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6-METHYLDEHYDRO-α-LAPACHONE FROM *FOMES ANNOSUS*

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Key Word Index—*Fomes annosus*; basidiomycete; 1,4-naphthaquinone; dehydro-α-lapachone; orange pigments.

Abstract—The fructification of *Fomes annosus* contains 6-methyldehydro-α-lapachone, the characterization of which relies on ^{13}C NMR and on synthesis. The synthesis of 7-methyldehydro-α-lapachone is also described.

INTRODUCTION

Fomes annosus (syn. *Heterobasidion annosum* Brief) is one of the Basidiomycete fungi that can cause the death of host cells in living trees, as well as extensive decay in the heartwood of diseased trees. It is not apparently a major cause of butt rot in natural stands of Sitka spruce but it is well established as a problem in plantations. The commercial implication of this disease emphasises the need to seek a control for this infection.

In parallel with our search for suspected phytopathogens [1] secreted by the fungus, we examined a fructification taken from a Sitka spruce at Avondale, Co. Wicklow. Two constituents were isolated from the ethanol extract of the powdered fructification: colourless needles $C_{15}H_{14}O_5$ [2] and bright orange needles $C_{16}H_{14}O_3$. The structure of the latter compound proved to be 6-methyldehydro-α-lapachone (2,2,7-trimethyl-2H-naphtho[2,3-b]pyran) (**1a**), a naphthaquinone not reported previously [3]. We describe here our investigations that led to the structure elucidation of the quinone; to its synthesis and to the synthesis of the isomeric 7-methyldehydro-α-lapachone (**1b**).

RESULTS AND DISCUSSION

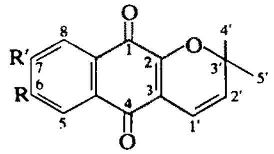
The orange colour and the absorption spectra of the natural product suggests a quinonoid structure. The molecular formula $C_{16}H_{14}O_3$ is expanded to $C_{13}H_5O_3(Me)_3$ upon inspection of the ^{1}H NMR spectrum. The protons of an AB quartet (δ5.75, 6.70, $J = 10.6$ Hz) and the geminal dimethyl (δ1.57) are assigned to a chromene ring which is placed in the α-position on the basis of the absorption in the UV. Two of the aromatic protons are deshielded due to the influence of the quinonoid carbonyl groups. One proton signal is a singlet (δ7.93), the second, *ortho*-coupled to the third aromatic proton. The aromatic methyl (δ2.51) must be at position 6 or 7 in the 1,4-naphthaquinone. It is not possible on the basis of the spectral data (see Table 1) of the natural product to assign the exact position of the aromatic methyl group; consequently the synthesis of 6-methyldehydro-α-lapachone and of the 7-methyl isomer was undertaken.

The synthesis of the 7-methyl isomer was approached through 7-methyltetralone [4]. Oxidation of the tetralone by O_2 in *t*-BuOK affords 2-hydroxy-7-methyl-1,4-naphthaquinone [5]. Acid-catalysed condensation of this quinone with 3-methylbutanal gives 7-methylisolapachol [6], the ^{1}H NMR spectrum of which has the signals characteristic of an ABX system ($\text{—CH}_x(\text{Me})_2\text{CH}_B\text{=CH}_A\text{—}$ δ1.14(6H, *d*, $J = 6.6$ Hz, Me_2) 2.51 (1H, *m*, H_x) 6.65(1H, *d*, $J = 17.0$ Hz, H_A) 7.10(1H, *dd* $J = 6.6$, 17.0 Hz, H_B)). Oxidative cyclization yields a mixture of α- and β-7-methyldehydrolapachone which on treatment with acid gives the dehydro-α-lapachone as the major product. The spectroscopic details presented in Table 1 closely resemble those of the natural product but the mp differs.

