DIFFERENT MODES OF ALKALINE HYDROLYSIS OF FERVENULIN AND ISOFERVENULIN

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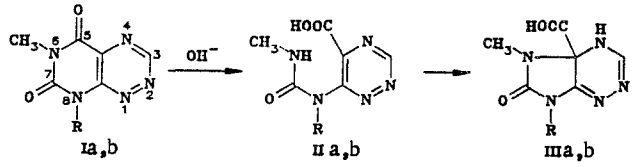
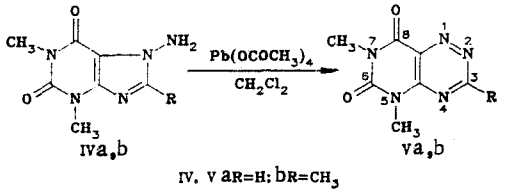
Unlike rheumycin and fervenulin, isofervenulin and 3-methylisofervenulin are hydrolyzed by aqueous alkalies at the $N_{(5)}$—$C_{(6)}$ bond. On acidification of the reaction mixture, the N-carboxy-N-methylcarbamoyltriazines formed are reconverted into the starting isofervenulins, and on basification (pH > 10) into methylaminotriazinecarboxamides. A by-product of the alkaline hydrolysis of isofervenulin is a product of the contraction of the uracil ring, namely imidazotriazinone-7a-carboxylic acid.

It has been reported [1, 2] that pyrimido[5,4-e]-1,2,4-triazine-5,7-diones, in particular the antibiotics rheumycin (Ia) and fervenulin (Ib), are hydrolyzed by aqueous alkali to 6-ureido-1,2,4-triazine-5-carboxylic acids (II). The latter are capable of undergoing cyclization to imidazo[5,4-3]-1,2,4-triazinone-4a-carboxylic acids (III):



I–III a R=H; b R=CH_3

The object of this investigation was to examine the alkaline hydrolysis of pyrimido-[4,5-e]-1,2,4-triazine-6,8-diones, which are structural isomers of (I). According to earlier findings [3], the course of hydrolysis could be different. As starting materials, we selected isofervenulin (Va) and its 3-methyl derivative (Vb), obtained by oxidizing 7-aminotheophyllin (IVa) and 7-amino-8-methyltheophyllin (IVb) with lead tetraacetate in methylene chloride [4]. The amines (IV) were prepared by N-aminating theophyllin and 8-methyltheophyllin with hydroxylamine-O-sulfonic acid in alkali.



IV, V aR=H; bR=CH_3

In the alkaline hydrolysis of (V), unlike that of rheumycin and fervenulin, the site of initial attack of the hydroxyl ion is the $C_{(6)}$ carbonyl of the ureido-group. Subsequent opening of the pyrimidine ring gives the acid (VI) (apparently as the anion). The end-products are the 5-methylamino-6-(N-methylcarbamoyl)-1,2,4-triazines (VIIa, b), obtained preparatively in yields of 55 and 82% respectively. In addition to these reactions, under these conditions a side reaction takes place to a small extent, involving nucleophilic attack by the hydroxyl ion on the carbonyl $C_{(8)}$ followed by contraction of the pyrimidine ring, to give (IXa, b).

The use of PMR spectroscopy allowed the sequence of reactions of pyrimido-[4,5-e]pyrimidines (Va, b) in alkali to be followed. The characteristic changes in the PMR spectra of

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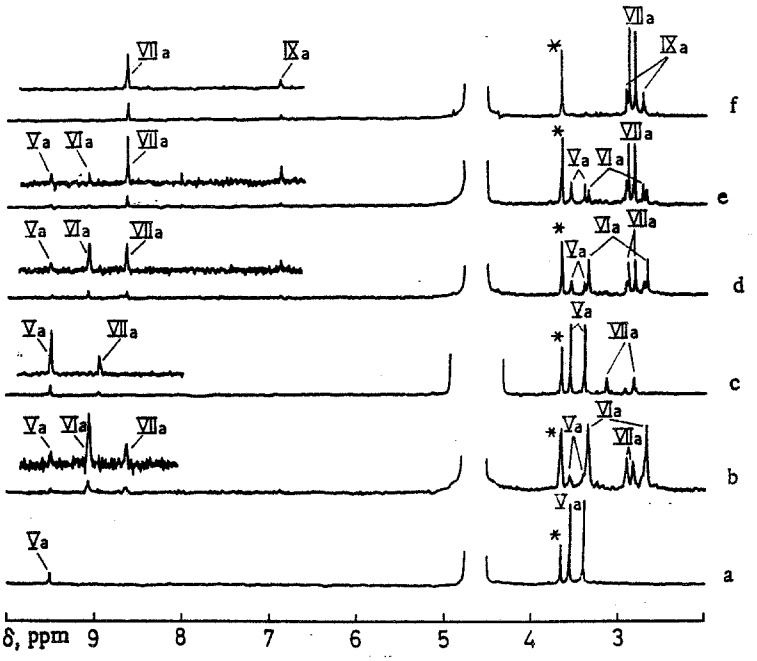


Fig. 1. PMR spectra of the reaction mixture of solutions of isofervenulin (Va) and NaOD in D_2O. The signal for the internal standard (dioxane, δ 3.65 ppm) is marked with an asterisk. a) Original solution of isofervenulin (Va), b) ten minutes after addition of alkali, pH 9, c) addition to reaction mixture ten minutes after commencement of reaction (spectrum (b)) of 1 N DCl, pH 4, d) 15 minutes after addition of alkali, e) 20 minutes after addition of alkali, f) 90 minutes after addition of alkali, pH > 10.

