the residue was distilled under vacuum. The yield of product was 35 g (45%), bp 129–135° (16 mm).

Butyl 4-Iodocrotonate. To a solution of NaI (19.3 g, 0.133 mol) in $(CH_3)_2CO$ (150 ml) at room temperature was added butyl 4-bromocrotonate (22.1 g, 0.1 mol). After stirring for 1 h, the NaBr formed was removed by filtration. The solids were washed with $(CH_3)_2CO$ and the combined filtrates were poured into 600 ml of Et_2O. The ether solution was washed with 5% $Na_2S_2O_3$ solution (100 ml), followed by H_2O (2 × 200 ml), and dried (Na_2SO_4). The solvent was removed in the rotary evaporator, and the residue was distilled under reduced pressure. The yield of product was 18.9 g (70%), bp 100–110° (0.1 mm).

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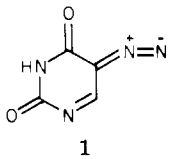
Synthesis and Antimicrobial Evaluation of Substituted 5,6-Dihydro-5-nitrouracils

Robert A. Long,* Thomas R. Matthews, and Roland K. Robins

ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, California 92715. *Received January 14, 1976*

Reaction of 5-nitrouracil derivatives with sodium borohydride in methanol–water, followed by neutralization of the product with acid, has produced 5,6-dihydro-5-nitrouracil (**5**), 5,6-dihydro-6-methyl-5-nitrouracil (**7**), 5,6-dihydro-5-nitro-1-(4-nitrophenyl)uracil (**10**), and 5,6-dihydro-5-nitro-1-(β-D-ribofuranuronic acid ethyl ester)uracil (**12**). In assays for antimicrobial activity using strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Trichophyton mentagrophytes*, significant inhibition of growth was not found.

It has been demonstrated that 5-diazouracil (**1**) inhibits cell division,[1] causes an increase in the mean cell volume,[2] and has a broad-spectrum in vitro activity against pathogenic bacteria.[3] The antimicrobial activity of **1** is, however, accompanied by severe mammalian toxicity. Since assigned structures[4] for the 5-diazouracils and 5-diazouridines indicate the presence of a substituent at C-6, as in the hydrated form of 5-diazouracil (**2**), we sought to prepare other uracil derivatives containing a 5,6-dihydro structure with a strongly electronegative group at C-5. The synthesis of 5,6-dihydro-5-nitrouracil and related derivatives by reduction of the corresponding 5-nitrouracil with sodium borohydride plus the evaluation for antimicrobial activity is herein reported.



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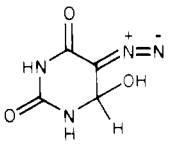

