*Table 1.* Oxygen uptake by mitochondria of red and white skeletal muscle given as μmoles per 10 mg of mitochondrial protein per hour. Rm = red muscle mitochondria. Wm = white muscle mitochondria. The results are given as mean values ± standard errors and have been compared by means of "students" t-test. Number of experiments within brackets.

| Substrate | Rm | Wm | P |
|---|---|---|---|
| 17 mM Pyruvate + 1 mM Malate | 29.2±3.2 (13) | 22.2±3.1 (13) | 0.2−0.1 |
| 17 mM DL-α-Glycero-phosphate | 12.4±1.5 (12) | 21.4±3.2 (12) | 0.02 |
| 17 mM β-Hydroxy-butyrate | 14.1±2.5 (5) | 0.0 (5) | <0.001 |

chondria. For the red-muscle mitochondria the oxygen consumption with β-hydroxybutyrate as a substrate is about half of that with pyruvate + malate, while in the white-muscle mitochondria β-hydroxybutyrate is not oxidized at all.

These findings may indicate that, while the α-glycerophosphate cycle is mainly operative in white skeletal muscle the β-hydroxybutyrate cycle is operative in rat skeletal muscle. Wirsen's finding,[12] that lipid deposition in muscle cells is correlated to the myoglobin content, is in favour of this hypothesis; red fibers store significantly more lipid than do intermediate and white fibers and this difference reflects a selective uptake of fatty acids from plasma. However, Lehninger *et al.*[13] have shown that the β-hydroxybutyrate dehydrogenase of the intact liver mitochondria does not react with acetoacetate and $NADH_2$, and no evidence for a soluble dehydrogenase was obtained. Devlin agrees that there is no soluble β-hydroxybutyrate dehydrogenase which functions in this cycle, but his recent work [14] suggests the presence of a β-hydroxybutyrate dehydrogenase on the surface of the mitochondria, which appears to have properties different from the well-known β-hydroxybutyrate dehydrogenase. It is quite apparent that the β-hydroxybutyrate-acetoacetate cycle requires further study before it can be established that it is at work in red skeletal muscle.


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# Influence of *cis-trans* Isomerism on the Kinetics of Vinyl Ether Hydrolysis

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The kinetics and mechanisms of protolytic cleavage of vinyl ethers were discussed in a recent paper.[1] The open-chain vinyl ethers studied were compounds which have no isomeric *cis* and *trans* forms, and therefore no conclusions could be drawn about the possible structural effect of this isomerism on the kinetics of the reactions. The present paper describes the results of experiments which were conducted in

order to obtain detailed information about this point. Jones and Wood [2] studied earlier the hydrolysis of three *cis-trans* isomeric vinyl ethers in a dioxane-water (80:20) mixture, but they did not isolate the isomers from their mixtures.

A mixture of the *cis* and *trans* forms of α-propenyl methyl ether, MeCH=CHOMe, was obtained by elimination of hydrogen chloride from α-chloropropyl methyl ether. The freshly distilled chloroether was added slowly dropwise to a boiling pyridine solution, and the fraction which distilled at temperatures below 70°C was collected for further purification. On gas-chromatographic analysis, the initial reaction product (not equilibrated) was found to contain approximately equal amounts of the both isomers. The separation of the isomers was effected by distilling the crude product four times in a Todd precision fractionation assembly. The purities and identities of the isomers were independently ascertained by gas chromatography and IR spectroscopy. The following physical constants were recorded for the isomers: *cis* form, b.p. 43.5–44.0°C/764.2 torr, $d_4^{20}$ 0.7758, $n_D^{20}$ 1.4012; *trans* form, b.p. 48.0–48.5°C/771.2 torr, $d_4^{20}$ 0.7805, $n_D^{20}$ 1.3942. Both isomers have been previously separated by Howard, Jacobsen and Newton,[3] whose *cis* and *trans* assignations are the same.

The isomers of α-butenyl methyl ether, EtCH=CHOMe, were prepared similarly starting from α-chlorobutyl methyl ether, and they were separated by repeated fractional distillation. *cis* Form, b.p. 71.0°C/756.2 torr, $d_4^{20}$ 0.7867, $n_D^{20}$ 1.3980; *trans* form, b.p. 76.0°C/765.0 torr, $d_4^{20}$ 0.7849, $n_D^{20}$ 1.4013.

Isobutenyl methyl ether, $(Me)_2C=CHOMe$, which has no *cis-trans* isomers, was also studied kinetically for a comparison of structural effects. It was prepared from α-chloroisobutyl methyl ether by the above-described method; b.p. 71.5°C/758 torr, $d_4^{20}$ 0.7859, $n_D^{20}$ 1.4042.

