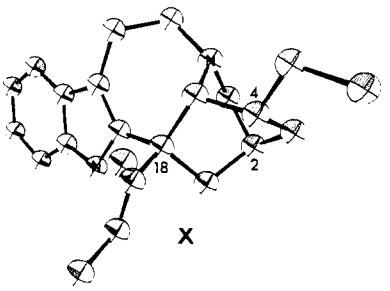
thine and (+)-coronaridine possessing the absolute configuration portrayed in III and VII, respectively, and in agreement with the previous proposal.[11,13] On this basis, ibogamine (now indicated as (+)-ibogamine in Table I) and epiibogamine obtained from dihydrocatharanthine[14] *must* also belong to this stereochemical series and possess the structures VIII and IV, respectively.

To eliminate any doubt as to the correctness of the above assignments, (+)-coronaridine (sample A) was converted by the British Columbia group to the crystalline hydrobromide salt, mp 225–226° dec, and submitted to X-ray analysis.

The hydrobromide crystallizes in the monoclinic space group $P2_1(C_2^2)$ with one molecule per asymmetric unit and $a = 14.16$ (1), $b = 9.667$ (7), $c = 7.421$ (5) Å, and $\beta = 80.31$ (4)°. A right-handed coordinate system was maintained throughout the analysis. The intensities of all reflections in the hkl, $\bar{h}kl$, $hk\bar{l}$, and $\bar{h}k\bar{l}$ octants with $\theta \leq 60°$ were measured using Cu Kα X-rays (1.5418 Å). After Lorentz polarization and background corrections a total of 2830, out of the measured 3230, were judged to be observed ($F_o \geq 3\sigma(F_o)$).

The structure was phased by the heavy atom procedure.[19] All 25 nonhydrogen atoms were located in the first two electron density syntheses and the 26 hydrogens appeared in a subsequent difference synthesis. Full-matrix least-squares refinements with anisotropic temperature factors for the heavy atoms and isotropic hydrogens reduced the conventional crystallographic discrepancy index to 0.047. The mirror image of the original structure could only be refined to a discrepancy index of 0.050. This statistically significant difference means that the correct absolute configuration was chosen at the outset.[20] Structure X depicts a computer-generated perspective drawing of the correct



X

absolute configuration of coronaridine. All bond distances and angles agree well with generally accepted values.[21]

It is now established that there are *two* stereochemical series possible within the Iboga alkaloid family although the results presented here are conclusive for only the alkaloids mentioned. Thus, the alkaloid (+)-catharanthine and the compounds, (+)-coronaridine and (+)-ibogamine, which have been interrelated with it during the various investigations in the Lilly and our laboratories, comprise members of one series while (−)-coronaridine and (−)-ibogamine studied by Blaha and coworkers[15] represent members possessing antipodal stereochemistry. We hasten to add that our previous and present results must not be taken as indicative of the absolute configuration of *all* the Iboga alkaloids which have been reported. Some of the confusion which has arisen in the literature has resulted from the assumption that our previous interrelationships, within a few members of the Iboga family, necessarily imply a distinct absolute configuration in other members which possess varying functionality particularly in the "nontryptophan" portion of the alkaloid system. We do not feel that a rotation value at one wavelength (usually 589 nm) is a conclusive criterion and even in the more preferable approach involving ORD and CD studies[15] caution must be exercised since the influence of substituents on the Cotton effect, for example, is not known accurately.

Finally, we would like to indicate that in the course of studies at Yale on the biosynthesis of Iboga alkaloids in *V. rosea* it was observed[22] that no measurable bioconversion of (+)-catharanthine to (−)-coronaridine took place, although both alkaloids were present at late and early stages of germination, respectively (with the chirality indicated), and *both* were efficiently biosynthesized from a common precursor, geissoschizine.

