vious data reported in the literature.[24-28] In this respect, however, further studies are in progress.

Experimental Section

Intrinsic viscosities were measured at 30°. Since the yields of all the poly(amide amines) listed were always practically quantitative, elemental analysis determinations were considered to be irrelevant. This polymerization being a polyaddition, in fact, the values obtained for the products could not be expected to be different from that of the monomeric mixtures. That under the conditions used no other reaction but polyaddition takes place between amines and compounds bearing activated double bonds has been previously demonstrated.[29]

Polymer G_1. To a cooled (5°) solution of 1.94 g of 1,4-bisacryloylpiperazine[30] in 10 ml of ethanol, 9 ml of aqueous 1 *M* ethanolamine and 1 ml of ethanolic 1 *M* *n*-dodecylamine were added under nitrogen. The mixture was thoroughly shaken and then kept in the dark at room temperature under a nitrogen atmosphere for 4 days. The solvents were then evaporated under reduced pressure and the product was dried at 45° under vacuum (0.1 mm). The yield was practically quantitative, apart from mechanical losses. The polymer had an intrinsic viscosity (in ethanol) of 0.21 dl/g.

Polymers G_2 and G_3. The same procedure was used as above, starting from the same quantity of 1,4-bisacryloylpiperazine solution, and 8 ml of 1 *M* aqueous ethanolamine and 2 ml of 1 *M* ethanolic *n*-dodecylamine (G_2), or 7 ml of 1 *M* ethanolamine and 3 ml of 1 *M* *n*-dodecylamine (G_3). The yields and the intrinsic viscosities of these polymers were similar to those of G_1.

Polymer G_4. To a cooled solution of 1.94 g of 1,4-bisacryloylpiperazine in 5 ml of water, 5 ml of aqueous 1 *M* piperazine and 0.375 g of solid glycine were added under nitrogen. The mixture was thoroughly shaken until all solids were dissolved and then kept in the dark at room temperature, under a nitrogen atmosphere for 1 week. The mixture was then poured into 250 ml of dry acetone and precipitated G_4 was collected by filtration and dried at 45° (0.1 mm). The yield was over 90%. The polymer had an intrinsic viscosity of 0.18 dl/g (in 0.1 *M* HCl/1 *M* NaCl).

Polymers G_5, G_6, and G_7 were prepared in precisely the same manner as already described for G_1, G_2, and G_3, starting with the same quantity of 1,4-bisacryloylpiperazine and the following quantities of amines: 5 ml of 1 *M* aqueous ethanolamine and 5 ml of 1 *M* ethanolic benzylamine for G_5; 9 ml of 1 *M* ethanolamine and 1 ml of 1 *M* benzylamine for G_6; and 7 ml of 1 *M* ethanolamine and 3 ml of 1 *M* benzylamine for G_7. The yields were over 90%. The intrinsic viscosities (in ethanol) ranged from 0.20 to 0.25 dl/g.

Polymer G_8 was prepared exactly as previously described in the case of G_7, but 7 ml of 1 *M* aqueous *as-N,N*-dimethylethylenediamine was substituted for the same quantity of ethanolamine.

Polymers G_9 and G_{10} were prepared as previously described in the case of G_5 and G_6 by substituting ethanolic 1 *M* *n*-amylamine for the same quantity of benzylamine.

Polymers G_{11} and G_{12} were prepared as previously described for G_9 and G_{10} by substituting aqueous 1 *M* *as-N,N*-dimethylethylenediamine for the same quantity of ethanolamine. The intrinsic viscosities of G_9–G_{12} (in ethanol) ranged from 0.22 to 0.26 dl/g.

