The Reaction of 4-(*p*-Nitrobenzyl)pyridine with Some Electrophiles (1)

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Received December 22, 1975

The nucleophile 4-(*p*-nitrobenzyl)pyridine was allowed to react with four carcinogenic alkylating agents, chloromethyl methyl ether, bis(chloromethyl) ether, glycol sulfate and propane sultone, one carcinogenic acylating agent, *N,N*-dimethylcarbamyl chloride, and one noncarcinogenic electrophile, perchlorocyclobutenone. The structures of the major products formed, which are substituted pyridinium salts or 1,4-dihydropyridines, were determined.

J. Heterocyclic Chem., **13**, 517 (1976).

In earlier studies (2-4) we have examined the chemical reactivity of a series of alkylating agents, some of which are direct-acting carcinogens (5). This report is a continuation of our work concerning the relationship between chemical structure, reactivity and potential carcinogenic activity.

The heterocyclic compound 4-(*p*-nitrobenzyl)pyridine (I) was selected for this study since previous workers have demonstrated that it is useful in assaying alkylating and acylating agents (6-9). Compound I is a relatively strong nucleophile and readily forms covalent bonds with a variety of electrophiles. Due to the extended chromophore in I the products formed from I and an electrophile have uv and sometimes visible absorption bands which are useful in analytical studies. The structures of the products formed when I and an electrophile react have not always been elucidated.

This report deals with the reaction of I with five alkylating agents and one acylating agent and the structure determination of the products formed.

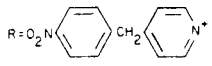
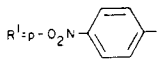
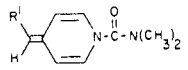
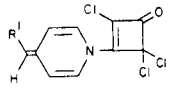
Chloromethyl methyl ether (10), bis(chloromethyl) ether (10), glycol sulfate (11) and propane sultone (12) have been shown to be carcinogenic alkylating agents. *N,N*-Dimethylcarbamyl chloride is a carcinogenic acylating agent (13), of which few are known (14). Perchlorocyclobutenone showed no carcinogenic potential (12).

Compound I reacted readily with chloromethyl methyl ether displacing the reactive halide to form Compound II. (See Table 1). Similarly, I reacted with bis(chloromethyl) ether to form III. Compound I also readily cleaved the five-membered ring in glycol sulfate to yield IV. The expected cleavage of the C-O bond in propane sultone (15) took place when it was allowed to react with I to yield V. The data in Table 1 and the information in the experimental section support the assigned structures. The products formed when I reacted with *N,N*-dimethylcarbamyl chloride or perchlorocyclobutenone were less predictable.

The reaction of *N,N*-dimethylcarbamyl chloride with I, rather than yielding an *N*-acylpyridinium compound, yielded the *N*-acyl-1,4-dihydropyridine, compound VI. The assignment of structure VI is based on the elemental analysis, the red shift in the uv, new visible absorption bands, and most importantly its nmr spectrum. The nmr spectrum of VI had no benzylic protons, but one vinyl proton singlet was present. In addition, the characteristic 1,4-substituted pyridinium proton doublets were absent, but the more coupled higher field dihydropyridine protons doublets were absent, but the more coupled higher field dihydropyridine protons were present (16-18).

The structure of the reaction product of I and perchlorocyclobutenone was also determined. The parent halo compound has been shown to react with nucleophiles in aqueous media to yield open-chain carbonyl compounds. With one equivalent of 1-butanol it yielded 1,3,3-trichloro-2-butoxycyclobutenone (19-20). However, no spectroscopic evidence was presented for the assignment of this structure. Compound VII (the product isolated when I was allowed to react with perchlorocyclobutenone) had an ir carbonyl absorption band at 1805 cm^{-1} compared to the starting material which had a carbonyl band at 1790 cm^{-1}. From this it was concluded that the four-membered ring ketone was intact in VII. The elemental analysis and mass-spectrum (parent ion m/e, 384) corresponding to $C_{16}H_9Cl_3N_2O_3$ indicated that compound VII was a dihydropyridine derivative. The nmr spectrum of VII in warm DMSO-d_6 showed two sets of aromatic protons, benzylic protons and no other protons indicating a pyridinium ion structure for VII. This anomaly was resolved when the nmr spectrum of

Table 1

	Product	Color	M.p.	λ, nm (ε x 10^{-3})(a)
				
II,	R-CH_2OCH_3, Cl^-	Yellow	154-155°	261 (14)
III,	$(R\text{-}CH_2)_2O$, $2Cl^-$	Off-white	169-172° dec.	264 (27)
IV,	R-$CH_2CH_2OSO_3^-\cdot H_2O$	White	215-216°	265 (18), 273 (sh)
V,	R-$CH_2CH_2CH_2SO_3^-$	White	290°	265 (19), 275 (sh)
				
VI,		Scarlet Red	172-173°	467 (15), 309 (12), 236 (11), 275 (sh)
VII,		Scarlet Red	169-171°	436 (23), 262 (13)

(a) The spectra of compounds II, VI and VII were measured in ethyl alcohol solutions; all other spectra were measured in water.

