New Syntheses of Fervenulin (1,3-Dimethyl-7-azalumazine) and Analogs

Fumio YONEDA and Tomohisa NAGAMATSU

Faculty of Pharmaceutical Sciences, Kumamoto University, Oe-honmachi, Kumamoto 862

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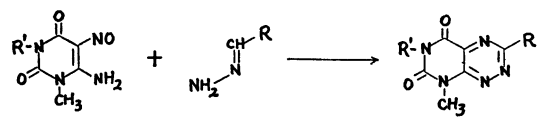
A new, convenient method for the synthesis of fervenulin and its derivatives on the basis of the condensation of 6-amino-5-nitrosouracils and aldehyde hydrazones is described. A transformation of toxoflavins into fervenulins *via* 1-demethyltoxoflavins is also described. The alkylation of 1-demethyltoxoflavin with several alcohols in the presence of diethyl azodicarboxylate and triphenylphosphine gave the corresponding fervenulin homologs.

Fervenulin, an antibiotic having pyrimido[5,4-*e*]-*as*-triazine ring system, was first synthesized in 1958 by Pfleiderer and Schündehütte.[1] Since then there has been considerable interest in the synthesis and the reaction of pyrimido[5,4-*e*]-*as*-triazine derivatives.[2] This paper will describe a new, convenient method for the synthesis of fervenulin and its derivatives on the basis of the condensation of 6-amino-5-nitrosouracils and aldehyde hydrazones; additionally, we wish to describe a transformation of toxoflavins into fervenulins *via* 1-demethyltoxoflavins and the synthesis of homologs of fervenulin by the alkylation of 1-demethyltoxoflavin.[3]

Reaction of 6-Amino-5-nitrosouracils with Aldehyde Hydrazones

Stirring a suspension of 6-amino-1,3-dimethyl-5-nitrosouracil (**1**) in aprotic solvents, such as dimethylformamide or dimethyl sulfoxide, with an equimolar amount of 37% aqueous formaldehyde and hydrazine hydrate under cooling, followed by a mild refluxing of the mixture, resulted in the formation of fervenulin (**2a**) in about a 40% yield. The product was found by IR and elemental analysis to be identical with an authentic sample prepared by an alternative route.[1] Similarly, the heating of **1** with other aldehydes and hydrazine hydrate in dimethylformamide or dimethyl sulfoxide gave the corresponding 3-substituted fervenulins (6- substituted 1,3-dimethyl-7-azalumazines) (see Table 1).



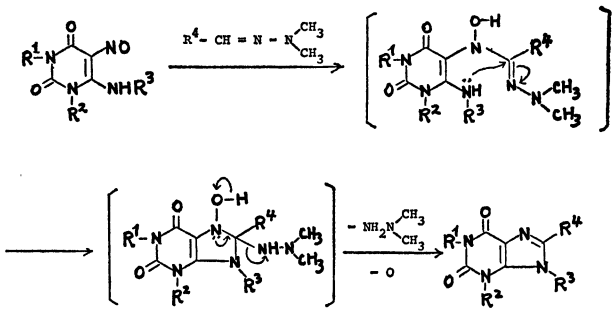
1 R′=CH$_3$ **2a-p** R′=CH$_3$
3 R′=H **4a,b** R′=H

Scheme 1.

The reaction essentially involves **1** and the aldehyde hydrazones, since aldehydes and hydrazine hydrate react immediately to give the hydrazones. In fact, the yields of the 3-substituted fervenulins increased when **1** was allowed to react with the aldehyde hydrazones.

