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The Structure and Properties of Some Sulfur Analogs of Acetoneuridine[1]

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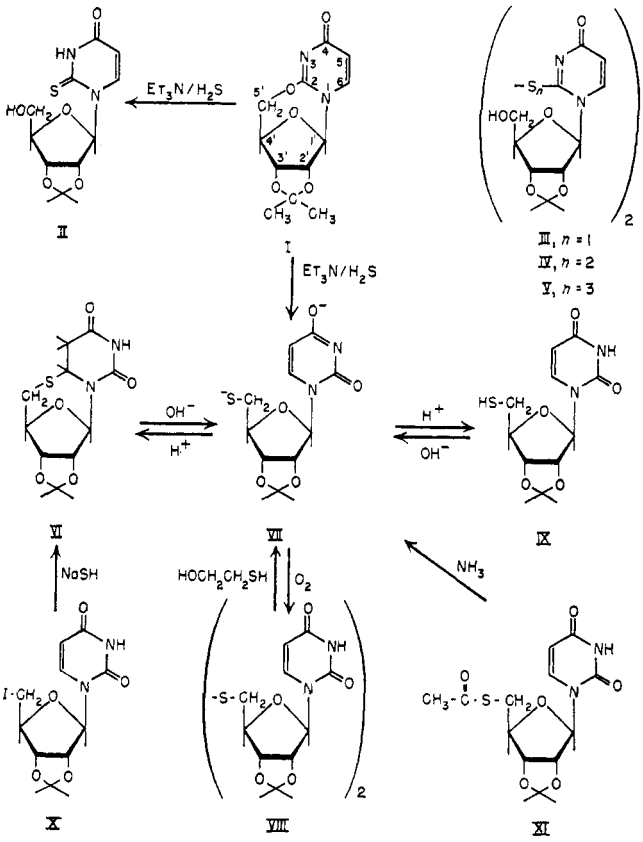
The compounds assigned the structures bis-(2-deoxy-2′,3′-*O*-isopropylidene-2-uridinyl) disulfide[2] (III) and 5′-deoxy-5′-thio-2′,3′-*O*-isopropylideneuridine[3] (IX) have been shown to be identical and they have been reassigned the structure 5′-deoxy-5′,6-epithio-5,6-dihydro-2′,3′-*O*-isopropylideneuridine (VI). In solution, VI is in equilibrium with the dianion of 5′-deoxy-5′-thio-2′,3′-*O*-isopropylideneuridine (VII). The position of this equilibrium is pH dependent. In alkali, slow oxidation of the dianion VII to bis-(5′-deoxy-2′,3′-*O*-isopropylidene-5′-uridinyl) disulfide (VIII) occurs. Authentic 5′-deoxy-5′-thio-2′,3′-*O*-isopropylideneuridine (IX) has been obtained by treating the episulfide VI with alkali to open the ring and then rapidly acidifying to trap mercaptan IX. The mercaptan can be converted back to the episulfide quantitatively by raising the pH and the *rate* of this conversion is pH dependent.

Todd and co-workers[2] have reported that several sulfur-containing nucleosides are formed when 2′,3′-*O*-isopropylidene-2,5′-*O*-cyclouridine (I, acetonecyclouridine) is treated with hydrogen sulfide in the presence of triethylamine. One of these products has been well characterized as 2-deoxy-2′,3′,-*O*-isopropylidene-2-thiouridine (II, acetone-2-thiouridine). One of the other products was designated A and tentatively assigned the structure bis-(2-deoxy-2′,3′-*O*-isopropylidene-2-uridinyl) disulfide (III). The other products were tentatively assigned as tetra- (IV) and hexa- (V) sulfide derivatives of III.



In order to obtain acetone 2-thiouridine for further synthetic work,[4] we repeated the synthesis described by Brown, *et al.*[2] In addition to the desired product, a crystalline material with the properties described for compound A was isolated in 42% yield.

In view of the disulfide structure III assigned to compound A, we attempted to reduce it to acetone-2-thiouridine. However, no reaction could be detected with sodium bisulfite,[5] sodium borohydride or thioethanol.

This rather puzzling result suggested that compound A was not the disulfide III. This was confirmed by a combination of molecular weight determination (by cryoscopic and isothermic methods) and elemental analysis which gave the empirical formula $C_{12}H_{16}N_2O_5S$ rather than $(C_{12}H_{15}N_2O_5S)_2$ as required by structure III.

These results prompted a closer examination of compound A and this paper describes the results of this work.

