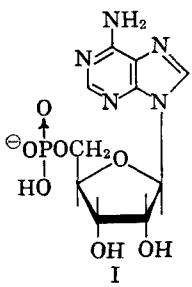
Nonclassical Antimetabolites XVIII

Simulation of 5′-Phosphoribosyl Binding II. ω-Uracil Alkanoic Acids Related to 2′-Deoxyuridylate

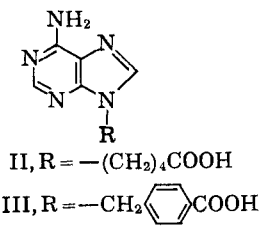
By B. R. BAKER and GIRISH B. CHHEDA

A series of ω-uracil-1-alkanoic acids was synthesized as potential inhibitors of thymidylate synthetase in order to study the mode of phosphate binding of 2′-deoxyuridylate to this enzyme. Preparation of 1-(*p*-carboxybenzyl)uracil (XII*c*) and 1-(carboxymethyl)uracil (XII*g*) from 2,4-diethoxypyrimidine by the Hilbert-Johnson reaction proved feasible. However, this approach was unsuccessful with the less reactive 5-bromovaleronitrile. Although uracil reportedly gives only a 1,3-dialkyl derivative on alkylation, suitable conditions have now been established for mono-alkylation of uracil on its 1 position in 35–50 per cent yields; in this way, the desired 1-(4′-carboxybutyl)uracil (XII*e*) was prepared *via* its nitrile (XII*d*) by alkylation of uracil with 5-bromovaleronitrile. None of these uracil-1-alkanoic acids (XII), including 10 of their derivatives, were inhibitors of thymidylate synthetase. The significance of these results in relation to further research in this area is discussed.

THE EARLIER exploration of the simulation of binding of the 5′-phospho-D-ribosyl moiety of nucleotides to some enzymic active sites was considered successful (1). Evidence was presented that 5′-adenylic acid (I) was bound to



I



II, R = —$(CH_2)_4COOH$

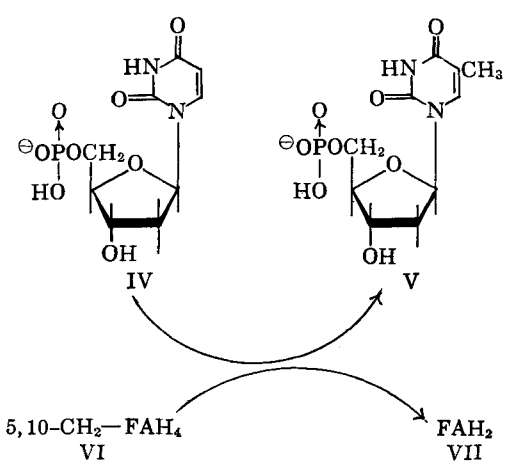
III, R = —CH_2⟨⟩—COOH

lactic dehydrogenase and glutamic dehydrogenase as an inhibitor, presumably binding to an area normally occupied by the 5′-adenylate moiety of DPN. Furthermore, 9-(4′-carboxybutyl)adenine (II) was bound to the two enzymes nearly as well as I. By a study of related compounds, such as III and the hypoxanthine analogs of II and III, the conclusion was made that the valeric acid side chain of II was indeed simulating the binding of the 5′-phospho-D-ribosyl moiety of I.

To determine whether the valerate group could simulate binding of the 5′-phosphoribosyl moiety of a nucleotide, the decision was made to investigate thymidylate synthetase (2), the enzyme that converts 2′-deoxyuridylate (IV) to thymidylate (V). This enzyme uses 5,10-methylene-tetrahydrofolate (VI) as the methyl source, the oxidation level of the one-carbon being changed by simultaneous formation of dihydrofolate (VII) (3, 4). Since this enzyme was already available to evaluate tetrahydrofolate analogs (5–7) as antagonists of VI, it was logical to extend the phosphate studies to anatagonists of 2′-deoxyuridylate (IV). The development of good synthetic routes to ω-uracil-alkanoic acids (XII) and certain of their derivatives as well as the enzymic evaluation of these compounds on thymidylate synthetase is the subject of this paper.

DISCUSSION

There are three basic routes to the synthesis of 1-alkyluracils (XII): (*a*) direct alkylation of a uracil or 2-alkylthiouracil (8), (*b*) alkylation of diethoxypyrimidine (VIII), the Hilbert-Johnson reaction (9), and (*c*) an assortment of ring closure methods (10).



