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# Nature's other greens

Apart from chlorophyll, which dominates the natural scene, there are several other green pigments.

The structure of one of them, xylindein, produced by a fungus that grows on dead wood, has recently been elucidated; it turns out to be quite different from that of chlorophyll

by Dr G. M. Blackburn

University Chemical Laboratory, Cambridge

THE abundance of the chlorophylls, the green leaf-pigment of plants, is so overwhelming that it almost becomes excusable to suppose that they uniquely provide green colour in nature. It is not so. Nature has other green pigments. Of those which bear some resemblance to the chlorophylls, the green bile pigment, biliverdin, is the best example: Figure 1 shows the similarity. Biliverdin is formed by biological degradation of the red blood pigment hemin and, in addition to bile, it colours such diverse objects as the placentas of dogs and eggshells of birds.

There are other natural green pigments that are not related in any way to the chlorophylls but are members of the third most numerous class of natural pigments—the quinones. These are less prolific than either the carotenoids (typified by the red-orange of carrots) or the melanins. And although the quinone pigments are distributed widely through nature, their colourful contribution is small because they are often located in micro-organisms or in the bark and roots of trees. Only those in the higher fungi and lichens catch the eye, and then mostly with yellows, reds, and a few purples. Of the greens, we should not count the green of the sea-urchins, which is composed of green calcium salts of reddish polyhydroxynaphthoquinones. Few are genuinely green. Two of these few, lo-kao and xylindein, have had commercial prospects, only to pass from interest with the advent of synthetic green pigments.

Lo-kao was used by the Chinese to dye silk and cotton a beautiful bluish-green. Stimulated by the high cost of the dye (224 francs per kilo), scientific study began in 1852 on a 1-g. sample of pigment sent by the American Consul in Canton to Persoz. Later information from missionary sources revealed that the green colour could be obtained by repeated dipping of silk in a colourless alkaline extract of broom followed by exposure to sunlight. The native

This article deals with a subject discussed at the International Congress of Pure and Applied Chemistry in London that ended last week. A paper entitled "The structure of xylindein," by G. M. Blackburn, A. H. Neilson and Lord Todd, was presented by Dr Blackburn.

plants "Hong pi lo chou" and "Pé pi lo chou" were identified as *Rhammus chlorophorus* and *R. utilis* respectively. As the latter was also found in France, interest transferred from the green material to the active colourless component of the alkaline extract.

Xylindein has a longer scientific history. The greenness in dead wood caused by the fungus *Peziza aeruginosa*, now known as *Chlorociboria aeruginosa*, was the object of scientific discussion as long ago as 1728. But the pigment responsible for the colour was only obtained in crystalline form in 1874 by Liebermann, who showed it to be a quinone. It was noticed that the dead wood which had been permeated by the coloured mycelium of the fungus was unaltered in texture and resisted decay. This green wood was used decoratively in Tunbridge ware, in which composite veneers were prepared from differently coloured woods, and a process was patented for the artificial colouring of wood with *Chlorociboria*.

In the determination of structure of a large quinonoid molecule, the investigation proceeds along three lines. It is necessary first to recognise the functional groups in the molecule so that its chemical manipulation can be understood, secondly to identify the major skeleton of the molecule, and thirdly to locate the functional groups

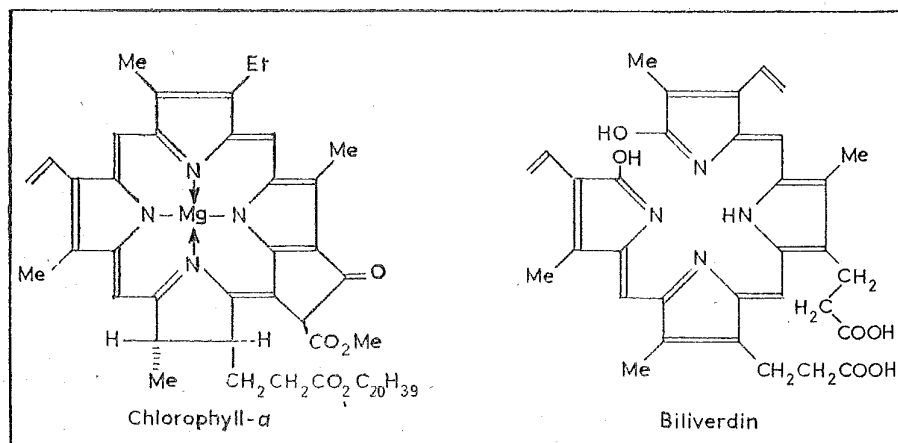


FIGURE 1. The chlorophylls, typified by a chlorophyll-a (left) are nature's most important greens—indeed, her most important pigments, because of their role in trapping the energy of sunlight for the growth of plants. The green bile-pigment, biliverdin (right) is one of the other natural greens and is closely related to the chlorophylls.