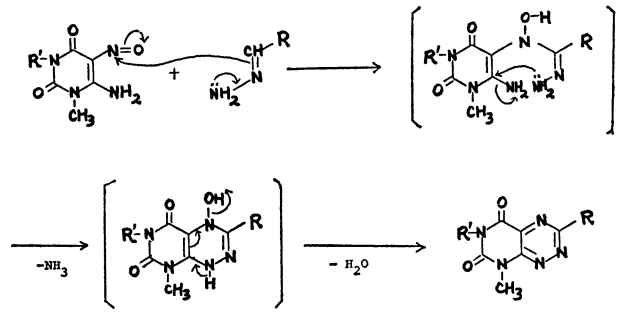
6-Amino-1-methyl-5-nitrosouracil (**3**) also gave the corresponding 7-azalumazine derivatives by the condensation with aldehyde hydrazones. For instance, the heating of **3** with formaldehyde and benzaldehyde hydrazones in dimethylformamide gave 1-methyl-7-azalumazine (**4a**) and 1-methyl-6-phenyl-7-azalumazine (**4b**) respectively. These 7-azalumazines gave the corresponding fervenulins (**2a**, **b**) by treatment with methyl iodide in dimethylformamide in the presence of potassium carbonate.

It is interesting to note that the heating of 6-amino-5-nitrosouracils with aryl aldehyde 1,1-dimethylhydrazones (instead of aryl aldehyde hydrazones) in dimethylformamide gave 8-arylxanthines. This purine synthesis can be explained as is depicted in Scheme 2.[4]



Scheme 2.

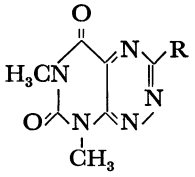
From the above facts, the reaction of the new fervenulin synthesis seems to proceed by the initial nucleophilic attack of the electron-rich-α-carbon of the hydrazones on the nitroso group of the uracils to form hydroxylamine intermediates, which are then converted into fervenulins by the elimination of the ammonia and water.



Scheme 3.

Another explanation of the mechanism would be initial transamination of the 6-amino-5-nitrosouracils by the aryl aldehyde hydrazones. It is possible that the previously formed 6-benzylidenehydrazino-5-nitrosouracils undergo intramolecular cyclization to give fervenulins. However, this mechanism seems unlikely, because the transamination of 6-amino-5-nitrosouracils with amines usually requires more drastic conditions

TABLE 1. SYNTHESIS OF FERVENULINS BY THE REACTION OF 6-AMINO-1,3-DIMETHYL-5-NITROSOURACIL (1) WITH ALDEHYDES AND HYDRAZINE HYDRATE

