the moment, since those configurations prevented by hindrance would have large moments.

It must be remarked that the observed low moment for methyl trisulfide does not exclude structure II as decisively as a high moment would have favored it. Whereas it is possible, in fact, to fix 2 $\times 10^{-18}$ as the upper limit for the moment of structure I if freedom of rotation exists, one can not determine the lower limit for structure II with equal certainty, since the actual polarity of the coordinate sulfur–sulfur bond involved in the latter formulation is not known, at least a certain amount of double bond character being possible in this bond.

The foregoing treatment has assumed the freedom of rotation around the S–S bonds normally associated with a single bond. If, as suggested by the electron diffraction results,[3] the C–S bonds oscillate about mean positions approximately 106° out of the plane of the three sulfur atoms, a *cis* form with the two alkyl groups on the same side of the plane and a *trans* form with the alkyl groups on opposite sides of the plane should exist. The *cis* form would have a moment larger than the mean value calculated for the case of free rotation, while the *trans* form would have a moment close to zero. A reasonable distribution of the molecules between the two configurations would give the observed moment. The observed dipole moment, therefore, does not rule out the existence of the molecules of the trisulfide in these two more or less fixed configurations. The dipole moment values do not decide unequivocally the structures of these polysulfides, but may be regarded as in accord with the other lines of evidence favoring an unbranched chain formulation.

Acknowledgment.—The assistance and advice of Professor Gregg Dougherty in the preparation of the compounds used in this investigation are gratefully acknowledged.

Summary

The dipole moments of methyl, ethyl and propyl disulfides, and of methyl trisulfide have been measured at 30° and found to be 1.95, 1.96, 1.96 and 1.66 $\times 10^{-18}$ respectively. The value for the trisulfide is consistent with an unbranched chain structure for that molecule.

PRINCETON, NEW JERSEY RECEIVED JULY 8, 1949

[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY OF POLAROID CORPORATION]

Absorption Spectra. VIII. The Infrared Spectra of Some Purines and Pyrimidines[1,2]

BY ELKAN R. BLOUT* AND MELVIN FIELDS†

The purine and pyrimidine bases are organic compounds of considerable interest because they are components of nucleic acids and various enzymes, because they exert powerful physiological actions, and because they pose some interesting problems of structure. In connection with our work on infrared spectroscopy of compounds of biological systems,[2,3,4] we have determined the spectra of a group of related pyrimidines and purines over the wave length region 2 to 15 μ (670 to 5000 cm.$^{-1}$). The differences in the infrared spectra of such compounds are so marked that infrared absorption techniques should become important in the identification of this class of compounds, especially since many of their other physical properties such as melting point, solubility and ultraviolet absorption may fail to differentiate among various members of the class or between mixtures.

Drastic acid hydrolysis of yeast ribonucleic acid yields cytosine, uracil, adenine and guanine; and similar treatment of thymus desoxyribonucleic acid gives the same bases, except that thymine is obtained in place of uracil.[5] The products of such hydrolyses are soluble in aqueous solutions, but insoluble in organic solvents. This situation is satisfactory for ultraviolet absorption spectroscopy, but makes infrared work very difficult except by the use of the materials in the solid state. We have found that the high vacuum sublimation of purines and pyrimidines onto rock salt disks yields films which are generally suitable for infrared spectroscopic work.[3,6,6a]

Pyrimidines from Nucleic Acids

The infrared spectra of cytosine (4-amino-2-

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(1) Supported in part by funds from the Office of Naval Research.

(2) For the last paper in this series see Blout and Fields, *J. Biol. Chem.*, **178**, 335 (1949.)

(3) Blout and Fields, *Science*, **107**, 252 (1948).

(4) Blout and Mellors, *ibid.*, **110**, 137 (1949).

(5) *Cf.* Levene and Bass, "Nucleic Acids," Am. Chem. Soc. Monograph Series, New York, N. Y., 1931; Vischer and Chargaff, *J. Biol. Chem.*, **176**, 715 (1948); Chargaff, Vischer, Doniger, Green and Misani, *ibid.*, **177**, 405 (1949), for a summary of previous references.

