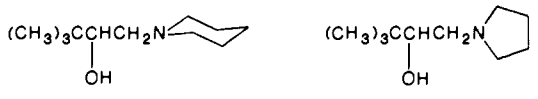
Table I. Asymmetric Amplification in Enantioselective Addition of Diethylzinc to Benzaldehyde[a]

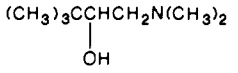
catalyst[b]	% ee of catalyst[c] ($[\alpha]^{24}_D$ (c 1.5, EtOH)) (deg)	product yield (%)	product $[\alpha]^{24}_D$ (c 2.0, EtOH) (deg)	product % ee[d] (confign)
1	3.1 (-2.3)	82	+17.6	36 (R)
	6.5 (-4.7)	95	+36.1	74 (R)
	10.7 (-7.8)	96	+40.1	82 (R)
	20.5 (-15.0)	96	+43.0	88 (R)
	59.8 (-43.6)	95	+45.0	92 (R)
	77.1 (+56.2)	96	-46.0	94 (S)
2	3.8 (+2.7)	78	-17.1	35 (S)
	7.4 (+5.3)	81	-25.4	52 (S)
	20.0 (+14.3)	92	-35.7	73 (S)
3	10.5 (-9.3)	88	+22.5	46 (R)
	19.8 (-17.5)	82	+29.4	60 (R)

[a] Reaction was carried out in degassed hexane at -10 °C by using 2 mol% of catalyst and 1.1 equiv of diethylzinc per benzaldehyde. [b] 1, 1-piperidino-3,3-dimethyl-2-butanol; 2, 1-pyrrolidino-3,3-dimethyl-2-butanol; 3, N,N-dimethyl-2-hydroxy-3,3-dimethylbutylamine. [c] Determined by HPLC (Sumipax OA 4000) of 3,5-dinitrophenylurethane derivatives. [d] Absolute configuration: Macleod, R.; Welch, F.; Mosher, H. S. *J. Am. Chem. Soc.* **1960**, *82*, 876.

group, for instance, 1-piperidino-3,3-dimethyl-2-butanol (PDB), **1**, 1-pyrrolidino-3,3-dimethyl-2-butanol, **2**, and *N,N*-dimethyl-2-hydroxy-3,3-dimethylbutylamine, **3**.[4] Thus, under the influence



1, PDB 2



3

of 2 mol% of (-)-PDB (10–20% ee), diethylzinc reacted with benzaldehyde (1.1:1 molar ratio) in hexane at -10 °C, and (*R*)-1-phenylpropanol was obtained in 80–90% ee and in high chemical yield. Table I exemplifies the asymmetric amplification. Wynberg and Feringa established the origin of the different chemical behaviors in reaction rates and product distribution of an enantiomerically pure compound and the corresponding racemic mixture in the absence of chiral reagents.[5] The asymmetric amplification observed in the reaction of diethylzinc with benzaldehyde will provide important insights into the reaction mechanism of the asymmetric alkylation. For instance, the equimolar reaction products of optically pure PDB and racemic PDB with diethylzinc both form dimers in benzene solution, as determined by cryoscopic molecular weight measurements. Also, the ee of the auxiliary used in the reaction has a marked effect on the reaction rate. For the typical example, the reaction rate with 60% ee of PDB was 5.5 times the one observed with completely racemic PDB under the same reaction condition of diethylzinc with benzaldehyde.

A typical experimental procedure is illustrated as follows: In a flame-dried Schlenk tube was placed (-)-PDB of 10.7% ee (167 mg, 0.9 mmol) and dry hexane (100 mL), and the whole mixture was degassed and covered with argon. Tc this solution was added diethylzinc (5.5 mL, 45.5 mmol), and the resulting solution was stirred at 20 °C for 15 min. After cooling to -10 °C, benzaldehyde (4.8 g, 45 mmol) was added, and the mixture was stirred for 12 h and quenched by adding 10% aqueous HCl. The usual extractive workup and distillation gave (*R*)-1-phenylpropanol in 82% ee (5.9 g, 96% yield), $[\alpha]^{22}_D$ +40.1° (*c* 2.26 $CHCl_3$).[6] The ee was determined by HPLC analysis (column, Daicel Chirapak OB; eluent, 0.2% 2-propanol in hexane; flow rate, 1.0 mL/min; detection, 254 nm light).