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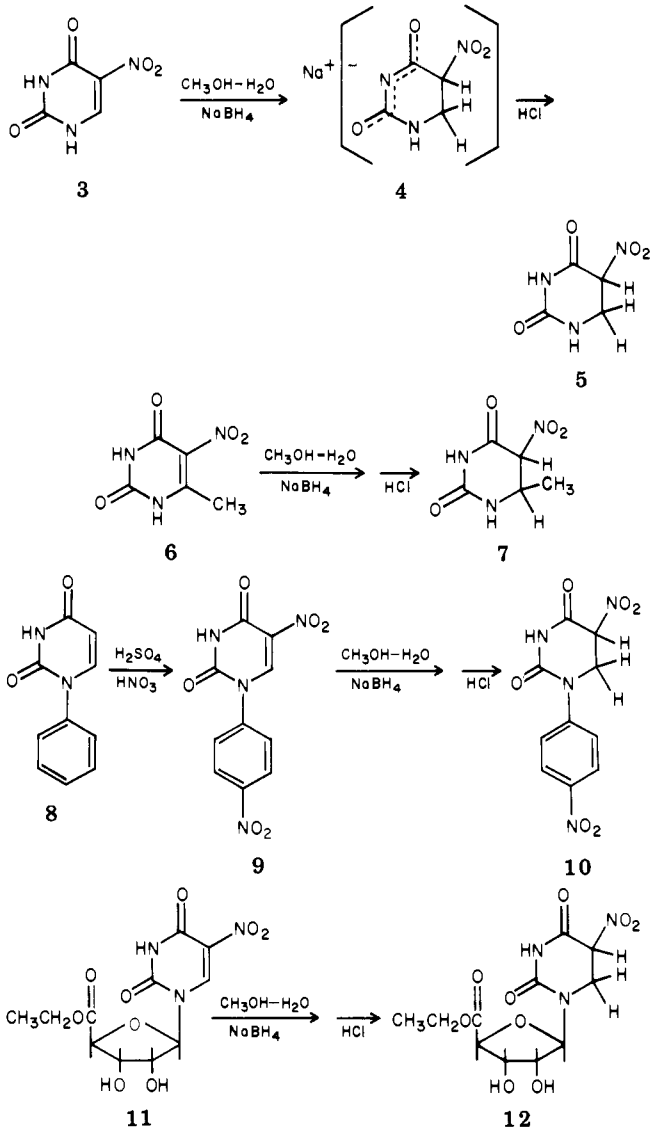
Chemistry. In an early investigation[5] of 5-nitropyrimidines, treatment of 5-nitro-1-(β-D-ribofuranuronic acid isopropyl ester)uracil with sodium borohydride and aluminum chloride or with potassium borohydride and lithium chloride yielded a new product which was identified as the 5,6-dihydro-5-nitro derivative. Reduction of the 5,6 bond in N^4-acetylcytidine[6] and 5-acetyluracil[7] has been reported using sodium borohydride. More recently it was found[8] that reduction of 6-nitroquinoxaline to give 6-nitro-1,2,3,4-tetrahydroquinoxaline occurred upon treatment with sodium borohydride in methanol. No reduction of quinoxaline itself under the same conditions was found, indicating the necessity of the nitro group for reaction with the hydride.

When a solution of 5-nitrouracil (**3**) in methanol–water was treated with sodium borohydride, a precipitate formed. This was identified as the sodium salt of 5,6-dihydro-5-nitrouracil (**4**). Treatment of a solution of **4** in water to pH 3 with 1 N hydrochloric acid gave 5,6-dihydro-5-nitrouracil (**5**). Similarly, 6-methyl-5-nitrouracil (**6**) yielded 5,6-dihydro-6-methyl-5-nitrouracil (**7**) (Scheme I). The ^1H NMR spectra of **7** in Me_2SO-d_6 revealed a mixture of two products, presumed to be the cis and trans isomers. The signal for the C_5H (δ 5.81 $J_{5,6}$ = 9.5 Hz) seems to indicate a predominance of the trans isomer,[9] which could be expected with the bulky methyl and nitro groups

Scheme I



preferring the trans equatorial positions. The addition of D_2O caused the disappearance of the signal for the C_5H, retaining the pair of doublets for the C_6 methyl (δ 1.25 and 1.18) unchanged. Similarly, deuteration of the Me_2SO-d_6 solutions of compounds **5**, **10**, and **12** simplifies each of the spectra by the removal of not only the NH and OH protons but also the C_5H.

Nitration of 1-phenyluracil[10] (**8**) with 90% nitric acid in sulfuric acid produced 5-nitro-1-(4-nitrophenyl)uracil (**9**). Treatment of this dinitro compound with sodium borohydride in methanol–water followed by acid gave 5,6-dihydro-5-nitro-1-(4-nitrophenyl)uracil (**10**).

Reaction of 5-nitro-1-(β-D-ribofuranuronic acid ethyl ester)uracil (**11**) with only sodium borohydride in methanol–water was found to yield the same result as previously described,[5] 5,6-dihydro-5-nitro-1-(β-D-ribofuranuronic acid ethyl ester)uracil (**12**). The ^1H NMR spectrum and analytical data of the isolated material confirm that reduction of the pyrimidine ring occurred without concomitant reduction of the ester function.

