Solvolysis of the Carcinogen *N*-Acetoxy-*N*-(4-stilbenyl)acetamide. Solvent Addition to an Intermediate Quinone Imide Methide[1]

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Received June 14, 1976

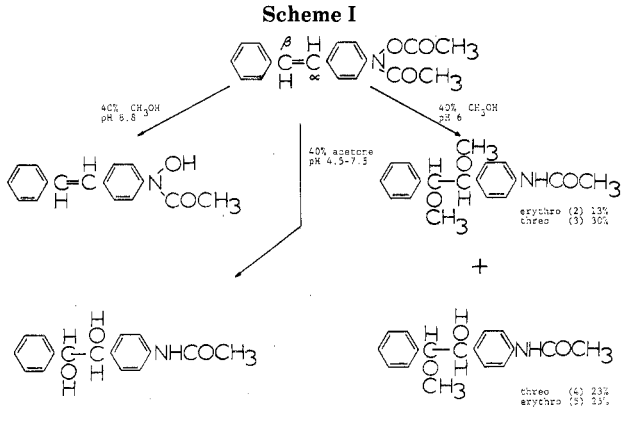
Decomposition of the carcinogen *N*-acetoxy-*N*-(4-stilbenyl)acetamide in 40% acetone yields 1,2-dihydroxy-1-phenyl-2-(4-acetamido)phenylethane as the only major product at pH values up to 7.5. Solvolysis in 40% methanol results in a mixture of isomeric 1,2-dimethoxy-1-phenyl-2-(4-acetamido)phenylethanes and 1-hydroxy-2-methoxy-1-(4-acetamido)phenyl-2-phenylethanes, plus a small amount of 1,2-dihydroxy-1-phenyl-2-(4-acetamido)phenylethane and minor amounts of other, unidentified substances. These products appear to result from nucleophilic attack on the β carbon of an intermediate delocalized nitrenium ion, followed by 1,6 addition of water or methanol to the quinone imide methide formed from the first step. In methanolic medium, increasing pH in the range 6.8–8.8 results in increasing replacement of bibenzyl derivatives by *N*-hydroxy-*N*-(4-stilbenyl)acetamide. These studies suggest a route by which related metabolites are formed in animals treated with *N*-(4-stilbenyl)acetamide, and offer clues to the structures of adducts between nucleic acid bases and *N*-acetoxy-*N*-(4-stilbenzyl)acetamide.

Studies in this laboratory are directed toward an understanding of the reactions of *N*-arylnitrenium ions with nucleic acid bases. Such ions are intermediates in the attack of nucleophiles on protonated *N*-arylhydroxylamines,[2] their esters,[3] or on esters of the corresponding hydroxamic acids.[4] Such molecules appear to be crucial intermediates in the metabolic activation of carcinogenic aromatic amines,[5] and adducts resulting from attack of nitrenium ions on nucleic acids in vivo have already been demonstrated.[6] We are making attempts to understand and predict the course of such reactions by the use of molecular orbital theory,[7] by a dialectical process of experimentally testing predictions from MO calculations, and modifying the calculations according to deviations from the original predictions. We are investigating the products of solvolysis of biologically relevant nitrenium ion precursors, in the expectation that the products obtained thereby will offer clues both to the success of our theoretical treatment and to the nature of complex adducts with nucleosides. In this paper is described the solvolysis of *N*-acetoxy-4-acetamidostilbene (**1**, a local carcinogen[5,8]), a model for active intermediates of the potent systemic carcinogen *N*-(4-stilbenyl)acetamide. Unique features of this particular compound are the facts that (1) it alone, of the four carcinogenic *N*-acetoxy-*N*-arylacetamides so far studied carefully, fails to react with the 8-carbon of guanine in the resulting adduct,[9] and (2) it is the only one of these four compounds which reacts significantly with cytidine.[5]

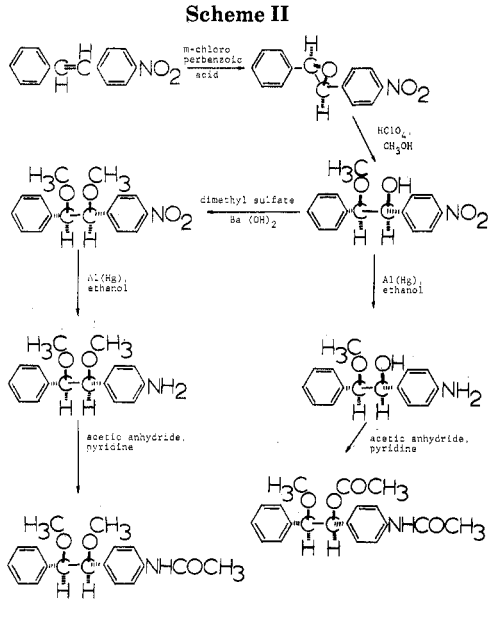
Results

A summary of the solvolysis products is presented in Scheme I. Structure proof and assignment of configuration were obtained by unambiguous synthesis of **2** from nitrostilbene oxide (Scheme II). Treatment of *trans*-4-nitrostilbene with *m*-chloroperbenzoic acid gave the trans epoxide in about

