16. *t*-Butyl Trifluoroacetylglycyl-L-prolinate.—Trifluoroacetylglycine[18] (1.71 g., 0.010 mole), *t*-butyl L-prolinate (1.71 g., 0.010 mole) and tetraethyl pyrophosphite (3 ml., 0.011 mole) were added to 7 ml. of dry dimethoxyethane. After 30 minutes of refluxing, the solution was added to 50 ml. of ice-water and the peptide derivative soon crystallized. It was collected, washed with water and air-dried; wt. 2.27 g. (69%), m.p. 89–90°. A 1-g. sample recrystallized from 10 ml. of methylcyclohexane yielded 0.85 g. having the same m.p. and $[\alpha]^{25}D$ −83° (*c* 2, ethanol). *Anal.* Calcd. for $C_{13}H_{19}F_3N_2O_4$: C, 48.1; H, 5.91; N, 8.64; F, 17.6. Found: C, 48.2; H, 5.78; N, 8.90; F, 17.8.

17. Ethyl Glycyl-DL-phenylalaninate. (Table IV).—Ethyl benzyloxycarbonylglycyl-DL-phenylalaninate[3] (3.84 g.) was added to 20 ml. of a solution of *N* hydrogen bromide in glacial acetic acid and the resulting solution was heated on a steam-bath for 15 minutes. Addition of a double volume of ether and chilling caused crystallization of 3.05 g. (93% yield) of ethyl glycyl-DL-phenylalaninate hydrobromide, m.p. 154–155°; calcd. 24.1% Br, found 24.0% (Volhard titration). The free base was obtained as an oil by adding anhydrous ammonia to a suspension of 1.66 g. (0.0050 mole) in 75 ml. of anhydrous ether, filtering off the precipitated ammonium bromide (Celite used) and evaporating the ether solution. The recovery was 90.4% for the dipeptide ester. Crystallization began in 38 minutes. After 5 hours at 25° the product was triturated with anhydrous ether (3 × 10 ml.) and air-dried; a 73% yield (670 mg.) of DL-3(6)-benzyl-2,5-diketopiperazine, m.p. 271–274° dec., was obtained. After a period of 23 hours an additional 150 mg. of diketopiperazine with the same m.p. was isolated, making a total recovery of 89% from the free base.

Comparative Stabilities of *t*-Butyl Glycinate and Ethyl Glycinate. (Table IV).—The free base of *t*-butyl glycinate was prepared by shaking 0.050 mole (10.65 g.) of the phosphite salt with a mixture of 50 ml. of 2 *N* sodium hydroxide and 100 ml. of ether. The ether layer was separated, washed with 10 ml. of water and dried with anhydrous sodium sulfate. Concentration of the solution and distillation of the residue at 2 mm. yielded 4.0 g. (62%), b.p. 30°, $n^{24}D$ 1.4227. The distillate was divided into two equal portions and each was placed in a screw-cap vial to exclude moisture and carbon dioxide.[19] One sample was kept at −20° and the other at room temperature. Freshly prepared ethyl glycinate was prepared and kept under identical conditions for comparison. The latter solidified completely during 4 days at room temperature, and was about one-third solid after 21 days at −20°. By contrast, *t*-butyl glycinate was unchanged after 30 days and only about one-fourth solidified after 325 days at room temperature; at −20°, no solid had formed in 700 days and the refractive index was unchanged. Reconversion to the phosphite salt, m.p. 157–159° dec., was quantitative.

TABLE IV

STABILITY OF AMINO ACID AND DIPEPTIDE ESTERS AS FREE BASES

	Amino ester	Solid formation,[a] % At rm. temp.	At −20°
1	H·gly·OEt[b]	100/4 days	35/21 d.
2	H·gly·O-*t*-Bu	25/325 d.	0/700 d.
3	H·pro·O-*t*-Bu(L)	Trace/150 d.	
4	H·pro·OEt(L)[c]	100/30 d.	
5	H·gly-phe·OEt(DL)	73/5 hr.	
6	H·gly-phe·O-*t*-Bu(L)	0/23 d.	Trace/540 d.

[a] The ether-insoluble solid from (5) was found to have the m.p. of the known diketopiperazine (see Exptl.). The trace of material from (6) had m.p. 95° dec., whereas the reported m.p. of the diketopiperazine is 260° (H. T. Huang and C. Niemann, THIS JOURNAL, **72**, 921 (1950)). [b] Prepared from the hydrochloride by NH_3 in ether and distilled under red ced pressure. [c] Prepared from the hydrochloride by Et_3N in ether; b.p. 53° at 2.5 mm., $n^{16}D$ 1.4500.

(18) F. Weygand and E. Leising, *Ber.*, **87**, 248 (1954).

(19) K. T. Poroshin, Yu. I. Khurgin and T. D. Kozarenko, *Bull. Acad. Sci. U.S.S.R.*, 1453 (1959), have shown that carbon dioxide catalyzes the self-condensation of ethyl glycinate.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN CO.]

The Synthesis of Nucleoside and Nucleotide Analogs Derived from Uridine*

BY BRIAN BANNISTER AND FRED KAGAN

RECEIVED SEPTEMBER 9, 1959

The synthesis of a diethyl 5′-deoxy-5′-phosphonate analog of uridylic acid is described, together with certain other 5′-substituted uridines. The 5′-deoxy-5′-mercapto analog of uridine exists in the solid state and in neutral and acid solutions as a 5,6-dihydro-5′,6-cyclic sulfide. In alkaline solution (0.01 *N*), ring opening occurs to regenerate the 5,6-double bond of an authentic uridine structure together with the formation of the 5′-mercaptide anion (*vide* XIII → XVI).

