The Journal of the Korean Chemical Society
Volume 8, Number 2.
Printed in Republic of Korea

Spot Test for Amins Acids with Alloxan

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(Received June 3, 1964)

Alloxan에 의한 Amino Acids의 Spot Test

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(1964, 6, 3 수리)

요 약

Alloxan에 의한 Amino acids의 spot test에 있어서, alloxan 수용액에 lactose를 가해 주면, alloxan 자신의 착색을 막을 수 있다. lactose를 포함하는 alloxan 수용액은 매우 안정하여서, 실온에서 수 개월 동안 두어도 착색되지 않으며, lactose의 존재로 말미암아, alloxan과 amino acids와의 색반응은 조금도 영향을 받지 않는다. 이 시약에 의한 amino acids의 spot test 법은 ninhydrin에 의한 spot test 보다 예민하고, 또 alloxan 또는 dimethyl-alloxan을 발색약으로 사용하는 paper chromatography 법에 비해, proline(1 γ), hydroxyproline (5 γ)를 확인할 수 있다는 장점을 가지고 있다.

Abstract

In order to stabilze alloxan as a reagent for detection of amino acids by spot test, sugars and other reductants were added to the aqueous alloxan solution. It was found that lactose was the best for the purpose. The alloxan reagent containing lactose did not give color change on blank test and was very stable that there was no color change even it was allowed to stand in room temperature for several months. The color reaction with amino acids and some amines was not affected by lactose. This spot test for amino acids is in sensitivity as comparable to that of the previously reported methods and gave color reaction with proline and hydroxyproline to 1γ and 5γ respectively.

Introduction

Previously numerous methods for the detection of amino acids on paper chromatogram, using alloxan as a reagent, have been reported [1], [2], [3], [4], [5], [6], [7], [8], [9], and recentlly Akabori *et al* [10] described that amino acids can be detected with dimethylalloxan instead of alloxan reagent. It is shown that these tests are in sensitivity comparable with that of ninhydrin and are well recognized usefulness of the detection on amino acid. However, alloxan and dimethylalloxan reagent are so unstable that they are colored within a few days when are exposed at room temperature or stocked in refrigerator, and it should be freshly prepared every time before use them. Also the former reagent does not give color with proline and hydroxyproline and the later reagent was negative with hydroxyproline.

In relation to studies on the toxic substances in korean scabby barley[11], the authors was attempted to

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detect choline, which is proposed to be a toxic principle of the infected grain, with alloxan by Klein's method [12]. But we could confirm that the spot test did not give a reliable result because alloxan is very unstable and on blank test it gave also color change.

In the detection of choline and amino acids by the spot test with alloxan, it was desirable to stabilize alloxan with some proper reductants, and for that purpose we tested the following compounds which were mixed with alloxan solution in turn, then it was used as a reagent, i.e., formalin, formic acid, ascorbic acid, hydroquinone and sugars (glucose, fructose, galactose, xylose and lactose). We found lactose was the best among those reductants for the purpose.

The prepared alloxan reagent, which contained lactose did not give color change on blank test of the spot test, was very stable that there was no color change of itself even it was allowed to stand in room temperature for several months, and was not affected by lactose on its color reaction with amino acids and some amines. By this method, proline and hydroxyproline could be also detected to 1γ and 5γ respectively.

Experimental

1. Reagent. With following method alloxan was purified and used throughout the experiments. Alloxan (25g), reagent grade and made by E. Merk AG, was dissolved in 32ml. of H_2O at 80°C. and filtered. The residue was discarded and the filtrate was in vaccum (15—20mm) concentrated. The resulting crystal was again dissolved in 32ml. of H_2O at 80°C. and the above process repeated twice, then the crystal was dissolved in minimum quantities of water, and the solution was poured into about three times of its volume of glacial acetic acid. This solution was allowed to stand in refrigerator for 24 hours and white needle crystals was obtained (mp. 244—245°C, dec.)

The purified alloxan did not contain alloxantin; its aqueous solution did not reduce silver ion, and there were no color change when $Ba(OH)_2$ solution was added.

2. Reagent solution. Aqueous solution of 0.08% alloxan containing 0.25% lactose.

3. Sample solution. At first 0.1% of each sample (E. Merk made, reagent grade) solution was prepared (if the samples was insoluble, added a few drops of 2N-HCl solution to dissolve.), then the solution was diluted to 50 times of its volume in turn and used as a sample solution.

4. Experimental method. On the steam bath, crucible lid was laid up side down, and two drops (one drop equals to 0.01 ml.) of alloxan reagent solution was separatory dropped on two spots by a capillary.

