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THE STRUCTURE OF XYLINDEIN

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Abstract—Xylindein has been isolated from infected wood and several new derivatives have been prepared. Spectroscopic examination of xylindein and its derivatives has established the orientation of the substituents on the central *peri*-xanthenoxanthene nucleus which has been identified by a mass spectral analysis of the zinc dust distillation product.

THE fungus *Chlorosplenium aeruginosum* (Oeder ex Fries) *Chlorociboria aeruginascens* (Nylander) imparts a characteristic green colour to infected dead deciduous wood. The green colouring matter was reported by Gumbels[1] who named it isoxylinic acid and by Bleys[2] who gave it the name xylochlor acid. Fordos[3] obtained a pigment identical with the above as an amorphous solid by extraction with chloroform; Rommier[4] claimed to have obtained a second green pigment which he named xylindein by extraction with alkali. Liebermann[5] was the first to obtain the pigment crystalline by extracting the green wood with phenol and recrystallization of the amorphous solid with aqueous phenol.

The first systematic chemical examination of xylindein was made by Kögl *et al.*[6,7] who ascribed a molecular formula $C_{34}H_{24}O_{11}$. They detected the presence of two acidic hydroxyl groups, an extended quinone system, two lactone rings and a phenanthrene nucleus.

Our investigations of the pigment produced by this fungus in dead wood has shown that in addition to xylindein there is present a yellow compound which has been identified as xylindein quinol, and also a considerable quantity of a green material which has not been crystallized. Shortly before the completion of this work Blackburn and Todd[8] published a short note on the structure of xylindein using material mainly derived from the fungus grown in culture. We now report the results of our own independent investigations which fully support the structure proposed by them for this compound.

For this investigation, infected ash wood was collected from Little Park Wood and Gundale Wood near Pickering, Yorks. The green colouring matter was completely removed from the finely powdered wood by extraction with chloroform. The extract, after concentration, yielded an amorphous precipitate, the bulk of which dissolved in phenol and crystallized from aqueous phenol as bronze plates. After the phenol extraction of the xylindein, a yellow material remained which was only sparingly

[1] Gumbels, *Uber das Grunfaule Holz Flora* Feb. (1858).
[2] Bleys, *Archiv. der Pharmacie* **94**, 129 (1858).
[3] Fordos, *C.R. Acad. Sci., Paris* **57**, 50 (1863).
[4] A. Rommier, *C.R. Acad. Sci., Paris* **66**, 108 (1868).
[5] C. Lieberman, *Ber. Dtsch. Chem. Ges.* **7**, 1102 (1874).
[6] F. Kögl and G. von Taeuffenbach, *Liebigs Ann.* **445**, 170 (1925).
[7] F. Kögl and H. Erxleben, *Liebigs Ann.* **484**, 65 (1930).
[8] G. M. Blackburn, A. H. Neilson and Lord Todd, *Proc. Chem. Soc.* 327 (1962).

TABLE I

	O—H	Carbonyl		
Xylindein	None	1726 cm^{-1}		1631 cm^{-1}
Dihydroxylindein	3445 cm^{-1} 3355 cm^{-1}		1660 cm^{-1}	
Dimethylxylindein		1730 cm^{-1}		1640 cm^{-1}
Dihydrodimethylxylindein	3280 cm^{-1}	1723 cm^{-1}		
Dihydrodiacetylxylindein	None	1775 cm^{-1}	1660 cm^{-1}	
Dihydrotriacetylxylindein	None	1779 cm^{-1}	1726 cm^{-1} 1661 cm^{-1}	
Dihydrotetra-acetylxylindein		1781 cm^{-1}	1725 cm^{-1}	
Diacetylxylindein		1775 cm^{-1}	1730 cm^{-1}	1640 cm^{-1}
Xylindein dimethyl ester	3350 to 3400 cm^{-1}		(1733 cm^{-1})	1630 cm^{-1}
Dihydrodimethyldiacetyl xylindein		1775 cm^{-1}	1725 cm^{-1}	
Xylindein sodium salt				1623 cm^{-1}

All measurements of O—H stretching absorption were made in Nujol mull.

soluble in hot phenol. Yellow phenolic solutions of this material slowly turned green and dihydroxy-xylindein tetracetate was rapidly formed by treatment with acetyl chloride in pyridine. Evaporation of the chloroform extract gave considerable quantities (up to 95%) of a green resinous material which could not be crystallized. The bulk of this material was soluble in petroleum ether and other non-polar solvents; extraction of a petroleum ether solution of the material with aqueous methanol gave a bright red methanol solution. It is interesting to note that Fordos[3] reported the removal of a red material by extracting the green wood with alcohol.

Xylindein, with acetic anhydride at 100° forms an insoluble red crystalline diacetate. No satisfactory recrystallizing solvent was found for this compound but analysis of the unpurified material was in best agreement with a molecular formula of $C_{38}H_{30}O_{13}$. Xylindein forms a red dimethyl ether with diazomethane. Analytical results for this compound were in fair agreement with Kögl's proposed formula of $C_{36}H_{30}O_{11}$ but fitted better the formula $C_{34}H_{28}O_{10}$. Acetylation at 100° over an extended period or reductive acetylation with zinc dust produced a mixture of acetates which were separated by repeated chromatography on magnesium carbonate. Three acetates were identified; a dihydrotetracetate $C_{40}H_{36}O_{14}$, a dihydrotriacetate $C_{38}H_{32}O_{13}$ and a dihydrodiacetate $C_{36}H_{28}O_{12}$. The formation of these compounds supports the presence of two hydroxyl groups and a quinonoid system. The presence of lactone rings was confirmed by hydrolysis with sodium hydroxide and the formation of a dimethyl ester. This latter compound could not be crystallized despite the claim by Kögl that it crystallized from chloroform. Analyses of the amorphous, chromatographically pure material, were inconsistent but its identity as an ester was confirmed by IR measurements. Oxidation of xylindein with dichromate, potassium permanganate and ozone failed to yield any useful crystalline material; butyric acid was detected as the only volatile product. Reaction with nitric acid at 100° yielded a crystalline dinitro derivative $C_{32}H_{22}O_{14}N_2$ but at higher temperature oxidation occurred and picric acid was produced. Sodium hydroxide fusion yielded a crystalline green quinonoid product which, on reductive acetylation gave a crystalline leuco tetra-acetate $C_{32}H_{26}O_{10}$.

