Phytochemistry, 1967, Vol. 6, pp. 1161 to 1163. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

LEAD ACETATE AS CHROMOGENIC REAGENT FOR ANTHOCYANINS*

TIBOR FULEKI† and F. J. FRANCIS

Department of Food Science and Technology, University of Massachusetts, Amherst, Mass., U.S.A.

(*Received* 25 *January* 1967)

Abstract—The color changes produced by treating anthocyanins on filter paper chromatograms with neutral and basic lead acetate are described. An ethanolic solution of neutral lead acetate is proposed as a chromogenic reagent to detect anthocyanins which contain a catechol group. The reaction with lead acetate is faster and the color change is more definite than that produced by $AlCl_3$.

INTRODUCTION

ANTHOCYANINS separated by paper chromatography are frequently treated with ammonia vapor, aluminium chloride and ferric chloride solutions as chromogenic reagents.[1] The color changes produced are useful in differentiating between the anthocyanins containing *o*-dihydroxy (catechol) group from those lacking such a reactive site. Basic and neutral lead acetate are widely used[2] to precipitate the anthocyanins. Both lead acetates are recommended as spray reagents for the yellow flavonoids,[3] but their utility as chromogenic reagents for anthocyanins has not been explored so far.

During the development of a method[2] for the quantitative determination of individual anthocyanins present in the American cranberry,[4, 5] neutral as well as basic lead acetate were tried as precipitants. While using lead acetate to precipitate the separated individual cranberry anthocyanins it was noticed that the color of the precipitated cyanidin monoglycosides was different from that of the peonidin monoglycosides. This observation suggested that lead acetate may be useful as chromogenic reagent for anthocyanins.

RESULTS AND DISCUSSION

The results obtained with the various anthocyanins representing the six important anthocyanidin types are presented in Table 1. The results show that the lead acetate treatment gave

* Part of a Ph.D. thesis by the senior author, carried out while on educational leave from the Canada Department of Agriculture, Research Station, Kentville, Nova Scotia. Contribution No. 1263 from the CDA Research Station, Kentville, N.S., and from the University of Massachusetts, Agricultural Experiment Station, Amherst, Mass.

† Present address: Research Station, Kentville, Nova Scotia.

[1] J. B. HARBORNE, *J. Chromatog.* **1**, 473 (1958).
[2] T. FULEKI, Ph.D. Thesis. University of Massachusetts, Amherst, Mass. (1967).
[3] T. B. GAGE, C. D. DOUGLASS and S. H. WENDER, *Anal. Chem.* **23**, 1582 (1951).
[4] S. SAKAMURA and F. J. FRANCIS, *J. Food Sci.* **26**, 318 (1961).
[5] C. ZAPSALIS and F. J. FRANCIS, *J. Food Sci.* **30**, 396 (1965).

a color change with every anthocyanin. However, the extent of the shift toward the blue hues was always greater with those pigments having a free *o*-dihydroxyl group in their structure, while the shift was less with the other anthocyanins. In general, the color of the anthocyanins containing an *o*-dihydroxyl group changed to blue while those lacking a free catechol group gave a violet color. The extent of the color change (Table 1) as shown by the hue numbers (the first or plate number for the color swatches) indicated that the shift was always 8 or higher for the pigments containing a catechol group and a 7 or less for the other anthocyanins. This indicated that, the "lead acetate shift" could probably be quantitated by measuring the shift of the wavelength of maximum absorption directly on the paper.