The importance of uridylic acid in ribonucleic acid synthesis and its determination as a constituent of the novel nucleotide shown by Park and Strominger[1] to be implicated in bacterial cell wall formation made it of interest to examine analogs of uridylic acid as potential antitumor, antiviral and antibacterial agents.

Although considerable data have been accumulated concerning nuclear derivatives of pyrimidine nucleosides,[2] relatively little information is available concerning pyrimidine nucleosides which incorporate substituted sugar moieties.[3] For this reason, attention in the present work was directed initially toward the synthesis of the phosphonic acid analog (V) of uridylic acid (VI). The phosphonic acid group bears an extremely close resemblance to the acid function of a monophosphate ester both sterically and in pK_a values, as has been pointed out previously.[2,4,5]

Burger, *et al.*,[6] have described the syntheses of "7-[2,3,4-tri-O-acetyl-6-deoxy-6-(diethyl phosphonate)-β-D-glucopyranosyl]-theophylline" and "6-benzamido-9-[2,3,4-tri-O-acetyl-6-deoxy-6-(diethyl phosphonate)-β-D-glucopyranosyl]-purine" in continuation of studies of phosphonate analogs of nucleotides, but no phosphonate analog of a naturally occurring nucleoside phosphate has been re-

* Presented at the Michigan-Toledo-South Bend Meeting in Miniature of the America Chemical Society, Wayne State University, Detroit, Michigan, on February 26, 1960.

(1) J. T. Park and J. L. Strominger, *Science*, **125**, 99 (1957).

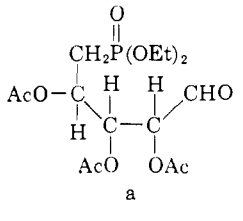
(2) See, *inter alia*, "Antimetabolites of Nucleic Acid Precursors," D. W. Visser in "Antimetabolites and Cancer," A.A.A.S. Symposium, A.A.A.S. publication, 1955, p. 47.

(3) J. J. Fox, J. F. Codington, N. C. Yung, L. Kaplan and J. O. Lampen, THIS JOURNAL, **80**, 5155 (1958), and earlier papers.

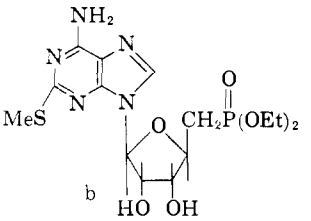
(4) S. Preis, T. C. Myers and E. V. Jensen, THIS JOURNAL, **77**, 6225 (1955).

(5) J. R. Parikh and A. Burger, *ibid.*, **77**, 2386 (1955); B. S. Griffin and A. Burger, *ibid.*, **78**, 2336 (1956).

(6) J. R. Parikh, M. E. Wolff and A. Burger, *ibid.*, **79**, 2778 (1957).

