with ammonium hydroxide. The product was extracted with chloroform and distilled, b.p. 220–225° (2 mm.), 25 g. (80%).

Anal. Calcd. for $C_{23}H_{29}N$: C, 86.47; H, 9.15; N, 4.38. Found: C, 86.17; H, 9.24; N, 4.36.

The hydrochloride, after recrystallization from ethanol–ether, melted at 225–227°.

Anal. Calcd. for $C_{23}H_{29}N \cdot HCl$: C, 77.61; H, 8.43. Found: C, 77.77; H, 7.63.

Chromatography of 10 g. of the free base in 75 ml. of pentane was carried out in a 25 × 900-mm. column packed with 300 g. of aluminum oxide. Fractions of approximately 200 ml. were collected (Table III).

TABLE III

Eluent	Fraction	Compd.	Yield, g.	M.p., °C.
Benzene(10%)–pentane	6–13	V	2.4	93–95
	14–20		Trace	
Ether(50%)–pentane	21–24	Isomer A	2.1	Oily solid
Ether(75%)–pentane	25–32	Isomer B	3.5	96–99

Compound V, after recrystallization from petroleum ether, melted at 100–102°. A mixture melting point with an authentic sample prepared above showed no depression.

The hydrochloride salt of isomer A was prepared, m.p. 274–275°.

Anal. Calcd. for $C_{23}H_{29}N \cdot HCl \cdot 0.5H_2O$: C, 75.68; H, 8.49; N, 3.83. Found: C, 75.22; H, 8.43; N, 4.32.

The salt was converted into the free base of isomer A, m.p. 90–91°, from hexane.

Anal. Calcd. for $C_{23}H_{29}N$: C, 86.47; H, 9.15; N, 4.38. Found: C, 86.76; H, 8.92; N, 4.29.

Isomer B was recrystallized from petroleum ether, m.p. 100–101°.

Anal. Calcd. for $C_{23}H_{29}N$: C, 86.47; H, 9.15; N, 4.38. Found: C, 86.80; H, 9.24; N, 4.09.

The hydrochloride salt was recrystallized from ethanol–ether, m.p. 272–273°.

Anal. Calcd. for $C_{23}H_{29}N \cdot HCl$: C, 77.60; H, 8.43; N, 3.90. Found: C, 77.35; H, 8.82; N, 4.06.

Reduction of IV in Ethanol at 60°.—The same procedure as above was used except that the reduction was carried out at 60–65°, b.p. 190–200° (2 mm.). The product did not show any absorption in the ultraviolet. Ten grams of this compound was chromatographed on 300 g. of alumina and fractions of approximately 180 ml. were collected (Table IV).

TABLE IV

Eluent	Fraction	Compd.	Yield, g.	M.p., °C.
Ether(10%)–pentane	13–28	Isomer A	2.8	81–87
	29–51	Mixture	2.1	Oily solid
Ether(15%)–pentane	52–57		Trace	Oil
Ether(20%)–pentane	67–69	Isomer B	2.4	HCl salt, 266–271
Ether	70–85	Isomer B	1.4	95–99

Reduction of IV in Acetic Acid.—Twenty-four grams (0.073 mole) of IV in 200 ml. of glacial acetic acid was hydrogenated in a Parr hydrogenator in the presence of 2 g. of platinum oxide catalyst at 4.2 kg./cm.² pressure at 55–60° for 20 hr. The catalyst was filtered and the solvent was removed on a steam bath *in vacuo*. Dilute (10%) hydrochloric acid (300 ml.) was added and the solution was extracted with ether. The acid solution was made basic with ammonium hydroxide, extracted with chloroform, and the residue after removal of the solvent was distilled, b.p. 185–200° (1 mm.), 18.6 g. (81%). Evaporation of the ether solution gave 2.1 g. (11%) of 5-cyclohexyl-10,11-dihydro-5H-dibenzo[*a,d*]cycloheptene, m.p. 76–79°, from petroleum ether.

Anal. Calcd. for $C_{21}H_{24}$: C, 91.25; H, 8.75. Found: C, 91.20; H, 8.90.

A Viscometric Study of Hydrogen-Bonding Properties of Carcinogenic Nitrosamines and Related Compounds[1]

MARY F. ARGUS, JOSEPH C. ARCOS, ASHRAFUL ALAM, AND JERRELL H. MATHISON

Seamen's Memorial Research Laboratory, U. S. Public Health Service Hospital, New Orleans, and Department of Medicine (Biochemistry), Tulane University, New Orleans, Louisiana

Received December 9, 1963

The viscosity of the hepatic carcinogens, dimethylnitrosamine and dioxane, in binary mixture with either water or propionic acid, shows a very large increase above the values of ideal mixing. No such increase is observed in the absence of a proton donor, as with mixtures of dimethylnitrosamine–dioxane, dimethylnitrosamine–propionic anhydride, or dimethylnitrosamine–benzene. The noncarcinogenic 1,1-dimethylhydrazine is considerably more potent in forming hydrogen bonds with water or propionic acid as measured by the extent of maximum viscosity increase. However, while the viscosity of dimethylnitrosamine–water is independent of the pH, neutralization of both basic groups in the hydrazine compound, as shown by its titration curve, causes considerable decrease in the viscosity of its water solutions. Hydrogen bonding with propionic acid approximately parallels carcinogenic activity of a small series of nitrosamines and dioxane. The carboxyl group of proteins appears to be the main participant in the hydrogen bonding of nitrosamines in the process of protein denaturation by these compounds. Hydrogen bonding with nitrosamines is through the nitroso oxygen and involves displacement of the amino electron doublet, resulting in lack of basicity of the amino nitrogen. Aryl substituents decrease hydrogen bonding by redirecting the displacement of the doublet.

