

The pooled PsCCP fractions were concentrated by ultrafiltration in a Diaflow ultrafiltration apparatus (Model 202, Aminco) and chromatographed on Sephadex G-100 Superfine (2.5 × 102 cm column, eluent 0.01 M sodium phosphate buffer pH 6.8, hydrostatic pressure 55 cm, flow rate 9.6 ml/h, 2.4 ml fractions). A single red band containing PsCCP was eluted (Fig. 1). Those fractions giving one protein band in disc electrophoresis (Fig. 1) were pooled.

The purification procedure is summarized in Table I.

Discussion. The last steps of the original procedure have been modified. The conditions for the CM-cellulose chromatography gave a quantitative retention of PsCCP during the passage of a large volume of solution through the column. This was achieved by lowering the ionic strength and pH of both equilibration buffer and preparation. Chromatography on Sephadex G-100 Superfine eliminated the need for rechromatography on CM-cellulose, which was found to be necessary sometimes with the earlier method.¹ Chromatography on Sephadex G-100 Superfine removed some minor impurities, as can be seen from Fig. 1. This large scale procedure gives a better recovery of PsCCP than does the original method; further, the degree of purification achieved is higher.

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General Acid Catalysis in the Hydrolysis of a Furan Derivative

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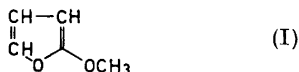
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Although reactions of furan and its derivatives have been thoroughly studied, only a few papers deal with the hydrolytic cleavage of the ring in these compounds. Stamhuis *et al.*¹ proposed that the rate-determining stage in the acid-catalyzed hydrolysis is proton transfer to the α -position of the ring. In the light of recent kinetic data on the hydrogen exchange of furans,^{2,3} this mechanism seems to be excluded because the rate of α -hydrogen exchange was found to be higher than that of the subsequent cleavage of the ring. The α -hydrogen exchange was studied using different isotopes of hydrogen and the kinetic data were found to be in accordance with a two-step $A-S_E2$ mechanism.³ As no β -hydrogen exchange was observed *prior* to further reactions, two alternatives remain. The first is that hydrogen exchange at the β -position of the ring takes place at a lower rate than the protolytic cleavage of the ring. Unfortunately, no verification is possible in aqueous solutions. Despite this, Ünverferth and Schwetlick⁴ recently concluded that β -hydrogen exchange is slower than the cleavage in the hydrolysis of furan derivatives. Consequently the hydrolytic decomposition proceeds by an α -protonation pre-equilibrium and a subsequent rate-determining cleavage of the ring. The second alternative is that the hydrolysis proceeds *via* a rate-determining β -protonation of the ring. Indeed, all the known facts seem to be in agreement with this alternative.³ It was therefore of considerable interest to study the possibility of general acid catalysis in the hydrolysis of furan derivatives. Unfortunately, the susceptibility of most furans to hydrolytic decomposition is so low that the rates in buffer solutions are too low to be experimentally studied. Therefore we investigated whether the hydrolysis of 2-methoxyfuran (I) is subject to general acid catalysis. The alkoxy substituent in this compound enhances the susceptibility

Table 1. Kinetic data for the hydrolysis of 2-methoxyfuran in light and heavy water at 25°C. The last three acids in the column headed HA are the acid components of the buffer systems.

HA	(HA) mol/l	10^3k s ⁻¹	k_{HA} M ⁻¹ s ⁻¹
H ₃ O ⁺	0.005	8.35 ± 0.12	1.67
		8.46 ± 0.09	1.69
		8.35 ± 0.18	1.67
		8.41 ± 0.16	1.68
D ₃ O ⁺	0.005	2.58 ± 0.05	0.515
		2.71 ± 0.09	0.542
CH ₃ COOH	0.2	1.85 ± 0.07	
	0.133	1.32 ± 0.02	
	0.0667	0.790 ± 0.012	0.00794 ± 0.00001
HCOOH	0.3	10.49 ± 0.15	
		10.15 ± 0.11	
	0.2	6.89 ± 0.11	
	0.1	4.19 ± 0.09	0.0310 ± 0.0017
ClCH ₂ COOH	0.1	15.59 ± 0.37	
		17.28 ± 0.32	
	0.0667	10.94 ± 0.29	
	0.0333	5.56 ± 0.11	0.163 ± 0.015

of the furan ring to electrophilic attack and hence the rates of its hydrolysis can be conveniently measured under various conditions.



Experimental. 2-Methoxyfuran was a product of Aldrich Chemical Company. The purity of the product was controlled by NMR-spectroscopy: 3 H at δ 1.74 ppm, 1 H at δ 2.98 ppm, 1 H at δ 4.14 ppm, and 1 H at δ 4.73 ppm (in carbon tetrachloride, tetramethylsilane as the standard). Other signals were not observed.