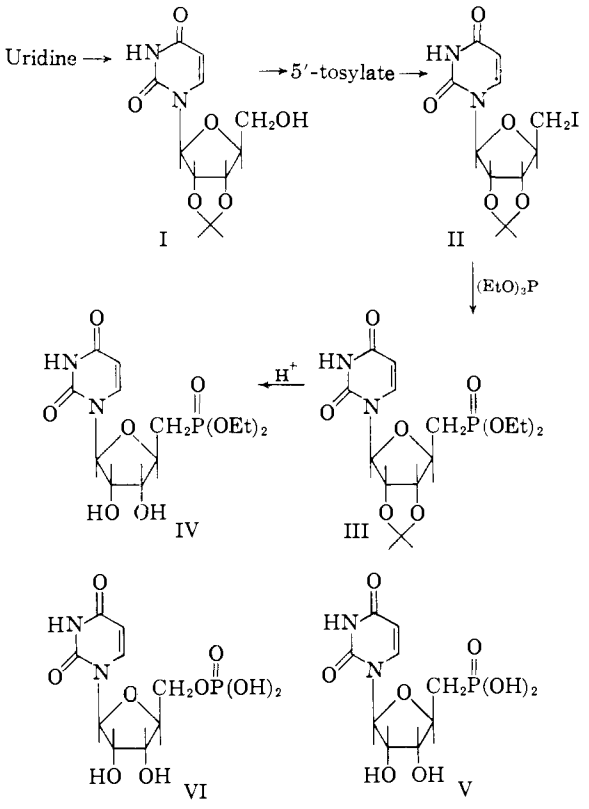


a



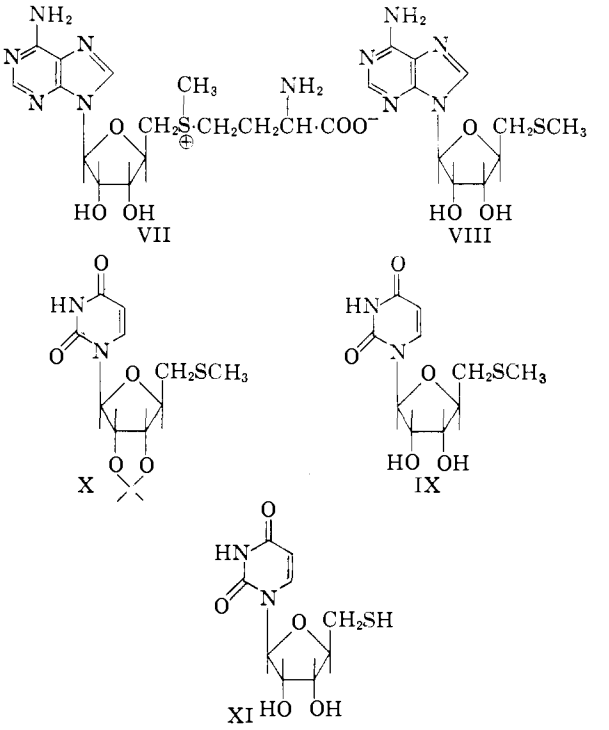
b

ported to date.[7] The conversion of uridine to 5′-deoxy-5′-iodo-2′,3′-O-isopropylideneneuridine (II) *via* the acetonide I, and the 5′-tosylate has been recorded by Levene.[8] This series of reactions was found to proceed in excellent yield in the present work. The iodide II underwent the Michaelis–Arbuzov reaction with triethyl phosphite to give the phosphonate ester III[9] in 49% yield, together with 25% of recovered iodide. Although hydrolysis of the acetonide group with dilute mineral acid was unsatisfactory, the method of Helferich, *et al.*,[10] using aqueous acetic acid gave the desired diol diester IV in good yield. No pure product could be isolated from the attempted acid hydrolysis of the diol diester IV to the free phosphonic acid V, and no reaction took place with dilute alkali (1 *N*) at room temperature. Studies on more vigorous alkaline hydrolysis conditions are being continued.



I II III IV V VI

Interest in 5′-substituted nucleosides is not restricted to nucleotides and their analogs. "Active methionine" (VII), the sulfonium salt formed enzymatically from ATP and methionine, is a key intermediate in the transfer of methyl groups in enzymatic methylations, and undergoes degradation to the nucleoside "adenine thiomethylpentoside" (VIII), the structure of which has been established by Baddiley.[11] It was of interest, therefore, to synthesize potential antimetabolites of "active methionine" in order to study their effects on tissue proliferation. Furthermore, unusual activities have been reported[12,13] for "adenine thiomethylpentoside" itself, making the synthesis of the corresponding uridine analog IX of interest for the determination of structure–activity relationships.



VII VIII X IX XI

The iodo derivative II reacted readily with potassium mercaptide in dimethylformamide solution or with sodium mercaptide in ethanol to yield the 5′-deoxy-5′-methylthioacetonide derivative X, which was hydrolyzed with dilute sulfuric acid to the desired 5′-deoxy-5′-methylthiouridine (IX).

As a thio analog of uridine, 5′-deoxy-5′-mercaptouridine (XI) was desired. Its acetonide XII was reported by Baddiley[14] as a model in a projected synthesis of S-(5′-deoxyadenosine-5′)-homocysteine by treatment of 2′,3′-O-isopropylideneuridine 5′-

(7) Since the completion of this work, Wolff and Burger [*J. Am. Pharm. Assoc.*, **48**, 56 (1959)] have published a "progress report" on further attempts to synthesize a nucleoside phosphonate and, by means of an elaborate partial synthesis from the aldehydo-ester, a, derived from D-ribose, have obtained "9-[5′-deoxy-5′-(diethylphosphonate)-β-D-ribofuranosyl]-6-amino-2-(methylthio)-purine," b. This, on removal of the methylthio- and ester groups, would furnish the phosphonic acid analog of adenylic acid, and is the closest recorded approach to the phosphonate of a naturally-occurring nucleoise.

(8) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **106**, 113 (1934).

(9) Phosphonic esters are formulated as containing the P=O linkage in this paper. For the preference for this coördinate structure to the dative (P → O) link, see L. E. Sutton, *et al.*, *J. Chem. Soc.*, 147 (1945), concerning dipole moment evidence, and references quoted in L. J. Bellamy's "The Infrared Spectra of Complex Molecules" (Methuen and Co. Ltd., 1958, Chapter 18, p. 311) concerning Raman spectral data.

(10) B. Helferich, H. Dressler and R. Grieber, *J. prakt. Chem.*, **153**, 286 (1939); B. R. Baker, R. E. Schaub and J. H. Williams, THIS JOURNAL, **77**, 7 (1955).

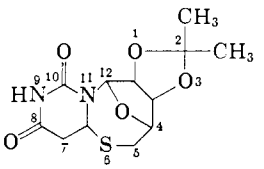
(11) J. Baddiley, *J. Chem. Soc.*, 1348 (1951).

(12) R. Kuhn and K. Henkel, *Z. physik. Chem.*, **269**, 41 (1941).

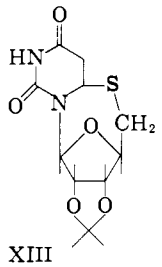
(13) W. Nakahara, F. Inukai, S. Ugami and Y. Nagata, *Sci. Jap. Inst. Phys. Chem. Res.*, **42**, 153 (1945).

(14) J. Baddiley and G. A. Jamieson, *J. Chem. Soc.*, 1085 (1955).

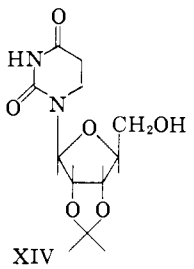
tosylate with potassium thiolacetate, followed by hydrolysis with methanolic ammonia. From the reaction between sodium hydrogen sulfide and the 5′-deoxy-5′-iodoacetonide (II), a crystalline solid was isolated in excellent yield in the present work, but was found to exhibit only end-absorption in the ultraviolet spectrum, as distinct from the characteristic 258 mμ absorption of uridine. The infrared spectrum indicated that the cyclic bisamide grouping was intact, and also that the sugar moiety was still present. The product contained sulfur, but was not a mercaptan. The analytical data were in perfect agreement with the expected 5′-deoxy-5′-mercapto structure (XII). The absence of absorption at 258 mμ and the absence of the 1617 cm.$^{-1}$ absorption assignable to the 5,6-double bond in the uracil nucleus were all in accord with the cyclized structure (XIII),[15] in which addition of the



-SH group across the activated 5,6-bond had occurred. Some confirmation of this proposed structure was obtained by a spectroscopic examination of 2′,3′-O-isopropylidene-5,6-dihydrouridine (XIV), a model 5,6-dihydro nucleoside, obtained by the catalytic reduction of 2′,3′-O-isopropylideneuridine. This product showed very similar ultraviolet and infrared spectra to those of the sodium hydrogen sulfide product. Baddiley[14] recorded an analysis



