Synthesis of the Carbocyclic Analogs of Uracil Nucleosides

Y. Fulmer Shealy and C. Allen O'Dell

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

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Treatment of the required hydroxyl derivatives of *cis*-3-aminocyclopentanemethanol with 3-ethoxyacryloyl isocyanate gave *N*-(3-ethoxyacryloyl)-*N'*-[hydroxy- or dihydroxy(hydroxymethyl)cyclopentyl]ureas. Cyclization of the ureas in dilute sulfuric acid afforded high yields of the carbocyclic analogs of uridine, 2'-deoxyuridine, and 3'-deoxyuridine. The uridine and 3'-deoxyuridine analogs were also obtained in good yields by cyclizing the ureas in concentrated aqueous ammonia. None of the three analogs showed activity in tests *versus* KB cells in culture or L1210 leukemia *in vivo*.

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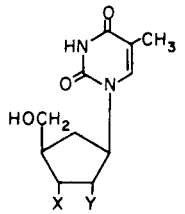
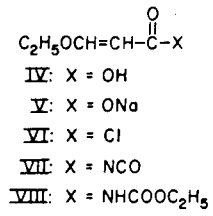
Carbocyclic analogs of many of the naturally occurring and biologically (or biochemically) active nucleosides of 6-substituted-purines and 2-amino-6-substituted-purines have been synthesized (1). Bennett and coworkers (2) have shown that the analogs of adenosine and 8-azaadenosine may function as inhibitors of, or as substrates for, certain nucleoside- or nucleotide-transforming enzymes. Furthermore, several of the carbocyclic analogs of purine nucleosides are cytotoxic to neoplastic cells growing in culture (1d,2a); some have demonstrated activity against certain bacteria and fungi *in vitro;* a few, notably the analog of 6-(methylthio)purine ribonucleoside, are active against DNA-viruses (3); and the carbocyclic analog of 8-azaadenosine is active against P388 leukemia in mice (4).

Several pyrimidine nucleosides, or heterocyclic analogs of pyrimidine nucleosides, have clinical anticancer activity; notable among these are 5-fluoro-2'-deoxyuridine (5), 1-(β-D-arabinofuranosyl)cytosine (6), and 5-azacytidine (7). Because of this association of clinical activity with the pyrimidine nucleoside structure and because of the demonstrated biological activity of carbocyclic analogs of purine nucleosides, we extended our studies to the synthesis of carbocyclic analogs of pyrimidine nucleosides. The carbocyclic analog (I) of thymidine (8,9) and carbocyclic analogs (*e.g.* II, III) of other thymine nucleosides (10) were prepared earlier. We now report the synthesis of the carbocyclic analogs of uridine, 2'-deoxyuridine, and 3'-deoxyuridine.

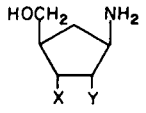
The cyclopentylamines (IX-XI) required for the synthesis of the uridine and deoxyuridine analogs were prepared by stereospecific syntheses described previously (1a-c). The synthesis route to the analogs (XV-XVII) was based on the acryloylurea variant (11) of the Shaw synthesis of 2,4(1*H*,3*H*)-pyrimidinediones, which requires 3-ethoxyacrylic acid (IV, 3-ethoxy-2-propenoic acid) for uracils. 3-Ethoxyacryloyl isocyanate (VII) (11) was prepared from IV *via* the sodium salt (V) and the acid chloride (VI) (12); benzene solutions of the isocyanate (VII) were prepared and used under rigorously anhydrous conditions. Satisfactory yields of the acryloylureas (XII-XIV) were obtained by treating the unprotected hydroxycyclopentylamines (IX-XI) with VII in benzene-dimethylformamide solution at low temperature (13). Some of the minute details of the procedures (see Experimental) for the preparation of VII and XII-XIV were incorporated after initial attempts to prepare XII and the ethyl urethane (VIII) were unsuccessful. The efficiency of the conversion of VI to VII was estimated in several subsequent preparations by adding ethanol to a portion of the isocyanate solution to form urethane VIII. The yields of VIII were 76-89%.



