FORMATION OF PROTON-TRANSFER COMPLEXES OF 2-AMINO-2-DEOXY-D-GLUCOSE AND OTHER SIMPLE ORGANIC BASES WITH VIOLURIC ACID

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(Received May 31st, 1972; accepted for publication, June 21st, 1972)

ABSTRACT

In the presence of 2-amino-2-deoxy-D-glucose or a number of other organic bases, violuric acid forms proton-transfer complexes which, in general, are pink in color. Some physicochemical characteristics of such complexes with 2-amino-2-deoxy-D-glucose, ethylenediamine, ethylamine, 2-aminoethanol, dimethylamine, trimethylamine, and pyridine are now reported. The structure of the complexes so formed is also mentioned. Several of these complexes (composition 1:1, except for that of ethylenediamine, which is 2:1) have been isolated in the solid state. Different tautomeric forms of violuric acid have been identified from visible, u.v., and i.r. spectral measurements; the tautomers formed depend on the pH of the solvent medium.

INTRODUCTION

Violuric acid [5-(hydroxyimino)barbituric acid] is blue in the presence of bases[1], and many of its salts are yellow or red[2]. In using violuric acid as a spray reagent for the chromatographic identification of calcium ion[3], it has been observed in this laboratory that this heterocyclic compound produces pink colors of different shades in the presence of various types of bases.

In the present paper, results of spectrophotometric (u.v., visible, and i.r.) investigations on the effect of the pH of the solution on violuric acid, and on its interaction with certain organic bases (ethylenediamine, ethylamine, 2-aminoethanol, dimethylamine, trimethylamine, 2-amino-2-deoxy-D-glucose, and pyridine), are reported. In each case, the reaction product is a proton-transfer type of complex[4–6].

EXPERIMENTAL

Materials. — The reagents used were all of pure grade (E. Merck), with the exception of ethylenediamine and 2-aminoethanol, which were products of Riedel, Germany, and were purified by distillation. The 2-amino-2-deoxy-D-glucose was generated from its hydrochloride in aqueous solution by the use of Amberlite IR-45 anion-exchange resin. Double-distilled, conductivity water was used as the solvent.

Spectral measurements. — U.v. and visible spectra were recorded with a Beckman DB apparatus employing silica cells (1-cm wide). The temperature of reaction and experimentation was maintained at 27 ±0.5°. A Perkin–Elmer infrared spectrophotometer Model 337 and the Nujol-mull technique were used for the i.r. measurements.

Isolation of the complex. — Upon addition of a solution of violuric acid to a solution of ethylenediamine, the product separated as fine, pink particles, only poorly soluble in water and in aqueous alcohol, and insoluble in almost all other solvents except amines. Products were also isolated from the reaction of violuric acid with ethylamine, 2-amino-2-deoxy-D-glucose, and pyridine, respectively. The procedure adopted was the following. A solution of violuric acid in aqueous ethanol was treated with an excess of the respective base. After allowing sufficient time for reaction at room temperature (~30°), the solution was concentrated under diminished pressure. To the concentrated solution was added *p*-dioxane to cause precipitation of the complex. The complex, which precipitated out immediately, was filtered off, washed with small portions of *p*-dioxane–water, and dried under vacuum. As all the components were pure and the amine had been added in large excess, it was assumed that only a trace of unreacted violuric acid was present in the product, and that any unreacted base was removed by the washing with *p*-dioxane–water and prolonged drying *in vacuo*.

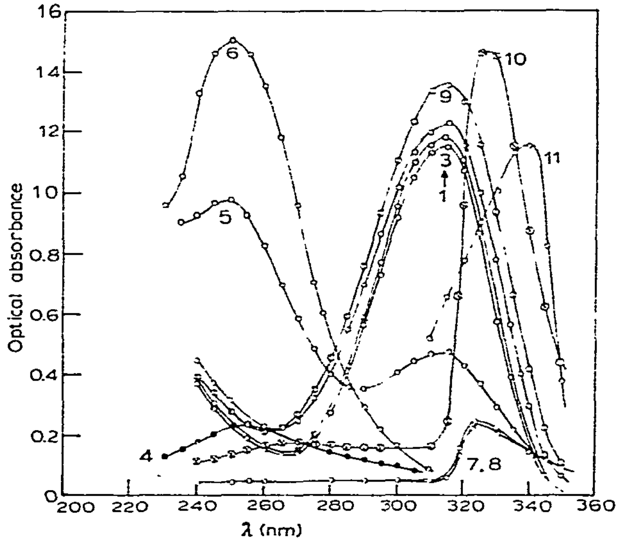


Fig. 1. Spectra of violuric acid (VA) under different conditions. (1, 100 μM VA; 2, 100 μM VA plus 100 μM 2-aminoethanol; 3, 100 μM VA plus 10 mM 2-aminoethanol; 4, 100 μM VA in 1M HCl; 5, 1 mM VA (0.1 cm path-length); 6, 1 mM VA in 1M HCl (0.1 cm path-length); 7, 100 μM VA in 80% acetone; 8, 100 μM VA plus 10 mM 2-aminoethanol in 80% acetone; 9, 1 mM VA plus 200 mM 2-aminoethanol; 10, 1 mM VA plus 200 mM 2-aminoethanol in 70% acetone; and 11, 10 mM VA plus 10 mM 2-aminoethanol.)

