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The formation of corydaline and related alkaloids in *Corydalis cava* in vivo and in vitro

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This paper is dedicated to Professors David B. MacLean and Ian D. Spenser

MARTINA RUEFFER, WOLFGANG BAUER, and MEINHART H. ZENK. Can. J. Chem. **72,** 170 (1994).

The biosynthesis of corydaline and thalictricavine has been investigated by application of [8-^{3}H]-labelled palmatine and berberine to *Corydalis cava* bulb tissue. In both cases, high incorporation into the respective (14*R*,13*S*)corydaline and -thalictricavine was observed. (*S*)-[14-^{3}H, 8-^{14}C]Scoulerine was also transferred into corydaline with all of the tritium being lost, indicating that a redox reaction had taken place in the transition of the (14*S*) to the (14*R*) product. A partly enriched protein fraction catalyzed the reduction of both protoberberine precursors to the 7,8-dihydro intermediate, which was subsequently methylated at C-13 at the expense of *S*-adenosylmethionine (SAM) and reduced at C-14 by a B-type reaction with NADPH as reductant. The identity of the product was unequivocally established by NMR, MS, and CD measurements. This biosynthetic route is in full agreement with previously published predictions based on in vivo experiments.

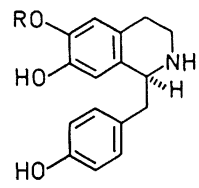
MARTINA RUEFFER, WOLFGANG BAUER et MEINHART H. ZENK. Can. J. Chem. **72,** 170 (1994).

On a étudié la biosynthèse de la corydaline et de la thalictricavine en appliquant de la palmatine et de la berbérine marquées au [8-^{3}H] à des tissus bulbaires de *Corydalis cava*. Dans les deux cas, on a observé une incorporation élevée dans les (14*R*,13*S*)-corydaline et -thalictricavine respectives. La (*S*)-[14-^{3}H, 8-^{14}C]scoulérine se transforme aussi en corydaline avec une perte des trois atomes de tritium; ceci indique qu'il se produit une réaction rédox lors de la transformation du produit (14*S*) en produit (14*R*). Une fraction partiellement enrichie en protéines catalyse la réduction des deux précurseurs protoberbérines en un intermédiaire 7,8-dihydrogéné qui est ultérieurement méthylé en C-13 aux dépens de la *S*-adénosylméthionine (SAM) et réduit en C-14 par une réaction de type B à l'aide NADPH qui agit comme réducteur. On a déterminé sans ambiguïté l'identité du produit en faisant appel à des mesures de RMN, de SM et de DC. Cette voie de biosynthèse est en parfait accord avec les prédictions publiées antérieurement et qui étaient basées sur des expériences in vivo.

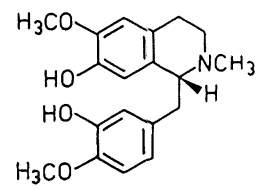
[Traduit par la rédaction]

The biosynthesis of select alkaloids of the protoberberine type has been clarified at the enzyme level using plant cell cultures both from the *Berberidaceae* and the *Ranunculaceae* (1–3). These alkaloids, formed from two molecules of tyrosine, posed an interesting biosynthetic problem, in that tyrosine labelled both the isoquinoline "upper" and the benzyl "lower" portion of the benzylisoquinolines, while the more immediate, dihydroxylated precursors DOPA or dopamine unpredictably labelled only the "upper" half of these molecules (4). This question was elegantly and expertly posed again more recently (5). This intriguing problem was solved when it could be demonstrated that the trihydroxylated (*S*)-norcoclaurine but not the tetrahydroxylated (*S*)-norlaudanosoline is the precursor of the benzylisoquinoline alkaloids (6). (*S*)-Norcoclaurine is derived from the enzyme-catalyzed stereoselective condensation of dopamine with *p*-hydroxyphenylacetaldehyde (1). The biosynthetic pathway to berberine is now completely known also at the enzyme level (1–3). One of the remaining questions was, however, by which mechanism the (*R*)-configurated tetrahydroprotoberberine molecules are formed. It was found that a highly stereoselective and reversible enzyme system using NADPH and protoberberine alkaloids as substrates reduced the C ring of protoberberines to yield the (*R*)-configurated tetrahydroprotoberberines (7). The hitherto claimed precursor role of (*R*)-reticuline (8) in the biosynthesis of these alkaloids is incorrect.

Corydalis cava is a rich source of benzylisoquinoline alkaloids and variously modified molecules (9), among them (+)-corydaline (10, 11). The structure of this compound had been elucidated (12, 13) and the stereochemistry of both asymmetric centers was solved (14). This alkaloid is of biosynthetic interest because it belongs to the (14*R*) series of the tetrahydroprotoberberines and, in addition, contains a rare one-carbon unit as methyl group at C-13.



