second sulfur atom. Further oxidation to the sulfenic acid derivative and lactonization would then yield thiotropocin.

Experimental Section

Methods and Materials. NMR spectra were obtained at 400.13 or 250.13 MHz (^{1}H) and 100.6 or 62.9 MHz (^{13}C). ^{1}H/^{13}C long-range HETCOSY spectral acquisition parameters: 20000-Hz sweep width in the F_2 dimension; 128 spectra (860 scans each) accumulated with a 2.0-s relaxation delay. Resolution was 9.766 Hz per point in the F_2 dimension and 13.378 Hz per point in the F_1 dimension. [1,2-$^{13}C_2$]Phenylacetic acid (99 atom %), DL-[3-^{13}C]phenylalanine (99 atom %) and D-[U-$^{13}C_6$]-glucose (90 atom %) were purchased from ISOTEC, Inc. Thiotropocin (**1**) was converted to the *p*-bromobenzyl thioether derivative **2** as previously described.[1b]

Fermentation of *Pseudomonas* CB-104. A loopful of cells from a cultivated slant was inoculated into 50 mL of seed medium in a 250-mL flask. The seed medium contained fructose (10 g), polypepton (BBL) (5 g), meat extract (Wako Pure Chemical Industries) (5 g), and NaCl (5 g) (pH 7.0) per liter. The inoculated seed culture was incubated at 28 °C for 2 days with reciprocal shaking at 100 rpm. The inoculum (0.8 mL) was transferred to 50 mL of the same seed medium and incubated at 24 °C for 1 day with 20 mL/min of aeration. The resulting culture (2.5 mL) was then transferred to 100 mL of production medium containing fructose (5 g) (sterilized separately), polypepton (0.5 g), meat extract (0.5 g), NaCl (0.5 g), and $Na_2S_2O_3$ (0.05 g) (pH 7.0). The production medium was incubated at 24 °C for 36 h with 44 mL/min of aeration and 100-rpm agitation.

Isolation and Purification of Thiotropocin. Cells from the above production medium were removed by centrifugation at 4000*g* for 15 min. The supernatant was then adjusted to pH 3.0 with sulfuric acid and extracted four times with ethyl acetate. The product was transferred to the aqueous phase by extraction with three portions of 2% $NaHCO_3$. The aqueous phase was then adjusted to pH 3.0 and reextracted three times with ethyl acetate. The crude product obtained after evaporation of the solvent was subjected to purification by preparative TLC (toluene–dioxane–acetic acid, 45:10:2) to yield 4 mg of thiotropocin crystals per 100 mL of fermentation medium.

Administration of Labeled Precursors. [U-$^{13}C_6$]Glucose. Feedings of labeled glucose were carried out with replacement cultures. Cells from a 12-h production culture were collected by centrifugation at 4000*g* for 15 min and resuspended in the same volume of a replacement medium of the same composition as the production medium except for glucose, which was at a concentration of 300 mg per 100 mL. After incubation of the resuspended cells at 24 °C for 0.5 h, 100 mg of [U-$^{13}C_6$]glucose and 200 mg of unlabeled glucose in 5 mL of water were introduced by sterile filtration. After an additional 24 h, the culture broth was extracted in the usual manner to yield, after purification, 2 mg of thiotropocin.

[3-^{13}C]Phenylalanine. A solution of 50 mg of [3-^{13}C]phenylalanine in 5 mL of nano-pure water, pH adjusted to pH 7.0, was administered by sterile filtration to 100 mL of a 12-h production culture. Incubation for an additional 24 h yielded 3.2 mg of purified thiotropocin.

[1,2-$^{13}C_2$]Phenylacetic Acid. A solution of 50 mg of [1,2-$^{13}C_2$]phenyl acetic acid in 5 mL of nano-pure water, pH adjusted to pH 7.0, was administered by sterile filtration to 100 mL of a 12-h production culture. Incubation for an additional 24 h yielded 6.7 mg of purified thiotropocin.

Acknowledgment. We would like to thank Dr. Setsuo Harada of the Central Research Division of Takeda Chemical Industries in Osaka, Japan, for bringing this problem to our attention and for kindly supplying us with authentic samples of thiotropocin and the producing strain. We would also like to thank Dr. Hideo Ono of Takeda for valuable technical advice. This work was supported by a grant from the National Institutes of Health, GM22172.

Registry No. **1**, 89550-93-6; **2**, 89553-81-1; **8**, 138-59-0; **11**, 156-06-9.

(−)-Cryptaustoline: Its Synthesis, Revision of Absolute Stereochemistry, and Mechanism of Inversion of Stereochemistry

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Abstract: The asymmetric synthesis of (*S*)-(+)-cryptaustoline was accomplished and found to differ in sign of rotation with the natural "*S*"-(−)-material. The previously assigned absolute configuration was found to be incorrect and is now corrected. The reversal in stereochemistry came about through an unusual manner involving (*S*)-(+)-laudanosoline (**3**) cyclizing to (*R*)-(−)-cryptaustoline ((−)-**1c**). The mechanism was explored by oxidation of a series of deuterio derivatives of laudanosoline, all of which retained deuterium in the resultant cryptaustoline. Both chemical and enzymatic oxidations yield the same stereochemical result which is dictated simply by the stereochemistry of the starting material **3**. A reverse Michael addition followed by readdition is considered the most plausible route to the stereochemical inversion of (+)-**3** to (−)-**1c**.

Introduction

As part of a program designed to further demonstrate the synthetic utility of chiral formamidines[1] toward the total synthesis of a variety of indole[2] and isoquinoline alkaloids,[3] we undertook the seemingly simple target in the dibenzopyrrocoline series, (−)-cryptaustoline (**1a**). Isolated[4a] in 1952 from *Cryptocarya bowiei* (Hook) Druce, indigenous to Queensland, **1a** was one of only two dibenzopyrrocoline alkaloids obtained from this plant, the other being (−)-cryptowoline (**2**). The water-soluble alkaloids were isolated as their sparingly soluble iodides and were both levorotatory.[4c] Biologically, their mode of action is still uncertain,

(1) Meyers, A. I.; Dickman, D. A.; Bös, M. *Tetrahedron* **1987**, *43*, 5095.
(2) Meyers, A. I.; Beard, R. *J. Org. Chem.* **1991**, *56*, 2091 and earlier references cited.
(3) Meyers, A. I.; Guiles, J. *J. Org. Chem.* **1991**, *56*, 6873 and earlier references cited.
(4) (a) Ewing, J.; Hughes, G. K.; Ritchie, E.; Taylor, W. C. *Nature* **1952**, *164*, 618. (b) Ewing, J.; Hughes, G. K.; Ritchie, E.; Taylor, W. C. *Aust. J. Chem.* **1953**, *6*, 78. (c) Elliott, I. W. *The Alkaloids*; Academic Press: New York, 1987; Vol. 31. (d) Shamma, M. *The Isoquinoline Alkaloids*; Academic Press: New York, 1972. (e) Shamma, M.; Moniot, J. L. *Isoquinoline Alkaloid Research 1972–1977*; Plenum Press: New York, 1978.