(6) As a check against decomposition during sublimation, samples of the compounds before and after sublimation were submitted to spectral measurements in aqueous solution in the vicinity of their ultraviolet absorption maxima. The results of these measurements are shown in Table II. In general, with fairly pure compounds the position of the maximum ultraviolet absorption was unchanged by this treatment, although in some cases an increase in the extinction coefficient was noted in the sublimed material. This increased ultraviolet absorption of some of the sublimed samples is probably indicative of higher purity obtained through the sublimation procedure.

(6a) Since samples prepared in this manner may show molecular orientation (personal communication from Dr. C. D. West), it is conceivably possible that certain frequencies would be diminished in intensity or not be observed at all when viewed only along one direction with collimated unpolarized normally incident light.

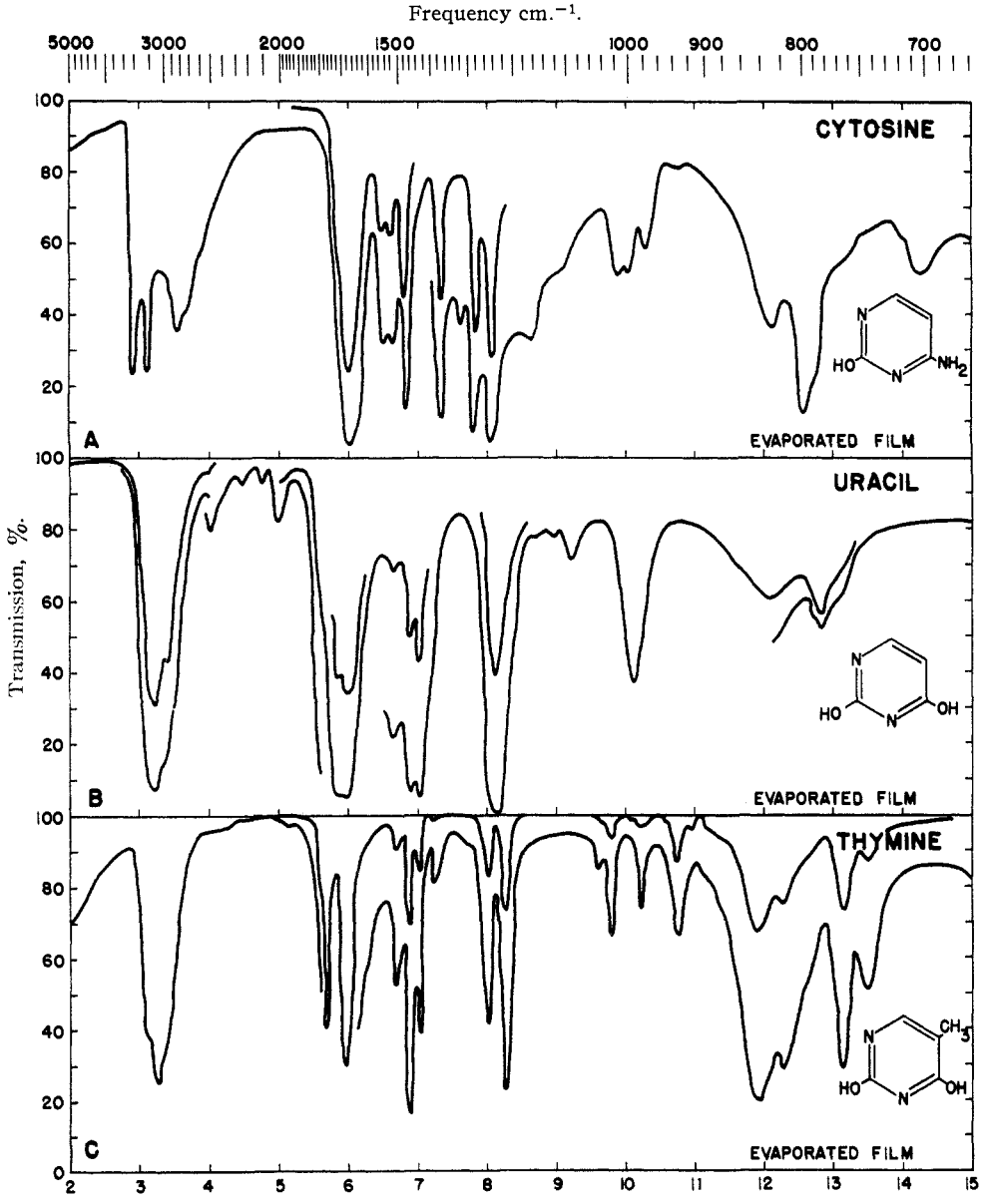


Fig. 1.

hydroxypyrimidine), uracil (2,4-dihydroxypyrimidine) and thymine (5-methyl-2,4-dihydroxypyrimidine) are shown in Fig. 1. Considering the spectra as a whole it is obvious that ready differentiation of the three compounds is possible. Several specific characteristics of the spectra are worth noting. All three compounds are characterized by intense absorption in the 3 μ region; however, it is only in cytosine that resolution into three absorption bands is seen. It is assumed on the basis of previous investigations[7] that these bands at 2.92, 3.12 and 3.55 μ correspond to O–H, N–H and C–H stretching vibrations, the location of the O–H and N–H bands being displaced toward lower frequencies (longer wave lengths) than usual by virtue of intermolecular hydrogen bonding.[8] Of particular interest is the fact that cytosine shows only one intense absorption band around 6 μ, the region of C=O, C=C and C=N stretching frequencies; while the uracil spectrum shows a doublet peaked at 5.85 and 5.90 μ and that of thymine shows two distinct bands at 5.68 and 5.96 μ. Since cytosine shows only a single intense absorption band in the

