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*.

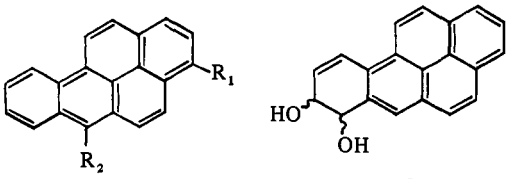
Aromatic hydrocarbons including the carcinogenic polycyclic aromatic hydrocarbons (PAH) are metabolized *in vivo* and *in vitro* by inducible enzymes which in general lead to the formation of more polar compounds such as phenols and dihydrodiols.[1] It is well established that these hydroxylated derivatives 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]

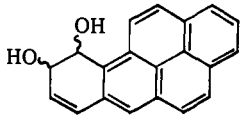
With the aid of mass spectral and uv analyses, we have identified as metabolites the phenolic compound **2** and three dihydrodiols, two of which have uv characteristics reported for the 7,8 and 9,10 derivatives **3** and **4**, respectively.[20] The third dihydrodiol has been shown to be the K-region 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

the complete system, this compound binds to DNA ten times more effectively than the parent hydrocarbon **1**.



1, $R_1 = R_2 = H$
2, $R_1 = OH$; $R_2 = H$
9, $R_1 = H$; $R_2 = OH$

3



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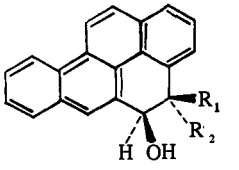
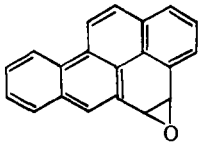


5a, $R_1 = H$; $R_2 = OH$
b, $R_1 = OH$; $R_2 = H$



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Experimental Section

Equipment. Radioactivity scans of tlc plates were performed on a Varian-Berthold scanner; uv spectra were recorded in absolute EtOH with a Cary Model 15 spectrophotometer; mass spectral analyses were obtained at 70 eV on an AEI MS 902 using the direct inlet probe at 225°. Isopycnic banding of DNA preparations in CsCl was done with a Spinco Model L using a 50 Ti rotor; absorbance measurements of fractionated CsCl gradients were made with a Beckman DU spectrophotometer; refractive indices of CsCl solutions were measured with a Bausch and Lomb Abbe-3L refractometer. Liquid scintillation counting was done with a Series 3000 Packard TriCarb, using a solution containing 4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-2-(5-phenyloxazolyl)benzene per liter of toluene.

Materials. Tritium-labeled BaP (13 Ci/mmol) was obtained from Amersham/Searle Corp. and was purified immediately prior to use by tlc on silica gel (Eastman Chromagram 6061) with C_6H_6 as solvent. The fluorescent band corresponding to BaP was eluted with absolute EtOH or Me_2CO. Upon rechromatography with C_6H_6 or C_6H_6-EtOH (9:1) a radioscan showed that the radioactivity corresponded to a single fluorescent spot. The uv spectrum in absolute EtOH of the purified BaP was identical with the reported spectrum.[22] Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was obtained from Sigma Chemical Co. RNase was from Worthington. Solvents used were spectral grade. Nonradioactive derivatives of BaP for use as chromatography standards were generously provided by Dr. H. Gelboin [3-hydroxybenzo[*a*]pyrene (**2**)] and Dr. P. Sims [(±)-*trans*-4,5-dihydroxy-4,5-dihydrobenzo[*a*]pyrene (**5a**) and benzo[*a*]pyrene-3,6-quinone (**7**)]. *rac-cis*-4,5-Dihydroxy-4,5-dihydrobenzo[*a*]pyrene (**5b**) was prepared by OsO_4 oxidation of BaP.[23]

Microsomal Incubation Procedure. Livers were removed from 3-month-old male Syrian hamsters weighing approximately 100 g each and washed with cold 1.15% KCl. Fractional centrifugation of the homogenate derived from this tissue by a previously described procedure[24] gave a microsomal pellet which was suspended in a volume of TKM-sucrose solution (0.05 *M* tris buffer, 0.02 *M* KCl, 0.005 *M* $MgCl_2$, and 0.25 *M* sucrose, pH 7.4) equal to the original weight of liver. For production of BaP metabolites the incubation system contained, per milliliter of SSC (0.14 *M* NaCl, 0.015 *M* Na citrate, pH 7.4), 4 nmol of ^{3}H-BaP, 1.2 μmol of NADPH, and 1 mg of microsomal protein (determined by the Lowry method[25]). In a typical large-scale preparation ten 30-ml incubation volumes containing the above proportions of ingredients were incubated with shaking at 37° for 30 min.

