcohol from (+)-14-epicorynoline. This finding means that the absolute configurations are 11*S* and 13*R*. As for the absolute configuration of C-14, it must be the opposite of (+)-14-epicorynoline, namely 14*R*. Thus the absolute configuration of (+)-corynoline was determined to be 11*S*, 13*R* and 14*R* (Scheme VI).¹⁵⁾

In 1970, Prof. Snatzke *et al.*¹⁶⁾ reported, that from the CD spectrum of (+)-chelidonine, its absolute configuration was 11*R*, 13*S* and 14*R*. But as described above, we determined the absolute configuration of (+)-14-epicorynoline to be 11*S*, 13*R* and 14*S* and for (+)-corynoline 11*S*, 13*R* and 14*R*. From our results, it seemed that the absolute configuration of (+)-chelidonine may be 11*S*, 13*R* and 14*S* (Scheme VII).^{16,17)} We have begun to study the absolute configuration of (+)-chelidonine with X-ray analysis.

Absolute configuration of (+)-chelidonineSnatzke et al. from CD-spectrum : 11 \bar{R} , 13 \bar{S} , 14 \bar{R} (1970)

Takao et al.

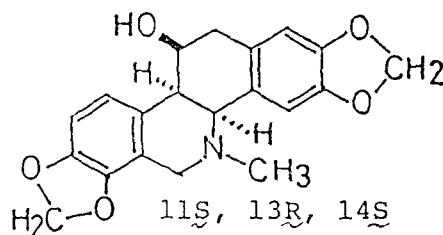
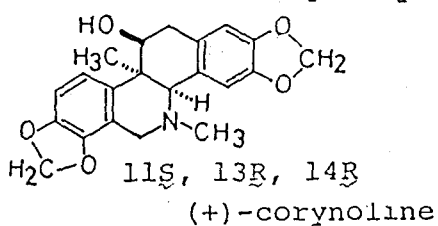
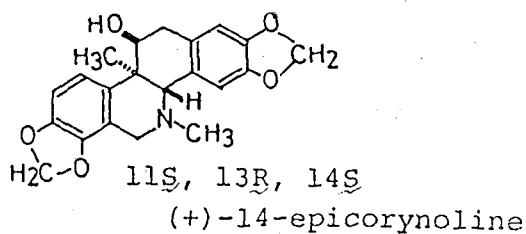
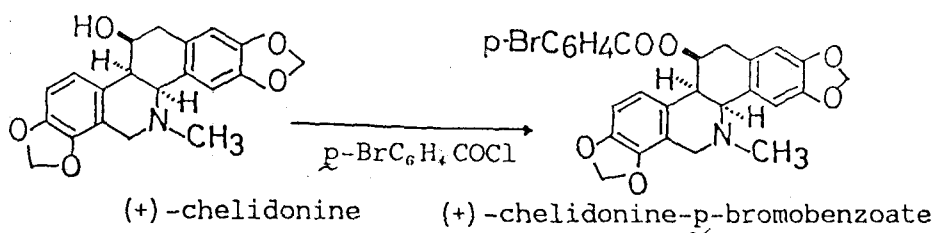
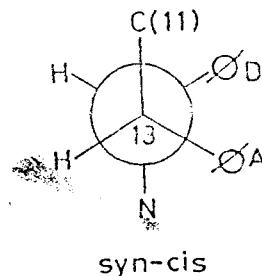
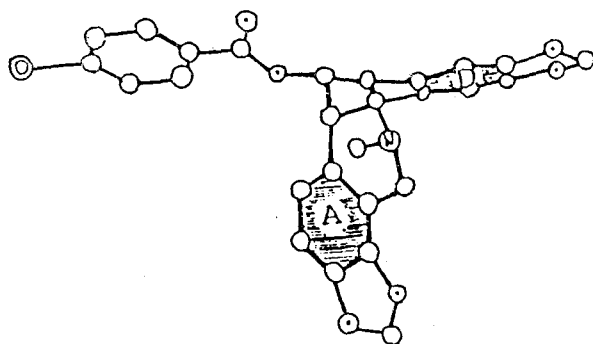
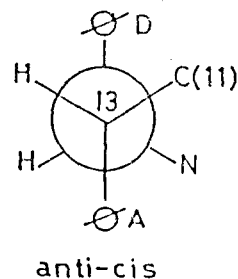
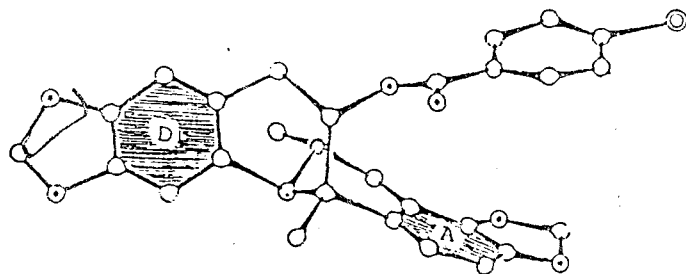
Stereostructure of (+)-chelidonine-p-bromobenzoate(+) -Chelidonine : main alkaloid of *Chelidonium majus* $C_{20}H_{19}O_5N$ mp. 135° $[\alpha]_D = +115^\circ$ 