TABLE 1. Chemical Shifts of the PMR Signals of (V-VII) and (IX) in Spectra of the Reaction Mixtures (D_2O/NaOD)

Compound	δ, ppm		
	3-R	$N_{(5)}$—CH_3	$N_{(7)}$—CH_3
Va	9,50	3,54	3,39
VIa	9,06	2,68	3,34
VIIa	8,62	2,82*	2,89*
IXa	6,87	2,72*	2,92*
Vb	2,81	3,53	3,37
VIb	2,59	2,66	3,33
VIIb	2,42	2,81*	2,88*
IXb	1,82	2,69*	2,91*

*Assignment of signals may be changed.

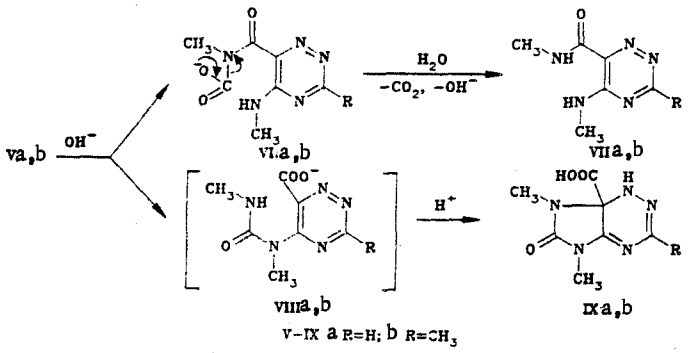
the reaction mixtures depending on the pH of the solutions and reaction time are shown in Fig. 1 for isofervenulin (Va). Basification of 1% solutions of (Va) and (Vb) in D_2O with 1 N solutions of NaOD or Na_2CO_3 to pH ~9 resulted in the appearance in the PMR spectra obtained after ten minutes of strong signals for groups of protons in the intermediates (VIa) with chemical shifts of 2.68, 3.34, and 9.06 ppm (Fig. 1, spectrum (b)) or (VIb) with shifts of 2.66, 3.33, and 2.59 ppm (Table 1), together with signals of lower intensity for the final reaction products (VIIa) (2.82, 2.89, and 8.62 ppm) (Fig. 1, spectrum (B)) or (VIIb) (2.81, 2.88, and 2.42 ppm)(Table 1). The spectra of the reaction mixtures also show PMR signals of low intensity for the starting materials (Va) with chemical shifts of 3.54, 3.39, and 9.50 ppm (Fig. 1, spectrum (b)) or (Vb) (3.53, 3.37, and 2.81 ppm) (Table 1). Assignment of the PMR signals to individual groups of protons in (VIa) and (VIb) in the reaction

products was carried out by examining the changes in the relative intensities of the signals in the spectra of the reaction mixtures with time (Fig. 1, spectra a, b, c, d) and the shift values of the N-methyl protons taking into account descreening by the α-carbonyl groups and the triazine ring. For instance, the signals for the $N_{(5)}$–CH_3 groups in (VIa, b) are shifted to higher field by ~0.9 ppm as compared with the signals of the analogous protons in the amide fragments in the starting materials (Va, b), and undergo little change in position when (VIa, b) are converted into (VIIa, b) as a result of the absence of a single α-carbonyl group.

Opening of the pyrimidine ring is reversible. Acidification of the reaction mixtures (Fig. 1, spectrum (c)) with 1 N DCl in D_2O to pH < 7 affords the starting materials (Va, b).

Basification of the reaction mixtures to pH > 10 gives predominantly the carbamoyltriazines (VIIa, b) (Fig. 1, spectrum f) (cf. [3]). In addition to signals for (VIIa, b), the spectra of the reaction mixture when the reaction is complete (1h 30 min at 30°C) contain signals for the by-products (IXa, b) (Fig. 1, spectrum (F)). The yields of (IXa, b) under these conditions do not exceed 10%. The compound (IXa) was isolated in the pure state, and its structure proved by UV, PMR, and high resolution mass spectrometry by comparison with the corresponding features of the imidazoltriazinone-4a-carboxylic acids (IIIa, b), which are the alkaline hydrolysis products of rheumycin and fervenulin [2].

