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Absolute configuration and tautomeric structure of xylindein, a blue–green pigment of *Chlorociboria* species

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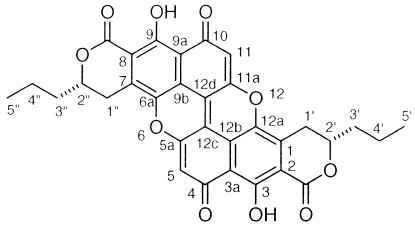
Abstract

The *S* absolute configuration of both chiral centers of xylindein was assigned using X-ray crystallographic heavy atom analysis after its conversion to a synthetic derivative. Crystallographic analysis of xylindein crystallized with phenols revealed that the proposed structure is the proper tautomer in the crystals. © 2000 Published by Elsevier Science Ltd.

Keywords: *Chlorociboria* species; Leotiaceae; Absolute configuration; Tautomeric structure; Pigment; Extended quinone; peri-Xanthenoxanthene-4-10-quinone; Xylindein

1. Introduction

Xylindein (**1**) is a blue–green pigment previously isolated from the fungi *Chlorociboria aeruginosa* and *aeruginascens* (reviews: Blackburn et al., 1965; Edwards and Kale, 1965, synthetic studies: Giles et al., 1979, 1990). Since these fungi are widely distributed and their fruiting bodies and the infected wood assume a beautiful blue–green color, the characteristic pigmentation has attracted attention for over 2 centuries. The insolubility of **1** in common organic solvents had prevented its isolation and structure determination; however, this was overcome by Liebermann and Fischer in 1874 who obtained crystals by extracting the green wood with aqueous phenol. After extensive work by many chemists, the planar structure of the extended quinone was independently revealed in 1965 by Blackburn et al. and Edwards and Kale, through chemical derivatization and spectroscopic analyses. We reinvestigated the xylindein structure to reveal its hitherto-unknown absolute configuration and its tautomeric structure.



Xylindein (**1**)

2. Results and discussion

The fruiting bodies of the *Chlorociboria* species and the infected wood were collected from the Okutama mountain system. Xylindein (**1**) was extracted from infected wood with hot chloroform (Edward and Kale, 1965). In order to determine the absolute configuration of **1** by X-ray crystallographic analysis using the anomalous scattering effect of a heavy atom, the conversion of **1** to an appropriate derivative containing some heavy atoms was examined. The direct introduction of some alkyl groups containing a heavy atom to the phenolic hydroxy groups in **1** was unsuccessful due to the insolubility of **1** in common organic solvents and, moreover, the direct halogenation to the aromatic nucleus (C5- and C11-positions) was a failure, giving a complex mixture. Accordingly, **1** suspended in chloroform was methylated with diazomethane via a procedure reported by

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Edwards and Kale (Fig. 1). The resulting dimethylxylindein (**2**), which is soluble in various organic solvents, was subjected to the hydrogenation conditions. The reaction mixture was rapidly filtered through Celite, and then the resulting dihydro derivative **3** was exposed to alkylation using *p*-bromobenzyl bromide and sodium hydride in DMF to give dihydrotetraalkylxylindein (**4**). The bromobenzyl derivative **4** was recrystallized from a mixture of 2-butanol and chloroform to give red needles which were not suitable for X-ray analysis. Next, crystallization was performed with hot 80% aqueous phenol to give red plates which were subjected to single-crystal X-ray diffraction analysis. The absolute configurations of both chiral centers of **4** were assigned as *S*. This crystal contains one molecule of **4** and four molecules of phenol per asymmetric unit (Fig. 2).

