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Study on the Color Reaction Mechanism of the Seliwanoff Reaction with 4-Ethylresorcinol as the Color-Developing Reagent

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4-Ethylresorcinol was found to be a very sensitive color-developing reagent for the detection of ketoses, and was used to elucidate the reaction mechanism of the Seliwanoff reaction.

The reaction product (I) responsible for the characteristic coloration of the Seliwanoff reaction was isolated and determined to be 2,7-diethyl-6-hydroxy-9-(5-methyl-2-furyl)-3*H*-xanthen-3-one. Compound I was also isolated as crystals from the reaction mixture obtained by Kulka's modified method using 4-ethylresorcinol as the color-developing reagent.

Keywords——Seliwanoff reaction; fructose; ketose; resorcinol; 4-ethylresorcinol; xanthene derivative; color reaction mechanism

The Seliwanoff reaction[1)] is a well-known color reaction of ketoses. However, the reaction product responsible for the coloration has not yet been isolated in crystalline form. We found that 4-ethylresorcinol produces a reddish-violet color with ketoses in boiling aqueous hydrochloric acid (HCl) somewhat more sensitively than and as selectively as resorcinol, and gives a crystalline color reaction product with a fairly good yield. This paper describes the results.

Results and Discussion

In the study of a color reaction mechanism of organic compounds from the standpoint of the chemical structure of the reaction product, difficulties can occur if the product responsible for the coloration is either unstable and is converted into other compounds fairly rapidly, or cannot readily be crystallized. In such cases, it may be possible to utilize a simple chemical conversion of the reaction product to a stable derivative, or to use a derivative of the original reagent as the color-developing reagent.

The first method is useful when both the reaction product and its stable derivative have the same characteristic color, or when the derivative having a different color can be reconverted easily to the original reaction product by a simple chemical treatment. This method has already been employed successfully.[2)] The second method is expected to be useful when the additional substituent in the derivative of the original reagent does not significantly influence the characteristics of the original reagent as a color-developing reagent, namely when the derivative develops the characteristic color with organic compounds to be tested as sensitively and selectively as the original reagent does.

Preliminary experiments showed that the isolation of the reaction product responsible for the characteristics coloration of the Seliwanoff reaction is extremely difficult. Moreover, all attempts at chemical conversion of the product to a derivative able to develop the same color

Table I. Limits of Detection (Miligram of Sugar in 1 ml of Reaction Mixture)

	Resorcinol	4-Ethyl-resorcinol	Orcinol	2-Methyl-resorcinol	4-Chloro-resorcinol
Fructose	0.13	0.08	0.25	0.15	0.35
Tagatose	0.10	0.02	0.20	0.08	
Sorbose	0.40	0.25	3.00	0.43	
Color	Red	Reddish-violet	Greenish-yellow	Yellowish-orange	Orange

as that of the product under the color reaction conditions were unsuccessful. Therefore, the second method was tried. Among several resorcinol derivatives commercially available, four compounds were found to be useful as color-developing reagents in preliminary tests. The selectivities and sensitivities of these four compounds were compared with those of resorcinol by the identification method for fructose given in the Japanese Pharmacopoeia X (JP X). The results showed that none of the aldoses (glucose, galactose, mannose, rhamnose, ribose, xylose and arabinose) developed any color with any of the four compounds even when the sugar was equimolar to the reagent, but all of the ketoses (fructose, tagatose and sorbose) developed the characteristic color with all four compounds very sensitively. The sensitivities of the compounds including resorcinol as color-developing reagents can be compared in terms of the limits of detection of ketoses [the minimum detectable amount (mg) of a sugar present in 1 ml of reaction mixture]. The results are shown in Table I together with the characteristic color developed by each of the compounds. Table I shows that 4-ethylresorcinol develops the chracteristic color (λ_{max} 494 nm) somewhat more sensitively than resorcinol with all kinds of ketoses tested. This result indicates that 4-ethylresorcinol not only can be used as a new color-developing reagent for ketoses, but also is a suitable derivative for use in the second method, because its ethyl group was found not to alter the characteristics as a color-developing reagent for the detection of ketoses. Therefore, the color reaction mechanism of the Seliwanoff reaction was studied by the use of 4-ethylresorcinol as the color-developing reagent.