XIII

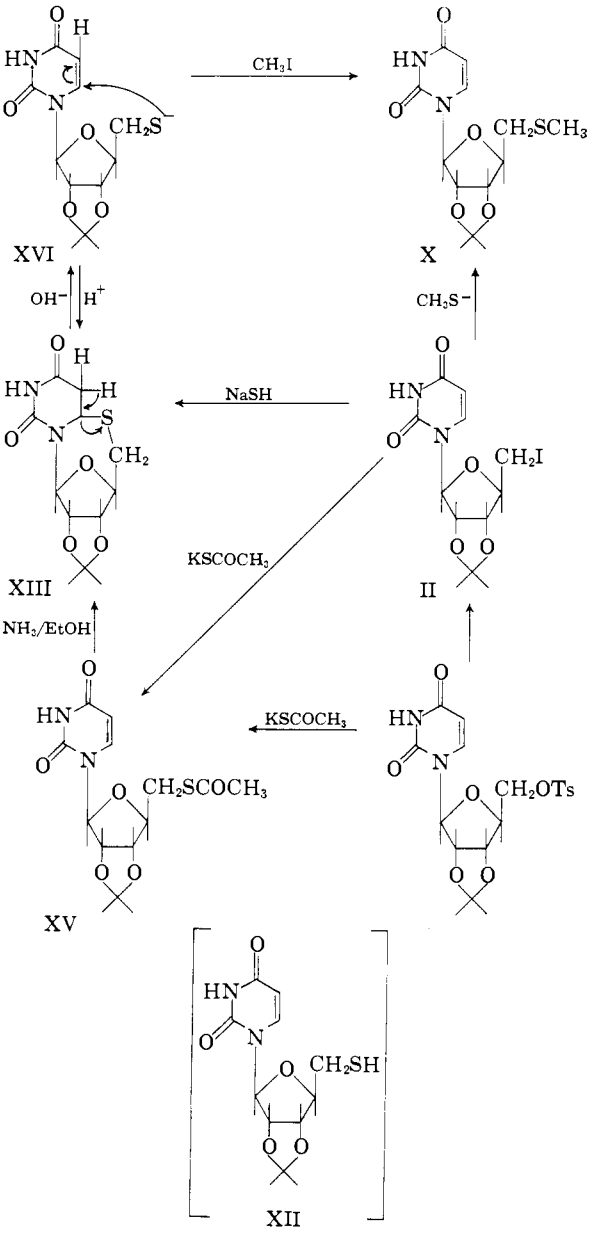


XIV

for his product, but no spectral data. The reported melting point was approximately that of the product XIII above. A repetition of Baddiley's reaction, using both the 5′-tosyl and 5′-iodo derivatives with potassium thiolacetate, yielded the same product, identical with that from the sodium hydrogen sulfide reaction by melting point, mixed melting point and ultraviolet and infrared spectra. It would appear, therefore, that the 5′-deoxy-5′-mercapto derivative XII is unknown, except in the form of the anion XVI. The acetylthio intermediate, not purified by Baddiley, was isolated as a homogeneous oil by chromatography in the present investigation. It showed the infrared and ultraviolet spectra to be expected of 5′-acetylmercapto-5′-deoxy-2′,3′-O-isopropylideneuridine (XV). It

(15) The naming of this cyclized structure presents certain difficulties. Alternatives would appear to be 5′-deoxy-5′,6-epithio-5,6-dihydro-2′,3′-O-isopropylideneuridine or, systematically, hexahydro-2,2 - dimethyl - 4,12 - epoxy - 1,3 - dioxolo[e]pyrimido[4,3-b][1,3]thiazocine-8,10(3aH, 9H)-dione.

is of interest that Baddiley's attempt to prepare a mixed sulfide from his "mercapto compound" with α-amino-γ-bromobutyric acid was unsuccessful, a failure which he ascribed to the acidity of uridine and its derivatives, since the reaction proceeded as expected in the corresponding adenosine series.



While the ultraviolet spectrum of the cyclic sulfide (XIII) in ethanol solution was unchanged in 0.01 *N* sulfuric acid–ethanol (Fig. 1), dissolution in 0.01 *N* potassium hydroxide–ethanol caused the reappearance of absorption at 260 mμ with a molar absorptivity of 7,750 (Fig. 1). This implies that reversal of cyclization occurs in solution in strong base to give the sulfide anion XVI in which the nuclear 5,6-double bond has been regenerated.[16] Acidification of the alkaline solution would have

(16) The high end-absorption of the cyclic sulfide, still present in alkaline solution (Fig. 1), appears to be exhibited frequently by sulfur compounds.

