

Kinetics of Formation of Anhydroretinol from Retinyl Acetate in Acetic Acid, and from Retinyl Acetate and Retinol in Ethanol and Ethanol—Water Mixtures Containing Hydrogen Chloride

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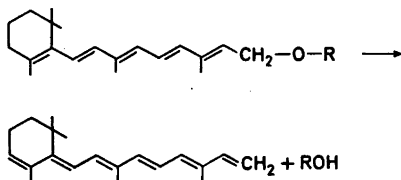
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The rate constant for the formation of anhydroretinol from retinyl acetate in acetic acid has been measured at 30, 40, 50 and 60 °C. In addition, the effects of sodium acetate, pyridine, acetic anhydride, and water on the rate constant have been studied at 50 °C. Only the addition of water had a pronounced effect, accelerating the rate of the reaction.

Also the rate constants for the HCl-catalyzed formation of anhydroretinol from retinyl acetate and from retinol in ethanol and ethanol-water mixtures have been measured at 15, 25, and 35 °C. The rate is about ten times higher with retinol than with retinyl acetate. In both cases the rate constants decrease markedly when water is added to ethanol. The changes in $\log k$ are correlated to changes in the acidity function H_0 (0.1 M HCl).

The mechanism of the reactions is discussed and the A-1 mechanism seems probable, the slowest stage being the dissociation of the protonated substrate to carbonium ion (retinyl cation) and acetic acid (with retinyl acetate) or water (with retinol).

Anhydroretinol is formed from retinol and retinyl acetate in an acid catalyzed reaction, which is typical of these compounds and which takes place easily.



R = H, retinol; R = Ac, retinyl acetate

This reaction was first studied by Edisbury and his co-workers.¹ They found that vitamin A₁ (retinol) in anhydrous ethanol and in the presence of hydrogen chloride is transformed to a product which was called cyclized vitamin A. Shantz² prepared a pure anhydrovitamin A₁ (anhydroretinol) using a reaction catalyzed by *p*-toluenesulfonic acid in benzene. Methods have been developed for the quantitative determination of vitamin A based on the formation of its anhydroform.³

The kinetics of the formation of anhydroretinol has been studied to only a limited extent. Barnholdt⁴ performed some measurements in the presence of HCl in ethanol with all-*trans* and 13-*cis* vitamin A₁. Higuchi and Reinstein⁵ investigated the kinetics of the formation of anhydroretinol from retinol and retinyl acetate in ethanol, ethanol-water mixtures and ethanol-heptane mixtures, usually in the presence of HCl. The rate constants, calculated on the basis of the initial rate of formation of anhydroretinol, were measured only in the case of retinyl acetate in ethanol, catalyzed by HCl. They found that the reaction catalyzed by HCl slowed down abruptly when water was added to the ethanol. As far as we know, no other kinetic studies have been carried out on the formation of anhydroretinol.

We have previously studied the polymerization of retinyl acetate in acetic acid-water mixtures.⁶ It was found that both the rate of polymerization and the rate of the formation of anhydroretinol were accelerated when water

was added to acetic acid. We present now, more precisely, measurements pertaining to the formation of anhydroretinol from retinyl acetate in pure acetic acid. The effect of the addition of water and some other compounds to acetic acid has also been studied. Experiments have been carried out with retinol and retinyl acetate in ethanol and ethanol-water mixtures with hydrochloric acid as catalyst. In particular, we wanted to compare the rate constants with the acidity function (H_0) because it is well known⁷ that H_0 changes greatly when water is added to ethanol. The purpose of this work has been to elucidate the mechanism of the formation of anhydroretinol from retinol and retinyl acetate.

EXPERIMENTAL

Method. The experiments were carried out in a special reaction vessel constructed so that flushing with nitrogen was possible. The nitrogen was saturated with the solvent. In the experiments in acetic acid, 1 ml samples were withdrawn at appropriate intervals, while nitrogen was flowing through the vessel. The 1 ml sample was added to 20 ml cold (about 0 °C) absolute ethanol and the absorbance was measured immediately at the wavelengths 390, 342, and 326 nm with a Beckman DU-2 spectrophotometer. In ethanol and in ethanol-water mixtures the progress of the reaction was followed by taking 5 ml samples and adding them to 20 w % water-ethanol mixtures and measuring the absorbance at the wavelengths 390, 342, and 324 nm or 326 nm. The absorbance reading at 342 nm (isosbestic point) did not change appreciably during the reaction, indicating that there were no significant side reactions. The rate constants were calculated from the absorbance readings measured at 390 nm according to the equation