Although the absolute configuration of xylindein (**1**), which had been unknown for a long time, was determined at this time by X-ray crystallographic analysis of its derivative **4**, ambiguity about the xylindein tautomeric structure still remains. X-ray crystallographic analysis of **1** would serve to elucidate this problem. The crystals for X-ray analysis of **1** were prepared according to the method of Edwards and Kale (1965) (recrystallized from hot 80% aqueous phenol) to give magenta plates. The crystals obtained were analyzed by X-ray crystallography without washing with organic solvent, because washing with EtOH resulted in damaging the crystal structure, removing phenols contained in the crystal and producing an amorphous solid. Also in this case, this crystal contains one molecule of **1** and four molecules of phenol per asymmetric unit (Fig. 3). The structure is not symmetrical when comparing the top and bottom halves. Accordingly, the bond lengths of the corresponding groups are not the same, which should be derived from the coordination effects of the phenols. Different coordination modes of two phenol moieties should bring about the extended and the shortened carbonyl bond lengths of the two lactone groups (1.179 and 1.311 Å). The bond lengths of the two quinone carbonyl groups (1.291 and 1.318 Å) are rather long and those of the two hydroxy groups (1.382 and 1.358 Å) are shorter compared to the standard geometries (C=O: 1.22Å, C−O: 1.43 Å, Hendrickson et al., 1970). Nevertheless, these data support that the represented tautomer is the proper one among the possible tautomers in the crystal state.

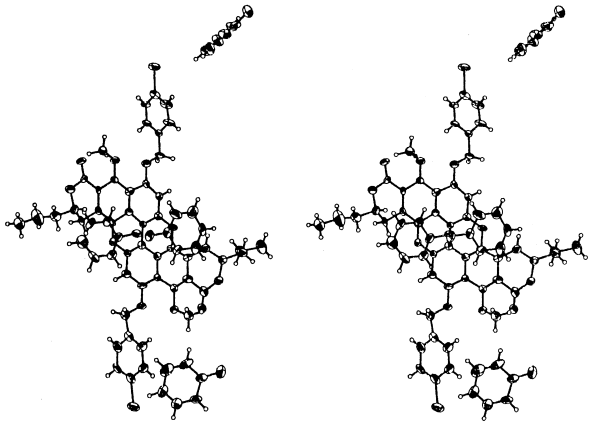

Fig. 2. A stereoview of compound **4**·4PhOH.

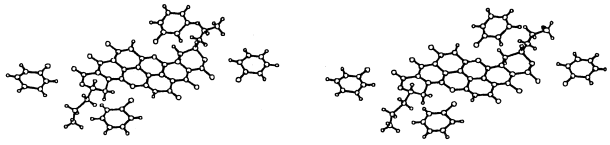

Fig. 3. A stereoview of compound **1**·4PhOH.

3. Experimental

3.1. General

Melting points are uncorrected. Optical rotations were measured on a JASCO DIP-360 polarimeter. UV spectra were recorded on a HITACHI U-2001 spectrometer. IR spectra were recorded on a JASCO FT-IR-200 spectrometer as a KBr pellet or on an NaCl cell with nujol. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL Lambda-300 (300 and 75 MHz, respectively)

