

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF CARNEGIE INSTITUTE OF TECHNOLOGY]

The Infrared Absorption Spectra of Liquid and Gaseous Morpholine¹BY R. A. FRIEDEL^{1a} AND D. S. MCKINNEY²

The infrared spectra of liquid and gaseous morpholine, $O(C_2H_4)_2NH$, between 1.4 and 15.0 μ have been investigated using a rock salt prism spectrometer. It was desired to observe the spectral differences between the two states due to any intermolecular association present, and also to compare the spectra with those of liquid and gaseous dioxane.³ This comparison would show the effects of (a) substitution of the NH group for one of the oxygens of dioxane, and (b) the existence of a larger dipole moment in morpholine than in dioxane.

Freyman^{4,5} has studied intermolecular association in secondary amines and consistently found that the bands due to the NH group undergo a shift to higher frequency upon going from the liquid to the gaseous or solution state. Gordy and Stanford⁶ observed the same frequency difference in the spectra of various liquid amines and their solutions in carbon tetrachloride. They noted further that solutions of these amines in *n*-

butyl ether produced spectra similar to the pure liquid, without appreciable shift to higher frequency. They concluded, therefore, that complex formation occurred between the NH group and the oxygen of the ether.

Buswell, Downing and Rodebush⁷ have pointed out that frequency shifts accompanying vaporization or dilution should not always be attributed to disruption of hydrogen bonds. In some cases the shift may be due to a change in the dielectric constant or to diminution of van der Waals forces. Conversely presence of a hydrogen bond may not be accompanied by a sufficiently large frequency shift to predict it. Finally these authors refute the formation of an NH-N bond unless two unlike molecules are present and one possesses a weaker acidic nitrogen; they also disclaim the existence of an NH-O bond with an ether. Both of these criteria deny the existence of hydrogen bonding in morpholine.

Experimental

The morpholine⁸ was prepared for use by refluxing over metallic sodium for an hour and then distilling onto fresh sodium in an atmosphere of pure dry nitrogen. The middle fraction was redistilled from sodium and collected under nitrogen; the middle fraction from this distillation boiled at 126.9° at a pressure of 738.8 mm.

The cells used were of the type described by Wright⁹; however, an amalgamated spacer was not used because complete sealing was not attained with such an arrangement and it was feared that water and carbon dioxide contamination of the sample would take place, with resultant formation of morpholine carbamate. So, after the cell was assembled, the edges of the rock salt plates and spacer were painted with Benolite,¹⁰ a bakelite-like substance which upon drying in air and baking for two hours at 150° produced a strongly adhering polymer which was impervious to the strong solvent action of morpholine. Filling of these cells was carried out in a stream of dry nitrogen.

The vapor determinations were made in a heated cell¹¹ consisting of a 10-cm. absorption tube, a side arm sample reservoir immersed in dibutyl phthalate, and mercury seal (Fig. 1). The whole assembly was placed in an asbestos board housing and heated with a nichrome coil. Pressure was varied in the absorption cell by regulating temperature. A barium oxide trap was attached at the mercury seal to prevent carbon dioxide and water contamination upon cooling the cell. The cement used to attach the rock salt windows to the cell had to withstand a temperature of 160°, to resist the strong solvent action of morpholine, and to adhere to rock salt without attacking it. Benolite was the only sealing compound tested which fulfilled these requirements.

Discussion

The spectral curves are shown in Figs. 2 and 3. Figure 2 includes the spectra of liquid and gaseous

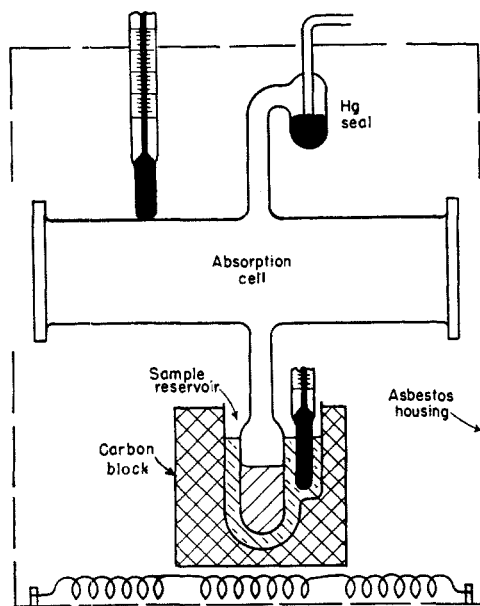


Fig. 1.—Heated gas cell for infrared spectroscopy.