Scheme I



Scheme II



50% yield. Treatment of this epoxide with dilute methanolic perchloric acid gave a single product, as assayed by silica gel TLC. This compound was methylated, then reduced with aluminum amalgam and acetylated, to give a single product identical with **2**, which had already been characterized by NMR and mass spectrometry. The product of methanolysis of the epoxide was also reduced directly, then acetylated, to yield **15**, which was identical with the acetate of **5**. Mass spectral analysis established that the methoxy group was on the β carbon, so that the two syntheses established the stereochemistry of the products as well. Although four isomers could conceivably arise from acid-catalyzed solvolysis of **10** in methanol, ring opening at the α position is deactivated by the *p*-nitro substituent.[10] The structure of **15** confirms the predicted selectivity of ring opening of the epoxide. With the lack of a second product, we must also conclude not only that β-attack occurred, but that it resulted in total inversion of configuration, leading to the final stereochemistry shown. Further reactions did not involve the asymmetric centers.

A phenomenon noted by the Millers is the pH-dependent competitive formation of a β-substituted acetamidostilbene and α,β-disubstituted acetamidobibenzyl.[11] They found that β-methylmercapto-*N*-(4-stilbenylacetamide) was the major

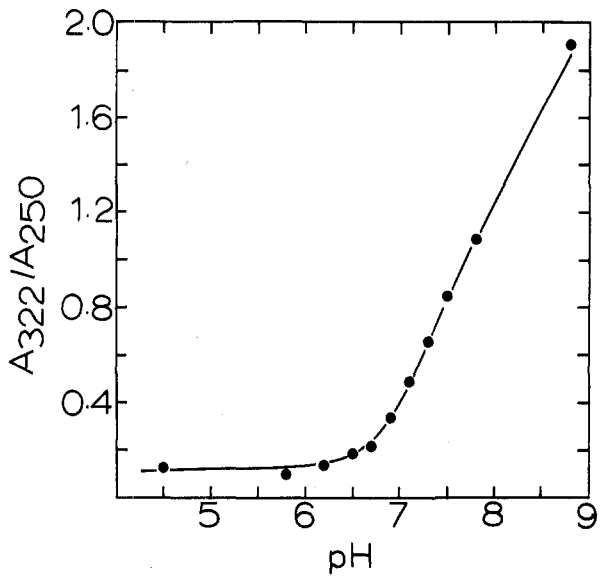


Figure 1. Effect of pH on ratio of stilbene to bibenzyl derivatives upon solvolysis of 1 in 40% methanol.

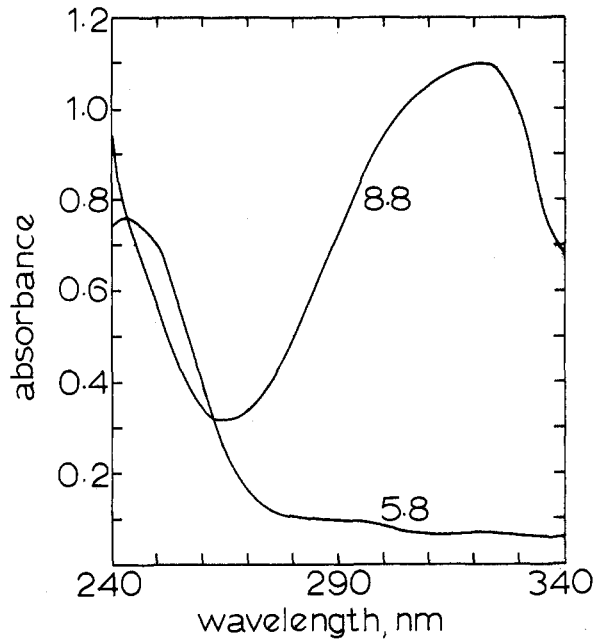


Figure 2. Ultraviolet spectra of product mixtures (after silica gel TLC) after solvolysis of 1 in 40% methanol at the pH values indicated.

product of reaction between 1 and methionine at pH 7.4, but noted a tenfold reduction in this compound at pH 6.5 concomitant with a corresponding increase in a hydrated methylmercaptoacetamidodibenzyl whose structure was not proven.[11] In the systems described here, however, a β-substituted acetamidostilbene was not observed. The yield of diols in 40% acetone does not change over a pH range of 4.5–7.5, nor is there any apparent change in the amount of the other minor products detectable by TLC. In 40% methanol, the mixture of **2–5** is increasingly replaced by 8 with increasing pH in the range 6.8–8.8 (Figure 1), as assayed by the change between the two spectra shown in Figure 2.

