SYNTHESIS AND PROPERTIES OF 4a-SUBSTITUTED 6,8-DIMETHYLPYRIMIDO-[5,5-e][1,2,4]-TRIAZINE-3,5,7-TRIONES

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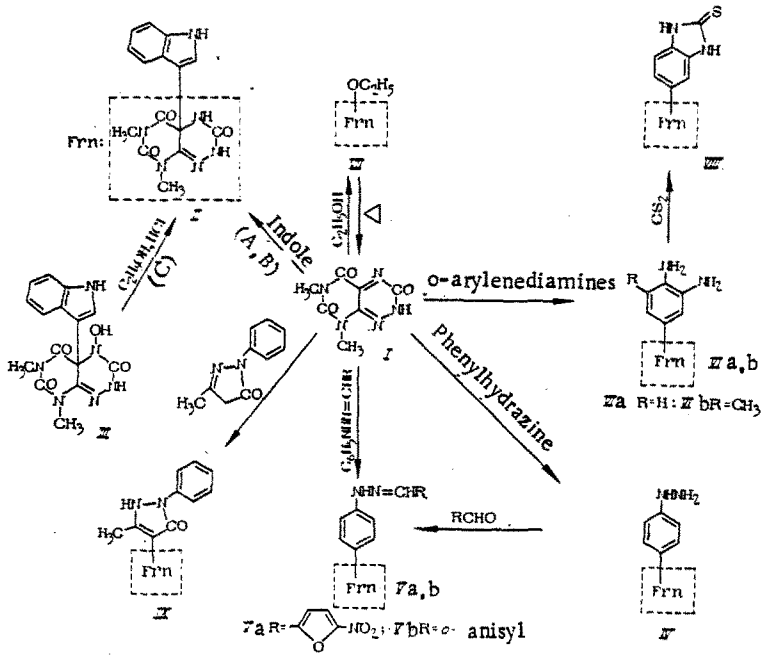
The pyrimidotriazine antibiotics possess a wide spectrum of antimicrobial and antitumor activity [3, 5]. The need for convenient methods of synthesis and the desirability of discovering novel drugs amongst analogs of the pyrimidotriazine antibiotics have stimulated the development of the chemistry of these compounds. The most easily accessible pyrimidotriazine antibiotic is 2,3,5,6,7,8-hexahydro-2,6,8-trimethylpyrimido[5,4-e][1,2,4]triazine]3,5,7-trione (2-methylfervenulone, MSD-92) [5, 6]. The chemical properties of MDS-92 are virtually unknown, although in view of the presence of oxogroups in the triazine ring this compound is of potential interest as a starting material for the preparation of novel analogs of the pyrimidotriazine antibiotics.

We have now examined the reactions of the desmethyl analog of anbibiotic MSD-92 [3-fervenulone (I)] with some nucleophiles. Reactions with both neutral and charge-activated compounds have been studied.



In a study of the reaction of 3-fervenulone with indole, it was found that on heating the reactants in boiling butanol, 2,3,4,4a,5,6,7,8-octahydro-4a-(3-indolyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-3,5,7-trione (II) is formed. When catalyzed by acids, this reaction takes place in ethanol at ambient temperatures. The structure of (II) was confirmed by direct synthesis from the known 2,3,4,4a,5,6,7,8-octahydro-4-hydroxy-4a-(3-indolyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-3,5,7-trione (III) [2] by heating in ethanolic hydrochloric acid. The PMR spectrum of the product (II) showed signals for protons in accordance with the proposed structure, the occurrence of spin coupling between the 2- and 4-NH protons (1.9 Hz) being characteristic.

