

# I. NOTES ON BIXIN.

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## INTRODUCTION.

*Isolation of Bixin.*—The colouring matters of crude anatto dye obtained from *Bixa orellana* (Linn.) were first investigated by Chevreul,<sup>1</sup> who isolated two substances: one yellow and soluble in water and termed orellin and a second, red and almost insoluble and termed bixin.

Bixin, the more important colouring matter, was subsequently examined by several chemists who succeeded in obtaining it in an amorphous form, and Etti<sup>2</sup> was the first to isolate crystalline derivatives by a relatively tedious process, which has subsequently been replaced by others. Zwick<sup>3</sup> in 1897 obtained bixin by extracting anatto with boiling chloroform for 24 hours, removal of the solvent and thorough exhaustion of the residue with light petroleum. The product was crystallised from chloroform and after washing with light petroleum was repeatedly crystallised from the former solvent. Van Hasselt's method,<sup>4</sup> which is the best, consists in a preliminary washing of the dyestuff with acetone to remove the resins and in subsequent extraction with chloroform. After removal of the solvent the residue is washed several times with acetone and repeatedly crystallised from chloroform. Various modifications of this method have been suggested, thus Riffart<sup>5</sup> first extracts the dye with chloroform, crystallises the residue from glacial acetic acid, and washes the crystals successively with acetone, alcohol and ether. Herzig, Faltis and Mizzan<sup>6</sup> prefer crystallising from ethyl acetate. In 1917 Heiduschka and Panzer<sup>7</sup> suggested a rearrangement of the extractions, washings and crystallisations. They wash with acetone, extract with chloroform, crystallise from acetic acid, wash successively with acetone, alcohol and ether and finally crystallise from ethyl acetate.

<sup>1</sup> Lecons de Chimie appliquée à la Teinture.

<sup>2</sup> *Ber.*, 1874, 7, 446; 1878, 11, 864.

<sup>3</sup> *Ibid.*, 1897, 30, 1972.

<sup>4</sup> *Chem. Weekblad*, 1909, 6, 480.

<sup>5</sup> *Inaug. Diss. Munich*, 1911.

<sup>6</sup> *Monatsh.*, 1914, 35, 997.

<sup>7</sup> *Ber.*, 1917, 50, 546.

*Composition of Bixin.*—Table I gives the various formulae which have been suggested from time to time together with the solvent used for crystallisation.

TABLE I.

*Formulae suggested for Bixin.*

Date	Authority	Solvent	Formula	Theoretic percentages for	
				Carbon	Hydrogen
1878	Etti <sup>1</sup> ... ..	Alcohol ... ..	$C_{28}H_{34}O_5$	74.66	7.56
1897	Zwick <sup>1</sup> ... ..	Chloroform ... ..	Do.	...	...
1905	Marchlewski and Matejko <sup>2</sup> ...	Chloroform, ethyl alcohol and acetic acid.	Do.	...	...
1909	Van Hasselt <sup>3</sup> ... ..	Chloroform ... ..	$C_{29}H_{34}O_5$	75.34	7.59
1911	Heiduschka and Riffart <sup>4</sup> ...	Do. ... ..	$C_{28}H_{34}O_5$	...	...
1914	Herzig, Faltis and Mizzan <sup>5</sup> ...	Ethyl acetate ... ..	$C_{26}H_{30}O_4$	76.81	7.44
1915	Reinkes <sup>6</sup> .. ..	Chloroform ... ..	$C_{27}H_{32}O_4$	77.13	7.62
		(a) Hasselt's method.	$C_{29}H_{30}O_5$	...	...
		(b) Riffart's method ...	$C_{28}H_{34}O_5$	...	...
1917	Heiduschka and Panzer <sup>1</sup> ...	(c) Pregl's micro-analysis.	$C_{28}H_{30}O_4$	76.11	7.61
1923	Herzig and Faltis <sup>7</sup> ...	Ethyl acetate ... ..	$C_{26}H_{30}O_4$	...	...