(7) See, for example, (a) Davies and Sutherland, *J. Chem. Phys.*, **6**, 767 (1938); (b) Thompson, *J. Chem. Soc.*, 328 (1948); (c) 301 (1944); and (d) Richards and Thompson, *ibid.*, 1248 (1947).

(8) It is possible, of course, that the shortest wave length band is a free NH_2 stretching vibration, although intermolecular hydrogen bonding (*vide infra*) makes this assignment unlikely.

region 5.0 to 6.4 μ, and shows three intense bands in the 3 μ region, it is suggested that the oxygen atom in this compound in the solid state exists completely in association with hydrogen—either through enolization or intermolecular hydrogen bonding. It is probable that the 5.68 μ absorption in thymine involves C=O stretching motions,[9,10,11,12] and that the methyl group extending out from the ring prevents complete hydrogen bonding of both the potential carbonyl groups in this compound. The bands at 6.02, 5.90 and 5.96 μ in cytosine, uracil and thymine, respectively, are probably associated with C=C and C=N stretching motions.[13]

In the region beyond 6 μ, each of the pyrimidines shows several strong bands, notably that in the neighborhood of 6.85 μ (C–H bending),[14] and those in the region 7.8 to 8.2 μ (C–N and C–O vibrations).[7b] The band at 7.24 μ in thymine is almost certainly associated with C–CH_3 group vibrations.[14,15] The bands that lie at wave lengths longer than 9 μ offer the best possibility for use in identification of the compounds, each of the materials showing highly characteristic, but as yet unassignable, absorption bands in this region.

Purines from Nucleic Acids

Adenine (6-aminopurine) and guanine (2-amino-6-hy-

(9) Thompson and Torkington, *J. Chem. Soc.*, 640 (1945).

(10) Jones, Williams, Whalen and Dobriner, THIS JOURNAL, **70**, 2024 (1948).

(11) Rasmussen, Tunnicliff and Brattain, *ibid.*, **71**, 1068 (1949).

(12) Barnes, Gore, Liddel and Williams, "Infrared Spectroscopy," Reinhold Publishing Corp., New York, N. Y., 1944.