(*S*)-Norcoclaurine: R = H
(*S*)-Coclaurine: R = CH_3



(*R*)-Reticuline

In an admirable set of experiments, MacLean, Spenser, and co-workers (5, 15) first found that corydaline was synthesized along the protoberberine route with its C-methyl group being supplied by methionine (15). Later, the important observation was made that palmatine and not (*R,S*)-tetrahydropalmatine was the immediate precursor of corydaline (15). Some uncertainty, however, remained as to the true intermediates and precursors of this alkaloid. We now set out to clarify at the enzyme level the route by which this (*R*)-configurated, C-methylated alkaloid is formed in the genus *Corydalis*. First, the incorporation experiments reported by Holland et al. (5) were repeated but, instead of *C. solida*, differentiated plants of *C. cava*, which contain (14*R*,13*S*)-thalictricavine (16) and (14*R*,13*S*)-tetrahydrocorysamine (17), in addition to (14*R*,13*S*)-corydaline (11), were used. Coptisine, palmatine, and berberine, all tritium-labelled in the 8-position, were separately supplied to bulbs of *C. cava* and, after being metabolized, the corresponding C-methylated end products were isolated. High relative incorporation rates of 24–38% were observed (Table 1). These data confirm and extend the previous results obtained with a different plant species by Holland et al. (5). The above incorporation experiment was

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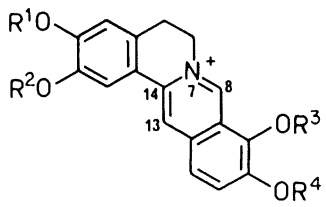
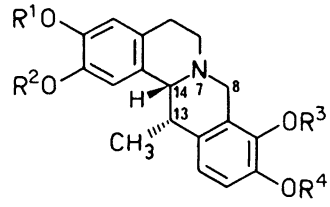


Berberine : $R^1 + R^2 = -CH_2-$; $R^3 = CH_3$; $R^4 = CH_3$
Columbamine : $R^1 = CH_3$; $R^2 = H$; $R^3 = CH_3$; $R^4 = CH_3$
Coptisine : $R^1 + R^2 = -CH_2-$; $R^3 + R^4 = -CH_2-$
Dehydroscoulerine : $R^1 = CH_3$; $R^2 = H$; $R^3 = H$; $R^4 = CH_3$
Jatrorrhizine : $R^1 = H$; $R^2 = CH_3$; $R^3 = CH_3$; $R^4 = CH_3$
Palmatine : $R^1 = R^2 = R^3 = R^4 = CH_3$

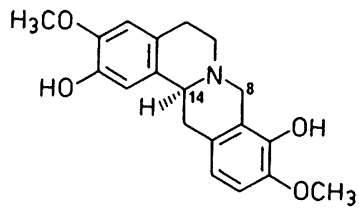


Corydaline : $R^1 = R^2 = R^3 = R^4 = CH_3$
Tetrahydrocorysamine : $R^1 + R^2 = -CH_2-$; $R^3 + R^4 = -CH_2-$
Thalictricavine : $R^1 + R^2 = -CH_2-$; $R^3 = CH_3$; $R^4 = CH_3$

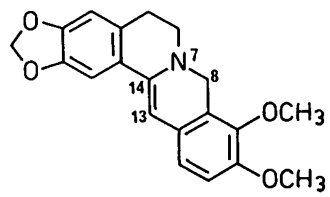
TABLE 1. Feeding of [8-^{3}H]protoberberines to *Corydalis cava* bulb tissue

Substrate	Product isolated	Total activity (dpm)	Product specific activity (dpm/μmol)	Relative incorporation (%)
Coptisine[a]	Tetrahydrocorysamine	9.60×10^5	3.07×10^6	32
Palmatine[a]	Corydaline	5.47×10^5	5.0×10^5	24
Berberine[a]	Thalictricavine	9.65×10^5	1.1×10^5	38

[a]Specific activity: 3.33×10^4 Bq/μmol; a total of 1.85×10^5 Bq was fed.



(*S*)-Scoulerine



7,8-Dihydroberberine

repeated using a mixture of (*S*)-[14-^{3}H]scoulerine and (*S*)-[8^{14}C]scoulerine, the $^3H/^{14}C$ ratio being 9.8:1. (+)-Corydaline was isolated from the tissue, diluted with unlabelled carrier corydaline, and recrystallized 6 times. Constant specific activity was attained already after the fourth recrystallization. A 2.6% relative incorporation of (*S*)-scoulerine into corydaline was found and the initial $^3H/^{14}C$ ratio of 9.8:1 was lowered to 0.01:1. This indicated clearly that the tritium label was almost completely removed from position 14 of the (*S*)-scoulerine molecule and substantiated the previously found reversal of configuration from (*S*) to (*R*) by an oxido-reduction process as established for the formation of (*R*)-tetrahydroprotoberberines from protoberberines (7).

