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METABOLITES OF PYRENOMYCETES II:[1] NECTRIAPYRONE, AN ANTIBIOTIC MONOTERPENOID

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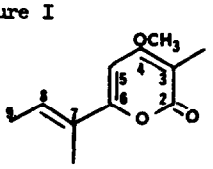
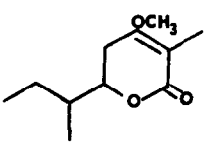
Gyrostroma missouriense Seeler, the imperfect stage of Thyronectria missouriensis (Ell and Ev) Seaver, when grown on a dextrose-yeast medium in still culture, produces an antibacterial culture liquid. From this, three compounds were isolated: succinic acid, bis-(2-ethyl-hexyl) phthalate, and a new antibiotic monoterpene, nectriapyrone. Both the phthalate and nectriapyrone incorporated 2-^{14}C mevalonic acid.

The spectral characteristics of the phthalate, especially its 220 MHz pmr spectrum [2] suggested its structure. Comparison of its ir spectrum with that of a commercial sample [3] confirmed the identification. The phthalate was known previously only as a synthetic compound. Its natural occurrence had not been reported. A 2% solution of our phthalate showed no optical rotation at the sodium D line.

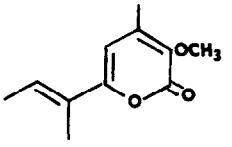
On the basis of the chemical and spectral evidence presented below, nectriapyrone was assigned structure I



