Environmental Health Perspectives
Vol. 49, pp. 223-231, 1983

In Vivo Metabolism of 3,2′-Dimethyl-4-Aminobiphenyl (DMAB) Bearing on Its Organotropism in the Syrian Golden Hamster and the F344 Rat.

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The *in vivo* metabolism of tritiated DMAB was examined in male Syrian golden hamsters, which are susceptible to both urinary bladder and intestinal carcinogenesis by this agent and in male F344 rats in which intestinal tumors represent the main lesions. Evidence was obtained for the presence of the *N*-hydroxy-*N*-glucuronide of DMAB as a major metabolite in hamster urine and bile and in rat bile but not urine. The routes of excretion of this metabolite, which may represent a transport form of the ultimate carcinogen, correlate well with the main tumor sites in the two species. Other metabolites partially identified were the sulfates and glucuronides of *C*-hydroxylated DMAB and *C*-hydroxylated-*N*-acetyl DMAB.

Introduction

During comparative carcinogenicity studies with various derivatives of 4-aminobiphenyl, Walpole et al. (*1-3*) observed that the introduction of a methyl group *ortho* to the amine function (3-methyl-4-aminobiphenyl) resulted in increased carcinogenicity towards the intestinal tract of rats. On the other hand, methyl substitution *meta* to the amine (2-methyl-4-aminobiphenyl) decreased the carcinogenicity and changed the organotropism, with the appearance of liver rather than intestinal tumors. The introduction of a second methyl group at the 2′ position to give 3,2′-dimethyl-4-aminobiphenyl (DMAB), enhanced the carcinogenicity toward the intestinal tract even further; moreover significant carcinogenicity appeared toward the ear duct, the salivary gland and other organs. Interestingly a methoxy group *ortho* to the amine produced a carcinogen with organotropism toward the urinary bladder. These relationships are summarized in Table 1. To explain the carcinogenicity of 4-aminobiphenyl, 3-methyl-4-aminobiphenyl and DMAB toward the small and large intestines, Walpole speculated (*1*) that the effective carcinogen of the amines was a metabolite excreted in the bile.

Because of the great interest in animal models for colorectal cancer which would accurately reflect the disease in man, DMAB was utilized by Spjut et al. (*4-6*), and So and Wynder (*7*) and others (*8*) to study the induction and development of colon cancer in rodents. In experiments designed to test Walpole's suggestion that metabolites of DMAB acted topically on the intestine, Navarrette-Reyna and Spjut (*9*) performed colostomies 4 cm above the rectum in rats. Following the SC administration of DMAB, they found that tumors were found exclusively in the intestine proximal to the colostomy. Other experiments by Cleveland et al. (*10*), involving the SC administration of DMAB to rats with surgically defunctionalized colon segments, similarly indicated that tumors appeared only in those segments which were in actual contact with the fecal stream. These experiments provided strong evidence that the induction of tumors in the intestine was related to the transport of some form of the carcinogen via the

