Hypoxyxylerone. A Novel Green Pigment from the Fungus *Hypoxylon fragiforme* (Pers.: Fries) Kickx

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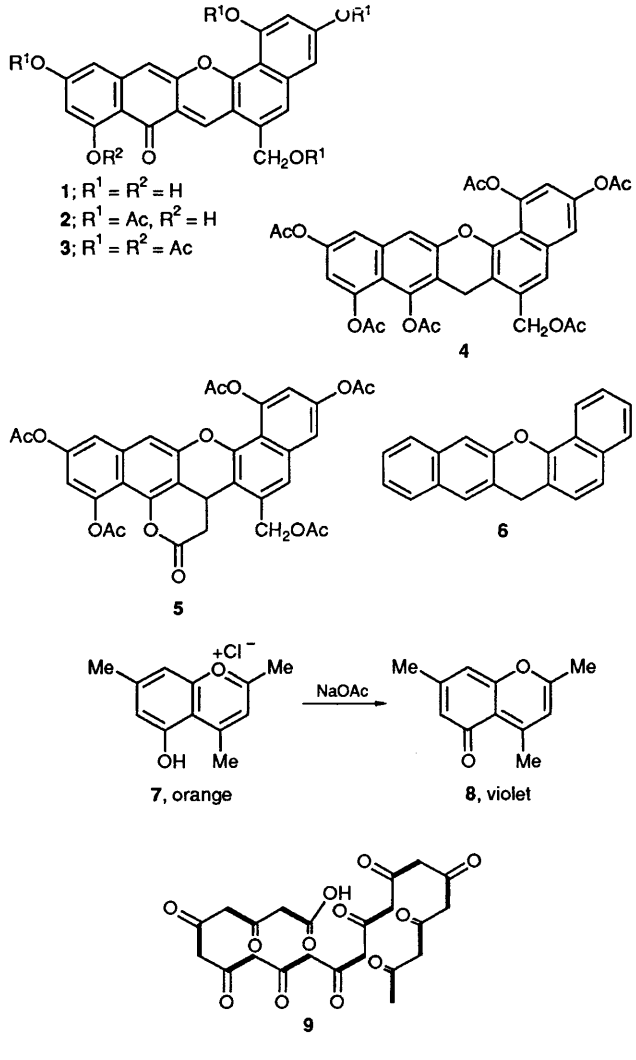
A novel green pigment from the fungus *Hypoxylon fragiforme* (Pers.: Fries) Kickx has been identified as 1,3,9,11-tetrahydroxy-6-hydroxymethyldibenzo[*b,h*]xanthen-8-one and named hypoxyxylerone; the X-ray crystal structure of a tetraacetate derivative has been determined.

The chlorophylls are the most important and widespread of the natural colourants. However, their green colour is exceptional and only a few other naturally occurring structures are green. These include the bile pigments (biliverdins) which are responsible for the colour of egg shells and the external cuticle of some insects, the aphinins[1] present in certain aphid species such as greenfly (*Macrosiphium rosae* L), xylindein[2,3] from the wood-staining fungus *Chlorociboria aeruginosa* (Oeder *ex.* S. F. Gray) Seaver, Lo-kao,[4] a historical dyestuff from *Rhamnus dahurica* (Pall), and *Rhamnus tinctoria* (Walldst & Kitt) apparently of unknown structure, used by the Chinese for dyeing silk, dehydrotechtol (technaquinone)[5] from teak wood (*Techtona grandis*), austrovenetin[6] from the toadstool *Dermocybe austroveneta* (Cleland) Moser & Horak, and arosine and arosinine[7] from the roots of *Glaucium flavum* Cr *var vestatum*. Chlorophyll and the bile pigments show close structural relationships; the aphinins, xylindein, dehydrotechtol, austrovenetin and perhaps Lo-kao are quinones, whereas arosine and arosinine are tetrasubstituted quaternary oxoaporphine alkaloids.

During our examination of members of the *Hypoxylon* genus,[8] several species were observed to produce an intensely green mycelium, when grown on malt extract medium. This green colouration is widespread in freshly isolated wild strains of *H. fragiforme* and *H. howieanum* Peck. We now report the isolation and structural elucidation of the compound, which we name hypoxyxylerone, that is responsible for this green colouration.

