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Mechanism of the Molisch Reaction

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2-Methyl-1-naphthol was found to develop characteristic color with hexoses and pentoses in concentrated sulfuric acid very sensitively, and was used to elucidate the reaction mechanism of the Molisch reaction.

The chemical structures of the reaction product of glucose (I) and that of rhamnose (II) were established to be 2-[(4-hydroxy-3-methyl-naphth-1-yl)methyl]-5-[(3-methyl-4-oxo-naphth-1-ylidene)methyl]furan and 5-[(3-methyl-4-oxo-naphth-1-ylidene)methyl]-2-methylfuran, respectively.

Keywords——Molisch reaction; glucose; rhamnose; hexose; pentose; 2-methyl-1-naphthol; 1-naphthol; color reaction mechanism

The color reaction of hexose or pentose with 1-naphthol in concentrated sulfuric acid (H_2SO_4), the Molisch reaction,[1] has long been known as one of the most familiar and sensitive color reactions of sugars. Bredereck investigated the mechanism of the Molisch reaction, and suggested that the product responsible for the characteristic coloration in the reaction of hexose is 2-[(4-hydroxy-3-sulfo-naphth-1-yl)-(4-oxo-naphth-1-ylidene)methyl]-5-hydroxymethylfuran.[2] No direct evidence was presented in support of this structure, though it has been used occasionally in discussions of the Molisch reaction.[3]

Recently, we found out that 2-methyl-1-naphthol reacts as sensitively as 1-naphthol with hexose and pentose in H_2SO_4, and the color reaction products crystallize very easily. Accordingly, it became possible to elucidate the reaction mechanism of the Molisch reaction. This paper describes the results of our investigation.

Results and Discussion

Isolation of the product responsible for the characteristics coloration of the Molisch reaction in a crystalline state has been considered to be almost impossible. The reason is the high probability of the formation of sulfonated products, because 1-naphthol is known to be sulfonated very easily at its $C_{(2)}$-position (and then $C_{(4)}$-position) in H_2SO_4. Sulfonation makes both the separation of the reaction product from the reaction mixture and the crystallization of the product very difficult. In such a case, it may be possible to use derivatives of the reagent of the reaction.[4] In the case of the Molisch reaction, derivatives of 1-naphthol having a substituent at the $C_{(2)}$-position are desirable. Among three such derivatives commercially available, 2-nitro- and 2-methyl-1-naphthol and 1-hydroxynaphthoic acid, only 2-methyl-1-naphthol was found to develop color with sugars in H_2SO_4 very sensitively. Its sensitivity to sugars was found to be almost identical with that of 1-naphthol (Table I). Thus, 2-methyl-1-naphthol could be suitable for the investigation of the mechanism of the Molisch reaction.

TABLE I. Limit of Identification (μg/1 ml H_2O)

	1-Naphthol		2-Methyl-1-naphthol	
		Color		Color
Glucose	10	Reddish-violet	10	Violet
Galactose	10	Reddish-violet	10	Violet
Mannose	10	Reddish-violet	10	Violet
Fructose	5	Reddish-violet	5	Violet
Tagatose	5	Reddish-violet	5	Violet
Sorbose	10	Reddish-violet	10	Violet
Ribose	10	Purple-red	5	Purple
Xylose	5	Purple-red	5	Purple
Arabinose	10	Purple-red	5	Purple
Rhamnose	10	Red	5	Reddish-violet

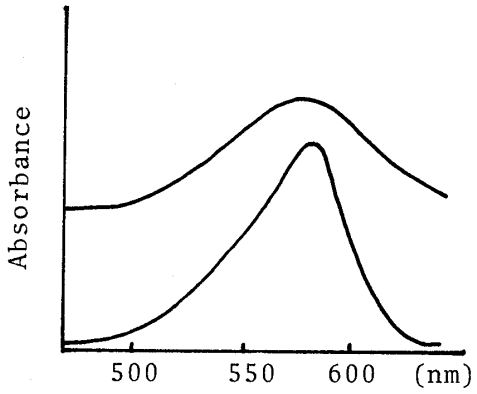


Fig. 1. Absorption Spectrum of the Molisch Reaction Mixture of Glucose (Upper Curve) and That of I Dissolved in a Mixture of H_2O, EtOH and H_2SO_4 (1 : 1 : 2) (Lower Curve)

