40. Biosynthesis of Benzo[*c*]phenanthridine Alkaloids Sanguinarine, Chelirubine and Macarpine

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Summary

The biosynthesis of the benzo[*c*]phenanthridine alkaloids was investigated in a cell suspension culture of *Macleaya cordata (papaveraceae)*. Feeding experiments define the biosynthetic pathway (–)-7,8,13,13a-tetrahydrocoptisine → (–)-*cis-N*-methyl-7,8,13,13a-tetrahydrocoptisinium salt **15** → protopine (**5**) → sanguinarine (**1**) → chelirubine (**3**) → macarpine (**4**).

Sanguinarine (**1**) and chelerythrine (**2**) belong to the benzo[*c*]phenanthridine alkaloids class [1]. These alkaloids occur widely in papaveraceous plants [2]. They have four oxygen functions at the positions 2, 3, 7 and 8. Besides **1** and **2**, the alkaloids chelirubine (**3**), chelirutine, sanguirubine and sanguirutine bearing five oxygen functions at C(2), C(3), C(7), C(8) and C(10) have been isolated from several plants [3]. Macarpine (**4**) bearing six oxygen functions at C(2), C(3), C(7), C(8), C(10) and C(12) has been also found [4].

Although biosynthetic studies on O_4-type alkaloids (sanguinarine and chelerythrine) have been described [5] [6], the biosynthetic route of the O_5- and O_6-type alkaloids has been unexplored, presumably due to their poor existence in plants. We now report a biosynthetic investigation on chelirubine (**3**) and macarpine (**4**) by using the callus tissues of *Macleaya cordata (papaveraceae)*. Moreover, the opportunity has been taken to investigate in detail the pathway of sanguinarine (**1**) and chelerythrine (**2**). A related experiment with the intact plants of *Macleaya cordata* is also described.

The callus tissues of *M. cordata* were derived from the stem and the root tissue, respectively. Each callus grew to form a mixture of the white and the red-orange colored colonies. The white-type and the red-orange-type colonies were selected visually, and two types of the colonies were subcultured under the same conditions. After subculturing for one year, the alkaloidal fraction of each callus tissue was examined. The alkaloidal components found in our callus tissues always involved the same four alkaloids, sanguinarine (**1**), chelirubine (**3**), macarpine (**4**) and protopine (**5**), which were also found in the intact plants [7]. Berberine (**6**), chelerythrine (**2**) and allocryptopine (**7**) were not detected in our callus tissues. These results are different from those reported by *Neumann et al.* [8] or by *Furuya et al.* [9].

Table 1. *Yield of alkaloids from the callus tissues of* M. cordata

Strain	Alkaloids [%][a])
Red-orange-type callus tissues from stem	0.316
White-type callus tissues from stem	0.061
Red-orange-type callus tissues from root	0.240
White-type callus tissues from root	0.058

[a]) Alkaloid yields are given as weight of basic fraction/weight of dry cells × 100%.

The yield of red-orange-type callus tissues was five times as high as that of white-type ones *(Table 1).* Such a variation of alkaloidal contents in the callus tissues derived from the same origin has been shown to occur in other plants [10]. The red-orange-type callus tissues derived from stems were used for our biosynthetic investigation.

Precursors were derived by partial conversion from berberine (**6**), coptisine (**8**) and (+)-chelidonine (**9**) (see *Scheme 1* and *Exper. Part*). A number of the precursors were used without being labelled with isotope for the incorporation experiments of chelerythrine (**2**) and allocryptopine (**7**) which were not found in our callus tissues.

In feeding experiments, the callus tissues were incubated in the *Murashige* and *Skoog's* medium containing the precursor with shaking in the dark at 24° for a definite time. After the incubation, the medium and cells were separated by filtration, and tertiary and quaternary alkaloids were fractionated in the usual manner. Each alkaloid was identified with the corresponding authentic sample by comparison of melting points, IR., ^{1}H-NMR. and mass spectra and elemental analyses. The isotope content (^{2}H and ^{13}C) was calculated [11] from the intensity of the molecular ions in the mass spectrum and by measuring the integral value and the peak heights of the *N*-methyl group of enriched and natural abundant bases in the ^{1}H-NMR. spectrum. The results are listed in *Table 2*.

Earlier work [6] [12] had shown the intermediacy of protoberberine alkaloids (scoulerine, tetrahydroberberine and tetrahydrocoptisine) on the pathway to benzo[*c*]phenanthridine alkaloids and protopine alkaloids. *Experiments 1* and *2* were planned to provide information about an enantiomeric specificity of the conversion of protoberberine skeleton into the benzo[*c*]phenanthridine skeleton. Whereas administration of (−)-(*S*)-7,8,13,13a-tetrahydroberberine (**10**) (as hydrochloride) to the callus tissues resulted in the formation of chelerythrine (**2**), allocryptopine (**7**) and berberine (**6**), a similar experiment with (+)-(*R*)-7,8,13,13a-tetrahydroberberine (**11**) yielded only dehydrogenated products, *i.e.* berberine (**6**) and 7,8-dihydroberberine (**12**) *(cf. Scheme 2).* Thus the (−)-(*S*)-enantiomer **10** was the precursor of chelerythrine (**2**) and allocryptopine (**7**). The dihydroberberine **12** (see *Exper. 1*) probably stands on the pathway between tetrahydroberberine and berberine.

Previous work [6] [12] had shown the incorporation of the *N*-methyl derivative of tetrahydrocoptisine into protopine (**5**) and sanguinarine (**1**). We undertook the incorporation experiments with pure *cis*- and *trans-N*-methyl derivatives of tetrahydroberberine and tetrahydrocoptisine to provide an insight into the discrimina-

