was extracted ×3 with 50 ml BuOH and the extract chromatographed on a column of Si gel to yield 350 mg **1**.

Partial hydrolysis. Compounds **1** and **2** (1.0 g) were heated in 50 ml 1.5 N HCl for 2 hr at +90° with H_2O and extracted with 3 × 30 ml BuOH. The BuOH extracts were chromatographed on Si gel ($CHCl_3$–MeOH–H_2O, 65:25:10). From **1** was obtained compound **8** (20 mg) mp 198–201°, $[\alpha]_D^{20} = -25°$ (DMSO; *c*1.0), **9** (70 mg) mp 251–253°, $[\alpha]_D^{20} = -5°$ (DMSO; *c*1.0), **10** (110 mg) mp 201–203°, $[\alpha]_D^{20} = -17°$ (DMSO; *c*1.0), **11** (320 mg) mp 224–226°, $[\alpha]_D^{20} = -8.5°$ (DMSO; *c*1.0), **12** (60 mg) mp 206–209°, $[\alpha]_D^{20} = -12°$ (DMSO; *c*1.0). From **2** beshornin was obtained in addition to **8–12**. Methylation of 0.05 g of each product (**9–12**) and methanolysis were carried out and the products identified by TLC and GLC.

Oxidation of compound **2**. Acetylated compound **2** (1.0 g), obtained by reaction with HOAc, was dissolved in 10 ml HOAc and 200 mg NaOAC was added [4]. The oxidation was carried out as described in ref. [4] to produce tetra-acetylglucoside methyl ester of δ-hydroxy-γ-methyl-*n*-valeric acid (**14**), which showed the characteristic MS peaks for acetylated glucose, as well as fragment peaks at *m/z* 331, 243, 242, 200, 169, 157, 145, 141, 115, 109 and peaks for the acidic residue at *m/z* 129, 97, 89, 81 [3–5, 9].

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STRUCTURE OF VERSICOLORONE ISOLATED FROM *ASPERGILLUS VERSICOLOR*

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Key Word Index—*Aspergillus versicolor*; fungal metabolite; anthraquinone derivative; structure elucidation.

Abstract—A new anthraquinone metabolite, versicolorone, has been isolated from *Aspergillus versicolor*.

In previous studies averufin[1], versicolorin B[1], averufanin[2], deoxyaverufinone[3], dehydroaverufin[3] and 1, 3, 6, 8-tetrahydroxyanthraquinone[4]were isolated from *Aspergillus versicolor* (Vuillemin) Tiraboschi (strain ATCC 34508). In a continuation of our investigation of anthraquinone metabolites produced by this fungus, a fourth new metabolite has been isolated and named versicolorone (**1**). The structure of versicolorone suggests a relationship between this metabolite and versiconal acetate[5].

Versicolorone, $C_{20}H_{16}O_7$, had UV and IR data which indicated the 1,3,6,8-tetrahydroxyanthraquinone structure[6]. The carbonyl region of the IR spectrum showed a non-chelated carbonyl band at 1670 cm^{-1}, a chelated carbonyl band at 1620 cm^{-1} and an additional carbonyl band at 1700 cm^{-1}. The electron impact mass spectrum of versicolorone lacked the expected $[M]^+$ ion (*m/z* 368, $C_{20}H_{16}O_7$). A prominent ion at *m/z* 310 ($[M-58]^+$, $C_{17}H_{10}O_6$) was initiated by a McLafferty rearrangement. In addition, other peaks of interest were observed at *m/z* 325 ($[M-43]^+$, $C_{18}H_{13}O_6$), 297 ($[M-71]^+$, $C_{16}H_9O_6$), 58 and 43.

