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A Spectrophotometric Method for the Identification of Lake Pigment Dyestuffs

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A lake may be defined as a pigment made by the precipitation of an organic dyestuff onto an inert, insoluble, inorganic substrate. The term traditionally refers to a transparent colour, in which the substrate is usually aluminium hydroxide, alone or mixed with another substance, such as the rather less transparent calcium sulphate; sometimes calcium sulphate or a similar salt may be used alone. Since the nineteenth century, however, a wider range of substrates has been used. The dyestuffs used in the preparation of lake pigments are today almost entirely synthetic, but before the discovery of the coal tar dyestuffs in the mid-nineteenth century they would have been derived from animal and vegetable raw materials; it is with these that the present investigation has been concerned.

There is no shortage of methods for the identification of natural dyestuffs; IR and UV/visible spectrophotometry and chromatographic analysis, for example, have been used for the identification of both lake pigment dyestuffs and textile dyes (1,2). Certain difficulties arise, however, in the application of such methods to the tiny fragments of paint that are usually available for analysis. Such a fragment may have a surface area of perhaps 0.25mm^2, for example, and consists as a rule of several layers of paint including a preparatory layer or ground. The paint layer containing the lake dyestuff to be identified may be, perhaps, 15–20μm thick. The actual weight of dyestuff available for analysis, allowing for the presence of the inorganic substrate and the paint binding medium, may thus be estimated to be probably in the region of tens of nanograms, unless the sample is larger than average or the paint layer unusually thick. It is by no means impossible to use the aforementioned methods on a sample of this size, although rather more sophisticated apparatus than usual may be required, but it is necessary to break up the paint fragment and extract the dyestuff before any such methods are possible, with the consequent risk of loss of some of the dyestuff during the process. The presence of other organic materials, from the paint binding medium, in great excess, may give rise to problems during chemical analysis by, for example, thin layer chromatography. Even after all precautions have been taken, there may be insufficient dyestuff in the extract to enable a positive identification to be made.

As the majority of lake pigments found in samples taken from paintings are naturally transparent, it seemed possible that a spectrophotometric method of identifying the dyestuff present might be devised exploiting this property, whereby extraction of the dyestuff would be unnecessary. In order to provide a sufficiently transparent sample, and one in which the paint layer containing the lake pigment alone may be investigated, a very thin cross-section of the fragment is cut, using a microtome. The cross-section is then examined under a microscope equipped with a microscope photometer fitted with a variable wavelength filter, covering, in this instance, the visible region of the spectrum. The transmittance of light at any given wavelength is shown as a deflection on a galvanometer caused by current from the photomultiplier; a spectral transmittance curve of the dyestuff may thus be plotted. Apart from the advantage that an indication of the dyestuff present may be obtained without destroying the paint structure, it is possible to prepare a number of cross-sections from a minute fragment, perhaps barely visible to the naked eye, and thus have suitable material available for tests on other paint constituents. There is also a great deal to be said for performing the analysis on the lake pigment in the position in which it is found on the painting, as it is possible to see precisely whereabouts the measurement has been made; the advantages of this are obvious in cases where more than one layer of lake pigment or a mixture of dyestuffs is present.

In order to be able to identify the dyestuffs, it is necessary to have a series of transmittance curves obtained under the same conditions from known dyestuffs for comparison with the unknown dyestuffs. To provide suitable standards, a number of lake pigments were prepared from natural dyestuffs. It is, however, necessary to take certain factors into account when comparing freshly prepared pigments with aged paint. These will be discussed in their appropriate place.

The dyestuffs

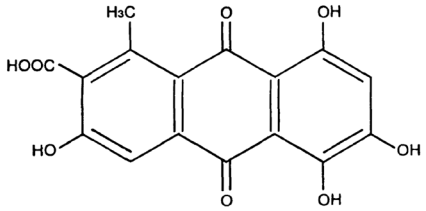
The lake pigments most commonly found in panel painting are those prepared from red and yellow dyestuffs. The investigation has concentrated up till now on the more frequently encountered red dyestuffs. It is not proposed to give a detailed account of the chemical nature of the dyestuffs, which is reasonably well documented (3–9); historical aspects of the use of the dyestuffs, particularly with reference to their use as textile dyes, have also been discussed at length (1,6,10–13). A brief account of the dyestuffs most likely to have been used in the preparation of lake pigments is, however, appropriate (Fig.2).

Some of the most beautiful and costly dyestuffs were extracted from scale insects, members of the family Coccidae, so named from the hard or waxy scale or other protective covering secreted by the insects. The dyestuffs they contain are chemically of the anthraquinone type and are extracted from the dried bodies of the wingless female insects, which, in their adult life, remain attached to a plant host. The best known are the kermes, cochineal and lac insects.

Kermes, *Kermes ilicis* L., is found on, for example, the

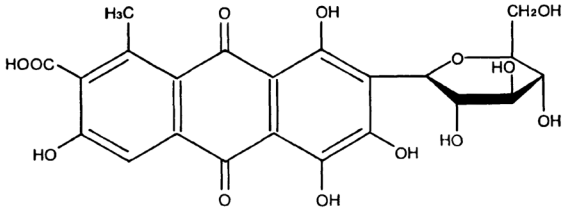
Kermes oak, *Quercus coccifera* L., in Southern France, Spain, the Near East and other areas around the Mediterranean Sea. The colouring matter consists principally of kermesic acid (1,5,6,9,14):

Kermesic acid, $C_{16}H_{10}O_8$



Cochineal, *Dactylopius coccus* Costa., is found on, for example, the nopal cactus, *Opuntia coccinellifera* Mill. Mexico is its country of origin. The colouring matter is carminic acid (1,5,6,9,14):