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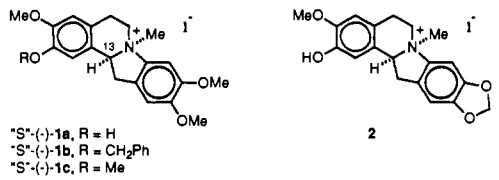
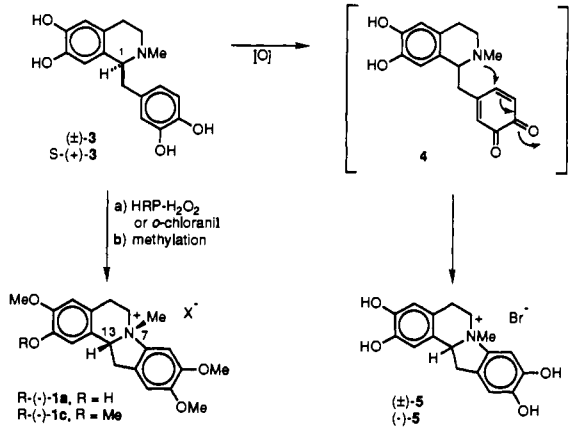


Figure 1. Proposed structures[11,12] for dibenzopyrrocolines **1** and **2**.

Scheme I

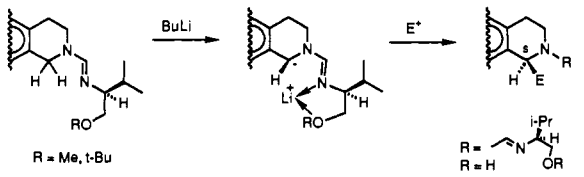


but they are known to cause neurological paralysis by acting as a respiratory poison.[4b]

The racemic versions of these dibenzopyrrocolines **1** and **2** were reported in 1932 by both Robinson[5] and Schöpf,[6] who accidentally prepared them through the oxidative cyclization of racemic laudanosoline **3** (Scheme I). These early workers suggested that the cyclization proceeded through an intermediate quinone (**4**) initially formed by oxidation, followed by a Michael addition to the tetracyclic salt **5**. The facility with which **5** was formed led Robinson to conclude that these tetracyclic systems may one day be found in nature originating from laudanosoline.

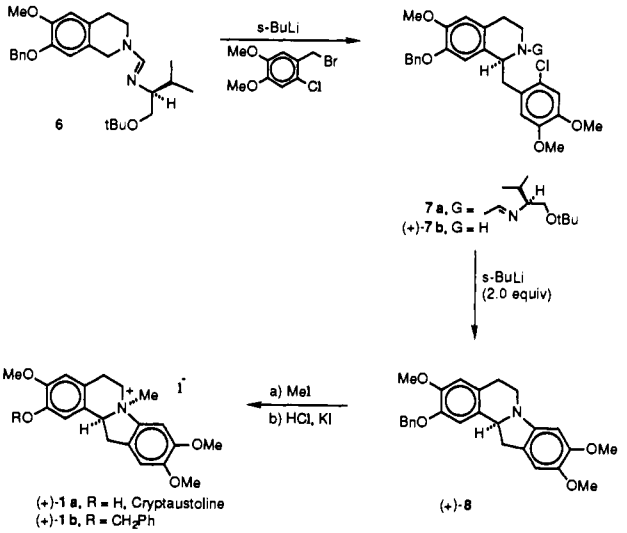
Since the Robinson and Schöpf groups described their findings, several other groups have reported the total synthesis of racemic cryptaustoline.[7-10] However, Brossi et al.[11] and Hughes et al.[12] prepared (-)-cryptaustoline starting from laudanosoline (+)-**3** by performing the oxidative cyclization using horseradish peroxidase–hydrogen peroxide or *o*-chloranil, respectively. From the established *S*-stereochemistry at C-1 in laudanosoline (+)-**3** both groups assigned the stereocenter (C-13) in cryptaustoline (-)-**1a** as *S*. The assignment was made by methylating the lone hydroxyl in (-)-cryptaustoline to the tetramethoxy derivative (-)-**1c** and comparing it to the tetramethoxy derivative obtained by oxidation of (+)-laudanosoline (**3**). Furthermore, NOE studies[8b] on both cryptaustoline **1** and cryptowoline **2** confirmed that the C-13 proton and the *N*-methyl group were cis-disposed although this did not confirm or dispute the absolute configurations at these centers. Proceeding with the assumption that the natural alkaloid possessed the *S*-configuration at C-13 (Figure 1), we embarked upon its synthesis[13] utilizing the chiral formamidines.

Scheme II



Results and Discussion

Synthesis. In all previous metalation–alkylation sequences involving *S*-formamidine auxiliaries, the resulting 1-substituted isoquinolines were found to possess the *S*-configuration when treated with *sec*-butyllithium and electrophiles (Scheme II).[1-3] The chiral isoquinoline formamidine **6**, previously prepared[14] in our laboratory, was treated with *sec*-butyllithium in THF at -78 °C to give the carbanion which was then subjected to 1.0 equiv of 2-chloro-4,5-dimethoxybenzyl bromide at the same temperature.



After aqueous quench the alkylated formamidine **7a** was isolated and immediately subjected to hydrazinolysis to afford (+)-**7b** which was isolated pure in 51% overall yield. The next step required that a benzyne precursor be prepared, and this was performed by adding 2.0 equiv of *sec*-butyllithium to **7b** at -100 °C and allowing the benzyne to form spontaneously, cyclizing within 15 min to give the tetracyclic product (+)-**8** in 98% yield as a crystalline material. All the physical properties of the latter were in agreement with those reported by Kametani[9] for the racemic material. N-Methylation was performed by adding excess methyl iodide to **8** and allowing the methiodide **1b** to crystallize. Finally, debenzylation was accomplished by heating **1b** in a heterogeneous mixture of concentrated hydrochloric acid and benzene which produced the methyl chloride salt of cryptaustoline **1a**. Treatment with potassium iodide in ethanol furnished cryptaustoline, the data for which were in good agreement with all physical data reported[4,11,12] *except the sign of rotation.* The $[\alpha]_D$ for the material obtained in the present synthesis was +141° (*c* 0.2, EtOH), whereas the specific rotation of the natural material reported as **1a** was -150° (Scheme I). On the basis of purely polarimetric data, the asymmetric synthesis was accomplished in 94% ee, but the more immediate question was the discrepancy in the sign of rotation. Had the synthesis we had just completed been accompanied by an anomalous change in stereochemistry? If so, the change in stereochemistry could only have occurred in one of two steps in the synthesis, namely, the alkylation to **7** or the benzyne cyclization to **8**.

(5) (a) Robinson, R. *The Structural Relations of Natural Products*; Oxford University Press: London, 1955. (b) Robinson, R.; Sugasawa, S. *J. Chem. Soc.* **1932**, 789. For an interesting addendum to this work, the reader is directed toward *Tetrahedron* **1992**, *48*, V, a memorial issue dedicated to the late Professor Sugasawa.

(6) Schöpf, C.; Theirfelder, K. *Liebigs Ann. Chem.* **1932**, *498*, 22.

(7) Yasuda, S.; Hirasawa, T.; Yoshida, S.; Hanaoka, M. *Chem. Pharm. Bull.* **1989**, *37*, 1682.

(8) (a) Takano, S.; Satoh, S.; Ogasawara, K. *Heterocycles* **1987**, *26*, 1483. (b) Takano, S.; Satoh, S.; Oshima, Y.; Ogasawara, K. *Ibid.* **1987**, *26*, 1487.

(9) Kametani, T.; Ogasawara, K. *J. Chem. Soc. C* **1967**, 2208.

(10) (a) Bennington, F.; Morin, R. D. *J. Org. Chem.* **1967**, *32*, 1050. (b) Strong bases have been reported to racemize 1-substituted isoquinolines: Brossi, A.; Rahman, M.; Rice, K.; Gehrig, M.; Bover. R.; O'Brien, J.; Teitel, S. *Heterocycles* **1977**, *7*, 277. Teitel, S.; O'Brien, J. *J. Org. Chem.* **1976**, *41*, 1657.

