

Bixin Content of *Bixa Orellana* in Papua and New Guinea.

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Introduction.

Bixa orellana (Annatto or Lipstick Pod Plant) is a large quick-growing shrub, native to tropical America but now growing in many tropical countries throughout the world. It is often grown in hedges and normally reaches about twelve feet in height, although isolated bushes sometimes grow to over 30 feet. It has glabrous caudiform leaves, and bears, on the end of branches, clusters of brown capsular, ovoid or spherical fruits with fleshy spines. These contain up to 50 small seeds coated with a bright red mass which darkens when the seeds are dried. This coating, which contains bixin, $C_{25}H_{30}O_4$, a carotenoid acid, is widely used by native communities as a dye. Bixin is not considered to be carcinogenic and, following the "Delaney" amendment to the U.S. Pure Food Act, which prohibits the use of carcinogens as food additives, it has been considered as a possible artificial colouring matter for foods.

With this in view, two Hawaiian workers (Moomaw and Matsumoto, 1961) recently investigated the bixin content of *Bixa orellana* in Hawaii. Two varieties occur in the Hawaiian Islands: one with a 'round pod' and the other with a 'pointed pod'. The whole seed was analysed by a modification of the method of Meyer and de Vos (1941) whereby the bixin was extracted from the whole seed with pyridine. It was found that the 'pointed pod' variety contained significantly more bixin than the 'round pod' variety.

The variation in shape of the capsules of different varieties of *Bixa* is considerable. The late Dr. P. J. Eyma made a survey of the forms at Bogor and found that, with regard to the shape of the fruit, its colour, the colour and length of the bristles on it, and the colour of the corolla, 18 combinations were represented (Backer, 1951). In Papua and New Guinea *Bixa* thrives in many areas, both on the coast (Port Moresby, Lae, Rabaul) and in the Highlands (Wau, Garaina, Goroka). On classification by capsular shape, three varieties occur:—

1. *Broadly Ovate*—from a subtruncate base broadly ovate with a broadly rounded abruptly and shortly acuminate apex. These are probably similar to those referred to as 'round pods' by the Hawaiian workers.
2. *Elongated*—from a subtruncate base elongate-ovate with a much narrowed rather long acuminate apex. These probably correspond to the 'pointed pod' variety in Hawaii.
3. *Spherical*—spherical capsules taken from trees at Garaina. These are of two types—those containing two segments and those with three segments.

Analysis.

Preliminary investigations showed that grinding does not destroy the bixin in the seed coat, but drying at 100 degrees C. tends to reduce the quantity of bixin recovered. All samples were therefore crushed through a 2.5 mm.

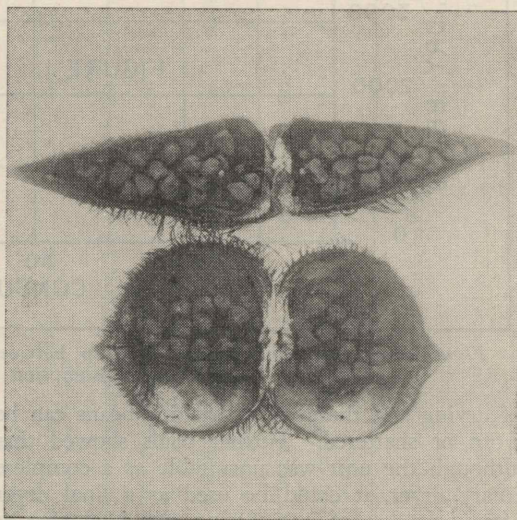


Plate I.—An open elongated capsule (top) and broadly ovate capsule (bottom).

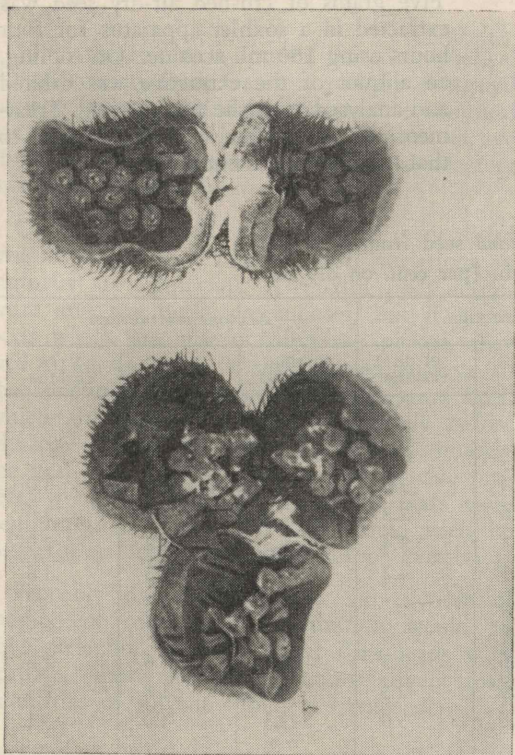


Plate II.—Open spherical capsules.

sieve and air-dried before extraction. Moisture content was determined on a separate portion of the sample so that all yields could be converted to an oven-dry basis. (The Hawaiian analyses refer to air-dry seed). Analyses were performed in duplicate and the average of the two values taken (these were always within 0.3 per cent.). Two methods of analysis were used:—

1. *Extraction with Pyridine* (Moomaw and Matsumoto). One gram of crushed air-dry seed was mechanically shaken with 30 ml. pyridine for half an hour. The dark red liquid was then poured through a glass wool plug into a 100 ml. volumetric flask, the residue thoroughly washed with pyridine and the washings added to the flask. An aliquot of the pyridine solution was dissolved in 70 per cent. ethanol and the transmittance read on a Beckman DU spectrophotometer.

