APPLICATION OF CHROMATOGRAPHY TO THE PURIFICATION OF DIRECT COTTON DYES AND VAT DYES, AND THE CONSTITUTION OF SOME VAT DYES.

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A Thesis
submitted by

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to the

UNIVERSITY OF BOMBAY

for the degree of

Ph.D
(Technology)

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December, 1958.

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PART I

DIRECT COTTON DYES:

CHROMATOGRAPHIC PURIFICATION & CRYSTALLIZATION OF CONGO RED, BENZOPURPURINE 4B & CHRYSOPHENINE G.

The preparation of dyes in analytically pure form is important in work on the mechanism of dyeing, the relation between absorption spectra and chemical constitution, and other fundamental problems. In view of the number and variety of the synthetic dyes it is obvious that a general procedure for the isolation of pure dyes from commercial products cannot be available. The methods which have been used for dyes of various classes have been surveyed elsewhere[1,2], and the present work is concerned with a few direct cotton dyes.

No direct cotton dye analysing correctly for all the elements within the limits of experimental error appears to have been described so far. The state of purity has usually been defined only in terms of the sodium content, determined as sodium sulphate by ashing with sulphuric acid. Direct cotton dyes in general are water-soluble sodium salts of sulphonic acids, and during manufacture they are precipitated by salting out from aqueous solution. More common salt, sodium sulphate and other diluents may be added for standardization and other purposes. The problem which has to precede attempts at crystallization of a commercial direct cotton dye is, therefore, the removal of sodium chloride and other inorganic electrolytes. Dialysis[3] and electrodialysis[4] lead to acid dye salts, which are often insoluble or sparingly soluble in water and cannot be neutralized exactly with caustic soda[2]. Continued washing in a pressure ultrafilter, until the conductivity of the ultrafiltrate is of the same order as that of the distilled water used[5], has been shown by Robinson and Mills[6] to be extremely slow and impracticable. In connection with pharmacological investigations Lubs[7] developed a general method for the purification of azo dyes which was first applied to Brilliant Congo R (C.I. 456; not mentioned in the new Colour Index, 1956). The dye was dissolved in boiling water and precipitated by sodium acetate; the precipitate was collected and digested with 95 per cent alcohol several times to remove sodium acetate. Azorubin (C.I.Acid Red 14; C.I. 14720) and Congo Red (C.I.Direct Red 28; C.I.22120) were purified similarly; the purity was estimated only by determining the sodium content, which differed from the required value by 0.3-0.4 per cent. Benzopurpurine 4B (C.I.Direct Red 2; C.I. 23500) of 100 per cent purity according to the sodium content was prepared by Robinson and Mills[6] by salting out an aqueous solution of the commercial dye with sodium acetate five times, when both the filtrate and dye were found to be free from chloride, and extracting the sodium acetate with boiling absolute alcohol five or six times. Boulton *et al.*[8] purified Benzopurpurine 4B, Chrysophenine G (C.I.Direct Yellow 12; C.I.24895), and Sky Blue FF (C.I.Direct Blue 1; C.I. 24410) by the sodium acetate method, and they have claimed to have achieved purities of very nearly 100 per cent, assayed by sodium content and titanous chloride titration. Ammonium carbonate, removed subsequently by sublimation at 110°, has been used[9] for salting out Benzopurpurine 4B and Diamine Blue 2B (C.I.Direct Blue 6; C.I.22610), but the products are largely the ammonium salts[6].

For the removal of inorganic salts from acid dyes, such as Orange R (C.I.Acid Orange 8; C.I.15575) and Azo Yellow (C.I.Acid Yellow 63; C.I.13095), two patents suggest dissolution in diacetone alcohol, tetrahydrofurfuryl alcohol, carbitols or cellosolves, precipitation of inorganic salts by the addition of solvents such as methanol and benzene, filtration and evaporation[10].

The sodium acetate method was found by Richardson[11] to be tedious because it often involved the filtration of near-colloidal precipitates, and prolonged extractions, which may result in considerable loss of dye, were necessary to remove the last traces of acetate. Based on the observation that ions of an average diameter of about 30A, corresponding approximately to the dimensions of benzidine dyes, were completely prevented from entering ion-exchange resins, Richardson described the purification of direct cotton dyes by ion-exchange techniques which were applied to Sky Blue FF, Benzopurpurine 4B, and Chlorazol Fast Red (C.I.Direct Red 1; C.I.22310). Purities were determined only in terms of the ash content; samples of Sky Blue FF, for instance, purified by the ion-exchange procedure, had ash contents of 25.5-27.2 per cent, the calculated value being 28.6 per cent; for Benzopurpurine 4B the found and calculated values respectively were 17.9 and 19.6 per cent. These differences were explained by the suggestion that the overall degree of sulphonation of the dye sample was less than theoretical, which appears to be unwarranted.

Aniline Black has been used as basic ion-exchanger for the purification of Sky Blue FF, which was thus obtained 94.3 per cent pure[12].

Aromatic sulphonic acids and arylamines form salts which crystallize well and have sharp melting or decomposition points, and the reaction can be used for characterizing both sulphonic acids and amines[13]. Rose used a similar method for purifying direct cotton dyes[14]. If a solution of the hydrochloride of diphenylguanidine or di-o-tolylguanidine is added to a hot solution of the dye, a tarry precipitate is formed at once and can be collected on a glass rod. It solidifies on cooling, and is then broken up, washed with water containing a little of the guanidine hydrochloride, and dissolved in methanol. The dye is then isolated as the sodium salt by adding the theoretical amount of sodium hydroxide or methoxide. Some of the guanidine salts can be recrystallized from absolute or aqueous alcohol. Rose has recorded no analytical data, but this procedure has possibilities which we are now exploring. Sclar[15] has used *n*-octadecylamine for precipitating the acid dye, Acid Fuchsine (C.I. Acid Red 33; C.I.17200); the salt was recrystallized from 50 per cent alcohol, decomposed with the calculated amount of caustic soda, the precipitated amine filtered off, and the solution concentrated under reduced pressure; the dye was precipitated by acetone, collected and dried at 135°. Elementary analyses of N, S and Na in close agreement with the theoretical values have been recorded; titanous chloride titration indicated a purity of 99.9 per cent.

Chromatography does not appear to have been applied for the removal of inorganic electrolytes from direct cotton dyes, although several chromatographic procedures have been described for the separation of mixtures of dyes. Thus Ruggli and Jensen[16] chromatographed water-soluble dyes from aqueous solution on alumina, and used it as a method of testing the homogeneity of dyes and the separability of dye mixtures. Cellulose in the form of powder and paper has been widely employed in chromatographic analysis, the separations being usually explained on the basis of partition on cellulose, which is assumed to function mainly as an inert support for the stationary phase. Lederer[17] separated acridine dyes on paper using water or dilute hydrochloric acid as developing solvent, referring to the mechanism of separation as "a clear case of adsorption chromatography".

The adsorption of direct cotton dyes on fibrous cotton material by chromatographic technique has been mentioned by Flugel and Koch[18,19]. The affinity of direct cotton dyes towards cellulose has been utilized in the present work for separating them from inorganic electrolytes and other impurities whose adsorption affinities for cellulose are of a much lower order.

On passing an aqueous solution of a direct cotton dye mixed with inorganic electrolytes through a column of powdered cellulose, the dye remains adsorbed on cellulose and the inorganic salts and other non-substantive impurities, if any, travel down the column with the solvent front. Elution with distilled water is continued until inorganic salts are completely removed. The chief difficulty during elution with distilled water is bleeding or tailing of the adsorbed band, involving in the case of some dyes a substantial loss of dye. This also makes it more difficult to test for inorganic electrolytes (e.g. sodium chloride) in the percolate. Bleeding of the dye is considerably reduced, if not totally eliminated, by introducing two modifications in the procedure; (a) carrying out the adsorption from a hot solution, and (b) eluting the inorganic salts with water at as low a temperature as possible, when the desorption of the dye from the substrate is at a minimum. Under these conditions Congo Red and Benzopurpurine 4B remain strongly adsorbed, but with Chrysophenine G, a dye of level-dyeing and non-aggregating character, bleeding can only be minimized. Another modification which increases the capacity of the column is to dye cellulose powder separately under the normal conditions of dyeing and then pack the dyed cellulose on top of a previously prepared column of untreated cellulose powder. A convenient general procedure is as follows. Cellulose powder (Whatman standard grade or powdered cellulose, 120 mesh, copper number 6-7, prepared from bleached pulp by acid treatment) is made into a slurry with distilled water and packed in a chromatographic column to about half the height. The column is then made up with cellulose powder dyed with the commercial dye and left in a cold room at 1°-2° until this temperature is attained. Water at 1°-2° is run through the column until the percolate does not answer tests for chlorides and sulphates. The column is then brought to room temperature (25°-28°) and washed with pure acetone to remove any organic impurities; a small amount of dye may be lost during this treatment. Elution of the dye is effected by means of a mixture of acetone and water (80 to 20 by volume). Separation of coloured impurities is noticed at this stage, and the eluant from the major zone of the pure dye is collected. The dye is recovered by azeotropic distillation with carbon disulphide or by distillation below 50° at about 30 mm. pressure.

As Robinson and Mills[6] have stated, direct cotton dyes in general cannot be crystallized from water. Quoting the statement of Pelet-Jolivet in _Die Theorie des Farbeprozesses_ that he purified

Benzopurpurine 4B by recrystallization from water, they conclude that he did not actually obtain crystals, since the cooling of a 2 per cent solution of the dye results in the separation of a tactosol. Weltzien and Gotze[19] have mentioned the crystallization of Brilliant Benzo Blue 6B (Sky Blue FF; C.I. Direct Blue 1), but not the procedure employed or the specific crystalline character of the product. Crystallization of Congo Red from 50 per cent alcohol for the purpose of obtaining it chromatographically homogeneous has been reported by Ruggli and Jensen[16]; Fig. 1 is a photomicrograph of Congo Red crystallized thrice from 50 per cent alcohol.

Chrysophenine crystallizes readily (Fig.2) from absolute or aqueous methanol; Standing and Warwicker[20] purified this dye by crystallizing several times from alcohol-water, after removal of inorganic salts by the sodium acetate method.

Benzopurpurine 4B has apparently not been obtained in a crystalline form so far. The commercial dye or the pure dye isolated by cellulose powder chromatography does not crystallize from aqueous alcohol, methanol or acetone[21]. However, if an aqueous solution of the pure dye is mixed with n-butanol (about 5 vols.) to form a clear homogeneous solution, filtered if necessary, and distilled at about 37°-40° and 40-50 mm. Hg. pressure, water is removed azeotropically, and the dye crystallizes in red needles (Fig. 3). After filtration, washing with acetone, and drying at room temperature in a vacuum, the crystalline dye appears reddish brown in colour and has a greenish metallic lustre. If distillation of butanol is continued after the azeotrope with water has distilled over, the crystalline dye crumbles to a fine powder. A remarkable property of crystalline Benzopurpurine 4B is its very sparing solubility in water, and this can be used for obtaining the dye free from inorganic salts without recourse to chromatography or sodium acetate treatment. The commercial dye is crystallized from butanol-water; the mixture of crystalline dye and inorganic salts that separate is filtered and washed with distilled water till the latter are completely removed. The crystalline dye is soluble in 80 per cent acetone, methanol, dimethylformamide and the cellosolves like the original amorphous dye; it also dissolves in water

FIG.1
Congo Red crystallized from
50 per cent Alcohol (X 200)

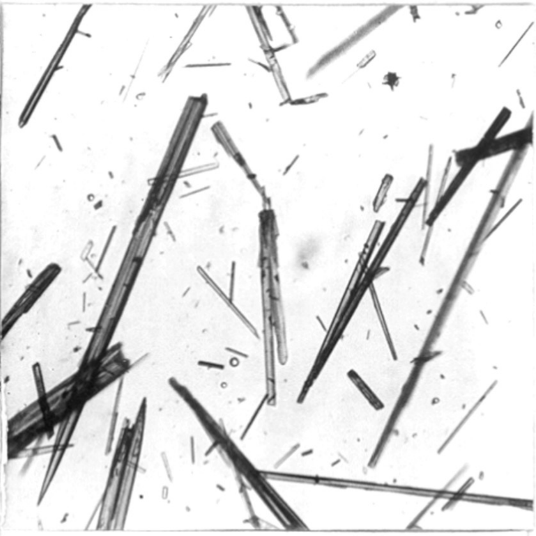

FIG.2
Chrysophenine G crystallized from
Methanol (X 200)

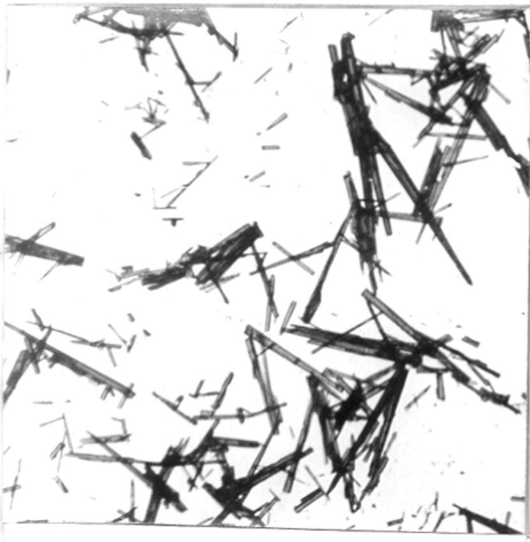

Fig.3
Benzopurpurine 4B crystallized from
n- Butanol - water (X 200)

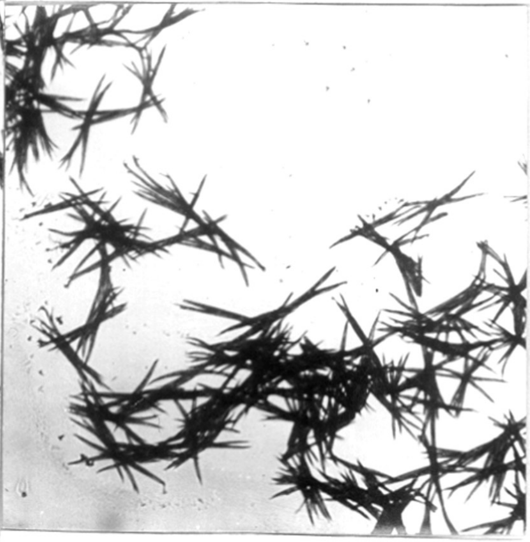

Fig.4
Congo Red crystallized from
n- Butanol- water (X 200)

if a trace of sodium carbonate or caustic soda is added. If the crystalline dye is submitted to distillation with butanol, it is transformed into a fine brownish red powder which now dissolves readily in water.

Congo Red (Fig.4) and Chrysophenine G (Fig.5) can also be crystallized by the butanol-water technique.

All the elements, except oxygen, were determined in the crystallized dyes after drying them for 4 hr. at the stated temperature in a vacuum (0.5 mm. Hg. pressure) over phosphorus pentoxide. Crystalline Benzopurpurine 4B, dried at 30°, contained $4H_2O$. When the crystalline substance was further distilled with butanol and dried at 110°, the product still contained $1H_2O$. Crystalline Congo Red, dried at 30°, contained $2H_2O$. Azeotropic distillation with butanol and drying at 105° failed to remove the water of crystallization, and the product still contained $1.5H_2O$, Crystalline Chrysophenine G contained $2H_2O$, not removed by drying at 105°; distillation with butanol yielded an amorphous orange powder which, after drying at 105°, contained $1H_2O$. The moisture content of each dye sample was estimated not only on the basis of the elementary analysis, but also by heating at 140°-45° in a current of nitrogen and absorbing the liberated water in anhydrone; the anhydrous dyes obtained in this manner were found to be extremely hygroscopic, increasing in weight during the processes of cooling to room temperature, transferring the boat to a balance and weighing. The crystalline dyes, containing co-ordinated water, were also hygroscopic if they were finely powdered and exposed to a humid atmosphere. Thus, crystalline Benzopurpurine 4B, finely powdered and exposed to the atmosphere at 28° and 68 per cent R.H. for 24 hr., was found to contain 13.5 per cent (corresponding to 6 mols) of H_2O, the moisture being determined, as mentioned above, by heating at 140°-45° in a current of nitrogen; elementary analysis of the sample, calculated on a bone-dry basis, gave values in close accord with the theoretical values. Crystalline Congo Red behaved similarly.

The purity of each dye was ultimately determined by heating the crystalline hydrated dye at 140°-45° in a current of nitrogen for 1 hr. in an electrically

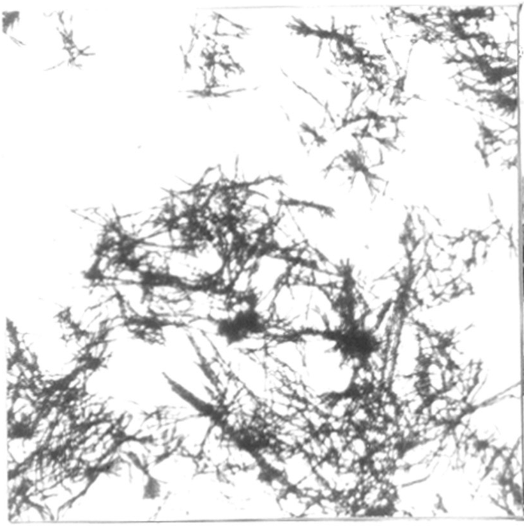

Fig. 5
Chrysophenine G crystallized from
η-Butanol-Water (X 200)

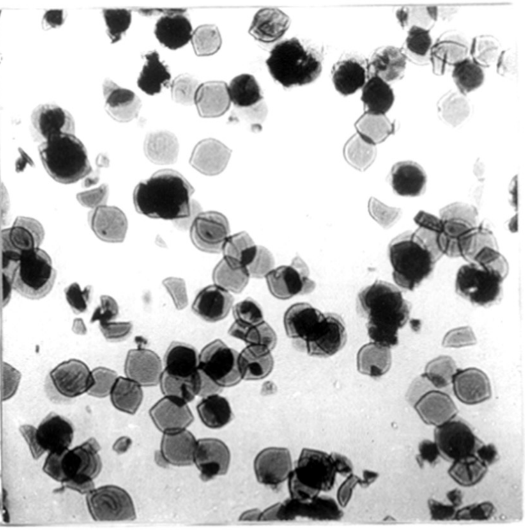

Fig. 6
Potassium salt of Congo Red (Mitchell) (X 200)

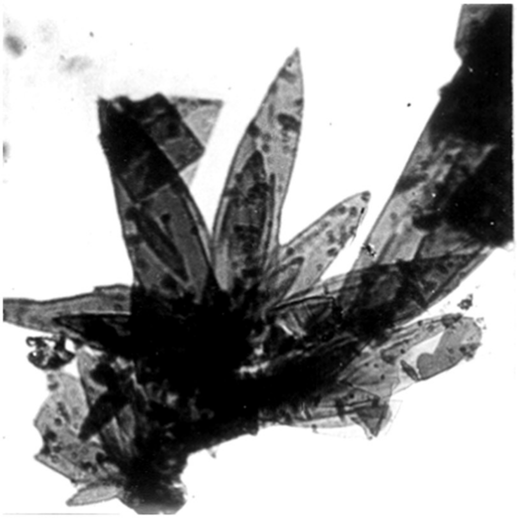

Fig. 7
Potassium salt of Congo Red Crystallized from
50 per cent Alcohol (X 200)

heated combustion unit for microanalysis of carbon and hydrogen. After noting the increase in weight of the anhydrone moisture-absorption tube, the temperature of the combustion furnace was raised to 750° and carbon-hydrogen estimation carried out as usual.

The potassium salt of Congo Red was obtained by Mitchell[22] in the form of very thin optically anisotropic plates by treating an aqueous solution of the dye at 95° with 40 per cent potassium acetate solution; the crystalline precipitate was collected, and recrystallized in the same manner four times; the crystals (Fig.6) were finally washed with boiling 98 per cent alcohol until free from potassium acetate. No analyses have been recorded. Mitchell also observed that the potassium salt of Congo Red crystallized in needles from alcohol-water mixtures, but only with difficulty and accompanied by much amorphous material. We have found that the potassium salt, prepared through the magnesium salt decomposed by means of potassium carbonate, can be crystallized (Fig.7) from 50 per cent alcohol in the normal way without salting out at 95° by potassium acetate. Unlike the sodium salt, the potassium salt (dried at room temperature) did not contain water of crystalization and analysed correctly for all the elements.

EXPERIMENTAL

Preparation of cellulose powder - Sheets of bamboo pulp (500 g.) were soaked in water (5 litres) and concentrated sulphuric acid (500 g.) added gradually under vigorous stirring, maintaining the temperature below 50°. The temperature was then raised to 60°-70° and stirring continued for 4 hr. After cooling overnight, the pulp was filtered off and washed throughly with water till free from acid. The pulp at this stage was cream coloured like the starting material, but considerably degraded. It was dried in air for about 2 days, then for a few hours at 110°, and powdered to 120 mesh in a disintegrator (Hammer mill type; 3000 r.p.m.).

The powder was then bleached for 2 hr. at 60°-70° with sodium chlorite (5g./litre) and hydrochloric acid to maintain the pH at 3-4. The cellulose powder was filtered off, washed with water till free from acid and chloride and then with acetone, and dried at 110° overnight. The material at this stage was white and in the form of small lumps which broke up to a fine powder on stirring with water. The copper number was 6.5.