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(19) The following library of crystallographic programs was used: C. R. Hubbard, C. O. Quicksall, and R. A. Jacobson, "The Fast Fourier Algorithm and the Programs ALFF, ALFFDP, ALFFT and FRIEDEL," U. S. Atomic Energy Commission Report IS-2625, Iowa State University–Institute for Atomic Research, Ames, Iowa, 1971; W. R. Busing, K. O., Martin and H. A. Levy, "A Fortran Crystallographic Least Squares Program," U. S. Atomic Energy Commission Report ORNL-TM-305, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965; C. Johnson, "ORTEP, A Fortran Thermal Ellipsoid Plot Program for Crystal Structure Illustrations," U. S. Atomic Energy Commission Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.

(20) W. C. Hamilton, *Acta Crystallogr.*, **18**, 502 (1965).

(21) O. Kennard and D. G. Watson, "Molecular Structures and Dimensions," Crystallographic Data Centre, Cambridge, England, 1970.

(22) Unpublished work by J. Michael, P. Reichardt, and J. G. Sweeny. Specific incorporations of ^{3}H-labeled catharanthine into coronaridine in *V. rosea* of less than 0.002% were obtained.

(23) Camille and Henry Dreyfus Teacher–Scholar Grant Awardee, 1972–1977; Fellow of the Alfred P. Sloan Foundation 1973–1975.

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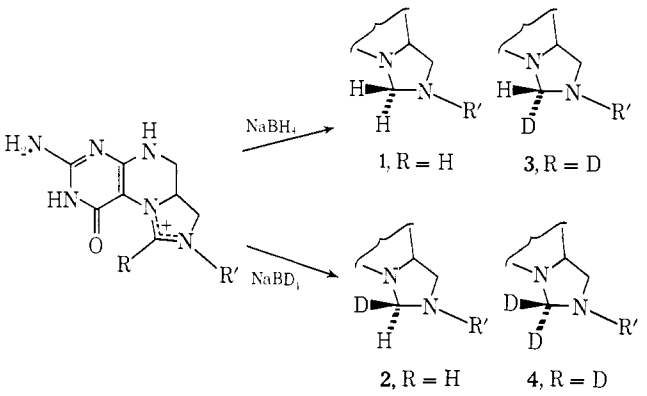
Stereoselective Chemical Reduction of 5,10-Methenyltetrahydrofolate

Sir:

We wish to report the stereoselective synthesis of diastereoisomeric 5,10-methylenetetrahydrofolate with deuterium substitution at the bridging carbon atom.

Reduction of (±)-L-5,10-methenyltetrahydrofolate[1,2] with sodium borodeuteride or of (±)-L-5,10-methenyltetrahydrofolate (deuterium substituted at the formamidinium carbon)[3] with sodium borohydride leads to the two diastereoisomers resulting from stereoselective deuterium substitution at the prochiral methylene carbon,[4] *i.e.*, **2** and **3**, respectively. Abbreviated structures for these products along with the dihydrogen **1** and dideuterio **4** isomers which also were synthesized are depicted below



R′ = *p*-$C_6H_4CONHCH(CO_2H)CH_2CH_2CO_2H$

This procedure permits the isolation of the above compounds as amorphous solids and in sufficient quantities to allow for nmr analysis. In addition, problems which are manifested by the use of tetrahydrofolate and formaldehyde[5-7] during *in situ* preparation—*i.e.*, hydroxymethylation *vs.* imidazolidine formation—can now be circumvented.

The method used for the preparation of **1** was as follows. (±)-L-5,10-Methenyltetrahydrofolic acid in DMSO–pyridine[8] (4/1, v/v), nitrogen atmosphere, undergoes reduction with sodium borohydride (6 equiv) to yield (±)-L-5,10-methylenetetrahydrofolate **1** upon precipitation with tetrahydrofuran. Purification by dissolution in cold 0.1 *N* KOH and rapid reprecipitation[9] with cold 0.1 *N* HCl gave, after freeze drying, solid **1** (50%) which was characterized in the following manner.

(1) The synthesis of 5,10-methenyltetrahydrofolate from *N*-5-formyltetrahydrofolate was made possible by generous gifts of this compound from Lederle Laboratories, Pearl River, N. Y.

(2) J. C. Rabinowitz, *Methods Enzymol.*, **6**, 814 (1963).