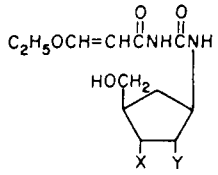
I: X = OH, Y = H
II: X = Y = OH
III: X = H, Y = OH



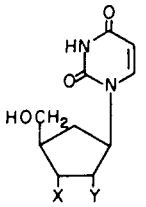
IV: X = OH
V: X = ONa
VI: X = Cl
VII: X = NCO
VIII: X = $NHCOOC_2H_5$



IX: X = OH, Y = H
X: X = Y = OH
XI: X = H, Y = OH

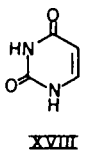


XII: X = OH, Y = H
XIII: X = Y = OH
XIV: X = H, Y = OH

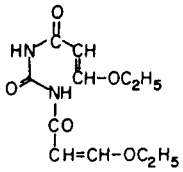


XV: X = OH, Y = H
XVI: X = Y = OH
XVII: X = H, Y = OH

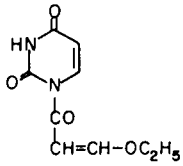
Conventionally, cyclization of acryloylureas has been effected in basic solution (11,14), but the yields of uracils and thymines were variable and frequently were low. However, acid-catalyzed cyclization of 3-methoxy-2-methylacryloylureas was shown to give high yields of 1-substituted thymines (9,10). This method also produced high yields of the uridine and deoxyuridine analogs (XV-XVII) when the acryloylureas were cyclized in 0.5*N*, 1*N*, or 2*N* sulfuric acid. Thus, cyclization of urea XIII in 2*N* sulfuric acid afforded a yield of 87% of pure uridine analog (XVI). Crude 2'-deoxyuridine analog (XV) was obtained similarly in yields of 82-84%, but exact yield data in the cyclization step were not available because impure acryloylurea (XII) was used. Cyclization of acryloylurea XIV in 1*N* sulfuric acid gave an apparent yield of 99.6% of the 3'-deoxyuridine analog (XVII), but this material contained a small amount of uracil (XVIII). By semi-quantitative thin-layer chromatography (tlc), the amount of uracil was determined to be 3-4%. After uracil had been removed by preparative tlc, the yield of pure XVII was 92.4%. Small amounts of uracil were also detected in XVI and XVII prepared by cyclizing the acryloylureas (XIII and XIV) in 15*N* aqueous ammonia and in some of the specimens of XVI prepared by acid-catalyzed cyclization. Since small amounts of uracil were formed in the base-catalyzed and in some of the acid-catalyzed cyclizations of two different acryloylureas, it probably arose from an impurity introduced during the preparation of the sensitive acryloyl isocyanate (VII). A possible explanation is that, despite precautions to exclude moisture and oxygen, a small amount of the bis(acryloyl)urea (XIX) (11) was formed. Cyclization of XIX should yield a 1-acyluracil (XX) which should readily lose the acyl group in either basic or acidic media.



XVIII



XIX



XX

The three carbocyclic analogs (XV-XVII) were not active (ED_{50} > 100 mcg./ml.) against KB cells in culture. All three were tested at a dose of 200 mg./kg./day, *q.d.* 1-9, against L1210 leukemia (3 mice per test). As the data in Table I show, there was no evidence of toxicity or activity in these tests.

EXPERIMENTAL

General.

Decomposition and melting temperatures were determined in capillary tubes heated in a Mel-Temp apparatus. Ultraviolet spectra (uv) were recorded with a Cary Model 17 spectrophotometer, and maxima are reported in nanometers. Solutions for ultraviolet spectral determinations were prepared by diluting a 5-ml. aliquot of an ethanol solution to 50 ml. with 0.1*N* hydrochloric acid, phosphate buffer (*p*H 7), or 0.1*N* sodium hydroxide; absorption maxima of these solutions are reported as being determined in 0.1*N* hydrochloric acid, at *p*H 7, or in 0.1*N* sodium hydroxide, respectively. Infrared spectra (ir) were recorded with Perkin-Elmer

Table I

Tests of Carbocyclic Analogs of Uracil Nucleosides

Compound	KB Cells ED_{50}	L1210 (a) Dose (mg./kg./day), *Q.D.* 1-9	L1210 (a) Mortality on Day 5	L1210 (a) ΔW(g.), (b) T-C	L1210 (a) T/C, %
XV	>100	200	0/3	+0.5	97
XVI	>100	200	0/3	+0.3	102
XVII	>100	200	0/3	+0.2	101

(a) T = treated mice; C = control mice. (b) ΔW = difference in average weights between the treated and control mice.

