δ 1.64–3.00 (m, 6 H, $CH_2CH_2CH_2$), 4.92 (t, 1 H, CH); ir (KBr) 3.40, 6.10, 6.53, 7.16, 9.20 μ. Anal. ($C_6H_{11}NO_3$) C, H, N.

In an effort to validate the integrity of the hydrolytic procedure, sample $B_2$ from the fractional precipitation of 1-amino-2-methoxycyclopentanecarboxylic acid (2.09 g) was cleaved in the presence of 16 ml of 48% HBr in the same manner. There was recovered 1.58 g of crude product which was recrystallized from $EtOH-H_2O$ to produce 771 mg of material, mp 286–287 dec. An amino acid analysis of this material indicated it to be 79.9% isomer 1 and 20.3% isomer **2**.

**Microbiological Assays.** Samples of 1 and 2 (99 and 80% pure by analysis on an amino acid analyzer, respectively) were examined for their inhibition to growth of *Escherichia coli* 9723 and *E. coli* W, *Streptococcus faecalis* 8043, *Leuconostoc mesenteroide* 10830, and *Lactobacillus arabinosus* 8014 using previously reported assay conditions.[2]

**Tissue Culture Studies.** Jensen sarcoma cells (ATCC-CCL-45) were seeded in replicate T-25 Falcon flasks using an initial inoculum of 400000 cells/flask in McCoy's 7a medium supplemented with 10% fetal calf serum. The flasks were gassed with 8% $CO_2$–92% air and incubated at 37° for 24 hr. After incubation, cell numbers were determined for zero time. The medium in the remaining flasks was replaced with a fresh solution containing the analog to be tested and incubation was continued. At the end of 48 hr, cell numbers were measured in replicate flasks. In the remaining flasks, the medium was again replaced with freshly prepared medium containing the analog and incubation was continued to a total of 72 hr when a final cell count was made. Cells were harvested and counted in a Coulter counter, Model B.[13] Highly purified samples of 1 and 2 (greater than 98% purity by analysis on an amino acid analyzer) were used for this study.

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# Synthesis of 5-Substituted Aminomethyluracils via the Mannich Reaction

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An extension of the Mannich reaction, in which aminomethylation of the five position of uracil, is reported. Thus, primary and secondary alkylamines and primary aromatic amines containing ring-activating groups led to the title compounds **3–10**. Compound **11** in which the aromatic ring contains the ring-deactivating nitro group was synthesized in an alternative way. All compounds were characterized by their elemental and spectral properties.

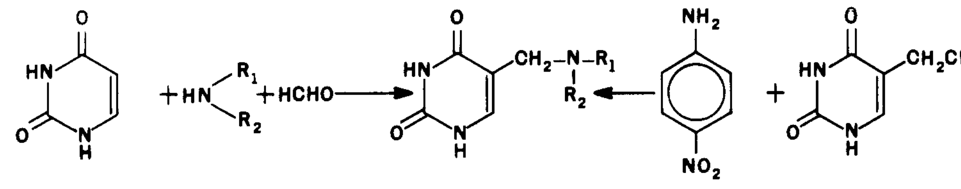
Over the years there has been a continuing interest in analogs of thymine which might have cytotoxic activity. Thus, compounds such as 5-fluorouracil,[2] 5-trifluoromethyluracil,[3] and 5-mercaptomethyluracil[4] are effective as inhibitors of cell growth. In view of the biological significance of these 5-substituted uracils we became interested in extending the derivatization at the 5 position of uracil by the use of the Mannich reaction. There exists a close parallel between in vivo thymidine 5-phosphate synthesis and the Mannich reaction. The biological requirements for thymidine 5-phosphate synthesis are formaldehyde, tetrahydrofolic acid, and deoxyuridine monophosphate[5] while those for the Mannich reaction are formaldehyde, an amine, and a compound containing a reactive hydrogen.