Antimicrobial. Broth concentrations of 5-diazouracil (**1**) and 5-diazo-6-hydroxy-1,6-dihydrouracil (**2**) required to inhibit clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were 0.02 μmol/ml or less. Antifungal activity against *Candida albicans* or *Trichophyton mentagrophytes* was not detected with either **1** or **2** at levels as high as 0.4 μmol/ml. This in vitro antimicrobial spectrum confirmed previous in vitro studies.[1-3]

Compounds **5–12** with the C-5 position of the uracil ring substituted with a nitro group were devoid of antimicrobial activity at concentrations of 0.4 μmol/ml or less. This effect was similar to that achieved by substitution of the C-5 position with the less electronegative amino group.[1] It is noteworthy that the diazo-substituted compounds which possess such strong in vitro and in vivo[3] antibacterial activity and are so toxic to the mammal do not inhibit the eucaryotic fungi.

Experimental Section

Melting points were determined on a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Analytical results are from Galbraith Laboratories, Inc., Knoxville, Tenn., and are within ±0.3% of the calculated values. NMR spectra were recorded on a Hitachi Perkin-Elmer R-20A.

General Method of Reduction. To a solution of the 5-nitrouracil derivative (1 mmol) in methanol (4 ml)–water (11 ml) at room temperature was added, in small portions so as to prevent excessive frothing, sodium borohydride (4 mmol). The mixture was stirred at room temperature for 1 h.

5,6-Dihydro-5-nitrouracil Sodium Salt (4). The treatment of **3** (6.28 g, 40 mmol) as described above produced a precipitate which was collected by filtration and recrystallized twice from MeOH–H_2O to yield 4.29 g (54%) of **4**, mp 298° dec. Anal. ($C_4H_4N_3O_4Na\cdot H_2O$) C, H, N.

5,6-Dihydro-5-nitrouracil (5). The sodium salt **4** (2.00 g, 12.7 mmol) was dissolved in the minimum amount of boiling H_2O and 1 N HCl was added to pH 3. After cooling the solution, the solid was filtered and dried to yield 1.40 g (70%) of **5**. An analytical sample was prepared by recrystallizing the solid from H_2O: mp 167° dec; uv λ_{max} (pH 11) 320 nm (ϵ 13 500); ^1H NMR (Me_2SO-d_6) δ 10.92 (s, N_3H), 7.95 (s, N_1H), 5.80 (t, C_5H, $J_{5,6}$ = 6 Hz), 3.88 ppm (2 d, C_6H). Anal. ($C_4H_5N_3O_4$) C, H, N.

5,6-Dihydro-6-methyl-5-nitrouracil (7). The precipitated salt from the reduction of **6** (4.28 g, 25 mmol) was treated as for **5**. The solid from the acidified solution was recrystallized twice from water and the resulting crystals were filtered and dried to yield 2.44 g (57%) of **7**: mp 209° dec; uv λ_{max} (pH 11) 321 nm (ϵ 13 000); ^1H NMR (Me_2SO-d_6) δ 10.99 (s, N_3H), 8.05 (s, N_1H), 5.81 (d, C_5H, $J_{5,6}$ = 9.5 Hz), 4.20 (m, C_6H), 1.25 (d, CH_3, J = 6.0 Hz), 1.18 ppm (d, CH_3, J = 6.0 Hz). Anal. ($C_5H_7N_3O_4$) C, H, N.

