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LAMELLICOLIC ANHYDRIDE, 4-O-CARBOMETHOXYLAMELLICOLIC ANHYDRIDE AND MONOMETHYL 3-CHLOROLAMELLICOLATE, METABOLITES OF *VERTICILLIUM LAMELLICOLA*

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Abstract—The fungus *Verticillium lamellicola* affords lamellicolic anhydride **1** (5 - methyl - 2,4,7 - trihydroxy - 1,8 - naphthalic anhydride) together with small amounts of the quinone **2**, the carbonate **3** and the chloro compound **4**. Selective reactions of the functional groups in these and their derivatives have been used to interrelate these and effect conversion of **1** into **18**, the anhydride obtained from *Penicillium herquei*.

We have reported the isolation of lamellicolic anhydride **1**, the major metabolite of *Verticillium lamellicola* together with small amounts of the quinone **2** which may be a product of further metabolism of **1**.[1] We now give details of the chemistry of **1** and report the isolation of two minor metabolites which are interesting relatives of **1**, namely 4-O-carbomethoxylamellicolic anhydride **3** and monomethyl 3-chlorolamellicolate **4**.

These metabolites were obtained by chromatography of broth extracts of the fungus grown in surface culture. Lamellicolic anhydride **1**, $C_{13}H_8O_6$ is a bicarbonate soluble compound containing three phenolic OH groups as indicated by the formation of a tri-O-methyl derivative **5**, and, using Ac_2O-pyridine, a tri-O-acetyl derivative **6**. Its acidity and twin IR absorption at 1700 and 1650 cm^{-1} changing to 1750 and 1715 cm^{-1} in **5** and to 1775 and 1730 cm^{-1} in **6** are characteristic of a chelated 1,8-naphthalic anhydride[2] (*cf* 2,7-dihydroxy-1,8-naphthalic anhydride which shows IR absorption at 1720 and 1685 cm^{-1} changing to 1750 and 1720 cm^{-1} upon O-methylation and to 1760 and 1720 cm^{-1} upon O-acetylation). Also characteristic of this system are the complex changes in the UV spectrum of **1** upon basification which are reversed upon reacidification (*cf* Fig. 1).[2]

Selective esterification of the free OH group at C-4 in **1** to give **7** and **8** was readily achieved by heating with Ac_2O or butyric anhydride and the monomethyl ether **9** was obtained as a minor product in the methylation of **1**. The ether **9** was also obtained from **5** by selective

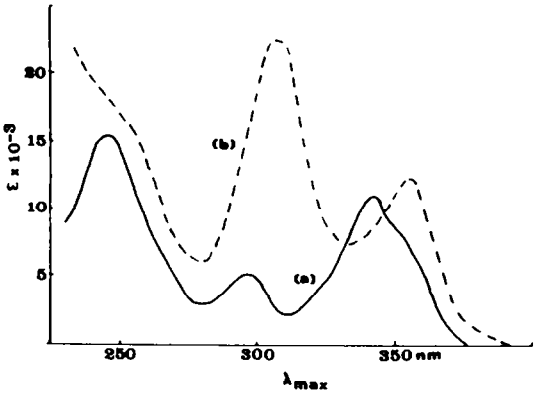
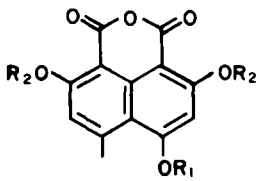


Fig. 1. UV spectrum of **1**: (a) in EtOH (b) in EtOH + NaOH.

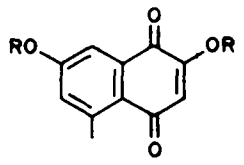
demethylation of the two methoxyl groups *peri* to the anhydride carbonyl groups using MgI_2-etherate.[3] The reactivity of these methoxyl groups, particularly that at C-2, was also evident in the reaction of **5** with $MeNH_2$ which afforded the yellow crystalline amino imides **10** and **11**. The IR and ^1H NMR spectra of these showed intramolecular hydrogen bonding of the NH groups and comparison of the chemical shifts of H-3 and H-6 in **5**, **10** and **11** shows the expected upfield shift of these protons on replacement of an ortho methoxy group by the methylamino group.[4]

In connection with biosynthetic studies, a number of reactions of the anhydride grouping in **1** were of interest with a view to chemical differentiation of the two carbonyl groups in **1**. The formation of the imides **10** and **11** must involve aminolysis of the anhydride function. It was also possible to effect ethanolysis of **5** to give an unstable acid ester **12** which showed a strong tendency to recyclize to **5** but could be esterified with CH_2N_2 to give the diester **13**. The most useful reaction of **1** involved treatment with aqueous NaOH. The dicarboxylic acid salt so formed readily underwent decarboxylation of one or both groups, in keeping with the presence of *ortho* phenolic OH groups. By carrying out this reaction under N_2 and treating the intermediate products with CH_2N_2 it was possible to obtain the trimethoxynaphthalenes **14** and **15** in modest yield. If, after treatment of **1** with alkali, the solution was allowed to stand in air, the orange naphthaquinone **2**, previously isolated from cultures of *V. lamellicola*, was obtained. The ^1H NMR spectrum of this compound in CD_3OD showed no signal for H-3 owing to facile deuterium exchange. However, the spectrum of the corresponding dimethyl ether **16** showed a singlet at 6.08 δ corresponding to this proton. *Meta* situated aryl protons in compounds **2**, **14** and **15** appeared as doublets of appropriate coupling constant.

