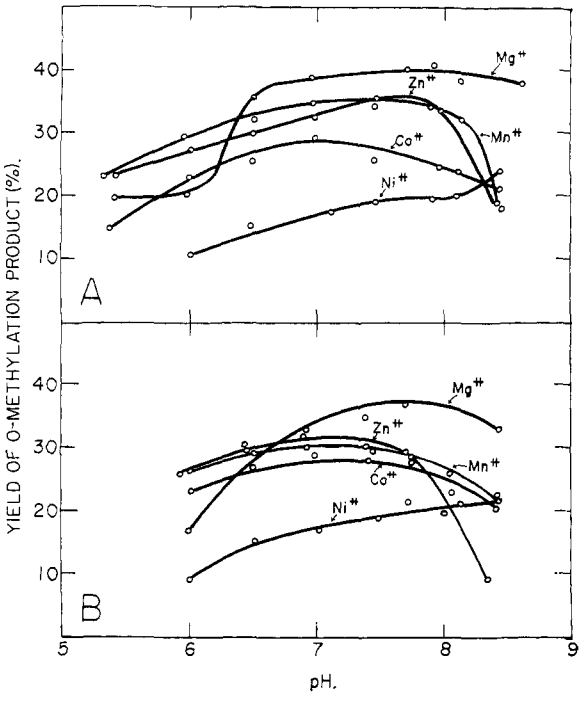
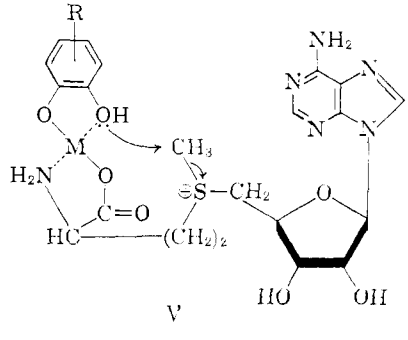


Fig. 6.—Yields of total enzymatic O-methylation products from 3,4-dihydroxyacetophenone (A) and from 3,4-dihydroxybenzaldehyde (B).

nesium consists in bringing substrate and enzyme together in the bridge complex V rather than in distorting the electron densities of the phenolic hydroxyls. In fact the transition metal cations that do withdraw electrons from the catechol hydroxyls most strongly prevent nucleophilic displacement of the methyl of adenosylmethionine (Fig. 6).



V

A second less likely explanation is the formation of the 2:1 catechol complex IV as a process competitive with the formation of the enzyme–metal–substrate complex V. Such an assumption fails to rationalize the observed order of activity of the various cations since both types of complexes would be expected to have the same relative stabilities.

TABLE VI

EFFECT OF THE CONCENTRATION OF CATIONS ON THE RATIO OF PRODUCTS IN THE ENZYMATIC O-METHYLATION OF 3,4-DIHYDROXYACETOPHENONE IN PHOSPHATE BUFFER AT *p*H 7.8

Metal,[a] μmoles		*m*-O-Methylation, %	Relative yield of O-methylated prod.,[c] %
Mg^{++}	0.2	55.0	66.2
	1.0[b]	55.3	100.0
	10.0	55.2	100.6
	100.0	55.4	68.7
Zn^{++}	0.2	63.3	82.1
	1.0	63.0	81.2
	10.0	62.0	81.3
	100.0	0	0
Mn^{++}	0.2	58.7	71.5
	1.0	58.0	76.2
	10.0	58.5	70.5
	100.0	54.5	21.8

[a] The figures express the molar ratio of added cations *versus* 1.0 μmole of substrate. [b] The yield obtained with 1.0 μmole of Mg^{++} ions was taken as standard (100%) for comparison. [c] The mixture containing 0.5 mg. (protein weight) of enzyme, 1 μmole of substrate, 150 μmoles of phosphate buffer (0.1 *M*, *p*H 7.8) and 0.1 μmole of EDTA was pre-incubated for 10 minutes at 37°. After the addition of 0.5 μmole of adenosylmethionine and 0.2 to 100 μmoles of metal salt and of sufficient water to a total volume of 3 ml., the mixture was incubated further for 50 min. and assayed for the ratio of *m*-isomer as well as for total yields of O-methylated product as described in the Experimental part.

[CONTRIBUTION FROM THE MIDWEST RESEARCH INSTITUTE, KANSAS CITY 10, MO.]

Antibiotics. I. Synthesis of 1,6-Dimethyl-5,7-dioxo-1,5,6,7-tetrahydropyrimido[5,4-e] *as*-Triazine (Toxoflavin) and Related Compounds[1a]

BY G. DOYLE DAVES, JR., ROLAND K. ROBINS AND C. C. CHENG[1b]

RECEIVED NOVEMBER 13, 1961

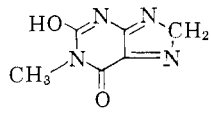
The synthesis of 1,6-dimethyl-5,7-dioxo-1,5,6,7-tetrahydropyrimido[5,4-e]-*as*-triazine (II) and related compounds is described. Compound II was found to be identical with the antibiotics toxoflavin and xanthothricin. The structure of a third related antibiotic, fervenulin, has been definitely established as 6,8-dimethyl-5,7-dioxo-5,6,7,8-tetrahydropyrimido[5,4-e]-*as*-triazine (XIX) which is isomeric with II.

The numerous mass fatal food poisonings in the province of Banjumas in central Java were found by van Veen and Mertens[2] to be due to a highly poisonous yellow crystalline substance called "toxoflavin," which periodically occurs in "bong-

(1) (a) This investigation was supported by research contract SA-43-ph-3025 from the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, Public Health Service. (b) To whom inquiries should be directed.

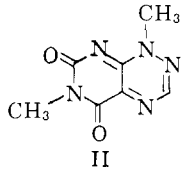
(2) (a) A. G. van Veen and W. K. Mertens, *Proc. Akad. Wetenschappen Amsterdam*, **36**, 666 (1933); (b) W. K. Mertens and A. G. van Veen, *Geneesk. Tijdschr. Ned. Indië*, **73**, 1223, 1309 (1933); (c) W. K. Mertens and A. G. van Veen, *Meded. Dienst Volksgezondheid Ned. Indië*, **22**, 209 (1933); (d) A. G. van Veen and W. K. Mertens, *Rec. trav. chim.*, **53**, 257, 398 (1934).

krek''—a popular and otherwise harmless native coconut product prepared by the action of certain fungi. The presence of toxoflavin has been shown to be due to contamination by the bacterium *Pseudomonas cocovenenans*.[3a] Since the isolation of toxoflavin in 1933, various efforts[3] have been made to elucidate its structure. The structure originally proposed by van Veen and Baars[3a] is the desmotropic tautomer of 1-methylxanthine (I)

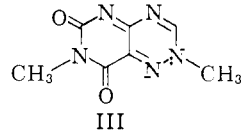


I

The original empirical formula $C_6H_6N_4O_2$, proposed by van Veen, *et al.*,[3a,b] has recently been shown to be in error. Hence it is quite understandable that Johnson and Ambelang[3c] failed to prepare toxoflavin on the basis of the proposed structure (I). Reinvestigation of this problem by van Damme, Johannes, Cox and Berends,[3f] resulted in the proposal of either structure II or III for toxoflavin. These investigators favored structure II on



II



III

the basis of degradation studies. Recent X-ray investigation[3h] has supported structure II for toxoflavin.

