

precipitation takes place from solutions either of α_s -casein alone or α_s - κ -casein mixtures. Over the Ca^{+2}/P range where micelles are present in the absence of any precipitate, interaction of calcium ion with phosphate groups is approaching completion. Over this latter Ca^{+2}/P range, but not before, are also observed altera-

tions in the absorption band of region V. Possibly interactions of calcium ions with group(s) responsible for absorption in this region are involved in micelle formation. Of course, protein-protein interactions or conformational changes not involving calcium ions or organic phosphate groups could be responsible.

2-Pyridone, 2-Pyridthione, and 2-Pyridselenone. Hydrogen-Bonding Ability as Determined by Dipole Moment and Molecular Weight Determinations¹

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The tendency of 2-pyridone, 2-pyridthione, and 2-pyridselenone, simple models of nucleic acid antagonists, to form hydrogen-bonded dimers was investigated by means of dielectric constant and molecular weight measurements in benzene and in dioxane. The propensity of 2-pyridone to dimerize exceeded that of its sulfur and selenium analogs; however, all these compounds must be considered as being powerful hydrogen bonders. The dipole moments of 2-pyridone, 2-pyridthione, 2-pyridselenone and their N-methyl derivatives were investigated. Polarization increased progressively as oxygen of 2-pyridone was replaced by sulfur and selenium.

Numerous heterocyclic sulfur compounds (e.g., 6-mercaptapurine, 2-thiouracil derivatives, 2-thiobarbiturates, penicillin) have found widespread application as medicinal agents. Because the often considerable differences in the biological activities of oxygen-, sulfur-, and selenium-substituted heterocyclic compounds are likely to be related to differences in their strengths of binding to receptor sites, rather than to differences in their abilities to fit receptor sites, a systematic investigation of the hydrogen-bonding and hydrophobic-bonding capacities of oxygen, sulfur, and selenium isologs was undertaken.

Interest in such a study was raised by the report³ that the antineoplastic activity of 6-thioguanine may be related to the ability of this compound to be incorporated into deoxyribonucleic acid (DNA). This observation led to the postulate⁴ that the antitumor action of 6-thioguanine⁵ and 6-selenoguanine^{4,6} might be due to the formation of unusual hydrogen bonds between the sulfur or selenium of the guanine analogs

and the amino group of cytosine, which would be facing these atoms within the double helix of DNA. A similar interaction has been proposed as taking place between the thiocarbonyl group of thionicotinamide and the amino group of adenine in the sulfur analog of dihydrophosphopyridine nucleotide.⁷

Since the complexity and insolubility in organic solvents complicate the study of the bonding tendencies of DNA bases and of their analogs, a series of simple heterocyclic compounds, 2-pyridone, 2-pyridthione, and 2-pyridselenone, was chosen for detailed investigation.

It was shown recently by comparison of the ultraviolet spectra and ionization constants of these compounds with those of their N-methyl, O-methyl, S-methyl, and Se-methyl derivatives that for 2-pyridone,⁸ 2-pyridthione,⁹ and 2-pyridselenone¹⁰ equilibrium was predominantly amidic rather than imidic tautomers. Molecular weight determinations carried out at moderately high concentrations showed 2-pyridselenone¹⁰ to be dimeric in benzene solution. Similarly, 2-pyridone and 2-pyridthione form hydrogen-bonded dimers in benzene¹⁰ and in chloroform.¹¹ To obtain information about the relative abilities of the above compounds to form hydrogen bonds, as well as about the relative degree of polarization of heterocyclic carbamyl, thiocarbamyl, and selenocarbamyl groups, a series of dipole moment and molecular weight measurements in benzene and in dioxane was undertaken.

Experimental

Materials. Reagent grade 2-pyridone was purified by two distillations followed by recrystallization from benzene. A commercial sample of N-methyl-2-pyridone was distilled twice *in vacuo* (b.p. 53.5–54.5 (0.2 mm.)). 2-Pyridthione,¹² N-methyl-2-pyridthione,¹³ 2-

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(3) G. A. Le Page and M. Jones, *Cancer Res.*, **21**, 1590 (1961); G. A. Le Page, *ibid.*, **20**, 403 (1960).