(11) Brossi, A.; Ramel, A.; O'Brien, J.; Teitel, S. *Chem. Pharm. Bull.* **1973**, *8*, 1839.

(12) Hughes, G. K.; Ritchie, E.; Taylor, W. C. *Aust. J. Chem.* **1953**, *6*, 315.

(13) The synthesis and revision of absolute configuration of (+)-cryptaustoline were reported in a preliminary communication: Meyers, A. I.; Sielecki, T. M. *J. Am. Chem. Soc.* **1991**, *113*, 2789.

(14) Guiles, J.; Meyers, A. I. *Heterocycles* **1989**, *28*, 295.

Scheme III

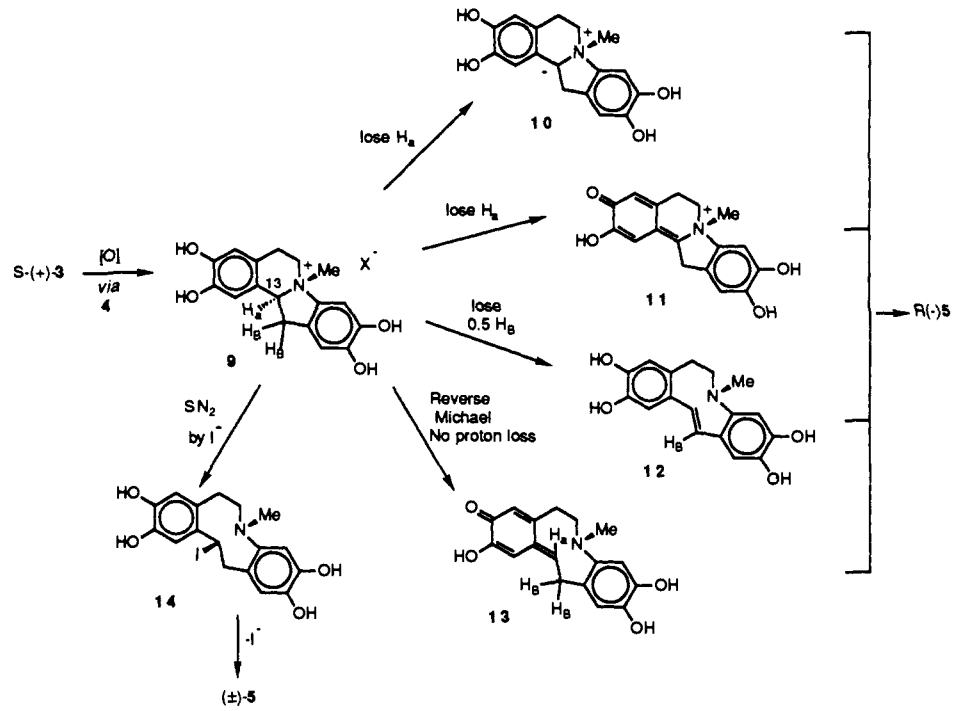


Table I. Cotton Effect of Intermediates in Cryptaustoline Formation

synthetic route		oxidative coupling route	
compd	λ, nm ($\Delta\epsilon$)[a]	compd[b]	λ, nm ($\Delta\epsilon$)[a]
(+)-**7b**	286 (+1.58)	(+)-**3**	298 (+1.67)
(+)-**8**	286 (+0.97)	(-)-**5**	298 (-0.94)
(+)-**1a**	290 (+1.45)	(-)-**1c**	293 (-1.18)

[a] Taken in 95% ethanol. [b] From refs 11 and 12.

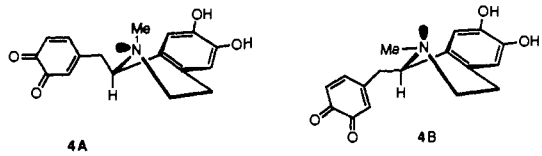


Figure 2. Lowest energy conformation for **4** prior to ring closure.

Although both instances were theoretically possible, we thought it highly unlikely that optically active isoquinoline **7b** would undergo a clean absolute stereochemical inversion to enantiomerically enriched **8** (in 94% ee) in the absence of any other optically active systems. If the strong base used in the benzyme cyclization abstracted the proton at C-1, there should have resulted, at best, considerable racemization. Actually, racemization did occur in an earlier attempt[10a] at asymmetric synthesis of cryptowoline **2** when a benzyne ring closure was carried out using optically active 1-benzylisoquinoline. The tetracyclic product analogous to **8** was completely racemic and was obtained in only 23% yield when excess potassium in liquid ammonia was employed to generate the benzyne intermediate from a compound analogous to **7**. To verify that the optically active intermediates in our synthesis had maintained their absolute configuration throughout the sequence **6–7–8–1**, we obtained their circular dichroism spectra which clearly showed that each Cotton curve for these intermediates was positive (Table I, synthetic route). Thus, we concluded that no configurational changes had occurred during the synthesis.

Assignment of Stereochemistry. This now caused us to question the reported absolute configuration of the "natural" cryptaustoline (-)-**1a** (Figure 1). The major difficulty with this aspect of the study was the ability to rationalize how two groups[11,12] starting with natural optically active laudanosoline (+)-**3** of known absolute stereochemistry at C-1 could have given the alkaloid (-)-**1a** with inverted configuration (Scheme I). First, it was critical that we confirm that an inversion at C-13 in cryptaustoline had indeed taken place. Thus, using authentic natural laudanosoline (+)-**3** we repeated the oxidation[11] using horseradish peroxidase–hydrogen peroxide[15] as well as the oxidation using *o*-chloranil.[12] Once again we examined the Cotton curves (Table I, oxidative coupling route) of both starting material (+)-**3**, the tetrahydroxy derivative (-)-**5**, and the methylated derivative (-)-**1c**. Furthermore, methylation of cryptaustoline (-)-**1a** gave the tetramethoxy derivative (-)-**1c** which had very similar Cotton curves. We were gratified to find that both of these products from the oxidative coupling route possessed negative Cotton effects, strongly implying that an inversion of configuration had indeed taken place during the oxidation step. The asymmetric synthetic route to (+)-cryptaustoline[16] gave the expected *S*-configuration and exhibited a sign of rotation opposite to that of the natural material, indicating that the natural (-)-alkaloid (Scheme I, (-)-**1a**) possessed the *R*-configuration at C-13. It also follows that, based on the NOE studies, the N-7 CH_3 group resides cis to C-13 (β-face of the molecule). Thus, the absolute stereochemistry of natural (-)-cryptaustoline may be assigned 13*R*,7*S*, whereas the synthetic material prepared herein is assigned 13*S*,7*R*.

Exploring Pathways to the Oxidative Inversion. We next address the question, How did inversion at C-13 occur during the oxidative coupling of (+)-**3** to (-)-**1c**? Additionally, is the cyclization mechanism facilitated by horseradish peroxidase related to that observed when facilitated by *o*-chloranil oxidation? Several reasonable mechanistic possibilities, shown in Scheme III, could all result in inversion of the stereochemistry. The routes shown start with optically active laudanosoline (+)-**3** and involve the initial oxidation (peroxidase–hydrogen peroxide or *o*-chloranil) to **4** continuing to the trans-ring closed product **9**.[17] It was deemed feasible to test the subsequent reaction leading to the inverted cis

(15) No differences in product formation were observed when oxidizing laudanosoline (+)-**3** with horseradish peroxidase–H_2O_2, from different sources using various isoenzymes. Differences in the rates of product formation were observed, with the Sigma Type II enzyme showing the greatest activity.

(16) Another more efficient asymmetric synthesis of (+)-cryptaustoline has been accomplished utilizing a novel benzyne-mediated indoline synthesis: Meyers, A. I.; Sielecki, T. M. *J. Org. Chem.* **1992**, *57*, 3673.

