[CONTRIBUTION FROM DEPARTMENTS OF CHEMISTRY AND BIOLOGY, SIENA HEIGHTS COLLEGE]

The Fluorescence of Some Purines and Pyrimidines

BY SISTER MIRIAM MICHAEL STIMSON AND SISTER MARY AGNITA REUTER

The known fluorescence of uracil and adenine[1] on the one hand, and of the factors promoting proliferation (at least in the crude state) on the other, and the possible relationships between these substances,[2] have led us to study the fluorescence of some twenty derivatives of uracil and adenine.

Procedure.—A series of twenty compounds in the solid state and seventeen in solution was investigated. The apparatus was enclosed in a light-proof box made with two sections so arranged that the source (a Sperti Mercolite 45 Q Z) was separated from the solution by means of a partition with the Wood's glass filter in the path of the light. The test and standard solutions in Pyrex tubes were placed at the principal focus of the lens (a 500-ml. Pyrex Florence flask).[3]

As a standard for comparison, the intensity of the fluorescence of xanthine in the solid state was taken as four. Both acid and basic solutions of the other compounds were estimated with reference to a basic solution of xanthine—fluorescence intensity of six.

The solutions used were made up at a concentration of 1 mg./ml.; xanthine, in acid solution, and 2-amino-6-chloropyrimidine, in basic solution, were used at a concentration of 0.5 mg./ml. At the concentration employed above, the two compounds and 2-oxy-6,8-diaminopurine, in acid solution were not completely dissolved and only the supernatant liquid was examined. Due to the negligible solubility of purines and pyrimidines in distilled water, neutral solutions could not be employed.

Materials.—Xanthine (Eastman Kodak Co.) was purified by recrystallization three times from dilute ammonium hydroxide, uracil five times from hot water, adenine sulfate (Eastman) repeatedly from concentrated aqueous solution to give four sided pyramids, barbituric acid (Eastman) from cold concentrated aqueous solution, adenosine phosphoric acid thrice from aqueous solution. 1,3-Dimethyluracil[4] was prepared by the methylation of uracil with dimethyl sulfate and recrystallized five times from water–alcohol mixture to long fine needles. 2-Chloro-6-aminopyrimidine, 2,6-dichloropyrimidine, 6-chloro-2-aminopyrimidine and isocytosine were prepared from uracil by the method of Johnson and Hilbert.[5] 2,6-Dichloropyrimidine was purified by distillation three times at 203° (uncor.). Isocytosine could not be so carefully purified because of the small quantity available. 2-Chloro-6-amino- and 6-chloro-2-amino-pyrimidines were recrystallized repeatedly to give fine needles and small glistening plates, respectively. Guanine hydrochloride (Eastman) was purified by recrystallization and the free base obtained therefrom. Yeast nucleic acid (Pfanstiehl) was reprecipitated from cold alcoholic sodium hydroxide made acid to congo red with glacial acetic acid, and gave no biuret reaction. β-Thymus nucleic acid was extracted from fresh thymus glands (calf) according to the modified method of Levene.[6] It was depolymerized with pancreatin according to Feulgen.[7] Hypoxanthine (Hoffman–LaRoche) was used without further purification. 2-Amino-6-methoxypyrimidine and cytosine monohydrate were supplied through the kindness of Dr. Francis F. Heyroth of the University of Cincinnati, isoguanine and 2-oxy-6,8-diaminopurine through Mr. Joseph R. Spies of the Bureau of Chemistry and Soils, United States Department of Agriculture.[8] The purity of these compounds was determined by means of extinction coefficients from ultraviolet absorption spectra.[9]

Discussion.—The materials dissolved in ammonium hydroxide were fluorescent more toward the blue than solutions in sodium hydroxide. Sulfuric acid tended to cause a shift toward the red, so that the fluorescence of some compounds, while not visible in hydrochloric acid, could be detected in sulfuric acid. It was previously reported in the case of quinine[10] that the fluorescence is stronger in sulfuric acid than in hydrochloric acid. No satisfactory explanation was advanced. It is not possible at this time to assign specific groups to account for the change. Constitutional relations can be assigned after the completion of the absorption spectra of these compounds (Loofbourow and Stimson, in preparation) and an extension of this work to include fluorescence spectra.