(4) H. G. Mautner and J. J. Jaffe, *Biochem. Pharmacol.*, **5**, 343 (1961).

(5) G. H. Hitchings and C. P. Rhoads, *Ann. N. Y. Acad. Sci.*, **60**, 183 (1954).

(6) H. G. Mautner, S. H. Chu, J. J. Jaffe, and A. C. Sartorelli, *J. Med. Chem.*, **6**, 36 (1963).

(7) S. Shifrin and N. O. Kaplan in "Light and Life," W. D. McElroy and B. Glass, Ed., The Johns Hopkins Press, Baltimore, Md., 1961, p. 145.

(8) A. Albert and J. P. Phillips, *J. Chem. Soc.*, 1294 (1956).

(9) A. Albert and G. B. Barlin, *ibid.*, 2384 (1959).

(10) H. G. Mautner, S. H. Chu, and C. M. Lee, *J. Org. Chem.*, **27**, 3671 (1962).

(11) A. R. Katritzky and R. A. Jones, *J. Chem. Soc.*, 2950 (1960).

pyridselenone,¹⁰ and N-methyl-2-pyridselenone¹⁰ were synthesized according to literature methods and recrystallized until maximum ultraviolet extinction coefficients were achieved. Since the selenium compounds are very susceptible to oxidation they were prepared just prior to use.

Solvents used for dielectric constant and molecular weight measurements were purified as follows. Analytical grade benzene was permitted to reflux over sodium wire for 24 hr. and fractionated prior to use through a 4-ft. vacuum jacketed column packed with glass helices. Dioxane was refluxed over molten sodium for 24 hr. and purified by fractional distillation. This procedure was carried out twice. Just prior to use dioxane was refractionated under nitrogen from a flask containing lithium aluminum hydride.

Dipole Moment Measurements. A WTW Dipolmeter Model DM-01 (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) with a reading sensitivity of 7×10^{-6} was used for all measurements. Two measuring cells, a DFL-1 cell with a capacity of 40 ml. or a DFL-2 cell with a capacity of 4 ml., were employed, depending on the availability and oxidizability of the solute. The cells were provided with thermostated jackets to maintain the temperature of the solutions at $30.00 \pm 0.05^\circ$. The instrument was calibrated periodically against cyclohexane, benzene, and carbon tetrachloride.

For each dipole moment measurement, the dielectric constants were determined for the pure solvent and for a series of six solutions of increasing concentrations; the weight fraction of solute in these varied from 0.0002 to 0.009. The rapid oxidation of 2-pyridselenone solutions in air necessitated the use of an apparatus in which the processes of preparing the solution, of introducing it into the measuring cell, and of measuring the dielectric constant could be accomplished under nitrogen. Ultraviolet spectra were checked before and after each measurement to verify that no oxidation had occurred.

The density at 30.0° of the pure solvent and of each of the solutions was ascertained using a pycnometer with a capacity of ca. 8 ml. Within the experimental error of the measurements, the density-concentration plots were linear over the range measured.

Dipole moments were calculated by the dielectric constant and density extrapolation method of Halverstadt and Kumler¹⁴ and found to be reproducible within ± 0.03 Debyes. Electronic polarizations were obtained from bond refraction values reported by Vogel¹⁵ and Smyth.¹⁶

Molecular Weight Determinations. Molecular weights were determined in benzene and in dioxane using a Mechrolab Model 301 A vapor pressure osmometer (Mechrolab Inc., Mountain View, Calif.). Measurements were carried out at 37.0° over the same concentration range as in the dipole moment determinations.

(12) M. A. Phillips and H. Shapiro, *J. Chem. Soc.*, 584 (1942).

(13) J. Renault, *Bull. soc. chim. France*, 1001 (1953).

(14) I. F. Halverstadt and W. D. Kumler, *J. Am. Chem. Soc.*, **64**, 2988 (1942).

(15) A. I. Vogel, *J. Chem. Soc.*, 514 (1952).

(16) C. P. Smyth, "Dielectric Behavior and Structure," McGraw-Hill Book Co. Inc., New York, N. Y., 1955, p. 409.