The detailed mechanism of the asymmetric amplifying phenomena observed in this work will be published in the near future.

Acknowledgment. We thank Dr. R. Noyori of Nagoya University for helpful dicussions of our results.

(4) 1-Piperidino-3,3-dimethyl-2-butanol was prepared by the following procedure. 1-Bromo-3,3-dimethyl-2-butanone was reacted with piperidine in benzene in the presence of triethylamine to give 1-piperidino-3,3-dimethyl-2-butanone. The product was reduced by $LiAlH_4$ (molar ratio, 1:0.5) in ether, which gave 1-piperidino-3,3-dimethyl-2-butanol in quantitative yield under usual experimental workup. The resulting racemic product was optically resolved by repeated crystallizations of a salt with (-)-dibenzoyl-L-tartaric acid (molar ratio, 1:1). The chemical analysis and NMR data of **1** coincided with its structure. $[\alpha]^{22}_D$ -71.5° (*c* 1.91, ethanol) for 98% ee's. The compounds **2** and **3** were also prepared similarly as the preparative method of **1**. $[\alpha]^{23}_D$ -14.3° *c* 2.13, ethanol) for 20% ee of **2**. $[\alpha]^{21}_D$ -17.5° (*c* 2.08, ethanol) for 19.8% ee of **3**. The ee's of the compounds **1–3** were determined by HPLC analysis of their 3,5-dinitrophenylurethane derivatives in comparison with racemic ones (column; Sumipax OA 4000; eluent, 0.2% methanol in hexane; flow rate, 1.0 mL/min; detection, 254 nm light).

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Stereochemistry of Enzymatic Formation of the Berberine Bridge in Protoberberine Alkaloids

Thomas Frenzel,[†] John M. Beale,[‡] Motomasa Kobayashi,[‡,§] Meinhart H. Zenk,*[,†] and Heinz G. Floss*[,‡,⊥]

Institut für Pharmazeutische Biologie der Universität München, Federal Republic of Germany
Department of Chemistry, The Ohio State University Columbus, Ohio 43210
Department of Chemistry BG-10 University of Washington Seattle, Washington 98195
Received April 25, 1988

An intriguing facet in the biosynthesis of the protoberberine family of benzylisoquinoline alkaloids is the formation of the "berberine bridge". Barton et al.[1] and Battersby and co-workers[2] demonstrated 25 years ago that the berberine bridge, C-8 of scoulerine (**4**) and columbamine (**7**) (Scheme I), arises by an oxidative cyclization from the *N*-methyl group of reticuline (**3**). The reaction sequence has since been elucidated in detail,[3] and the enzymes involved have been purified.[4] As shown in Scheme I, it leads from *S*-adenosyl-L-methionine (AdoMet, **1**) and *S*-*N*-

[†] Institut für Pharmazeutische Biologie der Universität München.
[‡] The Ohio State University.
[§] Permanent address: Faculty of Pharmaceutical Sciences, Osaka University, Osaka, Japan.
[⊥] University of Washington.

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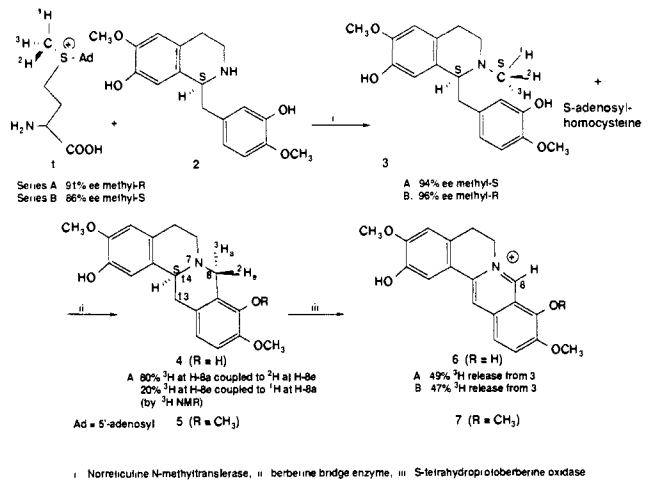
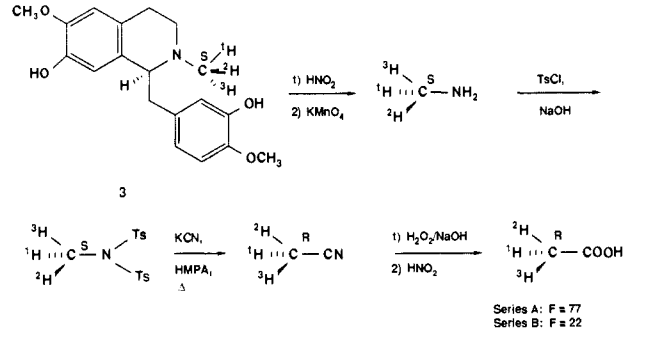
Scheme I