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A promising starting material for the synthesis of novel pyrimidotriazine antibiotics was obtained by reacting fervenulone with phenylhydrazine. On heating (I) with phenylhydrazine in boiling ethanol in the presence of hydrochloric acid, there was obtained 2,3,4,-4a,5,6,7,8-octahydro-4a-(p-hydrazinophenyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-3,5,7-trione hydrochloride (IV). Treatment of an aqueous solution of this hydrochloride with sodium acetate gave (IV) as the free base. The molecular mass of (IV), found by mass spectrometry, was in agreement with the calculated value. The PMR spectrum of (IV) showed spin coupling between the 2- and 4-NH groups analogous to that seen in (II). The hydrazino-derivative (IV) reacts smoothly with aldehydes to give the hydrazones (V). The latter can also be obtained directly, by the reaction between (I) and the appropriate phenylhydrazones in boiling ethanol in the presence of hydrochloric acid.

The reaction of (I) with o-phenylenediamines was carried out in the same way as with phenylhydrazine. The resulting 2,3,4,4a,5,6,7,8-octahydro-4a-(3,4-diaminophenyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-3,5,7-trione hydrochlorides (VIa, b) were also converted into the free bases by treatment with sodium acetate. The molecular masses of (VIa, b) (found by mass spectrometry) were in agreement with the calculated values. Addition of o-phenylenediamines at the 4a-carbon* is shown by the chemical shifts and coupling constants of the 2- and 4-NH protons in the PMR spectra of these compounds, which are similar to those of (II) and (IV). 4-Chloro-, 4-methyl-, 4,5-dimethyl-, and 3,6-dimethoxy-o-phenylenediamines fail to add to fervenulone as a result of steric hindrance. Under these conditions, the 4a-ethoxy compound (VII)* was isolated from the reaction mixture in yields of 65-70%. Heating the compound (VII) at 150-155°C for 3-4 h gives the starting compound (I) in quantitative yield. The o-diaminocompounds (VI) may be used for the synthesis of novel analogs of the pyrimidotriazine antibiotics. For example, treatment of (VIa) with carbon disulfide in pyridine gives the benzimidazole (VIII).

The formation of (IV), (V), and (VI) from fervenulone in the absence of acid has not been observed. It appears that the acid both activates the fervenulone towards nucleophiles by salt formation, and determines the orientation of the latter in the reaction.

Reaction of fervenulone with the carbanion from 1-phenyl-3-methylpyrazol-5-one at ambient temperature also affords the addition product at the 4a carbon (IX). Reaction in this instance takes place in dry dimethylformamide, triethylamine being used to generate the carbanion in the reaction mixture. The nature and positions of the signals for the 2- and 4-NH protons in the PMR spectrum of (IX) are similar to those of (II), (IV), and (VI).

Hence, when the substrate of the reactant is charge-activated, fervenulone adds nucleophiles at the 4a carbon. Indole adds in a similar way in boiling butanol, but without activation of the fervenulone with acid. However, this reaction proceeds readily in ethanol in the presence of acid, even at ambient temperatures.

EXPERIMENTAL CHEMICAL

PMR spectra were obtained on a Perkin-Elmer R-12B instrument, operating frequency 60 MHz, chemical shifts being given on the δ scale in ppm relative to tetramethylsilane. IR spectra were obtained on a UR-20 spectrometer (East Germany). The samples were prepared as mulls in vaseline oil. Mass spectra were obtained on a Variant MAT-311A instrument with an accelerating voltage of 75 V and ion temperature 200°C.

2,3,4,4a,5,6,7,8-Octahydro-4a-(3-indolyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-3,5,7-trione (II). A. A mixture of 0.209 g (1 mmole) of fervenulone (I) and 0.117 g (1 mmole) of indole was boiled in 5 ml of butanol for 1 h. The reaction mixture was cooled, and the (II) which separated was filtered off to give 0.248 g (76%) of (II), mp 269-270°C (butanol). Found, %: C 54.9; H 4.2; N 25.4. $C_{15}H_{14}N_6O_3$. Calculated, %: C 55.2; H 4.3; N 25.7. PMR spectrum (d_6-DMSO), ppm: 3.00 s ($N-CH_3$), 3.20 s ($N-CH_3$), 6.95-7.80 m (indole CH), 8.03 d (4-NH, J 1.9 Hz), 9.90 d (2-NH, J 1.9 Hz), 11.20 br. d (indole NH J 1.8 Hz). IR spectrum, ν_{max}, cm^{-1}: 1668, 1678, 1694, 1738 (CO), 3120, 3236, 3310, 3356, 3425, 3445 (NH).