(1) Paper presented before the Physical and Inorganic Division at Atlantic City, 1946.

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(3) McKinney, Leberknight and Warner, *THIS JOURNAL*, **59**, 481 (1937).

(4) Freyman, *Ann. chim.*, **11**, 11 (1939).

(5) Freyman, *Compt. rend.*, **205**, 852 (1937).

(6) Gordy and Stanford, *THIS JOURNAL*, **62**, 497 (1940).

(7) Buswell, Downing and Rodebush, *ibid.*, **62**, 2759 (1940).

(8) Sample kindly given by Mellon Institute Fellowship of Carbide and Carbon Corporation.

(9) Wright, *Rev. Sci. Inst.*, **12**, 204 (1941).

(10) Benolite Corporation, Manor, Pa.

(11) Essentially the cell described by McKinney, *et al.*, ref. 3.

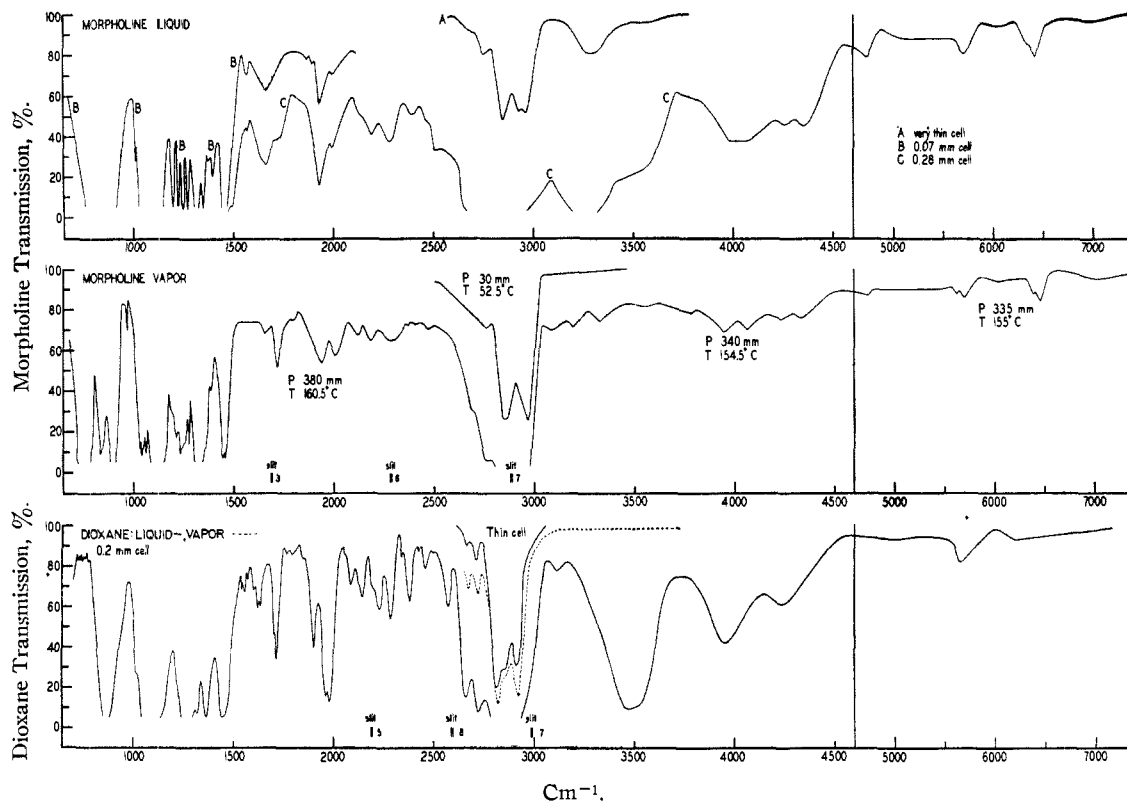


Fig. 2.—Morpholine, liquid and vapor, and dioxane liquid³ from 700 to 7000 cm^{-1} ; dioxane vapor in 3000 cm^{-1} region.

