Journal of Natural Products
Vol. 51, No. 6, pp. 1232-1235, Nov-Dec 1988

BIOTRANSFORMATION OF THE PROTOBERBERINES INTO BENZINDANOAZEPINE- AND SPIROBENZYLISOQUINOLINE-TYPE ALKALOIDS BY TISSUE CULTURES OF SEVERAL *CORYDALIS* SPECIES

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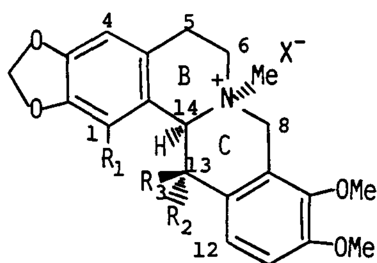
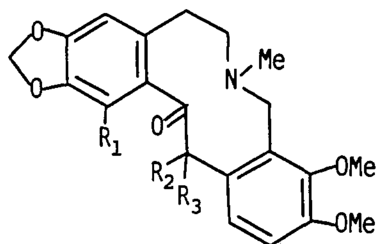
ABSTRACT.—It was demonstrated that a (−)-13-hydroxyprotoberberine *N*-methyl salt with the *cis*-quinolizidine system and bearing a C-1 methoxyl can be biotransformed into a benzindanoazepine via the 13-oxoprotopine analogue by cell cultures of several *Corydalis* species. In the corresponding protoberberine series devoid of a substituent at C-1, the (−) isomer is also converted effectively into the benzindanoazepine- and spirobenzylisoquinoline-type alkaloids.

We have previously demonstrated the bioconversion in callus cultures of *Corydalis* spp. (Fumariaceae) of the *N*-methyl quaternary salts of (±)-epiophiocarpine [**1**] and (±)-ophiocarpine [**2**] via 13-hydroxyallocryptopine [**3**] into 13-oxoallocryptopine [**4**], which is itself transformed into alkaloids **5** and **6** of the spirobenzylisoquinoline and benzindanoazepine types, respectively (1). It has been suggested that the (−) form of the epiophiocarpine *N*-methyl salt [**1**] would undergo conversion to 13-hydroxyallocryptopine [**3**] (1).

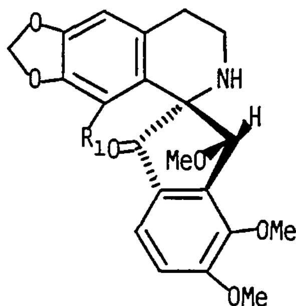
First experiments with the (−) enantiomer of **1** were undertaken in parallel with those with the racemic form. By comparison of experiments 1 and 3 or 2 and 4 (Table 1), it is shown that the (−) isomer of (−) and (+) forms of **1** is converted efficiently into



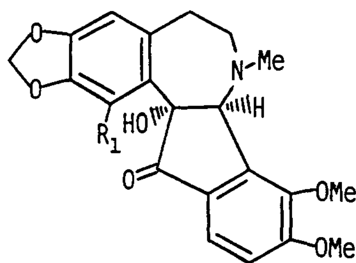
1 $R_1=R_3=H$, $R_2=OH$
2 $R_1=R_2=H$, $R_3=OH$
7 $R_1=OMe$, $R_2=OH$, $R_3=H$



3 $R_1=R_2=H$, $R_3=OH$
4 $R_1=H$, $R_2+R_3=O$
8 $R_1=OMe$, $R_2+R_3=O$



5 $R_1=H$
10 $R_1=OMe$



6 $R_1=H$
9 $R_1=OMe$

TABLE 1. Feeding Experiments in Liquid or Solid Medium Tissue Cultures of Several *Corydalis* Species.