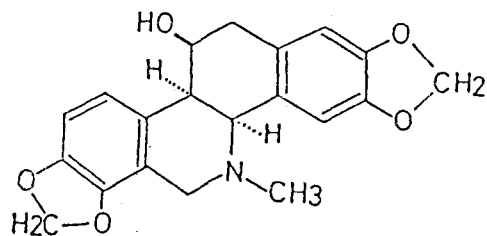
Table. Crystallographic data

$C_{27}H_{22}O_6NBr$ M.W. 536.36,	mp 208°
colourless, prisms, tricline	
$a=14.257(5)$,	$b=9.656(4)$, $c=10.21(5)\text{\AA}$
$\alpha=116.90(3)$,	$\beta=111.27(3)$, $\gamma=88.27(2)^\circ$
$V=1153.8\text{\AA}^3$,	$F(000)=548$,
$D_m=1.537$,	$D_x=1.544\text{Mgm}^{-3}$
space group P1	

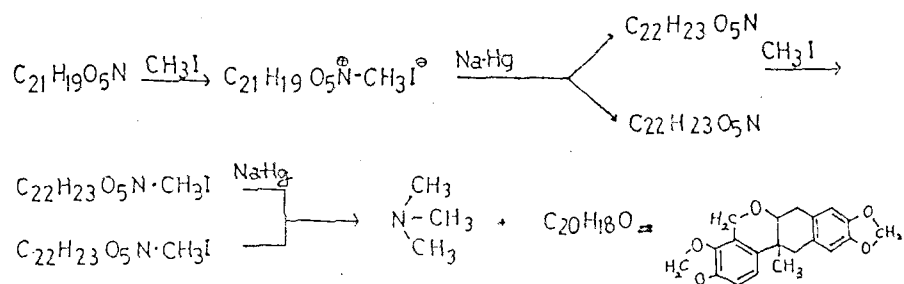
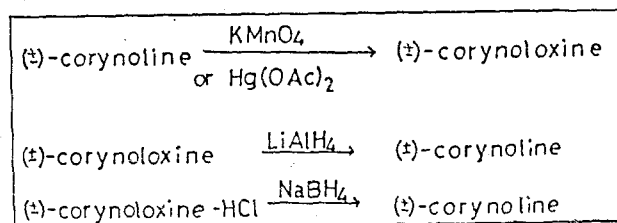
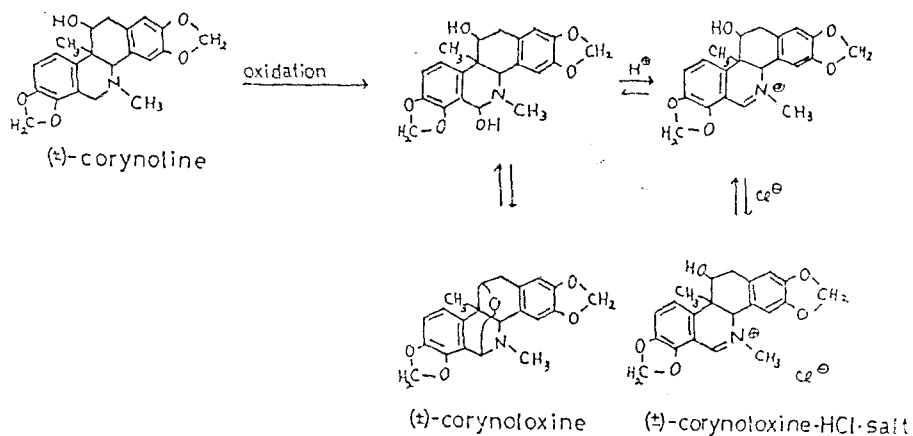
Scheme VII

Two conformations of *cis*-configuration (*syn-cis*, *anti-cis*) (+)-chelidonine-*O*-*p*-bromobenzoate(±)-corynoline-*O*-*p*-bromobenzoate (Kametani)Table. Absolute configuration of (+)-chelidonine
list of structure factors to determination of
absolute configuration

Index	$\frac{ F_c(hkl) }{ F_c(\bar{h}\bar{k}\bar{l}) } - 1$	$\frac{ F_c(hkl) }{ F_c(k\bar{k}l) } - 1$			
5-12	-0.180	-0.243	-2-21	-0.087	-0.082
4 11	-0.105	-0.092	-2-31	0.220	0.194
2 14	-0.106	-0.112	-3 01	0.066	0.061
2-12	0.204	0.194	-3 11	-0.103	-0.101
1 11	-0.094	-0.122	-3-11	-0.041	-0.038
1-11	0.220	0.244	-4 11	0.104	0.108
1-22	0.068	0.064	-4-15	-0.114	-0.115
0 20	-0.071	-0.089	-4-11	-0.088	-0.091
0 13	-0.115	-0.120	-6 02	-0.069	-0.056
0-17	0.084	0.160	-6-13	0.192	0.198
0-43	-0.090	-0.090	-6-12	0.167	0.207
-1-21	-0.043	-0.034	-7 12	-0.373	-0.321
-1-31	0.055	0.100			
-1-41	-0.105	-0.092			
-1-52	0.104	0.110			
-2 20	-0.122	-0.106			
-2-12	0.307	0.274			
-2-11	0.142	0.130			