(13) Blout, Fields and Karplus, THIS JOURNAL, **70**, 194 (1948).

(14) Thompson and Torkington, *Proc. Roy. Soc. (London)*, **A184**, 3 (1945).

(15) Fox and Martin, *ibid.*, **A175**, 208 (1940).

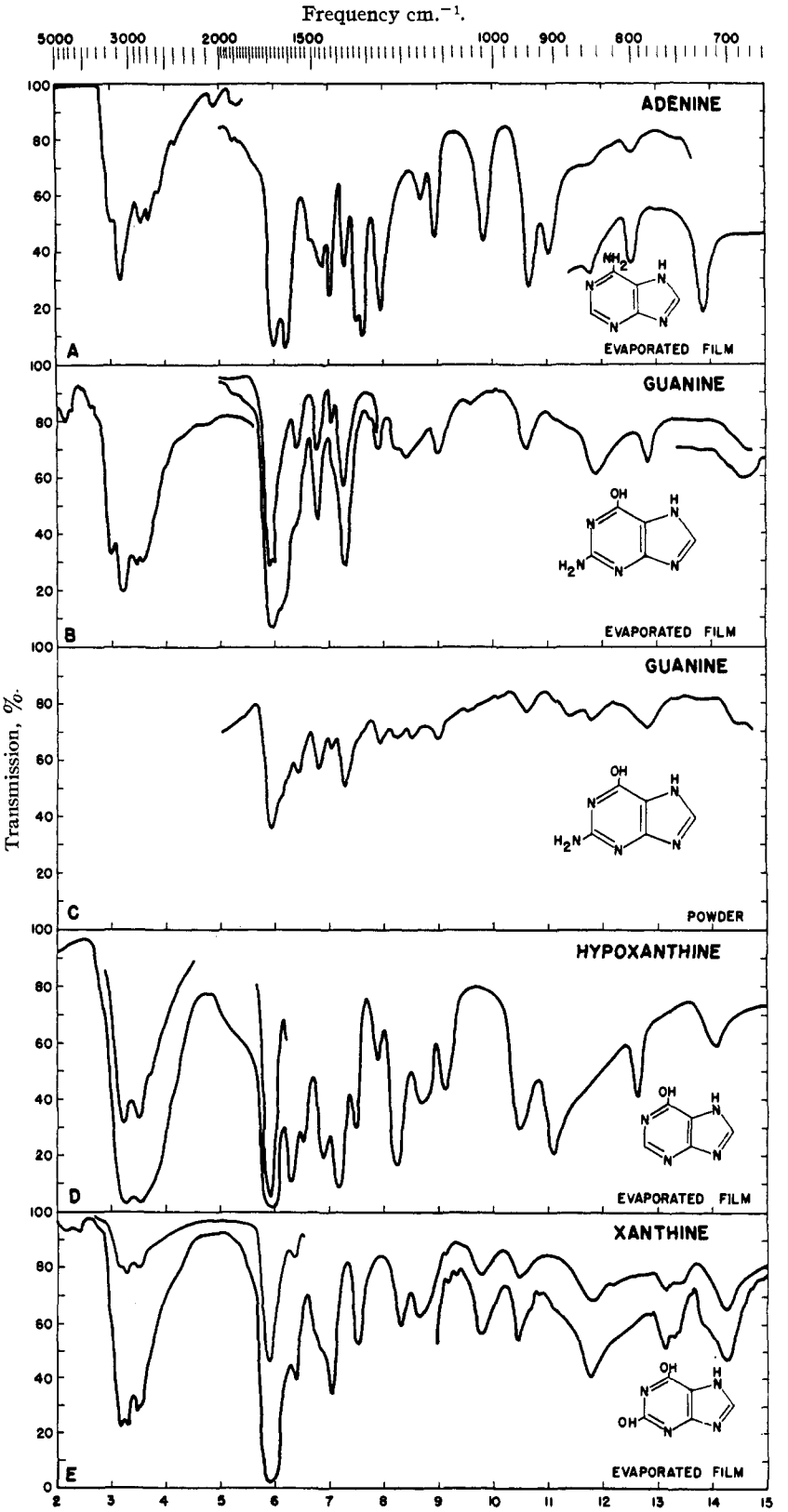


Fig. 2.

