The enzyme shows clear preference for ^3H transfer from the *R* position[16] but, on the surface, would appear not totally stereospecific as evinced by $^1/_8$th as much ^3H transfer from the *S* position. This unlikely result may have precedence with the flavoenzyme orcinol hydroxylase.[17]

Repetition of these experiments with dRF confirms the same stereoselectivity (but not complete stereospecificity) for *R*-^3H transfer from C_4 of NAD^3H now into a stable nonexchangable locus (presumably C_5) in reduced and subsequently air reoxidized dRF. In one experiment, the reisolated [^3H]dRF had a sixfold higher specific radioactivity from (*R*)-[^3H]NADH than from (*S*)-[^3H]NADH. In the experiment shown in Table I a fourfold differential occurred. The control incubation without enzyme demonstrates that both dRF reduction and tritium transfer are enzyme catalyzed. The [^3H]-dRF from the enzymatic incubations was purified with removal of radioactive pyridine nucleotides by batch treatment with DEAE-cellulose. The uv spectrum of the supernatant indicated presence of partially reduced dRF.

Table I. Enzymatic Tritium Transfer to Riboflavine and Deazariboflavine

Experiment	4-[^3H]-NADH[a]	Flavine	Enzyme	Product formation[b] (nm)	^3H_2O[c] (μCi/mol)	[^3H]dRF[d] (nCi/nmol)
1	4-*R*	RF	+	175	8	
2	4-*S*	RF	+	175	1	
3	4-*R*	dRF	+	31		1.40
4	4-*S*	dRF	+	56		0.34
5	4-*R*	dRF	−	0		0.05

[a] Incubations were as described for nonradioactive experiments.[14] The specific radioactivity of commercial 4-[^3H]NAD was 10 nCi/nmol. This was reduced enzymatically to the two chiral 4-[^3H]-NADH species. [b] With RF, product formation was measured by NADH oxidation at 340 nm; with dRF direct reduction of the deazaisoalloxazine chromophore was followed at 396 nm. [c] Measured as ^3H rendered volatile during enzymatic incubation and lyophilization. Values are corrected for a small nonenzymatic blank. Without any kinetic isotope selection,[16] a specific activity of 31.5 μCi/mol of ^3H_2O would be expected. [d] These values represent specific activity of isolated [^3H]dRF corrected for the different amounts of dRF reduced in experiments 3 and 4 but not for ^3H lost in nonenzymatic reoxidation of reduced [^3H]dRF included as part of the isolation.

The [^3H]dRF after air oxidation was passed through a 1 × 85 cm column of Biogel P_2 in 0.1 *M* NH_4HCO_3 and the fluorescent deazaflavine band was collected, lyophilized, redissolved in H_2O, and analyzed on silica gel tlc (as a single fluorescent spot). As a final identification, the [^3H]dRF samples were phosphorylated with partially purified riboflavine kinase[18] to form [^3H]dFMN cleanly separable from unreacted [^3H]dRF on silica gel tlc.[19]

These results demonstrate coenzymatic function for 5-deazariboflavine in an enzymatic oxidation, proving stereoselective, direct hydrogen transfer from NADH and establishing the biological relevance of the model system of Bruice and coworkers.[3] Observed direct hydrogen transfer is consistent with a hydride ion transfer in this enzymatic oxidation and further indicates the N_5 is not a unique electronic determinant for coenzymatic function, supporting the calculations of Song.[8] Given the greater resistance of reduced deazaflavines to air oxidation,[9] it may prove possible to use the reduced forms of the deazaflavines, chiral at carbon 5,[20] to probe stereochemistry of flavoenzyme reactions in a way not possible with the flavine coenzymes themselves.

Simultaneously with this work, Hersh, *et al.*, have found that dFMN functions coenzymatically with amino acid substrates for a bacterial *N*-methyl glutamate synthetase.[21] This parallels our finding that *M. smegmatis* apolactic oxidase reconstituted with dFMN undergoes enzyme-catalyzed reduction by L-lactate.[22]

Acknowledgments. We wish to gratefully acknowledge the valuable assistance of Drs. J. Becvar and J. W. Hastings for provision of crude extracts of the reductase and assistance in the enzyme purification. This research was supported by National Institutes of Health Grant No. 20011.

(16) We have not yet quantitated the kinetic isotope effects, implicit in Table I, by careful rate measurements.

(17) D. W. Ribbons, Y. Ohta, and I. J. Higgins in "The Molecular Basis of Electron Transport," Vol. IV, J. Schultz and B. F. Cameron, Ed., Academic Press, New York, N. Y., 1971, p 544. Alternatively and possibly more likely, the chiral purity of the 4-(*R*)- and 4-(*S*)-^{3}[H]-NADH samples may not have been absolute although they were generated by standard enzymatic means, or nonenzymatic deproportionation between oxidized and reduced nicotinamide coenzymes may do the same. This is under investigation.

(18) D. B. McCormick in "Methods in Enzymology," Vol. 18 B, D. B. McCormick and L. D. Wright, Ed., Academic Press, New York, N. Y., 1971, p 544.

(19) Brickman plastic-backed plates, H_2O as solvent, [^3H]dFMN had a mobility of 0.6, [^3H]dRF a mobility of 0.2.

(20) We have not yet determined that the enzymatic reduction proceeds with complete chiral transfer of ^3H to C_5.

(21) M. Jorns and L. B. Hersh, *J. Amer. Chem. Soc.*, **96**, 4012 (1974).

