HETEROCYCLES, Vol. 24, No.7, 1986

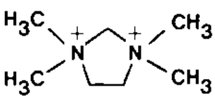
PREPARATION AND PROPERTIES OF METHYLENEBISPYRIDINIUM DERIVATIVES

Shekhar Munavalli, Edward J. Poziomek, and Wayne G. Landis

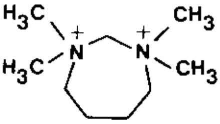
U.S. Army Chemical Research, Development and Engineering Center
Research Directorate
Aberdeen Proving Ground, MD 21010-5423, U.S.A.

Abstract - A number of N,N'-methylenebispyridinium halides have been synthesized and characterized. The toxicological properties, mass spectra and NMR spectra of this unusual group of compounds containing a reactive methylene group flanked by two positively charged nitrogen atoms have been examined.

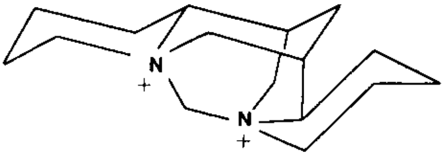
Methylenebisazonium compounds have attracted considerable attention in recent years[1-12]. Although the first example of the reaction of dibromomethane with alkylamines was reported at the turn of the century, no methylenebisazonium derivatives were characterized until later[13]. Stable methylenebisazonium compounds derived from tertiary amines had not been reported until 1970[7]. Since then several such compounds, including the cyclic derivatives (1-3), have been described[14-17]. Krohnke[18] was the first to synthesize methylenebispyridinium bromide (4a,R=H) via the decomposition of 1-phenacylpyridinium bromide (5). This served as the principal procedure for the preparation of N,N'-methylenebispyridinium derivatives[19-20]. Pyridine itself underwent bis-quaternization smoothly to yield (4a,R=H) when heated with methylene iodide[21]. The methylene protons of 4 are highly reactive and mobile[22]. The Krohnke synthesis has been extended to the preparation of high isotopically pure 1-deutero- and 1-tritiobenzaldehydes[23-25]. Thus, the "bisiminium cation" (6) exchanged with D_2O via the ylide(7). Because of the considerable "steric inaccessibility" of the nucleophilic center, the ylide failed to act as an alkylating agent. Our own interest in the chemistry of the methylenebispyridinium compounds stemmed from our efforts to identify and characterize the products obtained from the reaction of 4,4'-bipyridyl, 4-dimethylaminopyridine and 4-aminopyridine with $Br-(CH_2)_n-Br$. It was reported that the reaction of $Br(CH_2)_nBr$ *with 4,4'-bipyridyl in N-methylpyrrolidinone at 110 to 120°C for 24 h gave liquid* crystalline polymers containing the 4,4'-bipyridyl backbone[26]. Under these conditions, we were unable to isolate any solid compound. When the reaction time was shortened, an inseparable mixture of three or more compounds was obtained.[27] The formation and the composition of the *mixture depend on the time and the temperature of the reaction.*



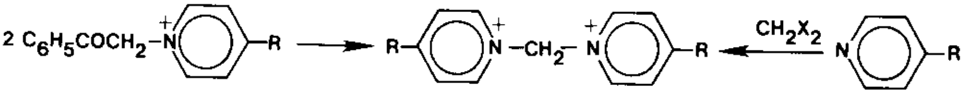
1



2



3



5

4

4a, R = H

b, R = $N(CH_3)_2$

c, R = NH_2

d, R = CH_3

e, R = CN

4f, R = COC_6H_5

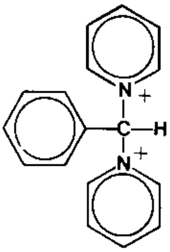
g, R = C_6H_5

h, R = CH = CH − C_6H_4 − NO_2(p)

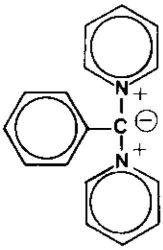
i, R = C_6H_4 − NO_2(p)