The leucoacetates of xylindein are deacetylated by hot alkaline solutions but are resistant to hydrolysis by dilute mineral acids. Hot formic acid was found to be the most convenient deacetylating medium for both the tri- and tetra-acetates. Deacetylation occurred readily at 100°, the acetates quickly dissolved in the formic acid and a yellow solid was precipitated within two to three minutes. Both the solution and the solid slowly turned green in air and the IR spectrum was identical with that of dihydroxylindein except for absorption at 1775 cm^{-1}. This band was weaker than that in diacetyldihydroxylindein and the acetyl analysis was low for the monoacetate. Elemental analysis was consistent with dihydroxylindein or dihydroxylindein monoacetate but its solubility in chloroform was much greater than that of authentic dihydroxylindein. On IR and solubility evidence we consider this compound to be dihydroxylindein monoacetate. Dihydroxylindeindiacetate was unaffected by hot formic acid.

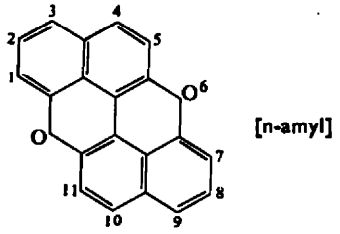
Dihydroxylindein is oxidized rapidly by lead tetra-acetate solution to xylindein. The leucodiacetate was rapidly oxidized within two to three minutes at room temperature, the triacetate within 30 minutes and the tetra-acetate in 16 hr to yield a red diacetate which was spectroscopically indistinguishable from xylindein diacetate produced by the acetylation of xylindein.

In the IR a feature common to the spectra of xylindein and its derivatives is a triplet of weak to medium intensity near 2970, 2940 and 2880 cm^{-1} often with a shoulder near 2840 cm^{-1}. These bands are due to the C—H stretching of an aliphatic portion of the molecule i.e. that part of the molecule yielding butyric acid on oxidation of xylindein. There is little evidence in any of the spectra of aromatic stretching near 3030 cm^{-1} but a weak absorption at 3070 cm^{-1} suggests the presence of unsaturation. Hydroxyl stretching absorption is notably absent in xylindein, its acetate, its dimethyl ether and the leuco acetates suggesting that the hydroxyl groups in these compounds are involved in hydrogen bonding. In the 1800–1600 cm^{-1} region all the non-reduced compounds, with the exception of the sodium salt, show a strong band at 1640–1630 cm^{-1}, this band is absent in all the reduced derivatives and must therefore be assigned to the quinone carbonyl stretching absorption. Quinones in which the carbonyl groups are in the same ring normally absorb in the region 1680–1660 cm^{-1} but extended quinones usually absorb in the range 1655–1635 cm^{-1};[9] from the position of the carbonyl absorption in xylindein and its derivatives we must conclude that an extended quinone is present in xylindein. The acetyl derivatives of xylindein all absorb strongly at 1780 cm^{-1}. Most xylindein derivatives, show one other strong absorption band in the carbonyl stretching region. This band is either at 1730–1724 cm^{-1} or 1662–1660 cm^{-1}. Of the exceptions, the dihydrotriacetate absorbs at both these frequencies, the dimethyl ester absorbs at 1735 cm^{-1} and the sodium salt absorbs strongly at 1570 and 1400 cm^{-1}. All xylindein derivatives except the last two would be expected to exhibit lactone carbonyl absorption and these remaining bands between 1800–1600 cm^{-1} have been assigned to this group.

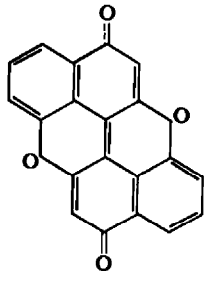
Xylindein, when fused with zinc dust, zinc chloride, sodium chloride melt gave a small yield of a yellow oil which yielded a yellow crystalline material $C_{30}H_{30}O_2$ after repeated chromatography on alumina. The UV spectrum of this material showed a marked resemblance to the spectra of some polynuclear aromatic hydrocarbons c.f.

[9] D. Hadzi and N. Sheppard, *J. Amer. Chem. Soc.* **73**, 5460 (1951).

perylene.[10] The small quantity of material available for investigation (2–3 mg) rendered a chemical investigation impracticable but a mass spectroscopic examination indicated a mol. wt. of 422 for the compound and isotopic abundance measurements on the parent ion and its satellite isotope peaks confirmed the molecular formula of $C_{30}H_{30}O_2$ obtained by combustion analysis. The fragmentation pattern suggested the presence of two unbranched amyl groups on a *peri*-xanthenoxanthene nucleus (I).[11] It was not possible to determine unequivocally the location of the substituents but most favoured were the 1,7 or 5,11 positions. Substituents at the 2,8 positions were definitely ruled out from the fragmentation pattern. Small traces of homologous compounds were detected of mol. wt. 408, 436, 450, 464, 478 and 492. Of these the compound of mass 450 was the most intense (2% of mass 422).[12*] The UV spectrum



I



II

of this experimentally derived compound was so similar to that of *peri*-xanthenoxanthene as to leave no doubt as to the nature of the nucleus of this compound (Fig. I).

