Hydrolysis reactions of the 4-and 5-Alkyl or Aryl Substituted 1,3(3H) Oxazine-2,6-Diones (Oxauracil) Ring System

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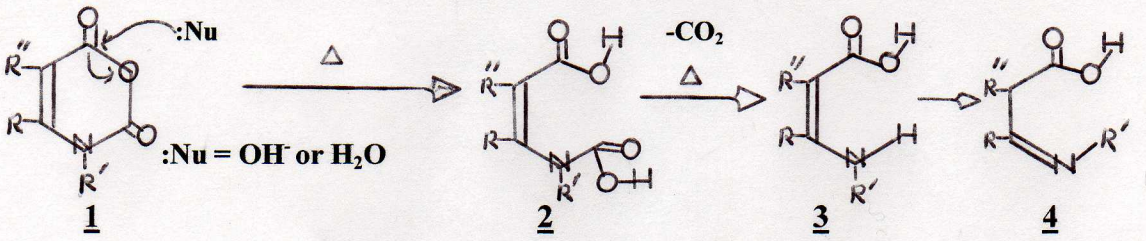
Abstract:

Acid or base hydrolysis of 4-Aryl or alkyl Substituted 1,3(3H) Oxazine-2,6-Diones (Oxauracils)[1,2] yield alkyl or aryl methyl ketones, 6, and ammonia. An intermediate beta keto acid, 5, could be isolated under mild conditions. N-alkylated oxauracils give the same methyl ketones and alkylamines. The N-alkylated oxauracils hydrolyze faster than the non alkylated oxauracils in competion experiments, probably due to electronic factors. 5-aryl oxauracils hydrolyze slower than the 4-isomers in direct competition experiments, probably due to steric factors at the C-6 carbonyl carbon. A mechanistic scheme is presented involving nucleophilic attack at the C-6 carbonyl, decarboxylation of the resulting amino acid 2 yielding enamine 3, which tautomerizes to imine 4. Hydrolysis of 4 and further decarboxylation of beta keto acid 5 give methyl ketones 6 in nearly quantitative yield. The in vitro toxicity of these products is low, thus is of little concern in genetic studies involving substitution of oxauracils for uracils in RNA or other nucleotides.



1 2 3 4

1a) R= Phenyl, R^1 = H

1b) R = Me, R^1 = H

1c) R = Me, R^1 = Me

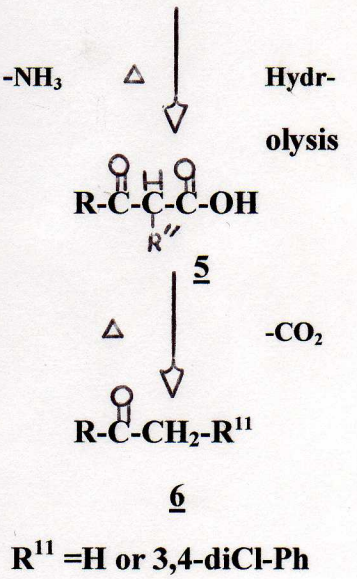
1d) R= p-Tolyl, R^1 = H

1e) R= p-Tolyl, R^1 = Me

1f) R= p-Chlorophenyl , R^1 = H

1g) R= 3,4-dichlorophenyl, R^1 = H

1h) R=H, R^{11} = 3,4-dichlorophenyl, R^1 = H



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b, Funded by:
- U.S. Army Medical Research and Development Command; Grant Number: DAMD 17-74-C-4100
- Army Research Program; Grant Number: 1381

Discussion

4-Aryl or alkyl substituted 1,3(3H) oxazine-2,6-diones (oxauracils)[1,2] are under active investigation in biochemical research as uracil oxa analogs with one N-H group replaced by oxygen. Since most of these studies involve RNA derivatives or drug candidates, in vitro stability of the oxauracils and their hydrolysis products are of interest. Consequently, the hydrolytic stability and reaction products of eight alkyl or aryl oxauracils were investigated. All gave methyl ketones and ammonia or alkylamines as final hydrolysis products (6), with the beta keto acid intermediate(5) isolable under mild conditions. At physiological pH (~ 7.2-7.4) and room temperature, hydrolysis was extremely slow, requiring over a week for full hydrolysis. Thus oxauracil drugs should remain active in the bloodstream long enough for their physiological action to occur before degradation. The products, beta keto acids, methyl ketones and ammonia, are of low toxicity, as desired in drug therapy.

N-methylated oxauracils hydrolyzed at a faster rate than their non-methylated counterparts under identical acidic conditions. This effect is probably electronic, the electron donating alkyl group making the carbonyl groups more negative, increasing the rate of carbonyl protonation under acidic conditions.