IV V

5,10-CH_2—FAH_4 VI

FAH_2 VII

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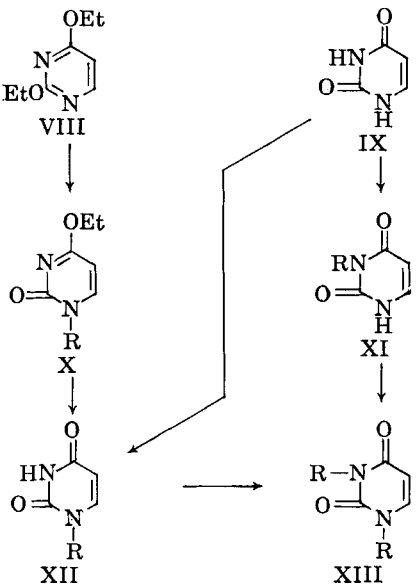
Previous paper: Baker, B. R., THIS JOURNAL, 53, 347 (1964). See *Reference 1* for first paper on phosphate simulation.

The direct alkylation of uracil (IX) to form 1-alkyluracils (XII) did not appear feasible since the main product was a 1,3-dialkyluracil (XIII) (8); apparently a 1-alkyluracil (XII) or 3-alkyluracil (XI) can be alkylated as rapidly as uracil (IX) itself. A suitable modification that made this approach feasible was alkylation of 2-ethylthio-4-pyrimidinol (8), whereby a mixture of 1- and 3-alkyl derivatives was obtained, which could be hydrolyzed to XI and XII, either before or after separation of the initial alkylation products.

The alkylation of 2,4-diethoxypyrimidine (VIII) to form a 1-alkyluracil-4-ethylether (X)—the Hilbert-Johnson reaction—is an elegant reaction that readily allows preparation of 1-alkyluracils (XII) by acid hydrolysis. This route was the method of choice for preparation of XII*a*, *b*, and *f*. It is clear that ethyl halide is a by-product in the Pinner rearrangement of the intermediate 1-alkyl-2,4-diethoxypyrimidine halide to X; therefore, for the



a Series, R = —Me
b Series, R = —CH_2⟨⟩COOCH$_3$
c Series, R = —CH_2⟨⟩COOH
d Series, R = —$(CH_2)_4C{\equiv}N$
e Series, R = —$(CH_2)_4$COOH
f Series, R = —CH_2COOEt
g Series, R = —CH_2COOH

reaction to be successful the attacking alkyl group must be more reactive than ethyl. For example, methyl, carbomethoxybenzyl, or carbethoxymethyl give XII*a*, *b*, and *f*, respectively. If the alkyl group is less reactive than ethyl, then the by-product ethyl halide can compete more or less favorably with the desired alkylation. In fact, attempts to prepare 1-(4′-cyanobutyl)uracil (XII*d*) were totally unsuccessful by this route, presumably due to the lesser reactivity of 5-bromovaleronitrile compared to ethyl bromide.

The ring closure method for synthesis of 1-alkyluracils developed by Shaw *et al.* (10) appeared to be more favorable for 1-aryluracils than 1-alkyluracils. The 9% yield of 1-phenyluracil from ethyl propionate reported by Shaw *et al.* (11, 12) was duplicated. However, use of 5-aminovaleric acid gave a maximum over-all yield of about 3%, based on the ultraviolet spectrum—a near hopeless yield for any quantity of this water soluble compound (XII*e*).

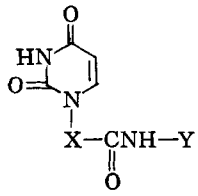
Therefore, attention was focused on the first route, *i.e.*, direct alkylation of uracil; even though poor yields and mixtures might be obtained, it would be only a one-step reaction. The first attempt with 5-bromovaleronitrile gave the nicely crystalline cyanobutyl-uracil (XII*d*), although only in about 3% yield. Since XII*d* was quite water soluble, questions arose of whether the poor yield was due to water solubility making work-up inadequate, or unsatisfactory experimental conditions, or both. Therefore, alkylation of uracil by *p*-carbomethoxybenzyl bromide to give XII*b* was selected for study since preparation of XII*b* *via* VIII and X showed XII*b* to be insoluble in water and readily isolatable.

After studying the reaction of methyl α-bromo-*p*-toluate with uracil under a variety of conditions, a satisfactory procedure was devised. With two equivalents of uracil, one equivalent of potassium carbonate, and one equivalent of bromo ester in dimethylsulfoxide at 80° for 4 hours, the desired XII*b* was obtained in 49% crude yield, but some 1,3-disubstituted-uracil (XIII*b*) was still obtained. If the ratio of uracil to bromo ester was reversed, then a 42% yield of 1,3-disubstituted-uracil (XIII*b*) was obtained, and no pure XII*b* could be isolated. With equimolar amounts of reactants, 33% of XII*b* and 14% of XIII*b* were obtained. The separation of XII*b* and XIII*b* was feasible with hot benzene; XII*b* was less soluble. However, a better separation was achieved by using aqueous alkali, XII*b* dissolving readily. The insoluble XIII*b* could be removed by filtration, and acidification of the filtrate gave the saponified product, XII*c*. That the disubstituted uracil had structure XIII*b* was readily shown by its ultraviolet maximum at 267 mμ in ethanol, in agreement with 1,3-dimethyluracil at 266 mμ and in disagreement with 1-methyl-4-ethoxy-2-pyrimidine at 275 mμ and 2,4-diethoxypyrimidine at 259 mμ (13). Furthermore, hot acid hydrolysis gave the corresponding diacid (XIII*c*) and not XII*b*, XI*b*, or uracil (IX) if one or two benzyl groups had been on oxygen rather than nitrogen.