Isolation Procedure for BaP Metabolites. At the end of the incubation period, the mixtures were combined and extracted with a mixture of 3 vol of hexane and 1 vol of Me_2CO, followed by a second extraction with 2 vol of EtOAc. The organic extracts were pooled, dried over Na_2SO_4, and taken to dryness under vacuum. The residue was extracted with a minimum volume of EtOAc and the soluble material was applied as a narrow band on a silica gel tlc plate. After ascending development in C_6H_6-EtOH (19:1) for approximately 15 cm, a narrow vertical strip of the plate was examined by uv light (254 and 365 nm) and by radioscanning. The various metabolites as indicated by the positions of fluorescent and radioactive bands were eluted separately with Me_2CO; the solutions were concentrated with a stream of dry N_2 and the individual metabolites rechromatographed twice more using the same system. The materials thus obtained appeared homogeneous on tlc using C_6H_6 or C_6H_6-EtOH (19:1) as solvents and were used for characterization of the metabolites and for binding studies.

Preparation of DNA and Binding Studies. DNA was prepared from cultured hamster embryo cells[26] by a modification of the Marmur technique.[27] Cell suspensions of 10^7 cells/ml (by hemocytometer count) were prepared by trypsinization of confluent monolayer cultures and lysed with 1% sodium dodecyl sulfate (total volume 3–4 ml). RNase (heated at 80° for 10 min to destroy DNase) was added to a concentration of 50 μg/ml and the mixture was incubated without shaking for 30 min at 37°. After addition of $NaClO_4$ to a concentration of 1 *M*, the mixture was shaken vigorously for 1 min with an equal volume of $CHCl_3$-isoamyl alcohol (24:1) and centrifuged (1500g, 15 min) to remove protein. This procedure was repeated three times. The final aqueous supernatant containing the DNA was dialysed in cellulose tubing against 100 vol of SSC for at least 24 hr. The concentration of DNA (approximately 1 mg/ml) was estimated by absorbance measurements at 260 nm.

Tests for binding of BaP and its metabolites to DNA were carried out in a total volume of 1.2 ml of SSC containing 200 μg of DNA. When microsomes were used, the mixture also contained 1.2 μmol of NADPH and 1 mg of microsomal protein. After 30 min of incubation, the DNA was reisolated from the mixture by precipitation of the protein with $CHCl_3$-isoamyl alcohol, separation and dialysis of the aqueous phase containing the DNA, as described above, and isopycnic banding of the DNA in CsCl (initial density 1.70 g/cm^3). After 48 hr of centrifugation at 40,000 rpm at 20°, the resulting CsCl density gradients were fractionated by puncturing the bottoms of the centrifuge tubes and collecting fractions dropwise. Absorbance measurements at 260 nm were made on undiluted fractions. Density was measured by comparing the refractive index of selected fractions with that of standard CsCl solutions. Radioactivity was measured by spotting an aliquot of each fraction on Whatman No. 3 paper, drying, and washing sequentially with cold 5% trichloroacetic acid, EtOH, and Me_2CO. After drying, the filter papers were immersed in scintillation solution and counted. DNA-containing fractions were pooled and dialysed against 100 vol of 1/10 diluted SSC prior to further analysis. In order to denature the DNA, the resulting solution was treated with 10% aqueous HCHO to yield a final concentration of 1% HCHO and then heated to 100° for 10 min.[28] After rapid cooling, the DNA samples were then centrifuged a second time in CsCl (initial density 1.75 g/cm^3).