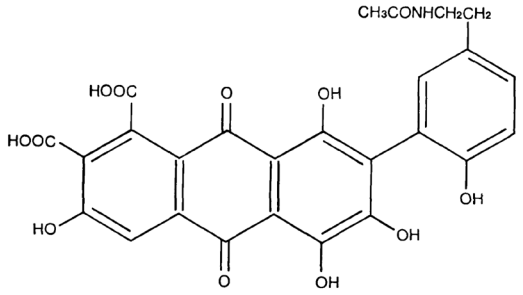
Carminic acid, $C_{22}H_{20}O_{13}$



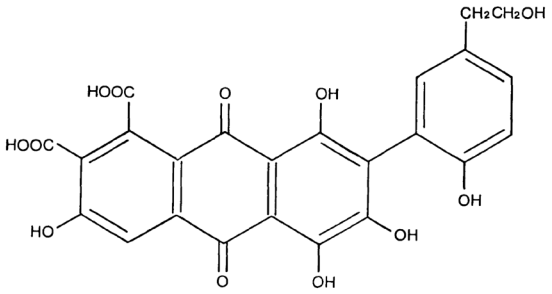
Unlike the other scale insects mentioned, which were available in Europe from quite early times, the use of cochineal would not be expected in Europe before the discovery of the New World. It was imported, by the Spaniards, from the early sixteenth century onwards, gradually supplanting kermes. There is, however, no reason to suppose that there were no Old World sources of carminic acid. Other local scale insects are known to have been used for textile dyeing in various parts of Europe and there may be more that are unknown to us today. Perhaps the most familiar of these lesser known insects is the Polish cochineal insect, *Porphyrophora polonica* L., or S. John's Blood, found on the roots of *Scleranthus perennis* in sandy regions of Eastern Europe, including Poland. The colouring matter contains carminic acid; analysis by thin layer chromatography suggests that it also contains a certain amount of kermesic acid, although this matter is still in need of clarification (1). *Porphyrophora hamelii* Brandt., Armenian or Ararat cochineal, from Armenia, and *Acanthococcus uvae-ursi* L. and *Coccus fragariae*, both used in Russia, are other lesser known varieties (1,6,14). Analysis has suggested that several varieties of kermes were used for textile dyeing (1) and a number of varieties of 'grana' and 'cremesino', apparently distinct dyestuffs, are listed in the *Plictho* of Gioanventura Rosetti, a mid-sixteenth-century dyeing handbook (15). The range of *Kermes* species and other scale insects available for use is thus larger than has, perhaps, been generally realized.

The resin-like protective coating secreted by the lac insects, found in Southern India, Bengal and other parts of Asia, completely envelopes the insects to the extent that the twigs of the host tree (*Butea* spp., *Ficus* spp., and others) are encrusted with the material, which is also the source of shellac. The most important species is the Indian, *Kerria lacca*, found on *Schleichera oleosa*, *Zizyphus mauritania*, *Butea monosperma* and others. The encrustation is crushed and treated with water or dilute sodium carbonate to extract the dyestuff, which, at one time, could be purchased dried in the form of a cake. The most important constituents of the dyestuff are the water-soluble laccaic acids, the chemistry of which is complex; other, less important, water-insoluble constituents, such as erythrolaccin, remain in the residual crushed material. The most important of the laccaic acids appear to be A and B (6,9,16):

Laccaic acid A, $C_{26}H_{19}NO_{12}$



Laccaic acid B, $C_{24}H_{16}O_{12}$

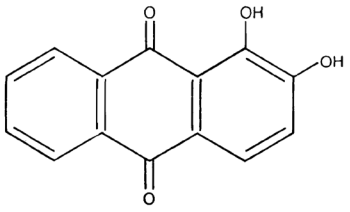


(The word 'lake' is derived from 'lacca', a word which, in mediaeval recipes at least, appears to have been used indiscriminately for 'lake pigment', 'lac' and similar materials, including a mysterious exudate obtained, it is said, from ivy; these and other examples of confused terminology have been discussed at length by other authors (12,13).)

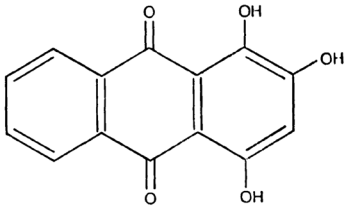
Of the red plant dyestuffs, madder and brazilwood have both been known in Europe from early times. Safflower, although an ancient dyestuff in the East, appears not to have been used for the preparation of a pigment in Europe before the nineteenth century (12). No other plant materials have the reputation of having been the sources of dyestuffs used in the preparation of red lake pigments for use in panel painting, though others may possibly occur in illuminated manuscripts (not necessarily in the form of true lakes).

Madder, *Rubia tinctorum* L., was cultivated for its dyestuff over a wide area of Europe, the Middle East and Asia; it may also be found growing wild. The dyestuff is extracted from the root. As many as nineteen anthraquinone pigments have been isolated from the mature root (17), of which by far the most important and best known are alizarin, the principal colouring matter, obtained synthetically today, and purpurin (1,5,6,9,18):

Alizarin, $C_{14}H_8O_4$



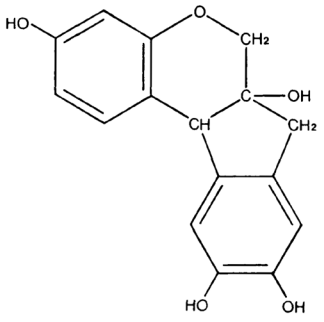
Purpurin, $C_{14}H_8O_5$



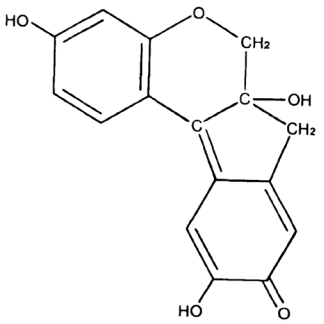
Alizarin is present in young plants as a glycoside, known as ruberythric acid, but a certain amount of the aglycone is also present in mature plants. From about the mid-nineteenth century, the ground root was often treated with dilute sulphuric acid to break up the glycoside molecule, thus releasing more alizarin (3); in earlier times this was not done. It is perfectly possible to obtain a madder lake containing little or no alizarin.

The name 'brazilwood' refers to a number of species of *Caesalpinia*, the so-called 'soluble' redwoods, a name derived from the fact that the colouring matter they contain is soluble in water. *Caesalpinia sappan* (Sappan wood, Lima wood), from parts of Eastern Asia, was used in Europe long before the discovery of America, when other species, for example *C. crista* and *C. braziliensis* became available. The colouring principle, as it is found in the wood, is brazilin, which must be oxidized, for example, by the air when the wood is rasped to shavings, to the actual colouring matter, brazilein (4,6–8,19,23).

Brazilin, $C_{16}H_{14}O_5$

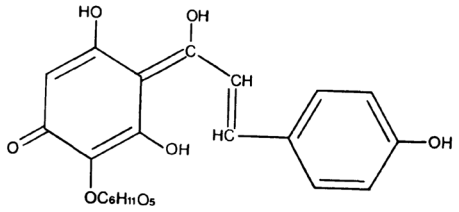


Brazilein, $C_{16}H_{12}O_5$



Safflower, *Carthamus tinctorius* L., a native of Southern Asia, was cultivated in Asia, the Near East and Southern Europe. The dried flowers are the source of two dyestuffs: a yellow dyestuff, very rarely used, and the alkali-soluble red dyestuff, carthamic acid or carthamin (4,6–8,23).

Carthamin, $C_{21}H_{22}O_{11}.H_2O$
(Mayer(7).)



In Rodd(23) the empirical formula $C_{21}H_{20}O_{10}.2H_2O$ is quoted.