The ^1H NMR spectrum of versicolorone confirmed the presence of three aromatic protons: an AX system at δ 6.65 and 7.17 ($^2J = 2.5$ Hz, 7-H and 5-H respectively) and a singlet at δ 7.88 (4-H). The spectrum further showed two sharp one-proton signals at δ 12.33 and 12.89 ascribed to strongly hydrogen-bonded hydroxyl groups (OH-8 and OH-1, respectively), and broad one-proton absorption at δ 11.81 attributed to an unbonded hydroxyl group (OH-6). The three-proton singlet at δ 2.07 was assigned to the methyl group (MeCO). H_2-1′, H-2′, H_2-3′ and H_2-4′ appeared as multiplets at δ 3.41, 3.80, 2.37 and 2.40 respectively.

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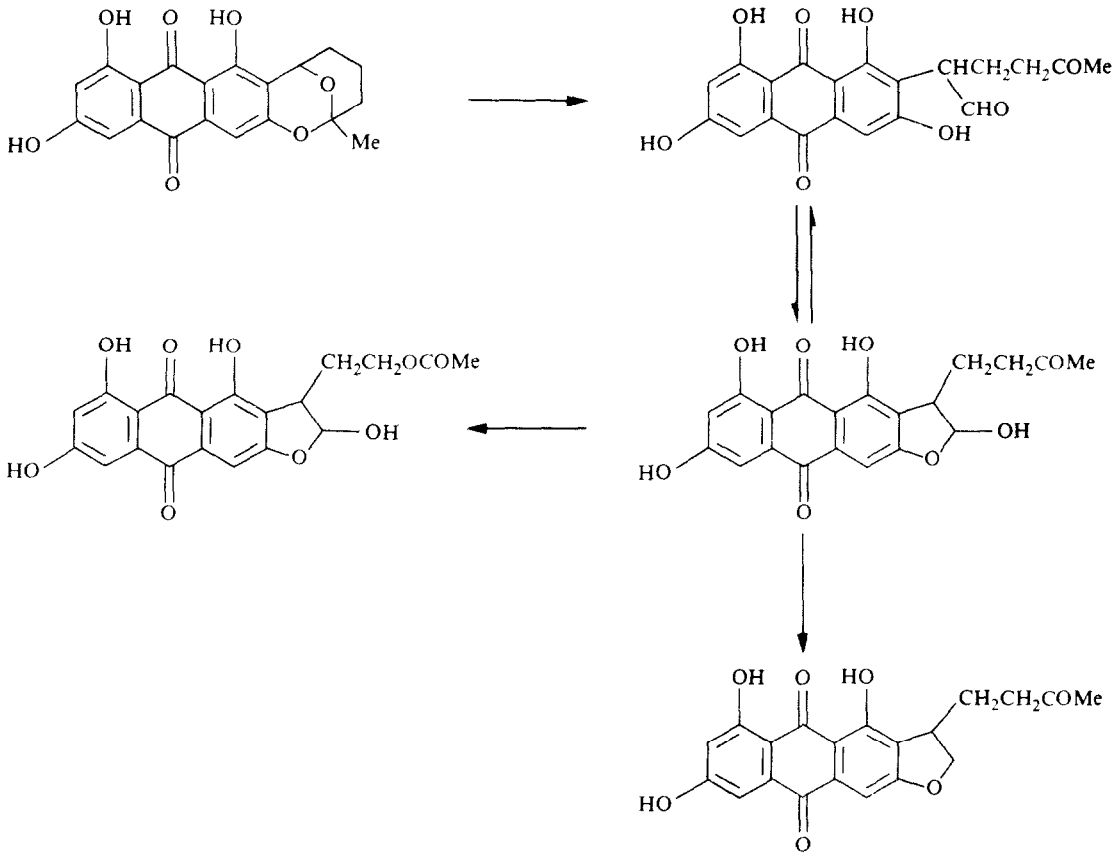


Scheme 1. Proposed relationship between averufin, versiconal acetate and versicolorone (i) ring opening-hydration; (11) dehydration; (iii) expoxidation; (iv) epoxide rearrangement; (v) Baeyer–Villiger oxidation; (vi) dehydration; (vii) hydrogenation.