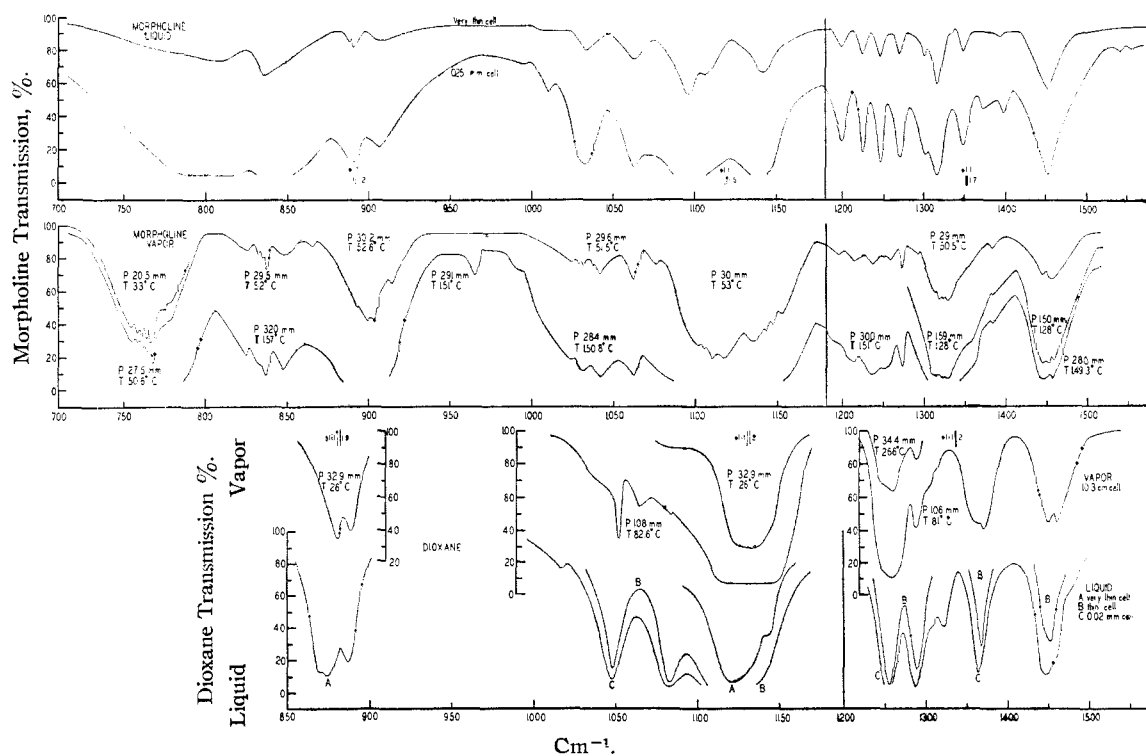


Fig. 3.—Morpholine and dioxane,³ liquid and vapor, from 700 to 1600 cm^{-1} .

morpholine and of liquid dioxane for the entire range of the spectrometer. Figure 3 presents the three spectra in greater detail in the region 6.5 to 15 μ , and in addition gives the partial spectrum of gaseous dioxane in this region. For morpholine 53 bands were found in the liquid and 78 in the vapor. Positions of the transmission minima for the principal bands are listed in the table along with the data for the morpholine Raman spectrum and the spectra of dioxane. For the latter spectra the frequencies from 2690 to 6200 cm^{-1} were determined on a grating instrument. Included with the vapor curves are the morpholine partial pressures in mm., as determined by the temperature of the liquid reservoir, and the temperature of the vapor in the absorption cell proper.

In liquid morpholine the last band found was at 809 cm^{-1} , a branch of the more intense band at 836 cm^{-1} . In the vapor the most intense band in this region was found with its peak at 768 cm^{-1} . Apparently this band is related to the liquid 809 cm^{-1} band; the resultant shift may be accounted for in various ways. One possible explanation is the removal of accidental degeneracy in going from the liquid to the vapor. Another explanation may be the removal of intermolecular association. If so, the particular kind of association does not appear to be hydrogen bonding, since removal of such a distorting influence upon the vibration of the six-membered ring should produce a shift to higher instead of to lower frequencies as is found in this case. Decreasing of van der Waals association, on the other hand, may result in a shift in either direction, depending upon the original and final relationships of the potential functions of neighboring molecules or groups of molecules. In going from dioxane liquid to vapor there is as expected no comparable shift, indicating the negligible association in dioxane liquid.

