EXPERIMENTAL

2-Mercapto-4-hydroxy-6-aminopyrimidine.[14] To a solution of sodium ethoxide (prepared by dissolving 9 g. of sodium in 200 ml. of ethanol) was added 22.6 g. of ethyl cyanoacetate. A white precipitate formed immediately. After 15 min., 15.2 g. of thiourea was added with shaking, and the mixture was allowed to stand at room temperature for 1 hr. with occasional shaking. It was then heated under reflux for 2 hr., cooled and filtered. The collected solid was dissolved in boiling dilute potassium hydroxide and reprecipitated by the addition of glacial acetic acid to give 28.4 g. (99%) of white crystals.

2-Mercapto-4-hydroxy-5-nitroso-6-aminopyrimidine.[14] To a solution of 20 g. of 2-mercapto-4-hydroxy-6-aminopyrimidine in 500 ml. of water containing 5.5 g. of sodium hydroxide and 10 g. of sodium nitrite and maintained at room temperature was added dropwise 15 g. of glacial acetic acid. The reaction mixture was stirred overnight and then filtered to give a brownish-red solid in 90% yield. The crude product was extracted with boiling acetone and then with boiling ethanol (thus removing a small amount of colorless impurity) and was then suitable for further reaction.

2-Mercapto-4-hydroxy-5,6-diaminopyrimidine. The procedure used was essentially the same as previously described by Albert *et al.*,[15] except that the temperature of the reduction mixture was maintained below 30° rather than below 50°, and the sodium hydrosulfite was added very slowly rather than all at once. Furthermore, the reduction mixture was stirred for 20 hr. at room temperature following addition of all of the hydrosulfite and was then decolorized with charcoal. The diaminopyrimidine was obtained in 96.5% yield.

4-Hydroxy-5,6-diaminopyrimidine-2-sulfinic acid. To a solution of 1 g. of 2-mercapto-4-hydroxy-5,6-diaminopyrimidine in 90 ml. of water containing 0.6 g. of sodium hydroxide and precooled to −3° was added dropwise 1.8 ml. of 30% hydrogen peroxide in 17 ml. of water. During the addition the temperature was carefully maintained below 0°. The reaction mixture was allowed to stir for 1.5 hr. following addition of the peroxide and was then acidified with glacial acetic acid. Filtration yielded 0.8 g. of a colorless solid, m.p. 188–190°.

4-Hydroxy-5,6-diaminopyrimidine hydrochloride. A mixture of 1.5 g. of 4-hydroxy-5,6-diaminopyrimidine-2-sulfinic acid and 30 ml. of ethanolic hydrogen chloride was stirred at room temperature for 20 hr. in a flask protected from atmospheric moisture by a calcium chloride tube. The reaction mixture was evaporated to dryness, the residue dissolved in water, filtered and the filtrate again evaporated to dryness to give 1.3 g. of a colorless solid, m.p. 249–251°d., identical in all respects with an authentic sample of 4-hydroxy-5,6-diaminopyrimidine hydrochloride prepared by Raney nickel desulfurization of 2-mercapto-4-hydroxy-5,6-diaminopyrimidine.[16]

Decomposition of 4-hydroxy-5,6-diaminopyrimidine-2-sulfinic acid with concentrated hydrochloric acid yielded 2,4-dihydroxy-5,6-diaminopyrimidine hydrochloride rather than the desired product.

Hypoxanthine. A mixture of 2 g. of 4-hydroxy-5,6-diaminopyrimidine hydrochloride and 30 ml. of an equimolar mixture of ethyl orthoformate and acetic anhydride was heated under reflux for 4 hr. and then evaporated to dryness. Recrystallization of the residual solid from aqueous ethanol gave 1.6 g. (95.5%) of pure hypoxanthine, identical in all respects with an authentic sample.

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(14) This preparation is a slight modification of the original method described by Traube (Ref. 9).