j, R = CH = NOH

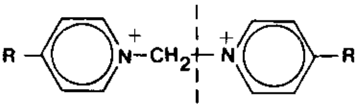
k, R = γ-pyridyl



6



7



8

In this context, it was considered desirable to examine the behavior of 4-dimethylaminopyridine under similar conditions. Thus refluxing a solution of equimolar amounts of 4-dimethylaminopyridine and dibromomethane in dry acetonitrile gave a solid in quantitative yield within a couple of hours. The mass spectra and elemental analysis indicated the molecular formula of the compound to be $C_{15}H_{22}Br_2N_4$. Although the NMR spectrum of this compound showed the product to be homogeneous, the chemical shift of the methylene protons was not consistent with that expected from the bisquaternization of the ring nitrogen [4b,R=N(CH_3)2], in that the chemical shift of the methylene protons was dramatically shifted upfield by more than 1.2 ppm as compared with that of the unsubstituted parent compound (4a,R=H). Similar anomaly was observed with the compound (4c,R=NH_2). Even assuming the possibility of solvent-solute interaction, the observed displacement of the NMR signal of the methylene protons of these compounds (4b,c) seemed highly unusual. Also, conspicuously absent from the NMR spectra of 4b and c was the low field shift of the α-protons usually observed on quaternization of the pyridine nitrogen. The chemical shift and splitting patterns of the α-pyridylhydrogens of a mono-substituted pyridine are characteristics for the position of the substituent[28,29]. Well defined changes occur on quaternization. The α-protons are easily recognizable by their downfield position in the salt in comparison with the free base[30-32]. This is because the proton chemical shifts are related to the π-electron density in aromatic molecules. The presence of a nitrogen atom causes a pronounced downfield shift of the ring protons, especially the α-protons[33]. Quaternization produces a positively charged nitrogen, which generally leads to lower screening for the neighboring protons. This results in the observed downfield displacement of the NMR signal due to the α-protons. This characteristic feature has often been used as a positive indication of quaternization of the nitrogen of the pyridine ring. In addition, compounds (4b and c) are electrochemically inert as compared with compounds (4d,g and k)[34]. Compounds 4b and c did not show any cathodic peak potentials, (Ep)c, vs Ag/AgCl reference electrodes of cyclic voltammograms of the compounds in 0.1 N HCl at a hanging mercury drop electrode; whereas compounds (4d, g, and k) did show them. To understand the basis of this large displacement of the methylene proton signal, a series of methylenebispyridinium derivatives (4d,e,f,g,h,i,j, and k) were synthesized. Our attempts to prepare the title compounds from 4-nitropyridine, 4-formylpyridine and 4-hydroxpyridine failed. The latter gave the O-alkylated product. The melting points, the chemical shift difference of the methylene protons, the mass spectral data and the elemental analysis of compounds (4a-k)are given in Table 1.

Careful examination of the NMR spectra of (4a-k revealed the subtle effect of the p-substituents on the chemical shift of the methylene protons in question. This effect has been attributed to synergistic exhaustive interaction[35].

Mass Spectrometry of Bispyridinium Salts.

Due to the low volatility of organic quaternary salts, their structure elucidation by mass spectromerty has proved to be a difficult process. Vaporization of the quaternary salts generally results in their degradation[36]. The structure of the molecular ion must then be deduced from the products of degradation. The electron impact or chemical ionization[32], field desorption[37] and laser desorption[38] techniques have been applied to improve the analysis of the mass spectra of quaternary salts. However, these techniques produce polymers of salts and thereby cause further complications. A recently introduced innovation involves heating salt coated wires inside the ion source to produce the molecular ion and the fragmentationof ions by direct thermal decomposition[39-42]. Diquaternary salts produce dication. Intact dications of diquaternary ammonium salts are rarely observed when secondary ion, fast atom bombardment and laser desorption techniques are used[43-45]. As in the case of monocationic salts, decomposition products of dications dominate their mass spectra. Recently, thermospray mass spectrometry has been applied to diquaternary salts and M^{2+} dication was observed to form the base peak of the mass spectrum[42].

We have applied conventional mass spectrometric, secondary ion and fast atom bombardment (FAB) techniques to study the structures of the methylenebispyridinium compounds for the first time. We were unable to observe the peak corresponding to the parent ion. However, the FAB gave $(M+H)^+$ peak in the case of 4b. Ions corresponding to the "benzylic type" cleavage of the methylenebispyridinium moiety was noted in all cases (cf.8). These ions were then further degraded according to the fragmentation pattern common to the pyridine ring system. Nonetheless, valuable information was obtained from the peaks formed due to the cleavage. This enabled the deduction of the structure of the molecular ions.

Toxicological Testing.

Although diquaternary azonium salts showed no observable antitumor activity[46], dipyroxide[47], and 4b[48,49] and 4a[50] did exhibit enhanced cholinesterase activity and chemisterilant properties respectively. The preliminary screening of compounds (4a-d, i-k) showed them to be slightly toxic to Daphnia, Salmonella and yeast; the lowest EC_{50} being 62.5 mg/liter. Even though no rigorous structure-activity relationship was observed, the presence of the nitro group appears to enhance the toxicity.