Results

Structure of Compound A.—Compound A in water or 95% alcohol shows mainly end absorption in the ultraviolet and very little absorption in the 250–290 mμ region (Fig. 1, curve A). This indicates that compound A is not a uridine-like compound possessing a 5,6-double bond conjugated to the carbonyl group. In fact, the spectrum closely resembles that of 1,3-dimethyl-6-hydroxy-5,6-dihydrouracil.[6]

Relatively little change in the spectrum of compound A occurs on heating in 0.025 *N* HCl at 100° (Fig. 1, curves B and C). In dilute alkali (0.01 *N*, room temperature) there is an immediate increase in the 260 mμ absorption (Fig. 1, curve D). This spectral change suggested that a new compound containing a conjugated 5,6-double bond had been formed. This new compound was clearly not acetone-2-thiouridine since it lacked the characteristic absorption maxima at 238 and 269 mμ.[7] Furthermore, paper chromatography in isopropyl alcohol–NH_4OH–H_2O indicated that the new compound (VII) was different from either acetone-2-thiouridine or acetoneuridine (the expected hydrolysis products if compound A had the disulfide structure III). Spraying the chromatograms with Grote reagent[8] showed that VII contained sulfur.

No attempt was made to isolate the alkaline reaction product VII, but evidence for its structure was obtained by treating compound A with alkali and allowing the reaction mixture to stand at room temperature for 120 hr. During this time, the spectral changes shown in Fig. 2 occurred and no further changes occurred on longer standing. When this reaction mixture was adjusted to pH 5 with hydrochloric acid, a new compound (VIII) crystallized. On the basis of its ultraviolet spectra (Fig. 3), elemental analysis and molecular weight determination (see Experimental), VIII was as-

(1) This work was supported by a grant from the National Science Foundation (NSF-G-10780).

(2) D. M. Brown, D. B. Parihar, A. Todd and S. Varadarajan, *J. Chem. Soc.*, 3028 (1958).

(3) J. Baddiley and G. A. Jamison, *ibid.*, 1085 (1955).

(4) P. Lengyel and R. W. Chambers, *J. Am. Chem. Soc.*, **82**, 752 (1960).

(5) This reagent has been used to reduce 2-thiouracil disulfide to 2-thiouracil: W. H. Miller, R. O. Roblin, Jr., and E. B. Astwood, *ibid.*, **67**, 2207 (1945). We repeated this procedure without difficulty.

(6) A. M. Moore and C. H. Thompson, *Science*, **122**, 595 (1955).

(7) J. B. Strominger and M. Friedkin, *J. Biol. Chem.*, **208**, 663 (1954); G. Shaw, R. N. Warrener, M. H. Maguire and R. K. Ralph, *J. Chem. Soc.*, 2294 (1958).

(8) I. M. Hais and K. Macek, "Handbuch der Papierchromatographie," Gustav-Fischer, Jena, 1958, Vol. 1, p. 737.

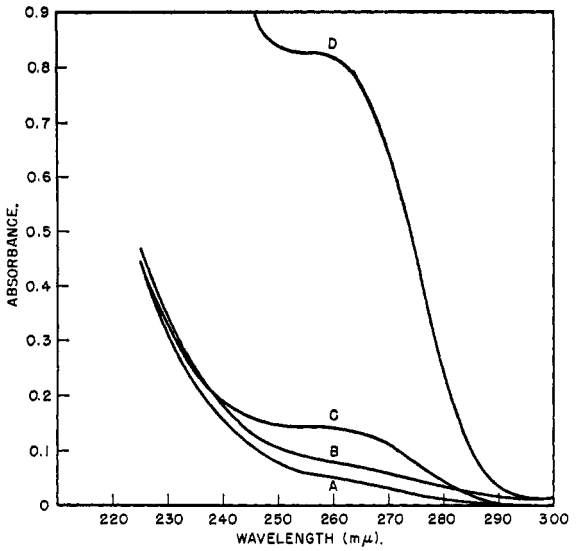


Fig. 1.—Spectra of compound A (IV, acetonedihydrouridine episulfide): curve A, in water or 95% alcohol; curve B, in 0.01 *N* HCl; curve C, after heating in 0.025 *N* HCl for 0.5 hr. at 100° and then diluting to give 0.01 *N* HCl; curve D, in 0.01 *N* NaOH.

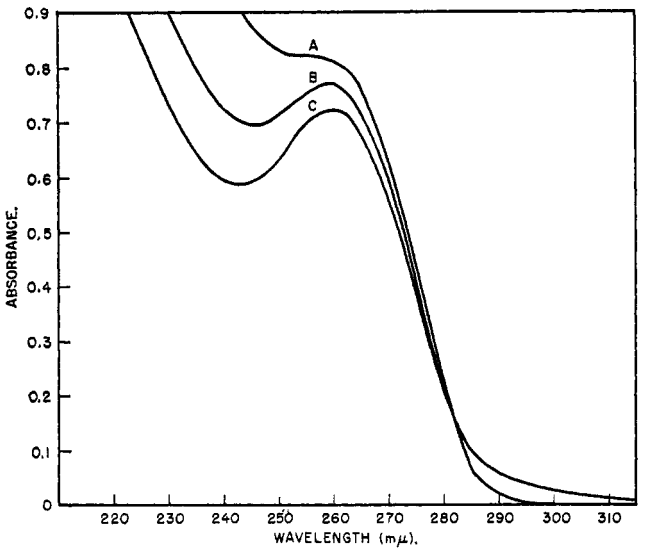


Fig. 2.—Spectra changes occurring when compound A is allowed to stand in pH 11 phosphate buffer: curve A, after 10 min.; curve B, after 24 hr.; curve C, after 120 hr.