The rates of hydrolysis of the compounds in water solution under the influence of acid catalysts were measured by spectrophotometry, using the procedure described in the earlier study of vinyl ethers.[1] The standard errors of the first-order rate coefficients obtained did not exceed 1 % in any run. The deuterium solvent isotope effect was also studied in $H_2O$-$D_2O$ mixtures. The individual rate coefficients of these experiments satisfied eqn. (3) of Ref. 1. To save space, the data for the reactions in $H_2O$-$D_2O$ mixtures are not given here, but are available from the authors.

It was observed that the *cis* and *trans* forms of the vinyl ethers are interconvertible in the presence of an acid catalyst. However, the equilibrium ratios of the two forms could not be measured in the pure liquid state owing to polymerization reactions brought about by the catalyst. The polymerization could be eliminated by studying the isomeric interconversion equilibrium in dilute solution (about 0.06 M) in dioxane with benzoic acid (about 0.02 M) as the catalyst. Under these conditions and at 25°C, the state of equilibrium was reached in a few days. The *cis-trans* ratios were determined by gas-chromatographic analysis. The following equilibrium values were obtained at 25°C: MeCH=CHOMe, $(cis/trans)_{equil.} = 2.45$; EtCH=CHOMe, $(cis/trans)_{equil.} = 4.26$. These correspond, respectively, to the values −0.53 and −0.86 kcal/mole for the differences in the standard free energies of the *cis* and *trans* forms. The observation that the *cis* form is favored at equilibrium is in accord with the results of Price and Snyder.[4]

The results of the kinetic measurements of the reactions in water are summarized in Table 1. It is seen that the *cis* forms of the studied vinyl ethers hydrolyze approximately three times faster than the *trans* forms under the same conditions. As the initial state stabilities have an opposite influence, the *cis* forms being more stable than the *trans* forms by 0.53 and 0.86 kcal/mole for the α-propenyl methyl and α-butenyl methyl ethers, respectively, the free energy differences of the transition states are larger than those of the initial states. From the rate coefficients at 25°C and from the differences in the initial state free energies one can calculate that the free energy levels of the transition states derived from the *cis* forms of the mentioned two compounds are lower by 1.2 and 1.6 kcal/mole, respectively.

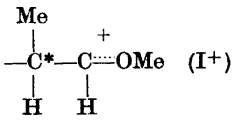
When the present values are compared with those for vinyl ethers of the type $CH_2=CHOR$,[1] it is seen that substitution of the hydrogens of the methylene group by methyl groups decreases the hydrolysis rate by factors of 3 to 10 per methyl group. These changes in the hydrolysis rates may be primarily attributed to the "hyperconjugative" stabilization energies, or, in the newer interpretation of Dewar and Schmeising,[5] to σ-bond energies which vary with the hybrid state of the carbon atom. Irrespective of any specific interpretation of this stabilization, its magnitude in the present system is well known from experiment. For example, the average value of the stabilization energy per methyl substitution at an ethylenic linkage is 2.3 kcal/mole,[5] as derived from the heats of hydro-

*Table 1.* Catalytic coefficients $k_{HA}$ and derived kinetic values for the protolytic cleavage of vinyl ethers in water solution.

| Vinyl ether | °C | HA | $k_{HA}$,$M^{-1}s^{-1}$ | $E$, kcal/mole | $\Delta S^{\neq}$, E.U. |
|---|---|---|---|---|---|
| MeCH=CHOMe (*cis*) | 25 | $H_3O^+$ | 0.245 | | |
| » | 35 | » | 0.562 | 15.6 | −11.1 |
| » | 45 | » | 1.280 | | |
| MeCH=CHOMe (*trans*) | 25 | » | 0.0818 | | |
| » | 35 | » | 0.199 | 16.3 | −10.8 |
| » | 45 | » | 0.461 | | |
| EtCH=CHOMe (*cis*) | 25 | » | 0.333 | 14.1 | −15.5 |
| » | 45 | » | 1.48 | | |
| EtCH=CHOMe (*trans*) | 25 | » | 0.0948 | 15.3 | −14.0 |
| » | 45 | » | 0.479 | | |
| $(Me)_2C$=CHOMe | 25 | » | 0.0252 | | |
| MeCH=CHOMe (*trans*) | 45 | HCOOH | 0.00258 | | |
| EtCH=CHOMe (*trans*) | » | » | 0.00282 | | |

genation of ethylene and methyl-substituted ethylenes.