Model 521 or 621 spectrometers from samples in potassium bromide disks; s = strong, sh. = shoulder. Mass spectral data were taken from low resolution spectra determined at 70 eV with a Hitachi-Perkin-Elmer Model RMU-7 double-focusing instrument or with a Varian MAT Model 311A spectrometer equipped with a combination electron-impact, field-ionization, and field-desorption ion source. The peaks listed are those due to the molecular ion (M^+), those attributable to the loss of certain fragments from the molecular ion (M - a fragment), and some other prominent peaks. Thin-layer chromatography (tlc) was performed on plates of fluorescing silica gel (15), and developed plates were examined with uv light (254 nm.) unless indicated otherwise in parentheses. Other pertinent information (amount applied, developing solvent, other methods of detection) is given parenthetically at the appropriate places in the experimental procedures. Preparative tlc was performed on Silica Gel 60 F-254 (16), and column chromatography was performed on Silica Gel H (17).

Intermediates.

Ethyl 3-ethoxy-2-propenoate was prepared by the procedure of Deno (18) except that stirring was continued throughout the Reformatsky reaction (manually, if necessary) and that elimination of ethanol from the crude acetal precursor required a longer period of heating at 200°, b.p. 191° [lit. (18) 189-193°]; yield of pure ester, 96 g. (23%) from 478 g. of ethyl bromoacetate; mass spectrum (RMU-7; indirect-inlet temperature, 100°) m/e 144 (M^+), 129 (M - CH_3), 116 (M - C_2H_5 + H), 115 (M - C_2H_5), 101, 99 ($C_2H_5OCH{=}CHCO^+$), 88, 71 ($C_2H_5OCH{=}CH^+$). The preceding distillation fraction, b.p. 189-191°, amounted to 67 g. (16%) and contained, according to vapor-phase chromatography, 5% of a mixture of ethyl bromoacetate and ethyl orthoformate.

3-Ethoxy-2-propenoic acid (IV) was prepared from the ethyl ester by the procedure of Shaw and Warrener (12), yields, 50-56%, m.p. 107-109°.

Sodium hydroxide (2*N*) was added dropwise to a suspension of 14.2 g. of 3-ethoxy-2-propenoic acid (IV) in 400 ml. of water until the *p*H was 7. The solution was treated with activated charcoal, filtered, and concentrated *in vacuo* at temperatures below 40°. The residual white solid (V) was dried to constant weight *in vacuo* over phosphorus pentoxide, weight 16.7 g.; ir: (2000-1400 cm^{-1} region) 1648s, 1630, 1614, 1545s, 1512, 1470, 1430s.

Anal. Calcd. for $C_5H_7NaO_3$: C, 43.48; H, 5.11. Found: C, 43.30; H, 5.07.

3-Ethoxy-2-propenoyl chloride (VI) was prepared from the sodium salt (V) and thionyl chloride by the procedure of Shaw and Warrener (12), yield, 80%, b.p. 105-107° at 37-38 mm. [lit. (12) 104° at 35 mm.]; mass spectrum: (RMU-7; indirect-inlet temperature, 100°) m/e 134 (M^+), 99 ($C_2H_5OCH{=}CHCO^+$), 71 ($C_2H_5OCH{=}CH^+$).

3-Ethoxy-2-propenoyl Isocyanate (VII) and 3-Ethoxy-*N*-(ethoxycarbonyl)-2-propenamide (VIII).

Silver cyanate (7.00 g., 49.6 mmoles), while protected from light, was dried *in vacuo* over phosphorus pentoxide at 100° for 3 hours and was then added to 50 ml. of dry benzene under an atmosphere of dry nitrogen. The benzene had been previously dried by distilling about 5 ml. under anhydrous conditions from a 55-ml. portion of benzene that had been stored over molecular sieves (19). The mixture was heated under reflux with vigorous stirring for 0.5 hour, and a solution of 3.35 g. (24.9 mmoles) of 3-ethoxy-2-propenoyl chloride (VI) in 10 ml. of dry benzene was added dropwise to the refluxing suspension. The vigorously stirred mixture was heated under reflux for an additional 0.5 hour and then stirred at room temperature for 2.5 hours. After the solid phase had settled, 8.3 ml. of the supernatant solution (theoretically containing 3.68 mmoles of VII) was transferred with a dry pipette to a dry dropping funnel and added dropwise to a dry dimethylformamide solution of IX, as described below.