The total synthesis of toxoflavin has now been accomplished in our laboratory and confirms the structure proposed by van Damme, *et al.*,[3f] as 1,6-dimethyl-5,7-dioxo-1,5,6,7-tetrahydropyrimido[5,4-e]-*as*-triazine.[4] A preliminary report in this work has already appeared.[5] It is of interest to note that one of the first recorded observations of van Veen and Mertens[2a] in 1933 was the close structural resemblance of toxoflavin to Warburg's "yellow pigment" and the "flavins." The actual structural relationship of toxoflavin to riboflavin is truly striking, since both compounds possess similar oxidation–reduction systems.

2-Thiobarbituric acid (IV)[6] served as the starting material in the present work. Methylation of IV with excess dimethyl sulfate in dilute base gave 6-hydroxy-3-methyl-2-(methylthio)-4- (3H)-pyrimidinone (V). Chlorination of V with a mixture of phosphorus oxychloride and N,N-dimethylaniline readily gave 6-chloro-3-methyl-2-(methylthio)-4-(3H)-pyrimidone (VI, $R_1 = CH_3S$) in 57% yield. Acid hydrolysis of VI ($R_1 = CH_3S$) produced 6-chloro-2-hydroxy-3-methyl-4(3H)pyrimidinone (VI, $R_1 = OH$). Methylhydrazine replaced the chlorine atom of VI ($R_1 = CH_3S$, OH) to yield VII ($R_1 = CH_3S$, OH). The position of the methyl group on the methylhydrazino moiety of VII ($R_1 = CH_3S$, OH) was verified readily by treating the latter with substituted aldehydes and ketones to form the corresponding hydrazone derivatives.

The introduction of a nitrogen atom at position 5 of compound VII ($R_1 = CH_3S$, OH) was first attempted by means of nitrosation. The nitrosation conditions of Pfleiderer and Schündehütte[4a] using amyl nitrite were applied on both VII ($R_1 = CH_3S$, OH) and their formylated derivatives (VIII, $R_1 = CH_3S$, OH). However, at lower temperature (<°) no nitrosated product could be obtained and at higher temperature, nitrosation with simultaneous cleavage of the N–N bond of the hydrazino group occurred to form 3-methyl-6-(methylamino)-2-(methylthio)-5-nitroso-4(3H)-pyrimidinone (IX, $R_1 = CH_3S$), and 2-hydroxy-3-methyl-6-methylamino-5-nitroso-4(3H)-pyrimidinone (IX, $R_1 = OH$), respectively. These compounds were also readily obtained from VII ($R_1 = CH_2S$, OH) by the action of aqueous nitrous acid. Reduction of IX ($R_1 = CH_3S$) followed by formylation and cyclization with triethyl orthoformate gave 1,9-dimethyl-2-(methylthio)-6-purinone (XI, $R_1 = CH_3S$). Similarly, from IX ($R_1 = OH$) was obtained 1,9-dimethylxanthine (XI, $R_1 = OH$).

Since benzenediazonium chloride coupling with a 5-unsubstituted pyrimidine followed by the reduction of the resulting 5-phenylazo derivative[7] is one of the standard methods to introduce an amino group at the 5-position of the pyrimidine ring, 2-hydroxy-3-methyl-6-(1′-methylhydrazino)- 5 - phenylazo-4(3H)-pyrimidinone (XII) was prepared from VII ($R_1 = OH$). Catalytic reduction of XII with 10% palladium-on-charcoal in formic acid, however, again resulted in the rupture of the N–N bond of the hydrazino group to give X ($R_1 = OH$). This reduced product was identical with that prepared by reduction and formylation of 2-hydroxy-3-methyl-6-methylamino - 5 - nitroso - 4(3H) - pyrimidinone (IX, $R_1 = OH$).

The ease of N–N cleavage under these conditions can be explained as follows: Compounds VII, VIII and XII are the vinylogs of acid hydrazide. The breaking of an N–N bond of an acid hydrazide in the presence of reducing agents is well known.[8] This bond rupture reaction in nitrous acid is enhanced by the presence of a methyl group at the nitrogen atom which is attached directly to the ring. With the substitution of a methyl group at the 1′-N atom, the N,N-disubstituted acid hydrazide VII reacts with nitrous acid to form a nitrosohydrazino derivative XIII, which is subsequently decomposed, with the liberation of nitrous oxide to

(3) (a) A. G. van Veen and J. K. Baars, *Proc. Akad. Wetenschappen Amsterdam*, **40**, 498 (1937); (b) A. G. van Veen and J. K. Baars, *Rec. trav. chim.*, **57**, 248 (1938); (c) T. B. Johnson and J. C. Ambelang, *J. Am. Chem. Soc.*, **61**, 2483 (1939); (d) D. H. Nugteren, Thesis, Delft, 1956; (e) D. H. Nugteren and W. Berends, *Rec. trav. chim.*, **76**, 13 (1957); (f) P. A. van Damme, A. G. Johannes, H. C. Cox and W. Berends, *ibid.*, **79**, 255 (1960); (g) H. E. Latuasan and W. Berends, *Biochem. Biophys. Acta*, **52**, 502 (1961); (h) A. S. Hellendoorn, R. M. T. Cate-Dhont and A. F. Peerdeman, *Rec. trav. chim.*, **80**, 307 (1961).

