Transformations of (±)-Salsolinol into Optically Active O- and/or N-Methylated Derivatives by Several Papaveraceae Plants and Their Tissue-Cultured Cells

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TRANSFORMATIONS OF (±)-SALSOLINOL INTO OPTICALLY ACTIVE *O*- AND/OR *N*-METHYLATED DERIVATIVES BY SEVERAL PAPAVERACEAE PLANTS AND THEIR TISSUE-CULTURED CELLS

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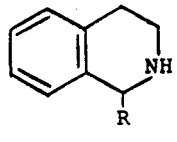
ABSTRACT.—(±)-Salsolinol [**3**], a substance possibly inducing parkinsonism, was biotransformed into optically active *O*- and/or *N*-methylated salsolinols **5**, **6**, and **7** by several Papaveraceae plants and tissue-culture cells derived from these plants. The bioconversion of racemic salsolinol into optically active tetrahydroisoquinolines has never been shown to occur, either in animals or in plants.

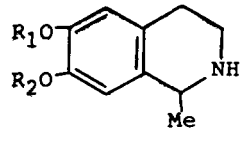
1,2,3,4-Tetrahydroisoquinoline [**1**] (TIQ) and 1-methyl-1,2,3,4-tetrahydroisoquinoline [**2**] (1MeTIQ) have been found in rat and human brain and in some foods (1–4). It is suggested that either TIQ or 1MeTIQ could be one of the candidates of endogenous or environmental factors inducing Parkinson's disease. It has been reported that TIQ produced parkinsonism in some kinds of monkeys (5,6). The metabolism of TIQ and 1MeTIQ is still poorly understood. It is important to elucidate the metabolism of TIQ and 1MeTIQ in order to clarify a mechanism of the manifestation of Parkinson's disease. Salsolinol [**3**], one of the most extensively studied TIQ derivatives, has been detected in human fluids and tissues as well as in some foods and beverages (7). Salsolinol was shown to be methylated almost exclusively to the 7-*O*-monomethyl derivative salsoline [**4**] by rat brain and heart (8,9).

In a preliminary report (10) we have demonstrated that (±)-salsolinol [**3**] was metabolized by various tissue-cultured cells of Papaveraceae to yield 6-*O*-monomethylated salsolinol, isosalsoline [**5**], and a small amount of salsoline [**4**] and isosalsoline [**5**] was further *N*-methylated to provide *N*-methylisosalsoline [**6**].

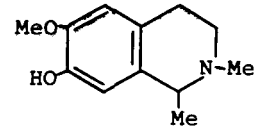
Metabolism of (±)-salsolinol in the tissue-cultured cells of *Corydalis pallida* var.

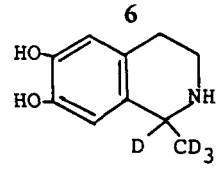


1 R=H
2 R=Me

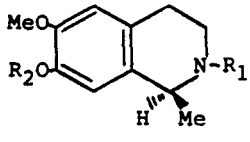


3 R_1=R_2=H
4 R_1=H, R_2=Me
5 R_1=Me, R_2=H
7 R_1=R_2=Me

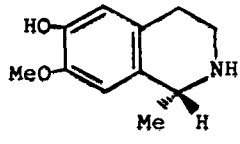




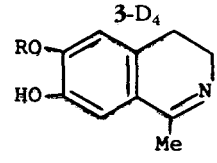
6



(−)-**5** R_1=R_2H
(−)-**6** R_1=Me, R_2=H
(−)-**7** R_1=H, R_2=Me
(−)-**8** R_1=R_2=Me



(+)-**4**



3-D_4

9 R=H
10 R=Me

tenuis Yatabe and *Corydalis incisa* Pers. (Papaveraceae) was further examined. The metabolism was compared with that in the corresponding intact plants.

Callus tissues from *C. pallida* var. *tenuis* and *C. incisa* were grown on an agar medium containing (±)-[1-D and 1-methyl-CD_3]salsolinol [**3**-D_4] hydrochloride at 25° for 3 and 4 weeks, respectively (Table 1). After incubation, the medium and cells were extracted for alkaloids, and the alkaloids were separated by preparative tlc. Isosalsoline-D_4 [**5**-D_4] mixed with a small amount of metabolite was obtained in addition to *N*-methylisosalsoline-D_4 [**6**-D_4] (Table 1). A small amount of metabolite has been concluded to be salsoline-D_4 [**4**-D_4] by ^{1}H-nmr analysis (10). However it was now determined to be salsolidine-D_4 [**7**-D_4] by comparison of retention time in hplc and by the protonated quasi-molecular ions observed in a liquid chromatography/atmospheric pressure ionization-mass spectrometry (lc/api-ms) (Table 2). In the lc/api-ms system

TABLE 1. Administration of Salsolinol D_4 [**3**-D_4] to Cell Cultures of *Corydalis pallida* var. *tenuis* and *Corydalis incisa*.