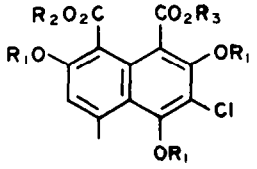
The fungus *Penicillium herquei* produces a number of phenalenone metabolites like herqueinone **17** together with the one other reported example of a fungal 1,8-naphthalic anhydride namely **18**[5] and this has also been obtained by chemical degradation of herqueinone.[6] In order to obtain this anhydride from **1**, etherification of the OH group at C-4 was effected with 3,3-dimethylallyl bromide giving **19**. When this was pyrolyzed at 160° the cyclic ether **18** formed the minor part of an inseparable mixture with **20**, the product from abnormal Claisen rearrangement. However the desired Claisen rearrange-



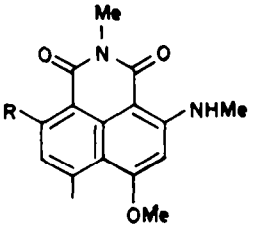
1 $R_1 = R_2 = H$
3 $R_1 = CO_2Me$, $R_2 = H$
5 $R_1 = R_2 = Me$
6 $R_1 = R_2 = Ac$
7 $R_1 = Ac$, $R_2 = H$
8 $R_1 = COCH_2CH_3$, $R_2 = H$
9 $R_1 = Me$, $R_2 = H$
19: $R_1 = CH_2CH{=}CMe_2$, $R_2 = H$
21 $R_1 = CO_2Me$, $R_2 = Me$



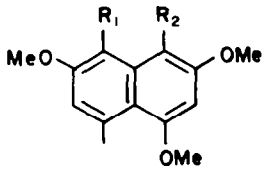
2 R = H
16 R = Me



4 $R_1 = H$, $R_2 = H(Me)$, $R_3 = Me(H)$
23 $R_1 = R_2 = R_3 = Me$



10 R = OMe
11 R = NHMe



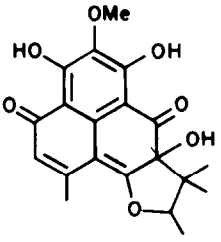
12 $R_1 = CO_2Et$, $R_2 = CO_2H$
13 $R_1 = CO_2Et$, $R_2 = CO_2Me$
14 $R_1 = CO_2Me$, $R_2 = H$
15 $R_1 = R_2 = H$

ment and cyclization to give **18** was effected cleanly by heating in $HCONMe_2$. This provided formal proof for the structure of **1** and this was also obtained by comparison with samples kindly supplied by Prof. B. W. Bycroft of **1** and **5** which had been synthesized via the phenalenone **22**.[7]

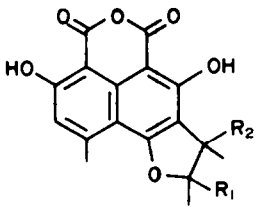
One of the two new minor metabolites of *V. lamellicola* proved to be the remarkable derivative **3** of lamellicolic anhydride. This showed the brick red colouration with $FeCl_3$ and the IR and UV absorption characteristic of the 2,7-dihydroxy-1,8-naphthalic anhydride system. This was supported by the ^1H NMR spectrum which showed the presence of two isolated aryl protons with an aryl methyl group ortho to one of them, and a signal at 3.90 δ typical of a methoxyl group. By contrast with the ether **9** and as for 4-O-acyl derivatives of **1**, e.g. **7**, the signal corresponding to H-3 was downfield relative to that in **1**. The presence of the O-carbomethoxy function was revealed by the presence of an extra carbonyl band in the IR at 1765 cm^{-1} and by appropriate mass spectral data. Confirmation was provided by ammonolysis of **3** to give **1** and methyl carbamate (as a volatile crystalline solid), and by synthesis of **3** from **1** using methyl chloroformate. Treatment of **3** with Me_2SO_4 and K_2CO_3 gave a dimethyl ether **21** together with some of the trimethyl ether **5**. Loss of the carbonate group was also observed under acetylation conditions, the triacetate **6** being obtained.

The second minor metabolite was a yellow chlorine-containing compound which was assigned the naphthalic acid mono-ester structure **4**. With diazomethane, this afforded the colourless diester **23** but with diethyl sulphate and potassium carbonate underwent anhydride formation along with alkylation to give the triethyl ether **24**. This and the corresponding trimethyl ether **25** were readily synthesized from **1** via the chloro derivative **26**. The ease with which **4** forms the anhydride **26** was evident from the UV spectrum which after addition of base and subsequent acidification was identical to the spectrum of **26** in base and acid respectively. By contrast the spectrum of the diester **23** was unaffected by addition of acid or base. The highest ions in the mass spectrum of the metabolite correspond to losses of water and methanol respectively from the parent ion. An alternative formulation which accommodates the data and reactions found for the metabolite would be **27**, the ring tautomer of **4**. The yellow colour might be due to a contribution from the quinone methide **28**.

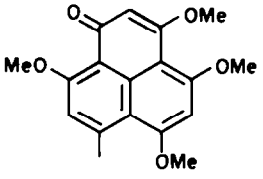
Formation of **4** may involve methanolysis of an anhydride precursor [cf formation of **12** from **5**] although both

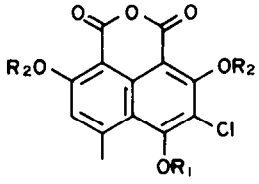


17

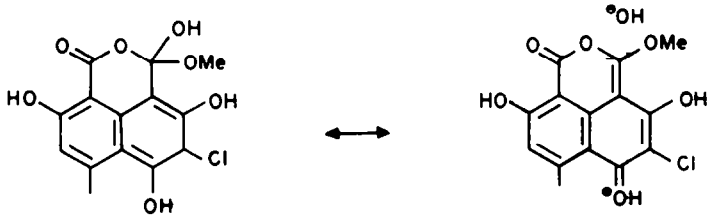


18 $R_1 = H$, $R_2 = Me$
20 $R_1 = Me$, $R_2 = H$





24 $R_1 = R_2 = Et$
25 $R_1 = R_2 = Me$
26 $R_1 = R_2 = H$



27 28

1 and 4 could be derived from naphthalic acid precursors. The carbonate 3 appears to be the first natural product which contains an $-O-CO_2Me$ function. The biogenesis of this grouping in 3 and the biosynthesis of 1 are discussed in a separate communication.

