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REACTIONS OF FERVENULIN AND ITS 3-SUBSTITUTED ANALOGS WITH INDOLE

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A study of the chemical reactions of antibiotics is of interest both in terms of research into effective drugs and for understanding the mechanism of the biological action which may be the result of biochemical reactions of these compounds in a living organism.

It is known that 3-substituted derivatives of the antibiotic fervenulin are converted into derivatives of xanthine [11] or 6-azapurine [12] as a result of nucleophilic "attack" by formamide on the C(8a) atom and by the OH^- ion on the C(5) atom of the pyrimidotriazine ring. At the same time the reactions of fervenulin and its analogs with C-nucleophiles have not been studied at all.

In the present work we have studied the conversions of the antibiotic fervenulin and its 3-substituted analogs with indole, the reactions investigated being carried out under conditions of acid catalysis, which is of interest as the latter can take place in some living tissues.

The synthesis of fervenulin (IIa) is carried out by reduction of fervenulin-4-oxide (I) according to the method in [7]. The 3-substituted derivatives are in turn obtained by heating I with the appropriate aldehyde in ethanol in the presence of hydrochloric acid. The formation of products IIb-f evidently occurs as a result of the decomposition of I to nitrosohydrazinouracil (III) as described in [3] and its subsequent cyclization with aldehydes according to the method reported in [6]. In fact, products IIc, f have also been prepared from a known sample of III according to the method of [6] and they match the products obtained from I.

It has been established during the investigation of the reaction of IIa with indole that even when the reagents are heated for a short time (15-20 min) in ethanol in the presence of hydrochloric acid, the hydrochlorides of the 4a-indolyl derivatives of 2,4,4a,5,6,7,8-hexahydro-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-5,7-dione (IVa,b) are formed. By treatment of aqueous solutions of the hydrochlorides obtained with sodium acetate, bases IVa, b are isolated in a free state.

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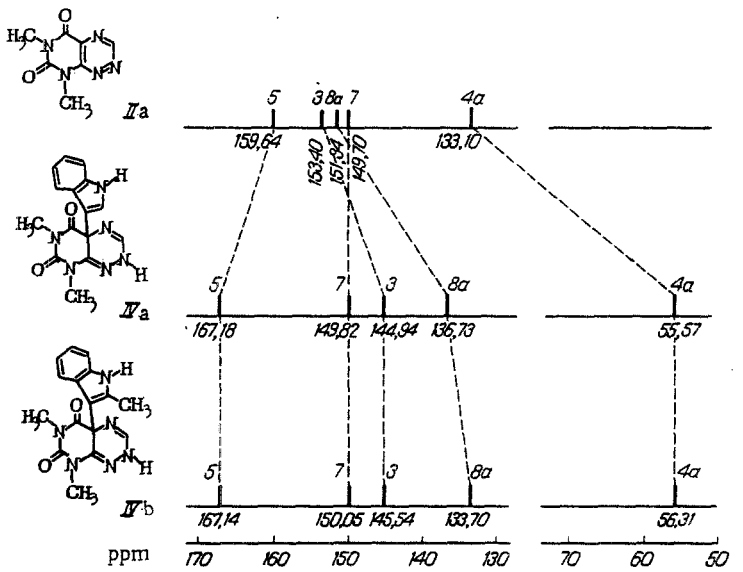
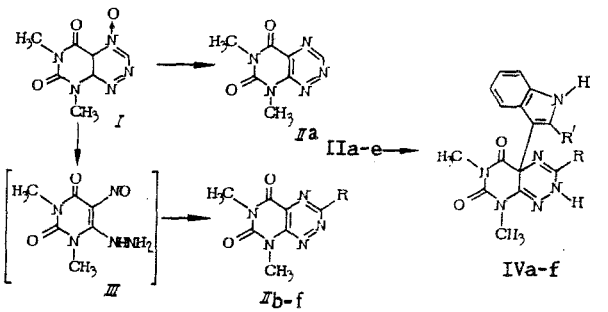


Fig. 1. Chemical shifts of ^{13}C atoms in pyrimidotriazine ring of compounds IIa, IVa, and IVb in DMSO-d_6.



