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TWO NEW METABOLITES, 2-NONAPRENYLPHENOL AND 2-NONAPRENYL-3-METHYL-6-METHOXY-1,4-BENZOQUINONE, FROM PSEUDOMANAS OVALIS

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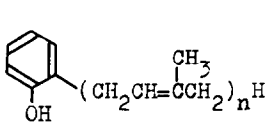
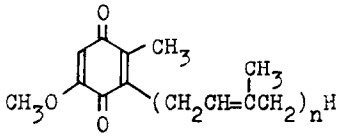
Two new metabolites have been isolated from the lipid fraction of the cells of Pseudomonas ovalis. Structural studies show these compounds to be 2-nonaprenylphenol (I, n=9) and 2-nonaprenyl-3-methyl-6-methoxy-1,4-benzoquinone (II, n=9), respectively.



I



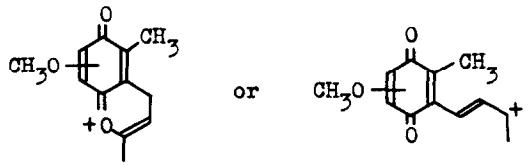
II

The wet cells of P. ovalis were extracted with acetone and the combined extracts were evaporated to dryness in vacuo. The hexane extract of the residual material was separated into two fractions by column chromatography over silica gel using hexane-chloroform (1:1) mixture as the solvent. The first fraction which contained the phenolic compound (I) was purified by preparative thin layer chromatography on silica gel G plates developed with benzene. The phenolic compound thus purified was shown to be a single substance by thin layer chromatography employing a number of solvent systems, and the following spectral data enabled one to characterize it as 2-nonaprenylphenol (I, n=9). The mass spectrum shows a molecular ion at m/e 706 and intense peaks at m/e 368 (base peak) and m/e 382. The ultraviolet absorption spectrum (λ_{max}^{hexane} 272 mμ (log ε 3.30) and 279 mμ (log ε 3.26)) and nmr spectrum (4H, multiplet at 7.1 - 6.5 ppm; 9H, multiplet at 5.08 ppm; 1H, singlet at 4.87 ppm; 2H, doublet (J=7 cps) at 3.31 ppm; multiplet

of alkyl hydrogens at 2.1 - 1.5 ppm) are very similar to that of 2-decaprenylphenol (I, n=10) which has been reported to be a precursor of ubiquinone-10 (1, 2).

The second yellowish fraction consisted of palmitic acid, ubiquinone-9 and a small amount of an unknown 1,4-benzoquinone derivative (II). Palmitic acid was removed by high vacuum sublimation. Separation of the two quinones was carried out by column chromatography over silica gel using 10% ethyl acetate in hexane as the eluent. Further purification of II was effected by thin layer chromatography on silica gel G plates developed with 20% ethyl acetate-hexane mixture and finally by recrystallization twice from ethanol, m.p. 52°C.

The infrared spectrum of II is similar to that of ubiquinone-9. The nmr spectrum of II shows 1H (ring), singlet at 5.69 ppm; 9H (vinylic), multiplet at 5.00 ppm; 3H (methoxyl), singlet at 3.73 ppm; 2H (allylic), doublet (J=7 cps) at 3.11 ppm; multiplet of alkyl hydrogens at 2.1 - 1.5 ppm. A nine-unit isoprenoid side chain was indicated by integration of the vinylic hydrogen regions at 5.00 ppm in the nmr spectrum and was confirmed by its mass spectrum; a molecular ion at m/e 764 and intense peaks at m/e 69 (base peak), m/e 81, m/e 167 and m/e 205. In view of fragmentation modes encountered in plastquinone-3 (3) and vitamin $K_{1(20)}$ (4), the fragment at m/e 205 can be assigned structure III.



III

These spectral data suggest that the structure of II is 5 or 6-methoxy-2-nonaprenyl-3-methyl-1,4-benzoquinone. Recently, the isolation of 2-decaprenyl-3-methyl-6-methoxy-1,4-benzoquinone (II, n=10) and its two lower isoprenylogs (II, n=8 and 9) as minor components from Rhodospirillum rubrum (5) has been reported; structural assignments were based on spectral data but differentiation between the 5 and 6-methoxy isomers rested on biogenetic considerations.