Since the *C. cava* tissue obviously has a high metabolic potential to convert protoberberines into the corydaline-type compounds, a cell-free system was tested to produce corydaline in vitro. A crude homogenate was prepared from *C. cava* bulbs, and was freed of contaminating low molecular weight compounds by treatment with Sephadex G 25. Unlabelled palmatine was incubated with NADPH and [C^{3}H$_3$]SAM in the presence of *C. cava* crude protein extract at pH 7.5. As shown in Fig. 1*A*, under these conditions a product was formed that had the same R_f value as authentic corydaline. Almost all radioactive SAM had been converted to this product. In control experiments either using heat-denatured enzyme (Fig. 1*B*) or omitting NADPH (Fig. 1*C*), no reaction occurred. In addition, the newly formed product had the same R_f value as corydaline in several other TLC solvent systems. To ascertain the identity of this product, the compound was diluted with unlabelled corydaline (calculated specific activity 1.5×10^4 dpm/μmol), and recrystallized 6 times to yield a specific activity of 1.06×10^4 dpm/μmol. This experiment proved that corydaline was indeed formed under the conditions given in Fig. 1. Extension of this type of experiment showed that neither (*R*)- nor (*S*)-tetrahydropalmatine could serve as acceptor for the activated methyl group of SAM, and verified the in vivo experiments of Holland et al. (5) with regard to the precursorship of palmatine at the in vitro level.

To obtain more insight into this rather complex reaction of reduction and C-methylation, we attempted to partially purify the corresponding enzyme. Since in preliminary experiments it was found that berberine was the best substrate, this substance was used for further experiments, (+)-thalictricavine being the end product of the reaction. The purification of the catalytic activity was carried out using 40 g of deep-frozen cells. Alkaloids and other low molecular weight material were removed from the protein solution by passage through an XAD column. The eluate was loaded directly onto a DEAE-Sephacel column and the enzyme eluted with KCl. The pooled fractions were chromatographed on a hydroxyapatite column at pH 6.5. The enzyme did not bind to this column but was eluted with the washthrough. This protein solution was subjected to affinity

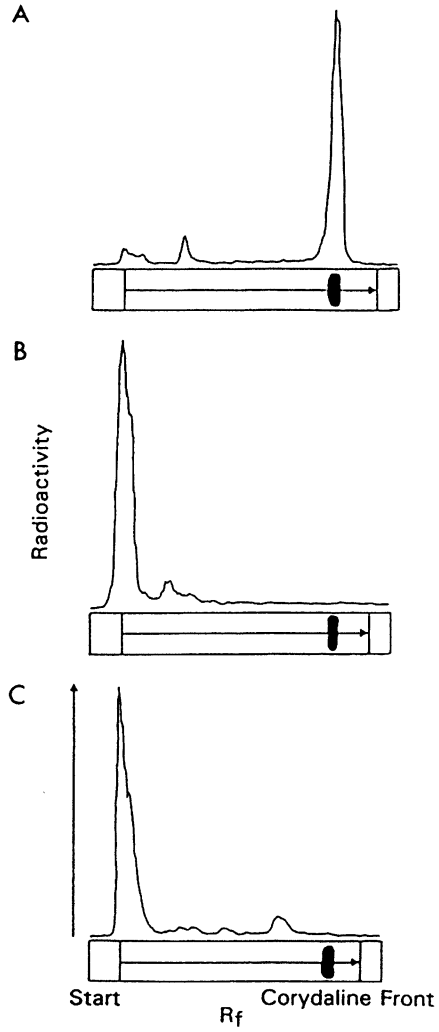


FIG. 1. Radioscan of an incubation mixture after TLC separation consisting of *A*: 25 nmol palmatine, 1 μmol NADPH, 27 pmol [C^3H_3]SAM (5 × 10^6 dpm), 860 μg *Corydalis cava* protein, in a total volume of 460 μL 50 mM KPO_4^{2-} buffer, pH 7.5; *B*: as in *A*, but with heat-denatured protein; *C*: as in A but without NADPH.

chromatography employing an SAH-AH Sepharose 4B matrix. Elution of the enzyme was achieved with KCl. This procedure yielded a 54-fold purified enzyme with 32% yield based on the crude homogenate. The purification procedure is summarized in Table 2. The enzyme is a cytosolic protein; no evidence for its occurrence in an organelle could be found. The enriched enzyme showed a pH optimum at 8.0 and a temperature optimum at 37°C (Figs. 2*A* and *B*). The M_r of the enzyme was determined by gel permeation HPLC (using appropriate marker proteins) and showed a distribution coefficient that corresponds to a M_r of 56 000, assuming a globular shape of the protein. Lineweaver–Burk plots showed apparent K_M values of 5 μM for berberine, 16 μM for NADPH, and 2.7 μM for SAM as substrates. Using the same three substrates, a K_i value of 2.6 μM was determined for SAH, which demonstrates that this homocysteine derivative, a reaction product, is a potent inhibitor of the methyltransferase reaction.

