THE CHEMISTRY OF DRUGS. III.[1] ACID HYDROLYSIS OF ANTIMALARIAL 5-ALKOXY-6,6-DIMETHYL-5,6-DIHYDRO-S-TRIAZINES

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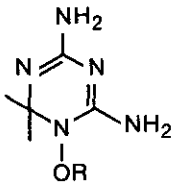
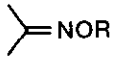
Abstract: 5-Alkoxy-6,6-dimethyl-5,6-dihydro-s-triazines undergo a fragmentation reaction in refluxing 1N HCl. The hydrolytic degradation of the antimalarial WR 99210 belonging to this class of compounds was investigated.

Study of the chemical compatibility of synthetic or natural drugs may provide useful information regarding purity, stability, mode of action and alternate synthesis. We considered the antimalarial 1a an interesting candidate to explore the validity of this concept and report here on the results obtained in studying its acid hydrolysis and its base catalyzed isomerization. Arylmethoxy-substituted biguanides and their dihydrotriazines obtained in their condensation with acetone, showed considerable antimicrobial activity in vitro.[3,4] Chlorophenoxyalkoxy-substituted representatives described later,[5] showed besides antimicrobial properties also marked antimalarial activity. In particular the 2,4,5-trichlorophenoxy-substituted compound 1a showed marked suppressive activity against Plasmodium berghei infections in mice.[6] Compound 1a is an antifolate,[7] effective against the drug-sensitive N strain, the cycloguanil-resistant B line, a pyrimethamine-resistant line of P. berghei,[8] and was found to be not mutagenic in the Ames test.[9] Compound 1a.HCl was for this reason selected by the Walter Reed Army Institute of Research for a human bioavailability study.[10] In this study 1a.HCl showed pronounced gastro-intestinal intolerance and poor bioavailability, and additional human studies were therefore not carried out. The excellent antimalarial properties of 1a, together with its poor bioavailability, poor stability at pH >8[3] and its rather unappealing structural features, made 1a an interesting candidate compound to investigate, hoping that such an endeavour would ultimately lead to the development of improved drugs.



1a, b, c

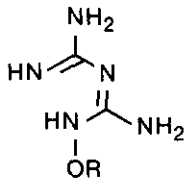


2, a, b

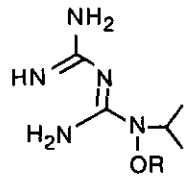
$RONH_2$

3a

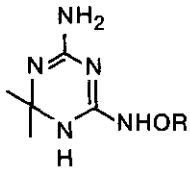
$RONHCH(CH_3)_2$



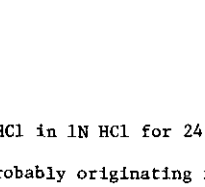
4a



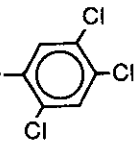
5a

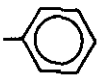


6a



7a

a, R = $(CH_2)_3O$- 

b, R = CH_2- 

c, R = H

Chemistry:

Refluxing 1a.HCl in 1N HCl for 24 hours gave beside ammonium chloride as the water soluble product and probably originating from guanylurea by further hydrolysis, the acetone oxime ether 2a in 64% yield. The structure of 2a is in agreement with its spectral properties, and is secured by its conversion into the O-substituted hydroxylamine 3a, isolated as its HCl salt.[5] Reduction of 2a with pyridine-borane complex,[11] afforded the N-isopropylated hydroxylamine ether 4a. Condensation of 3a and 4a with dicyanodiamide by the Beecham procedure,[5] gave the biguanides 5a and 6a. Condensation of the biguanide 5a with acetone in methanol in the presence of conc. HCl gave the s-dihydrotriazine 1a.HCl, readily isomerized to 7a.HCl with triethylamine.[3] A similar isomerization from 1a into 7a is noticable when ethanolic NaOH is added to an ethanolic solution of 1a.HCl, and the pH of the solution brought >8. The N,O-substituted hydroxylamine 4a could not be cyclized with acetone under similar reaction conditions. A comparison of the original dihydrotriazine hydrochloride (mp 187-189°C) with 1a.HCl prepared here (mp 205-207°C) by tlc revealed that the former sample was contaminated with about 10-20% of the isomer 7a.HCl.

Treatment of the known O-benzyl-substituted dihydrotriazine 1b[5] with 1N HCl afforded by a similar fragmentation the acetone oxime ether 2b[3] besides the water soluble N-hydroxydihydrotriazine 1c,[5] originating from 1b by cleavage of the benzyl ether group. The antimalarial activity of chemically pure 1a, its position isomer and it biguanidine precursor is being persued.

EXPERIMENTAL

M.p.s. were taken on a Fisher-John melting point apparatus and are uncorrected. Elemental analyses were performed by the Section on Microanalytical Services and Instrumentation of this Laboratory. IR spectra were recorded on Beckman 4230 instrument. NMR spectra were determiend by using a Varian HR-220 spectrometer with Me_4Si as internal reference. CI-MS spectra were obtained by using a Finnigan 1015 D spectrometer. Thin-layer chromatography plates were purchased from Analtech, Inc.

