p-hydroxybenzyl alcohol with N-acetylsuccinimide indicates that the latter possesses a selectivity comparable to Ac-TMH; however, N-acetylsuccinimide is a low-temperature melting solid not easily purifiable.[7] On the other hand N-acetylphthalimide[8] displays a very low reactivity; with 2-naphthol (0.001 mol) in benzonitrile (1 ml) at 80° for 8 hr, it reaches only 10% reaction, while in these conditions the consumption of Ac-TMH is complete.

Probably, the subtle selectivity of Ac-TMH is significantly dependent upon the operation of steric factors. Competition and kinetic measurements directed to study the mechanism of these reactions are in progress.

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[7] All the used samples of N-acetylsuccinimide, prepared as described by J. Tafel and M. Stern, *Ber.*, **33**, 2224 (1900), contained 10–15% succinimide according to nmr spectrometry.

[8] This reagent and closely related compounds have already been used for the acylation of several types of substances: N. Rabjohn, M. Drumm, and R. Elliot, *J. Am. Chem. Soc.*, **78**, 1631 (1956); J. Bosnjak, R. I. Mamuzic, and M. L. Mihailovic, *Glasnik. Khem. Drustva Beograd*, **27**, 313 (1962) (*Chem. Abstr.*, **60**, 5391*a* (1964)), and references cited.

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Stereochemistry of Addition of Deuterium Bromide to *cis*- and *trans*-2-Butene and the Control of Interfering Olefin Isomerization[1]

Sir:

The mechanistic details of the electrophilic addition of hydrogen halides to simple olefins has not received a great deal of attention despite the synthetic importance of these reactions.[2] The stereochemistry of these additions to nonconjugated olefins has been studied only with a relatively few olefins, these being cyclic olefins. The addition of hydrogen bromide to 1,2-dimethylcyclohexene[3] and cyclohexene-1,3,3-d_3,[4] deuterium bromide to cyclohexene[5] and cyclohexene-3,3,6,6-d_4,[6] and hydrogen chloride to 1,2-dimethylcyclopentene[7] all occur predominantly *trans*. The addition of hydrogen chloride in acetic acid to cyclohexene-1,3,3-d_3 is more complicated, giving a mixture of *cis* and *trans* addition products.[8] Similar additions to norbornene occur predominantly *cis-exo* and are accompanied by rearrangements.[9]

We have investigated some of the aspects of the addition of deuterium bromide in acetic acid-O-*d* to *cis*- and *trans*-2-butene. Employing the general procedure used by previous investigators (~1 *M* DBr in DOAc with inhibitor at room temperature and subject to normal laboratory atmosphere and light), *cis*- and *trans*-2-butene produce identical mixtures of 60% *threo*- and 40% *erythro*-3-deuterio-2-bromobutane.[10,11]

Treatment of *cis*- and *trans*-2-butene with a deficient amount of hydrogen bromide in acetic acid followed by immediate recovery of the excess 2-butene revealed that olefin isomerization had occurred ($cis \rightleftarrows trans\ K_{eq}$ at 25° is 2.80). Qualitative rate measurements demonstrated that the rate of olefin isomerization is much faster than the rate of addition. The olefin isomerization is not acid catalyzed, as indicated by the incorporation of only a single deuterium atom in the 3 position[12] of the bromide and the observation that similar concentrations of hydrogen chloride and *p*-toluenesulfonic acid in acetic acid do not lead to any substantial amounts of isomerization over a period of 14 days at 42°.

Various experiments indicate that the olefin isomerization reaction requires the presence of hydrogen bromide and oxygen or light.[13] These observations suggest a bromine atom catalyzed isomerization, the bromine atoms being produced in either of the following reactions.[15]

$$O_2 + HBr \rightleftarrows HOO\cdot + Br\cdot$$

$$HBr \overset{h\nu}{\rightleftarrows} H\cdot + Br\cdot$$

In our hands the presence of relatively large quantities of typical radical inhibitors used by previous investigators (hydroquinone, alkylphenols, etc.) has *no* effect on the olefin isomerization reaction.[16] Interestingly, the reaction of a mixture of 1-butene and an excess of *cis*-3-hexene with hydrogen bromide in acetic acid leads to isomerization of the *cis*-3-hexene ($cis \rightleftarrows trans\ K_{eq}$ at 25° of 10.0) but produces *none* of the radical addition product 1-bromobutane. Only when oxygen is bubbled through

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[1] Research partially supported by the National Science Foundation, Grant GP-4497, and the Alfred P. Sloan Foundation.

[2] For a recent review of electrophilic addition reactions see: R. C. Fahey in "Topics in Stereochemistry," Vol. 3, E. L. Eliel and N. L. Allinger, Ed., Interscience Publishers, New York, N. Y., 1968, pp 237–342.