(17) Oxidation of the 1-(*p*-hydroxybenzyl)tetrahydroisoquinolines (as in **3**) has been described on numerous occasions in the isoquinoline alkaloid literature, i.e., Kametani, T. Total Synthesis of Isoquinoline Alkaloids. In *Total Synthesis of Natural Products*; Apsimon, J., Ed.; Wiley Interscience: New York, 1977; Vol. 3, pp 1–273.

Table II. Global Energy Comparisons of *cis*-**5** vs *trans*-**5**[18]

strain type	*cis*-**5** energy (kcal)	*trans*-**5** energy (kcal)
bond	9.3	9.8
angle	16.4	23.2
torsion	8.0	8.4
inversion	0.1	0.1
van der Waals	41.0	41.7
electronic	20.6	21.4
total	95.4	104.7

product (−)-**5** by considering pathways involving potential intermediates **10–14** in the inversion process. Suitable labeling experiments should support or eliminate the plausible intermediates **10–13**, whereas the presence of intermediate **14** could be evaluated on a stereochemical basis (e.g., retention, inversion, or racemization at C-13).

First, the plausibility of the trans-ring closure of **4** to **9** was considered. Examination of models (Figure 2) showed that both conformations **4A** and **4B** were of comparable energy; however, **4B** is seemingly incapable of undergoing the Michael addition. On the other hand, **4A** is comfortably disposed to Michael addition and could readily lead to the kinetically favorable trans-fused tetracycle **9**. Although we were not able to obtain any spectral evidence for the formation of **9**, the inability of **4A** or **4B** to generate a cis-ring closed product, regardless of their stability, led us to discard a cis-ring closure as a likely possibility.

To determine their respective stabilities, the relative energies of the trans- and cis-fused ring systems were examined and the driving force to undergo the inversion was explored. Using annealed molecular dynamics,[18] a simplified model of cryptaustoline **5** with both cis- and trans-ring fusion was made the subject of the computations. Calculations showed that the trans ring was 9.3 (±2) kcal less stable than the cis-fused ring (Table II). The largest contribution to the energy difference between the two was angle strain (ΔE = 6.8 kcal). This is not surprising when recognizing the 1-azabicyclo 6–5-fused ring system contained four sp^2 carbons. We found that this energy difference was independent of variations in aromatic substitution and thus may be considered as the major driving force for the continued reaction of *trans*-**5** (or **9**) leading to the inversion.[19]

Attention was next turned to testing the hypothesis that the initially formed trans tetracycle **9** could undergo the inversion step. The possibility that the inversion of **9** might be occurring through ylide intermediate **10** was initially considered. An intermediate such as **10** should readily exchange with deuteriated solvents by loss of H_a. Without access to **9** but with access to the cis-fused systems **1a–c**, we attempted to incorporate deuterium by treating **1a** or **1c** with D_2O, $CDCl_3$, or CF_3CO_2D. No deuterium could be detected (mass spectrum) in the recovered materials, suggesting that a pathway involving intermediate **10** can be ruled out.

The possibility that the original H_a in **9** is lost via another pathway[13] enroute to (−)-**5** was explored by the preparation of the deuteriated precursor (*S*)-(+)-**3** and subjecting this material to the oxidative conditions. The C-1 deuteriated tetrahydroisoquinoline **20** was prepared as outlined in Scheme IV. The earlier synthetic sequence was repeated using the 1,1-dideuterioisoquinoline **15** prepared from the condensation of paraformaldehyde-d_2 with the dimethoxyphenethylamine. After affixing the chiral formamidine to give **16**, we performed the metalation–alkylation with *sec*-butyllithium and the appropriate benzyl halide to give **17**. Removal of the auxiliary furnished the amine **18** which was then transformed to the formamide **19** and reduced to the *N*-methyl deuteriated isoquinoline **20**. The proton NMR spectrum showed complete (>98%) absence of the C-1 proton. The four methoxy groups were removed[6] using 48% HBr, and the hydrobromide salt *d*-**21** after recrystallization was found to be identical spectroscopically to the protio version of commercially available laudanosoline, (*S*)-(+)-**3**·HBr. The deuteriated isoquinoline hydrobromide *d*-(*S*)-(+)-**21** was then subjected to the oxidative enzymatic treatment as reported by Brossi[11] (horseradish

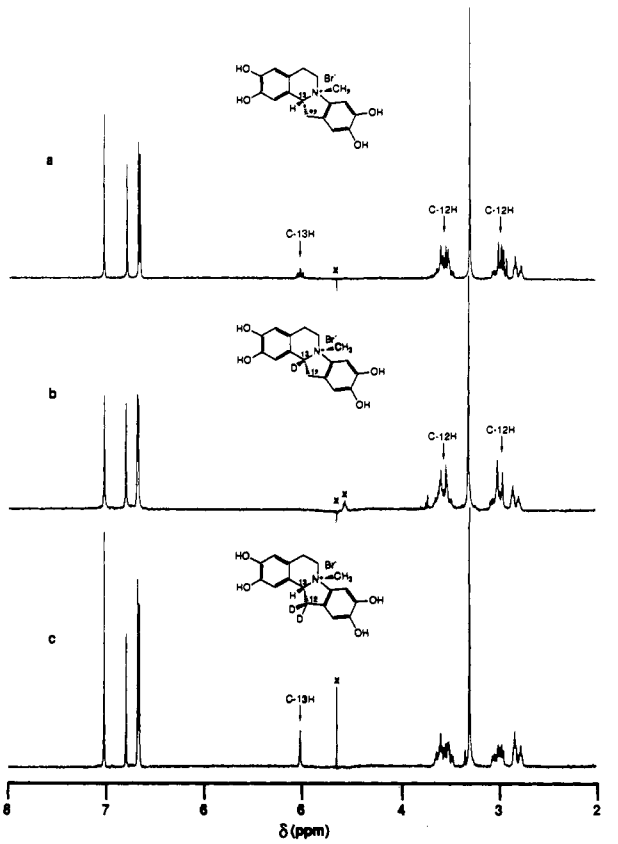
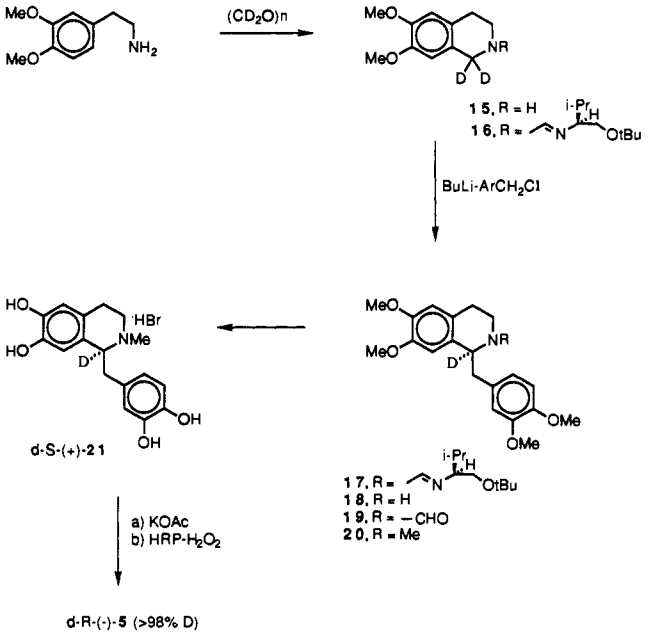


Figure 3. Proton spectra (300 MHz) of protio, deuterio, and dideuterio (*R*)-(−)-**5** after peroxidase–H_2O_2 oxidative cyclization.

