A Reinvestigation of the Structures for 5-Diazouracil, 5-Diazouridine, 5-Diazo-2′-deoxyuridine and Certain Related Derivatives by Proton Magnetic Resonance Spectroscopy (1).

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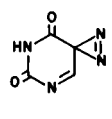
The structure of 5-diazouracil and several closely related derivatives have been revised on the basis of pmr spectroscopy. 5-Diazouracil, 5-diazouracil hydrate, 5-diazouracil methanol adduct, 5-diazouridine and 5-diazo-2′-deoxyuridine have been reassigned the structures 5-diazopyrimidin-2,4(3*H*)dione (XI), 5-diazo-6-hydroxy-1,6-dihydropyrimidin-2,4(1*H*,3*H*,6*H*)dione (XIII), 5-diazo-6-methoxy-1,6-dihydropyrimidin-2,4(1*H*,3*H*,6*H*)dione (XII), 1-(β-D-ribofuranosyl)-$O^{5'}$-6(*S*)cyclo-5-diazo-1,6-dihydropyrimidin-2,4(3*H*,6*H*)dione (XVII) and 1-(2-deoxy-β-D-ribofuranosyl)-$O^{5'}$-6(*S*)cyclo-5-diazo-1,6-dihydropyrimidin-2,4(3*H*,6*H*)dione (XIX), respectively. Treatment of XII with dimethylamine resulted in a coupling of the 5-diazo group with dimethylamine and a concomitant rearomatization of the heterocyclic ring by expulsion of the 6-methoxy group to furnish 5-(3,3-dimethyl-1-triazeno)uracil (XIV). A similar reaction of XIX and XVII with dimethylamine furnished the corresponding 5-(3,3-dimethyl-1-triazeno)derivatives. The effect which certain resonance hybrids of the diazo moiety may exert in reactions of the above heterocycles and the assignment of *S* configuration at C-6 for the nucleoside derivatives is also discussed.

The structures of 5-diazouracil, 5-diazouridine and 5-diazo-2′-deoxyuridine have been the subject of several investigations. 5-Diazouracil was originally prepared by decarboxylation of 5-diazoorotic acid (4). Several compounds were isolated in this investigation (4), including an anhydride, a hydrate, and an ethanol adduct of 5-diazouracil which were assigned the structures I, II, and III, respectively. 5-Aminouracil was subsequently diazotized (5) and the same structures were assigned to the products. A white form of 5-diazouracil was later isolated and postulated (6) to possess a different structure. This compound was assigned the structure IV, however, a subsequent investigation (7) established that this compound (IV) was in essence the same as the previously reported (4) 5-diazouracil (I).



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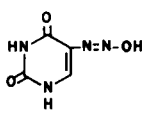

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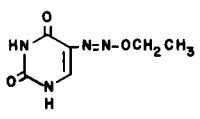

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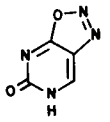

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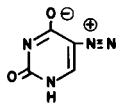

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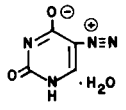

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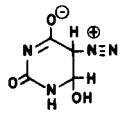
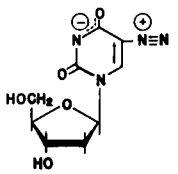
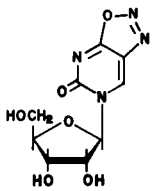


VII

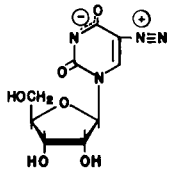
The structure of 5-diazouracil and certain related derivatives were reinvestigated by infrared spectroscopy and this study resulted in 5-diazouracil anhydride, 5-diazouracil hydrate and a 5-diazo-6-hydroxy-5,6-dihydrouracil being reassigned (8) the structures V, VI and VII, respectively. The product obtained by diazotization of 5-amino-2′-deoxyuridine (9) was assigned the structure VIII and the



VIII



IX



X

structure reported previously (10) for 5-diazouridine (IX) was also revised (9) to structure X on the basis of infrared spectroscopy (9). We have obtained spectral data in our laboratory which is inconsistent with the previously proposed structures I-X. This prompted us to initiate a reinvestigation of the structure previously assigned to 5-diazouracil and other closely related derivatives.

5-Aminouracil was treated with sodium nitrite in dilute acid to furnish a product which was recrystallized from methanol. The product obtained in this manner had been reported (8) to possess a structure similar to III. However, a pmr spectrum revealed a pattern of peaks which was inconsistent with the structures previously proposed, *e.g.*, the presence of two N-H absorption peaks (δ 10.25, singlet and δ 8.6, doublet, J = 3.7 Hz) and an absorption peak at δ 3.23 (3 proton singlet). The N-H absorption peak at δ 8.6 was coupled with a C-H proton at δ 5.72 (J = 3.7 Hz). This indicated that 5-diazouracil had crystallized and retained a mole of solvent (methanol) by association as indicated by elemental analysis (4). This possibility was eliminated since recrystallization from ethanol showed that the methyl group absorption had not been displaced by ethanol. The absence of an absorption peak at ≅ δ 4.9 for the hydroxyl proton (OH) of methanol also furnished strong evidence that the $-OCH_3$ group was bonded covalently to the heterocycle. A preliminary assignment for

