Phytochemistry, Vol. 30, No. 9, pp. 2973–2975, 1991
Printed in Great Britain.

0031-9422/91 $3.00+0.00

METABOLISM OF SALSOLINOL BY TISSUE CULTURES OF SOME PAPAVERACEAE

K. IWASA,* M. KAMIGAUCHI and N. TAKAO

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

(*Received in revised form* 15 *February* 1991)

Key Word Index—*Corydalis ochotensis*; *C. opliocarpa*; *Macleaya cordata*; Papaveraceae; tissue cultures; metabolism; isoquinoline alkaloid; salsolinol.

Abstract—(±)-Salsolinol, a substance possibly inducing Parkinsonism and alcoholism, was transformed into the 6- and 7-*O*-monomethylated salsolinols by various plant tissue cultures of the Papaveraceae. Only 6-*O*-methylsalsolinol was further *N*-methylated to provide *N*-methylisosalsoline.

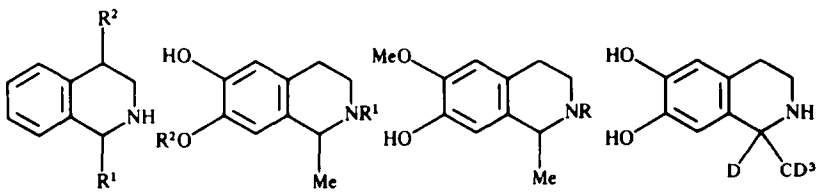
INTRODUCTION

It is known on the one hand that 1,2,3,4-tetrahydroisoquinoline (**1**) (TIQ) is present in parkinsonian as well as normal brain cells, but that the amounts of TIQ in the parkinsonian brain are markedly increased [1, 2]. On the other hand, Hirobe's group has found that the 1-methyl derivative (**2**) of TIQ exists in the human brain but is significantly reduced in the parkinsonian brain [3]. Other relevant findings concerning the presence of TIQ derivatives in animals are that the 4-hydroxy TIQ (**3**) has been detected in rat liver microsomes and rat urine [4], and that 6,7-dihydroxy-1-methylTIQ [salsolinol (**4**)] is present in rat brain [5] and in the urine of parkinsonian patients on L-DOPA treatment [6]. It has been suggested that TIQ derivatives may be possible candidates for inducing parkinsonism [1, 2]. The relationship between salsolinol (**4**) and alcoholism has been frequently discussed [7–9]. Further studies on the metabolism of TIQ derivatives seemed necessary in order to obtain further corroborating evidence for these assumptions.

RESULTS AND DISCUSSION

We began our studies on TIQ metabolism using plant tissue cultures of the Papaveraceae because these calli normally contain isosalsoline (**5**), salsoline (**6**) and *N*-methylisosalsoline (**7**) which are all structurally related to salsolinol (**4**) (Expts 2, 6 and 8) and also because details of the metabolism of TIQ derivatives is unclear in plants. Callus tissues from *Corydalis ochotensis* var. *raddeana* Ohwi was grown on an agar medium containing (±)-salsolinol (**4**) hydrochloride at 25° for three weeks (Expt 1). After incubation, the medium and cells were extracted for alkaloids and the alkaloids separated by preparative TLC. The *O*-methylated salsolinols, **5** and **6**, and *N*-methylisosalsoline (**7**) were obtained. The ^{1}H NMR analysis of crude *O*-methylated salsolinol, the chemical shifts and in particular *C*-methyl doublets, showed that the product was a mixture consisting of *ca* 83% isosalsoline (**5**) (δ 1.41) and 17% salsoline (**6**) (δ 1.47). The presence of **5** and **7** having laevorotatory ($[\alpha]_D$ −5.2) and dextrorotatory ($[\alpha]_D$ +14.7), respectively, was markedly increased compared with that from the control experiment (Expt 2), indicating that **5** and **7** are formed from **4**. A feeding experiment (Expt 3) with the same cultured cells with (±)-[1-D and 1-methyl-CD_3]salsolinol (**8**) hydrochloride (99%-D_4) produced labelled *O*-methylated salsolinol (77%-D_4, 83% isosalsoline and 17% salsoline, in addition to labelled *N*-methylisosalsoline (78%-D_4). Alternatively, when labelled isosalsoline hydrochloride (77%-D_4) obtained from Expt 3 was fed, only labelled *N*-methylisosalsoline (61%-D_4) and recovered isosalsoline (66%-D_4) were obtained (Expt 4). It follows that **4** was converted into **7** via 6-*O*-methylated salsolinol, namely isosalsoline (**5**).

