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16. **Takuo Okuda** : Color Reaction of Unsaturated Organic Compounds with Benzaldehyde and Sulfuric Acid.

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A sensitive color reaction of several toxic natural products, picrotoxin, morphine, codeine, thebaine, and delphine with benzaldehyde and sulfuric acid was found by Melzer[1] and has been used for identifying these toxic compounds.[2] Later, Kreis[3] found that this color reaction is also positive with phytosterol and cholesterol. This color reaction has also been described by Klein to be a test of alkaloids.[4] However, relationship between this color reaction and chemical structure of the compounds has not been mentioned.

In the course of studies on the chemical components of *Coriaria japonica* A. GRAY, it was found that with coriamyrtin, tutin, and ψ-tutin, these reagents gave a deep purplish red color which is almost identical with the color appearing with picrotoxin, and also that among two components of the molecular compound, picrotoxin, only picrotoxinin which has a double bond is positive to this color reaction. Hydrogenation and bromination products of these compounds were negative to this color reaction.

Taking the fact that all the compounds tested by Melzer and Kreis have double bond into consideration, this color reaction was tested with various kinds of compounds and this color reaction was found to be generally applicable to aliphatic compounds which have a double bond or a triple bond, except for certain types of compound:

(I) Compounds which are positive to the color reaction :

1) Compounds which have simple C=C double bond with at least one H :

Type of double bond	Compound
$H_2C=CH-$	Eugenol, safrole, and styrene.
$H_2C=C\langle$	Coriamyrtin, picrotoxinin, tutin, ψ-tutin, alantolactone, lupeol, and betulin.
$-CH=CH-$	Morphine, codeine, hydnocarpic acid, chaulmoogric acid, and chaulmoogryl alcohol acetate.
$-CH=C\langle$	Isoeugenol, isomyristicin, β-amyrin, thebaine, and cholesterol.

Compounds which have two of above types of double bond : Linalool, limonene, and aucubin.

2) Compound which readily forms a double bond by mineral acid : Terpine hydrate.

3) Compound which has a triple bond : Phenylacetylene.

4) Others : Acenaphthene.

(II) Compounds which are negative to this color reaction :

1) Saturated Compounds :

a) Hydrogenation and halogenation products of the compounds of (I): Dihydrocoriamyrtin, dihydropicrotoxinin, bromocoriamyrtin, bromopicrotoxinin, tetrachlorolimonene, and dihydrochaulmoogryl alcohol acetate.

b) Saturated natural products : Picrotin, coniin, pelletierine, atropine, scopolamine, cocaine, and deoxycholic acid.

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2) Compounds which have $\rangle C=O$ adjacent to one of the carbons of the double bond.
   a) Acids and esters : Maleic acid, dimethylacrylic acid, and methyl cinnamate.
   b) Lactones : Coumarine and esculetine.
   c) Others : Rapanone, santonin, meconic acid, myricetin, naringin, and acacetin.

3) Compounds with a substituted double bond :

Type of substitution	Compound
Halogen	Tetrachloroethylene and ω-bromostyrene.
NO_2	ω-Nitrostyrene.

4) Aromatic, heterocyclic, and other compounds : Papaverine, apomorphine, narcotine, emetine, naphthalene, anthracene (gives a faint green color), nicotine, caffeine, thiamine, hinokitiol, colchicine, ethylmercaptan, and *p*-chlorophenylmercaptan.

Jegosapogenol, which has a tetrasubstituted double bond, showed a faint coloration. Hydroxyl group attached to a carbon adjacent to the double bond seems not to hinder the color reaction, as in the case of morphine and codeine. Generally the color was faint when any element or group other than hydrogen is attached to one or both carbons of the double bond.

When this color reaction is compared with the color reaction of double bonds by tetranitromethane,[5,6] some similarity is observed although the latter is positive with aromatic compounds and mercaptans : 1) Both reactions are negative with α,β-unsaturated carboxylic acids. 2) Generally affected by substitution of the compound on a carbon adjacent to the double bond. 3) Remarkable red color appears with acenaphthene. As a method of identifying aliphatic compounds which have $C=C$ double bond, this color reaction with benzaldehyde and sulfuric acid gives clearer result because of its specificity and intensity of the color.