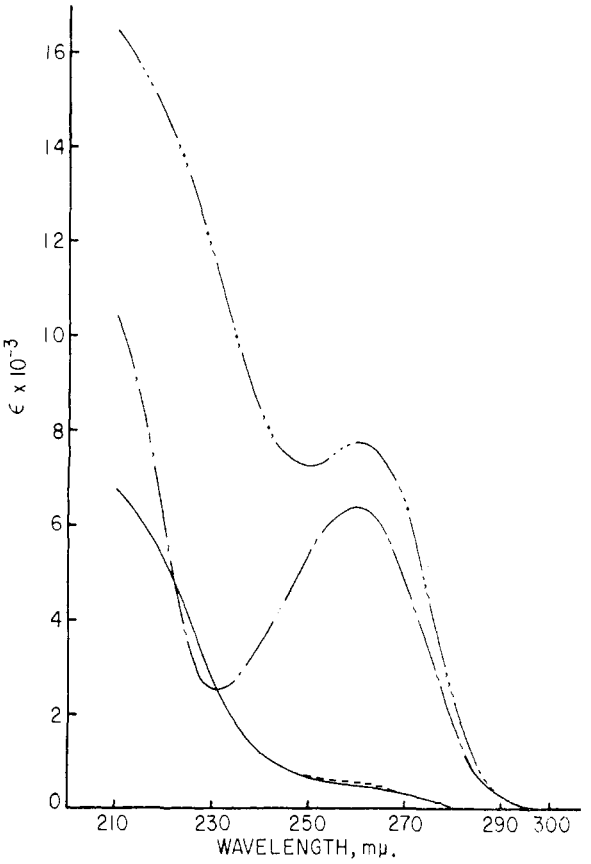


Fig. 1.—Effect of *p*H on ultraviolet spectrum of cyclic sulfide XIII: ——, 95% EtOH; ----, 0.01 *N* H_2SO_4–95% EtOH; — — —, 0.01 *N* KOH–95% EtOH; — - —, 0.01 *N* KOH–95% EtOH after acidification.

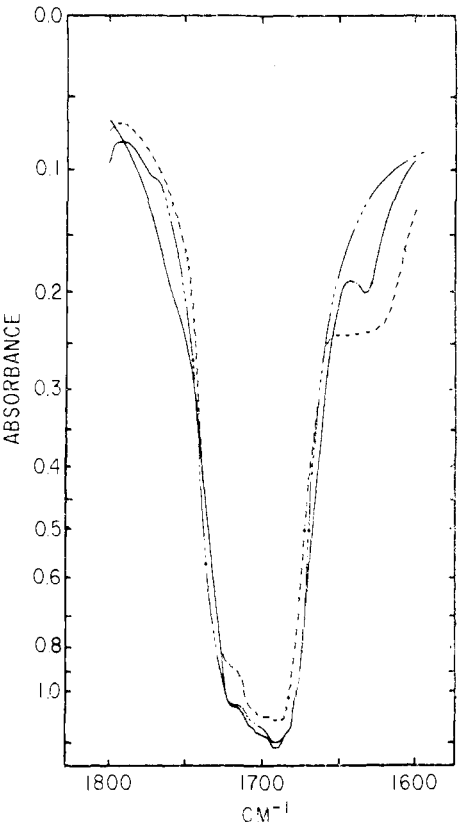
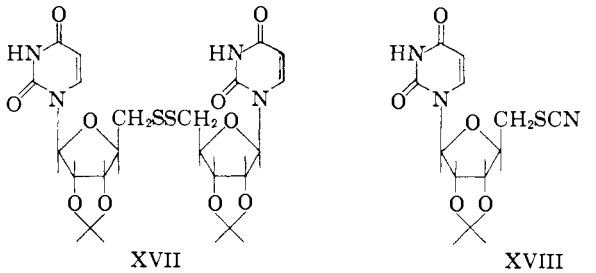


Fig. 2.—Effect of *p*H on carbonyl region of infrared spectrum of cyclic sulfide XIII: — - - - —, 90% $CHCl_3$ in EtOH; - - - - -, 0.01 *N* KOH–90% $CHCl_3$ in EtOH; ——, 5′-deoxy-5′-methylthio derivative X in 90% $CHCl_3$ in EtOH.

been expected to regenerate the original curve (high end-absorption, flex at 260 mμ with a molar absorptivity of 438) shown by the cyclic sulfide XIII. Instead, a discrete peak formed at 260 mμ (Fig. 1). This behavior suggests the formation of a stable, non-cyclized product from at least part of the intermediate mercaptide anion (XVI) probably the disulfide XVII formed by atmospheric oxidation. Additional spectral evidence concerning the reversibility of the reaction XVI ⇄ XIII is available from a study of the infrared absorption. In chloroform–ethanol solution, the cyclic sulfide XVI shows no absorption at 1617 $cm.^{-1}$ characteristic of the 5,6-double bond. On the addition of sodium hydroxide, however, a shoulder appears in this region (Fig. 2), comparable to that of the methylthio-derivative X.



XVII XVIII

Chemical evidence of the reversal of sulfide ring formation was obtained by treatment of the cyclic sulfide XIII with methyl iodide in the presence of sodium ethoxide in ethanol to yield 66% of the methylthio derivative X, identical with that derived by treatment of the iodo derivative II with methyl mercaptan and base. In the absence of methyl iodide, these same reaction conditions resulted in the recovery of 89% of the cyclic sulfide XIII after neutralization.

As expected, the iodo derivative II reacted normally with ethanolic sodium thiocyanate to yield 5′-deoxy-2′,3′-O-isopropylidene-5′-thiocyanatouridine (XVIII), but attempts to isolate the free diol by hydrolysis of the acetonide have been unsuccessful.

The biological evaluation of these nucleoside and nucleotide analogs is in progress, and any results of interest will be reported elsewhere when available.