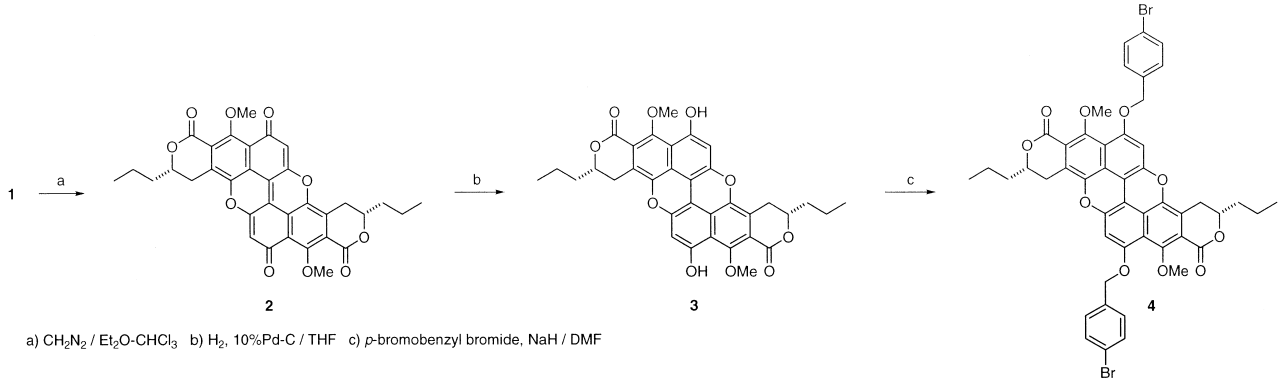

Fig. 1. Chemical conversions of xylindein (**1**) to a bromobenzyl derivative **4**.

spectrometer. The chemical shifts were reported as δ values in ppm relative to TMS as an internal standard, unless otherwise stated. Low and high resolution mass spectra were obtained on a JEOL GCmate (EI) and a Hewlett Packard HP5989A (TSI) mass spectrometers. Elemental analysis was performed on a YANACO, CHN-Corder-MT-5.

3.2. Xylindein (*1*, peri-xanthenoxanthene-2,8-dicarboxylic acid 4,10-dihydro-3,9-dihydroxy-1,7-bis(2 S-hydroxypentyl)-4,10-dioxo-di δ-lactone)

Xylindein (**1**) (45 mg) was extracted from 20 g of both the fruiting bodies of *Chlorociboria* species (*C. aeruginosa, C. aeruginascens*, and *C. omnivirens*) and the infected wood with refluxing $CHCl_3$ (500 ml) according to the procedure of Edwards and Kale (1965). The crude **1** was recrystallized from hot 80% aqueous PhOH to give magenta plates (28 mg). **1**•4PhOH: m.p. >200°C (lit; >300°C [Edwards and Kale, 1965)]; UV, λ_{max} 5% 2,2,2-trifluoroethanol–$CHCl_3$ nm (log ε): 658 (4.60), 611 (4.45), 488 (3.55), 428 (4.09), 405 (4.21), 384 (4.19), 348 (4.59), 256 (4.65); IR ν_{max}^{nujol} cm^{-1}: 1699, 1625, 1592, 1300, 1241, 1190, 1080, 1064, 980, 885, 842, 805; ^1H NMR (300 MHz, CF_3CO_2D, TMS) δ 1.10 (6H, *t, J*=6.3 Hz, H-5′, 5″), 1.72 (4H, *m*, H-4′, 4″), 1.98 (2H, *m*, H-3′, 3″), 2.09 (2H, *m*, H-3′, 3″), 3.33 (2H, *dd, J*=11.7, 17.3 Hz, H-1′, 1″), 3.91 (2H, *d, J*=17.3 Hz, H-1′, 1″), 4.91 (2H, *m*, H-2′, 2″), 7.32 (2H, *s*, H-5, 11), 6.92 (8H, *m*, PhOH), 7.03 (4H, *m*, PhOH), 7.27 (8H, *m*, PhOH); TSI–MS, *m/z* 569 $[M+H]^+$; Elemental analysis, found: C 70.63, H 4.87%, calcd for $C_{56}H_{48}O_{14}$: C 71.18, H 5.12%.

1 (free from PhOH): ^1H NMR (300 MHz, CF_3CO_2D, TMS) δ 1.11 (6H, *t, J*=6.9 Hz, H-5′, 5″), 1.71 (4H, *m*, H-4′, 4″), 1.98 (2H, *m*, H-3′, 3″), 2.09 (2H, *m*, H-3′, 3″), 3.33 (2H, *dd, J*=11.7, 17.5 Hz, H-1′, 1″), 3.90 (2H, *d, J*=17.5 Hz, H-1′, 1″), 4.92 (2H, *m*, H-2′, 2″), 7.32 (2H, *s*, H-5, 11).