(+) -chelidonine: 11*S*, 13*R* and 14*S*

Scheme VIII

Structure of corynoxineRelationship between (±)-corynoline and (±)-corynoxine

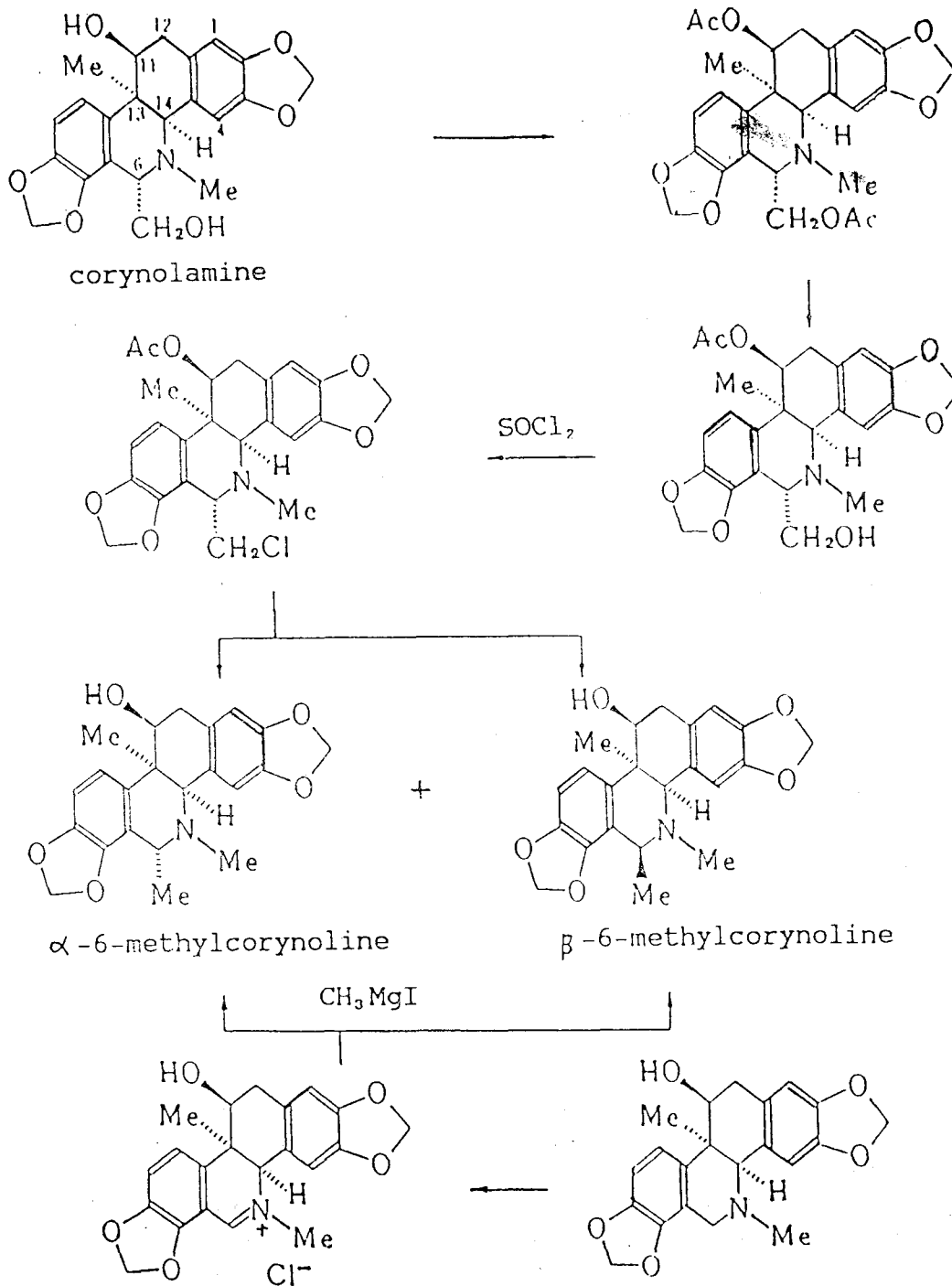
Scheme IX

(+)-Chelidonine is the famous main alkaloid of *Chelidonium majus*. Its melting point is 135°, $[\alpha]_{\text{D}} = +115^{\circ}$. We have synthesized *p*-bromobenzoate from (+)-chelidonine and *p*-bromobenzoylchloride. (+)-Chelidonine-*p*-bromobenzoate has a melting point of 208°, white prisms. The Table in Scheme VII shows the crystallographic data of (+)-chelidonine-*p*-bromobenzoate. The