Preparation of the lake pigments

Recipes for lake pigments present a most interesting study in themselves, to which, it is hoped, more attention will be devoted at a later date. For the preparation of the 'standard' lake pigments, recipes dating from *c.* 1400–1830 or thereabouts were consulted. The majority of paintings examined in the National Gallery Scientific Department fall within this range of dates. The range of recipes that could be tried was limited by the small size of the samples of raw materials available, some of which are extremely difficult to obtain today; in these cases at least it was deemed prudent to restrict experimentation to a minimum. Quite frequently, the information given in the recipes is scanty, with little or no practical detail; this may, in some instances, be attributable to the fact that the procedure was so well known that it was considered unnecessary to write it down. Examples of the sources consulted are given in the references (3,20). In earlier times, the making of lake pigments was closely connected with the textile dyeing industry, particularly, it would seem, in the case of the scale insects already discussed. It appears from the recipes that, before the seventeenth century, lakes were not made from scale insects directly (with the exception of lac lakes) but from clippings or shearings of dyed cloth, from which the dyestuff was extracted with alkali, 'ley' or 'lee', made from wood ash (in which case it would consist largely of potassium carbonate), or lime (12). In the case of kermes, Polish cochineal and some cochineal lakes, therefore, a standard procedure was adopted, based on the recipes, whereby the dyestuff was extracted from the crushed insects with distilled water, the necessary amount of potassium carbonate was added and the lake precipitated on a substrate of aluminium hydroxide by the addition of alum. (Essentially, many recipes for lakes of all types are based on this method, possibly adding the alum first, and there may be other additives.)

A curious point is the almost complete absence of recipes referring to madder before the early seventeenth century, which has been discussed by several authors (12,13). Among several possible explanations for this is the possibility that the colouring matter was also

extracted from dyed cloth. It is worth noting that, throughout the ages, it was realized that madder required careful treatment to obtain a good colour when dyeing cloth or preparing a pigment.

Certain points arising from the preparation of the standard lakes must be borne in mind when comparing them with aged pigments. It is impossible to reproduce exactly the conditions under which a lake pigment may have been made four or five hundred years ago. The presence of minor impurities in the water or chemicals used then, for example, may have affected the colour obtained. Differences in the lakes obtained then and in the laboratory today, if any, may also be due to the dyestuff materials themselves; it was unavoidable that some of the standards had to be prepared using dyestuff extracted from quite old materials, which may have deteriorated to some extent, whereas the material available to earlier pigment makers would probably have been relatively recently collected. Even such factors as the method of killing the scale insects may have some importance.

Preparation of paint cross-sections

In order to prepare paint cross-sections from the laboratory-prepared lakes, they were ground with linseed oil containing a lead drier and painted out onto a small gessoed hardboard panel (illustrated in Plate 5a). After about six months the paint was sufficiently hard for tiny fragments of paint to be removed with a scalpel, from which cross-sections were prepared after the paint fragments had been embedded in a suitable synthetic embedding resin.

Durcupan ACM Fluka Araldite base resin was used for embedding paint samples, both those of the standard lakes and those taken from paintings. It has been found that, if paint cross-sections up to 10μm thick are to be cut on a microtome, better results are obtained if the fragments are first impregnated with unpolymerized resin, even though impregnation is at best limited and uneven. The impregnation and embedding schedule followed was based on that suggested for biological material in the leaflet supplied with the resin, which has four components made up into two different mixtures with the following compositions:

Durcupan 1:
10ml epoxy resin; 10ml 964 hardener;
0.1–0.2ml plasticizer.

Durcupan 2:
10ml epoxy resin; 10ml 964 hardener;
0.3–0.4ml 964 accelerator;
0.1–0.2ml plasticizer.

In general the minimum amount of plasticizer was used for samples of hard aged paint; for the softer, more flexible paint of the standard lakes, more plasticizer was included in the mixture. Samples were impregnated in the following solutions:

(i) 3 parts dry toluene: 1 Durcupan 1:
2–4 hours at room temperature;

(ii) 2 parts dry toluene: 2 Durcupan 1:
2–6 hours at room temperature;

(iii) 1 part dry toluene: 3 Durcupan 1:
usually left overnight at room temperature;

(iv) Durcupan 1: 12 hours at 50°C;

(v) Durcupan 1: 12 hours at 50°C;

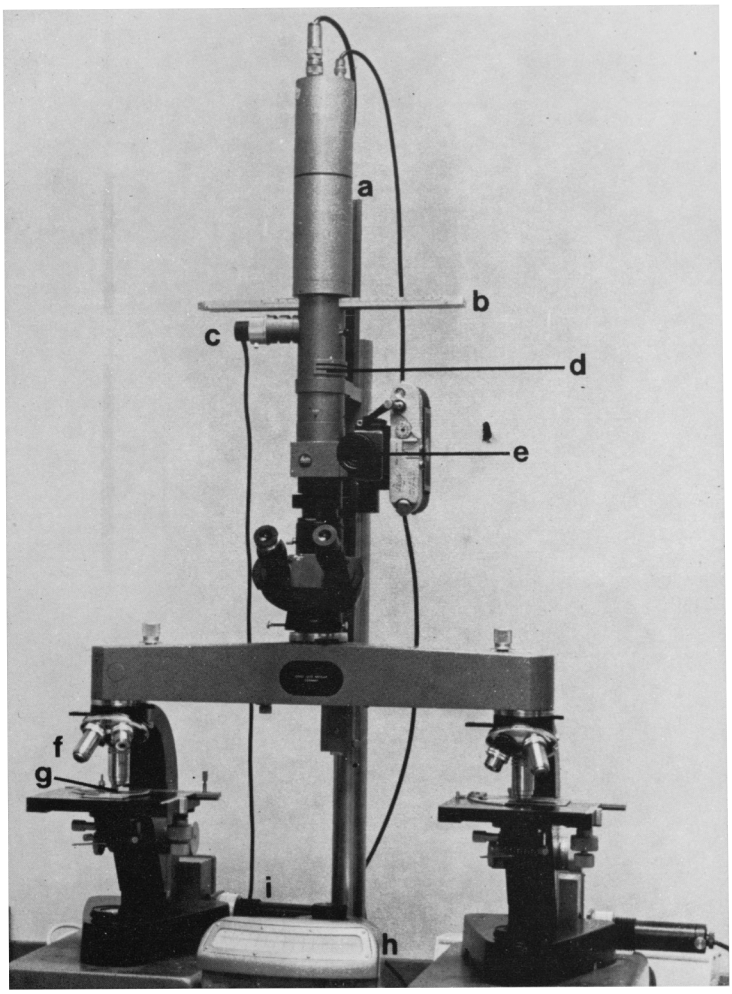
(vi) Durcupan 2: 2 hours at 50°C.

During stage (vi), suitable moulds were half filled with Durcupan 2 mixture, which was allowed to set at 60°C until the surface was just firm enough to take the weight of the paint samples. After the samples had been placed in position the moulds were filled and the resin left to harden at 60°C for at least two days. Using this method, the resultant blocks show a faint 'seam' where the two layers of resin meet, but this has never been found to be a source of weakness or in any way inconvenient. Sections were cut on an LKB Pyramitome glass knife microtome using 35° glass knives; usually sections of a range of thicknesses, between 1–10μm, were cut of each sample. It is important that the area of the face of the sample block should be small, but sufficient resin must be present around the sample in order to provide support for the cross-sections. Damaged cross-sections are hard to avoid as hard pigment particles damage the knife edge, causing scratches, and large particles may be knocked out altogether; lean friable paint is particularly vulnerable. However, when the cross-sections are examined, it is usually possible to find an area of paint suitable for measurement. The cross-sections were removed from the knife edge as they were cut, using a brush or tweezers, and were each placed on a minute drop of water on a clean glass microscope slide, which was then warmed to enable the sections to adhere to the slide. Finally, they were covered with cover glasses, generally using the same or another embedding medium (such as a polyester resin) as a mountant (21).