Discussion

The reactions of nitrenium ions derived from carcinogenic N-arylacetamides continue to provide useful insights into the chemistry of aromatic systems. In this case, the products obtained further confirm the previous prediction of the reactivity of the β carbon of the N-(4-stilbenyl)-N-acetylnitrenium ion. In fact, from these data, one can conclude that the delocalization is so extensive that the intermediate is better referred to as a highly stabilized carbonium ion. Hückel molecular orbital calculations predicted the β carbon to be by far the most reactive of the carbon atoms in this ion, based on frontier orbital coefficients. The same calculations, however, predicted that the nitrogen would be even more reactive. If this were so, one should expect that 1 would react with guanosine in the same manner as N-acetoxy-2-acetamidofluorene, N-acetoxy-4-acetamidobiphenyl, and N-acetoxy-2-acetamidophenanthrene. As mentioned briefly in the introduction, this is not the case, however.[9] Unlike what is found with the three compounds mentioned above, the reaction of 1 with [8-^{3}H]-guanosine yields a product mixture which retains tritium.[9] We have found that the major adduct from the reaction of 1 with guanosine lacks the stilbene chromophore (unpublished), which should not result from initial reaction at the nitrogen, but rather from reactions like those described in this paper. This same adduct does retain [8-^{3}H] from the starting guanosine. Furthermore, it shows no spectral change in the range pH 2–11, whereas 8-(N-2-fluorenylacetamido)guanosine[12] has a pK of 8.8. Finally, it is a highly polar product, as shown by its elution properties on Sephadex LH-20, suggesting minimization of the hydrophobic effect introduced by a large hydrocarbon group. These preliminary data thus suggest that the guanine adduct also results from reaction elsewhere than at the nitrogen of the intermediate nitrenium ion. More complete structural studies on the cytosine adduct (as the adduct with 1-methylcytosine) show that indeed substitution has taken place on the α and β carbons of the acetamidostilbene, yielding a hydroxy, (1-methyl)cytosylacetamidobibenzyl.

Thus, it appears that the simple HMO calculations carried out previously must be modified to recognize the large delocalization of charge into the aromatic system. The previous MO predictions in this series have been made on the basis of frontier orbital coefficients. If the calculated charge densities are instead used as the basis for predictions, the simple theory agrees entirely with our observations (Table I). From this table, it is clear that the "N-acetyl-N-4-stilbenylnitrenium ion" is no such thing, but is indeed a carbonium ion, with a small negative charge on the nitrogen. An apparent weakness with this approach is that it predicts greater reactivity at carbon in all of the ions presented. Whether this is in fact a weakness can only be determined by additional solvolysis studies on the other N-acetoxy-N-arylacetamides.

The ready formation of 8 in 40% methanol but not in aqueous acetone suggests that transesterification of 1 to an alcohol proceeds more readily than simply saponification. Comparable transacetylation of ribose in guanosine and of lysine in ribonuclease has been noted previously.[9,13]

The hydration of an intermediate quinone methide has been recognized previously,[14] but this may be the first instance of solvent addition to a quinone imide methide (Scheme III). It is surprising that no rearrangement of the type observed by the Millers was detected.[11] Although it is possible that p-phenacylacetanilide may have been a hidden product (its R_f on TLC was identical with that of 8), it was not noticeable in either solvolysis system. Similarly, in the methanolic system, there was no noticeable amount (pH 4.5–7.5) of any material which might have been characterized as β-methoxy-N-(4-stilbenyl)acetamide. Thus, solvent addition to the intermediate quinone imide methide appears to completely predominate over its rearrangement in this system. In the reaction with methionine, however, rearrangement is the major second step at pH 7.4.[11] A plausible explanation is that the

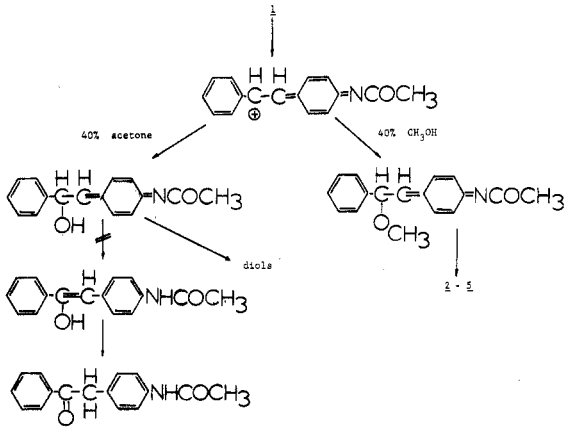
Table I. Charge Densities in *N*-Aryl-*N*-acetylnitrenium Ions[a]