Ether linkages have been observed by Clar[13] in the products of his modified zinc dust distillation, and condensation of hydrocarbon substituents on the nucleus have also been observed.[14] It therefore had to be established that the *peri*-xanthenoxanthene nucleus is present in the parent molecule and is not itself a condensation product. It is an accepted principle that the UV spectrum of the reduced acetyl derivatives of quinones bears a close similarity to that of the parent hydrocarbon.[15] The spectrum of dihydrotetra-acetylxylindein was therefore compared with that of *peri*-xanthenoxanthene. These spectra were similar in the region above 390 mμ, each exhibiting three bands, however the absorption of the xylindein derivative was shifted 5 mμ bathochromically (Fig. II). There was little resemblance at lower wavelengths. Since IR measurements had suggested the presence of an extended quinone chromophore in xylindein, the UV spectrum of *peri*-xanthenoxanthene 4,10-quinone (II) was compared with that of dimethylxylindein. The spectra were so much alike in form and intensity that there was no doubt that they arise from similar chromophores

* These results were determined by Dr. William shortly after a similar determination (in the same laboratory) had been made on a sample submitted by Dr. Blackburn. A comparison of these results with those obtained for Dr. Blackburn established the identity of the two compounds.

[10] A. D. Campbell, R. A. Elder, and G. W. Emerson, *J. Chem. Soc.* 3526 (1959).
[11] J. H. Beynon, *Proc. Xth. Colloquium Spectroscopium Internationale, Maryland*, 764 (1962).
[12] A. E. Williams, Private communication.
[13] E. Clar, *Ber. Dtsch. Chem. Ges.* **72**, 1655 (1939).
[14] H. Brockmann, F. Pohl, K. Maier and M. N. Haschad, *Liebigs Ann.* 553 (1942).
[15] H. Brockmann and G. Budde, *Chem. Ber.* **86**, 432 (1953).

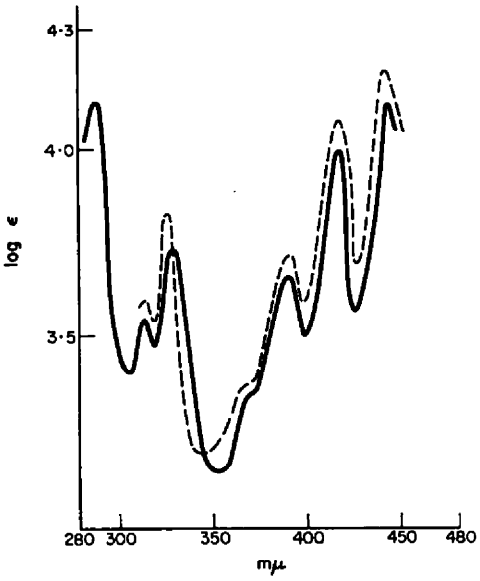


FIG. I. —— Zinc dust distillation product of xylindein
--- *peri*-Xanthenoxanthene

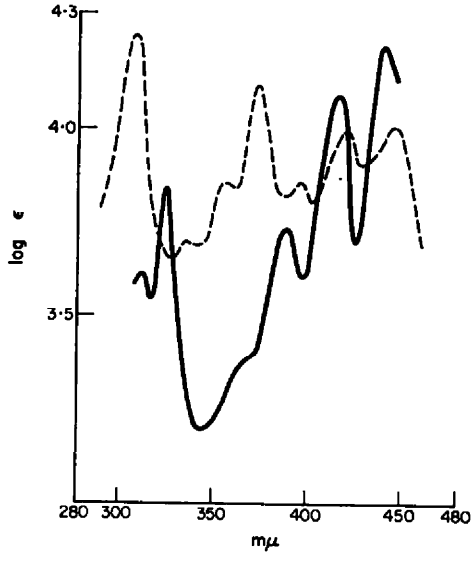


FIG. II. —— *peri*-Xanthenoxanthene
--- Dihydrotetra-acetylxylindein

(Fig. III). A hypsochromic shift occurs in the spectrum of dimethylxylindein, which is at a maximum of 22 mμ in the 600 mμ region compared with that of the synthetic quinone.

The absence of hydroxyl stretching absorption in the dihydrodi- and triacetates suggest that the free quinol hydroxyl groups in leucoxylindein derivatives are

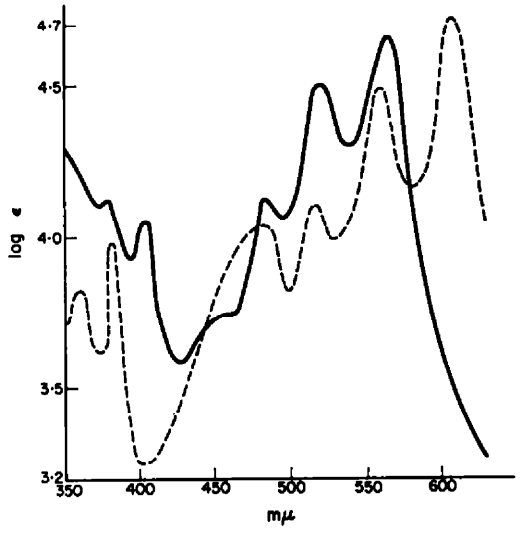
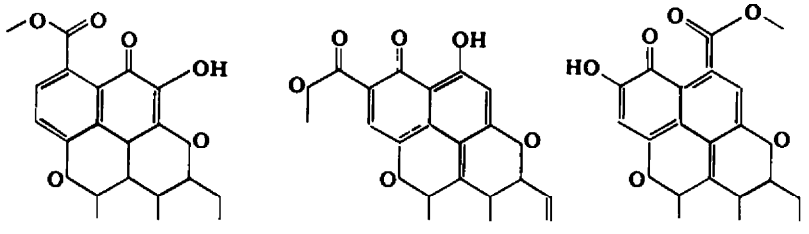


FIG. III. —— Dimethylxylindein
--- *peri*-Xanthenoxanthene-4,10-quinone

intermolecularly hydrogen bonded, also the lactone carbonyl absorption in dihydroxyxylindein and its diacetate is shifted from 1725 to 1660 cm^{-1} and in the triacetate appears as two bands at 1725 and 1660 cm^{-1}. These observations strongly suggest that the quinol hydroxyl groups are intramolecularly bonded with the lactone carbonyl group. Further support for the close proximity of these groups was obtained by oxidation of the quinol to xylindein when the lactone absorption band moves back to its normal position at 1725 cm^{-1}. Assuming xylindein to have a *peri*-xanthenoxanthenequinone nucleus those requirements are satisfied by three partial structures (III → V).