EXPERIMENTAL

General Remarks. All melting points were taken on a Thomas-Hoover Capillary melting point apparatus and are uncorrected. The infrared spectra were obtained as potassium bromide disks on a Perkin-Elmer Model 1420 spectrophotometer. The NMR spectra were obtained on a Varian EM-390 NMR spectrometer in D_2O at the probe temperature (34°C). Each reading is quoted to the nearest 0.05 ppm. The chemical shifts were relative to the internal standard, TSP (sodium 3-trimethylsilylpropionate). All signals were downfield from the reference and the values were obtained by direct measurement on 10 ppm sweep width. The chemical shift of the methylene protons of the unsubstituted parent compound (4a) occurred at 7.60 ppm downfield from the reference peak. According to the convention, the upfield displacements in comparison with the parent compound are indicated by a -ve sign, while the low field shifts by a +ve sign. Only those ions that correspond to the cleavage between the two positively charged nitrogens are given in Table 1. The solvents used to crystallize the compounds are indicated at the bottom of Table 1. The elemental analyses were obtained through the Analytical Division, Research Directorate, U.S. Army Chemical Research, Development and Engineering Center. The parent compound, namely N,N'-methylenebispyridinium halide has been described in the literature[18-20]. The 4-(p-nitrophenyl)pyridine and p-nitrostyrylpyridine required for the synthesis of 4h and 4i were themselves prepared by the nitration[51] of 4-phenylpyridine and by the base catalyzed reaction[52] of 4-picoline and p-nitrobenzaldehyde respectively. Bisquaternization of the substituted pyridines occurred smoothly within a short time, less than 30 min, when electron-donating substituents were present in the p-position. The yields of the expected products were generally quantitative. On the contrary, when electron-withdrawing groups were present on the pyridine ring, prolonged refluxing was necessary. The yields in this case were usually poor. The experimental procedure simply consisted of refluxing a solution of equimolar amounts of the substituted pyridines and dihalomethanes in dry acetonitrile or ethanol. The solvent was removed on the rotary evaporator, the residue triturated with ether or acetone, the resulting solid was filtered off and washed with excess ether or actone to remove the unreacted starting materials. The analytical samples were crystallized from the solvents indicated.

Toxicological Testing Protocol.

First instar *Daphnia magna* reared from at least third generation post acclimation adults were used as the experimental animals. Culture techniques were those described by Goulden et al[53]. Ten neonates were placed in each 250 ml glass beaker containing 100 ml of the sample. Two replicates of each concentration were used for each test. All bioassays conformed to current Organization of Economic Cooperation and Development and U.S. Environmental Protection Agency guidelines. Public drinking water which had been passed through particle filters, activated charcoal filters, and aged a minimum of 58 h in a 200 gallon polyethylene holding tank was used as a diluent. A 20 ±1°C temperature and 16:8 light-dark cycle were employed throughout the bioassay regime. Water hardness between 50-65 ppm $CaCO_3$ and a pH range of 6.1-7.0 were observed during testing. Seven of the title compounds (4a-d, i-k) were tested according to the above protocol. The results are summarized in Table 2.

ACKNOWLEDGEMENT

We thank Mr. Paul Cannon for cyclic voltametry data.