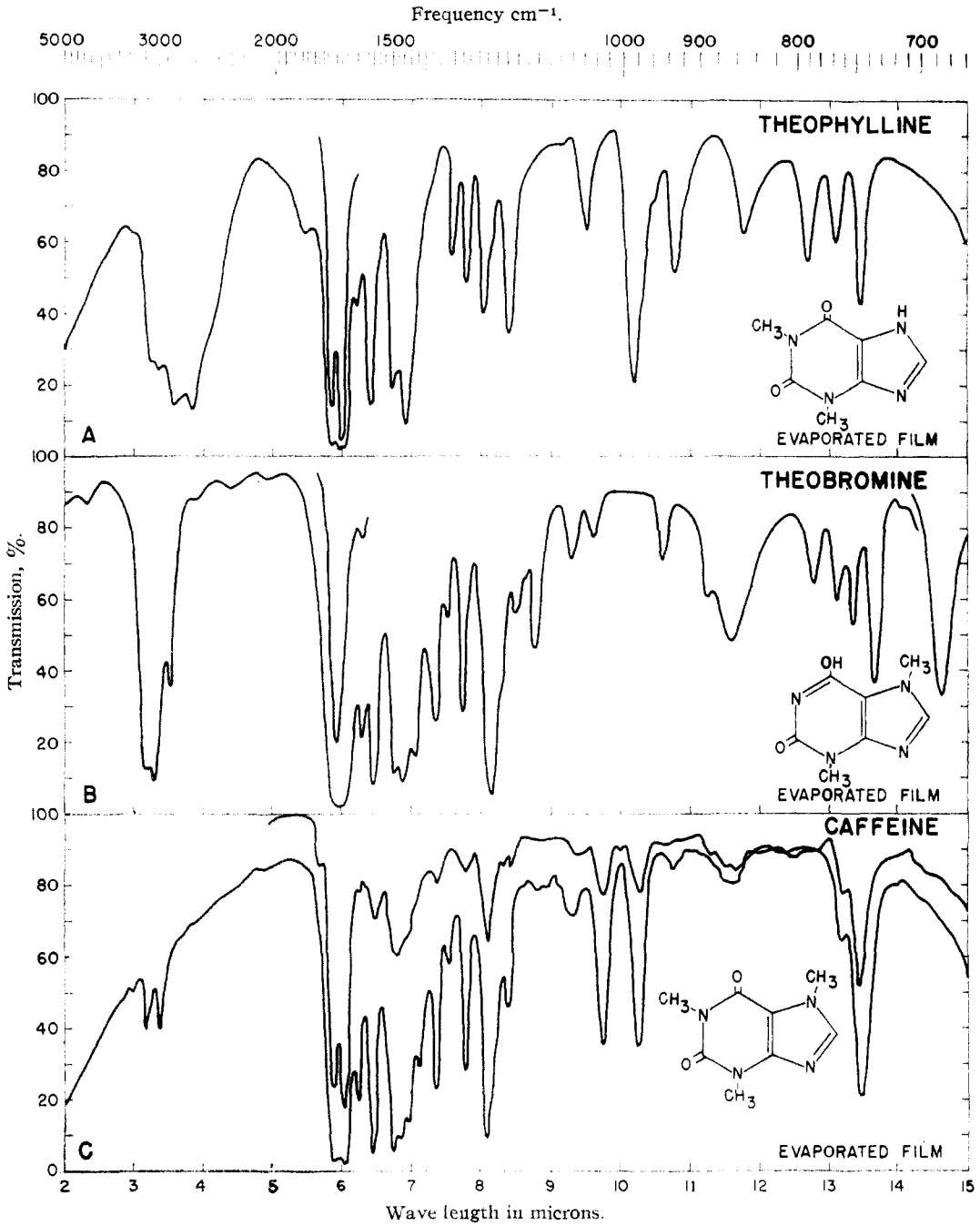


Fig. 3.