Registry no.	Aryl substituent	Position[b]	Net charge
60239-50-1	4-Xenyl	2,6	0.017
		3,5	0.116
		8,12	0.084
		9,11	0.025
		10	0.079
		N	0.090
60239-51-2	2-Fluorenyl	1	0.097
		3	0.121
		4	0.042
		5	0.071
		6	−0.005
		7	0.094
		8	−0.017
		N	0.055
60239-52-3	2-Phenanthryl	1	0.277
		3	0.054
		4	0.056
		5	0.048
		6	0.003
		7	0.061
		8	0.002
		9	0.000
		10	0.048
		N	0.120
60239-53-4	4-Stilbenyl	2,6	0.087
		3,5	0.114
		8,12	0.038
		9,11	0.025
		10	0.078
		α	0.118
		β	0.254
		N	−0.058

[a] Calculation method given in ref 4. [b] Numbering as in ref 7.

intermediate sulfonium ion facilitates removal of the β proton to form a quinone imide methide ylide, which can then accept a proton on the nitrogen to re-form the stilbene electronic system and the sulfonium ion.

Scheme III



The nature of these reactions suggests that the reaction of **1** with nucleosides or nucleic acids will also differ markedly from the reactions of other *N*-acetoxy-*N*-arylacetamides. The compounds already studied all effect attachment of the imide nitrogen to C-8 of guanine,[6,12,15] or attachment of the aromatic ring to an extranuclear amino group.[7,16] It appears likely, however, that reaction of **1** with nucleic acids at pH 7.4 will result in true alkylation of the nucleic acid, by reaction of the β carbon with extranuclear amino groups, or with oxygen.[17]

A clear relationship between these findings and carcinogenicity cannot be established at this point. All of the amides mentioned herein produce mammary tumors in female rats, with roughly equal potency.[18-20] The *N*-acetoxy-*N*-arylacetamides vary widely in the ability to induce tumors locally, with no apparent relationship between reactivity (expressed as reaction rate, product yield, or product distribution) and carcinogenicity.[5,8,9] On the other hand, **1** stands out clearly from this group as being highly toxic toward human fibroblasts in culture, an observation which may indeed be related to reactions with nucleic acids suggested by our findings.[21] Finally, Neumann, et al. have found that a major urinary metabolite of dimethylaminostilbene in the rat is α,β-dihydroxyacetamidobibenzyl.[22] Whether this results from a hydroxamic acid ester or epoxidation of the double bond is a question requiring further studies of other, similar metabolites.

Experimental Section

Unless noted otherwise, all reagents were obtained from J. T. Baker Chemical Co., and were used as received. Melting points were taken on a Fisher-Johns apparatus, and are corrected to standards. Infrared spectra were determined in KBR pellets on a Perkin-Elmer 257 instrument. Ultraviolet spectra were obtained with a Beckman ACTA III instrument. High-resolution mass spectra and 270-MHz NMR spectra were determined by Mr. F.-T. Liu at the University of Chicago.

Solvolysis of *N*-Acetoxy-*N*-(4-stilbenyl)acetamide (1). An acetone solution (80 ml) of **1** (400 mg, prepared in this laboratory[15,23]) was mixed with 120 ml of water. The mixture was heated until a clear solution was obtained, then maintained at 40 °C for another 3 h. The acetone was removed on a rotary evaporator, and the aqueous solution extracted with five 100-ml portions of ether. Chromatography of the product on a silica gel column in 5% CH_3OH in CH_2Cl_2 yielded a light brown resin which was homogeneous on silica gel TLC in the same system and in ethyl acetate–benzene (7:3). The ir spectrum of this material (neat, between NaCl plates) showed a broad, intense band with maximum at 3300 cm^{-1}. A 1% solution in CH_2Cl_2 gave two sharp bands at 3600 and 3430 cm^{-1}, corresponding to a free O–H and a free secondary amide N–H, respectively. The uv spectrum of the material gave λ_{max} 248 nm (log ε 4.28), which is representative of all of the bibenzyl derivatives reported here. This material could be resolved into two fractions by chromatography on Sephadex LH-20 in water. The two substances yielded acetates which melted sharply (162–163, 146–148 °C), had largely identical ir spectra, and yielded the same major fragments on low-resolution mass spectroscopy. Elementary analysis for the major acetate (mp 163 °C) was as follows. Anal. Calcd for 1,2-bis(acetoxy)-1-phenyl-2-(4-acetamido)phenylethane: C, 67.61; H, 5.92; N, 3.94. Found: C, 67.92, H, 5.99; N, 4.08. Ir maxima of major product: 3290, 3190, 3120, 3060, 3050, 3040, 2965, 2940, 1740, 1660, 1602, 1530, 1498, 1458, 1416, 1375, 1345, 1320, 1286, 1235, 1185, 1163, 1128, 1108, 1080, 1035, 980, 940, 868, 840, 772, 740, 710, 690, 672, 660, 640 cm^{-1}.