(15) A. Albert, D. J. Brown, and G. Cheeseman, *J. Chem. Soc.*, 474 (1951).

(16) R. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. P. Cole, and J. R. Vaughan, Jr., *J. Am. Chem. Soc.*, **67**, 290 (1945).

Potential Anticancer Agents.[1] XXVIII. Synthesis of 5-(Chloromethyl)uracil

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Both 5-fluorouracil[2] and 5-[bis(2-chloroethyl)-amino]uracil[3] have been shown to inhibit the growth of various tumors. Matthews[4] reported that all of the 5-halogenated uracils were incorporated into phage DNA, giving mutants. Hitchings *et al.*,[5] have shown 5-bromouracil to be a competitive thymine antagonist.

Efforts to find new anticancer agents could, therefore, be logically directed toward the preparation of various thymine derivatives such as 5-(fluoromethyl) uracil, 5-[bis(2-chloroethyl)aminomethyl]uracil, and other uracil derivatives containing potential alkylating groups attached to a 5-methyl grouping. The key intermediate to the synthesis of these agents would be 5-(chloromethyl)-uracil (IV). This compound has now been synthesized in 57% yield by the choromethylation of uracil (I).

Early attempts in this laboratory to prepare IV by the chlorination of thymine using *N*-chlorosuccinimide and benzoyl peroxide, as reported by West and Barrett,[6] failed to yield IV. Instead, a compound melting at 224.5–225.5° was obtained. West reported a similar melting point of 222–224° and an empirical formula of $C_5H_5ClN_2O_4$. The failure of this compound to react with alcoholic silver nitrate solution upon heating, its stability toward water (being recrystallized without change from hot water), its lack of absorption in the ultraviolet, and its liberation of iodine from an acetic acid solution of potassium iodide, are convincing

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, Contract No. SA-43-ph-1892. For the preceding paper in this series, *cf.* E. J. Reist, R. R. Spencer, and B. R. Baker, *J. Am. Chem. Soc.*, in press.

(2) C. Heidelberger, L. Greisbach, B. J. Montag, D. Mooren, O. Cruz, R. J. Schnitzer, and E. Grunberg, *Cancer Res.*, **18**, 305 (1958); R. Duschinsky, E. Pleven, and C. Heidelberger, *J. Am. Chem. Soc.*, **79**, 4559 (1957).

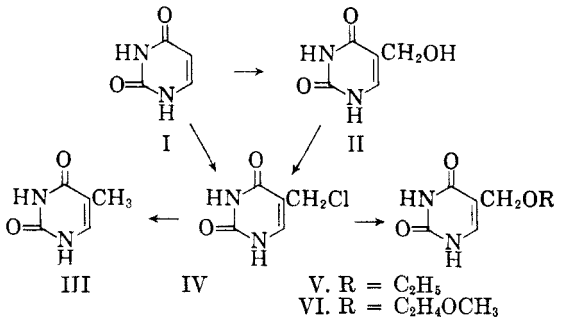
(3) D. A. Lyttle and H. G. Petering, *J. Am. Chem. Soc.*, **80**, 6459 (1958).

(4) R. E. F. Matthews, *Pharm. Reviews*, **10**, No. 3, 359 (1958).

(5) G. H. Hitchings, E. A. Falco, and M. B. Sherwood, *Science*, **102**, 251 (1945).

(6) R. A. West and H. W. Barrett, *J. Am. Chem. Soc.*, **76**, 3146 (1954).

evidence that it was not IV. The absence of absorption in the ultraviolet would suggest that the uracil ring had been either ruptured or else saturated. The iodide test indicates *N*-chlorination.



Efforts were shifted to the chlorination of 5-(hydroxymethyl)uracil (II), which was prepared by the method of Cline *et al.*[7] Attempts to chlorinate II in pyridine with thionyl chloride resulted in the formation of a compound that appeared to be the quaternary salt formed from IV and pyridine. When chlorination in dichloromethane with thionyl chloride was attempted, no reaction occurred, probably due to the limited solubility of II in that solvent.