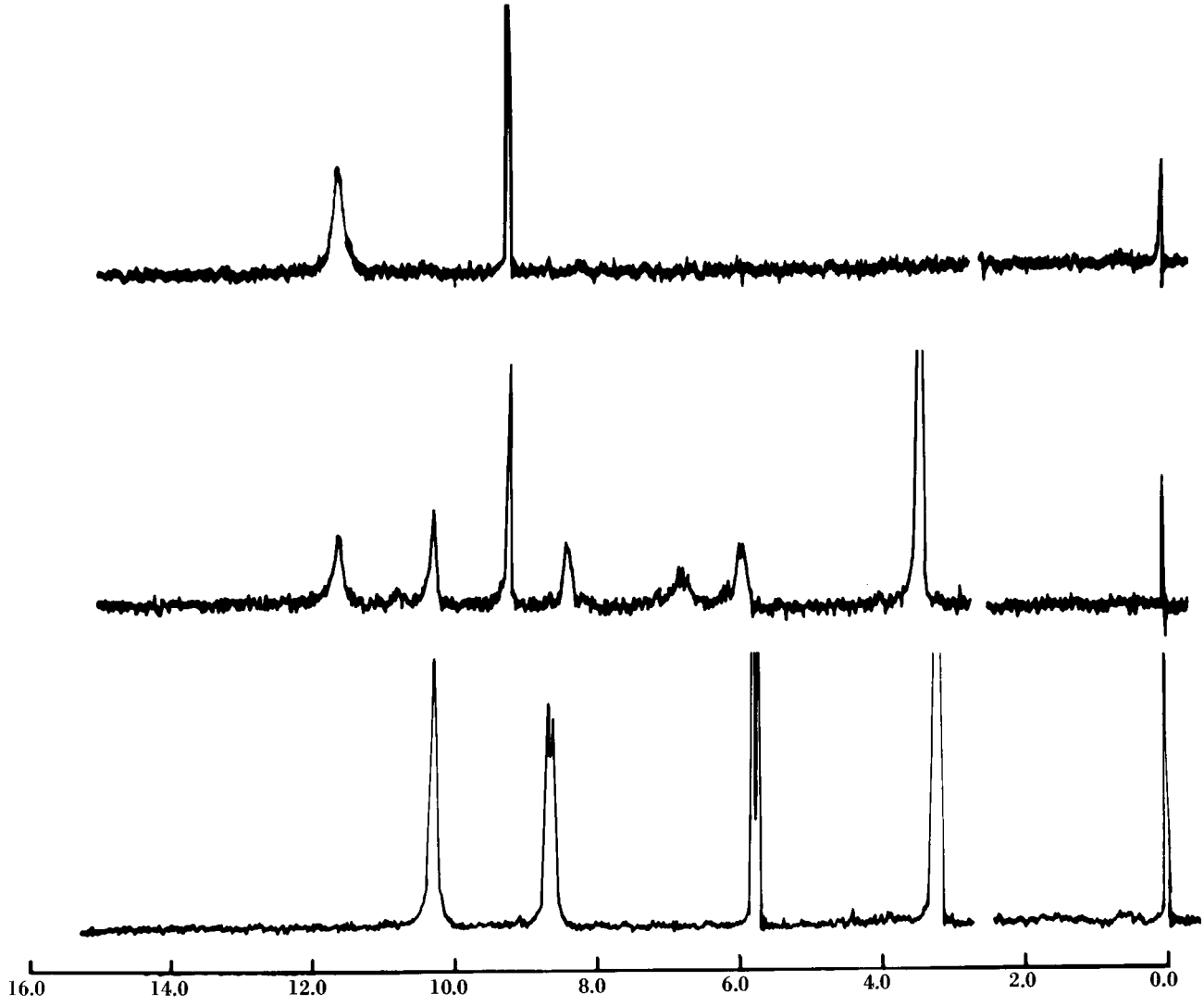
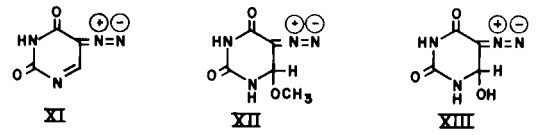


Figure 1. Pmr spectra (DMSO-d_6) of "anhydrous" 5-diazouracil (XI, top); mixture of XI and 5-diazouracilhydrate (XIII) obtained by drying the product from the reaction-mixture at ambient temperature for 18 hours (middle); 5-diazouracil-methanol adduct (XII, bottom).

the position of this attachment was at C-6 due to the significant upfield chemical shift observed for the C-6 proton (δ 5.72) (10). This compound will be subsequently referred to as the 5-diazouracil-methanol adduct.