EXPERIMENTAL

Isolation of the metabolites 1–4

The filtrates from 10-day old surface cultures of a strain of *Verticillium lamellicola* grown on Czapek-Dox/1% yeast extract were continuously extracted with CH_2Cl_2. The resulting mixture of metabolites (6 g) was chromatographed on a column of silica (600 g). After elution of lipids with $CHCl_3$, elution with $CHCl_3$–EtOAc (99:1) gave fractions which, using prep. TLC (EtOAc) gave 4-O-carbomethoxylamellicolic anhydride **2**, 50 mg). Further elution of the column with $CHCl_3$–EtOAc (19:1 to 10:1) gave the major component, lamellicolic anhydride **1** which was obtained pure by fractional crystallization from MeOH–$CHCl_3$ (600 mg, i.e. *ca* 6 mg/l.). Prep TLC of the mother liquors from these crystallizations gave the chloroacid **3**, *ca* 10 mg). Finally, elution of the column with $CHCl_3$–EtOAc (3:2) gave fractions which after prep TLC afforded the quinone **4**, 40 mg).

Lamellicolic anhydride **1**. This crystallized from MeOH–$CHCl_3$ as pale yellow needles, decomp. over 300°; R_f 0.35 on TLC with silica gel and $CHCl_3$–MeOH (9:1), IR (KBr) 1700, 1650, 1615, 1595, 1345, 1320, 1230, 1175, 1030, 880, 850, 810, 780, 755 cm^{-1}, UV (EtOH) λ_{max} 250 nm (ϵ 15000), 292 (5700), 352 (10,000), 368 inf (7800); UV (EtOH + NaOH) λ_{max} 251 nm inf (ϵ 15,000), 314 (23,000), 372 (11,000) reverting to EtOH spectrum upon acidification; ^1H NMR (DMSO-d_6) 2.74 δ (s, Ar-CH_3), 6.35 (s, H-3), 6.80 (s, sharpening upon irr at 2.74, H-6); MS *m/e* 260 (100%, M^+), 242 (8), 216 (38), 214 (8), 188 (8), 160 (29), 131 (2), 128 (8), 103 (8), 102 (8). (Found MS *m/e* 260.0323. $C_{13}H_8O_6$ requires 260.0320.)

Methylation of lamellicolic anhydride **1**. The anhydride **1** (100 mg) and an excess of Me_2SO_4 in dry acetone (10 ml) was refluxed and stirred under N_2 with anhydrous K_2CO_3 (100 mg) for 48 h. After addition of $CHCl_3$ (100 ml) the mixture was washed successively with dil. aq HCl and water. Evaporation and prep TLC ($CHCl_3$–MeOH, 49:1) of the residue gave the major product, the tri-O-methyl ether **5** (81 mg, 69%), m.p. above 290° from MeOH–$CHCl_3$; R_f 0.27 on TLC with silica gel using $CHCl_3$–MeOH (19:1); IR (KBr) 1750, 1715, 1595, 1562, 1358, 1295, 1245, 1218, 1172, 1064, 1042, 1010, 966, 817, 743, 730 cm^{-1}; IR ($CHCl_3$) 1750, 1715, 1595, 1562, 1295, 1065, 1040, 1010 cm^{-1}; UV (EtOH) λ_{max} 227 nm (ϵ 5900), 252 (13,000), 261 inf (12,000), 346 (5800), 363 (4900), 380 (3800); ^1H NMR (CF_3CO_2H) 3.06 δ (s, $ArCH_3$), 4.27 (s, OMe), 4.29 (s, 2 x OMe), 6.86 (s, H-3, enhanced by 37% upon irr at 4.27 δ), 7.27 (s, H-6, enhanced by 11% and 21% upon irr at 4.27 and 3.06 δ resp.); ^1H NMR ($CDCl_3$) 2.86 δ (s, $ArCH_3$), 4.10 (s, OMe), 4.13 (s, 2 x OMe), 6.49 (s, H-3), 6.88 (s, H-6); MS *m/e* 302 (100%, M^+), 287 (13), 258 (27), 229 (22), 228 (24), 195 (10). (Found: C, 63.9; H, 4.7. $C_{16}H_{14}O_6$ requires C, 63.6; H, 4.7% MS *m/e* 302.0803. $C_{16}H_{14}O_6$ requires 302.0790.) Also obtained was the monomethyl ether **9** (12 mg, 10%), m.p. above 260° from $CHCl_3$; R_f 0.48; IR (KBr) 1710, 1665, 1625, 1605, 1305, 1215, 1180, 1165, 1035 cm^{-1}; IR ($CHCl_3$) 3120–2800 (br), 1710, 1670, 1620, 1605, 1300, 1040, 990 cm^{-1}; UV ($CHCl_3$) λ_{max} 254 nm (ϵ 9900), 285 inf (4100), 290 (4200), 350 (8900), 367 (6700); ^1H NMR (CF_3CO_2H) 2.90 δ (s, $ArCH_3$), 4.15 (s, OMe), 6.81 (s, H-3), 7.06 (s, H-6); MS *m/e* 274 (100%, M^+), 230 (66), 187 (33), 185 (22), 174 (61), 159 (22),

157 (22), 131 (22), 116 (22), 115 (22). (Found: MS *m/e* 274.0476. $C_{14}H_{10}O_6$ requires 274.0477.)

Partial demethylation of the anhydride **5**. MgI_2–etherate was prepared by adding Mg (0.67 g), and I_2 (3.34 g) to a mixture of Et_2O (4.2 ml) and benzene (8.35 ml). The filtered solution (0.125 ml) was added under N_2 to a stirred suspension of **5** (40 mg) in benzene (10 ml) and the mixture refluxed for 3 h. After acidification, $CHCl_3$ (50 ml) was added and the organic layer washed successively with aq $NaHSO_3$ and water. Evaporation and prep TLC ($CHCl_3$–MeOH, 19:1) of the residue gave the anhydride **9** (16 mg, 44%), identical in all respects to the sample prepared in the foregoing experiment.