PURIFICATION OF BENZOPURPURINE 4B

Method (A): Chromatography on cellulose powder:

(a) Removal of inorganic electrolytes - Cellulose powder (100 g.) was made into a slurry with distilled water (400 ml.), poured into a chromatographic column, and packed uniformly (2.5 x 50 cm.) A boiling 2 per cent solution of sodium chloride (100 ml.) was run through the column, followed by a hot solution (90°-95°) of commercial Benzopurpurine 4B (250 mg.) in water (200 ml.). The column was left at room temperature for 1 hr. and then washed with 2 per cent salt solution (100 ml.). The column was then transferred to a cold room (0°-2°), and after 4 hr. distilled water at 0°-2° was run through the column until the eluant was found to be free from chloride; complete removal of chloride was usually attained after about 400 ml. of water had run through. Loss of dye in the aqueous percolate was slight, and the dye was retained on the column as a sharp red band (about 20 cm.) with a deep blue zone (1-2 cm.) at the top. The column was then allowed to attain room temperature (28°).

An alternative procedure for the cellulose powder chromatography was as follows. Cellulose powder (50 g.) was made into a slurry with distilled water (200 ml.) and packed uniformly inside a chromatographic column (2.5 x 25 cm.). Cellulose powder (30 g.) was separately dyed with Benzopurpurine 4B (1 g.) at the boil, using common salt as usual. The dyed cellulose was filtered off, and washed rapidly with distilled water (1 litre) at 5^{0}-10^{0}. This treatment removed most of the sodium chloride, together with a small amount of the dye. The wet cake was stirred into a slurry with cold water (100 ml. at 4^{0}-5^{0}) and poured into the chromatographic column. Thus a sharp red band of about 15 cm. was obtained over a colourless cellulose column (25 cm.). After transferring to a cold room (0^{0}-2^{0}), distilled water at 0^{0}-2^{0} was run through until the percolate was totally free from chloride, and the column was allowed to attain room temperature.

(b) Elution of the dye - The column was next washed with pure acetone (300 ml.) at room temperature to remove any organic impurity that may be present. During this treatment a small amount of dye was washed down with the solvent, but the major part of the acetone eluant was colourless. Elution of the dye was effected with aqueous acetone (acetone 80, water 20; 500-600 ml.). Three bands appeared on the column. The first, a reddish brown band (3-4 cm.) which came down with the solvent front, was discarded; the second and major red band (about 30 cm. long) was eluted by about 300 ml. of the solvent; the third band (3-4 cm.), which was also red in colour, was held strongly on the column and was not eluted. The last red band occupied the same position as the deep blue zone mentioned earlier, but during development with aqueous acetone, the colour changed from blue to red.

(c) Recovery of the dye: (1) Azeotropic distillation (using the ternary azeotrope of acetone, water and carbon disulphide, b.p.38.4°) - A mixture of carbon disulphide (250 ml.), acetone (200 ml.) and the dye solution in 80 per cent acetone (100 ml.) was submitted to azeotropic distillation in a recycling apparatus. In about 4 hr. all the water was removed and red Benzopurpurine precipitated from the organic solvent mixture. The dye was collected (50 mg.) and dried for 4 hr. at 30° and 0.5 mm.

pressure over phosphorus pentoxide. Unless otherwise stated, all samples for analysis in the present work were dried under these conditions. (Found: C, 52.0; H, 5.0; N, 10.7. Calculated for $C_{34}H_{26}O_6N_6S_2Na_2$: C, 56.4; H, 3.6; N, 11.6. Calculated for $C_{34}H_{26}O_6N_6S_2Na_2$, $4H_2O$; C, 51.3; H, 4.3; N, 10.5 per cent).

(2) Vacuum distillation - The dye solution in 80 per cent acetone (100 ml.) was distilled at 40 mm. in an atmosphere of nitrogen. Most of the acetone was removed at about 30°, and water removed at 50°-60°. The residue (50 mg.) was a bright red powder, which turned darker on drying. (Found: C, 49.2; H, 4.7; N, 9.6. Calculated for $C_{34}H_{26}O_6N_6S_2Na_2$, $6H_2O$: C, 49.0; H, 4.6; N, 10.1 per cent).

(3) Vacuum distillation and azeotropic distillation- To the dye solution (100 ml. of 80 per cent acetone solution) after the removal of acetone at 40-50 mm. dry benzene and absolute alcohol (50 ml.each) were added and the mixture distilled at 40-50 mm. The last traces of solvents were removed at 50°-60°; yield 50 mg. (Found: C, 49.3; H, 4.6; N, 9.4. Calculated for $C_{34}H_{26}O_6N_6S_2Na_2$, $6H_2O$: C, 49.0; H, 4.6; N, 10.1 per cent).

(4) Crystallization from butanol/water - The 80 per cent acetone eluant (300 ml.) collected after chromatography was distilled at 30° and 40-50 mm. pressure, and acetone recovered. To the residual aqueous solution of the dye (50-60 ml.), n-butanol (300 ml.) was added and the mixture vigorously shaken till a homogeneous solution was obtained. The clear red solution was filtered through a sintered glass filter and the filtrate distilled at 40-50 mm. pressure. The water was azeotropically removed and the dye gradually crystallized out in microscopic red needles. When the boiling point rose to 55°-60° the distillation was stopped. The solution was pale red in colour. The crystallized dye was filtered off, washed with acetone and collected as reddish brown needles with a greenish metallic lustre (100 mg.). Found: C, 51.6; H, 4.6; N, 10.7; S, 7.5; Na, 5.9; H_2O, 9.6. Calculated for $C_{34}H_{26}O_6N_6S_2Na_2$, $4H_2O$: C, 51.3; H, 4.3; N, 10.5; S, 7.8; Na, 5.8; H_2O, 9.0 per cent.)

(d) Dehydration of the crystalline dye - Crystalline Benzopurpurine 4B (0.2 g.) was mixed with n-butanol

(300 ml.) and distilled at 64°/50 mm. The crystalline dye changed to a fine reddish brown powder as distillation progressed. When 200 ml. of the solvent had distilled over, the precipitate was collected, washed with absolute ethanol, and dried at 105°. (Found: C, 54.4; H, 3.8; N, 11.8; S, 8.5; Na, 6.0; H_2O, 2.4, 2.7. Calculated for $C_{34}H_{26}O_6N_6S_2Na_2$, $1H_2O$: C, 55.0; H, 3.8; N, 11.3; S, 8.6; Na, 6.2; H_2O, 2.4 per cent.).

Method (B): Direct crystallization - Commercial Benzopurpurine 4B (1 g.) was dissolved in boiling water (100 ml.) and filtered. Pure dry n-butanol (600 ml.) was added to the filtrate and the mixture shaken for a few minutes. The cloudy solution was filtered under suction through a double filter paper to get a clear filtrate and a reddish black residue (0.1 g.) which was discarded. The filtrate was again filtered through a sintered glass filter into a distillation flask. The clear red solution was slowly distilled over a period of 3-4 hr. at 50 mm. The water was removed azeotropically (b.p.37°-40°) and the dye gradually crystallized out in shining reddish brown needles. When the b.p. rose to 55°-60° the distillation was stopped and the crystallized dye collected (0.5 g.). The dye was shaken with distilled water (20 ml.) for a few minutes, filtered, and washed with distilled water till the filtrate was free from chloride. The dye was finally washed with a little acetone, and dried as usual. (Found: C, 51.4; H, 4.6; N, 10.5; S, 7.8; Na, 5.7. Calculated for $C_{34}H_{26}O_6N_6S_2Na_2$, $4H_2O$: C, 51.3; H, 4.3; N, 10.5; S, 7.9; Na, 5.8 per cent).

A portion of the dye was finely ground and kept exposed to the atmosphere (28°, 68 per cent R.H.) for 24 hr., and analysed again. (Found: C, 48.1; H, 4.8; N, 9.7; H_2O, 13.5. Calculated for $C_{34}H_{26}O_6N_6S_2Na_2$, $6H_2O$: C, 49.0; H, 4.6; N, 10.1; H_2O, 13.0 per cent).

PURIFICATION OF CONGO RED

Method (A): Chromatography on cellulose powder -

(a) A cellulose column (3 x 25 cm.) was built up by wet packing with distilled water using 100 g. cellulose powder. Cellulose powder (50 g.) was dyed with commercial Congo Red (2 g.) in water (500 ml.) under the usual conditions, washed with cold water till the washing was free from chloride, and packed uniformly on top of the undyed cellulose. The column was left in the cold room (0^{o}-2^{o}) and washed with distilled water (1 litre) at this temperature. Three bands appeared on the column: (a) a top blue zone (2-3 cm.), which gradually turned red, but did not move down on washing successively with water and aqueous acetone as mentioned below; (b) a middle deep red zone (about 20 cm.), and (c) a lower reddish blue zone (about 10 cm.). Elution with distilled water removed all the electrolytes and the lower reddish blue zone. Elution with pure acetone (600 ml.) did not effect any change in the appearance of the bands, except that a small amount of red dye was removed along with the solvent front. Washing with aqueous acetone (80 per cent; 750 ml.) completely eluted the middle major red band. The percolate (500 ml.) was freed from acetone by distillation at 50 mm., the aqueous residue mixed with n-butanol, and water removed by azeotropic distillation as described for Benzopurpurine. Reddish brown needles (600 mg.) were thus obtained (Fig.4). (Found: C, 52.7; H, 3.6; Na, 6.0. Calculated for $C_{32}H_{22}N_6O_6S_2Na_2$, $2H_2O$: C, 52.5; H, 3.6; Na, 6.3 per cent). The sample was then distilled with n-butanol, dried at 105°, and the bright red powder analysed. (Found: C, 54.3; H, 3.8; N, 12.0; S, 8.8; Na, 6.3; H_2O, 4.0. Calculated for $C_{32}H_{22}N_6O_6S_2Na_2$, $1H_2O$; C, 53.8; H, 3.4; N, 11.8; S, 9.0; Na, 6.4; H_2O, 2.5 per cent).

Method (B):Crystallization - Commercial Congo Red (5 g.) was dissolved in hot water (60 ml.) and alcohol added (60 ml.). After boiling for a minute the mixture was filtered. From the filtrate a crystalline product (long needles under the microscope) separated slowly. This was collected, washed with alcohol (5 ml.) and acetone (5 ml.), shaken for 2 min. with distilled water (25 ml.), filtered, and again washed with alcohol and acetone. The red-brown crystalline product (1 g.) had a greenish lustre. (Found: C, 52.8; H, 3.8; N, 11.2; S, 8.6; Na, 5.8. Calculated for $C_{32}H_{22}N_6O_6S_2Na_2$, $2H_2O$: C, 52.5; H, 3.6; N, 11.5; S, 8.7; Na, 6.3 per cent).

The sample, if finely ground and kept exposed to the atmosphere (28°; R.H. 68 per cent) for 24 hr., absorbed moisture and gained in weight. (Found: H_2O, 14.5 per cent, and in the dried material: C, 55.3; H, 3.2; N, 12.3; S, 9.0; Na, 6.2. Calculated for $C_{32}H_{22}N_6O_6S_2Na_2$: C, 55.2; H, 3.2; N, 12.1;

S, 9.2; Na, 6.6 per cent).

Method (C): Potassium salt of Congo Red - To a boiling solution of commercial Congo Red (20 g. in 2 litres of water) magnesium sulphate solution (10 g. $MgSO_4$, $7H_2O$ in 20 ml. water) was added under stirring. The precipitated magnesium salt of Congo Red was filtered off (13 g.), washed with water, and suspended in boiling water (1 litre). Potassium carbonate solution (4 g. in 20 ml.water) was added under stirring, and the mixture boiled for a few minutes. The precipitated magnesium carbonate was filtered off, washed with hot water and the potassium salt of Congo Red salted out from the filtrate with potassium acetate (60 g. per litre). The red crystalline precipitate was collected, washed with alcohol and sucked dry. The product (8 g.) crystallized from 50 per cent alcohol in shining red plates (5 g.) with a green metallic lustre. (Found: C, 52.7; H, 3.1; N, 11.9; S, 8.5; K, 10.8. Calculated for $C_{32}H_{22}N_6O_6S_2K_2$: C, 52.9; H, 3.0; N, 11.5; S, 8.8; K, 10.7 per cent).

PURIFICATION OF CHRYSOPHENINE G

Method (A): Chromatography on cellulose - Following the procedure described for Benzopurpurine, 2 g. of commercial dye yielded 0.6 g. of yellow needles. (Found in material dried at 105°: C, 50.3; H, 4.5; N, 7.7; S, 8.4; Na, 5.9; H_2O, 5.6. Calculated for $C_{30}H_{26}N_4O_8S_2Na_2$: C, 53.0; H, 3.8; N, 8.2; S, 9.4; Na, 6.8. Calculated for $C_{30}H_{26}N_4O_8S_2Na_2$, $2H_2O$: C, 50.3; H, 4.2; N, 7.8; S, 9.0; Na, 6.4; H_2O, 5.0 per cent). The dye was distilled with <u>n</u>-butanol, dried at 105° and again analysed. (Found: C, 51.7; H, 4.3; N, 8.0; S, 8.9; Na, 6.3; H_2O, 3.0. Calculated for $C_{30}H_{26}N_4O_8S_2Na_2$, $1H_2O$: C, 51.6; H, 4.0; N, 8.0; S, 9.2; Na, 6.6; H_2O, 2.6 per cent).

<u>Method (B): Crystallization</u> - Commercial Chrysophenine G Crystallized readily from absolute methanol. After two crystallizations the shining golden yellow needles were free from inorganic salts. (Found in material dried at 105°: C, 50.5; H, 4.0; N, 8.4; S, 8.9; Na, 6.4; H_2O, 5.6. Calculated for $C_{30}H_{26}N_4O_8S_2Na_2$, $2H_2O$: C, 50.3; H, 4.0; N, 7.8; S, 9.0; Na, 6.4; H_2O, 5.0 per cent).

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PART II

CHROMATOGRAPHY OF VAT DYES ON ALUMINA AT HIGH TEMPERATURES.

Applications of chromatography to synthetic dyes have been limited in spite of its enormous scope. The chief difficulty in the chromatographic analysis of dyes appears to be the non-availability of suitable solvents for adsorption, development and elution. Broadly, dyes are divisible into two classes from the point of view of solubility: water-soluble and water-insoluble. The impurities present in water-soluble dyes and a method of purification of direct cotton dyes involving chromatography of an aqueous solution of the dye on a column of powdered cellulose is described in Part (I) of this thesis.

Vat dyes are insoluble in water and water-soluble impurities can therefore be readily removed. The non-vattable impurities are readily removed by vatting the dyestuff with alkaline sodium hydrosulfite, filtration and re-oxidation, but this method should be employed with caution since some dyestuffs thus undergo permanent chemical changes such as dehalogenation and reduction of nitro groups. The vattable portion of a commercial dye may contain any vattable intermediate from which the dye was made as well as vattable bye-products. One or more vat dyes added for shading purposes may also be present. The insoluble or very sparingly soluble nature of vat dyes in organic solvents has led to considerable difficulty in isolating them in a pure form. Conc. sulphuric acid dissolves all vat dyes, but it is unsuitable as a solvent for chromatographic analysis; however, methods involving fractional crystallisation by careful dilution of the sulphuric acid with water, alcohol or acetic acid have been mentioned.

The problem of separation of mixtures of vat dyes has not received adequate attention. Conflicting statements have been made on the separability of alkaline vats on alumina[1,2,3]. Vat dyes reduced by aqueous caustic soda and sodium hydrosulphite have been chromatographed on columns of bleached saw-dust or disintegrated cotton; the chromatogram was then developed in the colours of the oxidised dyes by means of potassium ferricyanide solution[1]. This method appears to be limited to the separation of dyes of widely different substantivity to cellulose.

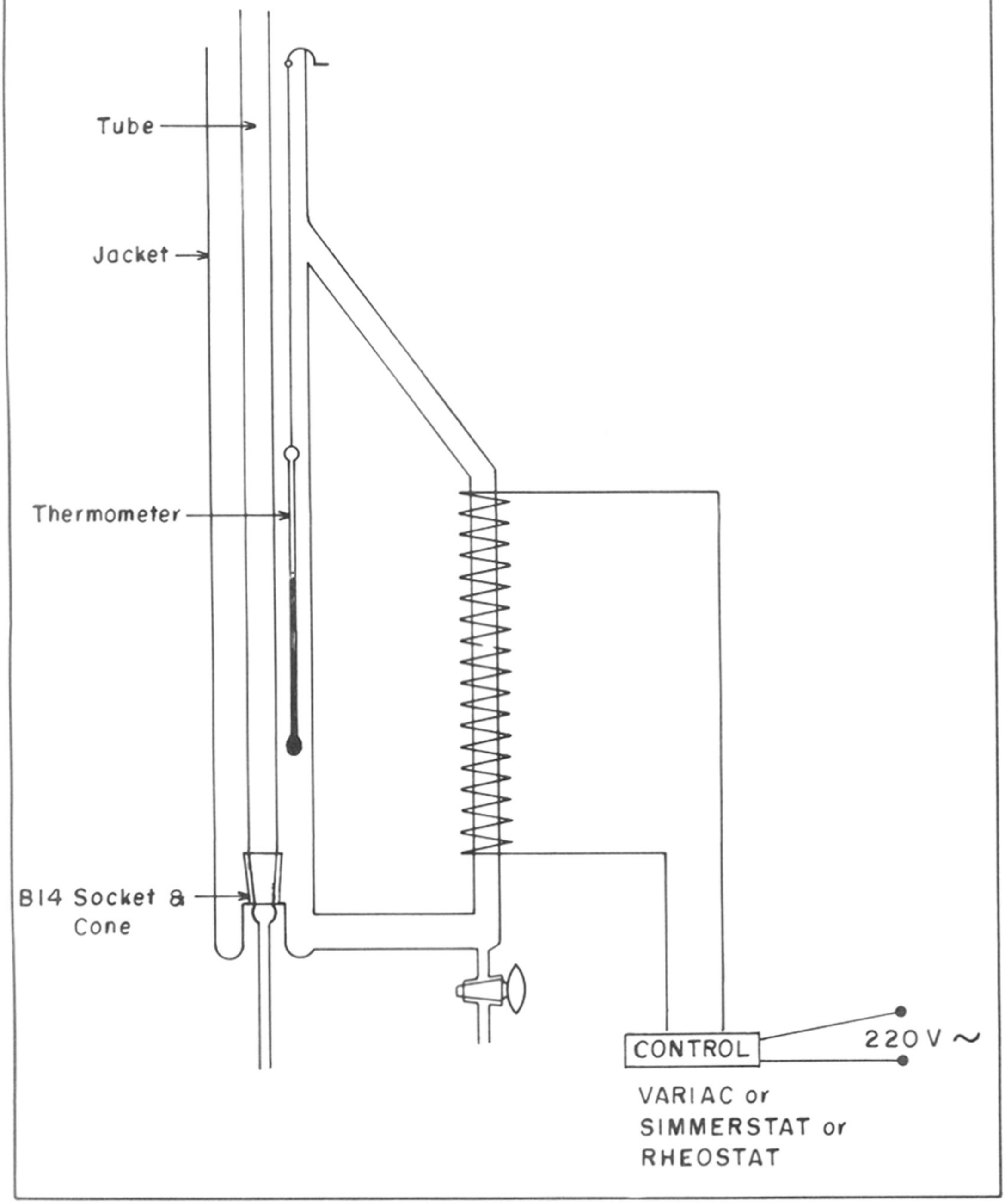
A generally applicable method for the separation of vat dyes[4] is to vat the mixture of dyes with aqueous tetraethylenepentamine, $NH_2(CH_2CH_2NH)CH_2$-CH_2NH_2, and sodium hydrosulphite, carry out the adsorption on a column of cellulose powder (80 mesh)[5] and develop the chromatogram with aqueous tetraethylenepentamine containing a little hydrosulphite[6]. One part of the organic solvent and four parts of water were employed both for adsorption and development. A clear and remarkably stable vat is obtained when a vat dye is made into a paste with tetraethylenepentamine and treated with aqueous sodium hydrosulphite at room temperature (20-30°). Employing these conditions several artificial mixtures of vat dyes were separated by Rao, et.al.[4] n-Butylamine which has been recommended as an effective solvent in conjunction with sodium hydrosulphite for stripping of vat dyes on cotton[7], gives a clear vat in the absence of caustic soda, but it is less effective than tetraethylenepentamine in separating mixtures of vat dyes. One difficulty with this method is the very slow rate of solvent flow; and further, since the leuco compounds of vat dyes have great affinity for cellulose, the movement of zones in the chromatographic column is extremely slow and elution is practically impossible. Addition of pyridine or cellosolve to the developing solvent cuts down the affinity of the leuco compounds considerably, and elution of some dyes can then be effected.

Using the vatting solution of Rao, et.al.[4], Klingsberg[8] has studied the chromatographic separation of mixtures of vat dyes on strips of paper and synthetic fibres. Operating in an atmosphere of nitrogen several artificial mixtures of vat dyes were quantitatively separated, employing spectrophotometric methods for the evaluation of the individual constituents after chromatographic separation. Twenty-two dyes were tabulated in the order of their adsorbability on cellulose and it was observed that the order of adsorption had no relation to the substantivity of the dyes under normal dyeing conditions. The descending technique was employed for separations and in the case of strongly held dyes it took days or weeks for separation.

It has been claimed that vat dyes in nitrobenzene solution can be separated on alumina[1], but many vat dyes are insoluble or very sparingly soluble in nitrobenzene in the cold. Bradley and Sutcliffe[9] used trichlorobenzene at 160°-170° for the chromatographic isolation of an isoviolanthrone derivative in the pure state. Varhman[10] has isolated the hydrocarbons in primary tars by chromatography in steam-jacketed columns of silica gel and elution with light petroleum (b.p.120°). Anthracene and chrysene were separated quantitatively from the corresponding quinones on alumina at the boiling point of toluene; the eluate was continuously distilled and the hot vapours passed around the column for heating purposes and later on condensed and fed to the top of the column[11].