5-Aminouracil was then diazotized in the same manner as above except for a slight modification of the isolation and purification procedure. The solid which separated from solution was collected by filtration, washed free of acid with cold water and then dried at 110° under high vacuum instead of recrystallization from methanol. The pmr spectrum (Figure I) of the product revealed a singlet (C-H) at δ 9.13 and only one N-H absorption at δ 11.60 (broad singlet). We have assigned structure XI to the "anhydrous" form of 5-diazouracil which was supported by the conversion of XI to the methanol adduct XII and the reactivity of XI towards the addition of water, *vide infra*. On the basis of the structure XI for "anhydrous" 5-diazouracil, a facile assignment of the structure XII for the methanol adduct was accomplished. There was only one C-H proton (excluding the methyl group) observed in the pmr spectrum (Figure I) and this proton was coupled with a N-H proton. This allowed us to assign the peak (doublet) at δ 8.6 to the N-1 proton, the peak (doublet) at δ 5.72 to the C-6 proton and the broad singlet at δ 10.25 to the N-3 proton. Therefore, the methoxy group must be attached to C-6 and this was corroborated by the significant upfield chemical shift observed between the C-6 proton of uracil (11) and the C-6 proton of the methanol adduct. It was also found that recrystallization of XI from methanol furnished a good yield of XII. This indicated that in aqueous solvents,



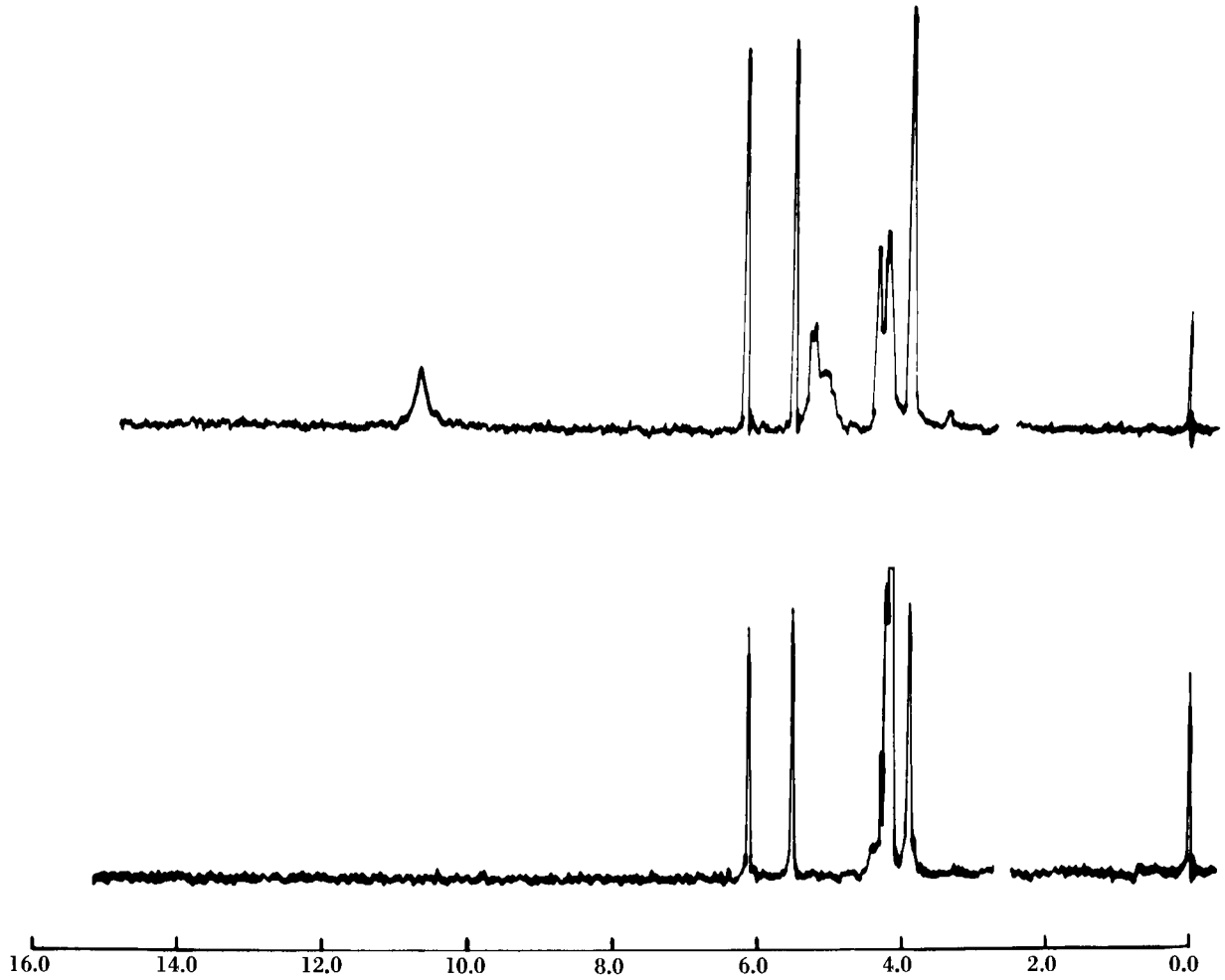
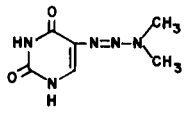