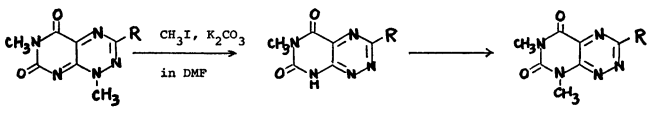


Aldehyde	3-Substituent (R) in product	Mp (°C)	Yield (%)	Formula	Analysis (%) Calcd (Found) C	H	N
Formaldehyde	Hydrogen (**2a**)[1]	175	40	$C_7H_7N_5O_2$	43.52 (43.23)	3.65 (3.60)	36.26 (35.98)
Benzaldehyde	Phenyl (**2b**)[2]	270	53	$C_{13}H_{11}N_5O_2$	57.98 (57.77)	4.12 (4.06)	26.01 (26.21)
4-Chlorobenzaldehyde	4-Chlorophenyl (**2c**)	280	62	$C_{13}H_{10}N_5O_2Cl$	51.41 (51.27)	3.32 (3.32)	23.06 (23.14)
3,4-Dichlorobenzaldehyde	3,4-Dichlorophenyl (**2d**)	249	67	$C_{13}H_9N_5O_2Cl_2$	46.17 (45.88)	2.68 (2.53)	20.71 (20.45)
4-Bromobenzaldehyde	4-Bromophenyl (**2e**)	303	48	$C_{13}H_{10}N_5O_2Br$	44.84 (44.78)	2.90 (2.93)	20.12 (20.02)
4-Nitrobenzaldehyde	4-Nitrophenyl (**2f**)[2]	323	62	$C_{13}H_{10}N_6O_4$	49.68 (49.44)	3.21 (3.20)	26.74 (26.38)
Salicylaldehyde	2-Hydroxyphenyl (**2g**)	282	85	$C_{13}H_{11}N_5O_3$	54.73 (54.87)	3.89 (3.69)	24.55 (24.53)
Anisaldehyde	4-Methoxyphenyl (**2h**)	268	78	$C_{14}H_{13}N_5O_3$	56.18 (56.22)	4.38 (4.37)	23.40 (23.33)
Veratraldehyde	3,4-Dimethoxyphenyl (**2i**)	305	58	$C_{15}H_{15}N_5O_4$	54.71 (54.39	4.59 (4.44)	21.27 (21.36)
Piperonal	3,4-Methylenedioxyphenyl (**2j**)	274	72	$C_{14}H_{11}N_5O_4$	53.67 (53.70)	3.54 (3.61)	22.36 (22.29)
4-Dimethylaminobenzaldehyde	4-Dimethylaminophenyl (**2k**)	>330	73	$C_{15}H_{16}N_6O_2$	57.68 (57.44)	5.16 (5.08)	26.91 (26.73)
Cinnamaldehyde	Styryl (**2l**)	263	45	$C_{15}H_{13}N_5O_2$	61.01 (60.89)	4.44 (4.42)	23.72 (23.82)
Picolinaldehyde	2-Pyridyl (**2m**)[2]	285	45	$C_{12}H_{10}N_6O_2$	53.33 (53·31)	3.73 (3.67)	31.10 (31.06)
Nicotinaldehyde	3-Pyridyl (**2n**)[2]	213	57	$C_{12}H_{10}N_6O_2$	53.33 (53.24)	3.73 (3.73)	31.10 (29.83)
Isonicotinaldehyde	4-Pyridyl (**2o**)[2]	262	47	$C_{12}H_{10}N_6O_2$	53.33 (53.38)	3.73 (3.77)	31.10 (31.46)
Thiophene-2-aldehyde	2-Thienyl (**2p**)	272	25	$C_{11}H_9N_5O_2S$	47.99 (47.83)	3.30 (3.32)	25.44 (25.27)

(*e.g.* fusion at a higher temperature in the presence of an acid catalyst).[5]

The Transformation of Toxoflavins into Fervenulins

Toxoflavin,[6,7] another antibiotic with pyrimido-[5,4-*e*]-*as*-triazine ring system, is isomeric with fervenulin. We are the first to have succeeded in the transformation of toxoflavin and its derivatives into fervenulin and its derivatives respectively. Heating toxoflavin (**5a**) under reflux with methyl iodide in dimethylformamide containing anhydrous potassium carbonate for 2 hr gave fervenulin (**2a**) in a moderate yield in a single step. Similarly, heating other toxoflavin derivatives with methyl iodide in dimethylformamide in the presence of potassium carbonate led to the formation of the corresponding 3-substituted fervenulins (Table 2).



5a-c, i, n **6** **2a-c, i, n**

Scheme 4.

The reaction essentially involves the demethylation of the toxoflavins (**5**) to 1-demethyltoxoflavins (**6**), and the subsequent methylation of **6** with methyl iodide. Both heating **5** with excess methyl iodide in dimethylformamide in the absence of potassium carbonate, and heating **5** alone in dimethylformamide in

Table 2. Conversion of toxoflavins into fervenulins

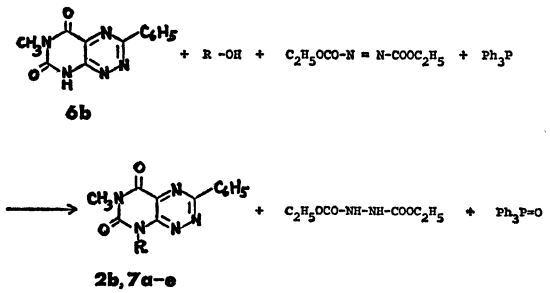
3-Substituent (R) of starting materials (Toxoflavins)	3-Substituent (R) of products (Fervenulins)	Yield (%)
Hydrogen (**5a**)	Hydrogen (**2a**)	35
Phenyl (**5b**)	Phenyl (**2b**)	52
4-Chlorophenyl (**5c**)	4-Chlorophenyl (**2c**)	61
3,4-Dimethoxyphenyl (**5i**)	3,4-Dimethoxyphenyl (**2i**)	57
3-Pyridyl (**5n**)	3-Pyridyl (**2n**)	66

the presence of potassium carbonate yielded only the corresponding **6** in almost quantitative yields.[8]

The Alkylation of 1-Dimethyltoxoflavins

Mistunobu *et al.*[9] have reported a convenient alkylation of phthalimide with alcohols in the presence of equimolar amounts diethyl azodicarboxylate (DAD) and triphenylphosphine. We have extended this reaction to the alkylation of **6**,[8] which was easily prepared by the demethylation of **5** with dimethylformamide.