During the past decade, the Mannich reaction has been applied to 6-methyluracil,[6–8] 2-thiouracil,[6–8] 6-aminouracil derivatives,[7] and 6-chlorouracil derivatives[9] in addition to uracil.[10,11] Accordingly, we have synthesized a series of 5-substituted aminomethyluracil derivatives (Scheme I, Table I) in order to ascertain the scope of the reaction as well as any differences the side chain might have on biological activity. Of particular emphasis during this investigation was the use of aromatic amines.

Uracil was treated with 2 equiv each of paraformaldehyde and the corresponding amine in aqueous ethanol. The products derived from the aliphatic amines were quite hygroscopic and consequently they were isolated as salts. Products derived from aromatic amines generally precipitated from the reaction mixture as nearly insoluble solids. These were conveniently purified by successive washings with aqueous ethanol, water, and acetone. In all cases the structural assignments were based on elemental analyses and $^1H$ NMR spectral data.

The $^1H$ NMR spectra of compounds **3–5**, as salts, and **6**, as free base, were obtained in $D_2O$ and the spectra of compounds **7–10** were obtained in trifluoroacetic acid. In all cases, the C-6 proton (δ 7.86–7.60), the protons of the

Scheme I



1 3-11 2

Table I

| Compd | $R_1$ | $R_2$ | Yield, % | Mp, °C | $^1$H NMR data$^a$ Solvent | $H_6$ | $-CH_2-$ | Formula$^b$ |
|---|---|---|---|---|---|---|---|---|
| 3 | -H | $-n-C_4H_9$ | 98 | 276 | $D_2O$ | 7.76 | 3.96 | $C_9H_{15}N_3O_2 \cdot HCl$ |
| 4 | -H | $-C_6H_{11}$ | 30 | 265-270 | $D_2O$ | 7.73 | 3.96 | $C_{11}H_{17}N_3O_2 \cdot HCl$ |
| 5 | $-CH_3$ | $-CH_3$ | 76 | 270-273 | $D_2O$ | 7.86 | 4.06 | $C_7H_{11}N_3O_2 \cdot HBr$ |
| 6 | -H | $-CH_2CH_2N(CH_2CH_3)_2$ | 66 | 179-181 | $D_2O$ | 7.60 | 3.55 | $C_{11}H_{20}N_4O_2$ |
| 7 | -H | $-C_6H_5$ | 71 | 240 dec | TFA | 7.80 | 4.56 | $C_{11}H_{11}N_3O_2$ |
| 8 | -H | $-p-ClC_6H_4$ | 70 | 255-257 | TFA | 7.90 | 4.56 | $C_{11}H_{10}N_3O_2Cl$ |
| 9 | -H | $-m-CH_3C_6H_4$ | 32 | 190 dec | TFA | 7.99 | 4.74 | $C_{12}H_{13}N_3O_2$ |
| 10 | -H | $-p-CH_3OC_6H_4$ | 77 | 255-258 | TFA | 7.84 | 4.60 | $C_{12}H_{13}N_3O_3$ |
| 11 | -H | $-p-NO_2C_6H_4$ | 63 | 263-265 | TFA | 7.90 | 4.73 | $C_{11}H_{10}N_4O_4$ |

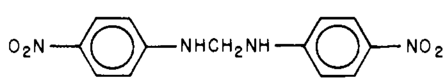
$^a$ Chemical shift of indicated protons in δ units. $^b$ Elemental analyses for C, H, and N are within ±0.4% of theoretical values.

methylene bridge at C-5 (δ 3.55–4.73), and the protons corresponding to $R_1$ and $R_2$ were observed in their respective ratios. The disappearance of the C-5 proton of uracil (1) was observed in all cases which offered further substantiation for the assignment of the substitution at the position.

It is significant that several aromatic amines were successfully employed in this reaction. There appears to be little modern work describing the use of aromatic amines in such reactions although older references describe some work with aniline[14] and *N*-methylaniline.[15–17] However, in our hands only anilines bearing H or a ring-activating group gave isolable products. Hence the aromatic derivatives 7–10 were obtained under Mannich conditions while anilines bearing the ring-deactivating substituents $-SO_2NH_2$, $-NO_2$, -COOH, and -CN failed to react.

