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HYPOXYLONE, A NAPHTHYL-NAPHTHOQUINONE PIGMENT FROM THE FUNGUS *HYPOXYLON SCLEROPHAEUM*

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Key Word Index—*Hypoxylon sclerophaeum*; Xylariaceae; naphthyl-naphthoquinone; stromal pigment; ^{1}H and ^{13}C NMR.

Abstract—Hypoxylone, the stromal pigment of *Hypoxylon sclerophaeum* was isolated and identified as 5-hydroxy-2-(1′,8′-dihydroxy-4′-naphthyl)-1,4-naphthoquinone. The structure was elucidated by a spectroscopic study and some chemical transformations.

INTRODUCTION

Hypoxylon are saprophytic fungi living on dead trees and producing coloured carpophores. In spite of their value for taxonomic purpose [1] few chemical studies are devoted to their pigments, except those of *H. fragiforme* which are mitorubrin derivatives [2]. We here report the isolation and structure determination of a novel naphthyl-naphthoquinone which we have named hypoxylone, and which is the main pigment of the dark purple carpophores of *H. sclerophaeum* collected in French Guyana.

RESULTS AND DISCUSSION

From the dichloromethane extract of *H. sclerophaeum* fruiting bodies, hypoxylone (**1**) was isolated by chromatography. The mass spectrum of **1** showed few ion fragments of low abundance but an intense molecular ion (m/z = 332.0684, calc.: 332.0685, $C_{20}H_{12}O_5$). This formula implies 15 unsaturated sites and suggests an aromatic structure.

The ^{1}H NMR spectrum displayed signals from nine aromatic protons between δ6.60 and 7.80 (Table 1) and three hydroxyl protons at δ8.36 (2H) and 12.18 (1H). The formation of a triacetyl derivative **2** was consistent with the presence of three hydroxyl functions. The ^{13}C NMR spectrum of **1** indicated that all of the 20 carbon atoms of the molecule were sp^2 (Table 3). The three hydroxyl substituted carbons were observed at δ155.3, 155.7 and 162.1, while signals at δ184.4 and 191.7 were attributed to two carbonyls, thus accounting for the five oxygen atoms of the molecular formula.

IR absorption bands of hypoxylone were due to hydroxyl groups (3290 cm^{-1}), a conjugated carbonyl group (1665 cm^{-1}), a conjugated and chelated CO group (1637 cm^{-1}) and aromatic double bonds (1608 and 1523 cm^{-1}).

All the spectroscopic data suggested the existence of a quinonoid structure in which one of the carbonyls was strongly hydrogen-bonded to a neighbouring hydroxyl group. The chemical shifts of the carbonyl carbons indicated their *para* distribution since *ortho*-quinones had carbonyl carbon signals more upfield [3]. The UV spectrum of **1** had the diagnostic bands of the 1,4-naphthoquinone nucleus (250 and 334 nm) as earlier described by Singh *et al.* [4], and considering the chelation of one of the carbonyls gave the first substructure, **I**. The remaining ten carbon atoms were used to construct a naphthalene ring (substructure **II**), in accord with the

Table 1. Aromatic part of ^{1}H NMR spectrum of hypoxylone (**1**) and its derivatives **3**–**6** [250 MHz, CD_3COCD_3, δ ppm and J (Hz) given in parentheses]