The remainder of the mixture was stirred while a solution of 5 ml. of ethanol in 10 ml. of benzene was added dropwise during 10 minutes. The mixture was stirred at room temperature overnight and filtered. The filtrate (plus ethanol washings) was concentrated to dryness *in vacuo*, and the residue was slurried with petroleum ether, collected by filtration, and dried *in vacuo* at 56°, weight of VIII, 3.5 g. (yield adjusted for removal of some of VII to prepare IX, 89%), m.p. 82-84° [lit. (11,20), 84°]; ir: (2000-1500 cm^{-1} region) 1765, 1675, 1605 broad, 1510; mass spectrum: (RMU-7; direct-inlet temperature, 40°) m/e 187 (M^+), 172 (M - CH_3), 158 (M - C_2H_5), 144, 130, 114 (M - $COOC_2H_5$), 99 ($C_2H_5OCH{=}CHCO^+$), 71 ($C_2H_5OCH{=}CH^+$).

Anal. Calcd. for $C_8H_{13}NO_4$: C, 51.33; H, 7.00; N, 7.48. Found: C, 51.05; H, 6.55; N, 7.31.

3-Ethoxy-*N*-[[[(1α,3β,4α)-(±)-3-hydroxy-4-(hydroxymethyl)cyclopentyl]amino]carbonyl]-2-propenamide (XII).

A solution of 483 mg. (3.68 mmoles) of cyclopentylamine IX in 17 ml. of previously dried (molecular sieves) dimethylformamide was dried further over molecular sieves (19) overnight. The filtered solution, protected from atmospheric moisture by a current of dry nitrogen and a tube of calcium sulfate, was cooled to -15°. To the cold, stirred solution was added, dropwise, 8.3 ml. of the benzene solution prepared as described above and containing, theoretically, 3.68 mmoles of isocyanate VII. During the addition of VII, the temperature of the mixture was not allowed to exceed -10°. The mixture was allowed to warm to room temperature during 1 hour, stirred at room temperature overnight, and filtered. The filtrate (including DMF washings of the residue) was concentrated at 40°, or less, under reduced pressure (oil pump), and several portions of ethanol were evaporated *in vacuo* from the residual oil. Trituration of the partially crystallized residue with ether gave a solid that was separated by filtration, washed with ether, and dried *in vacuo*, yield, 810 mg. (81%); mass spectrum (RMU-7; direct-inlet temperature, 320°) m/e 272 (M^+), 255 (M - OH), 254 (M - H_2O), 243 (M - C_2H_5), 225 (M - H_2O - C_2H_5), 213, 185, 159, 130, 116, 112, 99, 86, 71. This material was estimated to be about 90% pure XII by comparing its uv molar absorptivities with those of the analytical sample, and it was used without further purification for the preparation of XV (below).

A pure specimen was obtained by applying a methanol solution of 510 mg. of crude product from another run to a preparative tlc plate of silica gel, evaporating the methanol, and developing the chromatogram in chloroform-methanol (3:1). The band containing XII was extracted with methanol (3 x 50 ml.), the methanol was evaporated, the residue was slurried with ethanol, the mixture was filtered to remove residual silica gel, and the ethanol was evaporated. The residual syrup crystallized to a white solid, weight, 401 mg., m.p. 138-140°; tlc, 1 spot (80 mcg., 9:1 $CHCl_3$-CH_3OH); uv: max. 252 (ε 20,500) in 0.1*N* hydrochloric acid and at *p*H 7; ir: (2000-1400 cm^{-1} region) 1695s, 1675s, 1605s, 1535s, 1505, 1472, 1455, 1445; mass spectrum: (Varian MAT; direct-inlet temperature, 100°) m/e 273 (M + 1), 272 (M^+), 255 (M - OH), 254 (M - H_2O), 243 (M - C_2H_5), 225 (M - C_2H_5 - H_2O), 213, 185 ($C_2H_5OCH{=}CHCONHCONHC_2H_4^+$), 159 ($C_2H_5OCH{=}CHCONH$-CONH + 2H), 130 (M - $C_2H_5OCH{=}CHCONHCO$), 116, 113, 112, 100, 99 ($C_2H_5OCH{=}CHCO^+$), 96, 86, 83, 82, 72, 71 ($C_2H_5OCH{=}$

CH^+).