signed the structure bis-(5′-deoxy-2′,3′-*O*-isopropylidene-5′-uridinyl) disulfide (bis-acetoneuridine 5′-disulfide). Thus, the initial product formed by treating compound A with alkali is probably the dianion (VII) of 5′-deoxy-5′-thio-2′,3′-*O*-isopropylideneuridine. Additional evidence for this was obtained by potentiometric titration of compound A. Two equivalents of alkali were required to reach the pH where ring opening is complete.

These data suggest that compound A is actually 5′-deoxy-5′,6-epithio-5,6-dihydro-2′,3′-*O*-isopropylideneuridine (VI, acetonedihydrouridine episulfide). Further support for this structural assignment is provided by the work of Bannister and Kagen,[9] who synthesized the episulfide VI from 5′-deoxy-5′-iodo-2′,3′-*O*-isopropylideneuridine (X) and sodium hydrogen sulfide. The properties they describe for their product are identical with those we have found for compound A.

Reactions of Acetonedihydrouridine Episulfide (Compound A).—Conversion of acetonedihydrouridine episulfide (VI) to the dianion of acetone-5′-thiouridine is pH dependent. Since air oxidation of the dianion VII to its disulfide VIII is a fairly slow reaction, it is possible to study the ring opening (VI → VII) without serious interference from disulfide formation. This was accomplished by adding aliquots of a standard solution of acetonedihydrouridine episulfide to phosphate buffers at various pH values and, after exactly 2 min., determining the spectrum. In this way a family of curves showing an increase in A_{260} with increasing pH was obtained. When the % increase in A_{260} is plotted against pH (Fig. 4), the experimental points approximate a theoretical curve for the general reaction $HA + H_2O = H_3O^+ + A^-$, assuming a pK_a of 9.8, even though we have found 2 equivalents of OH^- are required for complete ring opening.

The reversibility of this reaction can easily be demonstrated by the spectral changes (Fig. 5) which occur when acetonedihydrouridine episulfide (curve A) is allowed to stand for 5 min. at pH 11 to form the dianion VII (curve B) and then adjusted to pH 5 and allowed to stand (curves C and D). The failure of curve D to return exactly to the original curve A even after 48 hr. at pH 5 may be due to a small amount of disulfide formation.

After demonstrating that the dianion VII could be converted back to the episulfide VI it was possible to obtain further evidence for the structure of VIII which we had assigned as bis-acetoneuridine-5′-disulfide. The crystalline material (VIII) obtained from air oxidation of the episulfide in alkaline solution was treated with excess mercaptoethanol at pH 11 to reduce the disulfide bond and then acidified to pH 5. Crystalline acetonedihydrouridine episulfide (VI) was isolated. In the absence of mercaptoethanol there is no conversion of the disulfide VIII to the episulfide VI under similar conditions. This experiment provides strong evidence for the 5′-disulfide structure of VIII.

According to the structures we have assigned and the reactions we have just discussed, one would predict that acidification of a solution of the dianion VII would give 5′-deoxy-5′-thio-2′,3′-*O*-isopropylideneuridine (IX, acetone-5′-thiouridine). When acetonedihydrouridine episulfide (VI) was dissolved in 0.01 *N* NaOH and then *rapidly* acidified to about pH 0.6 with 2 *N* HCl, a crystalline compound was obtained which had a spectrum in acid consistent with structure IX (Fig. 6, curve A).

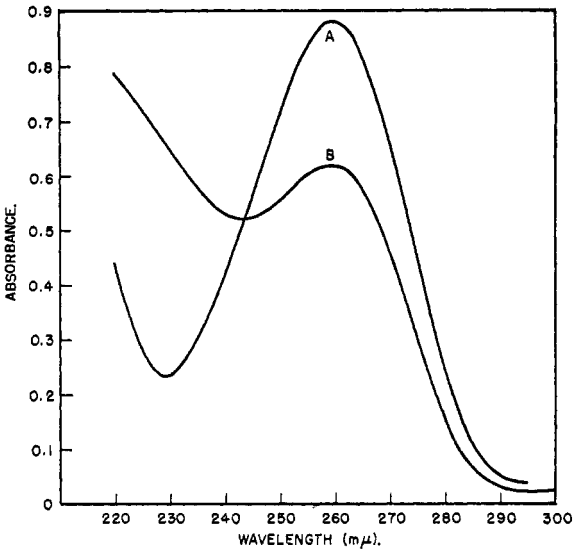


Fig. 3.—Spectra of bis-acetoneuridine 5′-disulfide (VIII): curve A, at pH 3 or 7; curve B, at pH 11.