Expt. No.	Cell Culture	Wt of dry cell (g)	Substrate and its amount (mg)	Amount of Medium (liters)	Incubation period (days)	Wt of Isolated Alkaloids (mg)						
						3	**4**	**5**	**6**	**1** or **7**	**8**	**9**
1	*Corydalis pallida* var. *tenuis*	25.0	(±)-**1** 500	4	41	24	11	3	5	142[c]		
2	*Corydalis ophiocarpa*[a]	4.9	(±)-**1** 300	2	40	30	10	1	6	18		
3	*C. pallida* var. *tenuis*[a]	11.3	(−)-**1** 250	2	39	22	8	2	8	70		
4	*C. ophiocarpa*[a]	3.8	(−)-**1** 150	1.2	39	23	10	5	7	18		
5	*C. ophiocarpa*[a]	7.5	(−)-**7** 375	2	43					37	10	4
6	*Corydalis platycarpa*[b]	19.6	(−)-**7** 600	3.2	16					315	15	5
7	*C. platycarpa*[a]	21.0	(−)-**7** 540	4	27					266	19	6
8	*C. pallida*[b] var. *tenuis*	7.0	(−)-**7** 600	3.2	19					31	4	4
9	*Corydalis ochotensis*[b] var. *raddeana*	19.9	(−)-**7** 582	3.6	14					96	6	—
10	*C. ochotensis* var. *raddeana*	20.0	(−)-**7** 592	3.2	28					282	17	—

[a]Solid medium.
[b]Liquid medium.
[c]This is present as 47% optical purity containing an excess of the (+) form.

the four alkaloids, and the (+) isomer is, for practical purposes, ineffective. This is in agreement with a previous suggestion (1).

In order to establish the validity and range of the conversions of the protoberberine skeleton into the spirobenzylisoquinoline or benzindanoazepine skeleton, the transformation of the corresponding protoberberinium chloride salt incorporating a methoxyl substituent at C-1 was examined. The callus from *Corydalis ophiocarpa* Hook. et Thoms. was grown on agar medium containing (−)-1-methoxyepiophiocarpine *N*-methyl chloride [7], which possesses a *cis*-fused quinolizidine system, for a period of 43 days (experiment 5). Compound **7** was found to possess a *cis*-fused quinolizidine and a *trans* configuration of the protons at C-13 and C-14 by comparison of the ^{13}C- and ^{1}H-nmr spectra with those of **1** and **2** (Table 2). At the end of this time, the spirobenzylisoquinoline alkaloid **10** was not detected. However, the 13-oxoprotopine **8** as well as the benzindanoazepine **9** were isolated.

TABLE 2. ^{13}C- and ^{1}H-nmr Data for the *N*-Methyl Salts of Protoberberines (2).

N-Methyl Salt	B/C-fused	^{13}C-nmr, δ ppm		^{1}H-nmr, δ ppm (*J*, Hz)
		C-6	N-Me	H-14
1	*cis*	54.2	52.0	4.42 dd (8.4, 1.2)
	trans	63.6	40.5	4.73 d (9.0)
2	*cis*	55.3	52.4	4.77 dd (4.0, 1.5)
	trans	63.6	44.2	5.04 d (3.0)
7		54.0	52.8	4.88 d (8.0, 1.0)

In a second experiment, the callus of *Corydalis platycarpa* Makino was cultivated both in liquid and in solid media containing salt **7** for 16 and 27 days, respectively (experiments 6 and 7). Again bases **8** and **9** were isolated in each instance.

In a third experiment, the callus from *Corydalis pallida* var. *tenuis* Yatabe was grown in liquid medium containing salt **7** for 19 days (experiment 8). Here also, bases **8** and **9** were obtained from the alkaloidal fraction.

In our fourth and last experiment, callus tissue from *Corydalis ochotensis* var. *raddeana* Ohwi was grown in a liquid medium containing salt **7** for 14 days (experiment 9). In this instance, only the 13-oxoprotopine **8** was obtained. Though **7** was incubated on agar medium for 28 days, **9** was still not detected (experiment 10).

