15. **Michiya Kimura**: Reaction between Polynitrobenzene and Active Methylene Groups. V. Jaffé and Baljet Reactions.

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The color reaction employing picric acid for the determination of creatinine or cardiac glycosides have long been known as the Jaffé reaction[1] or Baljet reaction,[2] respectively. These colorations are apparently based on the same mechanism covering the general reaction between polynitrobenzene and active methylene compounds, which had been pointed out by Reissert, *et al.*[3~5] However, the reaction and the colored substance produced are not simple so that for the accurate quantitative determination of the corresponding substances, a more critical control should be required. For this purpose the chemical as well as spectrochemical studies were carried out in the present series of experiments.

On the paper chromatogram of the colored solution derived from picric acid and acetone under the usual conditions, three distinct spots were separated: (a), Rf 0.4 (bright yellow), (b), Rf 0.5 (orange), and (c), Rf 0.6 (yellow). One spot (a) was found to be identical with that of picric acid. Substance (b), which was extracted with methanol, seemed to be that of the anticipated colored substance having two absorption

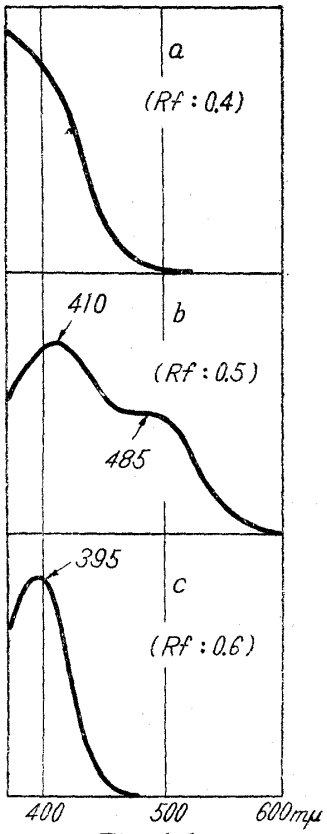

Fig. 1-1

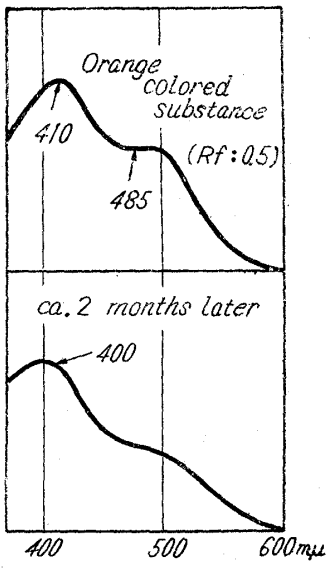

Fig. 1-2

Fig. 1-1. Absorption Curves of Three Spots (a, b, c) separated on the Paper Chromatogram from the Reaction between Picric Acid and Acetone in Alkaline Medium

Fig. 1-2. Absorption Curves of an Aqueous Solution of the Substance (b)

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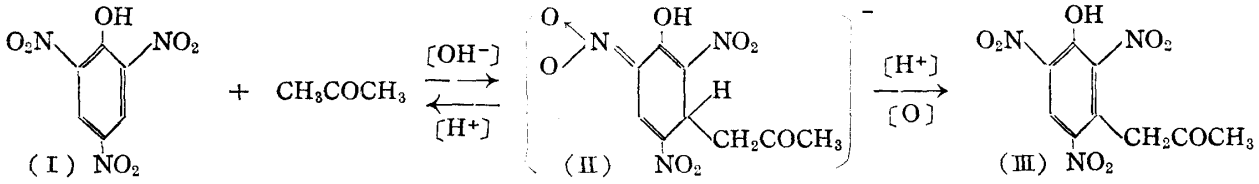
1) M. Jaffé: Z. physiol. Chem., **10**, 399(1886).
2) H. Baljet: Schweiz. Apoth. Ztg., **56**, 71(1918).
3) A. Reitsert: Ber., **37**, 831(1904).
4) W. Weise, *et al.*: Z. physiol. Chem., **178**, 125(1928).
5) T. Sasaki: Biochem. Z., **114**, 63(1921).

maxima at 410 and 485 mμ, as shown in Fig. 1. The substance from spot (c), which exhibits only one maximum at 395 mμ, is still undetermined. The substance (b) is fairly stable in a neutral aqueous solution and only a slight change takes place even after storage of about 60 days (Fig. 1-2). The absorption curve produced by a mixture of picric acid, sodium hydroxide, and acetone (1 : 2.5 : 10,000 in molar ratio) is shown in Fig. 2 (curve I) which suggests the shortage of the orange-colored substance (b). When alkali is used in excess, curves II and III are obtained after a lapse of 25 and 60 minutes, respectively, and finally reaches curve IV, which is closely similar to that of substance (c) (Fig. 1-1). It appears that the orange-colored substance converts gradually to the yellow substance (c) in alkaline medium. Moreover, when the aqueous solution of substance (b) is acidified, the chromatogram gives only one spot which is identical with that of picric acid, while in the presence of hydrogen peroxide, according to the previous procedures,[6] it gives two spots, the one corresponding to picric acid and the other likely to be the compound (III).