Plotting the transmittance curves

The transmittance curves were plotted using a microscope photometer mounted on a Leitz comparison microscope, illustrated in Fig.1. Only one of the two microscopes was used in this work, thus keeping the light source constant.

The light path first passes through the cross-section placed on the microscope stage and the microscope objective so that the sample can be viewed in the normal way through the binocular eyepiece. The position of the sample can be altered as necessary by means of the rotating stage of the microscope. If a prism is removed from the light path, the light is no longer diverted into the binocular eyepiece, but continues upwards to be brought to a focus at an adjustable rectangular aperture, by means of which that part of the sample whose transmittance is to be measured can be selected. By careful adjustment of the aperture it is possible to obtain measurements from thin paint layers or even individual particles or 'blobs' of lake pigment. In order to select and record the area measured, a second prism placed in the light path diverts the image of the sample, with rectangular aperture superimposed, to a camera, into the eyepiece of which is inserted a micrometer showing crossed scales (Fig.2). Above the rectangular aperture, the light beam converges onto a variable interference



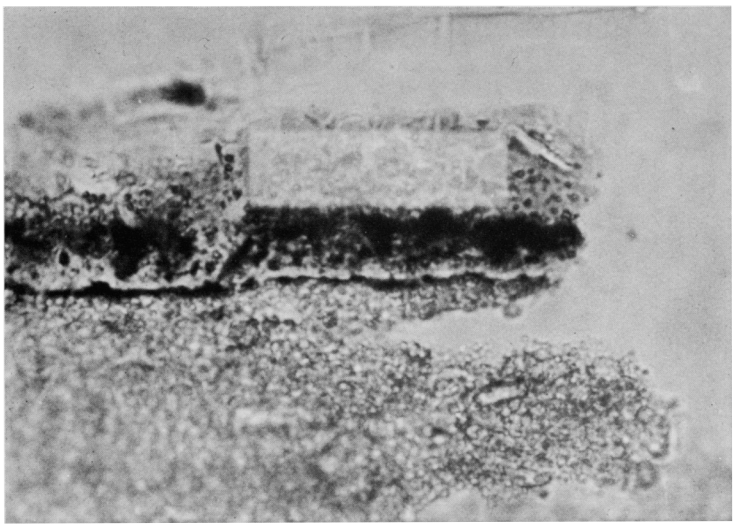


Figure 1 (*Top*). The apparatus used to plot the transmittance curves; only one of the two microscopes was necessary for the experiment. **(a)** photomultiplier; **(b)** variable interference filter; **(c)** light source for rectangular aperture; **(d)** rectangular aperture; **(e)** camera eyepiece containing micrometer scale; **(f)** selection of objectives on revolving nosepiece of microscope; **(g)** sample on rotating microscope stage; **(h)** galvanometer scale; **(i)** light source.

Figure 2. The rectangular aperture is seen here as a rectangle of light superimposed on the layer of the paint cross-section containing a red lake pigment. The cross-section was viewed, and photographed, at a magnification of 250×. Light reaching the photomultiplier has thus been transmitted through an area of paint measuring, in this case, about 15 × 52μm². The cross-section illustrated, which is 10μm thick, was cut from a sample of red paint taken from the cloak of a figure in *The Mass of S. Hubert* (N.G.253), a work from the Studio of the Master of the Life of the Virgin.

filter which can be moved laterally so that light of the wavelength selected emerges from it to reach the photomultiplier at the top of the instrument. Thus, after the light beam has passed through the sample, it is cut down by the rectangular aperture to that part passing through the area whose colour is to be measured. The interference filter then removes all light apart from that of the selected wavelength which finally reaches the photomultiplier, the current from which causes a deflection on the galvanometer.

The reading thus obtained is proportional to the transmission of the lake pigment in the cross-section together with that of the glass of the slide, the coverslip and the mounting resin. To obtain a relative transmittance of the sample alone, a reading was taken at each wavelength through an area free of pigment; this was taken as the reference for 100 per cent relative transmittance. A correction was made for the dark current from the photomultiplier.

Two or three readings were taken at 10nm intervals over the visible region of the spectrum, 400–700nm. With very small samples it was sometimes found impossible to take readings after about 650nm as too great a voltage was required to operate the photomultiplier.

It is important to choose a sample wherein the colour of the lake is not too saturated or too pale, that is, with transmittance in the middle of the range, since the characteristic features of the curves are obscured at transmittances which are too high or too low.

Results

Typical examples of transmittance curves obtained from the standard lake pigments may be seen in Fig. 3, showing curves plotted for kermes, cochineal, lac, madder and brazilwood lakes. Slight variations are seen in the curves obtained from the lake pigments made from any one dyestuff, but such curves almost always show certain invariable features. It is thus in general possible to distinguish between the closely related insect-derived dyestuffs as a group, madder, and brazilwood. Distinction between the various insect dyestuffs is less easy as their transmittance curves are very similar, whatever the method of plotting. It must be remembered that the paint medium has its own characteristic transmittance and absorbance features in the visible region of the spectrum, but in general the presence of the paint medium need not be taken into consideration unless it is very discoloured. It may, however, be necessary to make allowances for yellowed or browned paint medium if an appreciable amount is present in the area of lake pigment to be examined. Curves can be plotted of, for example, a standard browned oil film; better still, it may be possible to plot a transmittance curve of an area of discoloured paint medium within the paint sample itself. The curve for the discoloured material is then subtracted from the experimental curve to obtain a curve for the red pigment alone. This may be most accurately done by converting both sets of readings to optical densities, using the formula:

$$E = \log\ (100\ /\ (\text{transmittances})\ \text{per cent})$$
$$\text{where } E = \text{optical density} \quad (22)$$

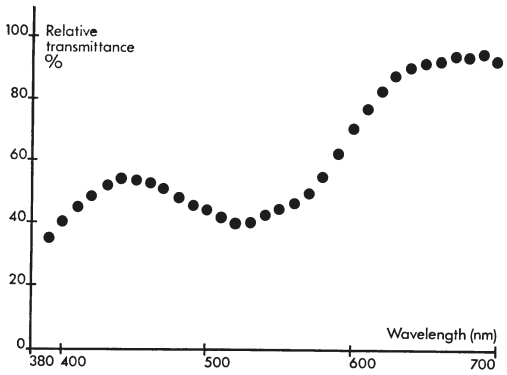
The optical densities may then be added and subtracted as desired and the resultant optical density may then be reconverted to percentage transmittance. This method may also be used if a mixture of dyestuffs is thought to be present.