Tri-O-acetyl-lamellicolic anhydride **6**. Prepared by acetylation of **1** using Ac_2O-pyridine, the tri-O-acetyl compound **6** crystallized from MeOH–$CHCl_3$ in colourless prisms, m.p. 177–179°; R_f 0.32 on TLC with silica gel using $CHCl_3$–MeOH (49:1); IR (KBr) 1775, 1730, 1598, 1580, 1370, 1355, 1278, 1172, 1158, 1075, 1058, 1035, 912, 688 cm^{-1}; UV (EtOH) λ_{max} 248 nm (ϵ 5700), 341 (2100); NMR ($CDCl_3$) 2.49 δ (s, 3 x OAc), 2.82 (s, $ArCH_3$) (H-3 and H-6 obscured by $CHCl_3$ signal at 7.25 δ); ^1H NMR (DMSO-d_6) 2.84 (s, $ArCH_3$). 7.58 (s, H-6), 7.62 (s, H-3) (OAc signals obscured by DMSO signal at 2.4; MS *m/e* 386 (3%, M^+), 344 (9), 302 (24), 260 (100), 216 (4). (Found: MS *m/e* 386.0645. $C_{19}H_{14}O_9$ requires 386.0638.)

4-O-Acetyl-lamellicolic anhydride **7**. The anhydride **1** (50 mg) was heated with Ac_2O (1 ml) at 110° for 2 h. Upon cooling, water (10 ml) was added and the mixture left for 15 h. The acetate **7** which separated, crystallized from $CHCl_3$–petrol in colourless plates (35 mg, 60%), m.p. 224°; R_f 0.5 on TLC with silica gel using $CHCl_3$–MeOH (49:1); IR (KBr) 1757, 1710, 1670, 1617, 1600, 1330, 1290, 1180, 1165, 1145, 1080, 1065, 1035, 1010, 910, 890, 885, 810, 785, 750 cm^{-1}; ^1H NMR ($CDCl_3$) 2.45 δ (s, OAc), 2.75 (s, $ArCH_3$), 6.95 and 7.0 (ea 1H, s, Ar–H). (Found: C, 59.03; H, 3.48. $C_{15}H_{10}O_7$ requires C, 59.60; H, 3.31%.)

4-O-Butyryl-lamellicolic anhydride **8**. An analogous procedure afforded the butyrate **8** (63%), m.p. 157° from $CHCl_3$; R_f 0.15 on TLC using $CHCl_3$; IR (KBr) 1757, 1710, 1667, 1618, 1605, 1340, 1290; 1185, 1170, 1120, 1095, 1070, 1040, 920, 880, 875, 810, 790, 765, 740 cm^{-1}; ^1H NMR ($CDCl_3$) 1.10 δ (t, J = 8 Hz, CH_2CH_3), 1.9 (m, CH_2Me), 2.72 (t, J = 8 Hz, $-CH_2CO$), 2.8 (s, $ArCH_3$), 6.9 and 7.0 (ea 1H, s, Ar–H). (Found: C, 62.70; H, 4.30. $C_{17}H_{17}O_4$ requires C, 63.03; H, 4.27%.)

The amino-imides **10** *and* **11**. The trimethyl ether **5** (100 mg) and 40% aq $MeNH_2$ (10 ml) was stirred and heated under N_2 at 80° for 48 h. After addition of $CHCl_3$ (100 ml) the mixture was washed successively with cold dil aq HCl (50 ml) and water. Evaporation gave a yellow solid prep TLC ($CHCl_3$–MeOH) of which gave the diamino-imide **10** (66 mg, 55%), m.p. 225–228° (MeOH–$CHCl_3$); R_f 0.80 on TLC with silica gel and $CHCl_3$–MeOH (49:1); IR ($CHCl_3$) 3240 br, 3005, 1635, 1605, 1595, 1275, 1195, 1148, 1070, 818 cm^{-1}; UV (EtOH) λ_{max} 246 nm (ϵ 39,000), 265 inf (30,000), 288 inf (12,000), 312 (7900), 370 inf (17,000), 390 inf (26,000), 403 (42,000); ^1H NMR ($CDCl_3$) 2.70 δ (s, $ArCH_3$), 3.05 (d, J = 4 Hz, irr 11.5 $\delta \rightarrow$ s, 2 x $ArNCH_3$), 3.50 (s, imide NCH_3), 3.95 (s, OMe), 5.85 (s, H-3), 6.39 (s, H-6), 11.5 (br, exchangeable with D_2O, 2 x NH); MS *m/s* 313 (100%, M^+), 312 (22), 298 (11), 296 (11), 285 (28), 269 (11). (Found: C, 65.15; H, 6.20; N, 13.40. $C_{17}H_{19}N_3O_3$ requires C, 65.16; H, 6.11; N, 13.41%.) Also obtained was the amino-imide **11** (24 mg, 20%), m.p. 244–246° (MeOH–$CHCl_3$); R_f 0.61 on TLC with silica gel and $CHCl_3$–MeOH (19:1); IR ($CHCl_3$) 3230, 3005, 1660, 1620, 1582, 1270, 1160, 1100, 1062, 818 cm^{-1}; UV ($CHCl_3$) λ_{max} 270 nm (ϵ 50,000), 302 (15,000), 329 inf (14,000), 251 (19,000), 426 (22,000); ^1H NMR ($CDCl_3$) 2.70 δ (s, $ArCH_3$), 3.05 (d, J = 4 Hz, irr 10.3 $\delta \rightarrow$ s, $ArNCH_3$), 3.48 (s, $-CONCH_3$), 3.95 (s, OCH_3), 4.08 (s, OMe), 6.08 (s, H-3), 6.75 (s, H-6), 10.3 (br, exchangeable with D_2O, NH); MS *m/s* 314 (90%, M^+), 313 (15), 299 (25), 297 (25), 286 (30), 285 (100), 271 (10), 270 (15), 260 (10), 268 (15), 256 (30), 228 (15). (Found: MS *m/e* 314.1264. $C_{17}H_{18}N_2O_4$ requires *m/e* 314.1266.)