However, it is possible to distinguish unambiguously between the two isomers by comparisons with synthetic models in the following manners. Thus a comparison of positions of the longest wave-length UV band of II and models clearly showed

that II belonged to the 6-methoxy series: II ($\lambda_{max}^{CCl_4}$ 269 mμ (log ε 4.28), 276^{sh} mμ (log ε 4.24) and ca. 320^{sh} mμ (log ε 2.98)); synthetic 2-phytyl-3-methyl-6-methoxy-1,4-benzoquinone (IV) ($\lambda_{max}^{CCl_4}$ 269 mμ (log ε 4.16), 276^{sh} mμ (log ε 4.13) and ca. 320^{sh} mμ (log ε 2.80)); synthetic 2-phytyl-3-methyl-5-methoxy-1,4-benzoquinone (V) ($\lambda_{max}^{CCl_4}$ 269 mμ (log ε 4.18), 276^{sh} mμ (log ε 4.15) and 317 mμ (log ε 2.94)). This was also confirmed chemically. The 5-methoxy derivative (V) was converted readily to its corresponding chromenol by treatment with sodium hydride or triethylamine, but the natural product (II) and 6-methoxy derivative (IV) were not derived to their chromenol derivatives under the same condition.

The synthesis of IV started from 2-methyl-5-methoxy-1,4-dihydroxybenzene, which was condensed with phytol in dioxane in the presence of boron trifluoride etherate. The reaction mixture was chromatographed over silica gel and eluted with 15% ethyl acetate in hexane to afford a light yellow oil. The oil obtained above was oxidized with ferric chloride and rechromatographed over silica gel column. Elution with 10% ethyl acetate in hexane yielded a reddish oily quinone (VI, 35% yield) and a yellowish oily quinone (IV, 11.5%). Spectral data allowed the assignment of the two quinones as 2-phytyl-3-methyl-6-methoxy-1,4-benzoquinone (IV) and 2-phytyl-6-methyl-3-methoxy-1,4-benzoquinone (VI) as follows. The nmr spectrum of IV (in CCl_4): 1H (ring), singlet at 5.63 ppm; 1H (vinylic), triplet (J=7 cps) at 4.78 ppm; 3H (methoxyl), singlet at 3.68 ppm; 2H (allylic), doublet (J=7 cps) at 3.05 ppm; 3H (ring methyl), singlet at 1.94 ppm; 3H (vinyl methyl), singlet at 1.66 ppm; multiplet of alkyl hydrogens at 1.4 - 0.8 ppm. The quinone VI: UV (in CCl_4), λ_{max} 264 mμ (log ε 4.23) and 384 mμ (log ε 2.86); NMR (in CCl_4), 1H (ring), quartet (J=1.5 cps) at 6.29 ppm; 1H (vinylic), triplet (J=7.5 cps) at 4.94 ppm; 3H (methoxyl), singlet at 3.98 ppm; 2H (allylic), doublet (J=7.5 cps) at 3.04 ppm; 3H (ring methyl), doublet (J=1.5 cps) at 1.99 ppm; 3H (vinyl methyl), singlet at 1.68 ppm; multiplet of alkyl hydrogens at 1.4 - 0.8 ppm.

By the same method, V (27%) and VII (5.6%) were synthesized from the condensation of 3-methyl-5-methoxy-1,4-dihydroxybenzene and phytol, and the oxidation of the condensates. Spectral data of V and VII indicate the structures 2-phytyl-3-methyl-5-methoxy-1,4-benzoquinone (V) and 2-phytyl-5-methyl-3-methoxy-1,4-benzoquinone (VII), respectively. The nmr spectrum of V (in CCl_4): 1H (ring),

singlet at 5.63 ppm; 1H (vinylic), triplet (J=7 cps) at 4.78 ppm; 3H (methoxyl), singlet at 3.68 ppm; 2H (allylic), doublet (J=7 cps) at 3.05 ppm; 3H (ring methyl) singlet at 1.94 ppm; 3H (vinyl methyl), singlet at 1.66 ppm; multiplet of alkyl hydrogens at 1.4 - 0.8 ppm. The quinone (VII): UV (in CCl_4), λ_{max} 264 mμ (log ε 4.21) and 380 mμ (log ε 2.84); NMR (in CCl_4), 1H (ring), quartet (J=1.5 cps) at 6.37 ppm; 1H (vinylic), triplet (J=7.5 cps) at 4.93 ppm; 3H (methoxyl), singlet at 3.94 ppm; 2H (allylic), doublet (J=7.5 cps) at 3.01 ppm; 3H (ring methyl), doublet (J=1.5 cps) at 1.97 ppm; 3H (vinyl methyl), singlet at 1.67 ppm; multiplet of alkyl hydrogens at 1.4 - 0.8 ppm. The nmr spectrum of the chromenol derivative of V (in CCl_4): 1H (vinylic), doublet (J=10 cps) at 6.40 ppm; 1H (ring), singlet at 6.14 ppm; 1H (vinylic), doublet (J=10 cps) at 5.37 ppm; 1H (hydroxyl), singlet at 5.03 ppm; 3H (methoxyl), singlet at 3.82 ppm; 3H (ring methyl), singlet at 2.14 ppm; multiplet of alkyl hydrogens at 1.5 - 0.8 ppm.

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