Lederer[12] has described a vapor-jacketed column suitable for high temperature chromatography. A column maintained at an elevated temperature by means of a jacket containing a self-circulating liquid has been used for hot chromatography by Schram and Prinosigh[13], and the apparatus employed in the present work, shown in Fig. 1 and described in the experimental part,

DIAGRAM OF THE APPARATUS FOR HOT CHROMATOGRAPHY



is a modification in which interchangeable ground-glass joints permit columns of different diameters to be used. Thus quantities of alumina varying from 5 g. to 100 g. can be conveniently handled. Because of the poor solubility of vat dyes in organic solvents in the cold, column chromatography necessitates the use of enormous volumes of solvents and becomes impracticable. At higher temperatures most vat dyes have considerably greater solubility, and the present investigation concerns the chromatographic separation of vat dyes at elevated temperatures.

In order to carry out hot chromatography, a column of alumina was built by the wet packing method and maintained at the proposed working temperature. The dye mixture was added to the column as a hot solution or suspension, and development and elution carried out as usual. Very often the compound crystallised out of the eluate as it cooled down to room temperature and mere filtration sufficed to isolate the product; however, concentration to a small bulk effected a more complete recovery. Following this method several model separations of mixtures of vat dyes were carried out quantitatively. Vat dyes of known constitution were purified by repeated crystallisation, their purity checked by elementary analysis and their chromatographic homogenity determined. Alumina columns maintained at about 110° were used for effecting separations of the mixtures and the individual components recovered in over 95% yield in most cases. The separations were observed visually as differently coloured bands, often with an intervening colourless zone. The isolated fractions were identified by the shades obtained on cotton and by colour reactions.

The solvents generally used for adsorption and development were <u>o</u>-dichlorobenzene or 1:2:4-trichlorobenzene, alone or mixed with increasing amounts of phenol or cresols to cut down eluate volumes and elution periods. <u>o</u>-Dichlorobenzene containing 3% phenol is a particularly useful solvent in the case of dibenzanthrone derivatives. Molten naphthalene (see Part IV) is of interest as a solvent for hot chromatography, but does not offer any distinct advantage over <u>o</u>-dichlorobenzene or trichlorobenzene.

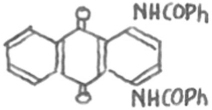
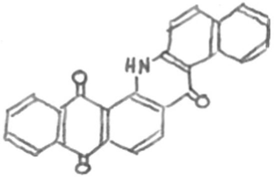
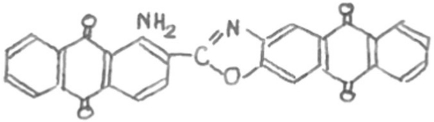
The working temperature of the column depends mainly on the solvents employed and the nature of the compounds to be separated. Very high temperatures (150° or above) tend to produce diffuse bands and therefore poor separability, while too low a temperature (below 70°) lengthens the time taken for development and elution; a temperature balance is therefore struck to combine separability with speed of development. In most cases a temperature of 110° was found to be satisfactory.

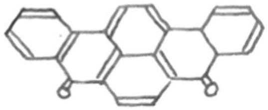
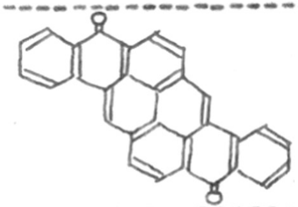
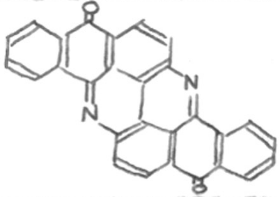
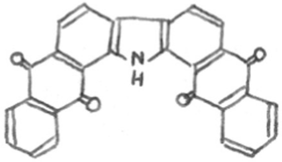
Thus isodibenzanthrone (XIV) separated readily from the isomeric dibenzanthrone (XIII); 16:17-dimethoxydibenzanthrone (VIII) from the dihydroxy compound (IX), the diamino derivative (X) or the parent quinone (XIII); mononitrodibenzanthrone (XV) from monoaminodibenzanthrone (XI) or the monoamino compound from its monobenzamido derivative (XVI). 3:4:9:10-Dibenzopyrene-5:8-quinone (IV) and 1:4-bisbenzamidoanthraquinone (1) were less strongly held on alumina than isodibenzanthrone or dibenzanthrone. The acridone (II) was separated from the carbazole (VII) and indanthrone (XII), and the oxazole derivative (III) from indanthrone (XII). Very good separations were observed between flavanthrone (VI) and indanthrone (XII) or pyranthrone (V). The separation of flavanthrone and indanthrone in presence of one another is of particular interest as both compounds are formed during the alkali fusion of 2-aminoanthraquinone at different temperatures.

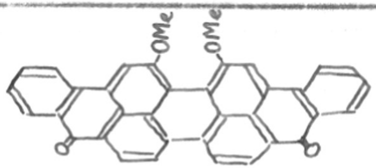
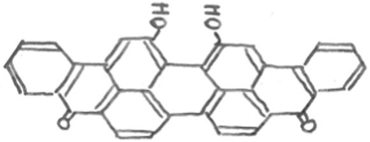
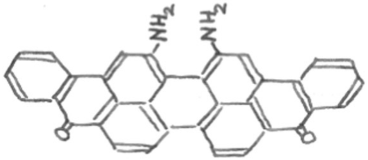
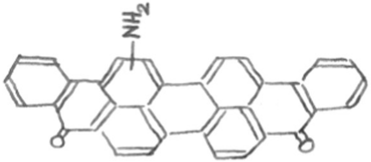
In Table I are listed the dyes used for separations with their commercial name, Colour Index number and structural formulae.

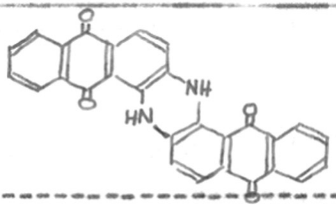
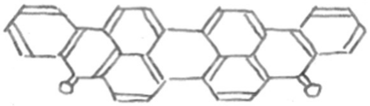
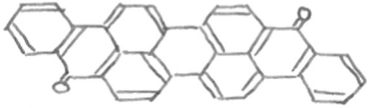
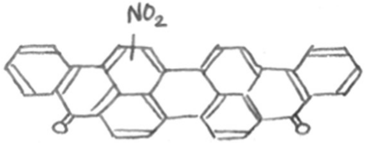
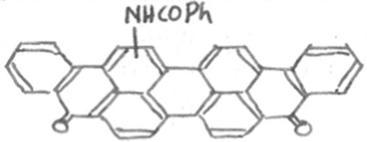
Table I

Vat Dyes used in model separations

No.	Commercial name and Colour Index number	Major chemical constituent	Structural formula
(1)	(2)	(3)	(4)
I	Indanthrene Red 5GK C.I. 61650	1:4-*bis*benzamido-anthraquinone	
II	Indanthrene Red RK C.I. 68000	1:2-benzo-5:6-phthaloyl-acridone	
III	Indanthrene Red FBB C.I. 67000	Oxazole derivative	

1	2	3	4
IV	- Halogenated derivatives are commercial dyes	3:4:9:10-dibenzopyrene-5:8-quinone	
V	Indanthrene Gold Orange G C.I. 59700	pyranthrone	
VI	Paradone Yellow G New C.I. 70600	flavanthrone	
VII	Indanthrene Yellow FFRK C.I. 69000	1:2:7:8-diphthaloyl-carbazole	

1	2	3	4
VIII	Caledon Jade Green C.I. 59825	16:17-dimethoxy-dibenzanthrone	
IX	-	16:17-dihydroxy-dibenzanthrone	
X	-	16:17-diamino-dibenzanthrone	
XI	-	15-or 16-amino-dibenzanthrone	

1	2	3	4
XII	Romanthrene Blue R C.I. 69800	indanthrone	
XIII	Indanthrene Dark Blue BOA C.I. 59800	dibenzanthrone	
XIV	Indanthrene Violet R (Halogenated derivatives are in use at present)	isodibenzanthrone	
XV	Caledon Black 2BM C.I. 59850	15-or 16-nitro-dibenzanthrone	
XVI	-	15-or 16-benzamido-dibenzanthrone	

EXPERIMENTAL

Purification of Samples: The commercial sample was freed from its ethanol and water-soluble matter by first extraction with ethyl alcohol and subsequently with hot water. The dye was then crystallised from a suitable solvent. Chromatographic purification at high temperature was employed, wherever necessary, and in all cases the chromatographic homogeneity of the purified sample was tested. The elementary analysis of the purified samples are recorded in Table II.

Solvents: The following solvents were used for carrying out adsorption and development:-

1 o-Dichlorobenzene (ODCB), b.p. 180-2°
2 1:2:4-Trichlorobenzene (TCB), b.p. 212-3°
3 Phenol, m.p. 41°; b.p. 182°
4 m-Cresol, b.p. 203°
5 o-Chlorophenol (OCP), b.p. 176°

All the solvents were dried and distilled before use.

Adsorbents: Alumina (Merck) was used throughout for carrying out the separations.

Table II

Elementary analysis of the pure dyes used in chromatographic separations

No.	Compound and molecular formula	% analysis		Method of purification
		Found	Calc.	
1	2	3		4
I	1:4-*bis*Benzamidoanthraquinone, $C_{28}H_{18}O_4N_2$	C, 75.4 H, 3.8 N, 6.5	75.2 4.0 6.3	chromatographed at 110° and cryst. from ODCB
II	1:2-Benzo-5:6-phthaloyl acridone, $C_{25}H_{13}O_3N$.	C, 80.3 H, 3.2 N, 4.1	80.0 3.5 3.7	chromatographed at 110° and cryst. from ODCB
III	Indanthrene Red FBB, $C_{29}H_{14}O_5N_2$	C, 77.3 H, 3.1 N, 6.4	77.4 3.1 6.2	chromatographed at 110° and cryst. from ODCB
IV	3:4:9:10-Dibenzopyrene-5:8-quinone, $C_{24}H_{12}O_2$	C, 85.9 H, 3.5	86.2 3.6	Cryst. from nitrobenzene
V	Pyranthrone, $C_{30}H_{14}O_2$	C, 88.2 H, 3.4	88.5 3.4	cryst. from diphenyl oxide
VI	Flavanthrone, $C_{28}H_{12}O_2N_2$.	C, 82.8 H, 2.9	82.5 2.9	cryst. from diphenyl oxide

1	2	3		4
VII	1:2:7:8-Diphthaloyl-carbazole, $C_{28}H_{13}O_4N$	C, 78.7 H, 2.8 N, 3.3	78.7 3.0 3.3	cryst. from diphenyl oxide
VIII	16:17-Dimethoxydibenzanthrone, $C_{36}H_{20}O_4$	C, 83.4 H, 3.5 OMe, 12.4	83.7 3.9 12.0	cryst. from mixed cresols
IX	16:17-Dihydroxydibenzanthrone	Water-soluble free product was used		
X	16:17-Diaminodibenzanthrone, $C_{34}H_{18}O_2N_2$	C, 83.1 H, 3.3 N, 5.3	83.7 3.7 5.8	chromatographed at 110° and cryst. from ODCB
XI	15-or 16-Aminodibenzanthrone, $C_{34}H_{17}O_2N$	C, 86.1 H, 3.5 N, 2.9	86.5 3.6 3.0	chromatographed at 110° and cryst. from ODCB
XII	Indanthrone, $C_{28}H_{14}O_4N_2$	C, 76.4 H, 2.8 N, 6.1	76.0 3.2 6.3	cryst. from m-Cresol
XIII	Dibenzanthrone, $C_{34}H_{16}O_2$	C, 88.9 H, 3.8	89.5 3.5	cryst. from mixed cresols

1	2	3		4
XIV	Isodibenzanthrone, $C_{34}H_{16}O_2$	C, 89.1 H, 3.7	89.5 3.5	cryst. from OCP
XV	15-or 16-Nitrodibenzanthrone, $C_{34}H_{15}O_4N$	C, 81.1 H, 3.1 N, 3.0	81.4 3.0 2.8	chromatographed at 110° and cryst. from ODCB
XVI	15-or 16-Benzamidodibenzanthrone, $C_{41}H_{21}O_3N$.	C, 85.3 H, 3.7 N, 2.8	85.5 3.7 2.4	chromatographed at 110° and cryst from ODCB

Description of the Apparatus:

The chromatographic column (see Fig.1) consists of a pyrex glass tube (2 x 60 cm.) with a standard B14 cone fused near the lower end. The B14 cone of the column rests on a standard B14 socket provided in the jacket which surrounds the column while in use. The jacket, which is 5 cm. in diameter and a few centimeters shorter in length than the column, is also made of pyrex glass tube and has a side-arm attached to it as in a Thiel's tube. The electrical heating of the self-circulating paraffin jacket is located in the side-arm and is controlled. Uniform bath temperature throughout the working length of the column is maintained at any desired point between 30^{0}-200^{0} by means of a control devise (a variac or a simmerstat or a rheostat) and the temperature is read off from thermometers suitably placed in the jacket. The paraffin can be removed, if necessary, through a tap provided in the side-arm.

Operation:

The column, plugged with a piece of cotton wool, is kept in place inside the jacket and the bath is heated to about 20° higher than the desired working temperature of the column. The required amount of alumina is made into a thin slurry with the solvent in a beaker and under vigorous mechanical stirring is heated and kept at near boil for 15-20 minutes. This pretreatment, which drives off the moisture and air pockets in the alumina, is very important to the satisfactory working of the column, especially when the proposed working temperatures are well above the boiling point of water. The alumina-solvent slurry, which may be cooled if necessary, is poured into the chromatographic column and the adsorbent allowed to settle down uniformly. A small amount of sand or powdered glass may be added to the top of the bed after it has settled down so as to prevent any disturbance during further addition of solvents. The temperature of the bath is finally adjusted to the working temperature and adsorption and development carried out as usual. The column may be run overnight without attention, if an adequate supply of developing solvent is provided through a constant level arrangement.

Separation of a mixture of Indanthrone (XII) and Flavanthrone (VI) on alumina at 120°.

A column of alumina (2 x 30 cm.) was built by the wet method of packing using o-dichlorobenzene and maintained at 120°. A mixture of flavanthrone (20 mg.) and indanthrone (20 mg.) was refluxed with o-dichlorobenzene (100 ml.) and the green solution was added to the column while still hot. During adsorption, part of the compound crystallised and settled on the top of the bed as a thin layer. On development with the same solvent at 120°, a lemon yellow band separated from the strongly held blue zone. The lemon yellow band was eluted out as a lemon yellow eluate (800 ml.) which on cooling to room temperature deposited yellow needles. The eluate was concentrated to small bulk (20-30 ml.) and after cooling, the yellow needles (20 mg.) were isolated and identified as flavanthrone. After discarding a colourless interval of eluate (1.2 l.) the blue band was eluted out as a blue solution (2 l.) out of which part of the dye crystallised on cooling to room temperature. The dye (19.6 mg.) was isolated by concentration to small bulk (30-40 ml.) and cooling and identified as indanthrone.

Table III gives the various model separations carried out together with experimental data. In all cases, alumina columns (2 x 30 cm.) built with o-dichlorobenzene were employed.

Table III

Chromatographic separations of vat dyes at high temperatures

o.	Components of the mixture in the order of elution	Weight in mg. Present	Found	Colour of band	Solvent	Working temperature °C	Remarks on Separation
	2	3		4	5	6	7
	Flavanthrone (VI) Indanthrone (XII)	20 20	20 19.6	Yellow Blue	ODCB ODCB	120	Excellent
	Flavanthrone (VI) Pyranthrone (V)	10 10	9.6 9.2	Yellow Orange	ODCB ODCB	120 120	Good
.	Acridone (II) Indanthrone (XII)	20 20	19.2 19.4	Red Blue	ODCB ODCB	120 120	Excellent
.	Oxazole (III) Indanthrone (XII)	20 20	19.8 19.6	Red Blue	ODCB ODCB	110 110	Excellent
.	Isodibenzpyrenequinone (IV) Indanthrone (XII)	20 20	20 19.4	Red Blue	ODCB ODCB	110 110	Excellent

2	3		4	5	6	7
Acridone (II)	10	9.6	Red	ODCB	110	Excellent
Carbazole (VII)	10	9.4	Yellow	ODCB	110	
Isodibenzanthrone (XIV)	25	20	Violet	TCB+3% Cresol	120	Fair
Dibenzanthrone (XIII)	25	15	Blue	TCB+3% Cresol	140	
1:4-*bis*-Benzamido-anthraquinone (1)	20	19.6	Red	ODCB	110	Excellent
Isodibenzanthrone (XIV)	20	17.4	Violet	ODCB+3% Phenol	120	
Isodibenzopyrene-quinone (IV)	10	10	Red	ODCB	110	Excellent
Isodibenzanthrone (XIV)	10	8.0	Violet	ODCB +3% Phenol	120	
Dibenzanthrone (XIII)	10	7.2	Blue	TCB+3% Cresol	140	Fair
16:17-Dimethoxy-dibenzanthrone (VIII)	20	18.4	Blue-green	ODCB+1% Phenol	110	Excellent
Dibenzanthrone (XIII)	20	15	Blue	TCB+3% Cresol	140	

1	2	3		4	5	6	7
11	16:17-Dimethoxy-dibenzanthrone (VIII)	20	18.8	Blue-green	ODCB+1% Phenol	110	Excellent
	Isodibenzanthrone (XIV)	20	17.0	Violet	ODCB+3% Phenol	120	
12	16:17-Dimethoxy-dibenzanthrone (VIII)	10	9.4	Blue-green	ODCB+1% Phenol	110	Excellent
	16:17-Dihydroxydibenz-anthrone (IX)	10	-	Green	Band was not eluted out.		
13	16:17-Dimethoxy-dibenzanthrone (VIII)	15	14.6	Blue-green	ODCB+1% Phenol	110	Excellent
	16:17-Diaminodibenz-anthrone (X)	15	13.6	Green	ODCB+3% Phenol	120	
14	Mononitrodibenzanthrone (XV)	10	9	Violet	ODCB+3% Phenol	110	Good
	Monoamino-dibenzanthrone (XI)	10	9	Green	ODCB+3% Phenol	110	

1	2	3		4	5	6	7
15	Monoamino-dibenzanthrone (XI)	10	9.2	Green	ODCB+3% Phenol	110	Good
	Monobenzamido-dibenzanthrone (XVI)	10	8.6	Violet	ODCB+3% Phenol	110	

Note:- Excellent: a clear colourless zone between bands.

Good: no interval, but complete separation.

Fair: slight overlapping of bands.

ODCB: <u>o</u>-Dichlorobenzene.

TCB: 1:2:4-Trichlorobenzene.

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PART III

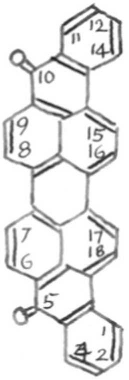
THE CONSTITUTION OF NITRATED DIBENZANTHRONE

Nitration of dibenzanthrone with nitric acid (1.1 mole) in acetic acid or chloracetic acid at 25-30° gives a mixture of mono and dinitrodibenzanthrones, dyeing an unlevel green (Indanthrene Green B; Bally 1904) of no practical value[1]. Since the nitro groups are reduced during vatting, the product on the fibre is a mixture of aminodibenzanthrones. When the green dye is oxidised on the fibre with hypochlorite (about 0.5% available chlorine) at room temperature a deep black shade with good fastness properties is obtained[2]. Nitrated dibenzanthrone (C.I.Vat Green 9; C.I. 59850) is marketed under the name of Indanthrene Black BB and BGA, Cibanone Black BB, Caledon Black NB, 2BM, etc., and is an important and largely used vat dye. The position of the nitro groups in the nitrated dibenzanthrones and the constitution of the black dye produced on the fibre are still undetermined.

The available data on the constitution of nitrated dibenzanthrone, obtained by nitration of dibenzanthrone, are conflicting and inconclusive. Maki et.al.[3] claims to have obtained dinitrodibenzanthrone (not crystalline) by nitration of dibenzanthrone by means of conc. nitric acid (60 moles) in acetic acid at 60° for 12 hours. They suggested that the nitro groups occupy the 16:17-positions in dibenzanthrone (I), since the corresponding diamino compound, on oxidation with chromic acid and sulphuric acid, first gave a brown alkali-soluble product (II) and ultimately a dicarboxylic acid (III); the proposed structures (II and III), however, were based mainly on nitrogen estimation and there was no real proof of the structures. On the assumption of the 16:17-orientation for the nitro groups, Maki[3] concluded that the black dye, produced by the hypochlorite oxidation of the diamine on the fibre, is the pyridazine derivative (IV). Maki[3] considered that the structure (IV) for the black dye was supported by its reversion to green on treatment with alkaline sodium hydrosulphite, followed by air-oxidation; the green shade could again be converted to black on treatment with hypochlorite. However, it appears unlikely that the pyridazine ring would undergo reductive fission by the usual vatting process. Maki excluded the orientation of the nitro groups in the 15:18-positions by synthesising

15:18-diaminodibenzanthrone from the corresponding dichloro compound, which was obtained by direct chlorination of dibenzanthrone[4]. 15:18-Diaminodibenzanthrone dyed cotton a dark violet-blue shade, unaltered by hypochlorite treatment.