An acetone solution (5 ml) of **1** (400 mg) was mixed with 80 ml of CH_3OH, then with 120 ml of water. This was heated as described above, the methanol removed on a rotary evaporator, and the products extracted with ether. The product mixture was chromatographed on a silica gel column, 1.5 × 100 cm, packed in CH_2Cl_2, and eluted with 500 ml each of 1, 2, and 5% CH_3OH in CH_2Cl_2. The first major products to emerge were **2** and **3**, which were mostly resolved from each other. A minor amount of an oil emerged, followed by the mixture of **4** and **5**. These were resolved by repeated rechromatography on a column of silica gel, 0.9 × 90 cm in 2% CH_3OH in CH_2Cl_2, and could be resolved, as well as **2** and **3**, by HPLC on a 1-m column of ODS Permaphase (Du Pont Instruments) eluted with water. The only remaining major band obtained from this column was the mixture of diols.

The above solvolysis was repeated using 0.1 M K_2HPO_4 instead of water. After heating as above and removal of methanol, a heavy precipitate was formed and was collected. TLC showed this to consist of mostly one substance. Recrystallization from methanol, column chromatography on silica gel, and recrystallization from ethyl acetate failed to remove a brown contaminant. Elementary analysis and comparison of the ir spectrum with that of authentic material showed this substance to be *N*-hydroxy-*N*-(4-stilbenyl)acetamide (**8**).

4-Nitrostilbene Oxide (10). 4-Nitrostilbene (**9**, 1.07 g, prepared in this laboratory[24]) and *m*-chloroperbenzoic acid (Aldrich, 85%, 1.00 g) were dissolved in 250 ml of CH_2Cl_2 at room temperature. After 6

days, TLC on silica gel (10% CH_2Cl_2 in petroleum ether) showed product in about 50% yield. After the solvent was removed, the mixture was chromatographed on 60 g of silica gel in 10–30% CH_2Cl_2 in petroleum ether. **9** emerged first, followed by **10**. Recrystallization of the epoxide from petroleum ether and benzene gave a first crop of 356 mg (mp 128–129.5 °C, lit. 125 °C)[25] and a second crop of 64 mg (mp 127–128 °C). Anal. Calcd for $C_{14}H_{11}NO_3$: C, 69.71; H, 4.56; N, 5.81. Found: C, 69.51; H, 4.65; N, 5.53. Uv maxima (95% ethanol) 281, 218 nm (log ϵ 3.14, 3.24); ir maxima at 3110, 3080, 3040, 2980, 2850, 1605, 1515, 1462, 1430, 1390, 1350, 1290, 1250, 1222, 1180, 1165, 1115, 1095, 1077, 1037, 977, 898, 871, 850, 837, 812, 768, 755, 715, 705 cm^{-1}.

1-Acetoxy-1-(4-acetamido)phenyl-2-methoxy-2-phenylethane (15). **10** (64 mg) was mixed with 10 ml of CH_3OH and 0.5 ml of concentrated $HClO_4$. Upon heating to 40 °C, all of the material dissolved. After 90 min, 0.8 g of $NaOCOCH_3 \cdot 3H_2O$ was added, and the solvent evaporated under reduced pressure. The residue was washed with five 2-ml portions of ether, and the combined ether extracts dried with Na_2SO_4 and evaporated. Acetic acid was removed with a stream of nitrogen, leaving 52 mg of an oil which crystallized on scraping, ir peaks at 3460, 2820 cm^{-1}. This material (50 mg, homogeneous on TLC on SiO_2 in CH_2Cl_2, and on HPLC on ODS Permaphase in H_2O) was dissolved in 3 ml of 95% ethanol and heated in a hot water bath (80–90 °C) with aluminum amalgam prepared from 2 cm^2 of minced Reynolds heavy duty aluminum foil,[26] with occasional replenishment of ethanol. After 45 min, 5 ml of ether was added, the mixture filtered, and the residue washed twice more with ether. The combined filtrate was evaporated under reduced pressure. Ir showed loss of $-NO_2$ with retention of $-OH$, $-OCH_3$. The crude product was treated overnight with 0.5 ml of pyridine and 0.3 ml of acetic anhydride, then diluted with 5 ml of water. The suspension was extracted with two 5-ml portions of ether, and the combined ether extracts washed with 10 ml of 0.5 M HCl, 10 ml of saturated $NaHCO_3$, and 10 ml of water, and dried over Na_2SO_4. Evaporation of the ether yielded a solid, mp 163–165 °C (petroleum ether/benzene). Anal. Calcd for $C_{19}H_{21}NO_4$: C, 69.72; H, 6.42; N, 4.28. Found: C, 69.69; H, 6.51; N, 4.13. Ir maxima at 3320, 3200, 3130, 3060, 3040, 2980, 2930, 2870, 2825, 1740, 1675, 1606, 1550, 1520, 1460, 1420, 1375, 1325, 1245, 1190, 1130, 1112, 1097, 1075, 1040, 975, 915, 885, 862, 842, 822, 766, 710, 637 cm^{-1}. Major peaks of mass spectrum: 327.1474 (calcd for molecular ion, 327.1470), 207.0850, 206.0814 (calcd for $CH_3CONHC_6H_4CHOCOCH_3^+$, 206.0817), 165.0731, 164.0690, 122.0649, 121.0621 (base peak; calcd for $C_6H_5CHOCH_3^+$, 121.0653), 120.0455, 105.0334.

