Yellow Pigments of *Dioscorea bulbifera*

Yellow pigments of aerial tubers of *Dioscorea bulbifera* L. were extracted with ethyl acetate, chromatogrammed on thin layers of silica gel, and eluted with solvents for spectrophotometry, with and without saponification. The pigments were provisionally identified from chromatographic mobilities, colors, stability, and absorption spectra, and in comparison with easily identified pigments of spinach leaves and carrot roots. The major part of the yellow color is due to the presence of saponifiable esters of xanthophylls. Small quantities of such xanthophylls also occur in the free state. The principal xanthophylls tentatively identified were lutein, neoxanthin, violaxanthin, zeaxanthin, auroxanthin, and cryptoxanthin. Other pigments present included chlorophylls, an anthocyanin, and unidentified phenolics. No carotenes were found.

The tuber of many yams (*Dioscorea* species) is yellow, but whether due to a nutritionally useful pigment, β-carotene, a related carotenoid, or a pigment of still another type has not been studied.

The chief yellow pigment of sweet potato (*Ipomoea batatas* (L.) Lam.) roots is β-carotene. Other carotenoids, chiefly carotenes, are found in much smaller quantities. The color of the root is generally accepted to be an indication of the β-carotene content (Villers *et al.*, 1944). The yellow color of the tuberous roots of cassava (*Manihot esculenta* Crantz) has recently been described as β-carotene (Guimaraes and De Barros, 1971).

According to Goodwin (1955), the most common carotenoids of roots are the carotenes, lycopene, prolycopene, and the following xanthophylls: lutein, chrysanthemaxanthin, and auroxanthin. In contrast, the common xanthophylls of leaves are cryptoxanthin, zeaxanthin, lutein, violaxanthin, chrysanthemaxanthin, and lutein 5,6-epoxide. Leaves also contain quantities of carotenes.

As a first step in the analysis of yellow pigments of yams, the aerial tubers of *Dioscorea bulbifera* L. were selected. Poisonous and nonpoisonous varieties of this species occur, often wild, throughout the tropics. Color of the tuber varies from creamish gray to dark yellow with a greenish tinge.

MATERIALS AND METHODS

The varieties of *D. bulbifera* studied, their recent geographic sources, colors, relative sizes, and bitterness of the flesh are given in Table I.

After preliminary trials, the peeled tubers were extracted by blending in ethyl acetate. A stream of nitrogen was maintained on the mixture. After extraction the macerated tuber tissue shrank, exuding the ethyl acetate and pigments. This extract was filtered rapidly, warmed in a water bath, and concentrated with a stream of nitrogen. The concentrated pigments were spotted in a long line on silica gel G thin layers (Eastman Chromagram Sheets, 6060) and chromatogrammed with a mixture of cyclohexane and ethyl ether (50:50). The ether was purified of peroxides by the method of Dasler and Bauer (1946). Chromatography was managed in a nitrogen atmosphere and in darkness. The proportions of the solvents were varied in order to vary the degree of separation of the pigment bands. The most useful mixtures were cyclohexane–ethyl ether 1:1, 9:1, and 1:9. With these, the pigment mixture could be separated into apparently all components. The colors of the various pigment spots were registered after they were viewed in normal and ultraviolet lights.

After development, the chromatograms were dried rapidly with nitrogen and the silica gel was scraped from the plates and transferred to small vials. The pigments were eluted from the silica gel with hexane–acetone (7:10) or with a single drop of methanol followed by hexane. The absorption spectra of the eluted pigments were determined in the above solutions, or after evaporation with nitrogen, in pure hexane.

The separation of pigments was compared before and after saponification. The pigment mixture was saponified by adding to the crude extract an equal quantity of methanolic *N* NaOH solution. After heating to drive off the ethyl acetate, the mixture was refluxed for 10 min or more. The methanol was then evaporated. The pigments were removed from the alkali by phase separation of ethyl acetate and water.

As a source of reference compounds, the leaves of spinach (Strain and Sherma, 1969) and roots of carrot were extracted in the same fashion, and the extracts were chromatogrammed similarly before and after saponification.

RESULTS

The pigments extracted from aerial tubers of ten varieties of *D. bulbifera* are given by number in Table II, together with their colors under regular light on wet plates and when dry under ultraviolet (uv) light. Their relative concentrations are estimated. R_f values are given for the most useful chromatographic system, equal parts of ethyl ether and cyclohexane. Other variations of the basic solvent systems were necessary to separate all the pigments. The influence on R_f of different ratios of cyclohexane and ethyl ether is illustrated in Table III. By varying the proportion of these two solvents, all of the pigments could be separated. Not all pigments were present in all varieties, and minor pigments were not always detected.

Of 17 spots that were isolated, three of these were easily identified as chlorophyll a, chlorophyll b, and phaeophytin (Table IV). These pigments were common to the yam tubers and to spinach leaves. They fluoresced red under uv light. When present, they served as convenient internal standards for comparing different chromatograms and treatments.