The product of the reaction was rigorously identified by physical methods. Large-scale standard incubations (total of 300 mL) were performed with [$^{13}CH_3$]SAM and a DEAE prepurified enzyme fraction. After 5 h of incubation at 37°C the mixture was passed through an XAD column that adsorbed the alkaloids. The column was washed thoroughly with distilled water and subsequently eluted with methanol. The eluate was evaporated and subjected to NMR analysis. Figure 3 shows a section of the NMR spectrum depicting clearly the ^{13}C-enriched carbon atom, which is attached to C-13 of thalictricavine (Fig. 3*B*). An aliquot of the enzymic reaction product was subjected to mass spectrometry (EI-mode) and yielded a mass peak at 354 *m/z* (Fig. 4*B*), one mass unit more than the unlabelled reference (Fig. 4*A*), as expected. Furthermore, a prominent benzylic fragment of *m/z* 179 was observed, which represents the D ring of corydaline with the $^{13}CH_3$ group originating from SAM attached to it (Fig. 4*B*). No doubt, the reaction product is thalictricavine. To ascertain the correct stereochemistry of the thalictricavine formed, a CD spectrum of the enzyme-synthesized product was recorded. The CD spectrum of this compound shown in Fig. 5 is identical with that one published previously (14). The enzyme-synthesized product is therefore (+)-thalictricavine, identical with that compound previously isolated from various *Corydalis* species (5, 10–13, 15, 16).

Finally, the substrate specificity of this enzyme was of considerable interest. Employing the reductive enzyme assay in the presence of [C^3H_3]SAM, NADPH, and various alkaloidal substrates using a 50-fold purified enzyme, the results shown in Table 3 were obtained. All protoberberines tested so far, except dehydroscoulerine, served as methyl group acceptor to varying degrees. Fully substituted protoberberines possessing no free OH group (berberine, palmatine) were the best substrates, followed by alkaloids having one unsubstituted OH group (columbamine, jatrorrhizine). The tetrahydroprotoberberines were completely inactive. Dehydroscoulerine was absolutely inactive in this system. This is in accord with the previously found reductase (7), which also is almost inactive with this substrate. It is most likely that this reductase, which operates in two steps (7), first reducing the 7,8 double bond and subsequently the 13,14 double bond, to yield the (*R*)-tetrahydroprotoberberines, is identical with that needed for (+)-thalictricavine biosynthesis. The assumption that this very enzyme is involved in thalictricavine biosynthesis is supported by the fact that 7,8-dihydroberberine serves indeed as an excellent substrate for the methyltransferase with concomitant reduction to the (*R*)-configurated (+)-thalictricavine (Table 3). Presuming that the previously found reductase (7) is involved in (+)-thalictricavine formation, the hydrogen transfer from NADP^{3}H should also follow a B-type reaction. Incubation of berberine in the presence of SAM and either 4A-^{3}H-NADPH or 4B-^{3}H-NADPH (18) under optimal conditions, and isolation of the (+)- thalictricavine formed, clearly showed that absolutely no tritium was transferred from 4A-^{3}H-NADPH, while 48.8% of the radioactivity of the 4B-^{3}H-NADPH was found in thalictricavine. This clearly demonstrated that the reductase involved is a B-type enzyme and most likely identical with the (*R*)-specific reductase found previously in *C. cava* (7). The (*R*)- or (*S*)-tetrahydroprotoberberines do not serve as acceptors for the methyl group of SAM.

