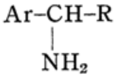
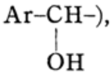
On the Specific Coloration of the Benzylamine Type Compounds in the Ninhydrin Color Reaction. I.[*]

By Eiichi TAKAGI, Mitsuo MANGYO, Masanobu SAWAI and Isao ENSAKA

(Received September 10, 1954)

During the course of an investigation concerning reactions between aromatic aldehydes and α-amino acids[1], the authors noticed the specific coloration of the benzylamine type compounds with ninhydrin in paper chromatography.

We thought that if the amines have the following constitution,  $\mathrm{Ar{-}\underset{NH_2}{\underset{|}{C}H}{-}R}$ (Ar = Aryl group; R = H or  $\mathrm{Ar{-}\underset{OH}{\underset{|}{C}H}{-}}$), yellow coloration will appear and then after an interval change to purple. Satake[2] pointed out a similar phenomenon, but details were not mentioned.

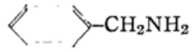
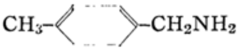
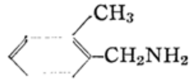
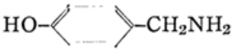
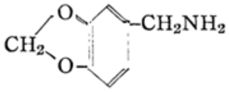
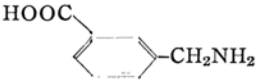
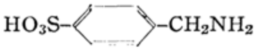
As the authors believed that this specific coloration could be a convenient and simple method for detecting a specific benzylamine-skeleton in a molecule, we studied several similar compounds and already published some of the results of the thesis[3].

Further studies were made on the same phenomenon using other substances and also using another reagent, alloxan. The present paper summarizes evidences obtained since the previous work.[1]

Coloration with Ninhydrin

The results with ninhydrin and alloxan color reactions are summarized in Table I.

TABLE I

No.	$\mathrm{Ar{-}\underset{\underset{|}{NH}}{\underset{|}{C}H}{-}}$ type compounds	Specific ninhydrin color reaction	Specific alloxan color reaction	*Rf* value	Synthetic methods of the compounds*
1		+	+	0.65	E. Takagi et al[4]
2		+	+	0.71	F. Lustig[5]
3		+	+	0.71	H. Strassmann[6]
4		+	+	0.55	M. Tiffeneau[7]
5		+	+	0.64	E. Takagi[8]
6		+	+	0.62	E. Takagi[8]
7		+	+	0.58	F. F. Blicke et al[9]
8		+	+	0.50	A. Einhorn[10]
9		+	+	0.09	Sample No. 10 is hydrolysed with sulfuric acid.

---

[*] Continuation of the paper: E. Takagi and M. Mangyo, *J. Pharm. Soc. Japan*, **72**, 812-5 (1952).

[1] loc. cit.

[2] K. Satake, "Chromatography" (Kyoritsu Zensho) (1951), p. 88.

[3] 6th Meeting of the Chemical Society of Japan, held at Kyoto on April 4, 1953.

[4] E. Takagi, I. Ensaka, M. Mangyo and M. Sawai, *J. Pharm. Soc. Japan*, **73**, 1086 (1953).

[5] F. Lustig, *Ber.*, **28**, 2988 (1895).

[6] H. Strassmann, *Ber.*, **21**, 577 (1888).

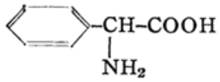
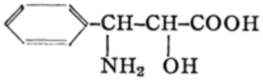
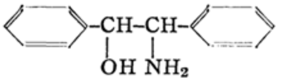
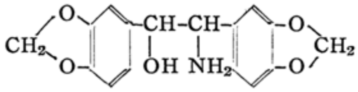
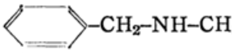
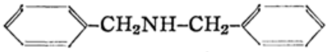
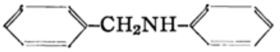
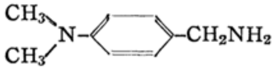
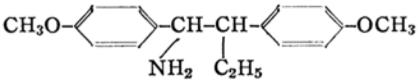
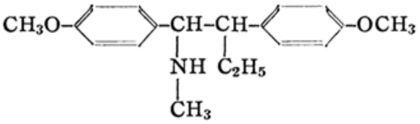
[7] M. Tiffeneau, *Bull. soc. chim. France*, **9**, 823 (1911).

[8] E. Takagi, *J. Pharm. Soc. Japan*, **71**, 656 (1951).

[9] F. F. Blicke and W.M. Lilienfeld, *J. Am. Chem. Soc.*, **65**, 2281 (1943).

[10] A. Einhorn, *Ann.*, **343**, 295 (1905).

