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THE STRUCTURE OF LUTEORETICULIN,
A NITRO-CONTAINING METABOLITE OF STREPTOMYCES LUTEORETICULI

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Luteoreticulin, a new toxic metabolite, m.p. 184.5-185°, $C_{19}H_{19}NO_5$ (Found : C, 66.61; H, 5.60; N, 4.12; M^+, 341.124; $C_{19}H_{19}NO_5$ requires C, 66.85; H, 5.61; N, 4.12; M^+, 341.126), λ_{max}^{EtOH} 225, 256 (infl.) and 368 mμ (ε 13,000, 6,000 and 14,400), $\nu_{max}^{CHCl_3}$ 3000, 1599, 1521 (aromatic), 1551, 1348 ($-NO_2$), 1688 and 1635 cm^{-1}, was isolated from acetone extracts of the mycelial cake of Streptomyces luteoreticuli Arai (1) by us and we wish to present its structure elucidation in this communication.

The n.m.r. (2) spectrum of luteoreticulin indicated the presence of one p-substituted phenyl group (1.88, d, 2H, J=9 c.p.s. and 2.62, d, 2H, J=9 c.p.s.), three vinylic protons (2.85, broad, 1H, half-height width 4.3 c.p.s., 3.42, broad, 1H, half-height width 4.3 c.p.s. and 3.81, s, 1H), one O-methyl group (6.13, s, 3H), and three C-methyl groups (7.90, broad, 6H, half-height width 4.1 c.p.s. and 8.10, s, 3H) in its structure. As the half-height width of the signal at 2.85 changed to 2.4 c.p.s. and that of the signal at 3.42 changed to 3.1 c.p.s. on irradiation at 7.90, it was revealed that each of the vinylic protons resonating at 2.88 and 3.42 couples with the methyl protons resonating at 7.90 in allyl-coupling fashion. Thus, those signals seemed to be attributable to the protons in two $-CH=C(CH_3)-$ groups.

On the hydrogenation over Adams' catalyst, luteoreticulin absorbed five moles of hydrogen to give an aromatic amine as a syrupy product (II), (M^+= 315),