Bennett, Pritchard and Simonsen[5] nitrated crystalline dibenzanthrone following Maki's conditions of nitration and obtained a product which dyed cotton a green shade changing to black on treatment with hypochlorite. From the nitrogen analysis of the amorphous product they assumed it to be a dinitrodibenzanthrone. In the course of the present work it was found that nitration of dibenzanthrone under the experimental conditions used by Bennett *et.al.*[5] yielded a mixture of nitrodibenzanthrone; the values for carbon, hydrogen and nitrogen varied on repeated crystallisation. It is important to note in this connection that the purity of a complex vat dye cannot be judged by the estimation of one element; in a nitrogen-containing dye carbon, hydrogen and nitrogen must be determined. While a nitrated dibenzanthrone analysing more or less correctly for nitrogen corresponding, for instance, to a dinitrodibenzanthrone, may be obtained readily, there is



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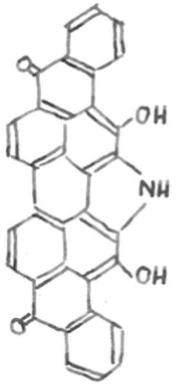

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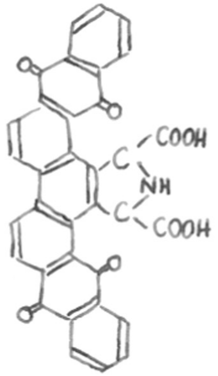

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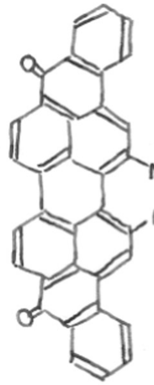

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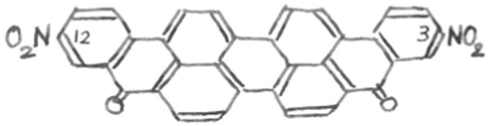
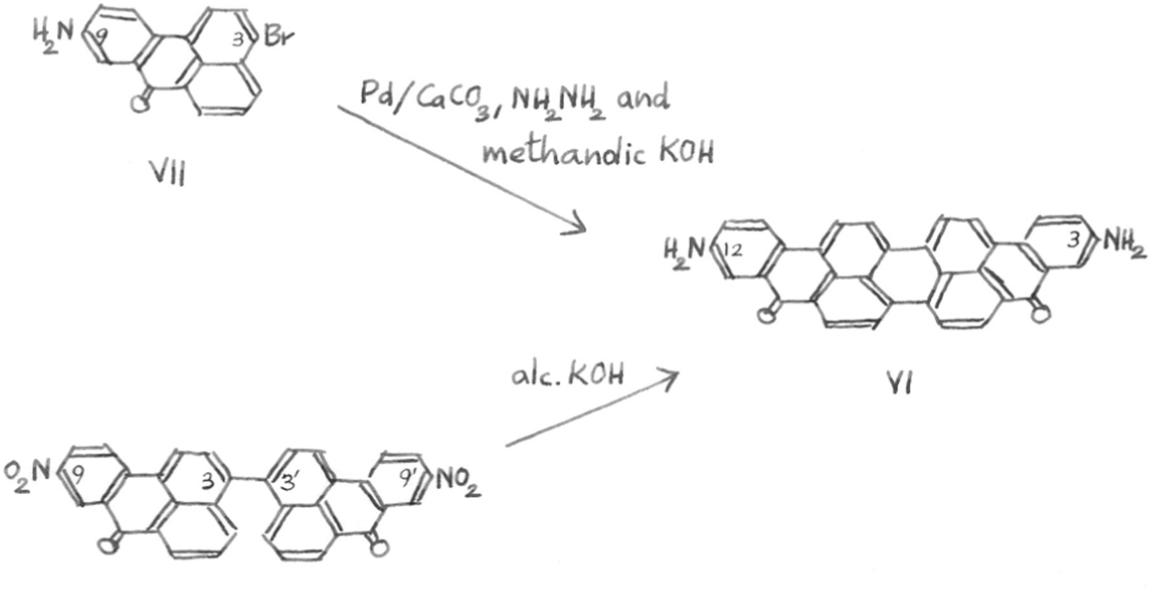


V



VIII

considerable difficulty in obtaining a compound whose C, H and N values all agree with a mono-, di- or tri-nitrodibenzanthrone. Bennett <u>et.al</u>.[5] ruled out the 16:17-orientation of the nitro groups in (I) because they obtained a nitrogen-containing carboxylic acid by chromic acid oxidation of their "dinitrodibenzanthrone". They obtained 0.5 g. of an amorphous dinitrodianthraquinonyl-1:1'-dicarboxylic acid from 6g. of "dinitrodibenzanthrone", but they did not disprove the formation of dianthraquinonyl-1:1'-dicarboxylic acid, which might have been eliminated during the elaborate purification. Reduction of the dinitrodianthraquinonyl-1:1'-dicarboxylic acid and deamination <u>via</u> the diazonium salt gave 2:2'-dianthraquinonyl-1:1'-dicarboxylic acid which was identical with the product of oxidation of dibenzanthrone. By analogy with other substitution reactions in the benzanthrone series they regarded their nitrated product as 3:12-dinitrodibenzanthrone (V).

Pandit, Tilak and Venkataraman[6] synthesised authentic 3:12-diaminodibenzanthrone (VI) by two unambiguous routes, and found that it dyes from a violet vat a dull-green shade which turned bright blue on hypochlorite oxidation. The synthesis of (VI) was achieved by the action of (a) palladised calcium carbonate, hydrazine and methanolic caustic potash on 3-bromo-9-aminobenzanthrone (VII) and (b) alcoholic potash on 9:9'-dinitro-3:3'-dibenzanthronyl (VIII). The constitution of (VIII) was proved by its oxidation to the known 6-nitroanthraquinone-1-carboxylic acid. In view of this unequivocal proof that the essential constituent of the nitrated dibenzanthrone, which dyes green turning to black by hypochlorite oxidation, cannot possibly be 3:12-dinitrodibenzanthrone, it is remarkable that in the new Colour Index, C.I.Vat Green 9 (C.I. 59850) is stated to be "possibly 3:12-dinitrodibenzanthrone".

Several other symmetrical diaminodibenzanthrones prepared by the alkali fusion of the appropriate aminobenzanthrone are described in the patent literature[7], but none of them has been reported to dye green changing to black by oxidation. By the alkali fusion of 5-aminobenzanthrone[8] Malhotra _et.al_.

prepared 7:8-diaminodibenzanthrone, which dyed cotton a dull bluish green from a blue vat, changed to a khaki shade on treatment with hypochlorite; it was of special interest to examine 7:8-diaminodibenzanthrone, because if this proved to dye green changing to black by oxidation, both Maki's and Simonsen's results could have been reconciled. The various diaminodibenzanthrones and their colour reactions are shown in the accompanying table. The list covers all the symmetrically substituted diaminodibenzanthrones except the 6:9-compound.

Colour reactions of diaminodibenzanthrones

Position of NH_2	Colour in sulphuric acid	Colour of vat	Shade on Cotton after		Ref.
			air-oxidation	NaOcl treatment	
1	2	3	4		5
16:17	reddish violet	blue	olive green	brown*	14
15:18	-	-	dark violet-blue	unaltered	4
1:14	green	greenish blue	brownish blue	darker	7,7(a)
2:13	bordeaux	-	violet	brown	7

1	2	3	4		5
3:12	-	violet	dull green	blue	6
4:11	bordeaux	greenish blue	blue	unaltered	7
7:8	reddish blue	dull blue	dull bluish green	khaki	

* The shade produced on treatment with hypochlorite is not mentioned in the patent.

Several polyamino-dibenzanthrones and isodibenzanthrones containing 3 to 5 amino groups made by amination of the corresponding bromo compounds, are reported to dye directly gray to black shades[9].

The product of the chromic acid oxidation of commercial Caledon Black 2B was examined by Pandit[10]. Decarboxylation of the acid gave 2:2'-dianthraquinonyl as the major product, thus demonstrating the presence in Caledon Black 2B of nitrodibenzanthrone containing only one or more nitro groups in the 15, 16, 17 or 18-positions or of unnitrated dibenzanthrone.

Kunz[11] has formulated Indanthrene Black BB as 16-nitrodibenzanthrone. In a private communication to Prof. K. Venkataraman Nawiasky[12] has stated that mononitration of dibenzanthrone in nitrobenzene yields 16-nitrodibenzanthrone, the proof of which was its conversion to 16:17-dimethoxydibenzanthrone through the following series of reactions. Reduction of the nitro to the amino group by vatting and air-oxidation, followed by diazotisation[13] and heating with dilute sulphuric acid, gave 16-hydroxydibenzanthrone. Further oxidation with manganese dioxide and treatment of the resultant quinone with bisulphite gave 16:17-dihydroxydibenzanthrone. Methylation yielded 16:17-dimethoxydibenzanthrone. However, no proof of the homogenity and analytical purity of the nitrated dibenzanthrone was offered and if unnitrated dibenzanthrone had been present this would also have led to 16:17-dimethoxydibenzanthrone.

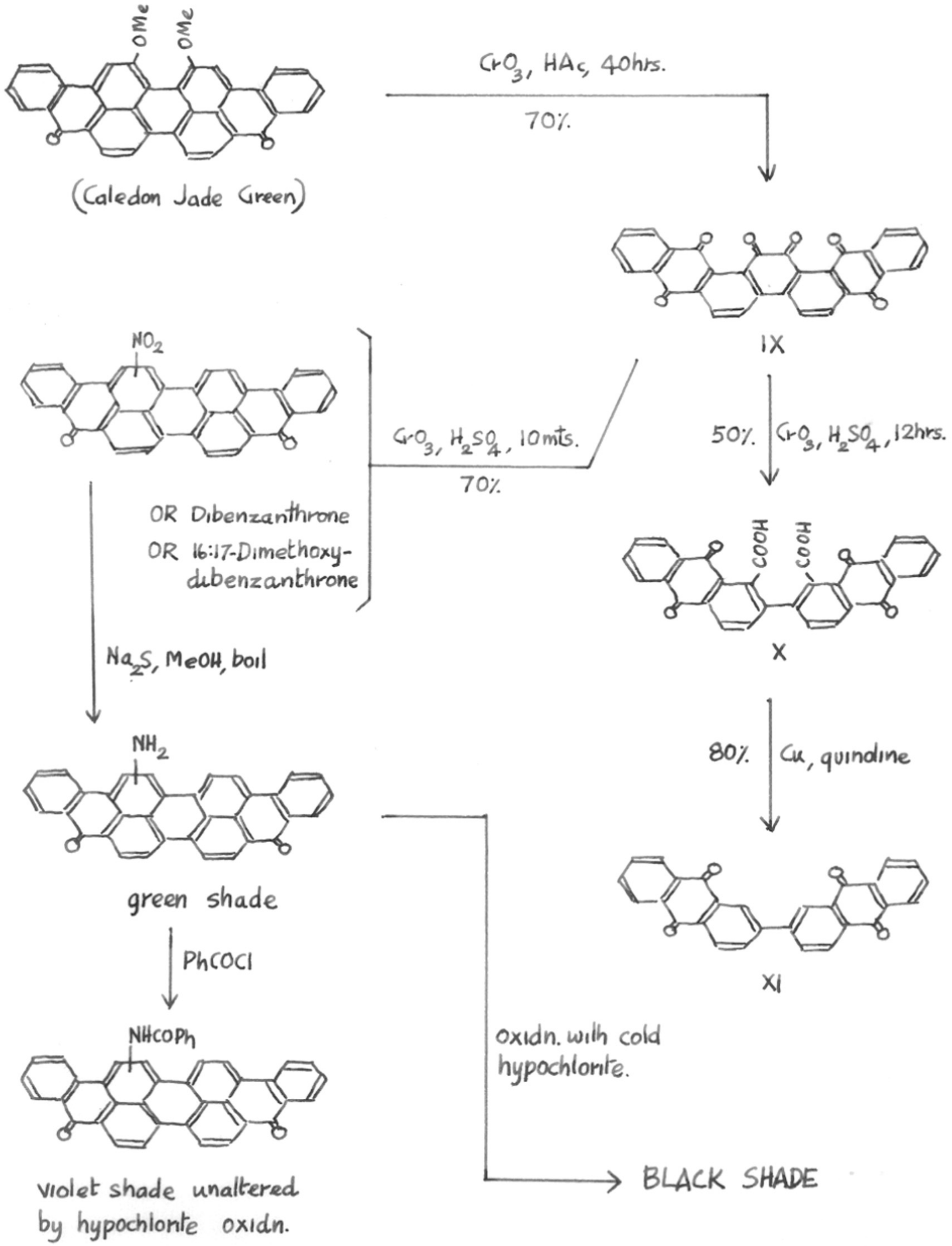
By the alkali fusion of 2-aminobenzanthrone[14] we obtained a dye in good yield, which is obviously 16:17-diaminodibenzanthrone. It was purified by hot chromatography over alumina and gave on crystallisation green needles. It dissolved in conc. sulphuric acid with a reddish violet colour and dyed cotton an olive-green shade from a blue vat; on treatment with cold hypochlorite a fast brown shade was obtained. Oxidation of 16:17-diaminodibenzanthrone in aqueous suspension with a solution of sodium hypochlorite gave a brown product which dyed a similar shade on cotton from a blue vat, unaffected by further hypochlorite treatment. This disproves the 16:17-positions of the amino groups proposed by Maki <u>et.al.</u>[3], for the production of black dye by the hypochlorite oxidation of the diamine on the fibre.

The conflicting results of Maki[3] and Simonsen[5] were apparently due to their handling nitrated dibenzanthrones, which were amorphous and of undetermined purity in terms of chromatographic analysis and carbon-hydrogen-nitrogen content. In the present work a few commercial nitrated dibenzanthrones were first submitted to chromatographic analysis with a view to isolate a homogeneous, crystalline and analytically pure constituent dyeing cotton a green shade turning to black after hypochlorite oxidation. A very convenient method for the chromatographic separation of mixtures of vat dyes on alumina at elevated temperatures (100-150°) has been described in Part II. The water-soluble matter from the commercial nitrated dibenzanthrone was removed by repeated extraction with boiling water. Caledon Black 2BM and Cibanone Black BB contained 50 and 60 per cent water-soluble matter respectively. A solution of the dye was prepared in boiling o-dichlorobenzene and chromatographed on alumina at 110°. Thus Caledon Black 2BM, free from water-soluble impurities was found to contain 4% of tribromopyranthrone (Caledon Brilliant Orange 4RN) and 5% of 16:17-dimethoxydibenzanthrone (Caledon Jade Green) added for shading purposes. Both these constituents were eluted with o-dichlorobenzene and were obtained in a crystalline state; their identity with tribromopyranthrone and dimethoxydibenzanthrone was established by elementary analysis, colour reactions and infrared spectra in comparison with the pure dyes isolated from Caledon Brilliant Orange 4RN and Caledon Jade Green. The main constituent of Caledon Black 2BM, which was eluted by o-dichlorobenzene containing 3% phenol, was obtained in 50% yield and crystallised in violet needles from o-dichlorobenzene. The substance gave a violet solution in sulphuric acid and dyed cotton a green shade from a blue vat, changing to a deep black on treatment with cold hypochlorite solution. The values for C, H, and N corresponded closely to a mononitrodibenzanthrone, and the infrared absorption at 1534 cm^{-1} and 1337 cm^{-1} was characteristic of a nitro compound.

Chromatographic analysis of water-soluble free Cibanone Black BB also gave a 50% yield of a mononitrodibenzanthrone, identical in its colour reactions, dyeing properties and infrared spectrum with the mononitrodibenzanthrone isolated from Caledon Black 2BM. Tribromopyranthrone and dimethoxydibenzanthrone were, however, absent in this commercial dye. From both the commercial samples several other fractions (amounting to about 20%) were obtained on continued elution; they were amorphous and dyed cotton green to greenish blue shades which changed to blackish violet on treatment with hypochlorite solution.

Reduction of the crystalline mononitrodibenzanthrone with alkaline sodium hydrosulphite or methanolic sodium sulphide, followed by hot chromatography on alumina gave the aminodibenzanthrone as green needles in 65 to 70% yield. The reduction products of the crystalline fractions from both the commercial dyes gave identical infrared spectra. The amine gave a violet solution in sulphuric acid, and dyed cotton a green shade changing to black on after treatment with hypochlorite. The N-benzoyl derivative, prepared by reacting the amine with benzoyl chloride, gave a blue vat and a violet shade on cotton, unaffected by hypochlorite treatment. Both the amine and the N-benzoyl derivatives analysed correctly for C, H and N.

Oxidation of the pure mononitrodibenzanthrone isolated from the commercial dyes was then studied in order to determine the position of the nitro group. When the mononitrodibenzanthrone was treated with chromic acid and sulphuric acid, the hitherto unknown 1:2:7:8-diphthaloylphenanthraquinone (IX) was first obtained in 70% yield as brown needles. Further oxidation of (IX) with chromic acid and sulphuric acid gave 2:2'-dianthraquinonyl-1:1'-dicarboxylic acid (X) in 65% yield, m.p. 360° (dec.); Scholl[15] quotes m.p. 355° (dec.). Decarboxylation of (X) with copper in boiling quinoline gave 2:2'-dianthraquinonyl (XI) as yellow needles, m.p.395-97°.


OMe
OMe
(Caledon Jade Green)
CrO_3, HAc, 40hrs.
70%
IX
NO_2
CrO_3, H_2SO_4, 10mts.
70%
OR Dibenzanthrone
OR 16:17-Dimethoxy-dibenzanthrone
50%
CrO_3, H_2SO_4, 12hrs.
COOH
COOH
X
Na_2S, MeOH, boil
80%
Cu, quinoline
NH_2
green shade
XI
PhCOCl
NHCOPh
oxidn. with cold hypochlorite.
violet shade unaltered by hypochlorite oxidn.
BLACK SHADE

(Scholl[16], m.p. 387-88°). It was identified by analysis m.p. and mixed m.p. with an authentic sample prepared by chromic acid oxidation of dibenzanthrone. The compounds (IX), (X) and (XI) also obtained by oxidative degradation of 16:17-dimethoxydibenzanthrone. It is therefore clear that the nitro group in mononitrodibenzanthrone is in the 15- or 16-position.

It was noticed that a 70% conversion of dibenzanthrone or its 16:17-dimethoxy derivative to (IX) could be achieved by dropwise addition of a solution of the compound in sulphuric acid to a boiling solution of aqueous chromic acid in 10 minutes and refluxing for 10 minutes more. Prolonged oxidation of (IX) gave the dicarboxylic acid (X). The yield and purity of the acid (X) obtained by the two stage oxidation was better than by the direct oxidation with a mixture of chromic and sulphuric acids. Oxidation of 16:17-dimethoxydibenzanthrone with chromic acid in boiling acetic acid gave (IX) in 70% yield but dibenzanthrone was unaffected under these conditions. The triquinone (IX) gave a red solution in sulphuric acid and dyed cotton a brown shade of poor fastness properties from a deep red vat.

The infrared absorption spectra of dibenzanthrone, 16:17-dimethoxydibenzanthrone, 16:17-diaminodibenzanthrone, mononitrodibenzanthrone and monoaminodibenzanthrone were determined with a Grub Parson double beam infrared spectrophotometer. The compounds are studied as nujol mulls and as KBr pressed discs. The principal absorption bands are marked in the accompanying table.

There is a marked similarity in the overall spectra of these molecules.

Medium strong bands are observed at 3333 and 3225 cm^{-1} in the amino substituted compounds due to the NH stretching vibrations. The carbonyl frequencies of dibenzanthrone and 16:17-dimethoxydibenzanthrone have appeared at 1645 and 1640 cm^{-1} respectively; that of nitrodibenzanthrone at 1647 cm^{-1} and those of amino- and 16:17-diaminodibenzanthrone at 1638 and 1632 cm^{-1} respectively. Strong absorptions at 1609 and 1593 cm^{-1} in the amino-, and 16:17-diamino compounds are due to the NH deformation vibrations of the NH_2 group. A strong band at 1527 cm^{-1} in nitrodibenzanthrone

Nitro- and Amino- Substituted Dibenzanthrone.

No.	Principal absorption bands in cm^{-1}				
	2000-1500	1500-1300	1300-1100	1100-900	900-650
I	1645 VS 1599 S 1587 S 1575 S	1455 S 1382 W 1336 VS 1302 VS	1284 shoulder 1250 W 1196 MS 1160 W	1082 M 950 S 1032 S	881 792 W 859 W 806 S 753 S 848 837
II	1599 S 1640 VS 1578 VS 1508 MS	1483)M 1387 S 1459) 1355 S 1300 VS	1268) W 1170MS 1245) 1124) 1228 S 1103 M	1088 S 971 S 1030 S 920 W	865 MS 786 W 834 802 S 754 S 823)W 733 M
III	1600 Sh 1647 VS 1587 S 1527 S	1465 M 1380) 1300 M 1350)M	1205 W 1103 M	1056 W 953 W 1032 M	882) 761)S 863) 811) 754) 852)W 788)MS 840)
IV	1609 S 1638 VS 1587 S 1561 S 1542 Sh	1465 M 1380) 1305 S 1346)M	1279 Sh 1103 M 1192 M	1056 W 953)W 935) 1032 M	882)W 755 S 846) 805M 723 W 723 W
V	1593 VS 1632 VS 1579 VS 1552 S 1529 M	1463 M 1305 S 1346 M	1279 Sh 1103 M 1192 M	1056 W 935 M 1032 M	882 M 755 S 805 M 840 W 692 S

Note:

I	Dibenzanthrone
II	16:17-Dimethoxydibenzanthrone
III	Mononitrodibenzanthrone
IV	Monoaminodibenzanthrone
V	16:17-Diaminodibenzanthrone

VS	.. very strong
S	.. strong
M	.. medium
W	.. weak
Sh	.. shoulder

is due to the assymetric stretching vibration of the nitro group.

Strong bands between 1575-1587 cm^{-1} are characteristic of in-plane skeletal aromatic ring C=C bond vibrations, the enhancement of the intensity being due to external conjugation with the carbonyl group. Absorptions in the regions 1200-1350 cm^{-1} are typical of polynuclear quinones, while those between 900-650 cm^{-1} are due to the out of plane bending vibrations of the aromatic ring CH groups.

EXPERIMENTAL

Chromatography of Caledon Black 2BM:

Caledon Black 2BM 800 powder (30 g.) was first extracted with ethanol and then with boiling water to give a violet black powder (15 g.) free from ethanol-and water-soluble matter. A suspension of crude dye (1 g.) and alumina (5 g.) in boiling o-dichlorobenzene (50 ml.) was poured over a column of alumina (2 x 30 cm.) maintained at 110°. The suspension settled down on the column as a black band (3 cm.). The different fractions were isolated in the following order using various developing solvents.

