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A Convenient Method for Detection of NH Containing Compounds on Thin Layer Chromatograms

Yasuo ARIYOSHI, Naotake SATO, Hideo ZENDA,* and Kikuo ADACHI*

Central Research Laboratories, Ajinomoto Co., Inc., Suzuki-cho, Kawasaki, Kanagawa
**Katsura Chemical Co., Inc., Kurihara, Zama, Kanagawa*

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The technique of thin layer chromatography is frequently used to detect various compounds in the course of studies of amino acids or peptides. The compounds with amino or imino group can be easily detected with ninhydrin at μg level on chromatogram. However, the compounds without amino or imino group, such as N-protected amino acids and cyclic peptides, cannot be detected with this reagent. It is known that the compounds with NH group can be detected by chlorination of NH to NCl and subsequent spraying with starch-potassium iodide. As chlorinating reagent, chlorine gas[1-4] and aqueous sodium hypochlorite[5-6] were used. In some cases, they cause background coloration and give indistinct chromatograms. This can be improved by use of tertiary butyl hypochlorite,[7] but this reagent is unstable and expensive.

We will report here a convenient method of chlorinating NH group on a thin layer chromatogram of silica gel. When the silica gel chromatogram was exposed to gas generated from bleaching powder (calcium hypochlorite) and hydrochloric acid, NH group was found to be easily chlorinated in a short period of time at room temperature. Subsequent sprayings of ethanol and aqueous starch-potassium iodide gave a clear violet spot on chromatogram without any background coloration.

This method is recommended for detection of peptides, cyclic peptides, amino acid esters, amino acid amides, N-protected amino acids. It is also applicable to other NH containing compounds such as nucleosides and related compounds and acid amides. The detectable threshold of amino acids or peptides is 0.1—0.25 μg after chromatography. Table 1 shows the results when glass plate coated with silica gel G of 0.25 mm thickness was charged with 0.5 μg of various compounds, developed in butanol-acetic acid-water (4:1:2) system, and stained by this method.

TABLE 1. COLOR INTENSITY AND R_f VALUE OF VARIOUS COMPOUNDS

Compound	R_f value	Color intensity
Ala	0.31	W
β-Ala	0.34	S
Arg·HCl	0.14	S
Asp	0.21	S
Cys	0.15	W
Gln	0.27	S
His	0.33	W
Hyp	0.43	S
Leu	0.53	W
Lys·HCl	0.10	S
Ser	0.29	S
Trp	0.65	M
α-Asp-Phe	0.58	M
β-Ala-Tyr	0.41	S
Gly-Leu	0.73	S
Cyclo-(α-Asp-Phe-)	0.78	S
Cyclo-(Phe-Ala-)	0.75	S
Cyclo-(Phe-Val-)	0.86	S
Z-Asp	0.90	M
H-Phe-OMe·HCl	0.67	S
Pyroglutamic acid	0.59	W
Hypoxanthine	0.52	W
5′-Cytidylic acid	0.38	W
Acetanilide	0.85	S

Abbreviations used are: S, strong; M, medium; and W, weak coloration

Experimental

Five μl of sample solution was spotted on a silica gel G plate (5×20 cm) of 0.25 mm thickness. The plate was developed with *n*-butanol-acetic acid-water (4:1:2 v/v). After being dried, the plate was put in a cylinder (22 cm in height and 7 cm in diameter), at the bottom of which a small beaker (1.5 cm in height and 3 cm in diameter) containing 3 g of calcium hypochlorite (available chlorine content is 60%) was placed. The back of the plate was preferably placed to face the beaker to keep the chromatogram from spots caused by

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dispersed bleaching powder on addition of hydrochloric acid. One m*l* of concentrated hydrochloric acid was added to the bleaching powder in the beaker, and the cylinder was covered loosely with a glass plate. Chlorination proceeded at room temperature in 7—10 min. The plate was removed from the cylinder, exposed to a stream of air for 1 min, and then sprayed with a small amount of ethanol. After being dried in a rapid current of air for 1—2 min, the plate was sprayed with a starch-iodide reagent prepared by dissolving 0.5 g of soluble starch and 0.5 g of potassium iodide in 100 m*l* of water. Violet spots of the compounds in the sample appeared immediately on the clear background.