(3) This compound was prepared according to a modification of a published procedure: P. B. Rowe, *Anal. Biochem.*, **22**, 166 (1968). Deuterioformic (Merck) and tetrahydrofolic acid (Sigma) were heated at 60° for 24 hr under nitrogen in glacial acetic acid solution containing 0.1% mercaptoethanol. The solution was lyophilized and product recrystallized from 0.1 *M* HCl containing 0.1 *M* mercaptoethanol.

(4) The configurations depicted for **2** and **3** represent the relative stereochemistry with respect to deuterium substitution and are not meant to define the absolute relationship of deuterium to the asymmetric C-6 proton which is presently unknown.

(5) S. B. Horwitz, G. Kwok, L. Wilson, and R. L. Kisliuk, *J. Med. Chem.*, **12**, 49 (1969).

(6) M. Viscontini and J. Bieri, *Helv. Chim. Acta*, **55**, 21 (1972).

(7) R. L. Blakley, "The Biochemistry of Folic Acid and Related Pteridines," North-Holland, Amsterdam, 1969.

(8) The employment of DMSO–pyridine circumvents the difficulties encountered in aqueous media caused by the instability of 5,10-methenyl- or 5,10-methylenetetrahydrofolate at a common pH.[7] The presence of pyridine allows the accumulation of **1** and prevents its further reduction to the *N*-methyl derivative *via* acid-catalyzed ring opening and subsequent reduction of the iminium species.

(9) The pH of the supernatant was usually between 3.8 and 4.5, suggesting that either the mono- or diacid had been precipitated. pK_a: α 3.5, γ 4.8 for dissociation of the carboxyls in the glutamyl side chain: R. G. Kallen and W. P. Jencks, *J. Biol. Chem.*, **241**, 5845 (1966).

(1) The FT nmr spectrum (100 MHz) of **1** displayed a doublet centered at 4.94 ppm which is assigned to the methylene bridge protons (Figure 1). Support for this designation is based on the observation of a similar resonance at 4.63 ppm (DMSO) for the methylene bridge protons of a tetrahydroquinoxaline analog.[10] In addition, **4**, formed by sodium borodeuteride reduction of 5,10-methenyltetrahydrofolate (70% deuterium substitution at the formamidinum carbon) leads to a corresponding loss in this resonance. The doublets at 6.50 and 7.82 ppm are assigned to the aromatic protons of the *p*-aminobenzoyl moiety. The signals at 6.2, 6.7, and 7.32 ppm are attributed to pyridinium and pyridine ^1H species present to preserve the structural integrity of **1**.

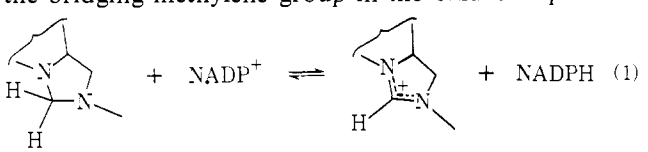
(2) At pH 7.5, the λ_{max} 294 nm (ϵ 28,700) of **1** is similar to that reported by Blakley, pH 7.2, λ_{max} 294 nm (ϵ 32,000).[11]

(3) The uv spectrum of **1** at pH 1.0 initially is identical with that reported by Huennekens[12] and coworkers and undergoes a time-dependent change to that characteristic for tetrahydrofolate. This behavior is consistent with the pH dependent equilibrium between tetrahydrofolate, formaldehyde, and **1**.[13] Correspondingly the dissociation of **1** at pH 4.0 was inhibited by the addition of excess formaldehyde.

(4) Compound **1** showed identical enzymic activity toward 5,10-methylenetetrahydrofolate dehydrogenase[14,15] (baker's yeast) when compared to an incubated sample of tetrahydrofolate and formaldehyde. The addition of excess formaldehyde to **1** (normal assay conditions) did not alter its activity. Enzymic assay of **1** indicated approximately 77 ± 5% purity (based on the diacid).[16] Uv spectrophotometric assay indicates that the starting material, (±)-L-5,10-methenyltetrahydrofolate, is 80 ± 5% pure, suggesting that purification of this reagent should lead to **1** of high purity.