Scheme IV

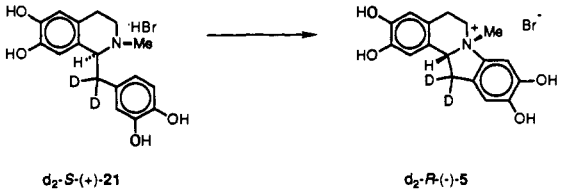


(18) The calculations were performed on the *cis* vs *trans* **9** (or **5**) using the forcefield DREIDING, with partial charges obtained with the OEq method in the molecular modeling package Biograf. After a preminimization of each trial structure, five cycles of annealed dynamics were carried out from 0 K to 600 K with a time step of 2.0 fs and a temperature increment of 10 K per 10 fs. The collection of structures for each conformation was minimized using a Fletcher–Powell minimization procedure.

(19) In a recent report, F. Zhang and G. Dryhurst (*J. Org. Chem.* **1991**, *56*, 7113) described both a peroxidase and an electrochemical oxidation of related tetrahydroxyisoquinoline (tetrahydropapaveroline) and proposed an analogous ring closure to a tetracyclic system. The latter did not possess any stereocenters, and, therefore, these authors were unaware of any stereochemical events that may be associated with this process.

peroxidase, hydrogen peroxide), and after workup the tetracyclic (*R*)-(-)-desmethylcryptaustoline ((*R*)-(-)-**5**) with all the deuterium at the C-13 position (H_a) was recovered intact (Figure 3b). The same results were obtained when the oxidation of *d*-(*S*)-(+)-**21** was carried out with *o*-chloranil. Thus, it is possible to eliminate both **10** and **11** as intermediates in the inversion process.

The possibility of a pathway involving **12** as the intermediate was examined by preparing the dideuterioisoquinoline d_2-(*S*)-(+)-**21**. This material was reached by simply adding the α,α-dideuteriobenzyl chloride to the lithio derivative of the protio version of the formamidine **16** and proceeding as described in Scheme IV. Once again, the dideuterioisoquinoline d_2-(*S*)-(+)-**21**



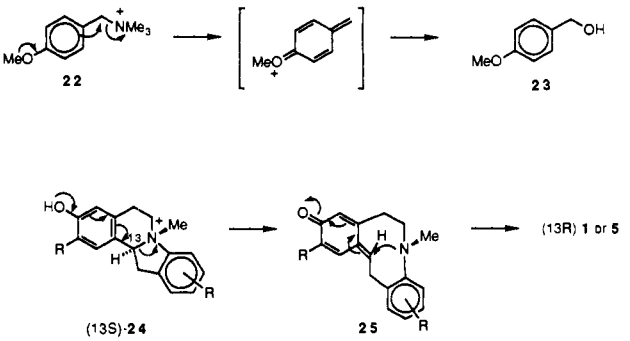
d_2-*S*-(+)-**21** d_2-*R*-(-)-**5**

was treated with the combination of peroxidase and hydrogen peroxide, producing the *R*-(-)-tetracyclic system d_2-(*R*)-(-)-**5** with all the deuterium still in place as determined by its NMR spectrum (Figure 3c). If an intermediate of type **12** had been produced, we should have seen loss of 50% of the deuterium in the elimination of d_2-**9** (D in place of H_b) to the alkene. Based on this result, it was possible to exclude a pathway involving **12** as the intermediate in the inversion process. Interestingly, the elimination to **12** had been suggested earlier to account for racemization in other isoquinoline alkaloids.[20,21]

We further considered the inversion pathway that could conceivably occur via the ring-opened intermediate **14**. First, however, it was necessary to assess the stability of **9** toward racemization. Since **9** was not accessible, we found that *R*-(-)-**5** completely racemizes by allowing it to sit overnight in an aqueous solution at pH 4. Alternatively heating this solution at reflux for 1 h gave complete racemization as did an aqueous solution containing 10% KOH at room temperature. On the other hand, an acetonitrile solution of *R*-(-)-**5** containing 10 equiv of sodium iodide at extended reflux was completely stable toward racemization.[21] This latter result, in our opinion, tends to rule out a mechanism involving the ring opening of **9** (Scheme III) by an S_N2 (A_ND_N) process to give **14**, which then may recyclize to **5** by ejection of iodide or bromide. Had the ring indeed opened, there is considerable expectation that racemization would occur upon ring closure.

On the basis of these considerations, the most reasonable pathway to inversion of **9** to (*R*)-(-)-**5** involves the intermediate **13** which arises from a reverse Michael addition and then recyclizes to the tetracyclic system *R*-(-)-**5**. Additionally, the natural alkaloid, (*R*)-(-)-**1a** should also have been formed by an analogous route. If indeed these considerations are correct, the stereochemistry of the starting material should dictate the stereochemistry of the product. It therefore follows that subjecting (±)-**3** to the horseradish peroxidase treatment should yield racemic product, and this was indeed observed. The possibility that different sources of horseradish peroxidase may show varying selectivities was examined, but Sigma's Type II, IV, and X and Worthington Biochemical Company's horseradish peroxidase *all proceed with the absence of enantiomeric selectivity*. The original conversion of *S*-(+)-**3** to the enantiomerically pure natural product using horseradish peroxidase[11] was accomplished because the stereochemical information was already present in the starting material and not because the enzyme facilitated an enantiomerically specific synthesis. Horseradish peroxidase is enantiomerically "blind" in these transformations.

A pathway involving an intermediate analogous to **13**, wherein the quaternary nitrogen acts as the leaving group, was previously reported by Openshaw and Norcross[23] during their structure determination of emetine. They reported that the methoxy group in **22** facilitates the loss of trimethylamine via an S_N1 ($D_N + A_N$) process, and rapid addition by the nucleophilic solvent then gives rise to the alcohol **23**. For the case at hand the reverse Michael



addition would occur without loss of any of the hydrogen atoms (H_a, H_b) in **9** or **13** (Scheme III), which was indeed observed when the deuteriated derivatives were subjected to the oxidative procedures. The reversion of **24** with the appropriate *p*-hydroxyl group should occur readily in an intramolecular sense and cause the C-13 H to rotate inward (Dreiding models) as the double bond forms in **25**. The 7-kcal difference between trans and cis should provide the driving force for the reverse Michael addition in **24**, and the rigidity of the nine-membered ring in **25** should be maintained by the presence of five sp^2 carbons. Michael addition of the *N*-methylamino group to the olefin would be expected to proceed in a fashion to afford the cis-ring juncture and place both the *N*-methyl and the benzylic proton on the β-face, as is the case with the natural alkaloid. The rigidity of the nine-membered ring in **25** is crucial to this mechanistic proposal, and there are earlier reports by both Mislow[24] and Magnus[25] suggesting that there is a sufficient energy barrier to inversion in such a ring system. It has been stated that the E_a for inversion for nine-membered rings can be as high as 23 kcal per mol at 25 °C as a result of nonbonded interactions between hydrogens oriented in direct opposition. Thus, if **25** does indeed form to relieve angle strain, as shown, models indicate that only a slight twist of the ring (minimum motion) will allow the *N*-Me group, already set in the proper position, to close at C-13. Furthermore, the nitrogen will have difficulty in inverting rapidly in **25** due to the nonbonded interactions between the olefinic hydrogen and the nitrogen lone pair.