Results and Discussion

Figure 1 is an example of a radioscan of ^{3}H-BaP metabolites indicating the relative positions of the various metabolites. The yields from different preparations were qualitatively similar, but quantitative variations of metabolite production and the presence of minor components were

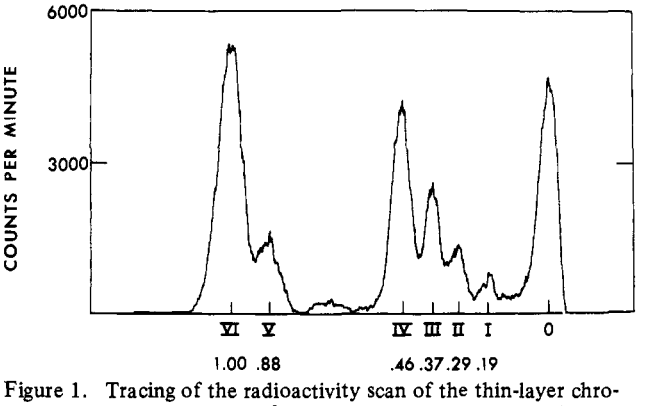


Figure 1. Tracing of the radioactivity scan of the thin-layer chromatogram of metabolites of ^{3}BaP. Migration values are relative to BaP at 1.00. Solvent was C_6H_6-EtOH (19:1), ascending 15 cm.

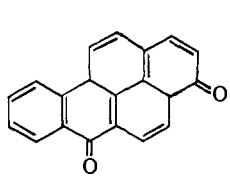
Table I. Metabolites of BaP Produced by Syrian Hamster Liver Microsomes

Compound	No.	Migration on tlc[a]	Band no.	Fluorescence at 254 nm	Formula	Mass spectra, m/e Calcd	Found	Comment
9,10-Dihydroxy-9,10-dihydrobenzo[a]pyrene	4	0.39	I	Violet	$C_{20}H_{14}O_2$	286.09937	286.09839	
7,8-Dihydroxy-7,8-dihydrobenzo[a]pyrene	3	0.43	II	Blue green	$C_{20}H_{14}O_2$	286.09937	286.08761	
trans-4,5-Dihydroxy-4,5-dihydrobenzo[a]pyrene	5a	0.46	III	Violet	$C_{20}H_{14}O_2$	286.09937	286.09998	Cochromatographs with std (±)-*trans*-4,5-diol
3-Hydroxybenzo[a]pyrene	3	0.79	IV	Blue	$C_{20}H_{12}O$	268.09998	268.08814	Cochromatographs with std, rapidly decompose in air to red-color substance chromatographing as quinone
Benzo[a]pyrene-3,6-quinone	7	0.87	V	Red	$C_{20}H_{10}O_2$	282.06837	282.06810	Cochromatographs with std
BaP	1	1.00	VI	Blue	$C_{20}H_{12}$	252.09390	252.09381	

[a] Tlc system: silica gel Analtech HR (0.25 mm) with benzene–ethanol (9:1) as solvent. Migration values are reported relative to BaP = 1.00.

observed. Except for what may be a decomposition product of the material found in band IV, these will not be dealt with in the present communication. No attempt has been made at present to obtain quantitative data regarding these metabolites. When boiled microsomes (5 min at 100°) were employed, only unchanged BaP was detected on tlc, confirming the enzymatic origins of the metabolites described.

The uv spectra of the purified materials isolated from bands I–VI were obtained and, in the case of the isolates corresponding to bands IV and VI, spectral comparisons with authentic samples established their identities as 3-hydroxybenzo[a]pyrene (**2**) and benzo[a]pyrene (**1**), respectively. Comparative tlc R_f values and high-resolution mass spectral analyses (Table I) confirmed these assignments. As previously reported,[9] the 3-hydroxybenzo[a]pyrene was unstable and after exposure to air and moisture showed upon rechromatography of the original fluorescent 3-hydroxybenzo[a]pyrene band, a red-colored substance which gave on high-resolution mass spectral analysis a parent ion corresponding to a benzpyrenequinone (see Table I). The uv-visible absorption spectrum of this substance corresponded to that obtained with authentic benzo[a]pyrene-3,6-quinone (**7**). Upon tlc of some incubation extracts, this red quinone was detected together with a yellow-colored band, presumably the 1,6-quinone, compound **8**.[9] These materials are present in band V (Figure 1) but were not resolved in this tlc system. It is not clear if these quinones were strictly artifacts resulting from atmospheric oxidation of phenolic substances or if they were actually produced enzymatically during the incubation.



