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NAPHTHOQUINONE-LACTONES AND EXTENDED QUINONES FROM *VENTILAGO CALYCULATA*

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Key Word Index—*Ventilago calyculata*; Rhamnaceae; benzisochromanquinone; ventilatone; ventilein.

Abstract—New quinones have been isolated from the root bark of *Ventilago calyculata*. Ventilatones A and B are benzisochromanquinones, related to the ventiloquinones, which have an additional fused lactone ring while ventileins A and B are benzisochroman dimers having a dihydroxy-*peri*-xanthenoxanthenequinone chromophore.

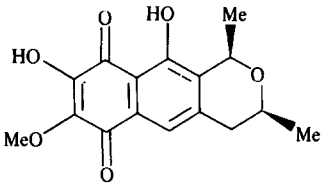
In continuing our search for quinonoid constituents in *Ventilago* species [1–6], we have isolated from the root bark of *V. calyculata* two quinone lactones, ventilatones-A and -B, and two extended quinones, ventileins-A and -B, which are related to the benzisochromanquinones described earlier [7].

Ventilatone-A has the molecular formula $C_{17}H_{12}O_6$, which suggests that it belongs to the benzisochromanquinone group [7], e.g. ventiloquinone H (**1**) a co-metabolite. It is reduced by dithionite, and shows quinone carbonyl absorption at 1675 and 1650 cm^{-1}. The ^1H NMR spectrum (in DMSO-d_6) includes signals for the part structure $ArCH_2CHMe$, with the methine carbon attached to oxygen (δ_{CH} 4.61), but there was no evidence for the usual ArCH(Me)O– group at C-1. The rest of the ^1H NMR spectrum comprises four singlets of which three could be assigned to methoxyl (δ3.85), a *peri*-proton (7.78) and a proton (δ6.30) on the quinone ring adjacent to the methoxyl (H-3 in 2-methoxy-1,4-naphthoquinone resonates at δ6.36 in DMSO-d_6). These data suggest the part structure **2**, and when that is expanded to **3** it accounts for the remaining C_3HO_2 moiety, the carbonyl absorption at 1730 cm^{-1}, and the 1H singlet at δ5.93 (H-3 in 4,6-dimethoxycoumarin resonates at δ5.91 in DMSO-d_6). The methoxyl can be assigned to C-7 as the C-6 signal at δ179.0 in the proton-coupled ^{13}C spectrum is a double doublet coupled to H-5 (J = 4.1 Hz) and H-8 (J = 8.2 Hz) whereas C-9 shows only a broad signal at δ182.1. The coupling constants for H-3 and H-4 show that H-3 is axial ($J_{3a,4a'}$ = 10.8 Hz) and the methyl is therefore equatorial so that ventilatone-A can be defined as **4**. The structure is fully supported by the ^{13}C NMR spectrum, and by the ^1H NMR spectrum of the leuco-acetate. The latter, in particular, shows long range coupling between H-4 and H-5 which confirms the orientation of the dihydropyran ring. Finally, an X-ray crystallographic analysis [Cowe, H. J. and Cox, P. J., unpublished results] has established the structure and relative stereochemistry of the leuco-acetate and so confirmed that the parent quinone has structure **4**.

^{13}C assignments for ventilatone-A were based mainly on the proton-coupled ^{13}C spectrum. In addition to the carbonyl carbon signals, the four resonances at low field (δ_C 150.7, 158.6, 161.0, 163.0) correspond to carbons bonded to oxygen. The C-7 carbon signal would be expected to show fine splitting as it is coupled to the methoxyl protons and to H-8, and hence the multiplet signal at 158.6 was assigned to C-7. The signal at 150.7 (*s*) was attributed to C-10 as it is removed by four bonds from the nearest proton, and those at 161.0 and 163.0 were assigned to C-12 and C-1, respectively, by analogy with 4-alkoxycoumarins [8, 9]. The signals at 116.1 and 116.3 can be attributed to carbons *ortho* to aroyloxy substituents, i.e. C-9a and C-10a. They were not well resolved but as the signal at 116.3 shows more fine structure than the other, it must correspond to C-10a which can couple with H-5 and H-13. The remaining signals at δ133.6 and 138.6 were assigned to C-5a and C-4a, respectively, as the latter appears as a broad triplet (J = 5.7 Hz) due to coupling with the H-4 protons.

Ventilatone-B, $C_{17}H_{12}O_7$, is hydroxyventilatone-A. The ^1H NMR spectrum of B is very similar to that of A, the significant differences being the replacement of the *peri*-proton by a *peri*-hydroxyl, and the absence of long range coupling to the H-4 protons. Whereas A has no maximum in the visible region B shows λ_{max} 460 nm shifting to 548 nm in alkaline solution. Thus ventilatone-B has structure **5**.

