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PREPARATION OF BIXIN AND METHYL BIXIN FROM INDIAN SEEDS OF *BIXA ORELLANA*

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BIXA ORELLANA is a shrub in Central and South America, and in India it grows into a tree. The plant is commonly associated with the Annatto dye obtained from the seeds; its earlier use for dyeing of fabrics has ceased with the development of synthetic dyes. Bixin is the main component and this is at present in considerable demand as a non-toxic fat-soluble food colour.

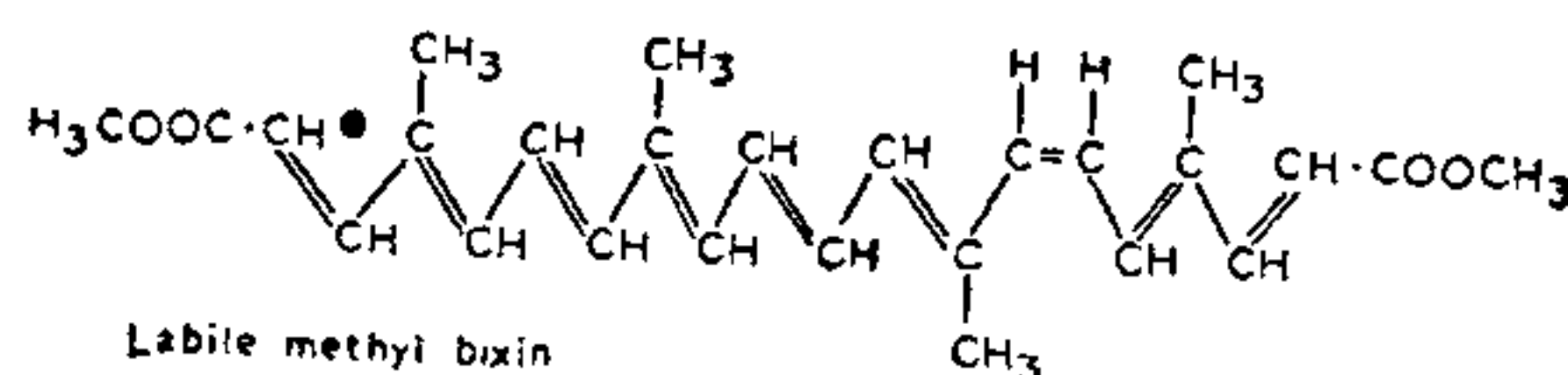
In the earlier method of isolation of Bixin,¹ the seeds were stirred with water for a few hours and filtered through a sieve in order to obtain crude annatto preparation which was later purified. The extraction is not efficient because the outer red coating of colouring matter is not detached fully even after shaking with water for six hours in a machine. Boiling the seeds with water brings in many impurities. Direct extraction of the seeds seemed to offer better possibilities and we have examined the use of various solvents. Further, a careful physical examination of the seeds showed that the seed-coat is quite hard and non-porous, that the kernel is also hard and the pigment is entirely on the seedcoat. Therefore, it is most convenient to extract the entire seeds without powdering; this reduces the difficulty of filtration of the extracts, and the extraction of other components present in the kernel. Petroleum ether and ether are not satisfactory as solvents; the best extraction could be carried out by means of acetone or ethyl acetate. Extraction in a Soxhlet apparatus is slow; direct boiling in a flask and decanting the extract through cotton is found to be most convenient. A typical experiment is given below:

The seeds of *Bixa orellana* (200 g.) were directly boiled with ethyl acetate (2 × 500 ml.), the solution decanted and concentrated. It deposited about 2.2 g. of pure crystalline labile bixin which was filtered off. The filtrate was poured into excess of petroleum ether with vigorous stirring, when 1.8 g. of a deep red solid was precipitated; it was filtered and mother liquor marked (A). On TLC, using silica gel as the adsorbent and employing the solvent (CHCl₃-MeOH, 94:6) for developing the chromatogram, the solid was found to be mainly bixin with a small amount of deep orange impurity that did not move. Purification could be effected by taking the ethyl acetate solution of the solid and diluting it with petroleum ether, when pure bixin (1.5 g.) was precipitated. The mother liquor (A) obtained earlier was concentrated. The solid residue (5.2 g.) obtained was taken up in ether and separated into alkali-soluble and neutral portions. The alkali-soluble portion, after acidification followed by column chromatography over silica gel yielded some more of bixin (0.8 g.).

