EXPERIMENTAL

The two anthraquinone sulphates were separated from leaf extracts of *Rumex pulcher* by electrophoresis on Whatman No. 3 paper at 400 V/cm for 2 hr in acetate formate buffer, pH 2.2. They were purified by standard PC procedures as quickly as possible to avoid loss of material due to hydrolytic breakdown. The spectral and R_f properties are recorded in Table 1. Emodin 1 (or 8)-glucosidesulphate was identified on the basis of the above properties and the fact that very mild acid hydrolysis gave emodin 1 (or 8)-glycoside, identified by direct comparison with an authentic sample. Location of the sulphate group on the glucose residue follows from the absorption spectral properties and from the fact that the IR spectrum of the sulphate was practically identical to that of the glucoside. Complete acid hydrolysis gave glucose, bisulphate, potassium and emodin, which was identified by comparison (IR, UV, MS, co-PC and co-TLC) with an authentic sample. Emodin dianthrone diglucosidesulphate was identified by similar procedures. On acid hydrolysis, it gave glucose, bisulphate, potassium and emodin dianthrone, which was identified by comparison with an authentic specimen. Location of the glucose and sulphate residues was based on the fact that on standing in methanolic solution, it gave rise to emodin 1 (or 8)-glucoside and emodin as a result of aerial oxidation. Emodin and its dianthrone were identified by PC (see Table 1) and also by TLC on Si gel in C_6H_6–EtOAc–HOAc (15 :4 :1) and in EtOAc–toluene–HOAc (49 :50 :1) and on polyamide in MeOH–C_6H_6 (4 :1).

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6,8-DIHYDROXY-3-HYDROXYMETHYLISOCOUMARIN, AND OTHER PHENOLIC METABOLITES OF *CERATOCYSTIS MINOR*

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Key Word Index—*Ceratocystis minor*; fungus; metabolites; isocoumarins; 6,8-dihydroxy-3-hydroxy-1*H*-2-benzopyran-1-one; 6,8-dihydroxy-3-methyl-1*H*-2-benzopyran-1-one; α-tetralones; 3,6,8-trihydroxy-3,4-dihydro-1(2*H*)-naphthalenone.

The fungus *Ceratocytis minor* (Hedgc.) Hunt is generally introduced into the phloem and xylem of southern pine trees during attack by the southern pine beetle *Dendrotonus frontalis* Zimmerman, and development of *C. minor* in the xylem is considered to be central to the death of beetle-attacked trees [1]. There are close parallels in both the cause and symptoms of this disease to those of the Dutch Elm disease in which bark beetles introduce the fungus *Ceratocystis ulmi* (Buism.) C. Moreau, into the xylem of Elms [2, 3]. Claydon *et al.* [4] showed that phenolic C-10 acids or their dihydroisocoumarin tautomers were produced in highest yields by the most virulent strains of *C. ulmi* and compounds of the isocoumarin class are known for their biological activity on plant growth [5–8]. The similarities in these two important tree diseases prompted us to examine the phenolic metabolites of *C. minor*.

3 major phenolic metabolites of *C. minor* grown on malt extract were indicated by PC and TLC of EtOAc extracts of culture filtrates. The spectral properties of the major phenolic compound **1** indicated a 6,8-dihydroxy-hydroxymeth-y-lisocoumarin. The vinyl proton (δ6.83, 1H, *s*) appeared further down-field than that reported for 3-Me substituted isocoumarins [5–10]. This shift would be expected because of the hydroxymethyl group (cf δ6.6 for the α-vinyl proton of *trans*-cinnamyl alcohol with 6.36 for *trans*-isoeugenol models [11]). The PMR shift for the vinyl proton supports a 3-hydroxymethyl substitution since a vinyl proton adjacent to the lactone would be expected to be much further down-field (cf δ7.75 for vinylacetate [12]). Based on the above evidence, we have concluded that **1** is a new isocoumarin 6,8-dihydroxy-3-hydroxymethyl-1H-2-benzopyran-1-one.

Compound **2** occurred in much smaller quantities than **1**. Comparison of mp and spectral properties of **2**

with reported data [5, 10] indicated that it was the isocoumarin 6,8-dihydroxy-3-methyl-1H-2-benzopyran-1-one. This compound is also a metabolite of *C. fimbriata* E11. and Halst [13], where it also occurs in small proportions along with major amounts of 8-hydroxy-6-methoxy-3-methylisocoumarin [9]. The closely related 3-methyl-3,6,8-trihydroxy-3,4-dihydroisocoumarin is a metabolite of *C. ulmi* [4] as well as a number of other fungi.

Compound 3 was identified as scytalone (3,6,8-trihydroxy-3,4-dihydro-1(2H)-napthalenone) by comparison of its mp and spectral properties with data reported for the α-tetralone found as a metabolite of a *Scytalidium* sp. fungus [14]. No species of *Ceratocystis* has previously been found to produce α-tetralones. Mixtures of α-tetralones and isocoumarins are, however, produced by *Pyricularia oryzae* Cavara [7]. The same metabolites were produced by *C. minor* grown on a defined medium although in lower yields. These compounds were not found when sterile malt extract solutions were incubated under comparable conditions.