It has already been mentioned that it is necessary to take certain factors into account when comparing the freshly prepared standards with aged paint. Some of these, connected with the actual preparation, have already been discussed. The aged pigments may have changed colour or faded as a result of the effects of light or chemical action: all the natural dyestuffs discussed are more or less fugitive, alizarin being the most permanent. It is, however, often possible to take a sample from an area of the picture that has been protected from light, for example, from the edge of the picture which has been protected by the frame. Even in cases where change is apparent on the surface of the sample, it is frequently found that, when the cross-section prepared from the sample is examined, the alteration is found to be confined to the upper part of the paint layer, the lower remaining unchanged. Even in the most deteriorated sample, it is usually possible to find a particle of unaltered pigment sufficiently large to be examined. The effects of several hundred years of aging and exposure to light can be simulated to some extent by carrying out, for example, fading tests on the standard pigments.

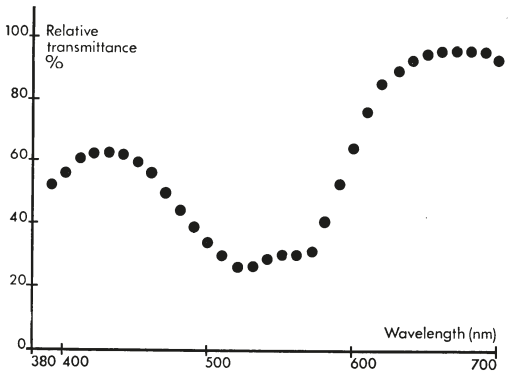
Relatively few samples of lake pigments from paintings have been investigated so far as the study is still in its infancy, but the results already obtained have been most encouraging. The experimental curves have been found to resemble the standard curves sufficiently closely to be able to indicate that the dyestuff present is brazilwood, madder, or one of the scale insect dyestuffs. Distinction between the various insect dyestuffs is less easy at present. Examples of the curves obtained are shown in Fig. 3; these may be compared with the curves from the known dyestuffs. The results obtained are shown in the table.

The method can give a good indication of the dyestuff present; for more positive identification a more thorough analysis, using, for example, chromatographic methods, is necessary. It is of great value to be able to say, from information gained from a sample perhaps too small for other methods of analysis to be convenient or, indeed, possible, that the lake pigment in a particular paint layer contains a particular dyestuff or type of dyestuff; even this much is rather more than was known before. There has been a tendency to assume that all lake pigments present in paintings must have been made using dyestuff extracted from madder. It is clear, even from the few results obtained so far, that this is far from being the case.

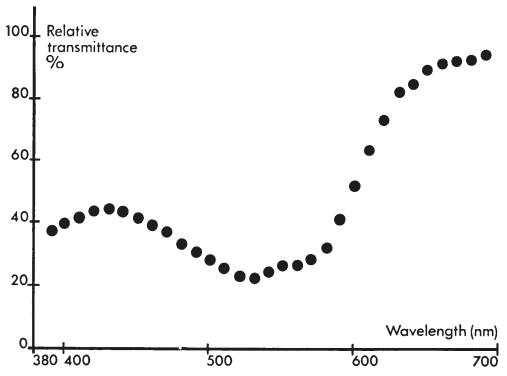
The preparation of thin cross-sections of the samples has enabled unexpected features of the paint to become apparent, in some cases. Until thin sections were cut of the samples from No.221 Rembrandt *Self portrait* and No.6432 Rembrandt *Hendrickje Stoffels*, the presence of a mixture of red and yellow dyestuffs in the layer of lake pigment, giving a resultant colour very close to that of a madder lake, had not been suspected. Careful inspection of the lake pigment in a thin cross-section, using a



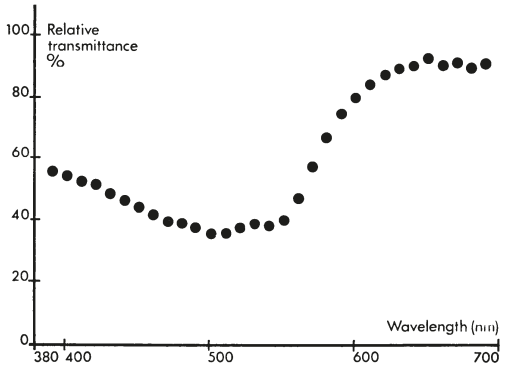
(a) Kermes Lake 2



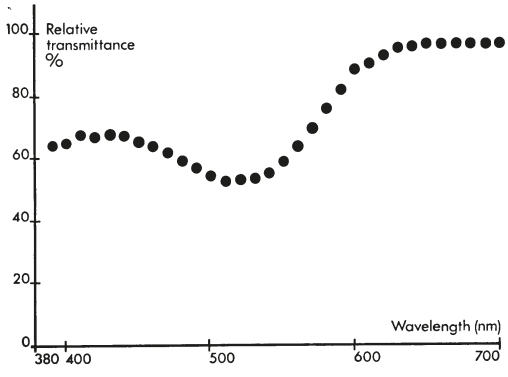
(b) Cochineal Lake 4a



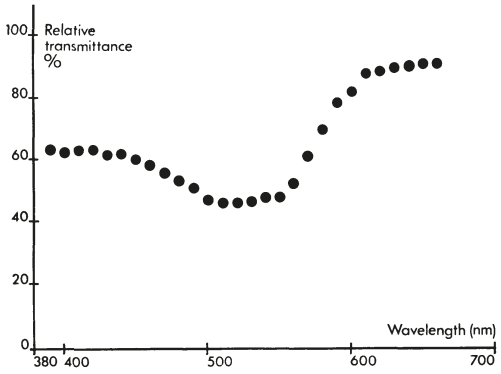
(c) Lac Lake 3a



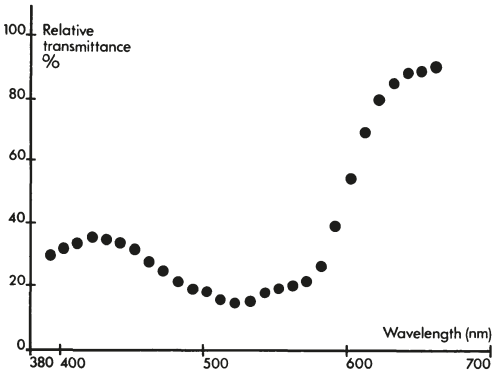
(d) Madder Lake 1



(e) Brazilwood Lake 1c



(f) 253 The Master of the Life of the Virgin



(g) 4757 Sassetta

Figure 3. Examples of spectral transmittance curves obtained. Relative transmittance % (ordinate) plotted against wavelength in nanometres (abscissa).

(a) Kermes lake 2: Lake prepared from dyestuff obtained from *Kermes ilicis* L., part of the material used by Professor O. Dimroth; sample obtained from the Courtauld Institute. (Standard method).