Interaction with functional groups that partake in intramolecular hydrogen bonding is commonly assumed to be involved in the mechanism of denaturation of proteins and nucleic acids by chemical agents. The carcinogenic N-nitrosodialkylamines,[2–5] N-nitroso-

(1) This investigation was supported by the U. S. Public Health Service Research Grants CA-05793 and CA-05431.

(2) P. N. Magee and J. M. Barnes, *Brit. J. Cancer*, **10**, 142 (1956).

(3) M. F. Argus and C. Hoch-Ligeti, *J. Natl. Cancer Inst.*, **27**, 695 (1961); **30**, 533 (1963).

(4) H. Druckrey, R. Preussmann, D. Schmähl, and M. Müller, *Naturwiss.*, **48**, 134 (1961); **49**, 19 (1962).

(5) H. Druckrey and R. Preussmann, *ibid.*, **49**, 111 (1962).

piperidine,[4] acetamide,[6] N-acyldialkylamines,[7] thioacetamide,[8] thiourea,[8,9] ethylcarbamate,[10,11] and dioxane,[7] that have been termed "hydrogen bond reactors,"[12a] were actually shown to be powerful agents of protein denaturation.[13,14] Urethane and the hydrogen-bonding carcinogenic aminophenols,[12b] 2-amino-1-naphthol and 1-amino-2-naphthol, were shown to produce lowering of the thermal denaturation temperature of DNA.[15,16] The effect of carcinogenic azo dyes on the conformation of proteins[17] and the enhancement of heat denaturation of DNA by carcinogenic polycyclic hydrocarbons[18] have been recently reported, although these interactions may involve valence forces other than hydrogen bonding.[12c,19] These findings are in line with the hypothesis, set forth by Rondoni as early as 1938,[20] that carcinogenesis and the denaturation of proteins are closely related processes; this hypothesis may now be extended to include nucleic acids.

The increase of viscosity of certain binary liquid mixtures has been known for some time, *e.g.*, ref. 21–23, and has been attributed to the formation of hydrogen-bonded molecular associations extending over many molecules.[24a] Because of these transient polymeric chains, radial distribution calculations may not be applied to hydrogen-bonded liquids. Furthermore, hydrogen bonding raises the mutual potential energy of pairs of molecules passing in contact with one another. For these reasons the viscosity of no hydrogen-bonded liquid is at the present time accessible to rigorous mathematical treatment.[25] Nevertheless the viscometry of liquid mixtures has been used recently by Bello and Bello[26] as an effective practical tool for studying the enhancement by Li^+ of hydrogen bonding between monomethyl- and dimethylacetamide and water.

In the present report the nature of viscosity increase of nitrosamine carcinogens in mixture with certain solvents has been investigated, the interaction of dimethylnitrosamine with amino acid side-chain models has been studied, and the comparative hydrogen bonding ability of various nitrosamines and related agents has been measured. The study of the pH dependence of viscosity of dimethylnitrosamine, 1,1-dimethylhydrazine, and related amines, and of the titration curve of these amines permitted delineation of the molecular site of hydrogen bonding and of the role of the amino doublet in hydrogen bonding with nitrosamine carcinogens.

Materials and Methods.—All compounds used, except N-methylindole,[27] were commercially available reagent grade products and were used without further purification. Binary or ternary mixtures were prepared in the proportions indicated in the figures and exactly 1-ml. volumes were used in all viscosity determinations. Viscosities were measured at 30, 45, or 65° with Cannon–Manning[28] Ostwald-type semimicro viscometers (sizes 50, 75, 100, and 150) in a constant temperature (±0.02°) water bath using a "Bronwill" circulator. The kinematic viscosity (ν) values obtained were plotted against the volume per cent of the mixtures. Viscosities representing ideal mixing were defined as the values along a straight line connecting the viscosity values of two single components.[29] Because of the nature of the conclusions drawn from this investigation it was not considered necessary to determine the densities of the various liquid mixtures for calculation of the absolute viscosities. Potentiometric titrations were carried out at 23–25° with a Beckman G pH meter fitted with a glass electrode and a calomel reference electrode. All values in this report are averages of four to six determinations.

