cipitate was obtained. The mixture was allowed to cool and filtered to give a tan powder. Recrystallization from 70% aqueous ethanol gave 0.63 g. (59%) of tan, plate-like crystals. Two recrystallizations from ethanol–water gave white plates, melting at 198–199°. This compound was soluble in cold bicarbonate solution, and in dilute base. It could be titrated as an acid with sodium hydroxide. Oxidation with alkaline permanganate gives benzoic acid. Potentiometric titration gave a pK_a value of 5.13.

Anal. Calcd. for $C_{12}H_9O_3N$: C, 66.97; H, 4.22; N, 6.51; neut. equiv., 215.2. Found: C, 67.05, 67.13; H, 4.17, 4.32; N, 6.64, 6.51; neut. equiv., 217.

5-Benzoyloxy-2-pyridone (V).—One gram of 5-benzoyl-2-pyridone was dissolved in 6 ml. of 37% hydrogen peroxide solution and 6 ml. of glacial acetic acid. The pyridone went into solution as the mixture was heated to and maintained at 55° for 12 hours. Water was added to the warm solution until a very slight cloudiness was noted. Fine tan needles separated and were filtered, washed with water several times and air dried to give 0.34 g. (31%) of 5-benzoyloxy-2-pyridone. This material gave a weak greenish-brown color with acidic ferric chloride. Upon addition of enough dilute sodium hydroxide to this test solution to make it basic, and then reacidifying with hydrochloric acid, a darker red-brown ferric chloride test was obtained.

Two recrystallizations of this material from alcohol–water mixture gave fine light tan needles, m.p. 187–188°. This compound was soluble in base, with the appearance of a mossy green color, insoluble in acid and water.

Anal. Calcd. for $C_{12}H_9O_3N$: C, 66.97; H, 4.22; N, 6.51; sapon. equiv., 107.5. Found: C, 67.31, 67.35; H, 4.12, 4.25; N, 6.43, 6.46; sapon. equiv., 109.

5-Benzoyl-2-pyridone Sodium.—One gram (0.005 mole) of 5-benzoyl-2-pyridone was stirred with 7 ml. of hot 10% sodium hydroxide. The pyridone dissolved and fine white needles reappeared immediately and upon cooling. These were filtered, washed with acetone and air dried giving 0.85 g. (71%). One recrystallization from large volumes of dry acetone gave short white needles, m.p. 261–262°. A sample of this material melted and resolidified when introduced into the melting point bath at temperatures above 140°. Upon slowly raising the melting point bath containing the salt, this change took place too gradually to be visible.

Both the crystalline material first obtained and a sample fused at 150° for an hour yielded the original pyridone on acidification of an aqueous solution. The compound gave a strong alkaline test with moist red litmus paper and burned to leave an alkaline ash. The analysis of the crystalline material first obtained indicated two molecules of water of crystallization.

Anal. Calcd. for $C_{12}H_8O_2NNa \cdot 2H_2O$: C, 56.04; H, 4.70. Found: C, 56.43, 56.37; H, 4.97, 5.19.

3-Bromo-5-benzoyl-2-pyridone.—One gram (0.005 mole) of 5-benzoyl-2-pyridone dissolved in 15 ml. of hot methanol was treated with liquid bromine until a permanent color of bromine remained. After about a minute a heavy crop of short white needles separated, were filtered, washed with water and methanol and air dried to give 1.1 g. (79%) of product melting at 248–255°. Two recrystallizations from large volumes of ethyl acetate gave short white needles, m.p. 255–257°. This compound was insoluble in water and only slightly soluble in all organic solvents tested, but dissolved readily in dilute sodium hydroxide.

Anal. Calcd. for $C_{12}H_8O_2NBr$: Br, 28.73. Found: Br, 28.72.

N-Methyl-3-bromo-5-benzoyl-2-pyridone.—One and two-tenths grams (0.0056 mole) of N-methyl-5-benzoyl-2-pyridone dissolved in 20 ml. of hot ethanol was treated with liquid bromine until the color of bromine persisted briefly. One and three-tenths grams (78%) of white crystals separated after a few minutes. Two recrystallizations from large volumes of ethanol gave white prisms melting at 201–202°. This material was insoluble in water and dilute sodium hydroxide, and only slightly soluble in the common organic solvents.

