Synthesis of 1-Demethyltoxoflavin (8-Demethylfervenulin)[1]

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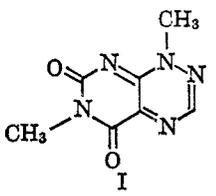
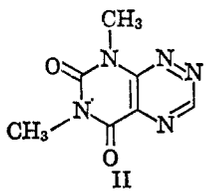
Chemical studies on a new class of antibiotics possessing the 5,7-dioxopyrimido[5,4-*e*]-*as*-triazine ring system have been continued since the original synthesis of toxoflavin (I) and the structural elucidation of fervenulin (II) were reported. The 1-demethyltoxoflavin (8-demethylfervenulin)—6-methyl-5,7-dioxo-1,5,6,7(5,6,7,8)-tetrahydropyrimido[5,4-*e*]-*as*-triazine (III)—has now been synthesized from 3-methyl-5-amino-6-(2-formylhydrazino)-uracil (XIII) by spontaneous cyclization and air oxidation. The latter was prepared *in situ* by catalytic hydrogenation of 3-methyl-5-nitro-6-(2-formylhydrazino)uracil (XI), which was in turn prepared from 3-methyl-6-chlorouracil (IV) *via* 3-methyl-5-nitro-6-chlorouracil (V). Ultraviolet absorption spectra studies revealed that compound III in acid and neutral media possesses the "fervenulin form" and in alkali medium possesses the "toxoflavin form." The major product isolated during the preparation of III was identified as 5,7-dioxo-6-methyl-8-amino-1,5,6,7-tetrahydro-*s*-triazolo[4,3-*c*]pyrimidine (XV).

The total synthesis of toxoflavin (I), an antibiotic isolated from *Pseudomonas cocovenenans*, was accomplished in 1961.[2] Shortly thereafter, the structure of fervenulin (II), an antibiotic isolated from *Streptomyces*



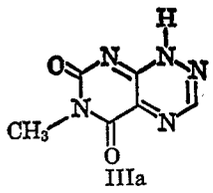
I



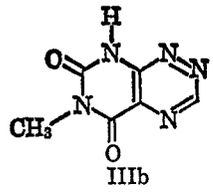
II

fervens n. sp., was found to be isomeric to that of toxoflavin.[3] The antibiotic xanthothricin[4] (from a culture of a member of the genus *Streptomyces*) was confirmed as being identical with toxoflavin,[3,5] and, more recently, a report stated that another antibiotic, planomycin[6] (from *Streptomyces rubrireticuli*), is identical with fervenulin. These facts suggested that 5,7-dioxopyrimido[5,4-*e*]-*as*-triazine is an important structural basis in a new class of antibiotics. The biological significance of this particular ring system is further illustrated by a recent report on the isolation and characterization of a new antibiotic, MSD-92.[7]

Therefore, it is of interest to investigate the synthesis of a compound without a methyl group at either position 1 of toxoflavin (as IIIa), or position 8 of fervenulin (as IIIb). It is known that the antibiotic activities and other physiological actions of toxoflavin and fervenulin are quite different.[2,3,8] A biological evaluation of compound III would, undoubtedly, be of particular value.



IIIa



IIIb

Theoretically, the synthesis of III (IIIa or IIIb) can be achieved by employing either the synthetic method for toxoflavin[2] or fervenulin[9] using 3-methyl-6-chlorouracil[10] (IV) as the starting material (see Scheme I). 3-Methyl-5-formylamino-6-chlorouracil (VI), for instance, reacted readily with methylhydrazine and, after oxidation, afforded toxoflavin (I) in 16% overall yield.[2] However, it was found that compound VI failed to react with hydrazine under similar and various other conditions to yield III. Pfleiderer and Schündehütte were able to introduce a 5-nitroso group into 1,3-dimethyl-6-(2-formylhydrazino)uracil for the synthesis of fervenulin (II).[9] The corresponding 3-methyl-6-(2-formylhydrazino)uracil (VIII) was therefore prepared from IV *via* 3-methyl-6-hydrazinouracil (VII). Attempted nitrosation of VIII under various conditions, however, failed to yield the desired nitroso compound IX.[11]