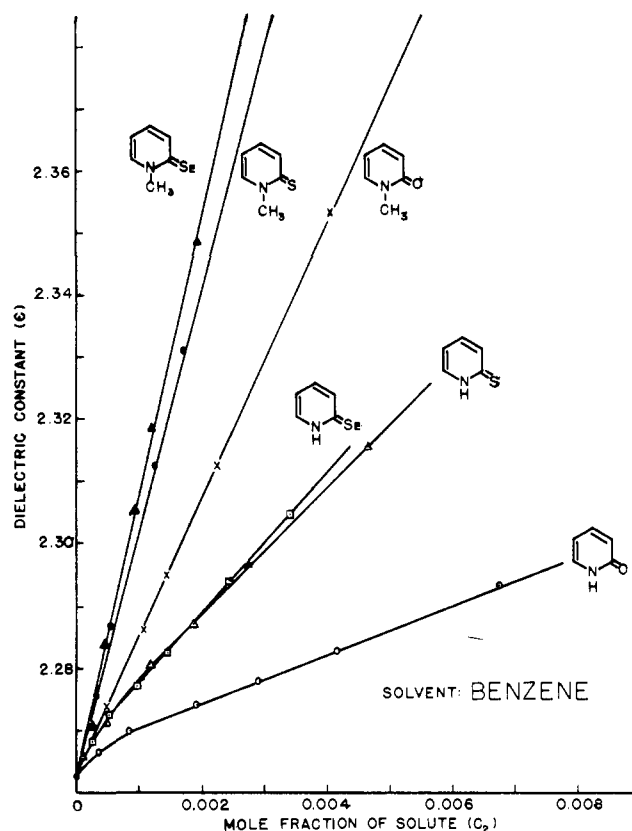


Figure 1. Plot of dielectric constant vs. mole fraction of solute in benzene.

From the molecular weight data association constants (K_{assoc}) were calculated using the following relationship:

$$K_{\text{assoc}} = (C_s - A)/(2A - C_s)^2$$

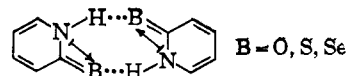
where A = measured molar concentration and C_s = molar concentration based on molecular weight of monomer.

Results

Dimerization Studies. The results of the dielectric constant measurements are shown in Figure 1 and 2. In benzene (Figure 1) plots of dielectric constant vs. mole fraction yielded linear relationships for the N-methyl derivatives. On the other hand, for 2-pyridone, 2-pyridthione, and 2-pyridselenone appreciable deviations from linearity were obtained.

In dioxane (Figure 2) the N-methyl derivatives and 2-pyridthione yielded linear dielectric constant-mole fraction relationships, while the corresponding plot for 2-pyridone showed a slight but significant deviation from linearity. The extreme oxidizability of 2-pyridselenone in dioxane prevented the collection of reliable data for this compound.

The curvature of the dielectric constant-mole fraction plots for the three nonmethylated compounds in benzene and for 2-pyridone in dioxane can be ascribed to the formation of hydrogen-bonded dimers.