According to Van Hasselt pure bixin melts at  $189^{\circ}$ , and analysis points to the formula  $C_{29}H_{34}O_5$ , whereas Heiduschka and Riffart prefer the old formula  $C_{28}H_{34}O_5$ , suggested by Etti. In a later paper Van Hasselt still supports the  $C_{29}$  formula and suggests that the discrepancy between his own and Heiduschka and Riffart's numbers arises from the fact that bixin is readily susceptible to oxidation with formation of amorphous products. He states that when prepared by their method the analyses agree with the  $C_{29}$  formula, and adds that the numbers given by Marchlewski and Matejko for hydrobixin are explicable if bixin is given the formula  $C_{29}H_{34}O_5$ .

<sup>1</sup> *loc. cit.*

<sup>2</sup> *Bull. Acad. Sci. Cracow*, 1905, 745.

<sup>3</sup> *Rec. trav. Chem.*, 1911, 30, 1; 1914, 33, 192. cf. also *Chem. Weekblad*, 1909, 6, 480; 1916, 13, 429, 436.

<sup>4</sup> *Arch. Pharm.*, 1911, 249, 43.

<sup>5</sup> *Monatsh.*, 1914, 35, 997; *Ber.*, 1917, 50, 927.

<sup>6</sup> *Chem. Weekblad*, 1915, 12, 998, cf. also 1916, 13, 244, 1224; 1917, 14, 888.

<sup>7</sup> *Annalen*, 1923, 431, 40.

When putting forward the formula  $C_{26}H_{30}O_4$ , Herzig, Faltis and Mizzan state that the real difficulty is the combustion, and that correct results can only be obtained when special precautions are taken.<sup>1</sup>

Heiduschka and Panzer, on the other hand, claim that the composition varies with the method of preparation and the crystallising medium used. When prepared by Van Hasselt's method the composition appears to be  $C_{29}H_{34}O_5$ , but by Riffart's method  $C_{28}H_{34}O_5$  and finally when prepared and purified by their own method the analyses agree best with the formula  $C_{25}H_{30}O_4$ .

*Constitution of Bixin.*—The dye contains one free hydroxyl group as it gives rise to a mono-sodium salt and a mono-methyl derivative. It does not yield an acetyl or benzoyl derivative and cannot be directly esterified. It has also been shown to contain one methoxy-group. It is readily reduced to colourless products and also takes up 10 atoms of bromine.

For many years it was regarded as the mono-methyl ether of an unsymmetrical dihydroxy-compound, but according to Herzig and Faltis<sup>2</sup> it is probably the mono-methyl ester of an unsymmetrical dicarboxylic acid,  $CO_2H \cdot C_{23}H_{26} \cdot CO_2Me$ . According to these authors the residue contains 9 olefine linkings as it absorbs 18 equivalents of hydrogen in the presence of palladium-barium sulphate. Although bixin itself cannot be esterified by means of alcohol and hydrogen chloride, its octadecahydride, hydrobixin, can be. Bixin is readily hydrolysed by alkalis to norbixin, and the proof that the two carboxyl groups are differently situated in the molecule is found in the fact that when norbixin is partially methylated the product is isobixin and not bixin. When the calcium salt of norhydrobixin is heated with lime, the hydrocarbon  $C_{23}H_{46}$ , is obtained as an oil.

Three mono-methyl compounds are known, viz., bixin, isobixin and  $\beta$ -bixin, and it is suggested that in the formation of one a transposition of an olefine linking in bixin occurs. Isobixin is not so readily hydrolysed by alkalis as bixin.

When bixin is heated in a current of hydrogen, *m*-xylene<sup>3</sup> is formed, but no palmitic acid as stated by Zwick. The ozonide when distilled gives methyl  $\beta$ -acetylacrylate,  $CH_3 \cdot CO \cdot CH : CH \cdot CO_2Me$ , together with other products.<sup>4</sup>

<sup>1</sup> viz., with lead chromate and a 10 cm. layer of platinised asbestos, cf. *Ber.* 1917, 50 927.

<sup>2</sup> *Annalen*, 1923, 431, 40.

<sup>3</sup> Van Hasselt, *Chem. Weekblad*, 1909, 6, 480.