(b) Cochineal lake 4a: Dyestuff obtained from *Dactylopius coccus* Costa, Silver grain insects, supplied by BDH Ltd. (Standard method).

(c) Lac lake 3a: Dyestuff obtained from *Kerria lacca*, broken from sticks of *Butea frondosa*; sample obtained from Royal Botanic Gardens, Kew, (Method: Bolognese Ms., Paragraph 129, in Merrifield Vol. 2, p. 446, Dover edition, 1967.)

(d) Madder lake 1: Dyestuff obtained from inner portion of root of *Rubia tinctorum* L., from Jollunder in the Punjab; sample obtained from Royal Botanic Gardens, Kew. (Method: Sir Henry Englefield's method, described in Tingry, 3rd. edition, 1830, p. 120).

(e) Brazilwood lake 1c: Dyestuff obtained from *Caesalpinia echinata* Lam.; sample obtained from Royal Botanic Gardens, Kew. (Method: Tingry, 3rd edition, 1830, p. 121.)

(f) The Master of the Life of the Virgin, Studio. *The Mass of S. Hubert*, Sample 19. Red glaze from shadow of priest's robe.

(g) 4757 Sassetta. *The Whim of the young S. Francis to become a soldier*. Sample 2. Dark red of robe from S. Francis's left sleeve, where lake still in good state of preservation.

Wavelength (nm)	**Relative transmittance % (average)**						
	(a) Kermes Lake 2	**(b)** Cochineal Lake 4a	**(c)** Lac Lake 3a	**(d)** Madder Lake 1	**(e)** Brazilwood Lake 1c	**(f)** 253 The Master of the Life of the Virgin	**(g)** 4757 Sassetta
390	34·6	52·1	38·1	55·5	64·9	64·1	30·5
400	39·4	56·3	40·4	54·8	65·9	63·7	32·5
410	44·1	60·7	42·4	53·2	68·1	64·7	34·2
420	48·3	62·3	44·2	52·3	68·0	64·9	36·4
430	51·9	62·4	45·1	50·0	68·8	63·6	36·0
440	53·9	62·1	44·7	47·9	68·7	63·6	35·6
450	53·8	60·2	43·3	46·2	67·3	62·5	33·8
460	53·3	57·3	41·3	44·3	66·7	61·2	30·2
470	52·3	51·5	39·5	42·5	64·8	58·9	27·5
480	50·0	46·2	36·0	41·6	62·5	56·7	24·1
490	48·0	40·9	34·1	40·5	60·0	54·2	22·4
500	47·0	36·6	32·1	38·8	57·9	50·5	21·5
510	45·0	32·3	30·5	38·8	56·5	49·3	19·2
520	44·0	28·8	28·2	40·4	56·7	49·7	18·4
530	44·2	28·5	28·2	42·0	57·1	50·0	19·0
540	46·4	31·7	29·9	41·5	59·0	50·8	21·9
550	48·4	33·1	31·6	43·1	62·5	51·4	23·2
560	50·2	33·6	32·1	50·2	67·3	55·8	24·2
570	53·4	34·3	33·4	60·5	72·8	64·5	25·7
580	59·1	43·6	37·5	70·4	79·3	72·7	30·4
590	66·5	56·0	46·4	78·1	85·3	81·4	43·3
600	74·9	68·0	57·1	83·7	91·9	84·8	58·6
610	80·9	79·4	68·6	88·2	93·6	90·8	73·6
620	86·7	88·5	78·2	91·6	96·2	91·7	83·9
630	91·4	92·5	87·5	93·5	98·6	92·6	89·3
640	94·3	96·2	90·0	94·4	99·3	93·2	92·5
650	96·3	98·1	94·8	96·6	99·9	93·8	93·0
660	97·0	99·4	96·5	95·3	100·0	94·0	94·0
670	98·5	99·8	97·7	96·0	100·0		
680	98·5	99·8	98·3	95·0	100·0		
690	99·0	99·5	99·5	96·0	100·0		
700	98·0	97·5			100·0		

TABLE OF RESULTS[1]

Gallery	School	Date	Artist	Title	Description of Sample	Type of Dyestuff
NG 4757	Sienese	1437–44	**Sassetta**	The whim of the young S. Francis to become a soldier	Sample 2: Red of Saint's left sleeve, where lake still in good state of preservation	Scale insect (Kermes?)[2]
NG 1128	Florentine	*c.* 1491	**Signorelli**	Altarpiece: The Circumcision	Sample 31: Green of priest's robe – crimson paint beneath green paint	Scale insect (Kermes?)[2,3]
NG 3943	Florentine	1st. decade 16th C.	**Raphael**	Altarpiece: The crucified Christ with the Virgin Mary, Saints and Angels	Sample 12: Deep crimson of S. John's robe – upper layer of bright bluish-red glaze	Scale insect (possibly of the kermes type)[2,4]
Venice, Accademia No. 404	Venetian	Mid 1560's.	**Tintoretto**	The Crucifixion	Sample 14: Crimson glaze on Virgin's dress	Scale insect (possibly of the kermes type)[2,4]
Venice, Scuola di San Rocco	Venetian	Mid 1570's	**Tintoretto**	The Brazen Serpent	Sample 13: Crimson drapery – (i) lower bright red layer	Scale insect (possibly of the kermes type)[2]
					(ii) upper purplish-red glaze	Scale insect (probably lac)
British Museum, Dept. of Mediaeval and Later Antiquities	Upper Rhenish	*c.* 1250	**Anon**	[Triptych; outer surface of wings painted with figures of saints]	Sample 11: Red shadow in fold of S. Martin's gown	Scale insect (lac?)[5]
National Portrait Gallery No. 362	[Flemish, working in England]	After *c.* 1585	Attributed to Marcus **Gheeraerts** the younger	William Cecil, 1st. Baron Burghley	Sample 3: Deeper red of robe, from bottom edge of picture where protected by frame	Scale insect (lac?)[6]
NG 6432	Dutch	*c.* 1659	**Rembrandt**	Hendrickje Stoffels	Sample 2: Red of drapery	Scale insect (probably cochineal)[7]
NG 221	Dutch	1669	**Rembrandt**	Self portrait aged 63	Sample 7: Red glaze on sleeve	Scale insect (probably cochineal)[7]
NG 253	German	*c* .1485–90	Studio of **The Master of The Life of the Virgin**	The Mass of S. Hubert	Sample 19: Red of robe of figure to the left of S. Hubert	Madder[8]
NG 4681	Early Netherlandish	*c.* 1500	**The Master of S. Giles**	The Mass of S. Giles	Sample 9: Red of carpet, from lower right hand corner	Probably madder[4]

Notes to the Table of Results

1. It was hoped that it would be possible to confirm the results by thin layer chromatography; unfortunately this was not always possible as the samples available were often too small for any result to be obtained.

2. It is felt that it is necessary to obtain further examples of curves from lakes where the dyestuff is known to be kermes before any serious attempt can be made at a positive identification of the dyestuff. In at least one of the National Gallery samples of the insect (and both samples were many years old), the dyestuff is known to have deteriorated. It seems essential that samples of fresh insects should be obtained if at all possible.