Refluxing a mixture of 3-phenyl-1-demethyltoxoflavin (**6b**) and an alcohol in dioxane in the presence of DAD and triphenylphosphine gave the corresponding 1-alkyl-3-methyl-6-phenyl-7-azalumazines (Table 3). Some of these compounds were identified with the authentic samples prepared by the alkylation of **6b** with the corresponding alkyl halides. When **6b** and ethylene glycol were used in this reaction, 1-(2-hydroxyethyl)-3-methyl-6-phenyl-7-azalumazine (**7e**) was obtained.



Scheme 5.

Experimental

The melting points were determined on a Yanagimoto Micro-melting Point apparatus and are uncorrected. The infrared spectra were determined on a Japan Spectroscopic Co., Ltd., spectrophotometer, Model IR-I A, from samples mulled in Nujol.

*Fervenulin (1,3-Dimethyl-7-azalumazine) (**2a**).* To a cooled suspension of 6-amino-1,3-dimethyl-5-nitrosouracil (**1**) (1.8 g, 0.01 mol) in DMF (50 ml), we added 37% formaldehyde (0.8 g, 0.01 mol) and 100% hydrazine hydrate (0.5 g, 0.01 mol). The mixture was stirred for about 10 min under cooling with ice water and then refluxed for 3 hr. After the solvent had been evaporated to dryness, the residue was extracted with chloroform. The chloroform extracts were evaporated, and the resulting residue was treated with 50% aqueous MeOH to cause the separation of 0.76 g (40%) of **2a** which was identical in all respects with an authentic sample.[1]

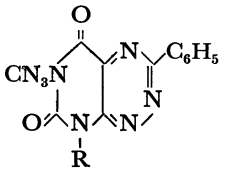
*3-Substituted Fervenulins (**2b-p**); General Procedure*: A mixture of **1** (0.01 mol), an aryl aldehyde (0.01 mol), and 100% hydrazine hydrate (0.01 mol) in DMF (50 ml) was refluxed for 3—5 hr. The reaction mixture was then evaporated to dryness, and the residue was diluted with EtOH in order to precipitate a crude product, which was recrystallized from EtOH to give the 3-arylfervenulin as yellow needles.

Sometimes a small amount of a by-product, the corresponding 8-aryltheophylline, was isolated from the mother liquor.

*1-Methyl-7-azalumazine (**4a**).* To a suspension of 6-amino-1-methyl-5-nitrosouracil (**3**) (2 g, 0.012 mol) in DMF (50 ml), we added 37% formaldehyde (0.96 g, 0.012 mol) and 100% hydrazine hydrate (0.6 g, 0.012 mol) under cooling in ice water with continuous stirring, after which the mixture was refluxed for 4 hr. The reaction mixture was then concentrated *in vacuo*, and the residue was diluted with EtOH to precipitate crystals, which were subsequently recrystallized from EtOH to give 1.25 g (58%) of pale brown crystals; mp>260 °C (decomp.). MS *m/e* 179 (M^+). Found: C, 40.20; H, 2.77; N, 38.87%. Calcd for $C_6H_5N_5O_2$: C, 40.23; H, 2.81; N, 39.10%.