The ventileins, present in very small amount in *V. calyculata*, are rare examples of blue natural quinones. Ventilein-A, $C_{30}H_{24}O_8$, turns yellow on reduction with dithionite, and forms a diacetate and a leucotetra-acetate. It shows only 15 signals in its ^{13}C NMR spectrum and is therefore symmetrical. The ^1H NMR spectrum shows that each half of the molecule contains a dihydro-1,3-dimethylpyran system (cf. **1**), a *peri*-hydroxyl group, and a quinonoid proton (δ6.21, *s*) next to oxygen. This suggests that ventilein-A is a dimer related to the benzoisochromanquinones present in *V. calyculata* [7]. The nature of the chromophore was revealed by the unstable yellow leucotetra-acetate whose visible spectrum closely resembles that of *peri*-xanthenoxanthene (**6**) showing the same fine structure above 390 nm with each peak shifted bathochromically by 11–19 nm due to substituent effects. This strongly implies that ventilein-A is a derivative of 3,9-dihydroxy-*peri*-xanthenoxanthene-4,10-quinone (**7**). As the



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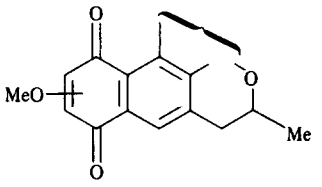

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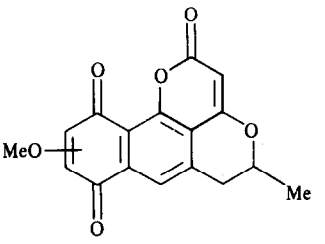

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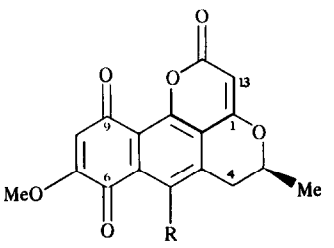
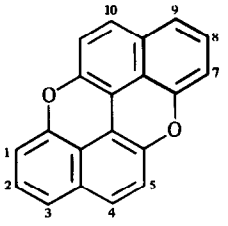


4 R = H
5 R = OH



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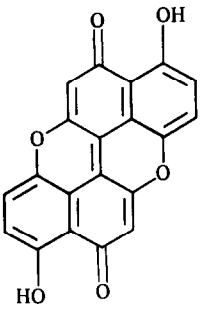

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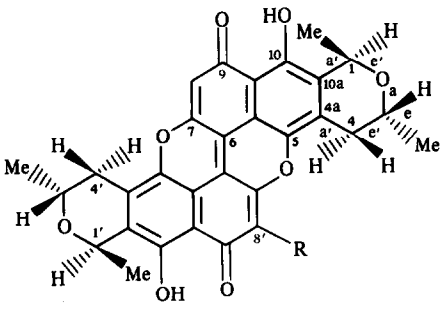
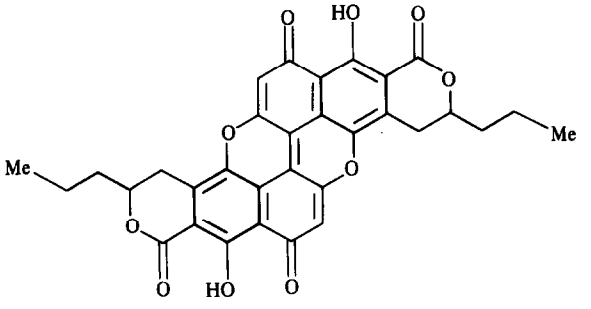


8 R = H
9 R = OMe



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blue pigment has no benzenoid protons the dihydropyran systems must be fused to the benzenoid rings of **7** and hence ventilein-A has the gross structure **8**. This has the same chromophore as the fungal pigment xylindein (**10**), from *Chlorociboria aeruginosum* [10, 11], and the two compounds have similar visible spectra and IR carbonyl absorption. The relative stereochemistry shown in **8** follows from the coupling constants of the pyran ring protons. As long range ($J_{1,4}$) coupling was not observed the proton at C-1 must be pseudo-equatorial and hence the methyl groups are *trans* [12, 13]. This is in contrast to all the *Ventilago* benzisochromanquinones which have *cis*-methyl groups [7], e.g. **1**, whereas in the aphin pigments they are invariably *trans* [14].