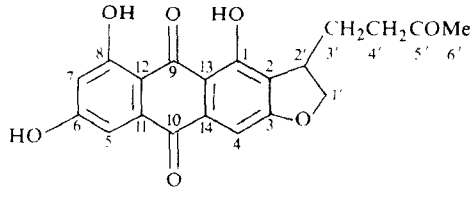
These assignments were verified by decoupling experiment.

Confirmation of the structure was obtained by ^{13}C NMR. The assignments of the anthraquinone signals were made on the basis of chemical shifts values of 1, 3,6,8-tetrahydroxyanthraquinone as well as on the basis of C–H coupling constants over one and more bonds[4]. The chemical shifts and the multiplicities observed in the NOE enhanced single frequency spectrum of versicolorone were used to assign the methyl (C-6′), methylene (C-1′, C-3′ and C-4′) and methine (C-2′) groups.

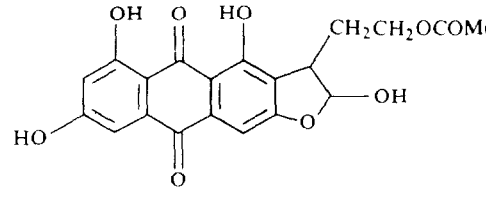
Knowledge of the structure of versicolorone helps to clarify the intriguing rearrangement of the C_6 side-chain of averufin into the C_4 bishydrofuran moiety of versicolorin A [7, 8]. In fact, Steyn's mechanism[7] involves versiconal acetate (**2**), a structurally related metabolite of versicolorone. The isolation of versicolorone indicates that the Baeyer–Villiger type oxidation of the terminal acetyl group leading to versiconal acetate, appears in the final step of the biotransformation. This observation leads us to propose a slightly modified relationship between averufin and versiconal acetate (Scheme I) which takes versicolorone into account.

EXPERIMENTAL

Isolation of versicolorone (**1**). *A. versicolor* (strain ATCC 34508 ex. IRSAC 2172) was cultured on Czapek-Dox formule II at 25° for 25 days. The dried pigmented mycelium (480 g) was extracted with petrol and Et_2O. The Et_2O extract was concd and chromatographed on Si gel (500 g) using toluene with increasing amounts of EtOAc as eluent. The main fractions obtained gave averufin (510 mg), versicolin B



(**1**) Versicolorone



(**2**) Versiconol acetate

(380 mg) and 1,3,6,8-tetrahydroxyanthraquinone (32 mg). The intermediate fraction between versicolorin B and 1, 3, 6, 8-tetrahydroxyanthraquinone was subjected to TLC (0.25 mm Si gel; toluene–EtOAc, 4 : 1). The orange material at R_f 0.44 was eluted with CH_2Cl_2 and re-crystallized (Me_2CO) to give pure versicolorone (135 mg).