Isolation and Structure of the Reaction Product

Both glucose and rhamnose were subjected to the Molisch reaction using 2-methyl-1-naphthol as the color-developing reagent. After repeated separation on silica gel using benzene–ethyl alcohol (EtOH) as the eluent, the ethyl acetate (AcOEt) extracts of the reaction mixtures afforded crystals in both cases.

5-Hydroxymethylfurfural and 5-methylfurfural were also subjected to the reaction with 2-methyl-1-naphthol in H_2SO_4. In both cases, crystals were obtained from the AcOEt extracts of the reaction mixtures after purification processes similar to those mentioned above.

The crystals (I) obtained from the reaction mixture of the Molisch reaction of glucose were proved to be identical with those obtained from the reaction mixture of 5-hydroxymethylfurfural, 2-methyl-1-naphthol and H_2SO_4.

The absorption spectrum of the Molisch reaction mixture of glucose (upper curve) and that of a solution of I dissolved in H_2O–EtOH–H_2SO_4 (1 : 1 : 2) (lower curve) are shown in Fig. 1. Figure 1 clearly indicates that I is the coloring material responsible for the characteristic coloration of the Molisch reaction of hexose. Compound I has the molecular formula $C_{28}H_{22}O_3$, and its infrared (IR) spectrum showed the presence of both a hydroxyl and a conjugated carbonyl group. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of I in *N*,*N*-dimethylformamide-d_7 (DMF-d_7) showed the presences of the following groups: two methyl groups with different chemical shifts; one methylene group; one furan ring; three methine groups with different chemical shifts; two phenyl rings; one hydroxyl group. From all of the physico-chemical properties mentioned above, the chemical structure of I was established to be 2-[(4-hydroxy-3-methyl-naphth-1-yl)methyl]-5-[(3-methyl-4-oxo-naphth-1-ylidene)methyl]furan.

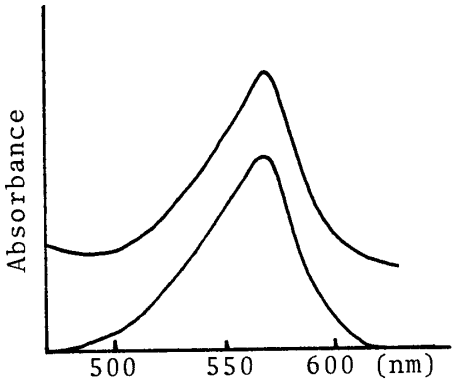
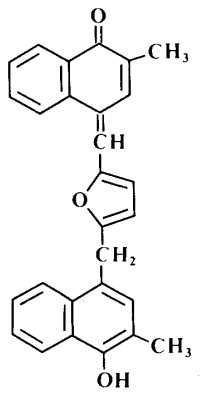
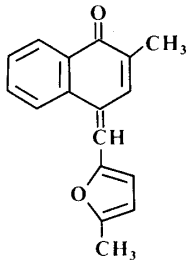


Fig. 2. Absorption Spectrum of Molisch Reaction Mixture of Rhamnose (Upper Curve) and That of II Dissolved in a Mixture of H_2O, EtOH and H_2SO_4 (1 : 1 : 2) (Lower Curve)



I



II

Chart 1

The crystals (II) obtained from the reaction mixture of the Molisch reaction of rhamnose were also proved to be identical with those obtained from the reaction mixture of 5-methylfurfural, 2-methyl-1-naphthol and H_2SO_4. A comparison of the absorption spectrum of the Molisch reaction mixture of rhamnose (upper curve) with that of a solution of II dissolved in H_2O–EtOH–H_2SO_4 (1 : 1 : 2) (lower curve) (Fig. 2) indicates clearly that II is the coloring matter responsible for the characteristic coloration of the Molisch reaction of rhamnose. Compound II has the molecular formula $C_{17}H_{14}O_2$, and its IR spectrum schowed the presence of a conjugated carbonyl group. The ^{1}H-NMR spectrum of II in DMF-d_7 showed the presence of the following groups: two methyl groups with different chemical shifts; one furan ring; two methine groups with different chemical shifts; one phenyl ring. All of these physico-chemical properties support the view that the chemical structure of II is 5-[(3-methyl-4-oxo-naphth-1-ylidene)methyl]-2-methylfuran.

