titanacyclobutanes, extrusion of cyclopropane provides a very fast intramolecular trap for the analogous 1,4-biradical intermediate.

Current efforts are directed at characterizing monomeric titanocene by low-temperature spectroscopy, developing the reaction chemistry of this and other photochemically generated intermediates, and further probing the mechanistic details. We are also investigating the photochemical reactivity of other group 4 metallacycles.

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## Stereochemical Fate of *O*-Methyl Groups in the Biosynthesis of Protoberberine Alkaloids

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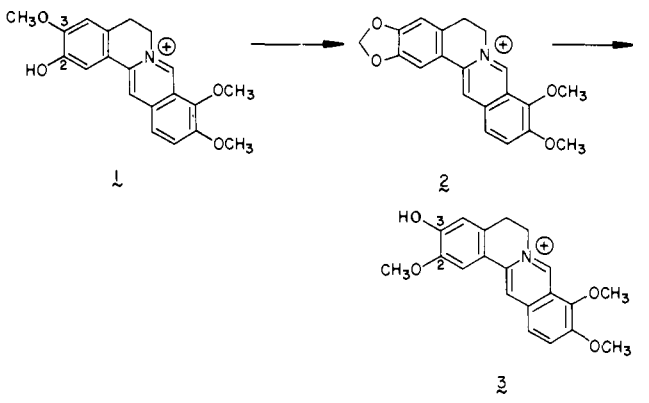
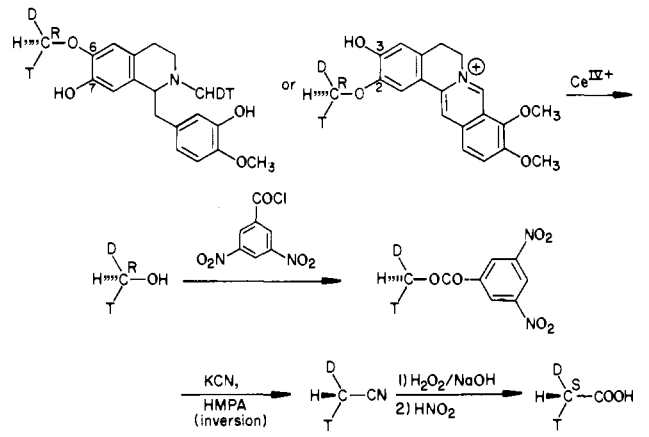
The formation of the medicinally important benzylisoquinoline alkaloid berberine (**2**) from one molecule each of dopamine and *p*-hydroxyphenylacetaldehyde, both in turn derived from tyrosine,[1] involves eight enzymes which have recently been purified and characterized.[2] The terminal step in this sequence is the formation of **2** from columbamine (**1**). This reaction, catalyzed by the $Fe^{2+}$-dependent enzyme berberine synthase,[3] is one of a number of examples of the formation of a methylenedioxy bridge by oxidative cyclization of an *o*-methoxy phenol precursor[1,4,5] either by an ionic or a radical mechanism.[1,4,6] An intriguing further transformation of **2** leads to jatrorrhizine (**3**),[7,8] the major alkaloid of *Berberis* cell cultures. This conversion was shown[8] to involve a ring opening of the methylenedioxy bridge to give the 2-methoxy group of **3** (Scheme I). To obtain more information on the

**Scheme I**



**Scheme II**



mechanisms of these transformations, we traced the stereochemical fate of the chiral methyl group of *S*-adenosylmethionine (AdoMet) through the reaction sequence leading to **3**.

Samples of (*methyl-R*)- and (*methyl-S*)-[*methyl*-$^2$H,$^3$H]-AdoMet[9,10] (100 and 160 mCi/mmol, 91% and 86% ee) were used as substrates in the *O*- and *N*-methylation of 4′-*O*-methylnorlaudanosoline catalyzed by the 6-*O*-[11] and *N*-methyltransferases[12] isolated from *Berberis*[12] cell cultures, respectively. The resulting samples of (1*S*)-[6-*O*,*N*-methyl-$^2$H,$^3$H]reticuline were degraded as shown in Scheme II[10] to convert the 6-*O*-methyl group stereospecifically into the methyl group of acetic acid for chirality analysis by the method of Cornforth[13] and Arigoni.[14] The *F* values[15] of the resulting acetic acid samples were 75.6 and 25.1, respectively. Since the degradation sequence involves one inversion of configuration, these values indicate 88% ee *S* configuration and 86% ee *R* configuration, respectively, for the 6-*O*-methyl group of the two reticuline samples. Hence, the transfer of the methyl group of AdoMet has occurred cleanly with inversion of configuration. This stereochemistry conforms to that established for most methyltransferases studied to date.[16,18,19]

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(14) Lüthy, J.; Retey, J.; Arigoni, D. *Nature (London)* **1969**, *221*, 1213-1215.

(15) For a definition of the *F* value see ref 16: % ee = (|50 - F|/29) × 100 (see ref 17).

(16) Arigoni, D. *Ciba Found. Symp.* **1978**, *60*, 243-261.

(17) Floss, H. G.; Tsai, M.-D. *Adv. Enzymol.* **1979**, *50*, 243-302.

(18) Floss, H. G. In *Mechanisms of Enzymatic Reactions: Stereochemistry*; Frey, P. A., Ed.; Elsevier: Amsterdam, 1986; pp 71-88.