It has been known for many years that an N-acyl-N′-(*o*-nitrophenyl)hydrazine can be converted into a benzo-*as*-triazine by means of reduction and subsequent acid-catalyzed cyclization.[12–14] Polya and Shanks[15] have recently reported the synthesis of several 1,2-dihydropyrimido[5,4-*e*]-*as*-triazines from 4-(2-acylhydrazino)-5-nitropyrimidines. However, oxidation of these dihydro derivatives, except in one instance, was not possible.[15] (Temple, McKee, and Montgomery[16] have earlier reported the same behavior in 5-chloro-1,2-dihydropyrimido[5,4-*e*]-*as*-triazine and its meth-

(1) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute of the National Institutes of Health, U. S. Public Health Service, Contract No. PH-43-65-94.

(2) (a) G. D. Daves, Jr., R. K. Robins, and C. C. Cheng, *J. Am. Chem. Soc.*, **83**, 3904 (1961); (b) G. D. Daves, Jr., R. K. Robins, and C. C. Cheng, *ibid.*, **84**, 1724 (1962), and references cited therein.

(3) G. D. Daves, Jr., R. K. Robins, and C. C. Cheng, *J. Org. Chem.*, **26**, 5256 (1961), and references cited therein.

(4) R. A. Machlowitz, W. P. Fisher, B. S. McKay, A. A. Tytell, and J. Charney, *Antibiot. Chemotherapy*, **4**, 259 (1954).

(5) H. E. Latuasan and W. Berends, *Biochem. Biophys. Acta*, **52**, 502 (1961).

(6) K. Tanabe, Y. Asahi, M. Nishikawa, T. Shima, Y. Kuwada, T. Kanzawa, and K. Ogata, *Takeda Kenkyusho Nempo*, **22**, 133 (1963); *Chem. Abstr.*, **60**, 13242 (1964).

(7) T. W. Miller, L. Chaiet, B. Arison, R. W. Walker, N. R. Trenner, and F. J. Wolf, *Antimicrobial Agents Chemotherapy*, 58 (1963).

(8) Professor W. Berends, Technological University of Delft, The Netherlands, has kindly informed us that the oral toxicity of toxoflavin is not very high because a major part of toxoflavin is apparently destroyed in the stomach. *Pseudomonas cocovenenans*, according to Professor Berends, produces another very toxic principle, bongkrekic acid, which is a very strong oral poison. *Cf.* D. H. Nugteren and W. Berends, *Rec. Trav. Chim.*, **76**, 13 (1957). We have found that the lethal dose of toxoflavin to BDF mice is 3 mg/kg intraperitoneally.[8b] Toxicity of xanthothricin (which is identical with toxoflavin) was reported by R. A. Machlowitz.[4] (b) Information furnished by contract screeners of the Cancer Chemotherapy National Service Center.

(9) W. Pfleiderer and K. H. Schündehütte, *Ann.*, **615**, 42 (1958).

(10) (a) H. Biltz and H. Wittek, *Ber.*, **54**, 1035 (1921); (b) G. Nübel and W. Pfleiderer, *ibid.*, **95**, 1605 (1962).

(11) The absence of 1-methyl group in this ring system probably hinders the nitrosation reaction. This is illustrated by the ease of nitrosation of 1,3-dimethyl-6-(2-formylhydrazino)uracil[3] and by the difficulty in nitrosating 3-methyl-6-(2-formyl-1-methylhydrazino)uracil.[2]

(12) (a) A. Bischler, *Ber.*, **22**, 2801 (1889); (b) A. Bischler and S. Brodsky *ibid.*, **22**, 2809 (1889).