Ethanolysis of **5** *and formation of the diester* **13**. A suspension of the trimethyl ether **5** (30 mg) in EtOH (5 ml) was stirred with $NaBH_4$ (30 mg) for 24 h. Addition of $CHCl_3$ (50 ml), filtration through glass paper and evaporation under reduced pressure gave the acid ester **12** as a colourless solid, R_f 0.2 on TLC using silica gel and $CHCl_3$–MeOH (9:1). This readily cyclized to **5**, R_f 0.5 by treatment with acid or by heating, e.g. in EtOAc. The product **12** in MeOH (10 ml) was treated with ethereal CH_2N_2 prepared from nitrosan (2.57 g) for 15 h. The solution was filtered, washed with water and evaporated, prep. TLC of the residue ($CHCl_3$) and crystallization from EtOAc–petrol then giving the diester **13** as colourless needles (29 mg, 81%), m.p. 96–97°; R_f 0.3 ($CHCl_3$); IR ($CHCl_3$), 1720, 1590, 1335, 1075, 1035 cm^{-1}; UV (EtOH) λ_{max} 254 nm (ϵ 24,000), 320 (5000), 341 inf (3700) unchanged upon addition of acid or base; ^1H NMR ($CDCl_3$) 1.35 δ (t, J = 6 Hz, irr 4.30 $\delta \rightarrow$ s, CH_3), 2.85 (s, Ar–CH_3), 3.85 (s, OMe), 3.90 (s, 3 x OMe), 4.30 (q, J = 6 Hz, irr 1.35 $\delta \rightarrow$ s, OCH_2-), 6.45 (s, H-3), 6.85 (s, H-6); MS *m/e* 362 (75%, M^+), 331 [17, $(M-OMe)^+$ 317 [58, $M-OEt)^+$], 303 [84, $(M-CO_2Me)^+$], 289 [100, $(M-CO_2Et)^+$], 275 (58), 259 (50). (Found: C, 63.04; H, 6.23%. $C_{19}H_{22}O_7$ requires C, 62.98; H, 6.12%.)

Preparation of the trimethoxynaphthalenes **14** *and* **15** *from* **1**. The anhydride **1** (106 mg) was refluxed under N_2 with 5N aq NaOH (5 ml) for 3 h. After acidification, extraction with EtOAc gave a mixture which was taken up in MeOH and allowed to stand with a large excess of ethereal CH_2N_2 for 15 h. Prep TLC of the product ($CHCl_3$–petrol, 1:1) gave the carbomethoxy-trimethoxynaphthalene **14** as colourless prisms (47 mg, 39%), m.p. 126–128° from EtOAc–hexane; R_f 0.45 on TLC using silica gel and $CHCl_3$; IR ($CHCl_3$) 1720, 1620, 1590, 1350, 1070, 1045, 1030, 990 cm^{-1}; UV (EtOH) λ_{max} 236 nm (ϵ 17,000), 245 inf (20,000), 249 (21,000), 307 (6200) unchanged upon addition of acid or base; ^1H NMR ($CDCl_3$) 2.86 δ (s, Ar–CH_3), 3.90 (s, OMe), 3.95 and 4.04 (ea 3H, OMe), 6.38 and 6.60 (ea 1H, d, J = 2 Hz, Ar-H), 6.82 (s, sharpening upon irr at 2.86 δ, H-6); MS *m/e* 290 (100%, M^+), 259 (88). (Found: C, 66.29; H, 6.27. $C_{16}H_{18}O_5$ requires C, 66.20; H, 6.25%.) Also obtained was the trimethoxynaphthalene **15** as colourless prisms (13 mg, 13%), m.p. 93–95° from hexane; R_f 0.55 on TLC with silica gel and $CHCl_3$–petrol (1:1); IR (KBr) 1615, 1595, 1250, 1208, 1160, 1055, 960, 835 cm^{-1}; UV (EtOH) λ_{max} 237 nm (ϵ 33,000), 249 inf (26,000), 269 (2800), 281 (3300), 297 (3500) unchanged upon addition of acid or base; ^1H NMR ($CDCl_3$) 2.80 δ (s, Ar-CH_3), 3.88 (s, 2 x OMe), 3.90 (s, OMe), 6.39, 6.69, 6.76 and 6.90 (ea 1H, d, J = 3 Hz, Ar-H); MS *m/e* 232 (100%, M^+), 217 (12), 189 (37), 175 (15). (Found: MS *m/e* 232.1099. $C_{14}H_{16}O_3$ requires *m/e* 232.1099.)

2,7 - *Dihydroxy* - 5 - *methyl* - 1,4 - *naphthaquinone* **2** (i). Isolated from cultures of *Verticillium lamellicola* as described above, this crystallized from MeOH–$CHCl_3$ as red brown prisms which decomposed above 250°; R_f 0.35 on TLC using silica gel and EtOAc; IR (KBr) 3210 br, 1660, 1590, 1570, 1360, 1320, 1205, 1095, 1060, 1005, 880, 868, 800, 740, 705 cm^{-1}; UV (EtOH) λ_{max} 266 nm (ϵ 10,000), 296 (6100), 346 (1600); UV (EtOH, NaOH) λ_{max} 286 nm (ϵ 14,000), 325 inf (4100), 378 (2800); ^1H NMR (CD_3OD), 2.60 δ (s, Ar–CH_3), 6.90 (d, J = 2 Hz, Ar-H), 7.35 (d, J = 2 Hz, Ar–H); MS *m/e* 204 (100%, M^+), 176 (61), 148 (17), 135 (78), 107 (22). (Found: MS *m/e* 204.0422. $C_{11}H_{18}O_4$ requires *m/e* 204.0423.)

(ii) The anhydride **1** (30 mg) in aq NaOH (5M, 10 ml) was heated at 80° under N_2 for 4h. After cooling and acidifying with dil aq HCl, extraction with EtOAc gave crude 4-methyl-1,3,6-trihydroxynaphthalene. A solution of this in MeOH (10 ml) was stirred with 5M aq NaOH (0.1 ml) for 48 h. After acidification with dil aq HCl and evaporation of the MeOH, extraction with EtOAc gave the quinone, identical (R_f, IR, UV, NMR, mixed m.p.) with the sample obtained as in (i).