Scheme 1

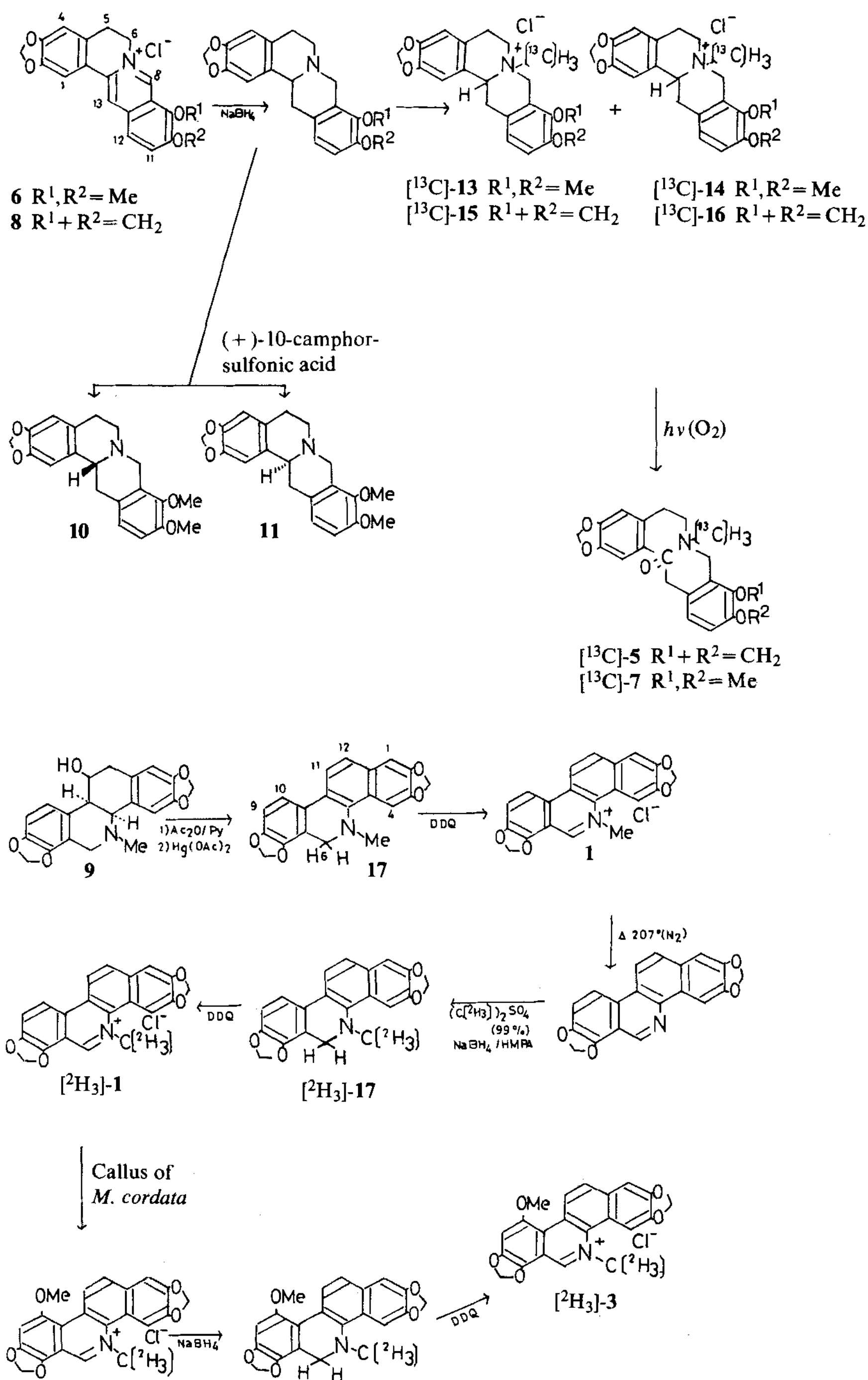
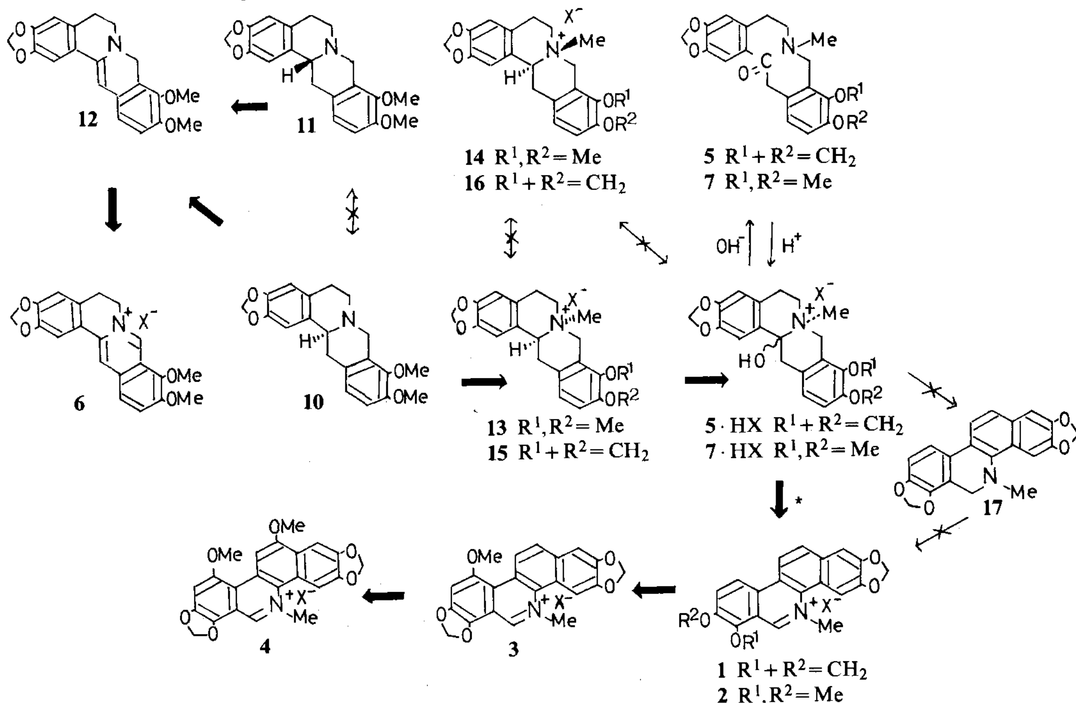


Table 2. *Incorporation experiments on* M. cordata

Experiment No.	Substrate	Amount [mg] (Enrichment [%])	Weight of cells [g]	Incubation time [day]	Metabolic product	Conversion (yield mg) [%]	Ratio of enrichment [%]	Ratio of recovery [%]	Optical purity [%] of recovered substrate
1	(+)-(*R*)-7,8,13,13a-Tetrahydroberberine hydrochloride (**11** · HCl)	88	108	6	7,8-dihydroberberine (**12**) berberine (**6**)	[3.7] [11.4]		42	100 ((+)-enantiomer)
2	(−)-(*S*)-7,8,13,13a-Tetrahydroberberine hydrochloride (**10** · HCl)	88	110	6	chelerythrine (**2**) allocryptopine (**7**) berberine (**6**)	[0.4] [1.7] [12.1]		25	100 ((−)-enantiomer)
3	(±)-*cis*-*N*-Methyl-7,8,13,13a-tetrahydroberberinium chloride (**13**)	70	70	8	chelerythrine (**2**) allocryptopine (**7**)	[1.4] [6.2]		17	26 ((+)-enantiomer)
4	(±)-*trans*-*N*-Methyl-7,8,13,13a-tetrahydroberberinium chloride (**14**)	70	70	8	–			29	4 ((−)-enantiomer)
5	(±)-*cis*-*N*-[^{13}C]Methyl-7,8,13,13a-tetrahydrocoptisinium chloride ([^{13}C]-**15**)	64(90)	52	7	sanguinarine (**1**) protopine (**5**)	[2.0] [9.6]	42 58	41	25 ((+)-enantiomer)
6	(±)-*trans*-*N*-[^{13}C]Methyl-7,8,13,13a-tetrahydrocoptisinium chloride ([^{13}C]-**16**)	81(90)	42	7	–			54	3 ((+)-enantiomer)
7	Allocryptopine hydrochloride (**7** · HCl)	70	18	7	chelerythrine (**2**)	[33.4]		30	
8	[*methyl*-^{13}C]Protopine hydrochloride ([^{13}C]-**5** · HCl)	23(90)	18	7	sanguinarine (**1**)	[24.6]	40	48	
9	5,6-Dihydro[*methyl*-$^{2}H_3$]sanguinarine hydrochloride ([$^{2}H_3$]-**17** · HCl)	40(99)	84	9	–			80	
10	[*methyl*-$^{2}H_3$]sanguinarine (chloride; [$^{2}H_3$]-**1**)	229(99)	370	7	chelirubine (**3**) macarpine (**4**)	4.8 12.0	44 15	18	
11	[*N*-*methyl*-$^{2}H_3$]chelirubine (chloride; [$^{2}H_3$]-**3**)	14(49)	84	13	macarpine (**4**)	1.5	45	14	
12	[*N*-*methyl*-^{13}C]allocryptopine hydrochloride ([^{13}C]-**7** · HCl)	20(90)	12.5	7	chelerythrine (**2**)	180.0	10	-	