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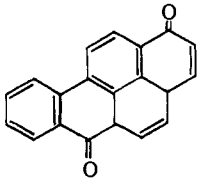

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The high-resolution mass spectra of the materials isolated from the close-running tlc bands I, II, and III (Figure 1) established that they were isomeric with molecular formulas corresponding to dihydrodiol derivatives of BaP (Table I). The uv spectra of the compounds isolated from bands I and II corresponded to the spectra reported by Waterfall and Sims[20] for compounds identified as 9,10-hydroxy-9,10-dihydrobenzo[a]pyrene (**4**) and 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (**3**), respectively. The compound obtained from band III was a substance not previously characterized. Synthetic (±)-*trans*-4,5-dihydroxy-4,5-dihydrobenzo[a]pyrene (**5a**) cochromatographed with this material. Furthermore, the uv spectra of the synthetic 4,5-dihydrodiol and the unknown compound both before and after boiling for 5 min in glacial acetic acid containing a few drops of concentrated HCl were superposable. Synthetic (±)-*cis*-4,5-dihydroxy-4,5-dihydrobenzo[a]pyrene (**5b**) displayed the same uv characteristics as the metabolite but did not cochromatograph with band III (C_6H_6–EtOH, 9:1). On the basis of these data we concluded that the structure of this metabolite was the *trans*-dihydrodiol **5a**. The trans stereochemistry of metabolically produced aromatic dihydrodiols was first observed by Boyland and coworkers[13] and has also been observed in the case of 1,2-dihydroxy-1,2-dihydronaphthalene.[2]

The spectrum of BaP metabolites produced by the hamster liver preparation resembled that previously reported by Sims[9,29] as being produced by rat liver homogenates and mouse cell cultures with the important exception that we have also detected the K-region dihydrodiol **5a**. An association between the presence in PAH of regions of high-electron density ("K regions") and carcinogenic activity was postulated some years ago.[30] The recent report by Marquardt, *et al.*,[31] that the K-region epoxides of benzanthracene transform cells more efficiently than the non-K-region epoxides tested provides a metabolic interpretation of this postulate. We have also been able to detect compound **5a** as a metabolite produced by rat liver microsomes.† However, it was present in much smaller quantities compared to the hamster preparation which may explain the inability of earlier workers to detect it.‡

The overwhelming evidence suggests that dihydrodiol metabolites of aromatic hydrocarbons are formed by hydration of the corresponding arene oxides.[2,34] The data obtained in these studies predict the formation of at least three arene oxides, the 4,5-, 7,8-, and 9,10-epoxides of BaP. The characterization of the 4,5-dihydrodiol **5a** (and by implication the 4,5-epoxide **6**) is of significance since, based on metabolic studies reported on related PAH,[2,12,18,20,34] this K-region double bond would be expected to readily undergo microsomal oxidation. Whether or not K-region

†I. Y. Wang, R. E. Rasmussen, and T. T. Crocker, unpublished data.

‡While this manuscript was in preparation, Grover, *et al.*,[32] informed the authors that they now have evidence for oxidation of the 4,5 double bond by rat liver microsomes. An earlier report[33] gave indirect evidence for the *in vivo* oxidation of the 4,5 double bond of BaP in the mouse.