Table 1

Compound	m.p.*	Chemical Shift Difference (ppm)**	Ionic Fragmentation of Dictations	Molecular Formula	Elemental Analysis
N,N'-Methylenebispyridinium bromide (4a)	255-9°	0	93,79	$C_{11}H_{12}Br_2N_2$	Ref. 18-20
N,N'-Methylene-(4,4'-dimethylaminobispyridinium) bromide (4b)	295-8° (decomp.)	-1.23	136,122	$C_{15}H_{22}Br_2N_4$	Found: C,43.1;H,5.3;N,13.5.Calculated: C,43.1;H,5.3;N,13.4%.
N,N'-Methylene-(4,4'-diaminobispyridinium) bromide (4c)	300°	-1.23	108,94	$C_{11}H_{14}Br_2N_4$	Found: C,36.3;H,3.9;N,15.2.Calculated: C,36.5;H,3.9;N,15.4%.
N,N'-Methylene-(4,4'dimethylbispyridinium) bromide (4d)	265-8°	-0.30	-	$C_{13}H_{16}Br_2N_2$	Found: C,43.1,H,4.8;N,7.5.Calculated: C.43.3;H,4.5;N,7.6%.
N,N'-Methylene-(4,4'-dicyanobispyridinium) bromide (4e)	192-5° (decomp.)	+0.11	-	$C_{13}H_{10}Br_2N_4$	Found: C,40.8;H,2.6;N,14.5.Calculated: C,40.9;H,2.6;N,14.7%.
N,N'-Methylene-(4,4'-dibenzoyl bispyridinium) bromide (4f)	220-2° (decomp.)	+0.26	-	$C_{25}H_{20}Br_2N_2O_2$	Found: C,55.6;H,3.9;N,4.9.Calculated: C,55.6;H,3.7;N,5.2%.
N,N'-Methylene-(4,4'diphenyl bispyridinium) bromide (4g)	266-8° (decomp.)	-0.15	169,155	$C_{23}H_{20}Br_2N_2$	Found: C,56.8;H,4.4;N,5.5.Calculated: C,57.0;H,4.2;N,5.7%.
N,N'-Methylene-(4,4'-di-[p-nitrostyryl) bispyridinium] bromide (4h)	300°	-0.37	-	$C_{29}H_{22}Br_2N_4O_4$	Found: C,51.5;H,3.4;N,8.8.Calculated: C,51.7;H,3.5;N,8.9%.
N,N'-Methylene-[4,4'-di-(p-nitrophenyl) pyridinium] bromide (4i)	230-2°	-0.07	-	$C_{23}H_{18}Br_2N_4O_4$	Found: C,47.8;H,3.3;N,9.8.Calculated: C,48.1;H,3.2;N,9.8%.
N,N'Methylene bis(4,4'-formylpyridinium bromide)oxime (4j)	229-32° (decomp.)	-0.12	136,122	$C_{13}H_{14}Br_2N_4O_2$	Found: C,37.3;H,3.4;N,13.2.Calculated: C,37.3;H,3.4;N,13.4%.
N,N'-Methylene-[4,4'-di(γ-pyridyl))bispyridinium] bromide(4k)	300°	0	171,157	$C_{21}H_{18}Br_2N_4$	Found: C,51.7;H,3.8;N,11.5.Calculated: C,51.9;H.3.7;N,11.5%.

*Compounds 4b,g-k crystallized from EtOH, 4d,f from MeOH, 4c from MeOH-CH_3COCH_3 and 4e from EtOH-CH_3COCH_3

**The NMR signal of the methylene protons of the parent compound is at 7.60 ppm.

Table 2

(EC_{50} Data)

Compound	Test Period (in hours)	EC_{50} (mg/liter)	Lower Limit*	Upper Limit*
4a	24	112.7	103.9	122.2
	48	70.7	64.6	77.4
4b	24	182.3	161.9	205.3
	48	84.4	77.6	98.1
4c	24	62.4	53.5	77.9
4d	24	107.8	71.5	162.5
	24	98.9	62.3	156.9
4i	24	60.3	44.0	82.6
4j	24	208.9	127.9	340.9
4k	24	94.7	82.5	108.6
	48	40.2	31.5	51.2

* 95% confidence limits from Probit analysis.

REFERENCES

1. W. C. Davies, E. B. Evans and F. L. Hulbert, J. Chem. Soc., 1939, 412.
2. L. J. Dry, M. J. Koebemoer and F. L. Warren, J. Chem. Soc., 1955, 59.
3. C. A. Caws and G. E. Foster, J. Pharm. Pharmac.,1956, 8, 790.
4. H. Bohme and E. Boll, Chem. Ber., 1957, 90, 2013.
5. C. C. J. Culvenor, L. W. Smith and W. G. Woods, Tet. Lett.,1965, 2025.
6. H. Williams, J. Org. Chem.,1964, 29, 2064.
7. D. A. Wright and C. A. Wulff, J. Org. Chem., 1970, 35, 4252.
8. R. Besseilievre, N. Langlois and P. Potier, Bull. Soc. Chim (France), 1972, 1477.
9. J. D. Phillpsen and N. G. Bisset, Phytochem., 1972, 11, 2547.
10. A. Vincze and L. Gef'en, Israel J. Chem., 1978, 17, 236.
11. A. H. Beckett and H. M. Ali, J. Chromatogr., 1979, 177, 255.
12. S. H. Hansen and L. Nordham, J. Chromatogr., 1981, 204, 97.
13. F. Schmidt and F. M. Litterscheid, Liebigs Ann. Chem., 1904, 337, 67.
14. H. Noguchi and A. Rembaum, Macromolecules, 1972, 5, 253.
15. A. Rembaum and H. Noguchi, Macromolecules, 1972, 5, 261.
16. R. M. Cosgrove, C. A. Maryanoff, D. McComsey, J. E. Mills, L. Scott and V. Paragamian, 17th Middle Atlantic Regional Meeting, White Haven (PA) Apr. 6-8, 1983, Abst. #316.
17. A. I. Ishbaev, A. N. Nizamkhodxhaeva and K. T. Il'yasova, cf. Chem. Abstr.,1980, 92, 129150c.
18. F. Krohnke, Chem. Ber., 1950, 83, 50.
19. K. G. Mizuch, N. M. Kasatkin and T. M. Gel'fer, cf. Chem. Abstr.,1957, 51, 12884.
20. V. Hach, V. Kvita, J. Kolinsky and K. Macek, Coll. Czech. Chem. Comm., 1963, 28, 266.
21. G. Scheibe, H. J. Friedrich, W. Guckelj and J. Smits, Angew. Chem., 1961, 73, 736.
22. V. N. Setkina and E. V. Bykova, cf. Chem. Abstr., 1955, 49, 10946.
23. R. A. Olofson and D. M. Zimmerman, J. Amer. Chem. Soc., 1967, 89, 5057.
24. T. Hase, Acta. Chem. Scand., 1970, 24, 2263.
25. R. A. Olofson, D. M. Zimmerman and R. C. Schnur, J. Labeled Compds., 1972, 8, 392.
26. E. T. Samulski and L-P. Yu, 183rd American Chemical Society National Meeting Las Vegas March 28 - April 2, 1982, Abstr. #170.
27. S. Munavalli and E. J. Poziomek (Unpublished results).
28. H. J. Bernstein and W. G. Schneider, J. Chem. Phys., 1956, 24, 469.
29. W. R. Anderson, Jr. and R. M. Silverstein, Anal. Chem., 1965, 37, 1417.
30. I. C. Smith and W. G. Schnieder, Can. J. Chem., 1961, 39, 1158.