Anal. Calcd. for $C_{12}H_{10}O_2NBr$: Br, 27.36. Found: Br, 27.36.

LOUISVILLE, KENTUCKY

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLLEGE OF ARTS AND SCIENCES, UNIVERSITY OF LOUISVILLE]

2-Pyrones. XIX. 3-Hydroxy-2-pyrone[1] and 4-Arylhydrazono-2,3-pyranones

BY RICHARD H. WILEY AND C. H. JARBOE

RECEIVED DECEMBER 12, 1955

3-Hydroxy-2-pyrone (isopyromucic acid) has been prepared by the potassium bisulfate dehydration of mucic acid and purified by a new technique as a colorless solid giving a blue ferric chloride test. Comparisons of its infrared absorption data with those of 3-methoxy-2-pyrone confirm the presence of a hydrogen-bonded hydroxyl group as in the structures IIa, IIb and IIc. Coupling with diazonium salts gives unstable, deeply colored products for which the infrared absorption characteristics are in accord with the 4-arylhydrazono-2,3-pyronone structure VI.

Although the acid-catalyzed transformation of mucic acid to pyromucic acid (furan-2-carboxylic acid) and dehydromucic acid (furan-2,5-dicarboxylic acid) has received continued attention since the reaction was originally observed by Scheele in the late eighteenth century,[2] the competitive conversion of mucic acid to 3-hydroxy-2-pyrone, originally reported by Limpricht,[3] who named the product isopyromucic acid, has received scanty attention. As a result little is known of the chemistry of this interesting structure and no reliable directions for its preparation are available. In this paper we wish to report the results of our studies on the preparation, isolation and characterization of this compound, our studies on its reactions with aryldiazonium compounds and our analysis of its structure in terms of comparisons of its infrared absorption spectrum with that of 3-methoxy-2-pyrone.

(1) The systematic *Chemical Abstracts* name for this structure (formulas I and IIa,b,c) can be 3-hydroxy-2-oxo-2H-pyran; 2H-pyran-2,3(4H)-dione; 2H-pyran-2-one, 3-hydroxy; or 5-hydroxy-2-oxo-4-pentenoic γ-lactone. It has also been known as isopyromucic acid. *Chemical Abstracts* indexing policy is not decisive for these structures and although there has been an announced policy to index these compounds as lactones, many have been indexed as hydroxypyranones or pyrandiones depending upon whether the keto or enol form is used as a basis for indexing. The pyrone nomenclature is used in this paper because of its relation to other compounds we are studying which also contain this structural unit.

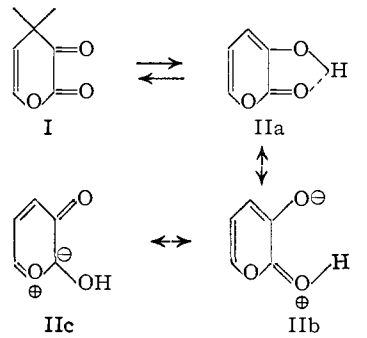
Selection of suitable nomenclature for the arylhydrazones (formula VI) poses similar problems. Consistent with the pyranone nomenclature these are 4-arylhydrazones which may properly be named 4-arylhydrazono-2,3-dioxo-3,4-dihydro-2H-pyrans or 4-arylhydrazono-2,3-dioxo-2,3-dihydro-4H-pyrans. The pyronone name represents a convenient extension of the pyranone–pyrone nomenclature which has previously been used for similar structures derived from dehydroacetic acid types. Systematically, replacement of "an" in a name by "on" implies replacement of $-CH_2-$ by $-CO-$ and in this sense pyranone is a contraction of a dihydropyranone, not of a pyranone structure. Again *Chemical Abstracts* will probably index these structures as γ-lactones of 3-arylhydrazono-5-hydroxy-2-keto-4-pentenoic acid.

