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IN VITRO OXIDATION OF THE 8-HYDROXYQUINOLINE MOIETY WITH METABOLIC ACTIVATION SYSTEM TO A MUTAGENIC QUINOLOQUINONE COMPOUND OF LAVENDAMYCIN ANALOGS

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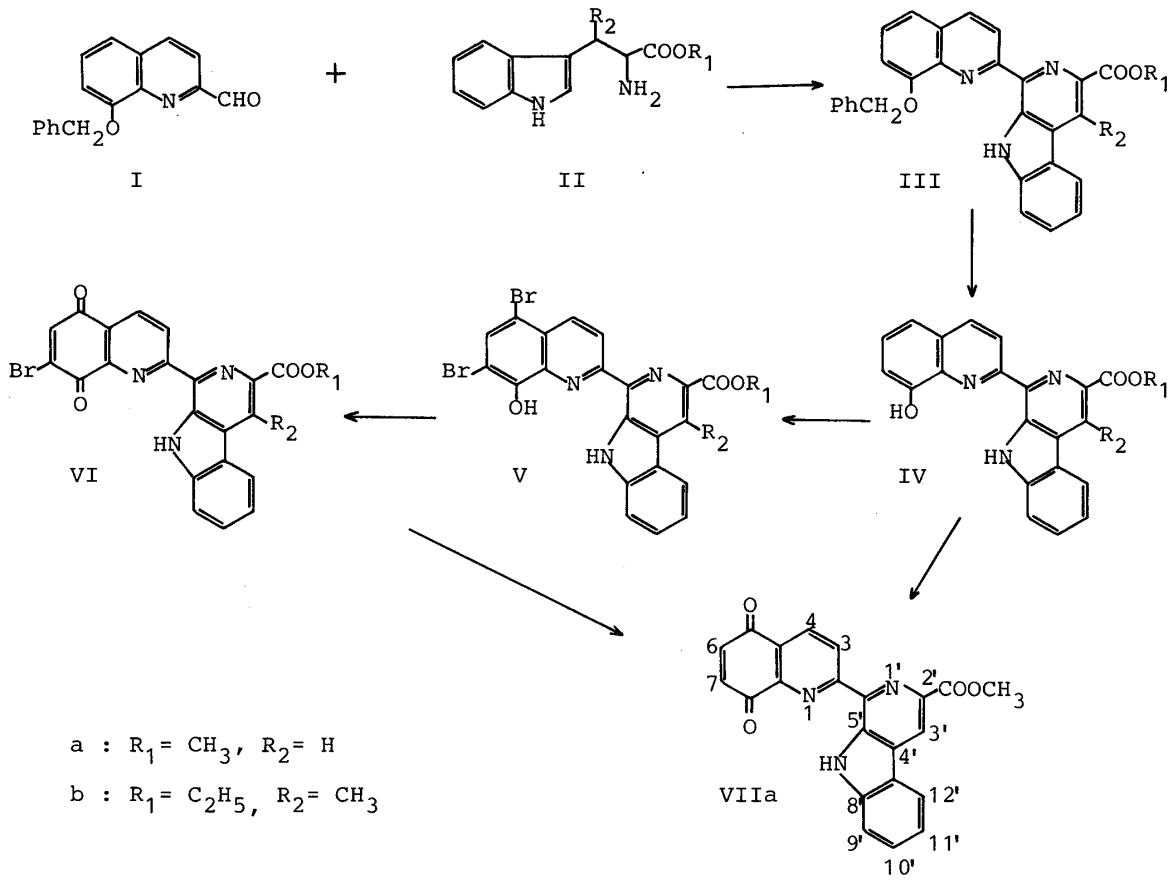
Intermediary products in the synthesis of lavendamycin were tested for mutagenic activities in Salmonella typhimurium TA 98 and TA 100 with and without a metabolic activation system. Lavendamycin analogs having a methyl group at the 3' position showed a significant mutagenicity to TA 100 after the metabolic activation using S9 mix prepared from rat liver homogenate. Oxidative products of the 8-hydroxyquinoline derivatives were mutagenic without the metabolic activation. Of these oxidative products, desamino-desmethyllavendamycin methyl ester was identified as a metabolic product obtained by the incubation of the 8-hydroxyquinoline derivative with mouse liver homogenate.