Anal. Calcd. for $C_{12}H_{20}N_2O_5$: C, 52.93; H, 7.40; N, 10.29. Found: C, 52.36; H, 7.63; N, 10.04.

N-[[[(1α,2β,3β,4α)-(±)-2,3-Dihydroxy-4-(hydroxymethyl)cyclopentyl]amino]carbonyl]-3-ethoxy-2-propenamide (XIII).

A benzene solution of isocyanate VII was prepared and transferred to a dropping funnel by the procedure described above. To a stirred solution, previously cooled to -15°, of 580 mg. (3.94 mmoles) of (1α,2α, 3β,5β)-(±)-3-amino-5-(hydroxymethyl)-1,2-cyclopentanediol (X) in 20 ml. of dry DMF was added 8.76 ml. of the benzene solution containing, theoretically, 3.94 mmoles of VII. The DMF solution of X had been prepared from dry DMF and had then been stored over molecular sieves (19) overnight, and the molecular sieves were not removed prior to the addition of VII. The isocyanate solution was added at a rate that did not cause the temperature to exceed -10°. The reaction mixture was stirred under dry nitrogen at -10 to -15° for 1 hour, allowed to warm to room temperature, stirred at room temperature overnight, filtered, and concentrated *in vacuo* (first with an aspirator, then with an oil pump) to a viscous yellow syrup. Several portions of ether and ethyl acetate were evaporated from the residue to complete the removal of DMF; tlc and a mass spectrum revealed several impurities in the residue. The crude product (976 mg.) was dissolved in 1.5 ml. of methanol, 10.5 ml. of chloroform was added, the solution was applied to a 30-g. column of silica gel, and the column was eluted with chloroform-methanol (7:1). Product-containing fractions, located by tlc (5:1 chloroform-methanol), were combined and concentrated to a colorless syrup that crystallized during trituration with ethyl acetate. Concentration of the mixture to dryness left 556 mg. (49% yield) of white crystalline solid, m.p. 135-137°; tlc, 1 spot (100 mcg., 5:1 chloroform-methanol); uv: max. at 252 (ε, 21,100) at *p*H 1, 252 (ε, 21,300) at *p*H 7; ir: (2000-1400 cm^{-1} region) 1690 sh., 1670s, 1602s, 1530s, 1505, 1470, 1450 sh.; mass spectrum: (Varian MAT; direct-inlet temperature, 70°) m/e 289 (M + 1), 288 (M^+), 270 (M - H_2O), 252 (M - $2H_2O$), 239, 234, 223, 213, 208 (M - CH_2OH - H_2O), 185 ($C_2H_5OCH{=}CHCONHCONHC_2H_4^+$), 184, 180, 178, 171, 168, 159 ($C_2H_5OCH{=}CHCONHCONH$ + 2H), 149, 141, 116, 113, 112, 99 ($EtOCH{=}CHCO^+$), 94, 86, 71 ($EtOCH{=}CH^+$).

Anal. Calcd. for $C_{12}H_{20}N_2O_6$: C, 49.99; H, 6.99; N, 9.72. Found: C, 50.08; H, 6.99; N, 9.62.

3-Ethoxy-*N*-[[[(1α,2β,4α)-(±)-2-hydroxy-4-(hydroxymethyl)cyclopentyl]amino]carbonyl]-2-propenamide (XIV).