(9) B. Bannister and F. Kagen, *J. Am. Chem. Soc.*, **82**, 3363 (1960).

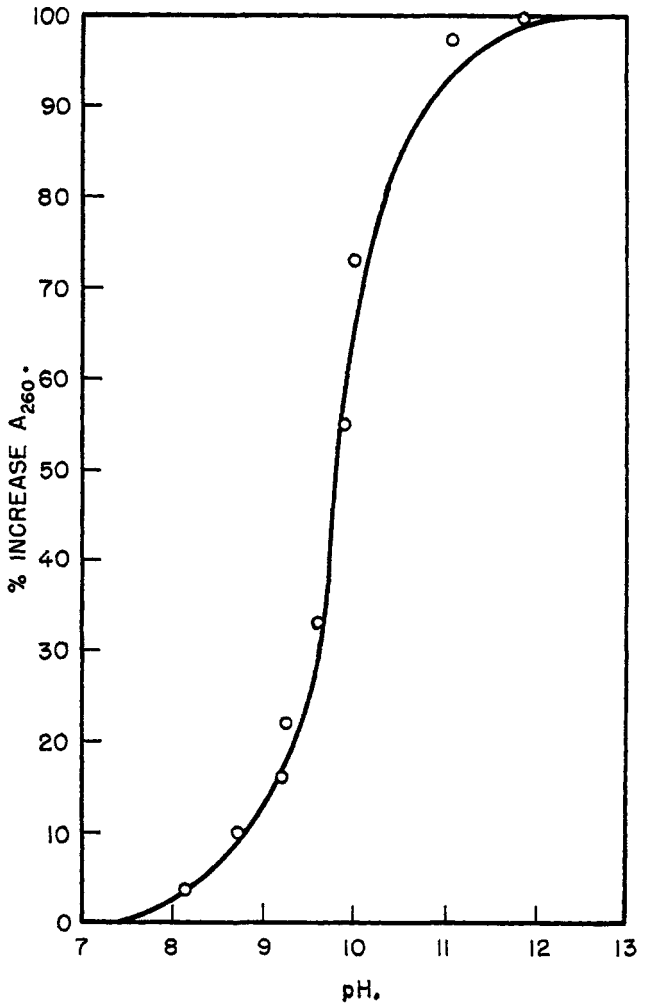


Fig. 4.—The effect of pH on acetonedihydrouridine episulfide. The solid line is a theoretical curve for the equilibrium HA $\rightleftarrows$ H^+ + A^- using an assumed p*K*a of 9.8. The experimental points were obtained from individual spectra run at the indicated pH values.

When this compound was dissolved in 95% ethanol, the spectrum (Fig. 6, curve C) resembled that of the episulfide VI. When it was dissolved in pH 11 buffer, the spectrum resembled that of the dianion VII. These results clearly indicate cyclization of acetone-5′-thiouridine to acetonedihydrouridine episulfide. The conversion is also pH dependent, but in this case the effect is exclusively kinetic. Thus, cyclization requires about 1 hr. at neutrality, but it requires several hours at pH 5 and about 5 days at pH 3 to reach completion.

The purity of the original sample of IX was not considered adequate for analysis and we have been unable to isolate any more solid material. Instead, the crystalline product which now invariably separates on acidification is acetoneuridine episulfide (VI). However, the presence of acetone-5′-thiouridine is easily demonstrated in solution by an ultraviolet spectrum similar to that shown in curve A of Fig. 6, and the original crystalline material shows no alteration in properties after storage for over a year.

On the basis of these results there is little doubt that the crystalline material IX is acetone-5′-thiouridine. This compound was previously reported by Baddiley and Jamison[3] as the product formed when acetoneuridine 5′-thioacetate (XI) was treated with alcoholic ammonia. When we checked the spectrum of Baddiley's compound,[10] we found that it differed from our

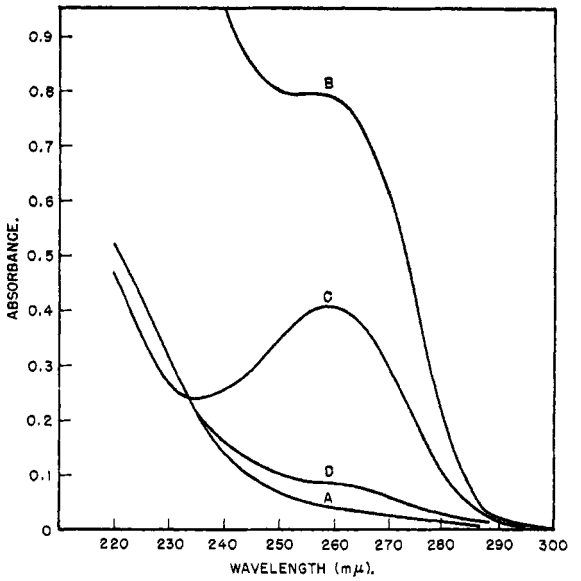


Fig. 5.—Demonstration of reversibility for the reaction VI $\rightleftarrows$ VII: curve A, acetonedihydrouridine episulfide after 5 min. at pH 5; curve B, after 5 min. at pH 11; curve C, after 5 min. at pH 11 and then at pH 5 for 15 min.; curve D, after 5 min. at pH 11 and then at pH 5 for 20 hr.