*1-Methyl-6-phenyl-7-azalumazine (**4b**).* A mixture of **3** (2 g, 0.012 mol), benzaldehyde (1.25 g, 0.012 mol), and 100% hydrazine hydrate (0.6 g, 0.012 mol) in DMF (50 ml) was refluxed for 5 hr. After the DMF had been evaporated, the residue was treated with EtOH to precipitate crude **4b**, which was collected by filtration and recrystallized from

Table 3. Preparation of 1-alkyl-3-methyl-6-phenyl-7-azallmazines



1-Alkyl (R)	Mp (°C)	Yield (%)	Appearance	Formula	Analysis (%) Calcd (Found) C	H	N
Methyl (**2b**)	270	51	yellow needles	$C_{13}H_{11}N_5O_2$	—	—	—
Ethyl (**7a**)	228	60	yellow prisms	$C_{14}H_{13}N_5O_2$	59.35(59.31)	4.63(4.70)	24.72(24.50)
n-Propyl (**7b**)	214	67	yellow needles	$C_{15}H_{15}N_5O_2$	60.59(60.57)	5.09(5.01)	23.56(23.60)
i-Propyl (**7c**)	226	76	yellow needles	$C_{15}H_{15}N_5O_2$	60.59(60.48)	5.09(5.08)	23.56(23.54)
Allyl (**7d**)	213	78	yellow needles	$C_{15}H_{13}N_5O_2$	61.01(61.09)	4.44(4.48)	23.72(23.79)
2-Hydroxyethyl (**7e**)	215	95	yellow prisms	$C_{14}H_{13}N_5O_3$	56.18(56.37)	4.38(4.36)	23.40(23.50)

DMF+EtOH to give 1.45 g (47%) of yellow crystals; mp> 280 °C (decomp.). MS *m/e* 255 (M^+). Found: C, 56.38; H, 3.67; N, 27.23%. Calcd for $C_{12}H_9N_5O_2$: C, 56.47; H, 3.55; N, 27.44%.

In the mother liquor, a trace of 3-methyl-8-phenylxanthine[10] was detected.

Methylation of ***4b*** *with Methyl Iodide.* A mixture of **4b** (0.5 g, 0.002 mol), CH_3I (1.4 g, 0.01 mol), and K_2CO_3 (0.7 g, 0.005 mol) in DMF (20 ml) was refluxed for 2 hr. The reaction mixture was then concentrated *in vacuo*, and the residue was diluted with H_2O to precipitate 0.3 g (56%) of 3-phenylfervenulin, which was found by IR to be identical with the **2b** prepared by the above method.

Conversion from Toxoflavins into Fervenulins; General Procedure: To a mixture of CH_3I (0.05 mol) and K_2CO_3 (0.01 mol) in DMF (50 ml), we added a toxoflavin (0.005 mol), after which the mixture was refluxed for 2—3 hr. After the solvent had been evaporated *in vacuo*, the resulting residue was diluted with H_2O to separate yellow crystals. Recrystallization from EtOH gave the corresponding fervenulin.

*Alkylation of 3-Phenyl-1-demethyltoxoflavin (****6b****) General Procedure*: To a suspension of **6b** (0.002 mol) and triphenylphosphine (0.03 mol) in dioxane (30 ml), we added diethyl azodicarboxylate (0.004 mol) and an alcohol (0.01—0.02 mol), after which the mixture was refluxed for 3 hr. The reaction mixture was then evaporated *in vacuo*, followed by dilution with benzene or EtOH to precipitate a crude sample, which was collected by filtration and recrystallized from EtOH to give the corresponding homologs of fervenulin (1-alkyl-3-methyl-7-azalumazines).

Ethylation of ***6b*** *with Ethyl Iodide.* A mixture of **6b** (1.5 g, 0.005 mol), C_2H_5I (3.9 g, 0.025 mol), and K_2CO_3 (3.5 g, 0.025 mol) in DMF (50 ml) was refluxed for 2 hr. After the insoluble material had been filtered off, the filtrate was concentrated to dryness *in vacuo* and the residue was diluted with H_2O to give crystals. Recrystallization from EtOH gave 1 g (71%) of 8-ethyl-3-methyl-6-phenyl-7-azalumazine (**7a**), which was found by IR and elemental analysis to be identical with the product prepared as has been described above.

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2) For references to this ring system, see Blankenhorn and W. Pfleiderer, *Chem. Ber.*, **105**, 3334 (1972).

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