Scheme 2. *Biosynthetic sequence for the alkaloids of* Macleaya cordata *as obtained by incorporation experiments* (*indicates the result obtained by the use of intact plants)



tion between the stereochemical *cis/trans* isomers. *Experiment 3* showed good incorporations of (±)-*cis*-*N*-methyl-7, 8, 13, 13a-tetrahydroberberinium chloride (**13**) into both chelerythrine (**2**) and allocryptopine (**7**). *Experiments 5* and *6* with (±)-*cis*- and (±)-*trans*-*N*-[^{13}C]methyl-7, 8, 13, 13a-tetrahydrocoptisinium chloride ([^{13}C]-**15** and [^{13}C]-**16**, respectively), confirmed that the *cis*-derivative is a good precursor of sanguinarine (**1**; enrichment 42%) and protopine (**5**; enrichment 58%) and showed that the *trans*-derivative is ineffective (s. also *Exper. 4*). Thus, only the *cis*-*N*-methyl derivatives of protoberberine can be stereospecifically metabolized into the benzo[*c*]phenanthridine-type and the protopine-type skeleton. In the *Experiments 3* and *5*, the recovered substrates contained much (+)-enantiomer for each substrate (optical purity 25 and 26%, resp.). This means that predominantly the (−)-enantiomer would be metabolized into the benzo[*c*]phenanthridine-type and the protopine-type skeleton. This is also supported by the results of the *Experiments 1* and *2*.

Results relevant to the intermediacy of protopine (**5**) on the pathway to sanguinarine (**1**) in intact plants of *Chelidonium majus* have been described [12]. To ensure the intermediacy of protopine type alkaloids into the benzo[*c*]phenanthridine type, we undertook incorporation experiments with the callus tissues of *M. cordata*. They showed that allocryptopine (**7**) (as hydrochloride) was transformed to chelerythrine (**2**) in *ca.* 33% conversion yield (metabolic product/substrate × 100%), and [*methyl*-^{13}C]protopine ([^{13}C]-**5**) (as hydrochloride) to sanguinarine (**1**; enrichment

40%) in *ca.* 25% (*Exper. 7* and *8*). These yields were much higher than the ones obtained in *Experiments 1–6*. In a biosynthetic pathway, protopine-type alkaloids (protopine (**5**) and allocryptopine (**7**)) should be situated between *N*-methyl-protoberberinium salts (*cis-N*-methyltetrahydrocoptisinium salt **15** and *cis-N*-methyltetrahydroberberinium salt **13**) and benzo[*c*]phenanthridine-type alkaloids (sanguinarine (**1**) and chelerythrine (**2**)).

The biosynthetic investigation of chelirubine (**3**) and macarpine (**4**) has not been reported. A proposal for the biogenetic conversion of sanguinarine (**1**) into chelirubine (**3**) had been made [13]. It involved oxidative fission of the C(6),N bond and recyclization to the benzo[*c*]phenanthridine-type skeleton. We assumed another biogenetic pathway based on that **1** was found at the initial stage of the callus culture period and that **4** was produced at the final stage instead of **1**. Direct evidence for the ($O_4 \rightarrow O_5 \rightarrow O_6$)-type alkaloidal sequence came from feeding experiments. Whereas [*methyl*-$^2H_3$]sanguinarine (chloride; [$^2H_3$]-**1**) was converted into chelirubine (**3**; enrichment 44%) in the callus tissues (*Exper. 10*), an analogous experiment with 5,6-dihydro[*methyl*-$^2H_3$]sanguinarine ([$^2H_3$]-**17**; as hydrochloride) did not yield **3** (*Exper. 9*). Therefore, 5,6-dihydrosanguinarine (**17**) is not effective as a precursor of chelirubine (**3**) and sanguinarine (**1**) is methoxylated at C(10). When [*N-methyl*-$^2H_3$]chelirubine (chloride; [$^2H_3$]-**3**) was administered into the callus tissues, it was metabolized into macarpine (**4**; enrichment 45%). These experiments confirm the sequence **1** → **3** → **4**. Based upon these results it is corroborated that the introduction of an oxygen function at C(10) and then at C(12) to form O_5- and O_6-alkaloids occurs after the formation of the benzo[*c*]phenanthridine skeleton.

Summarizing, the incorporation experiments with the callus tissues of *Macleaya cordata* define the biosynthetic pathway (–)-(*S*)-7,8,13,13a-tetrahydroberberine (**10**) → (–)-*cis-N*-methyl-7,8,13,13a-tetrahydroberberinium salt **13** → allocryptopine (**7**) → chelerythrine (**2**) and (–)-*cis-N*-methyl-7,8,13,13a-tetrahydrocoptisinium salt **15** → protopine (**5**) → sanguinarine (**1**) → chelirubine (**3**) → macarpine (**4**; *Scheme 2*). The conversion shown in *Scheme 2* may also take place on the intact plants of *Macleaya cordata* as indicated by the incorporation of [*N-methyl*-^{13}C]allocryptopine hydrochloride ([^{13}C]-**7** · HCl) by intact plants into chelerythrine (**2**; enrichment 10%; see *Exper. 12*).

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Experimental Part

1. *General remarks.* Melting points were taken on a *Kofler* hot stage apparatus, and are uncorrected. IR. spectra were recorded on an *EPI-G2 (Hitachi)* spectrophotometer in KBr or in $CHCl_3$ with micro cells. Mass spectra were run on an *JEOL-OIS* instrument (indication of *m/z*). ^1H-NMR. spectra were recorded on *NEVA-NV-21* (90 MHz, cw-technic) and *Varian-XL-200* (200 MHz, FT.-mode) spectrometers, with tetramethylsilane as an internal standard (= 0 ppm). Coupling constants *J* are given in Hz. ^{13}C-NMR. spectra were recorded on the *NV-21* (22.6 MHz) spectrometer. Optical rotations were measured using a *DIP-SL (JASCO)* polarimeter.

2. *Callus culture.* The callus were derived from the aseptic stem and the root of *Macleaya cordata* on *Murashige* and *Skoog's* medium (*M-S* medium) containing 2,4-dichlorophenoxyacetic acid (1 mg/l), kinetin (0.1 mg/l), yeast extract (0.1%) and agar (1%) in July 1974. The callus tissues from stem and root were subcultured every 3 weeks onto the fresh *M-S* medium at 24° in the dark for 1 year. The white-type and red-orange-type callus tissues were visually selected during the subculture.

3. *Extraction procedure.* A typical procedure is as follows: The red-orange-type callus from stem (113.6 g, fresh weight) was suspended in MeOH (500 ml) and extracted on a steam bath for 4 h. The suspension was filtered and the cells reextracted in MeOH (4 × 500 ml). The extracts were combined and evaporated *in vacuo.* The residue was extracted with tartaric acid (3%, 3 × 200 ml). This acid layer was washed with ether (2 × 150 ml), and its pH adjusted to *ca.* 10 (with 28% NH_4OH-solution). The alkaline solution was extracted with ether (3 × 200 ml). The combined ether layer were dried over K_2CO_3 and evaporated to give a red oil (24 mg; *tertiary-alkaloid fraction*). The aq. solution was adjusted to pH *ca.* 4 (with 35% HCl-solution). The acidic solution was extracted with $CHCl_3$ (twice 200 ml) after addition of KI (3 g). The org. layer was washed with $Na_2S_2O_3$-solution (10%) and then with sat. NaCl-solution, dried over $MgSO_4$ and evaporated *in vacuo.* The residue (*quaternary-alkaloid fraction*) was found in this case not to contain any alkaloids.