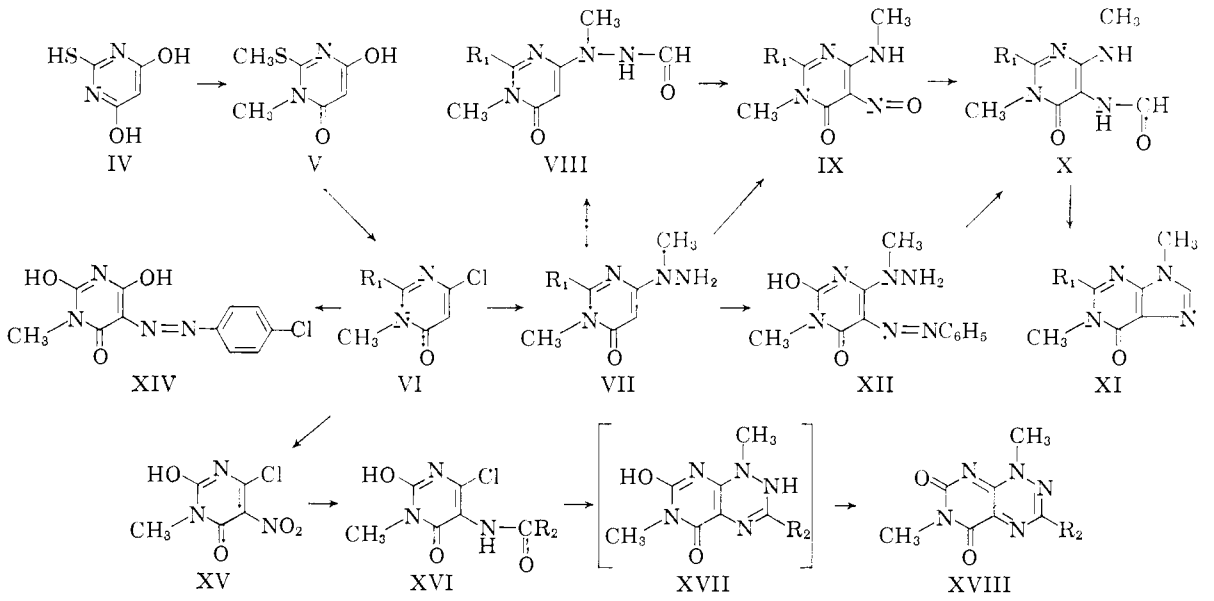
(4) The synthesis of this type of ring system has recently been reported by (a) W. Pfleiderer and K. H. Schündehütte, *Ann.*, **615**, 42 (1958); and (b) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **82**, 4592 (1960).

(5) G. D. Daves, R. K. Robins and C. C. Cheng, *ibid.*, **83**, 3904 (1961).

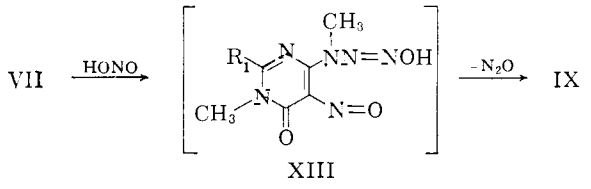
(6) H. Michael, *J. prakt. Chem.*, [2] **35**, 456 (1887).

(7) A systematic study of this reaction was made by B. Lythgoe, A. R. Todd and A. Topham, *J. Chem. Soc.*, 315 (1944).

(8) See, for example, (a) C. Ainsworth, *J. Am. Chem. Soc.*, **76**, 5774 (1954); **78**, 1635, 1636 (1956); (b) R. L. Hinman, *J. Org. Chem.*, **22**, 148 (1957); (c) A. Furst and R. E. Moore, *J. Am. Chem. Soc.*, **79**, 5492 (1957); (d) F. P. Robinson and R. K. Brown, *Can. J. Chem.*, **39**, 1171 (1961).



form the corresponding 6-methylamino-5-nitroso compound IX. Similar reactions of disubstituted hydrazine with nitrous acid have previously been observed.[9]



XIII

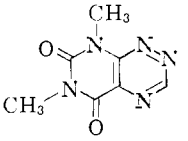
The coupling reaction was then attempted prior to the introduction of the hydrazino group. When benzenediazonium chloride failed to react with VI (R_1 = OH), the more reactive *p*-chlorobenzenediazonium chloride was utilized. The hydrolysis of the 6-chloro group during this coupling reaction, with the formation of 5-(*p*-chlorophenylazo)-2,6-dihydroxy-3-methyl-4(3H)-pyrimidinone (XIV), terminated this line of investigation.

Finally, nitration of 6-chloro-2-hydroxy-3-methyl-4(3H)-pyrimidinone (VI, R_1 = OH) at low temperature[10] gave a 45% yield of 6-chloro-2-hydroxy-3-methyl-5-nitro-4(3H)-pyrimidinone (XV). Catalytic reduction of XV in methanol in the presence of a trace of ammonia, followed by the addition of formic–acetic anhydride to the concentrated reaction mixture, gave 6-chloro-5-formamido-2-hydroxy-3-methyl-4(3H)-pyrimidinone (XVI, R_2 = H). This compound was then treated with methylhydrazine in ethanol. Cyclization and spontaneous oxidation of the intermediate XVII readily yielded 1,6-dimethyl-5,7-dioxo-1,5,6,7-tetrahydropyrimido-(5,4-e)-*as*-triazine (XVIII, R_2 = H) which was proved to be identical with toxoflavin (II).

Reduction of 6 - chloro - 2 - hydroxy - 3 - methyl-5-nitro- -4(3H)-pyrimidinone (XV) followed by the addition of acetic anhydride gave the corresponding 5-acetylamino derivative (XVI, R_2 = CH_3). This was treated with methylhydrazine to yield the 3-methyl homolog of toxoflavin (XVIII, R_2 = CH_3).

It has been suggested that toxoflavin was apparently identical with the antibiotic xanthothricin isolated by Machlowitz, *et al.*[11] This was confirmed in our laboratory by a comparison of the synthetic toxoflavin with an authentic sample of xanthothricin.

The structure of fervenulin, a new antibiotic recently isolated and studied by DeBoer, *et al.*,[12] and Eble, *et al.*,[13] has been elucidated and confirmed in our laboratory[14] as 6,8-dimethyl-5,7-dioxo-5,6,7,-8-tetrahydro-pyrimido[5,4-e]-*as*-triazine (XIX),[4a] which is isomeric with toxoflavin (II).



XIX

The structures of fervenulin and toxoflavin (xanthrothricin) provide a new class of antibiotics possessing the pyrimido[5,4-e]-*as*-triazine ring system. Further studies in this interesting area are currently in progress.

