ences observed correlate with a qualitative observation of the viscosity differences of the samples.

The runs of the asphalt sample and of the asphaltenes extracted from it are shown in Figure 9. Again one gets a distinct distribution of the different materials along the elution–volume axis.

Some comments are appropriate at this point on the expected properties of the high-ΔT TFFF in the characterization of complex materials. We note, first, that the elution sequence is from low to high molecular weight, unlike exclusion (gel) chromatography. The low molecular weight components tend to clump as a single peak while resolution improves continuously for the heavier components. Gel permeation chromatography (GPC) also shows better resolution for larger molecules, providing these are in the active partitioning range (*13*). However, resolution in GPC eventually declines with further increases in molecular weight, whereas with TFFF resolution is expected to reach a steady plateau (*10*).

More important are the molecular characteristics which determine elution. A steady and fairly predictable increase in elution volume occurs with increase in molecular weight for any homologous series. However, unlike GPC, TFFF exhibits a solvent effect, in which a change of solvent will alter the magnitude of retention but not the pattern with respect to molecular weight. Presumably a change of solvents will shift different solute families with respect to one another, although this has not been confirmed. If so, variations in solvent could become a useful tool in identifying different chemical families and the molecular weight distribution within families. This possibility is not immediately present in chromatographic methods. This results because the exclusion techniques are basically solvent independent and the other chromatographic techniques do not yield broad, readily-characterized molecular-weight spectrums. The TFFF method will, in all likelihood, permit the advantageous combination of these two characteristics.

LITERATURE CITED

(1) J. C. Giddings, *Sep. Sci.*, **1**, 123 (1966).
(2) J. C. Giddings, *J. Chem. Phys.*, **49**, 1 (1968).
(3) E. Grushka, K. D. Caldwell, M. N. Myers, and J. C. Giddings, *Sep. Purif. Methods*, **2**, 129 (1973).
(4) J. C. Giddings, *J. Chem. Educ.*, **50**, 667 (1973).
(5) J. C. Giddings, *Sep. Sci.*, **8**, 567 (1973).
(6) G. H. Thompson, M. N. Myers, and J. C. Giddings, *Anal. Chem.*, **41**, 1219 (1969).
(7) M. E. Hovingh, G. H. Thompson, and J. C. Giddings, *Anal. Chem.*, **42**, 195 (1970).
(8) J. C. Giddings, M. E. Hovingh, and G. H. Thompson, *J. Phys. Chem.*, **74**, 4291 (1970).
(9) M. N. Myers, K. D. Caldwell, and J. C. Giddings, *Sep. Sci.*, **9**, 47 (1974).
(10) J. C. Giddings, Y. H. Yoon, and M. N. Myers, *Anal. Chem.*, **47**, 126 (1975).
(11) J. C. Giddings "Dynamics of Chromatography. Part 1. Principles and Theory", Marcel Dekker, New York, 1965.
(12) "Handbook of Chemistry and Physics", 45th ed., Chemical Rubber Co., Cleveland, Ohio, 1964, p D119.
(13) J. C. Giddings, *Anal. Chem.*, **40**, 2143 (1968).

RECEIVED for review July 3, 1975. Accepted August 26, 1975. This investigation was supported by National Science Foundation Grant Number MPS74-05260 A03.

Proton Magnetic Resonance Study of the Effect of Water, Acetonitrile, and Benzonitrile on Diprotonated 2,2′-Dipicolylamine

M. R. Olds and R. T. Iwamoto

Department of Chemistry, University of Kansas, Lawrence, Kan. 66045

The protonation of 2,2′-dipicolylamine in water, acetonitrile, and benzonitrile has been examined by proton magnetic resonance. The protonation scheme of the amine in each of these solvents has been established, and by the use of substituent shielding constants for the methylene group, obtained from appropriate model compounds, the distribution of protons among the three basic sites for the diprotonated stage in each of the three solvents has been determined.

A significant number of compounds of biochemical importance are multibasic substances, which undergo successive protonation or metal ion coordination. It is generally accepted that the protonation scheme of multibasic compounds or the proton distribution pattern of some of the multiprotonated species will vary with the nature of the medium. There is, however, no information as to how large a variation can be expected. To obtain a better appreciation and understanding, in particular a quantitative picture, of the effect of solvent on the protonation of multibasic substances, we have examined the protonation of 2,2′-dipicolylamine, a tribasic amine, in water (D_2O) [dielectric constant, 78; dipole moment, 1.86 D], acetonitrile [dielectric constant, 37.5; dipole moment 3.44 D], and benzonitrile [dielectric constant, 25.2; dipole moment, 4.02 D]. Future studies with metal ions are planned.

The protons of a diprotonated symmetrical noncyclic tribasic compound can be located on neighboring or terminal basic sites. Differences in the ability of solvents to solvate the protonated sites should lead, through differences in relief of coulombic interaction, to varying extent of isomerism of position, i.e., varying percentages of the two diprotonated forms.