The biosynthesis of (+)-corydaline and congeners has now been worked out at the enzyme level. The protoberberine molecule is enzymically reduced by a specific dehydrogenase (7) to a 7,8-dihydro derivative. This intermediate (which can also be supplied in a synthetic form) acts at C-13 as an acceptor for the

TABLE 2. Purification of *S*-adenosylmethionine: protoberberine-13C-methyltransferase from 40 g *Corydalis cava* cultivated cells

Purification step	Total activity (pkat)	Total protein (mg)	Specific activity (pkat/mg)	Yield (%)	Purification factor (×)
Crude extract	261	440.0	0.6	100	1.0
XAD-2	240	242.0	1.0	92	1.7
DEAE-Sephacel	153	43.3	3.5	58	5.8
Hydroxyapatite	118	16.0	7.4	45	12.3
SAH-AH Sepharose 4B	84	2.6	32.3	32	53.8

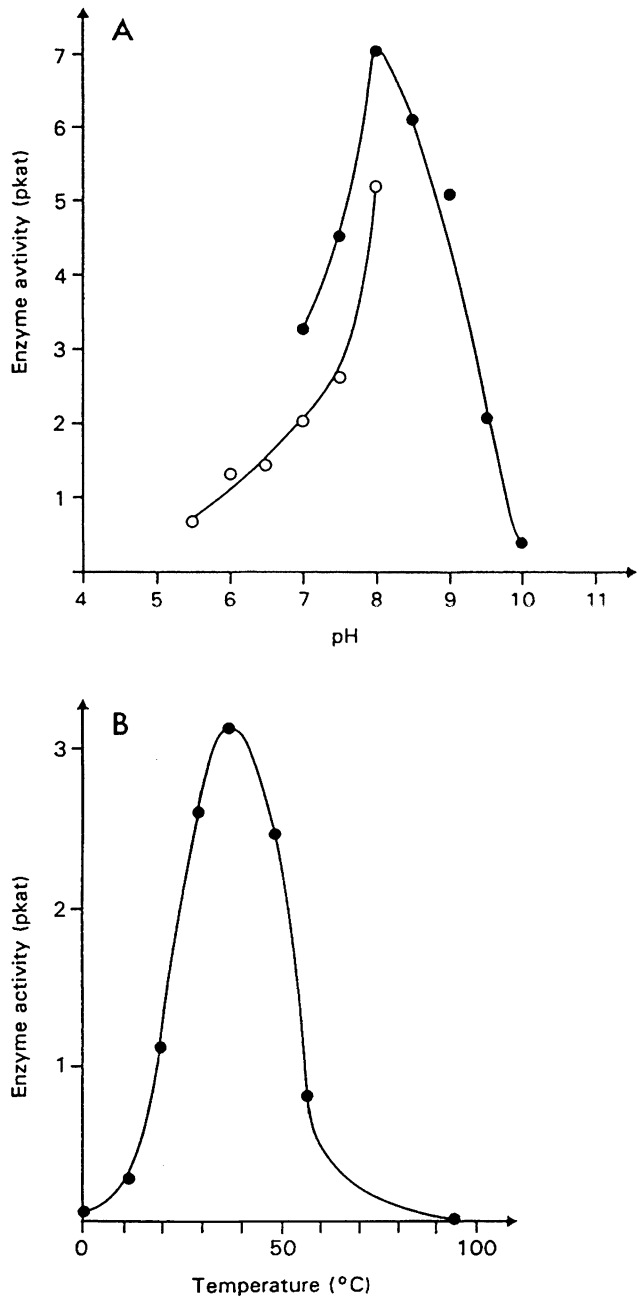


FIG. 2. *A*: pH profile of the catalytic activity of the 50-fold enriched enzyme system yielding thalictricavine using standard incubation mixtures with 100 μM each of (●—●) Tris-HCl buffer; (○—○) KPO_4^{2-} buffer, at 37°C. *B*: Temperature profile using the standard incubation assay at pH 8.0.

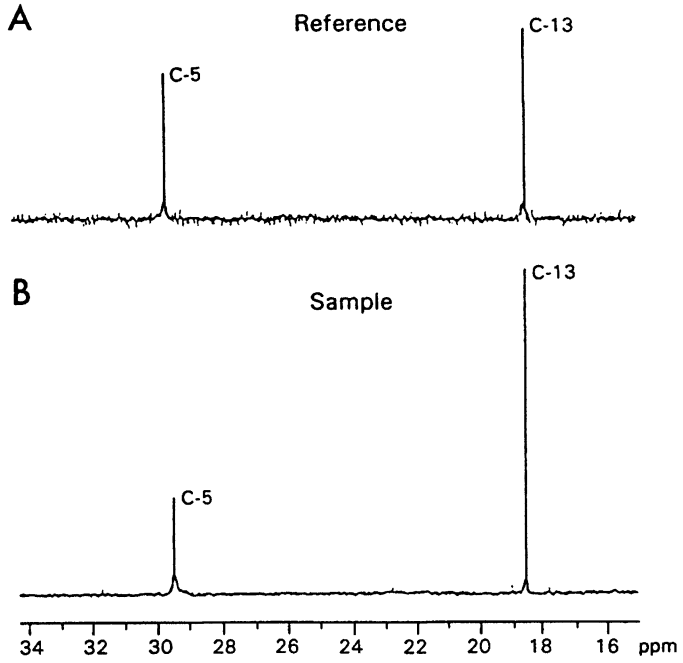


FIG. 3. Partial proton-decoupled ^{13}C NMR spectra of thalictricavine. *A*: unlabelled reference compound; *B*: enzyme product formed by transfer of the methyl group from [$^{13}CH_3$]SAM.