31. R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds", J. Wiley and Sons, Inc., New York (1967) p. 124.

32. J. L. Sudmeier and C. N. Reilley, Anal. Chem., 1964, 36, 1698.

33. H. Spiesecke and W. G. Schneider, Tet. Lett., 1961, 468.

34. S. Munavalli, P. Cannon and E. J. Poziomek (Unpublished results).

35. S. Munavalli, L. Szfraniec, W. Beaudry and E. J. Poziomek (submitted for publication).

36. J. Shabanowitz, P. Brynes, A. Madicke, D. V. Bowen and F. H. Field, Biomed. Mass Spectrom., 1975, 2, 164.

37. D. F. Hunt, J. Shabanowitz and F. K. Botz, Anal. Chem., 1977, 59, 1160.

38. F. W. Rollgen, U. Giessmann, H. J. Heinen and S. J. Reddy, Int. J. Mass Spectrom. Ion Phys., 1977, 24, 235.

39. R. J. Cotter and A. L. Yergey, J. Amer. Chem. Soc., 1981, 103, 1956.

40. R. Stoll and F. W. Rollgen, J. Chem. Soc., Chem. Comm., 1980, 789.

41. A. L. Yergey and R. J. Cotter, Biomed. Mass Spectrom., 1982, 9, 286.

42. G. Schmelzeissen-Redeker, U. Griessmann and F. W. Rollgen, Angew. Chem. Int. Ed. Engl., 1984, 23, 892.

43. T. M. Ryan, R. J. Day and R. G. Cooks, Anal.Chem., 1980, 52, 2054.

44. D. F. Barofsky and V. Giessmann, Int. J. Mass Spectrom. Ion Phys., 1983, 46, 359.

45. D. N. Hellor, H. Yergey and R. J. Cotter, Anal. Chem., 1983, 55, 1310.

46. H. Seigoro, F. Mitsuru, F. Yoko, I. Nobuchika and K. Yoichi, Chem. Pharm. Bull. (Japan), 1972, 20, 15.

47. S. N. Golikiv, V. I. Shchekoldins and I. G. Simonova, cf. Chem. Abstr. 1973, 78, 11613 p.

48. F. Hobbiger and P. W. Sadler, Brit. J. Pharmocol., 1959, 14, 192.

49. F. hobbiger, M. Pitman and P. W. Sadler, Biochem. J., 1960, 75, 363.

50. J. E. Oliver and M. M. Crystal, J. Econ. Entomol., 1972, 65, 303.

51. A. R. Katritzky and P. Simmons, J. Chem. Soc., 1960, 1511.

52. A. R. Katritzky, A. J. Boulton and D. J. Short, J. Chem. Soc., 1960, 1516.

53. C. E. Goulden, R. M. Comotto, J. A. Hendrickson, Jr., L. L. Horning and K. L. Johnson, American Society for Testing and Materials Special Technical Publication, 1982, 766, 139.

Received, 27th January, 1986