droxypurine) are the only purines which have shown to be actual components of the two known types of nucleic acids. The infrared spectra of these compounds are shown in Fig. 2, along with the spectra of hypoxanthine (6-hydroxypurine) and xanthine (2,6-dihydroxypurine), which are enzymatic and chemical transformation products of adenine and guanine. Curves B and C are both of guanine; C was obtained as a powder on rock salt,[2,16] and B, in the same manner as the other spectra recorded in this paper—by sublimation in high vacuum. There is a good correlation of both the location and the relative intensities of the bands obtained with the two techniques, except for the presence of a fairly weak band at 11.4 μ in the powder sample which is not present in the evaporated film. The scattering of the radiation by the powder particles at short wave lengths makes spectra obtained with samples prepared in this manner practically useless at wave lengths shorter than 5 μ.

(16) Lecomte, *Cahiers phys.*, **17**, 1 (1943).

The spectra of this group of purines are characterized by the fact that those of adenine and guanine show many similarities, as do those of hypoxanthine and xanthine, but only a few absorption bands are common to all four compounds. Adenine and guanine reveal four distinct absorption maxima in the 3 μ region, even with the rather low dispersion rock salt prism we used. These peaks are located at approximately 3.0 μ and 3.2 μ (N–H and O–H stretching), and 3.4–3.7 μ (probably C–H stretching modes). Hypoxanthine and xanthine show bands at no wave lengths shorter than 3.2 μ, indicating that the 3.0 μ band observed in adenine and guanine may be associated with the NH_2 group present in the latter compounds.

All four purines have an intense absorption band between 5.88 and 5.98 μ and another band between 6.22 and 6.42 μ, which bands are probably associated with C=C and C=N stretching modes in the purine ring system. One additional band that seems to be characteristic of this type of compound is located in the 10.45 to 10.70 μ region and is seen in all four spectra. Reference should be made to the original spectra for additional absorption similarities, especially between the pairs mentioned above. Finally the attention should be drawn to the similarities shown in the spectra of adenine and adenosine,[2] and xanthine and xanthosine,[2] especially at wave lengths up to 8 μ.

N-Methylated Purines

We have also examined the infrared spectra (Fig. 3) of three N-methylated purines, *viz.*, theophylline (1,3-dimethylxanthine), theobromine (3,7-dimethylxanthine) and caffeine (1,3,7-trimethylxanthine). This group of compounds offers particular interest since the placement of the methyl groups in theophylline and caffeine precludes enolization in these purines. The general absorption in the 3 μ region shows no abnormalities compared with the other purines, except that theophylline shows a strong band at 3.82 μ, a wave length much longer than usually associated with C–H stretching motions.

Of particular interest is the region around 6 μ. There, we find striking correlation between the spectra of all three compounds (Table I), except that the 5.86 μ absorption band of theophylline and caffeine is not shown by theobromine. Since this band is most likely associated with the C=O stretching vibration, we are led to suggest that the structure of theobromine (in the solid state) may be that in which one of the oxygens is bonded to an enolic hydrogen, which hydrogen atom is in turn associated with a carbonyl oxygen of another molecule of theobromine. The structure drawn in Fig. 3B is meant to be illustrative only, since no distinction can be made on the basis of presently available data as to which oxygen is enolic and which exists as a carbonyl oxygen.

TABLE I

ABSORPTION MAXIMA IN THE REGION 5.8–8.2 μ

Theophylline	Theobromine	Caffeine
5.86		5.86
5.98	5.98	6.02
6.20	6.26	6.24
6.40	6.45	6.46
6.74	6.76	6.77
6.92	6.86	6.93
7.58	7.49	7.36
7.78	7.72	7.77
8.03	8.15	8.09

In addition to the rather strict correspondence of absorption bands in the 5.8 to 8.2 μ region, there is a strong band around 13.55 μ which appears in the spectrum of all three compounds. The region between 8.5 and 13 μ is quite dissimilar and would prove useful for differentiation and identification purposes.