3.3. Xylindein dimethyl ether (*2*, peri-xanthenoxanthene-2,8-dicarboxylic acid 4,10-dihydro-3,9-dimethoxy-1,7-bis(2 S-hydroxypentyl)-4,10-dioxo-di δ-lactone)

Xylindein (**1**, 500 mg) suspended in $CHCl_3$ (360 ml) was methylated with CH_2N_2 (1 M in Et_2O, 20 ml) according to the procedure of Edwards and Kale (1965) and the resulting crude methyl ether was subjected to silica gel chromatography (eluted with 20% acetone–$CHCl_3$) to give **2** (300 mg) as a magenta amorphous solid.

UV, $\lambda_{max}CHCl_3$ nm (log ε): 570 (4.58), 525 (4.42), 488 (4.04), 455 (3.67), 405 (3.96), 382 (4.04), 339 (4.49), 259 (4.50); IR ν_{max}^{KBr} cm^{-1}: 3460, 2940, 2870, 1738, 1719, 1639, 1580, 1450, 1327, 1200, 1103, 1072, 1040, 978, 942, 850; ^1H NMR ($CDCl_3$, TMS) δ 1.04 (6H, *t, J*=7.6 Hz, H-5′, 5″), 1.65 (4H, *m*, H-4′, 4″), 1.83 (2H, *m*, H-3′, 3″), 1.94 (2H, *m*, H-3′, 3″), 2.91 (2H, *dd*, *J*=11.5, 16.8 Hz, H-1′, 1″), 3.42 (2H, *dd, J*=2.8, 16.8 Hz, H-1′, 1″), 4.08 (6H, *s*, 3-, 9-*O*Me), 4.56 (2H, *m*, H-2′, 2″), 6.48 (2H, *s*, H-5, 11); ^{13}C NMR ($CDCl_3$, TMS) δ 13.9 (C-5′, 5″), 18.2 (C-4′, 4″), 28.0 (C-1′, 1″), 36.8 (C-3′, 3″), 63.6 (3-, 9-*O*Me), 77.3 (C-2′, 2″), 111.0 (C-5, 11), 118.0 (*), 120.0 (*), 121.0 (*), 124.8 (*), 133.2 (*), 142.4 (*), 153.6 (*), 158.0 (C-3, 9), 159.9 (2-, 8-C=O), 180.7 (C-4, 10) [*: C-1, 7 or C-2, 8 or C-3a, 9a or C-5a, 11a or C-6a, 12a or C-9b, 12b or C-12c, 12d we could not assign these carbons because the sample could not withstand the long time measurement (irradiation in the strong magnetic field), such as INADEQUATE (measured in CD_3OD–$CDCl_3$ (1:5))]; EI–HR–MS *m/z* 598.1850 $[M+2H]^+$, calcd for $C_{34}H_{30}O_{10}$, 598.1838.

3.4. Dihydroxylindein dimethyl ether (*3*, peri-xanthenoxanthene-2,8-dicarboxylic acid 4,10-dihydroxy-3,9-dimethoxy-1,7-bis(2 S-hydroxypentyl)-di δ-lactone)

A suspension of xylindein dimethyl ether (**2**: 300 mg) and 10% Pd-C (30 mg) in THF (80 ml) was stirred for 0.5 h under an atmospheric pressure of H_2. The mixture was rapidly filtered through Celite and the filtrate was evaporated in vacuo, giving an orange amorphous solid (**3**, 301 mg). A small portion of this was subjected to silica gel chromatography in order to obtain an analytical sample.

