Synopsis of Original Research Paper

Metabolism of the Hair Dye Component

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Regioselectivity in *N*-acetylation of nitro-*p*-phenylenediamne (NPDA, 2-nitro-1,4-diammobenzene), a widely used hair dye component, by rat liver cytosolic *N*-acetyltransferases was studied in relation to its substituent effects on enzymatic *N*-acetylation of mono-substituted anilines. NPDA was acetylated specifically at the *N*4-position to afford the N^4-monoacetate, a maior urinary metabolite in the rat, when incubated with rat liver cytosol fortified with acetyl-coenzyme A. N^1-Acetylation of NPDA did not take place even when the N^4-monoacetate was used as a substrate, suggesting a strong steric hindrance effect of the *ortho* nitro group on the enzymatic N^1-acetylation. The steric hindrance effect of the nitro group on the cytosolic *N*-acetylation of the *ortho* amino group was revealed by a comparative study carried out by using aniline, three respective regioisomers of nitroanilines and phenylenediamine (PD)s as model substrates. The comparative study also indicated the enzymatic *N*-acetylation of the mono-substituted anilines to be strongly influenced by the electronic effect of the substituents.

Regioselective *N*-acetylation in the hepatic cytosol was also investigated with N^1 and N^4-monoacetates of 1,2,4-triaminobenzene (TAB). The monoacetates yielded the N^1, N^4-diacetate, another major urinary metabolite of the hair dye component, in the rat, without concomitant formation of the N^2, N^4-diacetate or the N^1, N^2, N^4-triacetate. The triacetate was formed only from the N^1, N^2-diacetate in the enzymatic reactions. A comparative study, carried out by using *N*-mono-acetates of three regioisometric PDs, indicated that the *N*-acetyl group had a potent steric hindrance effect on the primary amino group at the *ortho* position.

Thus, the present *in vitro* study strongly suggested that the two major urinary metabolites, NPDA N^4-acetate and TABN^1, N^4-diacetate, of the hair dye component could be formed, at least in the rat liver, by the enzymatic *N*-acetylation of the corresponding amines.