Conclusions

The oxidation of (*S*)-(+)-laudanosoline ((+)-**3**) proceeds through an unusual and unexpected pathway wherein the kinetically formed trans-ring system possesses significant strain and by virtue of a reverse Michael reaction reorganizes itself into the more stable thermodynamic cis product *R*-(-)-**5**. A remarkable aspect of this transformation is the ability of the free tertiary amino group to maintain the "memory" of the stereochemical center such that the other stereochemical configuration could be reached by both chemical or enzymatic transformations. Of further significance is the value of rational asymmetric synthetic processes to correctly predict the absolute configuration of the products from such reactions. Only in this manner was the unexpected reversal in stereochemistry in going from (*S*)-(+)-**3** to (*R*)-(-)-**1** first uncovered.

Experimental Section

General. All proton and carbon NMR spectra were taken on a Bruker 300 analytical instrument. NMR spectra shown in Figure 3 were re-

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corded on the Bruker ACP-300 in D_2O using solvent supression. The program PRESAT.AUR was used for solvent supression. Samples were saturated at the water frequency (5533 Hz) for 1.5 s at a power of 22 Hz before the application of the pulse and data aquisition; 90° pulse widths were used throughout. Integrations were carried out in spectra where no solvent had been suppressed. Exchange between protons was not observed due to exchange with D_2O. IR spectra were taken on a Perkin-Elmer FTIR using a Hewlett-Packard plotter, and melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Argon was used as the inert atmosphere and was passed through both a drying tube and an oxytrap (BASF catalyst) to remove moisture and oxygen. Tetrahydrofuran solvent was distilled from sodium, and benzophenone, dichloromethane, hexanes, and triethylamine solvents were distilled from calcium hydride.

Reactions were monitored by analytical thin-layer chromatography (TLC) using Merck TLC aluminum sheets precoated with silica gel 60 F254 (0.2 mm thick). Flash chromatography was performed using Aldrich grade 951 silica gel. Reaction temperatures are reported as bath temperatures. Elemental analyses were performed by Desert-Analytics, Tucson, AZ. High resolution mass spectral analyses were performed by Bristol-Myers Squibb Co.

1-(2′-Chloro-4′,5′-dimethoxybenzyl)-7-(benzyloxy)-6-methoxy-1,2,3,4-tetrahydroisoquinoline (7b). A solution of tetrahydroisoquinoline **6**[14] (65 mg, 0.15 mmol) in tetrahydrofuran (30 mL) was introduced into a 100-mL round-bottomed flask equipped with a magnetic stirrer. The flask was cooled in a −78 °C bath and purged with argon. *sec*-Butyllithium (0.14 mL, 0.17 mmol) was added over 5 min, the blood-red solution was stirred for 1 h at −78 °C, and then 2-chloro-4,5-dimethoxybenzyl bromide (44 mg, 0.17 mmol) in tetrahydrofuran (3 mL) was added dropwise. The color immediately became a faint yellow, and the solution was stirred an additional 15 min. The mixture was quenched (saturated NH_4Cl solution, 0.5 mL), warmed to room temperature, and concentrated. The residue **7a** was taken up in an ethanol/water/acetic acid/hydrazine (8:5:1:2) (16 mL) solution and stirred at room temperature overnight (12 h). After being poured over 10% sodium hydroxide, the mixture was extracted four times with methylene chloride. The combined organic phases were dried (K_2CO_3), and the solvent was removed in vacuo. Following column chromatography (sg, 85% ethyl acetate/10% hexane/5% triethylamine) the product **7b** was isolated as a clear oil, 38 mg (51% yield); $[\alpha]_D$ +28.3° (*c* 2.0, acetone). ^1H NMR (300 MHz, $CDCl_3$): δ 7.41–7.24 (m, 5 H), 6.86 (s, 1 H), 6.73 (s, 1 H), 6.64 (s, 1 H), 6.59 (s, 1 H), 5.05–5.04 (d, 2 H, *J* = 2.8 Hz), 4.23–4.15 (dd, 1 H, *J* = 5.1 Hz), 3.86–3.80 (m, 9 H), 3.28–2.83 (m, 6 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.1, 155.5, 154.8, 133.9, 125.9, 124.9, 124.8, 124.5, 122.1, 113.2, 112.3, 110.8, 110.5, 109.8, 109.0, 68.7, 53.4, 53.3, 53.2, 52.3, 38.1, 37.6. IR: cm^{-1} 3727, 2929, 1606, 1509, 1463, 1261, 1220, 735, 668.

Dibenzopyrrocoline (+)-8. A solution of **7b** (143 mg, 0.31 mmol) in 30 mL of tetrahydrofuran was prepared in a 25-mL round-bottomed flask equipped with a magnetic stirrer. The flask was placed in a −100 °C bath, and *n*-butyllithium (0.41 mL, 0.62 mmol) was added over 5 min. The reaction was stirred for an additional 15 min and concentrated. The residue was taken up in chloroform/water and extracted with chloroform (3 × 30 mL), the combined organic phases were dried (Na_2SO_4), and the solution was concentrated. Following column chromatography on silica gel (30% ethyl acetate/60% hexane/10% triethylamine) the product was isolated in 98% yield (114 mg) as off-white crystals, mp 131–133 °C (lit.[9] mp 126–128 °C); $[\alpha]_D$ +48.5° (*c* 1.0, acetone). ^1H NMR (300 MHz, $CDCl_3$): δ 7.44–7.25 (m, 5 H), 6.88 (s, 1 H), 6.73 (s, 1 H), 6.70 (s, 1 H), 6.60 (s, 1 H), 5.10 (s, 2 H), 4.14–4.09 (dd, 1 H, *J* = 7.7 Hz), 3.86 (s, 3 H), 3.85 (s, 3 H), 3.81 (s, 3 H), 3.25–3.15 (m, 2 H), 2.96–2.65 (m, 4 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 148.23, 148.19, 147.64, 146.14, 137.32, 130.64, 128.83, 128.48, 128.02, 127.75, 127.36, 125.23, 114.17, 112.91, 112.70, 112.28, 71.42, 56.15, 56.12, 55.97, 55.24, 40.49, 40.21, 29.51. IR: cm^{-1} 3588, 1605, 1577, 1508.

8-*O*-Benzyl-*N*-methylcryptaustoline ((+)-1b). A solution of (+)-**8** (50 mg) in methanol (1 mL) in a 25-mL round-bottomed flask was treated with methyl iodide (1 mL). Upon standing the product crystallized as white needles, mp 226–228 °C (lit.[9] mp 224–226 °C); $[\alpha]_D$ = +47.8° (*c* 1, acetone). ^1H NMR (300 MHz, $CDCl_3$): δ 7.35–7.25 (m, 5 H), 7.19 (s, 1 H), 6.85 (s, 1 H), 6.62 (s, 1 H), 6.42 (s, 1 H), 4.99–4.98 (d, 1 H), 4.93–4.92 (d, 1 H), 4.69–4.67 (m, 1 H), 3.91 (s, 3 H), 3.86 (s, 3 H), 3.79 (s, 3 H), 3.78–3.18 (m, 6 H), 1.53 (s, 3 H).