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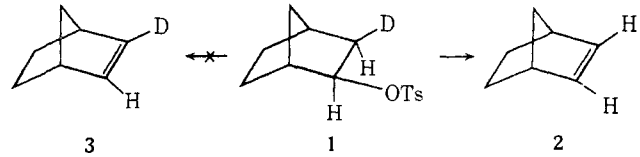
Stereochemistry in β Eliminations from *exo*-2-Norbornyl Tosylate. The Effect of Base Association

Sir:

In 1970, Brown and Liu[1] reported that eliminations from *exo*-2-norbornyl-*exo*-3-*d* tosylate, **1**, induced by the sodium salt of 2-cyclohexylcyclohexanol in triglyme produced norbornene, **2**, but no 2-deuterionorbornene, **3**. The observed exclusive exo–syn elimination stereochemistry was consistent with previous investigations of substituted norbornane reactions in which favoring of syn–exo elimination over anti–endo-H elimination by a factor of 100 or greater has been noted.[2]



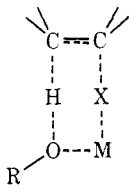
3 1 2

(1) H. C. Brown and K-J. Liu, *J. Amer. Chem. Soc.*, **92**, 200 (1970).

(2) For a review see N. A. LeBel, "Advances in Alicyclic Chemistry," Vol. 3, H. Hart and G. T. Karabatsos, Ed., Academic Press, New York, N. Y., 1971, pp 196–290.

As in earlier studies,[3] Brown and Liu chose a solvent of low polarity to suppress competing solvolysis. However, marked effects of base association upon elimination orientation and stereochemistry in such solvents have recently been recognized.[4-6] Associated alkali metal–alkoxide ion bases exhibit large steric requirements[5] and stabilize syn elimination transition states by simultaneous coordination of the metal cation with the base and a neutral leaving group,[4,6] as depicted in **4**. Both factors should favor exo–syn elimination from **1**.

In order to assess the effect of base association upon the stereochemistry of eliminations from *exo*-2-norbornyl tosylate, reactions of **1** and its undeuterated analog **5** with the sodium salt of 2-cyclohexylcyclohexanol in triglyme in the presence of a sodium ion complexing agent[7] 18-crown-6,[8] **6**, have been investigated.



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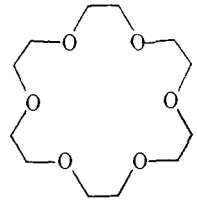

6

A comparison of our results with those of Brown and Liu is presented in Table I. The appreciable amount of **3** formed from **1** in the presence of **6** reveals the importance of base association in producing the exclusive syn–exo elimination stereochemistry observed earlier.

Table I. Products from Reactions[a] of *exo*-2-Norbornyl Tosylate with the Sodium Salt of 2-Cyclohexylcyclohexanol[b] in Triglyme at 80°

Compound	18-Crown-6 present	% of total hydrocarbons[c] 2	3	Nortricyclene
5[d]	No	99.5		0.5
1[d]	No	98.0	0	2.0
5	Yes[e]	99.5		0.5
1	Yes[e]	70.0	27.2	2.8

[a] [ROTs] = 0.1 *M*, [NaOR'] = 1.0 *M*. [b] A mixture of 70% *trans*- and 30% *cis*-2-cyclohexylcyclohexanol was used in the present investigation. [c] 60–65% yields of hydrocarbons were realized. [d] Reference 1. [e] 1.0 *M* **6** present.

Increase in the nortricyclene percentage from the deuterated compound presumably arises by decreased rate of **2** formation due to a primary deuterium isotope effect. On this basis, the relative product proportions indicate an isotope effect of 5–7 for syn–exo elimination from **1** promoted by the dissociated base.[9] If the relative proportions of **2** and **3** formed by reactions of the dissociated base with **1** are adjusted for an assumed isotope effect of **6** in the formation of **2**, a relative propensity for syn–exo and anti–endo-H eliminations of approximately 15:1 may be calculated. This ratio contrasts with >100:1 for the associated base. These results clearly demonstrate the heretofore unrecognized importance of base association upon the stereochemistry of base-promoted β eliminations from norbornyl derivatives.

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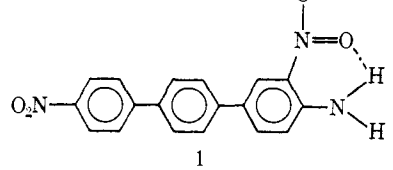
p-Phenylene Di-*p*-amino- and Di-*p*-hydroxybenzoate. Novel Mesomorphism of an Amine and a Phenol

Sir:

The molecular structural criteria for mesomorphism (liquid crystallinity)[1-3] are rigidity, rod shape, and polarity. Sometimes compounds that satisfy these criteria do not exhibit mesomorphism, phenols and amines being prime examples. In 1962, Gray[4] proposed that phenols had never been observed to be mesomorphic because intermolecular hydrogen bonding raises the melting point above the mesophase–isotropic liquid transition temperature and may also encourage the adoption of a nonlinear molecular arrangement. He further pointed out that, for the same reasons, primary or secondary amines are unlikely to be mesomorphic unless they are capable of intramolecular H bonding. To our knowledge, the only liquid crystalline primary amine (**1**)[5] and phenol (**2**)[6,7] presently described in the literature have this capability. Accordingly, we were surprised to find that *p*-phenylene di-*p*-aminobenzoate (**3**, Z = NH_2) and *p*-phenylene di-*p*-hydroxybenzoate (**3**, Z = OH) are mesomorphic. Their molecular



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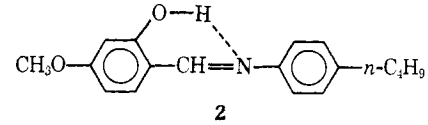

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