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TWO NEW NATURAL SUBSTITUTED HEXENOPHENONES FROM THE FUNGUS SCYTALIDIUM[1]

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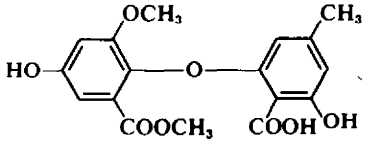
Abstract—Two metabolites of the fungus *Scytalidium Sp.* FY strain, have been shown to be 5'-formyl-2'-hydroxy-4'-methoxy-(E)-4-hexenophenone **3** and 5'-formyl-2'-hydroxyl-4'-methoxy-(E,E)-sorbophenone **4**.

Asterric acid[2] **1** and scytalidin[3] **2** have been previously isolated from the fungus *Scytalidium* sp. FY strain, an immunizing commensal of Douglas-fir utility poles.[4] We describe in this paper the isolation and structure assignment of two additional metabolites **3** and **4** from this fungus.

The fungus was grown on agar plates for two months and the plates were then dried and extracted. Column and preparative plate chromatography allowed isolation of the pure components. Details of growth, isolation and purification are given in the Experimental.

3 was obtained as a crystalline solid, m.p. 118–121°. High resolution mass spectrometry established the molecular formula as $C_{14}H_{16}O_4$. The base peak in the mass spectrum was due to a fragment ion $C_9H_7O_4$, apparently from loss of a C_5H_9 fragment. Compound **4** was isolated as a yellow solid, m.p. 154–157°. High resolution mass spectrometry established the molecular formula of **4** as $C_{14}H_{14}O_4$ and **4** yielded the same fragment ($C_9H_7O_4$) with loss of C_5H_7. The IR of **3** showed carbonyl absorptions at 1680 and 1650 cm^{-1}, while **4** had corresponding absorptions at 1680 and 1643 cm^{-1}. The NMR of **3** showed a singlet (1H) at 10·27 δ typical of an aromatic aldehyde and this was evident in the NMR of **4** as well.* Thus, the 1680 cm^{-1} IR peaks could be assigned to this group. The 1650 cm^{-1} band in **3** was then likely due to the carbonyl of an $ArC(=O)-CR_3$ moiety while that of **4** was probably from a $ArC(=O)-C=C-R$ moiety. This possibility was strengthened by the UV spectra since **3** had a long wavelength band at 321 nm while that of **4** was at 345 nm. Both compounds showed alkali shifts of the UV spectra typical of phenols and each showed a three proton singlet in the methoxy region of the NMR. Taken *in toto*, these data indicated part structures **5** and **5a** for **3** and **4**, respectively.



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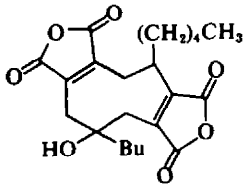

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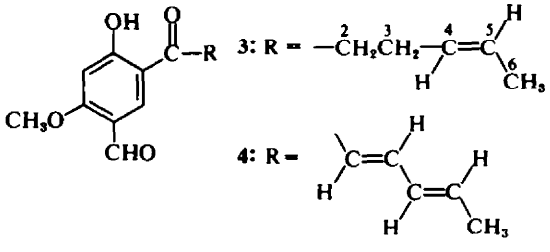
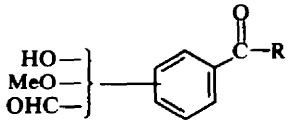


3: R = —CH₂CH₂—C(H)=C(H)—CH₃ (2,3,4,5,6)

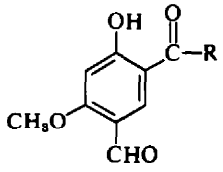
4: R =



5: R = C_5H_9
5a: R = C_5H_7

*Sufficient pure **3** was obtained for the mass spectral, ir, uv, and NMR studies reported here. **4** was difficult to free from traces of **3**, but sufficient pure **4** was obtained for the mass spectral, ir, and uv studies. The NMR data for **4** were obtained on samples containing impurities of **3**, but from which the absorptions due to **3** could be substracted easily.