Ventilein-B is the methoxyventilein-A (**9**). In the ^{1}H NMR spectrum there are signals for only one quino-

noid proton and one methoxyl group. At 500 MHz weak coupling (J = 1 Hz) is detectable between H-4_a and H-1_e in one of the dihydropyran rings, and the *peri*-hydroxyl signal is split. The optical rotation was not measured.

Naturally occurring extended quinones form a small group of about 20 pigments, the majority being of microbial origin. They have not been observed previously in Rhamnaceae. It is evident that all the *Ventilago* quinones [7] are biogenetically related. The octaketide **11** is the likely precursor of the ventilatones but in the conformation **12** it could also give rise to the 1,3-dimethylbenzisochromanquinones and also to the γ-lactone enantiomers kalafungin and nanaomycin D (**13**) [15]. The ventileins presumably arise by phenolic coupling probably at the naphthol stage, e.g. **14** or a glycoside thereof as in the formation of the aphin pigments [14].

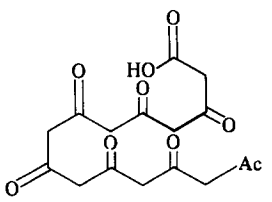
EXPERIMENTAL

For preliminary separation of the pigments from the Me_2CO extract of the root bark (4.2 kg) of *V. calyculata* see ref. [1]. Fractions 97–112 were subjected to CC (C_6H_6–EtOAc, 9:1). The earlier fractions gave ventilein-A (**8**) as dark blue needles (C_6H_6), mp > 275° (42 mg) and ventilein-B(**9**) as greenish blue needles (C_6H_6–MeOH), mp 240° (14 mg). Fractions 113–148 were rechromatographed (CC: C_6H_6–EtOAc, 9:1 and 4:1). The C_6H_6–EtOAc (9:1) eluate containing ventilatone-A (**4**) was purified by repeated crystallisation from C_6H_6; yellow needles, mp 284° (140 mg). The C_6H_6–EtOAc (4:1) eluate afforded ventilatone-B (**5**) after prep. TLC (C_6H_6–EtOAc, 4:1), orange-red needles (C_6H_6), mp 231° (65 mg).

Ventilatone-A (**4**). Found: C, 65.23; H, 3.74%; $[M]^+$, 312.0630. $C_{17}H_{12}O_6$ requires C, 65.39; H, 3.87%; $[M]^+$, 312.0634; $[\alpha]_D^{25}$ +133° (*c* 0.123, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 237 (4.43), 266 (4.21), 307 (4.02), 380 (3.75); IR ν_{max}^{KBr} cm^{-1}: 1648, 1652, 1675, 1730; ^1H NMR (360 MHz, DMSO-d_6): δ1.47 (3H, *d*, *J* = 6.3 Hz, 3-Me), 3.02 (1H, *dd*, *J* = 17.3, 10.8 Hz, H_a-4), 3.37 (1H, *dd*, *J* = 17.3, 2.9 Hz, H_e-4), 3.85 (3H, *s*, OMe), 4.61 (1H, *ddq*, *J* = 10.8, 6.3, 2.9 Hz, H-3), 5.93 (1H, *s*, H-13), 6.30 (1H, *s*, H-8), 7.78 (1H, *s*, H-5); ^{13}C NMR (90.56 MHz, $CDCl_3$): δ20.05 (Me), 33.33 (CH_2), 56.40 (OMe), 74.19 (C-3), 94.54 (C-8), 111.7 (C-13), 116.1 (C-9a), 116.3 (C-10a), 119.9 (C-5), 133.6 (C-5a), 138.6 (C-4a), 150.7 (C-10), 158.6 (C-7), 161.0 (C-12), 163.0 (C-1), 179.0 (C-6), 182.1 (C-9); MS *m/z* 312 (100%), 297.0399 ($C_{16}H_9O_6$ requires 297.0399, 16), 284.0688 ($C_{16}H_{12}O_5$ requires 284.0684, 20), 282 (25), 269 (12), 255 (18), 241 (14), 227 (10), 213 (10). The leucodiacetate (Zn–NaOAc–Ac_2O) was obtained as needles, mp 289° (MeOH). Found: C, 63.40; H, 4.52%. $C_{21}H_{18}O_8$ requires C 63.32; H, 4.55%; UV λ_{max}^{MeOH} nm (log ε): 237 (4.43), 266 (4.21), 307 (4.02), 350 (3.75); IR ν_{max}^{KBr} cm^{-1}: 1730; ^1H NMR (360 MHz, $CDCl_3$): δ1.53 (3H, *d*, *J* = 6.3 Hz, Me), 2.45 (3H, *s*, OAc), 2.63 (3H, *s*, OAc), 2.98 (1H, *ddd*, *J* = 16.7, 10.7, 1.6 Hz, H_a-4), 3.14 (*ddd*, *J* = 16.7, 3.2, 0.9 Hz, H_e-4), 3.96 (3H, *s*, OMe), 4.48 (1H, *ddq*, *J* = 10.7, 6.3, 3.2 Hz, H-3), 5.77 (1H, *s*, H-13), 7.05 (1H, *s*, H-8), 7.31 (1H, *dd*, *J* = 1.6, 0.9 Hz, H-5); MS *m/z* 398 (4%), 256 (28), 314 (100), 313 (35), 271 (9).

