A new color reaction with potassium permanganate and bromophenol blue on thin-layer chromatograms

A new color reaction was devised in the course of studies on thin-layer chromatography of antibiotics.[1] When organic compounds are detected by means of potassium permanganate alone, the sensitivity is not very high and the color becomes vague with passage of time. The authors found that, if 0.2% aqueous bromophenol blue solution is sprayed about 10 or 15 min after treatment with 0.5% aqueous potassium permanganate solution at room temperature, a blue or greenish blue color appears depending on the degree of acidity of the reaction products resulting from the oxidation, and that the coloration lasts more than a month with enough contrast to be directly photographed. Furthermore the sensitivity is enhanced more than ten fold.

For example, for the detection of amaromycin, a macrolide antibiotic, by potassium permanganate alone, more than 1 μg was necessary. When the chromatogram was sprayed with bromophenol blue solution after the treatment with permanganate, less than 0.1 μg of the material was sufficient for detection. Phenolphthalein, litmus, or crystal violet were tried but without success. Thymol blue could be used instead of bromophenol blue when the reaction products had a higher acidity.

All antibiotics studied by thin-layer chromatography by the authors[1] were detected by means of this color reaction, except antimycin A, griseofulvin, amidinomycin, and fradiomycin (neomycin); these were detected with a mixture of 1% methyl red and bromothymol blue solution instead of bromophenol blue solution.

The color reaction described in this paper can be generally applied to the detection of other organic substances, for instance, some amino acids, peptides, monosaccharides and unsaturated organic compounds. Results of the color reaction with sugars, organic acids, amino acids and others are shown in Table I. Since most of the known antibiotics are polyfunctional, we can expect that at least one of the functional

TABLE II

COLOR REACTIONS OF VARIOUS ORGANIC COMPOUNDS WITH POTASSIUM PERMANGANATE AND BROMOPHENOL BLUE

Tests were run with two kinds of plates: Silicagel (Wölm) and Alumina (pH 3.6) (Wölm)

	Potassium permanganate reaction	*Bromophenol blue coloration*
Octanol	—	no spot
Glycerol	+	pH 4.0–4.2
Glucose	+*	pH 4.4–4.8
Xylose	+*	pH 4.4–4.8
Galactose	+*	pH 4.4–4.8
Fructose	+*	pH 4.4–4.8
Sucrose	—	no spot
Lactose	—	no spot
Maltose	—	no spot
Stearic acid	—	pH 2–3**
Succinic acid	+	pH 2–3**
Tartaric acid	+	pH 2–3**
Citric acid	+*	pH 2–3**
Glycine	±	practically no spot
Alanine	±	practically no spot

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TABLE I (*continued*)

	Potassium permanganate reaction	*Bromophenol blue coloration*
Phenylalanine	±	practically no spot
Leucine	±	practically no spot
Valine	—	no spot
L-Cysteine	—	no spot
Aspartic acid	±	practically no spot
Glutamic acid	±	practically no spot
Lysine	±	practically no spot
Threonine	+*	pH 4.2–4.4
Serine	+*	pH 4.4
Proline	+*	pH 2–3**
Hydroxyproline	+*	pH 4.8
Methionine	+	pH 4.2–4.4
Tyrosine	+*	pH 4.0
Tryptophan	+	pH 4.4
Arginine·HCl	+*	pH 4.4
Histidine·HCl	+*	pH 4.0
Cholesterol	+*	pH 4.4
Acetylacetone	+	pH 4.4–4.8
Crotonic acid	+	pH 4.6
Xylol	—	no spot
Phenol	—	no spot
β-Naphthol	+*	pH 4.4–4.8
Anthraquinone	—	no spot
Anthrone	+	pH 3.6–4.0
Benzoic acid	±	pH 2–2.4**
p-Dimethylamino-benzaldehyde	+	pH 4.4–4.8
Benzidine	green	——
o-Dianisidine	green	——

* Spot appeared slowly.
** Detected by both bromophenol blue and thymol blue.

groups will react with potassium permanganate to give a detectable spot on the plate. Substances indifferent to potassium permanganate at room temperature, such as simple aromatic substances, disaccharides or straight chain saturated aliphatic acids cannot be detected. Depending on their acidity the acids can be detected either by thymol blue or by bromophenol blue.

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