BIOTRANSFORMATION OF THE 13-HYDROXYTETRAHYDROPROTOBERBERINE N-METHYL SALTS BY CALLUS CULTURES OF CORYDALIS SPECIES

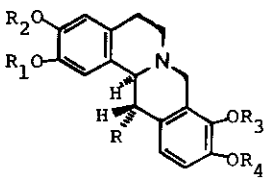
Kinuko Iwasa*, Akiko Tomii and Narao Takao
Kobe Women's College of Pharmacy, Motoyamakita-machi
Higashinada-ku, Kobe 658, Japan

Abstract— Each N-methyl salt of trans- and cis-13-hydroxytetrahydroprotoberberines, ophiocarpine and epiophiocarpine, was biotransformed via corresponding protopine-type alkaloid into a benzindanoazepine.

It has been found that the tetrahydroprotoberberine α-N-methyl salts (e.g. 3c) were transformed in intact plants and callus cultures via the protopines into the benzo[c]phenanthridines.[1] The only naturally occurring trans-13-methyltetrahydroprotoberberine, thalictrifoline (1b R_1= R_2= CH_3, R_3+ R_4= CH_2), and some corresponding cis isomers [e.g. cavidine (2b R_1= R_2= CH_3, R_3+ R_4= CH_2)] have been isolated from natural sources.[2] Both trans- and cis-13-methyltetrahydroprotoberberines (1b and 2b R_1+ R_2= R_3+ R_4= CH_2) are converted by Corydalis incisa plants and its cell cultures via the N-methyl salts to the protopine-type alkaloids which are intermediates in the biosynthesis of the benzo[c]phenanthridines.[1] Two cis-13-hydroxytetrahydroprotoberberines (2a R_1+ R_2= R_3+ R_4= CH_2 and R_1+ R_2 = CH_2, R_3= R_4= CH_3) have been isolated from Corydalis ophiocarpa while the corresponding trans isomer (e.g. 1a) has not been found from natural sources.[3] However, we became interested in the biosynthetic conversion of the cis- and trans-13-hydroxytetrahydroprotoberberines into the other class of alkaloids as demonstrated by the biotransformation of both cis- and trans-13-methyltetrahydroprotoberberines and their N-methyl salts.

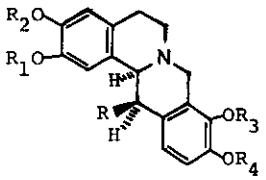
Calluses derived from the stems of Corydalis ophiocarpa, Corydalis ochotensis var. raddeana, and Corydalis platycarpa were used for present study. We have reported that the culture has good biosynthetic capabilities for transformation of exogenous alkaloids.[1b] Each callus from Corydalis ophiocarpa and Corydalis ochotensis var. raddeana was grown on agar medium containing non — labelled (±)-epiophiocarpine α-N-methyl chloride (3a) for 44 days. Bases A, B, C, and D were isolated from the former callus and its medium and base B was isolated from the latter callus and its medium. Alkaloids A, B, C, and D were not found in the callus and its medium grown

in the absence of the exogenous alkaloid under the same conditions. Alkaloid A, mp 179-180°C (MeOH), has absorptions ascribed to a hydroxyl (3600-3200 cm^{-1}) and a carbonyl (1690 cm^{-1}) group in its ir spectrum (nujol). The composition of the base, $C_{20}H_{23}O_6N$, was verified by high resolution mass spectrometry. In the ^1H nmr spectrum, the signal at δ2.22 is assigned to an N-methyl group, the signals at δ3.74 and 3.84 to methoxyl groups and that at δ5.94 to a methylenedioxy group. In the aromatic region of the spectrum, two singlets due to para protons and an AB quartet attributed to ortho protons were observed. The mass spectrum of the base had a parent peak at m/z 385, a base peak at m/z 206, and fragment peaks at m/z 208 and 178. The protopine-type structure (4a) was proposed for base A from the spectral data. Base B, mp 204-205°C (acetone), had a carbonyl absorption at 1660 cm^{-1} in its ir spectrum. In the high resolution mass spectrum, there is a parent peak of composition $C_{21}H_{21}O_6N$. The mass spectrum of base B showed a parent peak at m/z 383, a base peak m/z 178, and the fragment peaks at m/z 206 and 150. The ^1H nmr spectrum had the signal of an N-methyl group at δ1.95, the signals of two methoxyl groups at δ3.84 and 3.96, and that of a methylenedioxy group at δ6.00. The spectrum contained two singlets at δ6.71 and 7.44 assignable to para aromatic protons, and an AB quartet at δ6.79 and 7.70 due to adjacent aromatic protons. The ^{13}C nmr spectrum showed the signals for aliphatic carbons at δ29.69 (CH_2), 42.60($N-CH_3$), 50.84 (CH_2), 55.91 (CH_2), 55.91 (OCH_3), 60.81 (OCH_3), and 101.25 (OCH_2O) and the signals at δ177.0 and 190.95 attributable to carbonyl carbons. Structure (5) is fully compatible with the spectral data. The mercuric acetate oxidation[4] of allocryptopine (4c) produced 13-oxoallocryptopine (5) and its ir, mass, ^1H nmr spectra were identical with those of base B. 13-Oxoallocryptopine hydrochloride (5a)[5] was reduced with $NaBH_4$ to give 13-hydroxyallocryptopine (4a). The ir, mass, and ^1H nmr spectra of the synthetic 13-hydroxyallocryptopine were identical with those of base A. Thus, the structures of bases A and B were confirmed by each synthesis from allocryptopine (4c). Base C, mp 145-147°C ($MeOH-Et_2O$), had bands at 3500-3100 and 1710 cm^{-1} in its ir spectrum, suggesting the presence of a hydroxyl and a carbonyl group, respectively. The high resolution mass spectrum showed a parent peak of composition $C_{21}H_{21}O_6N$. The mass spectrum had a parent peak at m/z 383, a base peak at m/z 338, and the fragment peak at m/z 336 and 177. The ^1H nmr spectrum showed one AB quartet at δ7.60 and 7.06 assigned to ortho aromatic protons, and two singlets at δ6.63 and 6.27 due to para aromatic protons. A methylenedioxy signal at δ5.88, two methoxyl signals at δ3.98 and 3.95, and an N-methyl signal at δ2.20 were apparent in the spectrum. A total area of four



