Xylindein

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Deciduous wood which is infected with the fungus *Chlorociboria aeruginosa*,[1] formerly known as *Peziza aeruginosa*,[2] assumes a bright green hue. This green pigmentation has stimulated scientific inquiry for over two centuries. Thus, the brothers Tulasne comment:[3] "It was certainly our fungus which caused the greenness of that vineprop which Geoffroy le Jeune once found in a vineyard at St. Cloud, and of which he spoke a few words to his colleagues in the Parisian Académie des Sciences in the year 1728."

Following early work by Döbereiner,[4] Bley,[5] and Fordos,[6] the pigment was named xylindein by Rommier.[7] In 1874, Liebermann[8] overcame the problems of extraction and purification caused by the almost complete insolubility of xylindein in common organic solvents and obtained pure xylindein by crystallization from phenol as small, purple platelets with a coppery lustre.

Prior to our studies[9] in Cambridge the only substantial chemical work on xylindein was that of F. Kögl *et al.*, who published two papers[10, 11] on the subject in the period 1925–1930. Based on analytical results Kögl favoured a formula $C_{34}H_{26}O_{11}$ for xylindein and established its quinonoid nature. The intractable nature of the pigment, which is virtually insoluble in most organic solvents, severely hampered chemical investigation but on the basis of studies on methylation and acetylation Kögl concluded that it contained in addition to the quinone system 4 hydroxyl groups of which two at least were phenolic. Xylindein yielded a purple dimethyl ether which on acetylation gave a yellow diacetate, while direct acetylation gave a yellow tetraacetyl derivative. Reductive acetylation of xylindein and its dimethyl ether gave respectively a yellow tetra-acetate and a yellow diacetate very similar to but allegedly distinct from the substances obtained by simple acylation. From these results Kögl inferred that the phenolic groups produced by reduction of the quinonoid system were resistant to acylation although he had no satisfactory explanation to offer for the remarkable colour change noted in passing from xylindein dimethyl ether to its diacetate. He also established the presence of two lactone groupings in xylindein; these he formulated as enol-lactones since he was able to prepare a *bis*-semicarbazone from the dimethyl ester of the corresponding acid (xylindeic acid). Zinc dust distillation of a derivative of xylindeic acid was reported to yield phenanthrene in good yield and, although no structural formula was advanced, it was suggested that xylindein might be a derivative of 2,7-phenanthraquinone; it is of some interest in this connection that Liebermann, much earlier, had speculated[8] on a relationship between xylindein and coerulignone (3,5,3′,5′-tetramethoxydiphenoquinone).

Our interest in natural green pigments of a non-porphyrin character led us to take up the study of xylindein afresh. At first we used material extracted from infected wood with chloroform or phenol, but later discarded this in favour of the more convenient method of culturing the fungus on a sterile medium of cellulose powder and malt extract. Although the fungus does not form fruiting bodies under these conditions it produces a green pigmented mycelium from which by extraction with phenol or chloroform, xylindein is obtained identical in ultra-violet and infra-red absorption with that from rotting wood. Analysis of xylindein gave values similar to those recorded by Kögl but the formula allotted by him is only one of several which would fit with the analytical figures and indeed, as will be shown later, it became clear in the course of our work that the true formula of xylindein is $C_{32}H_{24}O_{10}$. By xylindein we mean the main component of the pigment as ordinarily isolated. There is some evidence, which will be mentioned later, that the pigment obtained especially from fungus-infected wood may contain small amounts of a closely related material almost certainly derived from the true xylindein. The study of xylindein proved to be of considerable complexity and although in attempting to review its course here it is not possible to adhere strictly to its chronological course this will be done as far as possible.

Preliminary studies showed that, as reported by Kögl, xylindein is a hydroxyquinone containing two lactone groups which can be opened by treatment with strong alkali to give the tetrasodium salt of xylindeic acid. The infra-red spectrum of xylindein shows no hydroxyl absorption and the two strong carbonyl absorptions at 1720 and 1625 cm^{-1} can be assigned respectively to the

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[1] F. J. Seaver, *North American Cup Fungi*, New York 1951.

[2] C. H. Persoon, *Synopsis Methodica Fungorum*, Göttingen 1801, p. *105*, 663.

[3] L. R. and C. Tulasne, *Selecta Fungorum Carpologia*, Paris 1865, translated W. B. Grove, Oxford 1931, Vol. III, p. 178.

[4] J. W. Döbereiner, *Schweiggers Journal 9* (1813) 160.