Grunwald, Lowenstein, and Meiboom (*1*), and Lowenstein and Roberts (*2*) reported that the chemical shift of methyl and methylene hydrogens adjacent to basic groups is sensitive to changes in the electronic environment brought about by protonation and that there is a linear relationship between the shielding contribution of a basic site and the fraction of time it is protonated. Furthermore, Shoolery has demonstrated the additive nature of the shielding effect of substituents on the signal of the methylene protons of compounds of the type XCH_2Y (*3*). Importantly, the chemical shift of the methylene hydrogens then should undergo a predictable change in going from XCH_2Y to $X'CH_2Y$, $X'CH_2Y'$, or XCH_2Y', where X′ and Y′ are designations for protonated basic sites.

For this study on the effect of solvent on the protonation scheme or more specifically on the nature of the diprotonated species of a noncyclic symmetrical tribasic amine, 2,2′-dipicolylamine was selected because of the relative basicity of its basic sites and the presence of methylene groups (which can be monitored) between the basic sites. The diprotonated species $PyH^+CH_2NH_2{}^+CH_2Py$ will be referred to as the adjacent charge diprotonated species and the species $PyH^+CH_2NHCH_2PyH^+$ as the separated charge species.

EXPERIMENTAL

Materials. Acetonitrile of reagent grade purity from Matheson Coleman and Bell was fractionally distilled from sodium hydride, phosphorus pentoxide, and then from calcium hydride, as described by Focier and Olver (*4*). A final distillation from phosphorus pentoxide was performed to eliminate any traces of amine formed during the distillation over calcium hydride. The distillate was protected from the atmosphere and light. Benzonitrile, superior grade from Matheson Scientific, was purified by distillation from phosphorus pentoxide, according to a procedure described earlier (*5*). In the final step, a fore cut of 25% was removed and the next 35% was collected. The purified material was stored in the dark and protected from the atmosphere. Deuterium oxide and deuteroacetonitrile from Stohler Isotope Chemicals were used as obtained. Perchloric acid (70%) and trifluoromethane sulfonic acid (TFMSA), both reagent grade, from Fisher Chemical Co. and Aldrich Chemical Co., respectively, were used without further treatment. The latter acid is *very* hygroscopic. The chemicals used in the examination of shielding effects of amine substituents were purchased in the purest form available and used without purification.

Samples of 2,2′-dipicolylamine were provided by Reilly Tar and Chemical Co. This material was used in some of the preliminary investigations without further purification. In the final studies, the base was outgassed at 1 mm for 30 min and vacuum distilled at 125 °C (0.1 mm). *Anal.* equiv wt. Calcd: 199. Found: 201.

Preparation of Compounds. *2-N-Ethylpicolylamine.* This compound was prepared by treating a mixture of 25 g of ethylamine hydrochloride and 4 g of 2-picolyl chloride in an Erlenmeyer flask with 30% sodium hydroxide solution. The flask was placed in a beaker of ice water, and the sodium hydroxide was added slowly while the solution was mixed with a magnetic stirrer. With the addition of excess sodium hydroxide, the solution turned from yellow to pink. The mixture was then poured into a larger beaker and treated with an excess of sodium hydroxide in the form of pellets (without cooling). This treatment caused the solution to boil and a red layer of oil formed. The oil was separated, dissolved in ether, and filtered. After evaporation of the ether, the oil was dried overnight with molecular sieves, and vacuum distilled at 95 °C (22 mm). The product was pale yellow, and the following analytical data were obtained. *Anal.* Calcd. for $C_8H_{12}N_2$: C, 71.11; H, 8.15; N, 20.74. Found: C, 69.64; H, 8.53; N, 20.44.

2-Ethoxypicoline. This amine was obtained by a method reported by Suzuki (*6*). *Anal.* Calcd. For $C_8H_{11}NO$: C, 70.07; H, 8.03; N, 10.22. Found: C, 70.60; H, 8.28; N, 10.55.

Procedure. Proton magnetic resonance spectra were obtained with a Varian A-60 and a Varian HA-100 spectrometer. Chemical shifts in acetonitrile and in benzonitrile were measured relative to tetramethylsilane (TMS) as internal standard, and in deuterium oxide to tertiary butyl alcohol. The chemical shift of the methyl resonance of tertiary butyl alcohol in deuterium oxide [1.29 ppm vs. 3-(trimethylsilyl)propane sulfonate] is independent of pH within experimental error (*7*). Chemical shift values are reported relative to tetramethylsilane and 3-(trimethylsilyl)propane sulfonate. In addition to a daily check of the calibration of the recorder bed of the NMR spectrometers, before each set of runs the spectrum of 2,2′-dipicolylamine was recorded and checked. All spectra were obtained at a probe temperature of 40 ± 2 °C.

