Organic Spot Tests. I. Detection of Acidic Compounds*

By Yujiro Nomura

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The familiar and sensitive Griess test for nitrites has been found to be used conversely for the detection of very small quantities of acidic compounds. Thus, the mixed solution of sodium sulfanilate, sodium nitrite and α -naphthylamine in dilute alcohol or dioxan produces intense red color on adding a tiny quantity of various acidic compounds. This color reaction depends on the formation of the red azo dye through the following successive reactions:

$$HA \rightleftharpoons H^{+} + A^{-} \qquad (1)$$

$$NaNO_{2} + H^{+} \rightleftharpoons HNO_{2} + Na^{+} \qquad (2)$$

$$NaO_{3}S - \bigcirc \longrightarrow -NH_{2} + HNO_{2} \rightarrow$$

$$NaO_{3}S - \bigcirc \longrightarrow -N \equiv N + OH^{-} + H_{2}O \qquad (3)$$

$$NaO_{3}S - \bigcirc \longrightarrow -N \equiv N + \bigcirc -NH_{2} \rightarrow$$

$$NaO_{3}S - \bigcirc \longrightarrow -N = N - \bigcirc -NH_{2} + H^{+} \qquad (4)$$

The reactions 3 and 4 proceed so rapidly and completely that the equilibria 2 and 1 are shifted toward the right successively. Accordingly, by virtue of the equilibrium disturbance it is possible to detect many organic acids which are not very soluble and are so weak that they do not affect usual dyestuff indicators at all or do indecisively at most.

Reagents.—1) 2.9 g. of sodium sulfanilate $NH_2C_6H_4SO_3Na\cdot 2H_2O$ and 0.7 g. of sodium nitrite are dissolved in 30 cc. of distilled water.

2) 1.8 g. of α -naphthylamine is dissolved in 40 cc. of ethanol or dioxane.

The reagent solution is prepared by

mixing two solutions 1) and 2) just prior to use.

Procedure.—A few drops of the reagent solution are placed in a depression of a spot plate. A small amount of the test material (a few crystals or the residue after evaporating a drop of a test solution) is added. Depending on the acidity and solubility of the acid present, a red or orange color appears at once or after a few minutes. A comparison blank test is advisable when dealing with a slight amount of the sample.

Results.—The limits of detection attained with various acids were $25 \sim 40 \gamma$. The following compounds were tested.

Immediate intense red color reaction.— Formic acid, acetic acid, propionic acid, butyric acid, isovaleric acid, caproic acid, oxalic acid, malonic acid, succinic acid, adipic acid, fumaric acid, maleic acid, lactic acid, thioglycolic acid, malic acid, citric acid, tartaric acid, benzoic acid, salicylic acid, anthranilic acid, sulfanilic acid, benzenearsonic acid, hippuric acid and picric acid.

Immediate orange to orange-red color reaction.—Caprylic acid, pelargonic acid, capric acid, palmitic acid, stearic acid, oleic acid, elaidic acid, erucic acid, cholic acid, aspartic acid and glutamic acid.

Orange color reaction within a few minutes.

-Lauric acid, myristic acid and 12-hydroxyoleic acid.

Negative reaction.—Phenol, cresol, catechol, resorcinol, hydroquinone, ethyl acetate, ethyl benzoate, diethyl malonate, benzoylacetone, ethyl acetoacetate, acetanilide, succinimide and ammonium salts or metal salts of carboxylic and sulfonic acids.

 α -Amino acids except aspartic acid and glutamic acid gave no response to the reagent solution. But after digesting with two or three drops of formaline they showed positive reactions. The following amino acids could be detected in this manner: glycine, alanine, cystine, valine, methionine, leucine, isoleucine, phenylalanine, tyrosine, histidine and asparagine.

Department of Chemistry College of General Education The University of Tokyo Meguro-ku, Tokyo

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