5-Nitro-1-(4-nitrophenyl)uracil (9). To a solution of concentrated H_2SO_4 (3 ml) and 90% HNO_3 (3 ml) at room temperature was added 1-phenyluracil[10] (0.564 g, 3 mmol) in small portions with stirring. After 0.5 h, the solution was poured onto ice (50 ml) and the mixture stirred until the ice melted. The solid was collected by filtration and washed well with H_2O. The resulting solid was filtered and purified by two recrystallizations from acetone–H_2O to yield 0.393 g (47%) of **9**: mp 271–272°; uv λ_{max} (pH 1) 304 nm (ϵ 14 150), 240 (9460); uv λ_{max} (pH 11) 325 nm (ϵ 19 500); ^1H NMR (Me_2SO-d_6) δ 12.45 (s, N_3H), 9.23 (s, C_6H), 8.42 and 7.85 ppm (d, phenyl, J = 8.5 Hz). Anal. ($C_{10}H_6N_4O_6$) C, H, N.

5,6-Dihydro-5-nitro-1-(4-nitrophenyl)uracil (10). The precipitated solid from the reduction of **9** (0.528 g, 1.9 mmol) was collected by filtration and dissolved in boiling H_2O. The resulting solution was adjusted to pH 3 with 1 N HCl and cooled. The filtered solid was purified by being recrystallized twice from acetone–H_2O, yielding 0.273 g (51%) of **10**: mp 181–182° dec; uv λ_{max} (pH 1) 289 nm (ϵ 10 580); uv λ_{max} (pH 11) 321 nm (ϵ 20 280); ^1H NMR (Me_2SO-d_6) δ 11.55 (s, N_3H), 8.30 and 7.64 (d, phenyl), 6.15 (t, C_5H), 4.62 ppm (d, C_6H, 2 H). Anal. ($C_{10}H_8N_4O_6$) C, H, N.

5,6-Dihydro-5-nitro-1-(β-D-ribofuranuronic acid ethyl ester)uracil (12). The reduction solution of **11** (1.45 g, 4.4 mmol) was stirred 1 h, neutralized to pH 3 with 1 N HCl, and reduced to dryness in vacuo. The solid was dissolved in a minimum of boiling H_2O, and the solution was filtered and cooled. The resulting solid was recrystallized twice from H_2O to give 0.368 g (25%) of **12**: mp 181–184° dec; uv λ_{max} (pH 11) 322 nm (ϵ

14 000); ^{1}H NMR (Me_2SO-d_6) δ 11.30 (s, N_3H), 5.90 (m, $C_1'H$, C_5H), 4.19 (m, $C_5'H$, $C_4'H$, $C_3'H$, $C_2'H$, CH_2, C_6H), 1.26 ppm (t, CH_3). Anal. ($C_{11}H_{15}N_3O_9$) C, H, N.

Antimicrobial. The compounds synthesized for this study were assayed for antimicrobial activity using strains of *Escherichia coli* (*Ec*), *Pseudomonas aeruginosa* (*Ps*), *Staphylococcus aureus* (*Sa*), *Candida albicans* (*Ca*), and *Trichophyton mentagrophytes* (*Tm*) isolated in the clinic. In vitro sensitivity of these organisms to this series of 5,6-dihydro-5-nitrouracils was quantitatively determined by broth dilution assay. Serial dilutions were prepared in chemically defined medium in a range from 0.4 to 0.005 μmol/ml. The minimal inhibitory concentration (MIC) was recorded as the highest dilution of compound which prevented visible growth of the pathogen. Bacterial and yeast MIC's were read following 24 h of incubation at 35°. Dermatophyte inhibition was read after 48 h of incubation at 30°.

Although 5-diazouracil (1) [MIC (μmol/ml) *Sa* 0.01, *Ps* 0.04, *Ec* 0.02] and 5-diazo-6-hydroxy-1,6-dihydrouracil (2) [MIC (μmol/ml) *Sa* 0.02, *Ps* 0.02, *Ec* 0.01] inhibited the in vitro growth of bacteria, none of the nitro-substituted compounds, **5**–**12**, were inhibitory at broth concentrations of 0.4 μmol/ml or less. Antifungal activity was not detected for any compounds of this series.

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Optical Isomers of 2-(2-Ethoxyphenoxymethyl)tetrahydro-1,4-oxazine (Viloxazine) and Related Compounds

Ralph Howe,* Thomas Leigh, Balbir S. Rao, and Alexander H. Todd

Imperial Chemical Industries, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, England.