Ventilatone-B (**5**). Found: C, 61.96; H, 3.71%; $[M]^+$, 328.0587. $C_{17}H_{12}O_7$ requires C, 62.20; H, 3.68%; $[M]^+$, 328.0583; $[\alpha]_D^{25}$ +125° (*c* 0.107, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε): 238 (4.33), 273 (4.10), 330 (3.84), 460 (3.75); (MeOH–HO^-) 548; IR ν_{max}^{KBr} cm^{-1}: 1610, 1648, 1653, 1720, 1734 sh; ^1H NMR (360 MHz, $CDCl_3$): δ1.59 (3H, *d*, *J* = 6.2 Hz, Me), 2.75 (1H, *dd*, *J* = 17.9, 10.7 Hz, H_a-4), 3.35 (1H, *dd*, *J* = 17.9, 3.4 Hz, H_e-4), 3.90 (3H, *s*, OMe), 4.48 (1H, *ddq*, *J* = 10.7, 6.2, 3.4 Hz, H-3), 5.92 (1H, *s*, H-13), 6.18 (1H, *s*, H-8), 12.38 (1H, *s*, exch. with D_2O, *peri*-OH); MS *m/z* 328 (100%), 313.0349 ($C_{16}H_9O_7$ requires 313.0348, 26), 285.0404 ($C_{15}H_9O_6$ requires 285.0399, 14).

Ventilein-A (**8**). Found: C, 70.60; H, 4.70; $[M]^+$, 512.1489. $C_{30}H_{24}O_8$ requires C, 70.31; H, 4.72%; $[M]^+$, 512.1471; $[\alpha]_D^{20}$ −1910° (*c* 0.05, $CHCl_3$); UV λ_{max}^{MeOH} nm 236, 259, 342, 410, 598, 645; (MeOH–HO^-) 265, 368, 800 (cf. xylindein, $\lambda_{max}^{CHCl_3}$ nm 380, 405, 423, 603, 647); IR ν_{max}^{KBr} cm^{-1}: 1620; ^1H NMR (360 MHz, $CDCl_3$): δ1.49 (6H, *d*, *J* = 6.0 Hz, Me-3, 3'), 1.68 (6H, *d*, *J* = 6.3 Hz, Me-1,1'), 2.58 (2H, *dd*, *J* = 16.7, 10.5 Hz, H_a-4,4'), 2.93 (2H, *br d*, *J* = 16.7 Hz, H_e-4,4'), 3.84 (2H, *m*, H-3,3'), 5.12 (2H, *q*, *J* = 6.3 Hz, H_e-1,1'), 6.21 (2H, *s*, H-8,8'), 13.24 (2H, *s*, exch. with D_2O, 2 × *peri*-OH); ^{13}C NMR (67.89 MHz, $CDCl_3$): δ20.77 (*q*, C-3a, 3'-a), 21.50 (*q*, C-1a,1'a), 30.90 (*t*, C-4,4'), 68.43 (*d*, C-3,3'), 71.31 (*d*, C-1,1'), 106.64 (*d*, C-8,8'), 108.08[a] (*s*, C-9a,9'a), 109.99[a] (*s*, C-5a, 5'a), 128.37[b] (*s*, C-10a, 10'a), 130.82[b] (*s*, C-4a, 4'a), 135.04 (*s*, C-6,6'), 140.81 (*s*, C-5,5'), 156.13 (*s*, C-7,7'), 156.37 (*s*, C-10,10') shifted to 156.10 on addition of D_2O), 186.74 (*s*, C-9,9'); MS *m/z*



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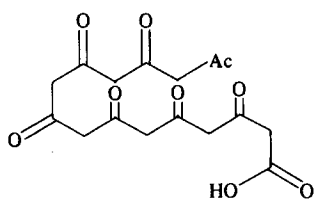

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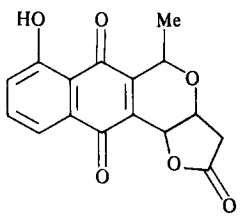