R=H (IVa,b); C_6H_5 (IIb, IVc); $C_6H_4N(CH_3)_2$-p (IIc, IVd); $C_6H_4OCH_3$-p (IIe, IVe); $C_6H_4OCH_3$-o (IIe, IVf); $C_6H_4NO_2$-p (IIf); R =H (IVa,c-f); CH_3 (IVb)

A doublet signal from the C(4a) atom ($^3J_{C_{4a}{}^3-H}$ = 8.6 Hz) in the high-resolution ^{13}C NMR spectrum of the initial compound IIa occurs at 133.10 ppm (Fig. 1), while the doublet from the C(4a) atom ($^3J_{C_{4a}{}^3-H}$ = 10.0 Hz) in product IVb occurs at 56.31 ppm, which is confirmation of its sp^3-hybrid character. The location of a proton on the N(2) atom (but not the N(4) atom) of molecule IVb is confirmed by the presence of the signal from the C(3) atom split into a doublet with a small spin-spin coupling constant of $^2J_{C_3{}^2-H}$ = 3.7 Hz (in addition to $^1J_{C_3{}^3-H}$ = 196.2 Hz) and the absence of such for the C(4a) atom. Product IVa has a similar structure, which is borne out by the similarity of the chemical shift values of ^{13}C atoms in the pyrimidotriazine ring of compounds IVa and IVb (see Fig. 1).

In the IR spectra of crystalline samples of IVa, b, in the regions 1610-1650, 3100-3300, and 3340-3370 cm^{-1} there are absorption bands that are absent in the spectra of the initial indoles and fervenulin (Fig. 2). The intense band at about 1640 cm^{-1} is evidently a feature of the stretching vibrations of the isolated C=N bonds in the triazine ring of compounds IVa, b. It is these bonds that are formed when a proton adds on to the N(2) atom (and not the N(4) atom) of products IVa, b. The accuracy of such an assignment is corroborated by the fact that for fervenulin, which has a system of conjugated C=C and C=N bonds, the absorption of the latter occurs at considerably lower frequencies (1583 and 1540 cm^{-1}). The intense sharp band in the region 3300-3370 cm^{-1} has an outline and position of maximum which are not very different from the characteristic absorption of the NH group in the initial indoles, and it can be related to the stretching vibrations of the amino group in the indole fragment of compounds IVa, b. At the same time the broad band in the region 3100-3300 cm^{-1} apparently corresponds to vibrations of the weaker (compared to N–H of the indole fragment) N–H bond of the electron-withdrawing triazine ring of products IVa, b.

Reaction of the 3-substituted derivatives of fervenulin IIb-e with indole under condi-

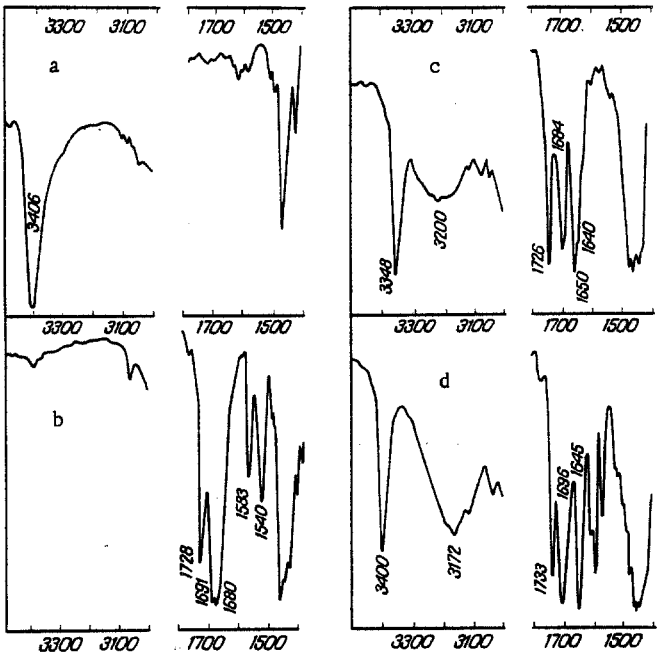
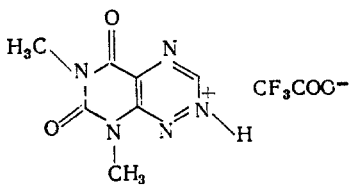