VII was determined at room temperature in deuteriochloroform, in which it is slightly soluble. This spectrum had the characteristic dihydropyridine resonance peaks, with no indication of benzylic protons. Thus, we concluded that the compound VII, upon being dissolved in warm DMSO, changes from the dihydropyridine to a pyridine moiety. That this spectrum is not I or the hydrochloride of I was evident by comparing their nmr spectra in the same solvent. The phosgene derivative of 4-(*p*-nitrobenzyl)pyridine (I), was recently reported to readily change from its dihydropyridine structure to the parent pyridine compound (21). Finally, substitution of I at the β-position of the cyclobutenone seemed most likely since the Michael addition of I to the conjugated carbonyl with subsequent loss of the β-chlorine would yield compound VII. Substitution at the α- or γ-position is less likely and would probably lead to additional changes in the butenone chromophore.

EXPERIMENTAL

Ir spectra were obtained with a Perkin Elmer 421 infrared spectrophotometer. Samples were run as potassium bromide pellets or as 10% solutions in chloroform. Nmr spectra were determined in deuterium oxide, deuteriochloroform and deuteriodimethylsulfoxide with tetramethylsilane as the internal standard. Micronalyses were performed by Sprang Microanalytical Laboratory, Ann Arbor, Michigan. The mass spectrum was determined by Morgan Schaffer Corp., Montreal, Canada. The melting points, and uv-visible absorption spectra of all the compounds prepared are given in Table 1.

Preparation of *N*-Methoxymethyl-4-(*p*-nitrobenzyl)pyridinium Chloride (II).

The preparation of this compound was carried out as previously described (22); its nmr spectrum in deuteriochloroform had peaks at δ 9.23 (d, J = 6.8 Hz, 2H), 8.37 (2 d, J = 9.0 Hz, 4H), 7.80 (d, J = 6.8 Hz, 2H) 6.18 (s, 2H), 4.92 (s, 2H), 3.78 (s, 3H).

Preparation of *N*-Methyl-4-(*p*-nitrobenzyl)pyridinium Chloride Ether (III).

This compound was prepared as described previously (22).

Preparation of *N*-Ethyl-β-sulfate 4-(*p*-Nitrobenzyl)pyridine (IV).

To 100 mg. (0.81 mmole) of glycol sulfate in 5.0 ml. of acetone, 355 mg. (1.66 mmoles) of 4-(*p*-nitrobenzyl)pyridine was added and the solution refluxed on a steam bath for 3 hours. Upon cooling 203 mg. (74% yield) of crystalline product was obtained; it was recrystallized from water. An ir spectrum (potassium bromide) showed bands at 1640, 1522, 1468, 1352, 1266, 1222, 1014, 905 and 752 cm^{-1}. The nmr spectrum (deuteriodimethylsulfoxide) showed peaks at δ 9.00 (d, J = 6.8 Hz, 2H), 8.28 (d, J = 9.0 Hz, 2H), 8.10 (d, J = 6.8 Hz, 2H), 7.69 (d, J = 9.0 Hz, 2H), 4.84 (t, J = 4.5 Hz, 2H), 4.52 (s, 2H), 4.28 (t, J = 4.5 Hz, 2H).

Anal. Calcd. for $C_{14}H_{14}N_2SO_6\cdot H_2O$: C, 47.18; H, 4.52; N, 7.86; S, 9.00. Found: C, 47.18; H, 4.52; N, 7.85; S, 8.99.

Preparation of *N*-Propyl-γ-sulfonate-4-(*p*-nitrobenzyl)pyridine (V).