Figure 2. Pmr spectra of $O^{5'}$-6(*S*)cyclo-5-diazouridine (XVII) in DMSO-d_6 (top) and DMSO-d_6/deuterium oxide (bottom).

water could add across the 1-6 double bond of XI to form a 5-diazouracil-hydrate (XIII) or a mixture of XI and XIII. Recrystallization of a mixture of XI and XIII from methanol could then afford XII as the major product. This prompted the diazotization of 5-aminouracil with the product being collected by filtration washed free of acid and then allowed to air dry rather than drying at 110°. A pmr spectrum (Figure I) of this solid indicated that it was indeed a mixture of XI and XIII by the appearance of three separate and distinct N-H absorptions (δ 11.6, 10.2 and 8.4). There was also an absorption peak at δ 3.35 which was attributed to water, *per se*, and a broad absorption (δ 6.7) assigned to an OH group. The addition of deuterium oxide to the pmr tube resulted in the disappearance of all N-H absorption peaks and the absorptions peak at δ 9.2 and 5.9 for the C-6 protons of the mixture were now observed as a singlet at δ 5.9.

There was also observed a conversion of XI, *per se*, to XIII-d_3 on the addition of deuterium oxide to a sample of XI in DMSO-d_6 as established by the upfield chemical shift (δ 9.2 to δ 5.9) for the absorption peak assigned to the C-6 proton (11a,11b). This established that XI was the only structure for anhydrous 5-diazouracil consistent with the hydration of XI to afford XIII and this assignment was corroborated by a facile dehydration of XIII to afford XI, *vide infra*. A crude mixture of XI and XIII was recrystallized from water to give only XIII. There was obtained a mixture of XI and XIII when XIII was dried at ambient temperature and pressure for 18 hours. However, when XIII was dried at 110°, 0.2 Tr. for 18 hours a facile dehydration furnished XI as the only product. A loss of methanol from XII to give XI under similar temperature and pressure but for 36 hours was found to afford a mixture of XI and XII with an approximate 1:1 ratio. This would indicate, as expected, that the expulsion of methanol from XI occurs less readily than dehydration of XIII.

In view of the structure XII assigned the 5-diazouracil methanol adduct, it was of considerable interest to establish whether a nucleophile would simply displace the 5-diazo group or couple to form a triazeno derivative and if there was a reaction at the 5 position, whether rearomatization would occur or if a 1,6-dihydro product would be obtained. The 5-diazouracil-methanol adduct (XII) was treated with dimethylamine to give a product with elemental analysis consistent with the empirical formula $C_6H_9N_5O_2$. A pmr (DMSO-d_6) spectrum revealed a loss of the absorption peak for the methoxy group at δ 3.23 and the appearance of a peak at δ 3.20 for the dimethylamino moiety, a broad singlet (2 protons) at δ 10.53 for the N-1 and N-3 protons and a downfield chemical shift (δ 7.10, singlet; Δδ, 1.38) for the C-6 proton which would indicate that C-6 was once again incorporated in a conjugated π electronic system. This established that the 5-diazo group had coupled with dimethylamine with the concomitant expulsion of methanol to afford a product which was assigned the structure 5-(3,3-dimethyl-1-triazeno)uracil (XIV).



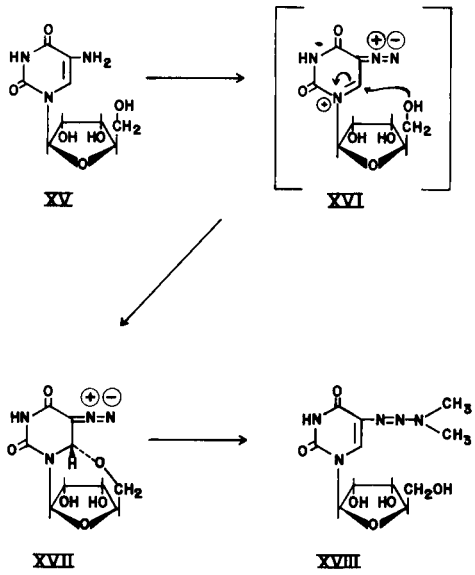
XIV

This compound (XIV) is of considerable interest due to the significant antitumor activity reported (12) for 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DIC) as well as the interest in related triazeno derivatives (13).