Fig. 2. IR spectra in petrolatum oil: a) indole; b) fervenulin (IIa); c) IVa; d) IVf. Frequency ν (in cm^{-1}) on the x-axis, transmittance (in %) on the y-axis.

tions similar to those described above gave the corresponding 4a-indolyl derivatives IVc-f. The IR spectra of products IVc-f contain characteristic absorption bands due to the C=N and N–H bonds similar to those of compounds IVa, b (see Fig. 2), which suggests an analogous structure for these compounds.

The 3-phenyl derivative IIb proved to be less reactive towards indole than fervenulin itself. At the same time the reactivity of derivatives IId, e, which contained electron-donating substituents in the benzene ring, was higher than for IIb, whereas the nitrophenyl derivative IIf appeared to be completely inert under these conditions. Thus, for the conversion of 3-phenyl derivative IIb it was necessary to heat the reagents for 3-4 h, whereas methoxy derivatives IId, e reacted completely after 1-1.5 h. The p-dimethylamino derivative IIc was somewhat less reactive than IId, e, which is evidently due to the decrease in electron-donating power of the dimethylamino group as a result of its protonation during the reaction. It appears that the effect of substituents at the C(3) atom of fervenulin on the rate of addition of indole is the result of a change in basicity of the triazine ring, which in turn affects the susceptibility of this heterocycle to activation via protonation. A similar anomalous effect of substituents is known for acid-catalyzed nucleophilic substitution reactions [5].

Addition of indoles to the C(4a) atom of compounds II is due to activation of this position because of protonation on the N(2) atom of the pyrimidotriazine ring during the reaction. In fact, when 4.5 ml of trifluoroacetic acid (TFAA) is added to a 0.2 M solution of IIa in 3 ml of $CDCl_3$ the chemical shift value of the C(3) atom decreases by 0.8 ppm and the chemical shifts of the C(4a), C(5), C(7), and C(8a) atoms increase by 2.2, 1.3, 1.3, and 0.8 ppm respectively. At the same time the direct spin-spin coupling constant $^1J_{C_3-H}$ increases by 5.8 Hz. Similar types of changes for the chemical shift of the ortho carbon atom [C(3)] and $^1J_{C_3-H}$ spin-spin coupling constant as well as a downfield shift of the para carbon atom [C(4a)] are a feature of azines [9], indicating that when TFAA is added the solution of compound IIa contains a given equilibrium quantity of its N(2)-protonated form.



Thus, it is obvious that acid catalysis plays a decisive role in the addition of indole to fervenulin and its 3-substituted derivatives, the more so that in its absence no covalent addition products are formed. In this case the conversions described are in themselves the first examples of addition of nucleophiles to the C(4a) atom of the pyrimidotriazine ring of fervenulin and its 3-substituted derivatives. At the same time conversions with a nucleophile such as indole are of special interest since the latter is amongst the most important products for the activity of an organism.

EXPERIMENTAL (CHEMISTRY)

^{13}C NMR spectra of the compounds were recorded on a WP-200-SY Bruker instrument with working frequency 50.32 MHz. Chemical shifts were determined relative to TMS as the internal standard. IR spectra were obtained on a UR-20 instrument (GDR).