Evidence for the stereoselective reduction is based on both nmr and enzymic analysis. The nmr spectrum of **2** exhibits a singlet at 4.90 ppm and is separated by 0.05 ppm from the singlet observed for **3** at 4.95 ppm (Figure 1). The sharp singlets indicate that isomers **2** and **3** are represented by upfield or downfield resonances of the AB pattern for the prochiral center observed in **1**.

Before employing the enzymic assay, it was necessary to establish that 5,10-methylenetetrahydrofolate dehydrogenase (baker's yeast) removed hydrogen from the bridging methylene group in the oxidative process.



Incubation of **4** with the enzyme followed by reoxidation of NADPH with glutamate dehydrogenase yielded

(10) S. J. Benkovic, P. A. Benkovic, and D. R. Comfort, *J. Amer. Chem. Soc.*, **91**, 5270 (1969).

(11) R. L. Blakley, *Biochem. J.*, **74**, 71 (1960).

(12) M. J. Osborn, P. T. Talbert, and F. M. Huennekens, *J. Amer. Chem. Soc.*, **82**, 4921 (1960).

(13) R. G. Kallen and W. P. Jencks, *J. Biol. Chem.*, **241**, 5851 (1966).

(14) B. V. Ramasastri and R. L. Blakley, *J. Biol. Chem.*, **237**, 1982 (1962).

(15) B. V. Ramasastri and R. L. Blakley, *J. Biol. Chem.*, **239**, 106 (1964).

(16) The reported purity includes a correction for the enzymatically inactive (−)-L isomer.

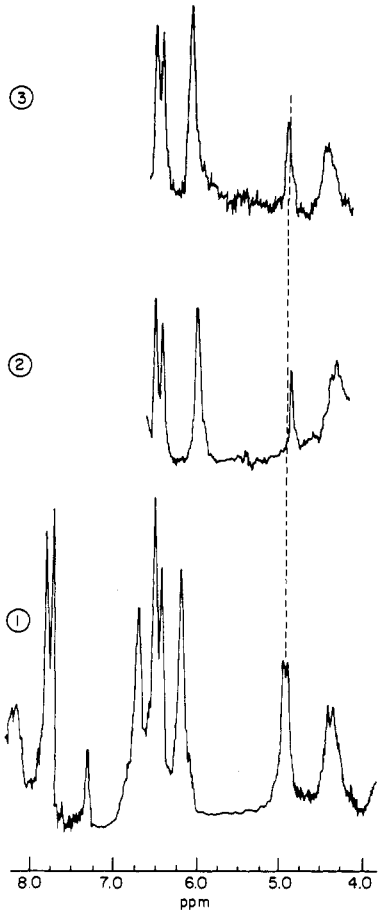


Figure 1. The nmr spectra of **1**, **2**, and **3** in DMSO-d_6:pyridine-d_5 (9/1, v/v). Diastereoisomer **3** contains *ca.* 30% of **2** owing to the presence of ^1H in 5,10-methenyltetrahydrofolate (deuterium substituted at formamidinium carbon).

NADP+. Ramasastri and Blakley previously had shown that hydrogen is transferred to the A side of NADP+ by the 5,10-methylenetetrahydrofolate dehydrogenase,[17] thus employment of glutamate dehydrogenase (specific for the B side)[18] should result in retention of deuterium. Conversion of NADP+ to nicotinamide was accomplished according to published procedures.[19,20] Fractions containing nicotinamide were concentrated, extracted with ether, and the latter evaporated to dryness. The residue was chromatographed on silica gel PF with either ethyl acetate or ethanol as solvents, and the uv absorbing band corresponding to nicotinamide was excised. Mass spectral analysis revealed 95 ± 5% D content, conclusively demonstrating that the hydrogen transferred originates from the bridging carbon.[21,22]

The rates of the dehydrogenase catalyzed oxidation of **1**, **2**, **3**, and **4** are displayed in Figure 2. The relative