The degree of purity of isocytosine as checked by absorption data is not so high as that of the

(1) Heyroth and Loofbourow, THIS JOURNAL, **53**, 3441 (1931).
(2) Loofbourow, Cook and Stimson, *Nature*, **142**, 573 (1938).
(3) Hand, *Ind. Eng. Chem., Anal. Ed.*, **11**, 306 (1939).
(4) Davidson and Baudisch, THIS JOURNAL, **48**, 2379 (1926).
(5) Johnson and Hilbert, *ibid.*, **52**, 1152 (1930).
(6) Levene and Bass, "Nucleic Acids," Chemical Catalog Co., New York, N. Y., 1931; Levene, *J. Biol. Chem.*, **53**, 441 (1922).
(7) Feulgen, *Z. physiol. Chem.*, **238**, 105 (1936).
(8) Spies, THIS JOURNAL, **61**, 350 (1939); **61**, 351 (1939).
(9) Loofbourow and Stimson, *J. Chem. Soc.*, 844 (1940); 1275 (1940).
(10) Dhéré, "Fluorescence en Biochemie," Paris, 1937.

FLUORESCENCE TABLE[a]

Material	Solids Color	Solids Intensity	NaOH soln. Color	NaOH soln. Intensity	NH_4OH soln. Color	NH_4OH soln. Intensity	H_2SO_4 soln. Color	H_2SO_4 soln. Intensity
Barbituric acid	Wh. vi.	2	Lt. gr.	3	Violet	2	Violet	1
Uracil	Violet	2	Lt. gr.	4	Bl. vi.	2	Bl. vi.	2
1,3-Dimethyluracil	Purple	1	Lt. gr.	3	Violet	2	Violet	1
2-Amino-6-methoxypyrimidine	Vi. gray	3	Lt. gr.	2	...	...	...	...
6-Chloro-2-aminopyrimidine	Bl. wh.	1	Lt. gr.	6	Bl. vi.	2	Violet	1
2-Chloro-6-aminopyrimidine	Vi. bl.	3	Lt. bl.	4	Blue	3	Violet	1
2,6-Dichloropyrimidine	Violet	1	Lt. gr.	1	Bl. vi.	1	Bl. vi.	1
Cytosine monohydrate	Bl. vi.	1	0	0	...	...	...	...
Cytosine (anhydrous)	Bl. vi.	2	0	...	...	...	...	...
Isocytosine	Vi. wh.	1	...	...	Lt. bl.	4	Lt. bl.	3
Xanthine	Vi. bl.	4	Bl. gr.	6	Lt. bl.	4	Lt. bl.	3
Hypoxanthine	Violet	1	0	0	0	0	0	0
Guanine (free base)	Purple	1	Lt. gr.	2	Violet	1	Violet	1
Guanine HCl	Purple	1	...	...	...	...	...	...
Isoguanine (crystals)	Pur. wh.	1	0	0	Violet	1	Violet	1
2-Oxy-6,8-diaminopurine	Gr. bl.	2	Green	8–10	Lt. bl.	4	Lt. bl.	3
Adenine sulfate	Bl. wh.	1	Lt. gr.	1	Bl. vi.	2	0	0
Adenosine phosphoric acid	Purple	1	0	0	0	0	0	0
Yeast nucleic acid	Lt. bl.	2	Green	6	Lt. bl.	2	Violet	1
β-Thymus nucleic acid	0	0	Yel. gr.	6	Yel. gr.	5	Yel. gr.	4

[a] In HCl solution, only 2-amino-6-methoxypyrimidine (lt. gr. 2), xanthine (blue 2), 2-oxy-6,8-diaminopurine (lt. bl. 4), yeast nucleic acid (lt. gr. 2) and thymus nucleic acid (yellow green) fluoresced in the visible region.

other members of the series. The sample used had an ultraviolet extinction coefficient of 4,790, whereas Heyroth and Loofbourow[11] give a value of 6100. Less pure preparations, which also were examined, showed a bright yellow fluorescence and had considerably lower extinction coefficients.