Table II. Binding of Metabolites of ^{3}H-BaP to DNA *in Vitro* with and without Syrian Hamster Liver Microsomes[a]

Compound	No.	Amt present in incubation mixture, μCi (Ci/mmol)	DNA samples			
			With microsomes		Without microsomes	
			Native	Denatured	Native	Denatured
BaP	1	15 (13)	11.4	13.9	0.6	1.6
7,8-Dihydroxy-7,8-dihydrobenzo[a]pyrene	3	15	168.4	168.0	2.8	3.9
trans-4,5-Dihydroxy-4,5-dihydrobenzo[a]pyrene	5a	85	5.1	6.8	2.1	3.0
9,10-Dihydroxy-9,10-dihydrobenzo[a]pyrene	4	4.2	3.2	4.0	0.3	1.3
3-Hydroxybenzo[a]pyrene	2	50	17.7	24.2	6.8	7.8

[a] Incubation conditions and analyses were as described in the text. Values are counts per minute per microgram of DNA, based on that DNA-containing CsCl gradient fraction having the highest absorbance value.

oxidation of PAH is specifically linked to carcinogenicity is still under debate.[16,18,31]

The ^{3}H-labeled metabolites of BaP produced and isolated as described were incubated with DNA prepared from cultured hamster embryo cells with and without the complete microsomal system. Table II shows that in the presence of microsomes the amount of [^{3}H]-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (**3**) bound to DNA was more than ten times greater than the amount of ^{3}H-BaP bound under similar conditions. Since the metabolites were prepared from the same lot of ^{3}H-BaP as that used in the present experiment, it was assumed that the [^{3}H]-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene had essentially the same specific radioactivity as the starting ^{3}H-BaP. If anything, one would have expected the specific radioactivity to have been lower than that of ^{3}H-BaP because of ^{3}H loss during the incubation due to metabolic processes.[35]

Tests of the binding of other ^{3}H-labeled metabolites were also done, but since the concentrations of metabolites in the incubation mixtures differed from that of BaP, quantitative comparisons are of limited value. Nevertheless, it seems clear that none of the other metabolites were activated by the microsomal preparation to bind to DNA to a greater extent than was ^{3}H-BaP (Table II).

The specific radioactivities of the DNA samples changed little when the DNA was denatured (Table II) providing further evidence that the radioactivity associated with the DNA was due to covalently linked metabolites of ^{3}H-BaP. The increase in absorbance due to denaturation of the DNA was estimated at approximately 20% and has been taken into account for the specific activity values given.[36]

It is apparent from the binding studies that in addition to epoxides and phenols, other metabolites of BaP formed in hamster liver microsome systems may not necessarily be end products but may be further metabolized to bind to DNA. The present finding that 7,8-dihydroxy-7,8-dihydrobenzo[a]-pyrene (**3**) was active in this regard suggests that this compound may be one intermediate in the pathway taken by BaP in the formation of covalent bonds to DNA.

There is some evidence for spontaneous binding of BaP metabolites in the absence of microsomes. Gelboin[37] has reported the presence of an unknown reactive intermediate which binds to DNA in microsomal incubation mixtures and we have previously confirmed this finding.[24] The present results (Table II) indicate that some spontaneous binding of the metabolite identified as 3-hydroxybenzo[a]pyrene (**2**) may have occurred. Since the available monohydroxy derivatives of BaP show very similar chromatographic behavior in our system and that of others,[9] it is possible that a contaminating metabolite, not detectable by uv, was responsible for the binding to DNA. In fact, evidence for the spontaneous binding of 6-hydroxybenzo[a]pyrene (**9**) to DNA has been reported.[38]

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Stereochemical Analogs of a Muscarinic, Ganglionic Stimulant. *cis*- and *trans*-4-[*N*-(3-Chlorophenyl)carbamoyloxy]-2-butenyltrimethylammonium Iodides[1,†]

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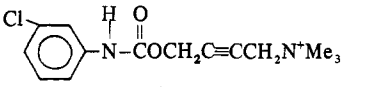
Synthesis and pharmacological properties of *cis*- and *trans*-4-[*N*-(3-chlorophenyl)carbamoyloxy]-2-butenyltrimethylammonium iodides (2 and 3) are reported. The trans compound 3 shows atropine-sensitive ganglion-stimulating properties similar to those previously reported for the acetylenic analog 1. The cis compound 2 shows much less ganglion-stimulating activity than 3. Muscarinic ganglion-stimulating properties of 1 and 3 are interpreted in terms of similar fit at the receptor level of the alkyltrimethylammonium ion, unsaturation at C-2, and the ether oxygen. Both 2 and 3 show certain nonmuscarinic properties of 1.