$$k = \frac{2.303}{t} \log \frac{A_e}{A_e - A}$$

where A is the absorbance reading at time t (initially equal to zero) and A_e is the final absorbance reading. It should be noted that A_e/A_r [A_r = absorbance of retinyl acetate or retinol at the beginning of the reaction at wavelength 326 nm (retinyl acetate) or 324 nm (retinol)] did not change significantly when the temperature was altered. The following values were obtained for the ratio A_e/A_r in the case of retinyl acetate in acetic acid: at 60 °C, 0.99 (15 experiments); at 50 °C, 1.1 (15 experiments); at 40 °C, 1.1 (4 experiments); at 30 °C 1.2 (2 experiments). These figures indicate that about 58–70 % of the retinyl acetate is converted into anhydroretinol according to the absorbance values given by Shantz⁸ and 70–85 %

according to those given by Oroshnik, Karmes and Mebane⁹ and Bulgrin and Lookhart.⁹

The initial concentration of retinyl acetate in acetic acid was varied from 0.7×10^{-4} M to 7×10^{-4} M. In this concentration range, the rate constant was independent of the initial concentration of retinyl acetate. In ethanol and in ethanol-water mixtures the initial concentrations of retinol and retinyl acetate were about 1.8×10^{-4} M.

Chemicals. Retinol and retinyl acetate were products from F. Hoffmann-La Roche & Co Ltd. Ethanol was dried using Al-powder and Hg_2Cl_2 as a catalyst. The water content of dried ethanol was less than 0.01 w % (Karl Fischer) and that of the acetic acid, on the average, 0.1 w %. When the reaction was carried out in pure ethanol, HCl gas was introduced to dry ethanol by the usual method.¹⁰ Solvent mixtures were made by weighing.

DISCUSSION

The rate constants k_1 ($\times 10^4/s^{-1}$) for the formation of anhydroretinol from retinyl acetate in pure acetic acid were found to be 0.372, 1.24, 3.85, and 11.9 at 30, 40, 50, and 60 °C, respectively. The corresponding activation energy was 97.0 kJ/mol and the value of $\log A$ 12.29.

Table 1 presents the other results pertaining to the formation of anhydroretinol from retinyl acetate in acetic acid. According to these results the effect of sodium acetate, acetic anhydride and pyridine on the rate of formation is slight. On the other hand, water has clearly an accelerating effect. Bruckenstein and Kolthoff¹¹ found that upon addition of water to a solution of a basic indicator like *p,p*-dimethylaminoazobenzene in acetic acid, the colour changes in favour of the acid species, even though water

Table 1. The formation of anhydroretinol from retinyl acetate in acetic acid at 50 °C.

Compounds added to acetic acid	M	H ₂ O (M)	Rate constant $k_1 \times 10^4/s^{-1}$
NaOAc	0.18	—	3.63
NaOAc	0.18	1.1	6.25
NaOAc	0.17	—	3.67
NaOAc	0.17	2.6	8.37
NaOAc	0.17	1.1	6.55
—	—	0.55	4.93
Ac ₂ O	0.085	—	3.77
Pyridine	0.24	—	4.17
Pyridine	0.24	1.1	6.30

is a base. This problem has been dealt with quite recently by de la Mare and Singh.¹² They studied, for example, the protonation of *p*-bromo-*N,N*-dimethylaniline in acetic acid when sodium acetate or sodium acetate and water were added. At the beginning, sodium acetate caused a slight decrease in the protonation power. When water was added to this solution, the protonation power clearly increased. These results suggest that the accelerating effect of water on the formation of anhydroretinol is caused by the stronger protonation of retinyl acetate and not by the other effects of water on the reaction. This interpretation is confirmed by the experiments in ethanol and ethanol-water mixtures, where the addition of water greatly retards the reaction catalyzed by HCl. Similar results have been obtained earlier by Higuchi and Reinstein.⁵ Water does not seem to be necessary for the formation of anhydroretinol from retinyl acetate. The reaction which takes place by acyl-oxygen fission producing first retinol is therefore not very likely, but the rate-determining stage would be the same as in the acid catalyzed ester hydrolysis which takes place by the mechanism A_{AC} -1. For vinyl acetate, evidence¹³ has been presented that the hydrolysis reaction is of A_{AC} -2 type, but the alcohol part of the ester in retinyl acetate is quite different. The assumption that the formation of anhydroretinol from retinyl acetate is an A -1 reaction is strengthened by the relatively high value of the activation energy and by the small negative value of the activation entropy (97.0 kJ mol⁻¹; -18 J mol⁻¹ K⁻¹).

Table 2. The formation of anhydroretinol from retinol in ethanol and in ethanol-water mixtures.