Polymers G_{13} and G_{14} were prepared according to Danusso, *et al.*[13]

Polymer G_{15} was prepared according to Tamikado, *et al.*[14]

Polymer G_{16} was prepared according to Ferruti and Marchisio.[15]

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N-Hydroxylation of *p*-Acetophenetidide as a Factor in Nephrotoxicity

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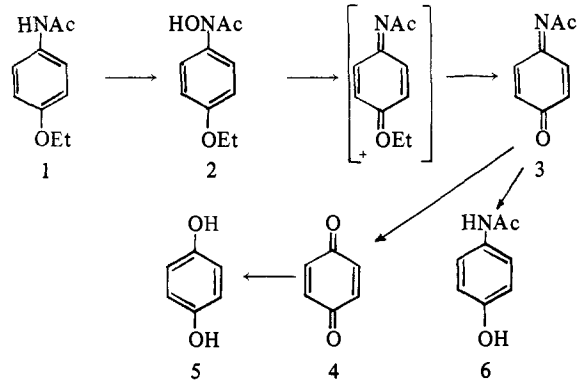
N-Hydroxy-*p*-acetophenetidide (**2**) has been synthesized by acetylation of *N*-hydroxy-*p*-phenetidine with 1 equiv of ketene. Also *N*-acetyl-*p*-benzoquinoneimine (**3**) was prepared and characterized as the cyclopentadiene adduct **13**. Both **2** and **3** give *p*-benzoquinone on hydrolysis. Intravenous injection of *N*-hydroxy-*p*-acetophenetidide, *p*-benzoquinone, and hydroquinone into rats has shown these compounds to be nephrotoxic. This study has implicated N-hydroxylation as a potentially nephrotoxic pathway of *p*-acetophenetidide metabolism.

The association between renal damage and excessive consumption of compound analgesic preparations is widely reported[1] but the underlying chemical factors are still unknown and even the nature of the renal lesion not precisely determined.[2-4] A study[2] of the acute nephrotoxicity of *p*-acetophenetidide (phenacetin) derivatives showed that a

number of compounds closely related to *p*-acetophenetidide (**1**) produce necrosis of proximal convoluted tubules in the rat, but the major urinary metabolite of **1**, 4-hydroxyacetanalide (**6**), is not nephrotoxic.[2] However, some of the more recently recognized[5] minor urinary metabolites of **1** have not been investigated and it is among these that the factor(s) responsible for the nephrotoxicity of the drug may be found.

N-Hydroxylation of free and *N*-acyl aromatic amines is now a well-recognized[6] metabolic pathway, and N-hydroxylation of *p*-acetophenetidide has been suggested[5] as the route to such minor urinary metabolites as hydroquinone and acetamide *via* *N*-acetyl-*p*-benzoquinoneimine (**3**) and *p*-benzoquinone (**4**) (Scheme I). Accordingly, we report the results

Scheme I

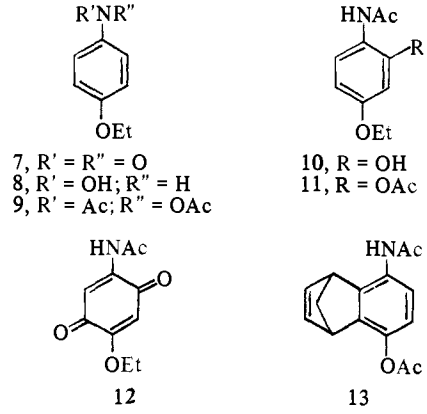


of nephrotoxicity studies on *N*-hydroxy-*p*-acetophenetidide (**2**) and compounds derived therefrom and some chemical reactions of these compounds which may help to elucidate the mechanism of the nephrotoxic action of **1**.