TABLE I

FREQUENCIES OBSERVED IN THE INFRARED ABSORPTION OF LIQUID AND GASEOUS MORPHOLINE. FREQUENCIES FROM RAMAN SPECTRUM AND THE SPECTRA OF DIOXANE GIVEN FOR COMPARISON

Dioxane frequencies		Morpholine frequencies			Raman ^b
Raman ^a	Infrared ^c	Infrared			
Vapor ^c	Liquid ^c	Liquid	Vapor		
432 (3)				171 (vw)	
485 (5)				422 (vw)	
				446 (vw)	
				476 (w)	
			755 (vs)		
			758 (vs)		
			761 (vs)		
			764 (vs)		
			766 (vs)		
			768 (vs)		
		809 (vs)	825 (s)		
835 (10)		836 (vs)	837 (s)	832 (vs)	
852 (0)			848 (s)		
	880	874 (vs)			
	889	887 (vs)			
		888 (s)	899 (vs)		
		891 (s)	903 (vs)		
		907 (s)	914 (s)		
			965 (w)		

1014 (6)	1017 (s)	1011 (w)	1031 (s)	1011 (m)
	1052	1048 (s)	1033 (m)	1042 (s)
	1065		1063 (m)	1062 (m)
				1102 (vs)
				1105 (vs)
1111 (3)	1084	1083 (s)	1097 (vs)	1110 (vs)
			1107 (vs)	1117 (vs)
1125 (3)	1133	1122 (vs)	1142 (vs)	1135 (vs)
1215 (4)		1142 (s)		1124 (vw)
			1200 (s)	1196 (w)
			1226 (s)	1214 (w)
	1255	1256 (vs)	1248 (s)	1237 (m)
				1259 (w)
			1271 (s)	1273 (m)
1304 (7)	1290	1289 (vs)	1302 (s)	1293 (w)
				1314 (vs)
				1319 (vs)
1331 (0)		1322 (vs)	1317 (vs)	1324 (vs)
				1329 (vs)
	1367	1367 (s)	1348 (s)	
			1373 (w)	
			1395 (w)	1384 (w)
				1445 (vs)
1443 (6)	1451	1452 (s)	1453 (s)	1451 (vs)
1457 (2)	1461			1458 (vs)
		1558 (w)		1460 (w)
		1624 (w)		
		1636 (w)	1649 (m)	1650 (w)
		1703 (m)	1692 (m)	1714 (m)
		1717 (m)		
		1899 (m)		
		1964 (m)	1929 (m)	1940 (m)
		1982 (m)	1994 (w)	2006 (m)
		2087 (w)		2119 (w)
		2143 (w)	2187 (w)	2179 (w)
		2234 (w)		
		2286 (w)	2278 (w)	2290 (w)
		2380 (w)	2390 (w)	2404 (w)
		2433 (w)	2505 (w)	2470 (w)
		2575 (w)		2684 (vw)
2663 (0)	2690 (m)	2748 (s)	2763 (m)	2741 (w)
2719 (2)	2752 (m)			2776 (vw)
2858 (8b)	2863	2855 (vs)	2848 (vs)	2854 (vs)
2890 (3)	2898	2891 (vs)	2928 (vs)	2840 (ms)
2904 (3)	2921	2917 (vs)	2958 (vs)	2969 (vs)
2967 (9b)	2970	2961 (vs)		2955 (vs)
2074 (1)		3118 (w)		
			3086 (w)	
			3189 (w)	
			3300 (s)	3302 (vw)
			3311 (s)	3326 (w)
			3513 (s)	3546 (w)
			3582 (s)	
			3988 (m)	3983 (m)
			4011 (m)	3909 (w)
			4085 (m)	4067 (w)
			4164 (m)	
			4224 (m)	4259 (m)
			4272 (m)	4234 (w)
			4343 (m)	4349 (m)
			4402 (m)	4737 (w)
				4739 (w)
				5627 (w)
			5652 (w)	5701 (w)
				5704 (w)
				6398 (w)
			6200 (w)	6414 (w)
				6464 (w)

(vs) very strong, (s) strong, (ms) moderately strong, (m) moderate, (w) weak, (vw) very weak.

^a Kahovec and Kohlrausch, *Z. physik. Chem.*, **35B**, 29 (1937). ^b Médard, *J. chim. phys.*, **33**, 626 (1936). ^c See ref. 3.

Another difference between the liquid and vapor spectra concerns the NH vibration band at 3300 cm^{-1} . In the liquid this is a strong band, barely resolved into a doublet of 11 cm^{-1} separation; in the vapor it is apparently split into three components at 3326, 3189 and 3086 cm^{-1} and the

intensity is decreased tremendously. Throughout the rest of the spectra there are no great differences between the spectra of the two states of morpholine other than the usual vapor shifts and general broadening of bands in the vapor spectrum.