The kinetic experiments were run in isotopically different waters. The deuterium oxide used was supplied by Norsk Hydro-Elektrisk Kvaestofaktieselskap. Lyonium ion catalysis was studied in dilute perchloric acid solutions (0.005 M). When catalysis by Brønsted acid was studied, carboxylic acid-carboxylate ion buffers were employed. The molar ratio of acid to base was unity in formic acid-sodium formate and acetic acid-sodium acetate buffers and 1:8 in the monochloroacetic acid-sodium monochloroacetate buffers. When the concentration of the undissociated acid was varied in the kinetic experiments, the ionic strength was maintained constant by adding sodium chloride.

All the kinetic experiments took place at 25°C. The initial concentration of 2-methoxyfuran in the reaction mixtures was about 0.01 M. The hydrolysis of 2-methoxyfuran was followed with a Perkin Elmer F 11 gas chromatograph. A 1 ml sample taken from the reaction mixture was run into a tube containing 1 ml of carbon tetrachloride and a drop of concentrated sodium hydroxide to neutralize the catalyst acid. To ensure that the reaction was arrested immediately, the tube had been cooled in an ice bath. The unchanged 2-methoxyfuran was extracted from the reaction mixture with carbon tetrachloride with vigorous shaking immediately after the sample had been taken, as a slow decomposition of 2-methoxyfuran was found to take place in aqueous solution. No decomposition was observed to occur in carbon tetrachloride. In addition, a relatively high accuracy could be achieved when the solutions in carbon tetrachloride were analyzed by gas chromatography. Xylene was used as an internal standard in these gas chromatographic analyses.

The rate coefficients for the hydrolysis of 2-methoxyfuran are collected in Table 1. The relative standard errors of the rate coefficients vary from 1 to 2%. When the rate coefficients of the hydrolysis of 2-methoxyfuran are compared with the overall rates of hydrolysis of alkyl-substituted furans,^{3,5} the accelerating effect

of the methoxy group is seen to be about four powers of ten. It was thus easy to study whether the hydrolysis of this furan derivative is subject to general acid catalysis.

The rate coefficients of the hydronium ion-catalyzed hydrolysis in light and heavy waters collected in Table 1 show that the deuterium solvent isotope effect, $k_{D_2O^+}/k_{H_3O^+}$, is 0.315. Isotope effects of this magnitude in the hydrolysis of vinyl ethers^{6,7} have been interpreted in terms of rate-determining proton transfer to a carbon base. Thus general acid catalysis should be detected in the hydrolysis of the studied furan derivative. Experiments in buffer solutions showed that this is really the case. When the first-order rate coefficients in acetic acid–sodium acetate buffers are plotted against the concentration of the acid component (Fig. 1), a linear

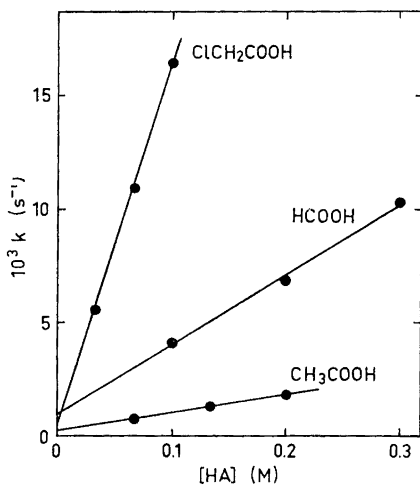


Fig. 1. Plot of the first-order rate coefficients against the concentration of the acid buffer component for the hydrolysis of 2-methoxyfuran in buffer solutions, 25°C.

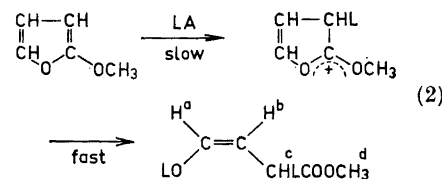
correlation in accordance with eqn. (1) is observed. The catalytic coefficient k_{HA} , the slope of the plot, was calculated by

$$k_{\text{obs}} = k_{HA}(HA) + k_{H_3O^+}(H_3O^+) \quad (1)$$

the method of least squares. General acid catalysis was detected also in the other buffer solutions. The calculated rate coefficients and their standard errors are collected in Table 1. When the logarithms

of the rate coefficients for the carboxylic acid-catalyzed reactions are plotted against the logarithms of the dissociation constants of the acids, a straight line results. The slope of this plot, i.e., the value of the Brønsted α , 0.69 ± 0.07 , was calculated by the method of least squares. Although this value may be only a rough estimate owing to the limited kinetic data, it is only a little higher than the value for the hydrolysis of the structurally related vinyl ethers.^{6,7} It is reasonable to assume that the protolytic cleavage of this furan derivative does not differ significantly from that of vinyl ether hydrolysis, i.e., proton transfer to the β -position of the carbon-carbon double bond is rate determining.