[3] G. S. Hammond and T. D. Nevitt, *J. Amer. Chem. Soc.*, 76, 4121 (1954).

[4] R. C. Fahey and R. A. Smith, *ibid.*, **86**, 5035 (1964).

[5] I. V. Smirnov-Zamkov and G. A. Piskovitina, *Ukr. Khim. Zh.*, **28**, 531 (1962).

[6] See p 248, ref 2.

[7] G. S. Hammond and C. H. Collins, *J. Amer. Chem. Soc.*, **82**, 4323 (1960).

[8] R. C. Fahey and M. Monahan, *Chem. Commun.*, 936 (1967); see also p 248 of ref 2.

[9] J. K. Stille, F. M. Sonnenberg, and T. H. Kinstle, *J. Amer. Chem. Soc.*, **88**, 4922 (1966); H. Kwart and J. L. Nyce, *ibid.*, 86, 2601 (1964); H. C. Brown and K. T. Liu, *ibid.*, **89**, 3900 (1967).

[10] The *threo*- and *erythro*-3-deuterio-2-bromobutanes were previously prepared (P. S. Skell and R. G. Allen, *ibid.*, **81**, 5383 (1959)) by the radical addition of DBr to *cis*- and *trans*-2-butene at −70°.

[11] The bromide mixtures derived from *cis*- and *trans*-2-butene contained 0.97 and 0.99 deuterium atom, respectively. The hydrogen magnetic resonance spectra of the two samples were identical in all respects and unambiguously demonstrated that all of the deuterium was at the 3 position. The stereochemical composition of the samples was determined by infrared analysis of the corresponding benzoates, prepared by conversion of the bromides to the acetates with silver acetate in acetic acid, reduction by lithium aluminum hydride to the alcohol, and esterification with benzoyl chloride, with known mixtures of authentic samples of *erythro*- and *threo*-3-deuterio-2-butyl benzoates (D. J. Pasto, C. C. Cumbo, and J. Hickman, *ibid.*, **88**, 2201 (1966)).

[12] The acid-catalyzed isomerization of the 2-butenes in deuterium bromide–acetic acid-O-*d* would require the formation of the 3-deuterio-2-butyl cation, which on regeneration of olefin would be expected to loose preferentially a proton to produce 2-deuterio-2-butene. Thus the product bromide would contain more than one atom of deuterium.

[13] Kharasch and coworkers[14] have reported that *cis*-stilbene does not isomerize in the presence of hydrogen bromide in air, but that isomerization does occur in the presence of peroxidic substances. Light was also implicated as catalyzing the isomerization in the presence of hydrogen bromide. The isomerization is completely stopped in the presence of hydroquinone or ethyl mercaptan.

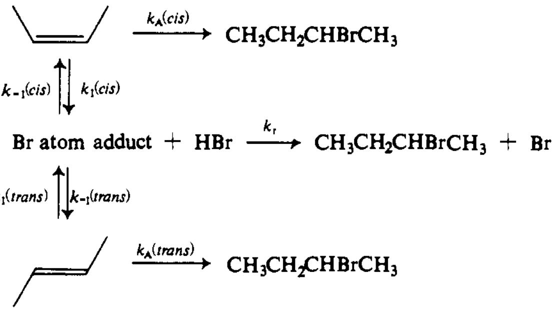
[14] M. S. Kharasch, J. V. Mansfield, and F. R. Mayo, *J. Amer. Chem. Soc.*, **59**, 1155 (1937).

[15] A similar suggestion has been made by Y. Urushibara and O. Simamura (*Bull. Chem. Soc. Japan*, **14**, 323 (1939)) to account for the isomerization of various olefins with hydrogen bromide in the presence of peroxidic substances and oxygen.

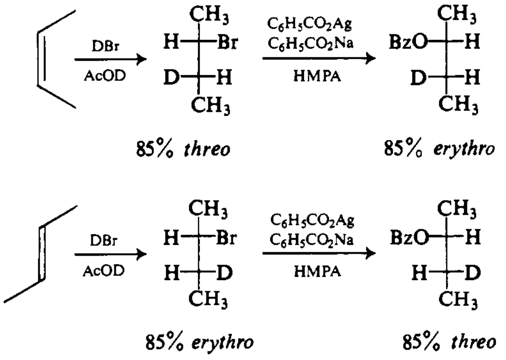
[16] See footnote 13.

the reaction mixture, or substantial quantities of benzoyl peroxide are added, is any substantial amount of 1-bromobutane formed.