TABLE I (Concluded)

No.	Ar-CH- type compounds NH	Specifiic ninhydrin color reacton	Specific alloxan color reaction	*Rf* value	Synthetic methods of the compounds*
10		+	+	0.37	Commercial substance made by the Wako Pure Chemical Industries, Ltd.
11		+	+	0.48	C. S. Marvel et al⁽¹¹⁾
12		+	+	0.59	M. O. Forster et al⁽¹²⁾
13		+	+	0.81	E. Takagi et al⁽⁴⁾
14		+	+	0.74	E. Takagi⁽⁸⁾
15		+	+	0.70	E. L. Holmes et al⁽¹³⁾
16		+	+	0.67	A. Galat et al⁽¹⁴⁾
17		−	−	**	Org. Synth⁽¹⁵⁾
18		(+)	−	0.65	***
19		+	−	0.87	***
20		+	−	0.88	***

* Literature cited for the synthesis.

** Clear spot is not obtained.

*** We are indebted to Mr. T. Takahashi of the Department of Chemistry, Faculty of Science, Tokyo University, for these substances.

According to results obtained, with the exceptions of *p*-dimethylaminobenzylamine (18) and benzylaniline (17), all substances caused specific colorations on filter paper with ninhydrin, i.e., first yellow coloration which then changed to purple. *p*-Dimethylaminobenzylamine (18) developed at first an orange red color, but being comparatively stable, changed to purple very slowly. In the case of benzylaniline (17), we could not obtain a clear spot and moreover the color developed was purple-without passing the yellow stage, i.e., this substance resembled aniline in its behaviour.

In the samples of Table I, if the *p*-position of aminomethyl group is substituted with hydroxyl or methoxyl group, or 3,4-positions are substituted with methylenedihydroxyl group, the rate of color change was slow. This was especially so in the case of *p*-hydroxybenzylamine (4), which took a very long interval (few weeks) to change its color.

We can show other kinds of substances, too, which cause similar specific coloration with ninhydrins. The results with these other kinds of substance are summarized in Table II.

---

11) C.S. Marvel and W.A. Noyes, *J. Am. Chem. Soc.*, **42**, 2264 (1920).

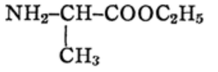
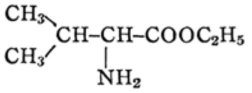
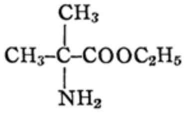
12) M.O. Forster and K.A. RaO, *J. Chem. Soc.*, **1926**, 1947; K. Vogler, *Helv. Chim. Acta.* **33**, 2111 (1950).

13) E.L. Holmes and K. Ingold, *J. Chem. Soc.*, **127**, 1813 (1925).

14) A. Galat, and G. Elion, *J. Am. Chem. Soc.*, **61**, 3585 (1939).

15) Org. Synth., Vol. 8, 38 (1928).

TABLE II

No.	Substances	Specific ninhydrin color reaction	Specific alloxan color reaction	*Rf* value	Synthetic methods of substances
1	NH_2–CH_2CO–$NHCH_2COOH$[2][20]	+	–	0.11	Commercial substance made by Wako Pure Chemical Industries, Ltd.
2	NH_2–CH_2–$COOC_2H_5$[2]	+	–	0.52	T. W. Curtius[16]
3	 $NH_2-\underset{\displaystyle CH_3}{\underset{\vert}{C}H}-COOC_2H_5$	+	–	0.64	T. W. Curtius[16]
4		–	–	0.85	T. W. Curtius[16]
5	 $CH_3-\overset{\displaystyle CH_3}{\overset{\vert}{\underset{\displaystyle NH_2}{\underset{\vert}{C}}}}-COOC_2H_5$	–	–	—	A. L. Barker et al[17]

Glycylglycine (1), glycine ethylester (2) and alanine ethyl ester (3) became yellow at first and then changed to purple.

Generally, the rates of the color changes of these substances in Table II are much greater than those of the substances in Table I.

Especially in the case of alanine ethyl ester (3), the color change occurred instantly. We could not find a yellow stage in the case of isoleucine ethyl ester (4) but it became purple directly, as normally occurs. Ethyl-α-aminoisobutyrate (5) gave no color with ninhydrin, which resembled that of the *tert*-butylamine[18] due probably to lack of hydrogen atom where carbon atom is linked to the amino group.

Coloration with Alloxan*

As the intensity of coloration with alloxan was much weaker than that of the ninhydrin, we were obliged to change experimental conditions (cf. experimental). The results obtained are shown in the section of alloxan in Table I and II.

Generally, in the samples of Table I, the color first became yellow, then changed to orange in the middle stage and finally to a pink color. The relations between the rates of the color changes and the constitutions of the substances seemed to be similar to the tendency in the case of ninhydrin. However the yellow stage of α-aminophenylacetic acid (11) and β-amino-β-phenyl-lactic acid (12) were relatively long (about one day), being different from that of ninhydrin.

Contrary to the case of ninhydrin, the substances (Nos. 1, 2, 3 and 4) in Table II developed an orange or pink color directly without first passing through the yellow stage. Ethyl α-aminoisobutyrate (5) developed no color as in the case of ninhydrin.

Factors Affecting Color Change

We sought the factor affecting color change to solve the problem, i.e., what causes color change.