Receptor categories based on classical autonomic agents are rather accommodating. Thus, for example, the population of β-adrenergic receptors blocked by propranolol may now be subdivided into β_1 and β_2 on the basis of specific agonists and antagonists.[2] Likewise, we have for years differentiated the nicotine-sensitive receptors into ganglionic nicotinic and neuromuscular nicotinic receptors, recognizing that these receptors, although related, are not identical. A similar subdivision of atropine-sensitive or so-called muscarinic receptors may be forthcoming based on work with specific subclasses of muscarinic agonists. The present work represents an attempt to explore one possible avenue toward more specific muscarinic agonists and/or antagonists.

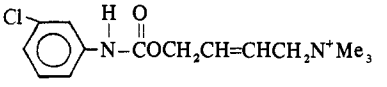
A detailed pharmacological study by Roszkowski[3] of 4-[*N*-(3-chlorophenyl)carbamoyloxy]-2-butynyltrimethylammonium chloride, McN-A-343 (1), showed that the compound possessed unique ganglionic-stimulant properties. Compound 1 excited ganglia, especially sympathetic ganglia, at muscarinic (*i.e.*, atropine-sensitive) sites. Other less potent muscarinic effects noted were vasodilitation and stimulation of intestinal smooth muscle. Direct nicotinic effects at the neuromuscular junction were also observed. More recently, other nonmuscarinic effects of 1 have been noted by other workers. These include antagonism of the amine uptake pump of the sympathetic nerve terminal[4,5] and a local anesthetic effect.[6]

Early work concerning structure-activity relationships in this series showed the olefinic analog to possess only weak pressor effects.[7] Since no stereochemistry was specified, our study began with preparation and testing of the cis and trans olefinic analogs of 1, compounds 2 and 3.

Synthesis. The cis olefinic analog 2 was prepared from 4-[*N*-(3-chlorophenyl)carbamoyloxy]-1-chlorobutyne (barban)‡ by reaction with dimethylamine, quaternization

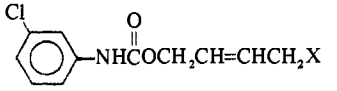


1



2 (cis)
3 (trans)

with iodomethane, and catalytic reduction on quinoline poisoned Pd·$BaSO_4$. Alternatively, *cis*-2-butene-1,4-diol (8) was converted to *cis*-4-chloro-2-buten-1-ol (10), treated with dimethylamine and iodomethane, and followed by esterification with 3-chlorophenyl isocyanate. The instability of the intermediate, *cis*-4-[*N*-(3-chlorophenyl)carbamoyloxy]-1-dimethylamino-2-butene (6), required this sequence of reactions to circumvent its preparation.



cis	trans
4, X = Cl	5, X = Cl
6, X = NMe_2	7, X = NMe_2

$HOCH_2CH{=}CHCH_2X$

cis	trans
8, X = OH	9, X = OH
10, X = Cl	11, X = Cl
12, X = NMe_2	13, X = NMe_2
14, X = $N^+Me_3I^-$	

The trans isomer 3 was prepared from *trans*-2-butene-1,4-diol (9), which is available from 1,3-butadiene by the method of V'yunova.[8] The diol was converted to chloro alcohol 11 and esterified with 3-chlorophenyl isocyanate to afford 5. Displacement with dimethylamine and quaternization completed the sequence.

All nmr data were consistent with the geometric assignment of cis and trans in each series of precursors. The cis compounds show non-first-order multiplets near δ 6.0 for the olefinic protons. The olefinic protons of the trans compounds show similar chemical shift and appear as multiplets resembling quintets. Similar observations were reported by Willette and Driscoll[9] in a series of 4-amino-2-buten-1-ol esters.

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