[5] L. Bley, *Arch. der Pharmacie 94* (1858) 129.

[6] M. Fordos, *C. R. Acad. Sci. 57* (1863) 50.

[7] M. A. Rommier, *ibid. 66* (1868) 108.

[8] C. Liebermann, *Ber. dtsch. chem. Ges. 7* (1874) 1102.

[9] G. M. Blackburn *et al.*, *Proc. Chem. Soc. London 1962*, 327.

[10] F. Kögl and G. von Taeuffenbach, *Liebigs Ann. Chem. 445* (1925) 170.

[11] F. Kögl and H. Erxleben, *ibid. 484* (1930) 65.

lactone and quinone groupings. The shift of the latter peak to 1640 cm^{-1} in the purple dimethyl ether and to 1638 cm^{-1} in the purple diacetate, which can be prepared by mild acetylation of xylindein, indicate clearly that we are dealing in xylindein with an extended quinone containing two phenolic hydroxyls *peri* to the quinone carbonyls.[12] Kögl's statements on the formation from xylindein of a yellow dimethyl ether diacetate and a tetra-acetyl derivative are correct, but the products obtained by him on reductive acetylation of the dimethyl ether and of xylindein are, in fact, identical with the above dimethyl ether diacetate and tetra-acetate. The fact that xylindein gives on vigorous acetylation a yellow tetra-acetate and that the purple dimethyl ether yields on similar treatment a yellow diacetate is due to these yellow compounds being derivatives of dihydroxylindein (i.e. the corresponding quinol) which originates from an intermolecular redox process. Evidence for this is provided by the facts that whereas the yellow tetra-acetate is the sole product of reductive (catalytic or zinc dust) acetylation of xylindein it is accompanied in the product of direct acetylation of xylindein by an orange tetra-acetate derived from a dehydro-xylindein; the nature of this latter product will be discussed later. Also in accordance with this view both the dimethyl ether diacetate and the yellow tetra-acetate are devoid of quinone carbonyl absorption in the infra-red, showing only the lactone carbonyl (at 1713 and 1726 cm^{-1} respectively) and phenolic acetate carbonyl (at 1775 cm^{-1}). There is thus no evidence for any hydroxyl groups in xylindein other than the two situated *peri* to the quinone carbonyls.

As has already been mentioned xylindein contains two lactone groupings which on the evidence of chemical and especially of spectroscopic properties must, like the hydroxyl groups, be symmetrically located. Three main lines of evidence indicate that the carbonyl group in these groupings must be directly attached to the main chromophore adjacent to the quinone carbonyl or *peri*-hydroxyl groups in xylindein. Firstly the opening of the lactone rings in xylindein derivatives is accompanied by marked changes in light absorption in the 350 mμ region; thus for example the peak at 350 mμ (log ε, 4.45) in xylindein changes to 337 mμ (log ε, 4.3) in the dimethyl ester of xylindeic acid. Secondly when the tetramethyl ether of dihydroxylindein (prepared by catalytic reduction of xylindein dimethyl ether and treatment of the product with diazomethane) is reduced with lithium aluminium hydride (i.e. conversion of 2-CO-O-C into 2-CH_2OH + 2 HOC-) and the product acetylated a tetramethyl ether tetra-acetate is obtained. This acetylated product shows a single acetate carbonyl absorption at 1727 cm^{-1} and hence the lactone groups of xylindein cannot involve phenolic hydroxyls. Moreover the n.m.r. spectrum of this substance also shows that it contains 4 aromatic methoxyls (6.3 τ) 4 aliphatic acetates (7.95 and 8.05 τ) and 4 protons (4.7 τ) corresponding to the benzyl protons in benzyl acetate (5.15 τ). Thirdly when the purple xylindein diacetate is reduced catalytically the yellow dihydro-xylindein diacetate shows carbonyl absorptions at 1770 cm^{-1} (acetate) and 1658 cm^{-1} with a broak weak hydroxyl band at 2680 cm^{-1}. Such absorption is characteristic of a carbonyl group hydrogen bonded with a hydroxyl in a chelate ring. Thus, for example, the carbonyl absorption of 8-hydroxy-6-methoxy-3-methyl-3,4-dihydro-*iso*-coumarin[13] (I) at 1660 cm^{-1} moves to 1720 cm^{-1} on methylation of the 8-hydroxy group. Dihydroxylindein diacetate does not react with diazomethane but on acetylation it yields the tetra-acetate with carbonyl absorption at 1726 cm^{-1}.