(2) A. P. Dunlop and F. N. Peters, "The Furans," Reinhold Publ. Co., New York, N. Y., 1953, pp. 486–487, 572–573.

(3) H. Limpricht, *Ann.*, **165**, 257 (1873).

Previous studies[3-5] of the potassium bisulfate dehydration of mucic acid to 3-hydroxy-2-pyrone have not provided entirely adequate descriptions of a reproducible, satisfactory synthesis. An unsuccessful attempt to bring about this reaction has been recorded[6] and our attempts to duplicate these[4,5] procedures were initially unsuccessful. Also our attempts to develop a satisfactory synthesis based on the procedure involving cyclization of tetraethyldioxalylsuccinate[7] were unrewarding. Our studies have been directed to two problems faced in the development of a reproducible and reliable procedure for the bisulfate dehydration. The first was that of developing reproducible conditions for obtaining a reasonable amount of the distilled crude product. This is a simple operational problem, our solution to which is stated in the Experimental section. The second was that of separating the acidic products formed in the process. This was resolved by ether extraction of the 3-hydroxy-2-pyrone, a weaker acid than either of the furan acid by-products also present, from an aqueous solution at a *p*H at which the stronger acids are in their salt forms. The procedure gave reproducible yields of 19% of a pure white, crystalline material, m.p. 92°, which gives a blue ferric chloride test. The product, obtained previously[4,5] in 9% yield, gave a blue-green ferric chloride test. We have noted that contamination with small amounts of furoic acid, which itself gives a bright yellow ferric chloride test, will change the blue color of the ferric chloride test to a blue-green.

The infrared absorption spectra of 3-hydroxy-2-pyrone show two significant features. The first is the presence of a single maximum in the carbonyl region at 1685 cm.$^{-1}$ and the second is the presence of a broad band in the O–H region at 3200 cm.$^{-1}$. It has been observed previously in our studies[8] that the carbonyl absorption in a wide variety of 2-pyrones is invariably present in the region of 1720 cm.$^{-1}$. The carbonyl absorption in 3-hydroxy-2-pyrone has been shifted 35 cm.$^{-1}$ toward lower frequencies. This is almost equivalent to the similar shift of 40 cm.$^{-1}$ in the normal carbonyl absorption noted in the hydrogen-bonded tropolones.[9] The broad absorption band in the 3200 cm.$^{-1}$ region is evidence of a strongly associated hydroxyl group and again is similar to that observed in tropolones. The infrared absorption characteristics of the methyl ether are those typical of other 2-pyrones. The carbonyl absorption is at 1725 cm.$^{-1}$ and the absorption in the OH–CH region is less intense and not nearly as broad. There is a shoulder at 2950 cm.$^{-1}$, characteristic of the C–H of the methyl groups. The shift in the carbonyl absorption band and the modification of the hydrogen-bonded hydroxyl band establish that 3-hydroxy-2-pyrone exists in a hydrogen-bonded enolic form (IIa, IIb, IIc) and not in the tautomeric 2,3-pyrononе structure (I). This is further substantiated by the absence of carbonyl absorption at 1750 cm.$^{-1}$ which is characteristic of unconjugated α-keto acids.[10] The comparisons with tropolone are evidence of considerable resonance stabilization in the pyrone which is perhaps surprising since the uncharged structures possible with tropolone do not exist.



I IIa IIc IIb

The reaction of 3-hydroxy-2-pyrone with aryldiazonium salts and the products obtained in the reaction are markedly different from those of the 6-hydroxy- and 4-hydroxy-2-pyrone structures found in β-methylglutaconic anhydride and triacetic lactone. The reaction must be run with sodium acetate as the base under conditions which are slightly acidic at the termination of the coupling because the products are decomposed by conditions of stronger alkalinity. The coupled products are also un-