Results and Discussion

Viscosity Increase and Hydrogen Bonding With Dimethylnitrosamine (DMN).—Figure 1 indicates that DMN is a potent agent to form hydrogen bond-linked molecular aggregates with both water and propionic acid, as shown by the large increase of viscosity above the values of ideal mixing. The maximum increase with dioxane–water is close to twice as large as with DMN–water which may be due to the presence in dioxane of two electron-donor oxygen atoms. That the interaction responsible for the viscosity increase is due to hydrogen bonding is shown by the observation that DMN and dioxane, both potent in forming hydrogen bonds with proton donors, fail to give increase of viscosity upon mixing. Actually, the curve

(6) B. Jackson and F. I. Dessau, *Lab. Invest.*, **10**, 909 (1961).
(7) C. Hoch-Ligeti and M. F. Argus, unpublished results.
(8) O. G. Fitzhugh and A. A. Nelson, *Science*, **108**, 626 (1948).
(9) A. Rosin and H. Ungar, *Cancer Res.*, **17**, 302 (1957).
(10) A. Nettleship, P. S. Henshaw, and H. L. Meyer, *J. Natl. Cancer Inst.*, **4**, 309, 523 (1943).
(11) C. D. Larsen, *ibid.*, **7**, 5(1946–1947); **8**, 99(1947–1948).
(12) J. C. Arcos and M. Arcos in "Progress in Drug Research," Vol. IV, E. Jucker, Ed., Interscience, New York, N. Y., 1962: (a) p. 505; (b) p. 468; (c) pp. 439, 485.
(13) M. F. Argus, C. J. Leotze, and J. F. Kane, *Experientia*, **17**, 357 (1961).
(14) M. F. Argus, J. C. Arcos, J. H. Mathison, and A. Alam, submitted for publication.
(15) A. M. Kaye, *Biochim. Biophys. Acta*, **61**, 615 (1962).
(16) S. Bellman, E. Levine, and W. Troll, *Federation Proc.*, **21**, 374 (1962).
(17) C. Watters and A. Cantero, *Proc. Am. Assoc. Cancer Res.*, **3**, 370 (1962).
(18) E. Boyland and B. Green, *Biochem. J.*, **87**, 14P (1963).
(19) J. C. Arcos and M. Arcos, *Arzneimittel-Forsch.*, **8**, 486, 643 (1958).
(20) P. Rondoni in "Advances in Cancer Research," Vol. 3, J. P. Greenstein and A. Haddow, Ed., Academic Press, New York, N. Y., 1955, p. 171.
(21) S. English and W. E. S. Turner, *J. Chem. Soc.*, **105**, 1656 (1914).
(22) N. Madsen, *Science*, **114**, 500 (1951).
(23) P. Pascal, "Chimie Générale," Vol. III, Masson & Co., Paris, 1951, p. 76.
(24) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," W. H. Freeman & Co., San Francisco, Calif., 1960: (a) p. 61; (b) p. 201.
(25) A. Bondi in "Rheology," Vol. I, F. R. Eirich, Ed., Academic Press, Inc., New York, N.Y., 1956, p. 321.
(26) J. Bello, and H. R. Bello, *Nature*, **190**, 440 (1961).
(27) Custom synthesis by the Chemicals Procurement Laboratories, College Point 56, N. Y.
(28) Cannon Instrument Co., State College, Pa.
(29) No satisfactory formula relating the viscosity of binary liquid mixtures to the composition of the mixture and the viscosities of the two components has been obtained as yet.[30] This is especially the case for mixtures of markedly dissimilar molecular species and/or when the two components interact with one another causing departure from ideality. Certain mixture fluidity equations[30,31] are in good agreement with selected experimental data; however, it must be emphasized that the correction term applied was arrived at empirically and therefore the observed correlation between calculated and experimental values is not entirely unexpected.[31] While it is true that several binary systems are known where viscosity is not a simple additive property, the assumption that a negative deviation relative to the line representing the ideal linear mixture law increases as the difference of the viscosities of the two components increases[32a] has not been substantiated in later work,[32b] and does not appear to be supported by viscosity data reported herein. It may be concluded, consequently, that while the positive increase of viscosity in certain binary mixtures can be accounted for by the formation of molecular associations,[24a] the particular parameter(s) of viscosity determining negative deviation from the linear mixture law has not been clearly determined. It is suggested tentatively that in certain binary systems where the molar volume of the two components (containing centers of high electron density) is not very different, marked negative deviation may be attributed to repulsion between the two molecular species. Moreover, the effect of such repulsion will necessarily enter into the correction term of the mixture fluidity equation[30,31] since the "excess free energy of mixing" is calculated from the vapor pressures of the mixtures.
(30) R. E. Powell, W. E. Roseveare, and H. Eyring, *Ind. Eng. Chem.*, **33**, 430 (1941).
(31) S. Glasstone, K. J. Laidler, and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941, pp. 516–517.
(32) (a) J. Kendall and K. P. Monroe, *J. Am. Chem. Soc.*, **39**, 1787 (1917); (b) *ibid.*, **39**, 1802 (1917).