Isolation and Structure of the Reaction Product

In the method of identification for fructose described by the JP X, fairly dilute HCl is used as the reaction medium, namely 1 ml of HCl is added to 10 ml of the aqueous solution containing both sugar and resorcinol. On the other hand, in the original Seliwanoff reaction, fairly concentrated HCl is used as the reaction medium, namely the volume of HCl to be added to the aqueous solution of sugar and resorcinol is about a half of or almost equal to the volume of the aqueous solution. In this study, the concentration of HCl used was that used in the Seliwanoff reaction, namely an aqueous solution of fructose, an alcoholic solution of 4-ethylresorcinol and HCl were mixed together in 3 : 24 : 13 ratio by volume, and the reaction was carried out at 80 °C in order to avoid the loss of alcohol by vaporization.

At the beginning of the reaction, the reaction mixture showed two absorption maxima at about 415 and 505 nm. The latter absorption band disappeared rapidly during heating, and a new absorption band appeared at about 470 nm (Fig. 1). With the prolongation of the heating time, the new absorption band became more and more intense, and the band having the maximum at about 415 nm became gradually weaker. This result indicates that the characteristic absorption of this new color reaction using 4-ethylresorcinol as the reagent is at about 470 nm. Although the use of longer reaction times yielded more intense characteristic absorption, the reaction was stopped after heating for 15 min, and the reaction product was extracted with AcOEt. The AcOEt extract afforded red prisms (I). Compound I showed an

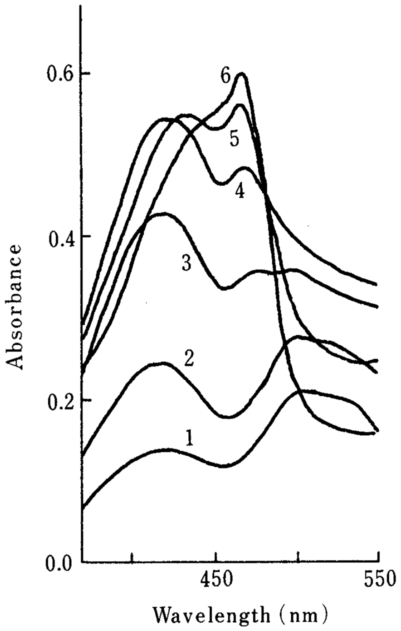


Fig. 1. Spectral Change of the Reaction Mixture Diluted 300-fold with the Solvent Mixture of H_2O, EtOH and HCl (3 : 24 : 13)

Curves 1—6: 3, 5, 10, 15, 30 and 60 min after the beginning of the reaction, respectively.

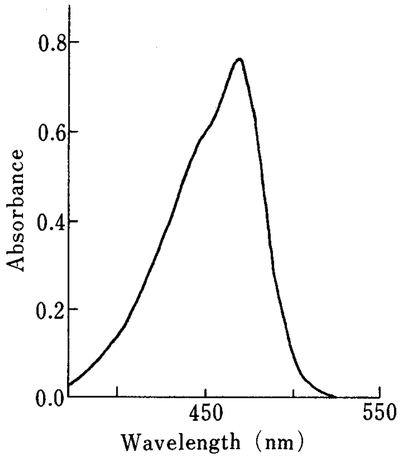
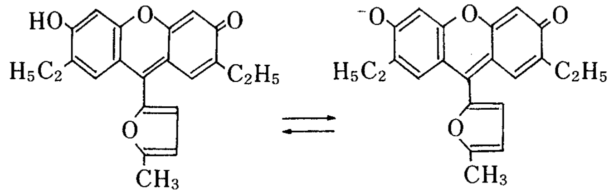


Fig. 2. Absorption Spectrum of the 1.25×10^{-5} M Solution of I in the Solvent Mixture of H_2O, EtOH and HCl (3 : 24 : 13)

$\lambda_{max}^{H_2O\text{-}EtOH\text{-}HCl}$ 469 nm (log ε = 4.79).



Ia

Ib

Chart 1

absorption maximum at 469 nm in the solvent mixture of water, alcohol and HCl having the same volume ratio as that of the reaction medium (Fig. 2). Figure 2 shows that I is the coloring matter responsible for the absorption maximum at about 470 nm of the reaction mixture. The molecular formula of I was determined to be $C_{22}H_{20}O_4$ from the results of elemental analyses and mass spectral (MS) measurement. The infrared (IR) spectrum of I showed that I has a carbonyl group and a hydrogen-bonded hydroxyl group. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of I in CF_3COOD showed the presence of two ethyl groups, one methyl group, one =CH–CH= group and two kinds of two magnetically equivalent protons. All of these results support the conclusion that the chemical structure of I is 2,7-diethyl-6-hydroxy-9-(5-methyl-2-furyl)-3*H*-xanthen-3-one.