***erythro*-1,2-Dimethoxy-1-(4-acetamido)phenyl-2-phenylethane (2).** After solvolysis of **10** in acidic methanol, 258 mg of *erythro*-1-hydroxy-1-(4-nitro)phenyl-2-methoxy-2-phenylethane was dissolved in 5 ml of dimethyl sulfoxide and 5 ml of dimethylformamide.[27] The flask was cooled in ice and 1.5 g of $Ba(OH)_2 \cdot 8H_2O$ was added with magnetic stirring. Dimethyl sulfate (Eastman, 2 ml) was added dropwise under nitrogen. After 3 h, the nitrogen was removed and stirring continued overnight at room temperature. Concentrated ammonia (2 ml) was then added slowly and stirring continued for 30 min. The solution was extracted with two 30-ml portions of $CHCl_3$, and the extract dried over Na_2SO_4, evaporated under reduced pressure, redissolved in $CHCl_3$, washed with water, dried, and evaporated. The product was reduced with aluminum amalgam from 6 cm^2 of minced foil, and the product acetylated. After the usual workup and recrystallization from methanol, a product was obtained identical with **2** (HPLC on Permaphase/H_2O, ir), mp 182–183 °C. Anal. Calcd for $C_{18}H_{21}NO_3$: C, 72.24; H, 7.02; N, 4.68. Found: C, 72.10; H, 7.15; N, 4.79.

Spectral Data for *erythro*- and *threo*-1,2-Dimethoxy-1-(4-acetamido)phenyl-2-phenylethane (2 and 3). Ir (**2**) 3300, 3260, 3190, 3120, 3060, 3030, 3000, 2930, 2900, 2880, 2820, 1670, 1610, 1555, 1530, 1515, 1457, 1450, 1415, 1375, 1320, 1270, 1245, 1235, 1210, 1190, 1108, 1040, 1020, 975, 945, 885, 862, 847, 837, 815, 780, 770, 740, 720, 690, 638 cm^{-1}; NMR (**2**, $CDCl_3$, Me_4Si reference) δ 2.21 (singlet, 3 H), 3.19 (singlet, 3 H), 3.20 (singlet, 3 H), 4.33 (quartet, 2 H), 7.17 (multiplet, 4 H), 7.30 (multiplet, 3+ H), 7.46 (doublet, 2+ H); mass spectrum (**2**) 268.1306 (calcd for M − OCH_3, 268.1337), 179.0916, 178.0866 (base peak; calcd for $CH_3CONHC_6H_4CHOCH_3^+$, 178.0868), 137.0676, 136.0750, 121.0612 (calcd for $C_6H_5CHOCH_3^+$, 121.0653), 120.0456.

Ir (**3**) 3310, 3190, 3110, 3060, 3030, 2970, 2930, 2900, 2825, 1690, 1602, 1530, 1517, 1495, 1470, 1457, 1410, 1370, 1312, 1280, 1253, 1220, 1188, 1160, 1115, 1090, 1080, 1055, 1012, 987, 970, 960, 868, 843, 775, 742, 716, 675, 641 cm^{-1}; NMR (**3**, $CDCl_3$, Me_4Si reference) δ 2.17 (singlet, 3 H), 3.28 (singlet, 3 H), 3.29 (singlet, 3 H), 4.32 (singlet, 2 H), 7.00 (multiplet, 4 H), 7.18 (multiplet, 3 H), 7.36 (doublet, 2 H), 7.56 (singlet, 1 H); mass spectrum (**3**) 268.1409 (calcd for M − OCH_3, 268.1337), 179.0893, 178.0842 (base peak; calcd for $CH_3CONHC_6H_4CHOCH_3^+$, 178.0868), 137.0698, 136.0777, 121.0614 (calcd for $C_6H_5CHOCH_3^+$, 121.0653), 120.0456.