<sup>4</sup> Reinkes, *loc. cit.*

## EXPERIMENTAL.

*Preparation of the crude dye.*—The seeds were used for the preparation of the dye and these were subjected to treatment resembling that devised by Dumontal in 1848. The seeds were rubbed by hand under water, the resulting product strained through a 10-mesh sieve and the operation repeated until practically all the dye was removed from the surface of the seeds. The coloured turbid liquid was allowed to settle and the upper layer then removed by decantation. The somewhat pasty, lower layer was heated to boiling to coagulate the small particles, then filtered, washed and dried in the sun on porous plates and finally ground and dried at 100–105°. The average yield of dry crude dye was usually about 6 per cent. of the weight of the seeds taken. It is stated that this method gives a product richer in bixin than the alternative method in which the seeds and pulp are macerated with water and allowed to ferment.

In order to deal with larger quantities of material, a modification of Dumontal's method was subsequently used. 5 lbs. of seeds together with 10 lbs. of water were placed in a small wooden barrel which was kept revolving on its longitudinal axis for a number of hours. The liquid was removed from time to time and fresh water introduced. With 5 lbs. of seeds a yield of 7 per cent. of crude dye was obtained, but with larger quantities, e.g., 10 or 15 lbs. of seeds the yield fell to 4.5 or even 3.0 per cent., largely owing to the dye forming balls about the same size as the seeds, and thus remaining behind with the seeds.

*Isolation of Bixin.*—Zwick's method was first tried, but it was found necessary to mix the crude dye with appreciable quantities of sand in order to prevent its forming a pasty mass and choking the extractor. Light petroleum does not appear to be an efficient solvent for removing resinous matter from the dye, and the final product obtained was sticky and impure. Much better results were obtained by using Heiduschka and Panzer's method, and working according to their directions a 6 per cent. yield of fairly pure bixin melting at 186–189° was obtained.

The process of washing the crude dye with acetone on an open filter proved to be very tedious, entailing considerable loss of solvent; the first washings were always turbid and appeared to hold a quantity of the dye in suspension.

Attempts to purify the crude product by extraction for an hour in a Soxhlet apparatus readily gave pure bixin, but about 33 per cent. of the dye was lost by solution in the acetone as the temperature was too high. A very convenient method of removing the resin by means of acetone is to close the tube which conducts the vapour of the

solvent to the top of the extractor and to lead these vapours from the flask to the top of the condenser by means of a long glass tube. By this means the acetone entering the extractor is maintained at the room temperature, and can be kept at this temperature by shielding the extractor from the heat of the water bath or electric heater. Extraction for half an hour in such an apparatus was sufficient to remove practically all resin.

After treatment with acetone the residue is extracted with chloroform until the extract becomes practically colourless; the chloroform is removed, the product weighed and then crystallised from hot chloroform or ethyl acetate.

By adopting this process it appears possible to eliminate the intermediate crystallisation from glacial acetic acid, the second washing with acetone and the washing with alcohol and ether, without affecting the purity of the product.

As a rule a single crystallisation from chloroform or ethyl acetate is sufficient to give a very pure bixin; ethyl acetate, which is recommended by Heiduschka as the best crystallising medium, is not very suitable as the solubility of bixin in the hot and cold solvent is much the same.

Table II gives the results obtained in a number of experiments.

TABLE II.

No.	Weight of dye	Conditions of Acetone Extraction	Weight in grams of material used	M. P. of crude bixin	Weight of bixin	Per cent. of bixin
1	in gr. 50	Washed in an open filter (Heiduschka).	8.4	...	in gr. 3	6
2	19	Hot extraction for 1 hour (ordinary Soxhlet).	8.7	...	0.7	3.7
3	23	Hot extraction for 1 hour (ordinary Soxhlet).	1.95	187-192°	0.95	4.1
4	20	Hot extraction for 2 hours (ordinary Soxhlet).	1.80	183-192°	0.80	4
5	20	Hot extraction for 3 hours (ordinary Soxhlet).	1.30	188-192°	0.45	2.25
6	20	Cold extraction for 1 hour (modified Soxhlet).	3.6	183-186°	1.6	8
7	20	Cold extraction for 1 hour (modified Soxhlet).	3.3	178-182°	1.4	7
8	20	Cold extraction for $\frac{1}{2}$ hour (modified Soxhlet).	4.4	178-185°	1.7	8.5
9	20	Cold extraction for $\frac{1}{2}$ hour (modified Soxhlet).	3.8	180-184°	1.8	9
10	20	Cold extraction for $\frac{1}{2}$ hour (modified Soxhlet).	3.6	178-184°	1.7	8.5
11	240	Cold extraction for $\frac{1}{2}$ hour (Copper Soxhlet).	42.4	185-190°	21.5	8.96

