suggested that the addition of a hydroxymethyl group to the C-5 carbon of the D-xylose ring shields the ring oxygen atom from the aqueous solvent thereby weakening or eliminating hydrogen bonding and shifting the band due to ring oxygen to the red. This interpretation is consistent with the idea that the sign of the first CD band in pyranoses is dependent on the rotamer population of the hydroxymethyl group. In the case of the pyranosides then, it must be the methoxy group which tends to shield the ring oxygen from the hydrogen bonding solvent, since methyl α- and β-D-xyloside as well as methyl β-L-arabinoside, which have no hydroxymethyl group, still display a long-wavelength CD band which is apparently due to the ring oxygen atom.

While the aldo- and ketopyranoses have at most one obvious band at wavelengths longer than 174 nm, the methyl pyranosides (see, for instance, D-xyloside, Figure 2) have two. The fact that the first two bands do not change between α-D-xyloside and β-L-arabinoside suggests that these bands are due to a chromophore which is not near the C-4 hydroxyl. Also, chromophoric nonbonding electrons on an ether are apparently more easily shielded from a hydrogen-bonding solvent than is the case for an alcohol chromophore. Thus, if the first band in methyl pyranosides is due to the ring oxygen, one expects the second band to be due to the methoxyl group. Actually, earlier work on the vacuum ultraviolet absorption spectra of model sugar compounds indicated that it might be best to treat acetal and hemiacetal groups as a single chromophore.[20] The results here indicate that one can treat the two oxygen atoms in these chromophores separately. Since the two oxygen atoms are only separated by a single carbon, some mixing undoubtedly takes place so that assigning bands simply to a ring ether oxygen or a methoxyl ether oxygen is probably something of an oversimplification.

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Biosynthesis of Ophiocarpine. Introduction of a Stereospecific Label through Transannular Cyclization[1]

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Abstract: Results from ^{14}C labeling indicate that (−)-ophiocarpine (**8**) is biosynthesized in *Corydalis ophiocarpa* plants from scoulerine (**2**) via tetrahydroberberine. Nandinine (**9**) is only poorly incorporated into (−)-ophiocarpine and the biosynthetic sequence, **2** → isocorypalmine (**12**) → **10** → **8**, is suggested. Methods based upon a highly stereoselective transannular cyclization of the *trans*-dibenzazacine (**15**) and its 13-^3H derivative are shown to provide an efficient route for the synthesis of [13α-^3H]- and [13β-^3H]tetrahydroberberines (**30** and **31**) with high configurational purity. Incorporation experiments with [8,14-$^3H_2$]-, [13α-^3H]-, and [13β-^3H]tetrahydroberberines have established that the hydroxylation of (−)-tetrahydroberberine to (−)-ophiocarpine in *C. ophiocarpa* proceeds with retention of configuration involving the removal of the pro-*R* hydrogen atom from the C-13 position of tetrahydroberberine.

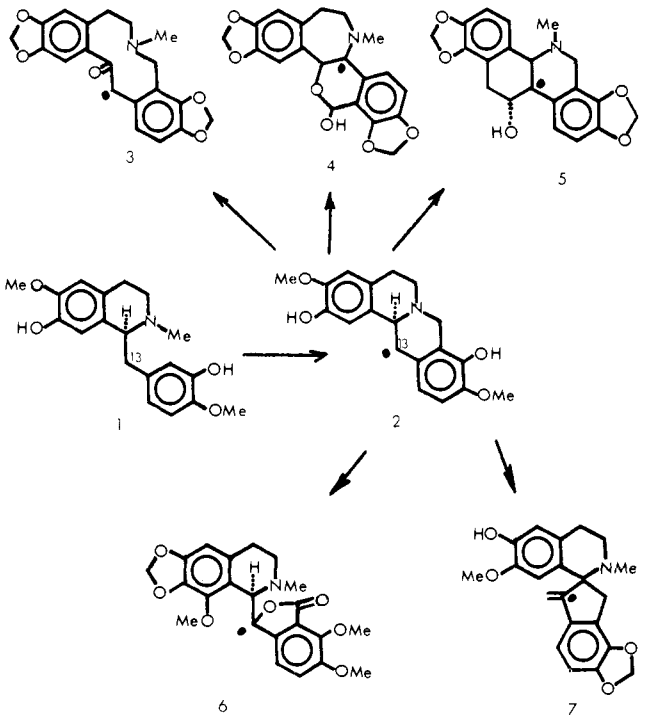
Studies of the biosynthesis of benzylisoquinoline alkaloids during the past 15 years have led to the elucidation of the major details of the steps involved in the construction of the skeletal framework of the principal classes of alkaloids of this family.[2] Despite the tremendous range in structural types presented by these alkaloids, their biosynthesis is linked by a common pathway from tyrosine to the simple benzylisoquinoline reticuline (**1**) which serves as the ubiquitous precursor of all members of this family so far studied. Second in importance to reticuline as a biosynthetic intermediate in this series are representatives of the protoberberine alkaloid series exemplified by scoulerine (**2**). Not only do these alkaloids constitute a large and important class of bases but certain members occupy a central position between reticuline and alkaloids of wide structural diversity representing the protopine (**3**), rhoeadine (**4**), benzophenanthridine (**5**) and phthalide isoquinoline (**6**) groups. In each case evidence supporting the intermediacy of the appropriate protoberberine base has been provided by tracer experiments.[3-6]

In addition to these alkaloid types, the spirobenzylisoquinolines such as ochotensine (**7**) are yet another class of alkaloid whose biosynthesis is thought to proceed via a proto-

berberine intermediate and although experimental support for the biotransformation is lacking, an ingenious chemical model for this conversion has been demonstrated.[7]

With the exception of the formation of the protopine bases, the biosynthetic conversion of the protoberberine ring system to each of these other classes of alkaloid involves either methylation or oxidation at the C-13 position at some stage during these transformations (see Scheme I). Since the C-13

Scheme I



center in the protoberberine system is prochiral with the 13α- and 13β-hydrogens being diastereotopic, enzyme-mediated reactions at this site have some stereochemical significance. Consequently, elucidation of the stereochemistry of these reactions is important if some insight is to be obtained into the mechanisms of the biosynthetic conversion of the protoberberine intermediates to the various classes of benzylisoquinoline alkaloids mentioned previously.

Battersby and co-workers[6] have provided evidence in preliminary reports using nonstereospecifically labeled compounds that a stereospecific oxidation at C-13 is involved in the conversion of scoulerine (**2**) to narcotine (**6**). In contrast, the possibility of a nonstereospecific removal of a hydrogen at C-13 during the bioconversion of scoulerine to chelidonine (**5**) was initially suggested by these authors.[5]

To undertake a complete investigation of the stereochemical details of reactions at C-13 which occur in the biosynthesis of these various alkaloids, suitable protoberberines containing an isotopic hydrogen label of known configuration at C-13 are required.

In this paper we describe a general method for the synthesis of such systems and report the elucidation of the stereochemical course of a hydroxylation step which occurs in the biosynthesis of ophiocarpine (**8**).

Post-Reticuline Intermediates in the Biosynthesis of Ophiocarpine. The alkaloid ophiocarpine was first isolated from the plant *Corydalis ophiocarpa* by Manske[8] in 1939. Of the more than 70 protoberberines which have been isolated from natural sources, this alkaloid was, until the recent report of 13β-hydroxystylopine,[9] the only member of the family to contain an oxygen function at the 13 position. Since hydroxylation at C-13 of the protoberberine system has been implicated in the biosynthesis of the phthalide isoquinoline and rhoeadine systems, a study of the biosynthesis of ophiocarpine was undertaken as a prelude to further studies of these other classes of alkaloid.

Initial consideration of the biosynthesis of ophiocarpine was concerned with identification of the particular post-reticuline intermediate which is hydroxylated at C-13.

In considering the conversion of reticuline to ophiocarpine there is ample precedent for a pathway in which reticuline is converted to a scoulerine as the first step.[5,6] Subsequent steps would then require O-methylation, oxidation at C-13, and formation of a methylenedioxy group but not necessarily in that order.

To investigate the sequence of these events, three radiolabeled compounds, (±)-[1,12-$^3H_2$]scoulerine (**2**), (±)-[12-^{3}H]nandinine (**9**), and (±)-[8,14-^{3}H,9-OMe-^{14}C]tetrahydroberberine (**10**), were synthesized as test precursors. (±)-Scoulerine, synthesized by modification (see Experimental Section) of the procedure of Kametani and co-workers,[10] was labeled at the 1 and 12 positions with T_2O in a base-catalyzed reaction in DMF. (±)-Nandinine, obtained from berberine by selective O-demethylation of the C-9 methoxyl and subsequent reduction of the resulting phenol betaine (**11**) with sodium borohydride,[3a] was tritiated at C-12 in similar fashion under basic conditions. (±)-[8,14-^{3}H,9-OMe-^{14}C]tetrahydroberberine was obtained by admixture of the ^{3}H- and ^{14}C-labeled compounds which were synthesized separately. Introduction of the ^{14}C label was accomplished by methylation of the phenol betaine (**11**) with $^{14}CH_3I$ followed by reduction of the product with borohydride. The tritiated compound was prepared by reduction of berberine with sodium borotritide in methanol.

Allocation of the tritium labels in compounds **2, 9,** and **10** to the positions indicated followed from the methods used for their introduction but was also verified in each case by a comparable experiment in which deuterium was employed as a label. Mass spectral and ^{1}H NMR spectral studies (see Experimental Section) of the deuterated products confirmed that the isotopic label was restricted to the expected positions.