From all of the experimental results mentioned above, the chemical structure of the compound responsible for the characteristic coloration of the Molisch reaction of pentose is expected to be 5-[(4-oxo-3-sulfo-naphth-1-ylidene)]-2-methylfuran.

Unfortunately, 2-methyl-1-naphthol is not suitable for routine work as the color-developing reagent of the Molisch reaction, because this compound cannot be stored for a long period in air.

Experimental

Ultraviolet (UV), IR and mass spectra (MS) were recorded on Hitachi 200-20, JASCO DS-701G and JEOL JMS-D300 spectrometers, respectively. ^{1}H-NMR spectra were recorded on a JEOL FX-100 spectrometer with tetramethylsilane (TMS) as an internal standard. Melting points (mp) were measured on a Yanaco MP-500 apparatus and are uncorrected. Guaranteed reagent grade chemicals were used, and EtOH for spectroscopic use was employed in the UV measurements.

Determination of the Limit of Identification——An aqueous sugar solution (1 ml), an EtOH solution of 1-naphthol (0.15%, w/v) or 2-methyl-1-naphthol (0.15%, w/v) and 2 ml of H_2SO_4 were added to each of three test tubes. After vigorous shaking, the reaction mixture was heated in a boiling-water bath for 5 min. The color developed by the sugar was compared with the color of the blank test, and the test was considered to be positive when the reaction solutions in all three tubes showed color definitely different from that of the blank solution. The limits of identification (μg in 1 ml of H_2O) of the sugars tested are given in Table I, together with the color developed by them.

The Molisch Reaction of Glucose, and the Isolation of I——A mixture of 45 ml of an aqueous solution of 4.5 g of glucose (0.025 mol) and 45 ml of an EtOH solution of 0.4 g of 2-methyl-1-naphthol (0.0025 mol) was stirred vigorously, and 90 ml of H_2SO_4 was dropped into the mixture slowly so as to maintain the reaction temperature below 60 °C. The reaction mixture was poured into about 2 l of an aqueous sodium carbonate solution, and the

reaction products were extracted with AcOEt. The AcOEt layer was washed with H_2O several times, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue (*ca.* 0.4 g) was dissolved in benzene–EtOH and separated on silica gel repeatedly with benzene–EtOH (5 : 1→200 : 1) as the eluent. Evaporation of the fraction containing a substance which develops a reddish-violet color with H_2SO_4 left crystals. Recrystallization of the separated crystals from benzene afforded 11 mg of 2-[(4-hydroxy-3-methyl-naphth-1-yl)methyl]-5-[(3-methyl-4-oxo-naphth-1-ylidene)methyl]furan (I) as reddish-brown needles of mp 221—223 °C. Compound I is soluble in ethyl ether, benzene, MeOH, EtOH, $CHCl_3$, acetone, THF and dioxane, and insoluble in H_2O. *Anal.* Calcd for $C_{28}H_{22}O_3$: C, 82.73; H, 5.45; mol wt., 406.46. Found. C, 82.53; H, 5.40; mol. wt., 406 (MS *m/e*, M^+). UV λ_{max}^{EtOH} nm (log ε): 434 (4.53); $\lambda_{max}^{H_2O-EtOH-H_2SO_4}$ nm (log ε): 580 (4.85). IR ν_{max}^{Nujol} cm^{-1}: 3400 (OH), 1630 (C=O). ^{1}H-NMR (DMF-d_7) δ: 2.08 (s, 3H), 2.49 (s, 3H), 4.57 (s, 2H), 6.51 (d, 1H, J=3.4 Hz), 7.04 (d, 1H, J=3.4 Hz), 7.45 (s, 1H), 7.77 (s, 1H), 7.47—8.43 (m, 8H), 8.39 (s, 1H), 9.29 (s, 1H).