The NMR spectra of the neutral and protonated amines and of related compounds were obtained in 0.1, 0.2, and 0.4 *F* solutions of the compounds in deuterium oxide, acetonitrile, and benzonitrile. Solutions of the protonated forms of the supportive compounds were prepared by combining trifluoromethane sulfonic acid (TFMSA) or perchloric acid and the compounds of interest in 10:1 mole ratio in the appropriate solvent. Where the signal of the hydrogens of acetonitrile obscured the region of interest, deuteroacetonitrile was used as solvent. Additions of TFMSA were carried out in a dry box. In preliminary investigations of the protonation scheme of dipicolylamine, 1.00-ml aliquots of a 0.100*M* solution of the amine were placed into a number of vials and a multiple of 10 μl of 1 *M* acid was added to each. [The acidic titrants were made up to be 1*M* and then checked vs. primary standard tris(hydroxymethyl)aminoethane (THAM, Fisher Chemical Co.)] (*8*). TMS or *tert*-butyl alcohol was then added, and the solutions were placed in NMR tubes and examined immediately.

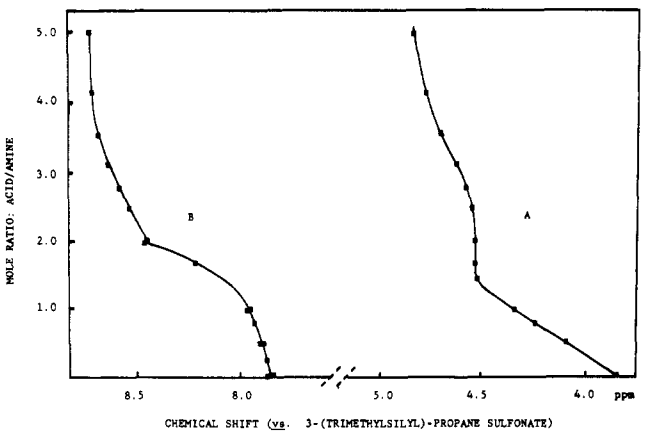

Figure 1. Shift in the proton NMR signal of the methylenic hydrogens (curve A) and the ring hydrogen in the 5-position (curve B) of 2,2′-dipicolylamine on protonation of the amine in water (D_2O) with 70% perchloric acid

In the final studies, the molar concentration of the stock solutions of 2,2′-dipicolylamine in deuterium oxide, acetonitrile, and benzonitrile was adjusted (0.232 *M* for titrations involving perchloric acid and 0.282 *M* for titrations with TFMSA) so that 500-μl aliquots of the stock base solutions would require additions of 20 and 25 μl of 70% perchloric acid and neat TFMSA, respectively, to provide 1:1 mole ratios of acid to amine. The stock dipicolylamine solutions were checked by potentiometric titration with standard hydrochloric acid solution (benzonitrile solutions were not checked because of problems arising from the immiscibility of the nitrile in water). Because the smallest volume increment of acid that could be handled was 5 μl, small changes in mole ratio of acid to dipicolylamine were attained by adding measured amounts of the stock base solution to the 1:1, 2:1, and 4:1 (or 5:1) mole ratio mixtures of acid to dipicolylamine. Fully protonated 2,2′-dipicolylamine was obtained by the addition of a tenfold excess of perchloric acid or TFMSA. The additions of acid and of the dipicolylamine were made directly into NMR tubes with Fisher micropipets. Neat TMS or *tert*-butyl alcohol was added and the solutions were mixed and run immediately.

Computations were handled with a GE-635 digital computer.

RESULTS

The shift in the signal of the methylene hydrogens in 2,2′-dipicolylamine on protonation of the amine in water (deuterium oxide) with perchloric acid is shown in Figure 1 and in acetonitrile in Figure 2. Similar protonation data of the amine in benzonitrile with trifluoromethane sulfonic acid (TFMSA) are shown in Figure 3. Interestingly, in acetonitrile there is a clearly noticeable *upfield* shift of the signal of the methylene hydrogens on diprotonation of the amine. The upfield shift is observed only in the 1:1 to 2:1 mole ratio range of perchloric acid to 2,2′-dipicolylamine; continued protonation beyond the 2:1 points results in reversal of the shift in signal from upfield to downfield. Importantly, the signal of the ring proton in the 5-position in 2,2′-dipicolylamine on protonation of the amine in the region from 1:1 to 2:1 mole ratio of perchloric acid to the amine (Figures 1 and 2) shows a larger downfield shift than that expected for protonation of only one of the ring nitrogens (based on the protonation data for 2-ethylpyridine). In benzonitrile, protonation studies involving perchloric acid to amine ratios >1:1 were plagued by precipitation of polyprotonated 2,2′-dipicolylamine salts. The variation in the chemical shift of the methylene hydrogens of 2,2′-di-

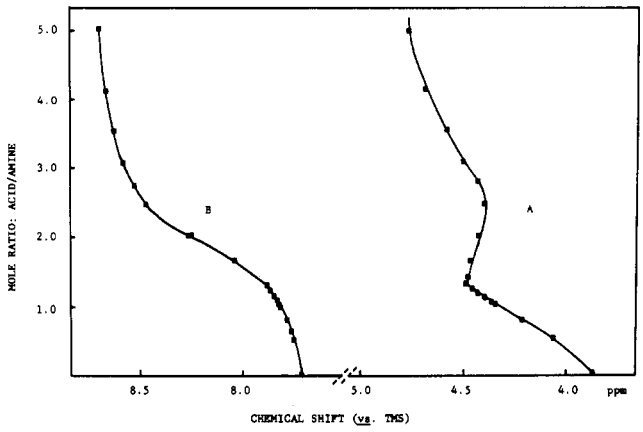

Figure 2. Shift in the proton NMR signal of the methylenic hydrogens (curve A) and the ring hydrogen in the 5-position (curve B) of 2,2′-dipicolylamine on protonation of the amine in acetonitrile with 70% perchloric acid