The labeled (±)-scoulerine, (±)-nandinine, and (±)-tetrahydroberberine were fed hydroponically in separated experiments to young shoots of *C. ophiocarpa*. After 1 week radiolabeled (−)-ophiocarpine was isolated by preparative layer chromatography from these experiments and the percentage incorporations obtained for each of the test precursors are summarized in experiments 1–3 in Table I. Comparison of the results from the first two experiments demonstrates that while scoulerine is only a moderately efficient precursor of ophiocarpine, nandinine does not appear to be incorporated to any significant extent into this alkaloid in *C. ophiocarpa*.

Based upon these results it appears that scoulerine undergoes O-methylation at the 9 position prior to formation of the methylenedioxy group at the 2,3 position. Verification of this suggestion would require a study of the incorporation of labeled isocorypalmine (**12**) (see Scheme II). Most importantly, however, experiment 3 demonstrates that tetrahydroberberine is incorporated relatively efficiently into ophiocarpine and it may be concluded that a major pathway in the biosynthesis of ophiocarpine in *C. ophiocarpa* involves the introduction of the C-13 hydroxyl as the terminal step.

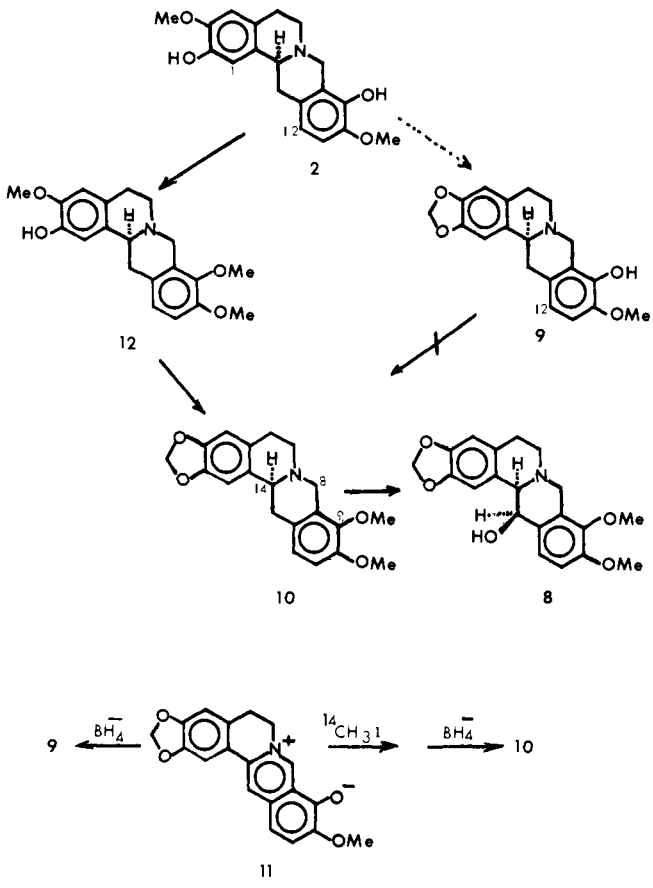
Synthesis and Cyclization of Medium-Ring Olefins. Having established that tetrahydroberberine is hydroxylated to ophiocarpine, we were in a position to consider procedures for the stereospecific introduction of a deuterium or tritium label at the C-13 position in the former compound. The availability of medium ring olefins such as **13** and their facile recyclization to the tetrahydroberberine ring system was first provided by the elegant work of Pyman[11] in his classic studies on the elucidation of the course of the Hofmann degradation of (−)-tetrahydroberberine. The ten-membered ring *trans*-azecine

Table I

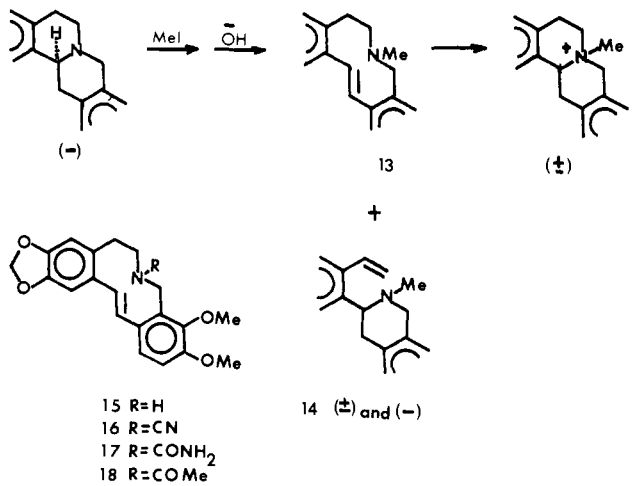
Expt	Precursor	T/^{14}C	% incorpn[a]	T/^{14}C	% retention of T
1	(±)-[1,12-$^{3}H_2$]Scoulerine (**2**)		0.05		
2	(±)-[12-^{3}H]Nandinine (**9**)		0.001		
3	(±)-[9-OMe-^{14}C,8,14-$^{3}H_2$]Tetrahydroberberine (**10**)	20.6	0.13	19.8	96
4	(±)-[9-OMe-^{14}C,13α-^{3}H]Tetrahydroberberine (**30**)	13.1	0.99	11.6	88
5	(±)-[9-OMe-^{14}C,13β-^{3}H]Tetrahydroberberine (**31**)	14.2	0.25	2.0	14

[a] Incorporations are based upon ^{14}C only for double labeled samples.

Scheme II



13, formed as one of the Hofmann products, was shown to recyclize to give the (±)-*N*-methyltetrahydroberberinium salt in order to account for the partially racemic nature of the second product **14.** Additional examples of transannular re-



actions between the nitrogen and the C-14 in the mesocycle **13** were later provided by Russell[12] and several related examples of transannular reactions between nitrogen and a carbon–carbon double bond have been well documented.[13]

Inspection of a model of **13** indicates the existence of two enantiomeric conformations in which the nitrogen atom is close to within bonding distance (1.60 Å) of the C-14 olefinic carbon and ideally positioned for maximum overlap of the nitrogen lone pair with the π bond. In view of this favorable arrangement we reasoned that the transannular cyclization of **13** to the *N*-methyltetrahydroberberinium salt might proceed by anti addition to the C-13–C-14 double bond and could, in principle, provide a simple route for the stereoselective introduction of a deuterium or tritium label at C-13. However, the amino olefin **13** is unsuitable for the synthesis of the required tetrahydroberberine because of the potential difficulty in removing the *N*-methyl group from the *N*-methotetrahydroberberinium salt which is formed on recyclization. To obviate this problem we undertook the examination of the routes to the corresponding secondary amine **15,** preferably in a suitably protected form so as to allow its release under controlled conditions.

A compound which appeared to fulfill the requirements was the *N*-cyanodibenzazecine **16** which is one of two products obtained directly from tetrahydroberberine on treatment with cyanogen bromide.[14] The *N*-cyano compound was obtained by essentially the same procedure as previously reported. The trans stereochemistry of the stilbenoid system in **16** which had been previously allocated[14] from uv spectral data was verified by its ^{1}H NMR spectrum which contained the olefinic proton signals as an AB spin system centered at δ 6.65 (J_{AB} = 16.0 Hz).

Attempts to hydrolyze the cyanamide to the secondary amine under a variety of basic conditions were unsuccessful. In each case, the urea **17**[14] was obtained. In contrast to these efforts, acid hydrolysis of **16** in 0.5 M HCl in acetic acid proceeded readily under reflux to give tetrahydroberberine in high yield. This result suggested that the secondary amine **15,** which presumably occurs as an intermediate in the hydrolysis, undergoes a transannular cyclization in the acidic medium to give tetrahydroberberine.

The ease with which this latter reaction occurred was evident when it was found that lithium aluminum hydride reduction of the cyanamide **16** when subjected to the normal aqueous workup of the reaction also led to tetrahydroberberine in high yield. Although the lability of the secondary amine precluded attempts to isolate this compound, its presence in the $LiAlH_4$ reduction of **16** was demonstrated by the isolation of the *N*-acetyl derivative **18** and the corresponding *N*-2,2,2-trichloroethoxycarbonyl derivative from separate trapping experiments with acetyl chloride and β,β,β-trichloroethyl chloroformate, respectively. In each case, the acyl derivative was accompanied by varying amounts of tetrahydroberberine and served to emphasize the facility with which **15** undergoes transannular cyclization in protic media, even under basic conditions.

Both the *N*-cyanoazecine **16** and the *N*-2,2,2-trichloroethoxycarbonyl derivative of **15** are readily converted directly to (±)-tetrahydroberberine by either mild acid hydrolysis of the nitrile or reductive elimination in acid of the urethane.