Condensation of excess uracil with 5-bromovaleronitrile in dimethylsulfoxide at 80° in the presence of potassium carbonate gave 46% yields of the beautifully crystalline 1-(4′-cyanobutyl)-uracil (XII*d*); although XIII*d* was probably present, it did not crystallize and was separated readily from the desired XII*d*. Hydrochloric acid hydrolysis of XII*d* gave crystalline 1-(4′-carboxybutyl)uracil (XII*e*) in 79% yield.

When XII*c* and XII*e* were assayed with thymidylate synthetase (2, 5) as possible antagonists of 2′-deoxyuridylate (40 μm.) in the presence of 214 μm. 5,10-methylene-*dl*-tetrahydrofolate, no inhibition was observed at a concentration of 3 m*M* (75 times substrate). This lack of inhibition can be attributed to (*a*) loss in binding from lack of the four oxygens of the ribose moiety or (*b*) possibly different binding of the phosphate from a simple anion, or (*c*) both. In fact, at least 20 more modes of binding of phosphate—other than simple anionic

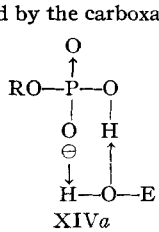
TABLE I.—PROPERTIES OF 1-URACIL-ALKANAMIDES



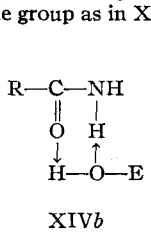
Compd.	X	Y	Method	Yield, %	M.p., °C.	Anal. Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
XV	$-CH_2-$	$-CH_2CONH_2$	*A*	34	270–276	42.5	4.46	24.8	42.3	4.55	24.5
XVI	$-CH_2-$		*A*[a]	93	275–279dec.[b]	51.2	3.63	13.8	51.4	3.81	13.4
XVII	$-CH_2-$		*C*[a,c,g]	23	292–295[d]	55.3	4.93	12.1	55.6	4.98	12.1
XVIII	$-(CH_2)_4-$		*A*[a]	58	250–252[e]	55.3	4.93	12.1	55.1	4.99	12.2
XIX	$-(CH_2)_4-$	H	*B*	35	163–164[e]	51.2	6.20	19.9	51.1	6.02	19.7
XX	$-CH_2$ 	H	*B*[f]	77	274–275[b]	58.8	4.52	17.1	58.9	4.62	17.0
XXI	$-CH_2-$		*C*[g,h]	28	367–370[i]	54.0	3.83	14.5	53.8	3.97	14.4
XXII	$-(CH_2)_4-$		*C*[g]	27	224–226[i]	55.3	4.93	12.1	55.6	4.98	12.1
XXIII	$-CH_2-$	$-CH_2COOEt$	*C*[g]	53	219–220[j,k]	47.1	5.13	16.5	47.0	5.12	16.3
XXIV	$-CH_2-$	$-C_6H_5$	*D*	52	327–330dec.[b]	58.8	5.21	17.3	58.7	5.00	17.0
XXV	$-CH_2-$		*D*[l]	31	320–323[e]	47.1	3.29	18.3	46.8	3.42	18.0
XXVI	$-CH_2-$		*D*[m]	56	305–306[b]	56.8	4.75	13.2	56.7	4.67	13.4
XXVII	$-CH_2-$	$-CH_2CH_2COOH$	*E*	57	254–256	44.8	4.60	17.4	44.6	4.59	17.3

[a] Product separated by trituration of spin-evaporation residue with 1% hydrochloric acid. [b] Recrystallized from *N,N*-dimethylformamide-water. [c] Attempted preparation *via* the nitrophenyl ester (XXVII) method failed. [d] Purified by recrystallization from *N,N*-dimethylformamide-acetone, then reprecipitation from aqueous sodium bicarbonate. [e] Purified by recrystallization from *N,N*-dimethylformamide-acetone. [f] Mixed anhydride prepared at −2°, then reacted at room temperature. [g] Same as *Method A*, except mixed anhydride prepared at −2° and coupling reaction performed at room temperature. [h] Also prepared by saponification of XXVI with 0.2 *N* NaOH at 25° for 7 hours in 86% yield. [i] Purified by reprecipitation from aqueous sodium bicarbonate. [j] Recrystallized from *N,N*-dimethylformamide-ethanol. [k] Reaction with alcoholic ammonia at room temperature failed to give XV, but XXIII was recovered unchanged. [l] Excess (20%) of amine and carbodiimide employed. [m] *N,N*-Dicyclohexylurea was removed by leaching with boiling ethanol rather than solution of the product in 1 *N* base.