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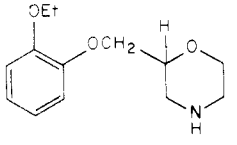
The optical isomers of 2-(2-ethoxyphenoxymethyl)tetrahydro-1,4-oxazine (viloxazine) and 2-(3-methoxyphenoxymethyl)tetrahydro-1,4-oxazine have been prepared and absolute configurations have been assigned. In their action on the central nervous system the *S* isomers are at least ten times more potent than the *R* isomers. The intermediate 4-benzyl-2-(*p*-toluenesulfonyloxymethyl)tetrahydro-1,4-oxazine has been resolved. Its isomers provide a convenient starting point for the preparation of optical isomers of viloxazine analogues of known configuration.

The synthesis of the psychotropic agent (±)-2-(2-ethoxyphenoxymethyl)tetrahydro-1,4-oxazine (**1**, viloxazine)[1] and its effects on laboratory animals have been described by Mallion et al.[2-4] Controlled studies have shown that viloxazine is an effective antidepressant in man.[5-7] It was of interest to resolve the racemate to provide the optical isomers for biological study and also to assign absolute configurations to the isomers. For clarity, the isomers will be referred to from the outset as *R* and *S* isomers rather than (+) and (−) isomers, before the proof of assignment is given. The reason is that for a given isomer the sign of rotation depends upon the solvent.



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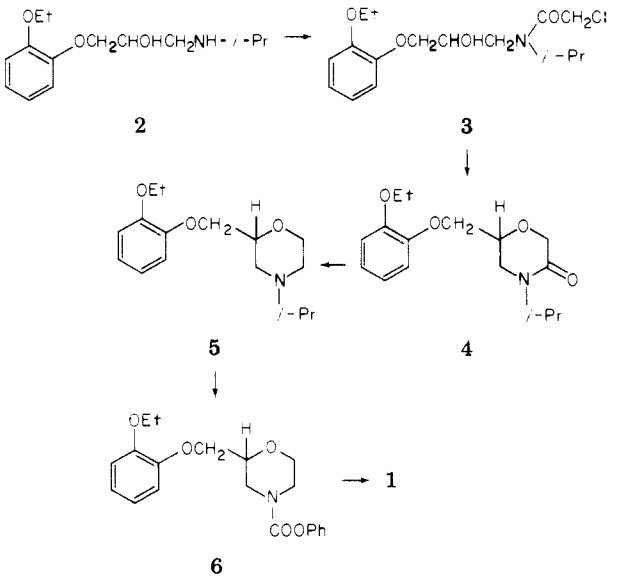
Resolution of (±)-**1** with (−)-*O*,*O*-di-*p*-toluoyltartaric acid[8] gave the salt (*R*)-**1** hydrogen (−)-acid.[9] The mother liquors remaining after the removal of that salt yielded (*S*)-**1** hydrogen (−)-acid. The free base (*R*)-**1** and (*R*)-**1** hydrochloride had low positive rotations when measured in MeOH. In H_2O (*R*)-**1** hydrochloride had a low negative rotation. Because of these low rotations and the small difference in rotation between the two diastereoisomeric salts it was not practicable to monitor the resolution by measuring optical rotation. However, it proved convenient to monitor progress in the research stage by following the change in melting point of the salt with the resolving acid

Scheme I



2 **3** **4** **5** **6**

or of the hydrochloride. Resolution of (±)-**1** with the more expensive (+)-*O*,*O*-di-*p*-toluoyltartaric acid was a more direct route to (*S*)-**1** derivatives.[9] The physical properties of the isomers are given in Table I.

The assignment of absolute configuration to (*R*)-**1** was made following its synthesis from (*R*)-(+)-3-(2-ethoxyphenoxy)-1-isopropylamino-2-propanol [(*R*)-(+)-**2**] by the route shown in Scheme I. The sequence rule is not