(+)-Cryptaustoline (1a). To (+)-**1b** (50 mg) in a 10-mL round-bottomed flask were added hydrochloric acid (2 mL) and benzene (2 mL). The solution was heated for 3 h and cooled. Crystals precipitated and after filtration were taken up in 95% ethanol (2 mL), and potassium iodide (50 mg) was added. After the flask was warmed (75 °C) the solution was filtered and the filtrate evaporated to dryness. Recrystallization from 95% ethanol afforded the product (+)-**1a** as an off-white powder, mp 261–263 °C dec (lit.[9,4a] mp 260 °C dec, $[\alpha]_D$ +141° (*c* 0.6, ethanol), natural material $[\alpha]_D$ −150°.[11,12]

1,1-Dideuterioisoquinoline (15). Following literature procedures,[14] 3,4-dimethoxyphenethylamine (1.18 g, 6.52 mmol), deuterioparaformaldehyde (0.21 g, 6.5 mmol), and acetic acid (10 mL) were warmed to 50 °C in a 50-mL round-bottomed flask for 48 h. The reaction mixture was allowed to cool to room temperature and concentrated, and the residue was diluted in absolute ethanol. This solution was then added dropwise to a solution of oxalic acid in ethanol. The oxalate was isolated as a white solid in 89% yield, mp 209–211 °C (oxalate). The material was identical (except for the 1-d_2) to that purchased from Aldrich, free base,[26] mp 83–85 °C, and was transformed to the oxalate. ^1H NMR: δ 6.56 (s, 1 H), 6.47 (s, 1 H), 3.87 (s, 3 H), 3.86 (s, 3 H), 3.09–3.05 (t, 2 H), 2.69–2.60 (t, 2 H), 1.82 (br s, 1 H). ^{13}C NMR: δ 147.69, 128.14, 126.93, 112.86, 110.15, 109.91, 56.07, 52.83, 43.94, 28.76. IR: cm^{-1} 3311, 2935, 1610, 1514, 1458, 1260, 1056.

1,1-Dideuterioisoquinoline Formamidine 16. Following literature procedures,[26] 1,1-deuterioisoquinoline **15** (0.55 g, 2.8 mmol), the chiral (dimethylamino)formamidine (0.60 g, 2.8 mmol), toluene (50 mL), and ammonium sulfate (25 mg) were combined in a 100-mL round-bottomed flask fitted with a reflux condensor. A flow of argon with a vent needle were set over the system allowing for the liberation of dimethylamine, and the flask warmed to reflux for 48 h. The solution was cooled and concentrated, and the residue was passed through a plug of silica (10% Et_3N/20% EtOAc/hexane), giving **16** in 91% yield as a pale yellow oil identical to the reported[26] protio material, except for the absence of a methylene signal at 4.1–4.3 ppm. ^1H NMR: δ 7.37 (s, 1 H), 6.61 (s, 1 H), 6.58 (s, 1 H), 3.83 (s, 3 H), 3.81 (s, 3 H), 3.51–3.45 (m, 4 H), 3.20–3.44 (m, 1 H), 2.78–2.69 (m, 3 H), 1.85–1.78 (m, 1 H), 1.15 (s, 9 H), 0.86–0.83 (dd, 6 H). IR: cm^{-1} 2969, 1645, 1517, 1465, 1362, 1262, 1056, 918.

(*S*)-(+)-Deuteriolaudanosine (20). Formamide **16** (1.59 g, 4.35 mmol) was weighed into a flame-dried 100-mL round-bottomed flask equipped with a magnetic stirrer. Tetrahydrofuran (50 mL) was added, the reaction vessel was cooled in a −78 °C bath, and *sec*-butyllithium (3.96 mL, 1.21 M) was added dropwise. Following stirring for 30 min, freshly distilled 3,4-dimethoxybenzyl chloride (0.74 g, 4.8 mmol) in 10 mL of tetrahydrofuran was added slowly to the bright red reaction mixture. After 30 min of additional stirring, the solution was quenched (saturated NH_4Cl solution) and concentrated, and the residue was extracted between H_2O and dichloromethane three times. The combined organic phases were dried (K_2CO_3) and concentrated to give crude **17**. The latter was stirred for 12 h in ethanol/H_2O/hydrazine/acetic acid (36 mL/4.5 mL/9 mL/4.5 mL), and the mixture was concentrated to a pale yellow residue (**18**) which was taken up in ethyl formate (20 mL) and warmed to reflux for 1 h, after which the solution was concentrated and the crude formamide (oil) **19** was taken up in tetrahydrofuran (50 mL). Lithium aluminum hydride was added, and the solution was warmed to reflux for 1 h. Quench (2.6 mL of H_2O, 5.2 mL of 10% NaOH, 10.4 mL of H_2O) was followed by filtration to remove aluminum salts. Volatiles were removed, and the residue was purified (sg 85% EtOAc/5% Et_3N/hexane), giving the title compound in 63% yield, $[\alpha]_D^{20}$ +51.6° (*c* 0.50, chloroform), mp 87–88 °C (lit.[27] mp 89–91 °C). ^1H NMR: δ 6.76–6.73 (d, 1 H), 6.63–6.57 (m, 2 H), 6.53 (s, 1 H), 6.02 (s, 1 H), 3.82 (s, 3 H), 3.81 (s, 3 H), 3.76 (s, 3 H), 3.15 (s, 3 H), 3.15–3.10 (m, 2 H), 2.81–2.73 (m, 4 H), 2.51 (s, 3 H). ^{13}C NMR: δ 148.86, 147.61, 147.60, 146.59, 132.86, 129.54, 126.35, 122.15, 113.29, 111.48, 111.38, 111.30, 56.23, 56.13, 56.08, 55.86, 47.18, 42.95, 41.12, 25.77. IR: cm^{-1} 2972, 1643, 1601, 1470, 1352, 1050, 917.[28]

Deuteriolaudanosoline Hydrobromide *d*-(*S*)-(+)-21. Following literature procedures,[6] isoquinoline **20** (0.66 g, 1.8 mmol) was weighed into a 50-mL round-bottomed flask containing 48% HBr (25 mL). The solution was warmed to reflux for 4 h, and the aqueous phase was distilled. The residue was diluted with H_2O and partially distilled eight times[6] to remove the HBr. The resultant oil crystallized from water as a pale brown powder in 72% yield, mp 122–124 °C (lit.[6] mp 124–125 °C), $[\alpha]_D$ +56.8° (*c* 1.0, H_2O).

Chloranil Oxidation. Following the literature procedure,[5] deuteriolaudanosoline hydrobromide *d*-(*S*)-(+)-**21** (71.3 mg, 0.18 mmol) was weighed into a flame-dried 25-mL round-bottomed flask. Potassium acetate (18 mg, 0.18 mmol) and ethanol (10 mL) were added, and the solution was warmed until all starting materials had dissolved. An ethanolic solution (10 mL) of *o*-chloroanil (46 mg, 0.18 mmol) was added dropwise, and the reddish-brown solution was stirred overnight. The

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product crystallized from solution and was recrystallized twice from ethanol, giving the pure material in 76% yield, mp 273–275 °C (lit.[11] mp 279–280 °C), $[\alpha]_D$ −142° (*c* 0.01, ethanol). ^{1}H NMR was identical to those reported below and indicated no loss of deuterium over the course of the reaction.

***d*-(*R*)-5.** Synthesis was performed as for racemic **5** using laudanosoline *d*-(*S*)-(+)-**21** with a deuterium atom substituted for hydrogen at the stereogenic center, producing 25.4 mg of product, $[\alpha]_D$ −140° (*c* 0.012, EtOH). ^{1}H NMR (300 MHz, D_2O, 296 K): 7.00 (s, 1 H), 6.78 (s, 1 H), 6.67 (s, 1 H), 6.66 (s, 1 H), 3.54 (m, 3 H), 3.29 (s, 3 H), 2.97 (m, 2 H), 2.83 (m, 1 H).

Horseradish Peroxidase Solution. Horseradish peroxidase suspension (Sigma, Type X, 5000 units in 2.0 mL) was spun for 3 min at 3000 rpm. The supernatant was removed, and the enzyme was dissolved in 1.00 mL of phosphate buffer (5 mM, pH 6.5). Similar solutions were prepared from horseradish peroxidase types VII and X and the peroxidase obtained from Worthington Biochemical Corporation.

Peroxide Solution. A 0.3% hydrogen peroxide solution was prepared by adding 4.95 mL of water to 48.2 μL of H_2O_2 (31.1%).

