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Biotransformation of Phthalideisoquinoline Alkaloids by Corydalis Tissue Cultures

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The phthalideisoquinolines (–)-α- and (–)-β-narcotine and (–)-β-hydrastine are metabolized to hydrocotarnine and hydrohydrastine, respectively, by Corydalis tissue cultures.

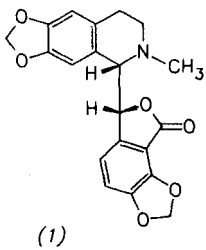
Biotransformation von Phthalidisochinolin-Alkaloiden durch Corydalis Zellkulturen

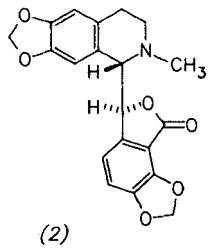
Die Phthalidisochinolinalkaloide (–)-α- und (–)-β-Narkotin und (–)-β-Hydrastin werden durch Corydalis Zellkulturen zu Hydrocotarnin und Hydrohydrastin metabolisiert.

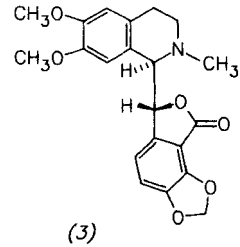
Biotransformations of 13-oxoprotopines to the spirobenzylisoquinoline- and benzindanoazepine-type alkaloids by cell cultures of *Corydalis* species have been reported[1]. Addition of the substrate to cell cultures had led to products not normally present in cell cultures and even in intact plants in certain cases[1, 2].

We have now investigated the biotransformation of phthalideisoquinoline alkaloids into other classes of alkaloids. The phthalideisoquinoline alkaloids, (+)-bicuculline **(1)** and (+)-adlumine **(2)**, have been isolated from *C. ochotensis var. raddeana*[3], **1** from *C. platycarpa*[4] and (–)-adlumine **(3)** from *C. ophiocarpa*[5]. We used (–)-α- and (–)-β-narcotine **(4** and **5)** and (–)-β-hydrastine **(6)** for biotransformation experiments by cell cultures of these plants. Hydrocotarnine **(7)**, meconine **(9)**[6], 1-methylcorypalline **(10)**[7], and N-nor-1-methylcorypalline **(11)**[7] were isolated from the calluses, to which **4** or **5** were administered besides a mixture[6] of **4** and **5** (Exper. 1 and 3–6 in Tab. 1).

In Exper. 2, only **4** and **7** were isolated. From calluses, to which **6** was fed, hydrohydrastinine **(8)**, **9**[6], **10**[7], and **11**[7] were obtained (Exper. 7–9). These experiments indicate that (–)-α- or (–)-β-narcotine and (–)-β-hydrastine can be metabolized to hydrocotarni-


(1)


(2)


(3)

**) Dedicated to Prof. Dr. Maurice Shamma, The Pennsylvania State University, USA, on the occasion of his 60th birthday.

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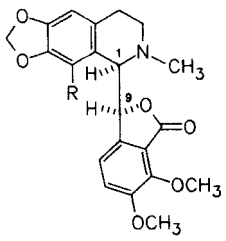
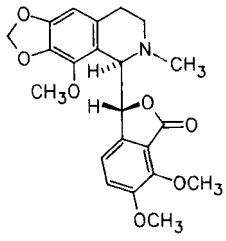
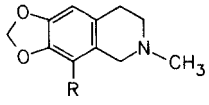
ne and hydrohydrastinine, respectively, by corydalis tissue cultures *via* reductive cleavage of the C-1 and C-9 bond. By a feeding experiment under mild conditions (Exper. 2) and by blank experiments, this conversion was confirmed to be due to an enzyme reaction. (–)-α- and (–)-β-narcotine were much more readily metabolized than (–)-β-hydrastine.



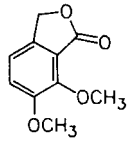
(4) R = OCH_3
(6) R = H



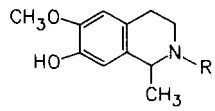
(5)



(7) R = OCH_3
(8) R = H



(9)