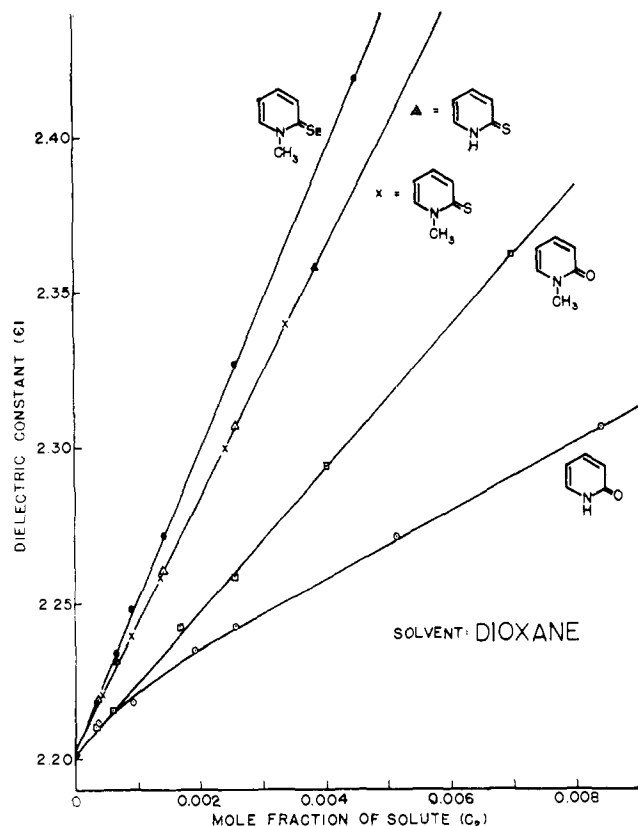


Figure 2. Plot of dielectric constant vs. mole fraction of solute in dioxane.

At very low concentration part of the hydrogen-bonding solute was present in the monomeric form, which has a high dipole moment. As concentration was increased the contribution of the solute to the dielectric constant stemmed increasingly from the considerably smaller, since internally compensated, dipole moment of the hydrogen-bonded dimers. The dipole moments of the N-methylpyridone analogs were unaffected by concentration over the range studied, since these compounds are incapable of dimer formation.

The nonlinearity of dielectric constant-mole fraction plots for amides in benzene has been used previously for the calculation of association constants.^{17,18} However, the pyridone analogs discussed in this paper were found to have a considerably higher tendency to form dimers than the straight-chain amides¹⁷ and aliphatic cyclic amides¹⁸ studied previously. Because of this high association tendency, significant amounts of monomer were present only in very dilute solutions. At these low concentrations the mathematical treatment¹⁹ employed by the above workers became inapplicable due to magnification of relatively minor experimental errors.

It seemed important to establish that association of the above compounds could lead to the formation of dimers, but not, as in the case of aqueous solutions of purine, cytidine, and uridine,²⁰ to the formation of

(17) M. E. Hobbs and W. W. Bates, *J. Am. Chem. Soc.*, **74**, 746 (1952).

(18) R. Huisgen and H. Walz, *Chem. Ber.*, **89**, 2616 (1956).

(19) H. A. Pohl, M. E. Hobbs, and P. M. Gross, *J. Chem. Phys.*, **9**, 408 (1941).

(20) P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, *J. Am. Chem. Soc.*, **85**, 1289 (1963).

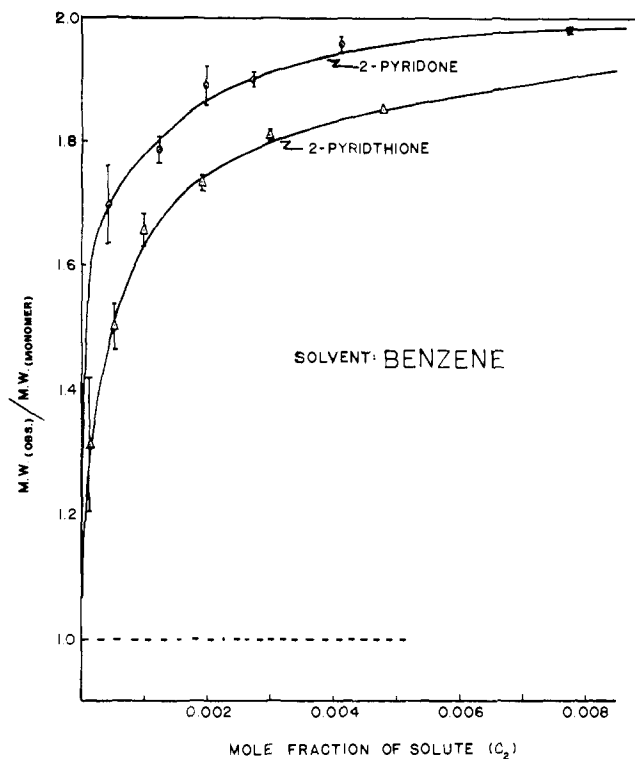


Figure 3. Plot of molecular weight vs. mole fraction of solute in benzene.

polymers. This aim was achieved by molecular weight measurements using a vapor pressure osmometer, carried out in benzene and in dioxane, over the same concentration range as in the dielectric constant determinations noted previously. The data are summarized in Figure 3 and 4.