This evidence clearly supports our contention that the lactone groups in xylindein are in the form of dihydro-*iso*-coumarin systems in which the carbonyl group is *peri* to one of the oxygens in the *peri*-hydroxyquinonoid system of the pigment i.e. that xylindein must incorporate the structural features shown in (II).

To complete the structural elucidation of xylindein it remains to determine the nuclear structure of the molecule and to account for the *n*-butyric acid which was obtained by Kögl by alkali fusion and (accompanied by some propionic and acetic acid) by us on chromic acid oxidation of the pigment.

The occurrence of extraneous absorption in the 350 mμ region associated with the lactone rings in xylindein prevented any useful comparison of the light absorption of its leuco-acetate or similar derivatives with that of possible chromophores. However the absorption spectrum of the yellow hexa-acetate of dimethyl dihydroxylindeate (Fig. 1a) shows a remarkable similarity to that[14] of *peri*-xanthenoxanthene[15] (III) (Fig. 1b) and strongly suggests that it contains this nucleus. It will be recalled that Kögl reported the production of phenanthrene on zinc dust distillation of xylindein derivatives; in view of our findings at this stage we carried out a zinc dust fusion on the yellow dihydro-xylindein tetra-acetate using the technique of Clar.[16] No phenanthrene was obtained but exhaustive purification of the product yielded a yellowish crystalline material m.p. 178–179° with the typical light absorption (Fig. 1c) of a *peri*-xanthenoxanthene. Examination of this product by high-resolution mass spectrometry identified it as 1,7-di-*n*-pentyl peri-xanthenoxanthene (IV) or any isomer in which both pentyl groups are restricted to the 1, 5, 7 and 11 positions.[17] In the light of the chemical evidence leading to the partial structure (II) we considered that only the 1,7-orientation (IV) need be considered. 1,7-di-*n*-pentyl-

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[12] A. Calderbank *et al.*, *J. Chem. Soc. 1954*, 1285.

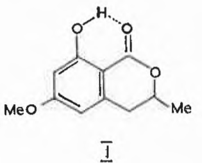
[13] E. Sondheimer, *J. Amer. Chem. Soc. 79* (1957) 5036.

[14] D. W. Cameron, unpublished results.

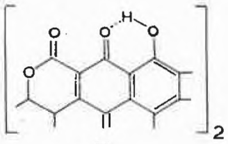
[15] R. Pummerer and A. Rieche, *Ber. dtsch. chem. Ges. 59* (1926) 2161.

[16] E. Clar, *ibid. 72* (1939) 1645.

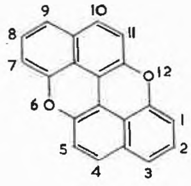
[17] J. H. Beynon, *Proceedings of Xth Colloquium Spectroscopicum Internationale, 1962.*



I



II



III

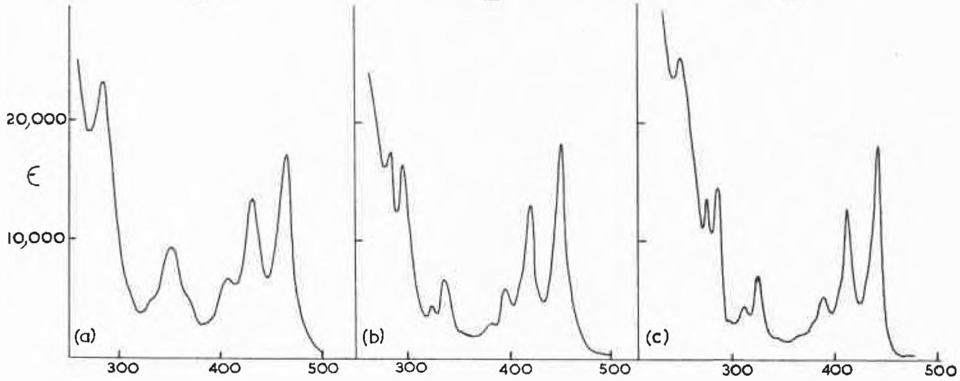
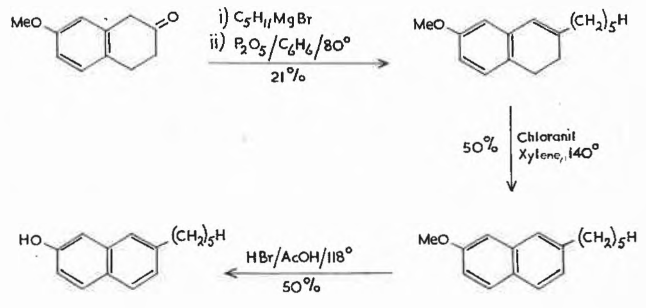
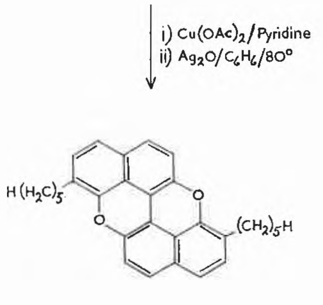