5-Aryl substituted oxauracils hydrolyzed at a slower rate than their non-methylated counterparts under identical basic conditions. This effect is probably steric, the bulky aryl group limiting accessibility to the C-6 carbonyl by the hydroxide nucleophile.

Conclusion:

Due to their very slow hydrolysis under in vitro conditions of pH and temperature and low toxicity of the hydrolysis products, 4-aryl or alkyl substituted 1,3(3H) oxazine-2,6-diones (oxauracils), and their N-methylated derivatives are usable for substitution for uracil in drug research, RNA or other bio-molecule incorporating uracil.

Experimental

Acid Catalyzed Hydrolysis of 4-Phenyl-1,3(3H) Oxazine-2,6-Dione (1a)

A 50 ml round bottom flask with heating mantel, magnetic stirrer and water condenser was charged with 350 mg (1a), 25ml of 50/50 water /ethanol and several drops of HCl. The pH was approximately 1. The mixture was refluxed and monitored by TLC (silica gel, ethyl acetate/hexane eluent). A new spot of high Rf value appeared. The solution was cooled and treated with 10ml of 2,4-dinitrophenyl hydrazine (DNPH)solution by the method of Shriner and Fuson[3]. A copious red precipitate formed, which on recrystallization from ethyl acetate yielded red orange crystals, mp 244-7°C, lit[3] : mp 250°C for the DNPH derivative of acetophenone. The product derivative showed no mp depression on admixture with an authentic sample of the DNPH derivative of acetophenone

Base Catalyzed Hydrolysis of 4-Methyl-1,3(3H) Oxazine-2,6-Dione (1b)

A 100 ml round bottom flask with heating mantel, magnetic stirrer and water condenser was charged with 3g (1b,) 57ml of 30% aqueous ethanol, and a KOH pellet. The pH was approximately 8. The mixture was refluxed and monitored by TLC (silica gel, ethyl acetate/hexane eluent). The odor of ammonia was detected.. A new spot of high Rf value appeared. After 3 hours no further 1b could be detected by TLC. The solution was cooled to room temperatures and made neutral with dilute sulfuric acid. The ethanol and other volatiles were removed on a rotovap and the collected volatiles were treated with 20ml DNPH solution. A copious yellow precipitate formed, wt 800 mg, 14% yield,of acetone, mp crude 117-22°C. Recrystallization from ethyl acetate gave yellow crystals, mp 121-4°C, lit[3]: mp 126°C, which gave no mp depression on ad admixture with an authentic sample of the DNPH of acetone.

An acid catalyzed reaction under similar conditions of 1b above also yielded acetone as the primary hydrolysis product.

Acid Catalyzed Hydrolysis of N-Methyl-4-Methyl-1,3(3H) Oxazine-2,6-Dione (1c)

A 100ml round bottom flask with heating mantel, magnetic stirrer and water condenser was charged with 0.6g 1c, 45ml of 50% aqueous ethanol, and 3 drops conc HCl. The pH was approximately 2. The mixture was refluxed and monitored by TLC (silica gel, ethyl acetate/hexane eluent). A new spot of high Rf value appeared. After 42 hours reflux no further 1c could be detected by TLC. The solution was cooled to room temperatures and the ethanol and other volatiles were removed on a rotovap. The collected volatiles were treated with 10ml DNPH solution. A copious yellow precipitate formed, wt 140 mg, (14%) mp 118-22°C. Recrystallization from ethyl acetate yielded yellow crystals, mp 123.5-5°C, lit: mp 126°C for the DNPH derivative of acetone. The product derivative showed no mp depression on admixture with an authentic sample of the DNPH derivative of acetone.

Base Catalyzed Hydrolysis of 4-(p-Tolyl)-1,3(3H) Oxazine-2,6-Dione (1d)

A 100ml round bottom flask with heating mantel, magnetic stirrer and water condenser was charged with 0.5g (1d,) 40ml of 95% ethanol, and 3 KOH pellets. The mixture was refluxed and monitored by TLC (silica gel, ethyl acetate/hexane eluent). The odor of ammonia was detected. The pH was approximately 11. A new spot of high Rf value appeared. After 7 hours no further 1d could be detected by TLC. The solution was cooled to room temperatures and acidified with dilute hydrochloric acid. One half of the solution was treated with 12ml DNPH solution. A copious red precipitate formed, which on recrystallization from ethyl acetate yielded red crystals, mp 250-5°C, lit: mp 260°C for the DNPH derivative of p-tolyl-methyl ketone. The product derivative showed no mp depression on admixture with an authentic sample of the DNPH derivative of p-tolyl methyl ketone. The other half of the reaction was concentrated on the rotovap yielding an aqueous later with an oily residue of pungent odor. The solution was extracted with ether and the ether extracts dried over anhydrous magnesium sulfate. Removal of the ether gave 20 mg of oil, which had an infrared spectrum identical to that of p-tolyl methyl ketone. The oil gave a positive iodoform test for a methyl ketone.