4. *Isolation of alkaloids.* A typical procedure is as follows: the tertiary-alkaloid fraction was chromatographed by prep. TLC. (MeOH) to yield *protopine* (**5**; Rf 0.22, 5.6 mg), and a mixture of other alkaloids (Rf 0.69) as an oil. **5**; m.p. 207-208° (MeOH). - IR. (KBr disc): 1670 (C=O). - ^{1}H-NMR. ($CDCl_3$)[1]: 1.92 (*s*, 3 H, CH_3N); 2.45 and 2.89 (2 *m*, 2 H each, 2 H−C(5), 2 H−C(6)); 3.58 (*s*, 2 H, 2 H−C(8)); 3.78 (*s*, 2 H, 2 H−C(13)); 5.90 and 5.92 (2 *s*, 2 H each, 2 OCH_2O); 6.63 and 6.89 (2 *s*, 1 H each, 2 arom. H); 6.65 (*s*, 2 H, 2 arom. H).

$C_{20}H_{19}NO_5$ (353.4) Calc. C 67.98 H 5.42 N 3.96% Found C 68.01 H 5.58 N 3.95%

The oil was dissolved in MeOH (10 ml) and reduced by addition of $NaBH_4$ (5 mg). After keeping at r.t. for 1 h, the mixture was evaporated, H_2O (100 ml) added to the residue, and the solution extracted with $CHCl_3$ (2 × 150 ml). The reduced alkaloids were chromatographed by prep. TLC. (benzene, 4 developments) to give *5,6-dihydrosanguinarine* (**17**; Rf 0.40; 8.4 mg), *5,6-dihydrochelirubine* (RF 0.33; 4.1 mg) and *5,6-dihydromacarpine* (Rf 0.26; 4.8 mg). 5,6-Dihydrosanguinarine (**17**): m.p. 190-191° (MeOH). - ^{1}H-NMR. ($CDCl_3$): 2.60 (*s*, 3 H, CH_3N); 4.14 (*s*, 2 H, 2 H−C(6)); 6.02 (*s*, 4 H, 2 OCH_2O); 7.10 and 7.72 (2 *s*, 1 H each, 2 arom. H); 6.83 and 7.30 (*AB*, *J* = 8, 2 H, 2 arom. H); 7.46 and 7.69 (*AB*, *J* = 9, 2 H, 2 arom. H).

$C_{20}H_{15}NO_4$ (333.2) Calc. C 72.06 H 4.54 N 4.20% Found C 72.20 H 4.54 N 4.18%

5,6-Dihydrochelirubine: m.p. 201-202° (MeOH). - ^{1}H-NMR. ($CDCl_3$): 2.54 (*s*, 3 H, CH_3N); 3.81 (*s*, 3 H, CH_3O); 4.07 (*s*, 2 H, 2 H−C(6)); 5.96 and 6.00 (2 *s*, 2 H each, 2 OCH_2O); 6.59, 7.11 and 7.72 (3 *s*, 1 H each, 3 arom. H); 7.47 and 8.35 (*AB*, *J* = 9, 2 H, 2 arom. H).

$C_{21}H_{17}NO_5$ (363.2) Calc. C 69.41 H 4.72 N 3.86% Found C 69.42 H 4.58 N 3.93%

5,6-Dihydromacarpine: m.p. 178-179° (ether). - ^{1}H-NMR. ($CDCl_3$): 2.53 (*s*, 3 H, CH_3N); 3.89 and 4.00 (2 *s*, 3 H each, 2 CH_3O); 4.11 (*s*, 2 H, 2 H−C(6)); 6.02 and 6.04 (2 *s*, 2 H each, 2 OCH_2O); 6.64, 7.54, 7.71 and 7.87 (4 *s*, 1 H each, 4 arom. H). - High resolution MS.: 393.121 (M^+, calc. for $C_{22}H_{19}NO_6$ 393.119).

5. *Preparation of (−)-(S)- and (+)-(R)-7,8,13,13a-tetrahydroberberine* (**10** *and* **11**, *resp.*). To a solution of berberine (chloride; **6**; 2 g) in MeOH (300 ml), $NaBH_4$ (0.5 g) was added, and the mixture kept at r.t. for 1 h. After concentration *in vacuo*, water (200 ml) was added, and the resulting solution extracted with $CHCl_3$ (2 × 200 ml). The org. layer was washed with sat. NaCl-solution, dried (K_2CO_3), and evaporated to give **10/11** (1.45 g) as a white crystalline solid. Recrystallization from MeOH/$CHCl_3$ gave **10/11** as prisms, m.p. 168-169°.

$C_{20}H_{21}NO_4$ (339.4) Calc. C 70.78 H 6.24 N 4.13% Found C 70.79 H 6.29 N 4.11%

A MeOH-solution of **10/11** (500 mg) was combined with (+)-10-camphorsulfonic acid (350 mg) in MeOH (20 ml). After concentration, (+)-10-camphorsulfonate of **10/11** (685 mg, m.p. 232-236°) was

[1] The numbering of protopine (**5**), a 7,13a-secocoptisine derivative, is the same as the one of coptisine (**8**; *Scheme 1*).

obtained. The diastereomeric salt were fractionally recrystallized as follows: After 3 recrystallizations from MeOH, needles of (+)-10-camphorsulfonate of **10** (240 mg, m.p. 248–253°) were obtained. The mother liquor was evaporated, and the residue gave (+)-10-camphorsulfonate of **11** as plates (195 mg, m.p. 237–242°) after 4 successive recrystallizations from MeOH/acetone/ether.

The (+)-10-camphorsulfonate of **10** was dissolved in water, and the solution made alkaline (with 28% NH_4OH-solution) and extracted with ether (2×200 ml). The org. extract was washed with sat. NaCl-solution, dried (K_2CO_3) and evaporated. Crystallization of the residue from MeOH/ether afforded 128 mg of **10** as needles, m.p. 134–135°, $[\alpha]_D^{20} = -310°$ ($c = 1.5$, $CHCl_3$).

$C_{20}H_{21}NO_4$ (339.4) Calc. C 70.78 H 6.24 N 4.13% Found C 70.82 H 6.32 N 4.15%

By the same procedure, the enantiomeric **11** was regenerated from (+)-10-camphorsulfonate of **11**, m.p. 133.5–135° (needles, MeOH/ether), $[\alpha]_D^{20} = +307°$ ($c = 1.5$, $CHCl_3$).

$C_{20}H_{21}NO_4$ (339.4) Calc. C 70.78 H 6.24 N 4.13% Found C 70.80 H 6.28 N 4.19%

6. *Preparation of (±)-*cis-, *and (±)-*trans-N-*methyl-7,8,13,13a-tetrahydroberberinium chloride (***13** *and* **14** *resp.).* CH_3I was added to a solution of **10/11** in acetone, and the mixture was kept at 80° for 6 h in a sealed tube. After cooling, precipitated crystals were filtered. The crystalline product was recrystallized from MeOH to give *(±)-*trans-N-*methyl-7,8,13,13a-tetrahydroberberinium iodide* (1.0 g) as transparent plates, m.p. 250–252°.