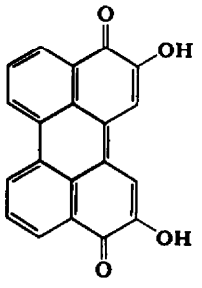
III IV V

Structure III has a counterpart in the structure assigned to xanthomegnin, a metabolite from the fungus Trichphyton megnini,[16] in which a similar shift in the lactone absorption band occurs on reduction to the quinol.

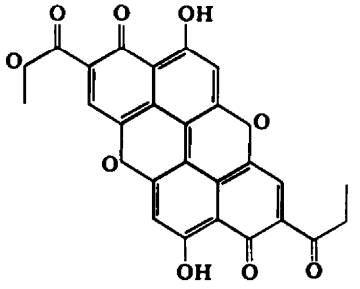
The IR spectrum of xylindein shows no absorption in the hydroxyl stretching region, implying that the hydroxyl groups present are internally hydrogen bonded and must, therefore, be close to the lactone or quinone carbonyl groups. In structures III and V there is the possibility of chelation between quinone carbonyl and hydroxyl

[16] G. Just, W. C. Day and F. Blanc, *Canad. J. Chem.* **41**, 74 (1963).

group on the same ring. However, the analogous 2,11-dihydroxyperylene-3,10-quinone (VI) shows bonded hydroxyl absorption at 3280 cm^{-1},[17] and 2-hydroxynaphthaquinone absorbs at 3020 cm^{-1},[18] it is therefore unlikely that such an arrangement of quinone and hydroxyl groups occurs in xylindein. On the other hand carbonyl groups and hydroxyl groups situated on different rings in a peri arrangement are known to show no hydroxyl stretching absorption and the normal quinone absorption

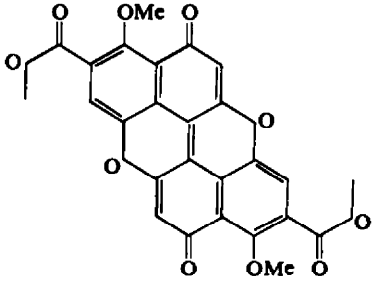


VI

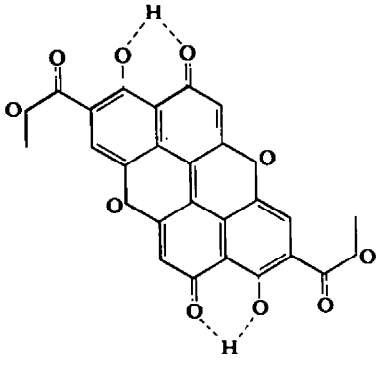


VII

is shifted to lower frequency. A comparison between the position of the carbonyl absorption in xylindein and its dimethyl ether shows a shift of 10 cm^{-1} to lower wavelength and we may therefore postulate a peri arrangement of these groups in xylindein. Only structure IV can accommodate such an arrangement and on this evidence we can write structure VII for xylindein which makes the molecule a derivative of the energetically less favoured 3,9-*peri*-xanthenoxanthenequinone. Although a compound believed to be the 3,9-quinone has been reported,[19] further tests for the validity of the proposed structure seemed necessary. Dihydrodimethylxylindein, prepared by catalytic reduction of the ether, showed no absorption at 1660 but absorbs strongly at 1724 cm^{-1} indicating that in this compound only unchelated lactone groups are present. This evidence supports the alternative and more favoured 4,10 arrangement of the quinone groups (VIII). Xylindein, from spectral evidence contains unchelated lactone groups so that



VIII



IX

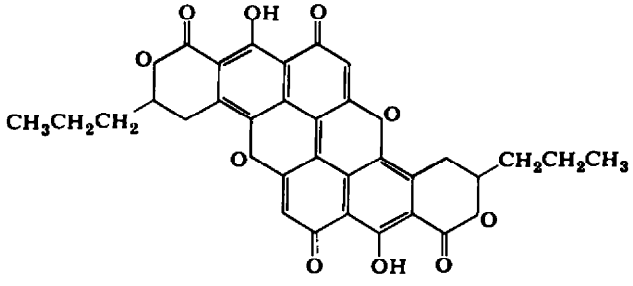
[17] A. W. Johnson, J. R. Quayle, T. S. Robinson, N. Sheppard and A. R. Todd, *J. Chem. Soc.* 2633 (1951).

[18] B. R. Brown and A. R. Todd, *J. Chem. Soc.* 1280 (1954).

[19] R. Pummerer, A. Reiche, G. von Krudener, H. Pfeiffer, E. Prell, W. Tuchmann and H. Wilsing, *Liebigs Ann.* **503**, 45 (1933).

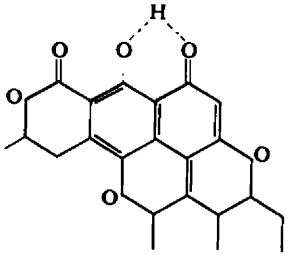
if we are to accept the latter evidence the hydroxyl groups must be preferentially bonded to the quinone carbonyl group, only becoming chelated with the lactone groups after reduction of the quinone carbonyl groups (IX).

The favoured location of the side chains, according to mass spectral evidence is at the 1,7 or 5,11 positions of the *peri*-xanthenoxanthene nucleus. Since the side chains are five membered, the detection of butyric acid among the products of sodium hydroxide fusion of dihydrotetra-acetylxylindein suggests that there is a point of weakness at the fourth carbon atom in the side chain of xylindein. If we are to accept the close proximity of lactone carbonyl and quinone carbonyl the only possible substitution positions are the 1,7; and inclusion of two of the amyl carbon atoms in the lactone ring would provide a point of attack by alkali at the fourth carbon atom (X).* Such an arrangement would mean that xylindein contains two δ lactone rings. The carbonyl groups of δ lactones normally absorb in the region 1750–1735 cm^{-1} while conjugated α, β unsaturation causes a decrease in the frequency of absorption of about 20 cm^{-1}. Thus the unchelated lactone carbonyl absorption of xylindein and its derivatives falls well within this range. The stability of chelated systems involving