Chemical. Previously, references[5,7,8] have been made to *N*-hydroxy-*p*-acetophenetidide (**2**) but details of a successful synthesis have not yet been reported.† Reduction of 4-nitrophenetole (**7**) according to the procedure of Rising[10] gave *N*-hydroxy-*p*-phenetidine (**8**). However, reaction of **8** with either acetyl chloride[11] or acetic anhydride[7] gave no useful amounts of **2**. The hydroxylamine **8** was cleanly monoacetylated by treatment with 1 equiv of ketene and the resulting *N*-hydroxy-*p*-acetophenetidide was obtained as a stable, highly crystalline compound, soluble in dilute base. Reaction of an excess of ketene with **8** gave[9] a mixture of 1-acetyl-5-ethoxyoxindole and the *N,O*-diacetate **9**. All attempts to isolate **9** gave a rearranged product, 2′-acetoxy-*p*-acetophenetidide (**11**) as a result of the well-precedented[12] migration of the acyloxy group. An independent synthesis of **9** was realized by treating **2** with acetic anhydride according to the procedure of Nery.[13] This smoothly formed the *N,O*-diacetate **9** which was identical by tlc with the product obtained from an excess of ketene on **8**. However, following the isolation procedure of Nery,[13] the product rearranged to **11**, mp 130°.

As an alternative to the above synthesis of **2**, direct oxidation of *p*-acetophenetidide with *m*-chloroperbenzoic acid was attempted, but no detectable amount of *N*-hydroxy-*p*-acetophenetidide was obtained. The products isolated suggest that N-hydroxylation may have been the initial step; with 1 equiv of peroxy acid the products consisted of small quantities of phenolic material, mainly 4-hydroxyacetanilide (**6**) and 2′-hydroxy-*p*-acetophenetidide (**10**). The major product, a yellow neutral compound, was isolated by preparative tlc and identified spectrally as 2-acetamido-5-ethoxy-1,4-benzoquinone (**12**), which presumably arose from further oxidation of **10**. The quinone **12** was found to be identical with a product from the $Pb(OAc)_4$ oxidation of *p*-phenetidine to which structure **12** had been tentatively assigned.[14] Oxidation of **10** with Fremy's salt provided an unambiguous synthesis of **12** (Chart I).

Chart I



p-Benzoquinoneimine has been previously prepared[15] but the *N*-acetyl derivative **3** was unknown. Oxidation of 4-hydroxyacetanilide with $Pb(OAc)_4$[16] in CH_2Cl_2 gave **3** as a highly reactive compound which was not isolated; Diels-Alder reaction of **3** with cyclopentadiene followed by acetylation facilitated characterization as the crystalline monoadduct **13**.

To establish the reactions in Scheme I, hydrolyses of *N*-hydroxy-*p*-acetophenetidide (**2**) and *N*-acetyl-*p*-benzoquinoneimine (**3**) were investigated. Treatment of **2** with dilute aqueous acid under a variety of conditions led to decomposition of the product without formation of any detectable quantities of *p*-benzoquinone. However, by passing steam through a suspension of **2** and then adding dilute perchloric acid, the steam volatile *p*-benzoquinone was removed from the acid media as it was formed and characterized in the distillate; the distillation residue was found to contain both **5** and **6**.

Similarly, if *N*-acetyl-*p*-benzoquinoneimine (**3**) is an intermediate formed from **2** as shown in Scheme I, then it should be possible to convert **3** to *p*-benzoquinone *in vitro*. Treatment of the CH_2Cl_2 solution of **3** with water gave mainly polymeric material but *p*-benzoquinone could be isolated by steam distillation. In the absence of water no *p*-benzoquinone was detected.

The final step in Scheme I, the reduction of *p*-benzoquinone to hydroquinone, has ample precedence both chemically and biologically.[17]

Biological. Compounds **2**, **4**, **5**, and **10** were administered to female hooded Wistar rats as single intravenous injections in aqueous solution, if necessary as the sodium salt, or as an alcohol–water–Tween 80 solution. The renal lesion was necrosis of proximal convoluted tubules and its severity was assessed 48 hr after intravenous injection and graded histologically,[2] grade 4 denoting the most severe lesion.

When administered to rats as single intravenous injections (Table I) *N*-hydroxy-*p*-acetophenetidide was nephrotoxic to 3 of 13 animals and *p*-benzoquinone and hydroquinone to all animals receiving the compounds. In contrast, 2′-hydroxy-*p*-acetophenetidide (**10**) was not nephrotoxic.