(In the earlier analyses an aliquot was first chromatographed on 1:1 sodium carbonate: filter cell, but as it was found that this purification did not appreciably influence the result, the shorter method which is described was used in later analyses. The maximum absorption of the non basic components is at 400 mu and these produce an error of less than 5 per cent. on measurements made at 455 mu. The bixin content was calculated using an

$$E \frac{1}{1 \text{ cm.}} \text{ of } 3200 \text{ at } 455 \text{ mu}$$

(Espoy and Barnett, 1955). The residue was collected, oven-dried and weighed. Nitrogen content was determined using the Kjeldahl method.

2. *Soxhlet extraction.*

Soxhlet extraction using various organic solvents was also investigated (Karrer & Jucker, 1959). Whereas ethanol and



Plate III.—Leaves and capsules of *Bixa orellana* (with elongated capsules).

petroleum ether were not very satisfactory solvents, extraction with acetone or chloroform proved very efficient. Comparison with yields from pyridine extraction (Table I) showed that these conditions of hot extraction with a low boiling solvent did not destroy the bixin.

Five grams of crushed air-dry seed was extracted in a soxhlet apparatus for four hours using 150 ml. acetone. On cooling, an aliquot of the extractive was diluted and analysed as in the first method. Treatment of the residue was also similar to that in the first method.

Table 1.
Comparative analyses of *Bixa orellana* seed from localities in
Papua and New Guinea, and Hawaii. (per cent. on dry basis).

				Pyridine extraction.			Acetone extraction.		
				Soluble material	Bixin	N in residue	Soluble material	Bixin	N in residue
<i>Garaina</i>									
Mature elongated									
(I)				36.4	3.4	3.0	12.9	3.6	3.0
(II)				34.6	3.6	2.8	12.3	3.8	2.6
Immature elongated				32.0	4.1	3.4	9.3	4.2	2.9
Immature spherical				29.1	5.3	4.2	11.5	5.3	3.8
<i>Wau</i>									
Mature broadly ovate									
(I)				22.6	1.6	2.3	5.1	1.6	2.3
(II)				20.0	1.9	2.3	8.9	1.8	2.3
Mature elongated				36.1	4.6	3.0	13.3	4.0	3.1
<i>Mageri</i>									
Mature elongated				33.3	4.2	3.2	10.4	4.3	2.1
<i>Goroka</i>									
Mature elongated				27.9	4.1	2.5	19.1	4.3	2.3
<i>Lae</i>									
Mature elongated				38.6	4.5	2.9	19.8	4.8	3.1
<i>Hawaii</i>									
'Round pod'				6.7 - 8.4	2.4 - 2.9	ca.2			
'Pointed pod'				7.4 - 9.5	3.2 - 4.1	ca.2			

Chromatographic Purification.

Several chromatographic adsorbents were used for purification, and of these sodium carbonate: filter cell (1:1) proved by far the most successful for analytical purposes. The non-acidic fraction was eluted with petroleum ether and acetone followed by the acidic fraction with aqueous ethanol. Deactivated alumina and mixtures containing this adsorbent tended to adsorb the bixin fraction very strongly and in any case the separation was not as satisfactory.

Silica gel was a better absorbent although the separation was not as sharp as with sodium carbonate: filter cell (1:1). An aliquot of the *Bixa* extract absorbed on sand was placed on a silica gel column prepared in petroleum ether. The bixin was eluted with chloroform: benzene (1:1) and chloroform. Chloroforms: ether (1:1) removed a mixture of bixin and other compounds, and acetone and ethanol were used to remove the remaining compounds (not containing bixin) from the column.

Discussion.

From the results it appears that the spherical capsule variety from Garaina, and the elongated capsule variety, would be the most economical sources of dye, whereas the other variety (broadly ovate capsules) is not as satisfactory owing to its low bixin content.

Contrary to some suggestions it is doubtful whether differences due to small variations in maturity are significant. Over-ripe pods deteriorate and the bixin decomposes, but if green pods which are just opening are picked, they will ripen and give a similar seed analysis to that obtained from pods from the same bush which are allowed to ripen completely before picking. Since the pods ripen very quickly the best time for picking, on a large scale project, would probably be when the pods were beginning to open. The seed could then be kept for as long as a week before extracting.

Probably the most efficient and economical method of commercial preparation would be cold extraction of the crushed *Bixa* seeds with acetone. Extraction is rapid and relatively small quantities of solvent would be required.

Summary.

The bixin content of the seeds of three varieties of *Bixa orellana*, from various localities in the Territory, has been determined by two

methods, pyridine extraction and acetone extraction. It was found that seeds taken from the spherical capsule variety had the highest bixin content (5.3 per cent. on an oven-dry basis), those from elongated capsules had 3.4—4.6 per cent. bixin and those from broadly ovate capsules only 1.6—1.9 per cent. bixin.

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