H	Band	Coupled with	**1**	**3**	**4**	**5**	**6**
3	(*s*)		7.06	7.08	7.07	7.01	7.03
6	(*dd*)	H-7, H-8	7.36 (8.6, 1.1)	7.37 (8.3, 1.1)	7.36 (8.3, 1.2)	7.60 (7.3, 2.0)	7.58 (7.6, 1.8)
7	(*dd*)	H-6, H-8	7.79 (8.6, 7.6)	7.80 (8.3, 7.5)	7.80 (8.3, 7.5)	7.96 (7.7, 7.3)	7.95 (7.6, 7.6)
8	(*dd*)	H-6, H-7	7.63 (7.6, 1.1)	7.63 (7.5, 1.1)	7.63 (7.5, 1.2)	8.09 (7.7, 2.0)	8.09 (7.6, 1.8)
2′	(*d*)	H-3′	6.87 (7.9)	7.08 (8.1)	6.88 (8.1)	7.11 (8.0)	7.20 (7.2)
3′	(*d*)	H-2′	7.34 (7.9)	7.42 (8.1)	7.41 (8.1)	7.52 (8.0)	7.55 (7.2)
5′	(*dd*)	H-6′, H-7′	7.24 (8.6, 0.8)	7.21 (8.3, 1.0)	7.36 (6.0, 0.1)	7.71 (8.4, 1.6)	7.43 (4.1, 0)
6′	(*dd*)	H-5′, H-7′	7.26 (8.6, 8.4)	7.32 (8.3, 7.8)	7.37 (2.8, 0.1)	7.47 (8.4, 7.2)	7.43 (5.0, 0)
7′	(*dd*)	H-5′, H-6′	6.84 (8.3, 0.8)	6.83 (7.8, 1.0)	7.04 (6.0, 2.8)	7.15 (7.2, 1.6)	7.07 (5.0, 4.1)

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primary UV absorption band of the naphthalene nucleus at 228 nm [5].

From the 250 MHz ^{1}H NMR spectrum (Table 1), vicinal and long range coupling constants allowed us to define two independent three-proton systems (H-6, H-7, H-8 and H-5′, H-6′, H-7′), a two-proton system (H-2′, H-3′) and a lone proton (H-3). The most downfield protons were placed on the benzene ring (H-6, H-8, H-7) of substructure **I**. This substructure could only be linked to **II** by either position -2 or -3 of the quinone ring, which implied that the lone proton (δ7.06) could equally have a dual localization (-3 or -2) (substructures **Ia** or **Ib**).

The location of the remaining five protons and two hydroxyl groups on substructure **II** was not possible until information about the relative position of the two hydroxyls was obtained from the methylation of hypoxylone.

Methylation of **1** with diazomethane formed two monomethyl ethers **3** (60%) and **4** (40%). Naphthalene-1,2-, naphthalene-1,8- and naphthalene-2,3-diols formed only monomethyl ethers with diazomethane [6], because of a hydrogen bond which prevented a second methylation. The chemical shift of the residual hydroxyl proton was characteristic of its position *peri* (~ δ9.30) or *ortho* (~ δ6.00) [7]. The chemical shifts observed for this hydroxyl proton in **3** (9.50) and **4** (9.73) implied that the two hydroxyl groups of **1** were *peri*.

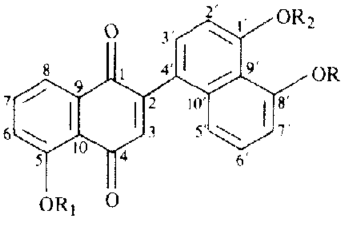
The acetylation of an aromatic hydroxyl group [8] led to a deshielded *ortho* proton (+δ0.22–0.29) and a more deshielded *para* proton (+δ0.33–0.49). The shift variations induced by acetylation of **3** and **4** (Table 2) indicated H-8, H-2′ as well as H-7′ to be *ortho* to a hydroxyl group in hypoxylone (**1**). Substructure **II** had to be linked by its 4-position (*para* to an hydroxyl) to position -2 or -3 of substructure **I**, leading to two possible formulae (**Ia** or **Ib**) for hypoxylone.

The structure was settled after a study of the carbonyl carbon signals in the ^{13}C NMR spectrum. Splitting of the carbonyl carbons of 2-amino- and 3-amino-5-hydroxy-1,4-naphthoquinone has been reported [9]: $^2J_{CH}$, as well as *peri* $^3J_{CH}$, is insignificant but $^3J_{CH}$ is significant (10–12 Hz). In the proton-coupled spectrum of **1**, we observed a sharp singlet for C-4 and a doublet (11.5 Hz) for C-1. Only structure **Ia** made a $^3J_{CH}$ possible for C-1 and not for C-4.