Since the bright yellow fluorescence has been noted in impure samples and gradually disappeared on successive recrystallizations, it is probably attributable to smaller amounts of impurity than would be sufficient to cause visible discoloration. Hence the fluorescence of solutions serves in this series of compounds as a rapid method for controlling purification.

In general fluorescence is greater in solution than in the solid state. This is more particularly true of basic solutions. Hausser and Kuhn[12] found in the case of mesoporphyrin dimethyl ester that at room temperature a solution fluoresces red whereas the solid shows no fluorescence. The same workers have also found the fluorescence in the adsorbed state to be less than in solution. The fluorescence of these compounds is not strong enough for detection by capillary analysis.

Euler[13] investigated xanthine, caffeine, guanine, guanosine, adenylic acid and uric acid, but reported their fluorescence too weak to be described. On the other hand, according to Grengross and Schulz[7] the fluorescence of xanthine is strong in the solid state, increases on alkalinization, and decreases on acidification. The conclusion of Grengross and Schulz has been confirmed. We have found, moreover, that 6-chloro-2-aminopyrimidine, 2-chloro-6-aminopyrimidine, xanthine, 2-oxy-6,8-diaminopurine, and the nucleic acids were sufficiently fluorescent to be used in ultrachromatograms. The work on the separation and purification of purines and pyrimidines by ultrachromatography is being continued and will be reported elsewhere.

It is not possible on the basis of fluorescence observations to ascribe the presence of any of these compounds to the crude proliferation promoting factors. The strong fluorescence of the latter is probably due in part to impurities.

(11) Heyroth and Loofbourow, THIS JOURNAL, **56**, 1729 (1934).
(12) Hausser and Kuhn, *Z. physik. Chem.*, **29B**, 363 (1935).
(13) Euler, *Biochem. Z.*, **281**, 206 (1935).

Summary

1. An investigation of the fluorescence of various purines and pyrimidines in the solid state and in acid and in basic solutions has been made.

2. In general the purines and pyrimidines investigated are more highly fluorescent in basic than in acid solution.

3. Applications have been suggested for use

in ultrachromatograms and in checking the purity of these compounds.

4. An inexpensive and rapid method for viewing fluorescent materials in solution and chromatograms has been given.

ADRIAN, MICHIGAN RECEIVED NOVEMBER 14, 1940

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF SOUTH CAROLINA]

Anhydrides of the Normal Aliphatic Saturated Monobasic Acids

BY JAMES M. WALLACE, JR., AND J. E. COPENHAVER

The alternation of the melting points of the acids of the acetic acid series is well known. It seemed of interest to make a comparison of the melting point pattern of these acids with that of the corresponding anhydrides. All but three of the anhydrides have been described but the data are from different sources and do not agree among themselves. Holde[1] and co-workers have prepared the most complete series, consisting of the even carbon acids and anhydrides from caprylic through stearic. In order to obtain consistent values, we have prepared all the anhydrides from heptylic to stearic, including the three not previously known, by the same method and have taken their melting points under the same conditions. These values, along with those obtained by previous workers, are given in Table I. The acids from capric to stearic were generously furnished from the extremely pure preparations of Meyer and Reid.[2] Heptylic, caprylic and pelargonic acids were obtained from the Eastman Kodak Company. The melting points of the complete series of the anhydrides, along with those of the corresponding acids are plotted in Fig. 1.