*Author to whom correspondence should be addressed.



1 R^1 = R^2 = H **4** R^1 = R^2 = H **5** R = H **8**
2 R^1 = Me, R^2 = H **6** R^1 = H, R^2 = Me **7** R = Me
3 R^1 = H, R^2 = OH **9** R^1 = R^2 = Me

Table 1. Administration of compounds **4**, **5** and **8** to cell cultures of *Corydalis ochotensis* var. *raddeana*, *C. ophiocarpa* and *Macleaya cordata*

Expt no.	Cell culture	Dry wt of cells (g)	Substrate applied (mg)	[D_4 distribution, %] Wt of isolated alkaloids (mg) **5**+**6**	([α]$_D$) **7**
1	*C. ochotensis*	4.62	**4** 400	35 (−5.2)*	34 (+14.7)
2	*C. ochotensis*	5.20	—	8	6.6
3	*C. ochotensis*	5.80	**8** 200	25 (−5.8)*	5.3 (+15.9)
			[99]	[77]	[78]
4	*C. ochotensis*	5.69	**5** 109	50	10
			[77]	[66]	[61]
5	*C. ophiocarpa*	5.20	**8** 200	32 (−1.0)*	5.6 (+13.7)
			[99]	[77]	[58]
6	*C. ophiocarpa*	5.90	--	5	3.6
7	*M. cordata*	2.24	**4** 400	17 (−11.6)*	24 (+3.3)
8	*M. cordata*	5.00	—	4	2.7
9	*M. cordata*	7.80	**8** 200	8.5 (−6.5)*	22 (+3.6)
			[99]	[88]	[78]

*The ratio between **5** and **6** is given in the text.

A similar experiment (Expt 5) in cultured cells of *C. ophiocarpa* Hook. et Thoms performed with (±)-(**8**) hydrochloride revealed that this substance was similarly converted to its *O*-methylated product (77%-D_4, 90% isosalsoline and 10% salsoline) and *N*-methylisosalsoline (58%-D_4).

In another feeding experiment (Expt 7), using cultured cells of *Macleaya cordata* R. Br, it was found that addition of **4** hydrochloride produced a mixture of two *O*-methylated products (60% isosalsoline and 40% salsoline) and *N*-methylisosalsoline in noticeably increased amounts as compared to the control experiment (Expt 8). Finally, a feeding experiment (Expt 9) with the same cultured cells with (±)-**8** hydrochloride was carried out in order to clarify transformation of **4** into **6**; *O*-methylated salsolinol (88%-D_4, 40% isosalsoline and 60% salsoline) and *N*-methylisosalsoline (78%-D_4) were obtained. There are apparent differences in the ratio of the formation of *O*-methylated salsolinols between calli of the two *Corydalis* species and that of *M. cordata*. It has been reported that salsolinol is mainly converted to its 7-*O*-methylated product in rat brain and heart [10, 11].

Our results demonstrate that (±)-salsolinol (**4**) is converted into the 6- and 7-mono-O-methylated salsolinols, **5** and **6**, and that only **5** is further metabolized into its *N*-methylated congener **7** by the plant cell cultures investigated. Salsolinol is thus similarly *O*-methylated by tissues of plants as well as those of animals [10, 11] and in the former further methylation on the nitrogen occurs. *O*- and *N*-Methylations at this stage have not been demonstrated at this time in plant tissues as well as in intact plants. Because salsolinol and its metabolites have been detected in plants [12], it is reasonable to assume that at least one metabolic pathway in intact plants is similar to that in cell cultures.

EXPERIMENTAL

General. Mps: uncorr. MS: 75 eV; CIMS: *iso*-butane. ^{1}H and ^{13}C NMR: 200 MHz and 125 MHz, respectively, using $CDCl_3$ except where noted. TLC and prep. TLC: silica gel 60F-254 (Merck) and silica gel 250F (Baker).