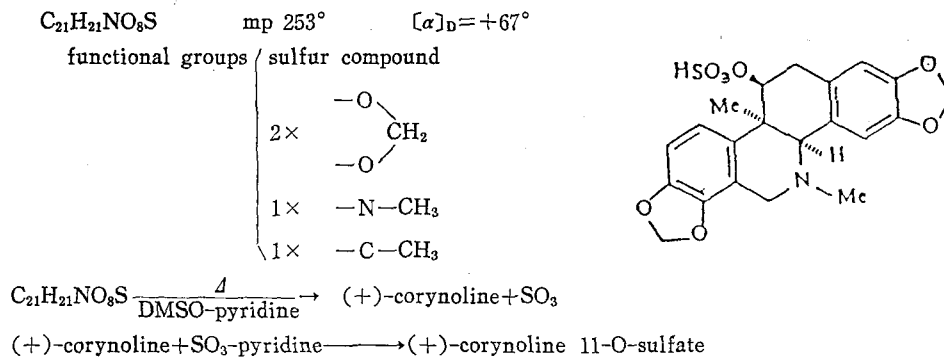
stereostructure was determined by the usual heavy atom method (Scheme VII).^{17,18)}

Our X-ray analysis took a very long time, because (+)-chelidonine-*p*-bromobenzoate is a rare case. As many people know, the *cis*-configuration has two conformations (*anti-cis* and *syn-cis*), with most *cis*-configurations preferring the *anti-cis* conformation. In this case, we

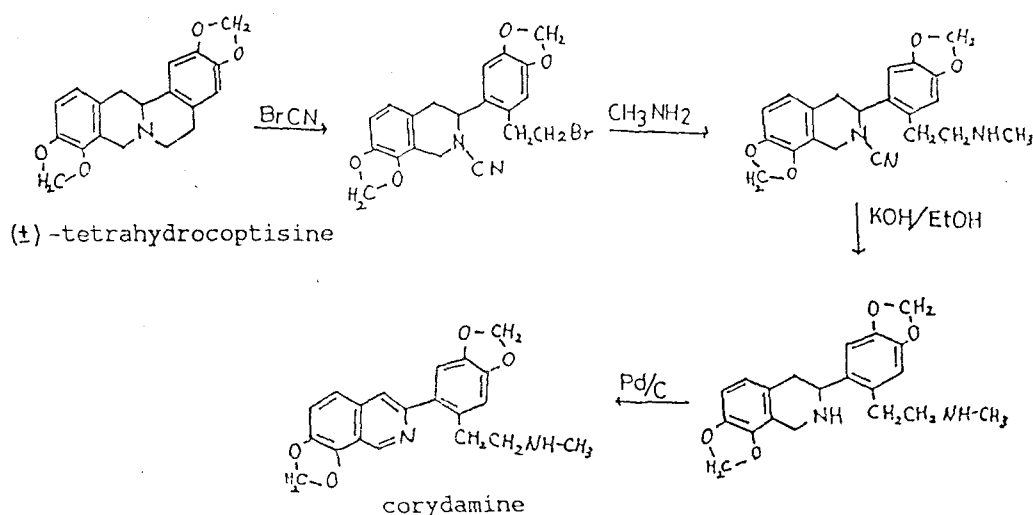
Corynolamine

Conversion of (\pm)-corynolamine to (\pm)-6-methylcorynoline

Scheme X

(±) -Corynoline 11-O-sulfate

Scheme XI

Corydamine-synthesis from (±)-tetrahydrocoptisine

Scheme XII

sought in vain the *anti-cis* conformation. The X-ray analysis could not go ahead. Then we happened to think of the *syn-cis* conformation and at last we were able to obtain a proper *R*-value. (+)-Chelidonine-*p*-bromobenzoate takes the *syn-cis* conformation. This is the first example of the *syn-cis* conformation in actual existence. The Scheme VIII^(ibid.) shows the stereostructure of (+)-chelidonine-*p*-bromobenzoate and its Newman projection (*syn-cis*) and the stereostructure of (±)-corynoline-*p*-bromobenzoate and its Newman projection (*anti-cis*). The

absolute configuration of (+)-chelidonine-*p*-bromobenzoate was determined using the anomalous dispersion effect of the bromine atom. The Table in Scheme VIII shows a comparison of the intensities with structure factors of Bijvoet-pair. As we assumed, the absolute configuration of (+)-chelidonine was determined to be 11*S*, 13*R* and 14*S* (Scheme VIII).^(ibid.)

We isolated a new alkaloid from a non-phenolic, tertiary base fraction. We named it corynoxine. This alkaloid has a melting point 207° and the formula $C_{21}H_{19}O_5N$ (two hydrogens

fewer than corynoline). It has no alcoholic hydroxyl group and its salt has a double bond between nitrogen and C-6.

The oxidation of (\pm)-corynoline with KMnO_4 or mercuric acetate gives (\pm)-corynoxine.¹⁹⁾ The reduction of corynoxine with LiAlH_4 and corynoxine HCl-salt with NaBH_4 give corynoline. After twice doing the Emde degradation, we obtained trimethylamine and neutral des-N-product. This product has no alcoholic hydroxyl group. This means that the fifth oxygen is ether-linked (Scheme IX).²⁰⁾ The relationship between corynoline and corynoxine was summarized in Scheme IX.^{ibid.)}

We were able to isolate a new alkaloid from a non-phenolic, tertiary base fraction, which was named corynolamine. Corynolamine has a melting point 199° and its formula is $\text{C}_{22}\text{H}_{23}\text{O}_6\text{N}$. From the various spectral data, it would seem as if corynolamine belongs to the hydrobenzo (c)-phenanthridine type alkaloids. It has two alcoholic hydroxyl groups. From the IR spectrum and mass spectrum, one is a secondary alcoholic hydroxyl group. The other is presumed to be a primary alcoholic hydroxyl group. By eliminating the methylol group from the mass spectrum, it was presumed that this hydroxyl group exists in the methylol group. Our study thus turned to the position of the methylol group. The acetylation with acetic anhydride gave diacetate. Hydrolysis of the diester with Al_2O_3 gave a monoester.