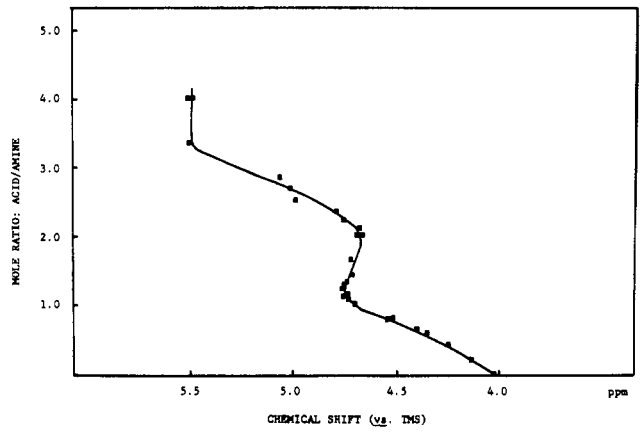

Figure 3. Shift in the proton NMR signal of the methylenic hydrogens of 2,2′-dipicolylamine on protonation of the amine in benzonitrile with trifluoromethane sulfonic acid

picolylamine on protonation in benzonitrile was, therefore, examined with TFMSA as titrant. No precipitation problems were encountered. When this protonation study was repeated in solutions of benzonitrile containing 2:1 mole ratio of water to added TFMSA (solutions of the same composition, except for the anion, as those examined when perchloric acid was the titrant), no precipitation of polyprotonated amine salt was encountered, and the chemical shift variation of the methylene hydrogen signal was identical with that obtained with no added water.

The variation in the chemical shift of the methylene hy-

Table I. Chemical Shift Values of Compounds Used to Obtain Substituent Shielding Constants for Methylenic Hydrogens in Water (D_2O), Acetonitrile, and Benzonitrile

Compounds[a]	Shielding components	Water		Acetonitrile			Benzonitrile	
		Neutral	Excess $HClO_4$	Neutral	Excess $HClO_4$	Excess TFMSA	Neutral	Excess TFMSA
Py-C^1-N-C-Py 2,2′-Dipicolylamine	(1) A + D	3.83	4.95	3.87	4.88	4.85	4.01	5.51
Py-C^4-C^3-N-C^2-C	(2) L + J	...	...	2.60	3.21	3.18	2.64	3.53
2-β-Ethylaminopyridine	(3) C + F	...	...	2.90	3.52	3.46	2.97	3.92
	(4) A + G	...	...	2.90	3.52	3.46	2.97	3.92
Py-C^5-C 2-Ethylpyridine	(5) A + J	2.84	3.14	2.79	3.11	3.12	2.80	3.18
Ph-C^7-N-C^6-C	(6) E + J	2.66	3.20	2.59	3.17	3.13	2.62	3.34
N-Ethylbenzylamine	(7) B + F	3.75	4.26	3.74	4.21	4.20	3.77	4.38
C-C^8-N-C^9-C^{10}-N	(8) K + J	2.62	3.28	2.59	3.20	3.20	2.62	3.54
N-Ethylethylenediamine	(9) F + I	2.68	3.48	2.62	3.40	3.37	2.73	4.02
	(10) G + H	2.68	3.48	2.62	3.40	3.37	2.73	4.02
N-C-C^{11}-N Ethylenediamine	(11) H + I	2.68	3.46	2.57	3.50	3.28	2.74	...
Ph-C-N-C^{12}-Ph Dibenzylamine	(12) B + E	...	...	3.75	4.29	4.29	3.81	4.46
Ph-C^{13}-C Ethylbenzene	(13) B + J	...	...	2.64	2.67	2.68	2.58	2.55
N-C^{14}-C Ethylamine	(14) H + J	2.69	3.13	2.63	3.08	3.09	2.72	3.33
N-C^{15}-C^{16}-C	(15) H + M	2.60	3.04	2.57	3.02	2.93	2.66	3.29
n-Propylamine	(16) I + J	1.48	1.72	1.41	1.71	1.70	1.43	1.94
C-C-N-C^{17}-C Diethylamine	(17) F + J	2.65	3.17	2.58	3.10	3.03	2.62	3.26
Ph-C^{18}-N Benzylamine	(18) B + H	3.82	4.25	3.79	4.23	4.18	3.91	4.49
Py-C^{19}-C^{20}-C	(19) A + M	...	3.08	2.74	3.05	3.07	2.77	3.12
2-*n*-Propylpyridine	(20) C + J	...	1.86	1.74	1.84	1.86	1.67	1.88
Ph-C^{21}-C-Ph Bibenzyl	(21) B + N	...	...	2.92	2.93	2.93	2.88	2.81
Py-C^{22}-C^{23}-N	(22) A + I	2.93	3.57	2.89	3.50	3.42	3.04	3.99
2-(2-Aminoethyl)pyridine	(23) C + H	2.93	3.57	2.92	3.55	3.48	3.10	4.04
N-C-C-N-C^{24}-C^{25}-N	(24) K + I	2.72	3.55	2.60	3.49	3.49	2.73	4.18
Diethylenetriamine	(25) H + O	2.72	3.55	2.60	3.49	3.49	2.77	4.27
Py-C^{26}-N-C^{27}-C	(26) A + F	3.86	4.87	3.84	4.67	4.64	3.95	5.13
2-*N*-Ethylpicolylamine	(27) D + J	2.66	3.38	2.63	3.32	3.28	2.68	3.71
Py-C^{28}-N 2-Picolylamine	(28) A + H	3.89	4.73	3.87	4.66	4.59	4.03	5.25
Ph-C^{30}-C^{29}-C	(29) N + J	...	...	1.63	1.64	1.65	1.56	1.55
n-Propylbenzene	(30) B + M	...	...	2.62	2.63	2.62	2.53	2.51