An acid catalyzed reaction under similar conditions of 1d above also yielded p-tolyl methyl ketone as the primary hydrolysis product.

Acid Catalyzed Hydrolysis of N-Methyl-4-(p-Tolyl)-1,3(3H) Oxazine-2,6-Dione (1e)

A 10ml round bottom flask with heating mantel, magnetic stirrer and water condenser was charged with 0.2g (1e,) 4ml of 95% ethanol, 3 ml H_2O and 2 drops conc HCl. The pH was approximately 4.The mixture was refluxed and monitored by TLC (silica gel, ethyl acetate/hexane eluent). A new spot of high Rf value appeared. After 23 hours no further 1e could be detected by TLC. The Rf value of the new strong spot was identical to that of authentic p-tolyl methyl ketone and the solution had the characteristic odor of this ketone. The solution was treated with 10ml DNPH solution. A copious red precipitate formed, which on recrystallization from ethyl acetate yielded red crystals, mp 252-5°C, lit: mp 260°C for the DNPH derivative. The product derivative showed no mp depression on admixture with an authentic sample of the DNPH derivative of p-tolyl methyl ketone.

Acid Catalyzed Hydrolysis of 4-(p-Chlorophenyl)-1,3(3H) Oxazine-2,6-Dione (1f)

A 10ml round bottom flask with heating mantel, magnetic stirrer and water condenser was charged with 0.2g (1f,) 7ml of 95% ethanol, 2 ml H_2O and 2 drops of conc HCl. The pH was approximately 2. The mixture was refluxed and monitored by TLC (silica gel, ethyl acetate/hexane eluent). A new spot of high Rf value appeared. After 48 hours reflux no further 1f could be detected by TLC. The solution was treated with 8ml DNPH solution. A copious red-orange precipitate formed, which on recrystallization from ethyl acetate yielded red crystals, mp 231-5°C, lit[3] : mp 231°C for the authentic DNPH derivative. The product derivative showed no mp depression on admixture with an authentic sample of the DNPH derivative of p-chlorophenyl methyl ketone.

Base catalyzed Mild Hydrolysis of 4-(p-Tolyl)-1,3(3H) Oxazine-2,6-Dione (1d), Isolation of the p-Toluoyl Acetic Acid Intermediate (5)

A 100 ml round bottom flask with magnetic stirrer and water condenser was charged with 0.4g (1d,), 40ml H_2O and 0.3g KOH. All 1d quickly dissolved. The mixture was stirred at room temperature and monitored by TLC (silica gel, ethyl acetate/hexane eluent). After 4 days unreacted 1d still remained. A new spot corresponding in Rf value to p-tolyl methyl ketone appeared along with much streaking just above the spotting point. (Typical acid behavior). After 6 days only traces of 1d remained. The solution was extracted with ether to remove p-tolyl methyl ketone and unreacted 1d. The aqueous layer was acidified with HCl yielding 30mg p-toluoyl acetic acid, mp 97-8°C (dec), lit:[4]mp 98-100°C. (dec). The compound was an acid and dissolved with gas evolution in $NaHCO_3$ solution. Beilstein[5] states "p-toluoyl acetic acid gives p-tolyl methyl ketone on melting or boiling with water".

IR ($CDCl_3$) 3400-3100 (broad, acid OH), 1795(m), 1720(m), 1680(s, C=O stretch), 1605(m), (aromatic C=C stretch) Cm^{-1}.

NMR 100MHz ($CDCl_3$), delta 7.6 (4H, d of d, aromatics), 4.0(s, 2H, CH_2), 2.6 (s, 1H, broad, collapses with D_2O, OH),), 2.45 (s, 3H, CH_3).

The intermediate hydrolysis product is thus unequivocally assigned the structure p-toluoyl acetic acid.

Conversion of the Intermediate p-Toluoyl Acetic acid (5) to p-Tolyl Methyl Ketone

The remaining 5 from above was dissolved in a test tube with 1 ml 50/50 ethanol/water. A small drop of KOH solution was added and the solution let stand overnight. The pH of the solution was approximately 12. The solution was then neutralized with several drops of conc HCl, decanted from solid KCl salt, and treated with one ml of DNPH solution. A copious red precipitate formed, which on recrystallization from ethyl acetate yielded red crystals, mp 253-6°C, lit[3]: mp 260°C for the authentic DNPH derivative. The product derivative showed no mp depression on admixture with an authentic sample of the DNPH derivative of p-tolyl methyl ketone