$[\alpha]_D^{27}$ −789.5 ($CHCl_3$, *c* 0.04); UV, $\lambda_{max}CHCl_3$ nm (log ε): 442 (4.62), 415 (4.63), 322 (4.52), 259 (4.57); IR ν_{max}^{KBr} cm^{-1}: 3320, 2959, 2938, 2877, 1708, 1619, 1608, 1582, 1499, 1417, 1381, 1362, 1238, 1150, 1104, 1063, 1019, 962, 940, 838, 762; ^1H NMR ($CDCl_3$, TMS) δ 1.02 (6H, *t, J*=7.0 Hz, H-5′, 5″), 1.60 (4H, *m*, H-4′, 4″), 1.73 (2H, *m*, H-3′, 3″), 1.85 (2H, *m*, H-3′, 3″), 2.42 (2H, *dd, J*=11.2, 16.8 Hz, H-1′, 1″), 2.90 (2H, *dd, J*=2.5, 16.8 Hz, H-1′, 1″), 4.06 (6H, *s*, 3-, 9-*O*Me), 4.39 (2H, *m*, H-2′, 2″), 6.12 (2H, *s*, H-5, 11), 9.33 (2H, *s*, 4-, 10-OH); ^{13}C NMR ($CDCl_3$, TMS) δ 13.9 (C-5′, 5″), 18.2 (C-4′, 4″), 27.2 (C-1′, 1″), 36.9 (C-3′, 3″), 64.3 (3-, 9-*O*Me), 77.2 (C-2′, 2″), 101.4 (C-5, 11), 102.6 (*), 112.3 (*), 113.5 (*), 118.5 (*), 124.3 (*), 143.6 (*), 146.8 (*), 155.2 (C-4, 10), 155.8 (C-3, 9), 162.3 (2-, 8-C=O) (*: C-1, 7 or C-2, 8 or C-3a, 9a or C-5a, 11a or C-6a, 12a or C-9b, 12b or C-12c, 12d); EI–HR–MS *m/z* 598.1841 $[M]^+$, calcd for $C_{34}H_{30}O_{10}$, 598.1838.

3.5. Dibromobenzyldimethyldihydroxylindein (*4*, peri-xanthenoxanthene-2,8-dicarboxylic acid 4,10-bis(4-bromobenzyloxy)-3,9-dimethoxy-1,7-bis(2 S-hydroxypentyl)-di δ-lactone)

To a solution of the above crude dihydroxylindein dimethyl ether (**3**, 301 mg) and 4-bromobenzyl bromide (377 mg, 1.5 equiv.) in DMF (10 ml, degassed and filled with Ar) was added a suspension of NaH (27 mg, 1.1 equiv.) in DMF (13 ml, degassed and filled with Ar).

After 1 hr the reaction was quenched with NH_4Cl aq and the mixture was extracted with EtOAc (10 ml×3). The extracts were washed several times with H_2O, and evaporated in vacuo. The residue was subjected to silica gel chromatography (eluted with 3% THF–benzene) to afford **4** (239 mg, 50.8%) as an orange amorphous solid. The resulting solid was recrystallized from hot 80% aq. PhOH to give orange plates.