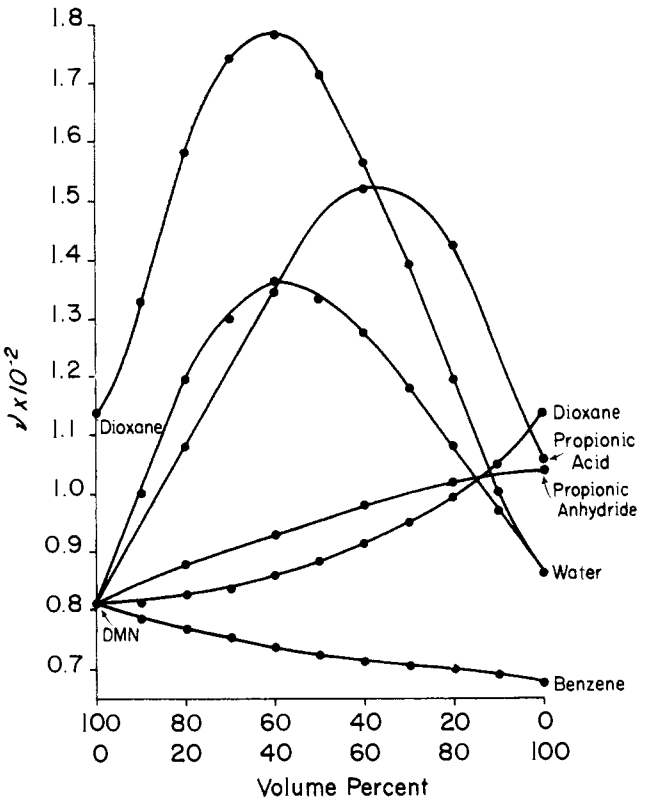


Fig. 1.—Kinematic viscosities of binary mixtures of dimethylnitrosamine (DMN) with water, propionic acid, propionic anhydride, dioxane, or benzene. The end points of the curves represent the viscosities of the single compounds. The proportions of the mixtures are given in volume per cent on two opposite scales in the abscissa. All determinations were carried out at 30°.

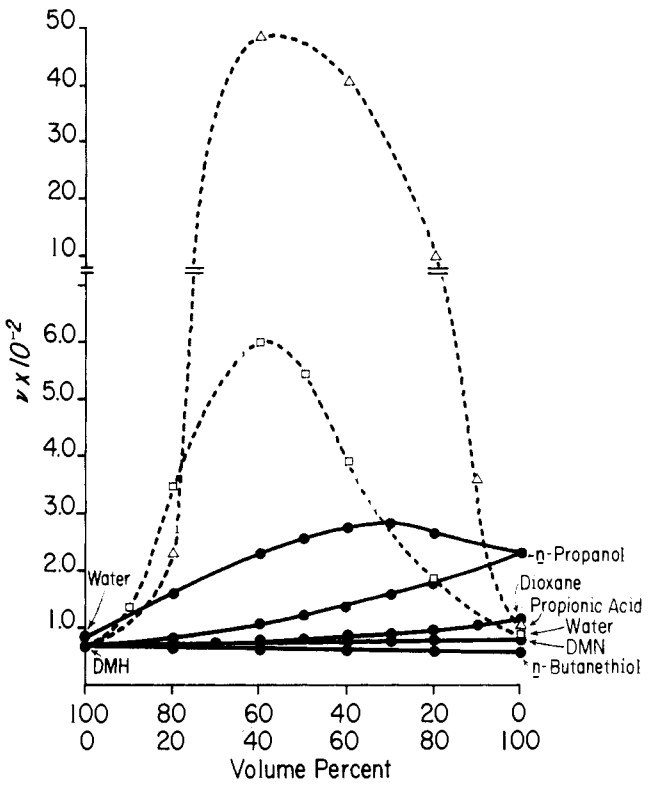


Fig. 2.—Kinematic viscosities of binary mixtures of 1,1-dimethylhydrazine (DMH) with water, propionic acid, DMN, 1-propanol, 1-butanethiol, or dioxane. The end points of the curves represent the viscosities of the single compounds. The proportions of the mixtures are given in volume per cent on two opposite scales in the abscissa. All determinations were carried out at 30°.

of viscosity change in the latter mixtures shows an appreciable negative increment of viscosity relative to the values of ideal mixing.[29]

That hydrogen bonding is responsible for the viscosity increase is further supported by the fact that "elimination" of the proton as in DMN–propionic anhydride mixtures causes almost total disappearance of the previously observed large viscosity increase. The values slightly higher than viscosities of ideal mixing may likely be due to the presence of small amounts of propionic acid. Similarly, there is no change or very small negative increment of viscosity relative to the values of ideal mixing with mixtures of DMN and benzene, indicating absence of appreciable interaction between the two components.

Hydrogen Bonding Ability of 1,1-Dimethylhydrazine (DMH).—Figure 2 shows mixture viscosity curves of DMH, the reduced, noncarcinogenic derivative of DMN. The figure indicates that replacement of the nitroso by an amino substituent does not lead to lesser viscosity increase when in mixture with either water or propionic acid, but on the contrary gives a considerably larger increase of viscosity above the values of ideal mixing than does DMN. In DMH–water and DMH–propionic acid mixtures DMH appears to be an exclusively electron-donating partner. This is suggested by the absence of hydrogen bonding as indicated by no viscosity change with mixtures of DMH–DMN and by negative viscosity increment with mixtures of DMH–dioxane. The absence of positive deviation from the values of ideal mixing with DMH–1-propanol