The cyclopentylamine (XI, 4.10 g., 31.2 mmoles) in dry DMF (140 ml.) was treated with 76.7 ml. of a benzene solution of isocyanate VII according to the procedure described for the preparation of XIII. The isocyanate solution, containing theoretically 31.2 mmoles of VII, was prepared by the procedure described above. After the reaction solvents had been evaporated, the residual syrup was dissovled in methanol (50 ml.), and the solution was treated with activated carbon, filtered, and concentrated *in vacuo*. Trituration of the residual tan syrup with acetonitrile furnished 3.65 g. (43%) of crude XIV; m.p., 119-125°. The crude product was chromatographed in chloroform-methanol (5:1) on a column of silica gel (120 g.). Product-containing fractions, located by tlc, were combined and concentrated to dryness *in vacuo*. Trituration of the residue with ethyl acetate provided 2.35 g. of white crystals (XIV), m.p., 126-128°; tlc, 1 spot (80 mcg., 5:1 chloroform-methanol); ir: (2000-1400 cm^{-1} region) 1710, 1680 sh., 1660s, 1600s, 1555s, 1470, 1450, 1437; uv: max. 252 (ε, 21,500) in 0.1*N* hydrochloric acid and in phosphate buffer (*p*H 7); mass spectrum: (Varian MAT; direct-inlet temperature, 20°) m/e 273 (M + 1), 272 (M^+), 254 (M - H_2O), 236 (M - $2H_2O$), 223 (M - CH_2OH - H_2O), 214, 197, 185 ($C_2H_5OCH{=}CHCONH$-$CONH$-$C_2H_4^+$), 171, 159 ($C_2H_5OCH{=}CHCONHCONH$ + 2H), 141, 126, 116, 113, 100, 99 ($C_2H_5OCH{=}CHCO^+$), 96, 94, 86, 83, 71 ($C_2H_5OCH{=}CH^+$).

Anal. Calcd. for $C_{12}H_{20}N_2O_5$: C, 52.93; H, 7.40; N, 10.29. Found: C, 52.85; H, 7.29; N, 10.12.

The filtrate from the crude product was concentrated to a syrup *in vacuo* and chromatographed in the same way. Fractions shown by tlc to be predominantly XIV were combined, concentrated to dryness *in vacuo*, and triturated with ethyl acetate. The white crystalline solid was collected by filtration, washed with ethyl acetate, and dried *in vacuo* at 78°, weight, 1.66 g. (total yield of XIV = 47%); m.p. 126-128°; identical by tlc (1 spot) with the specimen XIV described above.

(1α, 3β,4α)-(±)-1-[3-Hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4-(1*H*,3*H*)-pyrimidinedione (XV).

A solution of 750 mg. of crude XII (above) in 15 ml. of 2*N* sulfuric acid was heated under reflux for 3 hours and treated with activated carbon. The filtrate was neutralized (*p*H 7) with 2*N* sodium hydroxide and concentrated to dryness *in vacuo*, the residue was extracted with ethanol (3 x 15 ml.), the ethanol was evaporated *in vacuo*, and the residue (612 mg.) was stirred with ethyl acetate overnight. The ivory-colored solid was collected by filtration, washed with ethyl acetate, and dried *in vacuo:* weight, 514 mg. (82%); m.p. 158-160° dec. Recrystallization of this material from ethanol furnished white crystals, m.p. 160-163° dec. (inserted at 100°, 3°/minute); tlc, 1 spot (80 or 120 mcg., 5:1 chloroform-methanol, detection by uv light and by basic potassium permanganate spray); uv: max. 268 (ε, 10,400) in 0.1*N* hydrochloric acid and at *p*H 7, 266 (ε, 8,000) in 0.1*N* sodium hydroxide; mass spectrum: (21) (Varian MAT; direct-inlet temperature, 20°) m/e 226 (M^+), 209 (M - OH), 208 (M - H_2O), 196, 189, 177 (M - CH_2OH - H_2O), 169, 167, 152, 139 (U + C_2H_4), 134, 124, 113 (U + 2H), 112 (U + H), 96.

Anal. Calcd. for $C_{10}H_{14}N_2O_4$: C, 53.09; H, 6.24; N, 12.38. Found: C, 53.01; H, 6.51; N, 12.44.

(1α,2β,3β,4α)-(±)-1-[2,3-Dihydroxy-4-(hydroxymethyl)cyclopentyl]-2,4(1*H*,3*H*)-pyrimidinedione (XVI).

Cyclization of XIII in Sulfuric Acid.