3. An interesting point is that the priest's robe, originally red, was repainted green by the artist after a very short space of time indeed, thus, unusually, the red paint will have had very little exposure to the effects of light and atmosphere. This may have some bearing on the fact that the curve showed an unusually steep rise in percentage relative transmittance from 400–430 nm.

4. Suggested by TLC.

5. Particles of red lake mixed with a great deal of translucent brownish material.

6. In spite of the fact that the sample was taken from an area protected by the frame, the lake was beginning to change colour.

7. The red dyestuff was mixed with an unknown yellow dyestuff.

8. Confirmed by TLC.

microscope with facilities such that both transmitted and reflected light may be used, enables a good idea of the type of dyestuff that may be present to be obtained; the information that may be gained from such an apparently simple inspection should not be overlooked. It is also interesting to note that, in some samples examined, such as those taken from No.3943 Raphael *Altarpiece: The Crucified Christ with the Virgin Mary, Saints and Angels* and the Tintoretto *Crucifixion*, in the Accademia Gallery in Venice, the colour of the lake pigments was extraordinarily intense, even at a thickness of 2μm.

When a sufficiently large range of samples from paintings has been investigated, both by this method and by, for example, thin layer chromatography, sufficient experience should have been gained to enable a more positive identification of the insect dyestuffs to be made in most cases. It is undoubtedly necessary to obtain standards for comparison from samples taken from paintings, as well as the prepared standards, to enable this to be possible, using another method, such as chromatographic analysis, to identify the dyestuffs present.

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11. Several short but interesting articles on historical aspects of dyeing practice in different parts of Europe have appeared in Ciba Review over the years; these include 'Technical peculiarities of Flemish cloth-making and dyeing.' by A. L. Gutman, in Ciba Review No.14 1938, pp.484–487; 'Medieval dyeing technique', by M. C. Neuberger, in Ciba Review No.10, 1938, pp. 337–340; 'The Florentine textile industry of the Middle Ages', by W. Reininger, in Ciba Review No.27, 1939, pp.957–966.

12. R. D. Harley. 'Artists' pigments *c.*1600–1835: a study in English documentary sources.' Butterworths, 1970.

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16. The periodical literature on the chemistry of the pigments of stick lac is extensive; the following papers are some particularly relevant to laccaic acids A and B: (a) R. Burwood, G. Read, K. Schofield and D. E. Wright. 'The pigments of stick lac. Part I. Isolation and preliminary examination.' J.Chem.Soc., 1965, pp. 6067–6073. 'Part II. The structure of laccaic acid A.' J.Chem.Soc.(C), 1967, pp.842–851. (b) N. S. Bhide, E. D. Pandhare, A. V. Rama Rao, I. N. Shaikh and R. Srinivasan. 'Lac pigments: Part IV—Constitution of laccaic acid B.' Ind.J.Chem., **7**, 1969, pp.987–995.

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20. Examples of the more important sources consulted containing recipes for the preparation of lake pigments are as follows:
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V. Borradaile and R. Borradaile. 'The Strasburg Manuscript; a medieval painters' handbook.' Translated from Old German. Alec Tiranti, 1966.
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M. P. Merrifield. 'Original treatises dating from the XIIth to the XVIIIth centuries on the arts of painting.' 2 vols. John Murray, 1849, reprinted by Dover Publications, 1967.
A. Neri. 'The art of glass, wherein are shown the wayes to make and colour glass, pastes, enamels, lakes and other curiosities. . . .' English edition, London, 1662.

P. F. Tingry. 'The painter's and colourman's complete guide. . . .' Sherwood, Gilbert and Piper, 3rd edition, 1830.
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21. There are a number of standard texts describing methods of embedding and sectioning various types of material, most frequently biological material, for light and electron microscopy. Such methods may be adapted for use with samples of friable aged paint. The following books have been found useful:
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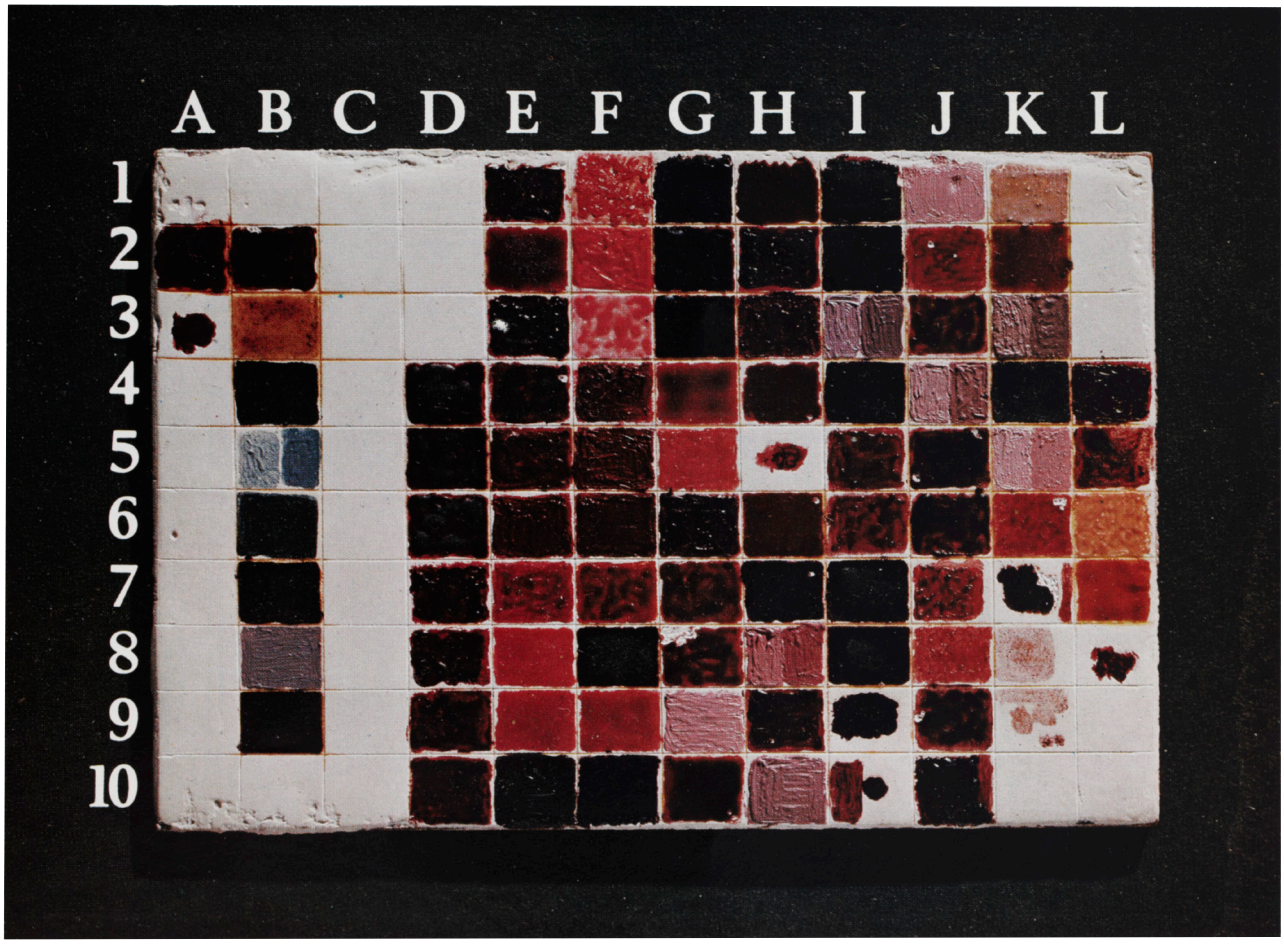
J. K.