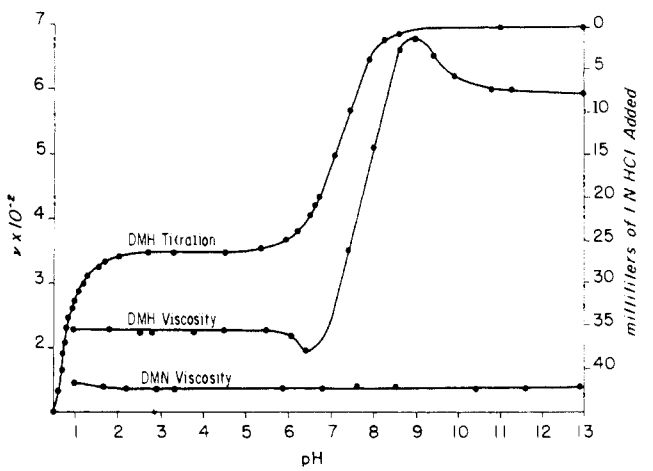


Fig. 3.—The pH dependence of kinematic viscosity of dimethylnitrosamine (DMN)–water and 1,1-dimethylhydrazine (DMH)–water mixtures as compared to the potentiometric titration curve of DMH. For the viscosity measurements (at 30°) the volume proportions were 60% DMN or DMH, and 40% water. This is the proportion for both mixtures to obtain maximum increment of viscosity above the values of ideal mixing (see Fig. 1 and 2). The pH was adjusted with HCl for DMH, and with HCl and NaOH for DMN, and account was taken of the water thus introduced when establishing the final proportion. Titration of DMH (2 ml. in 50 ml. of water) was carried out potentiometrically with 1 *N* HCl.

mixtures is unexpected and may be interpreted that the H–O···H–O hydrogen bonding in 1-propanol alone is energetically more favored than O–H···N hydrogen bonds in the mixtures. Mixtures of 1-propanol

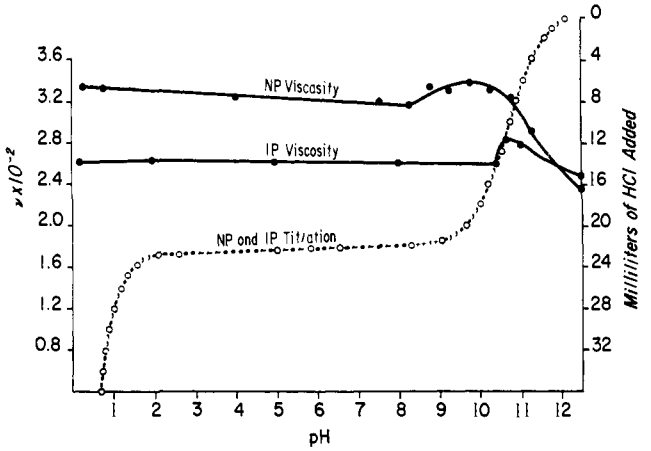
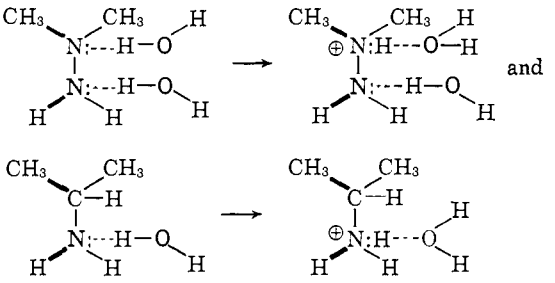


Fig. 4.—The pH dependence of kinematic viscosity of *n*-propylamine (NP)–water and isopropylamine (IP)–water mixtures as compared to the potentiometric titration curve of the amines. For the viscosity measurements (at 30°) the volume proportion was 60% of either amine and 40% water. This is the proportion to obtain maximum increments of viscosity above the values of ideal mixing, as established in experiments not presented in the figures. These experiments showed that at 30° the viscosity of NP singly was 0.52 centistokes and the viscosity of IP singly 0.45 centistokes. The maximum viscosity increment above the value of ideal mixing was 1.69 centistokes for NP–water mixtures and 1.89 centistokes for IP–water mixtures, at pH 12.5. The pH was adjusted with HCl and account was taken of the water thus introduced when establishing the final proportion. Titration of each amine (2 ml. in 50 ml. of water) was carried out potentiometrically with 1 *N* HCl.

water (Fig. 2) and of 1-propanol–propionic acid (not shown here) gave, on the other hand, viscosities appreciably higher than the values of ideal mixing. These results are consistent with observations indicating that for maximum stability of intermolecular hydrogen bonding between two different molecular species, the "bridgehead" atoms must have similar proton affinities (*i.e.*, electronegativities).[33,34] If the "bridgehead" atom of the proton donor partner is much less electronegative than the "bridgehead" atom of the electron-donor partner (*e.g.*, 1-propanol–DMIH mixtures), then polymolecular association composed of molecules of the proton donor alone will predominate; if, on the other hand, the relative electronegativity of the proton-donor "bridgehead" is high (*i.e.*, strong acids), onium-type salt linkages will be established.[33]

Effect of pH on Viscosity; the Molecular Site of Hydrogen Bonding.—Figures 3 and 4 show the pH-dependence of "maximum viscosity" water solutions of DMIH, DMIN, *n*-propylamine, and isopropylamine, and the potentiometric titration curves of DMIH, *n*-propylamine, and isopropylamine. Comparison of the DMIH titration and DMIH viscosity curves shows that high viscosity is maintained to the end of the neutralization of the dimethylamino group; then viscosity steeply decreases between about pH 8.6 and 6.6, leveling off at the neutralization of the primary amino group. The viscosity of isopropylamine is, on the other hand, almost completely independent of the pH. Totally neutralized DMIH has a viscosity comparable to that of isopropylamine. The high viscosity of non-neutralized DMIH–water mixtures is consequently due to the strong bifunctional hydrogen-bonding ability of the base. Neutralization in DMIH of only the dimethylamino group, or neutralization of isopropylamine, does not drastically affect the respective viscosities since the onium group may establish the same number of hydrogen bonds as the amino group from which it was formed.