[a] Py denotes the 2-pyridyl group and Ph the phenyl group.

drogens of 2,2′-dipicolylamine on protonation in acetonitrile with TFMSA was also examined. Although the magnitude of the upfield shift from the 1:1 to 2:1 protonation points was the same as that observed for protonation with perchloric acid, the changes in direction of the chemical shifts at the 1:1 and 2:1 points are more abrupt. In addition, with TFMSA the limiting value of the signal of the methylene hydrogens, reflecting complete protonation of the amine, is reached at a lower ratio of acid to amine than with perchloric acid titrant. The shift in the signal of the methylene hydrogens of 2,2′-dipicolylamine on protonation in benzonitrile with TFMSA (Figure 3) is similar to that in acetonitrile.

The chemical shift value of the methylene hydrogens of 2,2′-dipicolylamine on protonation can be accounted for by the following expression:

$$\delta \text{ (methylene hydrogens)} = C_1F_1 + C_2F_2 + C_3F_3 + 1.25 \text{ ppm} \quad (1)$$

C_1 is the chemical shift constant for total protonation of the central aliphatic amine, C_2 and C_3 are the chemical shift constants for total protonation of the pyridyl groups, F_1 is the average fraction of time during which the aliphatic amine is protonated, F_2 and F_3 are the average fraction of time during which the ring nitrogens are protonated, and 1.25 ppm is the normal chemical shift value of methylene hydrogens vs. TMS (7). Equation 1 is based on the assumptions that (a) the deshielding contribution of a particular amine site on protonation is linearly dependent on the fraction of time the site is protonated and (b) the deshielding contributions of different protonated sites are additive. These assumptions have been substantiated (*1, 2, 7, 9*) earlier. If values for appropriate methylenic substituent shielding constants could be determined, the resonance positions of the methylene hydrogens in adjacent charge and separated charge diprotonated 2,2′-dipicolylamine should be calculable. Table I contains the chemical shift values of the methylene hydrogens of a number of appropriate simple compounds in water (D_2O), acetonitrile, and benzonitrile. The shielding constants of interest are those which contribute to the establishment of the chemical shift of the methylene hydrogens for the adjacent charge and separated charge diprotonated 2,2′-dipicolylamine species. For the determination of the shielding constants, the observed chemical shift of the methylene hydrogens less the quantity 1.25 ppm was equated to the sum of the two methylenic substituent shielding constants. A large number of compounds were examined to provide a broad base on which to establish the substituent shielding constants.

The calculated values of the methylenic substituent shielding constants for acetonitrile, benzonitrile, and deuterium oxide media are listed in Table II. These constants were determined by the solution of a system of N equations and N unknowns which was obtained by finding a least squares analysis of M equations and N unknowns. The values in parentheses were reported in earlier work (7).

The chemical shift of the methylene hydrogens of 2,2′-dipicolylamine and its various protonated forms were simulated according to the following schemes.

$$\delta_{\text{Py-C-N-C-Py}} = \delta_{\text{Py-C-N-}_D} + \delta_{\text{Py-}_A} + \delta_{\overset{|}{C}} \quad (2)$$

$$\delta_{\text{Py-C-}\overset{H^+}{\text{N}}\text{-C-Py}} = \delta_{\text{Ph-C-}\overset{H^+}{\text{N}}\text{-}_{EH}} + \delta_{\text{Py-}_A} + \delta_{\overset{|}{C}} \quad (3)$$

$$\delta_{\text{Py-C-}\overset{H^+}{\text{N}}\text{-C-PyH}^+} = \frac{1}{2}\left(\delta_{\text{PyH}^+\text{-C-}\overset{H^+}{\text{N}}\text{-}_{DH}} + \delta_{\text{Py-}_A} + \delta_{\overset{|}{C}}\right) + \frac{1}{2}\left(\delta_{\text{Ph-C-}\overset{H^+}{\text{N}}\text{-}_{EH}} + \delta_{\text{PyH}^+\text{-}_{AH}} + \delta_{\overset{|}{C}}\right) \quad (4)$$

$$\delta_{\text{PyH}^+\text{-C-N-C-PyH}^+} = \delta_{\text{Py-C-N-}_D} + 0.06 + \delta_{\text{PyH}^+\text{-}_{AH}} + \delta_{\overset{|}{C}} \quad (5)$$

$$\delta_{\text{PyH}^+\text{-C-}\overset{H^+}{\text{N}}\text{-C-PyH}^+} = \delta_{\text{PyH}^+\text{-C-}\overset{H^+}{\text{N}}\text{-}_{DH}} + \delta'_{\text{PyH}^+\text{-}_{AH}} + \delta_{\overset{|}{C}} \quad (6)$$