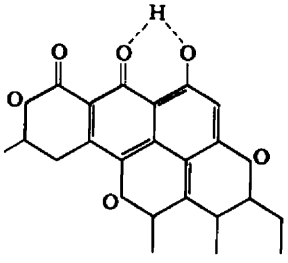


X

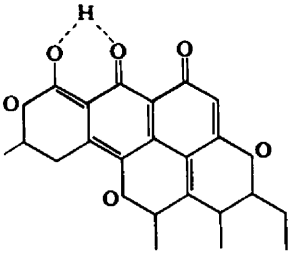
carbonyl and *peri* hydroxyl groups is due to contributions from more than one canonical form. Such forms lead to a weakening of the carbonyl bond and explain the observed shift of the carbonyl stretching frequency in the IR. A similar explanation has been put forward to explain the properties of the erythroaphins.[20] The hydrogen bonded form of xylindein might be expected to involve contributions XI and XII but it is not possible to envisage more than one fully conjugated contributing structure arising from the hydrogen bonded lactone form of xylindein, though the structure



XI



XII



XIII

* This structure proposed by Blackburn and Lord Todd.

[20] D. W. Cameron, R. I. T. Cromartie, Y. K. Hamied, P. M. Scott and Lord Todd, *J. Chem. Soc.* 62 (1964).

XIII might participate. Xylindein might therefore be expected to exist preferentially in the more highly stabilized quinone-bonded form.

The occurrence of acetates as derivatives of the 3,9 quinone, and ethers as derivatives of the 4,10 quinone might well be explained in terms of steric hindrance. The hydroxyl groups in the favoured 4,10 quinone are highly hindered and it might well be that the methyl groups are able to substitute in the more favoured 4,10 form, while the bulkier acetate groups substitute in the less favoured but less hindered 3,9 form.

The analytical results for xylindein and its derivatives are more consistent with the molecular formula $C_{32}H_{24}O_{10}$ for the parent pigment rather than the formula $C_{34}H_{26}O_{11}$ suggested by Kögl. No other functional groups have been detected and introduction of C_2H_2O into structure (X) would destroy the symmetry of the molecule. In addition, dinitroxylindein is readily prepared, presumably by substitution of the two free positions remaining on the *peri*-xanthenoxene nucleus. There is thus no other position at which the C_2H_2O residue might be attached.

The product from sodium hydroxide fusion of dihydrotetra-acetylxylindein apparently retains the xylindein chromophore intact. The green product readily formed a leuco acetate and the quinone carbonyl band at 1630 cm^{-1} disappeared. The reduced acetate has visible absorption maxima at 475, 436 and 407 mμ comparable with the spectrum of dihydrotetra-acetylxylindein. The absence of lactone carbonyl and carboxyl absorption in the IR suggests that ring opening and decarboxylation has occurred. Elemental analysis on the leuco acetate was in best agreement with the tetra-acetate of molecular formula $C_{32}H_{26}O_{10}$. The quinone could only be recrystallized from nitrobenzene and has an analysis in best agreement with the molecular formula $C_{20}H_{12}O_6$ attempts to further purify this compound were unsuccessful.

EXPERIMENTAL

All m.ps are uncorrected. Unless otherwise stated IR spectra were determined in KBr discs on a Perkin-Elmer 237 spectrophotometer and UV and visible spectra on a Unicam S.P. 500 instrument.

Xylindein. Finely ground green wood was air dried. The wood (1 kg) was extracted with hot $CHCl_3$ in a Soxhlet extractor until the siphoning solution, initially bottle green, became colourless. The same solvent (5 l.) was used to extract a further 9 kg of wood. The solution was then concentrated to half bulk, cooled and filtered. (Residue A and filtrate A.)

Residue A. The blue-black waxy solid (60 g) was extracted with aqueous phenol (500 ml; 80%) at 60°. The dark green extract, after cooling and filtering deposited rhombic plates of *xylindein.* The cycle was repeated 5 times until the residual solid was pale green. Typically, 5 to 10 g xylindein was obtained from 60 g crude and after recrystallizing 3 times from phenol had m.p. above 300°. (Found: C, 67·5; H, 4·4. Calc. for $C_{34}H_{26}O_{11}$: C, 66·9; H, 4·25. Calc. for $C_{32}H_{24}O_{10}$: C, 67·6; H, 4·3%.)

Xylindein is slightly soluble in hot $CHCl_3$, acetic acid, formic acid and high boiling solvents such as nitrobenzene, diethylene glycol and diethylene glycol dimethyl ether. It crystallized from 100% formic acid as small plates. λ_{max} (in $CHCl_3$) 647, 603, 423, 405, 380 mμ (log ε 4·46, 4·31, 4·06, 4·17, 4·19), ν_{max} 3070, 2965, 2940, 2880, 1726 (lactone C—O), 1631 (quinone C—O), 1608, 1480, 1421, 1353, 1280, 1193, 1108 cm^{-1}.

The pale green residue of *dihydroxyxylindein* was insoluble in most solvents but was slightly soluble in hot formic acid. Yellow solutions in this solvent turned green in air. ν_{max} 2965, 2940, 2880, 1660 (chelated lactone), 1639, 1608, 1503, 1450, 1390, 1303, 1265, 1248, 1191, 1149, 1125, 1070, 1035, 835 cm^{-1}.

Filtrate A. Was evaporated to dryness. The green waxy residue (80 g) was soluble in pet. ether, alcohol and acetic acid. Hot extracts of the material were brown and turned slowly green on cooling with precipitation of an amorphous green solid. The green waxy solid (10 g) was refluxed with acetic anhydride (100 ml), Zn dust (1 g) and anhydrous sodium acetate (0·3 g). After 1 hr the brown solution

was cooled, filtered and poured into water to yield a brown amorphous product (7·6 g) which was soluble in $CHCl_3$, alcohol and benzene but could not be crystallized.