Experimental[15]

6-Hydroxy-3-methyl-2-methylthio-4(3H)-pyrimidinone (V).—To 50 g. of 2-thiobarbituric acid[6] in 500 ml. of a 2 *N* sodium hydroxide solution cooled in an ice-bath was added, during 3 hr., 100 g. of dimethyl sulfate. The solution was allowed to stir at room temperature for 2 hr. and acidified with hydrochloric acid to *p*H 1–3. The precipitated product

(9) N. V. Sidgwick, "The Organic Chemistry of Nitrogen," Oxford University Press, New York, N. Y., 1942, pp. 379–380.

(10) (a) R. M. Cresswell and H. C. S. Wood, *J. Chem. Soc.*, 4768 (1960(; (b) J. Davoli and D. D. Evans, *J. Chem. Soc.*, 5041 (1960).

(11) R. A. Machlowitz, W. P. Fisher, B. S. McKay, A. A. Tytell and J. Charney, *Antibiotics & Chemotherapy*, **4**, 259 (1954).

(12) C. DeBoer, A. Dietz, J. S. Evans and R. M. Michaels, *Antibiotics Annual*, 220 (1959–1960).

(13) T. E. Eble, E. C. Olson, C. M. Lange and J. W. Shell, *ibid.*, 227 (1959–1960).

(14) G. D. Daves, R. K. Robins and C. C. Cheng, *J. Org. Chem.*, **26**, 5256 (1961).

(15) All melting points were taken on a Thomas–Hoover melting point apparatus. The infrared spectra were taken with a Perkin–Elmer infracord and the ultraviolet absorption spectra were determined with a Beckman DK-2.

was filtered, washed well with water and dried. Recrystallization from water several times gave 21 g. (35.2%) of white crystalline 6-hydroxy-3-methyl-2-methylthio-4-pyrimidinone, m.p. 195–197°; $\lambda_{max}^{pH\ 1}$ 243 mμ (ε 5,200), 276 mμ (ε 9,000); $\lambda_{max}^{pH\ 11}$ 265 mμ (ε 7,900).

Anal. Calcd. for $C_6H_8N_2O_2S$: C, 41.8; H, 4.6; N, 16.3. Found: C, 42.1; H, 4.6; N, 16.0.

6-Chloro-3-methyl-2-methylthio-4(3H)pyrimidinone (VI, $R_1 = CH_3S$).—A mixture consisting of 170 g. of unrecrystallized 6-hydroxy-3-methyl-2-methylthio-4(3H)-pyrimidinone, 500 ml. of phosphorus oxychloride and 75 ml. N,N-dimethylaniline was heated under reflux for 3 hr. The excess phosphorus oxychloride was then distilled under reduced pressure and the residue was poured on crushed ice. This crushed ice mixture was allowed to stand for 10 hr. under refrigeration. The precipitated product was then filtered and washed well with water and dried. The dry crude product was suspended in 1 l. of petroleum ether (b.p. 40–60°) and stirred for 30 min. The insoluble product was collected and dried to obtain 125 g. of product, m.p. 90–95°. Recrystallization from heptane gave 107 g. (57%) of product, m.p. 111–112°, λ_{max}^{EtOH} 299 mμ (ε 9,300).

Anal. Calcd. for $C_6H_7N_2OSCl$: C, 37.9; H, 3.7; N, 14.7. Found: C, 37.6; H, 3.8; N, 14.6.

3-Methyl-6-(1′-methylhydrazino)-2-methylthio-4(3H)-pyrimidinone (VII, $R_1 = CH_3S$).—To a stirred suspension of 5 g. of 6-chloro-3-methyl-2-methylthio-4(3H)-pyrimidinone in 25–50 ml. of ethanol was added 15 ml. of methylhydrazine. Complete solution with evolution of heat was immediately achieved and after a few minutes a heavy white precipitate was formed. After 30 min., this precipitate was filtered, washed with ethanol and dried. Recrystallization from ethanol gave 3.2 g. (61%) of long white needles, m.p. 190–191°; $\lambda_{max}^{pH\ 1}$ 236 mμ (ε 21,000), 283 mμ (ε 9,400); $\lambda_{max}^{pH\ 11}$ 243 mμ (ε 29,000), 283 mμ (ε 9,400).

Anal. Calcd. for $C_7H_{12}N_4OS$: C, 42.0; H, 6.0; N, 28.0. Found: C, 41.8; H, 5.8; N, 28.5.

3-Methyl-6-[1′-methyl-2′-(α-methyl-*m*-nitrobenzylidene)-hydrazino]-2-(methylthio)-4(3H)-pyrimidinone.—A mixture of 3 g. of 3-methyl-6-(1′-methylhydrazino-2-methylthio-4-(3H)-pyrimidinone and 3 g. of *m*-nitroacetophenone in 50 ml. of ethanol was heated under reflux for 1 hr. During this time solution was attained, which was soon followed by the formation of a heavy yellow precipitate. This product was filtered, washed with ethanol and dried to yield 5.1 g. (98%) of deep yellow crystals, m.p. 203–205°.

Anal. Calcd. for $C_{15}H_{17}N_5O_2S$: C, 51.8; H, 4.9; N, 20.2. Found: C, 52.3; H, 5.2; N, 20.0.

3-Methyl-6-(2′-formyl-1′-methylhydrazino)-2-methylthio-4(3H)-pyrimidinone (VIII, $R_1 = CH_3S$).—A solution of 3 g. of 3-methyl-6-(1′-methylhydrazino)-2-methylthio-4(3H)-pyrimidinone in 60 ml. of ethanol containing 15 ml. of formic–acetic anhydride[16] was stirred for 30 min. The product which precipitated during this time was filtered and recrystallized from ethanol to yield 1.9 g. (55%) of white crystals, m.p. 217–218°; λ_{max}^{EtOH} 223 mμ (ε 23,800), 237 mμ (ε 29,000), 281 mμ (ε 7,500).

Anal. Calcd. for $C_6H_{12}N_4O_2S$: C, 42.1; H, 5.3; N, 24.5. Found: C, 42.0; H, 5.0; N, 24.2.

3-Methyl-6-methylamino-2-methylthio-5-nitroso-4(3H)-pyrimidinone (IX, $R_1 = CH_3S$).—To an ice-cooled suspension of 2 g. of 3-methyl-6-(2′-formyl-1′-methylhydrazino)-2-methylthio-4(3H)-pyrimidinone in 40 ml. of 50% acetic acid was added 1.5 g. of sodium nitrite in 10 ml. of water. After the mixture was stirred for 4 hr. the blue precipitate was filtered and recrystallized from water to yield 0.9 g. (48%) of 3-methyl-6-methylamino-2-methylthio-5-nitroso-4(3H)-pyrimidinone as brilliant blue needles, m.p. 247–248°; $\lambda_{max}^{pH\ 1}$ 335 mμ (ε 16,500); $\lambda_{max}^{pH\ 11}$ 230 mμ (ε 10,500), 324 mμ (ε 20,500).