Table II. Methylenic Substituents and Constants

Substituent[a]		Acetonitrile			Benzonitrile		Water (D_2O)	
		Neutral	$HClO_4$	TFMSA	Neutral	TFMSA	Neutral	$HClO_4$
A	Py–	1.43			1.49		1.41	
AH	PyH^+–		1.74	1.73		1.92		1.74
B,BH	Ph–	1.30	1.29	1.30	1.28	1.22	1.33	1.19
C	PyC–	0.42			0.44		0.46	
CH	PyH^+C–		0.55	0.53		0.68		0.52
D	PyCN–	1.24			1.32		1.19	
DH	PyH^+CNH^+–		1.93	1.89		2.39		1.99
E	PhCN–	1.22			1.30		1.21	
EH	$PhCNH^+$–		1.78	1.75		2.03		1.84
F	C–CN–	1.21			1.27		1.19 (1.30)[b]	
FH	$C–CNH^+$–		1.70	1.69		1.97		1.84 (1.90)[b]
G	C–CNC–	0.18			0.18		0.21 (0.20)[b]	
GH	$C–CNH^+C$–		0.48	0.47		0.73		0.44 (0.50)[b]
H	N–	1.23			1.35		1.22 (1.35)[b]	
HH	NH^+–		1.71	1.66		2.06		1.79 (1.85)[b]
I	N–C–	0.13			0.19		0.25 (0.15)[b]	
IH	NH^+C–		0.45	0.44		0.75		0.39 (0.45)[b]
J,JH	C–	0.10	0.10	0.12	0.06	0.02	0.20 (0.05)[b]	0.11
K	NCCN–	1.23			1.30		1.20	
KH	$^+HNCCNH^+$–		1.82	1.84		2.22		1.92
L	PyCCN–	1.25			1.33			
LH	PyH^+CCNH^+–		1.86	1.81		2.26		
M,MH	C–C–	0.08	0.07	0.08	0.03	0.01		
N,NH	PhC–	0.32	0.34	0.33	0.30	0.31		
O	NCCNC–	0.12			0.17			
OH	$^+HNCCNH^+C$–		0.53	0.58		0.93		

[a] Py denotes the 2-pyridyl group and PyH^+ the corresponding protonated species, Ph denotes the phenyl group. [b] Value reported in Ref. 7.

Table III. Chemical Shift Data for the Protonation of 2,2′-Dipicolylamine in Water (D_2O), Acetonitrile, and Benzonitrile (ppm vs. TMS)

	Water (D_2O)[a] $HClO_4$ titrant		Acetonitrile $HClO_4$ titrant		Acetonitrile TFMSA titrant		Benzonitrile TFMSA titrant	
	Obsd	Calcd	Obsd	Calcd	Obsd	Calcd	Obsd	Calcd
Unprotonated	3.85	3.85	3.87	3.92	3.87	3.92	4.01	4.06
Monoprotonated	4.36	4.50	4.33	4.46	4.44	4.33	4.69	4.77
Triprotonated	4.97	4.98	4.88	4.92	4.85	4.87	5.51	5.56
Diprotonated	4.52	Adj[b]	4.42	Adj[b]	4.40	Adj[b]	4.68	Adj[b]
		4.74		4.69		4.65		5.16
		Sep[c]		Sep[c]		Sep[c]		Sep[c]
		4.24		4.29		4.28		4.55
	44% Sep species		68% Sep species		68% Sep species		79% Sep species	
	56% Adj species		32% Adj species		32% Adj species		21% Adj species	

[a] ppm vs. 3-(trimethylsilyl)propane sulfonate. [b] Adjacent charge species. [c] Separated charge species.

The substituent $PhCNH^+-$ was used for $PyCNH^+-$ because only effects owing to complete protonation of the aliphatic amine are desired. The shielding effect of PyH^+CN- cannot be determined experimentally because it requires complete protonation of a weak base, pyridine, in the presence of a stronger base, aliphatic amine. The shielding constant for this substituent was estimated by taking the shielding constant for PyCN– and adding an adjustment increment of 0.06. The adjustment increment was obtained through use of 2-*n*-propylpyridine and 2-ethoxypicoline and their protonated products. Protonation of 2-*n*-propylpyridine caused a downfield shift in the signal of the methylene hydrogens adjacent to the ring of 0.3 ppm and for that of the hydrogens of the methylene group one carbon removed from the ring of 0.1 ppm. The hydrogens of a methylene group two carbons removed from the ring should, therefore, show a downfield change in chemical shift of less than 0.1 ppm. Protonation of 2-ethoxypicoline caused a downfield shift of the signal of the methylenic hydrogens of the ethoxy substituent of 0.1 ppm. From the estimates above of the effect of atoms with lower and higher electronegativities (carbon and oxygen, respectively) than nitrogen on the signal of the hydrogens of a methylene group two positions removed from the 2-position of the protonated pyridine ring, an adjustment increment of 0.06 ppm was selected to be added to the substituent constant for PyCN.

