

In Vitro Hepatic Biotransformation of Aspalathin and Nothofagin, Dihydrochalcones of Rooibos (*Aspalathus linearis*), and Assessment of Metabolite Antioxidant Activity

abstract

Aspalathin (20,3,4,40,60-pentahydroxy-30-C- β -D-glucopyranosyldihydrochalcone) is the major flavonoid present in the South African herbal tea rooibos. In vitro metabolism of aspalathin and a structural analogue nothofagin, lacking the A ring catechol group, was investigated by monitoring the formation of glucuronyl and sulfate conjugates using Aroclor 1254 induced and uninduced rat liver microsomal and cytosolic subcellular fractions. Following glucuronidation of both aspalathin and nothofagin, HPLC-DAD, LC-MS, and LC-MS/MS analyses indicated the presence of two metabolites: one major and one minor. Only one aspalathin metabolite was obtained after sulfation, while no metabolites were observed for nothofagin. Two likely sites of conjugation for aspalathin are 4-OH or 3-OH on the A-ring. For nothofagin, the 4-OH (A-ring) and 60-OH (B-ring) seem to be involved. The glucuronyl conjugates of aspalathin lack any radical scavenging properties in online postcolumn DPPH radical and ABTS radical cation assays. Deconjugation assays utilizing glucuronidase and sulfatase resulted in the disappearance of the metabolites, with the concomitant formation of the unconjugated form in the case of the glucuronidated product. The balance between conjugated and unconjugated forms of aspalathin could have important implications regarding its role in affecting oxidative status in intra- and extracellular environments in vivo.