Preparations of (±)-salsolinol (**4**). Dopamine HCl (5 g) in H_2O (30 ml) was adjusted to pH 4.5 and cold MeCHO (1.6 ml) was added. The mixt. was left at room temp. for 3 days. After evapn to dryness, the product was dissolved in EtOH and $MeCO_2Et$ was added. The soln was concd and allowed to stand overnight to give salsolinol HCl (4.46 g) which was recrystallised from MeOH–EtOH–Me_2CO, mp 218–219°. ^{1}H NMR (CD_3OD): δ 1.62 (3H, *d*, J = 6.7 Hz, Me), 2.96 (2H, *m*), 3.38 (1H, *m*), 3.50 (1H, *m*), 4.45 (1H, *q*, J = 6.7 Hz, H-1), 6.61, 6.66 (1H, each, *s*, H-5, H-8). ^{13}C NMR (CD_3OD): δ 19.79 (Me), 25.81 (C-4), 40.97 (C-3), 52.40 (C-1), 113.57 (C-8), 116.20 (C-5), 123.49, 125.45 (C-1a, C-4a), 145.96, 146.60 (C-6, C-7). EIMS *m/z* (rel. int.): $[M]^+$ 179 (5), 178 (11), 164 (100). CIMS *m/z* $[M+1]^+$ 180.

Preparation of (±)-[1-D and 1-methyl-CD_3]-salsolinol [8]. Dopamine HCl (3 g) in H_2O (20 ml) was adjusted to pH 4.5 and cold CD_3CDO (99%-D_4, Aldrich) (1 g) was added. The mixt. was allowed to stand at room temp. for 4.5 days. After evapn to dryness, the residue was dissolved in MeOH–EtOH. The soln was concd and Me_2CO was added to give 8 HCl (1.93 g). A mixt. of **8** HCl and dopamine HCl (1, 4 g) was obtained from the mother liquid. **8** HCl: mp 218–219°. EIMS *m/z* (rel. int.): $[M]^+$ 183 (4.2), 182 (3.5), 181 (4.8), 165 (100). CIMS *m/z* $[M+1]^+$ 184.

O- and N-Methylation of salsolinol [preparations of **5**–**7** *and* **9**]. Compound **4** (500 mg) in MeOH (10 ml) was treated with CH_2N_2–Et_2O (10 ml, *ca* 120 mg CH_2N_2). The soln was allowed to stand overnight at room temp. Diluted HCl was then added and the solvent evapd to dryness. The residue was dissolved in H_2O, basified with NaOH and extracted with Et_2O (fr. 1, 203 g). The aq. phase was acidified with conc. HCl and then basified with conc. NH_4OH and extracted with $CHCl_3$ (fr. 2, 138 mg). Prep. TLC with MeOH of fr. 2 gave a 5:2 mixt. of **5** and **6** (30 mg) and a 5:3 mixt. of **7** and 7,*N*-dimethyl salsolinol (**9**) (62 mg). A mixt. of **5** and **6** (13 mg) was subjected to prep. TLC with MeOH to give **5** (11 mg) and **6** (2 mg). Compound **5**: ^{1}H NMR (assigned from two types of signals): δ 1.41 (3H, *d*, J = 6.7 Hz, Me), 3.88 (3H, *s*, OMe), 4.04 (1H, *q*, J = 6.7 Hz, H-1), 6.59, 6.74 (1H, each, *s*, H-5, H-8). ^{13}C NMR (assigned from two types of signals): δ 22.62 (Me), 29.48 (C-4), 41.92 (C-3), 51.18 (C-1), 55.93 (OMe), 111.11, 111.90

