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ANTHOCYANIDINS FROM LEUCOANTHOCYANIDINS

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Abstract—High acid strength and small amounts of iron have been effectively combined to improve the yield of anthocyanidins from leucoanthocyanidins over that obtained by currently used methods. An improved method for routine estimation of leucoanthocyanidins in plant extracts is outlined.

INTRODUCTION

LEUCOANTHOCYANIDINS (flavan-3:4-diols) have in recent years assumed economic importance in view of their role in the formation of tannins and in texture and flavour of foods. In the aqueous or alcoholic acid conversion of leucoanthocyanidins to anthocyanidins,[1] the yield is generally poor, being less than 10 per cent. The improvements in the method made by Swain and Hillis[2] gave a more uniform result, but the yield was still poor.[3] McFarlane[4] working with malt and beer found that the presence of small amounts of iron and copper in the acid reagent and purification of the leucoanthocyanidins by specific adsorbants improved the yield of anthocyanidins. High yields of anthocyanidins have been obtained from acetyl or methoxy derivatives[5,6] of monomeric leucoanthocyanidins or by using anhydrous conditions.[7] This paper records the results of a study of the variables and the standardization of an improved method for the estimation of leucoanthocyanidins.

RESULTS AND DISCUSSION

In view of the ease of experimentation, the procedure of Swain and Hillis[2] was used as the basic method. The nature of the conversion products was examined by paper chromatography using Forestal[1] and Roux's[8] solvents and by spectrophotometry. The effect of increasing acid concentration in the reagent of *n*-butanol-HCl on the conversion of areca leucocyanidin,[9] showed that the concentrations of acid (e.g. 1·18) of 20% and lower, gave clear and brilliant anthocyanidin colour, while at higher concentrations a brownish tinge from side reactions increased. Paper chromatography clearly separated the brown reaction products from the true anthocyanidin to show the relative intensity changes. Spectrophotometric observations confirmed the results by the reduction of specific adsorption at 545 mμ and

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increase in absorption in the region 400–450 mμ, with increased acid concentration (Fig. 1). With leucofisetinidin, similar effects were observed though the increase at around 400–450 mμ was much less.

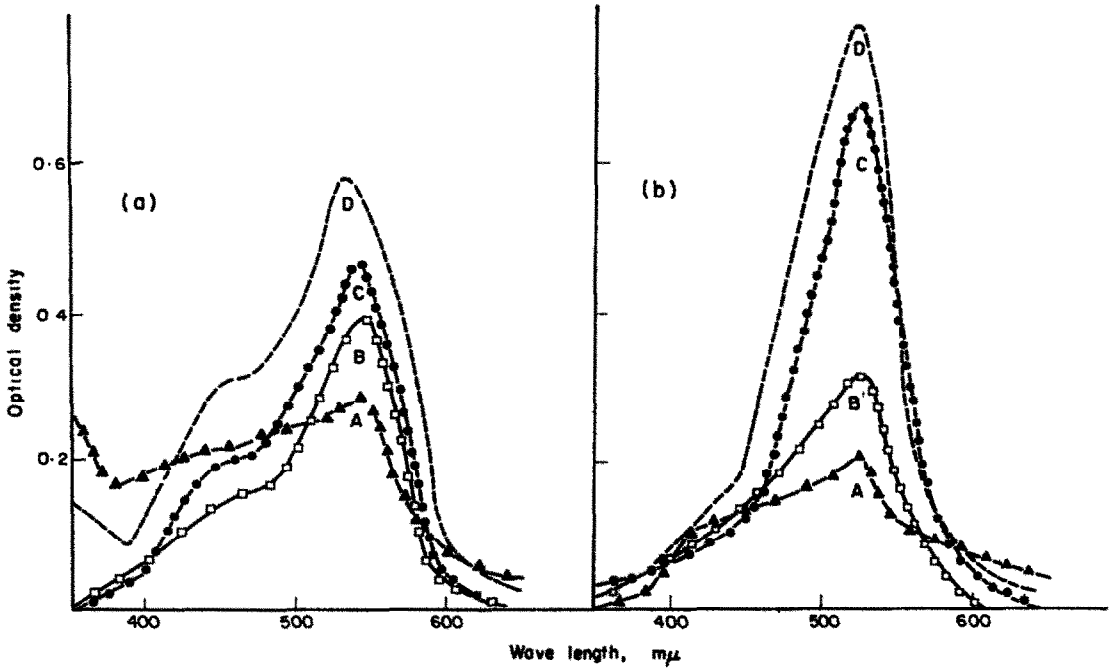

FIG. 1. ABSORPTION SPECTRA OF ANTHOCYANIDIN FROM (*a*) ARECA LEUCOCYANIDIN; (*b*) LEUCOFISETINIDIN WITH DIFFERENT REAGENTS.

Curves—A, *n*-butanol–HCl (40% acid); B, *n*-butanol–HCl (5% acid); C, *n*-butanol–HCl (5% acid) + Fe; and D, *n*-butanol–HCl (40% acid) + Fe.