In an attempt to effect a reaction with aromatic amines containing ring-deactivating substituents, we explored in some detail the reaction of *p*-nitroaniline to afford **11**. Besides the usual reaction conditions previously described, other conditions involving the use of dimethoxymethane or acetic acid as solvents and varying reflux times up to 72 hr were attempted. Also, the salt of *p*-nitroaniline was used as the amine source in each of the above solvents.

In dimethoxymethane only bis(*p*-nitroanilino)methane (**12**) was obtained. This was confirmed by $^1$H NMR spectra and comparison with an authentic sample prepared by a previously described method.[18]



**12**

In the mixture of those reactions employing either 95% ethanol or acetic acid, TLC indicated that the majority of the uracil was unreacted. However, an additional spot was observed in small quantity. We, therefore, undertook the synthesis of **11** via 5-chloromethyluracil (**2**) (Scheme I).

The $R_f$ value of **11** and its appearance under uv and iodine visualization corresponds to the unknown spot from the reaction mixture. This suggests that the Mannich reaction of *p*-nitroaniline does occur under the conditions described, but to such a small extent that it is not synthetically useful.

It has been proposed[19] that a possible intermediate in the Mannich reaction is a geminal diamine, such as **12**, and that under the proper conditions it can serve as the amine source. In an attempt to test this possibility, we added **12** to uracil in 95% ethanol and in acetic acid, resulting in no improvement in the result described above as indicated by TLC.

Compounds **4**, **5**, and **7** were evaluated by the National Cancer Institute against lymphoid leukemia (L1210) in mice. These compounds were found to be lacking in antitumor activity and to be nontoxic at levels up to 400 mg/kg. Antimalarial evaluation was performed by the Walter Reed Army Institute of Research on compounds **3**, **4**, **7**, **8**, **9**, and **10**. The results indicated (maximum dose in parentheses) that compounds **3** (100 mg/kg), **4** (160 mg/kg), **7** (120 mg/kg), and **10** (160 mg/kg) were inactive and nontoxic against *P. gallinacium* (strain B) in the chick while compounds **4**, **8**, and **10** at 320 mg/kg and compounds **3**, **7**, and **9** at 640 mg/kg were inactive and nontoxic against *P. berghei* in mice.

## Experimental Section

Analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Melting points were recorded on a Mel-Temp apparatus and are uncorrected. $^1$H NMR spectra were recorded on a Varian Model T-60, either in $D_2O$ or in trifluoroacetic acid using DSS and $Me_4Si$ as internal standards, respectively. TLC analyses were performed on silica gel using ethyl acetate–methanol (9:1) as the solvent system.

**General Procedure for Synthesis of Compounds 3–10.** A mixture of uracil (4.5 mmol), paraformaldehyde (9.0 mmol), and the corresponding amine (9.0 mmol) in 200 ml of 95% EtOH was heated under reflux for periods of 6–18 hr. Those compounds isolated as free bases were filtered, washed thoroughly with hot EtOH, $H_2O$, and acetone, and then dried. Those compounds isolated as salts were prepared by evaporation of the reaction mixture, dissolving the residue in absolute EtOH and passing the appropriate dry gas through the solution. The salts were then collected and recrystallized from EtOH. Physical data for each of the compounds may be found in Table I.

**5-(*p*-Nitroanilinomethyl)uracil (11).** To a suspension of 5-chloromethyluracil[10] (1.0 g, 6.2 mmol) in 75 ml of warm acetone was added a solution of *p*-nitroaniline (1.7 g, 12 mmol) in 25 ml of warm acetone with continual stirring. The mixture was heated under reflux for 2 hr, during which time a yellow, insoluble, high-melting material precipitated. This solid was filtered and the filtrate was returned to reflux overnight. During this time further product precipitated as a yellow solid (yield 1.0 g, 62%).

An analytical sample was prepared by washing with hot EtOH, hot $H_2O$, and hot acetone.