RESULTS

Effect of pH. — The pH of the medium has a marked influence on the spectra of violuric acid in solution (see Fig. 1). At moderate pH, bands at 252 and 315 nm are shown. At low pH, the 315 nm band disappears and that at 252 nm becomes prominent. Addition of base causes disappearance of the 252-nm band, and a band at 345 nm develops; another band, in the visible region between 540 and 550 nm, also appears, and the solution becomes pinkish. This behavior is presumed to be due to the formation of different tautomers of violuric acid in solution depending on the pH.

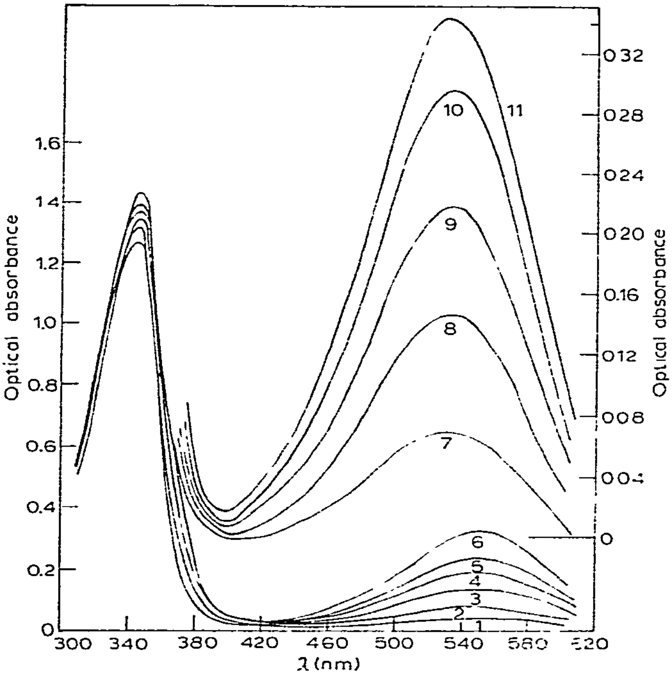


Fig. 2. Effect of 2-aminoethanol on the spectra of 10 mM violuric acid, and *vice versa*. (1, 10 mM VA; 2, plus 1 mM 2-aminoethanol; 3, plus 2 mM 2-aminoethanol; 4, plus 3 mM 2-aminoethanol; 5, plus 4 mM 2-aminoethanol; 6, plus 5 mM 2-aminoethanol; 7, 143 mM 2-aminoethanol plus 1.43 mM VA; 8, 143 mM 2-aminoethanol plus 2.86 mM VA; 9, 143 mM 2-aminoethanol plus 4.26 mM VA; 10, 143 mM 2-aminoethanol plus 5.72 mM VA; and 11, 143 mM 2-aminoethanol plus 7.15 mM VA.)

Interaction with organic bases. — In Fig. 2, results obtained on adding increasing amounts of 2-aminoethanol to a fixed concentration of violuric acid are shown in curves 1–6. In curves 7–11, the results of a reverse addition are depicted. The interesting feature in the first set is the increase in the height of the 345- and 540-nm bands; in the second, the visible band shifts towards the blue (530 nm). These results could not be fitted into the modified Benesi–Hildebrand equation[7] for the determination of the composition and stability of the complex.

TABLE I

I.R. SPECTRA OF VIOLURIC ACID AND ITS PROTON-TRANSFER COMPLEXES

Violuric acid		*Complex*	
Band (cm^{-1})	*Assignments*	*Band (cm^{-1})*	*Assignments*
3400(m)	NH stretching	3400(w)	NH removed or stretching lessened
3160(m)	NH stretching	3160(vw)	NH removed or stretching lessened
1700(s)	C=O stretching	1700(s)	C=O stretching
		1665(m)	C=N stretching in conjugated cyclic system
1600(m)	C=N stretching, open-chain		
		1575(w)	C=C stretching
1550(w)	C=N stretching, open-chain		
		1530(s)	N=O stretching
1330(s)	unbonded OH (enol form)	1320(w)	bonded OH
1260(s)	unbonded OH (enol form)	1260(s)	unbonded OH
1145(s)	C–O stretching (phenolic)	1145(s)	C–O stretching (phenolic)
1070(s)	ring vibration		
810(m)	ring vibration	810(m)	ring vibration
790(m)	ring vibration	790(m)	ring vibration