A solution of 375 mg. of XIII in 10 ml. of 2*N* sulfuric acid was heated under reflux for 3.5 hours, cooled, diluted to 25 ml. with water, and neutralized with 2*N* sodium hydroxide to *p*H 7. The solution was concentrated *in vacuo* to a pasty solid and was dried further (with the aid of an oil pump) to remove the last traces of water. The residual solid was extracted with three 15-ml. portions of ethanol, the ethanol solution was concentrated *in vacuo*, the residual syrup (307 mg.) was redissolved in ethanol (4 ml.), and the slightly cloudy solution was filtered. The colorless filtrate was heated to boiling, diluted with cyclohexane (15 ml.) to the cloud point, and allowed to stand at room temperature overnight. This mixture, containing white crystals, was diluted with 20 ml. of cyclohexane, chilled, and filtered. Crystalline XVI was collected by filtration, washed with cyclohexane, and dried *in vacuo*, yield, 275 mg. (87%), m.p. 176-179°, slight decomposition (inserted at 60°, 3°/minute); tlc, 1 spot (80 mcg., 9:1 or 3:1 chloroform-methanol, detection by uv light); ir: (2000-1400 cm^{-1} region) 1700-1665s (broad), 1615 sh., 1540 sh., 1460, 1440 sh., 1415, 1408; uv: max. 267 (ε, 10,700) in 0.1*N* hydrochloric acid and in phosphate buffer (*p*H 7), 266 (ε, 8000) in 0.1*N* sodium hydroxide;

mass spectrum: (21) (Varian MAT; direct-inlet temperature, 80°) m/e 243 (M + 1), 242 (M^+), 225 (M - OH), 224 (M - H_2O), 211 (M - CH_2OH), 206, 195, 193 (M - CH_2OH - H_2O), 167, 165, 155, 154, 150, 139 (U + C_2H_4), 134, 130, 122, 113 (U + 2H), 112 (U + H), 111 (U), 99, 96, 95.

Anal. Calcd. for $C_{10}H_{14}N_2O_5 \cdot 0.25H_2O$: C, 48.67; H, 5.92; N, 11.35. Found: C, 48.52; H, 6.00; N, 11.32.

Compound XVI was also prepared by cyclizing XIII in 0.5*N* sulfuric acid. Other modifications included neutralization of the reaction solution with 50% sodium hydroxide, lyophilization of the neutralized reaction mixture, and extraction of the lyophilization residue with several portions of ethanol or with ethyl acetate in a Soxhlet extractor. The crude product of one of the cyclizations in 0.5*N* sulfuric acid was shown by tlc to be contaminated with small amounts of uracil (XVIII) and a substance that moved between XVI and XVIII.

Cyclization of XIII in Aqueous Ammonia.

A solution of 500 mg. of urea XIII in 25 ml. of 15*N* aqueous ammonia was heated under reflux for 2.5 hours and concentrated *in vacuo* to a syrupy residue that crystallized after several portions of ethanol had been evaporated from it. The solid was triturated with 20 ml. of ethyl acetate, collected by filtration, washed with ethyl acetate, and dried *in vacuo* at 56°, weight, 371 mg. (88%), m.p. 164-169°. Tlc showed that this material was XVI contaminated with trace amounts of uracil (XVIII) and an impurity that moved between XVI and XVIII. (Concentration of the filtrate to dryness left 36 mg. of a white gum that was shown by tlc to be a mixture of XVI, XVIII, and the impurity with the intermediate R_f.) Recrystallization of 344 mg. of the crude product from ethanol-cyclohexane gave 253 mg. (74% recovery) of pure XVI, identified by m.p. (176-179°), tlc (1 spot, 80 or 120 mcg., 3:1 chloroform-methanol, detection by uv light and by basic potassium permanganate spray), and uv spectra.

(1α,2β,4α)-(±)-1-[2-Hydroxy-4-(hydroxymethyl)cyclopentyl]-2,4-(1*H*,3*H*)-pyrimidinedione (XVII).

Cyclization of XIV in Sulfuric Acid.