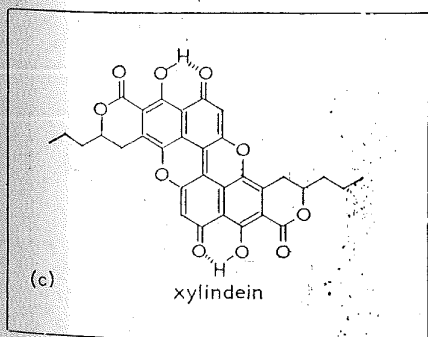
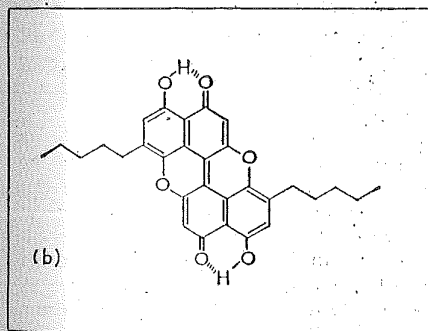
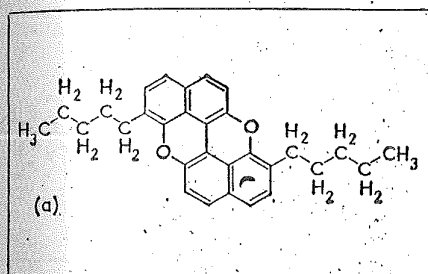
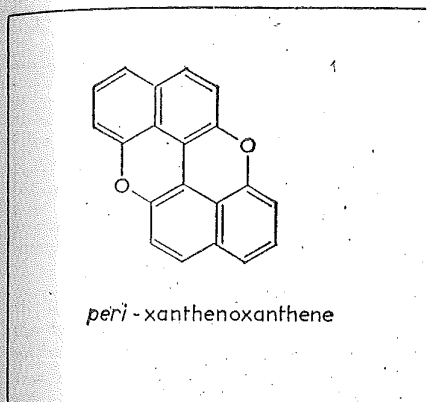


FIGURE 4. Steps in the elucidation of the structure of xylindein: (a) fragmentation by mass spectrometry reveals the presence of two side chains  $C_5H_{11}$  on the peri-xanthenoxanthene nucleus; (b) spectroscopic evidence points to the presence of the OH ... O bridges; (c) other evidence from degradation allows the completion of a pair of lactone rings.

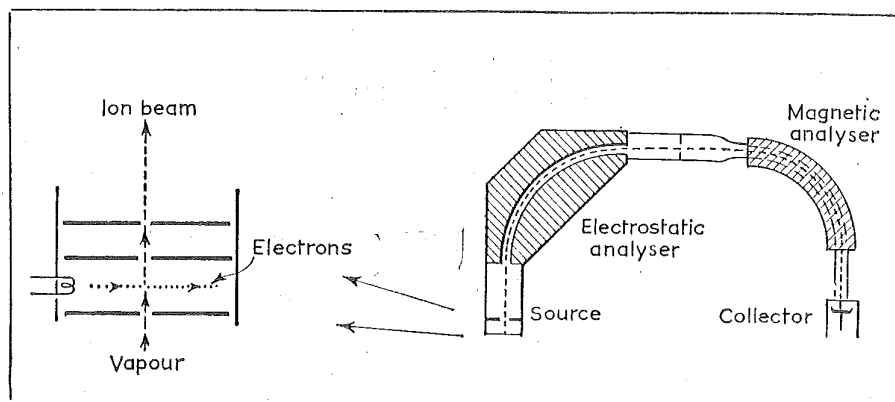


FIGURE 2. (Above, left). When xylindein, the green pigment found in dead wood, is broken down chemically, one fragment is this peri-xanthenoxanthene, which is a yellow material and provides the clue to the green of the parent molecule. FIGURE 3 (right). The mass spectrometer, which affords determinations of molecular weights to one part in 100,000.