Proton attack may take place at two β -positions in the hydrolysis of 2-methoxyfuran. If structural effects of the substituents are considered, the 3-position seems more susceptible to electrophilic attack as this route would lead in the rate-determining stage to a dialkoxy-carbonium ion intermediate which is much more stable than the monoalkoxy-carbonium ion which would be the intermediate if proton transfer to the 4-position took place. That the proton transfer really takes place at the position shown in eqn. (2) was confirmed by following the hydroly-



ysis of 2-methoxyfuran in isotopically different waters with a 60 MHz Perkin-Elmer R 10 NMR spectrometer. It was found that all the peaks of 2-methoxyfuran disappeared at the same rate and thus the proton attack must be the rate-determining stage of the hydrolysis. As shown in scheme (2), an unsaturated hydroxy ester with *cis* configuration is the primary product when the reaction proceeds by protonation at the 3-position. That the ester was present in the reaction mixture was revealed by the NMR spectrum. Chemical shifts, 3.0 ppm (H^d), 4.8 ppm (H^c), 6.0 ppm (H^b) and 7.8 ppm (H^a); coupling constants, $J_{ab}=5.7$ cps, $J_{ac}=1.6$ cps, and $J_{bc}=2.0$ cps. These values refer to the compound in a 50 mol % dimethyl

sulfoxide-heavy water mixture with tetramethylsilane as internal standard. When measurements were performed on the compound in the DMSO-protium oxide system, differences were found in the coupling of H^c and in addition the integral of the H^c signal was twice that in the DMSO-deuterium oxide mixture. Thus it is evident that the studied reaction proceeds by route (2), for another product would be obtained if proton transfer to the 4-position were rate determining.

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Formation of Conjugated Dienes on Lithium Aluminium Hydride Reduction of Allenic *tert.*-Alcohols*

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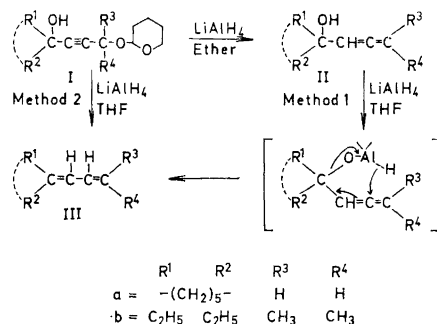
In connexion with the synthesis of allenic *tert.* alcohols for pharmacological evalu-

* Allenes and acetylenes I.

ation,¹ we noted that the reaction² of 1-[3-(tetrahydro-2-pyranyloxy)-1-propynyl]cyclohexanol (Ia) with $LiAlH_4$ to 1-propadienylcyclohexanol (IIa) upon prolonged reaction time afforded a by-product. This compound was identified as the conjugated diene IIIa (*cf.* Scheme 1). Treatment of the allenic alcohol IIa with $LiAlH_4$ in THF gave IIIa in 80% yield. Under similar conditions as in the latter experiment, the analogous allenic derivative IIb was also found to afford the corresponding diene IIIb.

The reactions were followed by GLC and GLC-MS in order to characterize all products formed. Only in the reaction of IIa to IIIa a by-product of appreciable amount (10%) was observed. This product was isolated by preparative GLC and, by spectroscopic methods (IR, NMR and MS), identified as 2-propynylidenecyclohexane.

In order to get insight into the mechanism of the reaction II→III we reacted IIa with $LiAlD_4$ in THF. This reaction yielded 2-propenylidenecyclohexane-2-*d* (IIIa-2-*d*), which indicates that the elimination of the hydroxyl group of the allenic alcohol IIa proceeds as outlined in Scheme 1. An analogous mechanism has been proposed³ for the $LiAlH_4$ reduction of tetraphenylbutyne-1,4-diol to 1,1,4,4-tetraphenylbutadiene-(1,3)^{3,4} and for the addition of Grignard reagent to 2,3-butadienol.⁵



Scheme 1.

To check the possibility of preparing the dienes in a one step reaction from the corresponding tetrahydro-2-pyranyloxy derivatives we carried out the reduction of Ia and Ib in THF and obtained 70% yields of IIIa and IIIb, respectively. This shows that the dienes can be prepared