TABLE 1. COLOR REACTIONS* OF ANTHOCYANINS WITH NEUTRAL AND BASIC LEAD ACETATE

Pigment†	Untreated	Neutral lead acetate	Basic lead acetate
Pl-3-Gl	scarlet red (9A8)	grayish ruby (12C4)	purplish gray (13C2)
Pl-3, 5-Gl	orange red (8A6)	purplish pink (14A5)	pastel violet (15A4)
Cy-3-Gl	pink (13A3)	pastel blue (21A4)	grayish blue (22B4)
Cy-3-Ga	pink (13A3)	pastel blue (21A4)	grayish blue (22B4)
Cy-3-Ar	pink (13A3)	pastel blue (21A4)	grayish blue (22B4)
Cy-3,5-Gl	pink (13A3)	pastel blue (21A4)	pale blue (23A3)
Pn-3-Ga	pink (13A3)	grayish violet (18B3)	grayish violet (18B4)
Pn-3-Ar	pink (13A3)	grayish violet (18B3)	grayish violet (18B4)
Dp-3-p-coumaroyl-rutinoside-5-Gl	grayish magenta (13B4)	pale blue (22A3)	pastel blue (22A4)
Pt-3,5-Gl	purplish pink (14A4)	pale blue (22A3)	pale blue (22A3)
Mv-3,5-Gl	purplish red (14B2)	grayish violet (19B3)	grayish violet (19C3)

* The values in parenthesis give the designation of the corresponding color swatches in the Reinhold Color Atlas.[7]

† Pl=pelargonidin, Cy=cyanidin, Pn=peonidin, Dp=delphinidin, Pt=petunidin, Mv=malvidin, Gl=glucoside, Ga=galactoside, Ar=arabinoside.

Since the lead acetate increased the pH considerably (the pH was 6·5 and 7·0 for the neutral and basic lead acetate reagents respectively) it was suspected that the color change for the anthocyanins lacking a free *o*-dihydroxyl group was caused by the increased pH alone. Indeed, similar colors could be produced by exposing the samples to ammonia vapor but the colors are difficult to reproduce. The color changes produced with anthocyanidins were similar to that with anthocyanins but the color faded rapidly due to the instability of anthocyanidins at the high pH.

The results showed that both neutral and basic lead acetate could be used as chromogenic reagents for anthocyanins. Neutral lead acetate is recommended because of the more pronounced differences between the colors of the pigments after treatment. This reagent can be applied by spraying, but the dipping technique or a spot test using a medicinal dropper, are favored because of the toxicity of lead. The lead acetate dip is used routinely in our laboratories as an aid in identification with pure anthocyanins as well as with chromatograms of crude extracts.

The lead acetate chromogenic reagent had two advantages over the conventional aluminum chloride:

1. The color developed within a few minutes with lead acetate whereas the color development took several hours with $AlCl_3$.

2. A color change occurred with every anthocyanin examined which eliminated the uncertainty experienced with $AlCl_3$, where it was not always clear whether the reaction was incomplete or the anthocyanin lacked the reacting site.

EXPERIMENTAL

Reagents

Saturated aqueous solutions of the lead acetates were used in the preliminary experiments. These solutions were replaced with ethanolic solutions because the drying of the treated chromatograms was slow. Both the neutral and basic lead acetate solutions were prepared by dissolving 1 g of the respective lead acetate in 100 ml 75% ethanol.

Procedure

Pure authentic individual anthocyanins[6] were applied as streaks across the width of Whatman No. 1 paper. The chromatograms were developed with the upper phase of 1-butanol-benzene-formic acid-water (100:19:10:25),[6] dried and sections from the anthocyanin containing bands were cut out. These anthocyanin containing paper chips served as samples for the color determinations. The "untreated" samples were fumed with hydrochloric acid to bring the pH to a uniform and low level. The chromogenic reagents were applied by dipping and the color was determined after the treated samples were dried. The color of the samples was determined by visual comparison of the anthocyanin containing chips with the color swatches from the Reinhold Color Atlas.[7] The color matching was carried out under artificial (daylight fluorescent light) illumination.

[6] T. FULEKI and F. J. FRANCIS, *J. Chromatog.* **26**, 404 (1967).
[7] A. KORNERUP and J. H. WANSCHER, *Reinhold Color Atlas*. Reinhold, New York (1961).