SAM-derived methyl group, and is subsequently reduced in a B-type reaction at the expense of NADPH to yield the (14*R*,13*S*)-(+)-corydaline-type molecule. The course of reaction is depicted in Scheme 1. It is most rewarding to see that the prediction of MacLean and Spenser and co-workers (5, 15) based entirely on in vivo feeding experiments and theoretical considerations could be verified by the results given here, which were obtained in vitro.

Experimental

Plant material

Plants of *Corydalis cava* were grown in pots in the greenhouse of this institute. Plant cell cultures of *Corydalis cava* were cultivated in 1-L Erlenmeyer flasks containing 250 mL Linsmaier and Skoog medium (19) over a period of 7 days at 23°C on a gyratory shaker (100 rpm) at 750 lux. The cells were then harvested by suction filtration, frozen in liquid nitrogen, and stored at −20°C.

Reference alkaloids

Thalictricavine, corydaline, and tetrahydrocorysamine were a kind gift of Prof. Slavik (Brno). Berberine and scoulerine were purchased from Roth (Karlsruhe). Coptisine and palmatine were donated by Dr. T. Tanahashi (Kobe). 7,8-Dihydroberberine was synthesized according to ref. 20.

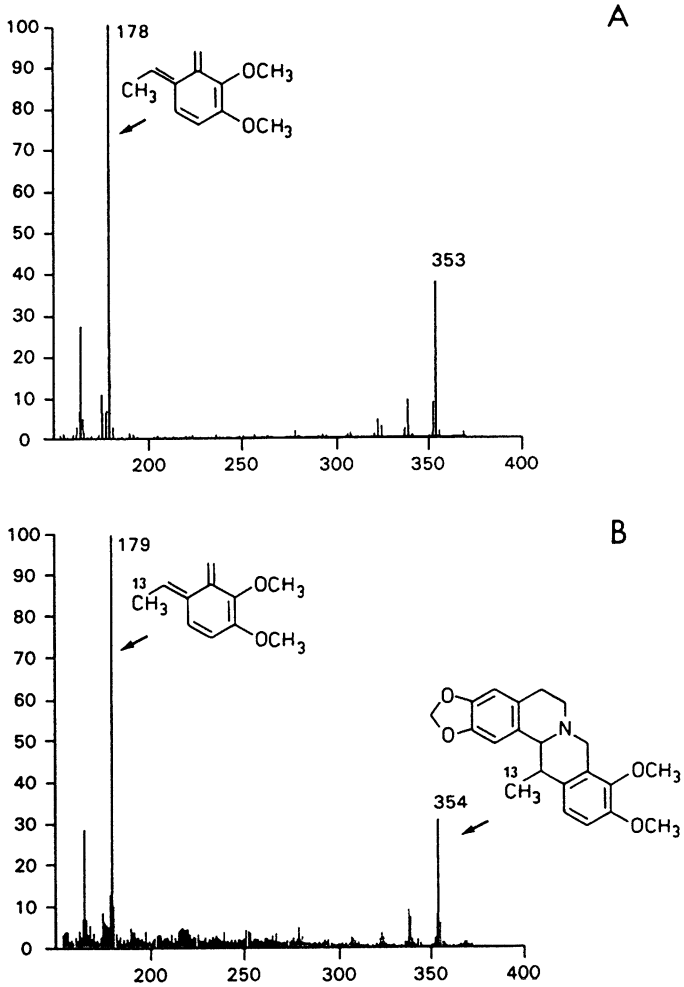


FIG. 4. Partial mass spectra (chemical ionization; isobutane) of thalictricavine. *A*: unlabelled reference compound; *B*: enzymic product formed by transfer of the methyl group from [$^{13}CH_3$]SAM.