Therefore, the principal reaction between picric acid and acetone in alkaline medium would be indicated as follows :



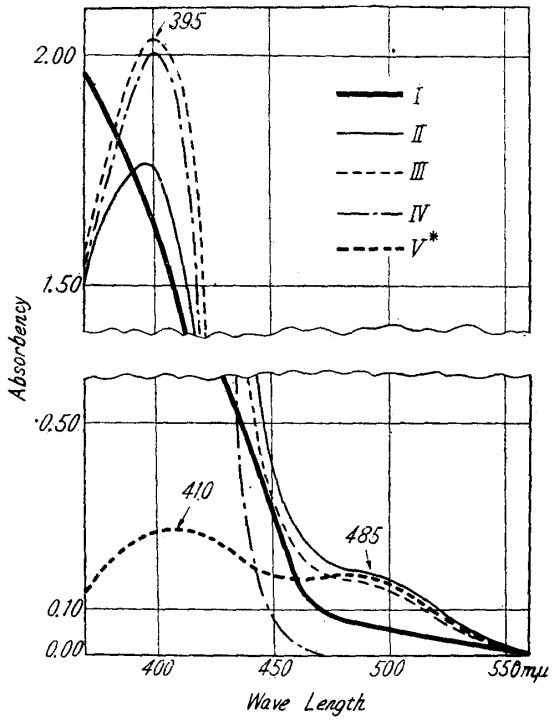


Fig. 2. Absorption Curves of the Reaction between Picric Acid and Acetone (1 : 10,000) in Alkaline Medium

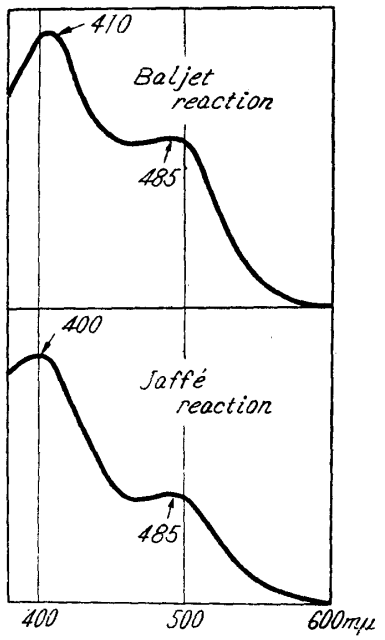
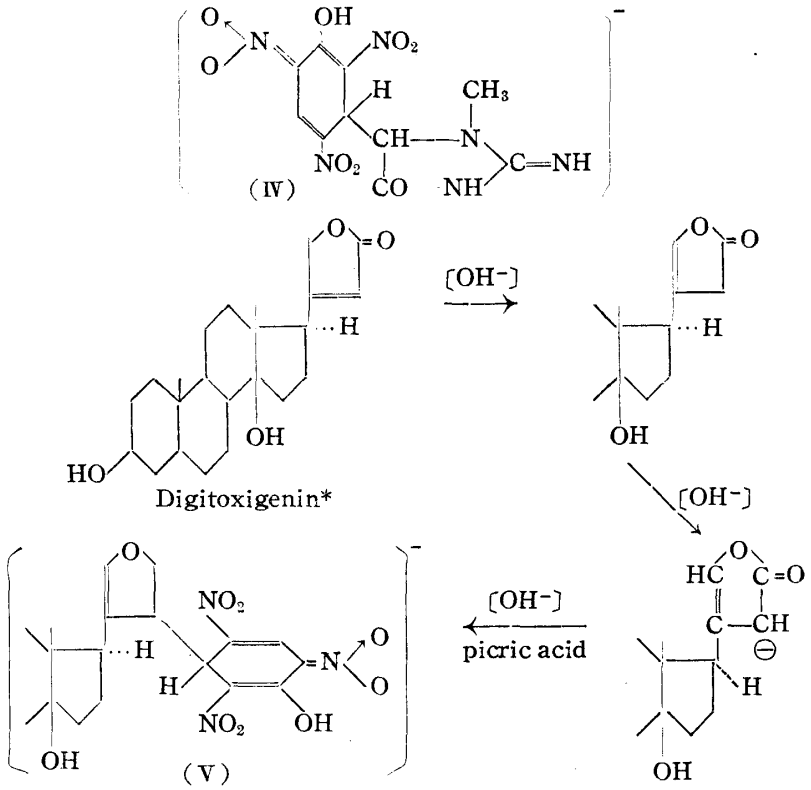


Fig. 3. Absorption Curves of the Colored Substances separated on the Paper Chromatogram

* The curve to be anticipated for the colored substance formed.

The absorption curves of the colored substances resulting from picric acid with creatinine (Jaffé reaction) and with digitoxin (Baljet reaction) are shown in Fig. 3. These absorption patterns are closely similar to that obtained with acetone (Fig. 1). The

6) M. Kimura : This Bulletin, **3**, 75 (1955).



probable structures of the colored complex anions may be illustrated as (IV) and (V).

From the above results it is clear that for the quantitative determination of active methylene compounds employing picric acid, the wave length at the second absorption maximum (485 mμ) should be taken, although it is not quite favorable in the absorbancy.

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Experimental

Paper chromatography—A paper strip, 2×15 cm., was developed by 5% aq. Na_2SO_4. The color reactions for this purpose were carried out as follows: About 2 mg. of the active methylene compounds (acetone, creatinine, or digitoxin) and about 10 mg. of picric acid were dissolved in about 3 cc. of 50% aq. MeOH and 3 drops of 10% aq. NaOH were added and allowed to stand for about 10 mins. at an ordinary temperature. Each of the color spots formed was dissolved in 50% aq. MeOH for the measurement of absorption spectrum.

Absorption spectrum—Measured by the Beckman Model DU spectrophotometer and by the Shimazu spectrophotometer. 50% aq. MeOH was used as the solvent.

Summary

The absorption spectra of the colored substances derived from the reaction between picric acid and active methylene compounds (acetone, creatinine, and digitoxin) in the presence of alkali were measured (cf. Fig. 1-1 and Fig. 3) and the chemical structures of these colored complex anions were proposed as shown by formulae (III), (IV), and (V).

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* The active methylene form of digitoxigenin was interpreted by T. Canbäck (cf. Svensk Farm. Tid., **54**, 201(1950).