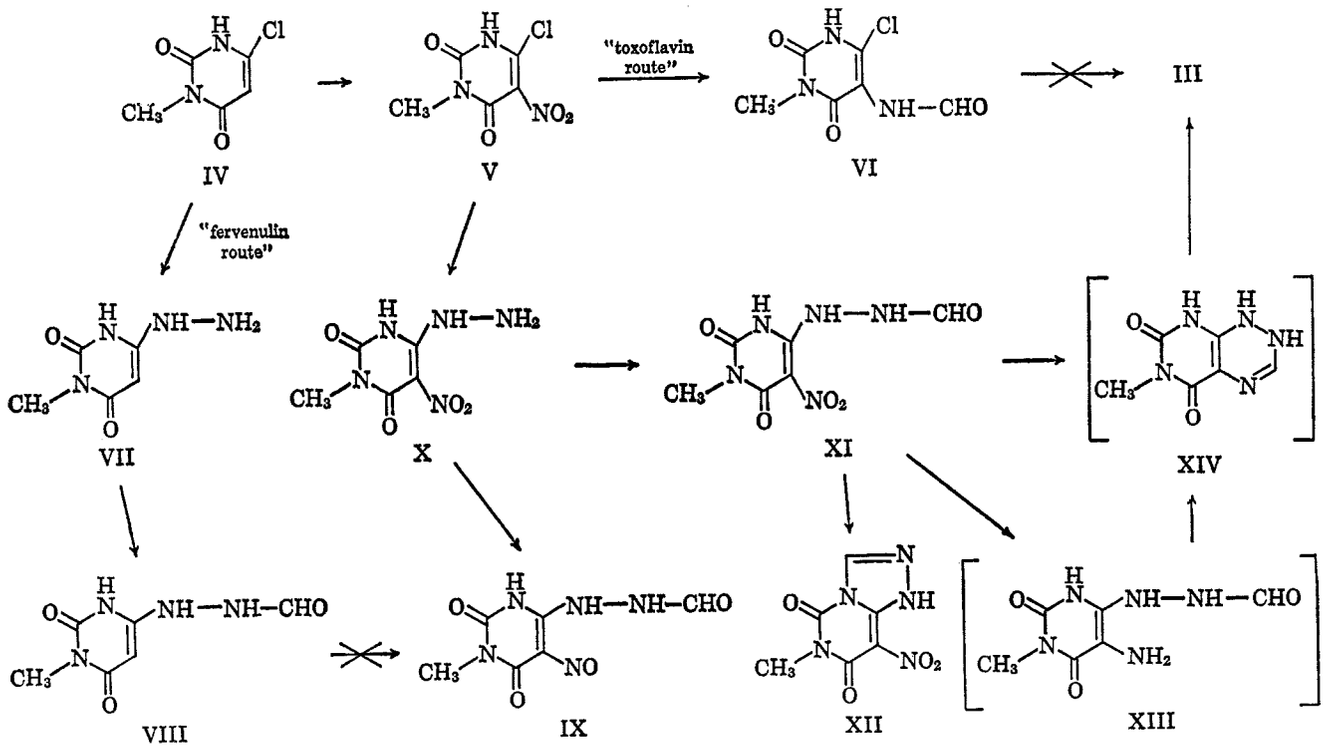
(13) R. A. Abramovitch and K. Schofield, *J. Chem. Soc.*, 2326 (1955).

(14) P. C. Guha and T. N. Ghosh, *J. Indian Chem. Soc.*, **5**, 439 (1928).

(15) J. B. Polya and G. F. Shanks, *J. Chem. Soc.*, 4986 (1964).

(16) C. Temple, Jr., R. L. McKee, and J. A. Montgomery, *J. Org. Chem.*, **28**, 923 (1963).

SCHEME I



ylated derivatives.) Nevertheless, this approach still seemed to be quite inviting since the key intermediate, 3-methyl-5-nitro-6-(2-formylhydrazino)uracil (XI), is readily accessible. When 3-methyl-5-nitro-6-chlorouracil[2] (V) was added to a cold solution of methanolic hydrazine, the corresponding 6-hydrazino derivative, X, was obtained. Treatment of compound X with excess formic–acetic anhydride at room temperature gave XI in good yield.[17,18] The desired compound III was finally obtained by stirring a dilute aqueous solution of 5-amino-6-(2-formylhydrazino)uracil (XIII)—prepared *in situ* by catalytic hydrogenation of XI in methanol—at room temperature in the air (*via* XIV) for 3–4 days. Compound III was isolated by repeated extraction of the aqueous solution with ethyl acetate. The acid-catalyzed cyclization commonly employed for the preparation of condensed *as*-triazine ring systems[12–16] did not prove to be successful for the preparation of III.[19,20]

During the preparation of III, a major side product isolated from the aqueous solution after ethyl acetate extraction was found to be 5,7-dioxo-6-methyl-8-amino-1,5,6,7-tetrahydro-*s*-triazolo[4,3-*c*]pyrimidine (XV).