$C_{21}H_{24}INO_4$ (481.3) Calc. C 52.40 H 5.03 N 2.91% Found C 52.41 H 5.04 N 3.91%

The residue from the mother-liquor was recrystallized from MeOH to afford *(±)-cis-*N-*methyl-7,8,13,13a-tetrahydroberberinium iodide* (180 mg) as needles, m.p. 240–242°.

$C_{21}H_{24}INO_4$ (431.3) Calc. C 52.40 H 5.03 N 2.91% Found C 52.69 H 4.98 N 3.09%

The above (±)-*trans*-iodide (100 mg) was dissolved in MeOH, the solution stirred with freshly prepared AgCl (100 mg) at r.t. for 1 h, the mixture filtered, and the filtrate concentrated to yield 92 mg of **14** as prisms, m.p. 245–251° (MeOH/acetone). – ^{1}H-NMR. (CD_3OD): 3.06 (*s*, 3 H, CH_3N); 3.99 and 4.03 (2 *s*, 3 H each, 2 CH_3O); 6.11 (*s*, 2 H, OCH_2O); 6.93 and 7.11 (2 *s*, 1 H each, 2 arom. H); 7.26 (*s*, 2 H, 2 arom. H).

By the same procedure, **13** was regenerated from the above (±)-*cis*-iodide, m.p. 214–218° (acetone/ether). – ^{1}H-NMR. (CD_3OD): 3.41 (*s*, 3 H, CH_3N); 3.97 and 4.00 (2 *s*, 3 H each, 2 CH_3O); 6.08 (*s*, 2 H, OCH_2O); 6.93 and 7.15 (2 *s*, 1 H each, 2 arom. H).

7. *Isolation of allocryptopine* (7). Allocryptopine was isolated from *Chelidonium majus*, m.p. 160–162° ($CHCl_3$/MeOH). – IR. (Nujol): 1650 and 1610 (C=O). – ^{1}H-NMR. ($CDCl_3$): 1.87 (*s*, 3 H, CH_3N); 3.78 and 3.85 (2 *s*, 3 H each, 2 CH_3O); 5.92 (*s*, 2 H, OCH_2O); 6.63 and 6.94 (2 *s*, 1 H each, 2 arom. H); 6.81 and 6.91 (*AB*, $J = 8$, 2 H, 2 arom. H).

$C_{21}H_{23}NO_5$ (369.4) Calc. C 68.28 H 6.28 N 3.79% Found C 68.48 H 6.28 N 3.78%

8. *Preparation of (±)-*cis- *and (±)-*trans-N-*[^{13}C]methyl-7,8,13,13a-tetrahydrocoptisinium chloride ([^{13}C]-***15** *and [^{13}C]-***16**, *resp.).* First *(±)-7,8,13,13a-tetrahydrocoptisine* (1.4 g) was prepared by reduction of coptisine (chloride, **8**; isolated from *C. majus*, m.p. 292–295°; 2 g) with $NaBH_4$ as described above in *5*, m.p. 217–219° ($CHCl_3$/MeOH). – ^{1}H-NMR. ($CDCl_3$): 5.91 and 5.96 (2 *s*, 2 H each, 2 OCH_2O); 6.62 and 6.73 (2 *s*, 1 H each, 2 arom. H); 6.67 (*s*, 2 H, 2 arom. H).

$C_{19}H_{17}NO_4$ (323.1) Calc. C 70.57 H 5.30 N 4.33% Found C 70.34 H 5.20 N 4.08%

To a solution of (±)-tetrahydrocoptisine in acetone, [^{13}C]H_3I (1 g, 90% enrichment; *MSD*, Canada) was added, and the mixture was kept at 80° for 6 h in a sealed tube. The mixture of iodides corresponding to [^{13}C]-**15**/[^{13}C]-**16** was separated by the procedure described in *6*. Iodide corresponding to [^{13}C]-**15**: m.p. 276–282° (MeOH). – ^{1}H-NMR. (CD_3OD): 3.30 (*d*, $J = 146$, 3 H, $^{13}CH_3N$); 6.00 (*s*, 2 H, OCH_2O); 6.06 (*d*×*d*, $J = 1$ and 8, 2 H, OCH_2O); 6.82 (*s*, 2 H, 2 arom. H); 6.79 and 6.93 (*AB*, $J = 8$, 2 H, 2 arom. H).

Iodide corresponding to [^{13}C]-**16**: m.p. 285–292° (MeOH). – ^{1}H-NMR. (CD_3OD): 3.00 (*d*, $J = 145$, 3 H, $^{13}CH_3N$); 6.03 (*s*, 2 H, OCH_2O); 6.09 (*d*×*d*, $J = 1$ and 8, 2 H, OCH_2O); 6.84 anf 7.03 (2 *s*, 1 H each, 2 arom. H); 7.69 and 7.70 (*AB*, $J = 7.5$, 2 H, 2 arom. H).

The anion moiety of the above iodides was changed into chloride as described in *6.* [^{13}C]-**15**: m.p. 238–245° (acetone). – ^{1}H-NMR. (CD_3OD): 3.31 (*d, J* = 144, $^{13}CH_3N$); 6.00 (*s,* 2 H, OCH_2O); 6.06 (*d*×*d, J* = 1 and 8, 2 H, OCH_2O); 6.82 and 6.84 (2 *s,* 1 H each, 2 arom. H); 6.79 and 6.87 (*AB, J* = 8, 2 H, 2 arom. H).

[^{13}C]-**16**: m.p. 273–275° (MeOH). – ^{1}H-NMR. (CD_3OD): 2.99 (*d, J* = 144, 3 H, $^{13}CH_3N$); 6.00 (*s,* 2 H, OCH_2O); 6.05 (*d*×*d, J* = 1 and 8, 2 H, OCH_2O); 6.80 and 7.00 (2 *s,* 1 H each, 2 arom. H); 6.90 and 6.93 (*AB, J* = 8, 2 H, 2 arom. H).

$C_{20}H_{20}ClNO_4 \cdot H_2O$[2]) (391.8) Calc. C 61.30 H 5.66 N 3.57% Found C 61.53 H 5.88 N 3.63%

9. *Preparation of [*methyl-*^{13}C]protopine ([^{13}C]-5)* [14]. A solution of [^{13}C]-**16** (800 mg) in MeOH/H_2O (500 ml) was irradiated with a high-pressure-Hg lamp (with *Pyrex* filter) at 5° under bubbling of O_2 for 26 h. After evaporation of solvent *in vacuo,* H_2O (200 ml) was added, and the resulting solution adjusted to pH *ca.* 10 (with 28% NH_4OH-solution) and extracted with ether (2×200 ml). The ether layer was dried (K_2CO_3) and evaporated to give [*methyl*-^{13}C]-**5** as an oil. The oil was chromatographed on a column of silicagel (benzene/ether 1:1) to give 22 mg of [*methyl*-^{13}C]-**5** as prisms, m.p. 205–206° ($CHCl_3$/MeOH). – ^{1}H-NMR. ($CDCl_3$): 1,92 (*d, J* = 134, 3 H, $^{13}CH_3N$). – High resolution MS.: 354.124 (M^+, calc. for $C_{19}{}^{13}CH_{19}NO_5$ 354.130).