†Klutch, *et al.*,[7,8] in a study of the urinary metabolites of *p*-acetophenetidide in cats, dogs, and humans, identified **2** by comparison with a sample supplied by H. C. White. The synthesis of **2** described here has been the subject of a preliminary communication.[9]

Table I. Histological Grade of Renal Tubular Necrosis Caused by Intravenous Injection of *p*-Acetophenetidide Metabolites in Rats

Metabolite and dose level	Number of animals showing necrosis				
	Grade 4	Grade 3	Grade 2	Grade 1	No necrosis
N-Hydroxy-*p*-acetophenetidide (**2**), 1.0 m*M*/kg			3		10
p-Benzquinone (**4**) 0.5 m*M*/kg			1	4	0
1.0 m*M*/kg		1	2		0
Hydroquinone (**5**), 1.8 m*M*/kg		4	1		0
2'-Hydroxy-*p*-acetophenetidide (**10**), 1.1 m*M*/kg					10

Discussion

Administration to rats of *N*-hydroxy-*p*-acetophenetidide and its proposed metabolites, *p*-benzoquinone and hydroquinone, indicates that all are nephrotoxic. 2-Hydroxy-*p*-phenetidine is also nephrotoxic[2] producing grade 1 lesions in similar conditions. These are the only known metabolites of *p*-acetophenetidide which have been shown to cause renal damage; 2'-hydroxy-*p*-acetophenetidide and the major metabolite 4-hydroxyacetanilide[2] are not nephrotoxic. The partial nephrotoxicity of *N*-hydroxy-*p*-acetophenetidide is consistent with its role as precursor of the more nephrotoxic *p*-benzoquinone and hydroquinone.

Acid hydrolysis of **2** *in vitro* provides supportive evidence for its role as precursor of *p*-benzoquinone and hydroquinone. The route of the acid hydrolysis is *via* quinoneimine (**3**) which has also been prepared and independently hydrolyzed to *p*-benzoquinone. The intermediacy of quinoneimine (**3**) would also account for the presence of 4-hydroxyacetanilide in the acid hydrolysate of **2**.

N-Hydroxylation generally leads to intermediates of high chemical reactivity which are often more toxic than the parent amine or amide.[18] In this case it has been shown that N-hydroxylation of *p*-acetophenetidide establishes a rational chemical pathway to *p*-benzoquinone and hydroquinone, both of which are acutely nephrotoxic in rats. Compounds such as *p*-benzoquinone or hydroquinone may be the common nephrotoxic factors for a wide variety of *p*-aminophenol derivatives with demonstrable nephrotoxic properties.

Experimental Section

Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained were within ±0.4% of the theoretical values and were obtained from the Australian Microanalytical Service. The nmr spectra were obtained on a Varian HA-100 spectrometer (TMS, $CDCl_3$ as solvent). Ir spectra were determined as KBr disks on a Perkin-Elmer 457 spectrophotometer. Uv determinations were made on a Unicam SP800 ultraviolet spectrophotometer. For glc–mass spectrometry a Perkin-Elmer 270B unit was used with a 6-ft, OV17 or OV101, column. Phenolic TMS derivatives were prepared with bis(trimethylsilyl)acetamide. Tlc was carried out on Merck silica gel GF, 0.25 mm for analytical plates and 1 mm for preparative plates; where solvent mixtures are given the proportions are by volume.