All of the foregoing observations are readily rationalized by Scheme I in which the rates of bromine atom isomerization are substantially greater than the rate of addition to *cis*-2-butene which in turn is greater than the rate of addition to *trans*-2-butene. Although the specific rate constants for the chain propagation steps for the radical chain addition of hydrogen bromide to the *cis*- and *trans*-2-butenes would appear to be much larger than the specific rate constants for the ionic addition reactions, the low concentration of radical intermediates, except in the presence of large quantities of oxygen or benzoyl peroxide, preclude the formation of substantial amounts of product *via* a radical chain mechanism. The interference of these radical-induced reactions would not be apparent in the additions to the unconjugated cyclic olefins because of the inability to observe olefin isomerization.[17]



The addition of *cis*- or *trans*-2-butene to a freeze-degassed acetic acid-O-*d* solution of deuterium bromide[18] maintained under a helium atmosphere in the dark produces a highly stereoselective *trans* addition (85 ± 1%) of deuterium bromide[19] as determined by the direct conversion of the bromides to the benzoates with silver benzoate–sodium benzoate in hexamethylphosphoramide.



The kinetics of the addition under various conditions is under study in an attempt to determine the mechanism of formation of the *cis* and *trans* addition products (Ad_E2 *vs.* Ad_E3 transition states).

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[17] A similar mechanistic scheme has been proposed by C. Walling and W. Helmreich (*J. Amer. Chem. Soc.*, **81**, 1144 (1959)) to account for the thiyl radical catalyzed isomerization of *cis*- and *trans*-2-butene.

[18] The acetic acid-O-*d* solution of deuterium bromide is prepared by the addition of deuterium oxide to phosphorus tribromide and bubbling the deuterium bromide into acetic acid-O-*d* prepared by the addition of deuterium oxide to distilled acetic anhydride, in a stream of nitrogen.

[19] The hydrogen nmr spectra of the *erythro*- and *threo*-3-deuterio-2-butanols are distinctly different: *erythro*, δ_{CHBr} 4.028, $J_{CHDCHBr}$ = 7.88 Hz, $J_{CHDCHBr}$ = 0.8 Hz; *threo*, δ_{CHBr} 4.075, $J_{CHDCHBr}$ = 5.16 Hz, $J_{CHDCHBr}$ = 1.2 Hz. The nmr spectra indicate stereochemical purities of >80%.

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A Urea-Specific Enzyme Electrode

Sir:

A prime objection to the use of enzymes as analytical reagents lies in the high cost of using large quantities of these materials. The immobilization or insolubilization of the enzyme eliminates this problem, because the enzyme could be used over and over again. The immobilization of several enzymes by various techniques has been recently reported. A review on the preparation of insoluble enzymes has been prepared by Chibata and Tosa.[1] Guilbault[2] has presented a more recent review of immobilized enzymes.

Very little work has been reported on immobilization of urease. In 1925, Sumner and Graham[3,4] obtained an enzymatically active water-insoluble urease preparation on adding small amounts of sodium chloride to neutral 30% alcohol urease. The water-insoluble product, which probably consists of intermolecularly disulfide cross-linked urease molecules, was only partially characterized. Its possible use as a heterogeneous catalyst was not investigated. Recently Riesel and Katchalski[5] reported the preparation and properties of water-insoluble derivatives of urease prepared by chemically binding urease with the diazotized copolymer of *p*-amino-DL-phenylalanine and L-leucine. The stability of the various urease preparations on prolonged storage at 4° varied from loss of 80% of the initial activity within a day to 35% of its initial activity within 5 months.

Bernfeld and Wan[6] and Hicks and Updike[7,8] have demonstrated the immobilization of enzyme activity in polyacrylamide gel. Our investigations have shown that urease immobilized in this gel possesses high activity. We wish now to report the preliminary results of our studies.

A urea transducer, suitable for rapid, continuous determination of urea in body fluids, has been developed. The urea transducer is called a urease electrode because

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(1) I. Chibata and T. Tosa, *Tampakushitu Kakusan Koso*, **11**, 23 (1966).

(2) G. G. Guilbault, *Anal. Chem.*, **40**, 459R (1968).

(3) J. B. Sumner and V. A. Graham, *Proc. Soc. Exptl. Biol. Med.*, **22**, 504 (1925).

(4) J. B. Sumner, *Science*, **108**, 410 (1948).

(5) E. Riesel and E. Katchalski, *J. Biol. Chem.*, **239**, 1521 (1964).

(6) P. Bernfeld and J. Wan, *Science*, **142**, 678 (1963).

(7) G. P. Hicks and S. J. Updike, *Anal. Chem.*, **38**, 726 (1966).

(8) S. J. Updike and G. P. Hicks, *Nature*, **214**, 986 (1967).