We wish to acknowledge the valuable technical assistance of Miss P. L. Snow and Miss A. P. Sutton in this work.

Experimental

Materials.—All the pyrimidines and purines were obtained from commercial sources and purified when necessary, as shown by measurement of ultraviolet absorption maxima or elementary analysis. The data are given in Table II.

Preparation of Samples.—All compounds were measured in the solid state as sublimed films on rock-salt disks. An apparatus was used in which the sample was sublimed, at approximately 10^{-5} mm., directly onto a clean, polished rock-salt disk held about three inches away from the material. In general films obtained by this method are uniform in thickness and often are completely transparent in the visible region. Absolute measurements of thickness in the thin layers used were not made. We collected sufficient sublimate so that the transmission in the 6 μ region was between 10 and 20%, and generally found such a sample gave satisfactory spectral data throughout the entire spectral range investigated.[17]

Ultraviolet absorption measurements were made on the samples before and after sublimation as a check as to whether decomposition had occurred. In some cases, as noted in Table II, the rate of sublimation was so slow that it was not practicable to collect sufficient material for that purpose. None of the pyrimidines or purines showed apparent evidence of decomposition under the conditions used for sublimation.

Instrumentation and Measurements.—The spectral measurements were made on a Perkin–Elmer infrared spectrometer, model 12A, with a ten-cycle chopper, a Strong bolometer, an alternating current amplifier, and a Brown Instru-

(17) NOTE ADDED IN PROOF.—Since the submission of this article an interesting note by Sinsheimer, Scott and Loofbourow has appeared (*Nature*, **164**, 796 (1949)) describing spectral changes occurring in evaporated films of certain purines and pyrimidines upon long standing in air. The measurements reported in the present article were made upon films either immediately after evaporation or else the evaporated samples were stored in a desiccator until measured. No evidence of spectral changes under such conditions was reported by Sinsheimer, Scott, and Loofbourow, or observed by us in the course of our work.

TABLE II

Compound	Melting point, °C. Reported	Found	Sublm. temp., °C. (at ~ 10^{-5} mm.)	Ultraviolet spectral data Reported $\lambda_{max.}$	log ϵ	Solvent[b]	Found λ_{max}	log ϵ	N, Analyses,[a] % Calcd.	Found
Cytosine	320–325 (dec.)[c]	313–315 (dec.)	200	265[h]	3.79	H_2O	265[m]	3.81	37.8	37.6
Uracil	335 (dec.)[c]	339 (dec.)	140	260[h]	3.95	H_2O	260[n]	3.90	25.0	25.0
Thymine	321 (dec.)[c]	320 (dec.)	100	263[h]	3.93	H_2O	265[m]	3.90	22.3	21.9
Adenine	360 (dec.)[d]	354 (dec.)	160	262[i]	4.09[j]	0.05 *N* NaOH	268[n]	4.08	51.8	51.7
Guanine	Not given[c]	>400 turns black	255	248	4.10	0.1 *N* HCl	248[m]	4.05	46.4	45.3
				274[h]	3.89		272	3.87		
Hypoxanthine	Not given[c]	>400 turns black	230	260[k]	4.04	0.1 *N* NaOH	263[n]	4.03	41.2	41.3
Xanthine	Not given[c]	379 (dec.)	200	278[k]	3.99	0.1 *N* NaOH	280[m]	3.99	30.3[o]	30.6[o]
Theophylline	264[e]	269–271	110			H_2O	272[n]	4.00	31.1	31.3
Theobromine	351[f]	354–355	115			H_2O	272[n]	4.01	31.1	30.8
Caffeine	235[g]	235–236	80	275[l]	4.03	0.1 *N* HAc	275[n]	3.99	28.9	28.7