Fervenulin-4-oxide (I). This was obtained according to the method in [2] from 1,3-dimethyl-4-hydrazinouracil [8].

5,6,7,8-Tetrahydro-3-(p-methoxyphenyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-5,7-dione (IId). Fervenulin 4-oxide (I) (0.418 g, 2 mmole) was dissolved in ethanol (40 ml) and 0.272 g (2 mmole) of p-methoxybenzaldehyde and 1 ml of conc. HCl were added. The reaction mixture was boiled for 15-20 min and cooled. The precipitate was filtered off, and 0.36 g (60%) of IId was obtained; mp 208-210°C (ethanol). Found: C 56.0; H 4.5; N 23.2%. $C_{14}H_{13}N_5O_3$. Calculated: C 56.2; H 4.4; N 23.4%.

5,6,7,8-Tetrahydro-3-(o-methoxyphenyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-5,7-dione (IIe). This was obtained in a similar manner to IId. Yield 65%; mp 250-252°C (ethanol). Found: C 56.0; H 4.5; N 23.1%. $C_{14}H_{13}N_5O_3$. Calculated: C 56.2; H 4.4; N 23.4%.

Products IIb, c, f were obtained in a similar manner to IId and matched the samples described in [6]. Yields of IIb, c, f were 70, 55, and 75% respectively.

2,4a,5,6,7,8-Hexahydro-4a-(3-indolyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-5,7-dione (IVa). A mixture of 0.193 g (1 mmole) of 5,6,7,8-tetrahydro-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-5,7-dione (IIa), 0.117 g (1 mmole) of indole, and 1 ml of conc. HCl in 15 ml of ethanol was boiled for 15-20 min, the solvent was evaporated, the solid precipitate was dissolved in the minimum amount of water, and a saturated aqueous solution of sodium acetate was added until pH 6.0-7.0 was reached. The precipitate of IVa formed was filtered off, and 0.268 g (86%) of IVa was obtained; mp 225-226°C (butanol). Found: C 58.3; H 4.5; N 26.9%. $C_{15}H_{14}N_6O_2$. Calculated: C 58.1; H 4.5; N 27.1%. IR spectrum, ν_{max}, cm^{-1}: 1640, 1650 (C=N), 1684, 1726 (C=O), 3200, 3348 (NH).

2,4a,5,6,7,8,-Hexahydro-4a-(2-methyl-3-indolyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-5,7-dione (IVb). This was obtained in a similar manner to IVa by reaction of IIa with 2-methylindole. Yield of IVb: 55%; mp 240-241°C (butanol). Found: C 59.8; H 5.3; N 25.7%. $C_{16}H_{16}N_6O_2$. Calculated: C 59.2; H 5.0; N 25.9%. IR spectrum, ν_{max}, cm^{-1}: 1612, 1634 (C=N), 1696, 1731 (C=O), 3200, 3368 (NH).

2,4a,5,6,7,8-Hexahydro-3-phenyl-4a-(3-indolyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-5,7-dione (IVc). A mixture of 0.269 g (1 mmole) of IIb, 0.117 g (1 mmole) of indole, and 4 ml of conc. HCl in 40 ml of ethanol was boiled for 3-3.5 h (until IIb had completely dissolved). The reaction mixture was cooled, diluted with water (1:1), and neutralized with a saturated aqueous solution of sodium acetate until pH 6.0-7.0 was reached. The precipitate was filtered off and reprecipitated from dimethylformamide with water. IVb was obtained, 0.316 g (82%); mp 229-230°C. Found: C 65.9; H 4.8; N 22.1%. $C_{21}H_{18}N_6O_2$. Calculated: C 65.3; H 4.7; N 21.8%. IR spectrum, ν_{max}, cm^{-1}: 1656 (C=N), 1679, 1723 (C=O), 3300, 3390 (NH).