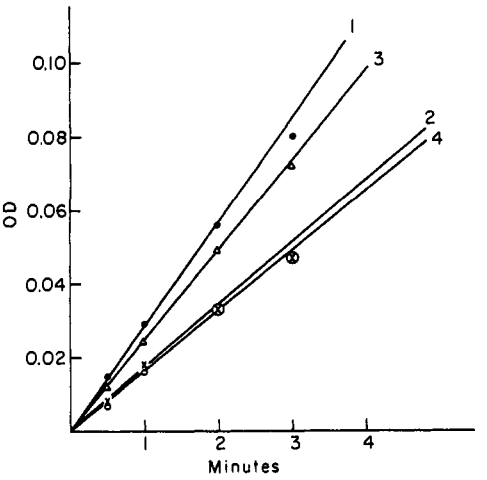


Figure 2. Enzymic utilization of diastereoisomers **1**, **2**, **3**, and **4** by 5,10-methylenetetrahydrofolate dehydrogenase. The assay system contained 0.05 *M* potassium phosphate buffer, pH 7.45, 0.2 m*M* NADP+, 1 m*M* dimercaptopropanol, 0.2 m*M* (±)-L-methylenetetrahydrofolate and enzyme. NADP+ and enzyme were incubated for 1 min, and the reaction was initiated by the addition of **1**, **2**, **3**, or **4** from a stock solution, pH 9.5 (0.05 *M* potassium phosphate), containing mercaptopropanol. The reaction was monitored by the increase in absorbancy at 340 nm with time at 25°. The final pH of the reaction was 7.55.

rate ratio for **1**:**4** is 1.7,[23] whereas **1**:**3** and **2**:**4** are 1.1 and 1.0. These data clearly suggest that the enzyme reacts stereospecifically with respect to the plane of the methylene bridge. Moreover, the fact that the pairs **1**:**3** and **2**:**4** assay identically supports the contention that the chemical reduction is highly stereoselective and that the hydrogen introduced chemically is that which is removed by the enzyme.

If **2** or **3** are preincubated in solution before addition of enzyme then the rate differences between the **2**:**3** pair decrease as expected for randomization of label brought about by equilibrium of **2** or **3** with formaldehyde and the parent cofactor. It should be noted that the stereochemistry of **2** and **3** remains to be related to the chiral center at C-6. In addition, the degree of chemical selectivity will require further examination. Nevertheless, the availability and stability[25] of **2** and **3** will permit investigation of the relative stereochemistry of one carbon unit transfer involving 5,10-methylenetetrahydrofolate.

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(17) B. V. Ramasastri and R. L. Blakley, *J. Biol. Chem.*, **239**, 112 (1964).
(18) T. Nakamoto and B. Vennesland, *J. Biol. Chem.*, **235**, 202 (1960).
(19) N. O. Kaplan, S. P. Colowick, and A. Nason, *J. Biol. Chem.*, **191**, 473 (1951).
(20) S. P. Colowick, N. O. Kaplan, and M. M. Ciotti, *J. Biol. Chem.*, **191**, 447 (1951).
(21) The possibility that the hydrogen at C-6 might be involved in the redox reaction (1) is suggested by its transfer in the thymidylate synthetase reaction.[22]
(22) For recent comments on the mechanisms of thymidylate synthetase see S. J. Benkovic and W. P. Bullard, *Progr. Bioorg. Chem.*, **2**, 133 (1973).
(23) The accuracy of the present data does not warrant comment on possible secondary isotope effects. The primary kinetic isotope effect is similar to that observed with yeast alcohol dehydrogenase, k_H/k_D = 1.8,[24] although its absolute value is certainly subject to further kinetic refinement.
(24) H. R. Mahler and J. Douglas, *J. Amer. Chem. Soc.*, **79**, 1159 (1957).
(25) Compounds **1**–**4** have been stored for 1 week at 0° *in vacuo* without any appreciable decomposition.
(26) National Institutes of Health Postdoctoral Fellow, 1972–1973.
(27) National Institutes of Health Career Development Awardee.

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