Scheme II



norreticuline (**2**) via **3** to **4**.[5,6] On the main pathway, **4** is first *O*-methylated to *S*-tetrahydrocolumbamine (**5**) which is then dehydrogenated by *S*-tetrahydroprotoberberine oxidase[7,8] (STOX) to **7**; however, STOX can also oxidize **4** to dehydroscoulerine (**6**).

To gain further insight into the mechanisms of these transformations we traced the fate of chiral methyl groups from AdoMet through this sequence. Starting from (methyl-*R*)- and (methyl-*S*)[methyl-$^2H_1$,^3H]AdoMet (100 mCi/mmol, 91% ee *R* and 160 mCi/mmol, 86% ee *S*, respectively) norreticuline *N*-methyltransferase[9] produced two samples of **3**. Aliquots of these were degraded as shown in Scheme II,[10] with one inversion of configuration, to give acetic acid for chirality analysis.[11-13] *F* values[13,14] of 77 and 22 for the latter indicated 94% ee *S* and 96% ee *R* configuration, respectively, for the *N*-methyl group in the two samples of **3**. Hence the methyl group of AdoMet is transferred to the nitrogen of **2** with clean inversion of configuration, consistent with the observations on most other methyltransferases.[14,15]

Next, we subjected the two samples of chirally labeled **3** to the sequential action of the berberine bridge enzyme (BBE) and STOX and monitored the release of tritium into the medium (Figure 1). BBE releases from each substrate about 8% of the tritium, pointing to a sizeable isotope effect in the abstraction of a hydrogen from the *N*-methyl group (cf. ref 5). Upon addition

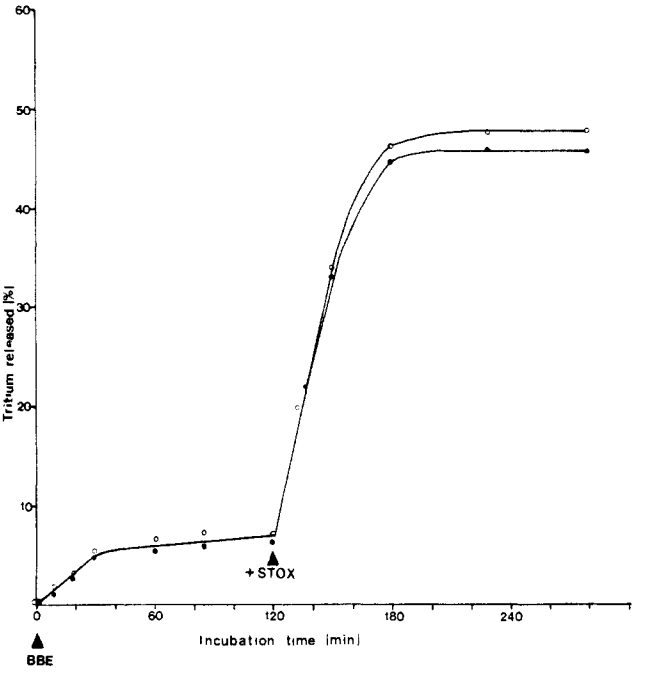
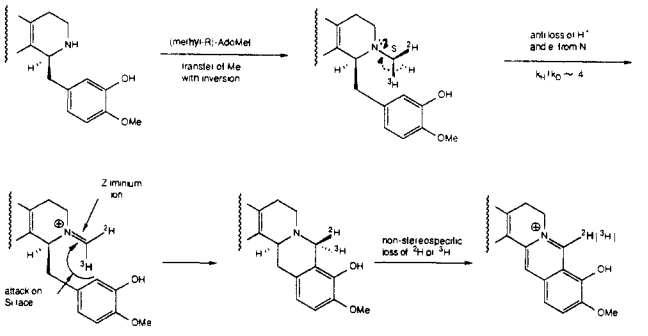