2,7-Dimethoxy-5-methyl-1,4-naphthaquinone **16**. The quinone **2** (35 mg) in dry acetone (20 ml) containing an excess of Me_2SO_4 was refluxed over anhydrous K_2CO_3 for 15 h. After evaporation of the acetone, the residue, in $CHCl_3$, was washed with brine, dried and evaporated to give the dimethyl ether **16** as long yellow needles (30 mg, 75%), m.p. 172–173° from $CHCl_3$–petrol; R_f 0.35 on TLC using silica gel and $CHCl_3$; IR ($CHCl_3$) 1680, 1640, 1625, 1595, 1560, 1345, 1305, 1275, 1155, 1080, 1035, 885 cm^{-1}; UV (EtOH) λ_{max} 263 nm (ϵ 18,000), 291 (10,000), 343 (1700), 384 (1300), unchanged on addition of acid or base; ^1H NMR ($CDCl_3$) 2.75 δ (s, Ar–CH_3), 3.90 and 3.95 (ea 3H, s, OMe), 6.08 (s, sharpening upon irr at 3.90 δ, H-3), 7.00 (d, J = 3 Hz, H-6), 7.58

(J = 3 Hz, H-8); MS *m/e* 232 (100%, M^+), 217 (27), 204 (20), 203 (20), 202 (37), 189 (13), 175 (17), 174 (17), 161 (47), 146 (20), 133 (80). (Found: C, 67.02; H, 5.20. $C_{13}H_{12}O_4$ requires C, 67.23; H, 5.21%; MS *m/e* 232.0731. $C_{13}H_{12}O_4$ requires *m/e* 232.0736.)

4 - *O* - (3,3 - *Dimethylallyl*)*lamellicolic anhydride* **19**. A mixture of the anhydride **1** (100 mg) and 3,3-dimethylallyl bromide (61 mg) in acetone (30 ml) was stirred and refluxed over anhydrous K_2CO_3 under N_2 for 15 h. After addition of $CHCl_3$ (50 ml) and cold dil aq HCl (25 ml), the organic layer was washed with brine to neutrality and evaporated to give the dimethylallyl ether **19** as colourless needles (67 mg, 53%), m.p. 192–193° from MeOH–$CHCl_3$; R_f 0.45 on TLC using silica gel and $CHCl_3$; IR (KBr) 3100 br, 1710, 1662, 1620, 1595, 1340, 1300, 1200, 1185, 1162, 1038, 958, 808, 755 cm^{-1}; UV (EtOH) λ_{max} 249 nm (ϵ 11,000), 290 (4100), 309 (3100), 348 (7400), 366 inf (6100); UV (EtOH, NaOH) λ_{max} 248 inf nm (ϵ 13,000), 302 (8400), 317 (11,000), 352 inf (4900), 384 (9200); ^1H NMR ($CDCl_3$) 1.84 δ (s, vinyl Me), 2.79 (s, Ar–CH_3), 4.70 (d, J = 6 Hz, irr 4.50 $\delta \rightarrow$ s, OCH_2–), 5.50 (br t, J = 6 Hz, irr 4.70 $\delta \rightarrow$ s, olefinic H), 6.42 (s, H-3), 6.82 (s, H-6), 11.25 and 11.50 (ea 1H, s, exchangeable with D_2O, OH); MS *m/e* 328 (7% M^+), 313 (7), 260 (100, M–C_5H_8), 242 (7), 231 (7), 216 (25), 214 (14). (Found: MS *m/e* 328.0936. $C_{18}H_{16}O_6$ requires *m/e* 328.0931.)

The cyclic ether **18**. The dimethylallyl ether **19** (35 mg) in dry $HCONMe_2$ (3 ml) was heated under N_2 at 120° for 15 h. After addition of EtOAc (100 ml), the mixture was washed with water, dried and evaporated to give a yellow solid. Prep TLC (benzene–EtOH, 24:1) gave the cyclic ether **18** which crystallized from MeOH–$CHCl_3$ in colourless needles (14 mg, 40%), subliming between 200 and 250°; R_f 0.69 on TLC using silica gel and $CHCl_3$–petrol (1:1); IR ($CHCl_3$) 3280–3000 br, 1710, 1670, 1625, 1615, 1330, 1305, 1060, 1040, 865 cm^{-1}; UV (EtOH) 256 nm (ϵ 21,000), 290 inf (6700), 360 (12,000), 322 (13,000), 376 inf (9500), 392 (14,000); UV (EtOH, NaOH) λ_{max} 250 inf nm (ϵ 17,000), 306 (12,000), 322 (13,000), 376 inf (9500), 392 (14,000); ^1H NMR ($CDCl_3$) 1.35 δ (s, Me), 1.55 (d, J = 6 Hz, irr 4.75 $\delta \rightarrow$ s, –CH–Me), 1.60 (s, Me), 2.85 (s, Ar–CH_3), 4.75 (q, J = 6 Hz, irr 1.55 $\delta \rightarrow$ s, –OCH–), 6.80 (s, H-6), 11.55 (m, exchangeable with D_2O, 2 × OH); MS *m/e* 328 (25%, M^+), 313 (100, M–CH_3), 295 (13), 285 (18), 269 (18). (Found: MS *m/e* 328.0947. $C_{18}H_{16}O_6$ requires *m/e* 328.0931). This was identical (R_f, mixed m.p., spectra) with a sample prepared from herqueinone.[6]

When the ether **19** was heated in a sublimation block at 160°/0.02 mm for 5 h, the product obtained after prep TLC appeared to be homogeneous by TLC and had UV and MS identical to that of **18**, but was probably a mixture (1:2) of **18** and **20**; NMR ($CDCl_3$) 1.25–1.55 (complex group of signals, 3 x Me), 3.38 (2/3 H, q, J = 6 Hz, Ar–CH), 4.75 (1/3 H, q, J = 6 Hz, OCH–), 6.85 (s, H-6), 11.30 and 11.50 (ea 1H, s, exchangeable with D_2O, OH).