Effect of pH on Solvolysis of 1. Stock solutions of 0.1 M K_2HPO_4 and 0.1 M KH_2PO_4 were mixed in the series 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, and 10:0 to give the pH values indicated in Figure 1. **1** was dissolved in acetone or methanol at a concentration of 150 μg/ml. Two milliliters of either stock solution of **1** was mixed with 3 ml of each buffer mixture. All samples were incubated at 37 °C for 24 h. Acetone was then removed by evaporation at reduced pressure, and the aqueous residue extracted with two 5-ml portions of ether. The methanol-containing samples were extracted directly with three 5-ml portions of ether. The ether was evaporated under nitrogen in a 10-ml test tube. Ethyl acetate (100 μl) was then added to each sample in an ice bath. The tubes were stoppered, the sides washed with the solvent, and 20-μl samples applied to a silica gel TLC plate. The plates were developed in 5% CH_3OH in CH_2Cl_2. Visual inspection under a uv hand lamp indicated no variation in the major spots between pH 4.5 and pH 7.8. The diol spots (from the acetone reaction mixtures) for pH values 4.5, 6.7, and 7.5 were scraped and eluted with 1 ml of 95% ethanol. Less than a 10% reduction in material was seen over this range. The combined spots for **2–5** were similarly assayed, and a major increase in stilbene compound detected. All spots from the methanol mixture assay plate were assayed (in 2 ml of 95% ethanol), and the results shown in Figures 1 and 2.

Acknowledgment. I thank Ms. Norma Naimy for repeated preparations of *N*-acetoxy-*N*-(4-stilbenyl)acetamide, and Ms. Becky Woodworth for performing the methylations. I am especially indebted to Professor N. C. Yang for offering the facilities available to him at the University of Chicago, and to Mr. F.-T. Liu for obtaining the NMR and mass spectra.

Registry No.—**1**, 26488-34-6; **2**, 60239-54-5; **3**, 60239-55-6; **9**, 1694-20-8; **10**, 14985-26-3; **15**, 60239-56-7; *erythro*-1,2-dihydroxy-1-phenyl-2-(4-acetamido)phenylethane, 60239-57-8; *threo*-1,2-dihydroxy-1-phenyl-2-(4-acetamido)phenylethane, 60239-58-9; *erythro*-1,2-bis(acetoxy)-1-phenyl-2-(4-acetamido)phenylethane, 60239-59-0; *threo*-1,2-bis(acetoxy)-1-phenyl-2-(4-acetamido)phenylethane, 60253-79-4; 1-hydroxy-1-(4-nitro)phenyl-2-methoxy-2-phenylethane, 60239-60-3; 1-hydroxy-1-(4-amino)phenyl-2-methoxy-2-phenylethane, 60239-61-4; 1,2-bis(methoxy)-1-phenyl-2-(4-nitro)phenylethane, 60239-62-5; 1,2-bis(methoxy)-1-phenyl-2-(4-amino)phenylethane, 60239-63-6.

References and Notes

(1) This is part 4 in the series "*N*-Aryl-*N*-acetylnitrenium Ions in Aromatic Amine Carcinogenesis". Reference 7 is the previous paper in this series. Supported by Grants CA-13155 and CA-18632 from the National Cancer Institute.
(2) (a) H. E. Heller, E. D. Hughes, and C. K. Ingold, *Nature (London)*, **168**, 909 (1951); (b) E. Kriek, *Biochem. Biophys. Res. Commun.*, **20**, 793 (1965); (c) C. M. King and B. Phillips, *J. Biol. Chem.*, **244**, 6209 (1969).
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The Iodomethylation of Nicotine. An Unusual Example of Competitive Nitrogen Alkylation[1]

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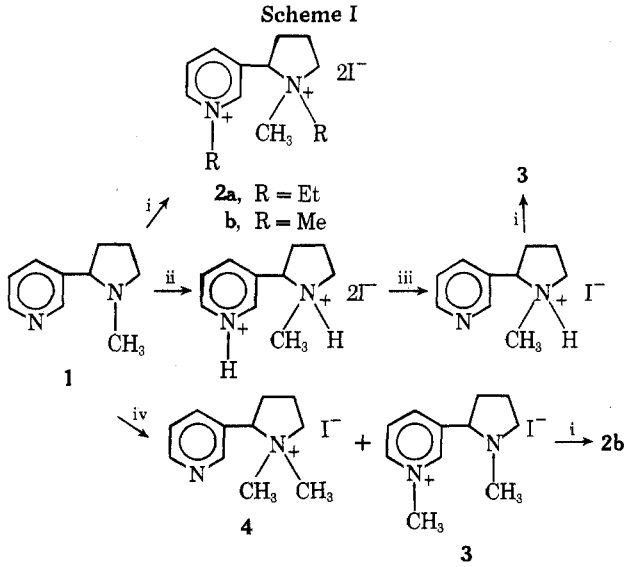
Received May 5, 1976