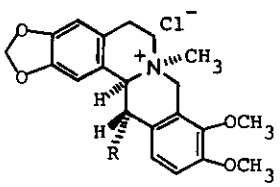
1a R = OH

1b R = CH_3



2a R = OH

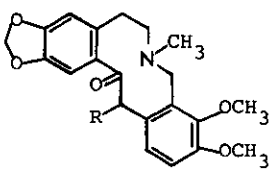
2b R = CH_3



3a R = OH

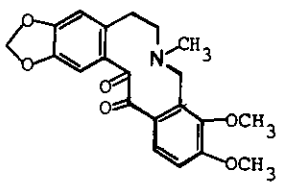
3b R = CH_3

3c R = H



4a R =OH

4c R = H



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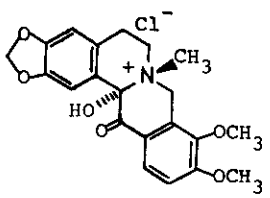
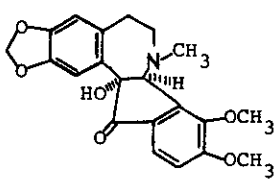


5a



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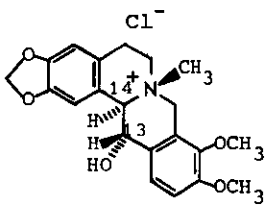

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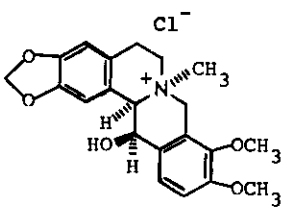

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protons is observed in the δ 2.77-4.58 region. The ^{13}C nmr spectrum had signals due to an N-methyl group (δ 35.58), two methoxyl groups (δ 56.33 and 60.87), two methylene carbons (δ 28.93 and 62.33), a methine carbon (δ 69.22), a quaternary carbon (δ 85.80), and one methylenedioxy group (δ 100.78). The eight signals due to the quaternary carbon and four signals due to the tertiary carbon were observed in the aromatic region. A signal of a carbonyl carbon was appeared at δ 201.93. A sufficient proof for structure elucidation of base C has not yet been provided, although it is apparently not a protoberberine, a protopine, or a benzo[c]phenanthridine type. Base D was identified as the administered material.

To clarify the relationship between bases B and C on the biosynthetic pathway, the biotransformation of 13-oxoallocryptopine (5) was examined. Each callus of Corydalis ophiocarpa, Corydalis ochotensis var raddeana, and Corydalis platycarpa was grown on agar medium containing non-labelled 13-oxoallocryptopine (5) during 35 to 47 days. Bases C and E were isolated from the alkaloid fraction of each callus and its medium. Bases C and E were not found from the same fraction in the absence of the precursor. Base E, mp 198-200°C, had hydroxyl and carbonyl bands at 3570 and 1720 cm^{-1}, respectively, in its ir spectrum ($CHCl_3$). The molecular formula $C_{21}H_{21}O_6N$ for this base was derived by high resolution mass spectral analysis. The mass spectrum had a molecular ion peak at m/z 383, and a base peak at m/z 177. The ^1H nmr spectrum showed an N-methyl signal at δ 2.62, two methoxyl signals at δ 3.93 and 4.00, a one-proton singlet at δ 4.56, and a two-proton AB quartet at δ 5.93 and 5.95 attributable to a methylenedioxy group. The spectrum contained four proton signals in the aromatic region as two singlets at δ 6.56 and 7.29 due to para protons and an AB quartet at δ 7.10 and 7.67 assigned to ortho protons. The ^{13}C nmr spectrum had the signals at δ 33.53 (CH_2), 45.76 ($N-CH_3$), 49.88 (CH_2), 70.80 ($-\overset{|}{C}H$) and 84.52 ($-\overset{|}{C}-$) due to the aliphatic carbons and a signal at δ 202.32 attributable to a carbonyl carbon. The ir, mass, and ^1H nmr spectra of base E were identical with those of a benzindanoazepine-type alkaloid (6).[6] The evidence shows that (±)-epiophiocarpine α-N-methyl salt (3a) was biotransformed via base A (4a) to base B (5) which was then converted into base C and base E (6). The callus from Corydalis ophiocarpa was grown on solid medium containing (±)-[$N-^{13}CH_3$]-epiophiocarpine α-N-methyl chloride (3a* ^{13}C enrichment 90%) for 42 days. Labelled bases A*, B*, C*, D*, and E* were isolated from the alkaloid fraction of the callus and its medium. The incorporation of each base was detected by mass, ^1H nmr and/or ^{13}C nmr analyses. The ^{13}C enrichment of each base was calculated from the intensity of satellites produced in ^1H nmr spectra by spin-spin coupling with ^{13}C (Table 1).