Diacetylxylindein. Xylindein (100 mg), acetic anhydride (20 ml) and H_2SO_4 (1 drop) was warmed at 100° for 30 min. The blue solution became red. The red *diacetylxylindein* (98 mg) which separated as rhombic crystals was filtered off and washed with EtOH and ether. (Found: C, 65·85; H, 4·48; $COCH_3$, 13·9. $C_{36}H_{28}O_{12}$ requires: C, 66·3; H, 4·29; $COCH_3$, 13·2. $C_{38}H_{30}O_{12}$ requires: C, 65·7; H, 4·23; $COCH_3$, 12·4%.)

The diacetate could not be recrystallized without some decomposition. It was almost insoluble in the common organic solvents but crystallized from warm nitrobenzene. ν_{max} 1775 (acetyl C—O), 1730 (unchelated lactone C—O), 1640 (quinone C—O) cm^{-1}.

Dihydrodi-, tri-, and tetra-acetylxylindein. A mixture of xylindein (100 mg), acetic anhydride (3 ml), Zn dust (10 mg) and anhydrous sodium acetate (trace) was refluxed for 15 min. The solution turned blue, then green and then golden brown. The reaction mixture was poured into water and the brown amorphous mixture of dihydrodi-, tri-, and tetra-acetates which separated was filtered off and dissolved in $CHCl_3$ (35 ml). The mixture was placed on a column of $MgCO_3$ and the column developed with $CHCl_3$. Three bands were resolved: a fast moving yellow band (I) and two slower moving orange bands (II and III). All three bands were eluted with $CHCl_3$ but bands II and III were not completely resolved. The solution containing the bulk of band III was evaporated to yield impure *dihydrodiacetylxylindein* which crystallized from $CHCl_3$ as yellow needles (10 mg) m.p. above 300°. (Found: C, 64·4; H, 3·9; $COCH_3$, 15·3. $C_{36}H_{30}O_{12}$ requires: C, 66·1; H, 4·6; $COCH_3$, 13·2%.) ν_{max} 1775 (acetate C—O), 1660 (chelated lactone C—O) cm^{-1}.

The solution containing band II was concentrated to 15 ml and re-applied to a column of $MgCO_3$. Elution with $CHCl_3$ gave two bands, the faster moving band gave an orange yellow solution which on evaporation and crystallization from $CHCl_3$ gave *dihydrotriacetylxylindein* (30 mg) as long yellow needles m.p. above 300° (dec). (Found: C, 65·7; H, 4·5; $COCH_3$, 18·9. $C_{38}H_{32}O_{13}$ requires: C, 65·5; H, 4·63; $COCH_3$, 18·5%.) ν_{max} 1779 (C—O acetate), 1726 (unchelated lactone C—O), 1661 (chelated lactone C—O) cm^{-1}. λ_{max} ($CHCl_3$) 457, 430, 393 mμ (log ε 4·00, 4·28, 4·01).

The eluents containing band I were combined, evaporated to 30 ml and re-applied to a column of $MgCO_3$. Evaporation of the eluent, containing the fastest moving band yielded *dihydrotetra-acetylxylindein* (46 mg) which crystallized from $CHCl_3$ as yellow needles m.p. >300°. (Found: C, 64·7; H, 4·4; CH_3CO, 21·0. $C_{40}H_{34}O_{14}$ requires: C, 65·0; H, 4·6; CH_3CO, 23·3. $C_{42}H_{36}O_{15}$ requires: C, 64·6; H, 4·6; CH_3CO, 22·0%.) ν_{max} 1781 (acetyl C—O), 1725 (unchelated lactone C—O) cm^{-1}. λ_{max} 446, 419, 395–397, 375, 357–358, 337–339, 311 mμ (log ε 4·05, 4·04, 3·86, 4·11, 3·86, 3·70, 4·25).

Dihydrodiacetylxylindein. A solution of diacetylxylindein (20 mg) in $CHCl_3$ (40 ml) was reduced with H_2 at room temp in the presence of Adams catalyst; 1 ml H_2 was absorbed and the acetate dissolved to yield a yellow solution which on evaporation and crystallization of the residue from $CHCl_3$ gave yellow needles of *dihydrodiacetylxylindein* identical with the product prepared by reductive acetylation of xylindein. ν_{max} 1775 (acetate C—O), 1660 (chelated C—O) cm^{-1}.

Dimethylxylindein. Powdered xylindein (1·0 g) was refluxed for 0·5 hr with $CHCl_3$ (500 ml). An excess of an etherial solution of diazomethane was added to the cooled suspension and the mixture set aside for 2 hr. There was slow evolution of N_2 and the solution became purple. Ether, excess diazomethane and $CHCl_3$ (300 ml) was distilled off and the solution filtered. The filtrate was separated on a column of $MgCO_3$ (40 × 2 cm) with $CHCl_3$ as eluent. Two bands were resolved; a green band, which remained at the top of the column and a purple band which was eluted with $CHCl_3$. Evaporation of the eluent gave an amorphous purple brown solid (0·2 g) which after crystallization and recrystallization from nitromethane gave *dimethylxylindein* as purple rods m.p. 270°d. (Found: C, 68·3; H, 4·7; OMe, 10·3. $C_{34}H_{28}O_{10}$ requires: C, 68·5; H, 4·7; OMe, 10·4%.) ν_{max} 1730 (unchelated lactone C—O), 1640 (quinone C—O). λ_{max} ($CHCl_3$) 566, 521, 485, 456–50, 403, 281 mμ (log ε 4·66, 4·51, 4·12, 3·74, 4·05, 4·13).

Dihydrodimethyldiacetylxylindein. Dimethylxylindein (70 mg) was refluxed with acetic anhydride (6 ml), Zn dust (20 mg) and anhydrous sodium acetate (trace) for 45 min. The purple solution turned yellow and was filtered hot. The filtrate was poured into water and the precipitate crystallized from benzene to yield *dihydrodimethyldiacetylxylindein* as yellow needles (55 mg) m.p. above 270 d. (Found: C, 66·7; H, 5·1; OMe, 9·1. $C_{38}H_{34}O_{12}$ requires: C, 66·9; H, 4·85; OMe, 9·1%.) ν_{max} 1775 (acetate C—O), 1725 (unchelated lactone C—O) cm^{-1}.