More detailed analysis of the spectral data allowed us to postulate the placement of the substituents on the aromatic ring. Thus, a one proton singlet (which disappeared upon the addition of D_2O) was present at 13·27 δ in **3** and 13·85 δ in **4**. This is typical[5] of phenolic protons *ortho* to side chain ketone functions and considerably farther downfield than for phenolic groups *ortho* to aldehyde functions. The loss of H_2O from the molecular ion of **3** in the mass spectrum was an indication that the methoxy group was also *ortho* to a carbonyl group.[6] In both **3** and **4**, the two aromatic protons were sharp singlets which indicated that they were *para* to each other. Thus, structures **6** and **6a** could

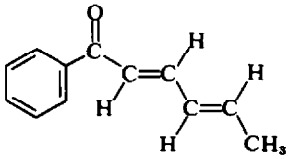


6: R = C_5H_9
6a: R = C_5H_7
7: R = CH_3

be postulated for **3** and **4**. This was confirmed by the synthesis of the model acetophenone **7** and spectral comparisons between it and **3** and **4**. The NMR chemical shifts of the aromatic protons and the protons of each substituent on the aromatic ring were identical for **3** and **7**. The IR spectrum of **7** showed carbonyls at 1687 and 1650 cm^{-1} and the UV spectrum of **7**, both neutral and with added base, was nearly identical with that of **3**. Both **3** and **7** show an aromatic proton at 8.3 δ which is characteristic of such a proton flanked by CO groups.

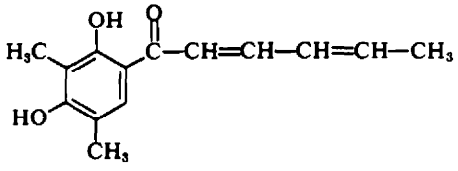
The side chain (C_5H_9 in **3** and C_5H_7 in **4**) structure was most easily approached through the simpler NMR spectrum for **4**. A three proton doublet (J = 5 Hz) at 1·94 δ and the presence of four vinyl absorptions left little choice other than —CH=CH—CH=CH—CH_3 for the C_5H_7 proton of **4**. In addition, the NMR showed that $J_{2,3}$ was 15 c/s and hence a *trans* configuration could be assigned to that double bond. The stereochemistry at the second double bond was not easily determined from the NMR and double irradiation experiments were not successful in simplifying the spectrum. Hence, we again turned to synthesis of a model compound, (E,E)-sorbophenone, **8**, whose stereochemistry was known.[7] The vinyl region NMR spectra of **8** and **4** proved to be identical and hence **4** had the E,E stereochemistry. Thus, structure **4** was unequivocally assigned to the $C_{14}H_{14}O_4$ metabolite.

The side chain fragment C_5H_9 in **3** contained only two vinyl hydrogen absorptions in the NMR and the carbonyl absorption position and UV spectrum indicated that the double bond was not conjugated with the carbonyl. These data together with the presence of a three proton doublet (J = 5 Hz) at 1·66 δ in the NMR, and more detailed NMR analysis showed that the side chain must be —CH_2—CH_2—CH=CH—CH_3. First order analysis of the vinyl multiplet at 5·50 δ as well as double resonance experiments showed a $J_{4,5}$ = 15 Hz and thus *trans* stereochemistry could be assigned to the side chain double bond. In addition, a series of nine compounds of differing known stereochemistry, each containing the —CH_2—CH=CH—CH_3 moiety, were investigated[8] and it was shown[8] that a clear assignment of *cis* or *trans* stereochemistry could be made based upon the differences in the NMR spectra of the isomers. The vinyl multiplet at 5·50 δ in **3** was identical to the same multiplet in the *trans* isomers investigated.[8] Thus, the **3** structure was unequivocally assigned to the $C_{14}H_{16}O_4$ metabolite.



8

As far as we are aware, only one other hexenophenone (sorbicillin, **9**, from *Penicillium chrysogenum*[9] and *P. notatum*[10] has been reported as a natural product. Neither **3** nor **4** was active against the wood decay fungus *Poria carbonica*.



9

EXPERIMENTAL

NMR spectra were recorded in $CDCl_3$ at 220 MHz with TMS as internal reference. UV data are in EtOH solvent and IR spectra in KBr. All m.ps are corrected.

Isolation of **3** *and* **4**. *Scytalidium* sp. FY strain was incubated at 30° for two months in 500 100 × 15 mm plates filled with malt extract agar. Then the plate contents were dried, ground, and continuously extracted with $CHCl_3$ for 2 days. After evaporation of the solvent, the extracted material was placed in a column containing aluminum powder (Baker). The column was eluted first with 1 l of $CHCl_3$ to remove the fatty material (2·1 g), then with 95:5 $CHCl_3$/EtOH solvent. The residue from this latter solvent system was placed on preparative TLC plates (Brinkman silica gel F-254) and developed with benzene. A band at R_f 0·20–0·25, which fluoresced blue under long wavelength UV, was eluted from the silica gel to yield 42 mg of **3**, which contained some **4**. A portion was rechromatographed to yield 10 mg of pure **3**: white solid; m.p. 118–121°; λ_{max} 255 (ε 24,800), 282 (ε 10,300) and 321