Isolation of I from the Reaction Mixture of 5-Hydroxymethylfurfural, 2-Methyl-1-naphthol and H_2SO_4——A mixture of 10 ml of an aqueous solution of 5-hydroxymethylfurfural (0.04 g, 0.0003 mol) and 10 ml of an EtOH solution of 2-methyl-1-naphthol (0.10 g, 0.0006 mol) was treated with H_2SO_4 (20 ml) under reaction conditions analogous to those used in the Molisch reaction of glucose. The AcOEt extract (*ca.* 0.10 g) was dissolved in benzene–EtOH (200 : 1) and separated on silica gel with the same solvent mixture as the eluent. Evaporation of the fractions containing a substance which develops reddish-violet color with H_2SO_4 left crystals. Recrystallization of the separated crystals from benzene afforded 27 mg of reddish-brown needles. The IR spectrum and mp of these crystals were identical with those of I.

The Molisch Reaction of Rhamnose, and the Isolation of II——A mixture of 40 ml of an aqueous solution of rhamnose (2 g, 0.01 mol) and 40 ml of an EtOH solution of 2-methyl-1-naphthol (0.40 g, 0.0025 mol) was treated with H_2SO_4 (80 ml) under reaction conditions analogous to those used in the Molisch reaction of glucose. The AcOEt extract (*ca.* 0.56 g) was dissolved in benzene–EtOH and separated on silica gel repeatedly with benzene–EtOH (30 : 1→200 : 1) as the eluent. Evaporation of the fraction containing a substance which develops violet color with H_2SO_4 left crystals. Recrystallization of the separated crystals from benzene afforded 23 mg of 5-[(3-methyl-4-oxo-naphth-1-ylidene)methyl]-2-methylfuran (II) as orange prisms of mp 138—139 °C. Compound II is soluble in ethyl ether, benzene, MeOH, EtOH, $CHCl_3$, acetone, THF and dioxane, and insoluble in H_2O. *Anal.* Calcd for $C_{17}H_{14}O_2$: C, 81.58; H, 5.64; mol. wt., 250.28. Found: C, 81.25; H, 5.79; mol. wt., 250 (MS *m/e*, M^+). UV λ_{max}^{EtOH} nm (log ε): 428 (4.56); $\lambda_{max}^{H_2O-EtOH-H_2SO_4}$ nm (log ε): 568 (4.88). IR ν_{max}^{Nujol} cm^{-1}: 1634 (C=O). ^{1}H-NMR (DMF-d_7) δ: 2.19 (s, 3H), 2.52 (s, 3H), 6.42 (d, 1H, J=3.2 Hz), 7.06 (d, 1H, J=3.2 Hz), 7.44—7.79 (m, 2H), 7.84 (s, 1H), 8.15—8.32 (m, 2H), 8.59 (s, 1H).

Isolation of II from the Reaction Mixture of 5-Methylfurfural, 2-Methyl-1-naphthol and H_2SO_4——A mixture of 40 ml of an aqueous solution of 5-methylfurfural (0.15 g, 0.0014 mol) and 40 ml of an EtOH solution of 2-methyl-1-naphthol (0.35 g, 0.0022 mol) was treated with H_2SO_4 (80 ml) under reaction conditions analogous to those used in the Molisch reaction of glucose. The AcOEt extract (*ca.* 0.4 g) was dissolved in benzene–EtOH and separated on silica gel repeatedly with benzene–EtOH (30 : 1→200 : 1) as the eluent. Evaporation of the fraction containing a substance which develops a violet color with H_2SO_4 left crystals (*ca.* 0.12 g). Recrystallization of the separated crystals from benzene afforded 49 mg of orange prisms. The IR spectrum and mp of these crystals were identical with those of II.

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