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Mechanism of the Color Reaction of 4,5-Dinitroveratrole with Reducing Sugars. (Organic Analysis. LXIV*3)

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4,5-Dinitroveratrole was first found by Wiggins[1] to give a sensitive color reaction with reducing sugars. Williams[2] determined 0.5～3.0 mg. of a reducing sugar with the reagent, and recently Bevenue, *et al.*[3] used the reagent to develop the color on a paper chromatogram, estimating 2～20 μg. of the sugar. This paper presents the mechanism of the color reaction with the experimental data of the isolated coloring matters.

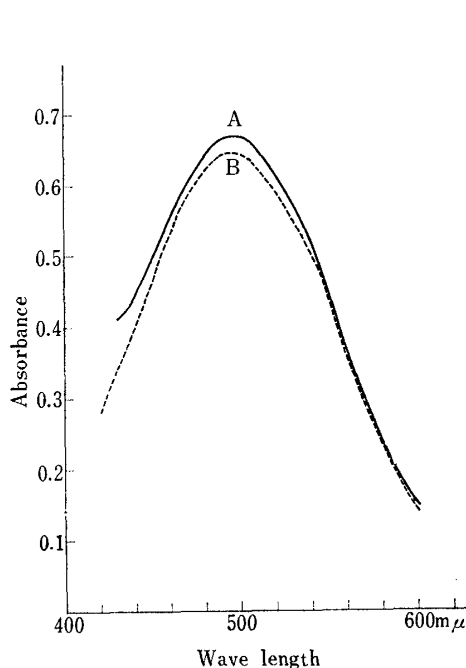

Fig. 1. Absorption Spectra for the Solution of the Color Reaction of Glucose with 4,5-Dinitroveratrole, and for the Isolated Coloring Matter (I) in Alkaline Medium

A : 1 ml. of 0.1% aqueous glucose, 1 ml. of 20% aqueous K_2CO_3, and 1 ml. of 0.5% 4,5-dinitroveratrole dissolved in EtOH were mixed and heated in a boiling bath for 2 min., cooled for 3 min. and 40 ml. of a mixture of H_2O, 20% K_2CO_3, and EtOH (1:1:1) was added.

B : 2 mg. of I was dissolved in 100 ml. of the same mixture.

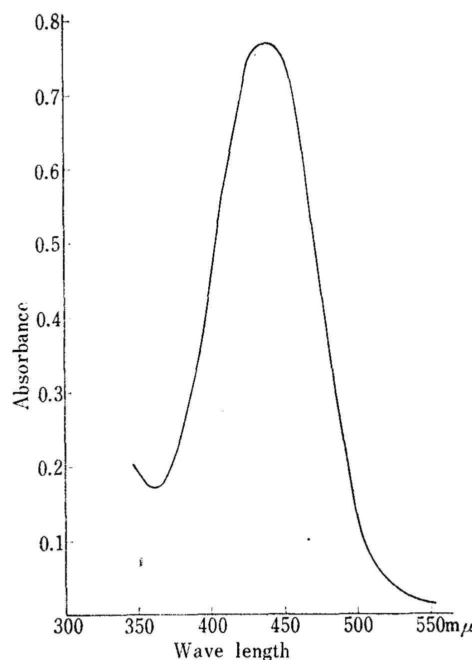

Fig. 2. Absorption Spectrum of Coloring Matter (II) in Alkaline Medium

2 mg. of II was dissolved in 100 ml. of a mixture of H_2O, 20% aqueous K_2CO_3, and EtOH (1:1:1).

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Coloring Matters of the Reaction

The reaction of 4,5-dinitroveratrole and glucose was carried out in a boiling water bath for three minutes in an alkaline medium. The deep red reaction mixture was neutralized with phosphoric acid, and extracted with ethyl ether. The etherial layer was extracted with aqueous potassium carbonate solution, and orange crystals (I) were separated when the solution was acidified with phosphoric acid. The remained etherial solution was chromatographed on alumina with benzene as eluant, and orange crystals (II) were separated along with the starting 4,5-dinitroveratrole and resinous substances.

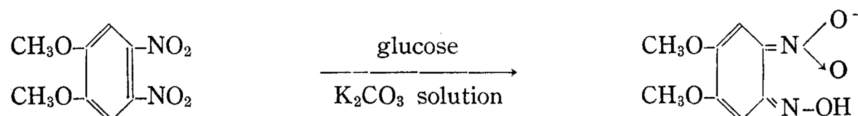
The coloring matter (I), orange prisms, m.p. 108°, was obtained in good yield, and considered to be the main product of the reaction. It gave a red color in an alkaline solution, and the absorption curve (Fig. 1-B) was similar to that of the developed color of glucose (Fig.1-A) in respect of the shape and absorption maximum, 497 mμ.