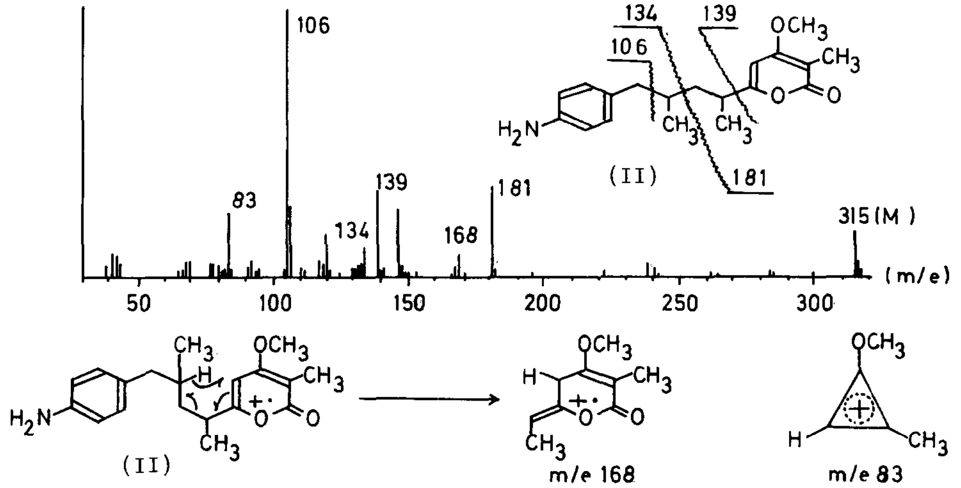
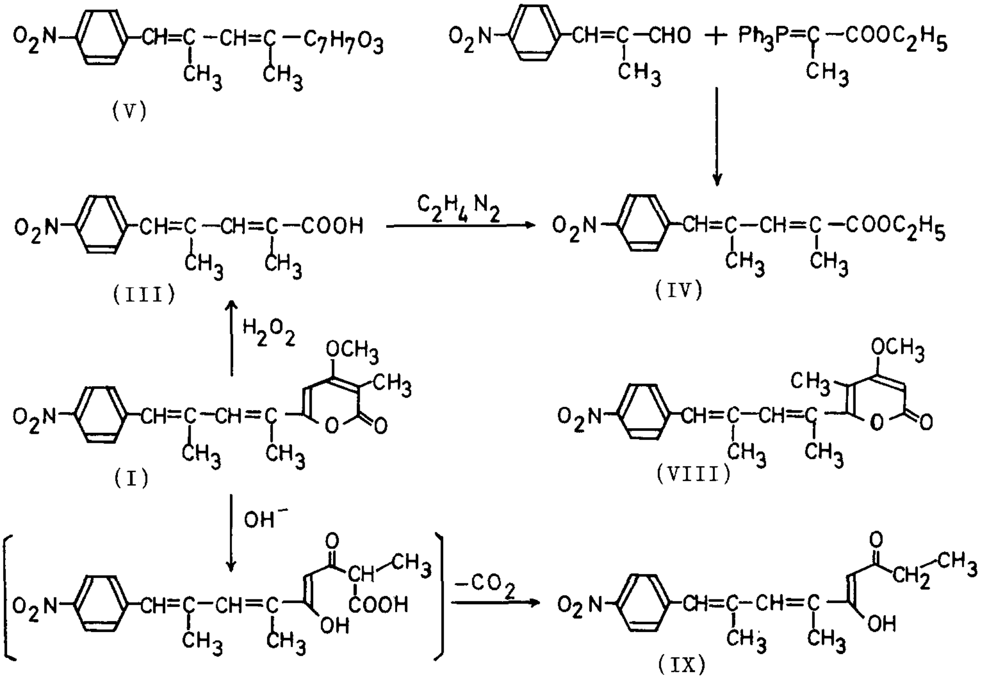


Fig. 1. Mass spectrum of II

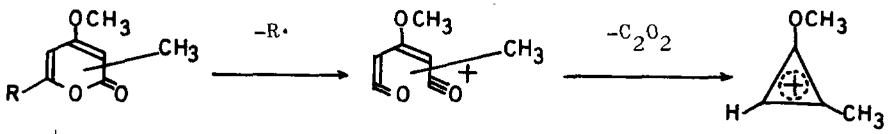


λ_{max}^{MeOH} 244 and 298 mμ, $\lambda_{max}^{0.1N\ HCl-MeOH}$ 298 mμ, $\nu_{max}^{CHCl_3}$ 3400 ($-NH_2$), 1688 and 1645 cm^{-1}. Further, oxidation of luteoreticulin with potassium permanganate gave p-nitrobenzoic acid. These findings revealed that a p-nitrophenyl group and two easily reductive double bonds exist in a luteoreticulin molecule.

Oxidation of luteoreticulin with alkaline hydrogen peroxide gave an unknown acid (III),(m.p. 199-201°, $C_{13}H_{13}NO_4$). Ethylation of III with diazoethane afforded an ethyl ester (IV),(m.p. 81-83°, $C_{15}H_{17}NO_4$), λ_{max}^{EtOH} 332 mμ(ε 18,000), $\nu_{max}^{CHCl_3}$ 1342 cm^{-1} ($-NO_2$), n.m.r.: 1.81 (d, 2H), 2.55 (d, 2H), 2.75 [broad, 1H, $-C\underline{H}=C(CH_3)-$], 3.38 [broad, 1H, $-C\underline{H}=C(CH_3)-$], 5.75 (q, 2H), 7.90 [broad, 6H, $-CH=C(C\underline{H}_3)-$] and 8.69 (t, 3H). Inspection of the fragmentation in the mass spectrum of II (Fig.1) permitted to assume that III is 2,4-dimethyl-5-(p-nitrophenyl)-2,4-pentadienoic acid. Ethyl 2,4-dimethyl-5-(p-nitrophenyl)-2,4-pentadienoate was prepared by Wittig reaction of α-methyl-p-nitrocinnamaldehyde with (1-ethoxycarbonylethylidene)triphenylphosphorane in absolute benzene and identified with IV derived from the natural product by mixed m.p., IR, UV, and n.m.r.. From the above evidences, partial structure V was deduced for luteoreticulin.