Only the acetate of primary alcohol was hydrolysed. SOCl_2 was added to this product to convert it from the hydroxyl group to chlorine. The catalytic reduction with PtO_2 gave two products, that were different with respect to the position of their methyl group, (α -position and β -position). The α -methyl product came from the original alkaloid.

The synthesis of α -6-methylcorynoline is achieved as follow: CH_3MgI was added to

corynoxine. Two 6-methylcorynolines (α - and β -6-methylcorynoline) arose. α -6-Methylcorynoline was identical with the above described product. Thus the position of the methylol group of corynolamine was determined. Corynolamine has the structure of α -6-hydroxymethylcorynoline (Scheme X).^{ibid.)}

During the isolation we could obtain a new alkaloid. This alkaloid has sulfur in the molecule, and its formula is $\text{C}_{21}\text{H}_{21}\text{O}_8\text{NS}$ (mp 253° , $[\alpha]_D^{25} = +67^\circ$). From the mass spectrum we could recognize the existence of the sulfur atom. At the same time from the peak of 367, we could presume the existence of the (+)-corynoline skeleton. When this alkaloid was heated in DMSO-pyridine, (+)-corynoline arose. Conversely, when (+)-corynoline was heated with pyridine- SO_3 complex, we could obtain this alkaloid. Thus, the structure of this alkaloid was determined as (+)-corynoline-11-O-sulfate (Scheme XI).²¹⁾ In steroid chemistry, many O-sulfates are observed to be metabolites, but in alkaloid chemistry it is a rare case. In this respect, this alkaloid is interesting.

From the non-phenolic, tertiary base fraction, we obtained an alkaloid in an oily state, but the HCl-salt (mp 255°) was obtained as crystals. This alkaloid is a new one, so we named it corydamine. It has two nitrogens and its formula for free base is $\text{C}_{20}\text{H}_{18}\text{O}_4\text{N}_2$, and for HCl-salt $\text{C}_{20}\text{H}_{18}\text{O}_4\text{N}_2 \cdot 2\text{HCl} \cdot \text{H}_2\text{O}$. Its functional groups are as follows, two nitrogens, one $-\text{NH}-\text{CH}_3$, the other tertiary nitrogen with one double bond, and two methylenedioxy groups. From the UV spectrum we could infer the existence of a conjugated system.

From spectral analysis we have presumed the structure and tried the corydamine synthesis from tetrahydrocoptisine. Tetrahydrocoptisine was heated with bromcyan, it was cleaved to a seco-type. This bromine compound was converted to a N-methyl compound with methyl-

mine. Further the nitril group was removed by heating in ethanolic caustic potash. The last step was the dehydrogenation with Pd-charcoal. The product is identical with corydamine (Scheme XII)²². This structure is interesting in connection with Robinson's biogenetic hypothesis.

The alkaloids from Japanese *Corydalis incisa* are summarized as follows: we isolated as known alkaloids—protopine, corycavine, coptisine and sanguinarine. As new alkaloids, we were able to obtain corysamine, (+)-tetrahydrocorysamine, (±)- and (+)-corynoline, (+)-corynoline-11-O-sulfate, (+)-14-epicorynoline, (±)- and (+)-corynoxine, corynolamine and corydamine.