Dihydrodimethylxylindein. Dimethylxylindein (25 mg) was reduced in $CHCl_3$ solution (50 ml) at room temp and atm. press. in the presence of Adams catalyst. The mixture absorbed 0·8 ml H_2 and the solution became golden yellow and exhibited an intense green fluorescence. The mixture was filtered and evaporated to yield *dihydrodimethylxylindein* as an amorphous yellow solid which crystallized from $CHCl_3$ as yellow needles. ν_{max} 3280 (broad OH in Nujol), 1723 (unchelated lactone) cm^{-1}.

Xylindein dimethyl ester. A suspension of powdered xylindein (0·55 g) in 2 N NaOH (30 ml) was shaken for 16 hr. The mixture was filtered and the residual dark green amorphous *sodium salt* was washed with EtOH and dried. Silver nitrate solution (100 mg; in 10 ml H_2O) was added to a solution of sodium salt (130 mg) in water (40 ml). An olive precipitate was immediately deposited. The mixture was set aside for 0·5 hr, filtration gave the silver salt (140 mg) which was washed with water and dried over NaOH *in vacuo*. The xylindein silver salt (130 mg), suspended in dry $CHCl_3$ (5 ml), was treated with MeI (3 ml) and the mixture set aside for 18 hr. A dark blue solution resulted. Filtration yielded a grey-green residue which was extracted with $CHCl_3$ and then treated with a similar mixture of $CHCl_3$ and MeI. Only slight colouration of the supernatant liquid occurred. The solution was filtered after 18 hr and the filtrates combined and evaporated to yield crude *xylindein dimethyl ester* (58 mg). Attempts to crystallize the ester from $CHCl_3$ and CCl_4 were unsuccessful. The material produced a homogeneous blue band on columns of $CaCO_3$ and $MgCO_3$ which was eluted with $CHCl_3$. (Found: C, 66·8; H, 5·7; OMe, 11·0. $C_{34}H_{32}O_{11}$ requires: C, 64·6; H, 5·1; OMe, 9·2%.) ν_{max} 1733 (ester C—O), 1630 (quinone C—O) cm^{-1}. ν_{max} 3400–3350 (OH) in Nujol.

Dihydroxyxylindein. Xylindein (50 mg) was dissolved in hot formic acid (300 ml). The solution was cooled and shaken with H_2 at room temp and atm. press. in the presence of Adams catalyst. The mixture absorbed 22 ml H_2 (1 mole equiv.) and yellow *dihydroxyxylindein* was deposited. (Found: C, 67·2; H, 4·5. $C_{32}H_{26}O_{10}$ requires: C, 67·4; H, 4·6%.) ν_{max} 1732 (very weak lactone C—O), 1660 (chelated lactone C—O). ν_{max} 3445, 3355 (OH) (nujol) cm^{-1}. λ_{max} 448, 422, 338–339, 325–330, 255 mμ (log ε 3·81, 3·74, 3·48, 4·17).

Formic acid hydrolysis of dihydroxylindein acetates

(i) *Dihydrotetra-acetylxylindein.* The acetate (23 mg) was refluxed with formic acid (100 ml). The solution darkened and after 7 min a pale yellow solid separated. Heating was continued for a further 13 min, then the mixture was cooled and filtered to yield *dihydroxylindein monoacetate* (16 mg). (Found: C, 67·2; H, 4·8; $COCH_3$, 1·3. $C_{34}H_{28}O_{11}$ requires: C, 66·6; H, 4·6; $COCH_3$, 7·0%.) ν_{max} 1775 (acetate C—O), 1660 (chelated lactone C—O) cm^{-1}. Solutions of this substance turned green in air.

(ii) *Dihydroxylindein.* The acetate (19·5 mg) was refluxed with formic acid (20 ml). The acetate quickly dissolved and *dihydroxylindein monoacetate* was deposited after 3 min (16 mg).

(iii) *Dihydrodiacetylxylindein.* The acetate quickly dissolved in hot formic acid but no precipitate was deposited. After 30 min, evaporation of the acid yielded unchanged dihydrodiacetylxylindein.

Oxidations with lead tetra-acetate

(i) *Dihydroxylindein monoacetate.* Dihydrotetra-acetylxylindein (500 mg) was refluxed with formic acid (500 ml; 100%). After 10 min the solution containing suspended dihydroxylindein monoacetate was concentrated to 200 ml, cooled to 60° and excess of a saturated solution of lead tetra-acetate in glacial acetic acid added. The mixture immediately turned green and a characteristic bronze lustrous solid floated on the surface of the solution. The solid was filtered off and identified as *xylindein* (350 mg). ν_{max} 1726 (lactone C—O), 1675 (quinone C—O) cm^{-1}.

(ii) *Dihydrotetra-acetylxylindein.* The acetate (500 mg) was refluxed with glacial acetic acid (500 ml) for 15 min. The mixture was cooled and excess of a saturated solution of lead tetra-acetate in glacial acetic acid was added. After 16 hr at room temp red diacetylxylindein was deposited (420 mg). ν_{max} 1775 (acetate C—O), 1730 (lactone C—O), 1675 (quinone C—O) cm^{-1}.

The triacetate and diacetate were similarly oxidized, the latter was completely oxidized to the red diacetate within 30 min. The IR spectra of all the oxidation products were identical.

Zinc dust distillation. 138 portions, each comprising a mixture of xylindein (20 mg), NaCl (200 mg), Zn dust (0·4 g) and $ZnCl_2$ (1·0 g) were heated at 230° for 10 min and at 290–300° for a further 15 min. Dense yellow and white fumes were evolved which condensed on the cool parts of the tubes. The tubes were crushed and the mixture digested with 3 N HCl (1 l.) for 2 hr. After filtration, the

residue was washed with water until the washings were neutral. After drying at 100° for 2 hr, the glass and reduced material was extracted with benzene (250 ml) until the extracting liquid was colourless. The green benzene extract was concentrated to 150 ml and washed with NaO Haq (2 × 200 ml; 5%). A small quantity of green solid was precipitated. The benzene layer was filtered, washed (3 times with water), dried (Na_2SO_4) and evaporated to 50 ml. This solution was separated on a column of alumina (7·5 g. Activity I) with benzene as developing solvent. Three bands were resolved; a yellow fluorescent band was eluted with benzene, an orange band with acetone and a brown band with MeOH. The benzene eluate after evaporation yielded a yellow gum (146 mg) which was dissolved in a benzene–pet. ether (1:9) and reseparated on alumina (75 g). The fractions (27) of 100 ml each were collected [21 fractions benzene–pet. ether (1:9), 3 fractions benzene–pet. ether (1:4) and 3 fractions benzene].