Propane sultone, 200 mg. (1.63 mmoles) and 350 mg. (1.63 mmoles) of 4-(*p*-nitrobenzyl)pyridine were dissolved in 5.0 ml. of acetone and heated for 3 hours at 50°. Upon cooling 165 mg. (59% yield) of V precipitated. The product was recrystallized from water/acetone. An ir spectrum (potassium bromide) showed bands at 3400 (brd), 1640, 1512, 1352, 1345, 1238, 1170, 1160 and 1052 cm^{-1}. The nmr spectrum (deuterium oxide) had the following peaks: δ 9.10 (d, J = 6.4 Hz, 2H), 8.22 (d of d, J = 6.4,

J = 8.6, 4H), 7.72 (d, J = 9.0 Hz, 2H), 5.02 (t, J = 6.8 Hz, 2H), 4.78 (s, 2H), 3.26 (t, J = 6.8 Hz, 2H), 2.70 (p, J = 6.8 Hz, 2H).

Anal. Calcd. for $C_{15}H_{16}N_2O_5S$: C, 53.57; H, 4.80; N, 8.32; S, 9.53. Found: C, 53.39; H, 4.63; N, 8.32; S, 9.51.

Preparation of *N,N*-(Dimethylcarbamyl)-4-(*p*-nitrobenzylidene)-1,-4-dihydropyridine (VI).

In a flask containing 5.0 ml. of acetone, 0.5 ml. of *N,N*-dimethylcarbamyl chloride (5.6 mmoles) and 1.19 g. of *p*-nitrobenzylpyridine (5.58 mmoles) were added. The solution was refluxed on a steam bath for 3 hours. The volume of solvent was then reduced under nitrogen, and 471 mg. (38% yield) of a solid precipitated. This was recrystallized from ethyl alcohol/water. The ir spectrum (chloroform) showed bands at 1685, 1655, 1575, 1565, 1495, 1486, 1377, 1325, 1313, 1252, 1164, 1107 and 847 cm^{-1}. The nmr spectrum (deuteriochloroform) had peaks at δ 8.27 (d, J = 8.6 Hz, 2H), 7.51 (d, J = 8.6 Hz, 2H), 6.92 (m, 2H), 6.31 (d, d 2H), 5.80 (s, 1H) and 3.12 (s, 6H).

Anal. Calcd. for $C_{15}H_{15}N_3O_3$: C, 63.17; H, 5.30; N, 14.73. Found: C, 63.23; H, 5.23; N, 14.80.

Preparation of *N*-3-(1,4,4-Trichloro)cyclobuten-2-one-4-(*p*-nitrobenzylidene)-1,4-dihydropyridine (VII).

To a round-bottomed flask containing 162 mg. of 4-(*p*-nitrobenzyl)pyridine (0.760 mmole), 10 ml. of acetone and 0.10 ml. of perchlorocyclobutenone (0.760 mmole) were added. The solution was refluxed for 2 hours. Upon cooling a small amount of 4-(*p*-nitrobenzyl)pyridine hydrochloride deposited. This was removed by filtration. Most of the filtrate was then evaporated and the crystals which appeared, 134 mg. (46% yield), were filtered and recrystallized from acetone. An ir spectrum (potassium bromide) had bands at 1788, 1660, 1605, 1578, 1555, 1368, 1320, 1295, 1182, 1132, 1102 and 830 cm^{-1}. The nmr (deuteriochloroform) had peaks at δ 8.32 (d, J = 9.0 Hz, 2H), 7.53 (d, J = 9.0 Hz, 2H), 7.24 (m, 2H), 6.67 (d, d, 2H), and 6.22 (s, 1H). The nmr (deuteriodimethylsulfoxide) of the pyridine compound derived from VII had peaks at δ 8.91 (d, J = 6.5 Hz, 2H), 8.27 (d, J = 9.4 Hz, 2H), 8.05 (d, J = 6.5 Hz, 2H), 7.66 (d, J = 9.4 Hz, 2H), and 4.51 (s, 2H).

Anal. Calcd. for $C_{16}H_9Cl_3N_2O_3$: C, 50.09; H, 2.36; N, 7.30; Cl, 27.72. Found: C, 50.04; H, 2.34; N, 7.31; Cl, 27.75.

Acknowledgement.

The authors wish to thank Mr. M. Yaslow and Dr. K. Frenkel for their fine technical assistance in the early phases of this research (23).

REFERENCES AND NOTES

(1) Research supported in part by USPHS Grant No. CA 133443 and USPHS Contract NO1 CP 43221 and is part of a Center Program supported by the National Institutes of Environmental Health Sciences, National Institutes of Health, Grant No. ES 00260.

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(23) Our colleagues, Dr. A. Segal and Dr. G. Loewengart have developed a personal monitoring badge which contains 4-(*p*-nitrobenzyl)pyridine for detecting direct-acting alkylating agents in ppb. Details of this development are being published elsewhere.