[a] We are greatly indebted to Dr. Earl D. Stewart of the Schwarz Laboratories for supplying us with some of the nitrogen analyses. [b] When no superscript appears with the log ϵ value under Reported, the solvent was the same as used in this investigation. [c] For references to the original literature, see Levene and Bass, "Nucleic Acids," A. C. S. Mon. Series, Reinhold Publ. Corp., New York, N. Y., 1931. [d] Traube, *Ann.*, **331**, 64 (1904). [e] Kossel, *Ber.*, **21**, 2164 (1888). [f] Kempf, *J. prakt. Chem.*, [2], **78**, 246 (1908). [g] Fischer and Bromberg, *Ber.*, **30**, 219 (1897). [h] Heyroth and Loofbourow, THIS JOURNAL, **56**, 1728 (1934). [i] Cavalieri, Bendich, Tinker and Brown, *ibid.*, **70**, 3875 (1948). [j] At *p*H 8.99. [k] Stimson and Reuter, THIS JOURNAL, **65**, 154 (1943). [l] Loofbourow, Stimson and Hart, *ibid.*, **65**, 148 (1943). [m] Spectral data on crystallized sample before sublimation. [n] Spectral data on sublimed sample. [o] With one molecule of water.

ment Company recording potentiometer. For initial observation, a complete spectrum from 1 to 15 μ was recorded continuously. For drawing the final curves in Figs. 1 to 4, the data were obtained on a point-to-point basis, the points being taken from 5 to 10 cm.$^{-1}$ apart at frequencies up to 1900 cm.$^{-1}$ (5.2 μ) and at larger intervals at higher frequencies.

Summary

The infrared spectra of the pyrimidines and purines obtainable by the hydrolysis of nucleic acids have been determined over the spectral region 2 to 15 μ. The infrared spectra of the related purines, hypoxanthine, xanthine, theophylline, theobromine and caffeine have also been determined. On the basis of these measurements, suggestions have been made concerning the molecular structure of certain of the compounds in the solid state.

Infrared spectroscopy offers a convenient method for the identification and differentiation of this class of compounds.

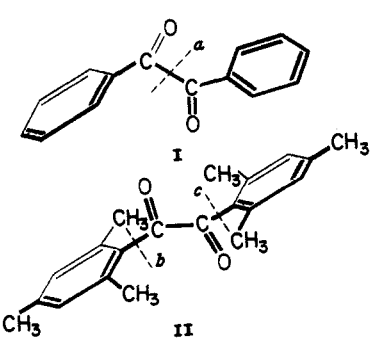
CAMBRIDGE 39, MASSACHUSETTS RECEIVED JUNE 9, 1949

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS, AND THE CHEMICAL RESEARCH LABORATORY OF POLAROID CORPORATION]

The Ultraviolet Absorption Spectra of Hindered Benzils

BY NELSON J. LEONARD AND ELKAN R. BLOUT*

In a previous paper[1] it was shown that the ultraviolet absorption maxima of certain alkoxy- and hydroxybenzils and the correspondingly substituted benzaldehydes lie at closely comparable wave lengths. This fact is consistent with the skew structure of benzil in which the two benzoyl units lie in planes approximately at right angles to each other (I) and the dicarbonyl system is not coplanar. Presumably the dicarbonyl absorbing unit would make a greater contribution to the total spectrum if the carbonyl groups could become coplanar. One method of permitting such coplanarity to develop is to provide hindering groups on the *ortho* carbons of benzil, thereby throwing each carbonyl group out of the plane of the attached ring. Thus, in contrast to the benzil molecule (I), which is twisted about *a*, the mesitil molecule (II) will be twisted about *b* and *c* and the dicarbonyl system is not prevented from becoming coplanar. Determination of the ultraviolet absorp-



* Harvard University National Research Fellow, 1942–1943.

(1) Leonard, Rapala, Herzog and Blout, THIS JOURNAL, **71**, 2997 (1949).