vibronically excited states which provide a mechanism for time-averaging the ligand environments and that this is the origin of fluorine atom equivalence, on the n.m.r. time scale, for IF_7 and ReF_7. A case for fast, intramolecular ligand exchange was made earlier for five-coordinate structures[7,8] and will be more thoroughly documented in another paper.[9]

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(8) E. L. Muetterties, W. Mahler, and R. Schmutzler, *Inorg. Chem.*, **2**, 613 (1963).

(9) E. L. Muetterties, K. J. Packer, W. Mahler, and R. Schmutzler, to be published.

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Biological Interconversions in the Amaryllidaceae. I. The Haemanthamine–Haemanthidine–Tazettine Sequence[1]

Sir:

Sprekelia formosissima (L.) contains three principal alkaloids[2]: haemanthamine (I),[3] haemanthidine (II),[4] and tazettine (III),[5] whose structures suggest the existence of the biological sequence I → II → III. An early indication of this was based on our observation that highest yields of I are obtained early in the flowering season while III predominates in later collections. Although the yield of II varies, its relationship to tazettine was considered on the basis of its facile conversion to the latter by the action of methyl iodide and dilute base[6] which must involve a hydride transfer.[4]

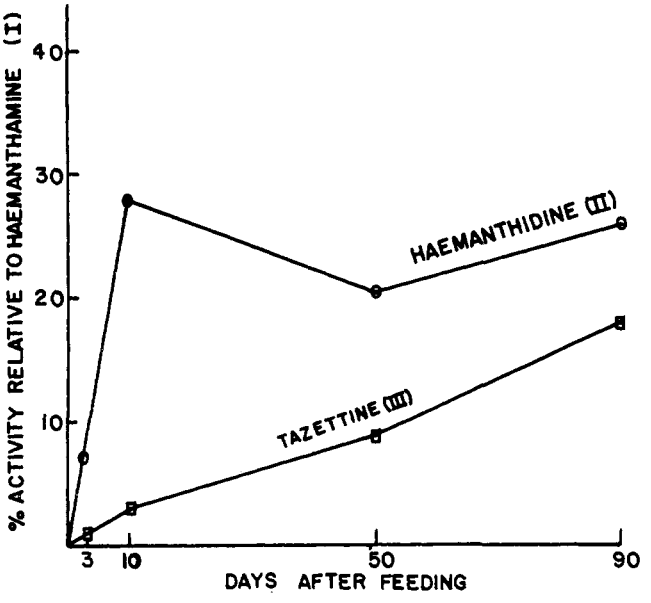
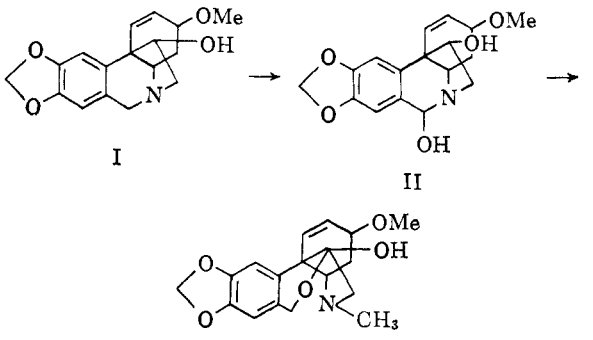


Figure 1.

We have shown now that the above sequence does in fact occur *in vivo*. Tritiated haemanthamine (I)[7] was administered to flowering *Sprekelia formosissima* by injection into the flower stems at pH 5.3–5.8. Samples were collected over 90 days, extracted, and the alkaloids were purified by thin-layer chromatography[8] and crystallization, affording the specific activities of II and III relative to I as a function of time indicated in Fig. 1. The rates of incorporation clearly support the sequence postulated, I → II → III.



In a confirmatory experiment radioactive I, II, and III were readministered to *S. formosissima*. The results (Table I) indicate that the conversion of I to II as well as II to III are essentially irreversible, although a small amount of III may have been recycled to I.[9]

TABLE I

Alkaloid fed (d.p.m./mmole)	—% dilution and conversion[a]— I	II	III	Activity of N-methylhydrastimide relative to alkaloid
I (16.7×10^6)	28.0	7.24	5.16	1.05
II (120×10^6)	0.00	18.0	6.69	0.97
III (352×10^6)	(1.14)[b]	0.077	13.1	0.98

[a] (D.p.m./mmole alkaloid recovered ÷ d.p.m./mmole alkaloid fed) × 100. [b] See Ref. 9.