Of the remaining pigment spots, 9 and 16 were held in common with spinach leaves. Spot 9 was identified as lutein by its chromatographic position, its orange-yellow color before drying, and its absorption spectrum (Table V). Band 16 was identified as auroxanthin by its high polarity, its yellow-green color, and its characteristic absorption maxima. It was seldom present. Zeaxanthin, present in some but not all extractions from yam tuber, was deduced from its chromatographic position only.

The pigments included an anthocyanin, probably of cyanidin, known to occur in other yams, and provisionally identified by its absorption maxima of 535 nm.

Several very polar compounds were not identified. These included no. 12, which was yellow on the wet chromatogram, disintegrated as it moved to a colorless compound, and later changed to an orange color, and in that form was given the no. 13. This compound was quite soluble in water, and its color was intensified by alkali treatment. It could be precipitated from the extract with lead acetate. It is most likely a phenolic substance. Another yellow compound, no. 15, showed absorption peaks typical of carotenoids but was not present in sufficient concentration to make identification possible.

Table I. Varieties of *Dioscorea bulbifera* Used, Their Source, Color of Tubers, Relative Size of Aerial Tuber, and Bitterness of Flesh

Identification numbers	Variety or type	Source	Internal color	Relative size	Bitterness
14861	Sharp angled	Puerto Rico	Yellow	Large	Slight
15216	Round	Puerto Rico	Light green	Large	None
15330	Sativa	India	Dull pink	Medium	Bitter
15335	Variant	Puerto Rico	Yellow	Large	Slight
15338	Smooth angled	Puerto Rico	Yellow	Large	Slight
15472	Poison	Hawaii	Dull pink	Small	Bitter
15492	Thuma	New Caledonia	Yellow-green	Medium	None
15500	Wild	Ivory Coast	Pink grey	Medium	Bitter
15501	Cultivated	Ivory Coast	Light yellow	Medium	None
15773	Cultivated	Nigeria	Yellow	Medium	Slight

Table II. Color, R_f Value, and Relative Concentration of All Pigments Extracted from Varieties of *D. bulbifera* and Separated on Silica Gel Thin Layers with Cyclohexane–Ethyl Ether (1:1)

Pigment no.	Color	Color uv	R_f	Concentrations[a]									
				14861	15216	15330	15335	15338	15472	15492	15500	15501	15773
1a	Dark yellow	Orange brown	0.98[b]	M	W	W	S	S	W	W	W	M	S
1b	Light yellow	Yellow	0.98[b]	W	S	W	S	S	W	M	?	W	S
2	Light yellow	Light brown	0.96	M	M		M	M		M		M	S
3	Light yellow	Brownish	0.78	W	W			W				W	M
4	Light yellow	Brownish	0.58	M	W			M				M	S
5	Dark yellow	Brown	0.45	M	W		M	S		M	M	M	S
6	Grey green	Red	0.29	M			M	S	W	M	M	M	S
7	Light yellow	Colorless	0.29	W	W			W				W	
8	Grey green	Brown	0.19				W	S					W
9	Orange yellow	Brown	0.13	M	M	W	M	M	W	M	W	M	S
10	Light yellow	None	0.09				W	W					
11	Green	Red	0.05	W	M		W	W		S		S	S
12	Light yellow	Green	0.03	W	W			W		M	W		S
13	Orange	Brown	0.00	S	M	M	M	S	M	M	S	M	S
14	Red	Purple	0.00	M				M					
15	Yellow	Brown	0.00	W				M					
16	Green	Brownish	0.00	W				W					

[a] S = strong, M = medium, W = weak. [b] Separated on silica gel with cyclohexane–ether (4:1).

Table III. Effects of Cyclohexane–Ethyl Ether Mixture on R_f Values of Principal Pigments of *D. bulbifera* Variety Sharp Angled

Pigment no.	Cyclohexane-ethyl ether ratio						
	20:0	16:4	13:7	10:10	7:13	4:16	0:20
1a	0.00	0.89	0.93	0.97	0.98	0.99	1.00
1b	0.00	0.72	0.85	0.95	0.97	0.98	1.00
4	0.00	0.26	0.44	0.69	0.81	0.92	1.00
5	0.00	0.23	0.34	0.61	0.71	0.89	0.99
6	0.00	0.14	0.24	0.39	0.50	0.71	0.99
9	0.00	0.04	0.12	0.23	0.32	0.50	0.98
15	0.00	0.00	0.00	0.00	0.00	0.02	0.97

No carotenes were present in the extractions. β-Carotene, extracted from carrots and from spinach leaves and also applied from an authentic sample, was much more mobile in all chromatographic systems, was easily separated by the cyclohexane–ether (9:1) system from yam pigments, and displayed a characteristic orange-yellow color that distinguished it from those compounds nearest to it on the chromatograms.