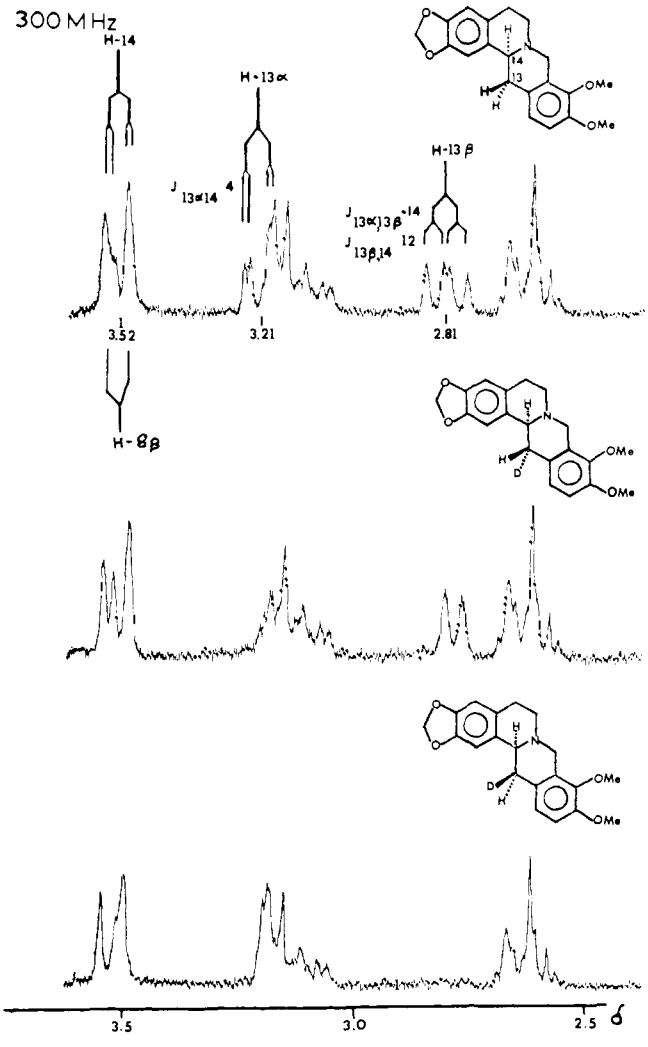
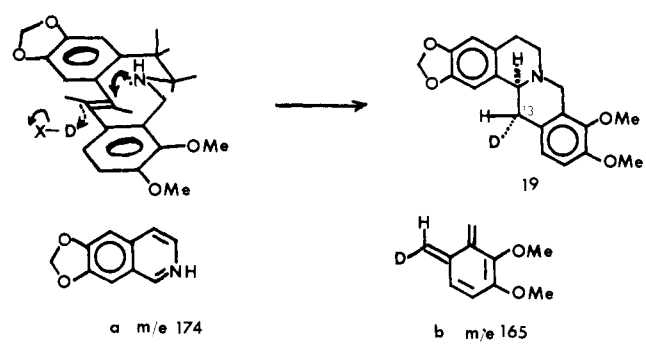


Figure 1.

Presumably the secondary amine **15** is generated in both of these reactions and undergoes cyclization to tetrahydroberberine. The cyclization step has to take place in a highly stereoselective manner if these reactions are going to be useful for the introduction of an isotopic hydrogen label at C-13 with high configurational purity. In this regard the facile cyclization of the secondary amine **15** in protic media at pH 7–8 observed in the $LiAlH_4$ reduction of **16** was encouraging in that it suggested the reaction was probably concerted and led us to expect that it should proceed by anti addition to the double bond.

Since the acid hydrolysis of the cyanamide **16** provided the more expedient route to tetrahydroberberine, it was examined in preference to the urethane. The mass spectrum of tetrahydroberberine obtained from this reaction when employing 0.5 M DCl in acetic acid-*O*-d_1 showed an $M^+ = 340$ in accord with a monodeuterated species. Furthermore, the fragment ions at *m/e* 174 (a) and *m/e* 165 (b) resulting from the retro-Diels–Alder cleavage of ring C[15] indicated that the deuterium was located exclusively in the CD portion of the molecule. However, this result did not allow the exact position of the label to be ascertained.

An unequivocal assignment of the location and stereochemistry of the label as indicated in structure **19** was based upon a detailed ^1H NMR study. The hydrogens at C-13 and C-14 in tetrahydroberberine constitute an ABX spin system which appears between δ 2.50 and 4.00 at 100 MHz. Unfortunately, some overlap of these signals occurs at this frequency with the resonances of methylene hydrogens at C-5 and C-6 and the 8α-hydrogen signal and makes a conclusive



analysis impossible. Fortunately the high-field spectrum of tetrahydroberberine at 300 MHz (see Figure 1) gave a clear separation of the 13β-hydrogen signal at δ 2.81 ($J_{13\beta,13\alpha} = 14.0$ Hz, $J_{13\beta,14} = 12.0$ Hz) in which the magnitude of the vicinal coupling is in accord with its trans relationship to the H-14 proton. A partially resolved quartet at δ 3.21 was assignable to the 13α-hydrogen signal and the 4.0-Hz vicinal coupling was in agreement with the cis relationship of this hydrogen with the neighboring hydrogen. The H-14 signal, which had been previously assigned[16] from a 100-MHz study of tetrahydroberberines deuterated at various positions in ring C, overlapped with the doublet from the 8β-hydrogen signal at δ 3.52; however, this did not interfere with the subsequent analysis and assignment of stereochemistry to the 13-deuterated compounds.

A comparison of the 300-MHz spectra of tetrahydroberberine and the D_1 derivative **19** obtained above shows clearly that the 13β-hydrogen quartet has collapsed to a doublet (J = 12.0 Hz) and that the H-13α signal is absent. This result establishes conclusively the location of the deuterium at C-13 and also defines the stereochemistry as being in accord with the expected anti addition to the double bond.

The success achieved in the transannular cyclization approach to the synthesis of **19** prompted us to examine a related scheme for the synthesis of [13β-^{2}H]tetrahydroberberine (**20**). The required stereochemistry at C-13 is generated from a transannular anti addition of the nitrogen (to C-14) and deuterium to the 13,14 double bond of the *cis*-dibenzazecine **21.**

It appeared that a reasonable approach to the synthesis of compounds in the *cis*-dibenzazecine series would be to attempt to photoisomerize[17] their readily available counterparts in the trans series. In preliminary studies of the photoisomerization reaction it was found that using the ten-membered *trans*-dibenzazepine **13,** irradiation of a hexane solution of this compound through a Pyrex filter with a medium pressure mercury lamp led to a photostationary state consisting of a 40:60 mixture of the cis and trans olefins. The required cis isomer could be readily separated from the mixture by chromatography. After a study of the absorption characteristics of the cis and trans isomers, it was possible to effect a quantitative conversion of the trans isomer **13** to **23** by eliminating light <305 nm (see Experimental Section). The structure of **23** was supported by its ^1H NMR spectral properties which included the presence of an AB system centered at δ 6.92 (J = 12 Hz) attributable to the cis-olefinic hydrogens and was verified by the identity of its dihydro compound[18] with the hydrogenation product of the trans olefin **13.**

In a similar reaction, photoisomerization of the *N*-cyano trans olefin gave the required cis isomer **22** in quantitative yield.[20] The structure of the *N*-cyano-*cis*-dibenzazecine **22** was established from its spectral and chemical properties (see Experimental Section) which paralleled those of the *N*-methyl analogue **23.**

Hydrolysis of **22** in acetic acid–hydrochloric acid under the same conditions used for the corresponding trans isomer gave

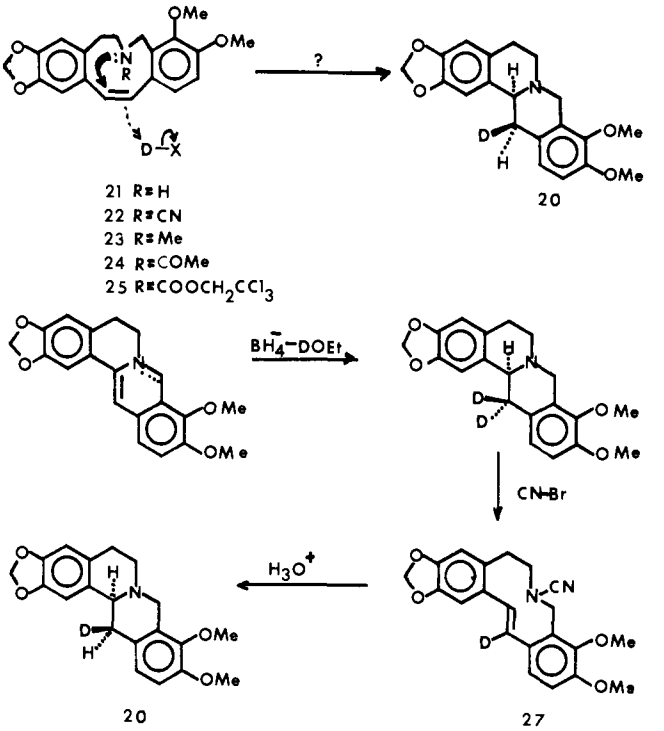
a basic compound which was not (±)-tetrahydroberberine. On the basis of ^{1}H NMR, ir, and uv spectral data (see Experimental Section), the hydrolysis product was assigned the secondary amine structure **21**. Lithium aluminum hydride reduction of the *N*-cyano compound also afforded the secondary amine. Confirmatory evidence for the structure of the secondary amine was provided by the formation and spectral properties of the *N*-acetyl derivative **24** and the crystalline urethane **25**.

The failure of the cis olefin to undergo transannular cyclization with the same ease as the trans isomer was not unexpected. Inspection of models shows that the *cis*-dibenzazecine ring system is much more flexible than the trans series. Of the various conformations of the cis form indicated from models, none approaches the favorable geometry for cyclization which exists in the trans isomer; yet, it would appear that there is at least one conformation which can assume the correct orientation for maximum overlap of the π bond and the nitrogen lone pair concomitant with a C-14–N internuclear distance of 2.40 Å.[21] To the extent that the conformation of the urethane **25** represents the ground state conformation of the parent secondary amine, some insight was provided by a detailed ^{1}H NMR study of **25**. Although the spectrum of **25** at 25° is complicated by slow interconversion of N–CO bond rotamers, it was possible to assign all the signals including those corresponding to the C-5 methylene at δ 2.80 and the C-8 methylene at δ 4.74 and 4.76. A double resonance experiment showed that irradiation at δ 2.80 resulted in a 15% enhancement of the C-8 methylene signals through a nuclear Overhauser effect. The close proximity of the hydrogens at C-5 and C-8 required by this result is uniquely described by the extended conformation **26**. While the analogous ground-state conformation of **21** is obviously incapable of undergoing transannular cyclization, the energy barriers separating it from other ring conformers although unknown are not expected to be so insurmountable as to preclude some finite concentration of a conformation which is more favorably disposed for cyclization. Despite this, all efforts to induce transannular cyclization of the *cis*-dibenzazecines **21** and **22** under a variety of conditions in protic media failed.