Figure 1. Tritium release in the formation of the berberine bridge by BBE and its dehydrogenation by STOX with (methyl-*R*)[methyl-$^2H_1$,^3H]-**3** (●) and (methyl-*S*)[methyl-$^2H_1$,^3H]-**3** (○) as substrate. The incubations contained 4.2 nmol of **3** (0.67 and 0.42 μCi, respectively) and 0.25 pkat BBE. Samples were analyzed at the times indicated. At 120 min 5 pkat STOX were added, and the analyses were continued.

Scheme III



of STOX nearly half of the remaining tritium is released from both substrates. It follows that either BBE or STOX or both enzymes must act nonstereospecifically at the labeled center and that hydrogen abstraction from C-8 proceeds with little or no isotope effect.

Finally, to establish the configuration of the tritiated methylene group (C-8) in **4**, we prepared a larger sample of tritiated **4** hydrochloride (153 μCi after purification) from (methyl-*R*)-[methyl-$^2H_1$,^3H]AdoMet (800 μCi, 86% ee *R*) by methylation of **2** with norreticuline *N*-methyltransferase followed by ring closure with BBE. Tritium NMR spectroscopy (IBM AF-300; 7.1 T, composite pulse ^1H decoupled) on this sample revealed the presence of two signals, one at 4.76 ppm (20%, ^3H-8e, $\delta_{^1H}$ 4.76) and the other at 4.29 ppm (80%, ^3H-8a, $\delta_{^1H}$ 4.34) (Figure 2). The proton NMR signals for the axial and equatorial protons were assigned unequivocally by using an identically prepared sample of scoulerine hydrochloride. A 2-D phase-sensitive NOESY[16] experiment as well as a 1-D steady-state NOE difference spectrum clearly showed only the signal for 8a (δ 4.34) to undergo cross-relaxation with H-14a and H-6a. These assignments are in accord with anisotropy arguments presented in the earlier literature,[17,18]

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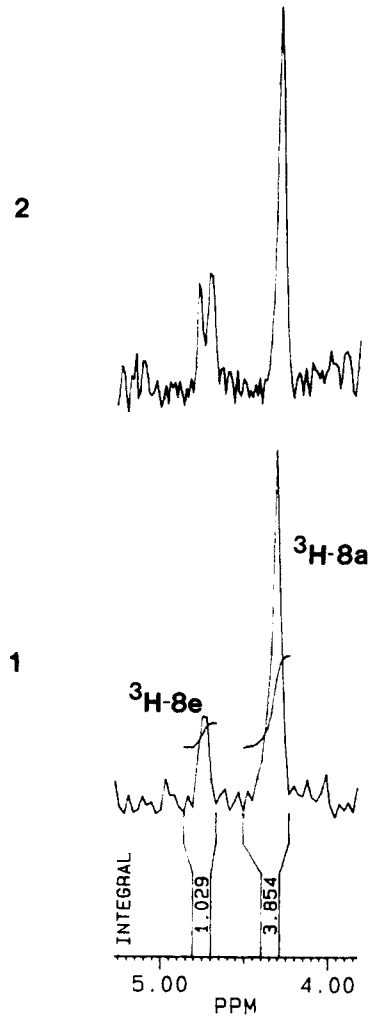

Figure 2. Tritium NMR spectra of **4** generated from (methyl-*S*)[methyl-$^2H_1$,^3H]-**3** with BBE. Sample contains 153 μCi ^3H, solvent CD_3OD, repetition time 1.0 s; spectrum 1, composite pulse broadband ^1H decoupled, 76 472 acquisitions; spectrum 2, ^1H gated decoupled, WALTZ-16 ^2H broadband decoupled, 47 347 acquisitions.

and coupling constants are consistent with the B/C *trans*-tetrahydroprotoberberine configuration. Upon removal of the broadband proton decoupling the ^3H NMR signal at 4.76 ppm split into a doublet (J = 15.3 Hz), whereas the signal at 4.29 ppm remained unchanged. Hence, 80% of the tritium is present in the *pro*-8*R* position flanked by deuterium (0.05 ppm isotope shift), and 20% is present in the *pro*-8*S* position coupled to ^1H.