The small viscosity peak toward the end of neutralization of the dimethylamino group in DMIH and toward the end of neutralization of isopropylamine (and *n*-propylamine) may be ascribed to somewhat greater hydrogen-bond strength in the water–onium than in the water–amine associations (*cf.* ref. 35). That addition of acid beyond the point of neutralization causes disappearance of these viscosity peaks may be the result of competition by excess acid for hydrogen bonding.

The observation that neutralization of the primary amino group in DMIH causes a drastic decrease of viscosity and establishment of a viscosity level comparable to neutralized isopropylamine indicates that total neutralization causes loss of bifunctionality of hydrogen bonding by DMIH. This in turn indicates steric hindrance of the formation of double onium-type, hydrogen-bonded, molecular associations.

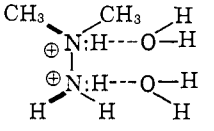


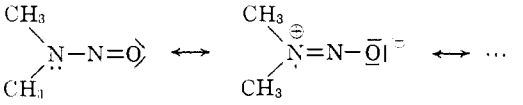
Figure 3 shows furthermore that the viscosity of the DMIN–water mixture is independent of the pH. The dimethylamino group in DMIN in aqueous solutions is totally devoid of basic properties, but the formation of crystalline DMIN·HCl by introducing dry hydrogen chloride in concentrated ether solution of DMIN has been described.[36] Carbon tetrachloride and benzene have now been found to be highly favorable for the formation of the hydrochloride from dilute solutions, but dioxane, acetonitrile, and propionic acid are increasingly inhibitory in that order. All these observations suggest that the viscosity increase of DMIN–water and DMIN–propionic acid mixtures is due to hydrogen bonding *via* the electron doublet(s) on the nitroso oxygen and not on the amino nitrogen as is the case with DMIH. The hydrogen-bonding ability of the nitroso oxygen is further enhanced by the increased electron density due to displacement of the amino nitrogen electron doublet in polar solvents and predominance or stabilization of the polar resonant limit structure.

(33) B. Eistert, "Tautomérie et Mésomérie," Presses Universitaires de France, Paris, 1949, p. 169.

(34) J. C. Dearden and W. F. Forbes, *Can. J. Chem.*, **38**, 896 (1960).

(35) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1960, p. 452.

(36) E. Renouf, *Ber.*, **13**, 2170 (1880).



The importance of electron-doublet displacement toward the nitroso oxygen for hydrogen bonding with nitrosamines is further supported by results shown in Fig. 5. The figure shows the maximum increments

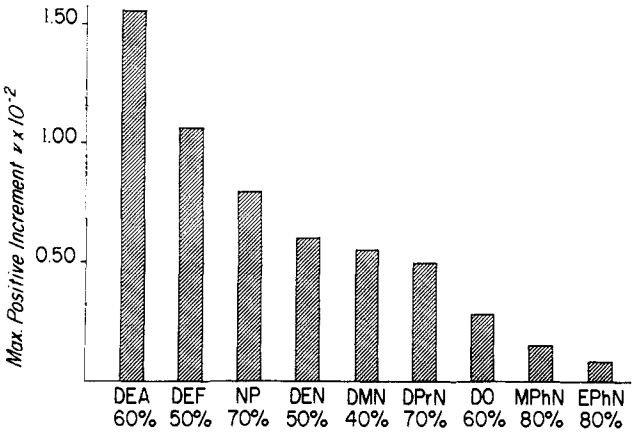
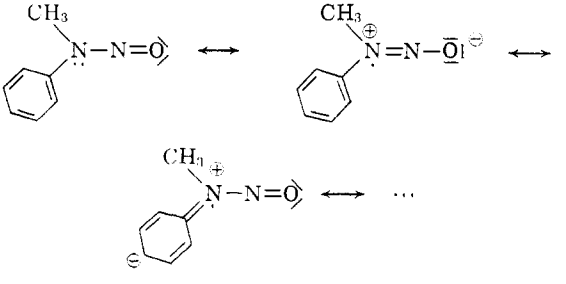


Fig. 5.—Maximum increments of viscosity above the values of ideal mixing in binary mixtures of propionic acid with diethylacetamide (DEA), diethylformamide (DEF), N-nitrosopiperidine (NP), diethylnitrosamine (DEN), dimethylnitrosamine (DMN), di-*n*-propylnitrosamine (DPrN), dioxane (DO), methylphenylnitrosamine (MPhN), or ethylphenylnitrosamine (EPhN). The percentage values in the figure indicate the volume fractions at which the respective agents gave the maximum positive viscosity increments in mixture with propionic acid. Viscosity determinations were carried out at 30°.

of viscosity above the values of ideal mixing in binary mixtures of propionic acid with diethylacetamide, diethylformamide, N-nitrosopiperidine, diethylnitrosamine, DMN, di-*n*-propylnitrosamine, dioxane, methylphenylnitrosamine, and ethylphenylnitrosamine. It may be seen that, when an alkyl group in di-*n*-alkylnitrosamines is replaced by a phenyl, hydrogen-bonding ability is considerably decreased because of participation of the amino doublet in the resonance of the aromatic substituent.