10. *Preparation of [*N-methyl-*^{13}C]allocryptopine ([^{13}C]-7).* By the same procedure as described in *9,* [^{13}C]-**7** (21 mg) was prepared from *(±)*-trans-N-*[^{13}C]methyl-7,8,13,13a-tetrahydroberberinium iodide* (90% enrichment, 600 mg; s. *Chap. 6*), m.p. 163–164° (MeOH). – IR. ($CHCl_3$): 1650 (C=O). – ^{1}H-NMR. ($CDCl_3$): 1.93 (*d, J* = 134, 3 H, $^{13}CH_3N$). – High resolution MS.: 370.154 (M^+, calc. for $C_{20}{}^{13}CH_{23}NO_5$ 370.158).

$C_{21}H_{23}NO_5$[2]) (369.4) Calc. C 68.28 H 6.28 N 3.79% Found C 68.35 H 6.29 N 3.76%

11. *Preparation of 5,6-dihydro[*methyl-*$^2H_3$]sanguinarine and [*methyl-*$^2H_3$]sanguinarine (chloride); ([$^2H_3$]-**17** and [$^2H_3$]-**1**, resp.)* [15]. (+)-Chelidonine (**9**; 10 g; isolated from *C. majus,* m.p. 135–136°) was dissolved in a mixture of acetic anhydride and 2 drops of pyridine and left at r.t. After 6 h, water was added, and the aq. solution adjusted to pH *ca.* 9 (with 28% NH_4OH-solution) and extracted with ether (2×300 ml). The ether layer was dried (K_2CO_3) and evaporated to give (+)-chelidonine acetate (9.8 g) as plates, m.p. 165–166° ($CHCl_3$/MeOH). – IR. ($CHCl_3$): 1720 (C=O).

$C_{22}H_{21}NO_6$ (395.4) Calc. C 66.82 H 5.35 N 3.54% Found C 66.68 H 5.38 N 3.82%

To a solution of (+)-chelidonine acetate (5 g) in acetic acid (5%, 1000 ml), HgO (15 g) was added and the mixture refluxed for 4 h. After cooling, the solid was filtered off. The filtrate was made alkaline (with 28% NH_4OH-solution) and extracted with ether (twice 300 ml). The org. layer was dried (K_2CO_3) and evaporated to give *5,6-dihydrosanguinarine* (**17**; 2.7 g) as prisms, m.p. 190–192° ($CHCl_3$/MeOH).

$C_{20}H_{15}NO_4$ (333.2) Calc. C 72.06 H 4.54 N 4.20% Found C 72.22 H 4.38 N 4.19%

To a solution of **17** (2 g) in benzene (80 ml) and aq. NaOH solution (5%, 36 ml), a solution of 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 1.96 g) in benzene (80 ml) was added dropwise. The mixture was stirred at r.t. for 1 h. Aq. NaOH solution (10%, 2000 ml) was added and the mixture extracted with ethyl acetate (2×300 ml). The org. layer was washed with sat. NaCl-solution, dried (K_2CO_3) and evaporated to give 2 g of an oil. The oil was suspended in MeOH (10 ml) and acidified with HCl solution (35%) to give 2.1 g of sanguinarine (chloride; **1**) as red-orange needles, m.p. 287–289°.

$C_{20}H_{14}ClNO_4$ (367.8) Calc. C 65.31 H 3.84 N 3.81% Found C 65.42 H 4.00 N 3.93%

Sanguinarine (chloride; **1**; 2 g) and sea sand (5 g) were mixed and kept for 7 min at 207° on a boiling tetraline bath under N_2. After cooling, the mixture was extracted with $CHCl_3$ (2×200 ml) and the org. solution evaporated *in vacuo* to give 1.43 g of *'norsanguinarine'* (= de-*N*-methylsanguinarine) as prisms, m.p. 280–283° ($CHCl_3$/MeOH). – ^{1}H-NMR. ($CDCl_3$): 6.13 and 6.28 (2 *s,* 2 H each, 2 OCH_2O);

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[2]) The calculated values for the ^{13}C-labelled products are based on the corresponding unlabelled compounds.

7.25 and 8.66 (2 *s*, 1 H each, 2 arom. H); 7.45 and 8.18 (*AB*, *J* = 8, 2 H, 2 arom. H); 8.33 and 7.86 (*AB*, *J* = 9, 2 H, 2 arom. H). – High resolution MS.: 317.073 (M^+, calc. for $C_{19}H_{11}NO_4$ 317.069).

$C_{19}H_{11}NO_4$ (317.1) Calc. C 71.92 H 3.49 N 4.41% Found C 72.03 H 3.25 N 4.30%

To 'norsanguinarine' (1.4 g) in hexamethylphosphortriamide (40 ml), [$^2H_6$]dimethyl sulfate (6 ml, 99% enrichment, *Merck*) and $NaBH_4$ (700 mg) were added at r.t., and the mixture was stirred at 50°. After 1 h, additional [$^2H_6$]dimethyl sulfate (4 ml) and $NaBH_4$ (700 mg) were added, the mixture was stirred for 1 h, then ice-cold water was added, and the solution made alkaline with aq. NaOH-solution (5 %) and extracted with ether (2×200 ml). The ether layer was washed with sat. NaCl-solution, dried (K_2CO_3) and evaporated to give 1.3 g of [$^2H_3$]-**17** as prisms, m.p. 188–189° ($CHCl_3$/MeOH). – ^{1}H-NMR. ($CDCl_3$): 4.15 (*s*, 2 H, 2 H–C(6)); 5.98 (*s*, 4 H, 2 OCH_2O); 7.10 and 7.75 (2 *s*, 1 H each, 2 arom. H); 6.83 and 7.30 (*AB*, *J* = 8, 2 H, 2 arom. H); 7.46 and 7.68 (*AB*, *J* = 9, 2 H, 2 arom. H). – High resolution MS.: 336.119 (M^+, calc. for $C_{20}H_{12}{}^2H_3NO_4$ 336.119).

When the dehydrogenation with DDQ was carried out under the condition described above, [$^2H_3$]-**17** (1.3 g) yielded [$^2H_3$]-**1** (998 mg) as red-orange needles, m.p. 286–288° (aceton/MeOH). – ^{1}H-NMR. (CD_3OD): 6.28 and 6.53 (2 *s*, 2 H each, 2 OCH_2O); 7.53, 8.12 and 9.92 (3 *s*, 1 H each, 3 arom. H); 7.94 and 8.52 (*AB*, *J* = 8.5, 2 H, 2 arom. H); 8.60 and 8.20 (*AB*, *J* = 8.5, 2 H, 2 arom. H).