Crude IV was prepared in 37% yield by heating 5-(hydroxymethyl)uracil (II) in concentrated hydrochloric acid. Although an analytical sample of IV could not be obtained by this procedure, the infrared absorption spectrum and paper chromatographic[8] behavior indicated that a product different from thymine (III), uracil (I), and 5-(hydroxymethyl)uracil (II) had been obtained. This crude IV was reduced to thymine in 62% yield by means of tin and hydrochloric acid. The thymine produced was identical with that of an authentic sample in its infrared absorption and paper chromatographic behavior.

Schmeds[9] reported the chloromethylation of 3,6-dimethyluracil to yield 3,6-dimethyl-5-(chloromethyl)uracil by heating the former in aqueous formaldehyde with excess concentrated hydrochloric acid. Attempts to prepare IV from uracil (I) using that procedure resulted in only a 13% yield of crude 5-(chloromethyl)uracil (IV). It was found, however, that extraction of crude IV with warm 1,2-dimethoxyethane and concentration of the extracts yielded a more pure sample of IV, as evidenced by paper chromatographic[8] behavior (disappearance of spots near the origin) and chloride analyses (closer to theoretical for IV).

It was then found that the best conditions for the synthesis of IV were continuous passage of hydrogen chloride through a solution of uracil and paraformaldehyde in concentrated hydrochloric acid while heating to 70–80°. The product was isolated in 57% yield and analyzed closely to the theoretical chlorine value for IV without extraction with 1,2-dimethoxyethane. Only a trace spot still remained at the origin on paper chromatographic[8] investigation. Proof that chloromethylation had occurred at the 5-position of uracil was shown by reduction of IV to thymine (III) in 82% yield by means of tin and hydrochloric acid. The thymine was characterized by the identity of its infrared absorption spectrum and paper chromatographic behavior[8] with those of an authentic sample of thymine. In addition, IV differs from West's compound[3] in that IV gives an immediate precipitate of silver chloride on treatment with alcoholic silver nitrate solution, and 5-(hydroxymethyl)-uracil (II) is produced upon reaction with water. The identity of II was proven by its agreement in behavior on paper[8] (R_f 0.19) and by its identical infrared absorption spectrum with that of II prepared by the method of Cline.[7]

5-(Chloromethyl)uracil (IV) also reacted readily with alcohols to yield ethers. A similar reaction has been reported by Cline[7] to occur with 5-(hydroxymethyl)uracil (II) and alcohols under somewhat more vigorous reaction conditions. The product obtained by heating IV with 2-methoxyethanol was isolated as a crystalline solid, m.p. >300°, and shown by analysis to be 5-[(2-methoxyethoxy)-methyl]uracil (VI). This material was homogeneous on paper chromatography,[8] showing only one spot, R_f 0.39.

EXPERIMENTAL

5-(Chloromethyl)uracil (IV). A suspension of 40 g. (0.36 mol.) of uracil (I) and 13.4 g. (0.44 mol.) of paraformaldehyde in 350 ml. of concentrated hydrochloric acid was stirred while gaseous hydrogen chloride was passed through the reaction mixture, which was then heated to 80°. When that temperature was reached, complete solution had been attained. The heat source was removed; the reaction temperature remained spontaneously at 80° for 0.5 hour, then subsided. After 4 hr. total time, the now heterogeneous reaction mixture was filtered through a glass sintered funnel and the precipitate dried over phosphorus pentoxide at 1 mm. pressure; yield, 32.6 g. (57%), m.p. >300°; $\lambda_{max\ (\mu)}^{Nujol}$ 3.05, 3.20 (NH), 5.70, 5.98 (uracil C=O), 6.70, 6.96, 8.24, 8.48 (uracil ring). The compound moved as two spots (R_f 0.70, 0.18)[8] provided the compound was dissolved in 1,2-dimethoxyethane for spotting on the paper. Uracil has R_f 0.28; thymine, R_f 0.42; and 5-(hydroxymethyl)uracil, R_f 0.19 in this system.[8] When IV was spotted in warm ethanol, the compound had R_f 0.53, and in warm methyl Cellosolve, R_f 0.39, corresponding to V and VI, respectively. In warm 1-butanol an R_f of 0.70 was obtained due to the formation of 5-(butoxymethyl)uracil.