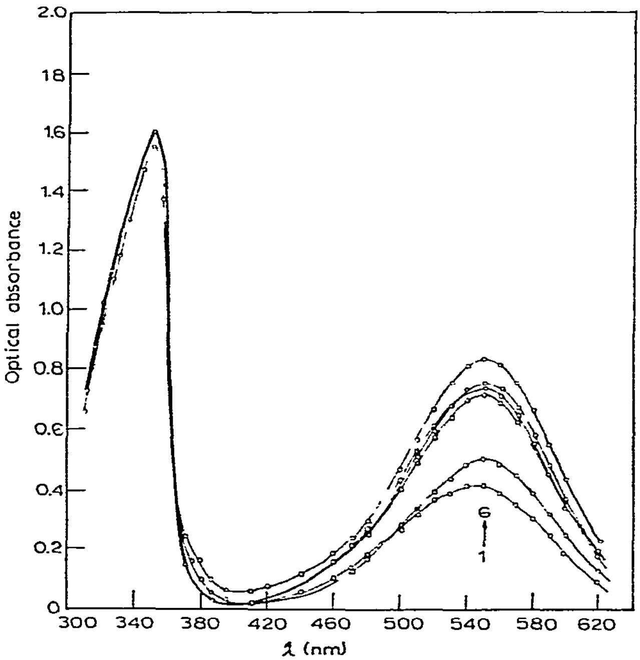


Fig. 3. Spectra of 1:1 mixtures of 15 mM violuric acid and 15 mM amines. [1, ethylenediamine; 2, pyridine; 3, ethylamine; 4, dimethylamine and trimethylamine; 5, 2-aminoethanol; and 6, 2-amino-2-deoxy-D-glucose (50 mM).]

I.r. spectra of the compounds. — The i.r. spectra of the violuric acid–base products also reveal association of the heterocyclic compound with the bases used. The characteristic bands of the compounds are listed in Table I. These results are discussed in the next section.

Composition of the complexes. — The compositions of the complexes were determined spectrophotometrically by the method of Job[8]. Measurements were taken at 540 nm, and, in every instance, a 1:1 complex was found. The spectra of 1:1 mixtures of violuric acid and the bases used are shown in Fig. 3.

In the presence of a large excess of a base (assuming complete complexation of the violuric acid), the extinction coefficients at 540 nm of the complexes were determined from the Beer's law plots. By using these extinction values and the optical absorbances of the 1:1 mixtures, the stability constants were calculated by the usual method[9]. The results are given in Table II.

TABLE II

PHYSICOCHEMICAL CHARACTERISTICS OF VIOLURIC ACID–ORGANIC BASE COMPLEXES

Complex with	$pK^{25°}$	*Extinction coefficient*[a]	*Stability constant* ($K \times 10^{-4}$)	*Frequency factor*[b] ($f \times 10^{2}$)	$-\Delta G$ (*kcal.mole*$^{-1}$)
2-Aminoethanol	4.56	51.0	1.20	2.12	5.64
Ethylamine	3.25	53.5	0.40	2.22	5.04
Dimethylamine	3.28	53.5	0.87	2.10	5.44
Trimethylamine	4.13	53.5	0.64	2.10	5.26
2-Amino-2-deoxy-D-glucose		53.5	0.13	2.10	1.54
Ethylenediamine	4.07	56.8	3.13	2.27	6.21
Pyridine	8.64	50.5	0.063	2.08	3.87

[a]At 27° and $\lambda_{max} = 540$ nm. [b]The frequency factor (f) was calculated by using the approximate formula $f = 4.319 \times 10^{-9}\, \varepsilon_{max} \nu_{1/2}$, where $\nu_{1/2}$ is the band width at half intensity (in cm^{-1}).

TABLE III

COLOR, MELTING POINT, AND ELEMENTAL ANALYSES OF THE ISOLATED COMPLEXES

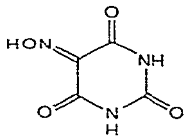
Complex of violuric acid with	*Color*	*Melting point* (°C)	*% C*		*% H*		*% N*	
			Calc.	*Found*	*Calc.*	*Found*	*Calc.*	*Found*
	white	203–205 (dec.)					26.73	26.82
2-Amino-2-deoxy-D-glucose (1:1)	dark pink	115–117 (dec.)	35.72	35.00	4.79	4.63	16.66	16.25
Ethylamine (1:1)	dark pink	135–140 (dec.)	35.62	34.86	4.95	4.65	27.71	27.00
Ethylenediamine (2:1)	bright pink	270–285 (dec.)	32.09	31.85	3.76	3.62	29.93	29.20
Pyridine (1:1)	light pink	195–197 (dec.)	45.76	45.30	3.41	3.16	23.71	23.21

The 1:1 composition of the complexes was further supported by the results of elemental analyses, given in Table III. For the solid violuric acid–ethylenediamine complex, the composition was 2:1, whereas the composition in solution was 1:1.

