Summary

The structures of three metabolites isolated from the urine of rabbits and dogs admintstered with meprobamate (2-methyl-2-*n*-propyl-1,3-propanediol dicarbamate) were established as keto-meprobamate [2-methyl-2-(2-oxo-propyl)-1,3-propanediol dicarbamate], hydroxy-meprobamate [2-methyl-2-(2-hydroxypropyl)-1,3-propanediol dicarbamate] and carboxy-meprobamate [2-methyl-2-(2-carboxyethyl)-1,3-propanediol dicarbamate].

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86. Tsutomu Momose and Masaru Nakamura : Organic Analysis. XXXIII.*1 Mechanism of the Color Reaction between Fructose and N-Methyldiphenylamine.

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N-Methyldiphenylamine gives a sensitive color reaction with fructose when heated in a mixture of hydrochloric acid and ethanol.[1] This reaction can successfully be used in the detection and estimation of the sugar in the presence of glucose, but the mechanism of the reaction has remained unknown. This paper describes a probable mechanism of the reaction, isolating the main dye in crystalline form.

Isolation of the Dye——When a definite amount of fructose was refluxed with varied amounts of N-methyldiphenylamine in a mixture of hydrochloric acid and ethanol, the intensity of the developed violet color increased with the increasing amount of the reagent. One mole of fructose needed two moles of N-methyldiphenylamine to give the maximum intensity (Fig. 1). But the isolation of the dye was carried out from the reaction mixture in which fructose was excess than the calculated amount.

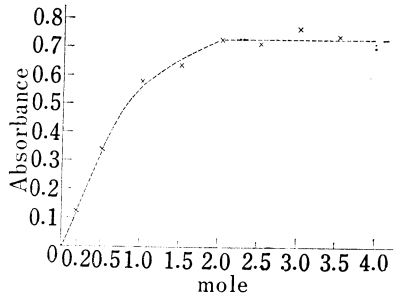

Fig. 1. Mole Ratio of N-methyldihenylamine to Fructose

540 mg. of fructose and varied amount of N-methyldiphenylamine were dissolved in a mixture of conc. HCl 30 ml.+EtOH 30 ml., refluxed for 5 min., diluted to 2500 times, and measured at 629 mμ.

To the developed mixture, a small amount of water was added to separate resinous substances. After a short time the precipitate was removed, a large amount of water was added to the filtrate, and allowed to stand overnight. The separated crystalline dye

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*1 Part. XXXII : Rinsho Kensa, **5** 451 (1961).
*2 Katakasu, Fukuoka (百瀬 勉, 中村 優).
1) G. Kallinich, H. Thies : Chem. Ber., **87**, 759 (1954).

was collected and recrystallized from 80% methanol in the presence of a small amount of hydrochloric acid to golden prisms, m.p. 145° (decomp.). This chloride was soluble in ethanol, methanol, and acetone, giving a deep blue solution, and was insoluble in water, benzene, and ether.

The sulfate of the dye could be obtained by the repeated recrystallization of the chloride from aqueous methanol in the presence of a small amount of sulfuric acid. It formed golden prisms of m.p. 155~157° (decomp.), and its solubility to the solvents was similar to that of the chloride.

The absorption spectrum of the chloride is shown in Fig. 2. This curve approximately agreed with that of the developed color in the shape, showing the adsorption maximum at 629 mμ. Therefore, the dye obtained might be the main coloring matter of the reaction.

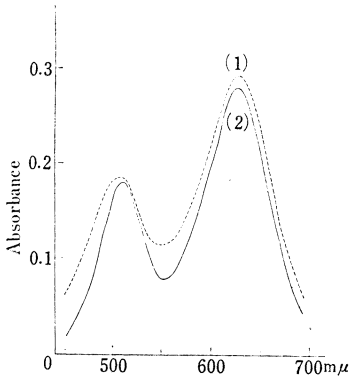


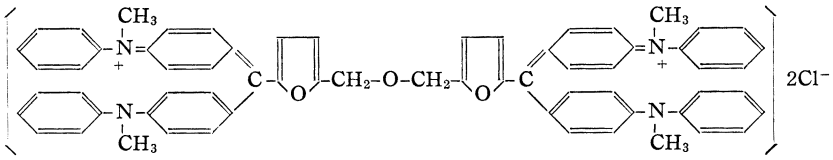
Fig. 2. Absorption Spectra of the Developed Color and the Dye

(1) Fructose was dissolved in a mixture of conc. HCl 2 vol.+EtOH 2 vol.+H_2O 1 vol. in 0.1 m. molar concentration, and refluxed for 5 min.
(2) The dye-chloride was dissolved in the same mixture in 0.0025 m. molar concentration.