2,4a,5,6,7,8-Hexahydro-3-(p-dimethylaminophenyl)-4a-(3-indolyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-5,7-dione (IVd). This was obtained in a similar manner to IVc by reaction of IIc with indole. Period of time for boiling the reaction mixture until IIc had completely dissolved was 3.5-4 h. Yield of IVd: 63%; mp 244-245°C. Found: C 64.1; H 5.4; N 22.7%. $C_{23}H_{23}N_7O_2$. Calculated: C 64.3; H 5.4; N 22.8%. IR spectrum, ν_{max}, cm^{-1}: 1644 (C=N), 1679, 1729 (C=O), 3200, 3325 (NH).

2,4a,5,6,7,8-Hexahydro-3-(p-methoxyphenyl)-4a-(3-indolyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-5,7-dione (IVe). This was obtained in a similar manner to IVc by reaction of IId with indole. Time of heating the reagents until IId until had completely dissolved

was 1-1.5 h. Yield of IVe: 73%; mp 260-261°C. Found: C 64.1; H 5.1; N 20.4%. $C_{22}H_{20}N_6O_3$. Calculated: C 63.4; H 4.8; N 20.2%. IR spectrum, ν_{max}, cm^{-1}: 1648 (C=N), 1681, 1722 (C=O), 3266, 3401 (NH).

2,4a,5,6,7,8-Hexahydro-4-(o-methoxyphenyl)-4a-(3-indolyl)-6,8-dimethylpyrimido[5,4-e][1,2,4]triazine-5,7-dione (IVf). This was obtained in a similar manner to IVc by reaction of IIe with indole. Time of heating the reaction mixture until IIe had completely dissolved was 1-1.5 h. Yield of IVf: 75%; mp 248-250°C. Found: C 63.7; H 4.9; N 20.3%. $C_{22}H_{20}N_6O_3$. Calculated: C 63.4; H 4.8; N 20.2%. IR spectrum, ν_{max}, cm^{-1}: 1645 (C=N), 1696, 1733 (C=O) 3172, 3400 (NH).

When IIf was heated with indole under similar conditions for 5 h, the initial compound was isolated in almost quantitative yield (IIf did not dissolve).

EXPERIMENTAL (BIOLOGY)

Pyrimidotriazine antibiotics have a wide spectrum of antimicrobial action and some antitumorigenic activity [10]. However, these compounds are noted for their very high toxicity. In this connection, it was useful to study the biological properties of analogs of these compounds in order to find less toxic effective drugs amongst them.

In the present work the antimicrobial, antiinflammatory, and analgesic activities of the compounds obtained were studied, particularly since such properties were previously [1] detected in some analogs of pyrimidotriazine antibiotics.

The acute toxicity (LD_{50}) of the compounds was studied on tetrahybrid mice of weight 18-20 g. The toxicity was determined by administration of a single dose per os and monitoring the death of the animals during five days. The compounds studied were introduced as a suspension in 2% starch slurry at 1/5 to 1/10 of the LD_{50}. The analgesic activity was determined by the "hot plate" test. The antiinflammatory activity was studied on Wistar white rats of weight 180-220 g using carrageenin inflammation as a standard. The magnitude of the inflammatory reaction was determined oncometrically in a similar manner to that in [3].

The antimicrobial action of the compounds was studied by means of double batch cultures relative to 209-P strain Staph. aureus and M17 strain E. coli bacteria. An 18-hour agar bacterial culture ($2.5 \cdot 10^5$ microbial bodies in 1 ml of medium) was used in the experiments. Solutions of the compounds in dimethyl sulfoxide were used in the studies. The highest concentration tested was 1000 μg/ml. The antimicrobial, bacteriostatic, and bactericidal activities were determined from the minimum effective concentration.