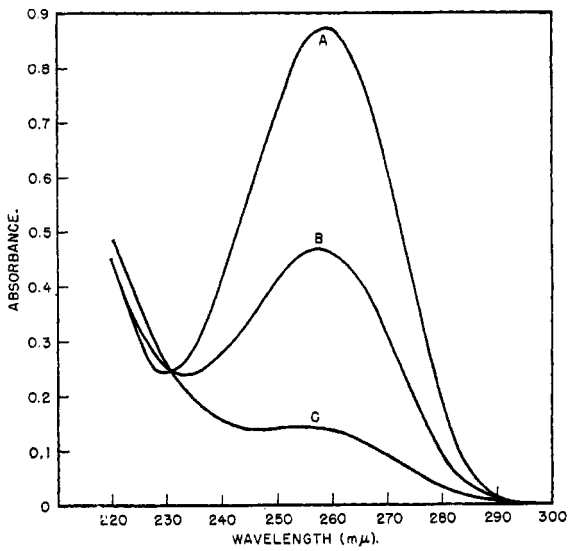


Fig. 6.—Conversion of acetone-5′-thiouridine (IX) to acetonedihydrouridine episulfide: curve A, spectrum of acetone-5′-thiouridine in 0.1 *N* HCl–ethanol (1:1, v./v.); curve B, after 10 min. in 95% ethanol; curve C, after 45 min. in 95% ethanol.

compound IX and was, in fact, identical with that of acetonedihydrouridine episulfide. This confirms the findings of Bannister and Kagen,[9] who repeated the reaction reported by Baddiley[3] and isolated the episulfide VI as the only product.

More recently, Michelson has reported[11] the synthesis of 5′-thiouridine 2′(3′)-phosphate. The conditions he describes are precisely those which would favor cyclization to the episulfide derivative by addition to the 5,6-double bond. Since no spectral data were presented, the 5′-thiouridine 2′(3′)-phosphate structure is suspect. The existence of an episulfide would not affect the use of this compound for the synthesis of poly 5′-thiouridylic acid because this reaction is carried out in the presence

(10) We are grateful to Professor Baddiley for a sample of his material. He warned us that the properties of this compound were not quite consistent with the structure assigned to it.

(11) A. M. Michelson, *J. Chem. Soc.*, 979 (1962).

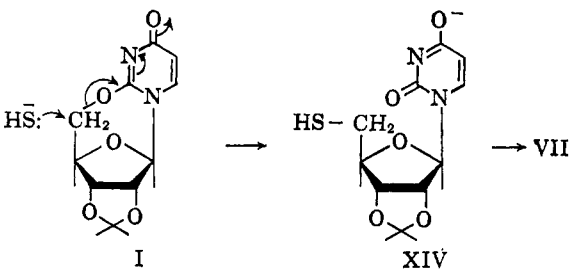
of tri-*n*-butylamine and this would favor opening of the episulfide.

Discussion

On the basis of these results, the compounds assigned the structures bis-(2-deoxy-2′,3′-*O*-isopropylidene-2-uridinyl) disulfide[2] (III) and 5′-deoxy-5′-thio-2′,3′-*O*-isopropylideneuridine[3] (IX) are identical and should be reassigned the structure 5′-deoxy-5′,6-epithio-5,6-dihydro-2′,3′-*O*-isopropylideneuridine (VI).[12]

It should be noted that formation of the sulfide bridge creates a new asymmetric carbon at position 6 of the dihydropyrimidine ring. Thus, two diastereoisomers of acetone dihydrouridine episulfide are theoretically possible. So far, we have no evidence for the actual existence of two isomers, although no concerted effort has been made to separate them. Since Bannister and Kagen[9] do not mention the possibility of these isomers, we assume they also failed to observe them.

The formation of acetonedihydrouridine episulfide from acetonecyclouridine (I) and H_2S in the presence of triethylamine can be rationalized as follows. Nucleophilic attack by the reactive species, $HS:^-$, on the anhydro ring can occur in two ways: (1) by attack at carbon 2 to give acetone-2-thiouridine (II) or (2) by attack at carbon 5′ to give first the anion of acetone-5′-thiouridine (XIV).