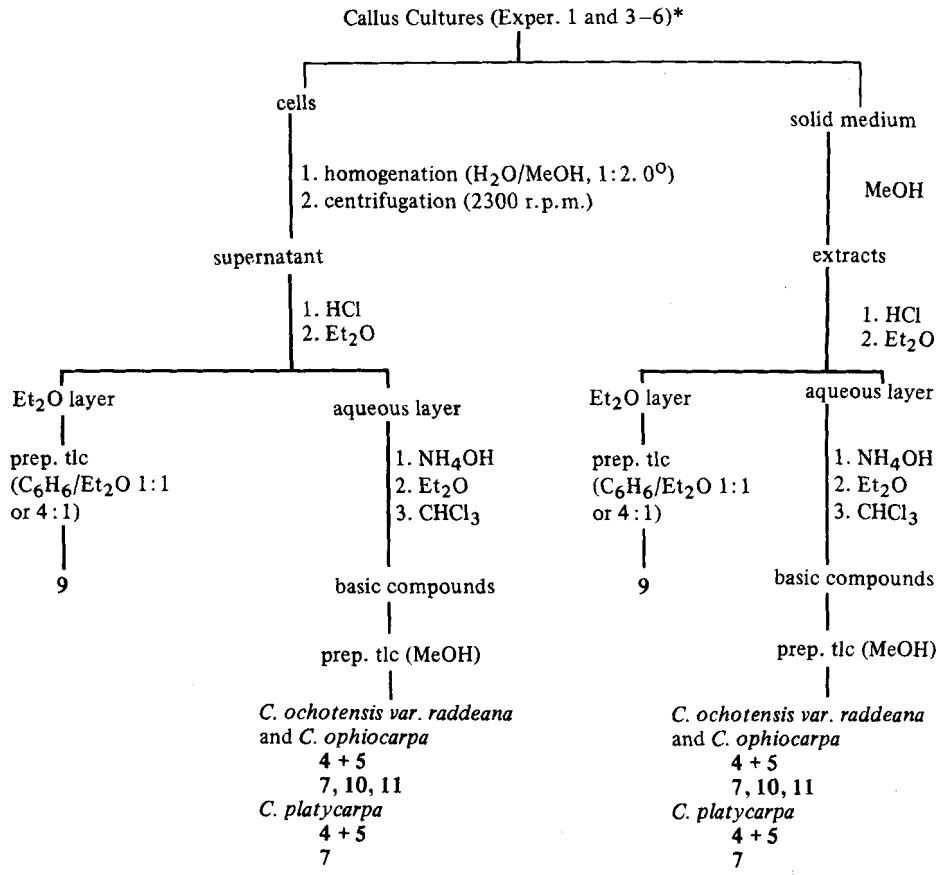
(10) R = CH_3
(11) R = H

Experimental Part

M.p.s. uncorr. – MS: Hitachi M80 (75 eV), Isobutane was used for chemical ionization. – ^{1}H-NMR-spectra: $CDCl_3$, Varian XL-200 (200.0 MHz), TMS as int. standard. – IR-spectra: EPI-G2 (Hitachi). – Optical rotations: Na-D, DIP-SL (JASCO) polarimeter. – Tlc and prep. tlc: Merck 60F-254, silica gel.

Callus Cultures and Extraction

The calluses of *C. ochotensis var. raddeana*, *C. ophiocarpa*, and *C. platycarpa* were subcultured on *Murashige* and *Skoog's* agar medium fortified with 2,4-dichlorophenoxyacetic acid (1 mg/l), kinetin (0.1 mg/l), and yeast extract (0.1 %). Hydrochlorides of **4**, **5**, and **6** (300 mg) were dissolved in the same agar medium (2 l) for experiments with *C. ochotensis var. raddeana* and *C. platycarpa* and in 1.6 l medium in the case of *C. ophiocarpa* and autoclaved (without Exp. 2). The calluses were incubated at 24° (at 22° in the case of *C. ophiocarpa*) for the appropriate time (Tab. 1). **4**-HCl (100 mg) in H_2O (3.6 ml) was injected into the autoclaved medium (400 ml) through a sterile bacterial filter. The callus was incubated at 24° for 12 days (Exp. 2). After incubation, the cells and solid medium were separated and worked up as shown in sheme 1.



* In Exp. 2, only **4** and **7** were isolated. In Exp. 7–9, **6** and **8** were isolated instead of **4** + **5** and **7**, respectively.

7: MS: m/z (rel. Int./%) = 221 ($M^{+\cdot}$, 100), CI-MS: m/z = 222 ($M^{+\cdot}$ + 1). – ^{1}H-NMR: δ (ppm) = 2.49 (s, 3H), 2.63 (t, 2H), 2.89 (br. s, 2H), 3.84 (s, 2H), 4.02 (s, 3H), 5.92 (s, 2H), 6.36 (s, 1H). – IR-spectrum identical with that of authentic **7**. **7**-HCl (MeOH/Me_2CO): m.p. 230–234° (decomp.).

8: CI-MS: m/z = 192 ($M^{+\cdot}$ + 1). – ^{1}H-NMR: δ (ppm) = 2.45 (s, 3H), 2.66 (t, 2H), 2.83 (t, 2H), 3.50 (s, 2H), 5.92 (s, 2H), 6.51 (s, 1H), 6.60 (s, 1H). Data agree with hydrohydrastinine **(8)**. **8**-HCl (Me_2CO): m.p. 227–235° (decomp.).