The complete assignment of the ^{13}C NMR spectrum of **1** was made by comparison of its spectrum with those of juglone and 1,8-dihydroxynaphthalene (Table 3). The spectrum of juglone was already assigned [10, 11] but with opposite assignments with regard to C-6 and C-8 (δ 119.4 and 124.9). This ambiguity was removed by studying the hyperfine coupling constants in the proton-coupled spectrum of juglone. The signal at δ124.9 appeared as a doublet ($^1J_{CH}$ = 164.5 Hz) of triplets ($^3J_{CH}$ = 7.5 Hz) which collapsed into a doublet of doublets ($^3J_{CH}$ = 7.5 Hz) by addition of D_2O. This signal was assigned to the C-6 which was located at three bonds from H-8 and from the hydroxylic proton.

The signal at δ119.4 was, in the proton-coupled spectrum, a doublet ($^1J_{CH}$ = 168.5 Hz) of doublets ($^3J_{CH}$ = 7.5 Hz), remained unchanged by addition of D_2O and was assigned to C-8.

Its structure indicated hypoxylone to be biosynthesized by phenolic coupling of a juglone-like precursor with itself.



1 $R_1 = R_2 = R_3 = H$

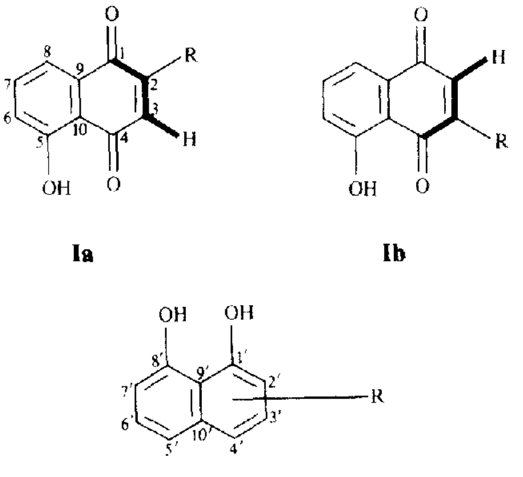
2 $R_1 = R_2 = R_3 = Ac$

3 $R_1 = R_3 = H$, $R_2 = Me$

4 $R_1 = R_2 = H$, $R_3 = Me$

5 $R_1 = R_3 = Ac$, $R_2 = Me$

6 $R_1 = R_2 = Ac$, $R_3 = Me$



Ia **Ib**

II

Fig. 1. Substructures of hypoxylone **1**. **Ia**, **Ib** (R = $C_{10}H_7O_2$); **II** (R = $C_{10}H_5O_3$); juglone (**7**) (**Ia**, **Ib** R = H).

Table 2. ^{1}H chemical shift variations (Δδ ppm) induced by acetylation of hypoxylone monomethyl derivatives **3** and **4**

	H-3	H-6	H-7	H-8	H-2′	H-3′	H-5′	H-6′	H-7′
δ (5) − δ (3)	0.07	+0.23	+0.16	+0.46	+0.03	+0.10	+0.50	+0.15	+0.32
δ (6) − δ (4)	−0.03	+0.22	+0.15	+0.46	+0.32	+0.14	−0.07	+0.06	+0.03

Table 3. ^{13}C FT NMR spectrum of hypoxylone (**1**), juglone (**7**) and 1,8-dihydroxynaphthalene (**8**) (25.115 MHz, CD_3COCD_3, δ ppm)

C	**1**	**7**	**8**
1	184.4 *s*	185.0	
2	152.1 *s*	140.7	
3	138.1 *d*	139.6	
4	191.7 *s*	191.8	
5	162.1 *s*	162.3	
6	124.6 *d*	124.9	
7	137.6 *d*	137.7	
8	119.0 *d*	119.4	
9	116.4 *s**	116.0	
10	133.7 *s*	133.0	
1′	156.9 *s*		154.1
2′	110.1 *d*†		109.0
3′	130.2 *d*		126.9
4′	129.8 *s*		119.7
5′	120.0 *d*		119.7
6′	127.5 *d*		126.9
7′	109.1 *d*†		109.0
8′	155.3 *s*		154.1
9′	136.1 *s*		137.3
10′	115.7 *s**		115.2

*,†May be reversed.

EXPERIMENTAL

Isolation of hypoxylone (**1**). *H. sclerophaeum* Berkeley & Curtis was collected in the Cabassou forest in the South of Cayenne and voucher specimens (HJ 2537) were deposited in the Herbarium of Muséum National d'Histoire Naturelle (Paris) and ORSTOM Centre (Cayenne). Well-crushed carpophores of this fungus (100 g) were extracted under stirring with CH_2Cl_2 (4 × 100 ml) at room temp.