KEYWORDS —— 8-hydroxyquinoline; lavendamycin analog; quinoloquinone; mutagenicity; metabolic activation; S9 mix; mouse liver homogenate

In 1981 lavendamycin was isolated by Doyle et al.[1] from fermentation of Streptomyces lavendulae. The substance was structurally and biogenetically related to the antitumor antibiotic streptonigrin.[2] During the past few years, Kende has successfully achieved a total synthesis of lavendamycin methyl ester,[3] and Boger has shown an approach to the tricyclic β-carboline moiety of lavendamycin.[4]

In continuation of studies of the synthesis of lavendamycin and related analogs, we recently developed methods for the nonacidic Pictet-Spengler type cyclization of an appropriately substituted quinoline-2-carbaldehyde (I) with tryptophan esters (II) to pentacyclic β-carboline systems (III). After hydrogenation of III, oxidation of the bromophenol systems (IV) with cerium ammonium nitrate gave bromoquinoloquinone structures (IV).[5] Then quinoloquinone (VIIa) was



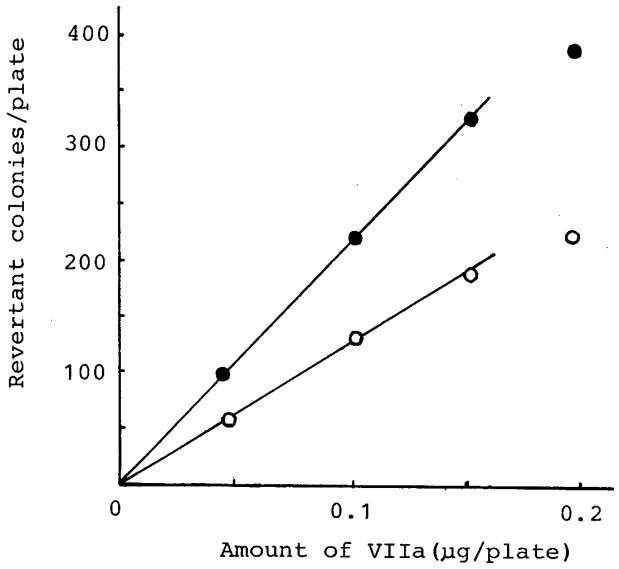


Fig. 1. Mutagenicity of VIIa against Salmonella typhimurium Strain TA 100 with S9 mix (●——●) and without S9 mix (o——o) Each point is the average of at least triplicate runs and the number of spontaneous revertants has been subtracted from each plotted value.

synthesized by direct oxidation of IVa with cerium ammonium nitrate in relatively low yield. VIIa was also derived from bromoquinoloquinone (VIa) by hydrogenolysis and oxidation. Here we evaluate the bacterial mutagenicity of these lavendamycin analogs and offer a possible inference regarding the nature of the oxidative intermediates involved in metabolic activation.