6-Methyl-1,4-naphthaquinone [7] when treated with aniline gives a mixture of 2 (and 3) -anilino-6-methylnaphthaquinone in a 3:2 ratio. Chromatographic separation affords the 2-anilino isomer with a mp 20° higher than that reported in the literature [18]. The 3-anilino derivative is identical with that obtained from reaction of 2-hydroxy-7-methylnaphthaquinone with aniline. The 2-anilino isomer gives 2-hydroxy-6-methylnaphthaquinone on acid treatment and the C_5 unit is introduced into this product with 3-methylbutanal. The resulting 6-methylisolapachol is oxidatively cyclized (DDQ) to a mixture of α- and β-6-methyldehydrolapachone. The dehydro-α-lapachone (**1a**) is the major product when the above mixture is refluxed in EtOH/HCl. The quinone (**1a**) is identical (UV; IR, ^{1}H NMR; ^{13}C NMR; mp, mmp) with the natural product.

The possibility that the natural 6-methyldehydro-α-lapachone is an artifact cannot be excluded, for it has been observed that lapachol undergoes partial conversion to dehydro-α-lapachone on prolonged storage or on exposure to light and air for a few days on a layer of silica [9]. It is also interesting to note that a 6-methyl-1,4-naphthaquinone is reported to occur in the related Basidiomycete, *Marasmius graminum* (Libert) Berk. [10].

Table 1. UV, IR, ^{1}H NMR and ^{13}C NMR spectra of lapachones



1a R = Me; R' = H
1b R = H; R' = Me

	Natural product	**1a**	**1b**
UV nm (log ε)	204 (4.52)	206 (4.47)	205 (4.38)
	265 (4.30)	265 (4.31)	260 (4.31)
	275 (4.26) sh	276 (4.27) sh	285 (4.12) sh
	333 (3.76) sh	333 (3.76) sh	338 (3.56) sh
	444 (3.14)	444 (3.14)	443 (3.08)
IR cm^{-1}	1668	1668	1675
	1648	1648	1655
^{1}H NMR			
4', 5' diMe	1.57	1.58	1.57
6(7)-Me	2.51	2.52	(2.53)
H-1'	*d* 5.75	*d* 5.76	*d* 5.77
H-2'	*d* 6.70	*d* 6.70	*d* 6.73
H-5	7.93	7.93	8.05
H-6(7)	*d* (7.55)	*d* (7.53)	*d* 7.6
H-8	*d* 8.02	*d* 8.01	*d* 7.97
^{13}C NMR ppm			
1	182.1*	182.1*	181.7*
2	152.6	152.6	152.3
3	117.7	117.7	117.8
4	179.7*	179.7*	180.0*
5	126.7†	126.7†	126.6†
6	145.1	145.1	134.4
7	133.8	133.8	144.2
8	126.5†	126.5†	126.4†
9	131.7‡	131.7‡	131.5‡
10	129.4‡	129.4‡	129.4‡
11	21.9	21.9	21.7
1'	130.6	130.6	130.7
2'	115.6	115.6	115.6
3'	80.3	80.3	80.3
4'	28.5	28.5	28.4
5'	28.5	28.5	28.4

J (Hz): 7, 8 = 5, 6 = 8.0. *J* 1' 2' = 10.6.

*†‡ These assignments may be reversed in each column.

EXPERIMENTAL

The reported mps were determined on a Köfler micro hot stage and are uncorr. Unless otherwise stated, spectra were measured in MeOH (UV), KBr (IR), $CDCl_3$ (60 MHz, ^{1}H NMR). The ^{13}C NMR data were determined with a Jeol FX60 instrument.

Isolation of 6-*methyldehydro*-α-*lapachone* (**1a**). A specimen of *Fomes annosus* fructification was collected from a *Picea sitchensis* at Avondale, Co. Wicklow. The hot EtOH extract (18 g) of the ground material (640 g), previously extracted with petrol is redissolved in cold EtOH. This soln is coated on Si gel then placed on a Si gel column eluting with $CHCl_3$ in 25 ml fractions. Fraction 5 (114 mg) and fraction 6 (126 mg) contain a distinctive red compound which is purified by PLC (developer: $CHCl_3$) to give an orange solid which crystallizes from petrol in orange needles of 6-methyldehydro-α-lapachone (**1a**), mp 130–132°. (Found: M$^+$ 254.0942 $C_{16}H_{14}O_3$ requires: M$^+$ 254.0942).