Cell culture	Dry wt of cells (g)	Substrate applied (mg)	Incubation period (weeks)	Wt of isolated alkaloids mg (D_4 distribution, %)[a]		
				5-D_4[b]	**7**-D_4[b]	**6**-D_4
C. pallida var. *tenuis*	10.1	200 (99)[c]	3	3.6 (99)	1.2 (99)	4.6 (99)
C. incisa	10.7	160 (99)	4	9.6 (52)[d]	1.6 (47)[d]	7.7 (37)[d]

[a]D_4 distribution was determined by ^{1}H-nmr spectra.

[b]This content was calculated from the ratio of two types of signals of the aromatic proton in ^{1}H-nmr spectra.

[c]D_4 distribution, %.

[d]D_4 distribution was reduced due to the mixtures of **5**-D_4 or **6**-D_4 or **7**-D_4 and **5**, or **6** or **7**, respectively.

TABLE 2. Major Observed ms Ions for Metabolites Obtained in Feeding Experiments of Salsolinol-D_4 [**3**-D_4].

Plant or cell culture	Main observed ion (retention time, min)[a] Metabolites		
	5-D_4	**7**-D_4	**6**-D_4
Corydalis pallida var. *tenuis*			
cell cultures	*m/z* 198 (7.8)	*m/z* 212 (13.6)	*m/z* 212 (8.6)
plants	*m/z* 198 (7.8)	*m/z* 212 (13.8)	*m/z* 212 (8.5)
Corydalis incisa			
cell cultures	*m/z* 198 (8.0)	*m/z* 208 (13.0)[b]	*m/z* 208 (8.5)[b]
plants	*m/z* 198 (8.3)	*m/z* 212 (13.8)	*m/z* 212 (8.6)
Macleaya cordata			
cell cultures	*m/z* 198 (8.2)	*m/z* 212 (13.8)	*m/z* 212 (8.6)
plants	*m/z* 198 (8.1)	*m/z* 212 (13.8)	*m/z* 212 (8.5)

[a]Analyses were performed with a Hitachi M-1000 lc/api-ms connected to a Hitachi L-6200 intelligent pump and a Hitachi L-4000 UV detector. Nebulizer and vaporizer temperatures were determined to be 260 and 395°, respectively. Drift voltage was 140 or 150 V. The column was a reverse-phase column. Cosmosil 5 C_{18}-AR (4.6 i.d. × 150 mm). The mobile phase was 0.1 M NH_4OAc containing TFA (pH 4.0), to which MeOH was added under a linear gradient (from 10 to 60%) over a 15 min period. The flow rate was 1 ml/min.

[b]Contents of **6** and **7** are larger than those of **6**-D_4 and **7**-D_4, respectively.

(11), protonated quasi-molecular ions, very useful for identifying compounds, are observed. (±)-Salsolinol was also metabolized to produce isosalsoline [**5**], *N*-methylisosalsoline [**6**], and salsolidine [**7**] in the tissue-cultured cells of *C. pallida* var. *tenuis* and *C. incisa* as well as *Corydalis ochotensis* var. *raddeana*, *Corydalis ophiocarpa*, and *Macleaya cordata* R. Br. (10) (Tables 1 and 2).

When (±)-[1-D and 1-methyl-D_3]salsolinol [**3**-D_4] hydrochloride was fed to intact plants of *C. pallida* var. *tenuis*, *C. incisa*, and *M. cordata*, deuterated metabolites, isosalsoline-D_4 [**5**-D_4], *N*-methylisosalsoline-D_4 [**6**-D_4], and salsolidine-D_4 [**7**-D_4] were obtained from all three kinds of plants (Tables 2 and 3). (±)-Salsolinol [**3**] was metabolized in the live whole plants with similar results to the tissue-cultured cells (Tables 1 and 3).

TABLE 3. Administration of Salsolinol D_4 [**3**-D_4] to Plants of *Corydalis pallida* var. *tenuis*, *Corydalis incisa*, and *Macleaya cordata*.