The failure to effect transannular cyclization of the *cis*-dibenzazepine prompted an investigation of an alternate route to the required labeled compound. The successful cyclizations achieved with the *trans*-dibenzazecines indicated that the required stereospecifically labeled tetrahydroberberine could be obtained from the 13-^{2}H analogue of **16** by cyclization in a protic medium.

Simple access to this compound was provided by the knowledge that [13,13-^{2}H]tetrahydroberberine was obtained, rather surprisingly, from the borohydride reduction of berberine or dihydroberberine in deuterioethanol or D_2O–dimethoxyethane.[16] Consequently, treatment of [13,13-^{2}H]tetrahydroberberine with cyanogen bromide afforded the required [13-^{2}H]-*trans*-*N*-cyanoazecine **27** which possessed the appropriate ^{1}H NMR and mass spectral properties. Its conversion to [13β-^{2}H]tetrahydroberberine **20** (Scheme III) proceeded in high yield on refluxing in 0.5 M hydrochloric acid in acetic acid. The mass spectral fragmentation pattern was in accord with the location of a single deuterium in the C:D portion of the molecule. Confirmation of the position and stereochemistry of the 13β-deuterium was again clearly evident from the 300-MHz ^{1}H NMR spectrum (Figure 1). The resonance at δ 2.81, which had been previously assigned to the 13β-hydrogen, was absent and the partially resolved doublet at δ 3.21 (J = 4 Hz) could be assigned to the 13α-hydrogen. The magnitude of the coupling constant of the latter to H-14 confirms the cis relationship between these protons, in accord with the expected stereochemistry resulting from a transannular anti-addition process.

Scheme III



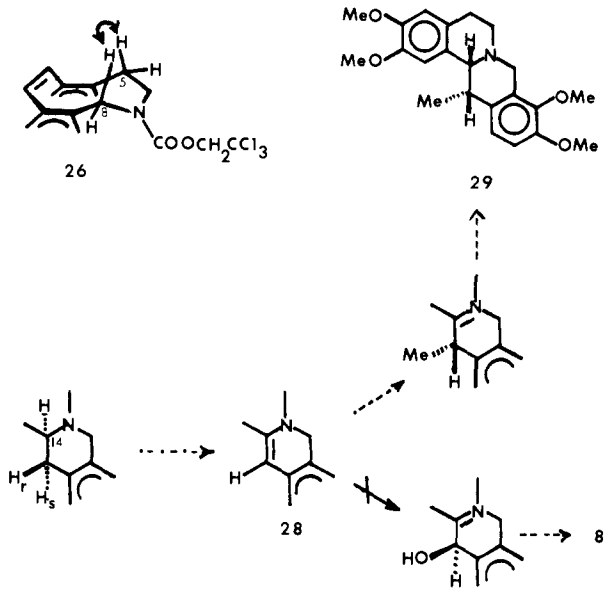
The high stereoselectivity of the transannular cyclization reaction is evident from the 300-MHz spectra of the 13α- and 13β-deuterated compounds. While the high stereoselectivity is in accord with a concerted process, a stepwise process involving a rapid and stereospecific protonation of the intermediate carbanion resulting from addition of the nitrogen to the double bond, although intrinsically unlikely, could also be invoked. Support for the concerted pathway was provided by an experiment in which the *trans*-*N*-cyanoazecine **16** was hydrolyzed in DCl–DOAc containing 30% of the protium species. The resulting tetrahydroberberine consisted of 60% D_0 and 40% D_1 species as determined by mass spectrometry.

The isotope discrimination revealed by this reaction rules out the carbanion pathway since the percentage label in the product should reflect the composition of the solvent if protonation is rapid. In contrast, a concerted addition is expected to show an isotope effect which is consistent with the observed discrimination.

A further point of interest concerned the possible reversibility of the transannular cyclization **15** → **19**. Our observation that equilibration of tetrahydroberberine (**10**) with DOAc–DCl under a variety of conditions failed to give any incorporation of deuterium at the C-13 position demonstrated that the conversion of **15** → **19** is irreversible under conditions which readily effect cyclization.

Stereochemistry of Hydroxylation of Tetrahydroberberine. The recent report[22] that the C-13 methyl group of corydaline (**29**) is introduced at a late stage in the biosynthesis of this alkaloid confirmed some earlier ideas which had prompted us to consider the possibility of an enamine (cf. **28**) as an intermediate to both **29** and ophiocarpine.

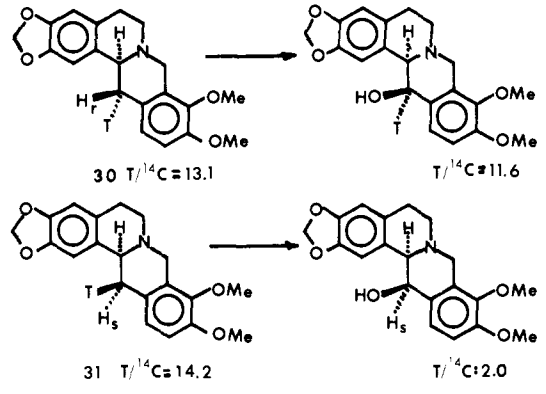
However, the formation of the enamine from the corresponding tetrahydroberberine necessarily involves the loss of **H**-14 (as well as the pro-13*R* or the pro-13*S* hydrogen) and, therefore, this pathway to ophiocarpine may be ruled out since the incorporation of (±)-[8,14-^{3}H,9-OMe-^{14}C]tetrahydroberberine occurs without loss of tritium from the 14 position. Consequently, it appeared that direct hydroxylation of the C-13 methylene group of tetrahydroberberine is involved in its conversion to ophiocarpine. A further point of importance established by this result is that it would appear unlikely that there is any biological interconversion of the (+) and (−) forms



of tetrahydroberberine such as occurs with the enantiomers of reticuline in certain *Papaver* species.[23] Therefore, it was possible to carry out experiments with the racemic forms of labeled tetrahydroberberine with the knowledge that only the (−) form would undergo conversion to ophiocarpine.

To study the stereochemistry of this reaction (±)-[13α-^{3}H]tetrahydroberberine and (±)-[13β-^{3}H]tetrahydroberberine were synthesized by analogous procedures reported for the corresponding deuterium-labeled compounds. While it would have been desirable to have a direct measure of the configurational purity of the isotopic label in each of these compounds, it was not essential, since each isomer is necessarily of the same configurational purity as a consequence of the complementary method used in each synthesis. Therefore, results of their separate incorporations into ophiocarpine still allow a rigorous conclusion to be drawn concerning the stereochemistry of the hydroxylation process.

Each of the chirally labeled compounds was mixed with (±)-[9-OMe-^{14}C]tetrahydroberberine, and the double labeled samples **30** and **31** were administered separately to excised cuttings of *C. ophiocarpa*. Isolation of the alkaloids and purification of ophiocarpine gave the incorporations indicated in Table I.



Clearly the results show that the hydroxylation of (−)-tetrahydroberberine at the C-13 benzylic methylene in affording (−)-ophiocarpine is proceeding with removal of the pro-13*R* hydrogen. This corresponds to an overall retention of configuration in the hydroxylation process and is in agreement with most of the previously studied examples of enzymatic hydroxylation at a saturated carbon.[24] The fact that only 88%, instead of the expected 100%, of the pro-*S* tritium remained after conversion of the 13α-^{3}H isomer **30** to (−)-ophiocarpine suggested that the starting material did indeed contain a small amount of the configurational isomer **31**. The corresponding loss of 86% of the pro-*R* tritium from the parallel experiment with the 13β-^{3}H isomer **31** was in agreement with this suggestion.

This simple and direct method[25] for the introduction of an isotopic hydrogen label at C-13, of either configuration, in a highly stereoselective manner by the transannular cyclization procedure, should now allow attention to be directed to some of the stereochemical processes involved at C-13 in the biosynthetic conversion of protoberberine intermediates to other related families of benzylisoquinoline alkaloids.[3-7]

Experimental Section

Melting points were determined on a Thomas-Hoover Mel-Temp apparatus and are uncorrected. Infrared spectra were determined on Perkin-Elmer Models 237 and 621 recording spectrophotometers. Nuclear magnetic resonance spectra were determined at 60 MHz on Varian A-60 and T-60 instruments, at 90 MHz on the Bruker HFX-10, and at 100 MHz on the JEOL MH-100 spectrometer. Chemical shifts are reported in δ units relative to internal Me_4Si. Ultraviolet spectra were recorded on Beckman DB-G and Cary Model 14 recording spectrophotometers. The ORD spectra were obtained on the Durrum-Jasco ORD-UV/5.

Mass spectra were recorded on the Bell and Howell CEC-21-490 and MS-902 instruments. High-resolution spectra were obtained on the MS-902 at the Research Triangle Institute Center for Mass Spectrometry through the courtesy of Dr. David Rosenthal and Mr. Fred Williams. The Center is sponsored by a Special Facilities Grant No. IR-0330-01, National Institutes of Health. Elemental analyses were performed by MHW Laboratories, Garden City, Mich.