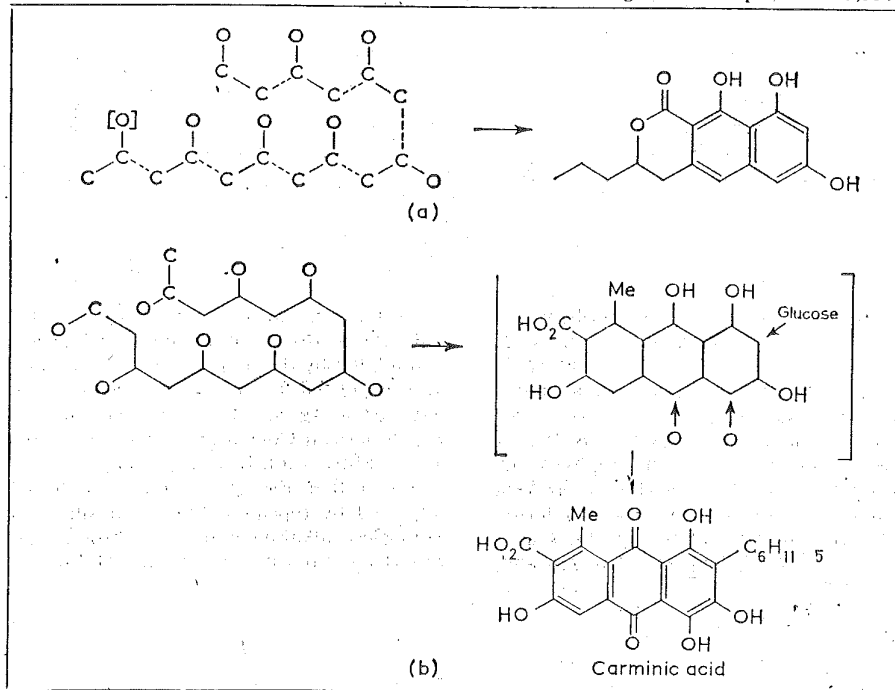


FIGURE 5. The xylindein molecule can be visualised as being composed of two identical parts built up from eight 2-carbon units, as in (a). If this 16-carbon unit is folded in a different way, it can lead to carminic acid (b), the coloured principle of cochineal.

on it. The first of these objectives was investigated by Fritz Kögl in the late 1920s with moderate success; he failed, however, to gain any knowledge of the skeleton other than a tentative identification of phenanthrene as a product of one degradation.

So when we began work on it in Cambridge the dominant question was "What is the chromophore of xylindein?" It is the nature of the chromophore of dyes and pigments which, augmented by the peripheral functional groups, governs the absorption of light energy by electrons whose orbitals extend across the molecule (see "Electrons in molecules" by Professor H. C. Longuet-Higgins, 14 March). The non-absorbed light determines colour. In

spite of recent advances in spectroscopy it was impossible to find a relation between the visible and ultraviolet absorption spectra of xylindein or its simple derivatives and those of known aromatic nuclei. So we needed a less complex, large fragment of the molecule.

The classical method of obtaining such a fragment is to deoxygenate the molecule by distillation with zinc powder in a melt of sodium and zinc chlorides. It looks, and is, very crude; but it worked. Our fragment was a yellow, crystalline material. Its ultraviolet spectrum showed it to be a peri-xanthenoxanthene (Figure 2), and further comparisons showed this skeleton almost certainly existed in xylindein as well. Here

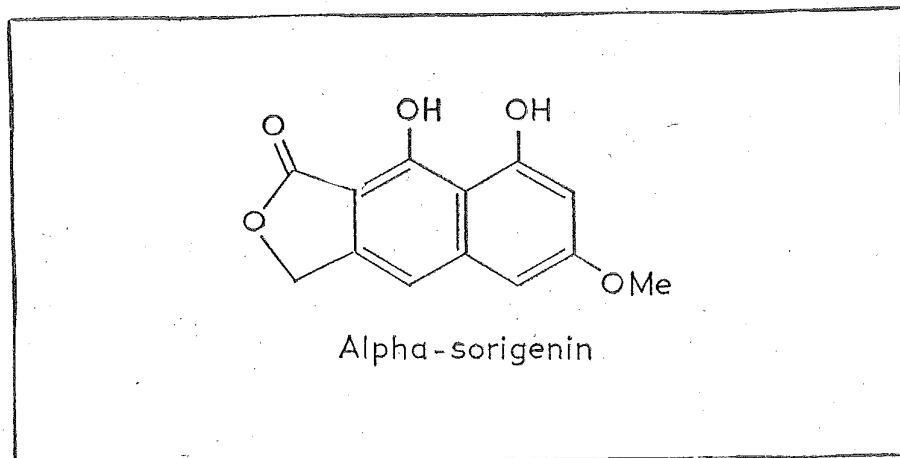
Nature's other greens *continued*

FIGURE 6. Another natural product, alpha-sorigenin, found in a species of broom, shows some similarity to the xylindein precursor of Figure 5. This in turn is probably closely related to lo-kao.

was the clue to the green colour. Xylindein had a chromophore similar to those of many of the synthetic dyestuffs. However, we needed more information and, as the fragment was only available in milligramme quantities, we resorted to mass spectrometry. Dr J. H. Beynon of ICI Dyestuffs Division kindly undertook this work for us.