nm (ε 6610); alkaline, λ_{max} 255, 314 and 350 nm; ν_{max} 1680 (Ar–CHO), 1650 (ArCOR), 1597, 1582 and 1134 cm^{-1}; NMR: δ 13·27 (1H, s, disappeared in D_2O, Ar–OH), 10·27 (1H, s, CHO), 8·34 (1H, s, Ar–H), 6·50 (1H, s, Ar–H), 5·50 (2H, m, Δ^4 bond protons), 3·96 (3H, s, OCH_3), 3·05 (2H, t, *J* 7 Hz, C-2 H's), 2·41 (2H, m, C-3 H's) and 1·66 (3H, d, *J* 5 Hz, vinyl Me); MS: *m/e* 248·1047 ($C_{14}H_{16}O_4$, 15%, M+), *m/e* 230·0942 ($C_{14}H_{14}O_3$, 10%, M+ $-H_2O$), *m/e* 215·0701 ($C_{13}H_{11}O_3$, 4% M+ $-H_2O-CH_3$), *m/e* 194·0579 ($C_{10}H_{10}O_4$, 8%) and *m/e* 179·0339 ($C_9H_7O_4$, 100%, M+ $-C_5H_9$). A yellow band at R_f 0·10–0·20 was eluted from the silica gel to yield 74 mg of **4**, which contained some **3**. A portion was rechromatographed to yield several mg of pure **4**; yellow solid; m.p. 154–157°; λ_{max} 256 (ε 21,300), 265 (ε 21,000; sh), 309 (ε 24,600) and 345 nm (ε 14,700; sh) alkaline, λ_{max}, 253, 285 (sh) 313 and 361 nm (sh); ν_{max} 1680 (Ar-CHO), 1643 (ArCOR) 1616, 1582, 1569 and 1133 cm^{-1}; NMR: δ 13·85 (1 H, s, disappeared in D_2O, Ar–OH), 10·27 (1H, s, CHO), 8·39 (1H, s, Ar–H), 7·51 (1H, m, C-3 H), 7·02 (1H, d, *J* 15 Hz, C-2 H), 6·50 (1H, s, Ar–H), 6·39 (2H, m, Δ^4 bond protons) 3·96 (3H, s, OMe) and 1·94 (3H, d, *J* 5 Hz, vinyl Me); MS: *m/e* 246·0891 ($C_{14}H_{14}O_4$, 58%, M^+), *m/e* 231·0662 ($C_{13}H_{11}O_4$, 100%, M^+-CH_3) and *m/e* 179·0356 ($C_9H_7O_4$, 50% $M^+-C_5H_7$).

Synthesis of 5'-formyl-2'-hydroxy-4'-methoxyacetophenone **7**. 2'-Hydroxy-4'-methoxyacetophenone (1 g), a NaOH aq (1·02 g in 5 ml water) and $CHCl_3$ (2·2 g) were refluxed together for 24 hr. The $CHCl_3$ was then distilled off and the remaining soln was acidified with H_2SO_4. This acid soln was continuously extracted for 8 hr with $CHCl_3$. The extracted residue, a dark brown oil, was chromatographed on a preparative TLC plate (Brinkman silica gel, F-254) with benzene as the solvent. A band at R_f 0·10–0·30 was eluted from the silica gel with $CHCl_3$ and this residue was sublimed at 50–55° at 20 mm pressure to yield 44 mg of **7**: white solid; m.p. 119–121°; λ_{max} 253 (ε 35,900) 283 (ε 14,900) and 325 nm (ε 7100); alkaline, λ_{max} 253,313 and 352 nm; ν_{max} 1687 (Ar–CHO), 1650 (ArCOR), 1610, 1590 and 1375 cm^{-1}; NMR: δ 13·05 (1H, s, disappeared in D_2O, Ar–OH), 10·25 (1H, s, CHO), 8·25 (1H, s, Ar–H), 6·40 (1H, s, Ar–H), 3·98 (3H, s, OMe) and 2·60 (3H, s, COMe); MS: *m/e* 194 (39%, M^+), *m/e* 179 (100%, M^+-CH_3) and *m/e* 176 (7%, M^+-H_2O). [Found: C, 62·4; H, 5·25%. $C_{10}H_{10}O_4$ requires: C, 61·9; H, 5·15%].

Synthesis of (E,E)-sorbophenone **8**. The method of Kluge and Lillya[7] was used, to give light yellow crystals: m.p. 47–49°; NMR: δ 8·0–7·2 (6H, m, Ar–H's + C-3 H), 6·82 (1H, d, *J* 15 Hz, C-2 H), 6·24 (2 H, m, Δ^4 bond protons) and 1·85 (3H, d, *J* 5 Hz, vinyl Me).

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