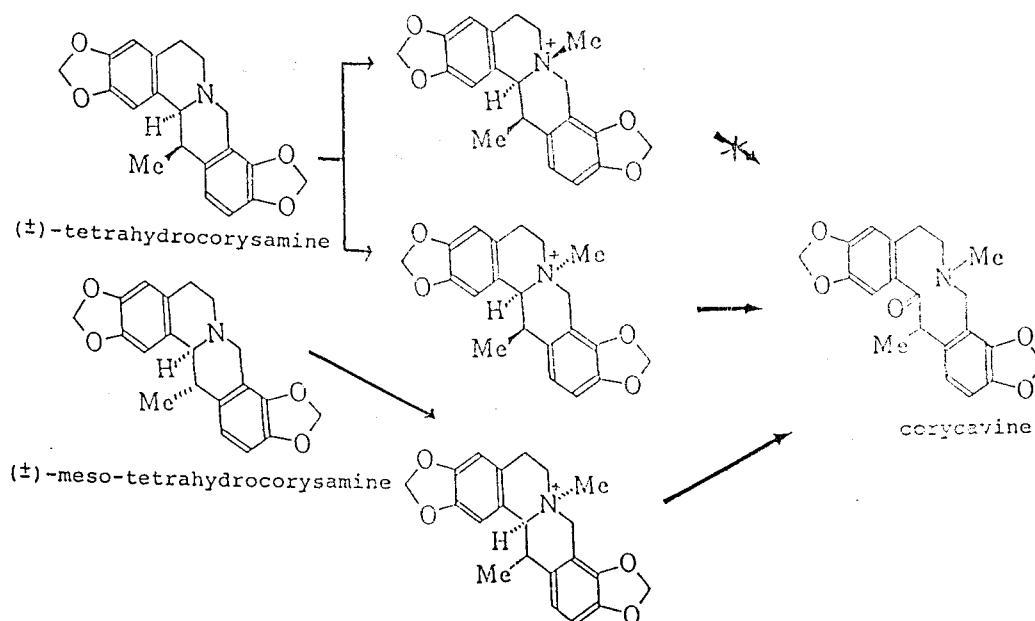
Part II

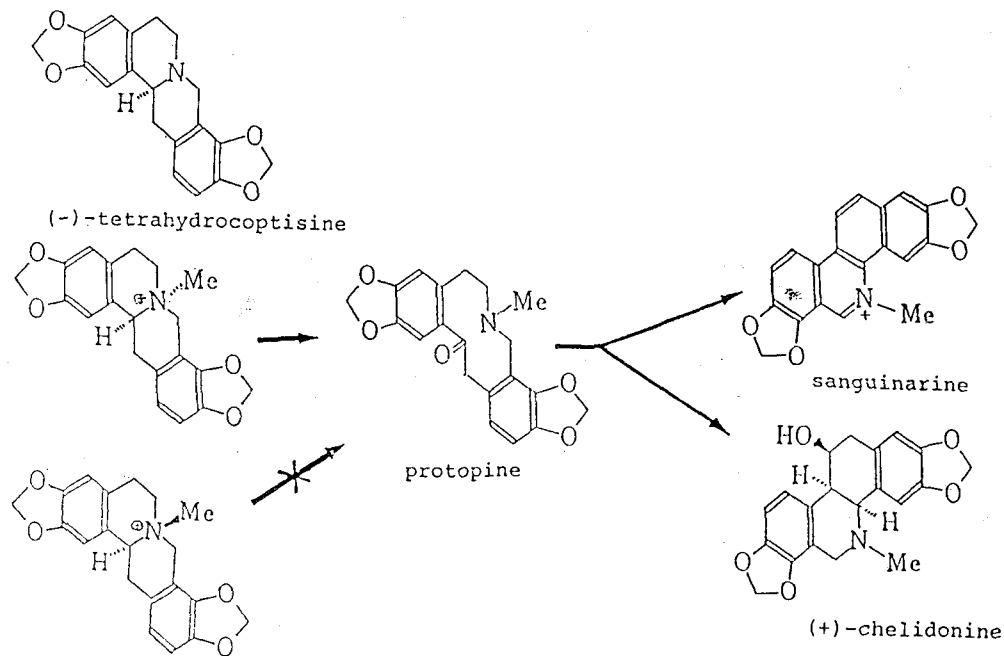
As described above, we used the biogenetic pathway of Robinson's hypothesis to presume the position of the angular methyl group and the position of the secondary alcoholic hydroxyl group in corynoline (Scheme II).

The tetrahydroprotoberberine skeleton was bioconverted to a protopine skeleton in *Corydalis incisa*. Corycavine (protopine type alkaloid) was bioconverted from tetrahydrocorysamine and *meso*-tetrahydrocorysamine. This bioconversion proceeds stereospecifically, because only α -N-methosalt and not β -N-methosalt was bioconverted. Another point, this conversion proceeds to the protopine type, and not to the benzo(c)-phenanthridine type. This bioconversion takes place with the introduction of oxygen-insertion at C-14 carbon of the tetrahydroprotoberberine skeleton (Scheme XIII).²³

In *Chelidonium majus*, only α -N-methosalt of optically active (-)-tetrahydrocoptisine was bioconverted via protopine to benzo(c)-phenanthridine type alkaloid sanguinarine and hydrobenzo(c)-phenanthridine type alkaloid (+)-chelidonine. These findings show that protopine is an intermediate (between protoberberine type and benzo(c)-phenanthridine type alkaloid). Until now, protopine type alkaloids have been treated as end-products in biogenetic pathways,

Corycavine-formation in *Corydalis incisa*



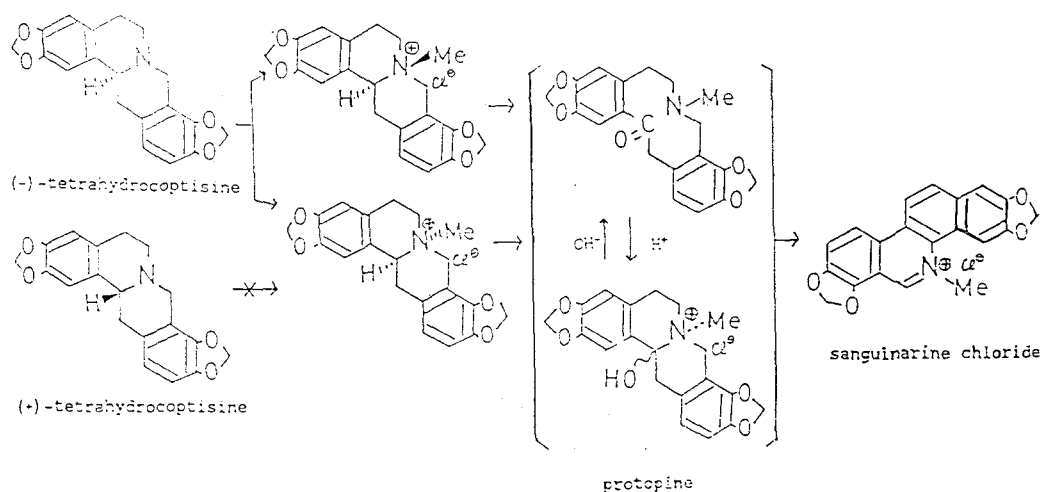
Sanguinarine- and (+)-chelidonium-formation in *Chelidonium majus*