Anal. Calcd. for $C_7H_{10}N_4O_2S$: C, 39.2; H, 4.7; N, 26.1. Found: C, 39.1; H, 4.5; N, 26.2.

In an analogous manner 5 g. of 3-methyl-6-(1′-methylhydrazino)-2-methylthio-4(3H)-pyrimidinone yielded 3.4 g. (63%) of 3-methyl-6-methylamino-2-methylthio-5-nitroso-4(3H)-pyrimidinone.

5-Formamido-3-methyl-6-methylamino-2-methylthio-4-(3H)-pyrimidinone (X, $R_1 = CH_3S$).—A suspension of 5 g. of 3-methyl-6-methylamino-2-methylthio-5-nitroso-4(3H)-pyrimidinone in 24 ml. of formamide and 16 ml. of formic acid was warmed on a water-bath to 90–100°. Sodium hydrosulfite was added portionwise until a light yellow solution was attained. The solution was heated for an additional 15 min. and then diluted with 200 ml. of water. After cooling, the product was filtered and recrystallized from water to yield 3.3 g. (62%) of 5-formamido-3-methyl-6-methylamino-2-methylthio-4(3H)-pyrimidinone, m.p. 273–275°; $\lambda_{max}^{pH\ 1}$ 238 mμ (ε 26,000), 275 mμ (ε 9,800); $\lambda_{max}^{pH\ 11}$ 238 mμ (ε 26,000), 277 mμ (ε 10,000).

Anal. Calcd. for $C_8H_{12}N_4O_2S$: C, 42.1; H, 5.3; N, 24.6; S, 14.0. Found: C, 42.3; H, 5.2; N, 24.7; S, 14.3.

1,9-Dimethyl-2-methylthio-6-purinone (XI, $R_1 = CH_3S$).—A mixture of 11 g. of 5-formamido-3-methyl-6-methylamino-2-methylthio-4(3H)-pyrimidinone, 35 ml. of formic acid and 35 ml. of ethyl orthoformate was heated under reflux for 3 hr. The resulting solution was evaporated to dryness and the residue recrystallized from a small volume of ethanol to yield 5.2 g. (51%) of 1,9-dimethyl-2-methylthio-6-purinone, m.p. 223–224°.

Anal. Calcd. for $C_8H_{10}N_4OS$: C, 45.7; H, 4.8; N, 26.7. Found: C, 45.3; H, 4.8; N, 26.7.

6-Chloro-2-hydroxy-3-methyl-4(3H)-pyrimidinone (VI, R_1 = OH).—A suspension of 50 g. of 6-chloro-3-methyl-2-2-methylthio-4(3H)-pyrimidinone in 500 ml. of 50% ethanol containing 75 ml. of concentrated hydrochloric acid was heated under reflux for 12 hr. The resulting solution was evaporated to dryness under reduced pressure and the residue was stirred with 250 ml. of ether for 2 hr. The crude product (12–19 g.), m.p. 270–274°, was filtered and after recrystallization from ethanol gave 10–16 g. (31–47%) of 6-chloro-2-hydroxy-3-methyl-4(3H)-pyrimidinone, m.p. 277–279°, $\lambda_{max}^{pH\ 1}$ 261 mμ (ε 8,700), $\lambda_{max}^{pH\ 11}$ 281 mμ (ε 11,000). Evaporation of the ether solution resulted in the recovery of 7–12 g. of starting material.

Anal. Calcd. for $C_5H_5N_2O_2Cl$ C, 37.5; H, 3.1; N, 17.4. Found: C, 37.3; H, 3.3; N, 17.4.

2-Hydroxy-3-methyl-6-(1′-methylhydrazino)-4(3H)-pyrimidinone (VII, R_1 = OH).—To a stirred suspension of 10 g. of 6-chloro-2-hydroxy-3-methyl-4(3H)pyrimidinone in 75 ml. of ethanol was added 30 ml. of methylhydrazine. Solution was achieved with evolution of heat. The solution was heated to boiling, stirred at room temperature for 1 hr., and then evaporated to dryness under reduced pressure. The residue was triturated with 50 ml. of ethanol and again evaporated to dryness. This process was repeated until most of the excess methylhydrazine was removed. The residue was then recrystallized from ethanol to yield 5.2 g. (49%) of 2-hydroxy-3-methyl-6-(1′-methylhydrazino)-4(3H)-pyrimidinone, m.p. 207–209°; $\lambda_{max}^{pH\ 1}$ 236 mμ (ε 19,400), 282 mμ (ε 4,800); $\lambda_{max}^{pH\ 11}$ 243 mμ (ε 25,700), 274 mμ (ε 4,700).

Anal. Calcd. for $C_6H_{10}N_4O_2$: C, 42.3; H, 5.9; N, 32.9. Found: C, 42.7; H, 6.1; N, 32.3.

6-(2′-Formyl-1′-methylhydrazino)-2-hydroxy-3-methyl-4-(3H)-pyrimidinone (VIII, R_1 = OH).—To a suspension of 2.5 g. of 2-hydroxy-3-methyl-6-(1′-methylhydrazino)-4-(3H)-pyrimidinone in 50 ml. of ethanol was added 8 ml. of formic–acetic anhydride. The resulting solution was stirred for 2 hr., then evaporated to dryness. The residue was recrystallized from ethanol to yield 1.8 g. of product, m.p. 248°; $\lambda_{max}^{pH\ 1}$ 268 mμ (ε 4,800); $\lambda_{max}^{pH\ 11}$ 224 mμ (ε 13,100), 270 mμ (ε 17,000).

Anal. Calcd. for $C_7H_{10}N_4O_3$: C, 42.4; H, 5.1; N, 28.3. Found: C, 42.3; H, 5.4; N, 28.2.

2-Hydroxy-3-methyl-6-methylamino-5-nitroso-4(3H)-pyrimidinone (IX, R = OH).—To an ice-cooled suspension of 2 g. of 2-hydroxy-3-methyl-6-(1′-methylhydrazino)-4(3H)-pyrimidinone in 30 ml. of 50% acetic acid was added 1.5 g. of sodium nitrite in 10 ml. of water. The mixture was stirred for 3 hr. and the red precipitate filtered and recrystallized from a mixture of dimethylformamide and water to yield 0.9 g. (32%) of bright red needles, m.p. 262–263° dec.; $\lambda_{max}^{pH\ 1}$ 226 mμ (ε 12,100), 314 mμ (ε 8,100); $\lambda_{max}^{pH\ 11}$ 225 mμ (ε 5,200), 245 (ε 5,000), 310 mμ (ε 15,100).