The spots were evaporated, almost to dryness, 0.01 % sample solution was then dropped by micropippet on it. Drying the spotted sample solution, red color was appeared, but there was no color change on the spot where blank test was pursued.

Repeated the procedure with the sample solution that are diluted to 50 times of its volume with H_2O and determined the limit of identification of the sample.

We also checked the stability of alloxan reagent and its sensitivity with time. The reagent was so stable that there were no color change, even it was allowed to stand in room temperature for 4 months, and the sensitivity of color reaction was not interfered when the reagent used after it was prepared and stocked in room temperature for 4 months.

Results

Table 1 shows limits of identification of some amino acids and other compounds by this method. The data are compared with those of paper chromatography by Saifer and Oreskes[8].

The detection of amino acids by this method is superior to that of spot test of amino acid by ninhydrin[13] in sensitivity, and need not to prepare buffer solution. The sensitivity of this test is as comparable to that of Saifer's paper chromatographic method in which alloxan is used as a color developing reagent. As shown in Table 1, this tests with as asparagine, glycine, leucine, threonine and tryptophane are more sensitive than color reaction on paper chromatogram. Moreover in contrast to previously reported tests [8],[10] in which the reagent show no reaction with proline and hydroxyproline, this spot test gives color reaction with them to 1γ and 5γ respectively. It will be cited that the alloxan reagent containg lactose could be used as color developer for paper chromatography of amino acids.

Table 1. Limits of Identification of Some Amino Acids with Alloxan as a Reagent of Spot Test.

Amino acids	Limit of identification		Color developed
	0.08% Alloxan & 0.25% Lactose	Alloxan in acetone[b]	
Alanine	0.5γ	0.4γ	Red-violet[b]
B-Alanine	0.5	0.5	Red-violet
Arginine HCl	0.5	1.0	Red
Asparagine	0.5	1.3	Red
Cystine[a]	0.5	0.8	Red
Ethionine	1.0	0.2	Red
Glycine	0.01	0.4	Red-violet
Glycylglycine	0.5	—	Violet
Histidine 2HCl	1.0	1.0	Red
Hydroxyproline	5.0	NR	Red-violet
Isoleucine	1.0	0.7	Red
Leucine	0.05	0.7	Red-violet
Lysine [a]	0.5	0.5	Red
Methionine	0.5	0.8	Rep
Phenylalanine[a]	1.0	0.8	Red
Proline	1.0	NR	Red-violet
Serine	0.5	0.5	Red
Threonine	0.05	0.6	Red
Thyroxine	1.0	—	Red
Tryptophane	0.1	1.0	Red-violet
Tyrosine [a]	1.0	2.0	Dark-yellow
Valine[a]	1.0	0.6	Red-violet
Acetylcholine	10.0	—	Violet
Choline	50.0	—	Violet
Creatine	10.0	—	Violet
Urea	5.0	—	Violet

a) 1—3 drops of 2N-HCl solution was added to dissolve.
b) The color was changed from red to violet according with the quantities of sample solution was diluted.

Acknowledgements

We are indebted to Dr. Kun Bae Lee, Chief of the Bilogical Division, the Institute of Atomic Energy Research, Republic of Korea, for his kind advice to carry out the experiments.

Reference

1). Hurtley, W. H., Wooton, W. O., *J. Chem. Soc.*, **99**, 288 (1911)
2). Lieben, F., Edel, E., *Biochem. Z.*, **244**, 403 (1932)
3). Abderhalden, R., *Z. Physiol. Chem.*, **252**, 81 (1938)
4). Copley, G. N., *Analyst*, **66**, 492 (1941)
5). Barakat, M. Z., Wahba, N., El-dada, M. M., *Analyst* **78**, 715 (1954)
6). Noworytko, J., Sarnecka-Keller, M., *Acta biochem. polon* **2**, 91 (1955)
7). Tagaki, E., Mangyo, M., *Bull. Chem. Soc. Japan* **28**, 212 (1955)
8). Saifer, A., Oreskes, L., *Anal. Chem.*, **28**, 501 (1956)
9). Ogawa, S., Kochi, Y., Nishimura, H., Eisei Shikenjo Hokoku No. **76**, 21 (1958)
10). Akabori, S., Narita, K., Fujii, Y., Ishii, *J., J. Biochem.* (Tokyo) **50**, 424 (1961)
11). T. B. Kim, B. S. Hahn, this journal **8**, 82~84 (1964)
12). Paech, K., Tracey, M. V., (Editor) *Moderne Methoden der Pflanzenanalyse* **4**, 562 (1955). Springer Verlag Berlin.
13). Feigl, F., Spot tests in Organic Analysis p. 293, 6th English Edition, 1960 Elsevier Publishing Co., New York.