(C-5, C-8). EIMS *m/z* (rel. int.): $[M]^+$ 193 (6), 192 (9), 178 (100), 163 (19). CIMS *m/z* $[M+1]^+$ 194. *Compound* **6**: mp 139–143°, 197–198° (HCl). ^{1}H NMR: δ 1.47 (3H, *d*, J = 6.7 Hz, Me), 3.86 (3H, *s*, OMe), 4.07 (1H, *d*, J = 6.7 Hz, H-1), 6.61, 6.64 (1H, each, *s*, H-5, H-8). ^{13}C NMR: δ 22.84 (Me), 29.21 (C-4), 41.72 (C-3), 51.30 (C-1), 56.03 (OMe), 108.31 (C-8), 114.73 (C-5), 127.47 (C-4a), 131.66 (C-1a), 144.03, 145.12 (C-6, C-7). EIMS *m/z* (rel. int.): $[M]^+$ 193 (7), 192 (8), 178 (100), 163 (19). CIMS: *m/z* $[M+1]^+$ 194. *Compounds* **7** and **9**. EIMS (mixt. of **7** and **9**) *m/z* (rel. int.): $[M]^+$ 207 (1.6), 206 (3.5), 192 (100), 177 (20). CIMS (mixt. of **7** and **9**) *m/z* $[M+1]^+$ 208. *Compound* **7**. ^{1}H NMR (assigned from two types of signals): δ 1.35 (3H, *d*, J = 6,5 Hz, Me), 2.45 (3H, *s*, NMe), 3.49 (1H, *q*, J = 6.5 Hz, H-1), 3.85 (3H, *s*, OMe), 6.45, 6.67 (1H, each, *s*, H-5, H-8). ^{13}C NMR (assigned from two types of signals): δ 19.62 (Me), 27.64 (C-4), 42.84 (NMe), 49.19 (C-3), 55.87 (OMe), 58.62 (C-1), 110.55 (C-8), 112.78 (C-5), 125.19 (C-4a), 132.34 (C-1a), 143.86, 145.09 (C-6, C-7). *Compound* **9**: ^{1}H NMR (assigned from two types of signals): δ 1.37 (3H, *d*, J = 6.5 Hz, Me), 2.46 (3H, *s*, NMe), 3.55 (1H, *q*, J = 6.5 Hz, H-1), 3.85 (3H, *s*, OMe). ^{13}C NMR (assigned from two types of signals): δ 19.86 (Me), 27.14 (C-4), 42.91 (NMe), 48.78 (C-3), 55.99 (OMe), 58.74 (C-1), 109.18 (C-8), 114.24 (C-5), 126.65 (C-4a), 130.99 (C-1a), 143.94, 145.13 (C-6, C-7). Fr. 1 was subjected to prep. TLC with MeOH to give 6,7-dimethylsalsolinol (**10**) (92 mg) and 6,7-*N*-methylated salsolinol (**11**) (73 mg). *Compound* **10**. mp 82–84°. ^{1}H NMR: δ 1.45 (3H, *d*, J = 6.7 Hz, Me), 2.6–2.9 (2H, *m*), 3.02 (1H, *m*), 3.28 (1H, *m*), 3.85 (6H, *s*, 2 × OMe), 4.08 (1H, *q*, J = 6.7 Hz, H-1), 6.58, 6.63 (1H, each, *s*, H-5, H-8). ^{13}C NMR: δ 22.89 (Me), 29.57 (C-4), 41.86 (C-3), 51.26 (C-1), 55.01, 56.01 (2 × OMe), 109.09 (C-8), 111.81 (C-5), 126.85 (C-4a), 132.45 (C-1a), 147.29, 147.36 (C-6, C-7). EIMS *m/z* (rel. int.): $[M]^+$ 207 (6), 206 (13), 192 (100). CIMS *m/z* $[M+1]^+$ 208. *Compound* **11**. Mp 116–118°. ^{1}H NMR: δ 1.4 (3H, *d*, J = 7.0 Hz, Me), 2.51 (3H, *s*, NMe), 2.66 (1H, *m*), 2.80 (2H, *m*), 3.05 (1H, *m*), 3.57 (1H, *q*, J = 7.0 Hz, H-1), 3.90 (6H, *s*, 2 × OMe), 6.62, 6.64 (1H, each, *s*, H-5, H-8). ^{13}C NMR: δ 19.8 (Me), 27.6 (C-4), 43.02 (NMe), 49.0 (C-3), 58.71 (C-1), 55.84, 56.0 (2 × OMe), 109.94 (C-8), 111.23 (C-5), 126.02 (C-4a), 131.75 (C-1a), 147.26, 147.29 (C-6, C-7). EIMS *m/z* (rel. int.): $[M]^+$ 221 (2.1), 220 (3.0), 206 (100). CIMS *m/z* $[M+1]^+$ 222.