(17) During the process of recrystallization, compound XI underwent cyclization to yield a substance having mp 251°. On the basis of elementary analysis and the ultraviolet absorption spectra,[18] structure XII was assigned for the product. Compound XII can be obtained directly by refluxing 3-methyl-5-nitro-6-hydrazinouracil (X) in excess formic–acetic anhydride.

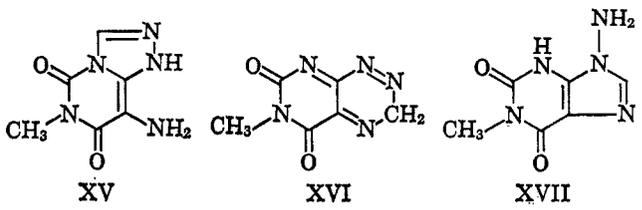
(18) (a) Similar cyclization of 4-chloro-5-amino-6-hydrazinopyrimidine to 8-amino-7-chlorotetrazolo[1,5-*c*]pyrimidine with nitrous acid was reported by C. Temple, Jr., R. L. McKee, and J. A. Montgomery, *J. Org. Chem.*, **27**, 1671 (1962); (b) C. Temple, Jr., R. L. McKee, and J. A. Montgomery, *ibid.*, **28**, 2257 (1963).

(19) The characteristic ultraviolet absorption for III was not detected after XI was stirred in saturated anhydrous methanolic hydrogen chloride at room temperature in the air for 5 days.

(20) Attempted preparation of III, according to the method of Temple, McKee, and Montgomery,[18] by stirring 3-methyl-5-amino-6-hydrazinouracil (prepared *in situ* by the catalytic hydrogenation of X) with ethyl orthoformate in the presence of a catalytic amount of concentrated hydrochloric acid for 4 days did indicate (ultraviolet) the formation of III. However, various efforts to purify this reaction product failed to yield compound III with satisfactory analysis.

Structure of XV was confirmed by comparison of this product with that obtained by the catalytic reduction of XII.

The nmr spectra of III indicated the presence of one single, sharp =CH– peak at 4.89 ppm having a peak height one-third that of the CH_3 peak, which is located at 1.65 ppm [d_5-DMSO (dimethyl-d_5 sulfoxide) as reference]. This would rule out the possibility of compound III existing in structures such as XVI. An alternate possible structure, XVII, was also eliminated by elemental analysis as well as comparison of the ultraviolet absorption spectrum of III with those of N-substituted purines.[21]



A comparison of the ultraviolet absorption spectra of III with those of toxoflavin and fervenulin (see Table I) revealed some interesting facts. In neutral and acidic media the spectra of III and fervenulin are strikingly similar. In the basic medium the spectrum of III (as the anion) resembles that of toxoflavin at pH 1.[22] The slight hypsochromic shift of the λ_{max} of compound III with respect to that of fervenulin and toxoflavin under the aforementioned conditions is in complete agreement with the general relationship between a hydrogen atom and a methyl group in a

(21) J. A. Montgomery and C. Temple, Jr. [*J. Am. Chem. Soc.*, **82**, 4592 (1960)] reported for 9-aminopurine λ_{max} 262.5 mμ (0.1 *N* HCl), 264 mμ (pH 7), and 265.5 mμ (0.1 *N* NaOH); for 9-aminohypoxanthine λ_{max} 250 mμ (0.1 *N* HCl), 249 mμ (pH 7), and 254 mμ (0.1 *N* NaOH).

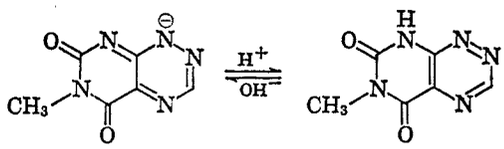
(22) The instability of toxoflavin in strong alkaline solution has been reported previously. See references cited in ref 2 and 8.