Chromatography was routinely performed on neutral Woelm aluminum oxide, grade III, or W. R. Grace silica gel unless otherwise indicated. Thin-layer (0.25 mm) and preparative layer (1 mm) chromatography using aluminum oxide, silica gel, or silica gel-5% potassium carbonate was used. Various solvent systems and multidevelopment techniques are noted. Visualization was achieved by ultraviolet light and by spraying with iodoplatinic acid or ceric ammonium sulfate in sulfuric acid.

Radiochemical procedures have been previously described[27] and radiochemical purity was assured by recrystallization of solid samples to constant activity and maintenance of constant molar activity during a synthetic sequence. Also, radiopurity was ascertained by scanning a silica gel (5 × 20 cm) thin-layer chromatographic plate with a Varian Model 6000-10 2 π thin-layer radioscanner using either methane or P-10 as counting gas. *Corydalis ophiocarpa* plants were grown in the Duke University phytotron.[9]

Feeding of Radiolabeled Precursors. The free bases were normally fed as solutions of their hydrochloride salts in a minimum amount of water (pH 5.5-6.0) and a few drops of a 1% aqueous solution of Tween 20 was added. For double-labeled experiments accurately weighed samples of the test precursors were combined such that a ^{3}H/^{14}C ratio of ca. 10:1 was obtained.

Solutions of these precursors were then administered to young shoots of *C. ophiocarpa* using a hydroponic feeding method. Young shoots were cut under water and placed in the solutions. As the precursor was taken up several small aliquots of distilled H_2O were added such that the open end of the shoots never became dry. After 7 days the shoots were homogenized in a Waring blender using 95% EtOH containing 200-300 mg of a mixture of crude representative alkaloids as carrier. After filtration the filter cake was extracted for several hours in a Soxhlet using 95% EtOH. The combined extracts were concentrated in vacuo, diluted with a large volume of hot H_2O (100-200 ml), and acidified to congo red end point with concentrated HCl. This solution was filtered through a Celite bed to remove some gummy insoluble deposits and then washed with several portions of Et_2O. After further cooling the solution was basified with concentrated NH_4OH and extracted with $CHCl_3$ (3 × 100 ml). The combined organic extracts were washed with H_2O (2 × 100 ml) and dried ($MgSO_4$) and the solvent was removed in vacuo to give the crude alkaloids. These were partially purified by PLC on Al_2O_3 [C_6H_6-EtOAc (4:1 v/v) once] to give two major fractions, R_f 0.88 [(−)-stylopine and (−)-canadine)] and R_f 0.50 [protopine, (−)-ophiocarpine, and (−)-13β-hydroxystylopine]. The fraction containing (−)-ophiocarpine was then further purified by PLC [SiO_2-5% K_2CO_3,

C_6H_6-EtOAc (4:1 v/v) three times] to give (−)-13β-hydroxystylopine, R_f 0.71; (−)-ophiocarpine, R_f 0.50; protopine, R_f 0.17.

The (−)-ophiocarpine was then recrystallized from 95% EtOH to constant specific activity.

(±)-12-Bromoscoulerine. (±)-1-(2-Bromo-5-hydroxy-4-methoxybenzyl)-1,2,3,4-tetrahydro-7-hydroxy-6-methoxyisoquinoline (0.51 mmol), prepared by the method of Jackson and Martin,[28] was dissolved in 95% EtOH (10 ml) and 38% aqueous formalin (15 ml). Concentrated HCl (10 μl) was added and the mixture was gently refluxed for 4 h. The alcoholic solvent was removed in vacuo and the solution was basified with 10% aqueous NH_4OH and extracted with $CHCl_3$ (4 × 25 ml). The combined organic extracts were washed with H_2O (3 × 20 ml) and dried ($MgSO_4$), and the solvent was removed in vacuo to give 178.7 mg (96%) of 12-bromoscoulerine as a pink resin. Recrystallization from 95% EtOH gave 133.5 mg of reddish prisms: mp 138–140° (lit.[10] 136–138°).

(±)-Scoulerine. The preceding 12-bromoscoulerine (0.25 mmol) was dissolved in EtOH-THF (1:1 v/v) (30 ml) and hydrogenolyzed over 30% Pd-$SrCO_3$ (5 mg) catalyst. The uptake of 1 mol of hydrogen was complete in ca. 3 h and the catalyst was then removed by filtration through Celite. Removal of the solvents in vacuo gave 81.1 mg (99%) of (±)-scoulerine (**2**) as a reddish solid which could not be recrystallized from 95% EtOH: mass spectrum *m/e* (rel intensity) 327 (77), 178 (100), 176 (31), 150 (58), 135 (28); R_f 0.50 [SiO_2, $CHCl_3$–MeOH (9:1 v/v) once], identical by TLC and MS with a sample of (−)-scoulerine provided by R. H. F. Manske.

Treatment of a small amount of this material with picric acid in 95% EtOH gave (±)-scoulerine picrate: mp 207–208° dec (lit.[29] 205–207°).

(±)-[1,12-$^2H_2$]Scoulerine (2). (±)-Scoulerine (0.030 mmol) was dissolved in a mixture of D_2O (0.2 ml) and DMF (0.2 ml) under N_2 and potassium *tert*-butoxide (0.015 mmol) was added in one portion. The tube was then sealed and heated at 100 °C in an oil bath for 5 days. After cooling to 0° the tube was opened and the mixture was immediately acidified by the addition of several drops of concentrated HCl. The mixture was then dissolved in H_2O (25 ml), basified with solid $NaHCO_3$, and extracted with $CHCl_3$ (2 × 20 ml). The combined organic extracts were then washed with H_2O (1 × 30 ml) and dried ($MgSO_4$), and the solvent was removed in vacuo to give 7.2 mg (72%) of (±)-[1,12-$^2H_2$]scoulerine (**2**) as a reddish powder: R_f 0.50 [SiO_2, $CHCl_3$–MeOH (9:1 v/v) once]; mass spectrum *m/e* (rel intensity) 329 (70), 328 (70), 327 (26), 179 (100), 178 (52), 177 (35), 176 (17), 151 (57), 150 (17), 136 (26).

(±)-[1,12-$^3H_2$]Scoulerine (2). This material was prepared exactly as described above by exchange with tritiated H_2O (1 Ci). Prior to $CHCl_3$ extraction during workup, 10 mg of inactive (±)-scoulerine was added as cold carrier. The material (16.9 mg) was isolated and examined by TLC in conjunction with radioscanning of the plate. A small amount of an impurity was present at the origin, but determination of relative peak areas indicated a radiochemical purity of >95% for the (±)-scoulerine (27.85 μCi/mg).

(±)-[12-$^3H_1$]Nandinine (9). The phenol betaine **11**[30] (0.2 mmol) was dissolved in MeOH-H_2O (9:1 v/v, 20 ml) and an excess of sodium borohydride was added in one portion. After stirring at room temperature for 3 h the mixture was carefully acidified with 10% HCl and MeOH was removed in vacuo. The resulting solid was partitioned between H_2O (30 ml) and $CHCl_3$ (30 ml) and the mixture was basified by the addition of solid $NaHCO_3$. The organic layer was separated, washed with H_2O (1 × 20 ml), and dried ($MgSO_4$). The solution was concentrated in vacuo to approximately 10 ml and chromatographed on Al_2O_3 (Woelm, III, neutral) (2 g) in $CHCl_3$. The eluent was collected (100 ml) and the solvent was removed in vacuo to give a tan solid. Recrystallization from MeOH gave 40.0 mg (61%) of (±)-nandinine as tan needles: mp 172–174° (lit.[3a] 170°); R_f 0.28 [SiO_2, $CHCl_3$-Me_2CO (19:1 v/v) twice].

The above phenol (0.10 mmol) was exchanged with tritiated water (500 mCi) in DMF (0.2 ml) containing potassium *tert*-butoxide (0.05 mmol) as described for (±)-scoulerine. The product was isolated after dilution with inactive carrier (10 mg) to give (±)-[12-$^3H_1$]nandinine (**9**, 31.5 mg) as a tan solid. Recrystallization from 95% EtOH gave 20 mg (42%) of chemically and radiochemically pure material as evidenced by TLC and radioscanning (18.7 μCi/mg).

(±)-[9-OMe-^{14}C]Tetrahydroberberine (10). The phenol betaine, berberrubine (**11**, 0.075 mmol), was dissolved in freshly distilled DMF (2 ml) and placed on a high vacuum manifold. After cooling of the reaction mixture in liquid N_2 and evacuation to a pressure of ca. 10^{-4} mmHg, carbon-14 labeled methyl iodide (250 μCi, 13 mCi/mmol) was allowed to distill into the reaction mixture. The reaction mixture was then sealed and kept at room temperature for 4 days. At the end of this time the mixture was cooled in dry ice-acetone and an additional aliquot (2 ml) of inactive MeI was added. After 3 days MeOH-H_2O (2:1 v/v, 30 ml) and excess sodium borohydride were added. The TLC of the reduction product indicated the presence of (±)-nandinine in admixture with tetrahydroberberine. Purification by PLC, R_f 0.55 [SiO_2, $CHCl_3$-Me_2CO (19:1 v/v) twice], gave 15.0 mg (60%) of chemically and radiochemically pure (±)-[9-OMe-^{14}C]tetrahydroberberine as a yellow oil which solidified. Recrystallization from aqueous ethanol (1:9 v/v) then gave 12.5 mg of light yellow needles (5.56 mCi/mmol).