In view of the revised structures for 5-diazouracil (XI) and the methanol adduct (XII), it was of considerable interest that 5-diazouracil had been shown to inhibit cell division in bacteria (14,15), inhibit virus production in animals (16), and exhibit significant activity against a variety of neoplasms (17). It has also been studied as a radiomimetic agent (18) and shown to irreversibly inhibit dehalogenation of 5-iodouracil (19). The irreversible inhibition of dehalogenation of 5-iodouracil was reported to occur with a commercial sample of 5-diazouracil. We purchased a sample of 5-diazouracil from the same commercial source and a pmr spectrum of this material showed it to be a mixture of several components including XI and presumably an ethanol adduct of XI similar in structure to XII. This would suggest that previous biological and chemotherapeutic studies on 5-diazouracil may have used a mixture. Therefore, it would be of considerable interest to determine the activity of pure XI, XII and XIII as well as XIV.

The results described above prompted us to initiate a reinvestigation of the structure reported (9) for 5-diazouridine. 5-Diazouridine was prepared by the diazotization of 5-aminouridine (XV) in dilute acid using the reported procedure (10) and the product recrystallized from methanol. A pmr spectrum (Figure II) of this nucleoside revealed an N-H absorption at δ 10.62 (broad singlet) which was inconsistent with the previously proposed structures IX and X. This absorption peak (δ 10.62) was assigned to a proton at N-3. There was observed an upfield chemical shift for the C-6 proton (δ 6.15) which suggested that the uracil ring had been perturbed in a fashion similar to that described for the 5-diazouracil-methanol adduct (XIII). Although this nucleoside was recrystallized from methanol, there was observed an absence of an absorption peak at δ 3.23 which indicated that methanol was not associated. However, if methanol was not associated with this nucleoside then the upfield chemical shift for the C-6 proton must be rationalized. The addition of deuterium oxide

to the pmr tube furnished a spectrum (Figure II) which showed an exchange for the N-3 proton but only two exchangeable protons for the sugar moiety rather than three. The anomeric proton appeared as a singlet instead of the usual doublet which is observed for pyrimidine nucleosides (11) (including XV). This suggested that a conformational change had occurred in the carbohydrate moiety. These data are consistent with cyclonucleoside formation through the C-6 position of the uracil derivative and the $O^{5'}$ position of the carbohydrate moiety. 5-Diazouridine was assigned the structure 1-(β-D-ribofuranosyl)-$O^{5'}$-6(*S*)cyclo-5-diazo-1,6-dihydropyrimidin-2,4(3*H*, 6*H*)dione ($O^{5'}$-6(*S*)cyclo-5-diazouridine, XVII) (20,21). This intramolecular cyclonucleoside formation explains why the association with methanol by recrystallization of the initial product was precluded. It is tempting to postulate that the formation of XVII from 5-aminouridine (XV) proceeds *via* an unstable intermediate of structure XVI which would create a tremendous increase in electrophilic character at the six position. The close proximity of the 5′-hydroxyl group could then allow cyclonucleoside formation to occur rather than cleavage of the glycosidic bond (NI/CI′). Treatment of XVII with dimethylamine furnished a good yield of nucleoside material which was assigned the structure 5-(3,3-dimethyl-1-triazeno)-uridine (XVIII). A pmr spectrum of XVIII revealed a downfield chemical shift from δ 6.15 to δ 7.8 for the C-6 proton, the appearance of an additional peak at ≅ δ 5.0 for the 5′-hydroxyl proton and a change in the anomeric proton from a singlet to a doublet ($J_{1',2'}$ = 5 Hz). This established that the cyclonucleoside linkage had been cleaved and that C-6 was once again incorporated into an aromatic system.



The product obtained from the diazotization of 5-amino-2′-deoxyuridine in dilute acid was recrystallized from aqueous ethanol and the structure established in precisely the same way. A pmr spectrum (Figure III) revealed the presence of an N-H absorption at δ 10.55, an upfield chemical shift for the C-6 proton (δ 6.15) and the appearance of only one exchangeable proton in the