TABLE I

SUBSTITUTED 4-PHENYLHYDRAZONO-2,3-PYRONONES

Substituent[a]	Color	M.p. (°C.)	Yield, %	Recrys. from[b]	Nitrogen analyses, % Calcd.	Found
...	Crimson	200[b]	90	E	12.96	12.94[c]
o-Nitro	Magenta	229	69	E	16.09	15.93
m-Nitro	Red	223	77	A	16.09	15.82
p-Nitro	Orange-red	233	77	A	16.09	16.14[d]
2-Methyl-4-nitro	Orange	226	65	C	15.27	15.32[e]
2,6-Dichloro-4-nitro	Yellow-brown	192	73	E	12.70	12.73
m-Chloro	Red	212[f]	88	T	11.19	11.08
p-Chloro	Red	220	77	T	11.19	11.27
m-Bromo	Red	211	81	A	9.50	9.19
o-Methyl	Red	120	87	E	12.17	12.20
p-Methyl	Red	194	85	A	12.17	12.04
o-Methoxy	Purple	179	81	E	11.38	10.73
p-Methoxy	Purple	197	95	T	11.38	11.16
2,5-Dimethoxy	Black	193	93	A	10.14	9.98
m-Trifluoromethyl	Orange	218	84	A	9.85	9.88
p-Aceto	Magenta	214	61	A	10.85	10.80[g]

[a] Substituents in phenyl ring. [b] E, ethyl acetate; A, anisole; C, chloroform; T, toluene. [c] *Anal.* Calcd. for $C_{11}H_8O_4N_2$: C, 61.11; H, 3.73. Found: C, 61.71; H, 3.77. [d] *Anal.* Calcd. for $C_{11}H_7O_5N_3$: C, 50.58; H, 2.70. Found: C, 50.73; H, 2.77. [e] *Anal.* Calcd. for $C_{12}H_9O_5N_3$: C, 52.37; H, 3.30. Found: C, 51.72; H, 3.45. [f] Darkens at 172°. [g] *Anal.* Calcd. for $C_{18}H_{10}N_4O_2$: C, 60.46; H, 3.90. Found: C, 60.71; H, 3.92.

(4) G. Chavanne, *Bull. soc. chim.*, [3] **29**, 337 (1903); *Compt. rend.*, **133**, 167 (1901); **134**, 1439, 1511 (1902); **136**, 49 (1903); **137**, 992 (1903); *Ann. chim. phys.*, [8] **3**, 507–574 (1904).

(5) L. Simon, *Compt. rend.*, **130**, 255 (1900).

(6) V. Oliveri and A. Peratoner, *Gazz. chim. ital.*, **19**, 633 (1889); M. Zenoni, *ibid.*, **20**, 517 (1890); *Ber.*, **23R**, 153c, 766 (1890).

(7) E. E. Blaise and H. Gault, *Compt. rend.*, **147**, 198 (1908); **148**, 176 (1909).

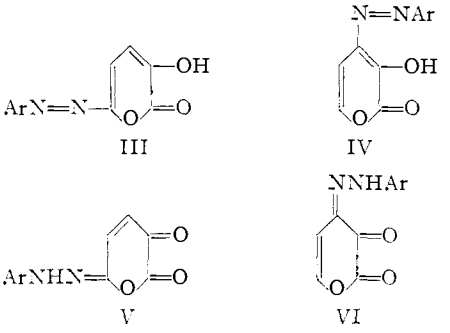
(8) Unpublished studies with Ellen V. Mochel.

(9) P. L. Pauson, *Chem. Revs.*, **55**, 9 (1955).

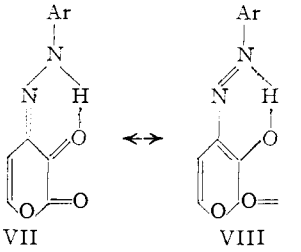
(10) L. J. Bellamy, "Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1954, p. 84.

stable in such solvents as methanol, ethanol, acetone and nitrobenzene. Those structures having electron attracting groups in the *para* position of the aryl ring are especially labile. The colors of the products, which are listed in Table I, are generally more dark than those of the other hydroxypyrone coupling products. No bright yellows were obtained and the dimethoxyphenyl derivative is a black solid. Since coupling does not occur with *p*-dimethylaminobenzenediazonium chloride as it does with the 6-hydroxy-2-pyrones and does not require the catalytic influence of pyridine in other couplings as is required by 4-hydroxy-2-pyrones, the 3-hydroxy isomer appears to be intermediate in reactivity between 6-hydroxy-2-pyrones and the 4-hydroxy-2-pyrones.