Each of the three isolated alkaloids was oxidized with potassium permanganate, and the hydrastic acid was converted to its N-methylimide with methylamine. The activity of the imide relative to the corresponding alkaloid is given in the last column of Table I, and illustrates that all of the tritium resides in the aromatic A ring and has not been scrambled during the experiment.

Biological hydroxylation α to an amino nitrogen is well known[10] and I → II appears straightforward. Whether II → III proceeds directly through reaction of II with the usual methyl donor, (+)-S-adenosyl-L-methionine,[11,12] or occurs *via* the methylation of nortazettine (III, no N-methyl)[4] remains unanswered. However, these experiments do show that the precursor of tazettine is haemanthidine rather than small fragments as originally proposed.[13]

(1) Presented in part at the 141st National Meeting of the American Chemical Society, Washington, D. C., March, 1962. The most recent paper on this subject is D. A. Archer, S. W. Breuer, R. Binks, A. R. Battersby, and W. C. Wildman, *Proc. Chem. Soc.*, 168 (1963).

(2) H.-G. Boit and H. Ehmke, *Chem. Ber.*, **88**, 1590 (1955).

(3) H. M. Fales and W. C. Wildman, *J. Am. Chem. Soc.*, **82**, 197 (1960).

(4) S. Uyeo, H. M. Fales, R. J. Highet, and W. C. Wildman, *ibid.*, **80**, 2590 (1958).

(5) T. Ikeda, W. I. Taylor, Y. Tsuda, S. Uyeo, and H. Yajima, *J. Chem. Soc.*, 80, 4749 (1956).

(6) W. C. Wildman, *Chem. Ind.* (London), 1090 (1956).

(7) Tritiation was conducted by combining 50 mg. of I with 0.2 ml. of tritiated acetic acid and 35 mg. of palladium-on-charcoal and heating the mixture at 80° overnight (New England Nuclear Corp., Boston, Mass.). Purification and dilution gave material of 16.7×10^6 d.p.m./mmole.

(8) Chloroform–ethyl–acetate–methanol (20:20:10) separated I and II (R_f 0.29) from III (R_f 0.57) on silica gel "G" while chloroform–methanol–diethylamine (92:3:2) separated I (R_f 0.33) from II (R_f 0.13).

(9) The sample of III fed was later analyzed and found to contain 0.88% of its radioactivity in the form of I.

(10) C. Mackenzie, "Amino Acid Metabolism," Johns Hopkins University Press, Baltimore, Md., 1955, p. 684.

(11) For leading references see S. H. Mudd and J. D. Mann, *J. Biol. Chem.*, **238**, 2164 (1963).

(12) O-Methylation requiring (+)-S-adenosyl-L-methionine has been observed in the Amaryllidaceae [H. Fales, J. D. Mann, and S. H. Mudd, *J. Am. Chem. Soc.*, **85**, 2025 (1963)].

(13) D. H. R. Barton and T. Cohen, "Festschrift A. Stoll," Birkhauser, Basle, 1957, p. 127.

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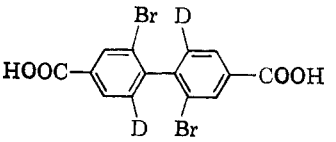
Steric Isotope Effects. The Isotope Effect on the Racemization of 2,2′-Dibromo-4,4′-dicarboxybiphenyl

Sir:

Experiments with sterically hindered biphenyl derivatives specifically designed to test the theory of steric isotope effects proposed by Bartell[1] have recently been reported by Mislow and his co-workers.[2,3] However, a quantitative comparison between the experimental observations and theoretical predictions has not been attempted, due to a lack of information about the detailed conformation of the transition state

We have now prepared a hindered biphenyl system for the appraisal of the importance of steric factors in secondary deuterium isotope effects which allows a comparison between theory and experiment, *viz.* optically active 2,2′-dibromo-4,4′-dicarboxybiphenyl specifically deuterated in the 6 and 6′ positions. The deuterium isotope effect on the racemization of this



compound should clearly reflect any effective size difference between protium and deuterium. Since it is known that in 2,2′-dihalobiphenyls the conformation with the two halogen atoms in van der Waals contact is energetically favored,[4] there is little reason to believe that steric crowding involving the isotope has any appreciable effect on the ground state. Accordingly, it is plausible to ascribe the entire steric isotope effect to crowding in the transition state.