N-Hydroxy-*p*-acetophenetidide (**2**). To a vigorously stirred solution of 4-nitrophenetole (16.5 g, 99 m*M*) in EtOH (250 ml) and 4% aqueous NH_4Cl solution (50 ml) was added pure Zn dust (13.0 g, 0.2 g-atom). The reaction mixture was maintained at 65° for 3.0 min, then cooled in an ice bath, and suction filtered. Dilution of the filtrate with twice its volume of saturated NaCl solution precipitated the product which was extracted into Et_2O (4 × 100 ml). After washing the Et_2O extract with H_2O and saturated NaCl solution, it was treated with ketene gas (30 m*M*) and then extracted with 10% NaOH solution (five times). The basic solution was neutralized with dilute H_2SO_4 at 5° and then extracted with CH_2Cl_2 (5 × 50 ml); this extract was washed (H_2O), dried ($MgSO_4$), and evaporated to give the crude crystalline product (9.1 g). A single recrystallization from C_6H_6–petroleum ether (bp 40–60°) gave pure **2**: 6.4 g (33%); mp 104°; ir λ max 3.22 (OH) and 6.19 μ (C=O); nmr δ 1.4 (t, *J* = 7 Hz, 3 H, ethoxylmethyl), 2.0 (s, 3 H, *N*-acetyl), 4.0 (q, *J* = 7 Hz, 2 H, ethoxylmethylene), 7.1 (q, 4 H, aromatic), 8.5 (br s, 1 H, OH). *Anal.* ($C_{10}H_{13}NO_3$) C, H, N. The residue in the Et_2O solution after base extraction consisted of unreacted **7** contaminated with a little 4,4'-diethoxyazoxybenzene.

Excess Ketene on **2**. *N*-Hydroxy-*p*-acetophenetidide (**2**) (500 mg, 2.5 m*M*) in Et_2O–$CHCl_3$ (25 ml, 1:1) was treated with ketene gas (30 m*M*) and the solution then evaporated *in vacuo*. Preparative tlc of the residue separated the two products of the reaction (solvent $CHCl_3$), 5-ethoxy-1-acetyloxindole[9] (R_f 0.7) and 2'-acetoxy-*p*-acetophenetidide (**11**, R_f 0.15). The latter was characterized by comparison with authentic material,[19] nmr, tlc, mp 130°, and mixture melting point.

Oxidation of **1** with *m*-Chloroperbenzoic Acid. A solution of **1** (2.0 g, 11.2 m*M*) and *m*-chloroperbenzoic acid (2.0 g, 11.6 m*M*) in $CHCl_3$ (125 ml) was refluxed for 5 hr. After concentrating (25 ml) the reaction mixture was separated by preparative tlc (solvent EtOH–$CHCl_3$, 5:95) into three bands: (a) lower R_f, a mixture of **10** (5 mg), **6** (10 mg), and **5** (5 mg) identified by glc–mass spectrometry of TMS derivatives; (b) middle R_f, **1**, (1.25 g); (c) high R_f, 2-acetamido-5-ethoxy-1,4-benzoquinone (**12**) (345 mg, 15%). Crystallization of **12** from EtOH gave yellow needles: mp 214–216°; ir λ max 2.95 (NH), 5.90 (C=O), 6.12 (C=O), and 6.32 μ; uv max (ε) 393 nm (4.6 × 10^2) and 297 (2.3 × 10^4); nmr δ 1.6 (t, *J* = 7 Hz, 3 H, ethoxylmethyl), 2.4 (s, 3 H, *N*-acetyl), 4.2 (q, *J* = 7 Hz, 2 H, ethoxylmethylene), 6.0 (s, 1 H) and 7.65 (s, 1 H) (quinone), and 8.3 (br, 1 H, NH). *Anal.* ($C_{10}H_{11}NO_4$) C, H, N.

2-Acetamido-5-ethoxy-1,4-benzoquinone (**12**). A. *p*-Phenetidine was oxidized with $Pb(OAc)_4$ according to the procedure of Pausacker and Scroggie[14] to yield **12**, mp 214–216°. The product was best purified by chromatography on silica gel.