Experimental[17]

Diethyl 5′-Deoxy-2′,3′-O-isopropylideneuridine 5′-Phosphonate (III).—A mixture of 10.0 g. of 5′-deoxy-5′-iodo-2′,3′-O-isopropylideneuridine[8] (II, 25.4 mmoles) and 43.5 ml. of triethyl phosphite (253 mmoles, freshly distilled) was heated in an oil-bath at 155° for 12 hours with exclusion of atmospheric moisture by means of a Drierite tube. The crystalline iodo compound dissolved within the first few minutes. The excess of triethyl phosphite was then removed under high vacuum in an oil-bath at 90–100° with magnetic stirring; 100 ml. of dry benzene was added and re-

(17) All melting points were taken on a Kofler block, and are uncorrected. Ultraviolet spectra were determined in 95% ethanol using a Cary model 11 or 14 recording spectrophotometer. Infrared spectra were determined in Nujol mulls with a Perkin-Elmer model 21 spectrophotometer.

moved by distillation at atmospheric pressure and finally at 40° (15 mm.). The fawn-colored viscous oily residue (11.7 g.) was dissolved in 30 ml. of benzene diluted with 15 ml. of Skellysolve B[18] and the solution placed on a column of 350 g. of Florisil,[19] made up in and washed with 33⅓% Skellysolve B in benzene. Elution with 25% ethyl acetate in benzene gave recovered iodo compound (2.65 g.), while the desired phosphonate was eluted with pure ethyl acetate. This crystalline fraction (5.03 g., 49%) gave colorless needles of diethyl 5′-deoxy-2′,3′-O-isopropylideneuridine 5′-phosphonate, m.p. 201–203° from ethyl acetate, λ_{max} 258 mμ, a_M 9,925; $\lambda_{max}^{0.01\ N\ KOH-EtOH}$ 256 mμ, a_M 6,575; infrared spectrum (cm.$^{-1}$): —OH/NH at 3180, 3070; C=O at 1690, 1675; conj. C=C and/or —C=N at 1625; P=O at 1220; C—O and P—O at 1085, 1045, 1020, 960; aromatic bands at 812,780.

Anal. Calcd for $C_{16}H_{25}O_8N_2P$: C, 47.53; H, 6.23; N, 6.93; P, 7.66. Found: C, 47.47; H, 6.17; N, 6.88; P, 7.78.

Diethyl 5′-Deoxyuridine 5′-Phosphonate (IV).—A solution of 690 mg. of the diethyl isopropylidene phosphonate (III) obtained above in 100 ml. of 70% aqueous acetic acid was heated at 54–58° in an oil-bath for 1.5 hours, the solid dissolving completely within the first 15 minutes. Removal of the solvent on a rotating evaporator at 25° (15 mm.) and finally under high vacuum gave a colorless glassy residue which crystallized slowly on standing with methanol. Recrystallization from aqueous methanol gave colorless prisms, m.p. 223–225° (466 mg., 75%). From aqueous acetic acid the phosphonate (IV) separated as small prisms, m.p. 224–225°; infrared spectrum (cm.$^{-1}$): —OH at 3260; P=O at 1212; P—O—Et at 1157; P—O at 1050, 1020; P^V at 957. Ultraviolet spectrum: λ_{max} 261 mμ, a_M 9800.

Anal. Calcd. for $C_{13}H_{21}N_2O_8P$: C, 42.85; H, 5.81; N, 7.69; P, 8.50. Found: C, 43.04; H, 5.86; N, 7.52; P, 8.47.

5′-Deoxy-5′-methylthio-2′,3′-O-isopropylideneuridine (X).—Potassium (3.99 g., 100 mmoles) was powdered under toluene and the toluene replaced by 100 ml. of ether. A solution of 19.8 ml. of methyl mercaptan (358 mmoles) in 100 ml. of dry ether was added dropwise. At the end of the addition the majority of the solvent was decanted from the white solid potassium mercaptide and the residual solvent removed by evacuating at 40° (15 mm.) on a rotating evaporator. This dry solid (8.6 g., 100 mmoles) was added rapidly to a solution of 3.94 g. (100 mmoles) of 5′-deoxy-5′-iodo-2′,3′-O-isopropylideneuridine (II) in 100 ml. of dry dimethylformamide, and the mixture heated on a steam-bath for 3 hours with the exclusion of moisture. The resulting pink solution was stored overnight at room temperature. Removal of the solvent at 30° (<1 mm.) gave a pale yellow solid residue which was cooled in an ice-bath and treated with ice-water. All of the solid dissolved and was reprecipitated by careful neutralization with 1 *N* sulfuric acid. The precipitate was extracted thoroughly with chloroform, the combined chloroform extracts washed with a little water, dried over anhydrous sodium sulfate, and the solvent removed at 25° (15 mm.), and finally at 25° (<1 mm.). The residual pale yellow solid (3.25 g.) crystallized from methanol in colorless chunky prisms, m.p. 156–157.5°. Two further recrystallizations from methanol gave the product in 86% yield (2.70 g.), m.p. 158–158.5°; ultraviolet spectrum: λ_{max} 258 mμ, a_M 10,200; $\lambda_{max}^{0.01\ N\ H_2SO_4-EtOH}$ 258 mμ, a_M 10,200; $\lambda_{max}^{0.01\ N\ KOH-EtOH}$ 256 mμ, a_M 5,775; infrared spectrum (cm.$^{-1}$): —NH at 3160; non-conj. >C=O at 1757, 1742, 1712; conj. >C=O at 1677, 1652; conj. C=C at 1617.

Anal. Calcd. for $C_{13}H_{18}O_5N_2S$: C, 49.65; H, 5.77; N, 8.91; S, 10.20. Found: C, 49.74; H, 5.61; N, 9.12; S, 10.20.

5′-Deoxy-5′-methylthiouridine (IX).—A solution of 1.00 g. of 5′-deoxy-5′-methylthio-2′,3′-O-isopropylideneuridine (X) in 10 ml. of glacial acetic acid was treated with an equal volume of 0.99*N* sulfuric acid and the mixture heated at 75° in an oil-bath for 1.5 hours. Fifty milliliters of water was added followed by 1.60 g. of barium hydroxide octahydrate, the mixture stirred for 10 minutes, and filtered. After washing the precipitate well with warm acetic acid, the combined filtrate and washings were taken to dryness on a rotating evaporator at 25° (<1 mm.). The crystalline residue was recrystallized from glacial acetic acid, giving 665 mg. (76% yield) of colorless flattened prisms, m.p. 191–193°, unchanged by further crystallization; ultraviolet spectrum: λ_{max} 260.5 mμ, a_M 9,825; infrared spectrum (cm.$^{-1}$)—NH/OH at 3160; non-conj. C=O at 1757, 1742, 1712: conj. C=O at 1677, 1652; conj. C=C at 1617.