A mass spectrometer is a device for measuring the masses (strictly, the mass: charge ratios) and relative abundances of positively charged ions. The vapour of the sample to be studied is bombarded with a beam of low energy electrons in a vacuum system (Figure 3). An electron striking a molecule tends to knock another electron out of it, producing a positive ion, but it often breaks chemical bonds, so that a charged fragment of the original molecule is produced. The positive ions are then accelerated by a high voltage and emerge from the ion in a fine beam. They are then subjected to the deflecting and focusing action of electrostatic and magnetic analysers: in general, the heavier ions are deflected less readily than the lighter ions. By varying the magnetic field the device directs one mass after another into a collector, and the signal obtained is amplified and recorded.

For the organic chemist the technique provides information of two types, the one precise, concerning the molecular weights of the parent and fragment ions, the other empirical, about the pattern of fragmentation involved. All this from a sample of less than a milligramme!

The possibility of measuring masses to an accuracy of 1 in 100 000 allows an actual molecular formula to be assigned to the parent ion. In the case of our xylindein, the measured molecular ion of mass  $422.223 \pm 0.004$  fits only the formula  $C_{30}H_{30}O_2$  (mass 422.224) and not  $C_{31}H_{34}O$

(mass 422.261) nor  $C_{33}H_{38}$  (mass 422.297).

Information about the structure of the molecule comes from collation of the fragments also recorded in the mass spectrometer. One of the best-known fragmentation processes involves the cleavage of the side chain at the carbon-carbon bond next but one to the aromatic system. This is recognised in the mass spectrum on finding a strong peak having the mass of the parent ion diminished by the mass of  $C_nH_{2n+1}$ . We found two such peaks in our spectrum corresponding to reductions of the parent mass by 57 and 114, or to the removal of one and two  $C_4H_9$  groups respectively. From this result we can deduce that there are two five-carbon side chains on a  $C_{20}H_{20}O_2$  nucleus. More complex deductions showed that they were unbranched and probably located as shown in Figure 4a.

When this structure was confirmed by synthesis, Kögl's molecular formula for xylindein,  $C_{34}H_{26}O_{11}$ , showed that we had only four carbon and nine oxygen atoms still to locate. Many of these fell rapidly into place by tacking on the functional groups. First, the quinone chromophore itself could now be recognised by comparison of spectra with that of the parent quinone. This, by one of those queer strokes of luck, had been synthesised unambiguously by Pummerer in 1933. Two hydroxyl (OH) groups had to be located as shown in Figure 4b, so as to provide six-membered rings containing a hydrogen bridging two oxygen atoms: this feature has been deduced from characteristic spectroscopic evidence.

The remaining functional groups comprised two six-membered lactone ( $C_5H_8O$ ) rings. Since other evidence from oxidative degradation suggested that the five-carbon side chains had oxygen atoms attached to the carbon next but one to the ring, we could use up two of the "spare" carbons

and four oxygens to give us the complete structure (Figure 4c).

This differed from Kögl's formula by a fragment  $C_2H_2O$  for which we had no other evidence, and the structure of Figure 4c appeared to be capable of explaining all the chemistry of xylindein. Did the fragment really exist?

There is often a certain amount of doubt about the correctness of big formulae. The percentages of carbon, hydrogen, and oxygen in the molecule determined by combustion analyses can only be found within experimental limits capable of encompassing several formulae. Only the mass spectrometer or careful X-ray determinations provide a molecular weight accurate enough to determine a unique formula in conjunction with analytical data. We thought it almost impossible to volatilise a molecule as big as xylindein into the electron beam.

We had underestimated the ability of the specialist mass spectrometer. When xylindein was cooked up at  $400^\circ\text{C}$  a spectrum was produced in which the predominant parent ion corresponded to the formula  $C_{32}H_{24}O_{10}$  of our structure (Figure 4c) but also showed the presence of other molecules in much smaller amounts with one or two carbon atoms more. We had not expected to find that xylindein was the major component of a series of homologous compounds!

We can speculate on how the fungus produces the pigment. While little is known of the function of the majority of the quinone pigments, it seems possible that they arise as an "overflow" of fatty acid metabolism when the enzymes concerned become overloaded. The xylindein molecule can be visualised as two identical parts which can be built up from eight two-carbon units (Figure 5a). However, speculation does not answer the question of how the halves are joined. That must be decided by experimentation.

This sixteen-carbon unit is quite common. If, for instance, it is folded in a different way, it can be seen as a precursor or carminic acid, the coloured principle of cochineal (Figure 5b). Materials of the general chemical type of xylindein are unusual and rare metabolites and it is, therefore, surprising to find that a similar one, alpha-sorigenin (Figure 6), is found in *Rhamnus japonica*. According to early work in the field, this species does not give rise to Lo-kao. But it is amusing to speculate that *R. utilis*, the source of Lo-kao, might contain a related metabolite, and that the chemical that the Chinese wrought by sunlight, *Chlorociboria* effects in the dark!

At the University Chemical Laboratory in Cambridge we have also been investigating another of nature's green pigments—the green colour of aphids. But that is another story, not yet quite ready to be told.