I reduced the Tollens' reagent, and its infrared spectrum suggested the presence of NO_2, OH and NH groups in its molecule. These data indicated that the compound should be 4-hydroxylamino-5-nitroveratrole, and then it was confirmed by direct comparisons with an authentic sample.[4]

The coloring matter (II), orange prisms, m.p. 169°, was obtained in poor yield, and identified as 4-amino-5-nitroveratrole by direct comparisons with an authentic sample[5].

The absorption curve of the product was shown in Fig. 2.

Those results revealed that 4,5-dinitroveratrole was mainly reduced to 4-hydroxylamino-5-nitroveratrole in the reaction, which showed a red color in an alkaline medium with a formation of the quinoidal anion.



Experimental

Isolation of Reaction Products——To a hot solution of 15 g. of glucose dissolved in a mixture of 120 ml. of H_2O and 210 ml. of aqueous 20% K_2CO_3, was added 6 g. of 4,5-dinitroveratrole dissolved in a mixture of 150 ml. of EtOH and 10 ml. of AcOEt, and the mixture was heated in a boiling water bath for 3 min. The mixture was immediately cooled in an ice bath, neutralized with H_3PO_4, and extracted with ether. The ether layer was separated and reextracted with aqueous 20% K_2CO_3. The aqueous layer was neutralized with H_3PO_4, and separated crystals (2.5 g.) were recrystallized from absolute EtOH to give orange prisms (I) of m.p. 108°. This substance showed no depression of the melting point on admixture with an authentic 4-hydroxylamino-5-nitroveratrole, and its IR spectrum was identical with the authentic one.

The remained ether layer was concentrated, and the residue was dissolved in benzene to chromatograph on 40 g. of Al_2O_3.

The successive elution of the column with benzene yielded in turn 4,5-dinitroveratrole, orange prisms (II) (0.1 g.), and resinous substances. II was recrystallized from benzene to give orange prisms, m.p. 169°, and identified as 4-amino-5-nitroveratrole by the mixed melting point and the comparison of IR spectra with an authentic sample.

Absorption Spectra——The visible light absorption spectra were measured by a Hitachi EPU-2A Spectrophotometer in a cell of 10 mm. optical length. The infrared spectra were measured by a Koken DS-301 Infrared Spectrophotometer in Nujol mull with NaCl prism, and a Hitachi EPI-S Infrared Spectrophotometer in KBr tablets with NaCl prism.

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Haruaki Yajima, Kazuo Kubo, Takao Oshima,*1 Kotobuki Hano, and Masao Koida*2 :

Studies on Peptides. VII.*3 Synthesis of Three Stereoisomeric Pentapeptides of Histidylphenylalanylarginyltryptophylglycine and Their Melanocyte-Stimulating Activities *in vitro.*

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In our recent communications,[*3,1~4] we have reported the syntheses and the physiological properties of stereoisomeric pentapeptides related to histidylphenylalanylarginyltryptophylglycine which corresponds to positions 6 to 10 in α-melanocyte-stimulating hormone (α-MSH). Of particular interest was the finding that the all-D-pentapeptide, D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycine[1,2] possessed an inhibitory action toward the physiological activity of the corresponding pentapeptide of all L-form.

We wish to record here the MSH activities of other three pentapeptide isomers which we have further synthesized. The method employed for the synthesis of these isomers are essentially the same as described in the preparation of L-histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine.[3,4] The MSH assays were performed according to the method of Shizume, *et al.*[5] using isolated pieces of frog-skins of *Rana pipiens.*

Experimental

The amino acid compositions of the acid and enzymatic hydrolysates were determined with a Hitachi amino acid analyzer, Model KLA-2 according to the method of Moore, *et al.*[6] The following abbreviations for the constituent amino acids, His=histidine, Phe=phenylalanine, Arg=arginine, Try=tryptophan, and Gly=glycine were used. Rf^1 values refer to the Partridge system[7]; Rf^2 values refer to the *sec*-BuOH-NH_4OH system[8] and were expressed as a multiple of distance traveled by Phe under identical conditions.

D-Histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine——$[\alpha]_D^{30}$ $-16.5°$ (c=0.5, 1*N* HCl), Rf^1 0.50, Rf^2 1.0. Amino acid ratios in acid hydrolysate, $His_{1.00}Phe_{1.00}Arg_{1.00}Gly_{0.94}$ (average recovery 89%, Try was destroyed during the hydrolysis).

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