I XIV

It is known that a strong nucleophile such as $C_2H_5S:^-$ attacks acetonecyclouridine to give acetone-5′-ethylthiouridine.[2] Apparently it has been assumed that HS^- is too weak a nucleophile to promote alkoxygen fission at C-5′. This conclusion is based on the fact that the products of the reaction were all believed to be derivatives of acetone-2-thiouridine. Our results show that acetone-2-thiouridine and acetonedihydrouridine episulfide are formed in similar amounts. Thus there is no pronounced selectivity in the attack of $HS:^-$ on the anhydro ring. The failure to isolate any acetone-5′-thiouridine from the reaction mixture is readily explained by our observation that acetone-5′-thiouridine cyclizes rapidly and quantitatively to acetonedihydrouridine episulfide at neutrality, the conditions existing during the work up.

This cyclization reaction is not reversible in the usual sense. That is, once cyclization has occurred, acidification of the solution does *not* effect ring opening to give back acetone-5′-thiouridine. pH does have an effect on the cyclization, but this appears to be entirely a *rate* effect.

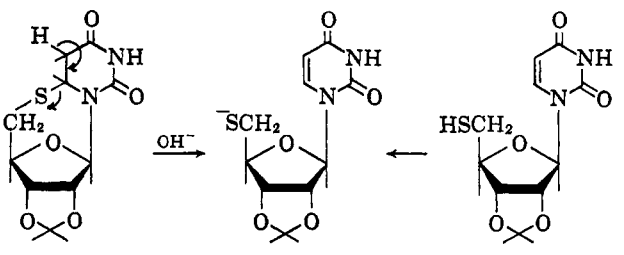
In contrast to this, acetonedihydrouridine episulfide is in equilibrium with the dianion of acetone-5′-thiouridine (VII) and the position of this equilibrium is pH dependent. At pH 9.8 equal amounts of the episulfide and the dianion are present. Below pH 9.8 the episulfide predominates and above pH 9.8 the dianion predominates. So far, the only way in which acetone-5′-thiouridine can be obtained is to open the episulfide ring with alkali and then trap acetone-5′-thiouridine by *rapid* acidification of the solution.

Our results differ in certain respects from those of Bannister and Kagen.[9] For example, they state that the cyclization is a reversible process, but they were unable to demonstrate the reformation of the episulfide after treating it with alkali to open the episulfide ring and then acidifying. They attributed this to the formation of a "stable, non-cyclized product, probably the disulfide." However, they did not isolate any disulfide nor did they provide any conclusive evidence that it was formed.

It is clear from the results reported here and the spectral curves recorded by Bannister and Kagen[9] that disulfide formation did actually occur under the conditions they used.[13] This can be seen from the pronounced minimum at 250 mμ (Fig. 1 in ref. 9, top curve) which is characteristic of bis-acetoneuridine 5′-disulfide and not of the dianion VII which shows only an inflection at 255 mμ (Fig. 2, this paper).

We can also conclude from Bannister and Kagen's data (Fig. 1 in ref. 9, middle curve) that actually they did get partial recyclization as well as disulfide formation. If disulfide formation had been complete, an ϵ_{max} of about 9×10^3 should have been observed instead of a little over 6×10^3 which they reported.

They have also stated that the episulfide ring opens in alkali to give the monoanion XV.

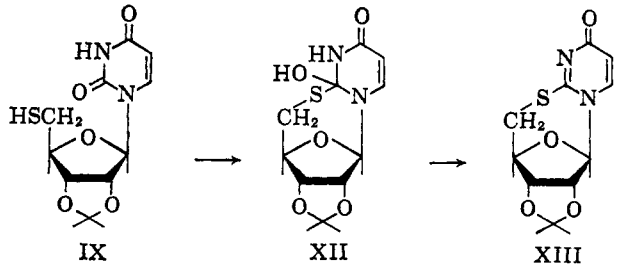


VI XV IX

However, titration of the episulfide VI clearly shows that 2 equivalents of alkali are necessary to effect complete ring opening. Thus, the dianion VII and not the monoanion XV is the predominant species at high pH. In fact, it appears that the monoanion XV is actually the kinetically active form in the cyclization process. This postulation serves to explain the effect of pH on the *rate* of ring closure of acetone-5′-thiouridine IX. Here as the pH is increased the amount of monoanion XV will increase and hence the rate of cyclization will increase.

Finally, it should be mentioned that all attempts to prepare authentic bis-(2-deoxy-2-thio-2′,3′-*O*-isopropylidene-2-uridinyl) disulfide (III) by oxidation of ace-

(12) The possibility that compound A has the structure XII or XIII has been considered. Both of these compounds have the 5,6-conjugated double



IX XII XIII

bond. Both infrared[9] and ultraviolet spectra indicate that this double bond is not present. For a discussion of this grouping as the chromophore in pyrimidines see L. F. Cavalieri and A. Bendich, *J. Am. Chem. Soc.*, **72**, 2587 (1950). Furthermore, the ultraviolet spectrum of XIII should resemble 2-ethylthio-1-methylpyrimidone-4 (D. Shugar and J. J. Fox, *Bull. soc. chim. Belges.*, **61**, 293 (1952)). The spectrum of compound A does not resemble that of 2-ethylthio-1-methylpyrimidone-4.