B. A solution of Fremy's salt[20] (550 mg, 2 m*M*) in H_2O (25 ml) buffered with NaOAc solution (1 ml, 1 *N*) was added dropwise to a stirred solution of **10**[21] (98 mg, 0.5 m*M*) in MeOH (10 ml). After 2 hr the yellow solution was extracted with Et_2O (5 × 50 ml), and the extracts were dried ($MgSO_4$) and evaporated *in vacuo*. The crude quinone was purified by preparative tlc ($CHCl_3$–EtOH, 95:5) to yield **12** (95 mg, 91%), mp 214–216°.

Acid Hydrolysis of **2**. *N*-Hydroxy-*p*-acetophenetidide (**2**) (1.0 g, 5.1 m*M*) suspended in H_2O (2 ml) was steam distilled for 1 min. $HClO_4$ solution (1 ml of 7%) was then added to the distillation flask and the distillation continued for a further 10 min. Examination of the distillate in the uv at 256 nm showed it to contain 40 mg of *p*-benzoquinone (7% conversion). The distillate was extracted with Et_2O (3 × 20 ml) and tlc of the extract showed *p*-benzoquinone to be the major component (solvent $CHCl_3$, R_f 0.5). Treatment of the ether extract with $NaBH_4$ reduced the *p*-benzoquinone to hydroquinone and this was confirmed by comparison with an authentic sample by tlc (solvent EtOH–$CHCl_3$, 7:93, R_f 0.1) and glc–mass spectrometry of the bis TMS derivative $C_{12}H_{22}O_2Si_2$, *m/e* 254.

The acidic distillation residue was extracted with CH_2Cl_2 (100 ml) and Et_2O (2 × 100 ml); the combined extracts were dried ($MgSO_4$) and evaporated. Preparative tlc (solvent $CHCl_3$–EtOH, 90:10) yielded **2** (370 mg), **6** (65 mg), and **5** (20 mg).

N-Acetyl-*p*-benzoquinoneimine Diels–Alder Adduct (**13**). 4-Hydroxyacetanilide (2 g, 13.2 m*M*) and $Pb(OAc)_4$ (4 g, 9 m*M*) were stirred in CH_2Cl_2 (50 ml) at −10°. After 10 min ethylene glycol (0.5 ml) was added and the mixture was stirred for a further 5 min and then filtered. Freshly distilled cyclopentadiene (10 ml, 120 m*M*) was added to the filtrate and the solution kept at −62° for 24 hr. The reaction mixture was extracted with NaOH (10%, 5 × 50 ml). The combined extracts were acidified with concentrated H_2SO_4 and extracted with Et_2O (5 × 50 ml) and CH_2Cl_2 (5 × 50 ml). The organic extract was dried ($MgSO_4$), evaporated to 5 ml, and separated by preparative tlc (solvent MeOH–$CHCl_3$, 5:95) into three bands. The major band (R_f 0.4) was eluted with MeOH to give an oil (200 mg 7%) which was acetylated with Ac_2O (2 ml) and pyridine (0.5 ml) overnight. Evaporation *in vacuo* and sublimation (150°, 10^{-3} mm) yielded **13** as a glass which crystallized from petroleum ether (80–100°): mp 146–148°; ir λ max 3.08 (NH), 5.65 (C=O), and 6.00 μ (C=O); nmr δ 2.13 (m, 5 H), 2.3 (s, 3 H), 3.9 (m, 2 H), 6.6 and 7.05 (AB q, *J* = 9 Hz, 2 H), 6.8 (m, 2 H), and 7.8 (b, 1 H). *Anal.* ($C_{15}H_{15}NO_3$) C, H, N.

Hydrolysis of *N*-Acetyl-*p*-benzoquinoneimine. 4-Hydroxyacet-

anilide (1 g, 6.6 m*M*) and $Pb(OAc)_4$ (2 g, 4.5 m*M*) were stirred together in CH_2Cl_2 (50 ml) for 10 min. The reaction mixture was then filtered and steam distilled. The distillate was extracted with CH_2Cl_2 to give *p*-benzoquinone [90 mg, 24% conversion based on $Pb(OAc)_4$] characterized by comparison with authentic material, tlc, uv, mp 113–114°, and mixture melting point.