Hypoxyxylerone **1**, $C_{22}H_{14}O_7$, m.p. >300 °C, *m/z* (EI) 390.07395 (M^+, 42%); ν_{max}(KBr)/cm^{-1} 3700–2400, 1659, 1629, 1590, 1535 and 1500; λ_{max}(EtOH)/nm 240 (log ε, 4.56), 306 (4.22), 319 (4.18), 330 (4.24), 338 (4.21), 342 (4.23), 348 (4.28), 354 (4.26), 424 (3.96) and 656 (4.06), crystallises from pyridine as dark green, thin, hair-like needles with a bronze lustre. The pigment, which is insoluble in non-polar solvents, was extracted from the mycelium with ethanol. The compound behaves like a quinone; the green alcoholic solution is decolourised by dithionite and the colour is restored on shaking in air. Acetylation with cold acetic anhydride and pyridine produces a bright blue crystalline tetraacetate **2**, $C_{30}H_{22}O_{11}$, m.p. >285 °C (decomp.), *m/z* (EI) 558 (M^+, 80%); ν_{max}(KBr)/cm^{-1} 3500, 1770, 1749, 1661, 1628, 1575, 1561 and 1542; λ_{max}($CHCl_3$)/nm 238 (log ε, 4.44), 255 (4.41), 295 (4.44), 313sh (4.47), 322 (4.55), 350 (4.33), 388 (4.03), 412 (3.95), 616 (4.04) and 670 (4.04), and a more intensely blue pentaacetate **3**, $C_{32}H_{24}O_{12}$, m.p. 265 °C, *m/z* (EI) 600 (M^+, 15%); ν_{max}(KBr)/cm^{-1} 1770, 1744, 1670, 1620, 1583, 1553 and 1544; λ_{max}($CHCl_3$)/nm 242 (log ε, 4.33), 302sh (4.35), 312 (4.43), 318 (4.47), 322 (4.49), 336sh (4.27), 368 (3.85), 374 (3.84), 386 (3.75), 410 (3.60), 572 (3.88) and 652 (3.82). The ^{1}H NMR spectrum [$(CD_3)_2SO$] of **1** shows five hydroxy resonances; one at δ 14.3 is strongly chelated. The other resonances are those of seven aromatic–alkene protons between δ 6.0 and 8.6, four of which are *meta* coupled (*J* 2.2 Hz) and a benzylic methylene attached to oxygen at δ 4.8. The ^{13}C NMR spectrum of **1** indicated the presence of 14 quaternary carbons, δ_C 180.4–106.56 (including a carbonyl group and six other carbons attached to oxygen), seven methine carbons, δ_c 136.06–95.4, and a methylene carbon bonded to oxygen at δ_c 60.95. In the ^{1}H NMR of the blue acetate **2** the chelated OH occurs at δ 13.39. Reductive acetylation, or catalytic reduction followed by acetylation, of **2** produces the colourless hexaacetate **4**, $C_{34}H_{28}O_{13}$, m.p. 238 °C, *m/z* (EI) 644 (M^+, 3.0%); ν_{max}(KBr)/cm^{-1} 1770, 1759, 1734, 1640, 1617, 1582 and 1574; λ_{max}($CHCl_3$)/nm 244 (log ε, 4.86), 312 (4.22) and 336 (4.04); λ_{min} 276 (4.11) and 332 (3.97); the large log ε values strongly suggested the presence of two α-hydroxynaphthalene type chromophores in each molecule. Acetylation of hypoxyxylerone **1** with acetic anhydride and pyridine over 24 h results in the formation of the lactone pentaacetate **5**, $C_{34}H_{26}O_{13}$, m.p. 279–291 °C, *m/z* (EI) 642 (M^+, 3.0%); ν_{max}(KBr)/cm^{-1} 1773, 1645, 1638, 1617, 1590