Fraction(i): On development with o-dichlorobenzene an orange-red band was eluted (800 ml.) from which orange-red needles (41 mg.) were isolated. This was identified as Caledon Brilliant Orange 4R (tribromopyranthrone) by shade matching and colour reactions. (Found: C, 55.6; H, 1.8; Br, 37.2. Calc. for $C_{30}H_{11}O_2Br_3$: C, 56.0; H, 1.7; Br, 37.4%).

Fraction(ii): Development with o-dichlorobenzene (250 ml.) containing 3% phenol gave a blue-green band from which green needles (56 mg.) were isolated. This was identified as Caledon Jade Green by shade matching, colour reactions and its infra-red spectrum.

(Found: C, 83.8; H, 3.7. Calc. for $C_{36}H_{20}O_4$: C, 83.7; H, 3.9%).

Fraction (iii): The violet band which appeared next was eluted with o-dichlorobenzene (1.9 l.) containing 3% phenol from which violet needles (490 mg.) were isolated. (Found: C, 80.9; H, 2.9; N, 2.9. $C_{34}H_{15}O_4N$ requires C, 81.4; H, 3.0; N, 2.8%). It dyed cotton a green shade from a blue vat, changing to black on treatment with hypochlorite. In sulphuric acid it dissolved with a violet colour.

Fraction (iv): Percolation with o-dichlorobenzene (1 l.) containing 10% phenol gave an amorphous black powder (72 mg.) (Found: C, 81.1; H, 4.0; N, 3.6%). It dissolved in sulphuric acid with a violet colour and dyed cotton a green shade which changed to black on hypochlorite oxidation.

Fraction (v): Pure m-cresol (1 l.) eluted out another blue fraction as an amorphous powder (47 mg.). (Found: C, 81.0; H, 4.0; N, 1.1 %). The compound gave a violet solution in sulphuric acid and dyed cotton a dull greenish blue shade from a blue vat. Treatment with cold hypochlorite gave a blackish violet shade.

Fraction (vi): The black band on the top of the column which could not be eluted out was scooped out, extracted with conc. sulphuric acid and isolated as a black powder (90 mg.).

Chromatography of Cibanone Black 2B:

The commercial product was freed from ethanol- and water-soluble matter when a 60% yield of the crude dye was obtained. It was chromatographed on alumina at 110° as in the case of Caledon Black 2BM. The following fractions were isolated from 1 g. of the crude dye.

Fraction (i): Development with o-dichlorobenzene gave a red fraction from which no crystalline compound could be isolated.

Fraction (ii): Development with o-dichlorobenzene containing 3% phenol gave a violet band from which mononitrodibenzanthrone was isolated in 49% yield. (Found: C, 81.1; H, 3.1; N, 3.0. $C_{34}H_{15}O_4N$ requires C, 81.4; H, 3.0; N, 2.8%). Its colour reactions and infra-red spectrum were identical with Fraction (iii) from Caledon Black 2BM.

Fraction (iii): A violet-black powder (107 mg.) was isolated on further development with o-dichlorobenzene (1.6 l.) containing 10% phenol. (Found: C, 80.8; H, 2.9; N, 2.4%). It dyed cotton a green shade from a blue vat which changed to black on hypochlorite oxidation.

Fraction (iv): Development with m-cresol (1.4 l.) gave a black powder (72 mg.) which dyed cotton a dull greenish blue shade which changed to a dark violet on hypochlorite treatment. (Found: C, 79.9; H, 3.9; N, 1.1 %).

Fraction (v): The black band which could not be eluted with solvents was extracted with sulphuric acid and isolated as a black powder (100 mg.).

Aminodibenzanthrone from mononitrodibenzanthrone.

(a) Reduction with methanolic sodium sulfide:

Freshly precipitated mononitrodibenzanthrone (200 mg.) was suspended in methanol (60 ml.) and a solution of fused sodium sulfide (500 mg.) in water (2 ml.) was added. The violet suspension slowly changed to a bluish green on refluxing for 4 hours. The product was filtered off, dissolved in o-dichlorobenzene and chromatographed on alumina at 110°. Development with o-dichlorobenzene containing 10% phenol eluted out a major bluish green band. Concentration of the eluate (900 ml.) gave the amine in green needles (130 mg.). (Found: C, 86.1; H, 3.5; N, 2.9. $C_{34}H_{17}O_2N$ requires C, 86.5; H, 3.6; N, 3.0%). The green shade on cotton changed to black on treatment with cold hypochlorite.

(b) Reduction with alkaline sodium hydrosulfite: Mononitrodibenzanthrone (200 mg.) was shaken with a 2% solution of sodium hydroxide (100 ml.) and sodium hydrosulfite (1 g.) at 80-85° for 30 minutes. The deep blue vat was air-oxidised and the green compound so obtained was collected as a black powder. Purification by hot chromatography on alumina at 110° and crystallisation from o-dichlorobenzene gave green needles (145 mg.) of monoaminodibenzanthrone. (Found: C, 86.2; H, 4.0; N, 2.5 %).

Monobenzamidodibenzanthrone:

A solution of the amine (500 mg.) in boiling o-dichlorobenzene (300 ml.) was refluxed with benzoyl chloride (0.5 ml.) for 30 minutes. The deep bluish green colour of the solution immediately changed to a bluish violet and remained unchanged throughout the period of refluxing. The product (500 mg.) was isolated by removal of the solvent and chromatographed on alumina at 110°. Development with o-dichlorobenzene containing 3% phenol removed the unconverted amine (35 mg.). The major violet band was eluted with o-dichlorobenzene containing 10% phenol and the compound isolated as violet needles (360 mg.). The violet shade on cotton from a bluish violet vat, on treatment with hypochlorite became slightly duller. (Found: C, 85.3; H, 3.7; N, 2.8. $C_{41}H_{21}O_3N$ requires C, 85.5; H, 3.7; N, 2.4%).

Oxidation of mononitrodibenzanthrone to 1:2:7:8-diphthaloylphenanthraquinone(IX):

Crystalline mononitrodibenzanthrone (1 g.) was dissolved in conc. sulphuric acid (20 ml.) and the violet solution was added dropwise to a boiling solution of chromic acid (5 g.) in water (100 ml.) during 10 minutes. The solution was refluxed for 10 minutes, cooled, filtered and washed free from acid when a brown product (720 mg.) was obtained.

The nitrogen-free product gave a red solution in sulphuric acid, a red vat with alkaline hydrosulphite and was insoluble in ammonia. It crystallised in brown needles from trichlorobenzene. (Found: C, 76.6; H, 2.5. $C_{30}H_{12}O_6$ requires C, 76.9; H, 2.6%).

Oxidation of (IX) to 2:2'-dianthraquinonyl-1:1'-dicarboxylic acid (X):

The triquinone (IX) (500 mg.) was dissolved in conc. sulphuric acid (20 ml.) and the deep red solution poured dropwise to a boiling solution of chromic acid (3 g.) in water (100 ml.). It was refluxed for 10 hours and the pale cream-coloured product was filtered off (470 mg.) and digested with 2% ammonia. The ammonia-insoluble portion (70 mg.) answered all the colour reactions of the starting quinone (IX). The reddish brown ammonical solution was norited and acidified when the dicarboxylic acid precipitated as a creamy white solid (340 mg.). On crystallisation from aqueous dimethyl formamide it gave colourless microscopic needles, m.p. 360° (dec.). (Found: C, 69.4; H, 3.9. $C_{30}H_{14}O_8$, 1 H_2O requires C, 69.2; H, 3.1%).

Decarboxylation of (X) to 2:2'-dianthraquinonyl (XI):

The dicarboxylic acid (X) (220 mg.) was refluxed with quinoline (20 ml.) and copper bronze (10-15 mg.) for 2 hours. At the end of the period most of the quinoline was distilled off and the contents poured into 2N hydrochloric acid (100 ml.). The solid product was filtered off, dissolved in boiling o-dichlorobenzene (250 ml.) and the pale-yellow solution after clarification with norit and concentration gave straw-yellow needles (180 mg.), m.p. 395-7°. The mixed melting point with an authentic sample of 2:2'-dianthraquinonyl was undepressed. (Scholl[17] cites m.p. 387-8° for 2:2'-dianthraquinonyl).

2:2'-Dianthraquinonyl (XI) from dibenzanthrone or 16:17-dimethoxydibenzanthrone:

A solution of dibenzanthrone (5 g.) or 16:17-dimethoxydibenzanthrone (5 g.) in sulphuric acid (100 ml.) was added dropwise to a boiling solution of chromic acid (25 g.) in water (500 ml.) and after refluxing for 10 minutes, the triquinone (IX; 3.7 g.) was isolated as usual. A solution of (IX; 1 g.) in sulphuric acid (20 ml.) was then refluxed with 3% aqueous solution of chromic acid (150 ml.) for

16 hours. The product (800 mg.) was digested with 2% ammonia to remove the unconverted quinone (300 mg.) and the ammonia-soluble portion (500 mg.) was isolated by acidification. The ammonia-soluble dicarboxylic acid (X) crystallised from aqueous dimethyl formamide in colourless needles, m.p. 360-2° (dec.). (Found: C, 69.2; H, 3.6. $C_{30}H_{14}O_8$, 1 H_2O requires C, 69.2; H, 3.1%). The acid (X; 400 mg.) on decarboxylation with boiling quinoline and copper bronze gave 2:2'-dianthraquinonyl (300 mg.); it crystallised from o-dichlorobenzene in straw-yellow needles, m.p. 396-7°. (Found: C, 81.0; H, 3.5. $C_{28}H_{14}O_4$ requires C, 81.3; H, 3.4%).

Oxidation of Caledon Jade Green with chromic acid in boiling acetic acid:

The compound (10 g.) was suspended in glacial acetic acid (200 ml.) and a solution of chromic acid (40 g.) in glacial acetic acid (125 ml.) was added under reflux in 5 equal portions, each at the end of 8 hours. After 40 hours of refluxing, the mixture was diluted with water (500 ml.) and the brown product (IX; 7.5 g.) collected, washed and dried. It was insoluble in ammonia, gave a deep red vat and dissolved in sulphuric acid with a deep red colour. It crystallised from trichlorobenzene in small brown needles. (Found: C, 76.4; H, 2.3. $C_{30}H_{12}O_6$ requires C, 76.9; H, 2.6%).

16:17-Diaminodibenzanthrone:

2-Aminobenzanthrone was prepared from 2-nitrobenzanthrone, m.p. 298-300°, by reduction with methanolic sodium sulphide. The product after chromatography and crystallisation from acetic acid had m.p. 234°.

An intimate mixture of 2-aminobenzanthrone (5 g.) and naphthalene (10 g.) was added in 10 minutes, in small lots at a time to a melt consisting of caustic potash (20 g.), caustic soda (5 g.), fused sodium acetate (2 g.) and naphthalene (40 g.) maintained at 220-25°. After maintaining the melt at 225° for 45 minutes with occasional stirring, it was poured into water. The deep blue solution was treated with sodium hydrosulphite (5 g.) at 60°, filtered and the filtrate air-oxidised. The deep green powder (3.78 g.), after chromatography on alumina, crystallised from o-dichlorobenzene in minute green needles. (Found: C, 83.1; H, 3.3; N, 5.3. $C_{34}H_{18}O_2N_2$ requires

C, 83.7; H, 3.7; N, 5.8%). It dissolved in sulphuric acid with a reddish violet colour, gave a blue vat and dyed cotton an olive-green shade changing to a fast brown on hypochlorite treatment.

Hypochlorite oxidation of 16:17-diaminodibenzanthrone:

Diaminodibenzanthrone (700 mg.), precipitated from sulphuric acid was added to a clear solution of sodium hypochlorite (freshly prepared from bleaching powder (5 g.) and the mixture was vigorously shaken for 1 hour in a mechanical shaker. The green colour of the diamine changed to brown almost immediately and remained unaltered. The product was filtered and collected (700 mg.). It contained nitrogen, gave a violet solution in sulphuric acid, a blue solution in alkaline sodium hydrosulphite and dyed cotton a fast bright brown shade, unaffected by hypochlorite treatment. It could not be obtained in crystalline form. (Found: C, 80.4; H, 3.2; N, 4.5%).

7:8-Diaminodibenzanthrone:

5-Aminobenzanthrone (300 mg.) was heated at 210-15^{o} for 45 minutes in a melt consisting of caustic potash (2 g.), caustic soda (1 g.), fused sodium acetate (200 mg.) and naphthalene (5 g.). After working up as usual the product consisted of an unvattable residue (200 mg.) and a vattable fraction (22 mg.). The vattable portion gave a reddish blue solution in sulphuric acid and dyed cotton a dull green shade from a dull blue vat, changing to a khaki shade on hypochlorite oxidation.

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7 D.R.P. 458,522.

7(a) Boys, Grieve and Gordon Rule, *J.Chem.Soc*., 1938, 1833, state that fusion of 0.5 g of 11-aminobenzanthrone, m.p. 215-17°, with KOH at 220° (30 mts.) gave 0.12 g. of dark blue-black powder, presumably diaminodibenzanthrone; the faint green vat dyed cotton a fast pale gray-green colour; the sulphuric acid solution is brown with a green fluorescence.

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PART IV

THE CONSTITUTION OF CYANANTHRENE O

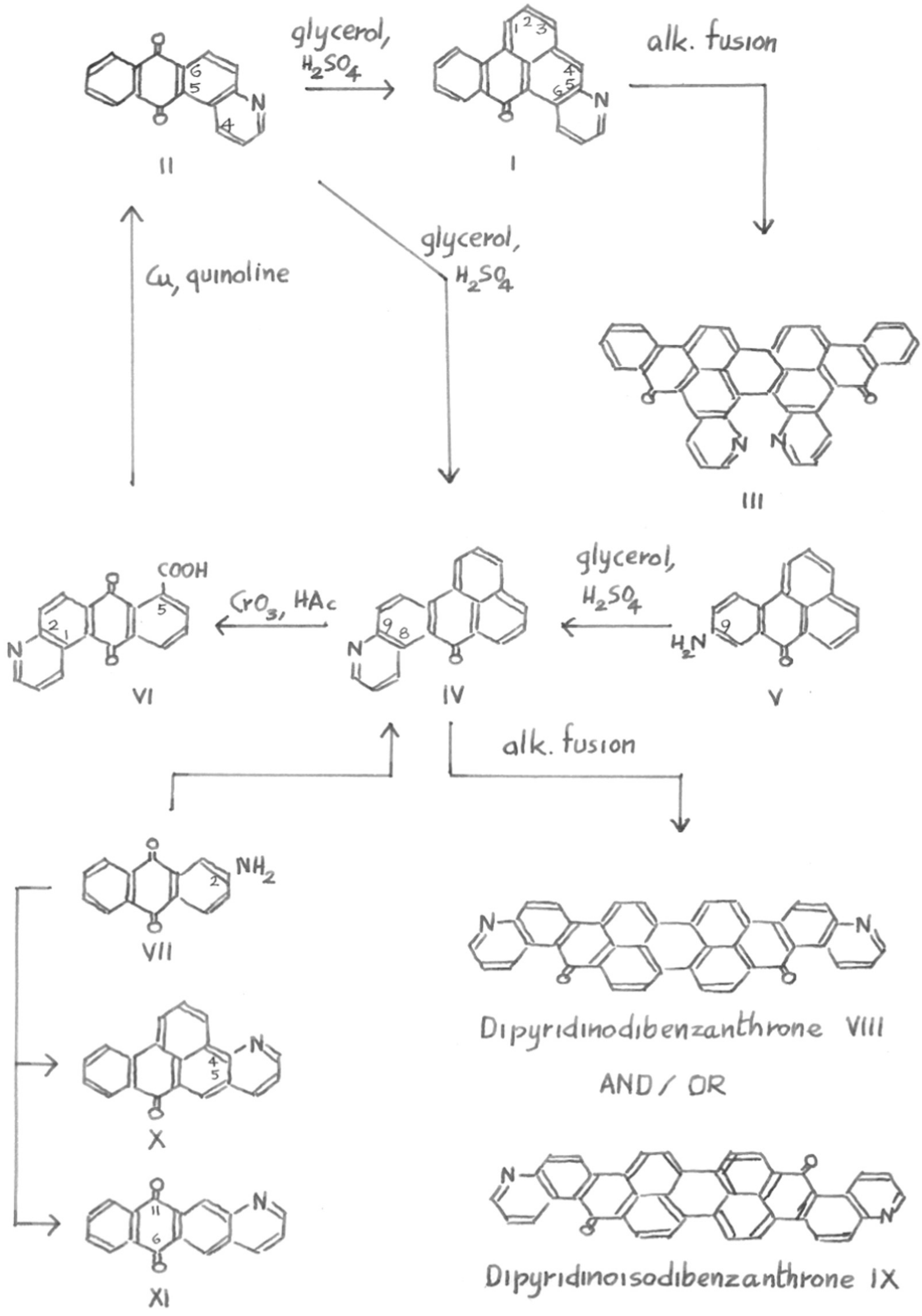
Cyananthrene O or Indanthrene Dark Blue BT (Bally and Isler, 1904; C.I. 68705*), the first vat dye of the violanthrone series, was prepared by the alkali fusion of "benzanthronequinoline" and is an obsolete vat dye. "Benzanthronequinoline", obtained by the action of glycerol and sulphuric acid on 2-aminoanthraquinone, was formulated by Bally[1] as being probably a 5:6-pyridino derivative (I) of benzanthrone. The angular orientation was proved by the fact that "benzanthronequinoline" (I) was obtained by the action of glycerol and sulphuric acid on 5:6-phthaloylquinoline (II); this angular pyridinoanthraquinone (II) was prepared earlier by Graebe[2] from 7:8-dihydroxy-5:6-phthaloylquinoline (Alizarine Blue; C.I. Mordant Blue 27; C.I. 67415) by zinc dust distillation to the pyridinoanthracene and subsequent oxidation. On the basis of Bally's structure for pyridinobenzanthrone (I), Cyananthrene O was formulated as (III) by Schultz[3].

Pandit, Tilak and Venkataraman[4] observed that Bally's "benzanthronequinoline" was not the 5:6-pyridino derivative (I), but the 9:8-pyridino derivative (IV) of benzanthrone. The Skraup reaction on 9-aminobenzanthrone (V) gave (IV), identical with Bally's "benzanthronequinoline"; the constitution was confirmed by its oxidation to 1:2(N)-pyridinoanthraquinone-5-carboxylic acid (VI). Decarboxylation of (VI) gave (II) which was also converted to 9:8-pyridinobenzanthrone (IV) by the action of glycerol and sulphuric acid. The dipyridinodibenzanthrone structure (VIII)[5] was then assigned to Cyananthrene O (Indanthrene Dark Blue BT) on the analogy of the alkali fusion of benzanthrone to dibenzanthrone; but Pandit *et al*. did not carry out any experimental work on Cyananthrene.

Following the demonstration by Pandit *et al*. of the correct structure of Bally's "benzanthronequinoline" as (IV), Bradley and Sutcliffe[6] submitted (IV) to alkali fusion and isolated from the crude product a 25% yield of a dye by extraction with boiling trichlorobenzene containing 10% phenol. This purified fraction on chromatography on alumina at 160-70° using trichlorobenzene containing 50% phenol gave a violet-black powder in 12% yield based on the purified sample. The ultraviolet and visible absorption spectrum of the chromatographed dye in sulphuric acid was much more similar to the spectrum of isoviolanthrone than that of violanthrone. This led to the formulation of Cyananthrene O as the dipyridinoisodibenzanthrone (IX).

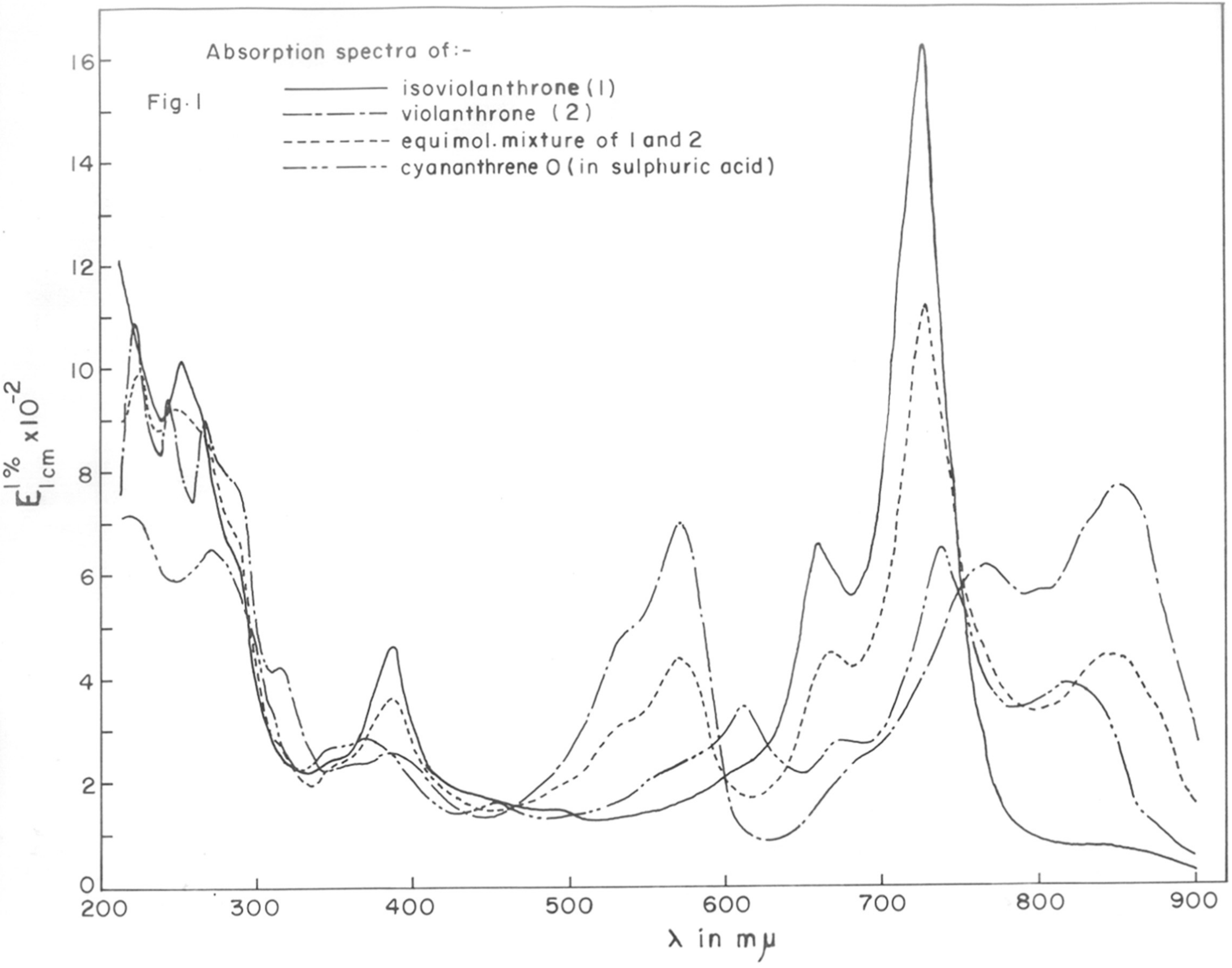
The spectrum of Cyananthrene O recorded by Bradley and Sutcliffe[6], although in many respects resembles the isoviolanthrone spectrum, shows the characteristics of the violanthrone spectrum beyond 780 mμ. The spectrum of an equimolecular mixture of violanthrone and isoviolanthrone was therefore determined and compared with that of Cyananthrene O (Fig. 1) which indicated that the dye is a mixture of isoviolanthrone and violanthrone structures. It was observed during our studies on the chromatographic separation of vat dyes on alumina at elevated temperatures (Part II of the thesis) that isoviolanthrone could be readily separated from violanthrone; and the present work is mainly an examination of the homogenity of commercial Cyananthrene O by methods involving hot chromatography.