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Metabolic Conversion of Benzo[*a*]pyrene by Syrian Hamster Liver Microsomes and Binding of Metabolites to Deoxyribonucleic Acid

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Analysis of metabolites of generally tritiated benzo[*a*]pyrene (^{3}H-BaP) produced by a Syrian hamster liver microsomal system has revealed the presence of a number of dihydrodihydroxy derivatives of BaP including 4,5-dihydroxy-4,5-dihydrobenzo[*a*]pyrene. Detection of this metabolite is taken as evidence that the K-region 4,5-double bond is acted upon by a microsomal hydroxylase to form the 4,5-epoxide of BaP which is subsequently converted *via* epoxide hydrase to the dihydrodiol. Incubation of several ^{3}H-BaP metabolites with DNA alone gave little evidence for spontaneous covalent binding. However, when hamster liver microsomes were present, a metabolite recently identified as 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene was found to covalently bind to DNA to a tenfold greater extent than BaP itself, suggesting that this compound may be an intermediate in the pathway leading to binding of BaP to DNA *in vivo*.

Aromatic hydrocarbons including the carcinogenic polycyclic aromatic hydrocarbons (PAH) are metabolized *in vivo* and *in vitro* by inducible enzymes which in general lead to the formation of more polar compounds such as phenols and dihydrodiols.[1] It is well established that these hydroxylated derivatives arise from unstable intermediate epoxides (arene oxides) which can undergo conjugation with glutathione, enzymatic hydration to form dihydrodiols, or rearrangement to yield phenolic products.[2] Additionally, it has been established that metabolic activation of aromatic systems by these microsomal enzymes results in their covalent linking to cellular macromolecules *in vivo*[3,4] and *in vitro*.[5-7]

The extensive studies of Sims and coworkers[8-12] on the metabolic conversion of PAH in cell-free rodent liver preparations have provided considerable experimental evidence in support of Boyland's proposal[13] that epoxidation is the key metabolic event leading to the binding of PAH to biologically important macromolecules. Furthermore, several authors have suggested that the macromolecular perturbations resulting from such binding may be responsible for the toxic,[14] mutagenic, and carcinogenic[15-17] properties of aromatic systems. Selkirk, *et al.*,[18] and Grover, *et al.*,[11] have shown that epoxides are formed during the metabolism of some carcinogenic PAH. Certain of these epoxides have been shown to transform rodent cells *in vitro*.[17]

In order to better understand the role metabolism may play in the carcinogenic properties of benzo[*a*]pyrene (BaP, **1**) many *in vitro*[8-12] and *in vivo*[19] studies of the metabolic fate of this substance have been undertaken. Several oxygenated metabolites have been completely or partially identified including 3-hydroxybenzo[*a*]pyrene (**2**) and two dihydrodiol derivatives, the 7,8 compound **3** and the 9,10 compound **4**.[9,20] In an effort to further characterize the biotransformation of BaP, we have examined its metabolic fate in liver microsome preparations obtained from Syrian hamsters, a species known to be susceptible to the carcinogenic effects of BaP.[21]

With the aid of mass spectral and uv analyses, we have identified as metabolites the phenolic compound **2** and three dihydrodiols, two of which have uv characteristics reported for the 7,8 and 9,10 derivatives **3** and **4**, respectively.[20] The third dihydrodiol has been shown to be the K-region derivative, *trans*-4,5-dihydroxy-4,5-dihydrobenzo[*a*]pyrene (**5a**) by comparative tlc, uv, and mass spectrum and presumably is derived from the corresponding epoxide **6**.[2] Additionally, we have examined the covalent binding potential to DNA of the several BaP metabolites isolated in this study, both in the presence and absence of the microsomal system. The results of this study provide evidence that 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (**3**) is further metabolized to an active alkylating agent since, in the presence of