It can be seen that in none of the cases studied was the molecular weight of the dimer exceeded; thus the formation of polymers through "stacking" can be eliminated. As in the dielectric constant measurements, dioxane was found to interfere more with the formation of hydrogen-bonded dimers than did benzene. The relatively high ability of dioxane, relative to benzene, to interfere with hydrogen bond formation has been noted previously.^{21,22} It is unfortunate that the extremely high oxidizability of 2-pyridoselenone in the equipment available prevented the determination of association constants for this compound.

From the molecular weight determinations the association constants for the formation of the dimers of 2-pyridone and 2-pyridithione could be calculated using the relationship

$$K_{\text{assoc}} = C_d / (C_m)^2$$

Here C_d and C_m are the molar concentrations at equilibrium of dimer and monomer, respectively. It is assumed that the differences between the activities and concentrations of the solutes are negligible. The results shown in Table I were obtained.

The association constant for the dimerization of 2-pyridone is strikingly higher than that for its saturated

(21) W. W. Bates and M. E. Hobbs, *ibid.*, **73**, 2153 (1951).

(22) L. E. Sutton in E. A. Braude and F. C. Nachod, "Determination of Organic Structures by Physical Methods," Academic Press Inc., New York, N. Y., 1955, p. 415.

Table I. Association Constants Derived from Molecular Weight Measurements

Solute	Solvent					
	Benzene			Dioxane		
	Moles/l.	% dimerized	K_{assoc}	Moles/l.	% dimerized	K_{assoc}
2-Pyridone	0.00475	82.2	2,720	0.00410
	0.01392	87.9	2,170	0.01354	9.5	4.3
	0.02229	94.1	6,130	0.02114	18.3	6.5
	0.03060	94.8	5,660	0.03723	34.0	10.5
	0.04648	97.6	18,000 ^a	0.05552	41.9	11.2
	0.08717	99.1	63,100 ^a	0.10242	55.9	14.0
2-Pyridthione	0.00164	47.3	520			
	0.00601	66.9	510			
	0.01120	79.4	830			
	0.02150	84.6	830		Monomeric	
	0.03346	89.4	1,200			
	0.05379	91.8	1,300			

^a Errors in calculation of K_{assoc} are greatly magnified as C_m approaches zero.

analog, 2-piperidone. The dielectric constant data of Huisgen and Walz¹⁸ yield an association constant ($K\gamma$)²³ of 315 for the latter compound in benzene. This value corresponds to a recalculated association constant (K_{assoc}) of 28 as compared to a minimum value of 2200 for 2-pyridone.

2-Pyridthione was found to have a lower ability to form dimers in either benzene or dioxane than its oxygen analog. While several references in the literature cite evidence for hydrogen bonding in thiocarbonyl compounds,^{7,10,11,24-26} little quantitative data regarding such binding appears to exist. It can be seen that in 2-pyridthione sulfur is acting as a powerful hydrogen bond acceptor, inferior to oxygen in 2-pyridone but superior to oxygen in 2-piperidone.

Dipole Moments. The results of the dipole moment studies are summarized in Table II. In either solvent moments increased progressively from N-methylpyridone to N-methyl-2-pyridthione to N-methyl-2-pyridselenone. This finding is in accord with the previously postulated increase in the contribution of charge-separated forms as oxygen in carbonyl groups²⁷ or ester groups²⁸ was replaced by sulfur or selenium. Certainly, the increase in dipole moment on descending the periodic table, as in the chalcogenic phenylureas and 2-phenylsemicarbazides studied previously,²⁷ was much too great to be attributed to changes in molecular size due to the introduction of sulfur and selenium instead of oxygen.

The moment of each member of the N-methylpyridone series was similar whether measured in benzene or in dioxane. This is not surprising since these compounds are incapable of dimerization and, therefore, should not be affected by the relative abilities of solvents to interfere with hydrogen bonding.