Scheme XIII

but this shows that protopine is important as an intermediate in the biosynthetic pathway (Scheme XIII).²⁴⁾

We have succeeded in a callus-induction from the *Macleaya cordata* plant. Using this callus, we conducted an incubation experiment. The

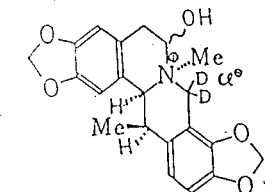
α - and β -N-methosalts of (\pm)-tetrahydrocoptisine were given to this callus. Only the α -N-methosalt of (-)-tetrahydrocoptisine was bioconverted via protopine to sanguinarine stereospecifically (β -N-methosalt was not). The callus method is more advantageous than the intact

Protopine- and sanguinarine-formation in callus of *Macleaya cordata*

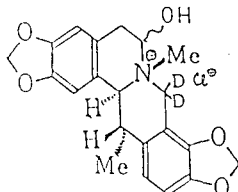
Scheme XIV

Biosynthesis of (±)-corynoline and (±)-corynoloxine in Corydalis incisa cell culture

a) Feeding of 6-hydroxy- and 6-hydroxy-meso- tetrahydrocorysamine N-methosalt

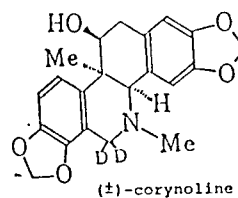


6-hydroxy-tetrahydrocorysamine N-methosalt

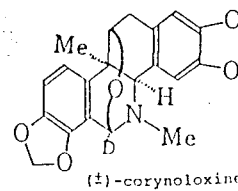


6-hydroxy-meso-tetrahydrocorysamine N-methosalt

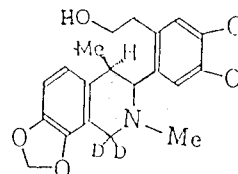
Callus of *Corydalis incisa*



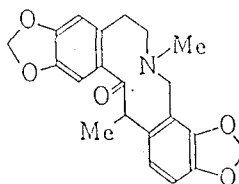
(±)-corynoline



(±)-corynoloxine

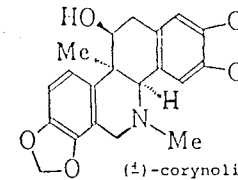


b) Feeding of corycavine



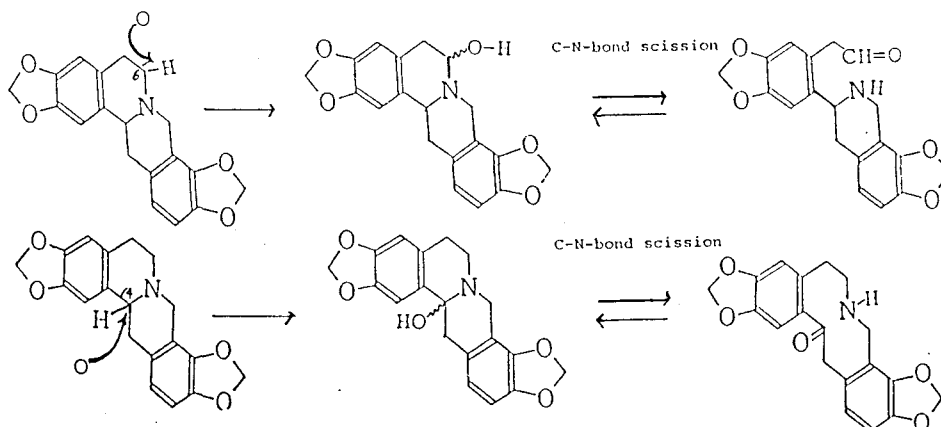
corycavine

Callus of *Corydalis incisa*



(±)-corynoline

Meaning of C-6-oxidation and C-14-oxidation



Conclusion : C-14-oxidation earlier step

Scheme XV

plant method, it being much simpler. These results agree with the results of our earlier experiment. This means, that the bioconversion proceeds, at first oxygen-insertion at C-14 (protopine formation)-via protopine to benzo(c)-phenanthridine type alkaloid sanguinarine (Scheme XIV).^{ibid.})