After filtration and evapn of the solvent the combined extracts left a residue (1.8 g). This crude solid was chromatographed on a silica gel column eluted with mixtures of hexane and increasing quantities of Et_2O. Hypoxylone was eluted with 60 % Et_2O. Dark violet crystals from Et_2O, decomposition 179°. Slow decomposition occurred even at room temp. to form a dark green solid. IR $\nu_{\mathrm{max}}^{\mathrm{KBr}}$ cm^{-1}: 3290, 1665, 1637, 1608, 1585, 1523, 1456, 1414, 1368, 1250, 1165, 1140, 1113, 1074, 1038, 964, 915, 835, 818, 807, 767, 643; ^1H NMR (250 MHz, CD_3COCD_3): δ12.18 (*s*, 1H, OH), 8.36 (*s*, 2H, OH), for aromatic part see Table 1; MS (70 eV, 200°) m/z (rel. int.): 333 (22), 332 $[M]^+$ (100), 315 (30), 304 (8), 303 (18), 287 (25), 166 (7), 92 (15). UV $\lambda_{\mathrm{max}}^{\mathrm{EtOH}}$ nm (log ε): 228 (4.4), 250 (3.9), 319 (3.7), 334 (3.7), 418.

5,1′,8′-*Tri-O-acetyl-hypoxylone* (**2**). $C_{26}H_{18}O_8$. Hypoxylone (**1**) (20 mg) was dissolved in Ac_2O (3 ml) and 1 drop conc. H_2SO_4 added. The mixture was poured into H_2O (50 ml). The orange crystals which formed were recrystallized in Me_2CO (mp 204–205°). IR $\nu_{\mathrm{max}}^{\mathrm{KBr}}$ cm^{-1}: 1775, 1768, 1745, 1665, 1627, 1600; ^1H NMR (CD_3COCD_3): δ8.10 (*dd*, 1H, J = 7.8 and 2.1 Hz), 7.96 (*dd*, 1H, J = 7.8 and 7.3 Hz), 7.86 (*dd*, 1H, J = 8.2 and 1.4 Hz), 7.63 (*d*, 1H, 7.9), 7.61 (*dd*, 1H, J = 7.3 and 2.1 Hz), 7.54 (*dd*, 1H, J = 8.2 and 7.5 Hz), 7.35 (*d*, 1H, J = 7.9 Hz), 7.29 (*dd*, 1H, J = 7.5 and 1.4 Hz), 7.10 (*s*, 1H), 2.46 (*s*, 3H, Me), 2.45 (*s*, 3H, Me), 2.42 (*s*, 3H, Me). MS (70 eV, 200°) m/z (rel. int.): 459 (2), 458 $[M]^+$ (7), 416 (15), 374 (41), 332 (100).

Methylation of **1**. To a soln of **1** (200 mg) in MeOH (50 ml) an Et_2O soln of CH_2N_2 was added repeatedly producing an orange yellow soln. After concn, the crude mixture was chromatographed (silica gel column, elution with mixtures of hexane with increasing proportions of Et_2O). A mixture of two monomethyl ethers, **3** and **4**, was collected which was separated by TLC (silica gel plates, toluene–Et_2O, 9:1) into **3** (R_f 0.59) and **4** (R_f 0.64).

1′-O-*Methyl-hypoxylone* (**3**), $C_{21}H_{14}O_5$. Brown orange crystals, mp 250° (Me_2CO). IR $\nu_{\mathrm{max}}^{\mathrm{KBr}}$ cm^{-1}: 3340, 1665, 1635, 1610, 1590, 1460, 1415, 1325, 1295, 1250, 1167, 1144, 1113, 1090, 1050, 1023, 835; ^1H NMR (CD_3COCD_3): δ12.05 (*s*, 1H, OH), 9.50 (*s*, 1H, OH), for aromatic part see Table 1, 4.34 (*s*, 3H, OMe); MS (70 eV, 220°) m/z (rel. int.): 347 (24), 346 $[M]^+$ (100), 331 (52), 329 (18), 314 (12), 303 (18), 173 (9).