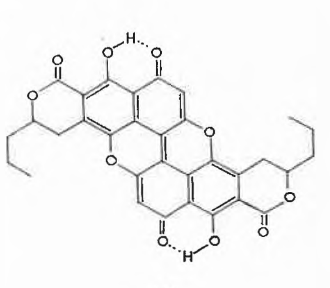
Fig. 1. Light absorption of (a) hexa-acetyldihydro-xylindein acid dimethyl ester, (b) *peri*-xanthenoxanthene (III), (c) product of zinc dust fusion of tetra-acetyldihydro-xylindein (IV)

perixanthenoxanthene was therefore synthesised and shown to be identical in every respect with the product obtained by zinc dust distillation; the synthetic route is outlined below.





IV



V

The production of *n*-butyric acid from xylindein by alkali fusion or by oxidation indicates that the *n*-pentyl residues in (IV) are oxygenated at the 2-position in the original pigment. The summation of available evidence thus leads to the conclusion that xylindein is represented by structure (V).

Structure (V) accounts satisfactorily for all the observed chemical facts and corresponds to a molecular formula $C_{32}H_{24}O_{10}$ for xylindein rather than the $C_{34}H_{26}O_{11}$ suggested by Kögl.[11] Despite the intractable nature of the pigment a mass spectrum of xylindein was obtained[18] using a direct inlet system at 400°.

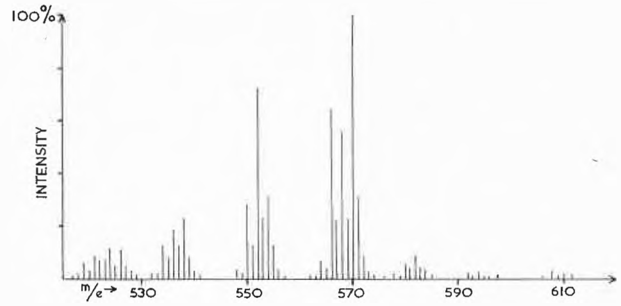


Fig. 2. Mass spectrum of xylindein

The spectrum observed (Fig. 2) varied with time due to decomposition in the crucible. In the high mass number region the most prominent group of peaks was that containing masses of 570, 568, 566 and 564. While at first the dominant peak is at 570 ($C_{32}H_{26}O_{10}$) the relative intensities change with time until the strongest peak is 566 ($C_{32}H_{22}O_{10}$). The suggested explanation is that at the high temperature of the source the quinone abstracts hydrogen intermolecularly so that the first observed species 570 is the quinol corresponding to xylindein. The dehydro- and *bis*-dehydro-xylindeins produced in this way would be expected to increase in amount with time