Peroxide Assay. The reaction mixture was periodically assayed for peroxide using a modified endpoint assay. Measuring production of tetraguaiacol, which has a strong absorbance in the visible region at 436 nm (ϵ = 2.55 L/mmol·min),[29] is based on published assays for horseradish peroxidase. The assay solution consisted of 2.83 mL of phosphate buffer (5 mM, pH 6.5), 0.25 mL of guaiacol (2.2 mg per mL), 0.02 mL of reaction mixture, and 0.01 mL of horseradish peroxidase. An aliquot of the reaction mixture was added to the phosphate buffer, and the absorbance was recorded. Guaiacol and the enzyme solution were quickly added, and the absorbance was monitored until a maximum was reached, which usually occurred after 15 s. The assay as described provides relative concentrations of hydrogen peroxide since laudanosoline is a better substrate for the enzyme than guaiacol and both laudanosoline and guaiacol are converted simultaneously. The absorbance change at each point is proportional to the overall peroxide concentration and converts to concentrations when using a standard curve.

Horseradish Peroxidase-Catalyzed Synthesis of (±)-5. The experimental procedure was based on that reported by Brossi et al. for preparation of (−)-**5**.[11] To a solution containing (±)-**3** (49.8 mg, 0.12 mmol) in 10 mL of 5 mM phosphate buffer was added 0.12 mmol of hydrogen peroxide. The reaction was started by adding 10 units of horseradish peroxidase (Type II), and was complete after 3 min. The solution was cooled in an ice bath for 1 h, and the colorless precipitate and green solid were removed by filtration. The product was isolated by washing the solids three times with 30 mL of water, collecting the filtrate (the green solid remains on the filter), and removing water by rotoevaporation. (*S*)-(±)-**5** (16 mg, 0.043 mmol) was isolated in a yield of 35.0%. Additional material (up to 90%) could be isolated from the original filtrate by extraction with ethanol, mp 272–275 °C, $[\alpha]_D$ 0° (*c*, 0.06, EtOH). The ^{1}H NMR spectrum was identical with an authentic sample.[11]

(*S*)-(+)-Dideuteriated Laudanosoline d_2-(*S*)-(+)-21. The formamidine **16** (1.13 g, 3.11 mmol) was taken up in 50 mL of THF and cooled to −78 °C. *sec*-Butyllithium (2.0 mL, 1.7 M) was then added dropwise until a reddish solution was attained. The solution was stirred for 30 min at −78 °C. Excess 3,4-dimethoxy-α,α-dideuterated benzyl chloride in 10 mL of THF was added slowly to the lithiated species to obtain a yellowish color. This solution was stirred an additional 30 min. The alkylated product was quenched with 4 mL of water and evaporated in vacuo to remove excess THF. The residue was extracted with CH_2Cl_2 (3 × 10 mL), and the organic extracts were dried (K_2CO_3) and concentrated. The residue was taken up in a mixture of ethanol/water/acetic acid (36 mL/4.5 mL/4.5 mL) and 9 mL of hydrazine and stirred under argon overnight. The peach-colored mixture was concentrated under reduced pressure (1 mmHg) to give a yellow oil, which was dissolved in excess ethyl formate (20 mL) and refluxed at 60 °C for 1 h. The mixture was concentrated and chromatographed over silica with a solvent solution made up of ethyl acetate, hexane, and triethylamine (85:10:5) which gave (0.30 g, 0.79 mmol) 75% yield. The *N*-formyl derivative (0.195 g, 0.522 mmol), dissolved in 25 mL of dry THF and 1.16 mL of 1 M of $LiAlH_4$, was added slowly through the top of the condenser. The mixture was heated at reflux for 4 h and quenched with 2.6 mL of water, 5.2 mL of 10% NaOH, and 10.4 mL of water. Ether was added, and the mixture was extracted (3 × 15 mL), dried, and evaporated to give laudanosine **20** as an off-white powder (0.169 g, 0.470 mmol, 90% yield).

Laudanosine (**20**, 130 mg, 0.362 mmol) was dissolved in 15 mL of 48% HBr and brought to reflux. The solution started to turn purple after 6 h, and it was cooled to room temperature and concentrated in vacuo. Water (3 × 10 mL) was added and then evaporated in vacuo. After the third time, the purple solution deposited a purple precipitate, which was cooled in an ice bath and filtered to give purple crystals of laudanosoline d_2-(*S*)-(+)-**21** (113 mg) 81.2% yield, mp 123–124°, $[\alpha]_D$ +56.9° (*c* 1.0, H_2O), identical in all respects to the protio compound except for the absence of the 1-benzylic protons in the NMR spectrum.

Horseradish Peroxidase-Catalyzed Synthesis of *d*-(*R*)-(−)-5. To a solution containing *d*-(*S*)-(+)-**21** (49.4 mg, 0.120 mmol) in 10 mL of 5 M phosphate buffer was added 0.122 mmol of hydrogen peroxide. The reaction was started using 19 units of horseradish peroxidase (Type II). The procedure described above afforded 25 mg (0.069 mmol) *d*-(*S*)-(−)-**5** with an optical rotation of −140° (*c* 0.059, EtOH). The product was isolated, corresponding to a yield of 57.5%. The ^{1}H NMR spectrum shows no signals at 5.03 ppm (Figure 3b).

Horseradish Peroxidase-Catalyzed Synthesis of d_2-(*R*)-(−)-5. To a solution containing d_2-(*S*)-(+)-**21** (as the HBr salt) (50.8 mg, 0.124 mmol) in 10 mL of 5 mM phosphate buffer was added 0.126 mmol of hydrogen peroxide. The reaction was started using 15 units of horseradish peroxidase (Type X). The procedure described above afforded 16 mg (0.044 mmol) of d_2-(*R*)-(−)-**5**, corresponding to a yield of 35.5%. The ^{1}H NMR shows the signal at 5.03 ppm as a singlet compared to the doublet of doublets in an authentic sample (Figure 3c).

***o*-Chloroanil Oxidation to d_2-(*R*)-(−)-5.** d_2-Laudanosoline (d_2-(*S*)-(+)-**21**, as the HBr salt) from above (20 mg, 0.05 mmol) and potassium acetate (5 mg) were dissolved in 2 mL of ethanol. *o*-Chloroanil (13 mg, 0.05 mmol) was dissolved in 15 mL of hot ethanol. This solution was cooled to room temperature and added slowly to the laudanosoline solution until a reddish-brown solution was observed. The solution was stirred overnight, after which it was filtered to remove impurities, and the filtrate was evaporated under reduced pressure (1 mmHg). The crude material was dissolved in water and filtered, and water was then removed under reduced pressure overnight. The desmethoxycryptaustoline d_2-(*R*)-(−)-**5** was dissolved in D_2O and, using solvent suppression, the ^{1}H NMR was recorded on the 300-MHz NMR. ^{1}H NMR: δ 2.70–2.90 (m, 1 H), 2.90–3.150 (m, 1 H), 3.35 (s, 3 H), 3.5–3.65 (m, 2 H), 5.08 (s, 1 H), 6.67 (s, 1 H), 6.70 (s, 1 H), 6.85 (s, 1 H), 7.10 (s, 1 H). The material was identical to (*R*)-(−)-**5** except for the methylene multiplets of C-12 (~3.6 and ~3.0), which were absent, and C-13, which appeared as a singlet at 5.08 ppm.

Acknowledgment. Financial support from the National Science Foundation and Bristol-Myers Squibb (A.I.M.) and the National Institutes of Health (D.C.C.) is gratefully acknowledged. Thanks are also due to Dr. Laurie Castonguay for her assistance with the molecular mechanics calculations.

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