TABLE I

ULTRAVIOLET ABSORPTION OF TOXOFLAVIN, FERVENULIN, AND III

Medium	λ_{max}, mμ (ε) Toxoflavin	Fervenulin	III
Ethanol	258 (22,500)	238 (18,500)	234 (15,800)
		275 (1600)	260 (sh) (3900)
	391 (5500)	340 (4200)	334 (2050)
pH 1	258 (18,400)	237 (17,000)	232 (17,350)
		275 (1950)	272 (2150)
	394 (4500)	341 (4250)	333 (5350)
pH 11		230 (sh) (4850)	253 (20,400)
		275 (1900)	
	320 (6400)		388 (4150)

homologous series.[23] Compound III is apparently more stable (anionic stabilization) than toxoflavin in basic medium, since reacidification of compound III from pH 1 restored the original absorption spectrum (λ_{max} 232, 272, 333 mμ) but reacidification of toxoflavin failed to do so.[22] These facts suggest that an equilibrium existed between 1-demethyltoxoflavin and 8-demethylfervenulin under different environmental conditions.



Experimental Section[24]

3-Methyl-6-hydrazinouracil (VII).—A solution of 16.1 g of 3-methyl-6-chlorouracil,[10] 5.0 g of 95% hydrazine, and 125 ml of 2-ethoxyethanol was refluxed for 20 min. The reaction solution was then chilled and the resulting precipitate was filtered with suction, washed with ethanol, and air dried to give 15 g of VII, mp 230–234° dec. The product was pure enough for the following experiment. An analytical sample was obtained by recrystallization from ethanol: mp 236–238°.

Anal. Calcd for $C_5H_8N_4O_2$: C, 38.5; H, 5.15; N, 35.9. Found: C, 38.4; H, 5.12; N, 35.9.

3-Methyl-6-(2-formylhydrazino)uracil (VIII).—To 5.0 g of VII in 85 ml of absolute ethanol was added 30 ml of formic–acetic anhydride. The mixture was then stirred vigorously at room temperature for 4 hr. The resulting precipitate was collected by filtration and recrystallized from a mixture of methanol and water to yield 3.5 g of VIII, mp 222–223°.

Anal. Calcd for $C_6H_8N_4O_3$: C, 39.1; H, 4.37; N, 30.4. Found: C, 39.3; H, 4.62; N, 30.2.

3-Methyl-5-nitro-6-hydrazinouracil (X).—A solution of 17.0 g of 3-methyl-5-nitro-6-chlorouracil[2] in 50 ml of anhydrous methanol was added dropwise with vigorous stirring to a chilled (0°) solution of 3.2 g of hydrazine in 300 ml of anhydrous methanol. After 1 hr the resulting precipitate was collected by filtration, washed successively with methanol and ether, then air dried. The yield of X, decomposing at 205°, was nearly quantitative. Recrystallization three times from water gave an analytically pure sample which decomposed at 211–211.5°.

Anal. Calcd for $C_5H_7N_5O_4 \cdot 0.5H_2O$: C, 28.6; H, 3.83; N, 33.3. Found: C, 28.7; H, 3.74; N, 33.6.

3-Methyl-5-nitro-6-(2-formylhydrazino)uracil (XI).—To 100 ml of formic–acetic anhydride cooled in an ice–water bath was added portionwise, with vigorous stirring, 10 g of X (the addition took *ca.* 30 min). After the addition, the reaction mixture was allowed to stir overnight under anhydrous conditions at room temperature. The resulting fine precipitate was collected by filtration, washed with ethanol, then ether, and finally air dried. The yield was 7.5 g: $\lambda_{max}^{pH\ 1}$ 227 mμ (ε 22,000), 318 mμ (ε 17,300); $\lambda_{max}^{pH\ 11}$ 230 mμ (ε 20,400), 260 (21,200), 320 (14,900). The product melted at 210°, followed by resolidification, and melted again at 251°.

Anal. Calcd for $C_6H_7N_5O_5$: C, 31.4; H, 3.08; N, 30.6. Found: C, 31.2; H, 3.13; N, 30.7.

When XI was heated with ethanol and water, the resulting material, mp 251°, was identified (see below) as XII.

5,7-Dioxo-6-methyl-8-nitro-1,5,6,7-tetrahydro-*s*-triazolo[4,3-*c*]pyrimidine (XII).—A mixture of 2 g of X, 15 ml of formic–acetic anhydride, and 300 ml of ethyl acetate was refluxed for 2 hr. The reaction solution was cooled and the resulting precipitate filtered and recrystallized from a mixture of dimethylformamide and water to give 2 g of product: mp 251°; $\lambda_{max}^{pH\ 1}$ 249 mμ (ε 17,400), 328 mμ (ε 16,700); $\lambda_{max}^{pH\ 11}$ 352 mμ (ε 15,100).