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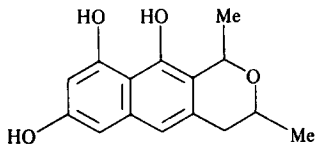

14

512 (100), 497.1244. ($C_{29}H_{21}O_8$ requires 497.1236, 82), 481 (13), 468 (24), 454 (45), 440 (17), 436 (19), 409 (14). *Leucotetra-acetate* (Zn–Ac_2O–Et_3N). The crude product was separated from a little red material (probably the diacetate) by prep. TLC ($CHCl_3$) to give a yellow solid, mp > 350°; Found: $[M]^+$, 682.2068. $C_{38}H_{34}O_{12}$ requires 682.2047; UV λ_{max}^{MeOH} nm: 236, 283, 293, 319, 334, 402, 426, 455; (*peri*-xanthenoxanthene λ_{max}^{MeOH} nm: 391, 410, 438; xylindeinleucotetra-acetate 396, 419, 446); ^1H NMR (360 MHz, $CDCl_3$): δ1.35 (6H, *d*, *J* = 6.1 Hz, Me-3,3'), 1.57 (6H, *d*, *J* = 6.3 Hz, Me-1,1'), 2.30 (2H, signal overlapped by acetate peaks, H_a-4,4'), 2.34 (12H, *s*, 4 × OAc), 2.78 (2H, *dd*, *J* = 16.4, 1.3 Hz, H_e-4,4'), 3.61 (2H, *m*, H-3, 3'), 4.74 (2H, *q*, *J* = 6.15 Hz, H-1,1'), 6.67 (2H, *s*, H-8,8'); (C_6D_6): δ2.33 (2H, *dd*, *J* = 16.2, 10.7 Hz, H_a-4,4'), 2.65 (2H, *dd*, *J* = 16.2, 2.1 Hz, H_e-4,4'), 3.47 (2H, *m*, H-3,3'), 4.87 (2H, *q*, *J* = 6.2 Hz, H-1,1'); MS *m/z* 682 (58%), 640 (36), 598 (63), 556 (57), 514 (100). *Diacetate* (Ac_2O–pyridine), purple needles (C_6D_6), mp 232°. Found: C, 68.12; H, 4.70. $C_{34}H_{28}O_{10}$ requires C, 68.45; H, 4.73%; ^1H NMR (100 MHz, $CDCl_3$): δ1.44 (6H, *d*, *J* = 6 Hz, Mc-3,3'), 1.64 (6H, *d*, *J* = 6 Hz, Me-1,1'), 2.48 (6H, *s*, 2 × OAc), 2.70 (2H, *dd*, *J* = 17, 10 Hz, H_a-4,4'), 3.08 (2H, *br d*, *J* = 17 Hz, H_e-4,4'), 3.72 (2H, *m*, H-3,3'), 5.04 (2H, *q*, *J* = 6 Hz, H-1,1'), 6.26 (2H, *s*, H-8,8').

Ventilein-B (**9**). Found: $[M]^+$, 542.1601. $C_{31}H_{26}O_9$ requires M, 542.1576; UV λ_{max}^{MeOH} nm: 236, 259, 342, 410, 604 sh, 645; ^1H NMR (500 MHz, $CDCl_3$): δ1.43 and 1.44 (each 3H, *d*, *J* = 6.03 Hz, Me-3 and 3'), 1.65 (6H, *d*, *J* = 6.35 Hz, Me-1 and 1'), 2.69 and 2.70 (each 1H, *ddd*, *J* = 16.25, 10.83, ~ 1.3 Hz, H_a-4 and 4'), 3.15 (± 1 Hz) (2H, *d*, *J* = 17.69 Hz, H_e-4,4'), 3.76 (1H, *dq*, *J* ~ 10, ~ 6 Hz, H_a-3 or 3'), 3.78 (1H, *dq*, *J* ~ 10, ~ 6 Hz, H_a-3 or 3'), 4.13 (3H, *s*, OMe), 5.14 (± 1 Hz), (2H, *dq*, *J* = 6.52, ~ 1 Hz, H_e-1,1'), 6.47 (1H, *s*, H-8), 11.92, 12.13 (each 1H, *s*, exch. with D_2O, 2 × *peri*-OH); MS *m/z* 542.1601. ($C_{31}H_{26}O_9$ requires 542.1576, 77), 527 (52), 512 (7), 485 (20), 452.0544 ($C_{26}H_{12}O_8$ requires 452.0532, 47), 425.0325 ($C_{24}H_9O_8$ requires 425.0297, 100), 379 (24), 351 (49).

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