Four structures—the 6-arylazo III, the 4-arylazo structure IV, and the 4- and 6-arylhydrazono structures (V, VI)—may be assigned to these coupling



products. The first two of these (III and IV) can be eliminated on the basis of the absence of a positive ferric chloride test and the absence of infrared absorption in the carbonyl region at 1685 cm.$^{-1}$ which is characteristic of the 3-hydroxy-2-pyrone structure. The arylhydrazono structure is indicated by the strong absorption in the carbon–nitrogen double bond region at 1655 ± 5 cm.$^{-1}$. This is one of the strongest and most consistently observed bands in the spectra of these compounds. Absorption in the carbonyl region at 1715 ± 15 cm.$^{-1}$ and at 1766 ± 4 cm.$^{-1}$ is consistent with the presence of a 2-pyrone and a ketonic carbonyl. The breadth of the former suggests the presence of hydrogen bonding. There is, however, absorption in the nitrogen–nitrogen double bond region (1408 ± 5 cm.$^{-1}$)[11] and in the region characteristic of the conjugated phenyl group (1581 ± 6 cm.$^{-1}$). There is also a binodal band at 3280 ± 120 cm.$^{-1}$ and 3003 ± 23 cm.$^{-1}$ in the C–H, N–H, O–H region. The 3003 cm.$^{-1}$ band is probably a C–H stretching frequency of the olefinic–aromatic type. The absorption at 3280 cm.$^{-1}$ is considered to be due to an O–H or N–H bond subjected to some sort of association slightly less effective than that observed with 3-hydroxy-2-pyrone itself for which this absorption was observed at 3200 cm.$^{-1}$. Since this can best be attributed to a replacement of the carbonyl hydrogen bonding with hydrogen bonding involving the N–H of the hydrazono structure, the 6-arylhydrazono structure in which this is not possible must be abandoned in favor of the 4-arylhydrazono structure in which the hydrazono N–H can participate in bonded structures (VII, VIII). This will also permit correlation with the observed absorption at frequencies characteristic of the conjugated phenyl and azo groups.



Acknowledgment.—The authors wish to acknowledge with appreciation support of this research under National Science Foundation grant NSF-G55.

TABLE II

IMPORTANT INFRARED ABSORPTION MAXIMA FOR SUBSTITUTED 4-PHENYLHYDRAZONO-2,3-PYRANONES[a]

Substituent[b]	CH, OH, NH	C=O	C=N	Phenyl	—N=N—
...	3175s	1768s	1650s	1595m	1410s
	3000s	1712m		1575m	
o-Nitro	3175m	1762s	1650s	1590s	1412m
	3000m	1716m		1575m	
p-Methyl	3275s	1765s	1660s	1600m,sh	1406w
	3000m	1712m		1575m	
p-Chloro	3275s	1763s	1655s	....	1412m
	3050m	1712m,sh		1580s	
p-Methoxy	3160m	1765s	1650s	1600m	1405m
	2980m	1700m		1580m	
2,5-Di-	3400m	1770s	1660s	1600m,sh	1403m
methoxy	3000m	1730m		1587m	

[a] W, weak; m, medium, s, strong; sh, shoulder. Values in wave numbers (cm.$^{-1}$). [b] Substituent in phenyl ring.

Experimental[12]

3-Hydroxy-2-pyrone.—The following procedure is an adaptation of that used previously.[3] Details are given since they are unavailable elsewhere and are critical.