*The 8a structure was earlier proposed [2] for (VI) and (VII), but the similarity of the PMR spectra of (VI) and (VII) to that of (II), the structure of which has been established by direct synthesis, and the similarity of the reaction conditions used to obtain (VI), (VII), and (II), indicate that these products are the 4a-derivatives.

B. To a solution of 0.021 g (0.1 mmole) of fervenulone (I) in 5 ml of ethanol was added a solution of 0.012 g (0.1 mmole) of indole in 3 ml of ethanol and 0.02 ml of conc. HCl. The mixture was kept for 1 h at 20-25°C. The precipitated (II) was filtered off, 0.025 g (77%).

C. (III) (0.171 g; 0.05 mmole) was boiled in 5 ml of ethanol and 1 ml of conc. HCl for 5 h. The mixture was cooled, and the precipitated (II) filtered off to give 0.098 g (60%) of (II).

2,3,4,4a,5,6,7,8-Octahydro-4a-(p-hydrazinophenyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]-triazine-3,5,7-trione (IV). A mixture of 0.084 g (0.4 mmole) of fervenulone (I) and 0.05 ml of conc. HCl was boiled with 0.043 g (0.04 mmole) of phenylhydrazine in 5 ml of ethanol for 2 h. The solid (IV) hydrochloride was filtered off, dissolved in 5 ml of water, and treated with saturated aqueous sodium acetate to pH 7.0. The precipitated (IV) was filtered off and washed with water to give 0.062 g (49%), mp 237-239°C. Found, %: C 49.4; H 4.9; N 30.4. $C_{13}H_{15}N_7O_3$. Calculated, %: C 49.2; H 4.8; N 30.9. PMR spectrum (d_6-DMSO), δ, ppm: 3.02 s (N–CH_3), 3.22 s (N–CH_3), 7.70 d (2'-H and 6'-H, J 9.2 Hz), 7.00 d (3'-H and 5'-H, J 9.2 Hz), 7.90 d (4-NH, j 1.9 Hz), 9.84 d (2-NH, J 1.9 Hz). IR spectrum, ν_{max}, cm^{-1}: 1683, 1700, 1710, 1740 (CO), 3105, 3205, 3350, 3385 (NH and NH_2).

2,3,4,4a,5,6,7,8-Octahydro-4a-[(5-nitrofurylmethylidene)-p-hydrazinophenyl]-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-3,5,7-trione (Va). A. A solution of 0.095 g (0.3 mmole) of (IV) in 10 ml of water and 0.5 ml of conc. HCl was prepared. To the resulting solution was added 0.042 g (0.3 mmole) of 5-nitrofurfural in 10 ml of ethanol, and the mixture boiled for 5 min. The precipitate of (Va) was filtered off, to give 0.125 g (95%) of product, mp 262-263°C (ethanol). Found, %: C 48.7; H 3.7. $C_{18}H_{16}N_8O_6$. Calculated, %: C 49.1; H 3.7.

B. A solution of 0.021 g (0.1 mmole) of fervenulone (I) in 3 ml of ethanol was boiled with 0.023 g of (5-nitrofurfurylidene)phenylhydrazine for 1 h, and the precipitated (Va) filtered off to give 0.042 g (95%) of product.

2,3,4,4a,5,6,7,8-Octahydro-4a-[o-methoxyphenylmethylidene)-p-hydrazinophenyl]-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-3,5,7-trione (Vb) was obtained as for (Va), by reacting (IV) with o-methoxybenzaldehyde. Yield of (Va) 95%, mp 247-248°C (ethanol). C 57.6; H 4.9. $C_{21}H_{21}N_7O_4$. Calculated, %: C 57.9; H 4.9.