Anal. Calcd. for $C_6H_8N_4O_3$. C, 39.1; H, 4.3; N, 30.5. Found: C, 39.2; H, 4.0; N, 30.3.

This compound was similarly prepared, in 34% yield, by the nitrosation of 6-(2′-formyl-1′-methylhydrazino)-2-hydroxy-3-methyl-4(3H)-pyrimidinone

(16) (a) A. Béhal, *Compt. rend. Acad. Sci.*, **128**, 1460 (1899); (b) A. Béhal, *Ann. chim. phys.*, [7], **20**, 411 (1900).

2-Hydroxy-3-methyl-6-(1'-methylhydrazino)-5-phenylazo-4(3H)-pyrimidinone (XII).—Concentrated hydrochloric acid was added dropwise, with stirring, to a suspension of 6.5 g. of 2-hydroxy-3-methyl-6-(1'-methylhydrazino)-4(3H)-pyrimidinone in 50 ml. of water until solution was complete. The *p*H of the solution was then adjusted to 4 by the addition of solid sodium acetate. Then an aqueous solution of benzene diazonium chloride, prepared from 3.7 g. of aniline in the usual manner, was added. After 3 hr. the yellow product was filtered and recrystallized from water to yield 4.3 g. (41%) of 2-hydroxy-3-methyl-6-(1'-methylhydrazino)-5-phenylazo-4(3H)-pyrimidinone, m.p. 215–216°; $\lambda_{max}^{pH\ 1}$ 252 mμ (ε 16,700), 299 mμ (ε 3,900), 400 mμ (ε 25,500); $\lambda_{max}^{pH\ 11}$ 231 mμ (ε 15,100), 270 mμ(s) (ε 8,500), 380 mμ (ε 13,700).

Anal. Calcd. for $C_{12}H_{14}N_6O_2$: C, 52.5; H, 5.1; N, 30.7. Found: C, 52.4; H, 4.9; N, 30.7.

5-Formamido-2-hydroxy-3-methyl-6-methylamino-4(3H)-pyrimidinone (X, R = OH). Method A.—A mixture of 4.3 g. of 2-hydroxy-3-methyl-6-(1'-methylhydrazino)-5-phenylazo-4(3H)pyrimidinone, 1.2 g. of 10% palladium-on-charcoal and 40 ml. of 80% formic acid was shaken at room temperature under 34–45 p.s.i. of hydrogen for 45 min. The catalyst was then filtered and the filtrate evaporated to dryness. The residue was recrystallized from water to yield 1.4 g. (45%) of 5-formamido-2-hydroxy-3-methyl-6-methylamino-4(3H)-pyrimidinone, m.p. > 360°, $\lambda_{max}^{pH\ 1}$ 269 mμ (ε 24,100), $\lambda_{max}^{pH\ 11}$ 267 mμ (ε 18,000).

Anal. Calcd. for $C_7H_{10}N_4O_3$: C, 42.4; H, 5.0; N, 28.3. Found: C, 41.9; H, 5.0; N, 28.8.

Method B.—A mixture of 2 g. of 2-hydroxy-3-methyl-6-methylamino-5-nitroso-4(3H)-pyrimidinone, 10 ml. of formamide and 8 ml. of formic acid was heated on a steam-bath. Sodium hydrosulfite was added portionwise until a clear light yellow solution was obtained. This solution was cooled and the product filtered and recrystallized from water to yield 0.9 g. product, identical with that prepared by method A.

1,9-Dimethylxanthine[17] (XI, R_1 = OH).—A mixture of 2 g. of 5-formamido-2-hydroxy-3-methyl-6-methylamino-4-(3H)-pyrimidinone, 50 ml. of dimethylformamide and 10 ml. of ethyl orthoformate was heated under reflux for 2 hr. The solution was evaporated to dryness and the residue recrystallized from water to yield 0.9 g. product, m.p. > 360°; $\lambda_{max}^{pH\ 1}$ 235 mμ (ε 6,700), 263 mμ (ε 9,900) $\lambda_{max}^{pH\ 11}$ 247.5 mμ (ε 9,000), 277 mμ (ε 9,000).

Anal. Calcd. for $C_7H_8N_4O_2$: N, 31.1. Found: N, 31.2.

5-(*p*-Chlorophenylazo)-2,6-dihydroxy-3-methyl-4(3H)-pyrimidinone (XIV).—To a solution of 2 g. of 6-chloro-2-hydroxy-3-methyl-4(3H)-pyrimidinone in 50 ml. of water containing 1.1 g. of sodium bicarbonate cooled to 0° was added a solution of *p*-chlorobenzenediazonium chloride prepared from 1.5 g. of *p*-chloroaniline, 7.5 ml. of concentrated hydrochloric acid in 100 ml. of water and 0.85 g. of sodium nitrite. After 30 min. the orange precipitate which had formed was filtered and washed with water, ethanol and ether. Recrystallization from ethanol gave 1.9 g. (54%) of yellow plates, m.p. 266–267°; $\lambda_{max}^{pH\ 1}$ 231 mμ (ε 9,000), 386 mμ (ε 16,200), 236 mμ (ε 8,400), 374 mμ (ε 12,600). (During recrystallization a marked decrease in solubility was noted, apparently due to *syn-anti* isomerization.)

Anal. Calcd. for $C_{11}H_9N_4O_3Cl$: C, 47.1; H, 3.2; N, 20.0; Cl, 12.7. Found: C, 46.8; H, 3.6; N, 20.1; Cl, 12.6.

6-Chloro-2-hydroxy-3-methyl-5-nitro-4(3H)-pyrimidinone (XV).—To 25 ml. of concentrated sulfuric acid cooled to 10–15° was added with stirring 8.5 g. of 6-chloro-2-hydroxy-3-methyl-4(3H)-pyrimidinone at such a rate that the temperature remained below 15°. After the addition was complete 8.5 ml. of fuming nitric acid was added dropwise at such a rate that the temperature remained below 15°. The mixture was then allowed to warm slowly to room temperature and was stirred for 30 min. The mixture was then poured on ice and stirred vigorously. After the ice had almost melted the light yellow crystalline product was filtered, washed well with water and dried to yield 6.3 g., m.p. 190–193°. Recrystallization from a benzene–methanol mixture gave 4.9 g. (45%) of light yellow plates, m.p. 195–197°, λ_{max}^{EtOH} 271 mμ (ε 7,000).