binding—can be envisioned. For example, a single enzymic OH group could form a six-membered binding complex involving two hydrogen bonds as shown in structure XIV*a*. This type of binding should be simulated by the carboxamide group as in XIV*b* but



XIV*a*



XIV*b*

not by carboxylate. The following groupings cover most, if not all, of the more than 20 types of phosphate binding: salicylate, *O*-carbamate, amide, carboxylate, and *o*-nitrophenol. A number of these groupings combined with uracil-1-acetic acid (XII*g*) and 1-valeric acid (XII*e*) through a peptide linkage have now been synthesized for enzymic evaluation. Four different methods were used for conversion of XII*e* and XII*g* to these amides: (*a*) the mixed anhydride method at −35°, (*b*) the mixed anhydride method at +2°, (*c*) the dicyclohexylcarbodiimide method, and (*d*) the *p*-nitrophenyl ester method.

The 10 final products prepared by these four methods are listed in Table I. It should be noted that attempts to convert uracil-1-acetic acid (XII*g*) to an acid chloride were unsuccessful.

There is a noteworthy difference in reactivity between an activated carboxyl group of uracil-1-acetic acid (XII*g*) and uracil-1-valeric acid (XII*e*). For example, the mixed anhydride of XII*g* was better prepared and reacted at −35°. At the usual 0°, fairly rapid disproportionation of the mixed anhydride to the ethyl ester (XII*f*) took place, whereas with the uracil-valeric acid (XII*e*) the mixed an-

hydride was fairly normal. Apparently uracil attached to the α-carbon of acetic acid greatly activates the carboxyl.

Of the compounds in Table I, all but the intermediates XXIII, XXIV, and XXVI were tested as reversible inhibitors of thymidylate synthetase and found inactive up to 1 m*M* (25 times substrate concentration). It is now obvious that one should not have assumed that these simple derivatives might simulate phosphate binding since other factors could lead to loss of binding such as (*a*) lack of bulk tolerance of the groups simulating phosphate, (*b*) the loss in binding from removal of the 4-oxygen functions of the D-ribose moiety, (*c*) the more than 20 different ways in which phosphate might bind.

A posteriori, it is now clear that a more systematic study starting with 1-(5′-hydroxypentyl)uracil and its phosphate should have been done initially to answer some of the above questions before anticipating simulation of phosphate binding to thymidylate synthetase. Such a study is now in progress.

EXPERIMENTAL

Melting points were taken in capillary tubes on a Mel-Temp block; those below 230° are corrected. Infrared spectra were determined in KBr disk with a Perkin-Elmer model 137B recording spectrophotometer. Ultraviolet spectra were determined with a Perkin-Elmer model 202 recording spectrophotometer.

1 - (p - Carbomethoxybenzyl) - 4 - ethoxy - 2 - pyrimidone (Xb).—Methyl α-bromo-*p*-toluate was made in 71% yield, m.p. 50–51°, by bromination of methyl *p*-toluate in boiling carbon tetrachloride with *N*-bromosuccinmide in the presence of a catalytic quantity of dibenzoyl peroxide.

A solution of 837 mg. (5 mmoles) of 2,4-diethoxypyrimidine and 2.29 Gm. (10 mmoles) of methyl α-bromo-*p*-toluate in 5 ml. of dimethyl sulfoxide was allowed to stand in a stoppered flask for 61 hours at room temperature. The solution was poured into 50 ml. of cold water, and the product was collected on a filter and washed with water. Recrystallization from methanol gave 671 mg. (47%) of first crop, m.p. 136–137°, and 169 mg. (total 58%) of second crop, m.p. 131–135°. Two more recrystallizations from methanol gave the analytical sample, m.p. 136°; $\nu_{max.}$ 1725 (ester C=O); 1660, 1630, 1535 (C=O, C=C); 1270 (ester C—O—C); 1310, 1030 cm.$^{-1}$ (ether C—O—C); $\lambda_{max.}^{EtOH}$ 278 mμ (ε 9900).

Anal.—Calcd. for $C_{15}H_{16}N_2O_4$: C, 62.5; H, 5.59; N, 9.71. Found: C, 62.4; H, 5.58; N, 9.68.