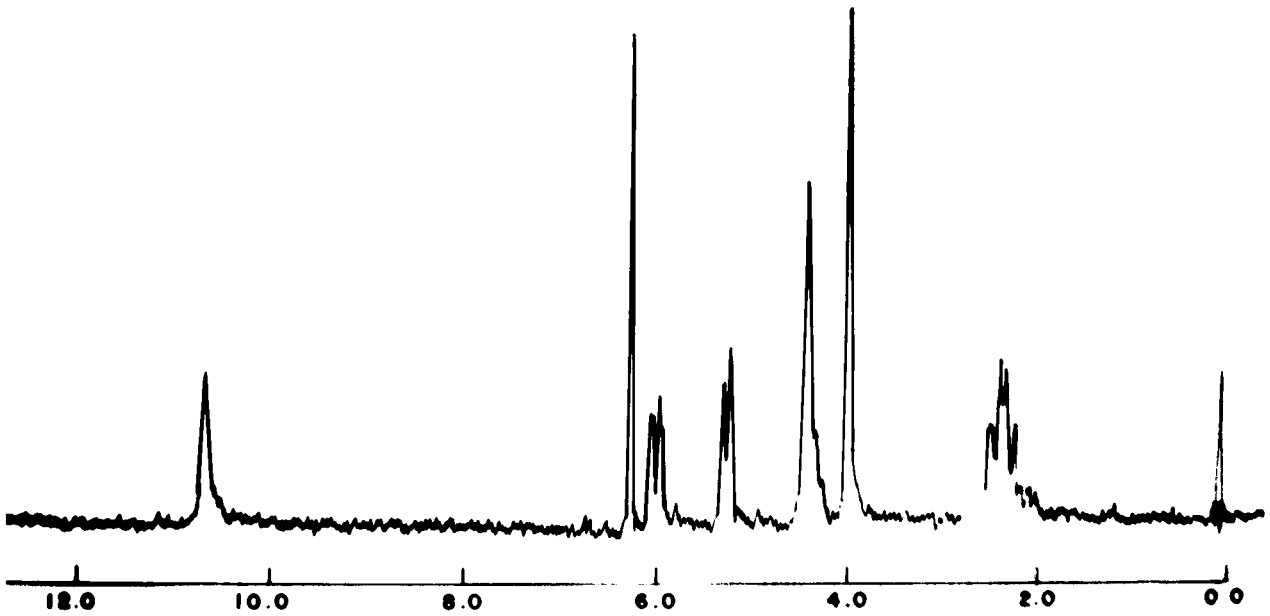
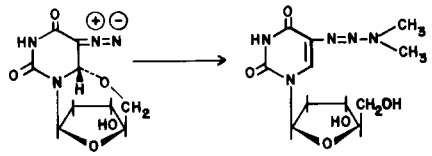


Figure 3. Pmr spectrum (DMSO-d_6) of $O^{5'}$-6(*S*)cyclo-5-diazo-2′-deoxyuridine (XIX).

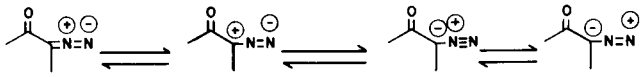
carbohydrate portion of the pmr spectrum. The anomeric proton appeared as a quartet instead of the expected triplet (11,22) and suggested that a conformational change in the carbohydrate moiety had occurred. On the basis of these data, this nucleoside was assigned the structure 1-(2-deoxy-β-D-ribofuranosyl)-$O^{5'}$-6(*S*)cyclo-5-diazo-1,6-dihydropyrimidin-2,4(3*H*,6*H*)dione, ($O^{5'}$-6(*S*)cyclo-5-diazo-2'-deoxyuridine, XIX) (20,21). Treatment of XIX with dimethylamine furnished a good yield of 5-(3,3-dimethyl-1-triazeno)-2'-deoxyuridine (XX).

A pmr spectrum of XX showed a downfield chemical shift of the C-6 proton to δ 7.80, the appearance of an additional absorption peak for a hydroxyl proton at ≅ δ 5.0 and a change in the pattern of peaks for the anomeric proton from a quartet to a triplet, (Pk Wd = 14 Hz) which established that cleavage of the cyclonucleoside linkage and rearomatization had occurred.



XIX XX

The coupling reactions of XII, XVII and XIX with dimethylamine would indicate that these compounds exist in a resonance state where some of the resonance hybrid XXId is present. This would also suggest that the 5-



XXIa XXIb XXIc XXId

diazouracil-hydrate (XIII), $O^{5'}$-6(*S*)cyclo-5-diazouridine (XVII) and $O^{5'}$-6(*S*)cyclo-5-diazo-2'-deoxyuridine (XIX) probably exist as a composite of several other resonance hybrids, *e.g.* XXIa, XXIb, and XXIc.

The configuration at C-6 of XVII and XIX was determined by a comparison of the chemical shifts observed for the C-6 proton of these two compounds with the chemical shift of the C-6 proton of XII. The absorption peak for the C-6 proton of XVII and XIX was observed at δ 6.15 while the peak for the C-6 proton of XII was observed at δ 5.72 (Δδ = 0.43). This downfield chemical shift difference was of such a magnitude that the electron withdrawing effect of the carbohydrate moiety could not be the only effect. Therefore, the additional deshielding effect must be due to the ring oxygen of the D-ribofuranose moiety. An examination of molecular models revealed that in the *S* configuration the C-6 proton of XVII and XIX lies directly over the ring oxygen atom of the D-ribofuranose moiety whereas in the *R* configuration, the C-6 proton lies in the region of the C2' and C3' protons and is well removed from the ring oxygen atom. Therefore, the assignment of *S* configuration would appear to be consistent with the pmr spectra of XVII and XIX, although it is not clear at this time why the 5'-hydroxyl group is situated so that formation of a cyclonucleoside with the *S* configuration should be favored.

It would be of considerable interest to reinterpret the reported biological and chemotherapeutic activity of these diazo compounds in view of the revised structures, *e.g.*, XVII was found (23) to be the only uracil riboside with sufficient activity against Leukemia L-1210 to be assigned to an active category.