2,3,4,4a,5,6,7,8-Octahydro-4a-(3,4-diaminophenyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]-triazine-3,5,7-trione (VIa). A solution of 0.042 g (0.2 mmole) of fervenulone in 4 ml of ethanol was boiled with 0.043 g (0.4 mmole) of o-phenylenediamine and 0.03 ml of conc. HCl for 3 h. The solid (VIa) hydrochloride was filtered off, dissolved in 5 ml of water, and the solution treated with a saturated solution of sodium acetate to pH 7.0. The precipitated (VIa) was filtered off and washed with water to give 0.041 g (65%) of product, mp 271-272°C. Found, %: C 49.0; H 4.9; N 30.9. $C_{13}H_{15}N_7O_3$. Calculated, %: C 49.2; H 4.8; N 30.9. PMR spectrum (d_6-DMSO), δ, ppm: 3.08 s (N–CH_3), 3.28 s (N–CH_3), 4.58 br. s (3- and 4-NH_2), 7.80 d (4-NH, J 1.9 Hz), 9.84 d (2-NH, J 1.9 Hz), the 2'-H (6.43), 5'-H (6.51), and 6'-H (6.33) protons forming a three-spin system with coupling constants $J_{5',2'}$ 0 Hz, $J_{6',5'}$ 8.2 Hz, $J_{6',2'}$ 2.2 Hz. IR spectrum, ν_{max}, cm^{-1}: 1670, 1696, 1738 (CO), 3190, 3308, 3373, 3427 (NH and NH_2).

2,3,4,4a,5,6,7,8-Octahydro-4a-(3,4-diamino-5-methylphenyl)-6,8-dimethylpyrimido[5,4-e]-[1,2,4]triazine-3,5,7-trione (VIb) was obtained as for (VIa), by reacting (I) with 3-methyl-o-phenylenediamine . Yield of (VIb) 75%, mp 297-298°C. Found, %: C 50.6; H 5.1; N 29.1. $C_{14}H_{17}N_7O_3$. Calculated, %: C 50.7; H 5.2; N 29.6. PMR spectrum (d_6-DMSO), δ, ppm: 1.96 s (CH_3), 3.00 s (N–CH_3), 4.31 br. s (NH_2), 4.50 br. s (NH_2), 6.24 s (2- and 6-CH arom.), 7.83 d (4-NH, J 1.8 Hz), 9.90 d (2-NH, J 1.8 Hz). IR spectrum, ν_{max}, cm^{-1}: 1685, 1696, 1742 (CO), 3158, 3193, 3321, 3395, 3428 (NH and NH_2).

2,3,4,4a,5,6,7,8-Octahydro-4a-ethoxy-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-3,5,7-trione (VII). A solution of 0.2 mmole of fervenulone (I) in 4 ml of ethanol was boiled with 0.2 mmole of 4-chloro-o-phenylenediamine and 0.03 ml of conc. HCl for 1.5-2 h. The mixture was cooled, and the solid (VII) filtered off and washed with ethanol to give 70% of product, mp 144-145°C (from ethanol). Found, %: C 42.1; H 5.0; N 27.7. $C_9H_{13}N_5O_4$. Calculated, %: C 42.4; H 5.1; N 27.4. PMR spectrum (d_6-DMSO), δ, ppm: 1.06 t (CH_3, J 6.7 Hz), 3.11 s (N–CH_3), 3.21 s (N–CH_3), 3.37 q (CH_2, J 6.7 Hz), 8.78 d (4-NH, J 1.9 Hz), 10.55 d (2-NH, J 1.9 Hz). IR spectrum, ν_{max}, cm^{-1}: 1686, 1699, 1710, 1742 (CO), 3100, 3150, 3235, 3310 (NH).

