the lactone could be an essential alkylating moiety of VP16-213. Further, the lactone is readily metabolized in vivo to the inactive ring opened hydroxy-acid:

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{OH} \\
\text{CO}_{2}H
\end{align*}
\]

In an attempt to eliminate this metabolic detoxification pathway and to examine whether the lactone is an essential reactive or structural feature for activity of VP16-213, we have synthesized the corresponding cyclic ether of the drug:

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

The cyclic ether has been tested in the mouse leukemia L1210 system and appears to retain activity relative to VP16-213. This suggests that the lactone is not an absolute requirement for activity of VP16-213.

In the synthesis of VP16-213 cyclic ether, considerable use was made of high resolution (470 MHz) nuclear magnetic resonance (NMR) spectroscopy, particularly in the determination of the cis- or trans-fused configuration of the ether at positions C-2 and C-3.

**Studies on the Metabolism of VP16-213 in the Rat**

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The metabolism of VP16-213 in vitro by rat liver systems and in vivo by the rat has been studied. VP16-213, specifically labelled with tritium at position C-1 and with a specific activity of 119.8 \(\mu\)Ci/mg, was incubated with rat liver microsomes and rat liver homogenates, and metabolism extracts analysed by high-performance liquid chromatography (HPLC) with UV- and radioactivity detection. For control incubations, VP16-213 was incubated only in buffer and in homogenates and microsomes inactivated by heating at 85–90° C for 20 min. After HPLC of metabolism extracts (ethanol/aqueous mixtures), eluate fractions were subjected to liquid scintillation counting, and percentage radioactivity distributions of chromatograms were calculated. It was found that VP16-213 was metabolised extensively by rat liver homogenates and by rat liver microsomes, with the formation of one major metabolite. After HPLC on a reversed-phase microparticle C\(_{18}\)-column using isocratic elution with a mixture of methanol-water, 40 : 60 (v/v%), buffered at pH 7.1 with 0.01 M potassium phosphate, VP16-213 showed a retention time varying between 8.5–9 min for the incubations in various systems. Incubations with rat liver homogenates showed the presence of a major radioactive product with short retention time (3 min), not present in control incubations. In one experiment this metabolite was present at a level of 39.5% of total radioactivity after 4 h of incubation with rat liver homogenate. Two other minor components with retention times 5 and 7 min were found to be produced after incubation with liver homogenates, but their formation was not reproducible. The major metabolite formed by liver homogenates was also present in methanol -, but not in chloroform extracts of plasma samples of rats, treated with VP16-213. Two hours after two consecutive i.p. injections, corresponding to doses of 1.1 mg/kg, with an interval of 3 h, the metabolite was found to be present in the plasma at a level of 45% relative to VP16-213. The short retention time of the metabolite in reverse-phase chromatography, which coincides with that of the synthetic cis-hydroxy acid derivative of VP16-213, and solubility properties, suggest the metabolite to be the cis- or trans-hydroxy acid derivative of VP16-213. On account of the performed experiments, the metabolite is formed by an enzyme present in the microsomal fraction of rat liver.

**Pharmacokinetics and Bioavailability of VP16-213 in Man**

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**VP16-213/Pharmacokinetics/Bioavailability/Analysis in Plasma and Urine**

Levels of VP16-213 have been measured in the plasma and urine of patients receiving the drug as part of chemotherapy regimes by high-pressure liquid chromatography. The analytical method involves extraction into chloroform using VM26 as internal standard and chromatography on a Hypersil-ODS column (100 × 5 mm) with 52% methanol as the eluting solvent.