Theoretical evaluations of the activation energy for the racemization of the protium compound by Westheimer[5] (18 kcal./mole) and by Howlett[6] (21.9 kcal./mole) are in good agreement with the experimental value[7] (19.0 kcal./mole). These calculations also provide a detailed picture of the equilibrium conformation of the transition state, which is assumed to be planar, and thus afford the input data necessary for the computation of the isotope effect according to Bartell.[1]

We have prepared the specifically deuterated compound in $\geq 97\%$ isotopic purity (according to n.m.r. analysis). The introduction of deuterium was accomplished *via* reduction of tetrazotized 2,2′-dibromo-4,4′-dicarbomethoxy-6,6′-diaminobiphenyl with D_3PO_2 prepared by repeated exchange of H_3PO_2 with 99.8% D_2O. The complete synthetic procedure is reserved for a forthcoming detailed paper.

The kinetics were followed on a Perkin-Elmer 141 automatic reading polarimeter, using the mercury lines at 436 and 546 mμ and the sodium D line. Due to the optical lability of these biphenyls, the measurements had to be made below 0°, and the results here reported were obtained at $-19.8 \pm 0.1°$ in ethanol solution. Difficulties due to the formation of frost were overcome by blowing streams of nitrogen through specially constructed brass mouthpieces onto the polarimeter cell windows. A comparison plot of representative kinetic runs (no. 9 and 14 in Table I) for the protium and deuterium compounds is shown in Fig. 1. Specific rates were obtained graphically from such plots. The results, together with the maximum deviation of four separate determinations for each compound, are given in Table I. The isotope effect ratio (k_D/k_H) calculated from these data is 1.19.

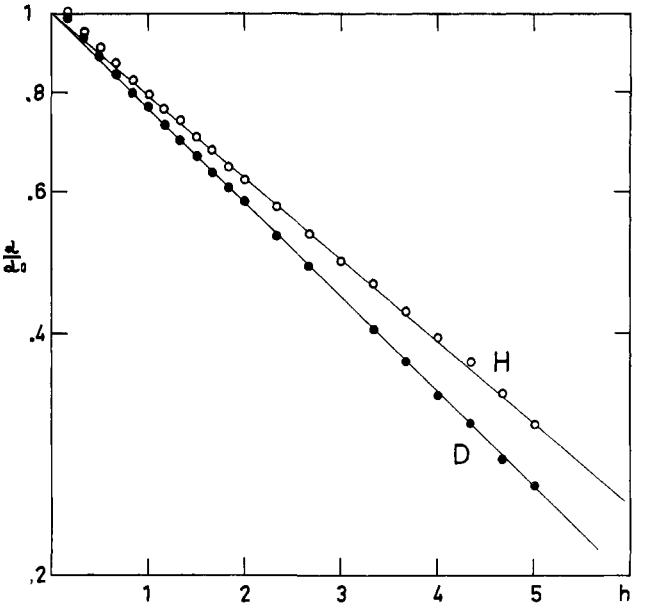


Fig. 1.—Semilogarithmic plot of relative optical rotation *vs.* time for runs 9 (H) and 14 (D) (see Table I).

TABLE I

RACEMIZATION RATES OF 2,2′-DIBROMO-4,4′-DICARBOXYBIPHENYL AND ITS 6,6′-DIDEUTERIO DERIVATIVE AT $-19.8 \pm 0.1°$ IN ETHANOL

Run	Isotope	Concn., mg./ml. EtOH	Rate constant, $10^5 k$ sec.$^{-1}$	Av. and max. dev., sec.$^{-1}$
4	H	27.2	6.31	
7	H	19.4	6.74	
9	H	21.4	6.53	
11	H	19.2	6.35	
				$6.48 \pm 0.26 \times 10^{-5}$
14	D	21.4	7.62	
15	D	20.0	7.58	
16	D	21.9	8.02	
17	D	21.7	7.60	
				$7.71 \pm 0.31 \times 10^{-5}$

In the absence of a study of the temperature dependence of the isotope effect, it is impossible to separate the observed effect into entropy and enthalpy components. However, if it is assumed that the observed effect is entirely due to a difference in enthalpy, $\Delta H_H^* - \Delta H_D^*$ would be equal to about 90 cal./mole. The data of Westheimer[5] combined with the computational procedure of Bartell[1] lead to a value of 506 cal./mole for this difference, whereas the data of Howlett[6] lead to a value of 100 cal./mole. The discrepancy arises mainly from the widely different nonbonded H···Br repulsion potentials used by these authors. Finally, it should be pointed out that the magnitude

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