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6-HYDROXYRAMULOSIN - A NEW METABOLITE FROM *PESTALOTIA RAMULOSA*

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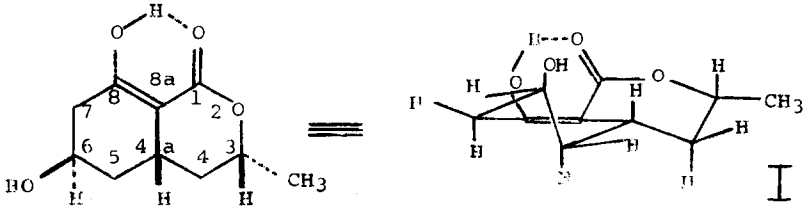
During an investigation of ramulosin production (1) by *Pestalotia ramulosa* several additional metabolites have been isolated. These include mellein (2) (R_f 0.55; $[\alpha]_D^{25}$ -95.4° (*c* 1, MeOH)), two ene-polyyne derivatives (R_f 0.10 and 0.15, respectively), and only from shake cultures, a new metabolite (I). The latter (R_f 0.02; $FeCl_3$ spray, deep violet; yield, 30 mg/l) appeared 4-5 days after inoculation of a malt extract-peptone-dextrose (3) medium. Chloroform extracts of agitated cultures maintained at 16° for 8 days were examined by tlc (Mallinkrodt AR sheets, benzene development) which revealed the presence of these ultraviolet-absorbing components, including the major metabolite, ramulosin (R_f 0.38). After solvent evaporation, the residue was crystallized from hexane, and further purified by chromatography on Florisil ($CHCl_3$ eluent). Recrystallization from $CHCl_3$-hexane provided (I), colorless needles, mp 132-133°, $[\alpha]_D^{25}$ + 91.6° (*c* 1, MeOH), λ_{max}^{EtOH} 262 mμ (ε 10,530), ν_{max}^{KBr} 3509 and 1070 cm^{-1} (nonbonded secondary -OH). Calcd for $C_{10}H_{14}O_4$: C, 60.60; H, 7.07; mol wt 198. Found: C, 60.74; H, 7.20; mol wt 206 by vp osmometry; *m/e* 198. Its uv maximum was shifted to 291 mμ with alkali and was restored by acidification, a change similar to the behavior of ramulosin.

Since I was comparable to ramulosin in many of its physical and chemical properties and contained but one more oxygen atom, placement of this function as a secondary hydroxyl group at the 4, 5, 6, or 7 position was indicated by the ir data. On biogenetic grounds the representation of I as 6-hydroxy-ramulosin is suggested by the occurrence of several 8-hydroxy-6-methoxy- and 6,8-dihydroxyisocoumarins(4). An attempt to confirm this structure by chemical conversion of I to the known 6,8-dihydroxy-3-methylisocoumarin(4a,5) by dehydrogenation with Pd/C in boiling p-cymene, led instead to a mixture of (-)-mellein (2) and 8-hydroxy-3-methylisocoumarin (6).



I

However, the 100 MHz nmr spectrum of I in comparison to that of ramulosin (Figure 1, b and a, respectively) provided proof for assignment of the additional oxygen at position 6, and gave an insight into the stereochemistry of the molecule. Thus, the H_3 multiplet at δ 4.46 (d of d of q, 1H, J_{ae} 2 Hz, J_{aa} 12 Hz, J_{CHCH_3} 7 Hz) indicates that a $-CH_2\overset{|}{C}HCH_3$ group is present, with the methyl group pseudoequatorial. The triplet (J_{vic} 12 Hz) pattern of the H_{4a} band at δ 2.85 indicates that the proton is transdiaxial to two other protons and thus excludes the possibility of an axial -OH at position 5. The presence of -OH at position 7 has been eliminated on the grounds that three allylic protons appear in the δ 2-3 region. A narrow band due to the carbinol methine proton at δ 4.29 (t of t, 1H, J_{vic} 2 and 4 Hz) indicates that the proton is equatorial, and therefore, the -OH group has the 6-axial conformation. This is further confirmed since the C_7 methylene protons at δ 2.39 and 2.60 (J_{gem} 19Hz) show no J_{aa} splitting.

The fact that I provided levorotatory mellein upon chemical dehydrogenation accords with the ancillary finding that (-)-mellein was isolated from the P. ramulosa fermentation. This, together with the assignment of the 3-methyl of I as pseudoequatorial (Figure Ib), strongly suggests the 3R absolute configuration for I, for the levorotatory of the two known (7) mellein enantiomers, and for

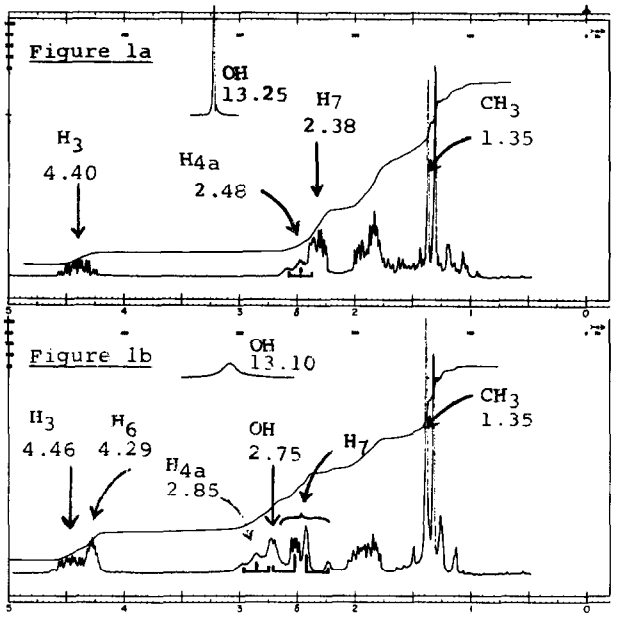


Figure I. 100 MHz Nmr Spectra of Ramulosin (a) and of I (b) in $CDCl_3$

ramulosin. This agrees with earlier work which has shown the 3R configuration for levorotatory 5-methylmellein (8) and for the 5-chloromellein-7-carboxylic acid derived from ochratoxin (9), by their degradations to D-β-hydroxybutyric acid. The metabolic branchpoints at which I and ramulosin, obviously biogenetically related not only to the isocoumarins but also to C-acetyl-o-orsellinic acid (4a,5), become transformed into hydroaromatic ring systems, are under further investigation.

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