Interaction of Dimethylnitrosamine with Model Compounds for Amino Acid Side Chains.—Viscosities of binary mixtures of DMN with agents which may be considered as model compounds for amino acid side chains have been investigated to delineate the functional groups which may be interacted with by means of hydrogen bonding in protein denaturation. Evidence for strong hydrogen bonding between the carboxyl group and nitrosamines, and for absence of interaction between benzene (for phenyl) and DMN, has been given above (Fig. 1 and 5). Figure 6 shows

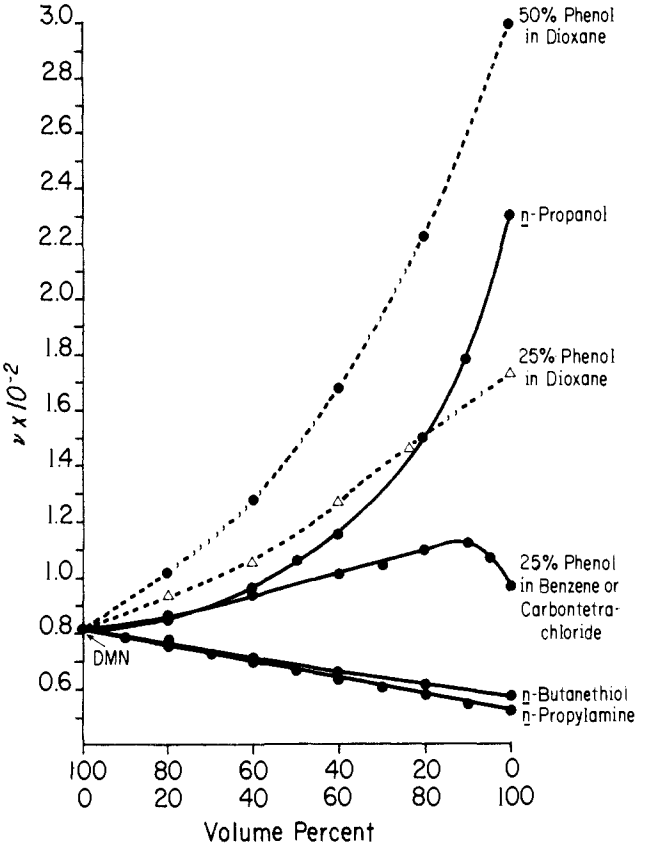


Fig. 6.—Kinematic viscosities of mixtures of dimethylnitrosamine (DMN) with compounds representing amino acid side chains. Viscosity determinations were carried out at 30°. The end points of the curves represent the viscosities of the pure compounds or their concentrated solutions as indicated. The proportions of the mixtures are given in volume per cent on two opposite scales in the abscissa.

that there is no change of viscosity relative to the values of ideal mixing with mixtures of DMN–*n*-propylamine and DMN–1-butanethiol, and a large negative increment with 1-propanol. The absence of interaction of DMN (and of DMH, see Fig. 2) with 1-butanethiol is consistent with the conclusion of a number of references that thiols are very weak proton donors and do not form hydrogen bonds in many systems (see ref. 24b). Phenol in 25% solution in benzene or in carbon tetrachloride gives a well-demonstrable positive viscosity increase. However, assayed in dioxane, or as pure phenol at 45°, there was lowering of viscosity below the values of ideal mixing; the lowering of viscosity was greater with the 50% than with the 25% solution of phenol in dioxane. Since in benzene and carbon tetrachloride the nonpolar, resonant limit structure of DMN is predominant, the observed positive viscosity increase must be ascribed to hydrogen bonding *via* the amino doublet. In the presence of phenol alone or dissolved in dioxane (*i.e.*, absence of nonpolar solvent) there is, however, predominance of the dipolar resonant structure of DMN, unfavorable to hydrogen bonding with comparatively weak proton donors. Neither is there hydrogen bonding between DMN and 1-propanol.

Imidazole gave no positive interaction when assayed in *n*-butylamine (Fig. 7). Control experiments with this amine indicated (as with *n*-propylamine, see Fig. 6) no change of viscosity relative to the values of