We now wish to refer the unestablished part of luteoreticulin, $C_7H_7O_3$, including O-methyl (1), C-methyl (1), carbonyl (1) and vinylic proton (1). The maximum at 298 mμ in the UV spectrum of II might be due to chromophore of 4-methoxy-2-pyrone system. However, the band at 1688 cm^{-1} in the IR spectra of luteoreticulin and II appeared to be somewhat lower for usual 4-methoxy-2-pyrones (1700-1730 cm^{-1}) and higher for 2-methoxy-4-pyrones (1645-1670 cm^{-1})(3, 4). For the purpose of more reliable comparison on this point of arguments, 6-alkyl (CH_3, C_2H_5)-4-methoxy-3-methyl-2-pyrones ($\nu_{max}^{CHCl_3}$ 1690 cm^{-1}, λ_{max}^{EtOH} 300 mμ) and 6-alkyl(CH_3, C_2H_5)-2-methoxy-3-methyl-4-pyrones ($\nu_{max}^{CHCl_3}$ 1670 cm^{-1}, λ_{max}^{EtOH} 260 mμ) were prepared as model compounds. The spectral data of the former were in good agreement with those of II confirming the presence of a methyl substituted 4-methoxy-2-pyrone moiety in luteoreticulin. Morever, the same prominent peaks at m/e 139 ($C_7H_7O_3^+$) and 83 ($C_5H_7O^+$) were observed in the mass spectra of luteoreticulin and II. From the consideration of the fragmentation patterns of known monocyclic 2-pyrones (5), the authors concluded that those peaks are attributed to cation VI and VII, respectively, which derived from 4-methoxy-3 or

5-methyl-2-pyrone moiety of luteoreticulin or II.



M$^+$ 341 m/e 139 (VI) m/e 83 (VII)

Although two possible structures (I and VIII) may be suggested for luteoreticulin from the above data, the following evidence clearly shows the position of methyl group in the 2-pyrone moiety of luteoreticulin. Hydrolysis of luteoreticulin with barium hydroxide in 50% ethanol gave a β-diketone (IX),(m.p. 82-83°, $C_{17}H_{19}NO_4$), λ_{max}^{EtOH} 237 and 351 mμ, $\nu_{max}^{CHCl_3}$ 1720 and 1590 cm^{-1}. In the n.m.r. spectrum of IX, the signals appeared at 8.80 (t, 3H, J=7 c.p.s.,-COCH$_2$C$\underline{H}_3$), 7.55 (q, 2H, J=7 c.p.s., -COC$\underline{H}_2$CH$_3$), 4.20 [s, 1H, -C(OH)=C$\underline{H}$-CO-], 6.12 (observed as a small singlet, -CO-C$\underline{H}_2$-CO-), 7.8-8.0 (6H), 3.39 (1H), 2.85 (1H), 2.51 (2H) and 1.80 (2H). In the mass spectrum of IX, the peaks appeared at m/e 276 (M$^+$- CH$_3$CH$_2$·), 245 (M$^+$- CH$_3$CH=C=O), 244 (M$^+$- CH$_3$CH$_2$CO·), 230 (M$^+$- CH$_3$CH$_2$COCH$_2$·) and 57 (CH$_3$CH$_2$CO$^+$), while a peak at m/e 258 (expected from M$^+$- CH$_3$CO·) was absent. On the basis of these data, it was confirmed that a -C(OH)=CH-CO-C$_2$H$_5$ group is present in IX. The presence of the terminal propanoyl group instead of acetyl group in IX shows that the position of methyl group in 2-pyrone ring of luteoreticulin should be C-3 position.

Consequently, the structure I was deduced for luteoreticulin. Studies on the geometric configuration of luteoreticulin will be published separately.

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