Through the courtesy of Dr. Waibel of Farbenfabriken, Bayer, Leverkusen, Germany, an authentic sample of Cyananthrene O was obtained.


II
glycerol, H_2SO_4
I
alk. fusion
glycerol, H_2SO_4
Cu, quinoline
III
COOH
CrO_3, HAc
glycerol, H_2SO_4
H_2N
VI
IV
V
alk. fusion
NH_2
VII
X
XI
Dipyridinodibenzanthrone VIII
AND / OR
Dipyridinoisodibenzanthrone IX

The sample which was free from water-soluble material, contained 14% of acetone-soluble substances, chromatography of which on alumina using benzene as solvent led to 4:5-pyridinobenzanthrone (X) (3%), 1-azanaphthacene-6:11-dione (XI) (2.3%), 2-aminoanthraquinone (1%) and several unidentified fractions which tatalled 7.7%. The residual dye after acetone extraction was a mixture of vattable (47%) and unvattable (35%) fractions, neither of which could be crystallised. The unvattable fraction, which does not vat even after dispersion from sulphuric acid or by the addition of pyridine to the vatting solution, gave colour reactions similar to the vattable portion. However, the absorption spectrum in sulphuric acid differed from the spectrum of the vattable fraction beyond 770 mμ and was similar to the isoviolanthrone curve (Fig. 1 and 2). This is in accordance with the fact that unlike violanthrone, isoviolanthrone is difficult to vat and the unvattable fraction of Cyananthrene O may probably represent the isoviolanthrone structure. The spectrum of the vattable portion shows an absorption maximum at 818 mμ; the spectrum of violanthrone has a peak at 848 mμ while

Fig. 1


Absorption spectra of:-
isoviolanthrone (1)
violanthrone (2)
equimol. mixture of 1 and 2
cyananthrene O (in sulphuric acid)
$E^{1\%}_{1cm} \times 10^{-2}$
0
2
4
6
8
10
12
14
16
200
300
400
500
600
700
800
900
λ in mμ

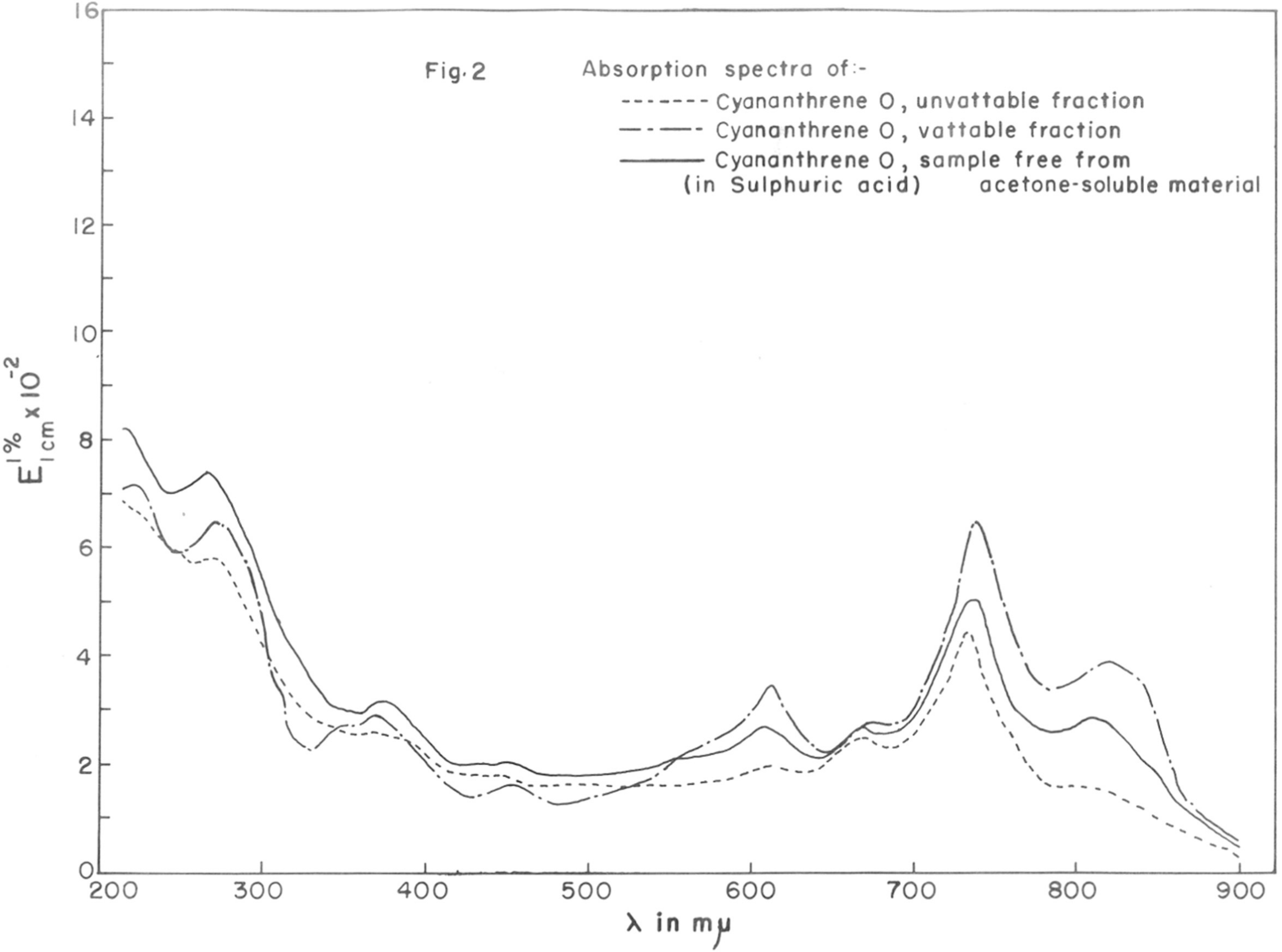


Fig. 2 Absorption spectra of:-

isoviolanthrone does not show any absorption at all beyond 790 mμ.

Chromatography of the vattable fraction on alumina at 110° using naphthalene, trichlorobenzene, mixtures of trichlorobenzene with increasing amounts of o-chlorophenol, and pure o-chlorophenol successively, yielded several fractions. The absorption spectra of these fractions in sulphuric acid showed remarkable similarity to one another which indicated that no separation had been achieved. In the chromatography of the vattable fraction 35% was very strongly held as a black band and could not be eluted or extracted.

In order to overcome the difficulty caused by the strong adsorption of the dye on alumina it was thought advantageous to reduce the quinone to the corresponding azahydrocarbon and then study the possibility of chromatographic separation. The reduction of Cyananthrene O using aluminium *cyclo*hexoxide in *cyclo*hexanol[7] proved ineffective; however, Clar reduction[8] afforded a 30% yield of the azahydrocarbon as a reddish brown powder. The azahydrocarbon dissolved in xylene, o-dichlorobenzene and other solvents with a red colour and a strong green fluorescence. The azahydrocarbon or its black picrate could not be obtained crystalline. The chromatographic analysis of the Clar reduction product is under investigation.

EXPERIMENTAL

Preliminary examination of Cyananthrene O .

The sample did not contain water-soluble matter. The dye (30 g.) was extracted with acetone in a soxhlet till the siphonings were practically colourless (30 hours). The deep reddish-brown acetone solution on evaporation to dryness gave a brown powder (4.2 g.). The acetone-insoluble fraction which was a violet-black powder (25.8 g.) was suspended in 3% sodium hydroxide solution (1.5 l.) warmed to 60° and treated with sodium hydrosulphite (35 g.). After stirring for 20 minutes the deep blue vat was filtered, the residue extracted with more alkaline sodium hydrosulphite (500 ml.) and the filtrates combined. The black unvattable residue was dried and collected (10.5 g.). The blue filtrate on air-oxidation gave violet-black powder which was filtered and dried (14.1 g.).

Examination of the acetone-soluble fraction (14%).

The brown powder (4.0 g.) was extracted with benzene when a benzene-insoluble residue (0.8 g.) was obtained. The brown-yellow benzene extract was chromatographed on alumina (3 x 60 cm.). On development with benzene several bands appeared and were eluted out and isolated. The fractions are recorded below in the order of elution.

(a) 4:5-Pyridinobenzanthrone (X) (0.9 g.), lemon yellow needles, m.p. 233°. It gave a cherry-red colour in sulphuric acid and did not vat. The mixed melting point with an authentic sample was undepressed.

(b) Unidentified (0.1 g.), red-brown needles, m.p.156-60°. It dissolved in sulphuric acid with a red colour and gave a crimson vat.

(c) Unidentified (0.8 g.), orange needles, m.p. 310°. It gave a brown-yellow solution in sulphuric acid and a yellowish green vat, turning brown on adding excess of hydrosulphite.

(d) Unidentified (0.1 g.), deep red needles, m.p. 315°. The sulphuric acid solution was brown-yellow and it gave a green vat.

(e) 1-Azanaphthacene-6:11-dione (XI) (0.7 g.), brown yellow needles, m.p. 320°. In sulphuric acid it gave brown-yellow solution and a green vat with alkaline sodium hydrosulphite. The mixed m.p. with an authentic sample was undepressed.

(f) 2-Aminoanthraquinone (0.3 g.), orange needles, m.p. 301°. The mixed m.p. with authentic sample was undepressed.

(g) Unidentified (0.2 g.), brown amorphous powder, m.p. 200-250°. This fraction was isolated by extraction of the alumina with methanol containing a little hydrochloric acid.

Examination of the unvattable fraction (35%).

The unvattable fraction (10.5 g.) contained nitrogen and could not be obtained crystalline from a variety of solvents. It dissolved in conc. sulphuric acid with a greenish blue colour. It did not sublime or melt below 420°. The spectrum of this compound was determined in sulphuric acid solution (Fig.2).

Examination of the vattable fraction (47%).

The vattable fraction (14.1 g.) contained nitrogen and could not be crystallised. It dissolved in conc. sulphuric acid with a blue colour. In cresol or *o*-chlorophenol it dissolved with a blue colour and the solution in trichlorobenzene was blue with an intense red fluorescence. It did not melt or sublime below 420°. It gave a deep blue vat and dyed cotton a deep reddish blue shade. The spectrum of this fraction, after purification by separation from *o*-chlorophenol, was determined in sulphuric acid and compared with the spectra of dibenzanthrone, isodibenzanthrone and an equimolecular mixture of the two (Fig.1).

Chromatographic analysis of the vattable fraction.

The dye (400 mg.) was made into a thin slurry with alumina (5 g.) and boiling trichlorobenzene (100 ml.) and the hot suspension was added to a column of alumina (2 x 40 cm.) maintained at 110°. The fractions were eluted out in the order given in the table below using a variety of solvents.

No.	Colour of band	Solvent	Volume of eluate in ml.	Weight of product in mg,	Appearance
1	2	3	4	5	6
1	Yellow	Naphthalene	150	4	Yellow needle
2	Three minor bands	"	200	not isolated	
3	Brownish violet	"	300	7	Black powder
4	Pale brown	TCB+ 1% OCP	350	10	"
5	Violet	TCB + 1% OCP	1000	20	"
6	Blue	TCB + 10% OCP	3000	32	"
7	Black	TCB + 10% OCP	5000	70	"
8	Black	OCP	4000	106	"
9	Black	could not be eluted out			

Note: TCB: 1:2:4-Trichlorobenzene

OCP: o-Chlorophenol

The absorption spectra of fractions 6,7 and 8 were determined in sulphuric acid and were similar in nature.

Clar reduction of Cyananthrene O.

The acetone-soluble free dye (5 g.) was mixed intimately with moist zinc chloride (25 g.), sodium chloride (5 g.) and zinc dust (5 g.) and heated to 290-300° under stirring. The black melt was maintained at the above temperature for 30 minutes, during which time the colour of the melt changed to a dirty brown. The melt was cooled and treated with dilute hydrochloric acid (500 ml.) to destroy the zinc. The red-brown residue was repeatedly extracted with boiling trichlorobenzene till the extract was faintly fluorescent. The combined extract on concentration gave a red-brown powder (1.2 g.) which did not vat. It contained nitrogen and dissolved in organic solvents to give a red solution with an intense green fluorescence. The compound was purified through its black amorphous picrate.

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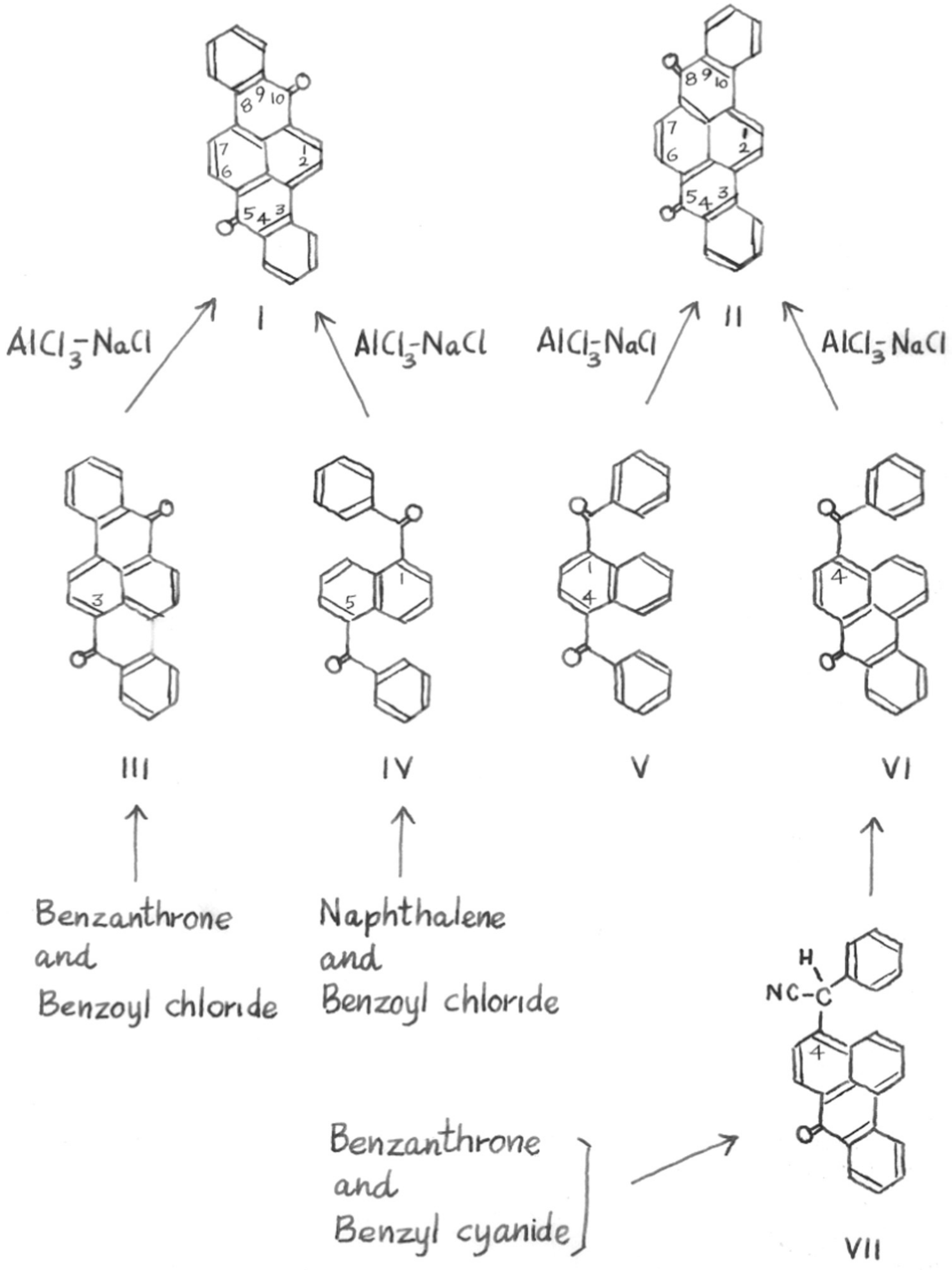
PART V

THE CONSTITUTION OF MAYVAT BRILLIANT RED AF.

Two types of dibenzpyrenequinones have been used as vat dyes, the 3:4:8:9-dibenzpyrene-5:10-quinone (I) and the isomeric 3:4:9:10-dibenzpyrene-5:8-quinone (II), but the second has little practical interest now[1]. The quinone (I), itself marketed as Indanthrene Golden Yellow GK (C.I.Vat Yellow 4; C.I. 59100), may be prepared by Scholl cyclisation of 3-benzoylbenzanthrone (III) or 1:5-dibenzoylnaphthalene (IV)[2]. The Friedel-Crafts reaction between benzanthrone and benzoyl chloride yields 3-benzoylbenzanthrone and the position taken up by the benzoyl group is clear from the general activity of the benzanthrone nucleus in the 3-position; thus mononitration of benzanthrone gives the 3-nitro compound which can be converted to the 3-benzoylbenzanthrone through the amine, nitrile and carboxylic acid, and condensation of the acid chloride with benzene[3]. The Scholl cyclisation of 1:4-dibenzoylnaphthalene (V) or 4-benzoylbenzanthrone (VI) yields isodibenzpyrenequinone (II)[4]. An excellent method for the preparation of 4-benzoylbenzanthrone is the condensation of benzanthrone with benzyl

CHART I


I
II
AlCl$_3$-NaCl
AlCl$_3$-NaCl
AlCl$_3$-NaCl
AlCl$_3$-NaCl
III
IV
V
VI
Benzanthrone and Benzoyl chloride
Naphthalene and Benzoyl chloride
NC-C-H
VII
Benzanthrone and Benzyl cyanide

cyanide in presence of an alkali, followed by oxidative hydrolysis of the product (VII)[5]. The various routes are presented schematically in Chart I.

Various halogenated derivatives of 3:4:8:9-dibenzpyrene-5:10-quinone find commercial use as vat dyes. Thus the dibromoderivative of (1) (Indanthrene Golden Yellow RK, C.I. Vat Orange 1; C.I. 59105) is much redder in shade than the parent quinone and is made by the addition of bromine to the quinone-aluminium chloride melt at 150-155° during 20 hrs.[6] Kunz[7] has made a careful study of the bromination of Indanthrene Golden Yellow GK and has shown the influence of the conditions of the reaction on the orientation of the halogen atoms, which in turn affects the shade and fastness properties. Halogenation in an organic solvent requires a carrier, such as iodine, and the shades are much redder and faster than the parent compound. Halogenated dibenzpyrenequinones can also be prepared by the cyclisation of 3-benzoylbenzanthrones containing halogen atoms in the benzene or benzanthrone residues[8].