Versicolorone (**1**). Orange-red needles (Me_2CO), mp 210°. Found: C, 65.1; H, 4.2. $C_{20}H_{16}O_7$ requires: C, 65.2; H, 4.3%. UV λ_{max}^{EtOH} nm (log ϵ): 224 (4.38) 254 (4.07), 263 (4.14), 291 (4.39), 320 (3.92) and 454 (4.03); IR ν_{max}^{KBr} cm^{-1}: 3350, 2920, 1700, 1670, 1620, 1395, 1320, 1260, 1170 and 758; ^1H NMR (250 MHz, DMSO-d_6, TMS): δ 2.04 (2H, *m*, H-4′) 2.07 (3H, *s*, H-6′) 2.37 (2H, *m*, *H*-3′), 3.41 (2H, *m*, H-1′), 3.80 (1H, *m*, J = 7.3 Hz, H-2′), 6.65 (1H, *d*, J = 2.5 Hz, H-7), 7.17 (1H, *d*, J = 2.5 Hz, H-5), 7.88 (1H, *s*, H-4), 11.81 (1H, *s*(*br*), 6-OH), 12.33 (1H, *s*, 8-OH), 12.89 (1H, *s*, 1-OH); ^{13}C NMR (62.86 MHz, DMSO-d_6, TMS): δ 23.75 (*t*, ^1J = 126 Hz, C-3′), 30.37 (*q*, ^1J = 126 Hz, C-6′), 38.32 (*d*, ^1J = 125 Hz, C-2′), 42.06 (*t*, ^1J = 122 Hz, C-4′), 63.56 (*t*, ^1J = 140 Hz, C-1′), 108.70 (*dd*, ^1J = 161 Hz, ^3J = 4 Hz, C-7), 108.85 (*d*, ^3J = 4 Hz, C-13), 109.20 (*t*, ^3J = 5 Hz, C-12), 109.22 (*d*, ^1J = 166 Hz, C-4), 109.38 (*dd*, ^1J = 166 Hz, ^3J = 5 Hz, C-5), 122.50 (*s*(*br*), C-2), 132.88 (*d*, ^2J = 4 Hz, C-14), 135.27 (*d*, ^2J = 4 Hz, C-11), 162.90 (*t*, ^2J = 4.5 Hz, C-6), 164.81 (*d*, ^2J = 5.5 Hz, C-8), 165.55 (*s*, C-1), 181.73 (*t*, ^3J = 4.5 Hz, C-10), 189.35 (*s*, C-9), 210.18 (*s*, C-5′); MS (probe) 70 eV *m/z* (rel. int.): 368 $[M]^+$ (5), 325(2), 310(32), 297(6), 58(46) and 43(100); high resolution MS *m/z* 368.0900 ($C_{20}H_{16}O_7$ requires 368.0895), 325.0713 ($C_{18}H_{13}O_6$ requires 325.0710), 310.0478 ($C_{17}H_{10}O_6$ requires 310.0476), 297.0401 ($C_{16}H_9O_6$ requires 297.0398).

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ISOLATION AND CRYSTAL STRUCTURE OF 5-HYDROXY-2,8-DIMETHYL-6,7-DIMETHOXYBENZOPYRAN-4-ONE FROM *COUEPIA PARAENSIS*

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Key Word Index—*Couepia paraensis*; Rosaceae; 5-hydroxy-2, 8-dimethyl-6, 7-dimethyloxychromone.

Abstract—A highly substituted chromone constituent of *Couepia paraensis* was isolated and identified as 5-hydroxy-2,8-dimethyl-6,7-dimethoxychromone by spectroscopic and X-ray crystallographic methods.

Couepia paraensis (M & Z) Benth. is a small tree belonging to the tribe Chrysobalanoideae of the Rosaceae. This plant has not previously been investigated for its chemical constituents. Members of the Chrysobalanoideae tribe have been reported to contain flavonoids and proanthocyanidins[1], and in this communication we wish to report the isolation and chemical characterization of a highly substituted chromone from *C. paraensis*.

The chloroform extract of *C. paraensis* upon column chromatography followed by prep. TLC gave a yellow crystalline compound (**1**), mp 125°, MW 250 (found: C, 62.36; H, 5.6; O, 31.98. $C_{13}H_{14}O_5$ requires: C, 62.5; H, 5.6; O, 31.9%). **1** gave a green color with $FeCl_3$, indicating the presence of one or more phenolic hydroxyl groups, which was also supported by the $AlCl_3$ induced bathochromic shifts in the UV spectra (λ_{max}^{MeOH} nm: 227, 264 and 330; $\lambda_{max}^{MeOH+AlCl_3}$ nm: 280 and 380). The IR spectrum (ν_{max}^{KBr} cm^{-1}: 3020, 2950, 1660 (>C=O), 1640 and 1575) indicated the presence of a γ-pyrone moiety in **1**[2]. The mass spectral fragmentation pattern of **1** [*m/z* (rel. int.) 250 $[M^+]$ (70), 235 $[M-CH_3]^+$ (94), 207 $[M-CH_3CO]^+$ (100), 192 $[M-C_3H_6O]^+$ (13); 164 $[M-C_4H_6O_2]^+$ (15), and 136 $[M-C_6H_{10}O_2]^+$ (44)] was characteristic of chromones and coumarins with methoxy groups at