1-(p-Carboxybenzyl)uracil (XIIc).—*Preparation A. (From Xb).*—A solution of 288 mg. (1 mmole) of X*b* in 10 ml. 12 *N* hydrochloric acid was refluxed for 3 hours. The cooled mixture was filtered and the product washed with water; yield, 238 mg. (97%), m.p. 316-320°. An analytical sample was obtained by recrystallization from 2-methoxyethanol-methanol, followed by reprecipitation from 5% aqueous sodium bicarbonate: white crystals, m.p. 315–316°; $\nu_{max.}$ 3150 (NH); 2680–2570 (broad acidic H); 1714–1660, 1600 cm.$^{-1}$ (C=O, C=C); $\lambda_{max.}^{pH\ 1}$ 265 mμ (ε 14,100); $\lambda_{max.}^{EtOH}$ 265 mμ (ε 11,000); $\lambda_{max.}^{pH\ 13}$ 265 mμ (ε 9100).

Anal.—Calcd. for $C_{12}H_{10}N_2O_4$: C, 58.5; H, 4.09; N, 11.4. Found: C, 58.3; H, 4.17; N, 11.1.

Preparation B. From Uracil (IX).—A mixture of 1.79 Gm. (16 mmoles) of uracil (IX), 0.916 Gm. (4 mmoles) of methyl α-bromo-*p*-toluate, 2.21 Gm. (16 mmoles) of anhydrous potassium carbonate, and 45 ml. of dimethyl sulfoxide was stirred magnetically in a bath at 80° for 20 hours. The pasty white reaction mixture was poured into 70 ml. of cold water. The insoluble crude bis-substituted uracil (XIII*b*) was collected on a filter; weight, 145 mg. The filtrate was acidified with 5% hydrochloric acid, then extracted with chloroform (5 × 60 ml.). The combined extracts were washed with water (2 × 50 ml.), dried with magnesium sulfate, then spin-evaporated *in vacuo* to residue, finally using an oil pump to remove dimethylsulfoxide.

The residue was combined with the above 145 mg. and suspended in 20 ml. of 0.05 *N* sodium hydroxide. After being stirred for 20 minutes at ambient temperature, the mixture was filtered, and the crude XIII*b* was collected on a filter; yield, 146 mg. (18%), m.p. 129–131°. The filtrate was acidified to pH 3, and the essentially pure XII*c* was collected on a filter and washed with water; yield, 490 mg. (50%), m.p. 314–315°, that was identical to material prepared *via* X*b*.

When the ratio of uracil to methyl α-bromo-*p*-toluate was 1:2, the yield of XIII*b* was 41%, m.p. 134–135°. Recrystallization from methanol gave white crystals of *1,3-bis-(p-carbomethoxybenzyl)uracil (XIIIb)*, m.p. 135–136°; $\nu_{max.}$ 1715, 1700 (ester C=O); 1655, 1600 (uracil C=O, C=C); 1270 cm.$^{-1}$ (ester C—O—C); $\lambda_{max.}^{EtOH}$ 267 mμ (ε 12,900).

Anal.—Calcd. for $C_{22}H_{20}N_2O_6$: C, 64.7; H, 4.93; N, 6.86. Found: C, 64.8; H, 4.83; N, 6.47.

1-(p-Carbomethoxybenzyl)uracil (XIIb).—*Preparation A. From Xb.*—A solution of 288 mg. (1 mmole) of X*b* in 3 ml. of methanol and 4 ml. of 1 *N* hydrochloric acid was refluxed for 1 hour, then cooled at 5–10°. The product was collected on a filter and washed with water; yield, 202 mg. (77%), m.p. 217–219°. Recrystallization from methanol gave white crystals, m.p. 224°; $\nu_{max.}$ 3150 (NH); 1725 (ester C=O); 1665, 1650, 1600 (C=O, C=C); 1280 cm.$^{-1}$ (ester C—O—C); $\lambda_{max.}^{pH\ 1}$ 266 mμ (ε 15,500); $\lambda_{max.}^{EtOH}$ 266 mμ (ε 13,000); $\lambda_{max.}^{pH\ 13}$ 266 mμ (ε 11,000).

Anal.—Calcd. for $C_{13}H_{12}N_2O_4$: C, 60.0; H, 4.65; N, 10.8. Found: C, 59.7; H, 4.42; N, 10.6.

Preparation B. From Uracil (IX).—The mixture of XIII*b* and XII*b*, obtained by alkylation of uracil, as described for preparation of XII*c*, could be separated by crystallization from benzene since XII*b* was less soluble. However, losses were high, and it was difficult to obtain pure XII*b* but easy to obtain pure XII*c* from uracil (IX).