Two hundred grams of powdered potassium bisulfate was intimately mixed with 200 g. of mucic acid in a 2-l. distilling flask attached to a condenser. The following operations must be carried out in a hood to dispose of the toxic fumes generated during the reaction. The mixture in the distilling flask was heated with a Meeker burner from the top in such a fashion that the mass melted from the top downward. The temperature read by the thermometer in the neck of the flask was held at 160–165° during the distillation which required about 4 hr. The distillate which consisted of approximately 100 ml. of light brown fluid was filtered, adjusted to a *p*H 7 with 6 *N* hydrochloric acid and extracted with ether in a Friedrichs continuous extractor for 12 hr. After drying the ether was removed from the ether extract to leave a yellow crystalline residue. This residue was distilled to give 22.0 g. (19.6%) of substantially pure white crystalline material. Recrystallization from petroleum ether gave pure 3-hydroxy-2-pyrone, m.p. 92°, which gave a blue ferric chloride test.

The infrared absorption spectrum for 3-hydroxy-2-pyrone showed absorption at the following wave numbers: 3200 (medium-broad), 1685 (strong), 1630 (medium), 1550 (medium), 1420 (medium), 1390 (medium).

3-Methoxy-2-pyrone.—Nine grams of diazomethane was added to a slurry of 1 g. (0.01 mole) of 3-hydroxy-2-pyrone in 250 ml. of ether. The mixture was held at −5° for 4 hr. and at room temperature for 12 hr. Petroleum ether was

(11) R. J. W. LeFevre, W. F. O'Dwyer and R. L. Werner, *Chem. and Ind.*, 378 (1953).

(12) Carbon, hydrogen and nitrogen analyses by Micro Tech. Laboratories, Skokie, Illinois.

added to precipitate the crude product. Recrystallization from petroleum ether gave 1.1 g. (87%) of 3-methoxy-2-pyrone as a hygroscopic solid, m.p. 61°; reported[4] m.p. 60°.

The infrared absorption spectrum for 3-methoxy-2-pyrone shows absorption at the following wave numbers: 3200 (medium-narrow), 1725 (strong), 1645 (strong), 1575 (strong), 1465 (medium), 1413 (weak).

4-Arylhydrazono-2,3-pyronones.—The procedure which follows for coupling of 3-hydroxy-2-pyrone with diazotized *p*-chloroaniline was typical of that used for the preparation of the 4-arylhydrazone derivatives listed in Table I with a few exceptions. *p*-Nitroaniline, 2-methyl-4-nitroaniline, *p*-aminobiphenyl and *p,p'*-methylenebisdianiline were diazotized as their hydrochlorides in acetic acid with solid sodium nitrite. 2,6-Dichloro-4-nitroaniline was diazotized by the addition of powdered sodium nitrite to a concd. sulfuric acid solution of the amine.

4-(*p*-Chlorophenylhydrazono)-2,3-pyronone.—A solution of 1.0 g. (0.007 mole) of *p*-chloroaniline in 8 ml. of hydrochloric acid and 15 ml. of water was cooled to 0°. To this solution was added enough 5% aq. sodium nitrite solution to give a positive starch–iodide test. This reaction mixture containing the diazotized amine was then added to a cold solution of 0.5 g. (0.005 mole) of 3-hydroxy-2-pyrone and 5 g. of sodium acetate in 500 ml. of water. The temperature was held at 0–5° at all times. The red precipitate which forms on mixing the two solutions was collected on a filter and recrystallized from toluene to give 1.0 g. (77%) of pure product, m.p. 220°.

In addition to the analytically pure products listed in Table I, products were obtained from the following amines which could not be purified or gave analytical data not in accord with the arylhydrazone structure: *p,p'*-bismethylenebisdianiline, 4-aminobiphenyl, α-naphthylamine and *p*-iodoaniline.

Absorption Data.—Infrared spectra were determined using potassium bromide pellets in a Baird double beam recording infrared spectrometer.

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The Binding of Safranine O by Tobacco Mosaic Virus[1]

BY WILLIAM GINOZA AND DANIEL E. ATKINSON

RECEIVED DECEMBER 20, 1955

Tobacco mosaic virus ("common" strain) contains about 3.4×10^3 apparently identical sites per particle which bind safranine O. Removal of C-terminal threonine residues does not affect the number of these sites, but exposes an approximately equal number of new sites which appear to be identical among themselves but have a higher affinity for the dye. In the presence of *p*H 7 phosphate buffer the native virus can bind about 1.0×10^4 dye ions. The results are discussed in terms of the repeating sub-unit hypothesis of tobacco mosaic virus structure.