In considering the tabulated moments of non-methylated pyridone and its sulfur and selenium ana-

logs, it must be remembered that only the value for 2-pyridthione in dioxane represents the dipole moment of a monomeric compound. The moment of 2-pyridthione is exceeded by that of its N-methyl analog

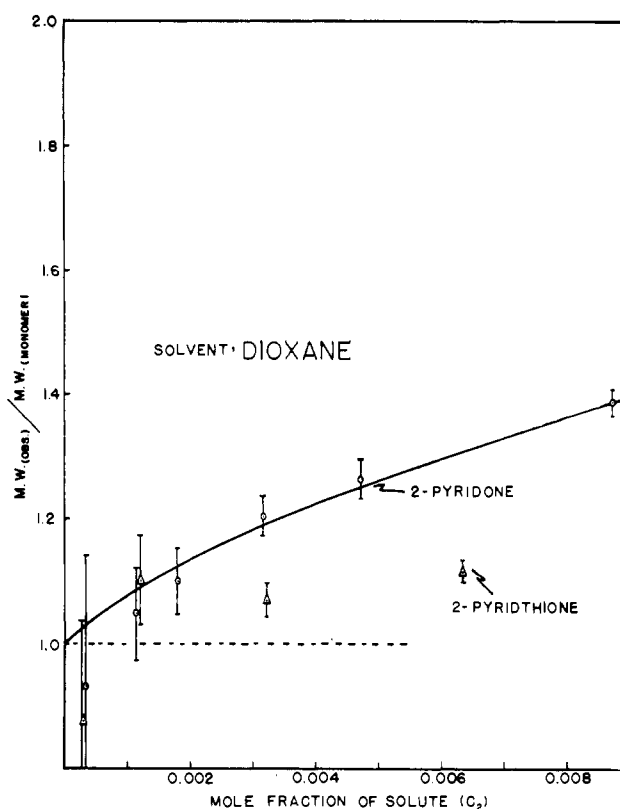


Figure 4. Plot of molecular weight vs. mole fraction of solute in dioxane. Since the difference between observed molecular weight and monomeric molecular weight of 2-pyridthione does not appear to be concentration dependent, this difference may be assumed to be due to oxidation rather than association.

by 0.2 Debyes, a value similar to differences noted previously in comparing the moments of lactams and of their N-methyl derivatives.²⁹

(29) C. M. Lee and W. D. Kumler *ibid.*, 83, 4595 (1961).

(23) $K\gamma = K_{\text{assoc}}/V$ where V (0.0889) equals the molar volume of benzene.

(24) B. R. Penfold, *Acta. Cryst.*, 6, 707 (1953).

(25) M. S. C. Flett, *J. Chem. Soc.*, 347 (1953).

(26) Y. Sheinker, I. Postovskii, and N. M. Voronina, *Zh. Fiz. Khim.*, 33, 302 (1959); *Chem. Abstr.*, 54, 4147 (1960).

(27) H. G. Mautner and W. D. Kumler, *J. Am. Chem. Soc.*, 78, 97 (1956).

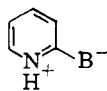
(28) H. G. Mautner, S. H. Chu, and W. H. H. Günther, *ibid.*, 85, 3458 (1963).

In contrast to 2-pyridthione, 2-pyridone in dioxane is partly associated. In benzene all three nonmethylated pyridone analogs are predominantly dimeric. It should be noted that the Halverstadt-Kumler method¹⁴ used in calculating dipole moments is based on the assumption that dielectric constant-weight fraction plots are linear. For those compounds where such plots yielded curved rather than straight lines, dipole moments calculated by this method must be considered to be hybrids of the moment of the monomer and the moment of the dimer. The molecular weight determinations quoted above indicate that the moments of nonmethylated compounds measured in benzene may be considered predominantly as the moments of the hydrogen-bonded dimers.