Table 1. Chemical Shifts (δ) of N-Methyl Group and ^{13}C Enrichment from ^{1}H nmr Spectra.

Isolated Alkaloids	Chemical Shifts of N-Methyl Group ($J_{^{13}C-H}$, Hz)	^{13}C Enrichment (%)
Base A•	2.26 d (136)	87
Base B•	1.92 d (136)	88
Base C*	2.21 d (133)	88
Base D*	3.28 d (145)	90
Base E*	2.62 d (135)	90

Base D had $[\alpha]_D^{25}$ + 60° (MeOH). This value was raised to + 142° after several recrystallizations. The callus from Corydalis ophiocarpa was grown on agar medium containing (±)-[N-$^{13}CH_3$]-epiophiocarpine β-N-methyl chloride (7* ^{13}C enrichment 90%). The formation of bases A, B, C, and E was not detected from the alkaloid fraction of the callus and the medium. The callus from Corydalis ochotensis var. raddeana was grown on solid medium containing (±)-[N-$^{13}CH_3$]-epiophiocarpine α-N-methyl chloride (3a* ^{13}C enrichment 90%) for 44 days and 22 days, respectively (experiments 1 and 2) Bases B• and C* and bases A* and B• were isolated from the alkaloid fraction of the callus and the medium in experiments 1 and 2, respectively. However, base E* was not detected in both experiments (Table 2).

Table 2. ^{13}C Enrichment from ^{1}H nmr Spectra.

Isolated Alkaloids	^{13}C Enrichment (%)	
	Experiment 1	Experiment 2
Base A•		83
Base B*	90	90
Base C•	83	
Base D*	90	90

Thus (-)-epiophiocarpine α-N-methyl salt (3a) was biotransformed via 13-hydroxyallocryptopine (4a) into 13-oxoallocryptopine (5) which was then converted base C and a benzindanoazepine-type alkaloid (6), respectively.

Corydalis ophiocarpa cells were grown in liquid suspension culture containing non-labelled (±)-ophiocarpine α-N-methyl chloride (8) with continuous shaking at 25°C for 43 days. Bases B and E were isolated from the alkaloid fraction of the callus and the medium. (±)-Ophiocarpine α-N-methyl salt (8) would be bioconverted via 13-oxoallocryptopine (5) into a benzindanoazepine-type alkaloid (6).

The following conclusions can be drawn from the present work. (a) (±)-Epiophiocarpine α-N-methyl salt (3a) bearing a cis-fused quinolizidine ring was biotransformed

via 13-hydroxyallocryptopine (4a) into 13-oxoallocryptopine (5). (b) (±)-Epiophiocarpine β-N-methyl salt (7) with a trans-fused quinolizidine ring was not be metabolited. (c) The (-)-form of (±)-epiophiocarpine α-N-methyl salt (3a) was transformed and the corresponding (+)-form was not. (d) 13-Oxoallocryptopine (5) was biotransformed into base C and a benzindanoazepine-type alkaloid (6). (e) (±)-Ophiocarpine α-N-methyl salt (8) having a cis-fused quinolizidine ring was biotransformed via 13-oxoallocryptopine (5) into a benzindanoazepine-type alkaloid (6). (f) Both α-N-methyl salts of (±)-epiophiocarpine and (±)-ophiocarpine with a trans- and cis-orientation of the protons, respectively, at C-13 and C-14 were converted into 13-oxoallocryptopine (5) by the cell cultures. (g) The pathway (3a) and (8) ⟶ (4a) ⟶ (5) ⟶ (6) and base C is thus defined. This is the first study on the biotransformation of the 13-hydroxytetrahydroprotoberberine type alkaloids. 13-Oxoallocryptopine and a benzindanoazepine-type alkaloid were first isolated from the callus cultures as the metabolite.

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