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# Products from Furans. 1. Synthesis and Anticoccidial and Antimicrobial Activity of 5-Amino-5,6-dihydro-6-methoxy-2-methyl-2-(4'-biphenylyl)-2*H*-pyran-3(4*H*)-ones and Related Compounds

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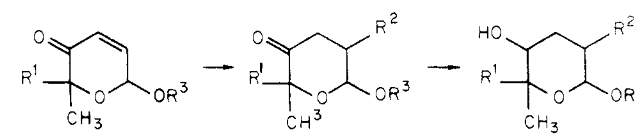
A Michael type addition of an amine to 6-methoxy-2-methyl-2-(4'-biphenylyl)-2*H*-pyran-3(6*H*)-one (1) dissolved in ether, benzene, or THF gave 5-amino derivatives of 5,6-dihydro-6-methoxy-2-methyl-2-(4'-biphenylyl)-2*H*-pyran-3(4*H*)-one (2). These by subsequent reduction with $LiAlH_4$ were converted to 5-amino derivatives of 6-methoxy-2-methyl-2-(4'-biphenylyl)tetrahydro-2*H*-pyran-3-ol (3). Both isomers A and B of 1 (in regard to the methoxy group at $C_6$) were used for the synthesis of 2 and 3. The in vitro antimicrobial activity of the amine adducts 2 was of the same order of magnitude as the starting material. Amine adducts in general, however, were by far more active as coccidiostats than the starting material and retained their activities when they were reduced. 5,6-Dihydro-6-methoxy-2-methyl-2-(4'-biphenylyl)-5-(dimethylamino)-2*H*-pyran-3(4*H*)-one hydrochloride (A) and 5,6-dihydro-6-methoxy-2-methyl-2-(4'-biphenylyl)-5-(dimethylamino)-2*H*-pyran-3(4*H*)-one hydrochloride (B), prepared from isomer A and B of 1, respectively, were the most active as coccidiostats. These compounds when administered orally to chickens 1 day prior to infection at a concentration 0.05% in their diet gave them total protection against *Eimeria tenella*.

The conversion of furans to pyran derivatives had previously been reported[1,2] when Lefebvre announced his synthesis of 6-hydroxy-2*H*-pyran-3(6*H*)-ones 1 by oxidation of 2-furanmethanol.[3] His procedure has been extensively used for the syntheses of compounds for biological screening.[4] However, the versatile 6-hydroxy-2*H*-pyran-3(6*H*)-ones 1 may be used as starting material for the synthesis of a variety of products.[5] In this note the synthesis of 5-amino-5,6-dihydro-6-methoxy-2-methyl-2-(4'-biphenylyl)-2*H*-pyran-3(4*H*)-ones 2 and 5-amino-6-methoxy-2-methyl-2-(4'-biphenylyl)tetrahydro-2*H*-pyran-3-ols 3 will be reported and the biological properties of the prepared compounds will be discussed.

**Synthesis.** The synthesis of the reported compounds is illustrated in Scheme I. A Michael type addition of amine to 6-methoxy-2-methyl-2-(4'-biphenylyl)-2*H*-pyran-3(6*H*)-one (1) gave 5-amino-5,6-dihydro-6-methoxy-2-methyl-2-(4'-biphenylyl)-2*H*-pyran-3(4*H*)-one derivatives 2 and these, by a subsequent reduction, yielded

Scheme I




1 2 3

5-amino-6-methoxy-2-methyl-2-(4'-biphenylyl)tetrahydro-2*H*-pyran-3-ols.

**Biology. 1. Methods.** Methods used in the biological evaluation of these compounds were the same as those used for 6-hydroxy-2*H*-pyran-3(6*H*)-ones.[4]

**(a) Antibacterial Screening.** The antibacterial properties of the compounds were tested in vitro by halving dilutions in nutrient broth (Difco). The following gram-positive organisms were used: *Staphylococcus pyogenes* S (penicillin-sensitive), *Staphylococcus pyogenes* R (penicillin-resistant), and *Streptococcus faecalis*. The gram-negative organisms were *Salmonella pullorum*, *Pseudomonas aeruginosa*, *Escherichia coli* No. 198, *Aerobacter aerogenes*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Serretia marcescens*. The results are ex-

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