Benzaldehyde itself turns yellow when mixed with sulfuric acid and changes to faint pink in 20~30 minutes. However, these colors can be easily discriminated from the color appearing in the presence of aliphatic unsaturated compounds. Some unsaturated compounds like coriamyrtin and picrotoxinin give reddish color with sulfuric acid. The color in this case is not so marked as the color which appears when benzaldehyde is added with sulfuric acid.

Similar color reactions of picrotoxin with benzotrichloride and sulfuric acid,[1] and also with benzalchloride and sulfuric acid[7] have been reported. Attempt was made to apply these color reactions to the compounds which were positive to the color reaction with benzaldehyde and sulfuric acid, and it was found that the former two reactions are not so clear as that with benzaldehyde.

Isolation of the colored substance could not be made due to its unstableness against moisture. The color disappers slowly when exposed to air and disappears rapidly when a few drops of water is added. It can be kept longer in a desiccator.

Experimental

Color Reaction with Benzaldehyde and Sulfuric Acid——A drop of benzaldehyde was added to cover a piece of crystal or a drop of liquid sample on a watch glass and then a drop of sulfuric acid was added. The color appeared around the sample generally in half a minute. In order to prevent appearance of the yellow color which comes out by the action of sulfuric acid to benzaldehyde, a solution of benzaldehyde in EtOH (20~50%) can be used instead of benzaldehyde.

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Summary

The color reaction with benzaldehyde and sulfuric acid known to identify picrctoxin was found to be a sensitive color reaction common to aliphatic compounds in general which have a C=C double bond. This color reaction and the color reaction of double bond by tetranitromethane are similar in some respects, however, the former is specific to aliphatic unsaturated compounds.

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17. **Masuo Akagi, Setsuzo Tejima, and Masanobu Haga :** Reaction Products of Glucuronic Acid with Phenylhydrazine.

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Since, in 1878, D-glucuronic acid was found in nature, it has been studied by many investigators chemically and biochemically.

Hitherto several reports dealing with the glucuronic acid phenylosazone also have been published, however, there are numerous discrepancies among them (Table I). For the cause of these discrepancies, it seemed that, in that time, glucuronic acid was prepared from natural products so the reported osazone might be contaminated, or that glucuronic acid is able to react with phenylhydrazine in various ways so the osazone might be corresponded to one of the isomers.

Previously, two kinds of crystals[1] (m.p. 170° and m.p. 124°) were obtained in our laboratory during the identification of glucuronic acid through its osazone. In this paper, the structures of crystals aforesaid are discussed and another crystalline derivative is also described.

Generally, glucuronic acid has two functions to be able to react with phenylhydrazine : formyl and carboxyl group; and on one hand, glucuronic acid exists in two states: glucofuranurono-γ-lactone (I) and glucopyranuronic acid (II). It is sufficiently probable that the reaction products vary according to (I) or (II), since carboxylic acid does not form phenylhydrazide by only heating on a steam bath, while lactone is able to form it. From this assumption, the authors examined the reaction of (I) and (II) with phenylhydrazine individually.

One part of sugar, two parts of phenylhydrazine and two parts of 50% acetic acid were dissolved in twenty parts of water and resulting solution was heated on a steam bath, the reaction was controlled by varying the heating period. As to (I), the mixture changed its color to yellow after *ca.* 5 minutes, to turbid red after *ca.* 15 minutes and yielded dark red syrup ultimately. In the course of reaction, the yellow solution gave pale yellow needles (S_1) m.p. 170~171° by cooling, but the authors failed to obtain constitutionally definite substance from aforesaid dark red syrup. Likewise, (S_1) was obtained either from methyl glucuronate (III) under similar conditions as stated above, or by

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