Solvent wt % H ₂ O	Conc. of HCl M	Rate constant $k_2 \times 10^3 / M^{-1} s^{-1}$			E kJ/mol
		15 °C	25 °C	35 °C	
0	0.002	—	230	—	—
0	0.004	89.0	269	655	74.0
5	0.025	—	8.30	—	—
5	0.050	1.95	8.77	29.1	99.9
10	0.050	—	4.82	—	—
10	0.10	—	4.85	—	—
20	0.050	—	3.85	—	—
20	0.075	—	3.79	—	—
20	0.100	1.06	3.81	12.5	91.1

Table 3. The formation of anhydroretinol from retinyl acetate in ethanol and in ethanol-water mixtures.

Solvent wt % H ₂ O	Conc. of HCl M	Rate constant $k_2 \times 10^3 / M^{-1} s^{-1}$			E kJ/mol
		15 °C	25 °C	35 °C	
0	0.010	—	23.7	68.3	—
0	0.012	—	21.9	—	—
0	0.020	6.95	24.3	67.5	84
0	0.052	—	25.4	—	—
0	0.089	—	27.3	—	—
5	0.250	—	1.37	4.36	—
5	0.360	0.403	1.60	4.96	92.8
10	0.250	—	0.741	—	—
10	0.500	—	1.01	—	—
20	0.250	—	0.631	—	—
20	0.500	0.214	0.813	2.45	90.3
20	0.670	—	0.839	—	—

The results for the hydrogen-chloride-catalyzed formation of anhydroretinol from retinol and from retinyl acetate in ethanol and in ethanol-water mixtures are presented in Tables 2 and 3. The rate of the formation of anhydroretinol is about ten times higher with retinol than with retinyl acetate. It is further interesting to note that, in the case of retinol, when the concentration of HCl is low, a rather good second-order rate constant is obtained. In the case of retinyl acetate, HCl concentrations were higher and the second-order rate constant increased with the HCl concentration, especially in solvent mixtures where water was present. This may be due to the fact that, at higher concentrations of acid, the rate is no longer proportional to the molar concentration of acid but the rate follows the acidity function.

With retinol and retinyl acetate the rate decreases greatly when water is added to ethanol. Taking the H_0 values from the work of

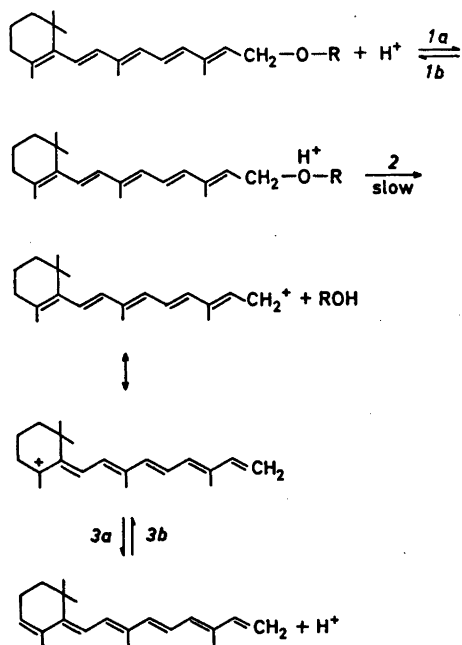
Table 4. Values $\log k_{0,1} + H_0$ for retinyl acetate and retinol in ethanol and ethanol-water mixtures at 25 °C.

Solvent wt % H ₂ O	H_0	$\log k_{0,1} + H_0$	
		Retinol	Retinyl acetate
0	0.61	-1.01	-2.01
5	1.99	-1.11	-1.84
10	2.15	-1.18	-1.91
20	2.26	-1.16	-1.88

Braude and Stern,⁷ we obtained the values presented in Table 4 for $\log k_{0,1} + H_0$ ($\log k_{0,1}$ represents the first-order rate constant when the hydrochloric acid concentration is 0.1 M, H_0 refers to the same concentration of HCl, $t = 25^\circ\text{C}$).

It can be seen that $\log k_{0,1} + H_0$ is constant, and it can therefore be assumed that the rate constant is proportional to the acidity function h_0 in the case of both retinol and retinyl acetate. Because the rate of formation of anhydroretinol is higher with retinol, one might assume that retinyl acetate first hydrolyses to retinol, but this is improbable on the basis of the results presented in Table 4.

The formation of anhydroretinol from retinol and retinyl acetate most probably follows the reaction scheme (R = H or Ac):



The carbonium ion in the above reaction scheme is the retinyl cation, a coloured compound absorbing at about 600 nm. This has been studied by Blatz *et al.*¹⁴ We have obtained the spectra of anhydroretinol and not observed any colour formation in the reactions. Consequently, we assume that the concentration of retinyl cation was low. It seems probable, therefore, that the rate-determining stage is

the dissociation of the protonated form (reaction 2) and that the mechanism is the A-1.

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Received November 14, 1975.