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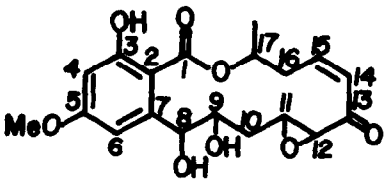
METABOLITES OF PYRENOMYCETES XIII:[1] STRUCTURE OF (+) HYPOTHEMYCIN, AN ANTIBIOTIC MACROLIDE FROM *HYPOMYCES TRICHOTHECOIDES*.

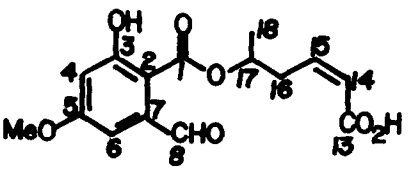
M. S. R. Nair* and Susan T. Carey
The New York Botanical Garden, Bronx, New York 10458

(+) Hypothemycin, an antibiotic metabolite of Hypomyces trichothecoides, *has been assigned the macrolide structure I, based on chemical evidence and spectroscopic data.*

Recently, we reported the isolation of the taxonomically significant pigment, skyrin, from a strain of *Hypomyces trichothecoides.*[2] The same strain of the fungus when grown in a dextrose-yeast medium in still culture in the dark at 25° produced an antibiotic metabolite, hypothemycin. This compound was active against *Tetrahymena furgasoni* at concentrations of 30 ppm (LD_{100}) and 1 ppm (LD_{50}). It was also moderately active against *Ustilago maydis.*



I



II

Hypothemycin, $C_{19}H_{22}O_8$ (elemental analysis), MW 378 (ms) had mp. 173-4°, [α] 365 = +109° (0.136% MeOH), CD curve (MeOH) [θ] 335 (+ 3,351) [θ] 305 (-8,987) [θ] 262 (-40,200) [θ] 234 (+ 29,600), and [θ] 212 nm (+ 77,600), λ_{max}^{MeOH} 220 (38,000), 267 (14,000) and 307 nm (7000), ν max (KBr) ∿ 3350 (b) 1695, 1653, 1620, 1593 and 1250 cm^{-1}. UV and IR spectra suggested a resorcylic acid macrolide structure.[3] In agreement with this its ^{13}C NMR spectrum showed signals at δ 21.0 (C-Me), 34.6 and 36.9 (two methylenes), six sp_3 carbons carrying oxygen at 55.5 (OMe) 57.9 (C_{11}), 62.6 (C_9) 70.7 (C_{12}) 73.2 (C_8) and 81.0 (C_{17}), and sp_2 carbons at 101.1 (C_4) 103.6 (C_6) 104.0 (C_2) 126.4 (C_{15}) 142.3 (C_{14}) 145.3 (C_7) 165.2 (C_5), 166.2 (C_3) 171.2 (COO) and 199.9 (C_{13}). ^1H NMR spectrum showed 3 protons exchangable with D_2O. Thus, three hydroxyls, one OMe, one lactone moiety and one carbonyl, account for seven of the eight oxygens. The eighth oxygen has to be in an oxiran ring to explain the ^{13}C NMR, as well as the molecular formula.

On oxidation with $NaIO_4$ in aqueous methanol, hypothemycin gave an aldehyde acid II, MW 308 (ms). ^1H NMR spectrum ($CDCl_3$) of this acid showed signals at δ 1.41 (3H, J=6.5) for the C-Me, 3.1 (2H dd J=6.5,8) for the C_{16} protons, 3.83 (3H, s) for the OMe, 5.45 (1H, q t J=6.5,6.5) for the C_{17} proton, 5.95 (1H, d

(J=11) for the C_{14} proton, 6.4 (1H, dd J=8,11) for the C_{15} proton and an AB quartet at 6.6 and 6.8 (J=2) for the aromatic protons. Formation of this degradation product defined all but four of the carbons in the macrolide ring. Detailed analysis of ^1H NMR using sequential decoupling defined the structure of hypothemycin as in I. NMR signals of protons on carbons 8 through 12 were: 8H, δ 4.58, dd ($J_{8,OH}$ =4.5; $J_{8,9}$ =2.0) 9H, δ 3.93m ($J_{9,OH}$ =9; $J_{9,8}$ =2.0; $J_{9,10}$ =4; $J_{9,10'}$ =8.7) 10H, δ 1.12m ($J_{10,10'}$ =15; $J_{10,9}$ =4; $J_{10,11}$ =8.7), 10'H, δ 2.05m $J_{10'10}$ =15; $J_{10',11}$ =2; $J_{10',9}$ =8.7), 11H, δ 2.89m ($J_{11,12}$ =2; $J_{11,10'}$ = 2) 12H 4.41 d ($J_{12,11}$ =2). The phenolic proton signal at 12.1 showed that it was chelated to the lactone carbonyl. Coupling constant of 2Hz between the aromatic protons in II showed them to *meta* to each other. Therefore, the OH and OMe are on carbons 3 and 5, respectively.

The coupling constant 11 Hz between the olefinic protons in I showed them to be *cis* since the *trans* coupling constant in comparable 7-dehydro zearalenone[4] is 16Hz. The degradation product II also showed J=11 between the olefinic protons confirming this assignment. The coupling constant of 2 Hz between the oxiran ring protons showed them to be *trans*; in comparable radicicol the *trans* coupling constant is reported as 3.0[3] and 2.8 Hz.[5] In oxiran rings the *cis* coupling constant is always much larger than the *trans* coupling constant.[6] The vicinal hydroxyls in the lactone ring are *threo*, since the coupling constant of the protons on those carbons is 2 Hz (J gauche).

The mass spectrum is in complete agreement with this structure. The major fragments are at m/e 180 (80%) and 179 (100%) formed by cleavage of bonds at C_8 to C_9 and C_1 to oxygen (Compound I).

We are trying to establish the stereochemistry by x-ray crystallography, but we have as yet been unable to obtain large enough crystals for this purpose.

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Footnotes and References

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