The greatest differences between morpholine and dioxane spectra exist in the same two regions discussed above. Dioxane liquid has no band at 3300 but does produce a band at 3500 cm^{-1} which morpholine does not show. However, Errera¹² has pointed out that vapor or pure dry liquid dioxane do not produce this band. The determinations by McKinney, *et al.*, were made with open cells so that absorption of water by hygroscopic dioxane was probable. Errera's statements have been checked briefly by determining the dioxane vapor spectrum in this region (Fig. 2); the 3500 cm^{-1} band is not found. The morpholine, which is also hygroscopic, may possibly have taken up water but pains were taken to prevent this occurrence by keeping the sample desiccated and filling the sealable cells in a stream of dry nitrogen. Other differences in the two spectra occur at: (a) the long wave length region

(12) Errera, *Physica*, **4**, 1097 (1937).

in which dioxane vapor exhibits no band comparable to the 809 and 768 cm^{-1} morpholine bands, (b) the 1200–1300 cm^{-1} region in which four strong bands occur in morpholine to only two in dioxane, and (c) the band at 1649 cm^{-1} in morpholine which is attributable to NH bending.

The Raman spectra values for morpholine in the table show close comparison with the infrared values, particularly at longer wave lengths.

Summary

The infrared absorption of liquid and gaseous morpholine has been investigated between 1.4 and 15.0 μ with a rock salt prism spectrometer. Several important differences exist between the two spectra and indicate that intermolecular association probably of the van der Waals type, is quite strong in the liquid state. Comparison with liquid and gaseous dioxane enables detection of bands produced in morpholine by the substitution of the NH group for one of the ether linkages in dioxane, and indicates the weaker intermolecular association present in the latter. Frequencies found for morpholine compare closely with those of the Raman spectrum.

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[CONTRIBUTION FROM STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

The Heat of the Inactivation of Pepsin¹

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The general problem of the structure of proteins is of the greatest importance in biology, as evidenced by the tremendous literature on this subject. Relatively little information is available concerning the energetics of reactions involving these important substances. It is with the hope of gaining new insight into the protein problem that we have undertaken calorimetric measurements of the heats of protein and allied reactions. The present paper reports data obtained three years ago on the inactivities of pepsin by alkali, using a calorimeter described in previous papers.³ We have chosen this reaction as a first point of attack chiefly because the very careful work of Steinhardt⁴ has demonstrated that it is characterized by relatively clean-cut first order kinetics. The present work is to be considered as preliminary in character; we feel that the results, though not as extensive as might be desired, are nevertheless of considerable interest. Certain weaknesses in our apparatus which appeared in the course of this work have led us to propose an ex-

tensive redesign before further work in this field is undertaken.

Experimental

Preparation of Pepsin.—Parke, Davis 1:10,000 pepsin was purified and crystallized essentially as described by Northrop.⁵ After four crystallizations the pepsin was dialyzed against distilled water until no further sulfate or chloride was removed, and the pepsin suspension was then lyophilized. Dried pepsin was weighed out for each run.

Analytical Methods. Nitrogen Determinations.—All nitrogen determinations were made by the Kjeldahl method, in some cases macro⁶ and in some semimicro. Non-protein nitrogen (NPN) was estimated in the filtrate from the treatment of a sample with 25 ml. of 0.3 *M* trichloroacetic acid after heating to boiling. Protein nitrogen (PN) was either taken by difference or determined in the trichloroacetic acid precipitate with the application of a suitable blank.

Determination of Peptic Activity.—The enzymatic activity of the pepsin solutions was determined essentially as described by Anson.⁷ The tyrosine⁸ formed by digestion of hemoglobin by the pepsin under carefully standardized conditions was estimated colorimetrically. Our

(5) J. H. Northrop, "Crystalline Enzymes," Columbia University Press, New York, N. Y., 1939, pp. 128 ff.

(6) The macro Kjeldahl determinations were performed at the Connecticut Agricultural Experiment Station through the courtesy of E. M. Bailey, to whom the authors wish to express their indebtedness.

(7) M. L. Anson, *J. Gen. Physiol.*, **22**, 79 (1938).

(8) Strictly speaking, one estimates unspecified products formed in the digestion which are not precipitated by trichloroacetic acid and which give the same color as tyrosine solutions.

(1) Calorimetric Investigations of Organic Reactions. V. Previous paper in this series. *THIS JOURNAL*, **64**, 762 (1942).

(2) Present address: Madison, Wisconsin.

(3) J. M. Sturtevant, *J. Phys. Chem.*, **45**, 127 (1941); *THIS JOURNAL*, **68**, 88 (1941); **64**, 762 (1942).

(4) J. Steinhardt, *Kgl. Danske Videnskaberne Selskab. Math. fys. Medd.*, **XIV**, 11 (1937).