Synthesis of 7-*methyldehydro*-α-*lapachone* (**1b**). *Formation of* 7-*methylisolapachol*. The starting material 2-hydroxy-7-methyl-1,4-naphthaquinone is prepared as reported in the literature [4, 5] via 7-methyltetralone. The 1,4-naphthaquinone is obtained as yellow needles ($CHCl_3$), mp 203–206°. (Found: C, 69.86; H, 4.27. Calc. for $C_{11}H_8O_3$: C, 70.21; H, 4.29%). ^{1}H NMR: δ2.53 (3H, *s*, Me), 6.37 (1H, *s*, H-3), 7.66 (1H, *d*, *J* = 8.0 Hz, 6-H), 7.98 (1H, *s*, H-8), 8.06 (1H, *d*, *J* = 8.0 Hz, H-5). The quinone (200 mg) and 3-methylbutanal (0.7 ml) in HOAc (3.5 ml) is heated with HCl (1 ml) at 100° for 20 min. The reaction mixture is extracted with Et_2O and the extract purified on PLC (developer: $CHCl_3$). The product crystallized in red needles (petrol) (185 mg). (Found: C, 75.07; H, 6.23. $C_{16}H_{16}O_3$ requires: C, 74.98; H, 6.29%). UV λ_{max} nm (log ε): 205 (4.23), 238 (4.22), 267 (4.35), 282 (4.28) sh, 307 (4.16) sh, 442 (3.34). IR ν_{max} cm^{-1}: 3320 (OH), 1660, 1645, 1630. ^{1}H NMR: δ 1.14 (6H, *d*, *J* = 6.6 Hz, *gem*-Me_2), 2.51 (3H, *s*, Me), ABX system 2.51 (1H, *m*, H_x), 6.65 (1H, *d*, *J* = 17.0 Hz, H_A), 7.10

(1H, *dd*, $J = 6.6$, 17.0 Hz, H_B), 7.61 (1H, *d*, $J = 8.0$ Hz, H-5), 7.93 (1H, *s*, H-8) 8.07 (1H, *d*, $J = 8.0$ Hz, H-6), 7.81 (1H, *s*, OH exchangeable D_2O).

Formation of 2-anilino-7-methylnaphthaquinone. 2-Hydroxy-7-methylnaphthaquinone (50 mg) and aniline (0.3 ml) in EtOH (4 ml) are refluxed for 3 hr. Purification by PLC (developer: $CHCl_3$–petrol, 4:1) gives a red solid (42 mg) which is recrystallized from EtOH as red needles, mp 232–233°. (Found: C, 77.32; H, 4.78, N, 5.60. $C_{17}H_{13}NO_2$ requires: C, 77.55; H, 5.03; N, 5.27%). IR ν_{max} cm^{-1}: 3365 (NH), 1670, 1635 (CO). ^1H NMR: δ 2.51 (1H, *s*, Me), 6.42 (1H, *s*, H-3), 7.22–7.66 (6H, *m*, aromatic-H's + NH), 7.95 (1H, *s*, H-8), 8.04 (1H, *d*, $J = 8.0$ Hz, H-5).

Formation of 7-methyldehydro-α-lapachone. The 7-methylisolapachol (185 mg) dissolved in C_6H_6 (3 ml) is added to DDQ (190 mg) in C_6H_6 (3 ml) and stirred for 12 hr. The filtrate from the reaction gives a mixture of α- and β-7-methyldehydrolapachone. The brown coloured solid (170 mg) is dissolved in EtOH (3 ml) and HCl (0.1 ml) and refluxed for 45 min. Purification by PLC (developer: $CHCl_3$) yields the α-isomer (110 mg) as the major product which is recrystallized from petrol in orange clusters, mp 116–118°. (Found: C, 75.08; H, 5.59. $C_{16}H_{14}O_3$ requires: C, 75.57; H, 5.55%).