Anal. Calcd. for $C_5H_5ClN_2O_2$: C, 37.5; H, 3.14; Cl, 22.1. Found: C, 37.7; H, 3.54; Cl, 21.1.

The compound is very sensitive to moisture, fuming in air, thus making an accurate analysis difficult.

5-[(2-Methoxyethoxy)methyl]uracil (VI). Crude IV (1 g.; Cl, 13.2%), prepared from uracil (I), was extracted with 2-methoxyethanol by heating to 70° and filtering hot.

(7) R. E. Cline, R. M. Fink, and K. Fink, *J. Am. Chem. Soc.*, **81**, 2521 (1959).

(8) Paper chromatograms were run by the descending technique on Whatman No. 1 paper with 1-butanol–water, unless otherwise indicated. Spots were detected by their ultraviolet absorption.

(9) K. Schmeds, *Ann.*, **441**, 192 (1925).

The filtrate upon concentration *in vacuo* yielded 0.46 g. of VI, m.p. >300°, silver nitrate test negative; λ_{max}^{Nujol} (μ) 3.12, 3.25 (NH), 5.72, 5.44 (C=O of uracil), 9.00, 9.10 (C—O—C of ether).

Anal. Calcd. for $C_8H_{12}N_2O_4$: C, 48.0; H, 6.05; N, 14.0. Found: C, 47.7; H, 6.00; N, 14.0.

Reduction of 5-(chloromethyl)uracil (IV) *to thymine* (III). A suspension of 5.00 g. (0.03 mol.) of 5-(chloromethyl)uracil (IV), prepared by chloromethylation of uracil, in 150 ml. of concentrated hydrochloric acid was heated to 60°. The reaction mixture was kept at 60° and 35.0 g. of tin was added over a period of 20 min. with stirring. After being stirred and heated for 3 hr., the reaction mixture was decanted to remove the excess tin. The solution was evaporated to one third its volume under reduced pressure on a water bath at 60°. The solution was diluted with 750 ml. of hot, distilled water and filtered. The filtrate was treated with hydrogen sulfide for 15 min., then heated for 30 min. at 80°, the mixture cooled to room temperature, and the tin sulfide removed by filtration. The filtrate yielded 3.49 g. of white crystals when concentrated *in vacuo*. This crude thymine was recrystallized from dilute aqueous ethanol to give 3.2 g. (82%) of thymine; λ_{max}^{Nujol} (μ) 3.15 (NH), 5.68, 5.94 (C=O of uracil), 6.65, 7.00 (ring). The spectrum was identical with that of an authentic sample of thymine.

The compound had the following R_f values as compared with thymine: in benzene-methanol-water (2:1:6) on Schleicher and Schuell No. 2043B acetylated paper, R_f 0.64 (thymine, 0.64); in 1-butanol–water on Whatman No. 1 paper, R_f 0.45 (thymine, 0.45); in isopropanol–ammonium hydroxide–water (70:5:25), R_f 0.79 (thymine, 0.79); in isopropanol–2*N* hydrochloric acid (65:35), R_f 0.85 (thymine, 0.83).

Acknowledgments. The authors wish to thank Dr. Peter Lim for interpetation of the infrared absorption spectra and his staff for the paper chromatography and spectrophotometry.

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New Di- and Tetrahydropyran Derivatives

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In connection with another study, certain di- and tetrahydropyran dibasic acids and amino acids were desired whose preparation is described below.