Table 2 gives the free energies of activation for some vinyl ethers along with the stabilization energies relating to methyl substitutions at the $sp^2$-carbon. The value for methyl vinyl ether, which corresponds to a rate coefficient of 0.83 $M^{-1}s^{-1}$ at 25°C, has been calculated from the correlation between structures and rates of hydrolysis of vinyl ethers and acetals discussed in the previous paper.[1] The hydrolysis mechanism of vinyl ethers implies that the stabilization energy in question is wholly lost in the intermediate ($I^+$) that is generated in the rate-determining proton-transfer step, because the



carbon atom C* becomes $sp^3$-hybridized in this intermediate. As may be inferred from the Brønsted $\alpha$ values,[1] the transition states of these proton transfer reactions are roughly half-way between the initial states and the intermediates, and thus it may be expected that an energy amounting to about half of the stabilization energy of the methyl substitutions will be lost in the transition states. The values of the free energies of activation relative to that of the unsubstituted vinyl ether, $\Delta$ ($\Delta G^{\neq}$), when compared with the stabilization energies of the initial states, are in satisfactory agreement with this conclusion.

The fact that the *cis* forms hydrolyze faster than the *trans* forms is probably

*Table 2.* Free energies of activation, $\Delta G^{\neq}$, of vinyl ethers at 25°C, and the stabilization energies, $SE$, relating to methyl substitutions at the $sp^2$-carbon.

| Vinyl ether | $\Delta G^{\neq}$ kcal/mole | $SE$ kcal/mole | $\Delta(\Delta G^{\neq})/SE$ |
|---|---|---|---|
| $CH_2$=CHOMe | 17.56 | — | — |
| MeCH=CHOMe (*cis*) | 18.28 | 2.3 | 0.3 |
| MeCH=CHOMe (*trans*) | 18.93 | 2.3 | 0.6 |
| $(Me)_2C$=CHOMe | 19.63 | 4.6 | 0.45 |

*Table 3.* Deuterium solvent isotope effects for the lyonium ion-catalyzed hydrolysis of vinyl ethers at 25°C.

| Vinyl ether | $k_{H_3O^+}/k_{D_3O^+}$ |
|---|---|
| $CH_2$=CHOEt | 2.95 (Ref. 7) |
| MeCH=CHOMe (*cis*) | 2.45 |
| MeCH=CHOMe (*trans*) | 2.41 |
| $(Me)_2C$=CHOMe | 1.66 |

largely due to conformational energy differences between the transition states in question. The preferred conformation of the intermediate $I^+$ should be similar to that of propionaldehyde, in which the methyl group is eclipsed with the double bond,[6] or, in our case, with the partial double bond of the intermediate. In the case of a *cis* compound, this conformation is already present in the transition state, whereas in the transition state derived from the *trans* form, it is a hydrogen atom that is eclipsed with the partial double bond, and the more favorable conformation is not achievable in the transition state because the central carbon-carbon linkage has not completely lost its double bond character.

Table 3 shows values of the deuterium solvent isotope effect for some of the vinyl ethers studied and the value of Kresge and Chiang[7] for ethyl vinyl ether. It is interesting to note that the isotope effect decreases with increasing methyl substitution. This results probably from the above-mentioned factors that bring about the changes in the hydrolysis rates with methyl substitutions, although more extensive information is needed to elucidate this point in detail.

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# The Purification of N,N-Dimethylformamide and Acetonitrile for Polarographic Use

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The sensitivity of the polarographic method makes the purity of the solvent a question of importance. As regards N,N-dimethylformamide and acetonitrile, the most widespread solvents in non-aqueous polarography, several methods of purification are reported in the chemical literature.[1-4] However, as a rule these methods are not entirely satisfactory. Either the product is not very pure or the method is laborious. The methods of purification developed by the author yield, with very little work, products with a high degree of purity. As a consequence, very pure solvents can be used even in preparative electrochemistry thus facilitating the identification of the products.

*N,N-Dimethylformamide (DMF).* This solvent is purified by treatment with active alumina: A separatory funnel with a wide bore stopcock is connected with a ground glass joint to a chromatography column, the stem of the funnel intruding approximately 15 cm into the column. The column is equipped with a fine porosity sintered glass filter and a Teflon key stopcock, leading through a ground glass joint into the receiving flask. Rubber tubing connects the receiving flask and the upper part of the column to a drying tower.

The apparatus is thoroughly dried and filled with dry nitrogen. The column is then filled with alumina. Woelm aluminium oxide, activity grade one, is the only one found effective. The separatory funnel is mounted and filled with DMF, previously dried over molecular sieves (Linde, 4A). After removal of the nitrogen with a vacuum pump, the column is filled with DMF from the funnel. During this operation dry nitrogen is led to the top of the funnel. The funnel is stoppered, and a constant niveau of DMF in the column is maintained by leaving the funnel stopcock open. If the first filling of the column is made with high purity DMF, the bottom stopcock of the column can be left open. If ordinary DMF is used, the bottom stopcock should be closed for a day or two before the column is