(±)-[8,14-$^2H_2$]Tetrahydroberberine (with Dr. L. T. Lytle). Berberinium chloride (108 mg, 0.291 mmol) was dissolved by heating in 2 ml of water and 59.6 mg of sodium borodeuteride added. After stirring for 15 min a fine slightly yellow solid precipitated which was collected by suction filtration, purified by column chromatography, and crystallized from aqueous methanol to give 66.7 mg (67.2%) of (±)-[8,14-$^2H_2$]tetrahydroberberine: mass spectrum *m/e* (rel intensity) 341 (84), 175 (13), 165 (100); NMR (100 MHz) ($CDCl_3$) 6.83 (d, 1, J = 10 Hz, H-12), 6.81 (s, 1, H-1), 6.67 (d, 1, J = 10 Hz, H-11), 6.56 (s, 1, H-4), 5.88 (s, 2, OCH_2O), 4.24 (br s, 0.5 H, H-8α), 3.82 (s, 6, $ArOCH_3$'s), 3.56 (s, 0.5 H, H-8β), 3.28 (d, 1, J = 16 Hz, H-13α), 2.82 (d, 1, J = 16 Hz, H-13β).

(±)-[8,14-$^3H_2$]Tetrahydroberberine (10). Anhydrous berberinium chloride (0.054 mmol) was dissolved in a mixture of H_2O (3 ml) and absolute MeOH (0.3 ml). To this was added sodium borotritiide (0.055 mmol, 200 mCi/mmol) in one portion with stirring. After 30 min the mixture was briefly refluxed and then cooled to room temperature. Acidification with 3 N HCl dissolved the precipitated product and the solution was transferred to a separatory funnel, basified with concentrated NH_4OH, and extracted with $CHCl_3$ (2 × 10 ml). After drying, the solvent was removed in vacuo to give 10.0 mg (55%) of a light yellow oil which solidified. Addition of cold carrier (10 mg) and recrystallization from 95% ethanol gave (±)-[8,14-$^3H_2$]tetrahydroberberine (**10**) as yellow needles (46.5 mCi/mmol).

Von Braun Degradation of Tetrahydroberberine. (a) Tetrahydroberberine (0.023 mol) was dissolved in dry benzene (750 ml) and cyanogen bromide (0.03 mol) in dry benzene (30 ml) was added dropwise under nitrogen. The reaction mixture was then refluxed for 24 h, cooled, and filtered to remove tetrahydroberberine hydrobromide (3.05 g). The filtrate was washed with 10% HCl (2 × 200 ml) and water (2 × 200 ml) and dried ($MgSO_4$), and the solvent was removed in vacuo to give a yellow foam. This was dissolved in methylene chloride and chromatographed on Florisil (100 g). The first 700 ml of eluent contained the bromocyanamide which was recrystallized from 95% ethanol to give 1.6 g of fluffy white crystals: mp 177–178 °C (lit.[14] 175°); mass spectrum *m/e* (rel intensity) 446 (8), 444 (8), 217 (13), 164 (100), 149 (31).

The next 100 ml of eluent gave 1.5 g of a mixture of the bromocyanamide and the desired *trans*-dibenzazecine cyanamide (**16**). Further elution with methylene chloride (2 l.) gave the pure unsaturated cyanamide which was recrystallized from 95% ethanol to give 1.2 g of white plates: mp 216–217 °C (lit.[14] 217°); ir λ_{max} ($CHCl_3$) 2200 cm^{-1} (CN); mass spectrum *m/e* (rel intensity) 364 (100), 349 (48); NMR (100 MHz) ($CDCl_3$) δ 7.00 (s, 1, H-1), 6.90 (q, 2, J = 8.5 Hz, H-11 and H-12), 6.72 (q, 2, J = 16 Hz, *trans*-stilbene), 6.67 (s 1, H-4), 5.90 (s, 2, OCH_2O), 4.14 (br s, 2, H-8), 3.94 (s, 3, OCH_3), 3.84 (s, 3, OCH_3), 3.40 (m, 2, H-5 or H-6), 2.89 (m, 2, H-6 or H-5); uv λ_{max} (95% EtOH) 286 nm (ε 12 700).

(b) The following is a modification of the reported[14] workup procedure which facilitated the isolation of the unsaturated cyanamide (**16**). The above procedure was used to the point where the crude mixture of products was isolated. The crude reaction mixture was then redissolved in dry benzene (100 ml) and freshly distilled diethylamine (50 ml) and gently refluxed under nitrogen for 24 h. The mixture was filtered to remove some solid material and the excess diethylamine was removed in vacuo. The resulting brown oil was dissolved in benzene (500 ml) and washed with 10% HCl (2 × 250 ml) and water (3 × 300 ml) and dried ($MgSO_4$), and the solvent was removed in vacuo to give a yellow solid (1.7 g). Thin-layer chromatography indicated the presence of some remaining bromocyanamide and the mixture was then chromatographed on Florisil as before. The isolated cyanamide **16** was recrystallized from 95% ethanol to give 1.5 g of white plates: mp 218–219 °C. The conversion of the bromocyanamide to the di-

ethylamine hydrobromide allows the facile removal of unwanted material in the H_2O wash.

***N*-2,2,2-Trichloroethyloxycarbonyl and *N*-Acetyl Derivatives of 75.** The *trans*-dibenzazecine **9** (145 mg, 0.4 mmol) was dissolved in dry benzene (10 ml) and added dropwise to a stirred suspension of $LiAlH_4$ (150 mg) in dry Et_2O (10 ml) under N_2. The mixture was stirred at room temperature for 1 h, cooled to 0°, and quenched by the slow dropwise addition of a 5% K_2CO_3 (ca. 5 ml) solution. Aluminum salts were then removed by filtration and an additional 25 ml of K_2CO_3 solution was added to the filtrate. This biphasic solution was rapidly stirred and treated as follows.

(a) A solution of 2,2,2-trichloroethyl chloroformate (0.10 ml) in benzene (10 ml) was added. After stirring for 3 h the reaction was worked up to give a brown oil (152 mg). PLC on SiO_2 [C_6H_{12}-EtOAc (4:1 v/v) twice] gave tetrahydroberberine (70 mg) and the *trans*-urethane, as an oil which solidified (75.0 mg, 37%): ir λ_{max} ($CHCl_3$) 1710 cm^{-1} (amide C=O); mass spectrum *m/e* (rel intensity) 515 (100), 513 (100), 338 (86); NMR (100 MHz) ($CDCl_3$) δ 6.84 (q, 2, J = 8 Hz, H-11 and H-12), 6.65 (s, 1, aromatic), 6.58 (s, 3, aromatic and olefinic), 5.86 (s, 2, OCH_2O), 4.58 (s, 2, OCH_2CCl_3), 3.92 (s, 2, H-8), 3.80 (s, 6, OCH_3), 3.64 (t, 2, J = 6 Hz, H-6), 3.04 (t, 2, J = 6 Hz, H-5).

(b) A solution of acetyl chloride (28.4 μl, 0.4 mmol) in 15 ml of benzene was added and the reaction stirred under N_2 for 15 h. A standard workup of the reaction gave a brown solid (112 mg, 79%). PLC on SiO_2 [SiO_2Bz-EtOAc (1:1 v/v) twice] gave tetrahydroberberine (35 mg), R_f 0.7, and the *N*-acetyl compound **18** (55 mg), R_f 0.38, which crystallized from benzene-hexane: mp 189-191°; ir λ_{max} ($CHCl_3$) 1630 cm^{-1} (amide CO); mass spectrum *m/e* (rel intensity) 381 (100), 338 (55), 178 (35). Anal. Calcd for $C_{22}H_{23}NO_5$: M^+, *m/e* 381.1576. Found: M^+, *m/e* 381.1572.

(±)-[13α-^{2}H]Tetrahydroberberine (19). Freshly distilled acetic anhydride (0.5 ml) was heated with D_2O (0.2 ml) for 10 min under dry N_2 and allowed to cool to room temperature. Freshly distilled acetyl chloride (35.6 μl) was then added and the mixture was heated for an additional 10 min to ensure complete hydrolysis. The *trans*-cyanamide (0.1 mmol) was then added in one portion to this solution (0.5 M in DCl) and the mixture was refluxed for 3 h, cooled, and basified by pouring into excess 10% NaOH. The turbid solution was then extracted with benzene (2 × 15 ml), the combined organic layers were washed with H_2O and dried (Na_2SO_4), and the solvent was removed in vacuo to give 30.9 mg (91%) of a yellow oil which solidified. Recrystallization from 95% ethanol gave (±)-[13-^{2}H]tetrahydroberberine (25.1 mg) as yellow needles: mp 173-174 °C; mass spectrum *m/e* (rel intensity) 340 (86), 174 (13), 165 (100), 150 (35); NMR (100 MHz) ($CDCl_3$) δ 6.83 (d, 1, J = 8.5 Hz, H-12), 6.80 (s, 1, H-1), 6.67 (d, 1, J = 8.5 Hz, H-11), 6.56 (s, 1, H-4), 5.88 (s, 2, OCH_2O), 4.24 (d, 1, J = 16 Hz, H-8β), 3.82 (s, 6, OCH_3); NMR (300 MHz) ($CDCl_3$) δ 3.52 (d, 2, $J_{13\beta,14}$ = 12 Hz, H-14 which is partially obscured by H-8α), 2.81 (d, 1, $J_{13\beta,14}$ = 12 Hz, H-13β). The mole percent deuterium obtained from the intensities of the *m/e* 164 and 165 ions indicated D_1 (85%) and D_0 (15%).