8′-O-*Methyl-hypoxylone* (**4**), $C_{21}H_{14}O_5$. Brown orange crystals, mp 236–237° (Me_2CO). IR $\nu_{\mathrm{max}}^{\mathrm{KBr}}$ cm^{-1}: 3410, 1665, 1640, 1605, 1590, 1460, 1410, 1310, 1255, 1167, 1113, 815, 770; ^1H NMR (CD_3COCD_3): δ12.05 (*s*, 1H, OH), 9.73 (*s*, 1H, OH), for aromatic part see Table 1, 4.20 (*s*, 3H, OMe); MS (70 eV, 220°) m/z (rel. int.): 347 (27), 346 $[M]^+$ (100), 331 (40), 329 (12), 314 (13), 303 (14), 173 (11).

5,8′-*Di-O-acetyl*-1′-O-*methyl-hypoxylone* (**5**), $C_{25}H_{18}O_7$. Monomethyl ether **3** (40 mg) was dissolved in Ac_2O (3 ml) and conc. H_2SO_4 (1 drop) was added. The mixture was poured into H_2O (50 ml). Orange crystals (30 mg) were formed, filtered and recrystallized in EtOH, mp 234–235°. IR $\nu_{\mathrm{max}}^{\mathrm{KBr}}$ cm^{-1}: 1775, 1763, 1680, 1658, 1627, 1600; ^1H NMR (CD_3COCD_3): δ for aromatic part see Table 1, 4.05 (*s*, 3H, OMe), 2.42 (*s*, 3H, Ac), 2.36 (*s*, 3H, Ac); MS (70 eV, 200°) m/z (rel. int.): 431 (11), 430 $[M]^+$ (38), 389 (15), 388 (57), 348 (38), 347 (45), 346 (100), 331 (47), 329 (19).

5,1′-*Di-O-acetyl*-8′-O-*methyl-hypoxylone* (**6**), $C_{25}H_{18}O_7$. By acetylation of **4**. Orange crystals. IR $\nu_{\mathrm{max}}^{\mathrm{KBr}}$ cm^{-1}: 1768, 1667, 1617, 1600; ^1H NMR (CD_3COCD_3): δ for aromatic part see Table 1, 4.01 (*s*, 3H, OMe), 2.42 (*s*, 3H, Ac), 2.37 (*s*, 3H, Ac); MS (70 eV, 200°) m/z (rel. int.): 431 (8), 430 $[M]^+$ (19), 389 (25), 388 (83), 347 (19), 346 (100), 331 (48), 329 (19).

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REFERENCES

1. Greenhalgh, G. N. and Whalley, A. J. S. (1970) *Trans. Br. Mycol. Soc.* **55**, 89.
2. Steglich, W., Klaar, M. and Furtner, W. (1974) *Phytochemistry* **13**, 2874.
3. Levy, G. C. and Nelson, G. L. (1980) *^{13}C NMR for Organic Chemists*, 2nd edn. Wiley Interscience, New York.
4. Singh, I., Ogata, R. T., Moore, R. E., Chang, C. W. J. and Scheuer, P. J. (1968) *Tetrahedron* **24**, 6053.
5. Rao, C. N. R. (1975) *UV and Visible Spectroscopy*, 3rd edn. Butterworth, London.
6. Coffey, S. (1978) *Rodd's Chemistry of Carbon Compounds*, Vol. III G. Elsevier, Amsterdam.
7. Brown, A. G., Lovie, J. C. and Thomson, R. H. (1965) *J. Chem. Soc.* 2355.
8. Massicot, J., Marthe, J. P. and Heitz, S. (1963) *Bull. Soc. Chim. Fr.* 2712.
9. Castillo, G., Ellames, G. J., Osborne, A. G. and Sammes, P. G. (1978) *J. Chem. Res.* (*S*) 45.
10. Kobayashi, M., Terui, Y., Tori, K. and Tsuji, N. (1976) *Tetrahedron Letters* 619.
11. Stack, M. E., Mazzola, E. P. and Eppley, R. M. (1979) *Tetrahedron Letters* 4489.