Competitive Acidic Hydrolysis between 4-(p-Tolyl)-1,3(3H) Oxazine-2,6-Dione (1d) and N-Methyl-4-(p-Tolyl)-1,3(3H) Oxazine-2,6-Dione (1e)

A 10ml round bottom flask with heating mantel, magnetic stirrer and water condenser was charged with 32mg (0.16 mmol) 1d and 34 mg (0.16 mmol) 1e. 2ml 95% ethanol and 2 ml water were then added together with 3 drops conc HCl. The solution was heated to reflux and monitored by TLC (silica gel, ethyl acetate/hexane eluent). After 9 hrs TLC analysis showed the N-methyl derivative 1e spot to be markedly lesser in intensity than the non methylated 1d. After 11 hrs the 1e spot had completely disappeared while substantial 1d remained, thus demonstrating that the N-methyl derivative hydrolyzed at a faster rate than its non-methylated counterpart under identical acidic conditions.

Competitive Base Catalyzed Hydrolysis of 4-(3,4-Dichlorophenyl)-1,3(3H) Oxazine-2,6-Dione (1g) and 5-(3,4-Dichlorophenyl)-1,3(3H) Oxazine-2,6-Dione (1h)

A 10ml round bottom flask with heating mantel, magnetic stirrer and water condenser was charged with 40mg 1g and 40 mg 1h (equimolar amounts). Two ml of 95% Ethanol and two ml of mildly alkaline (pH 8) water were added and the solution heated to reflux. After 18 hrs reflux TLC (silica gel, ethyl acetate/hexane eluent) showed the spot for the 4-isomer to have greatly reduced in intensity compared to that for the 5-isomer. After 20 hrs no 4-isomer spot remained with a still substantial 5-isomer spot present, thus demonstrating that the 4-isomer hydrolyzed at a faster rate than its isomeric 5-isomer under identical basic conditions.

Identification of Ammonia as a Hydrolysis Product in the Base Catalyzed Hydrolysis of 4-Methyl-1,3(3H) Oxazine-2,6-Dione (1b)

A 50 ml round bottom flask with heating mantel, magnetic stirrer and water condenser was charged with 2g (1b), 30ml of water and 6 KOH pellets. An exhaust tube from the reflux condenser lead to an exhaust trap containing 3 drops of concentrated nitric acid. The pH of the reacting solution was approximately 11.The mixture was refluxed and monitored by TLC (silica gel, ethyl acetate/hexane eluent). After 3 hrs 20 ml of the solution was distilled into the nitric acid trap and the trap contents evaporated on a watch glass, yielding 540 mg (54%) ammonium nitrate, mp 165-8°C, lit[3] : mp 169.6°C for ammonium nitrate. The salt gave no melting point depression on admixture with an authentic sample of ammonium nitrate.

Identification of Methylamine as a Hydrolysis Product in the Base Catalyzed Hydrolysis of N-Methyl-4-Methyl-1,3(3H) Oxazine-2,6-Dione (1c)

A 50 ml round bottom flask with heating mantel, magnetic stirrer and water condenser was charged with 1.3 g (9.2 mmol) (1c), 20ml of water, 10ml 95% ethanol and 3 pellets KOH. An exhaust tube from the reflux condenser lead to a trap containing 0.7g picric acid in 30 ml 95% ethanol. The pH of the reacting solution was approximately 10. After 3 hrs reflux the aqueous layer was distilled into the picric acid trap until the droplets were neutral to litmus, and the trap contents evaporated on a watch glass, yielding yellow crystals, wt 440 mg, mp 206-10°C, lit:[3] mp 215 °C for methylammonium picrate. Authentic methylammonium picrate was prepared and also melted at 206-10°C. The product picrate showed no melting point depression on admixture with the synthetic sample of methylammonium picrate.

References:

1) **J.D. Warren, J.H. MacMillan and S.S. Washburne "Synthesis of Substituted 2H-1,3-Oxazine-2,6-Diones by Reaction of Trimethylsilyl Azide with Maleic Anhydrides" J.Org.Chem., Vol. 40, p743 (1975).**

2) **J.H. MacMillan and S.S. Washburne "Further Investigation of the Interaction of Trimethylsilyl Azide with Substituted Maleic Anhydrides, Synthesis of 4-and 5-Aryl Substituted 1,3(3H) Oxazine-2,6-Diones" J.Heterocyclic Chemistry Vol. 12, p1215 (1975).**

3) The Systematic Identification of Organic Compounds R.L. Shriner, C.K.F. Hermann, T.C. Morrill, D.Y. Curtin & R.C. Fuson John Wiley & Sons, Inc., 1997 656 pp., ISBN 0-471-59748-1

4) Duff, J. Chem. Soc, 105, p 2186

5) Beilstein, Band X, 702, p334.