EXPERIMENTAL

IR spectra were measured as KBr discs. PLC was performed on 0.75 mm Si gel 7GF and compounds were eluted with MeOH. Analytical and preparative PC were conducted on Whatman No. 1 and 3. PMR spectra were recorded on a 60MHz instrument in Me_2CO-d_6 with TMS as an internal standard. High resolution MS was done by the High Resolution MS Laboratory, Florida State University, Tallahassee. Isolates of *C. minor* were grown on malt extract and the defined medium described in ref. [4]. Cultures were harvested when the pH had increased to 5.5 and residual reducing sugars were low (normally 3–4 weeks on malt extract). Filtrates were saturated with NaCl, and extracted with $CHCl_3$ followed by EtOAc.

6,8-Dihydroxy-3-hydroxymethyl-1H-2-benzopyran-1-one (*Compound* 1). PC R_f values of 0.78 in *n*-BuOH–HOAc–H_2O (6:1:2) and 0.47 in 6% HOAc. It absorbed UV light after exposure to NH_3 vapour, gave a weak blue coloration with $FeCl_3$–$K_3Fe(CN)_6$, and a yellow–orange color with diazotized sulfanilic acid which slowly turned to a dark tan after back-spraying with base. 1 was isolated by PLC (diisopro ether–HCO_2H–H_2O (90:7:3), R_f 0.7) and PC (6% HOAc). It was crystallized from Me_2CO to give needles (mp 220–225° decomp.). The UV (λ_{max} MeOH 236, 245, 277, 290, 329; MeOH + NaOAc 244, 254, 275sh, 309, 334; and MeOH + NaOH 244, 254, 306, 336, 354 nm) and IR (ν_{max} 3400, 3260br, 2950w, 1695, 1660, 1630s cm^{-1}) spectra suggested a 6,8-dihydroxyisocoumarin [5, 9]. High resolution MS showed $C_{10}H_8O_5$ (M^+, 65%, found 208.0366, requires 208.0371) and fragmentation to $C_9H_5O_4$ (79%, M^+—CH_2OH), $C_8H_5O_3$ (20%, M^+—CH_2OH, —CO), and $C_7H_5O_2$ (100%, M^+—CH_2OH,—CO,—CO). PMR showed δ11.4 (1H, *s*, H bonded OH), 6.63 (1H, *s*, C-4), 6.5–6.4 (2H, *dd*, J = 2.5 Hz, C-5 and C-7) and 4.35 (2H, *s*, C-9).

6,8-Dihydroxy-3-methyl-1H-2-benzopyran-1-one 2. PC R_f values of 0.85 in *n*-BuOH–HOAc–H_2O (6:1:2) and 0.41 in 6% HOAc. It absorbed UV light after exposure to NH_3 vapour, gave a weak blue coloration with $FeCl_3$–$K_3Fe(CN)_6$ and a yellow–orange color with diazotized sulfanilic acid which faded to a light tan after back-spraying with base. It was isolated by PLC ($CHCl_3$–Me_2CO, (9:1), R_f 0.75) and PC (6% HOAc). After a second purification by PLC, it was crystallized from Et_2O–hexane to give small prisms (mp 240–250° decomp.). UV spectra indicated a 6,8-dihydroxyisocoumarin. High resolution MS showed $C_{10}H_8O_4$ (M^+, 100%, found 192.0422, requires 192.0422), and fragmentation to $C_9H_5O_5$ (72%, M^+—Me), $C_8H_5O_3$ (9%, M^+—Me, —CO) and $C_7H_5O_2$ (55%, —Me, —CO, —CO). Insufficient amounts were isolated for PMR.

3,6,8-Trihydroxy-3,4-dihydro-1(2H)-naphthalenone (3). Bright-yellow fluorescent compound on PC at R_f values of 0.77 in *n*-BuOH–HOAc–H_2O (6:1:2) and 0.63 in 6% HOAc. It gave a weak violet-blue reaction with $FeCl_3$–$K_3Ke(CN)_6$ and a strong yellow-orange color with diazotized sulfanilic acid after back spraying with base. It was isolated by PLC (diisopro ether–HCO_2H–H_2O (90:7:3), R_f 0.57) two to 3 times, after which crystals were formed on evaporation of an EtOAc soln. Recrystallization from Et_2O–petrol gave needles (mp 160–162° decomp.). The UV and IR spectra indicated an α-tetralone [7, 14]. High resolution MS showed $C_{10}H_{10}O_4$ (M^+, 100%, found 194.057, requires 194.0578), and fragmentation to $C_{10}H_8O_3$ (86%, M^+ —H_2O), $C_8H_6O_3$ (84%, M^+ —H_2O, —C_2H_2), $C_9H_8O_2$ (21%, M^+, —H_2O, —CO) and $C_7H_6O_2$ (20%, M^+, —H_2O, —C_2H_2, —CO). The PMR spectrum was identical with data reported for scytalone in ref. [14].

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