Anal. Calcd. for $C_{10}H_{14}O_5N_2S$: C, 43.78; H, 5.14; N, 10.21; S, 11.69. Found: C, 43.69; H, 5.41; N, 10.35; S, 11.35.

Reaction between 5′-Deoxy-5′-iodo-2′,3′-O-isopropylideneuridine (II) and Sodium Hydrogen Sulfide.—Sodium metal (1.68 g., 73 mmoles) was dissolved in 150 ml. of commercial "absolute" ethanol and a stream of dry hydrogen sulfide was passed through the solution for 4 hours at room temperature. A solution of 2.87 g. of the 5′-deoxy-5′-iodo nucleoside (II, 73 mmoles) in 30 ml. of tetrahydrofuran and 30 ml. of ethanol was added slowly to the sodium hydrogen sulfide solution from a pressure-equalized dropping funnel, a vigorous stream of hydrogen sulfide being used to stir the reaction mixture. The dropping funnel was washed with a further 50 ml. of ethanol and the mixture allowed to stand at room temperature for 18 hours with a slow stream of hydrogen sulfide passing through. A colorless crystalline solid (650 mg., m.p. 193–196°) separated from the pale yellow solution. The filtrate and washings were combined and concentrated to approximately 100 ml. on a rotating evaporator at 25° (15 mm.). After neutralization with 5 ml. of glacial acetic acid, the remainder of the solvent was removed in the same manner. The solid residue was dissolved in a mixture of water and chloroform, the layers separated, and the aqueous layer extracted twice with chloroform. The combined chloroform extracts, after washing with water, dilute sodium bicarbonate, water, and drying over anhydrous sodium sulfate, were taken to dryness on a rotating evaporator at 25° (15 mm.) and gave a colorless solid residue, weight 1.71 g. Crystallization from ethanol yielded colorless prismatic needles, m.p. 200–215°, the melting point being dependent on the temperature at which the crystals were introduced. A mixture of the two samples melted at 195–205°; their ultraviolet and infrared spectra were identical, and the two were combined. Two further crystallizations from ethanol gave XIII as very small colorless needles, m.p. 209–220° (placed on Kofler block at 200°. When heated on the block from room temperature, the crystals melted at approximately 190–220°); ultraviolet spectrum: λ_{max} high end-absorption, flex at 260 mμ, a_M 438; $\lambda_{max}^{0.01\ N\ H_2SO_4-EtOH}$ high end absorption, flex at 260 mμ, a_M 598; $\lambda_{max}^{0.01\ N\ KOH-EtOH}$ high end-absorption, max. at 260 mμ, a_M 7,750; infrared spectrum (cm.$^{-1}$): —NH/OH at 3160, 3120, 3025; C=O at 1732, 1712, 1677; C—O/C—N at 1280, 1222, 1210, 1190, 1052, 1037.

Anal. Calcd. for $C_{12}H_{16}O_5N_2S$: C, 48.00; H, 5.37; N, 9.33; S, 10.68. Found: C, 47.99; H, 5.29; N, 9.28; S, 10.70.

Reaction between 5′-Deoxy-5′-iodo-2′,3′-O-isopropylideneuridine (II) and Potassium Thiolacetate.—To a solution of 18.2 g. of anhydrous potassium carbonate (132 mmoles) in 500 ml. of distilled water was added 10.0 g. of redistilled thiolacetic acid (132 mmoles). The resulting solution was evaporated to a small volume under a stream of nitrogen on the steam-bath. Treatment with animal charcoal, filtration, and re-evaporation gave a pale yellow solution which crystallized on cooling to give 11.0 g. of colorless crystalline solid. A mixture of 3.94 g. (100 mmoles) of 5′-deoxy-5′-iodo-2′,3′-O-isopropylideneuridine (II) and 4.56 g. (400 mmoles) of dry potassium thiolacetate was dissolved in 200 ml. of 95% ethanol and 50 ml. of acetone. After heating on the steam-bath for 3 hours, the solution was stored at room temperature overnight. The solvent was removed on a rotating evaporator at 25° (15 mm.), giving a gummy residue which was dissolved in chloroform, washed with water, dried over anhydrous sodium sulfate, and the solvent removed as above. A pale yellow, very viscous residue remained which could not be induced to crystallize. Chromatography over Florisil showed the oil to be homogeneous, being eluted as a sharp band with 25% ethyl acetate in benzene. The ultraviolet (λ_{max} 259 mμ, a_M 9,950) and infrared (cm.$^{-1}$: —NH at 3165; conj. C=C at 1617) spectra were in agreement with the postulated structure (XV).

(18) A saturated hydrocarbon fraction, b.p. 60–71°, Skelly Oil Co., Kansas City, Mo.

(19) A synthetic magnesia–silica gel manufactured by The Floridin Co., Warren, Pa.

A solution of this oil in methanol (50 ml.) was cooled in an ice-bath and saturated with dry ammonia. The mixture was allowed to stand at room temperature for 30 minutes and the solvent removed as before, giving an oil which crystallized on treatment with ether. Recrystallization from ethanol gave colorless needles (2.8 g., 93.5%), m.p. 193–200°, raised to 200–215° (placed on Kofler block at 190°), undepressed by admixture with the product of the previous reaction (XIII) and giving identical infrared and ultraviolet spectra (Baddiley[14] quotes colorless needles from 1:1 methanol–ethanol, m.p. 200–215°).