The extent of isomerism of positions in diprotonated 2,2′-dipicolylamine in acetonitrile, benzonitrile, and water (deuterium oxide) is reported in terms of the percentage of adjacent charge and separated charge diprotonated species in Table III. The percentage of separated charge diprotonated 2,2′-dipicolylamine was calculated by comparing the actual resonance position of the methylene hydrogen signal at 2:1 mole ratio of acid to amine with those calculated for adjacent charge and separated charge diprotonated species.

DISCUSSION

Protonation of the aliphatic amine group of 2,2′-dipicolylamine should have a deshielding effect on the hydrogens of both methylene groups, resulting in a downfield shift of the methylenic hydrogen signal. If on diprotonation there is (a) substantial shift in proton density from the aliphatic amine site to the terminal pyridine nitrogen basic site to relieve the strain of electrostatic repulsion and (b) pyridine nitrogen protonation causes little change in the shielding of the methylene hydrogens, there should be an upfield shift of the signal of the methylene hydrogens. Triprotonation should again result in deshielding of the methylene hydrogens and a downfield shift of the signal of the hydrogens of the methylene group should result. The surprisingly clear evidence of this series of shifts in the signal of the methylene hydrogens on protonation of 2,2′-dipicolylamine in water (Figure 1), acetonitrile (Figure 2), and benzonitrile (Figure 3) is noteworthy. In addition, of special interest are the ratios of adjacent charge and separated charge diprotonated 2,2′-dipicolylamine of 6:4 in water, 3:7 in acetonitrile, and 2:8 in benzonitrile. This *definitive* response of the distribution of the two diprotonated species to change in solvent was felt to be possible, but indeed not totally expected. It should be noted that the distribution of adjacent charge and separated charge diprotonated 2,2′-dipicolylamine in acetonitrile which contains water, because of the use of 70% perchloric acid as titrant, is essentially identical with that in acetonitrile with no added water (where TFMSA was the titrant). Because of the pronounced difference in the compatibility of water and benzonitrile as opposed to water and acetonitrile, it was of interest to carry out a protonation–hydration study in benzonitrile in which TFMSA and water (2:1 mole ratio of water to TFMSA) were added, duplicating the conditions if perchloric acid were the titrant. No difference in results was noted from those obtained with only addition of TFMSA. In view of the difficulty encountered in the protonation study in benzonitrile with perchloric acid (70% reagent), the results of this study do, however, raise the matter of possible interaction of the anions perchlorate and trifluoromethane sulfonate with the protonated species of 2,2′-dipicolylamine in benzonitrile. From the electrostatic viewpoint, any interaction of anions with the diprotonated species would be to promote the adjacent charge species over the separated charge, causing the observed ratio of adjacent charge to separated charge (2:8) to be larger than it should be.

For mono-, and adjacent charge and separated charge diprotonated 2,2′-dipicolylamine, intramolecular hydrogen bonding involving the aliphatic amine and a pyridine nitrogen is highly unlikely. This is based on the smaller pK_a of monoprotonated 2-picolylamine in water, 8.70 (*10*), than that of benzylammonium ion, 9.46 (*11*). Internal hydrogen bonding in monoprotonated 2-picolylamine would have resulted in its pK_a being greater than that of the latter acidic species.

Likewise, there does not appear to be the likelihood of internal hydrogen bonding involving the two pyridine nitrogens in adjacent charge diprotonated 2,2′-dipicolylamine. This view is based on the pK_a of monoprotonated 1,2-di(2-pyridyl)propane, 6.15 (*12*), being essentially identical with that of 2-methylpyridine, 6.20 (*13*).

Although only three solvents were used in this study, it is interesting to note that the percentages in the three media of the diprotonated species with the higher energy of electrostatic repulsion, in the order water ≫ acetonitrile >

benzonitrile, correlate with the order of the dielectric character of the solvents [water (D_2O) (dielectric constant, 78) ≫ acetonitrile (dielectric constant, 37.5) > benzonitrile (dielectric constant, 25.2)]. The dipole moments of the three solvents are 1.86, 3.44, and 4.02 D for water (D_2O), acetonitrile, and benzonitrile, respectively, and as a set do not appear to be directly accountable for the variation in the distribution of diprotonated 2,2′-dipicolylamine in the three different media. However, to understand fully the detailed aspects of the nature of this solvent effect, particularly that of hydrogen bond interactions in protic solvents and other specific interactions contributing to the mediation of the charges in the diprotonated species, studies in additional solvent systems will be necessary.