1,3-Bis-(p-carboxybenzyl)uracil (XIIIc).—A solution of 300 mg. (0.73 mmole) of XIII*b* in 7 ml. of 12 *N* hydrochloric acid was refluxed for 4 hours with magnetic stirring. Within 15 minutes the product began to separate from the hot solution. The cooled mixture was filtered, and the product was washed with water; yield, 270 mg. (97%), m.p. 255–256°. For analysis the material was reprecipitated several times from 5% aqueous sodium bicarbonate by addition of acetic acid to give white crystals, m.p. 261–262°; $\nu_{max.}$ 2680–2550 (acidic H); 1710–1650 (broad C=O, C=C); 1600, 1580 cm.$^{-1}$ (C=C); $\lambda_{max.}^{EtOH\ or\ pH\ 13}$ 269 mμ.

Anal.—Calcd. for $C_{20}H_{16}N_2O_6$: C, 63.1; H, 4.24; N, 7.36. Found: C, 63.1; H, 4.33; N, 7.20.

Uracil-1-acetic Acid (XIIg).—*Preparation A. From 2,4-Diethoxypyrimidine (VIII).*—A mixture of 1.68 Gm. (10 mmoles) of VIII and 3.34 Gm. (20 mmoles) of ethyl bromoacetate was allowed to stand in a stoppered flask for 36 hours, then excess bromo ester was removed by spin-evaporation in high vacuum. The residual oily X*f* was refluxed in 8 ml. of 12 *N* hydrochloric acid for 3 hours, then diluted with 5 ml. of water and chilled. The product was collected on a filter and washed with water; yield, 1.36 Gm. (80%), m.p. 292–293°. An analytical sample was prepared by reprecipitation from aqueous sodium bicarbonate to give white crystals, m.p. 295–296°; $\nu_{max.}$ 1700 (acid C=O); 1630 cm.$^{-1}$ (C=C of uracil).

Anal.—Calcd. for $C_6H_6N_2O_4$: C, 42.4; H, 3.55; N, 16.5. Found: C, 42.6; H, 3.70; N, 16.7.

Preparation B. From Uracil (IX).—Reaction of 2.24 Gm. (20 mmoles) of uracil with 837 mg. (5 mmoles) of ethyl bromoacetate in the presence 2.76 Gm. of anhydrous potassium carbonate and 40 ml. of dimethylsulfoxide, as described for the preparation of XII*d*, gave 350 mg. (35%) of ethyl ester (XII*f*), m.p. 141–142°, when the crude product was crystallized from ethanol. Recrystallization from ethanol gave the analytical sample of XII*f*, m.p. 143–144°; $\nu_{max.}$ 3190 (NH); 1735, 1210 (ester); 1695, 1620 cm.$^{-1}$ (uracil).

Anal.—Calcd. for $C_8H_{10}N_2O_4$: C, 48.5; H, 5.09; N, 14.1. Found: C, 48.6; H, 5.15; N, 14.0.

Hydrolysis of XII*f* with boiling 12 *N* hydrochloric acid for 3 hours proceeded in good yield to the acid XIIg, identical to *Preparation A*. If the residue from the chloroform extraction was hydrolyzed with 12 *N* hydrochloric acid, the over-al yield from uracil to XIIg was 43%.[1]

1-(4′-Cyanobutyl)uracil (XIId).—A mixture of 1.97 Gm. (17.5 mmoles) of uracil (IX), 0.816 Gm. (5.0 mmoles) of 5-bromovaleronitrile, 2.07 Gm. (15 mmoles) of anhydrous potassium carbonate, and 35 ml. of reagent grade dimethyl sulfoxide was stirred magnetically in a bath at 80° for 24 hours. The thick, still warm reaction mixture was diluted with 60 ml. of cold water, acidified with 5% hydrochloric acid, then extracted with chloroform (5 × 60 ml.). The aqueous solution deposited 993 mg. (50%) of unreacted uracil on standing at 3°.

The combined chloroform extracts were dried with magnesium sulfate, then spin-evaporated *in vacuo;* the last of the dimethyl sulfoxide was removed at 1 mm. Crystallization of the residue from chloroform–petroleum ether gave 0.438 Gm. (46%) of product, m.p. 112–116°. Several more recrystallizations gave the analytical sample as white crystals, m.p. 115°; $\nu_{max.}$ 3600 (NH); 2230 (C≡N); 1690–1645 cm.$^{-1}$ (broad uracil); $\lambda_{max.}^{pH\ 1,\ 13,\ EtOH}$ 267 mμ.

Anal.—Calcd. for $C_9H_{11}N_3O_2$: C, 55.9; H, 5.74; N, 21.7. Found: C, 55.7; H, 5.70, N, 21.8.

[1] A referee has pointed out that "Rabinowitz and Gorin (14) synthesized thymine-1-acetic acid by reaction of thymine (1 mole) with chloroacetic acid in the presence of 2 moles of KOH and obtained a decent yield of product. The proof of structure of their product is not too conclusive, but this referee has repeated their reaction and Rabinowitz and Gorin are correct." Whether uracil-1-acetic acid can be prepared in an identical manner cannot be automatically assumed since the mercury salt of thymine can be converted to a nucleoside, but the mercury salt of uracil cannot.