The demonstration that some strains of tobacco mosaic virus (TMV) bind a host nucleoprotein while others do not[2] suggested the desirability of quantitative studies on the binding of ions of known structure by the virus. Its large size makes TMV particularly well suited for such studies, since the virus and any bound component can be removed by centrifugation after equilibration. No dialysis is required and no membrane effects are involved. Despite these advantages and the wide application of ion-binding methods in the study of small protein,[3] there appears to be only one reported study of ion binding by TMV. Oster and Grimsson[4] reported that the virus bound 1.3×10^{-4} mole of acriflavin per g. of virus and presented a plot of extent of binding against free dye concentration.

Safranine O has been used in purification of TMV.[5] An insoluble complex was formed which was dissociated readily by addition of competing anions. The virus activity was fully restored on dissociation, implying that the dye–virus interaction was reversible and produced no permanent chemical or structural change in the particles. This dye has also been used at high *p*H for the determination of total acidic groups in TMV and other proteins.[6] It appeared to combine stoichiometrically with such groups, since the results with small proteins agreed well with the number of acidic groups indicated by amino acid analysis.

This paper reports quantitative studies on the binding of safranine O by native TMV and by the same strain of TMV after enzymic removal of its C-terminal threonine residues.

Experimental

Virus.—TMV strain U-1 ("common TMV") was purified by differential centrifugation.[7] Samples were stored either in distilled water or in phosphate buffer at 4° or at −30°. No observable change in binding behavior resulted from storage under any of these conditions. Virus concentration was estimated by nitrogen analysis (Nessler), using a factor of 6.25 g. of protein per g. of nitrogen[8] and a particle weight of 50,000,000.[9] Samples were dialyzed against dilute phosphate buffer, *p*H 7.0, or distilled water before use. When phosphate-stored samples were to be used for equilibrations in water, they were sedimented by centrifugation, resuspended in distilled water, and dialyzed against several changes of distilled water for six days on a shaker at 4°. The specific conductivity of the resulting solutions was about 15 μmhos per cm.

Terminal threonine residues were removed by treatment with carboxypeptidase.[10] After alternate slow and fast centrifugation to remove the enzyme, the virus was resuspended in distilled water and dialyzed against several changes of distilled water. Samples so treated will be referred to as "dethreonized" virus.

Dye.—Safranine O (National Aniline) was recrystallized once from water and dried under vacuum with sulfuric acid

(1) This work was supported in part by a contract between the Office of Naval Research, Department of the Navy, and University of California, NR 120-271, and in part by a grant from the Atomic Energy Commission, AT(11-1)-34, Project 8. (The AEC grant is under the direction of Dr. S. G. Wildman.)

(2) W. Ginoza, D. E. Atkinson and S. G. Wildman, *Science*, **119**, 269 (1954).

(3) I. M. Klotz in "The Proteins," H. Neurath and K. Bailey, Eds., Vol. I, Part B, Academic Press, New York, N. Y., 1953, pp. 727–806.

(4) G. Oster and H. Grimsson, *Arch. Biochem.*, **24**, 119 (1949).

(5) C. G. Vinson, *Phytopathology*, **22**, 965 (1932).

(6) (a) H. Fraenkel-Conrat and M. Cooper, *J. Biol. Chem.*, **154**, 239 (1944); (b) G. A. Kausche and F. Hahn, *Z. Naturforsch.*, **3b**, 437 (1948).

(7) W. M. Stanley, THIS JOURNAL, **64**, 1804 (1942).

(8) W. M. Stanley, *Physiol. Rev.*, **19**, 524 (1939).

(9) R. C. Williams, R. C. Backus and R. L. Steere, THIS JOURNAL, **73**, 2062 (1951).

(10) J. I. Harris and C. A. Knight, *Nature*, **170**, 613 (1952).