(13) The exact conditions were not reported so it is impossible to interpret their curves exactly.

tone-2-thiouridine have failed. Furthermore, no attempt has been made to isolate compounds B and C, which have been assigned the structures IV and V, respectively.[2] In view of the spectral properties reported for these compounds[2] and the results reported here, there is considerable doubt concerning the correctness of these structural assignments.

Experimental

Reaction of 2′,3′-*O*-isopropylidene-2,5′-*O*-cyclouridine (I) with Hydrogen Sulfide. (A). Isolation of 5′-Deoxy-5′,6-epithio-5,6-dihydro-2′,3′-*O*-isopropylideneuridine (VI).—The reaction was carried out essentially as described by Brown, *et al.*[2] 2′,3′-*O*-Isopropylidene-2,5′-*O*-cyclouridine was dried over P_2O_5 *in vacuo* at room temperature.[14] Three grams of the dried material (11.2 mmoles) was dissolved in 40 ml. of freshly distilled dimethylformamide and 2 ml. of triethylamine (distilled from KOH) was added. Dry hydrogen sulfide was bubbled through the solution under anhydrous conditions for 10 hr. The colorless solution turned dark green. The reaction flask was tightly stoppered and allowed to stand an additional 16 hr. at room temperature. Nitrogen was passed through the solution until the odor of H_2S was no longer detectable. The solvent was removed under reduced pressure using an oil-pump and a bath temperature of about 48°. The yellow sirup was mixed with 100 ml. of absolute ethanol. Crystal formation began almost immediately and was allowed to proceed overnight at room temperature. The crystals were removed by filtration and air-dried (830 mg.). A second crop of the same material was obtained by evaporating the filtrate to 22 ml. (575 mg.). The combined yield of acetonedihydrouridine episulfide was 4.7 mmoles (42% of theory), m.p. 235–237°. The ultraviolet spectrum of the product in 95% ethanol or water showed mainly end absorption (see Fig. 1 for a complete spectrum). Paper chromatography gave a single spot, R_f 0.81 in solvent I.

Anal. Calcd. for $C_{12}H_{16}N_2O_5S$ (300.33): C, 47.99; H, 5.37; N, 9.33; S, 10.67. Found: C, 48.16; H, 5.27; N, 9.49; S, 10.50; mol. wt., 294,[15] 299.[16]

(B) Isolation of 2-Deoxy-2-thio-2′,3′-*O*-isopropylideneuridine.—The solvent was evaporated from the filtrate described above and the residue was dissolved in 10 ml. of boiling chloroform. Crystals formed. The solution was cooled and the crystals were removed by filtration, washed with chloroform and air-dried (1.113 g., 3.7 mmoles, 33% of theory). The product was identified by its spectrum[7]; R_f 0.88, solvent I; 0.66, solvent II.

The chloroform filtrate was concentrated under reduced pressure and the crystalline 2′,3′-*O*-isopropylidene-2,5′-*O*-cyclouridine which formed was removed by filtration (136 mg., 0.5 mmole, 4%).

Evaporation of this filtrate to dryness gave an oily residue. All of the solids isolated and identified account for 79% of the starting material.

5′-Deoxy-5′-thio-2′,3′-*O*-isopropylideneuridine (IX).—5′-Deoxy-5′,6-epithio-5,6-dihydro-2′,3′-*O*-isopropylideneuridine (25 mg.) was dissolved in 1 ml. of 0.1 *N* NaOH (prepared from boiled, distilled water saturated with N_2) and allowed to stand for 2.5 min. at room temperature. Hydrochloric acid (0.5 ml. of 2 *N*) was added to the reaction mixture to give a pH of about 0.6. The solution became turbid and after a few minutes at room temperature, crystals began to separate from the solution. The solution was chilled in ice and after about a half-hour the crystals (rosettes of thin prisms) were collected by filtration and dried over P_2O_5 *in vacuo* at room temperature (14 mg.). This material gave a positive test for a sulfhydryl group when it was dissolved in acid and then nitroprusside added followed by alkali. The red color faded rapidly. A_{280}/A_{260} = 0.24, A_{250}/A_{260} = 0.83 in ethanol–0.1 *N* HCl (1:1, v./v.) (for a complete spectrum see Fig. 6, curve A).