4 - *O* - *Carbomethoxylamellicolic anhydride* **3**. (i) Isolated from cultures of *V. lamellicola* as described above as colourless prisms from $CHCl_3$, m.p. 202–204°; R_f on TLC using silica gel and $CHCl_3$–MeOH (19:1); IR (KBr) 1765, 1725, 1675, 1615, 1605, 1460, 1435, 1270, 1190, 1165, 1065, 1040, 930, 880, 870, 805 cm^{-1}; UV (EtOH) λ_{max} 226 nm (ϵ 4200), 247 (ϵ 4900), 282 (1200), 313 (1700), 341 (1900), 363 (2200), 395 (1100); UV (EtOH, NaOH) λ_{max} 251 inf nm (ϵ 8000), 314 (11,000), 372 (6000) reverting to spectrum of **1** in EtOH upon reacidification; ^1H NMR ($CDCl_3$) 2.80 δ (s, $ArCH_3$), 4.00 (s, OMe), 7.04 (s, H-6), 7.08 (s, H-3), 11.60 and 11.70 (ea 1H, s, exchangeable with D_2O, OH); MS *m/e* 318 (100%, M^+), 275 (13), 274 (100), 259 (13), 256 (13), 230 (50), 213 (25), 186 (13), 184 (13), 174 (37). (Found: C, 56.49; H, 3.24. $C_{15}H_{10}O_6$ requires C, 56.61; H, 3.17%.)

(ii) The anhydride **1** (100 mg) in dry acetone (20 ml) was stirred and refluxed under N_2 with anhydrous K_2CO_3 (100 mg) and $ClCO_2Me$ (29 μl) for 18 h. The mixture was poured into cold dil aq HCl and extracted with EtOAc (50 ml). The organic layer after washing to neutrality and evaporation gave yellow solid from which the carbonate **3** was obtained by crystallization ($CHCl_3$–MeOH) (75 mg, 61%), identical (m.p. R_f, spectral data) to a sample obtained as in (i).

Acetylation of **3** under standard conditions gave the triacetate **6**.

Methylation of the carbonate **3**. The carbonate **3** (58 mg) in dry acetone (20 ml) was stirred and refluxed under N_2 with anhydrous K_2CO_3 (58 mg) and an excess of Me_2SO_4 for 24 h. After addition of $CHCl_3$ (50 ml) the solution was washed with dil aq HCl and evaporated. Prep TLC gave the trimethyl ether **5** (14 mg, 26%) together with the less polar dimethyl ether **21** as colourless prisms from MeOH–$CHCl_3$ (22 mg, 35%), which sublimed slowly between 200 and 210°; R_f 0.36 ($CHCl_3$–MeOH, 19:1); IR ($CHCl_3$) 1760, 1720, 1605, 1560, 1460, 1435, 1360, 1290, 1065, 1040, 985, 935, 840 cm^{-1}; UV (EtOH) λ_{max} 257 nm (ϵ 15,000), 323 inf (2300), 340 (3800), 370 (3700), 384 (3300); UV (EtOH, NaOH) λ_{max} 249 nm (ϵ 20,000), 304 (2900), 312 (2800), 345 inf (1300); ^1H NMR ($CDCl_3$) 2.82 δ (s, Ar–CH_3), 4.02 (s, OMe), 4.16 (s, 2 x OMe), 7.10 (s, H-6), 7.18 (s, H-3); MS *m/e* 346 (100%, M^+), 331 (33), 302 (16), 301 (16), 287 (16), 259 (50), 257 (33). (Found: C, 58.87; H, 4.03. $C_{17}H_{14}O_8$ requires C, 58.96; H, 4.08%.)

Ammonolysis of the carbonate **3**. The carbonate **3** (30 mg) was treated with liquid NH_3 (1 ml) and the excess of NH_3 allowed to evaporate at room temp. Sublimation of the residue gave methyl carbamate as colourless plates (7 mg, 60%), m.p. and mixed m.p. 53–54°. The non-volatile residue consisting of **1** (16 mg, 64%) crystallized from $CHCl_3$–EtOH in yellow needles decomp over 300° (identified by mixed m.p., TLC, IR spectra).

Monomethyl 3-chlorolamellicolate **4**. Isolated from cultures of *V. lamellicola* as described above as orange prisms from MeOH–$CHCl_3$ which did not melt < 300° but sublimed slowly < 250°/0.03 mm Hg; R_f 0.20 ($CHCl_3$–MeOH, 4:1); brick red with $FeCl_3$; IR (KBr) 1675, 1640, 1610, 1580, 1535, 1490, 1460, 1320, 1265, 1200, 1045, 810, 735 cm^{-1}; UV (EtOH) λ_{max} 270 nm (ϵ 4000), 322 (3000), 400 (2000); UV (EtOH, NaOH) λ_{max} 316 nm (ϵ 5000), 378 (3000); λ_{max} (EtOH, NaOH then aq HCl), 254 nm (ϵ 7000), 356 (4000); ^1H NMR (DMSO-d_6) 2.75 δ (s, $ArCH_3$), 3.70 (s, OMe), 6.50 (s, H-6); MS *m/e* 310 and 308 (7 and 21%, M^+-18), 296 and 294 (33 and 100), 278 and 276 (7 and 21), 264 and 262 (6 and 17), 254 and 252 (12 and 36), 236 and 234 (5 and 14), 196 and 194 (8 and 24). (Found: MS *m/e* 308.0085. $C_{14}H_9ClO_6$ requires *m/e* 308.0088.)