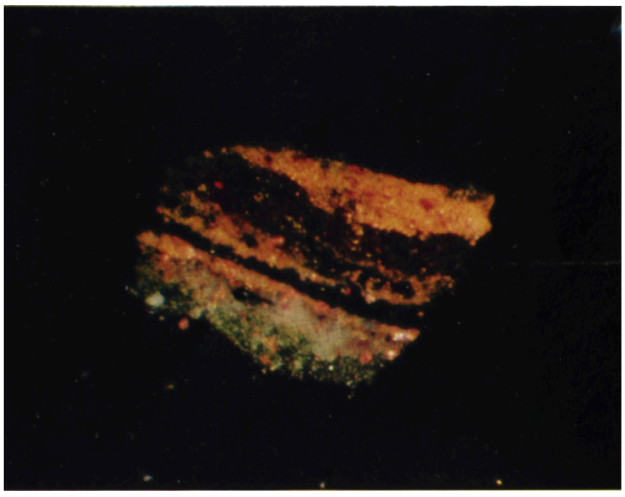
Plate 5a
A selection of red lake pigments, the majority prepared in the laboratory but also including some commercially-prepared lakes for comparison, painted out on a gessoed panel of area *c.*10× 15cm.[2] Lake K7 was prepared from kermes insects; K6 is one of those prepared from brazilwood, mixed with lead white in K5. Lake J10 was prepared from Polish cochineal. The remaining lakes in row J, some mixed with lead white, were prepared from lac. Rows H and I show cochineal lakes, including several examples of cochineal carmine, consisting essentially of the dyestuff precipitated as a salt, rather than on a substrate in the usual way; I10 is an example prepared in the laboratory, while H5 is from a water-colour cake dating from the late eighteenth or early nineteenth century. Rows E(5)-G show lakes prepared, in the laboratory and commercially, from madder root and from synthetic alizarin; Rows D and E(1-4) show lakes made in the laboratory from alizarin, purpurin and from mixtures of the two. Natural madder lakes prepared in the laboratory are seen in row G6-10, the remainder, although deriving their colouring matter from the natural madder root, were manufactured —G4, for example, is a sample of Winsor & Newton's Rose Madder. Most so-called madder lakes available today are prepared from synthetic alizarin as is alizarin crimson itself, seen in F4-6, F5 having been prepared in the laboratory.

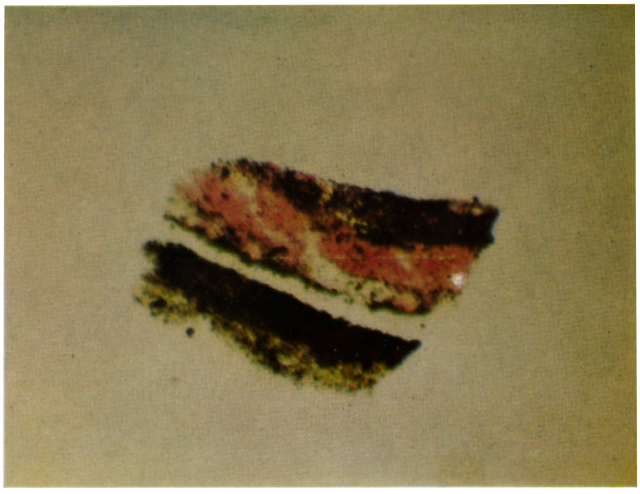
Plate 5b (*Centre left*)
10μm cross-section cut from a sample taken from *The Mass of S. Giles* by the Early Netherlandish painter known as the Master of S. Giles (NG 4681). The sample was taken from a red portion of the carpet in the lower right hand corner of the picture. The layer of deep red paint containing the lake pigment to be investigated may be seen sandwiched between two orange-red layers, containing the pigment vermilion. The layer of lake pigment is approximately 20-25μm thick and is easily accommodated by the rectangular aperture of the microscope photometer, as described in the text. The lake dyestuff present is probably that extracted from the madder root. In the lower of the two orange-red layers, vermilion is mixed with the same red lake pigment (this is more easily seen in 5c). The lighter orange layer below, divided horizontally by a crack, also contains vermilion, mixed with a little lead-tin yellow and black. The light-coloured layer below contains the pigment lead white, mixed with a very little vermilion and black; a faint line of black particles may be seen at the top of the layer. The lowest paint layer contains the green pigment malachite, mixed with black and a little lead-tin yellow. The lowest layers of cream underpaint and chalk ground are missing. The cross-section was photographed by reflected light. Magnification 270×.

Plate 5c (*Centre right*)
The same cross-section, photographed at the same magnification but by transmitted light. The layer of lake pigment, a slightly orange red by transmitted light, is transparent; the layers containing vermilion (red mercuric sulphide) and lead white are so opaque, even at a thickness of 10μm, that they appear black. The green pigment malachite is fairly transparent so that the lowest layer appears translucent.

Plate 5d (*Below*)
The raw materials. Upper row, from left to right: brazilwood (in this case *Caesalpinia echinata* Lam., Pernambouco wood), madder root, dried safflower petals. Lower row, from left to right: stick lac (it is possible to see fragments of twig and the purplish bodies of the insects embedded in the secreted resin-like material), cochineal, Polish cochineal, kermes.







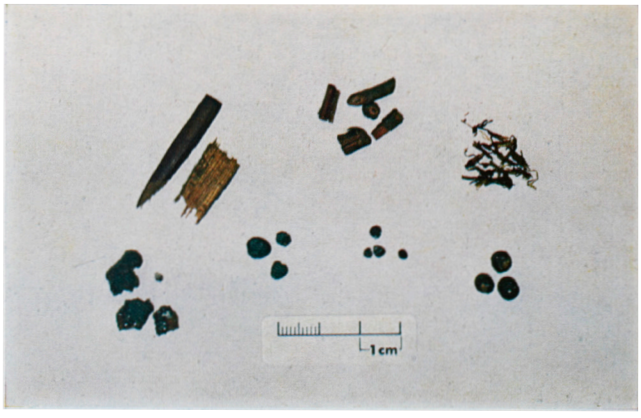


Plate 5 (Full captions on facing page)