The results were:

(1) After having removed the specimens to a dark place at the first color stage, further color change took place.

(2) When kept in a vacuum desiccator, they did not change color but when they were brought into contact with air, they began to change color.

(3) When kept in an atmosphere of nitrogen gas with some water vapour, normal color change occurred.

(4) If the specimens were exposed to steam, the color change occurred very rapidly.

Accordingly, the rate of the color change is dependent on the degree of the humidity of air and to the chemical structure of the substances at a given temperature*. The mechanism of the color change will be discussed later[19].

---

[16] T.W. Curtius, *J. prakt. Chem.*, (2) 37, 159 (1888).

[17] A.L. Barker and G.S. Skinner, *J. Am. Chem. Soc.*, 46, 405 (1924).

[18] R. Moubasher and A. M. Othman, *J. Am. Chem. Soc.*, 72, 2666 (1950).

* We are indebted to Prof. K. Miyagi of the College of Pharmacy, Chiba University for this reagent.

[19] Paper II. *J. Chem. Soc. Japan* (in press).

* When the samples were treated with water, the color change occurred comparatively slowly, showing that the rate of color change is affected by temperature.

Experimental

I. On the Paper Chromatography.—The authors carried out the paper chromatography in the usual manner. Details are as follows.

Sample: 0.5% aqueous solutions of the amino-carboxylic acids or the amino sulfonic acid. In the case of amines, 0.5–1% aqueous solutions of their hydrochlorides.

1/1000 cc. of the samples are spotted on the filter paper at the point, 3 cm from the end.

Filter paper: Toyo Roshi No. 2, 2 cm × 20 cm.

Developing method: One dimensional ascending method.

Developing solvent: Mixture of *n*-butanol, acetic acid and water (4 : 1 : 1).

Developing temperature and time: 15–18°C, 3–4 hrs.

Developing distance to the solvent front from the original point: 13–15 cm.

Reagent: 0.2% ninhydrin solution in *n*-butanol.

In the case of alloxan the coloration was very weak as mentioned before, so we examined the color reaction by spot tests on filter paper using 0.5% alloxan solution in *n*-butanol.

II. On the Factor Affecting Color Change.—We prepared five series of the same specimens of a substance to seek the factor affecting color change and examined their behaviours as follows.

Samples: The positive substances of the specific color reaction in the Tables I and II were used. The tests of the special substances, which took a very long time or a very short time for the color change, were excluded.

Filter paper: Toyo Roshi No. 2.

Reagents; 0.2% ninhydrin and 0.5% alloxan solutions in *n*-butanol.

Color tests:

A) *Ninhydrin test.*—0.01 cc. of a sample was spotted in the same manner on five filter papers, airdried, sprayed with the ninhydrin reagent, and heated on an electric heater to develop the yellow color.

The five yellow colored specimens were examined for five series of the following tests.*

The first series of the specimens was kept in room air for control. The second was kept in a desiccator at an atmospheric pressure filled with air on calcium chloride and the third in an evacuated desiccator on concentrated sulfuric acid.

The fourth series was kept under an atmospheric pressure filled with nitrogen gas on some water, using a vacuum desiccator as the vessel, and the fifth was enclosed in a piece of black paper and was kept in a dark place.

We observed that the color change of the first series took place relatively rapidly (in several minutes to several hours); the second series caused slight color change in 100 hours, yellow-brown to brown but not to purple; the third series maintained its yellow to yellow-brown color for 100 hours; the fourth and the fifth series were similar to the first series, changing their color rapidly.

We could not observe remarkable differences among the first, the fourth and the fifth series.

B) *Alloxan test.*—In the case of the alloxan reagent, although the color changes were not identical to that of the ninhydrin, being orange in the middle stage in general, results obtained were similar. In the cases of both the ninhydrin and alloxan tests, when the specimens of the second and the third series were brought out to room air, they began to change their color in the normal manner, i.e., in ninhydrin, the color stabilized to purple and in alloxan, the color stabilized to pink.

When the specimens of the first color stage or the middle color stage were exposed to steam, they changed their color very rapidly to the stabilizing color.

The authors wish to acknowledge the support of Dr. F. Nagasawa, The Director of the Laboratory.

Summary

The specific coloration of the benzylamine type compounds in the ninhydrin color reaction is extended to other kinds of compounds. Alloxan resembles ninhydrin in its behaviour as reagent. The fact that the specific coloration reaction seems to be common to the benzylamine type compounds, is verified.

However a few exceptions are observed as in the case of benzylaniline (17) and *p*-dimethylaminobenzylamine (18). The fact that the color change of these compounds in the usual paper chromatography is due to moisture in the air, is confirmed.

Research Laboratory, Mitsubishi
Chemical Industries Ltd.
Kawasaki, Kanagawa

---

20) Akabori and Narita, *Seni Kagaku Kenkyusho Nenpo*, 5, 76 (1950).

* The room temperature was about 26°C and the humidity of the air was about 70%.