*Composition of Bixin.*—Bixin crystallised from ethyl acetate and from chloroform has been subjected to combustion and the results are given in Table III together with the values obtained by Heiduschka.

The combustion of bixin is slow and in the actual analysis was continued until the absorption tubes showed no further increase in weight.

The results obtained are the same whether the material is crystallised from chloroform or ethyl acetate.

TABLE III.  
*Combustions of bixin.*

Serial No.	Crystallising medium	Per cent. hydrogen	Per cent. carbon	Hydrogen	Carbon
1	Ethyl acetate	7.35	75.80	7.48	76.01
2	Do.	7.58	76.28		
3	Do.	7.51	75.93		
4	Chloroform	7.66	75.58	7.51	75.93
5	Do.	7.48	76.23		
6	Do.	7.48	75.75		
Heiduschka's results	...	{ 7.86 7.68	{ 75.71 75.60	7.77	75.66
Calculated for $C_{26}H_{30}O_4$	...	...	...	7.44	76.81
Do. $C_{25}H_{30}O_4$	...	...	...	7.61	76.11
Do. $C_{24}H_{28}O_4$	...	...	...	7.36	75.80

The values agree with Heiduschka's formula  $C_{25}H_{30}O_4$  better than Heiduschka's own analytical figures. They agree, however, equally well with the formula  $C_{24}H_{28}O_4$  and it is impossible to distinguish between two such formulae by simple determination of carbon and hydrogen.

*Methoxyl estimations.*—The methoxyl value was determined by Hewitt and Moore's method<sup>1</sup> using material crystallised from chloroform and also from ethyl acetate. The latter gave a value 7.36 and

<sup>1</sup> *J. Chem. Soc.*, 1902, 81, 318.

the former a value 7.49, as compared with 8.03 obtained by Heiduschka and Panzer<sup>1</sup> and the values 6.71 and 7.86 required by the formulae  $C_{29}H_{34}O_5$  and  $C_{25}H_{30}O_4$ .

*Reduction of bixin.*—Several attempts were made to determine the degree of unsaturation of bixin by Fokin's method<sup>2</sup> but difficulties were experienced in obtaining an equilibrium between the catalyst, hydrogen and solvent before the introduction of the bixin. According to Herzig and Faltis<sup>3</sup> bixin in the presence of palladinised barium sulphate absorbs 18 equivalents of hydrogen.

*Attempted benzoylation of bixin.*—If bixin contains a free hydroxyl group it should be possible to prepare a benzoyl derivative. Attempts using both the Schotten-Baumann and the pyridine methods gave negative results. Experiments on acetylation also gave negative results.

*Hydrolysis of bixin.*—When bixin was boiled for 4 hours with a 5 per cent. solution of sodium carbonate and then distilled, methyl alcohol could be detected in the distillate and from the alkaline solution norbixin was precipitated. This reaction agrees with Herzig and Faltis' view that bixin is an ester and not a methyl ether.

*Oxidation with permanganate.*—No definite products were obtained by oxidising bixin with hot alkaline permanganate solution using 2, 5, 10 and even 20 atoms of oxygen for each molecule of bixin. With ferricyanide a small amount of a colourless product melting at 90-95° was isolated.

This work was undertaken before Herzig and Faltis' paper appeared in 1923 and was discontinued in July, 1923, owing to my leaving Bangalore for England.

In conclusion I have to thank Dr. J. J. Sudborough for suggesting the investigation and for his kindly guidance during the course of the experiments.

<sup>1</sup> *Ber.*, 1917, 50, 548.

<sup>2</sup> *J. Russ. Phys. Chem. Soc.*, 1908, 40, 700.

<sup>3</sup> *Annalen*, 1923, 431, 41.