Even though the molecular weights of the monomers had been used in calculating the dipole moments in benzene as well as in dioxane, the moments of the dimers were found to be surprisingly large. Huisgen and Walz¹⁸ attributed the rather high moments of their dimeric saturated cyclic lactams to the possibility of the rings not being planar and to the possibility of "chain association" by *trans*-amides. These explanations cannot be applied to the pyridone dimers studied here, 2-pyridone and its isologs being flat *cis*-amides. The possibility of more than two solute molecules interacting in benzene within the concentration range studied may be excluded on the basis of the molecular weight determinations reported above which show that the molecular weights of the dimers were approached asymptotically as concentration was increased. The relatively high moments of the dimers of carboxylic acids have been attributed³⁰ to a large atomic polarization. The observation that the moments of dimeric pyridthione and pyridselenone in benzene exceed that of the pyridone dimer by more than 1 Debye would seem to indicate that the high polarizability of sulfur and selenium relative to oxygen plays a part in determining the moment of the dimer as well as in determining the moment of the monomer.

It seems worth noting that while 2-pyridone will dimerize in dioxane, its saturated analog, 2-piperidone, is monomeric in that solvent. For that reason the calculated dipole moments of 2-pyridone and N-methyl-2-pyridone in dioxane differ by 1.13 Debyes, while N-methylation of 2-piperidone increases the moment in that solvent by only 0.2 Debyes.²⁶

Since 2-pyridone and its sulfur and selenium analogs are known to exist primarily in the lactam form, the increase in dipole moment from the oxo to the thio to the seleno compound is presumably related to increasing contributions of the polarized forms



(30) I. E. Coop, N. R. Davidson, and L. E. Sutton, *J. Chem. Phys.*, **6**, 905 (1938).

This factor has been used to account for the observation that 2-pyridselenone is a stronger acid than 2-pyridthione, which, in turn, is a stronger acid than 2-pyridone.^{9,10} However, the increasing contribution of charge-separated forms which may be related to the possibility of octet expansion in sulfur and selenium compounds does not increase the relative ability of sulfur and selenium to be protonated. Measurements carried out in sulfuric acid solution showed 2-pyridone to be a stronger base than its chalcogenic analogs.⁸⁻¹⁰ The observation that 2-pyridthione and 2-pyridselenone, in spite of the high polarization of the $-N^+H=C(B^-)$ -grouping, evidenced by dipole moment measurements and potentiometric titrations, are poorer bases and poorer hydrogen bonders than 2-pyridone is interesting.

Table II. Dipole Moments at 30° (in Debyes)

	B	Dioxane	Benzene
	O	(2.94) ^{a,b}	(1.73) ^{a,b}
	S	5.29	(2.78) ^b
	Se	...	(2.87) ^b
	O	4.07	4.04
	S	5.49	5.26
	Se	5.92	5.73

^a Literature values⁸ of 2.95 and 1.95 have been reported for 2-pyridone in dioxane and benzene, respectively. ^b Values in parentheses represent the moments of appreciably dimerized solute.

A recent paper³¹ discussed the interaction of "hard" and "soft" acids and bases and made the generalization that "hard" acids bind strongly to "hard" bases and "soft" acids bind strongly to "soft" bases. The observation that sulfur and selenium in the 2-position of pyridine, although highly polarized, have a weaker ability to accept hydrogen bonds or protons ("hard acids") than does oxygen makes it convenient to think of sulfur and selenium in compounds of this type as having a greater "soft base" and a smaller "hard base" character than oxygen. This idea is reinforced by the finding that thio- and seleno-substituted heterocyclic compounds exhibit greater chelating abilities for "soft" metals³² and show anomalously greater solubilities³³ in "soft" organic solvents than their oxygen analogs.

It is tempting to speculate that the highly polarized sulfur and selenium in thio- and selenopyrimidines^{6,34} and -purines³⁴ might confer a greater ability to form hydrophobic bonds to such compounds than their oxygen analogs possess.

Acknowledgments. We are indebted to Professor William von E. Doering for the generous long-term loan of his dipole meter and to Dr. Walter Lwowski for making available an osmometer.

(31) R. G. Pearson, *J. Am. Chem. Soc.*, **85**, 3533 (1963).

(32) H. G. Mautner, *ibid.*, **78**, 5292 (1956).

(33) H. G. Mautner and E. M. Clayton, *ibid.*, **81**, 6270 (1959).

(34) H. G. Mautner and G. Bergson, *Acta Chem. Scand.*, **17**, 1694 (1963).