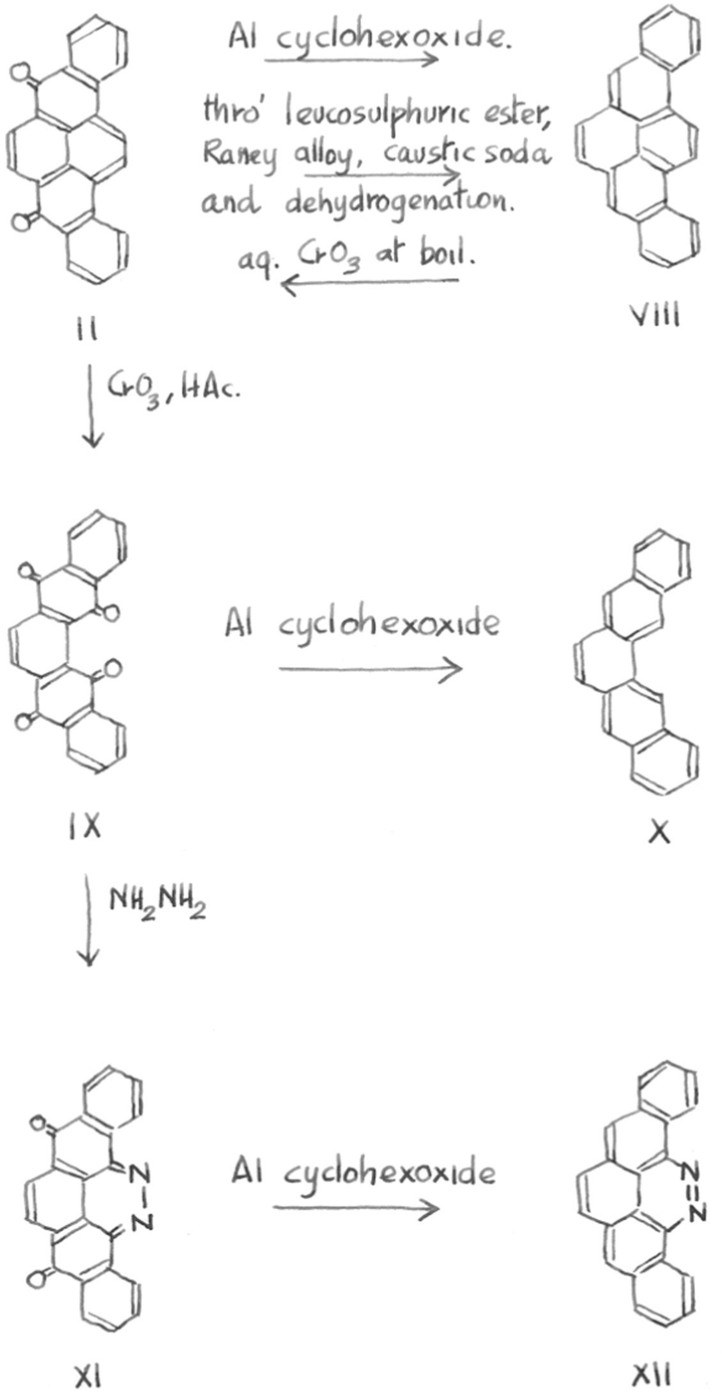
The isodibenzpyrenequinone (II) which is a red compound has not found application as a vat dye, but Indanthrene Scarlet 4G (C.I. 59115*; Vat dye described as x:x-dibromo-γ:γ'-dichloro-3:4:9:10-dibenzpyrene-5:8-quinone), stated to be the dibromoderivative of (II)[9] was marketed by the I.G.Farbenindustrie about 30 years ago and is not included in the commercial range of Indanthrens. Kunz[10] has described Indanthrene Scarlet 4G as a γ:γ'-dichloroderivative of isodibenzpyrenequinone; but a sample of Indanthrene Scarlet 4G which we obtained through the courtesy of Casella Farbwerke, Mainkur, contained both chlorine and bromine and analysed for $C_{24}H_{10}O_2Cl_{2.4}Br_{1.6}$. U.S.P. 1,942,761 describes the preparation of several halogenated derivatives of isodibenzpyrenequinone using a variety of halogenating agents and catalysts, whereby shades ranging from yellow-red to clear Turkey red are obtained.

Mayvat Brilliant Red AF Double Paste (not mentioned in the new Color Index (1956), recently introduced in United States by Otto B.May & Co. in pilot plant quantities, has been described by them as an "entirely homogeneous anthraquinone vat colour" which "is an original invention of our laboratories and is a distinct contribution to the range of anthraquinone vat dyes presently available." "Outstanding dyeing and fastness properties" were claimed. A preliminary examination of Mayvat Brilliant Red AF which dyes cotton a brilliant bluish red shade from a blood-red vat, showed that it was a hologenated quinone containing chlorine and bromine, but no nitrogen or sulphur. The dye after removal of water-soluble matter was chromatographically homogeneous, using molten naphthalene as solvent and alumina as absorbent. It crystallised from o-dichlorobenzene in red needles and sublimed without decomposition. Dehalogenation of the dye by shaking with caustic soda solution, Raney nickel and hydrogen gave the parent quinone (II) crystallising in red needles, m.p. 373°. Anthraquinone undergoes reduction to 1:2:3:4-tetrahydroanthraquinone by heating with Raney alloy and caustic soda solution at water-bath temperatures[11], but the quinone (II) was unaffected under these conditions. Indanthrene Scarlet 4G and Mayvat Brilliant Red AF were merely dehalogenated to isodibenzpyrenequinone on heating with Raney alloy and caustic soda solution. Treatment with caustic soda solution and an excess of sodium hydrosulfite at 90-95° did not effect dehalogenation of the dye, but the action of sodium methoxide in methanol in a sealed tube at 170° for 12 hrs. resulted in the complete removal of halogen atoms. The dehalogenated quinones gave identical derivatives by reductive acetylation and by reductive benzoylation.

Reduction of the quinone (II) with aluminium *cyclo*hexoxide[12] in *cyclo*hexanol gave 3:4:9:10-dibenzpyrene (VIII), m.p. 281°, in 70% yield and was identified by melting point and absorption spectrum. The general method for the reduction of quinones to hydrocarbons by treatment of the sulphuric ester of the leuco derivative with Raney alloy and caustic soda solution followed by dehydrogenation was also applicable to obtain the hydrocarbon in good yield[13]. 3:4:9:10-Dibenzpyrene (VIII) was prepared by Scholl and Newman[4] by distilling the 5:8-quinone with zinc dust; Clar[14] obtained a yield of about 85% by his technique of distillation with a mixture of zinc dust, zinc chloride and sodium chloride, but Buu Hoi and Lavit[15] have reported a yield of 53.3%. Oxidation of the hydrocarbon with boiling aqueous chromic acid gave back the quinone (II).

The quinone (II) from Mayvat Brilliant Red AF was unaffected by oxidation with chromic acid in boiling aqueous sulphuric acid or treatment with alkaline permanganate; but oxidation with chromic acid in boiling acetic acid converted (II) to a yellow crystalline quinone (IX), m.p. 332°, which gave an orange vat and was identified as pentaphene-5:14:8:13-diquinone[4]. Reduction of (IX) with aluminium *cyclo*hexoxide[12] gave a 91% yield of pentaphene (X) identified by the m.p. and absorption spectrum. This route to pentaphene is much more convenient than the earlier methods such as the Elbs pyrolysis of 2:4-dibenzoyl-*m*-xylene[16] or the synthesis starting from the Grignard reaction between phthalic anhydride and *o*-tolyl magnesium bromide[17]. After the present work was completed, Badger *et.al*. have reported a synthesis of pentaphene (X) by cyclisation of 3:3'-*bis*-bromomethyl-2:2'-dinaphthyl, followed by dehydrogenation, but this method did not give a higher yield than the earlier synthesis of Clar[16,17].

CHART II


II
Al cyclohexoxide.
thro' leucosulphuric ester, Raney alloy, caustic soda and dehydrogenation.
aq. CrO$_3$ at boil.
VIII
CrO$_3$, HAc.
IX
Al cyclohexoxide
X
NH$_2$NH$_2$
XI
Al cyclohexoxide
XII
N
N

Reductive acetylation of pentaphene diquinone (IX) gave the tetracetate of the leuco compound. Interaction of (IX) with hydrazine hydrate in boiling pyridine gave the reddish orange azaquinone (XI)[4] which dyed cotton a brilliant golden orange shade from a cherry-red vat. Reduction of (XI) with aluminium *cyclo*hexoxide gave 1:2-diaza-3:4:9:10-dibenzpyrene (XII), lemon yellow needles, m.p.304°, in good yield. The light absorption spectrum of the azahydrocarbon was determined in alcohol and compared with that of 3:4:9:10-dibenzpyrene (Fig.1).

According to a report which appeared in Indian newspapers in February 1957, Buu Hoi isolated 3:4:9:10-dibenzpyrene (VIII) from tobacco smoke and found that "when the substance was injected into 4000 mice, each of them developed cancer. In some cases the growth took only 42 days to show itself. Half a mg. was the amount given". The presence of 3:4:9:10-dibenzpyrene in considerable quantity in the neutral fractions of tobacco smoke was in fact demonstrated by Bonnet and Neukomm[19]; Lacassagne, Zaideta, Buu Hoi and Chalvet[20] compared the carcinogenic activities of the hydrocarbon, its 5-methyl

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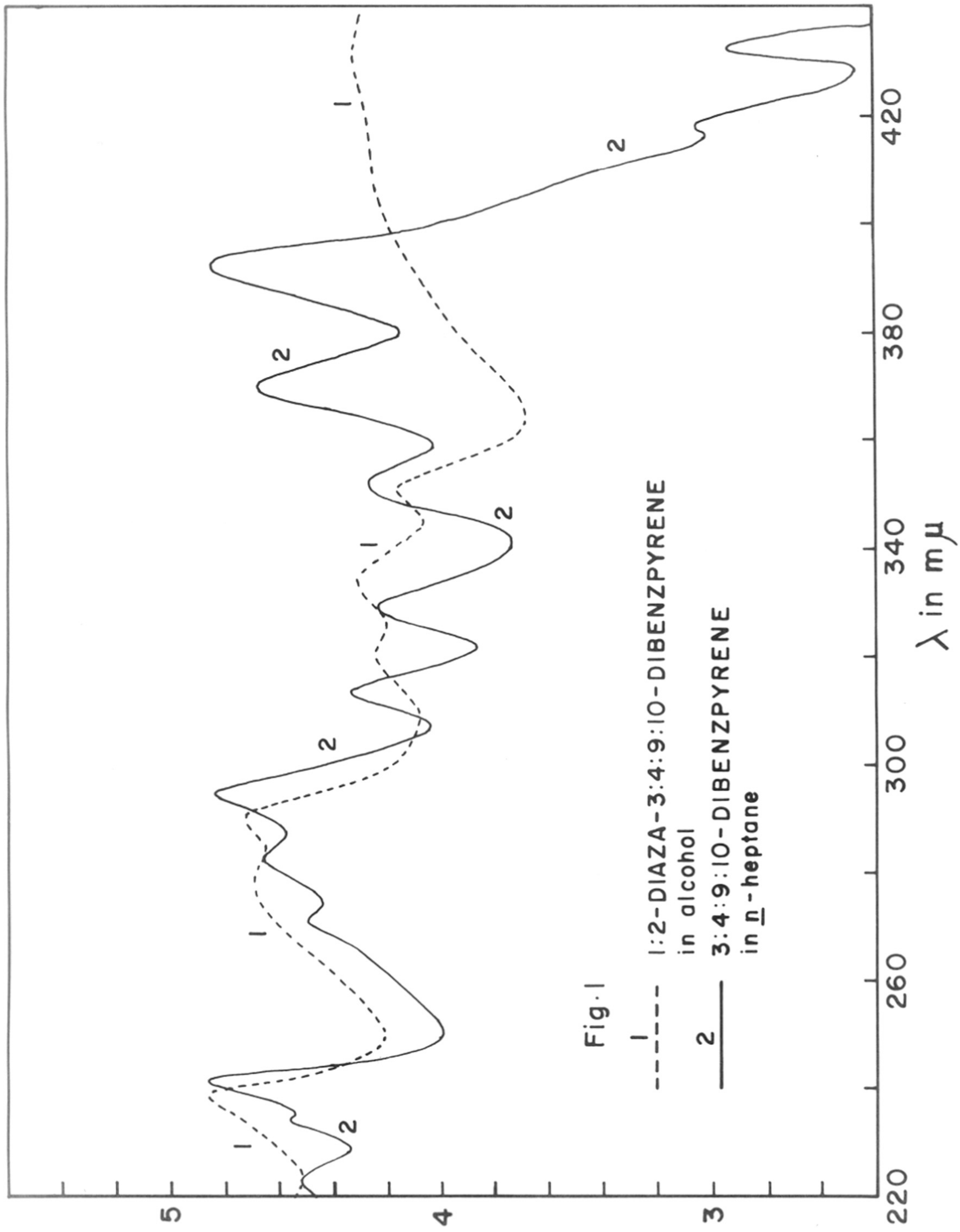
Fig. 1
1
2
1:2-DIAZA-3:4:9:10-DIBENZPYRENE in alcohol
3:4:9:10-DIBENZPYRENE in n-heptane
log ε
5
4
3
λ in mμ
220
260
300
340
380
420

and 5:8-dimethyl derivatives and observed that after a subcutaneous injection of 0.6 mg. of one of the three components, the hydrocarbon produced sarcoma on the 42nd day in 11 mice. The 5-methyl derivative produced sarcoma in 3 out of 10 mice after 150 days, and the 5:8-dimethyl derivative was inactive or feebly active. The carcinogenic properties of 3:4:9:10-dibenzpyrene (VIII) and 1:2 diaza-3:4:9:10-dibenzopyrene ~~[illegible] and 1:2 diaza-3:4:9:10-dibenzopyrene~~ (XII) are being examined at the Indian Cancer Research Centre. Subcutaneous injections of a ball-milled suspension of 2 mg. of (VIII) or (XII) in 0.2 ml. of propylene glycol were given to 16 mice; the hydrocarbon (VIII) produced first tumor in 90 days, and tumors developed in all the 16 mice after 120 days; the azahydrocarbon (XII) produced no definite tumor in 105 days, but a specific reaction at the site of injection was noticed in 10 mice after 90 days.

Mayvat Brilliant Red AF and Indanthrene Scarlet 4G dye substantially different shades and they exhibit different colour reactions in sulphuric acid; the number and position of the halogen atoms, which must be responsible for these differences, are under investigation.

EXPERIMENTAL

Purification and properties of Mayvat Brilliant Red AF: Mayvat Brilliant Red AF Double Paste (50 g.) was dried at 110° for 4 hrs. and the reddish black powder (11 g.) was dissolved in sulphuric acid (200 ml.). The deep olive-green solution was poured into ice water (1 l.), and the deep red flocculent precipitate was collected, washed free from acid, and dried (10 g.). The chromatographic homogeneity of the dye was examined by dissolving 150 mg. in molten naphthalene (50 g.) and running the solution through a jacketed column of alumina (2.5 x 30 cm.) maintained at 95-100°. Development with molten naphthalene produced a deep orange-red band, which was eluted with the same solvent. Naphthalene was distilled off and the dye recovered as a red powder (120 mg.) which crystallised from o-dichlorobenzene in red needles. (Found: C, 60.3; H, 1.8; Cl, 2.4; Br, 28.1. $C_{24}H_{10}O_2Cl_{0.3}Br_{1.7}$ requires C, 60.4; H, 2.1; Cl, 2.2; Br, 28.5%). The dye before chromatography also crystallised in red needles from o-dichlorobenzene. (Found: C, 57.9; H, 2.4; Cl, 8.7; Br, 24.9. $C_{24}H_{10}O_2Cl_{1.3}Br_{1.6}$ requires

C, 57.3; H, 2.0; Cl, 9.2; Br 25.2%). Indanthrene Scarlet 4G crystallised from the same solvent in deep orange needles (Found: C, 53.6; H, 1.7; Cl, 15.3; Br, 24.5. $C_{24}H_{10}O_{2}Cl_{2.4}Br_{1.6}$ requires C, 53.2; H, 1.7; Cl, 15.6; Br 23.8 %).

Cotton yarn dyed a 2% shade with Mayvat Red and Indanthrene Scarlet 4G respectively gave the following colour reactions[20]: (1) alkaline hydrosulphite: bluish red, red; (2) acid hydrosulphite: both yellow-brown; (3) conc.nitric acid: rubine, scarlet; (4) conc.sulphuric acid: black, deep rubine. Both Mayvat Red and Indanthrene Scarlet 4G dissolve in organic solvents such as o-dichlorobenzene or xylene to form an orange solution with a strong yellow fluorescence. The solution of Mayvat Red in conc. sulphuric acid is olive green and in 16% oleum straw-yellow; Indanthrene Scarlet gives a deep brownish red solution in conc. sulphuric acid and a deep red solution in 16% oleum.

Dehalogenation of Mayvat Brilliant Red AF:

(i) The finely powdered dye (1 g.) was precipitated from sulphuric acid and washed free from acid. It was suspended in 5% caustic soda solution (100 ml.)

in a pressure bottle, Raney nickel (1 g.) added, and the mixture shaken with hydrogen at 38 lbs. pressure for 6 hrs. The mixture was filtered, the nickel residue washed with 2% caustic soda solution containing a little glucose, and the combined deep red filtrate air-oxidized. The red precipitate was collected, washed and dried. The quinone (II; 0.58 g.), which was halogen-free crystallised from o-dichlorobenzene in deep red shining needles, m.p.373° (Found: C, 85.9; H, 3.5. Calc. for $C_{24}H_{12}O_2$: C, 86.2; H, 3.6%). The m.p. of 3:4:9:10-dibenzpyrene-5:8-quinone quoted in the literature[4] is 365°. This parent quinone, by halogenation of which both Mayvat Red and Indanthrene Scarlet 4G are prepared, gives the same colourations as Mayvat Red in o-dichlorobenzene, conc. sulphuric acid and 16% oleum.

(ii) Action of sodium methoxide on Mayvat Brilliant Red AF: Freshly precipitated Mayvat Brilliant Red AF (1.5 g.) was suspended in a solution of sodium (1 g.) in absolute methanol (20 ml.) kept in a pyrex tube. The tube was sealed and heated to 170-75° for 12 hrs. The deep red solution was air-oxidised and the red precipitate (.75 g.) which was halogen-free, was purified by sublimation and crystallised in red needles from o-dichlorobenzene, m.p. 372-3°, undepressed by quinone (II) from Mayvat Brilliant Red AF.

Reductive benzoylation of (II)

The quinone (II) (50 mg.) was shaken with 5% caustic soda solution (20 ml.) and sodium hydrosulphite (0.2 g.) at 70-80° for 10 mins. Benzoyl chloride (0.3 ml.) was then added to the clear red vat and the mixture shaken vigorously for 5 mins., when a pale yellow granular precipitate separated (60 mg.). Crystallisation from benzene gave a pale yellow needles, m.p. 343° (Found: C, 84.4; H, 4.1. $C_{38}H_{22}O_4$ requires C, 84.1; H, 4.0%).

Reductive acetylation of (II)

A mixture of the quinone (II) (100 mg.), acetic anhydride (15 ml.) and zinc dust (1 g.) was refluxed for 2 hrs. and filtered hot. The residue was extracted with hot acetic anhydride (10 ml.) and the combined filtrates concentrated to small bulk and poured into crushed ice under stirring. The precipitate (100 mg.) crystallised from benzene in pale yellow needles, m.p. 310-15° (dec.)

(Found: C, 79.7; H, 4.2. $C_{28}H_{18}O_4$ requires C,80.3; H, 4.3%).

Reduction of the quinone (II) to 3:4:9:10-dibenzpyrene (VIII). Aluminium turnings (1 g.) were refluxed with *cyclo*hexanol (20 ml.) after adding a few mg. of mercuric chloride as catalyst. The quinone (II) (0.5 g.) was added to the opalescent solution and mixture refluxed for 48 hrs. *Cyclo*hexanol was distilled off and the pasty mass stirred into a 5% aqueous solution of sodium hydroxide (200 ml.). The last traces of *cyclo*hexanol were removed by steam distillation, and the solution was then treated with sodium hydrosulphite (1 g.) to dissolve out any unconverted quinone. The mixture was filtered and the residual dark brown powder (0.7 g.) was dissolved in warm benzene (250 ml.) and chromatographed on alumina. The yellow fluorescent percolate was collected, concentrated to small bulk, and cooled. Pale yellow plates of the hydrocarbon (VIII) were obtained (0.32 g.). After recrystallisation from benzene-hexane the substance had m.p. 281° (Found: C, 94.9; H, 4.6. Calc. for $C_{24}H_{14}$: C, 95.4; H, 4.6%). The absorption spectrum of the compound was taken in n-heptane solution. (λ max. in mμ: 223, 234, 241, 271, 282, 294, 313, 329, 352, 370, 393, 409, 418, 432). The hydrocarbon dissolves in benzene to an orange-yellow solution with a blue fluorescence. The solution in conc. sulphuric acid is greenish blue, changing rapidly to blue and finally to violet; the solution first exhibits a blue fluorescence, which immediately changes to red, and disappears in a minute.

Reference 4 cites m.p. 281.5-282°. Identity with 3:4:9:10-dibenzpyrene was proved by the absorption spectrum, which was identical with that recorded by Clar[22].

Oxidation of the hydrocarbon (50 mg.) by refluxing with chromic acid (500 mg.) in water (10 ml.) gave a product which crystallised from o-dichlorobenzene in red needles, m.p. 373°, alone or mixed with the quinone (II). (Found: C, 86.6; H, 3.7. $C_{24}H_{12}O_2$ requires C, 86.2; H, 3.6%).

Oxidation of quinone (II)

The quinone (0.5 g.) was refluxed with glacial acetic acid (20 ml.), and a solution of chromic acid (2 g.) in glacial acetic acid (20 ml.) was added in five equal lots, each at the end of 8 hrs.

After refluxing for 40 hrs. the solution was diluted with water, and the yellow crystalline precipitate was collected, washed and dried (440 mg.). Crystallisation from chlorobenzene gave yellow needles of quinone (IX), m.p. 332° (Found: C, 78.3; H, 3.1. $C_{22}H_{10}O_4$ requires C, 78.1; H, 3.0%). Reference 4 cites m.p. 325° for pentaphene 5:14:8:13-diquinone (IX). The substance gives an orange vat which has no affinity for cotton. The solution in *o*-dichlorobenzene is yellow, and in conc. sulphuric acid lemon-yellow.

Reductive acetylation of quinone (IX):

The quinone (IX) (70 mg.), acetic anhydride (15 ml.) and zinc dust (1 g.) were refluxed for 2 hrs. and worked up as usual. The product crystallised from benzene-hexane in pale yellow needles, m.p. 295-300°(*dec.*). (Found: C, 71.2; H, 4.2; $C_{30}H_{22}O_8$ requires C, 70.6; H, 4.3%).