TABLE I

MELTING POINTS OF THE ANHYDRIDES, °C.

Anhydrides	This research	Prior values
Heptylic	−10.8	−12.4[a]
Caprylic	+ 0.9 ± 0.1	−1.0[b]
Pelargonic	14.8	16[c]
Capric	24.7 ± .2	23.9[b]
Undecylic	36.7	35[d]
Lauric	42.1 ± .1	41,[c] 41.8,[b] 44[e]
Tridecylic	49.9 ± .2	...
Myristic	53.5 ± .1	51,[c] 53.4[b]
Pentadecylic	60.6	...
Palmitic	63.9 ± .1	63–64,[b] 63,[f] 63.5–64[g]
Margaric	67.6	...
Stearic	70.7	71–71.5,[b] 70.5,[f] 71.5–72,[g] 70–71[h]

[a] Deffet, *Bull. soc. chim. Belg.*, **40**, 385–402 (1931). [b] Holde and Gentner, ref. 1. [c] Kraft and Rosing, *Ber.*, **33**, 3577 (1900). [d] Backer and van der Baan, *Rec. trav. chim.*, **56**, 1161–1174 (1937). [e] Mannick and Nadelmann, *Ber.*, **63**, 796 (1930). [f] Whitby, *J. Chem. Soc.*, **128**, 1462 (1926). [g] Rankov, *Ann. univ. Sofia, II, Faculté phys.-math.*, Livre 2, **33**, 221–227 (in German 228–229) (1937). [h] Autenrieth and Thomae, *Ber.*, **57B**, 423–437 (1924).

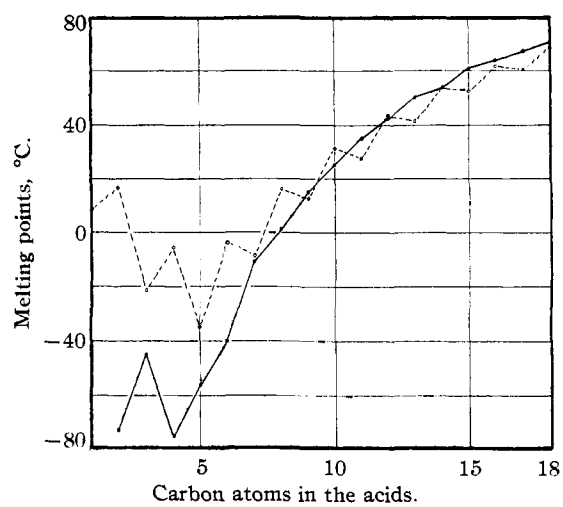

Fig. 1.—Melting points of the acids and anhydrides: acids ---; anhydrides —.

In general the method of Holde and Tacke was used. The acids were refluxed for six to eight hours in a flask, with ground-in condenser, with three equivalents of acetic anhydride. The acetic acid and excess acetic anhydride were distilled off under vacuum and the resulting anhydrides purified by vacuum distillation or by recrystallization from a suitable solvent. The anhydrides from heptylic through pelargonic were distilled under vacuum and capric through stearic were recrystallized three or four times from either diethyl or petroleum ether by cooling in an ice–salt-bath or with dry-ice. The yields ranged from 50 to 80%.

The melting points were taken, with mechanical stirring in a 200-cc. bulb, fitted with a side arm for the thermometer and melting point tube. Standard Anschütz thermometers were used, so the melting points may be regarded as corrected.

Carbon and hydrogen analyses are given in Table II

(1) Holde and Smelkus, *Ber.*, **53**, 1889–1897 (1920); Holde and Tacke, *Ber.*, **53**, 1898–1907 (1920); *Chem.-Ztg.*, **45**, 949–950, 954–956 (1921); Holde and Gentner, *Ber.*, **58**, 1418–1424 (1925).

(2) Meyer and Reid, THIS JOURNAL, **55**, 1574–1584 (1933).