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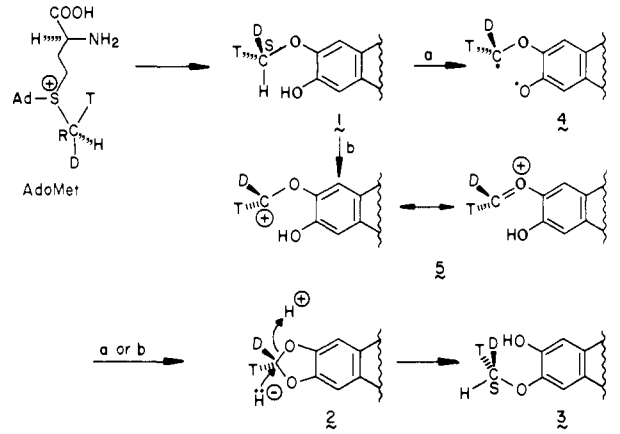
**Scheme III**



The formation of the 2-*O*-methyl group of **3** was studied in vivo in callus cultures of *Berberis koetineana*, because the enzyme catalyzing the opening of the methylenedioxy bridge has not yet been isolated. To this end, (1*S*,*methyl-S*)- and (1*S*,*methyl-R*)-[6-*O*-methyl-$^2$H,$^3$H]reticuline were synthesized from the above samples of chiral AdoMet with porcine liver catechol *O*-methyltransferase (COMT) (Sigma), known to transfer the methyl group with inversion of configuration.[10] The two substrates were purified to remove any traces of the 7-*O*-methyl isomer and fed to the callus cultures. The resulting samples of **3**, purified to remove traces of other alkaloids, particularly **1**, were again degraded as shown in Scheme II. *F* values of 55.4 and 44.2 for the acetic acids obtained indicated 19% ee *S* and 20% ee *R* configuration, respectively, for the methyl groups of the two samples of **3**. Hence the conversion sequence **1** → **2** → **3** has produced a methyl group in **3** of the same configuration as that in **1**.

The abstraction of a hydrogen from the 3-*O*-methyl group of **1** proceeds without an isotope effect[3] and will thus produce equal amounts of methylene groups in **2** containing $^3$H plus $^2$H and $^3$H plus $^1$H. In the subsequent opening of the methylenedioxy bridge, the former will give chiral and the latter achiral methyl groups in **3**. If both steps were completely stereospecific, the enantiomeric purities of the methyl groups in the two samples of **3** would be 44% and 43% ee, respectively.[24] The significantly lower observed values indicate that at least one of the two steps must be accompanied by substantial racemization. The stereochemical results are consistent with, although do not prove, a mechanism as shown in Scheme III. Abstraction of H• from the methyl group and the adjacent OH gives diradical **4**, which collapses with C–O bond formation on the same side on which H• has been removed (path a). Competing rotation and inversion of the methylene radical accounts for the observed racemization. An equally plausible alternative is ring closure and racemization via the cationic intermediate **5** (path b). Opening of the methylenedioxy bridge occurs by attack of H$^-$ on a trajectory opposite the $CH_2$–O-3 bond, possibly assisted by conjugation to the positively charged nitrogen of **2**,[7] replacing O-3 by H with inversion of configuration.

(19) However, several examples of methyl transfer with net retention of configuration have recently been uncovered,[20-23] including two cases involving transfer of the methyl group of methionine.[22,23] These are thought to represent two-step processes.

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(23) A methionine-dependent methylation in the biosynthesis of the antibiotic thiostrepton also proceeds with net retention of methyl group configuration (Yuen, L.-D.; Floss, H. G., unpublished results).

(24) This prediction makes the reasonable assumption that COMT methylates *O*-6 with the same degree of stereospecificity as the plant enzyme. The slightly lower *F* values reported[10] almost certainly are due to the stereochemically less stringent degradation procedure used in the earlier work.

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## Observation of Medium Chain Length Polymethylene Biradicals in Liquid Solutions by Time Resolved EPR Spectroscopy[1]

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In recent years much effort has been expended on the detection of unstable biradicals by physical methods.[2] Because of its superior time resolution, optically detected laser flash photolysis has been the method of choice for obtaining kinetic information. However, the broad solution spectra have little structural content, and the method fails entirely when the biradical has no useful absorption band. Consequently, no spectroscopic measurements of any kind have been reported on the fundamental polymethylene chain biradicals.[3] In this communication we report the first EPR spectra of localized polymethylene biradicals obtained in liquid solution under conditions where they undergo normal fast reactions.

The biradicals were generated in hydrocarbon solvents by photolysis with an excimer laser operating at 308 nm from cyclic $\alpha,\omega$-methylated cycloalkanones **1** by photochemical Norrish type I cleavage followed by fast thermal decarbonylation as shown in Scheme I. The reactions were run in the optical transmission cavity of an X-band EPR spectrometer modified for direct detection and boxcar averaging, operating with a gate of 100 ns at temperatures ranging from −10 to 60 °C with use of a flow system to prevent sample depletion.[4]

Figure 1A shows a series of spectra obtained from the photolysis of tetramethylcycloalkanones (**1**, $R_1 = R_2 = CH_3$; $n$ = 10, 9, 8) observed at 0.25 and 1.0 μs. It is clear from inspection that the two spectra obtained from the cyclododecanone ($n$ = 9) and measured at different delay times are caused by two different carriers. They are assigned to the acyl–alkyl biradical **2** at early times, subsequently evolving into the dialkyl biradical **3**. Although not shown, the other ketones give similar spectra at early times. Also, with decreasing chain length, the spectra take on a net emissive character.

The model used to interpret the strongly polarized spectra and their time evolution is shown schematically in Figure 2 and is essentially identical with the quantitative model developed recently for radical pairs in micelles.[5] Nonadiabatic bond breaking in

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(1) Work supported by NSF Grant CHE-8520326.

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