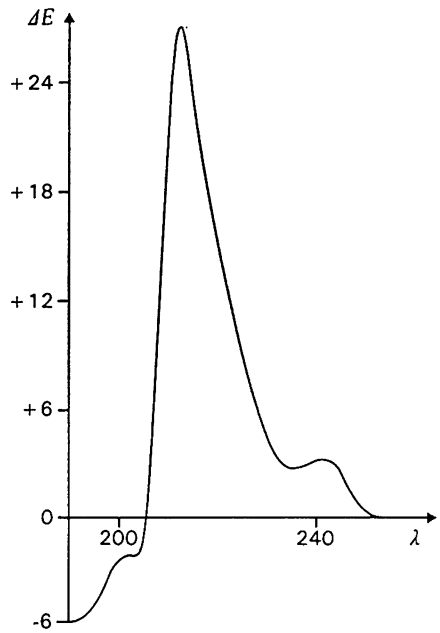


FIG. 5. CD spectrum of enzymically formed thalictricavine (concentration: 0.92 mg/2 mL methanol). Note positive value at 236 nm and 206 nm, negative at 192 nm analogous to (+)-corydaline (14).

TABLE 3. Substrate specificity of *S*-adenosylmethionine: protoberberine-13C-methyltransferase

Substrate[a]	Product formed (pmol)	Relative activity (%)
Berberine	900	100
Dihydroberberine	837	93
(*R,S*)-Canadine	0	0
Columbamine	243	27
Coptisine	189	21
Dehydroscoulerine	0	0
Jatrorrhizine	603	67
Palmatine	837	93

[a]The respective substrate (10 nmol) and 1 pkat of the enzyme were added to the standard assay and incubated for 15 min at 30°C.

Labelled chemicals

[C^3H_3]Methionine (3.15×10^9 Bq/μmol) was purchased from Amersham (U.K.) and transformed to *S*-adenosyl-(L)-methionine (SAM) by SAM-synthetase, isolated from *Escherichia coli* EWH 47 according to ref. 21. [$^{13}CH_3$]SAM was synthesized by the same procedure using [$^{13}CH_3$]methionine. Stereospecifically labelled 4A- and 4B-^{3}NADPH were synthesized according to the method of Moran et al. (18). [8-^{3}H]-Labelled coptisine, palmatine, and berberine were synthesized by simultaneous reduction of all three protoberberines with NaB^3H_4 and reoxidation of the corresponding (*S*)-tetrahydroprotoberberines was performed with (*S*)-tetrahydroprotoberberine oxidase (22). (*S*)-[8-^{14}C]Scoulerine was synthesized enzymatically in a two-step reaction by incubating (*S*)-norreticuline and [$^{14}CH_3$]SAM, first with *S*-adenosylmethionine:coclaurine-*N*-methyltransferase (23) and then with the berberine bridge enzyme (24). (*S*)-[14-^{3}H]Scoulerine was produced by incubation of (*R,S*)-[1-^{3}H]reticuline with the berberine bridge enzyme (24).

Application experiments

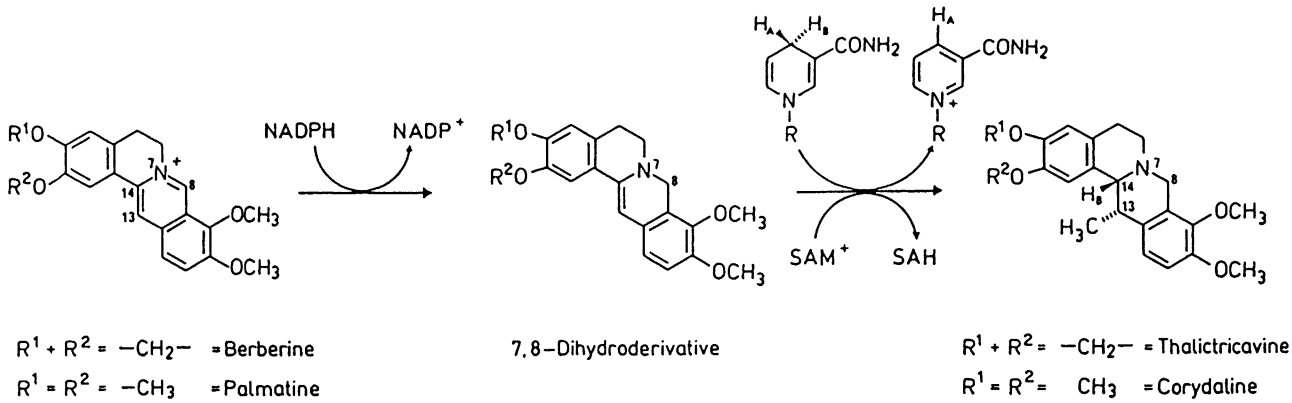
Young bulbs of *Corydalis cava* were cut into small pieces of 0.5 g fresh weight and incubated in 1 mL Knop's nutrient solution with berberine, coptisine, or palmatine (each 7.83×10^4 Bq/μmol). After extraction with methanol the labelled alkaloids were purified on TLC plates in three solvent systems: (1) ethyl acetate:2-butanone:formic acid:H_2O (50:30:10:10), R_f thalictricavine = 0.89, R_f corydaline = 0.86, R_f tetrahydrocorysamine = 0.94; (2) cyclohexane:diethylamine (9:1), R_f thalictricavine = 0.63, R_f corydaline = 0.55, R_f tetrahydrocorysamine = 0.71; (3) dioxane:cyclohexane:diethylamine (10:20:0.5), R_f thalictricavine 0.84, R_f corydaline = 0.75, R_f tetrahydrocorysamine = 0.77.