EXPERIMENTAL

Ultraviolet spectra were recorded on a Beckman DK-2 recording spectrophotometer. Infrared spectra (potassium bromide pellet) were determined on a Beckman IR-8 spectrophotometer and pmr spectra on a Varian A-60 instrument in DMSO-d_6 or DMSO-d_6/deuterium oxide solution. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses (CHN) were performed by Heterocyclic Chemical Corporation, Harrisonville, Missouri. The methanol and ethyl acetate used in the coupling reactions were dried by distillation from magnesium sulfate and acetonitrile was dried by distillation from phosphorus pentoxide. All solvents were stored over activated "Linde" Type 4A 4-8 mesh molecular sieves.

5-Diazopyrimidin-2,4(3*H*)dione ("Anhydrous" 5-Diazouracil, XI).

5-Aminouracil (24) (2.0 g.) was added to 1 *N* hydrochloric acid (40 ml.) and the solution cooled to 0° in an ice-salt bath. A 6.9% sodium nitrite solution (18 ml.) was then added dropwise over a period of 30 minutes while maintaining the temperature between 0° and 3° (25). The mixture was stirred for an additional 5 minutes after the final addition of sodium nitrite. The solid which had separated from solution was collected by filtration and washed with ice water until a silver nitrate test for chloride ions was negative (approximately 6 x 50 ml.). The remaining solid was added to boiling water (50 ml.) and stirred at reflux temperature until solution resulted. Norit (150 mg.) was added to this solution and then removed by filtration. The filtrate was allowed to stand at room temperature for 18 hours and the light yellow crystals were then collected by filtration and dried *in vacuo* over toluene at reflux temperature for 8 hours to give 570 mg. (26%) of XI, m.p. 210° exp., (lit. (8) 210°); uv: λ max (acetonitrile) 306 nm (ε, 12,500); ir, 2150 cm^{-1} (diazo). The low yield in this preparation was attributed to the insolubility of XI in water.

5-Diazo-6-methoxy-1,6-dihydropyrimidin-2,4(1*H*,3*H*,6*H*)dione (5-Diazouracil-Methanol Adduct XII).

The procedure described above was followed until after the additional 5 minutes of stirring at which time the ice bath was replaced by a dry ice-acetone bath at approximately -10°. Ethanol (54 ml.) which had been previously cooled to -10° was then added dropwise to the mixture while maintaining the temperature near 0°. The reaction mixture was cooled to -15° and the solid collected by filtration. The product was washed with ethanol (8 ml.) which had been previously cooled to -15° and then recrystallized from boiling methanol (120 ml.) to give 2.04 g. (94%) of large

yellow crystals, m.p. 198° exp. (lit. (4) 198° exp); uv: λ max (methanol) 262 nm (ε, 12,800); ir, 2150 cm^{-1} (diazo).

1-(β-D-Ribofuranosyl)-$O^{5'}$-6(*S*)cyclo-5-diazo-1,6-dihydropyrimidin-2,4(3*H*,6*H*)dione ($O^{5'}$-6(*S*)cyclo-5-diazouridine XVII).

5-Aminouridine (26) (XV, 10 g.) was added to 100 ml. of 1 *N* hydrochloric acid and the solution cooled to 0° in an ice-salt bath. To this solution was added 40 ml. of a 6.9% aqueous sodium nitrite solution dropwise over a period of 20 minutes (25) while maintaining the temperature below 3° and the isolation performed as in the preparation of XII. The filter cake was washed with 75 ml. of ethanol (cooled previously to -15°) and then recrystallized from 600 ml. of boiling methanol to give 8.7 g. (84%) of light yellow crystals, m.p. 190-193° (lit. (9) 178-182°); uv: λ max (methanol) 261 nm (ε, 13,300) (lit. (27) λ max (methanol) 262 nm (ε, 12,400)); ir, 2150 cm^{-1} (diazo).

1-(2-Deoxy-β-D-ribofuranosyl)-$O^{5'}$-6(*S*)cyclo-5-diazo-1,6-dihydropyrimidin-2,4(3*H*,6*H*)dione ($O^{5'}$-6(*S*)cyclo-5-diazo-2′-deoxyuridine) (XIX).

5-Amino-2′-deoxyuridine (28) (2 g.) was added to 20 ml. of 50% aqueous acetic acid at 0°. To this solution was added 8.2 ml. of a 6.9% aqueous sodium nitrite solution dropwise over a 5 minute period (25) while maintaining the temperature between 2° and 4°. The solid which separated was collected by filtration and washed with 5 ml. of ethanol (previously cooled to -15°) and then recrystallized from 80 ml. of boiling ethanol with the minimum amount (approximately 4 ml.) of water added to effect solution. This furnished 1.43 g. (68%) of light yellow crystals, m.p. 184-185° turns orange with foaming (lit. (8) 163-165° dec.); uv: λ max (methanol) 262 nm (ε, 16,200) (lit. (9) uv: λ max (methanol) 261 nm (ε, 12,600)); ir, 2150 cm^{-1} (diazo).

5-(3,3-Dimethyl-1-triazeno)uracil (XIV).