Structure of the Dye and Reaction Mechanism——From the data of microanalyses, it is more probable that the dye has the formula of $C_{65}H_{56}O_3N_4 \cdot X_2$, where X=Cl or HSO_4. One mole of fructose may need two moles of N-methyldiphenylamine as stated before, and then the reaction may occur as follows:

$$2C_6H_{12}O_6+4C_{13}H_{13}N+2HCl+O_2=C_{65}H_{56}O_3N_4 \cdot Cl_2+11H_2O$$

The infrared spectrum of the chloride of the dye shows neither hydroxyl nor carbonyl absorption band, but shows five bands at 3.19, 6.33, 6.65, 9.85 and 13.33 μ, which may be caused by the furan ring.[2] These results are quite similar to those of the reaction between fructose and diphenylamine,[3] and again 5-(hydroxymethyl)furfural and 5,5′-oxydifurfural give the same absorption spectrum with the dye when heated with N-methyldiphenylamine in a mixture of hydrochloric acid and ethanol, respectively. Thus, the mechanism of the present reaction quite resembles the reaction mechanism of diphenylamine, and the structure of the dye may be written as in the following.



2) M. Yamaguchi: Bunseki Kagaku, **7**, 210 (1958).
3) T. Momose, Y. Ueda, M. Nakamura: This Bulletin, 8, 827 (1960).

Experimental

Isolation of the Dye——To 5 g. of N-methyldiphenylamine dissolved in a mixture of 115 ml. of MeOH and 115 ml. of conc. HCl, 5 g. of fructose dissolved in 10 ml. of H_2O was added, and the mixture was refluxed on a water bath for 5 min. After cool at room temperature, 100 ml. of H_2O was added, and separated resinous precipitate was removed by filtration. To the filtrate, 200 ml. of H_2O was added, and allowed to stand overnight. The separated crude chloride of the dye (1.8 g.) was collected, and recrystallized from 80% MeOH in the presence of a small amount of HCl to golden prisms, m.p. 145° (decomp.). *Anal.* Calcd. for $C_{64}H_{56}O_3N_4Cl_2$: C, 76.86; H, 5.64; N, 5.60; Cl, 7.09; H, 5.88; N, 5.54; Cl, 6.88; N-CH_3, 5.48.

The sulfate of the dye was obtained as golden prisms, m.p. 155~157° (decomp.), by the repeated recrystallization of the above chloride from 40% MeOH in the presence of a small amount of H_2SO_4. N-CH_3, 6.00. Found: C, 76.32; *Anal.* Calcd. for $C_{64}H_{58}O_{11}N_4S_2$: C, 68.43; H, 5.21; N, 4.99; S, 5.71; N-CH_3, 5.34. Found: C, 68.35; H, 5.55; N, 5.11; S, 5.52; N-CH_3, 4.93.

Absorption Spectra——The visible-light spectra were measured by a Hitachi EPU-2 Spectrophotometer in a cell of 10 mm. optical length. The infrared spectra were measured by a Koken DS-301 Infrared Spectrophotometer in a Nujol mull with about 0.01 mm. thickness.

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Summary

The main dye of the color reaction between fructose and N-methyldiphenylamine was isolated in crystalline form as its chloride and sulfate. Its probable structure was presented and the reaction mechanism was assumed as the same as in the reaction of fructose with diphenylamine.

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87. Tsutomu Momose, Yo Ueda, and Masatake Iwasaki: Organic Analysis. XXXIV*1. Reaction Mechanism of Glucuronic Acid with 1,3-Naphthalenediol.

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In a previous paper[1] of this series, the mechanism of the Tollens reaction,[2] in which glucuronic acid gave a violet coloration when heated with 1,3-naphthalenediol in moderately concentrated hydrochloric acid, was clarified by isolating the main dyes in crystalline form. On the other hand, Guerrero and Williams[3] found that the same coloration was also given by the fusion of glucuronic acid with 1,3-naphthalenediol, and stated that the dye formed in the reaction was difficult to purify. This paper describes

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*1 Part XXXIII: This Bulletin, **10**, 544 (1962).
*2 Katakasu, Fukuoka (百瀬 勉, 上田 陽, 岩崎正武).
1) T. Momose, Y. Ueda, M. Iwasaki: This Bulletin, **4**, 49 (1956).
2) B. Tollens, F. Rorive: Ber., **41**, 1783 (1908).
3) A. H. Guerrero, R. T. Williams: Nature, **161**, 930 (1948).