1-(4′-Carboxybutyl)uracil (XIIe).—A solution of 218 mg. (1.13 mmoles) of XII*d* in 5 ml. of 12 *N* hydrochloric acid was refluxed for 2 hours, then spin-evaporated *in vacuo*. The residue was extracted with hot acetone and filtered from the insoluble ammonium chloride. The acetone solution deposited, on standing, 131 mg. (55%) of product in two crops, m.p. 134–135°. Recrystallization from ethanol–chloroform gave white crystals of unchanged melting point; $\nu_{max.}$ 2600–2500 (acidic H); 1710 (acid C=O); 1650 cm.$^{-1}$ (uracil C=O, C=C); $\lambda_{max.}^{pH\ 1}$ 268 mμ (ε 10,700); $\lambda_{max.}^{EtOH}$ 268 mμ (ε 9900); $\lambda_{max.}^{pH\ 13}$ 268 (ε 7600).

Anal.—Calcd. for $C_9H_{12}N_2O_4$: C, 50.9; H, 5.70; N, 13.2. Found: C, 50.7; H, 5.49; N, 13.0.

The yield was 79% on a larger scale.

Uracil-1-acetylglycinamide (XV).—A mixture of 340 mg. (2 mmoles) of XIIg and 4 ml. of *N,N*-dimethylformamide containing 0.28 ml. (2 mmoles) of triethylamine was stirred magnetically until solution was complete. The mixture was chilled to −35° protected from moisture in a dry-ice–chloroform bath, then 0.19 ml. (2 mmoles) of ethyl chloroformate was added. After being stirred at −30 to −40° for 30 minutes, a solution of 220 mg. (2 mmoles) of glycineamide hydrochloride in 2 ml. of *N,N*-dimethylformamide containing 0.28 ml. of triethylamine was added. After being magnetically stirred at −30 to −40° for 2 hours, then in a 6° chill room for 22 hours, the precipitated triethylamine hydrochloride was removed by filtration, and the filtrate was spin-evaporated to dryness *in vacuo*. Recrystallization of the residue from 1:3 water–acetone gave 155 mg. (34%) of product, m.p. 270–276°, in two crops. The compound is quite water soluble and had $\nu_{max.}$ 3300 (NH); 1750–1650 (C=O, NH); 1525 cm.$^{-1}$ (amide II). (See Table I for analytical data.)

Compounds prepared in a similar fashion are listed in Table I under *Method A*.

1-(4′-Carbamoylbutyl)uracil (XIX).—The mixed anhydride from 212 mg. (1 mmole) of XIIe and 0.095 ml. of ethyl chloroformate was prepared in *N,N*-dimethylformamide at −30 to −40° as described under *Method A* in Table I. A slow stream of ammonia was passed through the suspension for 30 minutes at such a rate that the temperature was −20 to −15°. After being stirred an additional 2.5 hours at −20° and 20 hours at 6° protected from moisture, the mixture was filtered; the filtrate was evaporated to dryness *in vacuo*. Trituration of the residue with 1:5 *N,N*-dimethylformamide–acetone gave 156 mg. (74%) of crude product, m.p. 142–144°. Recrystallization from the same solvent gave the pure product. (See Table I for further details.)

Other compounds prepared by this method are listed under *Method B* in Table I.

α-(1-Uracil)acetanilide (XXIV).—To a solution of 170 mg. (1 mmole) of XIIg and 94 mg. (1 mmole) of aniline in 5 ml. of *N,N*-dimethylformamide was added 207 mg. (1 mmole) of dicyclohexylcarboimide in 1 ml. of *N,N*-dimethylformamide. After standing for 18 hours in a stoppered flask, the mixture was filtered and the precipitate (240 mg.) washed with water; an additional 40 mg. of crude product was obtained from the filtrate. The combined precipitates were stirred with 1 *N* sodium hydroxide for about 10 minutes, then filtered from *N,N′*-dicyclohexylurea. The filtrate was acidified

to give 121 mg. (52%) of product, m.p. 330–333° dec. Recrystallization from aqueous *N,N*-dimethylformamide gave white crystals, m.p. 327–330° dec.; $\nu_{max.}$ 3300 (NH); 1700–1650 (broad C=O); 755–720 cm.$^{-1}$ (C_6H_5—). (See Table I for analytical data.)

