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5-METHYLMELLEIN, A NEW NATURAL DIHYDROISOCOUMARIN

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While purifiyng on a Florisil column Fusicoccin A, the main phytotoxic metabolite of Fusicoccum amygdali Del. (1), a number of fractions giving a violet ferric chloride reaction were observed. From these fractions a white crystalline product, m.p. 126-127°, was isolated and shown to be 5-methyl-8-hydroxy-3,4-dihydro-3R-methylisocoumarin (5-methylmellein), a new member of the small group of natural dihydroisocoumarins. The new metabolite, $C_{11}H_{12}O_3$ (2), contains a phenolic hydroxyl group (positive ferric chloride reaction, formation of a monomethyl ether, m.p. 93°, and a monoacetate, m.p. 132°, batochromic shift of λ_{max} from 323 mμ to 343 mμ on addition of alkali to a methanolic solution (3), broad absorption between 2700 and 3200 cm^{-1} (6) which is absent in the monomethyl ether and in the monoacetate, and a lactone (ν_{max} 1661 cm^{-1} (7), positive hydroxamic acid test for the monomethyl ether at pH 9.0). The phenolic hydroxyl group must be peri to the lactone carbonyl, as shown by the shift of the CO band to 1697 cm^{-1} in both the monoacetate and the monomethyl ether (9) and by the impossibility of preparing the last derivative with diazomethane.

A further insight into the structure of the new metabolite was yielded by its NMR spectrum (10). This showed signals centered at δ 1.53 (three protons; doublet; J = 6.3 c/sec; CH-CH_3), 2.19 (three protons; singlet; aromatic CH_3), 2.79(two protons; eight-line pattern of

an AB group of an ABX system; J_{AB}=16.9; J_{AX}=3.7; J_{BX}=11.1 c/sec; CH-C<u>H</u>$_2$-Ar), 4.68 (one proton; complex pattern of the X proton coupled with an adjacent methyl group; O-C<u>H</u>(CH$_3$)-CH$_2$), 6.70 and 7.18 (two doublets of one proton each; J=8.6 c/sec; 2 <u>H</u>-Ar ortho to each other), 10.98 (one proton; singlet which disappears on addition of D_2O; intramolecular hydrogen-bonded <u>H</u>O-Ar). Very similar spectra were obtained with the monomethyl ether and the monoacetate of the metabolite; of course, the <u>H</u>O-Ar signal was absent and peaks for C<u>H</u>$_3$O-Ar (three protons; singlet at 3.90 δ) or C<u>H</u>$_3$COO-Ar (three protons; singlet at 2.32 δ) were present. The NMR spectrum of 8-hydroxy-6-methoxy-3,4-dihydro-3-methylisocoumarin (11) showed chemical shifts due to the O-CH(CH$_3$)-CH$_2$ group very close to those of the new metabolite; the signal of <u>H</u>O-Ar fell at 11.25. The substitution pattern on the aromatic ring (hydroxyl group placed at position 8, <u>i.e. peri</u> to the carbonyl group, and a methyl group either <u>ortho</u> or <u>para</u> to it) suggested by the above mentioned IR and NMR spectroscopic data, was further clarified by negative Gibbs and Liebermann colour tests, indicating substitution <u>para</u> to the phenolic hydroxyl group; this situation was corroborated by the low value of one of the aromatic protons, which must therefore be <u>ortho</u> to the hydroxyl group (12).

The stereochemistry of the asymmetric carbon atom at position 3 (13) was established by exhaustive ozonolysis of the metabolite in acetic acid solution, followed by H_2O_2 treatment (15). As expected, β-hydroxybutyric acid was formed (paper chromatography in the system 2-butanone-acetic acid-water, 4:1:1, v/v/v); the acid was purified by partition chromatography on a Celite column (16), its concentration determined by titration with standard sodium hydroxide and the optical rotation measured in aqueous solution. $[\alpha]_D^{15}$ was -25.5°; D-β-hydroxybutyric acid is reported (17) to have $[\alpha]_D^{15}$ -25.3°. The formation of D-β-hydroxybutyric acid indicates the 3<u>R</u> configuration for the metabolite; this result together with the values of the coupling constants J_{AX}=3.7 and J_{BX}=11.1 c/sec, consistent with 3(ax)4(eq) and 3(ax)4(ax) couplings

respectively, demonstrates that the 3R-methyl substituent is in the equatorial position. The same stereochemistry of the lactone ring has recently been demonstrated for ochratoxins A, B and C, three interrelated dihydro-3R-methylisocoumarins produced by Aspergillus ochraceous Wilh. (18), a fungus which also makes mellein (19).

5-Methylmellein has no detectable phytotoxicity in vitro; it inhibits conidia germination in some fungi (20).

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2) Satisfactory elementary analyses were obtained for all the new compounds reported in this paper. Melting points were determined on a Kofler block and have not been corrected.

3) 5-Methylmellein has λ_{max}^{MeOH} 210, 247 and 323 mμ (ε_{max} 22,500, 6500 and 4100 respectively), as compared to mellein which has λ_{max}^{EtOH} 212, 246, and 314 mμ (ε_{max} 20,000, 6500 and 4100 respectively) (4). The absorption maximum at 247 mμ is in fair agreement with the value calculated with the empirical rules of Scott (5) for the position of the main electron transfer band (243 mμ).

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6) IR Spectra were determined for solid samples in KBr disc.

7) 8-Hydroxy-6-methoxy-3,4-dihydro-3-methylisocoumarin has the car-

bonyl stretching frequency at 1661 cm^{-1} (KBr) (8).

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9) O-Methylmellein has the carbonyl stretching frequency at 1716 cm^{-1} in the solid state (4), 6,8-dimethoxy-3,4-dihydro-3-methylisocoumarin at 1709 cm^{-1} (KBr) (8) and 8-acetoxy-6-methoxy-3,4-dihydro-3-methyl= isocoumarin at 1695 cm^{-1} (KBr) (8).

10) NMR spectrum determined at 100 Mc/sec for $CDCl_3$ solution with Me_4Si as internal standard.

11) NMR spectra determined at 60 Mc/sec for $CDCl_3$ solutions with Me_4Si as internal standard.

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