Anal. Calcd for $C_6H_5N_5O_4$: C, 34.2; H, 2.38; N, 33.2. Found: C, 34.5; H, 2.46; N, 33.4.

5,7-Dioxo-6-methyl-8-amino-1,5,6,7-tetrahydro-*s*-triazolo[4,3-*c*]pyrimidine (XV).—A mixture of 3 g of finely pulverized XII in 200 ml of anhydrous methanol was hydrogenated with 0.4 g of platinum oxide at room temperature and 60 psi of hydrogen. The hydrogen uptake was completed after 20 hr. The reaction mixture was immediately acidified with an ethereal solution of hydrogen chloride and the catalyst was removed by filtration. The resulting pale yellow filtrate was evaporated to dryness at room temperature under reduced pressure. Two grams of solid was collected. Trituration of the product from a mixture of propanol and methanol afforded a light tan powder, which turned red at 150°, became white at 240°, partially melted at 260°, and finally melted with decomposition at 280°: $\lambda_{max}^{pH\ 1}$ 264 mμ (ε 11,100), $\lambda_{max}^{pH\ 11}$ 281 mμ (ε 12,000). The product was dried at 100° (0.03 mm) for 15 hr before analysis.

Anal. Calcd for $C_6H_7N_5O_2 \cdot HCl$: C, 33.1; H, 3.71; Cl^-, 16.3; N, 32.2. Found: C, 33.4; H, 4.00; Cl^-, 16.6; N, 31.6.

6-Methyl-5,7-dioxo-1,5,6,7-(5,6,7,8)-tetrahydropyrimido[5,4-*e*]-*as*-triazine (III).—A mixture of 5.0 g of XI, 0.5 g of 5% platinum on charcoal, 0.5 ml of 28% aqueous ammonia, and 150 ml of methanol was hydrogenated under 60 psi of hydrogen at room temperature until the theoretical amount of hydrogen was absorbed (*ca.* 2.5 hr). The reaction mixture was diluted with 250 ml of water, and the catalyst was filtered. The filtrate was then stirred at room temperature for 4 days (the color of solution gradually changed from colorless to fluorescent yellow) and was then extracted repeatedly with sixteen 250-ml portions of ethyl acetate; the extract was evaporated to dryness *in vacuo* to yield 1.9 g of a yellow residue. Recrystallization of the residue from propanol gave 1.1 g of yellow solid which decomposed sharply at 230°. Two more recrystallizations from a mixture of water and ethanol yielded yellow crystals which decomposed at 239–240°.

Anal. Calcd for $C_6H_5N_5O_2$: C, 40.2; H, 2.81; N, 39.1. Found: C, 40.4; H, 2.93; N, 38.9.

The aqueous layer was acidified with dilute hydrochloric acid and then evaporated to dryness. After trituration from a mixture of propanol and methanol, 3.0 g of light tan powder was obtained. This by-product was found to be identical with XV in its melting behavior as well as in the ultraviolet absorption maxima in both pH 1 and pH 11.

Acknowledgments.—The authors wish to express their appreciation to Professor Roland K. Robins, Professor Wolfgong Pfleiderer, and Dr. G. Doyle Daves, Jr., for their continued interest and encouragement; to Dr. John A. Montgomery and Professor James A. Moore for their suggestions; to Mr. Wayne H. Nyberg, Mr. Leland R. Lewis, Mr. Hal P. Van Fossen, Mrs. Margaret L. Rounds, and Mr. John R. Gravatt for their valuable assistance in performing technical, analytical, and instrumental measurements; and to Dr. James J. Downs for the nmr discussions.

(23) *Cf.* R. N. Jones, *Chem. Rev.*, **32**, 11 (1943), and references cited therein.

(24) All melting points were taken on a Thomas–Hoover melting point apparatus. The ultraviolet absorption spectra were determined with a Beckman DK-2 spectrophotometer. The nmr spectra were run on a Varian A-60 high-resolution nmr spectrophotometer.