Roux and Bill[7] suggested that the high yields they obtained were due to the use of anhydrous conditions. We have found (Table 1), however, that addition of water up to 20 per cent of the reagent did not adversely affect the yield. The optimum amount of water is

TABLE 1. YIELD OF ANTHOCYANIDINS FROM LEUCOANTHOCYANIDINS—EFFECT OF DIFFERENT AMOUNTS OF WATER IN REACTION MIXTURE

Material	Reagent	Absorptivity* Volume of water in ml			
		0	0·25	0·5	1·0
Leucocyanidin, 0·050 mg	5 ml *n*-butanol–HCl (5%)	0·16	0·19	0·21	0·22†
Leucofisetinidin, 0·062 mg	5 ml of *n*-butanol–HCl (5%)	0·10	0·13	0·18	0·22†
Leucofisetinidin, 0·062 mg	5 ml of *n*-butanol–HCl (40%) + Fe (3 mg %)	0·34	0·43	0·46	0·45

* At respective λ_{max}.

† At this level of water there was phase separation.

10 per cent of reaction mixture and this, incidentally, is the usual amount introduced when making measurements on plant extracts.

Following McFarlane's findings,[4,10] the effect of small amounts of iron on the yield of anthocyanidin was studied. The conversion to anthocyanidins reached a maximum at 3 mg iron per 100 ml of reagent, with both leucoanthocyanidins studied (Fig. 1). Treatment of the sample with iron prior to heating with *n*-butanol–HCl did not give increased yields. But heating with the reagent containing iron or addition of iron after the initial colour development gave the maximum yield. These observations do not support the suggestion of McFarlane[4] that the increased yield is due to the formation of a chelate of iron with the leucoanthocyanidin.

The effect of increased absorption at higher acid levels in the 450 mμ region was again observed markedly with areca leucocyanidin but only slightly with leucofisetinidin. Paper chromatography of the reaction mixtures confirmed these results. While leucofisetinidin showed flavonols besides brown products, leucocyanidin from areca gave predominantly brown condensation products as the secondary reaction products thus exhibiting differences in reactivities of different leucoanthocyanidins. The absorption in the region 410–450 mμ due to the presence of a free 5-hydroxyl group[11] and increased acid catalysed self condensation similar to that of catechin[2,12] may explain the difference in the results obtained with the two leucoanthocyanidins studied. Roux and Bill[7] obtained yields of 40 per cent with leucoanthocyanidins lacking 5-hydroxyl groups, but only yields of 5–16 per cent with other leucoanthocyanidin extracts. Several anthocyanidin spots were formed from the same leucoanthocyanidin, confirming earlier findings[7,10] and emphasizing the need for caution in the interpretation of results.

The rate of conversion of areca leucocyanidin into cyanidin was studied with varying time and temperature at two concentrations of acid and using the reagents with and without

TABLE 2. YIELD OF ANTHOCYANIDIN FROM PLANT MATERIALS BY THE SWAIN AND HILLIS AND IMPROVED PROCEDURES

		Optical density at 540 mμ	
		n-Butanol–HCl (5%) (40 min heating)	*n*-Butanol–HCl (40%) + iron (3 mg%) (15 min heating)
1. Immature sapota	(fresh)	0·88	1·79
2. Immature guava	(fresh)	0·24	0·32
3. Raw cocoa beans	(fresh)	0·09	0·13
4. Fermented cocoa beans	(dried	0·08	0·11
5. Ripe arecanut	(dried)	0·54	0·74
6. Tea leaf	(dried)	0·04	0·08
7. Groundnut cuticle	(dried)	0·18	0·27
8. Green cavendish banana	(dried)	0·005	0·01

Fresh materials (1–3)—1 g material, hot extracted and made up to 100 ml. Dried material (4–8)—0·1 g material, hot extracted and made up to 100 ml.

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iron. The results obtained extend earlier observations of McFarlane and Vader[10] and of Joslyn and Goldstein.[13] The maximum yield of anthocyanidin was obtained with a reagent *n*-butanol-HCl (40%) containing iron at 3 mg per 100 ml. With this reagent the time for maximum colour formation at 100° could be reduced to 10–15 min as against 30 to 40 min necessary in Swain and Hillis procedure.[2] Furthermore easily reproducible results were obtained by using the iron-containing reagent. Table 2 records the yields of anthocyanidins from aqueous extracts of different plant materials using the method of Swain and Hillis[2] and the improved method. The increase in yields are however not constant with different plant extracts, probably because the samples represent mixtures of varying degrees of polymerization[14] and of several different leucoanthocyanidins.[15]

EXPERIMENTAL

Reagents. (*a*) *n*-butanol-HCl (40%): mix 2 vol of A.R. grade hydrochloric acid (sp. gr. 1·18) with 3 vol of *n*-butanol; (*b*) concentrated iron solution: dissolve 77 mg of A.R. grade $FeSO_4 \cdot 7H_2O$ in 100 ml of (*a*).

Improved anthocyanidin reagent. Mix 80 ml of (*a*) and 20 ml of (*b*).

Method. Transfer 0·5 ml of aqueous extract under test and 5 ml of the reagent into a 150 × 25 mm uniform test-tube and mix thoroughly by shaking. Cover the mouth with a loosely fitting glass closure to prevent development of pressure and heat in a constant level water bath for 15 min. Cool the tube and measure the optical density against a similarly heated reagent blank in a photoelectric colorimeter using a filter of maximum transmission between 525 mμ and 550 mμ.

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