Alkylation of nicotine with 1 equiv of iodomethane in either methanol or acetonitrile leads to ca. 2.5:1 mixtures of *N'*-methylnicotinium iodide (**4**) and *N*-methylnicotinium iodide (**3**), and not to only **4** as previously indicated in the literature. The alkylation results reflect kinetic control of product. Control experiments indicate that the products are formed irreversibly under the reaction conditions. Alkylation of the nicotine analogue *N,N*-dimethyl-3-aminomethylpyridine with 1 equiv of iodomethane led only to trimethyl-3-picolylammonium iodide. Competitive alkylation experiments between nicotine and pyridine and *N*-methylpyrrolidine indicate that alkylation on nicotine's pyrrolidine nitrogen is decelerated, and the causes for this anomalous example of competitive nitrogen alkylation are discussed.

Nicotine (**1**) and its nitrogen alkylated products (e.g., **2–4**) have been of considerable biological and chemical interest for many years.[2-4] The earliest reported work on the alkylation of nicotine was published in 1853 by Kekule[5a] and in 1854 by Stahlschmidt[5b] who treated the alkaloid with iodoethane and iodomethane and obtained nicotine diethiodide (**2a**) and dimethiodide (**2b**), respectively. In 1897, Pictet and Genequand[6] reported their preparation of the two monomethiodides of nicotine, *N*-methylnicotinium iodide (**3**) and *N'*-methylnicotinium iodide (**4**), as shown in Scheme I. It is of interest to

Scheme I



Reagents: i, excess CH_3I; ii, HI; iii, 1 equiv of nicotine; iv, 1 equiv of CH_3I.

note that recent investigators[7] have reported the preparation of these compounds, in some cases with much difficulty, following the old literature procedures.

As a part of our interest in nicotine structure[8] and reactivity,[9] we now report that alkylation of this alkaloid with 1 equiv of iodomethane in either methanol or acetonitrile, following the literature procedures,[6,7c] leads to ca. 2.5:1 mixtures of **4**:**3** and not to only **4** as previously reported[6,7] (see Figures 1–3).[10] However, we have isolated **4** (68%) uncontaminated with either nicotine or **3** by continuous extraction of the aqueous solution of the **3** + **4** mixture with chloroform followed by removal of water from the aqueous phase. Rotary evaporation of the chloroform phase followed by ether trituration led to the isolation of **3** (28%). Alternatively, treatment of an acetic acid solution[11] of nicotine with 2 equiv of iodomethane at room temperature for 3 days followed by removal of the acetic acid and trituration with ether yields (58%) pure **3**.

The identity of these compounds is evident from their ^1H NMR spectra (see Figures 1–3 and data cited in the Experimental Section), elemental analyses, and mode of synthesis. In addition, treatment of either **3** or **4** with iodomethane leads quantitatively to *N,N'*-dimethylnicotinium diiodide (**2b**). Thus, simple high-yield procedures for the preparation of the two nicotine monomethiodides, uncontaminated with each other, are now available.

The Menschutkin reaction has been shown to be reversible in some cases, generally under forcing conditions.[13] Treatment of pure **3** or a 4:1 mixture of **4**:**3** at 120 °C in acetonitrile in a sealed, degassed NMR tube resulted in no discernible chemical change after 36 h as judged by ^1H NMR of the total reaction mixture. Thus, the reaction product ratios in the nicotine alkylations are not complicated by the potential equilibration of products and starting material following selective quaternization; i.e., the alkylation results reflect kinetic rather than thermodynamic product control.

The iodomethylation of nicotine at pH >6 is an unusual example of competitive nitrogen quaternization,[13,14] especially since the pyrrolidine nitrogen of nicotine is almost three orders of magnitude more basic than nicotine's pyridine nitrogen (see Table I). While many factors other than basicity have a kinetic influence on the Menschutkin reaction, e.g., steric hindrance and solvation,[15] two limiting conditions could explain the nicotine alkylation results: (1) a rate decrease in pyrrolidine alkylation caused by the pyridine ring; and (2) a pyridine nitrogen alkylation rate enhancement due to the presence of the pyrrolidine ring. In an effort to distinguish between these two possibilities, two competitive alkylation experiments were performed. Treatment of a 1:1 mixture of nicotine and *N*-methylpyrrolidine with 1 equiv of iodomethane in methanol resulted in the formation of only *N,N*-dimethylpyrrolidinium