Enzyme assay

Berberine (10 nmol), 1 nmol [C^3H_3]SAM (20 000 cpm), and 100 nmol NADPH were incubated with 20 μmol KPO_4^{2-} buffer (pH 8.0) and 0.1–1 mg protein in a total volume of 250 μL for 30 min at 37°C (standard incubation mixture). The reaction was stopped by the addition of 200 μL sodium carbonate buffer (pH 9.5) and the reaction product extracted with 400 μL isoamyl alcohol for 10 min in a rotary shaker. After centrifugation (3 min in an Eppendorf centrifuge) the radioactivity of a 200-μL aliquot was determined in a scintillation counter.

Enzyme purification

Corydalis cava deep-frozen cultivated cells (40 g) were ground with a chilled mortar and pestle in 80 mL 10 mM KPO_4^{2-} buffer (pH 6.5, 10 mM β-mercaptoethanol) for 15 min, filtered through four layers of cheesecloth, and centrifuged for 10 min at 20 000 × *g*. The supernatant was passed through an XAD-2 column (Serva, Heidelberg, 2.5 cm × 20 cm) to remove the alkaloids and pumped onto a DEAE column (DEAE-Sephacel, Pharmacia, 2.5 cm × 25 cm, equilibrated with the extraction buffer) at a flow rate of 1 mL/min. Elution was per-



SCHEME 1

formed with a gradient from 0–1 M KCl over 8 h in fractions of 2.5 mL. The active fractions (98–115) were combined (57 mL) and pumped onto a hydroxyapatite column (Biorad, 1 cm × 15 cm, equilibrated with 10 mM KPO_4^{2-} buffer, pH 6.5, 10 mM β-mercaptoethanol) at a flow rate of 0.3 mL/min. The enzyme did not bind to the column but was eluted with the washthrough in 55 mL. This solution was slowly (0.2 mL/min) loaded onto an SAH-AH-Sepharose column (Pharmacia 1 cm × 5 cm, equilibrated with 10 mM KPO_4^{2-} buffer, pH 7.5, 10 mM β-mercaptoethanol) and eluted with a linear gradient up to 1 M KCl in 4 h. Fractions of 1.5 mL were collected. Enzyme activity was eluted with 1 M KCl in 7.5 mL.

Product identification

For identification of the product, four of the following large-scale assays were prepared: 10 mmol KPO_4^{2-} buffer, pH 8.5, 1 μmol [$^{13}CH_3$]SAM, 10 μmol berberine, 50 μmol NADPH, 50 μmol NADP, 50 μmol glucose-6-phosphate, 5 units glucose-6-phosphate-dehydrogenase (Serva, Heidelberg), and 75 mL enzyme solution (DEAE- eluate, 100 mg protein) in a total volume of 250 mL. After incubation (5 h at 37°C) the mixtures were passed through an XAD-2 column (2 cm × 10 cm) and the alkaloids eluted with methanol. After concentration under a stream of nitrogen, the product was purified by TLC (Macherey & Nagel, Polygram SIL G/UV 254, solvent system cyclohexane:diethylamine (9:1), R_f thalictricavine = 0.6, R_f SAM = 0.0), eluted with methanol, and subjected to mass spectrometry (MS 80 RFA, Kratos, EI, 70 eV) and ^{13}C NMR (in deuterated methanol–DMSO, AM, 360, Bruker). The CD spectrum was measured in ethanol with a CD-6 (Jobin-Yvon) instrument.

Stereospecificity of H-transfer from 4A- and 4B-^{3}H-NADPH

To test the stereospecificity of the reduction reaction 100 000 cpm 4A- or 4B-^{3}H-NADPH (18) (1.63×10^8 Bq/μmol), 10 nmol berberine, and 1 nmol SAM were incubated with 0.2 pkat of the enzyme in 50 μmol KPO_4^{2-} buffer, pH 8.0, at 30°C for 1 h. The reaction product was purified by TLC (cyclohexane:diethylamine (9:1), R_f thalictricavine = 0.6) and eluted with methanol. Scintillation counting of the methanol eluate showed no incorporation with 4A-^{3}H-NADPH, but with 4B-^{3}H-NADPH 48775 cpm (48.8%) could be detected in thalictricavine.

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