Callus cultures and extraction. Each callus was subcultured on Murashige and Skoog's (MS) agar medium fortified with 2,4-D (1 mg l^{-1}), kinetin (0.1 mg l^{-1}), and yeast extract (0.1%). Substrates were dissolved in H_2O (2–4 ml) and introduced into 100 ml conical flasks (20) containing 40 ml of autoclaved MS medium through a sterile bacterial filter. Calli (*ca* 4–5 g) were transferred to each conical flask and incubated at 25° in the dark for 3 weeks. In Expt 9, 1.6 l of MS medium was used and calli incubated for 5 weeks. Cells and solid medium were sepd and extracted with H_2O-MeOH several times at room temp. and 50°. Extracts were worked-up as reported in ref. [13]. The tertiary-alkaloid frs sol. in Et_2O and $CHCl_3$ were subjected to prep. TLC with MeOH to give a mixt. of **5** and **6** and **7**. Products, **5** and **7**, from Expt 1: ^{1}H and ^{13}C NMR spectra of **5** and **7** were identical with those of synthetic compounds. *Compound* **5**: mp 124–125°. IR ν^{CHCl_3} cm^{-1}: 3560 (OH). $[\alpha]_D$ −5.2° ($CHCl_3$; *c* 2.17). EIMS *m/z* (rel. int.): $[M]^+$ 193 (7), 192 (24), 178 (100), 163 (19). CIMS *m/z* $[M+1]^+$ 194. *Compound* **7**: mp 144–146°. IR ν^{CHCl_3} cm^{-1}: 3560 (OH), 2800 cm^{-1} (NMe). $[\alpha]_D$ + 14.7° ($CHCl_3$; *c* 1.1). EIMS *m/z* (rel. int.): $[M]^+$ 207 (1.5), 206 (2.8), 192 (100), 177 (18). CIMS *m/z* $[M+1]^+$ 208.

Deuterated products from Expts 3–5 and 9. The deuterium distribution of the products was determined by ^{1}H NMR (Table 1). *Compounds* **5** and **6**. Mixt. from Expt 3, EIMS *m/z* (rel. int.): $[M]^+$, 197 (6), 179 (100), 164 (13). CIMS *m/z* $[M+1]^+$ 198. *Compound* **7**. From Expt 3, EIMS *m/z* (rel. int.): $[M]^+$ 211 (1.7), 193 (100), 178 (17), 149 (5). CIMS *m/z*: $[M+1]^+$ 212. The MS of deuterated products from Expts 4, 5 and 9 were identical to those from Expt 3.

REFERENCES

1. Niwa, T., Takeda, N., Kaneda, N., Hashizume, Y. and Nagatsu, T. (1987) *Biochem. Biophys. Res. Commun.* **144**, 1084.
2. Kohno, M., Ohta, S. and Hirobe, M. (1986) *Biochem. Biophys. Res. Commun.* **140**, 448.
3. Ohta, S., Kohno, M., Makino, Y., Tachikawa, O. and Hirobe, M. (1987) *Biomed. Res.* **8**, 453.
4. Ohta, S., Tachikawa, O., Makino, Y., Tasaki, Y. and Hirobe, M. (1990) *Life Sci.* **46**, 599.
5. Nesterick, C. A. and Rahwan, R. G. (1979) *J. Chromatogr. Biomed. Appl.* **164**, 205.
6. Sandler, M., Carter, S. B., Hunter, K. R. and Stern, G. M. (1973) *Nature* **241**, 439.
7. Davis, V. E. and Walsh, M. J. (1970) *Science* **167**, 1005.
8. Cohen, G. and Collins, M. (1970) *Science* **167**, 1749.
9. Collins, M. A. and Bigdeli, M. G. (1975) *Life Sci.* **16**, 585.
10. Ball, M., Miller, S. and Cohen, G. (1980) *Life Sci.* **26**, 2051.
11. Origitano, T. C. and Collins, M. A. (1980) *Life Sci.* **26**, 2061.
12. Lundstrom, J. (1983) in *The Alkaloids* Vol. XXI (Brossi, A., ed.), p. 255. Academic Press, New York.
13. Iwasa, K., Kamigauchi, M., and Takao, N. (1987) *Arch. Pharm.* **320**, 693.