The absorption maximum of I was also observed at 536 nm in alcohol and at 469 nm in dioxane. Since I shows its absorption maximum at 538 nm in the medium of dioxane and aqueous 0.75 N NaOH solution (1 : 1) and at 467 nm in the medium of dioxane and aqueous 10 N H_2SO_4 solution (3 : 2), the absorption maximum in alcohol and that in dioxane can be assigned to the maximum of the anionic form of I (Ib) and to the maximum of the neutral form of I (Ia), respectively (Chart 1). 6-Hydroxy-9-phenyl-3*H*-xanthen-3-one was reported to show a quite similar phenomenon.[3]

Compound I was also isolated as crystals from the reaction mixture obtained by Kulka's modified method[4] which involves the use of ammonium ferric sulfate.

Experimental

Ultraviolet (UV), IR and MS spectra were recorded on Hitachi 200-20, JASCO DS-701G and JEOL JMS-D 300 spectrometers, respectively. ^{1}H-NMR spectra were recorded on a JEOL FX-100 spectrometer with tetramethylsilane (TMS) as an internal standard. Guaranteed reagent grade chemicals were used in the reaction, and solvents for spectroscopic use were employed in the UV measurement.

Determination of the Limit of Identification——Each of three test tubes containing 5 ml of an aqueous sugar solution, 5 ml of an aqueous solution of resorcinol derivative (0.5%, w/v) and 1 ml of HCl was shaken vigorously, then the reaction mixture was heated in a boiling water-bath for 3 min, and the color developed by the sugar was compared with the color of the blank test. The test was regarded as positive when the reaction solutions in all three tubes showed color definitely different from that of the blank solution. The limits of identification (mg in 1 ml of reaction mixture) of all sugars tested are given in Table I, togehter with the color developed by them.

Isolation of I from the Reaction Mixture of Fructose, 4-Ethylresorcinol and HCl——A mixture of 30 ml of an aqueous solution of 4 g (0.022 mol) of fructose, 240 ml of an alcoholic solution of 3.2 g (0.023 mol) of 4-ethylresorcinol and 130 ml of HCl was heated at 80 °C for 15 min, and the reaction product was extracted with AcOEt. The AcOEt layer was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated to dryness. The addition of a small volume of EtOH to the residue afforded crystals, which were separated and recrystallized several times from EtOH to afford 0.05 g of I as red prisms of mp 266—269 °C (dec.). Compound I is insoluble in H_2O, ether and benzene, slightly soluble in $CHCl_3$, acetone and dioxane, and soluble in MeOH, EtOH and tetrahydrofuran (THF). *Anal.* Calcd for $C_{22}H_{20}O_4$: C, 75.84; H, 5.79; mol. wt., 348.38. Found: C, 75.70; H, 5.80; mol. wt., 348 (MS *m/e*, M^+). UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 536 (4.49); $\lambda_{\max}^{\mathrm{dioxane}}$ nm (log ε): 469 (4.58); $\lambda_{\max}^{\mathrm{dioxane\text{-}NaOH}}$ nm (log ε): 538 (4.89); $\lambda_{\max}^{\mathrm{dioxane\text{-}H_2SO_4}}$ nm (log ε): 467 (4.78). IR $\nu_{\max}^{\mathrm{Nujol}}$ cm^{-1}: 2520 (OH), 1632 (C=O). ^{1}H-NMR (CF_3COOD) δ: 1.44 (6H, t, J=7.3 Hz), 2.76 (3H, s), 2.96 (4H, q, J=7.3 Hz), 6.77 (1H, d, J=3.5 Hz), 7.37 (2H, s), 7.56 (1H, d, J=3.5 Hz), 8.52 (2H, s).

Isolation of I from the Reaction Mixture of Fructose, 4-Ethylresorcinol, Ammonium Ferric Sulfate and HCl——A mixture of 100 ml of an aqueous solution of 3.6 g (0.02 mol) of fructose, 150 ml of an alcoholic solution of 2.8 g (0.02 mol) of 4-ethylresorcinol and 150 ml of a solution of 9.6 g of $Fe_2(SO_4)_3(NH_4)_2SO_4 \cdot 24H_2O$ in HCl was heated at 80 °C for 40 min. The reaction mixture was made weakly acidic with an aqueous $NaHCO_3$ solution, and the reaction product was extracted with AcOEt. The AcOEt layer was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated to dryness. Alcohol was added to the residue to give crystals, which were recrystallized several times from EtOH to afford 0.015 g of red prisms. The IR spectrum and the melting point of these crystals were identical with those of I.

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