It follows from the data that BBE operates highly or completely stereospecifically, replacing an *N*-methyl hydrogen by the phenyl group in an inversion mode. Consistent with earlier findings[5] the hydrogen abstraction involves a primary kinetic isotope effect ($k_H/k_D \sim 4$). It is proposed that the enzyme abstracts an electron from the nitrogen and a hydrogen atom from the methyl group in an anti fashion to generate, from (methyl-*S*)[methyl-$^2H_1$,^3H]-**3**, the (*Z*)-methyleneiminium ion which is then attacked by C-2 of the phenyl ring on the *si* face (Scheme III). The observation of nonstereospecific hydrogen removal from C-8 in the aromatization of ring C supports existing notions about the mechanism of this process. On the basis of the observed stoichiometry, 1.5 mol of O_2 consumed and 1 mol of H_2O_2 produced per mol of substrate, it has been suggested[7,8] that the enzyme STOX only catalyzes the dehydrogenation of the substrate to the 7,14-iminium ion. The latter than undergoes spontaneous air oxidation to **6** or **7**, respectively. This proposal is supported by the finding by Battersby and co-workers[19] that scoulerine tritiated stereoselectively at C-13 is converted in *Chelidonium majus* plants into berberine and coptisine with "extensive and nonstereospecific loss of hydrogen from C-13".[20]

In addition to unraveling the steric course of berberine bridge formation, the above data attest to the remarkable sensitivity of tritium NMR. In the present work, even on a medium field instrument 30 μCi of tritium in a single position were readily detected in an overnight run, suggesting that on a high field instrument under optimal conditions the detection limit can probably be pushed below 10 μCi per position.

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(20) It should be noted, however, that the loss of tritium from C-13 of **4** is much greater than expected [82-86% versus at most 50%].

The Reaction of 2,3-Diazabicyclo[2.2.2]oct-2-ene with Stable Cation Radical Salts

Paul S. Engel,*,† A. K. M. M. Hoque,‡ John N. Scholz,† Henry J. Shine,‡ and Kenton H. Whitmire†

Departments of Chemistry, Rice University Houston, Texas 77251
Texas Tech University, Lubbock, Texas 79409
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Whereas the free-radical chemistry of azoalkanes, initiated by photochemical and thermal decompositions, is richly documented,[1] their oxidative chemistry has begun to emerge only in the last few years.[2-11] For example, 1,1'-azoadamantane is oxidized by thianthrene cation radical ($Th^{\bullet+}$) perchlorate in acetonitrile solution, affording primarily nitrogen and cation-derived products.[2] We report here a novel reaction in the oxidative chemistry of azoalkanes.

Reaction of 2,3-diazabicyclo[2.2.2]oct-2-ene (DBO) with tris(*p*-bromophenyl)aminium ($TBPA^{\bullet+}$) hexachloroantimonate in degassed acetonitrile at 25 °C took place rapidly, but no N_2 was produced. The solution deposited a red solid, λ_{max} 523 nm, ϵ 24 300 in CH_2Cl_2, whose structure was determined by X-ray crystallography as the diazenium salt **1**.[12] As seen from the

† Rice University.
‡ Texas Tech University.

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(12) Crystal data are as follows: monoclinic, space group $P21/n$, a = 11.974 (2) Å, b = 20.208 (2) Å, c = 12.507 (2) Å, β = 96.77 (1)°, Z = 4, D_{calcd} = 1.87 g cm^{-3}. Intensity data were collected on a Rigaku AFC-5S diffractometer with graphite-monochromated Mo Kα radiation in the $2\theta \leq 50°$ range. The structure was solved with the direct methods program MITHRIL in the TEXSAN (v 2.0) Structure Analysis Package (Molecular Structure Corporation). Refinement converged with R = 0.033, R_w = 0.043 for 3073 (4806 collected) independent reflections with $I > 3\sigma(I)$.

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