4·4PhOH: m.p. 102–106°C (two molecules of PhOH were removed by heating), $[\alpha]_D^{27}$ −89.6 ($CHCl_3$, *c* 0.5); UV, $\lambda_{max}CHCl_3$ nm (log ε): 442 (4.61), 415 (4.65), 393 (4.64), 318 (4.66), 259 (4.76); IR ν_{max}^{KBr} cm^{-1}: 3322, 2960, 2930, 2870, 1690, 1600, 1490, 1477, 1440, 1400, 1379, 1345, 1330, 1252, 1170, 1118, 1065, 1010, 968, 950, 883, 821, 809, 757, 690; ^{1}H NMR ($CDCl_3$, TMS) δ 0.98 (6H, *t*, *J* = 7.3 Hz, H-5′, 5″), 1.58 (4H, *m*, H-4′, 4″), 1.69 (2H, *m*, H-3′, 3″), 1.84 (2H, *m*, H-3′, 3″), 2.47 (2H, *dd*, *J* = 11.8, 16.8 Hz, H-1′, 1″), 3.00 (2H, *dd*, *J* = 1.4, 16.8 Hz, H-1′, 1″), 3.74 (6H, *s*, 3-, 9-*O*Me), 4.35 (2H, *m*, H-2′, 2″), 4.94 (2H, *d*, *J* = 11.6 Hz, BrPh−C$\underline{H}_2$–), 5.06 (2H, *d*, *J* = 11.6 Hz, BrPh−C$\underline{H}_2$–), 6.23 (2H, *s*, H-5, 11), 7.37 (4H, *d*, *J* = 8.4 Hz, Br–C–C$\underline{H}$–), 7.51 (4H, *d*, *J* = 8.4 Hz, Br–C–CH–C$\underline{H}$–),4.84 (4H, *s*, Ph-OH), 6.84 (8H, *m*, HO-C–CH–C$\underline{H}$–), 6.93 (4H, *m*, HO–C–CH–CH–C$\underline{H}$–), 7.25 (8H, *m*, HO–C–C$\underline{H}$–); ^{13}C-NMR ($CDCl_3$, TMS) δ 13.9 (C-5′, 5″), 18.2 (C-4′, 4″), 27.5 (C-1′, 1″), 36.9 (C-3′, 3″), 63.5 (4-, 10-*O*Me), 70.7 (BrPh–$\underline{C}H_2$–), 77.2 (C-2′, 2″), 99.1 (C-5, 11), 103.2 (*), 115.2 (*), 115.3 (HO–C–CH–$\underline{C}$H–), 117.2 (*), 119.3 (*), 120.6 (HO–C–CH–CH–$\underline{C}$H–), 122.1 (Br–C–CH–CH–$\underline{C}$–), 125.4 (*), 129.1 (Br-C-CH–$\underline{C}$H–), 129.6 (HO–C–$\underline{C}$H–), 131.7 (Br–C–$\underline{C}$H–), 134.9 (Br–$\underline{C}$–), 142.9 (*), 145.9 (*), 155.6 (HO–$\underline{C}$–), 156.4 (C-4, 10), 156.8 (C-3, 9), 163.0 (2-, 8-C = O) (*: C-1, 7 or C-2, 8 or C-3a, 9a or C-5a, 11a or C-6a, 12a or C-9b, 12b or C-12c, 12d); Elemental analysis, found: C 65.31, H 4.65%, calcd for $C_{72}H_{64}O_{14}Br_2$: C 65.86, H 4.91%.

3.6. X-ray crystallographic analysis of *4·4PhOH*

Monoclinic, space group $P2_1$, $a = 12.677(2)$ Å, $b = 11.141(4)$ Å, $c = 22.048(1)$ Å, $\beta = 99.117(6)°$, $V = 3074.7(9)$ $Å^3$, $Z = 2$, $D_{calc} = 1.418$ g/cm^3. Reflection data were collected on a Rigaku AFC5R diffractometer with graphite-monochromated Cu–Kα to $2\theta_{max}$ 120.3°; an empirical absorption correction based on azimuthal scans of several reflections was applied. A final refinement gave $R(R_w) = 0.048$ (0.066) for 2529 reflections with $I > 3\ \sigma(I)$. The absolute configuration was determined by comparison of intensity of Friedel pairs which were the largest 14 reflections in a value of $|Fc(h)–Fc(h)|/\sigma(Fo)$. The signs of Fo(h)–Fo(h) of all Friedel pairs were consistent with one derived from an absolute configuration proposed in this paper.

3.7. X-ray crystallographic analysis of *1·4PhOH*

Monoclinic, space group $P2_1$, $a = 8.434(1)$ Å, $b = 24.027(2)$ Å, $c = 11.621(1)$ Å, $\beta = 102.222(9)°$, $V = 2301.7(4)$ $Å^3$, $Z = 2$, $D_{calc} = 1.363$ g/cm^3. Reflection data were collected on a Rigaku AFC5R diffractometer with graphite-monochromated Cu–Kα to $2\theta_{max}$ 120.3°; an empirical absorption correction based on azimuthal scans of several reflections was applied. A final refinement gave $R(R_w) = 0.091$ (0.115) for 1257 reflections with $I > 3\ \sigma(I)$.

The crystallographic data of **4**·4PhOH and **1**·4PhOH have been deposited at the Cambridge Crystallographic Data Centre.

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