Other observations. — The complexes isolated are sparingly soluble in water and alcohol, and practically insoluble in all nonaqueous solvents tried, but are readily soluble in many pure amines. All of the amine-treated solutions have a deep-blue color which becomes pink on dilution with water or on the removal of the free base by physical or chemical means. The precise colors of the isolated complexes differ (see Table III).

DISCUSSION

The heterocyclic ring-structure of violuric acid is in many respects identical with those of barbituric acid and of uracil, a nucleic acid base-constituent. Its u.v. and i.r. spectra will be discussed with reference to those of these compounds[10,11]. The u.v. and visible spectra of violuric acid in solution at different pHs, as well as the i.r. spectra of the isolated complexes and of violuric acid, suggest that one (or more) of the following tautomeric forms is present, depending on the conditions.



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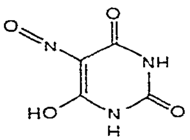

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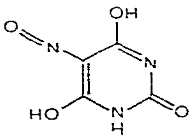

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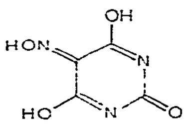

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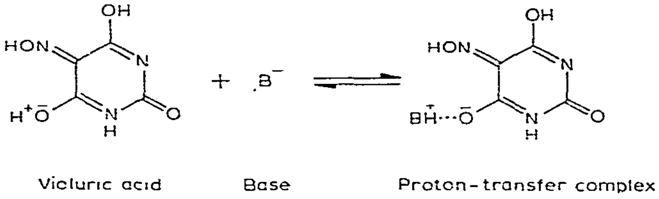
At moderate acidity, violuric acid shows two bands (at 252 and 315 nm). According to Besnik and Wolff[12], the chromophore –N=C–C=O is responsible for a band at 312 nm at 40 μM. Tautomer **1** has this chromophore. The skeleton structure of tautomer **2** is the same as the preponderant structure of barbituric acid, which absorbs[10] in the u.v. at 256.5 nm. We observed that, at low pH, violuric acid shows maximum absorption at 252 nm only; therefore, tautomer **2** preponderates in acidic medium. In the solid form, violuric acid exists mainly in form **1**; form **2** is present to only a slight extent, as revealed by the appearence of a weak i.r. band at 1530 cm^{-1} characteristic of the –N=O group.

Tautomers **3** and **4** are possible in basic environment. Condensation of violuric acid with aromatic amines[13] suggests formation of tautomer **4**; or any other form that differs only in the position of the C=O group may also be present. The u.v. peak at 345 nm of violuric acid in basic medium is presumed to indicate the C=N–C=O chromophore (present in tautomers **3** and **4**). The presence of the C=N group is also indicated by the i.r. spectra of the isolated complexes. The increasing sharpness and intensity of the 1530-cm^{-1} band in the i.r. spectra of the complexes with organic bases of increasing basicity are evidences for the presence in the complex of the –N=O group present in tautomer **3**. It is, therefore, reasonable to conclude that the violuric acid portion of the solid complexes has the structure of tautomer **3**. However, as primary organic nitroso compounds are unstable, tautomer **4** is more probable. Tautomers **3** and **4** may exist in solution depending on the conditions.

In tautomer **4**, the acidity of violuric acid is caused by the -OH groups present; their relative strengths would decide how many base molecules can be bound by the parent molecule. The spectrophotometric method has now revealed a 1:1 ratio. This has also been observed by us during titration (pH meter) of violuric acid with a base. The following reaction is suggested for formation of the complex.



Physicochemical characteristics of this proton-transfer complex (hydrogen-bonded ion-pair) are given in Table II, and the results are in accordance with those usually observed for complexes of this type. The ionized form of violuric acid (the violurate anion) in solution is responsible for its color and for the 540-nm peak. The solids isolated are not all of similar color and shade, possibly because of differences in the crystalline structure.

As regards the formation of a deep-blue solution upon addition of a large excess of base to solutions of the complexes, Schneider[1] had already indicated that addition of a base to a solution of violuric acid gave this color. In the present work, we have found that, so long as the base is not very much in excess, the interaction produces a pink color.

ACKNOWLEDGMENTS

Financial support of the U. S. D. A., Foreign Agricultural Research Service, in the form of a PL-480 grant is thankfully acknowledged. Stimulating discussion with Dr. N. Roy is greatly appreciated, and we are grateful to Dr. R. S. Tipson for taking a keen interest in our work. Thanks are due Dr. T. Chakrevarti of Calcutta University for the microanalyses.

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