12. *Preparation of [*N-methyl-$^2H_3$*]chelirubine (chloride; [$^2H_3$]-*3). Callus of *M. cordata* (1.4 kg) was incubated in the *M-S* medium containing [$^2H_3$]-**1** (700 mg, 99% enrichment) on a shaker under the conditions described in *13*. After 1 week, cells were harvested, and the 5,6-dihydrochelirubine fraction (20 mg) was isolated by the procedure used in *3* and *4*. For further purification the dihydrochelirubine fraction was chromatographed on a column of silicagel (benzene) giving 18 mg of *5,6-dihydro-[*N-methyl-$^2H_3$*]chelirubine* as prisms, m.p. 204–205° (MeOH/ether). – ^{1}H-NMR. ($CDCl_3$): 2.54 (*s*, 1.5 H, CH_3N); 3.80 (*s*, 3 H, CH_3O); 4.07 (*s*, 2 H, 2 H–C(6)); 5.98 and 6.01 (2 *s*, 2 H each, 2 OCH_2O); 7.11, 7.72 and 6.60 (3 *s*, 1 H each, 1 arom. H); 7.46 and 8.33 (*AB*, *J* = 9, 2 H, 2 arom. H). – High resolution MS.: 363.114 (calc. for $C_{21}H_{17}NO_5$ 363.111); 366.130 (calc. for $C_{21}H_{14}{}^2H_3NO_5$ 366.130).

When the dehydrogenation with DDQ was carried out under the same conditions as in *11*, [$^2H_3$]-**3** (14 mg) was regenerated as red needles, m.p. 245–250° (MeOH/acetone). – ^{1}H-NMR. (CD_3OD): 3.30 (CH_3N and solvent); 4.21 (*s*, 3 H, CH_3O); 6.25 and 6.47 (2 *s*, 2 H each, 2 OCH_2O); 7.49, 7.77, 8.01 and 9.81 (4 *s*, 1 H each, 4 arom. H); 8.09 and 9.40 (*AB*, *J* = 9, 2 H, 2 arom. H).

13. *Administration of (+)-(*R*)-7,8,13,13a-tetrahydroberberine* (**11**) (Exper. 1, Tab. 2). A typical procedure is as follows: A solution of **11** (88 mg) in MeOH was acidified with HCl solution (5%) and evaporated *in vacuo*. To the residue, sterilized water (15 ml) was added, and aliquots of 1 ml of this aq. solution were each poured into 100-ml-*Erlenmeyer* flasks containing callus (5 g, red-orange-type from stem, 2 weeks old) in *M-S* medium (40 ml). The cultures were incubated on a rotary shaker (70 rpm) at 24° in the dark for 6 days. After harvest, the callus (fresh weight 108 g) and the medium (15×40 ml) were separated by filtration through a glass filter. The medium was acidified with HCl-solution (5%), washed with ether, adjusted to pH *ca.* 10 (with 28% NH_4OH-solution), and extracted with ether. The ether layer was dried (K_2CO_3), and evaporated to give a *tertiary-alkaloid fraction from medium*. The fraction was chromatographed by prep. TLC. (benzene/ether 1:1) giving recovered **11** (28 mg), m.p. 133–135° (MeOH), $[\alpha]_D^{20} = +300°$ (*c* = 1, $CHCl_3$). – High resolution MS.: 339.145 (M^+, calc. for $C_{20}H_{21}NO_4$ 339.147).

The aq. solution was adjusted to pH *ca.* 4 (35% HCl-solution), KI (3 g) was added and the solution extracted with $CHCl_3$. The org. layer was washed with $Na_2S_2O_3$-solution (10%) and then with sat. NaCl-solution, dried ($MgSO_4$) and evaporated *in vacuo* giving a *quaternary-alkaloid fraction from medium*. In this case no alkaloid was found.

The cells were treated as described in *3* to give tertiary- and quaternary-alkaloid fractions. The *tertiary-alkaloid fraction from cell* was chromatographed by prep. TLC. (benzene/ether 9:1) giving *7,8-dihydroberberine* (**12**, 3.2 mg), m.p. 140–142° (MeOH)/ether). – ^{1}H-NMR. ($CDCl_3$): 2.96 (*m*, 4 H, 2 H–C(5), 2 H–C(6)); 3.84 (*s*, 6 H, 2 CH_3O); 4.32 (*s*, 2 H, 2 H–C(8)); 5.91 (*s*, 2 H, OCH_2O); 5.93, 6.57 and 7.17 (3 *s*, 1 H, each, 1 arom. H); 6.72 (*t*-like, *J* = 6.5, 2 H, 2 arom. H). – High resolution MS.: 337.129 (M^+, calc. for $C_{20}H_{19}NO_4$ 337.131).

The *quaternary-alkaloid fraction from cell* was chromatographed on a column of alumina (acetone/MeOH) to give *berberine* (iodide; **6**; 13.7 mg), m.p. 242–245° (MeOH). – ^{1}H-NMR. (CD_3OD): 3.26 (*t*-like, *J* = 7, 2 H, 2 H–C(5)); 4.11 and 4.21 (2 *s*, 3 H each, CH_3O); 4.94 (*t*-like, *J* = 7, 2 H, 2 H–C(6));

6.12 (*s*, 2 H, OCH_2O); 7.66, 8.71 and 9.79 (3 H, 1 H each, 3 arom. H); 8.02 and 8.13 (*AB*, $J = 9$, 2 H, 2 arom. H).

14. *Administration of (–)-(S)-7,8,13,13a-tetrahydroberberine* (**10**) (Exper. 2, Tab. 2). Callus (110 g) was incubated with **10** · HCl (from 88 mg of **10**) for 6 days. The cells and medium were treated as described in *3* and *4*. The *tertiary-alkaloid fraction from medium* gave *allocryptopine* (7, 1.6 mg), m.p. 160–161.5° (MeOH/ether).

The *tertiary-alkaloid fraction from cell* gave *5,6-dihydrochelerythrine* (0.4 mg), m.p. 143–144° (MeOH/ether). – ^{1}H-NMR. ($CDCl_3$): 2.56 (*s*, 3 H, CH_3N); 3.85 and 3.88 (2 *s*, 3 H each, 2 CH_3O); 4.29 (*s*, 2 H, 2 H–C(6)); 6.00 (*s*, 2 H, OCH_2O); 7.10 and 7.69 (2 *s*, 1 H each, 2 arom. H); 6.93 and 7.49 (*AB*, $J = 8$, 2 H, 2 arom. H); 7.47 and 7.70 (*AB*, $J = 8.5$, 2 H, 2 arom. H). – High resolution MS.: 349.135 (M^+, calc. for $C_{21}H_{19}NO_4$ 349.136).

The *quaternary-alkaloid fraction from cell* gave *berberine* (iodide; **6**; 14.5 mg), m.p. 242–245° (MeOH).

15. *Administration of (±)-*cis-N-*methyl-7,8,13,13a-tetrahydroberberinium chloride* (**13**) (Exper. 3, Tab. 2). Callus (70 g) was incubated with **13** (70 mg) for 8 days. The cells and medium were treated as described in *3* and *4*. The *tertiary-alkaloid fraction from medium* gave *allocryptopine* (**7**; 4.1 mg), m.p. 160–162° (MeOH/ether).

The *tertiary-alkaloid fraction from cell* gave *5,6-dihydrochelerythrine* (1.4 mg), m.p. 143–144° (MeOH/ether).

The *quaternary-alkaloid fraction from medium and cell* was chromatographed on a column of alumina (acetone/MeOH) to give recovered iodide corresponding to **13** (12 mg), m.p. 265–271° (acetone/MeOH), $[\alpha]_D^{20} = +44°$ ($c = 1.2$, MeOH).