The investigations showed that the compounds had low toxicity, as their LD_{50} was greater than 500 mg/kg. As a result of the experiments, weak antiinflammatory activity was detected in compounds IIc, IIe, and IVf. The percentage inhibition of edema by these compounds was 32, 15, and 10% of peak effect respectively. No antiinflammatory effect was registered with compounds IIf, IVa, and IVd. Out of all the compounds studied only compound IIf displayed weak analgesic activity. Compounds IIc, IId, and IIf had antimicrobial activity. Thus, compound IIc at a concentration of 1000 μg/ml inhibited the growth of Staph. aureus, while IIf at this concentration suppressed the growth of E. coli. Compound IId at a concentration of 100 μg/ml had a bacteriostatic effect on Gram-positive and Gram-negative flora.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 5-OXY-6-BROMOINDOLE SUBSTITUTES

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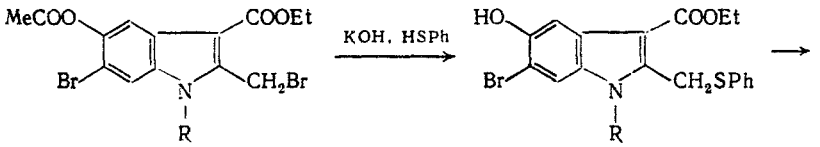
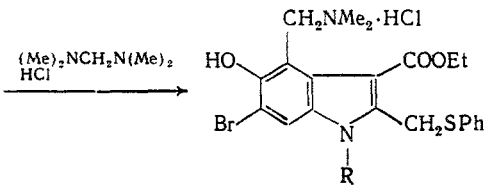
Aminoalkyl derivatives of 5-oxyindole represent considerable interest in the search for biologically active compounds [3-7]. Thus, for example, the 2-aminoalkyl, 2,4-bis, and 4,6-bis(alkylamino)methyl derivatives of 5-oxyindole exhibit pharmacological and antiviral activity [5-7]. Antiserotonin activity equivalent to the activity of 1-benzyl-2-methyl-5-methoxytryptamine (BAS) has been found in the 4-dialkylaminomethyl derivatives of 5-oxyindole [3], and antitubercular activity has been found in a number of dialkylaminoethyl derivatives of 5-oxyindole [4].

In that connection, we felt it would be of interest to study the biological activity of new derivatives of 1-alkyl(aryl)-2-phenylthiomethyl-3-ethoxycarbonyl-4-dimethylaminomethyl-5-oxy-6-bromoindole. The indicated compounds were obtained from 1-alkyl(aryl)-2-bromomethyl-3-ethoxycarbonyl-5-acetoxy-6-bromine indoles [5] through a series of reactions with thiophenol, deacylation, and aminomethylation.



I—VII



VIII—XIV

R = C_2H_5 (I, VIII), CH_2Ph (II, IX), Ph (III, X), $C_6H_4CH_3$-p(IV, XI), $C_6H_4OCH_3$-p(V, XII), C_6H_4Br-p(VI, XIII), C_6H_4Cl-p(VII, XIV).

EXPERIMENTAL (CHEMISTRY)

1-Phenyl-2-phenylthiomethyl-3-ethoxycarbonyl-5-oxy-6-bromoindole (III). A solution of 2.2 g (0.02 mole) of thiophenol in 5 ml of abs. ethanol was added to a solution of 3.4 g (0.06 mole) of KOH in 70 ml of abs. ethanol. The reaction solution was stirred for 5 min at room temperature after which a suspension of 10 g (0.02 mole) of 1-phenyl-2-bromomethyl-3-ethoxycarbonyl-5-acetoxy-6-bromoindole in 15 ml of abs. ethanol was added. The reaction mixture was then stirred for 1 h at room temperature, and then stirred for 1 h upon boiling in a water bath. The end of the reaction was determined chromatographically. The solvent was vacuum distilled off from the reaction mixture to 2/3 of the volume. A 5% aq. solution of acetic acid (1.3 g, 0.06 mole) was added to the residue. The resultant precipitate was

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