9: m.p. 96–97° (lit.[8] 102–103°). – MS: m/z (rel. Int./%) = 194 ($M^{+\cdot}$, 100), CI-MS: m/z = 195 ($M^{+\cdot}$ + 1). – IR (Nujol) 1765 cm^{-1}. – ^{1}H-NMR: δ (ppm) = 3.96 (s, 3H), 4.14 (s, 3H), 5.23 (s, 2H), 7.12 (d, J = 8/0 Hz, 1H), 7.27 (d, J = 8/0 Hz, 1H). – IR spectrum identical with that of authentic **9**.

10: CI-MS: m/z = 208 ($M^{+\cdot}$ + 1). – IR ($CHCl_3$): 3545 cm^{-1}. – ^{1}H-NMR: δ (ppm) = 1.38 (d, J = 6/5 Hz, 3H), 2.50 (s, 3H), 2.6–4.0 (m, 4H), 3.56 (q, J = 6/5 Hz, 1H), 3.88 (s, 3H), 6.59 (s, 1H), 6.71 (s, 1H). Data agree with 1-methylcorypalline **(10)**[2].

Tab. 1: Administrations of (-)-α-narcotine (**4**), (-)-β-narcotine (**5**), and (-)-β-hydrastine (**6**) to cell cultures of *C. ochotensis var. raddeana*, *C. ophiocarpa*, and *C. platycarpa*.

Experiment No.	Cell culture	Wt. of dried cells g	Substrate (300 mg each)	Incubation time day	Wt. of crude alkaloid fraction, mg cell	medium	4 + 5	7	10	11 (in mg)	9	6	8
1	*C. ochotensis var. raddeana*	15.1	4	36	99	48	40	36	3	1	46		
2	*C. ochotensis var. raddeana*	3.4	4[a)]	12	64	14	55[a)]	2					
3	*C. platycarpa*	11.1	4	31	93	41	45	42			47		
4	*C. ochotensis var. raddeana*	14.4	5	38	49	64	38	39	7	5	38		
5	*C. ophiocarpa*	6.8	5	46	65	59	57	34	2	4	28		
6	*C. platycarpa*	10.5	5	29	72	41	36	36			44		
7	*C. ochotensis var. raddeana*	14.0	6	38	102	30			11	11	18	77	11
8	*C. ophiocarpa*	8.8	6	46	58	53			2	5	6	83	14
9	*C. platycarpa*	14.4	6	37	86	45					18	48	14

a) **4** (100 mg) was administered and **4** (55 mg) was recovered.

11: CI-MS: m/z = 194 ($M^{+\cdot}$ + 1). – IR ($CHCl_3$): 3560 cm^{-1}. – ^{1}H-NMR: δ (ppm) = 1.46 (d, J = 7/0 Hz, 3H), 2.6–4.0 (m, 4H), 3.88 (s, 3H), 4.10 (q, J = 7/0 Hz, 1H) 6.60 (s, 1H), 6.73 (s, 1H). Data agree with N-nor-1-methylcorypalline (**11**). 11-HCl (MeOH-Me_2CO): m.p. 175–180° (decomp.). $[\alpha]_D$ ± 0° (c 0/3; $CHCl_3$-MeOH).

The ^{1}H-NMR-spectrum of the substrate recovered from Exper. 1 and 3 showes a *ca.* 3:1 mixture of **4** and **5**. – The ^{1}H-NMR-spectrum of the substrate recovered from Exper. 4–6 showes a *ca.* 1:2 mixture of **4** and **5**. – **6** recovered from Exper. 7–9 was characterized by direct comparison ($[\alpha]_D$, IR, and ^{1}H-NMR) with an authentic sample of (–)-β-hydrastine (**6**).

Blank Experiments

The medium (400 ml) containing **4**-HCl (50 mg) was autoclaved and incubated under the conditions of Exp. 1: a mixture of **4** + **5** (3:1, 18 mg) was obtained besides **9** (16 mg). **6**-HCl treated as in Exp. 7 gave 29 mg **6** and 9 mg **9** from 50 mg **6**-HCl.

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References and Notes

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6 Isomerizations of **4** to **5** and of **5** to **4** and formation of **9** occured under autoclave conditions (120°), used in the feeding experiments.
7 Compounds, **10**[2] and **11** are found in the tissue cultures of *C. ophiocarpa* and *C. ochotensis var. raddeana* without addition of the phthalideisoquinolines. – For **10** see S. Naruto and H. Kaneko, Phytochemistry **12**, 3008 (1973); for **11** see J. Strömborn and J. G. Bruhn, Acta Pharm. Suec. **15**, 127 (1978).
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