Thus, labile bixin was obtained as red prismatic needles, m.p. 197-99°, $\lambda_{\text{max}}^{\text{CHCl}_3}$ 502 (log ϵ , 4.49), 471 (4.46) and 446 (4.35) nm.; $\nu_{\text{max}}^{\text{KBr}}$ 3300 (broad), 1725 (s), 1660 (w), 1615 (m), 1575 (s), 1430 (w), 1290 (m), 1260 (m), 1210 (m), 1165 (s), 1010 (w) and 965 (s) cm⁻¹. It gave cornflower blue colour when treated with concentrated H₂SO₄.

Following the earlier procedure,² labile bixin has been converted into its methyl ester by shaking its solution in methanolic potassium

hydroxide with dimethyl sulphate. It is better prepared in good yield by the easier method of refluxing the acetone solution of bixin with dimethyl sulphate and potassium carbonate. After filtering off the potassium salts, the solution is concentrated, stirred with excess of water and allowed to stand when the crystalline labile methyl bixin is obtained. The ester is also easily formed when bixin is treated with ethereal diazomethane. It is obtained as red plates, m.p. 161–62°, $\lambda_{\text{max}}^{\text{CHCl}_3}$ 502 (log ϵ , 2.72), 472 (2.79) and 446 (2.65) nm; $\nu_{\text{max}}^{\text{KBr}}$ 1720 (s), 1650 (w), 1615 (s), 1560 (m), 1430 (w), 1310 (s), 1290 (m), 1265 (s), 1190 (m), 1165 (s), 1010 (m), 985 (m), and 965 (s) cm^{-1} . This gives a blue colour with conc. H_2SO_4 .



The nmr spectrum of labile methyl bixin in CDCl_3 showed a broad signal at 1.98 δ (12H) indicative of four C-Methyl protons. A sharp singlet at 3.77 δ (6H) was due to the protons of the two carbomethoxyls. In the olefinic region, a broad band centred at 6.57 δ (10H) was observed. Apart from this, there were three doublets, each centred at 5.89 (2H), 7.41 (1H)

and 7.98 δ (1H). The first doublet belongs to the 2 α protons merging together and the β protons differ, one being at 7.41 and the other at 7.98 δ . Barber *et al.*³ have earlier compared the nmr spectra of labile and stable methyl bixin. They noted that the two spectra agreed in all respects except one. In the stable methyl bixin, both the β -protons gave the same signal at 7.39 δ (2H). The shifting of a β -proton in labile methyl bixin by 0.56 δ to the lower field was attributed to the proximity of the β -proton at one end of the molecule to the cis 6, 7 double bond. Thus, the nmr supports earlier fixation of the cis double bond by chemical methods.⁴

The greater solubility of methyl bixin in fats and fat solvents as compared to bixin which is rather sparingly soluble would suggest the use of ester as more suitable for colouring butter. Further, it will not add to the acid value of the butter. By the simplified method given above, it is very easily prepared. However, its tinctorial value is poor; though λ_{max} is the same as for bixin, log ϵ is markedly low and hence it is not suitable.

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INHERITANCE OF 'DYE BINDING CAPACITY' VALUE IN RICE

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RECENT work done at the International Rice Research Institute¹ and at the Indian Agricultural Research Institute² has convincingly proved that, in rice, considerable genetic variation exists for protein content and quality. A number of high protein genotypes have been identified at both places in the world germplasm collection. However, before such variation can be profitably exploited the estimation of heritability and the gene control mechanisms should be worked out under varying conditions to gain maximum genetic advance, through hybridization. Although numerous such studies have been performed in wheat,³⁻⁶ there is no report available in rice

on this aspect. In the present study, an attempt was made to evaluate the inheritance of protein quality and quantity using the micro dye binding technique described earlier.⁷ The single grains of F_2 populations, derived from single plants from crosses between Tainan-3 \times IR-8 (*japonica* \times *indica* of subsp. *Oryza sativa*) and IR-8 \times Basmati-370 (*indica* \times *indica*) were studied along with their respective parents. Means and variances were obtained and the frequency distribution was plotted (Fig. 1). Parental variances were used to estimate the environmental component of variation. Before the data was used for estimating the heritability, scale was tested.⁸ Means, vari-