(17) (a) H. Biltz and K. Strufe, *Ann.*, **423**, 200 (1921); (b) E. S. Golovchenskaya and E. S. Chaman, *Zhur. Obschei Khim.*, **30**, 1873 (1960).

Anal. Calcd. for $C_5H_4N_3O_4Cl$: C, 29.3; H, 2.0; N, 20.5. Found: C, 28.8; H, 2.1; N, 20.2.

6-Chloro-5-formamido-2-hydroxy-3-methyl-4(3H)-pyrimidinone (XVI, R_2 = H).—A mixture of 5 g. of 6-chloro-2-hydroxy-3-methyl-5-nitro-4(3H)-pyrimidinone, 0.5 g. of platinum oxide, 5 ml. of 28% aqueous ammonia and 150 ml. of methanol was shaken under 30–40 p.s.i. of hydrogen until the theoretical amount had been absorbed. The catalyst was then filtered and the filtrate was concentrated to a thick paste below 25°. To this paste was added 15–20 ml. of formic–acetic anhydride. Complete solution was attained and after 5–10 min. a precipitate began to form. After 30 min., 50 ml. of ethanol was added and the product filtered and dried. The crude product (2.7 g., m.p. 220–225°) was recrystallized from a small amount of ethanol to yield 2.1 g. (43%), m.p. 225–226°; $\lambda_{max}^{pH\ 1}$ 268 mμ (ε 7,800); $\lambda_{max}^{pH\ 11}$ 230 mμ (ε 5,600), 286 mμ (ε 1,300).

Anal. Calcd. for $C_6H_6N_3O_3Cl$: C, 35.4; H, 3.0; N, 20.6. Found: C, 35.4; H, 3.2; N, 20.8.

1,6-Dimethyl-5,7-dioxo-1,5,6,7-tetrahydropyrimido(5,4-e)*as*-triazine (XVIII, R_2 = H).—A mixture of 1.5 g. of 6-chloro-5-formamido-2-hydroxy-3-methyl-4(3H)-pyrimidinone and 0.34 g. of methylhydrazine in 30 ml. of ethanol was heated under reflux on the steam-bath for 2 hr. The dark reddish-brown precipitate was filtered and stirred for 2 hr. in 50–75 ml. of saturated ammonium sulfate solution. The mixture was filtered and the filtrate was extracted five times with 50-ml. portions of chloroform. The chloroform extract was dried over anhydrous magnesium sulfate, filtered and evaporated to dryness. The yellow residue was recrystallized from a small volume of *n*-propyl alcohol to yield 0.4 g. (28%) of 1,6-dimethyl-5,7-dioxo-1,5,6,7-tetrahydropyrimido-(5,4-e)-*as*-triazine, m.p. 172–173° dec. (lit.[3a] m.p. 171° dec.); $\lambda_{max}^{pH\ 1,7}$ 257.5 mμ (ε 16,400), 394 mμ (ε 2,500); λ_{max}^{KBr} (μ) 3.4(w), 5.9(s), 6.0(s), 6.25(s), 6.6(s), 6.95(m), 7.05-(m), 7.25 (w), 7.4(w), 7.8(s), 8.1(s), 8.35(w), 8.8(m), 9.6-(m), 10.4(w), 10.9(s), 11.55(w), 12.35(m), 13.0(s), 13.8(m) and 14.15(m).

Anal. Calcd. for $C_7H_7N_5O_2$: C, 43.5; H, 3.7; N, 36.3. Found: C, 43.7; H, 3.6; N, 36.1.

The ultraviolet and infrared absorption spectra are identical with those of toxoflavin[3b,f] and xanthothricin.[11]

R_f-Values of synthetic toxoflavin (25°, descending) are 0.35 (95% ethanol), 0.29 (1-butanol–10% urea); R_f-values of xanthothricin[18]: 0.35(95% ethanol), 0.29 (1-butanol–10% urea).

5-Acetamido-6-chloro-2-hydroxy-3-methyl-4(3H)-pyrimidinone (XVI, R_2 = CH_3).—A mixture of 10 g. of 6-chloro-2-hydroxy-3-methyl-5-nitro-4(3H)-pyrimidinone, 1 g. of platinum oxide, 1 ml. of 28% aqueous ammonia and 100 ml. of methanol was shaken at room temperature under 35–45 p.s.i. of hydrogen until the theoretical amount was absorbed. The catalyst was filtered and the solvent evaporated under reduced pressure. Twenty-five milliliters of acetic anhydride was added to the residue and this mixture heated in a water-bath for 30 min. The mixture was cooled, 50 ml. of ethanol added, and the solvent evaporated to dryness. The residue was recrystallized from an ethanol–heptane mixture to yield 4.3 g. (40%) of 5-acetamido-6-chloro-2-hydroxy-3-methyl-4(3H)-pyrimidinone, m.p. 217–218°.

Anal. Calcd. for $C_7H_8N_3O_3Cl$: C, 38.7; H, 3.8; N, 19.4. Found: C, 38.8; H, 4.0; N, 19.2.

1,3,6-Trimethyl-5,7-dioxo-1,5,6,7-tetrahydropyrimido[5,4-e]-*as*-triazine (XVIII, R_2 = CH_3).—A mixture of 2 g. of 5-acetamido-6-chloro-2-hydroxy-3-methyl-4(3H)-pyrimidinone and 0.5 g. of methylhydrazine in 30 ml. of ethanol was refluxed on a water-bath for 2 hr. The insoluble precipitate was then filtered and stirred for 2 hr. in 75 ml. of a saturated ammonium sulfate solution. The solution was filtered and the filtrate was extracted with five 50-ml. portions of chloroform. The chloroform extract was dried over sodium sulfate and evaporated to dryness. The residue was recrystallized from a heptane–ethanol mixture to yield 0.3 g. of brilliant yellow plates, m.p. 181–182° dec.; $\lambda_{max}^{pH\ 7}$ 258 mμ (ε 17,400). 399 mμ (ε 1,800).

Anal. Calcd. for $C_8H_9N_5O_2$: C, 46.4; H, 4.4; N, 33.8. Found: C, 46.2; H, 4.7; N, 33.6.