Quantities of material from each fraction were:— 8·7, 11·9, 1·8, 0, 2·8, 4·0, 12·5, 13·8, 5·8, 7·5, 3·5, 3·6, 3·2, 1·2, 2·6, 3·5, 6·5, 1·5, 2·3, 1·2, 1·5, 2·0, 2·3, 1·2, 21·9, 13·2, 2·7 mg respectively.

λ_{max} Fraction 8, 443, 417, 391, 371, 329, 314, 289, 273 mμ.

Fraction 10, 446, 419, 393, 374, 332, 319, 294, 281 mμ.

Fraction 17, 446, 419, 394, 331, 317, 292, 280 mμ.

Fraction 25, 440, 419, 392, 371, 350, 336, 306, 288 mμ.

Fractions 7, 8 and 9 were combined (32 mg), dissolved in pet. ether (b.p. 40–60°) and separated on alumina (75 g; Grade I). Seven fractions were collected (2 × 100 ml [pet. ether], 5 × 100 ml [benzene–pet. ether 1:10]). The bulk of material occurred in fractions 3–6. These fractions were combined and after crystallization from abs. alcohol yielded yellow needles of 1,7 *di-n-amyl* peri-*xanthenoxanthene* (9·6 mg) m.p. 181–183°. (Found: C, 85·25; H, 7·0, M.W. [Rast] 379, 388. $C_{30}H_{30}O_2$ requires: C, 85·3; H, 7·2%.) λ_{max} 442, 414, 390, 370, 314, 289, 277 (log. ε 4·14, 4·01, 3·68, 3·75, 3·56, 4·13, 4·17) mμ.

The mother liquors from the crystallization of the analytical sample were evaporated and combined with fractions 11–21. The material, in benzene solution, was applied to a silica gel column and the column was developed with benzene. A yellow fraction with an intense green fluorescence was eluted and, after evaporation yielded yellow needles which melted over a wide range above 115°. This material was not examined further.

Alkali fusion. A mixture of dihydrotetra-acetylxylindein (1 g), NaOH (1 g) and water (1 ml) was heated at 240° in a stream of N_2 for 30 min. The fused mixture was cooled and water (30 ml) added. The resulting green mixture was acidified (H_2SO_4; 100 ml; 2N), and the dark green insoluble precipitate filtered off and crystallized from nitrobenzene to yield rods (560 mg) which decomposed without melting above 230°. (Found: C, 68·9; H, 3·6. $C_{20}H_{12}O_6$ requires: C, 69·0; H, 3·45%.) ν_{max} 3570, 2965, 2940, 1630, 1485, 1382, 1345, 1235, 1210, 1060, 1035 cm^{-1}.

The yellow filtrate which smelled strongly of butyric acid was continuously extracted with ether; vapour phase chromatography showed the presence of n-butyric and acetic acids.

The green alkaline fusion product (120 mg) was refluxed with acetic anhydride (3 ml), anhydrous sodium acetate and Zn dust for 1·5 hr. The brown mixture was filtered hot, cooled and the yellow solid (45 mg) which was deposited filtered off. The filtrate was hydrolysed with water to yield a brown solid (95 mg). Each of these fractions was dissolved in $CHCl_3$ (5 ml) and separated on a column of $MgCO_3$ (2 × 35 cm) using $CHCl_3$ as developing phase. The yellow material contained 4 components separating as a yellow band which remained at the top of the column, two orange bands which moved down the column but were not eluted, and a diffuse lemon-yellow band which was eluted to give a solution with an intense green fluorescence. The brown material behaved similarly but in addition produced a brown and green band which remained at the top of the column. The lemon eluates were combined and evaporated and the residue crystallized from benzene–EtOH to yield red needles. This material was reseparated on $MgCO_3$ (35 × 2 cm) from benzene solution. The yellow fluorescent eluate yielded yellow needles m.p. 250°. (Found: C, 67·45; H, 4·6; $COCH_3$ 28·5. $C_{32}H_{36}O_{10}$ requires: C, 67·4; H, 4·6; $COCH_3$ 29·5 [4 acetyl]%.) ν_{max} 1770, 1613, 1385 cm^{-1}.

Dinitroxylindein. Xylindein (1 g) was refluxed with HNO_3 (*d.* 1·42; 6 ml) for 45 min. Filtration of the reaction mixture yielded *dinitroxylindein* (350 mg) which crystallized from 20% aqueous phenol as plates m.p. > 250°d (Found: C, 58·3; H, 3·3; N, 4·5. $C_{32}H_{32}N_2O_{14}$ requires: C, 58·4; H, 3·3;

N, 4·3%.) ν_{max} 2960, 2940, 2880, 1730, 1675, 1631, 1605, 1565, 1531, 1483, 1415, 1378, 1334, 1208, 1125, 1010, 983, 828, 211 cm^{-1}.

Nitric acid oxidation. Xylindein (0·5 g) was refluxed with HNO_3 (*d.* 1·42; 10 ml) at 130–150° for 5·5 hr. The clear yellow solution and a small quantity of white solid resulted. The mixture was diluted and continuously extracted with ether (25 ml) for 16 hr. The extract was washed with 2 N NaOH (5 ml), the washings made just acid with 2 N H_2SO_4 and extracted with ether (4 × 10 ml). Evaporation of the ether at room temp gave yellow needles of *picric acid* which was recrystallized from EtOH, m.p. and mixed m.p. with picric acid 121°.

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