1:2-Diaza-3:4:9:10-dibenzpyrene-5:8-quinone (XI) from quinone (IX): A mixture of quinone (IX) (100 mg.), 85% hydrazine hydrate (1 ml.) and pyridine (10 ml.) was refluxed for one hour. The mixture was diluted with water, aerated, and the orange-yellow precipitate collected (100 mg.). Crystallisation from *o*-dichlorobenzene gave reddish orange needles, m.p. (*dec*.) above 360°. (Found: C, 79.0; H, 3.4; N, 8.7. Calc. for $C_{22}H_{10}O_2N_2$: C, 79.1; H, 3.0; N, 8.7. Scholl and Neumann[4] describe this compound as orange-red needles, *dec.ca*. 440°). The substance dyes cotton an orange-yellow shade from a cherry red vat.

Reduction of the azaquinone (XI) to 1:2-diaza-3:4:9:10-dibenzpyrene (XII): Refluxing overnight a mixture of the quinone (XI) (1 g.) and a solution of aluminium turnings (1 g.) in *cyclo*hexanol (20 ml.) and working up as usual, gave an unvattable product (0.8 g.); after chromatography on alumina and crystallisation from benzene the azahydrocarbon (XII) was obtained as lemon-yellow needles (0.6 g.), m.p. 304° (Found: C, 87.0; H, 4.2; N, 9.1. $C_{22}H_{12}N_2$ requires C, 86.8; H, 4.0; N, 9.2 %). The absorption spectrum of the azahydrocarbon was taken in alcohol. (λmax. in mμ: 238, 278, 290, 320, 335, 352, 432).

Reduction of quinone (IX) to pentaphene (X):

Quinone (IX) (0.2 g.) was refluxed with a solution of aluminium turnings (0.5 g.) in *cyclo*hexanol

(10 ml.) for 12 hrs. and the product worked up as usual. Chromatography on alumina and crystallisation from benzene-hexane gave pale yellow needles (0.15 g.), m.p. 262° (reference 16 cites m.p.257°, and reference 18 m.p. 255-57°). (Found: C, 94.8; H, 5.4. $C_{22}H_{14}$ requires C, 95.0; H, 5.0%). The absorption spectrum of the hydrocarbon was determined in heptane solution, and was identical with that of pentaphene[23]. (λ max. in mμ: 228, 246, 257, 288, 301, 314, 329, 346, 355, 378, 398). The substance dissolves in benzene to a very pale yellow solution with a blue fluorescence, and in conc. sulphuric acid to a pale red solution on warming.

Dehalogenation of Indanthrene Scarlet 4G:

The purified dye (5 g.) was dehalogenated, using Raney nickel (3 g.), and hydrogen at 40 lb. pressure as in the case of Mayvat Brilliant Red AF. The halogen-free quinone (3 g.) crystallised from o-dichlorobenzene in red needles, m.p. 373°, undepressed by quinone (II). (Found: C, 86.0; H, 3.6. $C_{24}H_{12}O_2$ requires C, 86.2; H, 3.6%). The quinone (0.3 g.), on reduction with aluminium cyclohexoxide (1 g. of aluminium turnings in 20 ml. cyclohexanol), gave the hydrocarbon (160 mg.), pale yellow needles or plates from benzene-hexane mixture, m.p. 281°, undepressed by 3:4:9:10-dibenzpyrene prepared from Mayvat Brilliant Red AF. (Found: C, 95.1; H, 4.5. $C_{24}H_{14}$ requires C, 95.4; H, 4.6%).

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PART VI

THE CHROMATOGRAPHIC SEPARATION OF ALIPHATIC ACIDS AS THE 2-ANTHRAQUINONYLAMIDES.

The isolation and characterisation of mixtures of acids obtained by oxidative or hydrolytic degradation of natural products are of interest to this laboratory. The complete separation of short chain fatty acids by adsorption chromatography is generally difficult, especially when the amounts available are relatively small; however, in the field of paper partition chromatography[1,2] and gas-liquid chromatography[3] elegant methods are available even with micro quantities. Several authors have published detailed reviews on the chromatography of fatty acids and related compounds[4-10].

A summary of separations of fatty acids using various chromatographic techniques is given by Lederer[11].

Separations of all the straight chain fatty acids from C_1 - C_{20} were reported by Holman and Hagdahl[12], using a mixture of one part Darco G-60 and two parts of Hyflo supercel as adsorbent. They were able to obtain quantitative recoveries by employing the technique of carrier displacement[13].

Several separations of aliphatic acids have been reported by methods of liquid-liquid partition chromatography on columns of silica gel[14,15], gas-liquid partition on columns of size-graded Celite 545[16] and by reversed phase partition on rubber columns[17] saturated with benzene and eluting with aqueous methanol. Asselineau[18] has reviewed the paper chromatography of fatty acids and their derivatives. Fatty acids from C_1 - C_9 were separated by Brown and co-workers[19] on paper using alcohols containing ammonia.

Several colourless derivatives of fatty acids have been used for separations. Methyl[20] and ethyl[21] esters of stearic, oleic, linoleic and linolenic acids have been fractionated on alumina and silicic acids[22]. Fluorescent p-phenylphenacyl esters of straight chain fatty acids from C_2 - C_6 were separated on silicic acid by Kirchner, et.al.[23] .

Chromatography of coloured compounds has the obvious advantage that the development of the chromatogram can be observed visually. p-Phenylazophenacyl esters of aliphatic fatty acids were prepared by Masuyama[24] and Sugiyama[25] and the chromatographic separation of the bright orange coloured esters on silicic acid is described by Ikeda, *et.al.*[26]. Good separations between adjacent members were observed early in the series, but higher up a four-carbon difference was necessary for good separations. The derivatives passed down the column in the reverse order of molecular weights with benzene and petroleum ether as solvents. Their attempts to separate the derivatives on alumina were unsuccessful. Rao[27] observed that coloured 1-acylamidoanthraquinones separate on a column of alumina from toluene solution. Good separations were obtained between acetic, propionic, *n*-butyric and *n*-valeric acid amides; the adsorptive power decreased with increasing molecular weight.

In the present work we hoped to take advantage of the ease of formation and colour of the 2-acylamidoanthraquinones of fatty acids for their chromatographic separations. By the treatment of the sodium salts of the aliphatic acids with phosphorous oxychloride, followed by 2-aminoanthraquinone, the amides were obtained in 90-95% yield. The amides of the first ten members of the series were thus prepared, chromatographed and analysed. Excellent separation on alumina between the earlier members of the aliphatic series (up to n-valeric) was observed, the higher acid amide being less strongly held and therefore eluted first. Separation between valeryl- and caproylamidoanthraquinone was only partial, and further up the series there was no separation between two successive members. 2-n-Butyramidoanthraquinone was eluted first from its mixture with 2-isobutyramidoanthraquinone, but the isomeric valeryl- and caproylamidoanthraquinones could not be separated on alumina.

For the separation of the acylamidoanthraquinones, a solution of the crude reaction product in benzene (see experimental) was added on to a column of alumina and the chromatogram developed with benzene. The orange coloured unreacted amine was separated, the yellow bands isolated and their identity confirmed by mixed melting point with an authentic sample of the acylamidoanthraquinone. The strength of adsorption of the acylamidoanthraquinones decreased with increasing molecular weight; 2-aminoanthraquinone was less less strongly held on alumina than the acyl derivatives.

The possibility of using the deeper coloured 2-*N*-methylaminoanthraquinone or 3-aminobenzanthrone instead of 2-aminoanthraquinone for the above separations was examined with the acetyl and propionyl derivatives, but they offered no advantage.

The separation of mixtures of fatty acids as acyl derivatives of 2-aminoanthraquinone is a satisfactory method with acids from C_2 to C_5 and was employed in this laboratory for the identification of aliphatic acids obtained as a result of degradative experiments on natural colouring matters. Thus in the work on the constitution of artocarpin, a new flavone colouring matter isolated from the heartwood of *Artocarpus integrifolia*, the alkali fusion of the dimethyl ether of artocarpin yielded acetic acid and isovaleric acid; they were identified as their 2-anthraquinonoylamides[28] after chromatographic analysis on alumina. Alkali fusion of the cyclohexanol-Raney nickel reduction product of morellin, the main colouring matter of *Garcinia morella*, gave a steam-volatile fatty acid. The

2-aminoanthraquinone and after chromatographic isolation was identified as *n*-valerylamidoanthraquinone by melting point and mixed melting point with an authentic sample[29]. Propionic acid was likewise identified as one of the products of oxidation of morellin with alkaline potassium permanganate.

EXPERIMENTAL

General method for the preparation of the 2-acylamidoanthraquinones.

The dry sodium salt of the aliphatic acid (1 g.) and phosphorous oxychloride (40% excess) were kept at room temperature for 12 hours. Toluene (250 ml.) and 2-aminoanthraquinone (1 g.) were added and the solution refluxed for 3 hours, during which time the colour of the solution changed to pale brown. The black insoluble residue was filtered off, the filtrate concentrated and the condensation product collected. After washing with dilute sodium carbonate solution to remove the acidic fractions, the product was dissolved in toluene and chromatographed on alumina. The lemon yellow band of the amide was eluted out and crystallised in lemon yellow needles.

Following the above general method several acylamidoanthraquinones were prepared, chromatographed and analysed. Table I lists the m.p. and elementary analysis of the various derivatives. Only the acetyl and propionyl derivatives of the amines have been reported before.

Table I

2-Acylamidoanthraquinones.

Acid	m.p. °C	Elementary analysis %	C	H	N
Acetic	264	Found:	72.3	4.5	5.2
		Calc.for $C_{16}H_{11}O_3N$:	72.4	4.2	5.3
Propionic	238	Found:	73.0	4.6	5.5
		Calc.for $C_{17}H_{13}O_3N$:	73.1	4.7	5.0
<u>n</u>-Butyric	226-7	Found:	73.9	5.2	4.7
		Calc.for $C_{18}H_{15}O_3N$:	73.7	5.0	4.8
Isobutyric	209	Found:	74.1	5.1	4.5
		Calc.for $C_{18}H_{15}O_3N$:	73.7	5.0	4.8
<u>n</u>-Valeric	210	Found:	73.9	5.3	4.3
		Calc.for $C_{19}H_{17}O_3N$:	74.3	5.5	4.6
Isovaleric	223-4	Found:	74.4	5.4	4.1
		Calc.for $C_{19}H_{17}O_3N$:	74.3	5.5	4.6
<u>n</u>-Caproi	203	Found:	75.5	5.5	4.9
		Calc.for $C_{20}H_{19}O_3N$:	74.9	5.9	4.4
Isocaproic	214	Found:	74.8	5.8	4.2
		Calc.for $C_{20}H_{19}O_3N$:	74.9	5.9	4.4
<u>n</u>-Octoic	198	Found:	75.6	6.7	3.9
		Calc.for $C_{22}H_{23}O_3N$:	75.6	6.6	4.0
<u>n</u>-Decoic	191	Found:	76.5	6.8	4.5
		Calc.for $C_{24}H_{27}O_3N$:	76.4	7.2	4.7

Table I
(continued)

2-Acylamide-N-methylanthraquinones

Acid	m.p. °C	Elementary analysis %			
			C	H	N
Acetic	155	Found:	72.8	4.4	4.9
		Calc.for $C_{17}H_{13}O_3N$:	73.1	4.7	5.0
Propionic	165-6	Found:	73.5	4.7	4.7
		Calc.for $C_{18}H_{15}O_3N$	73.7	5.0	4.8

3-Acylamidobenzanthrones

Acid	m.p. °C		C	H	N
Acetic	277		-	-	-
Propionic	269		-	-	-

General method for the separation of acylamidoanthraquinones.

The mixed sodium salts of the aliphatic acids (20 mg. or more) was dried and treated with an excess of phosphorous oxychloride (0.3 - 0.4 ml.) and kept at room temperature for 12 hours. Dry benzene (200 ml.) and 2-aminoanthraquinone (50 mg. or more) were added and after refluxing for 3 hours, it was filtered hot and washed with a dilute solution of sodium carbonate to remove the acidic components. The benzene solution was dried over anhydrous sodium sulphate and chromatographed on a column of alumina (1.5 x 20 cm.). The orange band which appeared first on development with benzene was unreacted 2-aminoanthraquinone. The yellow bands which appeared next were collected separately, the 2-acylamidoanthraquinones isolated and their identity confirmed by mixed melting point with authentic samples.

The separation between two successive members were excellent upto valeric acid amide; separation was only partial between valeric and caproic acid amide and further up the series there was no separation at all. The various separations are presented in Table II.

Table II

Table of separation of acylamidoanthraquinones.

	Propionyl	n-Butyryl	Isobutyryl	n-Valeryl	Isovaleryl	n-Caproyl	Isocaproyl	n-Octoyl	n-Decoyl
Acetyl	C.S.								
Propionyl		C.S.							
n-Butyryl			C.S.	C.S.					
Isobutyryl					C.S.				
n-Valeryl					N.S.	P.S.			
Isovaleryl							N.S.		
n-Caproyl							N.S.	N.S.	
Isocaproyl								N.S.	
n-Octoyl									N.S.

Note:

C.S: Complete separation

P.S: Partial separation

N.S: No separation

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SUMMARY

PART I. Direct Cotton Dyes: Chromatographic purification and crystallisation of Congo Red, Benzopurpurine 4B and Chrysophenine G.

The application of chromatography for the removal of inorganic electrolytes from direct cotton dyes has not been described so far, and a convenient method using a column of cellulose powder has now been developed. The dye is adsorbed from a hot aqueous solution, the inorganic salts are removed by percolation with water at 1-2°, and the pure dye is eluted with 80% acetone. The dye is recovered by azeotropic distillation or by distillation of solvents at reduced pressure. Chrysophenine G crystallises readily from methanol and Congo Red from 50% alcohol. Benzopurpurine 4B, which has apparently not been obtained crystalline so far, can be crystallised by azeotropic removal of water from a solution in a mixture of water and <u>n</u>-butanol. Crystalline Benzopurpurine 4B is very sparingly soluble in water, and this property can be used for obtaining the dye free from inorganic salts without recourse to chromatography.

Chrysophenine G and Congo Red can also be crystallised by the butanol-water technique. The crystalline dyes are hydrated; complete dehydration can be effected by heating at 140° in a current of nitrogen; the anhydrous dyes are extremely hygroscopic. The potassium salt of Congo Red, which crystallises readily from aqueous alcohol, does not contain water of crystallisation. Complete analytical data and photomicrographs of the crystalline dyes are presented.

A satisfactory method has been developed for the preparation of cellulose powder suitable for chromatography from bamboo pulp by treatment with dilute sulphuric acid, washing, drying and powdering to required mesh size.

PART II. Chromatography of vat dyes on alumina at high temperatures.

The chromatographic purification and separation of mixtures of vat dyes is difficult because of their relative insolubility in organic solvents that are suitable for chromatography. The possibility of carrying out chromatographic analysis at high temperatures was therefore investigated. A suitable apparatus for carrying out chromatographic adsorption analysis of vat dyes on alumina at temperatures between 30-200° was constructed. A number of vat dyes of known constitution were crystallised, their chromatographic homogenity determined and analysed. Several model separations were carried out in a quantitative manner and in most cases, over 95% recovery of the components of the mixture was obtained. Thus isodibenzanthrone separated readily from the isomeric dibenzanthrone; 16:17-dimethoxydibenzanthrone from the dihydroxy compound, the diamino derivative or the parent quinone; mononitrodibenzanthrone from monoaminodibenzanthrone or the monoamino compound from its monobenzamido derivative. 3:4:9:10-Dibenzopyrene-5:8-quinone and 1:4-*bis*benzamidoanthraquinone were less strongly held on alumina than isodibenzanthrone or dibenzanthrone. The acridone was separated from the carbazole and indanthrone. Very good separations were observed between flavanthrone and indanthrone or pyranthrone.

PART III. The constitution of nitrated dibenzanthrone.

The success of the separation of vat dyes at high temperatures led to the investigation of the chromatographic homogenity of nitrated dibenzanthrone (C.I.Vat Green 9; C.I.59850) in which the number and position of the nitro groups are still undetermined. The chromatographic separation of a commercial sample of nitrated dibenzanthrone (Caledon Black 2BM) free from water-soluble substances, on alumina at 110° yielded the following fractions: (a) tribromopyranthrone (4%), (b) 16:17-dimethoxydibenzanthrone (5%), (c) crystalline mononitrodibenzanthrone (50%) and (d) three amorphous fractions (20%); Cibanone Black BB likewise gave a 50% yield of crystalline mononitrodibenzanthrone. Oxidation of mononitrodibenzanthrone with chromic and sulphuric acid gave the hitherto unknown 1:2:7:8-diphthaloylphenanthraquinone; this was further oxidised to 2:2'-dianthraquinonyl-1:1'-dicarboxylic acid and decarboxylated to 2:2'-dianthraquinonyl. The same oxidation products were also obtained from dibenzanthrone and 16:17-dimethoxydibenzanthrone.

ODCB + 3% phenol

This indicated that the nitro group is in the 15 or 16 position, the latter being much more probable. Mononitrodibenzanthrone dyed cotton a green shade from a blue vat and changed to black on treatment with cold hypochlorite solution. Reduction of mononitrodibenzanthrone with methanolic sodium sulphide gave the amine in crystalline form. The <u>N</u>-benzoyl derivative of monoaminodibenzanthrone is a violet dye and is unaffected by hypochlorite oxidation.

The alkali fusion of 2-aminobenzanthrone gave 16:17-diaminodibenzanthrone which after purification by hot chromatography crystallised in green needles. It dyed a green shade from a blue vat and changed to bright brown on treatment with hypochlorite. This disproved the 16:17-orientation of the nitro groups proposed by Maki for nitrated dibenzanthrone. The hitherto unknown 7:8-diaminodibenzanthrone which dyed cotton an olive green shade changing to khaki on hypochlorite oxidation was synthesised by the alkali fusion of 5-aminobenzanthrone.

PART IV. The constitution of Cyananthrene O.

Cyananthrene O, which is prepared by the alkali fusion of 9:8-pyridinobenzanthrone is regarded as a dipyridino derivative of isoviolanthrone. A comparative study of the ultraviolet and visible absorption spectra of Cynanthrene O and an equimolar mixture of violanthrone and isoviolanthrone in sulphuric acid indicated that the dye is a mixture of dipyridinoviolanthrone and dipyridinoisoviolanthrone. A sample of the dye, which we obtained through the courtesy of Dr.Waibel of Farbenfabriken, Bayer, Germany, contained 14% of acetone soluble substances, 35% unvattable and 47% vattable fractions. The spectrum of the unvattable fraction had a closer resemblance to the isoviolanthrone curve than the vattable fraction. The chromatographic analysis of the vattable fraction on alumina at high temperatures using various developing solvents did not effect a separation; 35% of the dye was strongly held on the alumina and could not be eluted or extracted. Reduction of the vattable fraction of Cyananthrene O with aluminium *cyclo*hexoxide in *cyclo*hexanol was unsuccessful, but Clar reduction gave the azahydrocarbon as a red-brown powder. The chromatographic analysis of the amorphous Clar reduction product is in progress.

PART V. The constitution of Mayvat Brilliant Red AF.

Mayvat Brilliant Red AF, recently introduced by Otto B.May,Inc., Newark 5, N.J., which dyes cotton a brilliant scarlet-red shade from a blood-red vat, has been identified as a halogenated derivative of 3:4:9:10-dibenzopyrene-5:8-quinone, the latter being obtained by dehalogenation of Mayvat Brilliant Red AF or Indanthrene Scarlet 4G with Raney nickel, aqueous caustic soda and hydrogen. Reduction of the parent quinone with aluminium *cyclo*hexoxide gave a 70% yield of 3:4:9:10-dibenzopyrene, a potent carcinogen, which was isolated by Bonnett and Neukomm from tobacco smoke. Oxidation of the parent quinone with chromic acid in boiling acetic acid gave pentaphene-5:14:8:13-diquinone which was reduced to pentaphene in 90% yield using aluminium *cyclo*hexoxide. Interaction of pentaphene-diquinone with hydrazine hydrate gave

1:2-diaza-3:4:9:10-dibenzopyrene-5:8-quinone, reduction of which gave 1:2-diaza-3:4:9:10-diphenzopyrene. Mayvat Brilliant Red AF and Indanthrene Scarlet 4G dye remarkably different shades and exhibit different colour reactions in sulphuric acid.

PART VI. The chromatographic separation of aliphatic acids as the 2-anthraquinonylamides.

By the treatment of the sodium salts of aliphatic acids with phosphorous oxychloride followed by 2-aminoanthraquinone, the amides are obtained in 90-95% yield. The amides of the first ten members of the series were thus prepared, chromatographed and analysed. Excellent separations between the earlier members (up to *n*-valeric) were observed on alumina, the higher acid amide being less strongly held and therefore eluted first. Separation between *n*-valeric and *n*-caproic acid derivatives was only partial and higher up the series there was no separation between the adjacent members. Separation between the derivatives of isomeric acids was not satisfactory.

The possibility of using the deeper coloured 2-*N*-methylaminoanthraquinone or 3-aminobenzanthrone instead of 2-aminoanthraquinone for the above separation was tried with the acetyl and propionyl derivatives, but they offered no advantage.

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ACKNOWLEDGMENT.

I wish to express my sincere thanks to Prof.K.Venkataraman for suggesting the problem and for his inspiring guidance during the progress of the work.

My sincere thanks are due to Prof.B.D.Tilak, Professor of Dyestuff Technology, University of Bombay, and to Dr.B.S.Joshi, Senior Scientific Officer, National Chemical Laboratory, Poona, for their keen interest and helpful suggestions; to Dr.(Mrs.) Das Gupta for her help in the determination and interpretation of infrared spectra; and to all my friends and colleagues for their co-operation. I am grateful to Dr.T.S.Gore, Dr.G.D.Shah and Mr.V.S.Pansare for the micro analyses recorded in the thesis.

I am indebted to the Council of Scientific and Industrial Research for the award of a Fellowship, which made the present investigation possible.

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