Acid hydrolysis of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-[3'-(2,4,5-trichlorophenoxy)-propyloxy]-1,3,5-triazine hydrochloride (1a.HCl). Triazine hydrochloride (1a.HCl) (925 mg, 2.03 mmol) was taken up in 8 ml of 1N HCl and heated on an oil bath at 120-125°C for 22 h under nitrogen atmosphere. After diluting with 5 ml of water, the reaction mixture was extracted with ether (4x25 ml). The combined ether extract was dried (Na_2SO_4) and evaporated to obtain the oxime ether 2a as an oil (504 mg, 89.) which was crystallized from cold petroleum ether as colorless flakes, m.p. 50-51°C. ^1H NMR ($CDCl_3$). δ 1.85, 1.87 (each s, each 3H, 2xCH_3), 2.18 (m, 2H, CH_2-2'), 4.09, 4.20 (each t, J = 6Hz, each 2H, CH_2-1' and CH_2-3'), 6.99 and 7.42 (each s, each 1H, ArH); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2960, 2880, 1750, 1710; MS (Cl/NH_3) m/e 310, 312 (QM). Anal. Calcd. for $C_{12}H_{14}NO_2Cl_3$: C, 46.46; H, 4.54; N, 4.51; Cl 34.25. Found: C, 46.18; H, 4.07; N, 4.46; Cl, 34.56.
The aqueous solution was concentrated under reduced pressure and water was removed completely by azeotropic distillation with benzene. The residue thus obtained was found to be ammonium chloride.

Acid hydrolysis of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-benzyloxy-1,3,5-triazine hydrochloride (1b.HCl): Triazine hydrochloride (1b.HCl) (500 mg, 1.77 mmol) was hydrolysed in 1N HCl (10 ml) under identical conditions as described for 1a.HCl. The ether extract of the reaction mixture after evaporation afforded an oil identical with known benzyloxyoxime[3] 2b (TLC, NMR, IR).
The aqueous portion on concentration under reduced pressure afforded a colorless solid (methanol-acetone) m.p. 238-40° (dec.) identical with known triazine[5] 1c (TLC, IR).

3'-(2,4,5-Trichlorophenoxy)-propyloxyamine hydrochloride (3a.HCl). Oxime ether (2a) (500 mg, 1.6 mmol) was refluxed in ethanolic HCl (50 ml) (conc. HCl:EtOH::1:1) under nitrogen atmosphere for 4 h. The mixture was evaporated and the residual water was removed by azeotropic distillation with benzene to afford 3a.HCl as a gum which crystallized from methanol-ether as colorless prisms (227 mg, 47%), m.p. 154-156°C (lit.[5] m.p. 134-136°C). MS (Cl/CH_4) m/e 269, 271 (OM); Anal. Calcd. for $C_9H_{10}NO_2Cl_3$.HCl: C, 35.22; H, 3.58; N, 4.56; Cl, 46.21. Found: C, 35.18; H, 3.88; N, 4.68; Cl, 46.31.

3'-(2,4,5-Trichlorophenoxy)-1'-(isopropylamino)-propane (4a). A mixture of ether oxime (2a) (309.5 mg, 1 mmol) and pyridine-borane complex (280 mg, 3 mmol) was cooled in an ice-bath. To this solution was added 20% aqueous HCl (5 ml) dropwise over a period of 5 minutes, and the reaction mixture was stirred at ambient temperature for 30 minutes followed by stirring at 65-70°C for another 30 minutes. After diluting with water (10 ml), the cooled solution was basified with aqueous Na_2CO_3 solution and extracted with $CHCl_3$ (2x20 ml). The organic layer was dried (Na_2SO_4) and solvent evaporated to obtain 4a as an oil (290 mg, 93%). MS (Cl/NH_3) m/e 311, 313 (QM); ^1H NMR ($CDCl_3$): δ 1.02 (d, J = 7Hz, 6H, $2xCH_3$), 2.09 (m, 2H, CH_2-2'), 3.13 (m, J = 6Hz, 1H, CH), 3.86, 4.05 (each t, each J = 6Hz, each 2H, CH_2-1', CH_2-3'), 6.95 and 7.38 (each s, each 1H, ArH); Hydrochloride of 4a: colorless prisms (methanol-ether), m.p. 132-133°C; Anal. Calcd. for $C_{12}H_{16}NO_2Cl_3$. HCl: C, 41.30; H, 4.87; N, 4.01; Cl, 40.64. Found: C, 41.35; H, 5.22; N, 4.00; Cl, 40.49.