Compound (VII) was also obtained when the reaction was carried out with 4-methyl, 4,5-dimethyl-, and 3,6-dimethoxy-o-phenylenediamine . On drying (VII) at 150-155°C over P_2O_5, (I) was obtained in quantitative yield.

2,3,4,4a,5,6,7,8-Octahydro-4a-(2-thionobenzimidazol-5-yl)-6,8-dimethylpyrimido[5,4-e]-[1,2,4]triazine-3,5,7-trione (VIII). A solution of 0.063 g (0.2 mmole) of (VIa) in 4 ml of dry pyridine was boiled with 1 ml of carbon disulfide for 2 h. The solvent was dissolved off, and the residue treated with 5 ml of water and acidified to pH 3.0-4.0. The solid (VIII) was filtered off to give 0.060 g (80%) of product, mp 260-262°C (from ethanol). Found, %: C 46.3; H 3.9; S 8.9. $C_{14}H_{13}S_7O_3$. Calculated, %: C 46.8; H 3.6; S 8.9.

2,3,4,4a,5,6,7,8-Octahydro-4a-(1-phenyl-3-methyl-5-oxopyrazol-4-yl)-6,8-dimethylpyrimido-[5,4-e][1,2,4]triazine-3,5,7-trione (IX). Fervenulone (0.042 g, 0.2 mmole) was kept with 0.035 g (0.2 mmole) of 1-phenyl-3-methyl-5-pyrazolone and 0.03 ml of triethylamine in 3 ml of dry dimethylformamide for 12 h at 20-25°C. The mixture was diluted with water (1:1), and actified with conc. HCl to pH 3.0-4.0. The precipitated (IX) was filtered off to give 0,059 g (70%) of product, mp 170-172°C (from ethanol). Found, %: C 48.7; H 4.9; N 23.2. $C_{17}H_{17}N_7O_4 \cdot 2H_2O$. Calculated, %: C 48.7; H 5.0; N 23.4. PMR spectrum (d_6-DMSO), δ, ppm: 2.30 s (CH_3), 3.15 s (N—CH_3), 3.23 s (N—CH_3), 7.18-7.80 m (arom. CH), 8.00 br. s (4-NH), 10.00 br. s (2-NH).

EXPERIMENTAL BIOLOGICAL

Although it possesses high biological activity against bacterial cultures outside the living body, the antibiotic MSD-92 was found to be ineffective in infected mice as a result of its high toxicity (LD_{50} 2.5 mg/kg) [5]. For this reason, it was of interest to examine the biological activity of the analogs of the antibiotic MSD-92 with a view to identifying novel drugs with lower toxicity.

The acute toxicities of the compounds obtained in the present investigation were determined in mice weighing 18-20 g. Toxicity was measured following a single oral dose, and deaths of the animals noted over a period of five days.

The compounds were found to be of low toxicity, their LD_{50} values not exceeding 200 mg/kg.

The antimicrobial activity of the compounds was studied by double serial dilution [4] against gram-positive (Staph. aureus strain 209P) and gram-negative (E. coli strain M-17) bacteria. It was found that (IV) and (Vb) had bacteriostatic effects against gram-positive bacteria only. The minimum bacteriostatic concentrations (MBC) of these compounds were 250 and 1000 μg/ml respectively. Bactericidal activity against gram-positive and gram-negative bacteria was shown by (IV) only. The MBC of this compound against E. coli and Staph. aureus were 1000 and 500 μg/ml respectively. The remaining compounds showed no antimicrobial activity in the concentrations tested.

The reactions of 3-fervenulone with nucleophiles described here have opened up new routes for the modification of the pyrimidotriazine antibiotics. At the same time, studies of the biological properties of the 4a-derivatives of pyrimido[5,4-e][1,2,4]-triazine obtained here are important for understanding the nature of the biological activity of the pyrimidotriazine antibiotics which, as shown above, can be transformed under mild conditions into similar compounds.

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