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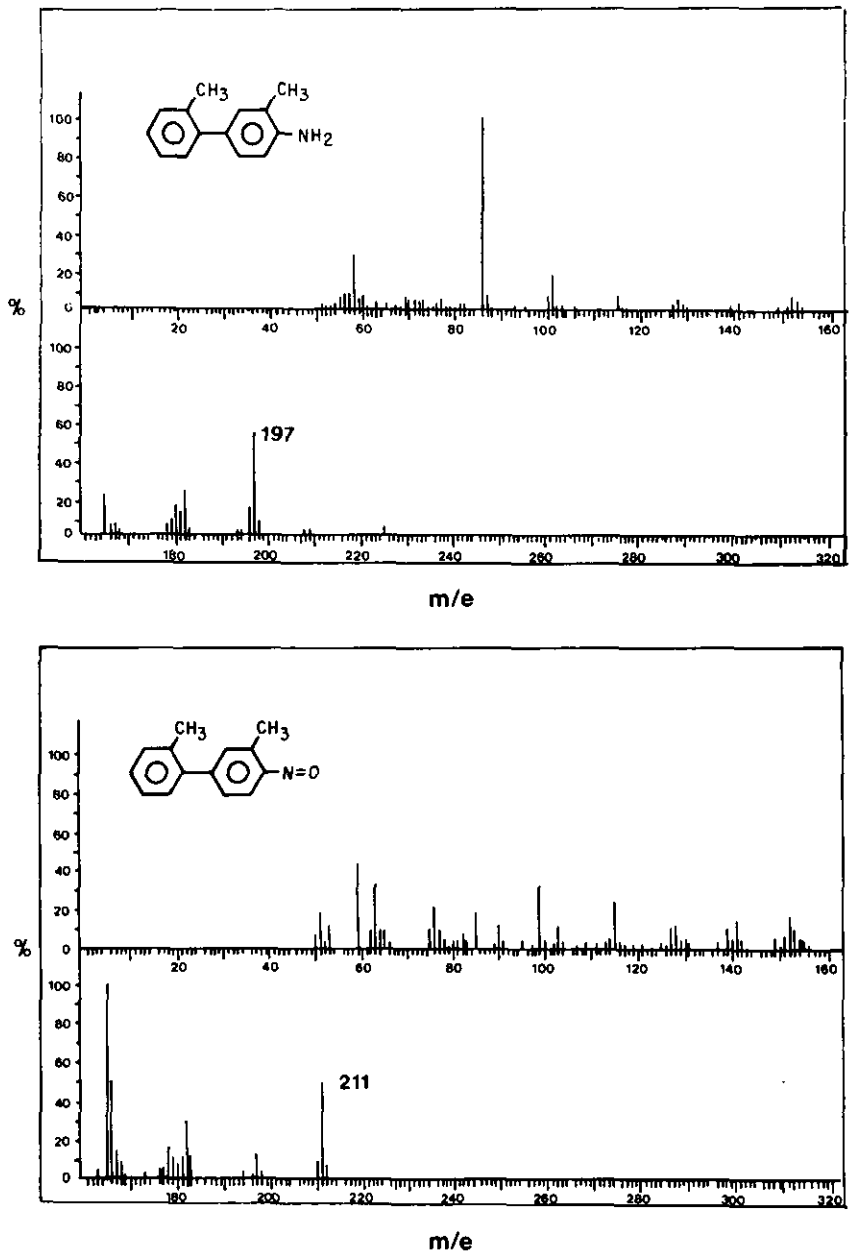

FIGURE 5. Mass spectra of aglycones recovered from mild acid (pH 5) hydrolysis of Sephadex LH-20 peak α.

yielded DMAB as the only component (Fig. 8, upper trace) whereas hydrolysis and HPLC of α_2 yielded 3,2'-dimethyl-4-nitrosobiphenyl as the major component and a small amount of DMAB (Fig. 8, lower trace). This indicates that the Sephadex LH-20 α peak was in fact composed of two glucuronic acid conjugates: the *N*-glucuronide of DMAB (α_1) and the *N*-hydroxy-*N*-glucuronide of DMAB (α_2). The approximate ratio of α_2 to α_1 was 5:1 in rat bile, 1:3 in hamster bile and 1:2 in hamster urine, as determined by HPLC.

Sephadex LH-20 peak β was present in the urines and biles of both hamsters and rats (Figs. 2 and 3). On TLC with solvent system A, a naphthoresorcinol-positive, radioactive band with an R_f = 0.74 was noted in all four cases. A strong immediate reaction with Ehrlich reagent spray indicated the presence of a free amine group. Following β-glucuronidase hydrolysis, the aglycone was recovered by ether extraction and purified by TLC with solvent system B (R_f = 0.46). The purified aglycone yielded a mass spectrum (Fig. 9) which is compatible with that of a

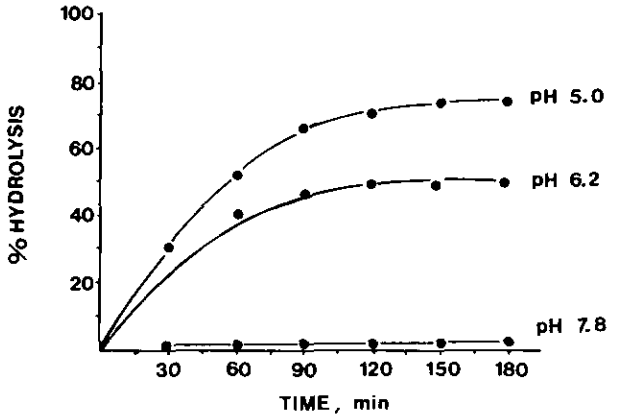

FIGURE 6. Time course of hydrolysis of Sephadex LH-20 peak α at pH 5, pH 6.2 and pH 7.8.