3'-(2,4,5-Trichlorophenoxy)-propyloxydiguanidine (5a): 3'-(2,4,5-Trichlorophenoxy)-propyloxy-amine hydrochloride (3a.HCl) (307 mg, 1 mmol) was refluxed with dicyanodiamide (126 mg, 1.5 mmol) in ethanol (10 ml) for 4 h. After removal of the solvent under reduced pressure the gummy residue was diluted with ice-cold water. The aqueous solution was basified with concentrated NH_4OH. The solid collected by filtration was crystallized from ethyl acetate-petroleum ether yielding 375 mg of (5a) as colorless prisms, m.p. 131-132°C (lit.[5] m.p. 125-126°C). IR ν_{max}^{KBr} cm^{-1}: 3415, 3360, 2900 (br), 1672, 1650, 1615; MS (CI/CH_4) m/e 354, 356 (QM); Anal. Calcd. for $C_{11}H_{14}N_5O_2Cl_3$: C, 37.27; H, 3.95; N, 19.75; Cl, 30.00; Found: C, 37.23; H, 4.12; N, 19.63; Cl, 30.09. Hydrochloride of 4a, m.p. 117-18°C. Anal. Calcd. for $C_{11}H_{14}N_5O_2Cl_3.2HCl$: C; 30.91; H, 3.74; N, 16.38; Cl, 41.47. Found: C, 30.99; H, 3.42; N, 16.03; Cl, 41.11.

3'-(2,4,5-Trichlorophenoxy)propyloxy-N-isopropyldiguanidine (6a). This compound was prepared in 52% yield according to the procedure described for 5a, m.p. 138-140°C (ether). ^1H NMR ($CDCl_3$ + $DMSOd_6$): δ 1.17 (d, J = 7Hz, 6H, $2xCH_3$), 2.25 (m, 2H, CH_2-2'), 4.15 (m, 4H, CH_2-1', CH_2-3'), 4.37 (m, 1H, CH), 7.18 and 7.47 (each 2s, each 2H, ArH); IR ν_{max}^{KBr} cm^{-1}: 3415, 3320, 2975, 2200, 2160, 1675, 1660, 1610; MS (CI/NH_3) m/e 396, 398 (QM) Anal. Calcd. for $C_{14}H_{20}N_5O_2Cl_3$: C, 42.41; H, 5.04; N, 17.66; Cl 26.82. Found: C, 42.12; H, 5.25; N, 17.54; Cl, 26.98. Hydrochloride of 6a, m.p. 198-199° (methanol-ether). Anal. Calcd. for $C_{14}H_{20}N_5O_2Cl_3.2CH1$: C, 35.82; H, 4.69; N, 14.92; Cl, 37.76. Found: C, 35.77; H, 4.95; N, 14.73; Cl, 37.88.

4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-[3'-(2,4,5-trichlorophenoxy)-propyloxy]-1,3,5-triazine hydrochloride (1a.HCl). The diguanidine (5a) (500 mg, 1.17 mmol) was taken up in methanol (2.5 ml) and treated with acetone (2.5 ml) and conc. HCl (0.5 ml). After stirring at room temperature for 48 h, the reaction mixture was concentrated under diminished pressure. The gum thus obtained was crystallized from methanol-acetone as colorless prisms of 1a.HCl (420 mg, 83%), m.p. 204-206°C (lit.[5] m.p. 187-189°C). ^1H NMR ($DMSOd_6$): δ 1.36 (s, 6H, $2xCH_3$) 2.16 (m, 2H, CH_2-2'), 4.12 (m, 4H, CH_2-1', CH_2-3') 7.44 and 7.76 (each 2s, each 2H, ArH) IR ν_{max}^{KBr} cm^{-1}: 3240, 2935,

1690, 1610; MS (CI/NH_3) m/e 394, 396 (QM). Anal. Calcd. for $C_{14}H_{18}N_5O_2Cl_3$.HCl: C, 39.01; H, 4.41; N, 16.25; Cl, 32.90. Found: C, 38.67; H, 4.38; N, 16.59; Cl, 32.94.

4-Amino-1,2-dihydro-2,2-dimethyl-2-[3'-(2,4,5-trichlorophenoxy)propyloxyamino]-1,3,5-triazine hydrochloride (7a.HCl): The triazine hydrochloride (1a.HCl) (500 mg, 1.17 mmol) was dissolved in warm ethanol (7 ml) and triethylamine (2 ml) added to the clear solution. After stirring the solution at ambient temperature for 45 min, it was diluted with 40 ml of water and stirred for another 1 h. The solid was collected and crystallized from alcohol to afford 7a.HCl as colorless prisms; yield 310 mg (62%) m.p. 179-180°C. ^{1}H NMR (DMSOd_6): δ 1.32 (s, 6H, 2xCH_3), 2.16 (m, 2H, CH_2-2'), 4.12 (m, 4H, CH_2-1', CH_2-3') 7.44 and 7.96 (each 2s, each 1H, ArH), IR ν_{max}^{KBr} cm^{-1}: 3440, 3380, 3325, 3000-2940, 1665, 1615. MS (CI/NH_3) m/e 394, 396 (QM). Anal. Calcd. for $C_{14}H_{18}N_5O_2Cl_3$.HCl: C, 39.01; H, 4.41; N, 16.25; Cl, 32.90. Found: C, 39.37; H, 4.01; N, 16.37; Cl, 32.99.

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