ACKNOWLEDGMENT

We wish to thank the Reilly Tar and Chemical Company for the generous gift of 2,2′-dipicolylamine.

LITERATURE CITED

(1) E. Grunwald, A. Lowenstein, and S. J. Meiboom, *J. Chem. Phys.*, **27**, 641 (1957).
(2) A. Lowenstein and J. D. Roberts, *J. Am. Chem. Soc.*, **82**, 2705 (1960).
(3) J. N. Shoolery, *Varian Tech. Inform. Bull.*, **2**, No. 3 (1959).
(4) G. A. Focier and J. W. Olver, *Anal. Chem.*, **37**, 1447 (1965).
(5) R. C. Larson and R. T. Iwamoto, *J. Am. Chem. Soc.*, **82**, 3239 (1960).
(6) I. Suzuki, *Pharm. Bull., (Tokyo)*, **4**, 211–16 (1956).
(7) J. L. Sudmeier and C. N. Reilley, *Anal. Chem.*, **36**, 1698 (1964).
(8) R. B. Fischer and D. G. Peters, "Quantitative Chemical Analysis", W. B. Saunders Co., Philadelphia, Pa., 1968, p 277.
(9) B. P. Dailey and J. N. Shoolery, *J. Am. Chem. Soc.*, **77**, 3977 (1955).
(10) H. Sigel, P. R. Huber, and R. F. Pasternack, *Inorg. Chem.*, **10**, 945 (1971).
(11) E. Gonick, W. C. Fernelius, and B. E. Douglas, *J. Am. Chem. Soc.*, **76**, 5253 (1954).
(12) H. Buhler and G. Anderegg, *Chimia*, **24**, 433 (1970).
(13) R. J. Bruehlman and F. H. Verhock, *J. Am. Chem. Soc.*, **70**, 1401 (1948).

RECEIVED for review July 21, 1975. Accepted September 12, 1975. The authors gratefully acknowledge the support of the National Science Foundation (Grants GP-11313 and GP-28332).

Rapid Radiochemical Separation of Selected Toxic Elements in Environmental Samples Prior to Gamma Ray Spectrometry

Frank W. Wilshire, Joseph P. Lambert, and Frank E. Butler

Analytical Chemistry Branch, Quality Assurance and Environmental Monitoring Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, N.C. 27711

Toxic elements in a variety of environmental samples are activated by neutron bombardment for subsequent analysis by γ-ray spectrometry. The radioactive isotopes of arsenic, cadmium, mercury, selenium, iodine, and zinc are chemically separated by using the liquid anion exchanger, triisooctylamine, following sample combustion. Significant improvements over currently used procedures are: a radiochemical separation that is quantitative in that all of the products trapped during combustion are contained in one of three sample fractions; an isolation of elements of interest from those elements with interfering γ energies; a procedure that has few chemical manipulations, thus minimizing related errors; a separation that is simple and requires less than 5 minutes to complete following combustion. In addition, the elements contained in liquid samples can be separated directly by liquid anion exchange, eliminating the combustion procedure. The methodology was tested by radiotracer experiments and by analyzing independently tested and certified coal and fly-ash samples.

Increased emphasis has been placed on the measurement of toxic elements in emission sources and ambient air following the formation of the U.S. Environmental Protection Agency (EPA) and the passage of the Clean Air Act of 1970 (*1*). Recently, von Lehmden, Jungers, and Lee investigated toxic elements of interest and compared results from six analytical techniques (*2, 3*). One of these techniques, neutron activation analysis (NAA), is the method discussed here.

A need exists to remove interferences from many samples that are to be sensitively analyzed by γ-ray spectrometry. Pollutants of interest to this laboratory (As, Cd, Hg, Se, U, and Zn) are usually a mixture of volatile compounds in a variety of matrices. The detection of these pollutants is often difficult because of interferences or masking effects. Bromine, present in many environmental samples, was the major interference limiting our NAA capabilities because of the 36-hour half-life and multiple γ rays of the ^{82}Br isotope.

A series of radiochemical separation procedures developed by the National Bureau of Standards (NBS) (*4*) was used to separate these elements of interest. These procedures involve the combustion and subsequent reduction of irradiated samples to remove the more volatile elements from less volatile interferences. Distillates are trapped in a liquid nitrogen-cooled condenser, dissolved in a mixed acid, and precipitated twice as sulfides to remove the bromine interference.

In an effort to minimize time requirements and to ensure against any losses of the desired elements by incomplete sulfide precipitation, the postcombustion radiochemical separation procedure was modified. Space and equipment were other parameters considered, while still maintaining a high level of bromine decontamination.

Experiments were conducted to develop a liquid anion exchange procedure for the separation of the desired radionuclides collected during sample combustion by the NBS procedure. Triisooctylamine (TIOA) diluted to 10% in xylene had been used previously in the rapid radiochemical separation of several of these elements (*5*) and was therefore chosen for this work. The goal then was to develop a rapid separation of the radioactive isotopes of arsenic, cadmium, mercury, selenium, iodine (as an indicator of naturally occurring uranium), and zinc into several fractions containing no appreciable interferences from isotopes emitting unwanted γ energies.