The dibasic acids were obtained through the addition of phosgene to the requisite 2,3-dihydro-4*H*-pyran derivative, followed by the elimination of the elements of hydrogen chloride, saponification of the acid chloride and, if desired, by hydrogenation. Alternatively, the acid chlorides were converted into amides which, on dehydration, yielded nitriles. An amino acid derivative was also prepared through the addition of *N*-substituted 2-aminoethanol to 5-carbethoxy-2,3-dihydro-4*H*-pyran.

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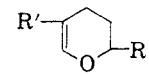
It is known that when a mixture of 2,3-dihydro-4*H*-pyran and phosgene is allowed to react and the resulting addition product is subsequently heated under reduced pressure, hydrogen chloride is eliminated and 2,3-dihydro-4*H*-pyran-5-carbonyl chloride is formed.[2] By adapting this method, Ia was obtained in 42% yield. Attempts to add phosgene to 2-formyl-2,3-dihydro-4*H*-pyran failed to produce an acid chloride, the phosgene reacting instead with the formyl group. However, acid chloride Ig was obtained from compound Ii in which the aldehyde function was masked through formation of the acetal derivative. In the absence of interfering substituents, the addition of phosgene may be a general method for the introduction of a carbonyl chloride group into the dihydropyran nucleus.

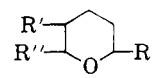
With Raney nickel catalyst, dihydropyran derivatives show a wide variation of susceptibility to hydrogenation, ranging from ready reactions at room temperature[3] to slow hydrogenations at elevated temperatures and pressures.[4,5] 2,5-Dicarbethoxy-2,3-dihydro-4*H*-pyran (Id) was difficult to reduce over Raney nickel at 150° and at 300 atm. pressure. However, over rhodium or palladium catalysts in glacial acetic acid, hydrogenation of Id could be effected with ease at room temperature and ordinary pressure.

Substituted 2,3-Dihydro-4*H*-pyran



Ia. R = $COOC_2H_5$, R′ = COCl; Ib. R = $COOC_2H_5$, R′ = COOH; Ic. R = R′ = COOH; Id. R = R′ = $COOC_2H_5$; Ie. R = $COOC_2H_5$, R′ = $CONH_2$; If. R = R′ = $CONH_2$; Ig. R = $CH(OC_2H_5)_2$, R′ = COCl; Ih. R = $CH(OC_2H_5)_2$, R′ = $COOC_2H_5$; Ii. R = $CH(OC_2H_5)_2$, R′ = H; Ik. R = $CONH_2$, R′ = H; Il. R = CN, R′ = H; Im. R = R′ = CN; In. R = $COOC_2H_5$, R′ = CN.

Substituted Tetrahydropyrans



IIa. R = R″ = H, R′ = $COOC_2H_5$; IIb. R = R″ = H, R′ = COOH; IIc. R = R′ = $COOC_2H_5$, R″ = H; IId. R = CHO, R′ = H, R″ = OC_2H_5; IIe. R = $OCH_2CH_2NHCOCH_3$, R′ = R″ = H; IIf. R = $OCH_2CH_2NHCOCH_3$, R′ = H, R″ = $COOC_2H_5$; IIg. R = $OCH_2CH_2NHCOC_6H_4NO_2$, R′ = R″ = H.

The replacement of the carbethoxy by a carboxamide group occurred easily when dihydropyran derivative Ia was treated with ammonium hydroxide at 0°. The carboxamides were converted to the nitriles by *p*-toluenesulfonyl chloride and

(2) P. A. Hawkins and N. Bennett, British Patent 570,974.

(3) M. Delépine and A. Horeau, *Bull. soc. chim. France*, (5) **5**, 339 (1938).

(4) J. G. M. Bremner and D. G. Jones, British Patent 612,314.

(5) R. R. Whetstone and S. A. Ballard, *J. Am. Chem. Soc.*, **73**, 5280 (1951).