Synthesis of 6-methyldehydro-α-lapachone (**1a**). *Formation of 2-anilino-6-methylnaphthaquinone.* 6-Methyl-1,4-naphthaquinone (3 g) and aniline (1.5 ml) in EtOH (20 ml) are refluxed for 1 hr. The product is a mixture of 2- and 3-anilino-6-methylnaphthaquinone. Purification by PLC (double development, $CHCl_3$–petrol) gives two bands. Band (i) on elution with $CHCl_3$ affords a red solid, which recrystallized from $CHCl_3$–petrol as needles of 2-anilino-6-methylnaphthaquinone, mp 223–225° [lit. [8] mp 203°]. (Found: C, 77.95; H, 5.03; N, 5.27. $C_{17}H_{13}NO_2$ requires: C, 77.55; H, 4.98; N, 5.32%). IR γ_{max} cm^{-1}: 3335 (NH), 1680 (>C=O). ^1H NMR: δ 2.51 (3H, *s*, Me), 6.42 (1H, *s*, H-3), 7.22–7.71 (6H, *m*, aromatic —H's + NH), 7.97 (1H, *s*, H-5), 8.06 (1H, *d*, $J = 7.8$ Hz, H-8). Band (ii) affords 2-anilino-7-methylnaphthaquinone as red needles (EtOH), mp 232° (see above). Hydrolysis of 2-anilino-6-methylnaphthaquinone (250 mg) with H_2SO_4 (3 ml) and H_2O (3 ml) at 100° for 1 min gives 2-hydroxy-6-methylnaphthaquinone, mp 201–202° (lit. [8] mp 198°).

Formation of 6-methylisolapachol. 2-Hydroxy-6-methylnaphthaquinone (145 mg) and 3-methylbutanal (0, 7 ml) in HOAc (3.5 ml) are heated with HCl (1 ml) at 100° for 20 min. The product (118 mg) obtained has mp 130–132°. (Found: C, 75.48; H, 6.54. $C_{16}H_{16}O_3$ requires: C, 74.98; H, 6.29%). IR ν_{max} cm^{-1}: 3342 (OH), 1662, 1633 (C=O). UV λ_{max} nm (log ε): 214 sh (4.29), 271 (4.42), 291 sh (4.39), 296 (4.40), 303 (4.37), 437 (3.43). ^1H NMR: δ 1.12 (6H, *d*, $J = 6.6$ Hz, *gem*-Me), 2.50 (3H, *s*, Me), 2.51 (1H, *m*, H_X), 6.61 (1H, *d*, $J = 16.6$ Hz, H_A), 7.05 (1H, *dd*, $J = 6.6$, 16.6 Hz, H_B part of ABX system), 7.51 (1H, *d*, $J = 8.0$ Hz, H-6), 7.83 (1H, *s*, OH exchangeable D_2O), 7.93 (1H, *s*, H-5), 7.95 (1H, *d*, $J = 8.0$ Hz, H-7).

Formation of 6-methyldehydro-α-lapachone (**1a**). The 6-methylisolapachol (110 mg) is dissolved in C_6H_6 (3 ml) and added to DDQ (190 mg in C_6H_6 (3 ml)). The filtrate from the reaction gives a mixture of the α- and β-isomers of 6-methyldehydrolapachone. The isomeric mixture is refluxed in EtOH (3 ml) and HCl (0.1 ml) for 45 min. Purification by PLC (developer: $CHCl_3$) yields the α-isomer as the major product (**1a**) which is recrystallized from petrol (70 mg, 64%), mp 128–130°. (Found: C, 75.57; H, 5.45. $C_{16}H_{14}O_3$ requires: C, 75.57; H, 5.5%).

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