6,8-Dimethyl-5,7-dioxo-5,6,7,8-tetrahydropyrimido[5,4-e]-*as*-triazine (fervenulin, XIX) was prepared by the reductive cyclization of 1,3-dimethyl-2,4-dioxo-5-nitroso-6-formyl-

(18) Kindly provided by Dr. F. J. Wolfe of Merck and Co., Inc., Rahway, N. J.

hydrazino-1,2,3,4-tetrahydropyrimidine with sodium hydrosulfite in the presence of formic acid and formamide,[4a] m.p. 178–179° (lit.[4a] m.p. 178–179°); $\lambda_{max}^{ethanol}$ 238 mμ (ε 18,500); 275 mμ (ε 1,600) and 340 mμ (ε 4,200); the infrared absorption λ_{max}^{Nujol} (μ); 3.0(w), 3.4(s), 3.8(w), 5.8(s), 6.0(s), 6.4(s), 6.55(s), 6.8(s), 7.0(s), 7.1(m), 7.2(w), 7.4(s), 7.8(s), 8.0(w), 8.25(s), 8.75(w), 9.0(w), 9.2(s), 9.6(s), 10.05(m), 10.4(w), 10.7(m), 11.3(m), 12.2(m), 12.45(w), 13.4(s), 13.55(m), and 13.9(s). The ultraviolet and infrared absorption spectra are identical to those of an authentic sample of fervenulin.[19]

(19) Kindly provided by Dr T. E. Eble, of the Upjohn Co., Kalamazoo, Mich.

R_f Values of synthetic fervenulin (25°, descending) are: 0.82 (96% water–4% butanol), 0.81 (25% acetic acid–50% butanol–25% water); R_f-values of natural fervenulin: 0.81 (96% water–4% butanol), 0.81 (25% acetic acid–50% butanol–25% water).

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Solvent Isotope Effects in Catalyzed Hydrolysis of Carboxylic Acid Derivatives

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Kinetic solvent isotope effects for N-methylimidazole and acetate catalysis of benzoic anhydride hydrolysis, and for N-methylimidazole and 4-methylpyridine catalysis of *p*-nitrophenyl acetate hydrolysis in light and heavy water have been measured. The isotope effects for these reactions fall in the range 1.1 to 1.5, indicating that nucleophilic catalysis is operative. The two mechanistic possibilities for general base catalysis, (1) the general base acting directly on a water molecule in the transition state or (2) prior equilibrium of the ester with hydroxide ion and a general acid-assisted decomposition of the intermediate, are discussed. The latter mechanism is discarded for carboxylic acid derivatives with good leaving groups (phenyl esters and anhydrides).

Solvent isotope effects obtained from rate of reaction in light and heavy water (k_{H_2O}/k_{D_2O}) may in certain cases be used to differentiate between nucleophilic catalysis and true general base catalysis for catalyzed hydrolysis of carboxylic acid derivatives. The reason for this is that general base mechanisms involve a slow proton transfer, therefore, a sizable kinetic isotope effect: small isotope effects (1.0–1.5) will be expected for nucleophilic catalysis by analogy with the small isotope effects for the hydrolysis of alkyl halides and sulfonates, and the acetate-catalyzed enolization of methylacetylacetone.[1,2]

Existing examples of general base catalysis in carboxylic acid derivatives are the hydrolysis of acetylimidazole catalyzed by imidazole,[3a] of 1-acetyl-3-methylimidazolium chloride catalyzed by N-methylimidazole,[3b] of acetic anhydride catalyzed by acetate,[4] of ethyl haloacetates catalyzed by various bases,[5] and of acetylserine derivatives catalyzed by various bases[6]; also, the general base-catalyzed aminolysis of esters.[7] The general base mechanism is enhanced by electron-withdrawing groups on either the alkyl group or the acyl group.

It is possible that carboxylic acid anhydrides could have the properties which make general base catalysis possible even for heterocyclic nitrogen bases, which tend to react by nucleophilic interaction on unsaturated centers. However the general base mechanism in this case is not kinetically distinguishable from nucleophilic catalysis. Therefore in the present work use is made of isotope effects in order to differentiate between these two mechanisms; benzoic anhydride hydrolysis catalyzed by N-methylimidazole in light and heavy water was investigated. In order to compare this result with reactions which in all likelihood react by nucleophilic catalysis, benzoic anhydride hydrolysis catalyzed by acetate and *p*-nitrophenyl acetate hydrolysis catalyzed by N-methylimidazole and 4-methylpyridine were also studied in light and heavy water. The rate of disappearance of both *p*-nitrophenyl acetate and benzoic anhydride can be simply and accurately followed spectrophotometrically allowing increased acccuracy over use of aliphatic anhydrides as substrates. N-Methylimidazole was chosen as the catalytic base instead of imidazole because it was desired to avoid secondary isotope effects due to the NH group of imidazole. In formate-, acetate- and pyridine-catalyzed hydrolysis of acetic anhydride, Gold and Butler have observed solvent isotope effects of 1.1, 1.5–1.7 and 5 ± 1, respectively.[8,9] These results were explained as nucleophilic catalysis by formate,[8] general base catalysis by acetate[8] and a rate-controlling attack of water on the acetylpyridinium ion, the concentration of which is limited by the acetate present in the buffer used.[9]

Experimental

Materials.—*p*-Nitrophenyl acetate was prepared from acetic anhydride and *p*-nitrophenol,[10] and recrystallized several times from petroleum ether; m.p. 77.5–78.0°. Eastman Kodak Co. white label benzoic anhydride was recrystallized three times from benzene–petroleum ether, and

(1) R. E. Robertson and P. M. Laughton, *Can. J. Chem.*, **34**, 1714 (1956); R. E. Robertson and P. M. Laughton, *ibid.*, **35**, 1319 (1957).

(2) F. A. Long and D. Watson, *J. Chem. Soc.*, 2019 (1958).

(3) (a) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1280 (1959); (b) W. P. Jencks and R. Wolfenden, *J. Am. Chem. Soc.*, **83**, 4390 (1961).

(4) M. Kilpatrick, *ibid.*, **50**, 2891 (1928).

(5) W. P. Jencks and J. Carriuolo, *ibid.*, **83**, 1743 (1961).

(6) W. P. Jencks, E. Cordes and B. Anderson, *J. Biol. Chem.*, **236**, 455 (1961).

(7) J. F. Bunnett and G. T. Davis, *J. Am. Chem. Soc.*, **82**, 665 (1960); W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 675 (1960).

(8) V. Gold and A. R. Butler, *Proc. Chem. Soc.*, 15 (1960); V. Gold and A. R. Butler, *J. Chem. Soc.*, 2305 (1961).

(9) V. Gold and A. R. Butler, *ibid.*, 4362 (1961).

(10) F. Chattaway, *ibid.*, **134**, 2495 (1931).