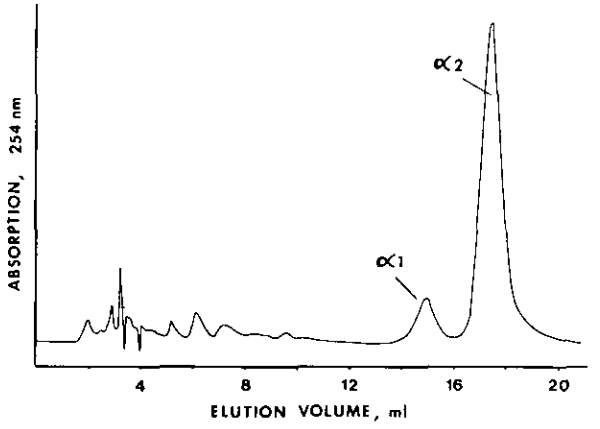

FIGURE 7. Resolution of Sephadex LH-20 peak α (rat bile) into glucuronides (α_1 and α_2) by HPLC. A μBondapak C_{18} column was eluted with 30% methanol, 0.005 M sodium phosphate, pH 8.0.

ring hydroxylated metabolite of DMAB.

Sephadex LH-20 peak γ, present in both rat urine and bile and in hamster urine was positive for glucuronic acid. After β-glucuronidase hydrolysis of γ obtained from rat urine, TLC in system B gave a major radioactive zone with an R_f of 0.27. Mass spectral analysis of the aglycone yielded distinct peaks at m/e = 255 (M^+), 213, 198, 181 and 163. We infer that peak γ represents the glucuronide of N-acetyl ring-hydroxylated DMAB.

Sephadex LH-20 peak δ, a major metabolite in rat urine and possibly a minor metabolite in rat bile and hamster urine gave an R_f of 0.60 on TLC using solvent system A. The metabolite was negative for glucuronic acid and gave a yellow color with Ehrlich reagent which developed only after a period of time. The greater elution volume of δ compared to the glucuronides α and β on Sephadex LH-20, which

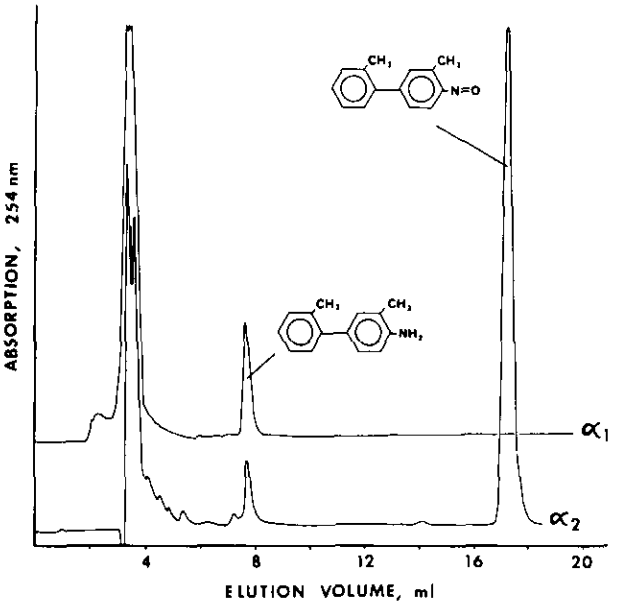

FIGURE 8. HPLC of pH 5 hydrolysis products of α_1 and α_2. A μBondapak C_{18} column was eluted with 75% methanol.

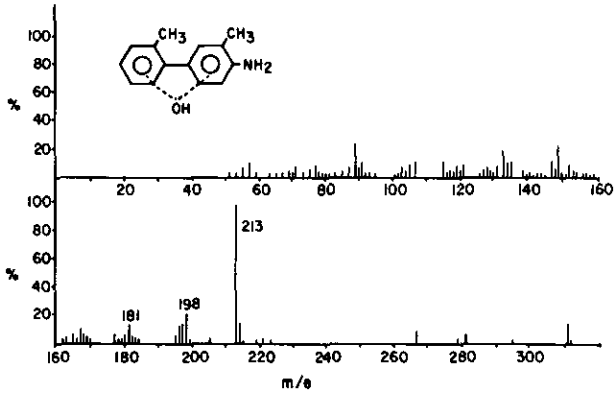

FIGURE 9. Mass spectrum of the aglycone of Sephadex LH-20 peak β. A ring hydroxylated metabolite is indicated; the position of the hydroxyl group cannot be determined by mass spectral analysis alone.

effects separations mainly by molecular sieving, suggested that peak δ might be a sulfuric acid conjugate. In fact after incubation with aryl sulfatase at 37°C for 4 hr at 37°C, more than 80% of the total radioactivity was extractable into ether. The concentrated ether extract gave a single radioactive band on TLC with system B with an R_f of 0.27. Submission of the eluted aglycone to mass spectrometry gave a spectrum essentially identical to that obtained using the aglycone of peak γ. Thus peak δ represents the sulfate ester of N-acetyl-C-hydroxy DMAB.