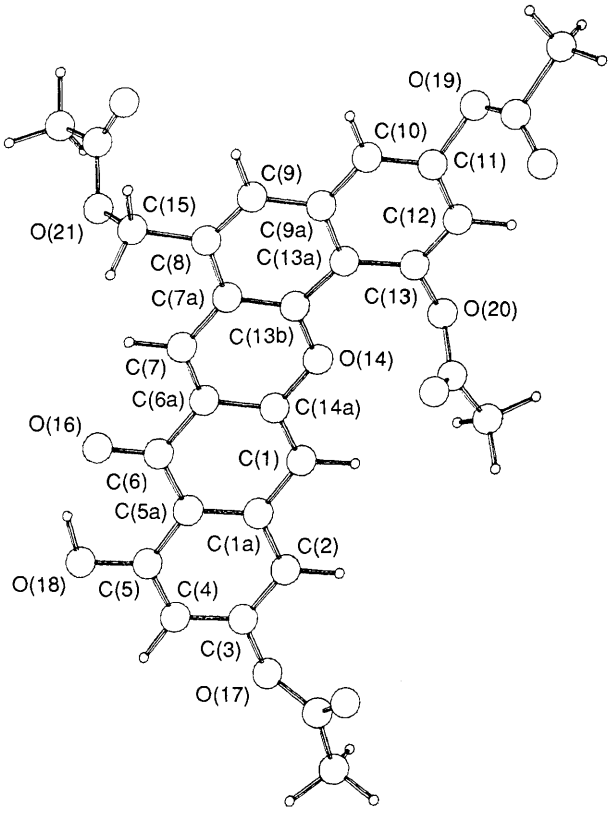


Fig. 1 The structure of the tetraacetate **2** showing the atom numbering scheme (which differs from that adopted in the systematic labelling of the compound); selected bond lengths (Å) and angles (°): C(1)–C(1a) 1.443(11), C(1a)–C(2) 1.394(11), C(1a)–C(5a) 1.422(8), C(2)–C(3) 1.386(12), C(3)–C(4) 1.361(12), C(4)–C(5) 1.378(12), C(5)–C(5a) 1.401(12), C(5a)–C(6) 1.453(12), C(6)–C(6a) 1.476(11), C(6a)–C(7) 1.340(11), C(6a)–C(14a) 1.437(8), C(7)–C(7a) 1.436(11), C(7a)–C(8) 1.437(11), C(7a)–C(13b) 1.388(8), C(8)–C(9) 1.348(11), C(9)–C(9a) 1.412(11), C(9a)–C(10) 1.416(12), C(9a)–C(13a) 1.424(8), C(10)–C(11) 1.342(12), C(11)–C(12) 1.395(12), C(12)–C(13) 1.353(12), C(13)–C(13a) 1.424(11), C(13a)–C(13b) 1.416(11), C(13b)–O(14) 1.367(9), O(14)–C(14a) 1.380(9), C(1)–C(14a) 1.343(10), C(6)–O(16) 1.246(10), C(5)–O(18) 1.350(11), C(3)–O(17) 1.412(10), C(11)–O(19) 1.490(12), C(13)–O(20) 1.400(10), C(8)–C(15) 1.518(11), C(15)–O(21) 1.451(10); C(13b)–O(14)–C(14a) 121.2(6), C(7a)–C(13b)–O(14) 121.2(7), C(7)–C(7a)–C(13b) 117.5(8), C(6a)–C(7)–C(7a) 121.6(8), C(14a)–C(6a)–C(7) 119.6(8) and O(14)–C(14a)–C(6a) 118.8(7)

and 1575; λ_{max}($CHCl_3$)/nm 244 (log ε, 4.78), 284 (4.23) and 312 (4.22); λ_{min} 278 (4.22). The formation of **5** must involve attack by an anhydride anion similar to that seen in the Perkin reaction. In this instance however, addition occurs across the 1,4-position of the enone. Structure **5** is confirmed by the appearance in the ^1H NMR ($CDCl_3$) spectrum of a pair of single-proton doublet of doublet resonances at δ 2.80 and 3.55 which together constitute a methylene group, the protons of which are coupled to a methine proton resonance at δ 4.84. The UV spectrum resembles that of the reduced pigment, *i.e.* that of a substituted naphthalene.

The skeleton of hypoxyxylerone is that of a substituted dibenzo[*b*,*h*]xanthene **6**, and is the first naturally occurring example reported; the parent compound is colourless and dissolves in concentrated sulphuric acid to yield a greenish brown solution.[9] The presence of the carbonyl group in hypoxyxylerone at the 8-position obviously has a profound effect on the colour and gives rise to a system which has some analogy with the anhydro-bases of the anthocyanidin series, which are formed when 5-hydroxybenzopyrylium chlorides are treated with base. Compounds of this type are capable of occurring in the *o*-quinonoid form and as such are intensely coloured. Thus 5-hydroxy-2,4,7-trimethyl-1-benzopyrylium hydrochloride **7** is orange, yielding a violet anhydrobase **8** on treatment with base.[10] Similarly 5-hydroxy-7-methyl-2,4-diphenyl-1-benzopyrylium chloride is brown-red, yielding a blue anhydro-base with sodium acetate.[11] The hypoxyxylerone system is remarkably stable compared with the 5-hydroxybenzopyrylium system, the stability of which is dependent on the presence of other substituents in the system and on the solvent.

Inspection of the hypoxyxylerone structure **1** suggests a biosynthetic derivation by folding of an undecaketide chain as depicted in **9**. Some polycyclic compounds have been shown to be derived from a folded decaketide chain, *e.g.* aflatoxins, but the use of larger polyketide chains is relatively uncommon and leads to simpler cyclic compounds, where a large proportion of the polyketide chain is used to produce a long straight-chain fragment.[12] If hypoxyxylerone is derived from a single long-chain polyketide, it affords a unique example of undecaketide chain folding.

We have confirmed the structure by a single crystal X-ray analysis (Fig. 1) of the tetraacetate **2**.†

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† *Crystal data* for **2**: $C_{30}H_{22}O_{11}$, M = 558.5, triclinic, space group $P\bar{1}$, a = 8.014(2), b = 10.980(9), c = 15.208(9) Å, α = 91.60(9), β = 92.14(7), γ = 101.24(4)°, V = 1310.9(6) Å^3, $F(000)$ = 580, D_c = 1.414 g cm^{-3}, Z = 2. The data for 2556 unique reflections [$I > 2.0\sigma(I)$] were measured on a Stoe automatic 4-circle diffractometer. The structure was elucidated by direct techniques (SHELX-86) and refined by least squares calculations (SHELX-76) to a final unweighted R of 0.063. Several refinement strategies were tried including a weighted I in proportion to the inverse $\sigma(I)$ and blocked full-matrix anisotropic least-squares refinement separating the fused ring section from the more flexible acetate groups. R-factor convergence occurred with final shifts/e.s.d. factors less than 0.1. The lowest R was obtained from unweighted intensities with the hydrogen atoms placed and riding on their conjugated atoms and least-squares variance of the anisotropic parameters of the acetate groups. A final Fourier difference-map revealed less than 0.5 e Å^{-3} residual electron density. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Data Centre. See Notice to Authors, Issue No. 1.