We conclude that (−)-1-methoxyepiophiocarpine *N*-methyl chloride [7] with the *cis*-quinolizidine system can be transformed by several *Corydalis* spp. into the corresponding 13-oxoprotopine **8**, which may be further converted into benzindanoazepine **9**. The fact that the spiro compound **10** was not observed may be due to steric interaction of the C-1 methoxyl with the ring-C substituent in such a structure. We have presently demonstrated that protoberberines bearing a C-1 methoxyl can be biotransformed into benzindanoazepines via the 13-oxoprotopine analogue. This result parallels that obtained at the present time and previously (1) in the protoberberine series devoid of a C-1 substituent. The fact that conversion of the 13-hydroxyprotoberberine *N*-methyl salt into the spirobenzylisoquinoline or benzindanoazepine type alkaloid was established in several kinds of cell cultures suggests strongly the generality of these ring rearrangements including migration of the methyl groups from *N* to *O* in vivo.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. Ir spectra were recorded on an EPI-G2 (Hitachi) spectrophotometer. Mass spectra were determined on an Hitachi M80 instrument at 75 eV. Isobutane gas was used for chemical ionization. ^{13}C- and ^{1}H-nmr spectra were obtained

on a Varian XL-200 spectrometer operating at 50.3 MHz and 200.06 MHz, respectively. Chemical shifts are reported in ppm relative to TMS as an internal standard. Preparative tlc was performed on Si gel 60F-254 Merck glass plates. Optical rotations were measured using a DIP-SL (JASCO) polarimeter.

(±)-Epiophiocarpine *N*-methyl chloride [**1**] was prepared under the same conditions as described in a previous paper (2).

(−)-Epiophiocarpine *N*-methyl chloride [**1**] was prepared according to the procedure of Ohta *et al.* (3): [α]D −171° (c = 0.36, MeOH) [lit. (3) −169.5° (c = 0.99, EtOH)].

(−)-1-Methoxyepiophiocarpine *N*-methyl chloride [**7**] was prepared from (−)-α-narcotine via (−)-α-narcotinediol according to the procedure of Ohta *et al.* (4).

(−)-α-*Narcotinediol*.—Mp 130–132° [lit. (4) 133–134°]; ir ($CHCl_3$)3600–2300 cm^{-1} (br, OH); ms *m/z* 220 (100 base peak), cims *m/z* $[M+1]^+$ 418; [α]D +55.1° (c = 0.74, $CHCl_3$) [lit. (4) +63.5° (c = 1.00, $CHCl_3$)]; ^1H nmr ($CDCl_3$) δ 2.0 (3H, s, NMe), 2.35–2.5 (1H, m), 2.55–2.9 (2H, m), 3.1–3.23 (1H, m), 3.88, 3.90, and 4.12 (each 3H, s, 3 × OMe), 4.05 (1H, d, J = 8.5 Hz, H-1), 4.58 and 4.96 (each 1H, d, J = 11.5 Hz, CH_2OH), 4.74 (1H, d, J = 8.5 Hz, H-9), 5.93 and 5.94 (each 1H, d, J = 1.5 Hz, OCH_2O), 6.42 (1H, s, H-5), 7.02 and 7.38 (each 1H, d, J = 8.6 Hz, H-2′ and H-3′); ^{13}C nmr ($CDCl_3$) δ 27.24 (C-4), 44.18 (NMe), 49.95 (C-3), 54.74 (CH_2OH), 55.87, 59.64, and 61.85 (3 × OMe), 65.53 (C-1), 73.73 (C-9), 100.87 (OCH_2O), 102.93 (C-5), 112.31 (C-3′), 118.61 (C-1a), 122.49 (C-2′), 130.74 (C-6′), 134.05 (C-4a), 134.54 (C-1′), 136.63 (C-7), 141.65 (C-8), 146.58 (C-5′), 148.32 (C-6), 151.77 (C-4′).