The following pigments showed mobilities intermediated between most free xanthophylls and carotenes: 1a, 1b, 2, 3, 4, 5, and 7. Reproducible absorption spectra were obtained from five of the seven pigments. The rest of the pigments were not obtained in sufficient quantity to make determination of spectra possible. Pigments 1a, 1b, and 5 absorbed light at approximately the same wavelengths as lutein (Table V). The absorption spectrum of pigment 4 is about the same as that of neoxanthin. The absorption peaks of pigment 6 did not correspond closely with the absorption peaks of other common xanthophylls. These absorption maxima are not typical of monohydroxy xanthophylls (of similar polarity). The assumption was made that these compounds were xanthophyll esters, and they were then analyzed after saponification of total extracts and of individually eluted pigments.

Saponification, accomplished with difficulty and seldom complete, resulted in the removal of several of the more polar compounds in the aqueous phase (Table IV). In addition, the chlorophylls were destroyed. The less polar compounds believed to be esters resisted saponification, but were modified in chromatographic response. Saponification of the total extracts revealed four pigments, cryptoxanthin, neoxanthin, auroxanthin, and violaxanthin, the latter in only a very small quantity. The nature of the compounds formed by saponification was deduced from their chromatographic mobilities in at least two solvent systems, in comparison with the known compounds from spinach leaves. Cryptoxanthin was deduced only from its apolar nature and its pale orange color.

Individually eluted pigments were sometimes saponified satisfactorily. Pigment 1a, when saponified, yielded more polar bands still in the area of suspected esters and complete saponification resulted in conversion to lutein. Pigment 1b resisted saponification but in some cases yielded small amounts of lutein. Pigment 4 yielded only neoxanthin. Pigment 5 yielded small amounts of violaxanthin and auroxanthin.

The nature of the faint spots 2, 3, and 7 was not determined. These bands resisted saponification and their corresponding free xanthophylls were not isolated. Nevertheless, because crude extracts were completely saponified and the ester spots were thus removed, the xanthophylls

Table IV. Fate of Principal Pigments upon Saponification and Principal Compounds Produced

Pigment no.	Suspected nature	Result of saponification	New compounds produced
1a	Ester	Destroyed	Lutein, other esters
1b	Ester	Destroyed	Lutein, other esters
2	Ester	Resistant	
3	Ester	Resistant	
4	Ester	Destroyed	Neoxanthin
5	Ester	Destroyed	Violaxanthin, auroxanthin
6	Phaeophytin	Destroyed	
7	Ester	Resistant	
8	Chlorophyll a	Destroyed	
9	Lutein	Unchanged	
10	Zeaxanthin	Unchanged	
11	Chlorophyll b	Destroyed	
12	Phenolic	Removed	
13	Phenolic	Removed	
14	Anthocyanin	Removed	
15	Unknown	Removed	
16	Auroxanthin	Unchanged	
Total extract			Cryptoxanthin, neoxanthin, violaxanthin, auroxanthin

Table V. Absorption Maxima of Principal Pigments of *D. bulbifera*

Pigment no.	Compound	Absorption maxima, nm					
		In hexane			In hexane–acetone		
1a	Ester	420	443	474	419	441	470
1b	Ester	419	440	469	420	440	470
4	Ester	412	435	464	414	437	464
5	Ester	415	441	468	418	442	471
6	Ester	412	435	472			
9	Lutein	418	440	470	420	443	473
14	Anthocyanin	(in methanol)		535			
15			432	455		430	454
16	Auroxanthin				378	398	426

of these esters appear to be those previously obtained and not new compounds.

DISCUSSION

The chromatography of carotenoid pigments on silica gel has its risks. As studied by Strain *et al.* (1967), isomerization may occur, particularly that of violaxanthin and neoxanthin, to provide bands of cis isomers not normally encountered. Furthermore, the degradation of violaxanthin to auroxanthin is so common that the two pigments commonly occur together. In the current study these free xanthophylls did not occur in the crude extracts. The positions of the difficult-to-identify bands did not correspond to those that would be expected from isomers. We have no reason to believe that any of the pigments were artifacts nor that uncommon cis isomers of some pigments were confused with the normal trans isomers of others.

The use of spinach leaf and carrot root as sources of common readily identified compounds has adequate precedence. When known and unknown pigments show the same color properties and the same mobilities in several solutions, the evidence of their correspondence is quite strong.

Among the free xanthophylls, lutein was easily and definitely identified. Saponification, although accomplished with difficulty, showed that at least two of the principal apolar bands also yielded lutein. Once the xanthophylls were released from their esters, it became apparent that the tubers of *D. bulbifera* contain the same pigments that are commonly found in green leaves but that these pigments were frequently in the form of esters.

This study did not reveal carotenes, even in trace amounts. The techniques revealed quantities of carotene in the spinach and carrot controls, however. Most of the xanthophylls and their esters have no known nutritional value (Bauernfeind, 1972), but cryptoxanthin (only tentatively identified, and then in small amounts) is an exception. Thus, the yellow pigments of *D. bulbifera* are apparently of little or no value in the diet.

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