A solution of 890 mg. of XIV in 17.8 ml. of 1*N* sulfuric acid was heated under reflux for 2.5 hours, cooled, and neutralized (*p*H 7) with 50% sodium hydroxide. The neutral solution was saturated with sodium chloride and was extracted with ethyl acetate (300 ml.) in a continuous liquid-liquid extractor for 22 hours. The ethyl acetate extract was concentrated *in vacuo* to a white solid; weight, 696 mg. (94.2%), m.p. 172-174°. Tlc, uv data, and the mass spectrum indicated that this material was XVII containing a small amount of uracil (XVIII). Aqueous solutions containing 100, 200, and 300 mcg. of this material and similar solutions containing 2, 4, and 6 mcg. of authentic uracil (XVIII) were applied to a thin layer of silica gel on a standard tlc plate. The chromatogram was developed in chloroform-methanol (5:1) and examined under uv light (254 nm.). Comparison of the spots of authentic uracil with the uracil spots in the three portions of the white solid showed that 3-4% of the white solid was uracil and the remainder was XVII.

A portion (250 mg.) of the white solid was applied in methanol solution to a preparative tlc plate, and the chromatogram was developed twice in chloroform-methanol (5:1). Pure XVII was recovered as follows: the product band (R_f about 0.25) was removed from the plate and extracted with hot methanol (3 x 30 ml.), the methanol was evaporated, the residue was dissolved in ethanol, the ethanol solution was filtered, and the ethanol was evaporated. The white residue was dried *in vacuo* at 56° for 2 hours and at room temperature overnight, weight, 231 mg. (92.4% recovery), m.p. 174-176°; tlc, 1 spot (40 or 80 mcg., 5:1 chloroform-methanol, detection by uv light and by basic potassium permanganate spray). The amount of pure XVII isolated by preparative tlc corresponds to a yield of 87% of XVII.

The original aqueous layer was subjected to continuous liquid-liquid extraction for 24 hours with a second portion of ethyl acetate. Evaporation of the solvent left 40 mg. (5.4% yield) of white solid that was homogeneous according to tlc (80 mcg., 5:1 chloroform-methanol) and produced a good mass spectrum (compared with that of the analytical sample), m.p. 173-176°. The combined yield of the two portions, the first of which contained 3-4% uracil, was 99.6%; the yield of pure XVII, isolated by preparative tlc from the first ethyl acetate extract plus the specimen from the second ethyl acetate extract, amounted to 92.4%. A sample for analysis was prepared by recrystallizing a specimen from an earlier experiment from ethanol-cyclohexane (1:2), m.p. 174-176°; tlc, 1 spot (80 or 120 mcg., 5:1 chloroform-methanol); ir: (2000-1400 cm^{-1} region) 1705s, 1660s (broad), 1635, 1520, 1485, 1420; uv: max. 268 (ε, 10,300) in 0.1*N* hydrochloric acid and in phosphate buffer (*p*H 7), 266 (ε, 7600) in 0.1*N* sodium hydroxide; mass spectrum: (21) (Varian MAT; direct-inlet temperature, 20°) m/e 227 (M + 1), 208 (M - H_2O), 198, 189, 180, 177 (M - CH_2OH - H_2O), 168, 167, 165, 152, 151, 139 (U + C_2H_4), 134, 126, 125, 113 (U + 2H), 112 (U + H), 96.

Anal. Calcd. for $C_{10}H_{14}N_2O_4$: C, 53.09; H, 6.24; N, 12.38. Found: C, 52.88; H, 6.29; N, 12.18.

Cyclization of XIV in Ammonia.

Acryloylurea XIV was treated with 15*N* aqueous ammonia by the procedure described for the cyclization of XIII except that trituration of the crude product (100% yield, calculated as XVII) with ethyl acetate was omitted. A water solution of the crude product (385 mg.) was applied to a preparative tlc plate, and the chromatogram was developed in chloroform-methanol (5:1). The major band (R_f about 0.2) was removed and extracted with ethanol (3 x 30 ml.). Evaporation of the solvent *in vacuo* left 282 mg. (73% yield) of white crystalline XVII, which was identified by uv and mass spectra and was shown to be homogeneous by tlc (80 mcg., 5:1 chloroform-methanol). From another band (R_f about 0.4), 5 mg. of a solid was isolated by the same procedure. A mass spectrum, tlc alongside an authentic specimen of uracil, and uv spectra showed that this material was predominantly uracil (XVIII). Two very weak bands near the front of the preparative tlc plate were discarded.

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(14) See, also, publications cited in reference 9.

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