(−)-*1-Methoxyepiophiocarpine* N-*methyl chloride* [**7**].—Mp 243–246° dec [lit. (4) 241–242° dec]; ir (Nujol) 3160 cm^{-1} (br OH); [α]D −174.4° (c = 0.41, EtOH) [lit. (4) −191.5° (c = 1.00, EtOH)]; ^1H nmr (CD_3OD) δ 3.1–3.3 (2H, m), 3.23 (3H, s, NMe), 3.4–3.6 (1H, m), 3.66–3.84 (1H, m), 3.89, 3.91, and 4.05 (each 3H, s, 3 × OMe), 4.77 (1H, d, J = 8.0 Hz, H-13), 4.88 (1H, dd, J = 8.0, 1.0 Hz, H-14), 4.95 and 4.97 (each 1H, d, J = 15.5 Hz, H-8), 5.99 and 6.00 (each 1H, d, J = 1.0 Hz, OCH_2O), 6.54 (1H, s, H-4), 7.16 and 7.29 (each 1H, d, J = 8.5 Hz, H-11 and H-12); ^{13}C nmr (CD_3OD) δ 24.43 (C-5), 52.84 (NMe), 54.0 (C-6), 56.53, 60.34, and 61.31 (3 × OMe), 61.78 (C-8), 67.39 (C-14), 71.56 (C-13), 102.94 (OCH_2O), 104.19 (C-4), 114.74 (C-11), 116.78 (C-1a), 121.35 (C-8a), 124.56 (C-12), 125.13 (C-4a), 128.41 (C-12a), 136.76 (C-1), 142.82 (C-2), 146.10 (C-10), 151.73 (C-3), 153.64 (C-9).

CALLUS CULTURES AND EXTRACTION.—Each callus of *Corydalis* spp. was subcultured on Murashige and Skoog's agar medium fortified with 2,4-dichlorophenoxyacetic acid (1 mg/liter), kinetin (0.1 mg/liter), and yeast extract (0.1%). The substrate was dissolved in H_2O and introduced into 100-ml Erlenmeyer flasks, which contained liquid medium without agar or solid medium (each ca. 40 ml) containing the same components as the medium used for subculture, through a sterile bacterial filter. The callus (ca. 3.5–4.0 g) was transferred into each Erlenmeyer flask and then incubated in a liquid or solid medium at 23° in the dark for the appropriate time period (Table 1). The rotary shaker (100 rpm) was used for suspension cultures. Extraction was carried out as shown in earlier work (5). The tertiary basic fractions were subjected to preparative tlc using C_6H_6-Et_2O (7:3) to give **5**, **6**, and **9**. An extract from the band having the low R_f value in tlc using C_6H_6-Et_2O (7:3) was further purified by preparative tlc (MeOH) to afford **3**, **4**, and **8**. The administered material was recovered from the quaternary-alkaloid fraction.

Biotransformation products **3**, **4**, **5**, and **6** were identical with the samples previously isolated (1).

Compound **8**.—Mp 204–205° (from MeOH); ir ($CHCl_3$) 1678 (C=O) cm^{-1}; ^1H nmr δ 1.91 (3H, s, NMe), 3.82, 3.90, and 4.02 (each 3H, s, 3 × OMe), 5.95 and 5.97 (each 1H, br s, OCH_2O), 6.42 (1H, s, Ar-H), 6.89 and 7.32 (each 1H, d, J = 8.3 Hz, Ar-H); ms *m/z* (rel. int.) $[M]^+$ 413 (5), 206 (100), 178 (71); cims *m/z* $[M+1]^+$ 414; hrms *m/z* $[M]^+$ 413.1480 (calcd for $C_{22}H_{23}NO_7$, 413.1473); 206.0629 (calcd for $C_{11}H_{10}O_4$, 206.0579), 178.0658 (calcd for $C_{10}H_{10}O_3$, 178.0629).

Compound **9**.—Mp 211–214° (from MeOH); ir ($CHCl_3$) 3400 (br OH), 1718 (C=O) cm^{-1}; ^1H nmr ($CDCl_3$), δ 2.82 (3H, s, NMe), 3.92, 4.00, and 4.13 (each 3H, s, 3 × OMe), 4.63 (1H, s, N-CH-Ar), 5.95 and 5.97 (each 1H, d, J = 1.4 Hz), 6.35 (1H, s, Ar-H), 7.05 and 7.63 (each 1H, d, J = 8.4 Hz, Ar-H); ms *m/z* (rel. int.) $[M]^+$ 413 (41), 207 (100); cims *m/z* $[M+1]^+$ 414; hrms $[M]^+$ 413.1477 (calcd for $C_{22}H_{23}NO_7$, 413.1473), 207.0645 (calcd for $C_{11}H_{11}O_4$, 207.0656).

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Received 5 July 1988