Plant	Dry wt of plant (g)	Substrate applied (mg)	Feeding period (week)	Wt of isolated alkaloids mg (D_4 distribution, %)[a]		
				5-D_4[b]	**7**-D_4[b]	**6**-D_4
C. pallida var. *tenuis*	3.90	200 (99)[c]	1	6.4 (99)	1.4 (99)	4.0 (99)
C. incisa	4.97	200 (99)	1	5.5 (99)	0.8 (99)	5.0 (99)
M. cordata	6.19	200 (99)	1	3.4 (99)	0.7 (99)	1.5 (99)

[a] D_4 distribution was determined by ^{1}H-nmr spectra.
[b] This content was calculated from the ratio of two types of signals of the aromatic proton in ^{1}H-nmr spectra.
[c] D_4 distribution, %.

In our experiments, *O*-methylation of salsolinol at the 6- rather than at the 7-hydroxy group occurred in both plants and their cultured cells. It has been reported that 7-*O*-methylated salsolinol was obtained in vivo in rat brain or heart, while the 6- and 7-*O*-methylations occurred in vitro with the slices of rat liver (8). These unexpected different results should be further pursued, comparing the metabolism of animals and plants under similar experimental conditions.

Isosalsoline [**5**] (containing a small amount of salsolidine [**7**]) and *N*-methylisosalsoline [**6**], obtained from feeding experiments in both intact plants and tissue-cultured cells, were levorotatory and dextrotatory, respectively, although their values varied (Table 4). Naturally occurring (+)-salsoline [**4**], (−)-salsolidine [**7**], and (−)-carnegine [**8**] have been shown to possess the *R*, *S*, and *S* configurations, respectively (12). Isosalsoline [**5**] and *N*-methylisosalsoline [**6**] obtained from feeding experiments may be shown to possess predominantly enantiomers having the *S* and *R* configurations, respectively. This is the first report of bioconversion of racemic salsolinol into optically active TIQs in animals or in plants. The proportion of the *R* and *S* enantiomers of salsolinol as well as 1MeTIQ in mammalian tissue and foods has been examined (7, 13). It has been suggested that an unequal abundance of the two enantiomers could be due to oxidation of salsolinol [**3**] into 1,2-dehydrosalsolinol [**9**], followed by an asymmetric reduction. It was now shown that a conversion of racemic salsolinol into optically active isosalsoline and *N*-methylisosalsoline does not occur via the intermediacy of 1,2-dehydroisoquinoline. If there is a contribution of 1,2-dehydrosalsolinol [**9**] or 1,2-dehydroisosalsoline [**10**] in the metabolic route, isosalsoline-D_3 and *N*-methylisosalsoline-D_3 [**6**-D_3] instead of isosalsoline-D_4 [**5**-D_4] and *N*-methylisosalsoline-D_4 [**6**-D_4] should be isolated. In lc/api-ms, the protonated quasi-molecular ions (*m/z* 197 and 211)

TABLE 4. [α]D Values of Metabolites Obtained from Feeding Experiments in Plants and in Cell Cultures.

Plant or cell culture	[α]D (*c*) Metabolites	
	5-D_4 and **7**-D_4	**6**-D_4
Corydalis pallida var. *tenuis*		
cell cultures	−8.1 (0.35)	+9.0 (0.40)
plants	−5.7 (0.35)	+9.4 (0.34)
Corydalis incisa		
cell cultures	−1.9 (0.68)	+23.0 (0.43)
plants	−1.8 (0.56)	+7.0 (0.37)
Macleaya cordata		
cell cultures	−6.5 (0.49)[a]	+3.6 (0.52)[a]
plants	−3.8 (0.46)	—
Corydalis ochotensis var. *raddeana*		
cell cultures	−5.8 (1.06)[a]	+15.9 (0.44)[a]
Corydalis ophiocarpa		
cell cultures	−1.0 (0.4)[a]	+13.7 (0.35)[a]

[a]These data are cited from Iwasa *et al.* (10).

due to **5**-D_3 and **6**-D_3 were not observed. A preferential *N*-methylation of the *R* enantiomer of isosalsoline [**5**] might explain the predominance of the *S* enantiomer and the *R* enantiomer in isosalsoline [**5**] and *N*-methylisosalsoline [**6**], respectively. An unequal abundance of the two enantiomers reflects stereoselective formation and is indicative of the participation of an enzymatically mediated biosynthetic reaction.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were determined on a Hitachi M80 instrument at 75 eV. ^{1}H-nmr spectra were obtained on a Varian XL-200 200 MHz spectrometer in $CDCl_3$ solvent. Optical rotations were determined with an Na-D. DPI-SL (Jasco) polarimeter in $CDCl_3$ solvent. Tlc and preparative tlc were performed on Si gel 60F-254 (Merck) glass plates. Hplc was performed using Cosmosil 5C_{18}-AR (4.6 i.d. × 150 mm) reversed-phase column in which temperature was maintained at 40° by a Jasco 860-CO column oven. A Jasco hplc system was employed incorporating 880-02 ternary gradient unit with 880-50 3-line degasser, 880-PU intelligent pump, 875-UV intelligent UV/VIS detector, 807-IT integrator, and 802-SC system controller.