Methylation of Cyclic Sulfide XVI to 5′-Deoxy-5′-methylthio-2′,3′-O-isopropylideneuridine (X).—To a solution of 255 mg. (11.1 mmoles) of sodium in 50 ml. of dry ethanol, cooled to room temperature, was added 1.0 g. (33.3 mmoles) of the cyclic sulfide XVI, which dissolved readily on swirling by hand, followed by a solution of 1.42 g. (0.63 ml., 10 mmoles) of methyl iodide in 20 ml. of dry ethanol. After standing overnight, the colorless solution was cooled in ice-water, and neutralized by the careful addition of 1 *N* sulfuric acid.

The neutral reaction product was extracted thoroughly with chloroform, the extract washed with water, and dried over anhydrous sodium sulfate. Removal of the solvent on a rotating evaporator at 40° (15 mm.) gave 1.102 g. of a colorless oil, which crystallized from methanol solution, giving 688 mg. (66%) of colorless prisms, m.p. 158–159°, undepressed on admixture with an authentic sample of 5′-deoxy-5′-methylthio-2′,3′-O-isopropylideneuridine (X), m.p. 158–158.5°. The ultraviolet spectrum of this material (λ_{max} 258 mμ, a_M 10,150) corresponded to that of X, and its infrared spectrum was indistinguishable from that of the authentic material.

A repetition of this reaction under identical conditions but with the omission of methyl iodide led to a pale yellow solution which was neutralized carefully with 1 *N* sulfuric acid. Extraction with chloroform followed by washing the extract with water and drying over anhydrous sodium sulfate gave a colorless solid residue (985 mg.) on removal of the solvent on a rotating evaporator at 30° (15 mm.). Crystallization from ethanol gave 889 mg. of colorless needles, m.p. 215–230°, undepressed on admixture with the starting material, and giving an identical infrared spectrum.

5′-Deoxy-2′,3′-O-isopropylidene-5′-thiocyanatouridine (XVIII).—To a solution of 5.15 g. (636 mmoles) of sodium thiocyanate in 100 ml. of methyl ethyl ketone was added 5.0 g. (12.7 mmoles) of 5′-deoxy-5′-iodo-2′,3′-O-isopropylideneuridine (II), and the mixture was heated under reflux on the steam-bath for 18 hours. Removal of the solvent from the cooled solution on the rotating evaporator at 30° (15 mm.) gave a pale yellow semi-solid residue, which was dissolved in a mixture of water and chloroform. The combined chloroform extracts were washed three times with water, dried over anhydrous sodium sulfate, and the solvent removed as above, giving 3.98 g. of an amorphous solid. Two crystallizations from methanol gave 3.42 g. (83%) of XVIII as colorless prisms, m.p. 170.5–171.5°; infrared spectrum (cm.$^{-1}$): —NH at 3120; C=O at 1760, 1707, 1685, 1670, 1665 and 1652; C=C at 1625; ultraviolet spectrum λ_{max} 256 mμ, a_M 10,150.

Anal. Calcd. for $C_{13}H_{15}O_5N_3S$: C, 47.99; H, 4.65; N, 12.92; S, 9.86. Found: C, 47.94; H, 4.44; N, 13.08; S, 10.17.

Acknowedgments.—We should like to express our thanks to the staffs of Dr. J. L. Johnson and Mr. W. A. Struck of The Upjohn Co. for the analytical and physical data recorded and to Dr. B. R. Baker, Stanford Research Institute, for helpful suggestions.

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[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

The Stereochemistry of Amaryllidaceae Alkaloids Derived from 5,10b-Ethanophenanthridine[1]

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RECEIVED NOVEMBER 20, 1959

The Amaryllidaceae alkaloids crinine, powelline, buphanisine, buphanidrine, buphanamine, crinamidine and undulatine have been related to the (−)-crinane nucleus. Vittatine, haemanthamine, haemanthidine, haemultine, crinamine, 6-hydroxycrinamine and (+)-epicrinine are shown to be based on the enantiomorphic (+)-crinane nucleus. A method for the O-methylation of several of these alkaloids without concurrent N-methylation is described.

In 1951, the known alkaloids of the Amaryllidaceae were comprised of twelve bases in varying degrees of characterization and purity.[2] At the present time, the number of pure alkaloids isolated from plants of this family approaches one hundred. One of the most rapidly expanding groups of alkaloids within the family is that derived from a 5,10b-ethanophenanthridine (crinane) nucleus (I). This ring system was demonstrated first for the alkaloid crinine (II) in 1956.[3] Since that time, powelline (VI),[4,5] buphanisine (III),[4,5] buphanidrine (VII),[4,5] buphanamine (IX),[4] undulatine (XI),[6] haemanthamine (XVIIa),[7] crinamine (XVIIc),[7] haemanthidine (XVIIb),[8] haemultine (XXII),[9] vittatine (XIXa),[10] (+)-epicrinine (XIXb)[1] and 6-hydroxycrinamine (XVIId)[11] have been added to it. Because subsequent papers of this series will expand the group by at least four more alkaloids, it seems desirable to pause at this point and classify the known alkaloids of this ring system according to the enantiomorphic nature of the basic ring system and to complete the stereochemistry of the functional groups in the light of recent experimental evidence.

(1) Paper XVI in a series on the alkaloids of the plant family Amaryllidaceae; for the previous paper see: R. E. Lyle, E. A. Kielar, J. R. Crowder and W. C. Wildman, THIS JOURNAL, **82**, 2620 (1960).

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(3) W. C. Wildman, THIS JOURNAL, **78**, 4180 (1956).

(4) W. C. Wildman, *Chemistry & Industry*, 1090 (1956).

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(10) H.-G. Boit and H. Ehmke, *ibid.*, **90**, 369 (1957).

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