16. *Administration of (±)-*trans-N-*methyl-7,8,13,13a-tetrahydroberberinium chloride* (**14**) (Exper. 4, Tab. 2). Callus (70 g) was incubated with **14** (70 mg) for 8 days. The cells and medium were treated as described in *3* and *4*. No fraction contained any metabolites.

17. *Administration of (±)-*cis-N-*[^{13}C]methyl-7,8,13,13a-tetrahydrocoptisinium chloride ([^{13}C]-***15**) (Exper. 5, Tab. 2). Callus (52 g) was incubated with [^{13}C]-**15** (64 mg) for 7 days. The cells and medium were treated as described for *3* and *4*. The *tertiary-alkaloid fraction from medium* gave *[*methyl-*^{13}C]protopine* ([^{13}C]-**5**; 9 mg), m.p. 203–204.5° (MeOH/ether). – ^{1}H-NMR. ($CDCl_3$): 1.92 (*s*, 1.4 H, CH_3N); 1.92 (*d*, $J = 135$, 1.6 H, $^{13}CH_3N$). – High resolution MS.: 353.126 (calc. for $C_{20}H_{19}NO_5$ 353.126); 354.134 (calc. for $C_{19}{}^{13}CH_{19}NO_5$ 354.130).

The *tertiary-alkaloid fraction from cell* gave *5,6-dihydro[*methyl-*^{13}C]sanguinarine* ([^{13}C]-**17**; 2.5 mg), m.p. 187–189° (ether). – ^{1}H-NMR. ($CDCl_3$): 2.59 (*s*, 1.8 H, CH_3N); 2.59 (*d*, $J = 135.5$, 1.2 H, $^{13}CH_3N$). – High resolution MS.: 333.104 (calc. for $C_{20}H_{15}NO_4$ 333.100); 334.101 (calc. for $C_{19}{}^{13}CH_{15}NO_4$ 334.103).

The *quaternary-alkaloid fraction from medium and cell* was chromatographed on a column of alumina (acetone/MeOH) to give recovered iodide corresponding to **15** (26 mg), m.p. 264–271° (MeOH/acetone), $[\alpha]_D^{20} = +54°$ ($c = 1.3$, MeOH).

18. *Administration of (±)-*trans-N-*[^{13}C]methyl-7,8,13,13a-tetrahydrocoptisinium chloride ([^{13}C]-***16**) (Exper. 6, Tab. 2). Callus (42 g) was incubated with [^{13}C]-**16** (81 mg) for 7 days. The cells and medium were treated as described in *3* and *4*. No fraction contained any metabolites.

19. *Administration of allocryptopine* (7) (Exper. 7, Tab. 2). Callus (18 g) was incubated with **7** · HCl (70 mg) for 7 days. The cells and medium were treated as described in *3* and *4*. The *tertiary-alkaloid fraction from cell* gave *5,6-dihydrochelerythrine* (22 mg), m.p. 143–144.5° (MeOH/ether).

$C_{21}H_{19}NO_4$ (349.4) Calc. C 72.19 H 5.48 N 4.01% Found C 72.00 H 5.41 N 4.07%

20. *Administration of [*methyl-*^{13}C]protopine ([^{13}C]-5)* (Exper. 8, Tab. 2). Callus (18 g) was incubated with [^{13}C]-5 (23 mg) for 7 days. The cells and medium were treated as described in *3* and *4*. The *tertiary-alkaloid fraction from cell* gave *5,6-dihydro[*methyl-*^{13}C]sanguinarine* ([^{13}C]-**17**; 12 mg), m.p. 186–189.5° (MeOH/ether). – ^{1}H-NMR. ($CDCl_3$): 2.60 (*s*, 1.8 H, CH_3N); 2.60 (*d*, $J = 135.5$, 1.2 H, $^{13}CH_3N$). – High resolution MS.: 333.102 (calc. for $C_{20}H_{15}NO_4$ 333.100); 334.099 (calc. for $C_{19}{}^{13}CH_{15}NO_4$ 334.103).

$C_{20}H_{15}NO_4$[2]) (333.3) Calc. C 72.00 H 4.54 N 4.20% Found C 72.12 H 4.43 N 4.18%

21. *Administration of 5,6-dihydro[*methyl-$^2H_3$*]sanguinarine ([*$^2H_3$*]-***17***)* (Exper. 9, Tab. 2). Callus (84 g) was incubated with [$^2H_3$]-**17** · HCl (40 mg)[3]) for 9 days. The cells and medium were treated as described in *3* and *4*. No fraction contained any metabolites.

22. *Administration of [*methyl-$^2H_3$*]sanguinarine (chloride; [*$^2H_3$*]-***1***)* (Exper. 10, Tab. 2). Callus (370 g) was incubated with [$^2H_3$]-**1** (229 mg) for 7 days. The cells and medium were treated as described in *3* and *4*. The *tertiary-alkaloid fraction from cell* gave *5,6-dihydro[*N-methyl-$^2H_3$*]chelirubine* (4.8 mg) and *5,6-dihydro[*N-methyl-$^2H_3$*]macarpine* (12.0 mg). 5,6-Dihydro[*N-methyl*-$^2H_3$]chelirubine: m.p. 200–201° (ether). – High resolution MS.: 363.114 (calc. for $C_{21}H_{17}NO_5$ 363.111); 366.130 (calc. for $C_{21}H_{14}{}^2H_3NO_5$ 366.130).

5,6-Dihydro[*N-methyl*-$^2H_3$]macarpine: m.p. 178–179° (ether). – High resolution MS.: 393.126 (calc. for $C_{22}H_{19}NO_6$ 393.121); 396.142 (calc. for $C_{22}H_{16}{}^2H_3NO_6$ 396.140).

23. *Administration of [*N-methyl-$^2H_3$*]chelirubine (chloride; [*$^2H_3$*]-***3***)* (Exper. 11, Tab. 2). Callus (84 g) was incubated with [$^2H_3$]-**3** (14 mg) for 13 days. The cells and medium were treated as described in *3* and *4*. The *tertiary-alkaloid fraction from cell* gave *5,6-dihydro[*N-methyl-$^2H_3$*]macarpine* (1.5 mg), m.p. 177.5–179° (ether). – High resolution MS.: 393.124 (calc. for $C_{22}H_{19}NO_6$ 393.121); 396.143 (calc. for $C_{22}H_{16}{}^2H_3NO_6$ 396.140).

24. *Administration of [*N-methyl-^{13}C*]allocryptopine [*^{13}C*]-***7***)* (Exper. 12, Tab. 2). Stems (12.5 g, dry weight) of *M. cordata* were dipped into an aq. solution (100 ml) of [^{13}C]-**7** · HCl (20 mg) in small beakers. After 7 days, the plants were harvested and treated as described in *3* and *4*. The *tertiary-alkaloid fraction* gave *5,6-dihydro[*N-methyl-^{13}C*]chelerythrine* (23 mg), m.p. 143–144° (MeOH/ether). – ^{1}H-NMR. ($CDCl_3$): 2.56 (*s*, 2.7 H, CH_3N); 2.56 (*d*, *J* = 135, 0.3 H, $^{13}CH_3N$).

$C_{21}H_{19}NO_4$[2]) (349.4) Calc. C 72.19 H 5.48 N 4.01% Found C 72.39 H 5.45 N 4.05%

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[3]) This substrate was dissolved in 0.5 ml of dimethyl sulfoxide and water (200 ml). The solution was adjusted to pH 6.5 with HCl-solution (5%).