Dimethyl 3-chloro-O,O,O-trimethylamellicolate **23**. The chloro compound **4** (30 mg) in dry MeOH (5 ml) was treated with ethereal CH_2N_2 prepared from nitrosan (5.1 g) and the mixture allowed to stand at room temp for 15 h. The product **23**, purified by prep TLC ($CHCl_3$–MeOH, 24:1) was obtained as a colourless gum; R_f 0.58 ($CHCl_3$–MeOH, 49:1); IR ($CHCl_3$) 1730, 1590, 1570, 1340, 1070, 1005, 980 cm^{-1}; UV (EtOH) λ_{max} 246 nm (ϵ 57,000), 285 inf (4600), 298 (5700), 311 (5700), 344 (3100), unchanged upon addition of acid or base; ^1H NMR (CCl_4) 2.85 δ (s, $ArCH_3$), 3.80 (s, OMe), 3.85 (s, OMe), 3.90 (s, OMe), 3.95 (s, 2 x OMe), 6.95 (s, sharpening upon irr at 2.85 δ, H-6); MS *m/e* 384 and 382 (M^+, $C_{18}H_{19}ClO_7$, 29 and 86%), 353 and 351 (14 and 43%), 338 and 336 (5 and 14), 325 and 323 (33 and 100), 310 and 308 (9 and 28), 295 and 293 (19 and 57).

3-Chloro-O,O,O-triethyllamellicolic anhydride **24**. (i) The chloro compound 4 (50 mg) in dry acetone (20 ml) containing an excess of Et_2SO_4 was stirred and refluxed over K_2CO_3 for 15 h. After addition of $CHCl_3$ (100 ml) and washing successively with dil aq HCl and water, evaporation and prep TLC ($CHCl_3$) gave the triethyl ether **24** as colourless prisms (20 mg, 38%), subliming above 170° and m.p. 195–197°; R_f 0.23 ($CHCl_3$); IR ($CHCl_3$) 1760, 1725, 1595, 1570, 1375, 1345, 1340, 1095, 1030, 970, 880 cm^{-1}; UV (EtOH) λ_{max} 257 nm (ϵ 14,000), 323 inf (3100), 339 (4000), 366 (3300), 384 (3000); UV (EtOH, NaOH) λ_{max} 249 nm (ϵ 17,000), 308 (3100), 345 inf (1900); ^1H NMR ($CDCl_3$) 1.50 δ (t, J = 6 Hz, 2 x C–Me) 1.55 (t, J = 6 Hz, C–Me), 2.95 (s, ArMe), 4.10–4.45 (br m, 3 x OCH_2), 7.10 (s, H-6); MS *m/e* 380 and 378 (M^+, 20 and 60%), 362 and 360 (30 and 90), 351 and 349 (33 and 100), 323 and 321 (20 and 60), 296 and 294 (27 and 80). (Found: C, 60.52; H, 5.16. $C_{19}H_{19}ClO_6$ requires C, 60.32; H, 5.03%.)

(ii) Ethylation of the chloro-anhydride **26** in similar fashion afforded a sample of the triethyl ether **24** identical (R_f, spectral data, mixed m.p.) with that prepared as in (i).

3-Chloro-O,O,O-trimethylamellicolic anhydride **25**. (i) The anhydride **5** (30 mg), suspended in CCl_4 (10 ml) was treated with a 1.2 M soln of Cl_2 in CCl_4 (0.17 ml) and the mixture stirred for 15 h. After addition of $CHCl_3$ (75 ml) the mixture was washed successively with aq $NaHSO_3$ (25 ml) and water and then evaporated. Prep TLC ($CHCl_3$) gave the chloro compound **25**

which crystallized from $CHCl_3$–petrol as pale yellow needles (15 mg, 44%) which sublimed slowly above 200°, R_f 0.31 on silica using $CHCl_3$; IR ($CHCl_3$) 1760, 1725, 1595, 1570, 1035, 965, 945 cm^{-1}; UV (EtOH) λ_{max} 256 nm (ϵ 13,000), 323 inf (3800), 338 (4600), 368 inf (3800), 386 inf (3100); UV (EtOH, NaOH), λ_{max} 248 nm (ϵ 18,000), 303 (4000), 313 (4000), 345 (2800); ^1H NMR ($CDCl_3$) 3.00 δ (s, Ar–CH_3), 4.10 (s, 2 x OMe), 4.15 (s, OMe), 7.20 (s, sharpening upon irradiation at 3.00 δ, H-6), MS *m/e* 338 and 336 (33 and 100%, M^+), 323 and 321 (27 and 80), 320 and 318 (13 and 40), 305 and 303 (7 and 20), 293 and 291 (20 and 60). (Found: C, 56.94; H, 3.76. $C_{16}H_{13}ClO_6$ requires C, 56.80; H, 3.84%.)

(ii) The chloro-compound **26** (35 mg), suspended in acetone (20 ml) was stirred and refluxed with an excess of Me_2SO_4 and anhydrous K_2CO_3 (35 mg). After addition of $CHCl_3$ (100 ml) and successive washing with dil HCl (25 ml) and water (3 × 25 ml), the soln was evaporated. Prep TLC ($CHCl_3$) then gave the chloro compound **25**, a sample being identical in all respects (R_f spectral data and mixed m.p.) to a sample prepared as in (i).

3-Chlorolamellicolic anhydride **26**. The anhydride **1** (58 mg) suspended in CCl_4 (10 ml), was treated with a 1.2 M solution of Cl_2 in CCl_4 (0.32 ml) and the mixture stirred for 15 h. The resulting insoluble chloro compound **26** crystallized from a large volume of MeOH in colourless needles (46 mg, 71%), decomposing slowly above 250°; IR (KBr) 1710, 1660, 1610, 1590, 1455, 1310, 1190, 1150, 1035, 805, 765 cm^{-1}; UV (EtOH) λ_{max} 254 nm, 276 inf, 290 inf, 322 inf, 355, 371 inf; UV (EtOH, NaOH) λ_{max} 253 inf nm, 316, 376 reverting to EtOH spectrum upon acidification; MS *m/e* 296 and 294 (33 and 100%, M^+), 278 and 276 (7, 21), 252 and 250 (14, 42), 196 and 194 (12, 36). (Found: C, 52.59; H, 2.32. $C_{13}H_7ClO_6$ requires C, 53.06; H, 2.45%.)

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