***trans*-9,10-Dimethoxy-7-methyl-2,3-methylenedioxy-5,6,7,-8-tetrahydrodibenzo[*c,g*]azecine (13).** (±)-Tetrahydroberberine (0.024 mol) was dissolved in dry acetone and methyl iodide (40 ml) and refluxed for 30 min. Excess methyl iodide was removed in vacuo and the product was removd by filtration to give a yellow-white solid (10.5 g). The methiodide was converted to the azecine **13** by the procedure of Pyman[11] to give, after crystallization from dry ethyl acetate, white needles (1.04 g) (44%): mp 129-131° (lit.[12] 132°); NMR (100 MHz) ($CDCl_3$) δ 7.00 (s, 1, H-1), 6.95 (q, 2, J = 10 Hz, aromatic), 6.84 (q, 2, J = 16 Hz, *trans*-stilbene), 6.70 (s, 1, H-4), 5.96 (s, 2, OCH_2O), 3.90 (s, 3, OCH_3), 3.84 (s, 3, OCH_3), 3.71 (s, 2, H-8), 2.75 (s, 4, H-5 and H-6), 2.24 (s, 3, N-CH_3); uv λ_{max} (C_6H_{12}) 284 nm (ϵ 11 850) [lit.[12] λ_{max} 285-290 (12 000)].

***cis*-9,10-Dimethoxy-7-methyl-2,3-methylenedioxy-5,6,7,-8-tetrahydrodibenzo[*c,g*]azecine (23).** The preceding *trans*-stilbene (0.4 mmol) was dissolved in cyclohexane (250 ml) in a Pyrex flask and the solution was flushed with dry nitrogen for 5 min. This mixture was then irradiated with a 450-W high-pressure mercury lamp (Hanovia) fitted with a Pyrex filter and placed in a double walled quartz immersion well containing a solution of potassium acid phthalate (5 mg/ml) at a thickness of 1 cm in the outer jacket. After 3 h the mixture was filtered and the solvent was removed in vacuo to give a light yellow oil which solidified. Recrystallization from MeOH-H_2O gave 120 mg (86%) of fine white needles: mp 121-122°; NMR (90 MHz) ($CDCl_3$) δ 6.92 (q, 2, J = 12 Hz, *cis*-stilbene), 6.91 (s, 2, aromatic), 6.75 (s, 1, aromatic), 6.67 (s, 1, aromatic), 6.03 (s, 2, OCH_2O), 4.00 (s, 6, OCH_3), 3.76 (s, 2, H-8), 2.62 (s, 3, NCH_3); uv λ_{max} (C_6H_{12}) 292 nm (ϵ 6710). Anal. Calcd for $C_{21}H_{23}NO_4$: C, 71.35; H, 6.56; N, 3.96. Found: C, 71.10; H, 6.64; N, 3.71.

9,10-Dimethoxy-7-methyl-2,3-methylenedioxy-5,6,7,8,13,-14-hexahydrodibenzo[*c,g*]azecine. The preceding cis olefin **23** (0.1 mmol) was dissolved in 3 N HCl (5 ml) and hydrogenated over platinum oxide. The product, 35.1 mg (99%), was obtained as a light oil, which on recrystallization from methanol gave the dihydro derivative as a white powder: mp 129-131 °C (lit.[12] 129°).

The melting point was undepressed upon admixture with the dihydro derivative prepared from the trans olefin by the same procedure.[12]

***cis*-9,10-Dimethoxy-7-cyano-2,3-methylenedioxy-5,6,7,8-tetrahydrodibenzo[*c,g*]azecine (22).** The *trans*-cyanamide **16** (0.69 mmol) was dissolved in methylene chloride (200 ml) in a Pyrex flask and the solution was flushed with nitrogen. This was then irradiated for 4.5 h with a 450-W high-pressure mercury lamp (Hanovia) using a Pyrex filter. Thin-layer chromatography indicated the absence of starting material (R_f 0.33) and the appearance of a new substance (R_f 0.25). The solvent was removed in vacuo to give a yellow oil which was recrystallized from EtOH-H_2O (1:1) to give 230 mg (92%) of small white crystals: mp 163-165°; R_f 0.25 [SiO_2, C_6H_6-EtOAc (1:1 v/v), once]; ir λ_{max} ($CHCl_3$) 2210 cm^{-1} (CN); NMR (100 MHz) ($CDCl_3$) δ 6.81 (q, 2, J = 8.5 Hz, H-11 and H-12), 6.76 (q, 2, J = 12 Hz, *cis*-stilbene), 6.57 (s, 1, 1-H or 4-H), 5.83 (s, 2, OCH_2O), 3.99 (br s, 2, H-8), 3.81 (s, 6, OCH_3), 3.24 (t, 2, J = 7 Hz, H-5 or H-6), 2.88 (t, 2, J = 7 Hz, H-6 or H-5); uv λ_{max} (95% EtOH) 286 nm (ϵ 7860). Anal. Calcd for $C_{21}H_{20}N_2O_4$: C, 69.21; H, 5.53; N, 7.68. Found: C, 69.03; H, 5.56; N, 7.62.

9,10-Dimethoxy-7-cyano-2,3-methylenedioxy-5,6,7,8,13,14-hexahydrodibenzo[*c,g*]azecine. The *cis*-cyanamide **22** (0.1 mmol) was dissolved in ethyl acetate (10 ml and hydrogenated over 10% Pd/C (4 mg). The catalyst was removed by filtration through Celite and the solvent was removed in vacuo to give a white solid. Recrystallization from 95% ethanol gave 34.0 mg (94%) of white crystals of the dihydro derivative: mp 228-230 °C (lit.[14] 224-226 °C). This material was identical by TLC and mixture melting point to the dihydro derivative prepared by hydrogenation of the *trans*-cyanamide **16.**

***cis*-9,10-Dimethoxy-2,3-methylenedioxy-5,6,7,8-tetrahydrodibenzo[*c,g*]azecine (21).** (a) The *cis*-cyanamide **22** (0.2 mmol) was dissolved in glacial acetic acid-d_1 (10 ml) and 0.5 M DCl (5 ml) and refluxed under dry N_2 for 3 h. After cooling to room temperature the solution was poured into excess 10% NaOH and extracted with $CHCl_3$ (2 × 20 ml). The combined organic extracts were washed with H_2O (2 × 50 ml) and dried ($MgSO_4$), and the solvent was removed in vacuo to give 60.0 mg (89%) of the secondary amine **21** as a yellow oil: R_f 0.17 [SiO_2, $CHCl_3$-EtOAc (1:1 v/v)]; ir λ_{max} ($CHCl_3$) 3400 cm^{-1} (NH); NMR (100 MHz) ($CDCl_3$) δ 6.84 (d, 4, olefinic and aromatic), 6.60 (s, 1, aromatic), 6.52 (s, 1, aromatic), 5.92 (s, 2, OCH_2O), 3.86 (d, 8, OCH_3 and H-8), 2.84 (br s, 4, H-5 and H-6), 2.00 (s, 1, NH, exchangeable with D_2O).

(b) A solution of the *cis*-cyanamide **22** (109 mg, 3 × 10^{-4} mol) in dry benzene (10 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (150 mg) in dry ether (10 ml). After 45 min the reaction was quenched with NH_4OH and on the usual washup afforded **21** (104 mg) which proved identical in its spectral and chromatographic properties with that of a sample prepared by method a.

Characterization of **21** was achieved as its *N*-acetyl derivative **24** and its β,β,β-trichloroethoxycarbonyl derivative **25.**

The *N*-acetyl compound **24** was obtained as an oil: ir ($CHCl_3$) 1630 cm^{-1} (amide C=O); mass spectrum *m/e* (rel intensity) 318 (100), 338 (82); NMR (100 MHz) ($CDCl_3$) δ 7.04-6.32 (m, 6, aromatic and olefinic), 5.98 (s, 2, OCH_2O), 4.74 (d, 2, H-8), 3.80 (m, 6, OCH_3), 3.00 (m, 4, H-5 and H-6), 2.22 (d, 3, $NCOCH_3$). Anal. Calcd for $C_{22}H_{23}NO_5$: M^+, *m/e* 381.1576. Found: M^+, *m/e* 381.1582.

The urethane **25** was prepared by adding a solution of the amine **21** (1.5 mmol) in dry benzene (25 ml) to a stirred solution of β,β,β-trichloroethyl chloroformate in benzene (25 ml) in the presence of K_2CO_3 (1 g) under a N_2 atmosphere. The crude product, a yellow oil, was purified by chromatography or on silica gel (40 g) in benzene. Elution of the column with benzene-ethyl acetate (4:1 v/v) gave a foam which crystallized from 95% ethanol to give 706 mg (60%) of the pure urethane **25:** mp 152.5-153.5°; ir λ_{max} ($CHCl_3$) 1700 cm^{-1} (amide C=O); mass spectrum *m/e* (rel intensity) 515 (100), 513

(100), 338 (87); NMR (100 MHz) ($CDCl_3$) δ 6.84-6.35 (m, 6, aromatic and olefinic) 5.80 (s, 2, OCH_2O), 4.76 (s, OCH_2CCl_3), 4.74 (s, OCH_2CCl_3), 4.56 (s, H-8 methylene), 4.54 (s, H-8 methylene), 3.72 (d, 6, OCH_3), 3.08 (br t, 2, H-6 methylene), 2.80 (t, 2, J = 6 Hz, H-5 methylene). Anal. Calcd for $C_{23}H_{22}Cl_3NO_6$: C, 53.66; H, 4.30; N, 2.71; Cl, 20.66. Found: C, 53.86; H, 4.07; N, 2.64; Cl, 20.76.