I



II

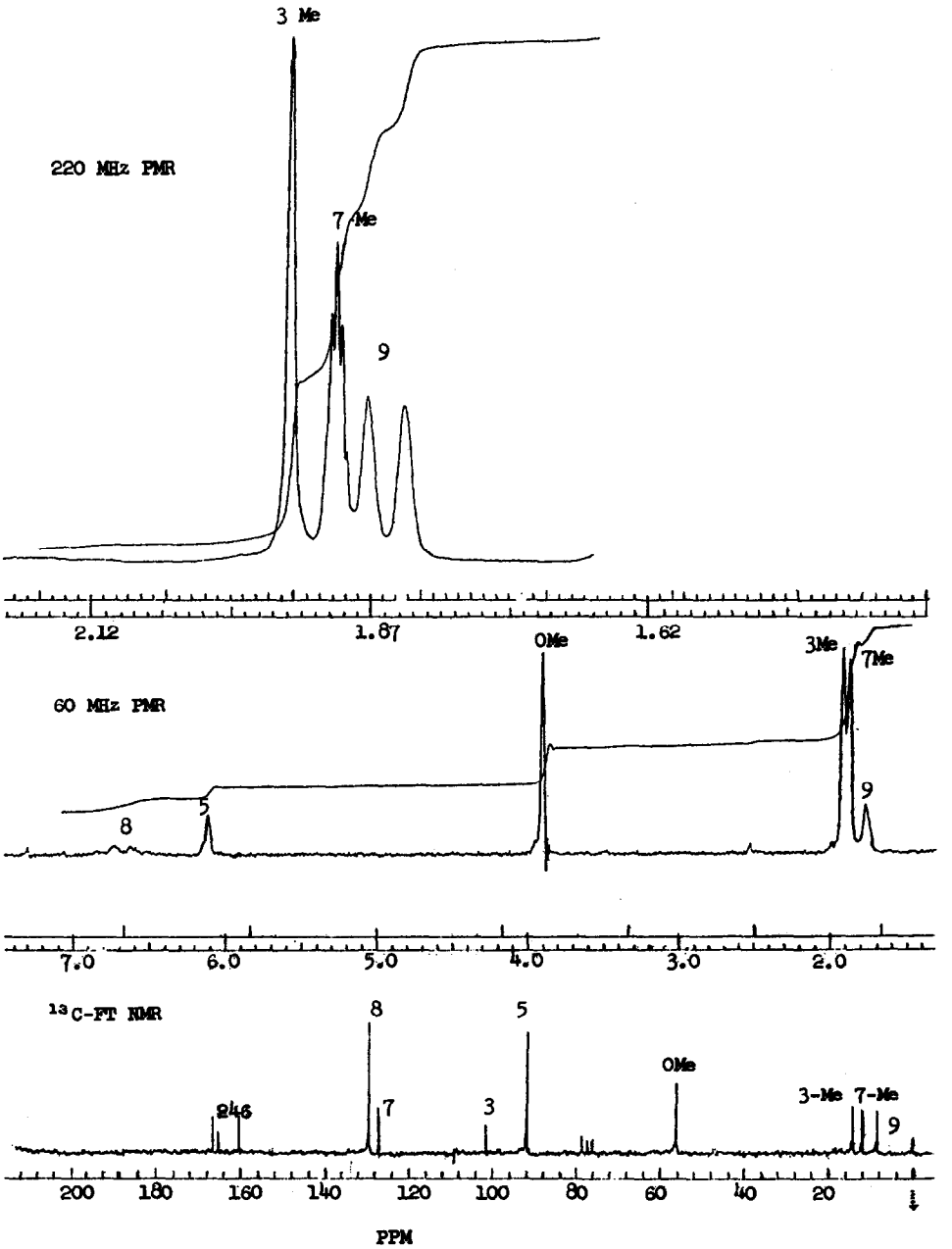


III

Nectriapyrone, $C_{11}H_{14}O_3$ (elemental analysis), MW 194 (ms) had mp 100-102°; λ_{max} 330, 227 nm (ε 10,400, 28,000); ν_{max} 1718 (sh), 1700, 1658, 1629, 1553, 1170, 802, 746 cm^{-1}. The ir spectrum suggested an α-pyrone system,[4] and the uv spectrum was in agreement with an extended α-pyrone chromophore.[5] The 60 MHz pmr spectrum showed two singlets at δ 1.87 and 1.90 for protons of the C-7 Me and C-3 Me, respectively. Only one component of the doublet for C-9, that at δ 1.75, could be seen; the other was masked by the singlets. The 220 MHz pmr spectrum [6] of this region clearly showed the doublet (J 7Hz). The peak at δ 1.87 appeared as a multiplet (J ca. 1Hz) due to allylic coupling with the C-8 proton and homoallyic coupling with the C-9 protons. This coupling also explained the broadening of the doublet peaks ($w_{\frac{1}{2}}$, 3Hz). The remaining peaks, a singlet at δ 3.88 (3H) and one at 6.10 (1H) and a quartet at 6.67 (1H, J 7Hz) were assigned to the protons of the OMe, C-5, and C-8, respectively.

Mass spectral fragmentation of nectriapyrone, 194 (M^+, 65%), 166 (M-CO, 100%), 151 (M-CO-CH_3, 30%) 139 (M-C_4H_7, 10%), 111 (M-CO-C_4H_7, 10%), 83 (1 methoxy, 2-methyl-cyclopropenium

NMR Spectra of I



ion, 70%), 55 ($C_4H_7^+$, 45%) and 43 ($CH_3C{=}O^+$, 80%) was in good agreement with the assigned structure.[7]

On hydrogenation of I over palladized charcoal in EtOAc, the main product was the tetrahydro derivative (II), mp 39-45° (pet. ether); λ_{max} 253 nm (ε 8,600); ν_{max} 1705, 1655 cm^{-1}. The pmr spectrum showed a doublet at δ 1.1 superimposed on a triplet at 1.06 for the C-7 methyl and the C-9 protons, respectively. The C-8 protons appeared as a multiplet at δ 1.23-1.66. A triplet at δ 1.9 (3H, J 1.5Hz), a doublet at 2.6, with fine splitting due to allylic coupling (2H, J 9Hz, 1.5 Hz), a singlet at 3.93 (3H), and a multiplet at 4.13-4.50 (1H) were assigned to the C-3 methyl, (splits with C-5 protons), the C-5 protons, the OMe, and the C-6 proton, respectively. The ms of II showed peaks at 198 (M^+, < 5%), 141 (M-C_4H_9, 100%), 113 (141-CO, 90%), 85 (141-CO-CO, 80%).

The ^{13}C nmr spectra[2] of I and II showed the following signals (assignments are in parentheses): For I, δ 8.6 (C-7 Me), 12.1 (C-9), 14.4 (C-3 Me), 56.0 (OMe), 91.8 (C-5), 101.6 (C-3), 127.2 (C-7), 129.5 (C-8), 160.3 (C-6), 165.4 (C-4) and 166.4 (C-2); for II, δ 8.8 (C-9), 11.3 (C-7 Me), 14.6 (C-3 Me), 24.7 and 26.4 (C-8 and C-5), 38.3 (C-7), 55.4 (OMe), 77.6 (C-6), 102.6 (C-3), 166.0 (C-4) and 169.0 (C-2). In II, all of the signals except those for C-2, C-3, C-4, C-9 and OMe, showed a set of very close peaks. This was not unexpected, since on hydrogenation two asymmetric centers are created. As hydrogenation is not stereospecific, four diastereoisomers may be present. Olefinic carbon chemical shifts of the pyrone ring in I were similar to that reported for 4-methoxy-2-pyrones.[8] Structure III, biogenetically possible but less likely, was considered for nectriapyrone, but was rejected on the basis of shifts in the 100 MHz pmr spectrum[3] induced by $Eu(FOD)_3$: On addition of $Eu(FOD)_3$ to nectriapyrone, the signal for the OMe shifted by 0.1 ppm and that of the C-3 Me by 0.3 ppm which showed that the methyl group was nearer to the carbonyl than the OMe. Therefore, nectriapyrone has structure I. Decoupling experiments established the homoallylic coupling constant between the two methyls on the side chain as 1 Hz: In the 100 MHz pmr spectrum[2] of I containing $Eu(FOD)_3$, the signals for the C-7 Me and C-9 protons became a quartet (J 1Hz) and broad singlet ($w_{\frac{1}{2}}$ 3.4Hz) respectively, on decoupling the C-8 proton. This suggested that the two methyls were cis rather than trans to each other, since in comparable systems, the cisoid homoallylic coupling constant is usually 1.0-1.2 Hz, and the transoid, 1.4 to 1.6 Hz.[9] Examination of a molecular model showed that the $Eu(FOD)_3$ induced shifts in the pmr, C-7 Me, 0.07 ppm, C-8 proton, 0.07 ppm, and C-9 protons, 0.0 ppm could be explained by only a configuration in which the methyl groups are cis.

It is of interest that in all known naturally-occurring methoxy α-pyrones, the methoxyl is in the 4-position.[8],[10] Monoterpenes are rarely produced by fungi[11] and to our knowledge, nectriapyrone is the only monoterpenoid α-pyrone reported.

Nectriapyrone is antibacterial to Staphylococcus aureus at a concentration of 30 ppm.

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