FEEDING EXPERIMENTS IN TISSUE-CULTURED CELLS.—Feeding experiments with (±)-[1-D and 1-methyl-CD_3]-salsolinol [**3**-D_4] hydrochloride in the tissue-cultured cells of *C. pallida* var. *tenuis* and *C. incisa* and extractions were carried out as described in a previous paper (10). The tertiary-alkaloid fractions soluble in Et_2O and $CHCl_3$ were subjected to preparative tlc with MeOH to give **6**-D_4 and a mixture of **5**-D_4 and **7**-D_4. Products **5**-D_4, **6**-D_4, and **7**-D_4 were identified by comparison of their mass spectra, ^{1}H-nmr spectra, and retention times on hplc with those of authentic samples (10). Hplc analysis was carried out as follows. The mobile phases were 0.1 M NH_4OAc adjusted to pH 4.0 with TFA (A) and MeOH (B), and they were delivered in a linear gradient: initial 5 min A-B (9:1): 20 min A-B (6:4): 15 min A-B (9:1). The flow rate was 1 ml/min. The retention times of metabolites **5**-D_4, **6**-D_4, and **7**-D_4 were 7.1, 7.8, and 15.5 min, respectively. Those of the standard compounds **5**, **6**, and **7** were 7.2, 8.0, and 15.6 min, respectively.

FEEDING EXPERIMENTS IN PLANTS.—(±)-[1-D and 1-methyl-CD_3]salsolinol [**3**-D_4] hydrochloride was fed as an aqueous solution into each plant of *C. pallida* var. *tenuis*, *C. incisa*, and *M. cordata* according to the following procedure. Several pieces of each plant were dipped into an aqueous solution of the substrate in small tubes, which were repeatedly refilled with distilled H_2O after the original solution had been absorbed. Then the plants were transferred to the container of distilled H_2O. After 1 week, the plants from the individual feeding experiments were homogenized in H_2O and centrifuged (2300 rpm). The supernatant liquid was separated, and the residue was extracted repeatedly with MeOH and H_2O. Extracts were concentrated and worked up as reported in a previous paper (10). The tertiary-alkaloid fractions solu-

ble in Et_2O and $CHCl_3$ were subjected to preparative tlc with MeOH to give **6**-D_4 and a mixture of **5**-D_4 and **7**-D_4. These products were identified by comparison of their mass spectra, ^{1}H-nmr spectra, and hplc retention times with those of authentic samples (10).

LITERATURE CITED

1. M. Kohno, S. Ohta, and M. Hirobe, *Biochem. Biophys. Res. Commun.*, **140**, 448 (1986).
2. S. Ohta, M. Kohno, Y. Makino, O. Tachikawa, and M. Hirobe, *Biomed. Res.*, **8**, 453 (1987).
3. Y. Makino, S. Ohta, O. Tachikawa, and M. Hirobe, *Life Sci.*, **43**, 373 (1988).
4. T. Niwa, H. Yoshizumi, A. Tatematsu, S. Matsuura, and T. Nagatsu, *J. Chromatogr.*, **493**, 345 (1989).
5. T. Nagatsu and M. Yoshida, *Neurosci. Lett.*, **87**, 178 (1988).
6. M. Yoshida, T. Niwa, and T. Nagatsu, *Neurosci. Lett.*, **119**, 109 (1990).
7. M.S. Benedetti, V. Bellotti, E. Pianezzola, E. Moro, P. Carminati, and P. Dostert, *J. Neural Transm.*, **77**, 47 (1989).
8. M. Bail, S. Miller, and G. Cohen., *Life Sci.*, **26**, 2051 (1980).
9. T.C. Origitano and M.A. Collins, *Life Sci.*, **26**, 2061 (1980).
10. K. Iwasa, M. Kamigauchi, and N. Takao, *Phytochemistry*, **30**, 2973 (1991).
11. M. Sakairi and H. Kambara, *Anal. Chem.*, **60**, 774 (1988).
12. J. Lundstrom, in: "The Alkaloids." Ed. by A. Brossi, Academic Press, New York, 1983, Vol. XXI, p. 302.
13. Y. Makino, Y. Tasaki, S. Ohta, and M. Hirobe, *Biomed. Environ. Mass Spectrom.*, **19**, 415 (1990).

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