Bis-(5′-deoxy-2′,3′-*O*-isopropylidene-5′-uridinyl) Disulfide (VIII).—5′-Deoxy-5′,6-epithio-5,6-dihydro-2′,3′,-*O*-isopropylideneuridine (100 mg.) was dissolved in 6.6 ml. of 0.01 *N* NaOH, adjusted to pH 11 and allowed to stand at room temperature. The reaction was followed by removing 20-λ aliquots, diluting to 10.0 ml. with pH 11 phosphate buffer and determining the spectrum. The results are shown in Fig. 2.

After 120 hr., the reaction mixture was adjusted to pH 5 with 2 *N* HCl. The crystalline solid which formed was removed by filtration, washed with cold water and dried over P_2O_5 *in vacuo* at room temperature (80 mg.). This material was dissolved in a minimum amount of hot 95% ethanol and water was added until turbidity could be detected. On cooling, 62 mg. of crystalline material was recovered, m.p. 183–185°; λ_{max} 259 mμ, ϵ_{max} 19.1 × 10^3; λ_{min} 229, ϵ_{min} 4.5 × 10^3; A_{280}/A_{260} = 0.24, A_{250}/A_{260} = 0.80 at pH 3; λ_{max} 259, ϵ_{max} 13.4 × 10^3; λ_{min} 243.5, ϵ_{min} 11.2 × 10^3; A_{280}/A_{260} = 0.23, A_{250}/A_{260} = 0.89 at pH 12. The spectra are shown in Fig. 3.[17]

Anal. Calcd. for $C_{24}H_{30}O_{10}N_4S_2$ (598.62): C, 48.15; H, 5.05; N, 9.36; S, 10.71. Found: C, 47.56; H, 5.02; N, 8.99; S, 10.20; mol. wt., 578.[16]

Conversion of Bis-(5′-deoxy-2′,3′-*O*-isopropylidene-5′-uridinyl) Disulfide to 5′-Deoxy-5,6-epithio-5,6-dihydro-2′,3′-*O*-isopropylideneuridine.—Bis-(5′-deoxy-2′,3′-*O*-isopropylidene-5′-uridinyl) disulfide (10 mg.) was dissolved in 0.5 ml. of 0.3 *N* NaOH. After 5 min., 1.6 ml. of 0.1 *N* mercaptoethanol (~10 equiv.) was added. A 0.2-ml. aliquot was removed, diluted to 10.0 ml. with phosphate buffer (pH 5) and the spectrum determined using mercaptoethanol of the same concentration in the reference cell. Partial conversion to the cyclosulfide VI had occurred. The process was repeated until the spectrum indicated conversion was complete. A total of 6.4 ml. (~40 equiv.) of mercaptoethanol was required. The pH of the solution was adjusted to 7 and the solution was concentrated to a small volume under reduced pressure. The crystalline material which separated was collected by filtration (2 mg.) and shown to be identical with 5′-deoxy-5′,6-epithio-5,6-dihydro-2′,3′-*O*-isopropylideneuridine.

Spectral Data.—The spectral constants reported for individual compounds were obtained with a Beckman DU spectrophotometer. The spectra shown in the figures were obtained with a Beckman DK-2 recording spectrophotometer and replotted manually using a linear wave length scale. Phosphate buffers (0.22 *M*) were used for all spectral determinations. The spectra were determined at concentrations of *about* 0.1 μmole/ml. except for the disulfide (Fig. 3) which was about 0.05 μmole/ml.

Chromatography.—Chromatograms were run by the descending technique on Whatman No. 1 paper: solvent I, *n*-butyl alcohol–acetic acid–water (4:1:5)[18]; solvent II, isopropyl alcohol–ammonia–water (7:1:2).[19]

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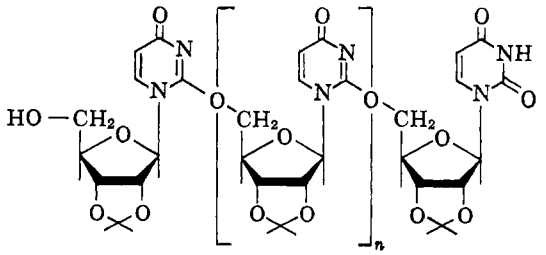
(14) When the compound was dried over P_2O_5 *in vacuo* at 100°, the solid became insoluble in all solvents tested except 90% formic acid. No marked change in the crystal form, elemental analysis or infrared spectrum was observed, but other properties (chromatographic mobility and sedimentation coefficient) indicated polymerization had occurred. This may have been initiated by traces of water (or ethanol) in the crystals to give a polymer of the type



(15) R. L. Shriner, R. C. Fuson and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, p. 55. This value is the average of two determinations made by Dr. Peter Lengyel using borneol as a solvent.

(16) Schwarzkopf Microanalytical Laboratories, Woodside, N. Y. (isothermic method).

(17) Since the analytical data are not really satisfactory, the spectral constants should be regarded as tentative. Further purification of this compound has proved difficult.

(18) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(19) R. Markham and J. D. Smith, *ibid.*, **52** (1952).