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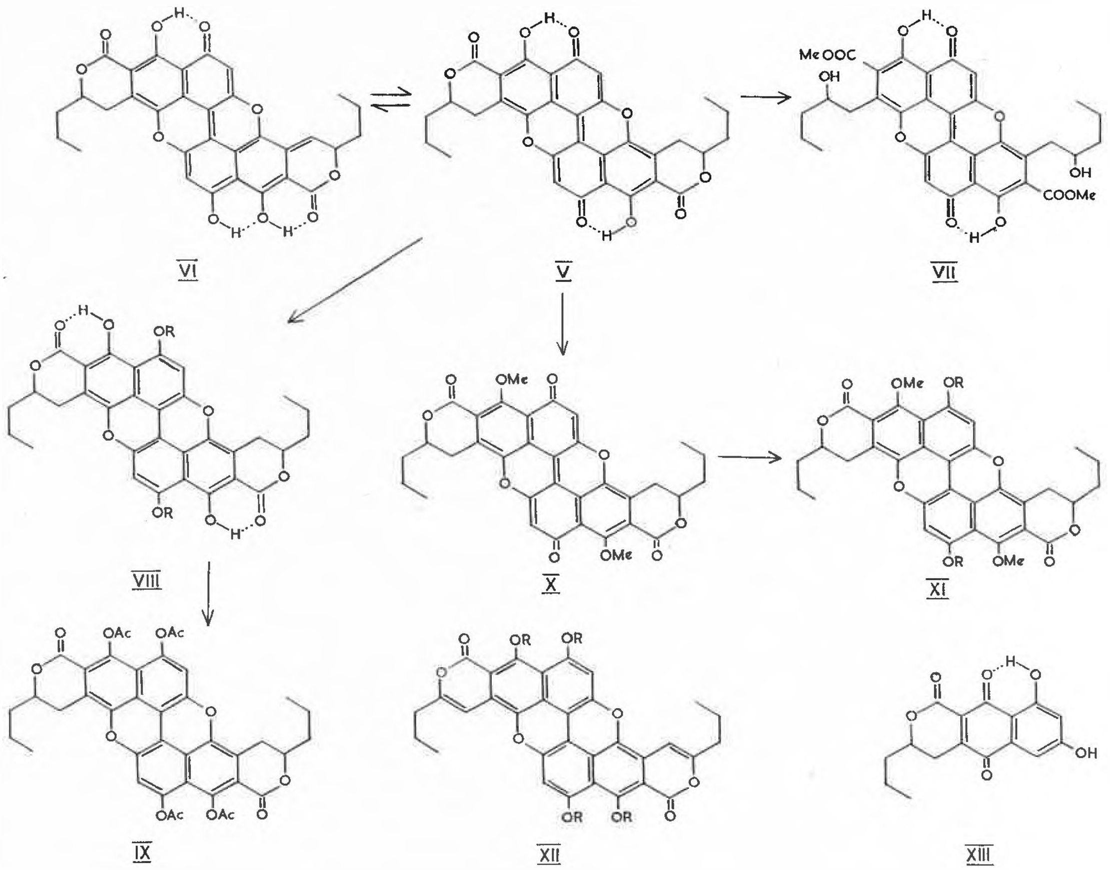
[18] We are indebted to Dr. J. H. Beynon for mass-spectrometric data.



relative to the 570 species. In the spectrum a few small peaks above 570 were observed; their origin is unknown but the suggestion that they may arise by methyl transfer[19] is perhaps more attractive than the alternative that they are due to traces of higher homologues in natural xylindein.

It is now appropriate to discuss some of the complex aspects of xylindein chemistry in the light of structure (V) and to allot definite structures to some of its derivatives. Perhaps the most striking feature of xylindein is the «auto-reduction» it undergoes on vigorous acylation, when the quinone carbonyls are converted to acyloxy groups. This reduction could be achieved by direct hydrogen transfer between two molecules in form V or, perhaps more likely, *via* the tautomeric mono-enolised form VI. Several features of xylindein suggest strongly that the hydrogen atoms at the 3′ and 4′ positions in the lactone rings are involved in this type of behaviour. We have already mentioned that when xylindein is acetylated with sodium acetate—acetic anhydride it yields in addition to the yellow dihydro-tetra-acetate (IX) an orange tetra-acetate. Analysis of the latter gives values which do not with any certainty indicate a difference from (IX) in molecular formula, but its ultra-violet absorption spectrum shows absorption at 470 mμ which does not occur in that of (IX). This suggests an extension of the chromophoric system such as would result from the introduction of one or two double bonds conjugated with the aromatic system as in (XII; R = Ac), which probably represents the orange tetra-acetate of the quinol corresponding to a *bis*-dehydroxylindein whose formation provides the hydrogen necessary for the production of (IX) from xylindein. Again, the mass spectrum of xylindein already discussed is indicative of intermolecular hydrogen-transfer to give a mixture of xylindein quinol (VIII; R = H) and other quinols containing 2 or 4 hydrogen atoms fewer.

The *bis*-dehydroxylindein quinol (XII; R = H) would contain two enol-lactone groupings as part of two *iso*-coumarin systems. In his work on xylindein Kögl described[11] xylindein as containing two enol-lactone groups whereas according to structure (V) it contains two saturated lactone groups. This raises a point of considerable interest for in our experience xylindein as isolated from wood or from the mycelium of the cultured fungus is not always homogeneous, although (V) is undoubtedly the major component. Thus for example in one instance the crude dimethyl ester of xylindeic acid prepared from

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[19] Cf. G. Büchi *et al.*, *J. Amer. Chem. Soc.* *85* (1963) 1893.