(±)-[13-$^2H_2$]Tetrahydroberberine. Dihydroberberine (1.2 mmol) was dissolved in dry dimethoxyethane (15 ml) and MeOD (5 ml). To this was added 0.25 M DCl (1.5 ml) under dry N_2 and the mixture was refluxed for 10 min. After cooling to room temperature the mixture was neutralized by the addition of solid $NaHCO_3$. Sodium borohydride (70 mg) was added and stirring continued overnight. The mixture was carefully acidified with concentrated HCl and MeOD-DME was removed in vacuo. The remaining solid in H_2O was basified by the addition of 10% NaOH (10 ml) and extracted into $CHCl_3$ (2 × 30 ml). The combined organic extracts were washed with H_2O (1 × 30 ml) and dried ($MgSO_4$), and solvent was removed in vacuo to give a yellow solid which was pure by TLC. Recrystallization from 95% ethanol gave 390 mg (94%) of yellow needles: mp 172-174°; mass spectrum *m/e* (rel intensity) 341 (100), 174 (7), 166 (93), 151 (36); NMR (100 MHz) ($CDCl_3$) δ 6.83 (d, 1, J = 8.5 Hz, H-12), 6.80 (s, 1, H-1), 6.67 (d, 1, J = 8.5 Hz, H-11), 6.56 (s, 1, H-4), 5.88 (s, 2, OCH_2O), 4.24 (d, 1, J = 16 Hz, H-8β), 3.82 (s, 6, OCH_3), 3.56 (δ, 1, H-8α), 3.52 (s, 1, H-14); mole percent of deuterium from MS data D_1 (3.90%), D_2 (85.35%), D_3 (10.73%).

***trans*-9,10-Dimethoxy-7-cyano-2,3-methylenedioxy-5,6,7,8-tetrahydro[13-$^2H_2$]dibenzo[*c,g*]azecine (27).** [13-$^2H_2$]Tetrahydroberberine (0.8 mmol) was dissolved in dry benzene (25 ml) containing anhydrous K_2CO_3 (110 mg) and was allowed to react with CNBr (1.0 mmol) according to procedure b to give 61.4 mg of a yellow oil. PLC on SiO_2 [$CHCl_3$-EtOAc (95:5 v/v) twice] gave the 13-^2H olefin (40 mg) as a white solid: mass spectrum *m/e* (rel intensity) 365 (100), 350 (60); NMR (100 MHz) ($CDCl_3$) 6.98 (q, 3, J = 8.5 Hz, H-11 and H-12 overlapping with 1-H), 6.80 (s, 1, H-14), 6.64 (s, 1, H-4), 5.96 (s, 2, OCH_2O), 4.20 (s, 2, H-8), 3.96 (s, 3, OCH_3), 3.88 (s, 3, OCH_3), 3.44 (t, 2, J = 6 Hz, H-5 or H-6), 2.93 (t, 2, J = 6 Hz, H-6 or H-5).

(±)-[13β-$^2H_1$]Tetrahydroberberine (20). The above deuterated olefin (0.20 mmol) was refluxed for 4 h under nitrogen in a mixture of glacial HOAc (5 ml) and 0.5 M HCl (4 ml). After cooling to room temperature the mixture was diluted with H_2O (30 ml) and basified with excess 10% NaOH. The precipitated solid was extracted with $CHCl_3$ (3 × 20 ml) and the combined organic extracts were washed with H_2O (2 × 50 ml) and saturated NaCl (1 × 50 ml) and dried ($MgSO_4$), and the solvent was removed in vacuo to give 67.1 mg (89%) of a yellow oil which solidified. Recrystallization from 95% ethanol gave yellow needles: mp 172-174 °C; mass spectrum *m/e* (rel intensity) 340 (70), 174 (20), 165 (100), 150 (55); NMR (100 MHz) ($CDCl_3$) δ 6.83 (d, 1, J = 8.5 Hz, C-12H), 6.80 (s, 1, C-1H), 6.67 (d, 1, J = 8.5 Hz, C-11H), 6.56 (s, 1, C-4H), 5.88 (s, 2, OCH_2O), 4.24 (d, 1, J = 16 Hz, C-8βH), 3.82 (s, 6, OCH_3); NMR (300 MHz) ($CDCl_3$) δ 3.52 (d, 2, $J_{13\alpha,14}$ = 4 Hz, C-14H partially obscured by C-8αH), 3.21 (d, 1, $J_{13\alpha,14}$ = 4 Hz, C-13αH).

Isotope Discrimination on Transannular Cyclization of 16. The cyanamide **16** (36.4 mg, 1 × 10^{-4} mol) was added to a solution prepared from 0.2 ml of D_2O-H_2O (7:3), 0.52 ml of acetic anhydride, and 35.6 μl of acetyl chloride and the solution was refluxed for 6 h. (±)-Tetrahydroberberine (21.0 mg, 62%), mp 171-172°, was subjected to mass spectral examination. The mole percent deuterium found was D_0 (60.39), D_1 (34.91), and D_2 (4.69).

(±)-[13α-^3H]Tetrahydroberberine. A solution of TCl-TOAc was prepared by the addition of freshly distilled acetic anhydride (5.5 mmol) and acetyl chloride (35 μl) to tritiated water (10.0 mmol, 1 Ci) under dry N_2. After stirring for 30 min the *trans*-cyanamide **16** (0.10 mmol) was added and the mixture was then gently refluxed for 3 h. After cooling to room temperature the mixture was basified by the addition of 10% NaOH and extracted with C_6H_6 (2 × 15 ml). The combined organic extracts were washed with H_2O (1 × 15 ml) and dried (M_2SO_4), and the solvent was removed in vacuo to give a light yellow oil. This was dissolved in $CHCl_3$ and chromatographed in Al_2O_3 (Woelm, III, neutral, 1.5 g) using $CHCl_3$ as solvent. The eluent (30 ml) was concentrated in vacuo to give 28.0 mg (83%) of a yellow oil. Recrystallization from 95% EtOH gave 20.1 mg of small needles. Inactive carrier (5 mg) was added to the filtrate and a second recrystallization gave an additional 4.0 mg of material which was combined with the first crop to give a total of 24.1 mg of (±)-[13α-^3H]tetrahydroberberine (4.55 mCi/mmol).

(±)-[13-$^3H_2$]Tetrahydroberberine. Dihydroberberine (0.2 mmol) was dissolved in dry dimethoxyethane (5 ml), MeOH (0.1 ml), and T_2O (0.1 ml, 500 mCi) and refluxed for 1 h. After cooling to room temperature sodium borohydride (20 mg) was added and the mixture was stirred overnight. An additional amount of borohydride (20 mg) was added and the mixture was refluxed for 30 min, cooled to room temperature, and carefully acidified with concentrated HCl. After dilution with H_2O (50 ml) and basification with 10% NaOH (pH 10), the product was extracted into C_6H_6 (2 × 40 ml). The combined organic extracts were washed with H_2O (1 × 50 ml) and dried ($MgSO_4$), and the solvent was removed in vacuo to give 48 mg of a yellow oil. The product was purified by PLC [SiO_2, $CHCl_3$-EtOAc (4:1 v/v) once] to give 27.9 mg (41%) of (±)-[13-$^3H_2$]tetrahydroberberine as a light yellow solid (49.4 mCi/mmol).

***trans*-9,10-Dimethoxy-7-cyano-2,3-methylenedioxy-5,6,7,8-tetrahydro[13-$^3H_1$]dibenzo[*c,g*]azecine.** The [13-$^3H_1$]tetrahydroberberine (0.2 mmol) was diluted with cold carrier (0.8 mmol) and dissolved in dry C_6H_6 (50 ml) and a solution of CNBr (1 mmol) in dry C_6H_6 (10 ml) was slowly added dropwise under dry N_2. After stirring at room temperature for 1 h the mixture was refluxed for 48 h and cooled to room temperature and tetrahydroberberine hydrobromide was removed by filtration. The filtrate was washed with 10% HCl (1 × 40 ml), H_2O (2 × 40 ml), and saturated NaCl (2 × 40 ml) and dried ($MgSO_4$), and the solvent was removed in vacuo to give 182.8 mg of a yellow oil. Purification was achieved by PLC [SiO_2-5% K_2CO_3, C_6H_6-etOAc (95:5 v/v) three times] and the product was isolated in the usual manner. Recrystallization from 95% EtOH gave 46.3 mg (<10%) of the labeled cyanamide as white plates (3.42 mCi/mmol).

(±)-[13β-^3H]Tetrahydroberberine. The tritiated cyanamide (0.1 mmol, 3.42 mCi) was refluxed for 4 h in a mixture of glacial HOAc (5 ml) and 0.5 M HCl (3 ml). After cooling to room temperature the mixture was diluted with H_2O (30 ml) and basified with 10% NaOH. This was then extracted with $CHCl_3$ (3 × 30 ml) and the combined organic extracts were washed with H_2O (1 × 50 ml) and saturated NaCl (1 × 50 ml) and dried ($MgSO_4$), and the solvent was removed in vacuo to give 27.1 mg (80%) of a yellow oil which solidified. The TLC and radioscan verified the chemical and radiochemical purity of this material. Recrystallization from 95% EtOH gave 25.9 mg of (±)-[13β-^3H]tetrahydroberberine as yellow needles (2.78 mCi/mmol).

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