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### Rapid Screening of the antimicrobial activity of microbial extracts by microdilution assay

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The screening system for bioactive compounds from various samples must be simple and rapid, can generate accurate and reproducible data also. In general the methods for testing of antimicrobial activity were divided into three groups, disc diffusion assay, dilution plate and dilution broth assay. However these methods are not suitable for screening because it is too laborious and only comparable among antimicrobial agents with similar physical properties.

Therefore we applied microdilution assay for screening of antimicrobial agents. We isolated microorganisms from several soil samples and prepared assay samples by solvent extraction. The 200 samples were tested by microdilution assay and the strains RJ0252, RJ0002, RJ0189, RJ0230, RJ0179, RJ0191 and RJ0228 were selected as potent candidates.

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### Characterization of pyranosone dehydratase involved in the biosynthetic pathway of the antibiotic cortalcerone

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The molecular mass of pyranosone dehydratase was determined as 95 kDa on SDS/PAGE and 192 kDa by gel filtration chromatography. 1,10-Phenanthroline and nitrilotriacetate showed the marked inhibition effect on the enzyme activity. The metal analysis showed that pyranosone dehydratase has 1.14 mol of zinc per mol of subunit.

Biosynthesis of cortalcerone was studied on the *in vitro* enzyme system containing pyranose oxidase, catalase, and pyranosone dehydratase by UV-visible absorption, $^{13}C$, and $^{1}H$ NMR spectroscopy and chemical derivatization. Pyranosone dehydratase produced cortalcerone from D-*arabino*-hexos-2-ulose through a double dehydration. As a first dehydration step, the enzyme could eliminate one $H_2O$ from D-*arabino*-hexos-2-ulose and produced 4-deoxy-D-*glycero*-2-hexos-3-enosulose having absorptional maximum at 265 nm. 4-Deoxy-D-*glycero*-2-hexos-3-enosul-ose rearranged chemically to other intermediate (Int III), having no absorbance in UV-visible range, through a tricarbonyl compound (Int II), 4-deoxy-D-*glycero*-hexos-2,3-ulose. Pyranosone dehydratase can convert this intermediate (Int III) to cortalcerone with an absorptional maximum at 230 nm. Among the tested analogues, 4-fluoro-4-deoxy-, 6-deoxy-, and 6-fluoro-6-deoxy-D-*arabino*-hexos-2-ulose were served as substrates for pyranosone dehydratase.