The mutation test was carried out to detect the his^+ revertants with Salmonella typhimurium TA 98 and 100 using method of Ames et al.[6]: the test compound was dissolved in dimethylsulfoxide (DMSO), and an overnight nutrient broth culture of the bacterial suspension (approximately 6 X 10^7 cells in 0.075 ml) was mixed with 2.5 ml of molten top agar by agitation, and poured onto base agar plates. The S9 fraction was prepared from the liver of rats treated with polychlorinated biphenyls (PCB), and 50 µl of liver supernatant (S9) was used per plate as a metabolism inducing system. Mutagenic activity was calculated from the linear portion of the dose-response curve. Among the 9 lavendamycin analogs (a and b types), VIb exhibited the strongest mutagenicity to TA 100 (1041.7 rev./n mol) with S9 mix and (975.0 rev./n mol) without S9 mix, but its mutagenicity to TA 98 was weak with S9 mix. The next strongest mutagenic effect on TA 100 with S9 mix was VIIa (838.5 rev./n mol). Without S9 mix the value was 511.5 rev./n mol (Fig. 1). With VIIa, toxicity tended to increase up to a dosage of 1 µg/plate. Without S9 mix IVa did not mutate to either TA 100 or TA 98. This compound was strikingly lethal to both strains with S9 mix at 0.5 µg/plate. The relative mutagenic potency was drastically influenced by the nature of the substituent, as is the case with the methyl and/or bromine groups. Compounds with CH_3 at the 3' position were more mutagenic in the following order: VIb > Vb > IIIb > IVb. These macromolecular metabolizing studies in conjunction with enhancement of the mutagenicity by S9 mix demonstrate that oxidative products such as semiquinone radicals may be responsible for the reactive intermediates in the metabolism of the 8-hydroxyquinoline structure.

The biosynthesis and isolation of the metabolically active intermediates of 8-hydroxyquinoline (IVa) were attempted using liver homogenate obtained from mice. Male ddy mice weighing 21-23g were fed a pellet diet (CE-2, Clea Japan Co.) for at least three days before use. Livers (13g) were excised from 11 mice and homogenized with a Potter-Elvejhem homogenizer in 100 ml of a modified Krebs-Ringer phosphate buffer, pH 7.4, in an ice-water bath. A solution of compound IVa (20 mg) in DMSO (1 ml) was added to the homogenate and incubated at 37° C for 30 min. After incubation, the reaction mixture was extracted 3 times with 5% MeOH in chloroform (100 ml). The extract was concentrated in vacuo below 40° C and chromatographed on preparative TLC using ethyl acetate, then extracted with 5% MeOH in chloroform. Next the crude material was rechromatographed on preparative TLC using 1% MeOH in chloroform as the development solvent to separate the Rf values 0.25 of IVa and 0.34 of the unknown metabolite.[7] After development, a light-brown metabolite at Rf 0.34 was scraped from the plates and extracted with 5% MeOH in chloroform. The extract was concentrated in vacuo below 40°C to give yellowish brown crystals; mp 257-259°C. This metabolite was identical with chemically synthesized quinoloquinone (VIIa) on the basis of following spectroscopic evidences.

The structure of VIIa was thus determined by the high resolution MS [m/z: 383.0905 for $C_{22}H_{13}N_3O_4$ (calcd: 383.0907)] and ^1H - NMR [8] [δ:4.11 (3H, s, OCH_3), 7.08 (1H, d, J=10.30Hz, C_6 or C_7 - H), 7.16(1H, d, J=10.30Hz,

C_6 or C_7 - H), 7.34 (1H, dd, J=8.05Hz, 7.08Hz, $C_{11'}$ - H), 7.62 (1H, dd, J=8.30Hz, 7.08Hz, $C_{10'}$ - H), 7.75 (1H, d, J=8.30Hz, $C_{9'}$ - H), 8.18 (1H, d, J=8.30Hz, $C_{12'}$ - H), 8.52 (1H, d, J=8.30Hz, C_3 - H), 8.93(1H, s, $C_{3'}$ - H), 9.14(1H, d, J=8.30Hz, C_4 - H), and 11.97(1H, s, NH disappeared with D_2O)], which was assigned on pattern similar to that of lavendamycin methyl ester described by Kende.[3)]

These results demonstrate that compound VIIa is a metabolic product of IVa and possesses the mutagenic activity on the quinone moiety.

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7) The preparative TLC was done on silicagel plates prepared with Kieselgel 60 PF_{254} (Merck, Art 7747).

8) ^1H -NMR was done on a JEOL JNM GX - 400 in $CDCl_3$ with tetramethylsilane as an internal standard.

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