a crystalline sample of xylindein by KÖGL's method showed in the infra-red strong ester carbonyl absorption at 1730 cm^{-1}, strong hydroxyl absorption at 3400 cm^{-1} and a weak absorption at 1710 cm^{-1} corresponding to a ketonic carbonyl. Careful chromatographic purification showed that this material was a mixture of two components. One of these the true dimethyl ester of xylindeic acid (VII) was the major constituent and could be obtained in a pure state; its infra-red spectrum showed peaks at 1730 cm^{-1} and 3400 cm^{-1}. The second which was present in smaller amount and which could not be wholly freed from the other had only weak hydroxyl absorption but strong absorption both at 1730 cm^{-1} (ester) and 1712 cm^{-1} (dialkyl ketone). In other words the xylindein used as starting material contained some of the quinone form of compound (XII; R = H) or of the related quinone containing one saturated and one unsaturated lactone group. Whether the dehydro-xylindein present in the extracted material is produced from xylindein during the extraction process, is produced together with xylindein by the fungus, or is formed from xylindein in wood or in the mycelium as result of an ageing process we do not as yet know although we think the third possibility is the most likely in view of the variable amount of dehydro-xylindein present in different samples.

The pigment studied by KÖGL was isolated from rotting wood and in the light of our experience would be expected to contain a certain amount of *bis*-dehydro-xylindein. That the major constituent was xylindein (V) would seen to follow from the fact the dimethyl ether diacetate and tetra-acetates he described were undoubtedly derived from (V). Nevertheless his starting material may well have contained sufficient of the *bis*-dehydro-xylindein (quinone corresponding to XII; R = H) to give, as a result of concentration in the intervening steps, an apparently reasonable yield of a *bis*-semicarbazone on treating his dimethyl xylindeate with semicarbazide, so causing him to conclude that xylindein contained two enol-lactone groupings.

Xylindein is correctly formulated as (V) in which both quinone carbonyls and phenolic hydroxyls are strongly hydrogen bonded. As in other cases of *peri*-hydroxyquinones attempts to ascribe one or other of the various possible tautomeric hydroxy-quinone formulae to it are meaningless. It is, however, possible on the available evidence to put forward structures for a number of xylindein derivatives. Dihydroxylindein dimethyl ether shows the expected infra-red absorption at 3340 and 1720 cm^{-1} and it reacts with diazomethane to give the yellow tetramethyl ether (XI; R = Me). Thus as in xylindein itself the lactone carbonyls are not hydrogen-bonded, and it can therefore be allotted structure (XI; R = H), and xylindein dimethyl ether must have structure (X).

By constrast the purple xylindein diacetate is catalytically reduced to a yellow dihydro-xylindein diacetate in which the lactone carbonyl absorbs at 1658 cm^{-1} indicating that the lactone carbonyls are hydrogen-bonded and that the dihydro-diacetate has structure (VIII; R = Ac); similar catalytic reduction of xylindein itself gives a yellow dihydroxylindein (VIII; R = H) showing absorption at 1650 cm^{-1}. The dihydro-diacetate unlike the above dihydro-dimethyl ether does not react with diazomethane but vigorous acetylation yields the corresponding yellow tetra-acetate (IX). This evidence does not, of course, rigidly fix the structure of xylindein diacetate itself since it is at least conceivable that acetyl migration might occur during reduction to the dihydro-compound.

Xylindein (V) represents the first recorded case of a *peri*-xanthenoxanthene derivative occurring in nature but we consider it likely that others may exist. Examination of structure (V) suggests that xylindein could arise by a dimerisation of the naphthaquinone derivative (XIII) together with a double dehydration to form the two ether linkages.

Although we have not yet synthesised xylindein itself, we have established that such reactions are realisable in the laboratory. Thus 5,7-dihydroxynaphthaquinones substituted in the 2 and 3 positions pass readily under mild conditions in the absence of air into *peri*-xanthenoxanthenequinone derivatives of the xylindein type. Moreover, evidence as yet incomplete suggests that xylindein is hydrolytically cleaved in strong aqueous alkali to a naphthoquinone derivative which under acidic conditions reverts to a green pigment almost indistinguishable from xylindein. 5,7-Dihydroxynaphthaquinone[20] itself under similar conditions also gives a green insoluble product but this appears to be polymeric in character and may arise from an alternative oxidative process in a manner reminiscent of the production of Lokao (Chinese Green).

Our work on reactions of this nature, which seems *inter alia* to open a way to the production of a variety of green colouring matters, will be described in detail elsewhere.

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[20] Sample provided by Prof. R. H. THOMSON.