Other compounds prepared by this method are listed in Table I under *Method D*.

p-Nitrophenyl Uracil-1-acetate (XXVII).—To a solution of 680 mg. (4 mmoles) of XIIg and 612 mg. (4.4 mmoles) of *p*-nitrophenol in 13 ml. of *N,N*-dimethylformamide was added a solution of 912 mg. (4.4 mmoles) of dicyclohexylcarbodiimide in 3 ml. of the same solvent. After standing at room temperature for 20 hours protected from moisture, the separated *N,N'*-dicyclohexylurea was removed by filtration. The filtrate was spin-evaporated to residue *in vacuo* and crystallized from acetone; yield, 803 mg. (69%) of product, m.p. 191–194°, which was slightly contaminated with the urea. Recrystallization from *N,N*-dimethylformamide–ethanol gave 547 mg. (47%) of white needles, m.p. 213°; the analytical sample obtained in one more recrystallization had the same melting point and $\nu_{max.}$ 1750 (ester C=O); 1670 (broad C=O, C=C); 1520, 1335 cm.$^{-1}$ (NO_2).

Anal.—Calcd. for $C_{12}H_9N_3O_6$: C, 49.5; H, 3.11; N, 14.4. Found: C, 49.7; H, 3.30; N, 14.3.

Uracil-1-acetyl-β-alanine (XXVIII).—To a solution of 45 mg. (0.5 mmole) of β-alanine in 6 ml. of water containing 0.55 mmole of sodium hydroxide was added a suspension of 155 mg. (0.53 mmole) of XXVII in 10 ml. of chloroform. The three-phase system was stirred magnetically in a stoppered flask for 24 hours during which time the *p*-nitrophenyl ester (XXVII) had dissolved. The separated aqueous phase was adjusted to pH 3 with 5% hydrochloric acid, then spin-evaporated to dryness *in vacuo*. The residue was extracted with a large volume of hot 95% ethanol to remove sodium chloride. The insoluble residual product weighed 69 mg. (57%), m.p. 250–255°. Recrystallization from *N,N*-dimethylformamide–acetone gave the pure product, m.p. 254–256°; $\nu_{max.}$ 3300 (NH); 2600–2500 (broad acidic H); 1700 (carboxyl C=O); 1650 cm.$^{-1}$ (uracil C=O). (See Table I for analytical data.)

This reaction failed with *p*-aminosalicylic acid in place of β-alanine.

REFERENCES

(1) Baker, B. R., and Sachdev, H. S., THIS JOURNAL, **52**, 933(1963).
(2) Wahba, A. J., and Friedkin, M., *J. Biol. Chem.*, **237**, 3794(1962).
(3) Pastore, E. J., and Friedkin, M., *ibid.*, **237**, 3802 (1962).
(4) Blakley, R. L., and McDougall, B. M., *ibid.*, **237**, 812(1962).
(5) Baker, B. R., Ho, B.-T., and Neilson, T., *J. Heterocyclic Chem.*, **1**, 79(1964).
(6) Baker, B. R., Ho, B.-T., and Chheda, G. B., *ibid.*, **1**, 88(1964).
(7) Baker, B. R., and Ho, B.-T., THIS JOURNAL, **53**, 1457 (1964).
(8) Brown, D. J., "The Pyrimidines," John Wiley and Sons, Inc., New York, N. Y., 1962, pp. 360–362.
(9) *Ibid.*, p. 372.
(10) *Ibid.*, pp. 89–90.
(11) Atkinson, M. R., *et al.*, *J. Chem. Soc.*, **1957**, 2363.
(12) Atkinson, M. R., Shaw, G., and Warrener, R. N., *ibid.*, **1956**, 4118.
(13) Shugar, D., and Fox, J. J., *Biochim. Biophys. Acta*, **9**, 199(1952).
(14) Rabinowitz, J. L., and Gorin, S., *J. Am. Chem. Soc.*, **75**, 5758(1953).

Interaction Between Poly-*N*-vinyl-5-methyl-2-oxazolidinone and Certain Pharmaceuticals in Aqueous Solution

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The interaction between certain pharmaceuticals and poly-*N*-vinyl-5-methyl-2-oxazolidinone is reported. Compounds studied include aromatic hydroxyl compounds, aromatic amino compounds, *p*-hydroxybenzoic acid esters, barbiturates, plant growth hormones, and sulfonamides. A dialysis method is used to study the complexing reaction. Data indicate that, in general, *p*-substituted compounds exhibit a degree of binding greater than their *m*-isomers. The *o*-substituted compounds appear to interact less than the *p*- or *m*-substituted compounds with poly-*N*-vinyl-5-methyl-2-oxazolidinone. 5,5-Substitution of barbituric acid greatly reduces its ability to associate with the polymer. Data are reported to show the value *K*, the ratio of the total drug concentration in solution to the concentration of the unbound drug as a function of the polymer concentration.

CONTINUING advances in the areas of pharmaceutical product development and research have shown that some organic polymeric substances are suitable for incorporation in medicinal preparations as auxiliary agents and/or as vehicles. Observations of the nonionic polymers in the presence of certain drug molecules showed that there was a tendency for some medicinals to form molecular association complexes with several different macromolecules (1–3, 6–20). In some cases the solubility of the medicinal was

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