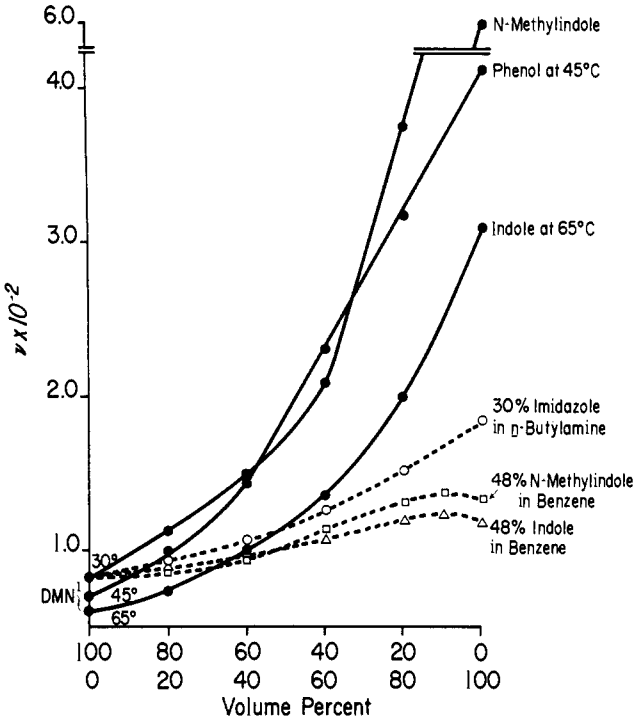


Fig. 7.—Kinematic viscosities of mixtures of dimethylnitrosamine (DMN) with compounds representing amino acid side chains. Viscosity determinations were carried out at 30°, except pure phenol–DMN at 45° and pure indole–DMN at 65°. The end points of the curves represent the viscosities of the pure compounds or their concentrated solutions as indicated. The proportions of the mixtures are given in volume per cent on two opposite scales in the abscissa.

ideal mixing when in combination with DMN. The viscosity behavior of indole was similar to that of phenol except that the maximum positive viscosity increment in benzene was appreciably lower. The positive viscosity increment observed with N-methylindole in benzene is surprising since the only proton which may be involved in hydrogen bonding in indole is assumed to be the one linked to the nitrogen atom, on the ground that it can be replaced by alkali metals. Therefore, the possibility that carbon-linked hydrogen of indole may partake in weak intermolecular association with DMN in certain conditions must be considered. The results with amino acid side-chain model compounds indicate that carboxyl groups are overwhelmingly the main participants in hydrogen bond-mediated interactions of proteins with nitrosamine carcinogens.

Relation between Hydrogen-Bonding Ability and Carcinogenic Activity.—In view of the relationship between protein denaturing ability and carcinogenic activity,[12–14,20] it is of significance that the hydrogen-bonding abilities of N-nitrosopiperidine, diethylnitrosamine, DMN, di-*n*-propylnitrosamine, dioxane, and methylphenylnitrosamine decrease in the same order (Fig. 5) as their approximate carcinogenic activities.[2–5,7,37] The carcinogenic activity of ethylphenylnitrosamine remains to be tested. It may be recalled that the ability of dioxane to act as a mitotic disorganizer at levels comparable to that of the lung carcinogen, ethyl carbamate, has been attributed to its surface active properties.[38] Diethylacetamide and diethylformamide, which are structural analogs of the nitrosamines, appear to possess only low carcinogenic activities when tested at levels equimolar to DMN,[7] yet they show greater viscosity increase with propionic acid than the more carcinogenic nitrosamines or dioxane. These two apparent exceptions may possibly be due to only partial electron displacement toward the acyl groups and, thus, to contribution of a *nitrogen-localized* electron doublet to hydrogen bonding. It is significant in this respect that the increment of viscosity was notably greater with diethylacetamide, containing the less electronegative acetyl groups, than with diethylformamide, containing, the more electronegative formyl group (Fig. 5). Comparison of the maximum viscosity increases observed with DMH (Fig. 2) and those found with nitrosamines (Fig. 1 and 5) shows, *in fact*, that hydrogen bonding *via* an amino electron doublet produces a much greater viscosity increase than hydrogen bonding *via* a nitroso or acyl group. This is further indicated by the considerable viscosity gain (of respective mixtures with propionic acid) when the intranuclear $-CH_2-$ in the 4-position of N-nitrosopiperidine is replaced by a monomethylamino group, resulting in 1-methyl-4-nitrosopiperazine. Thus, the maximum viscosity increment of 0.79 centistoke with the nonbasic N-nitrosopiperidine rose to 21.97 centistokes with the monobasic piperazine compound, which is roughly half the value found with the dibasic DMH (Fig. 2).

With respect to the relation between hydrogen-bonding ability and carcinogenic activity (Fig. 5) it must, of course, be remembered that in some instances breaking of hydrophobic bonds, dipole–dipole interactions, etc., may be more important in bringing about denaturation and cellular alterations than interactions by means of hydrogen bonding.

The apparent paradox that the powerful hydrogen-bonding agent, DMH, is inactive both as a carcinogen[3] and as an agent of protein denaturation[13,14] may be the consequence of three mutually not exclusive situations: (a) in the physiological pH range bifunctionality of hydrogen bonding responsible for high viscosity is almost totally lost; (b) the pH sensitivity observed with DMH, but not with DMN, may cause considerable instability of hydrogen bonding with the former agent because of local pH changes in the microenvironment of the functional groups involved; and (c) orientation of the hydrogen bond(s) with respect to the N–N axis in DMH may result in steric hindrance of interaction with functional groups.

(37) H. Druckrey, R. Preussmann, D. Schmähl, and G. Blum, *Naturwiss.*, **48**, 722 (1961).
(38) K. Hohl, *Experientia*, **3**, 109 (1947).