The 5-diazouracil-methanol adduct (XII, 2.5 g.) was added to 84 ml. of ethyl acetate at room temperature in a pressure bottle. This suspension was cooled to 0° and 10 ml. of anhydrous dimethylamine was then added. The pressure bottle was sealed and heated in an oil bath at 40-43° for 4 hours and then allowed to stand at 5° for 18 hours. The white solid was collected by filtration, washed with 10 ml. of ethyl acetate at room temperature, dissolved in 125 ml. of methanol at room temperature and then stirred with Norit (100 mg.) for 15 minutes. The Norit was removed by filtration and the filtrate concentrated (not to dryness) keeping the temperature below 20° to give a thick suspension. The precipitate was collected by filtration, washed with ethyl acetate (15 ml.) and then allowed to dry at room temperature to give 1.9 g. of product, m.p. 162-164° dec. with explosion. An additional 0.56 g. of product (29) was obtained from the methanol filtrate by evaporation to dryness *in vacuo*, to give a total yield of 2.46 g. (90%). A small sample was dissolved in methanol, treated with Norit, the Norit removed by filtration and the filtrate evaporated to dryness. This solid was dried at 100° under 0.2 Tr vacuum over phosphorus pentoxide to afford an analytical sample, m.p. 166-167° dec.; uv: λ max (methanol) 263 nm (ε, 16,500).

Anal. Calcd. for $C_6H_9N_5O_2$: C, 39.34; H, 4.95; N, 38.23. Found: C, 39.39; H, 4.98; N, 38.39.

5-(3,3-Dimethyl-1-triazeno)uridine (XVIII).

$O^{5'}$-6(*S*)cyclo-5-diazouridine (XVII, 10.0 g.) was added to 200 ml. of ethyl acetate and this reaction mixture divided equally into two separate pressure bottles. These reaction mixtures were then cooled to 0° in an ice-salt bath and 25 ml. of anhydrous dimethylamine added to each vessel. The pressure bottles were sealed, covered with aluminum foil and allowed to stand at room temperature for one hour with occasional shaking. They were then heated in an oil bath at 52-55° with vigorous stirring for 4 hours. The source of heat was removed, the oil baths allowed to slowly cool and the reaction mixtures stirred for an additional 16 hours.

The pressure bottles were cooled to 0° and the solutions combined. The oil which remained in the reaction vessels was dissolved in the minimum amount of methanol (approximately 50 ml.) and this was added to the ethyl acetate solution. This solution was evaporated *in vacuo* to an oil while maintaining the temperature below 30°. The oil was triturated with ethyl acetate (50 ml.) and scratching with an aluminum spatula produced a light yellow solid which was collected by filtration and washed with ethyl acetate (25 ml.). An additional quantity of product was obtained from the filtrate by repeating the above procedure (evaporation and trituration) to give a total yield of 10.6 g., m.p. 122-126° softens, 130-132° melts. This solid was recrystallized from boiling acetonitrile (500 ml.) with sufficient methanol added to effect a clear solution. Two crops were obtained by isolation, volume reduction to 250 ml. *in vacuo* and the addition of 20 ml. of ethyl acetate to give 9.2 g. of product (79%), m.p. 136-138°. An analytical sample was obtained by recrystallization from acetonitrile and dried in an Abderhalden apparatus over 2-propanol at reflux temperature for 1.5 hours over phosphorus pentoxide, m.p. 170-172°; uv: λ max (methanol) 322 nm (ε, 11,800), 271 (ε, 10,700).

Anal. Calcd. for $C_{11}N_{17}N_5O_6$: C, 41.91; H, 5.43; N, 22.21. Found: C, 41.96; H, 5.49; N, 22.12.

5-(3,3-Dimethyl-1-triazeno)-2′-deoxyuridine (XX).

Anhydrous dimethylamine (10 ml.) was added to 150 mg. of $O^{5'}$-6(*S*)cyclo-5-diazo-2′-deoxyuridine (XIX) and the solution stirred and allowed to reflux (dry ice-acetone condensor) at room temperature for 1.5 hours. The solution was evaporated to dryness *in vacuo* (38°) to give a fine white crystalline material. Ethyl acetate (3 ml.) was added and the crystals collected by filtration, washed with 5 ml. of ethyl acetate and dried at room temperature to give 162 mg. (92%) of product, m.p. 168-169° dec. A small sample (155 mg.) was recrystallized, with charcoal, from 15 ml. of acetonitrile. This was dried under high vacuum at room temperature for 20 hours using Drierite as the dessicant to give 146 mg., m.p. 170-171° dec.; uv: λ max (methanol) 330 nm (ε, 9,400) 271 nm (ε, 8,000).

Anal. Calcd. for $C_{11}H_{17}N_5O_5$: C, 44.15; H, 5.73; N, 23.40. Found: C, 44.20; H, 5.79; N, 23.77.

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(3a) A preliminary report of this research was presented by T. C. Thurber and L. B. Townsend at the Third International

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