Then, we studied the oxidation-order. We synthesized as the precursor, deuterated N-methosalt of 6-hydroxy-tetrahydrocorysamine and N-methosalt of 6-hydroxy-meso-tetrahydrocorysamine. These deuterated precursors were incubated in the cell culture of *Corydalis incisa*. This callus is not sensitive to the stereospecific process. Deuterated (\pm)-corynoline and deuterated (\pm)-corynoxine arose. One deuterated aminoalcohol was obtained, too. This showed that 6-hydroxylated precursors are the effective precursor of corynoline and corynoxine (Scheme XV).^{25,26)}

In parallel with this incubation experiment, we carried out a feeding experiment with corycavine. As described already, the protopine type alkaloids are effective precursors (or intermediates) of the hydrobenzo(c)-phenanthridine type alkaloids. In this case, corycavine was bioconverted to (\pm)-corynoline. In this feeding experiment, the metabolic yield was several times (5~6 times) better than the yield from 6-hydroxy-tetrahydrocorysamine and 6-hydroxy-meso-tetrahydrocorysamine (Scheme XV).^{ibid.})

Scheme XV shows the meaning of the C-6-oxidation and C-14-oxidation. C-6-oxidation means a C(6)-N bond fission. Then via aldehyde, recyclization to hydrobenzo(c)-phenanthridine skeleton may arise. On the other hand, C-14-oxidation means a C(14)-N bond fission. This means the formation of protopine skeleton. As described above, the better yield of (\pm)-corynoline (from corycavine) means that the first oxidation at C-14 is the main biosynthetic pathway in this plant.

Our studies on the biosynthesis of the alkaloids in *Corydalis incisa* have reached this point. There remain problems regarding the detailed mechanisms of the biosynthetic pathway of these alkaloids.

Literature Cited

1. Ohwi, J.: *Flora of Japan*, Smithsonian Institution, Washington, D.C., U.S.A., p. 426 (1965).
2. Preininger, V.: *The Alkaloids* (Manske) (edited by A. Brossi) 29, Academic Press Inc., pp. 51-58 (1986).
3. Manske, R.H.F.: *J. Am. Chem. Soc.* 72, 3207 (1950).
4. Tani, C. and Takao, N.: *J. Pharm. Soc. Japan* 82, 594 (1962).
5. Tani, C. and Takao, N.: *J. Pharm. Soc. Japan* 82, 598 (1962).
6. Tani, C., Takao, N. and Takao, S.: *J. Pharm. Soc. Japan* 82, 751 (1962).
7. Tani, C., Takao, N., Takao, S. and Tagahara, K.: *J. Pharm. Soc. Japan* 82, 751 (1962).
8. Takao, N.: *Chem. Pharm. Bull.* 11, 1306 (1963).
9. Takao, N.: *Chem. Pharm. Bull.* 11, 1311 (1963).
10. Takao, N., Takao, S. and Tagahara, K.: *J. Pharm. Soc. Japan* 84, 1212 (1964).
11. Robinson, R.: *The Structural Relation of Natural Products*, Oxford, Clarendon Press, p. 89 (1973).
12. Takao, N., Bersch, H.W. and Takao, S.: *Chem. Pharm. Bull.* 21, 1096 (1973).
13. Takao, N., Kamigauchi, M., Iwasa, K., Tomita, K., Fujiwara, T. and Wakahara, A.: *Tetrahedron Letters* 805 (1974).
14. Takao, N., Kamigauchi, M., Iwasa, K., Tomita, K., Fujiwara, T. and Wakahara, A.: *Tetrahedron* 35, 1099 (1979).
15. Takao, N., Kamigauchi, M. and Iwasa, K.: *Tetrahedron* 35, 1977 (1979).
16. Snatzke, G., Hrubek, J., Hruban Jr., Hruban, L., Horeau, H. and Santavy, F.: *Tetrahedron* 26, 5013 (1970).
17. Takao, N., Bessho, N., Kamigauchi, M., Iwasa, K., Tomita, K., Fujiwara, T. and Fujii, S.:

- Tetrahedron Letters* 1495 (1979).
18. Takao, N., Morita, N., Kamigauchi, M. and Iwasa, K.: *Acta Cryst.* B37, 2043 (1981).
 19. Takao, N.: *Chem. Pharm. Bull.* 19, 247 (1971).
 20. Takao, N. and Iwasa, K.: *Chem. Pharm. Bull.* 27, 2194 (1979).
 21. Iwasa, K., Takao, N., Nonaka, G. and Nishioka, I.: *Phytochemistry* 18, 1725 (1979).
 22. Takao, N. and Iwasa, K.: *Chem. Pharm. Bull.* 21, 1587 (1973).
 23. Takao, N., Iwasa, K., Kamigauchi, M. and Sugiura, M.: *Chem. Pharm. Bull.* 24, 2859 (1976).
 24. Takao, N., Kamigauchi, M. and Okada, M.: *Helv. Chim. Acta* 66, 473 (1983).
 25. Iwasa, K., Kamigauchi, M., Takao, N., Cushman, M., Wong, W.C. and Chen, J.: *Tetrahedron Letters* 6457 (1988).
 26. Iwasa, K., Kamigauchi, M., Takao, N., Cushman, M., Wong, W.C. and Chen, J.: *J. Am. Chem. Soc.* 111, 7925 (1989).