Contraction of the pyrimidine ring in isofervenulins (Va, b) requires prior cleavage of the uracil ring at the $N_{(7)}$–$C_{(8)}$ bond to give the ureidotriazine-6-carboxylic acids (VIIIa, b). It was not, however, possible to detect the presence of these compounds in the reaction mixtures from the PMR spectra, perhaps owing to their low stability under the reaction conditions.



A possible reason for the different course of alkaline hydrolysis of (I) and (V) would be the presence of differing positive charges on the carbonyl carbon atoms. This assumption is to some extent supported by ^{13}C NMR spectral data (Table 2). There is known to be an approximate correlation between the chemical shifts of carbon atoms and the electron density localized thereat [5]. The greater the shift, the more descreened is the nucleus, and the lower the electron density at the atom. It will be seen from Table 2 that in the spectra of rheumycin and fervenulin, the signals for $C_{(5)}$ are especially shifted to low field, their shifts (161.8 and 166.9 ppm) being much greater than those of the other carbons, including $C_{(7)}$ (150.8 and 151.0 ppm). It is not surprising that it is the carbonyl $C_{(5)}$ in (I) is also the site of nucleophilic attack. In isofervenulins, the differences between the shift values of the carbons of both carbonyl groups are much smaller, and hence nucleophilic attack is directed towards both $C_{(6)}$ and $C_{(8)}$. However, the preferred attack at $C_{(6)}$ is not in accordance with the relative shift values for $C_{(6)}$ (151.5 and 150.0 ppm) and $C_{(8)}$ (159.7 and 158.1 ppm). This shows that in addition to the effective charges, the course of alkaline hydrolysis is affected by other factors, one of which appears to be covalent hydration of (I), a discussion of which will form the subject of a subsequent communication.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were obtained on a Bruker WH-90 spectrometer [90 (^1H) and 22.62 (^{13}C) NHz] at 30°C. The ^1H chemical shifts are given on the δ scale relative to dioxane as internal standard for solutions in D_2O (δ 3.65 ppm) and to TMS for solutions in $CDCl_3$. ^{13}C chemical shifts were measured from the signals for the solvents $CDCl_3$ (δ 77.0 ppm) and DMSO-d_6 (δ 39.7 ppm), given on the δ scale. Mass spectra were obtained on a MAT-311A spectrometer with direct introduction of the sample into the ion source; accelerating voltage 3.0 kV, cathode emission current 1.0 mA. The UV spectra of (IVa) and (Va, b) were obtained on a

TABLE 2. Chemical Shifts and Coupling Constants in the ^{13}C NMR Spectra of Pyrimidotriazinediones

Compound	δ, ppm (J, Hz), in DMSO-D_6						
	$C_{(3)}$	$C_{(4a)}$	$C_{(5)}$ [a]	$C_{(7)}$ [b]	$C_{(8a)}$	$N_{(6)}CH_3$ [c] q	$N_{(8)}CH_3$ [d] q
Ia	155,1 d (^1J=213,3)	133,5 d (^3J=8,8)	161,8 q (^3J=2,9)	150,8 q (^3J=2,9)	152,9 s	29,0 (^1J=142,7)	—
Ib	154,7 d (^1J=212,5)	134,3 d (^3J=8,1)	166,9 q (^3J=3,0)	151,0 m	152,6m	29,7 (^1J=142,6)	30,2 (^1J=142,7)
Va	158,9 d (^1J=208,5)	151,5m	159,7 m	151,5 m	139,2d (^4J=2,9)	29,6 (^1J=142,6)	29,6 (^1J=143,1)
Vb [e]	169,0 q (^2J=7,0)	149,6 q (^3J=2,7)	158,1 q (^3J=2,7)	150,0 m	134,4s	28,6 (^1J=143,0)	28,4 (^1J=143,5)

[a] For $C_{(8)}$ in (Va, b). [b] For $C_{(6)}$ in (Va, b). [c] For $N_{(7)}$–CH_3 in (Va, b). [d] For $N_{(5)}$–CH_3 in (Va, b). [e] In $CDCl_3$, $\delta_{3\text{-}CH_3}$ = 24.0 ppm, ^1J = 129.4 Hz.

Specord-M40 in methanol, and those of (VIIa, b) and (IXa) on a Pye-Unicam SP8-100 spectrophotometer. IR spectra were obtained on a UR-20 spectrometer in vaseline oil. Melting points were measured on a Boetius hot plate.

7-Aminotheophyllin (IVa). In 250 ml of water there were successively dissolved 18.5 g (0.33 mole) of KOH and 30 g (0.15 mole) of theophyllin monohydrate. The solution was heated to 50°C, and a solution of sodium hydroxylamine-O-sulfonate (obtained by neutralizing 21 g (0.2 mole) of 95% hydroxylamine-O-sulfonic acid with $NaHCO_3$ in 25 ml of water) added over 5 min with stirring. After 20 min, 10 g (0.1 mole) of similarly neutralized hydroxylamine-O-sulfonic acid in 10 ml of water was added. The mixture was stirred for a further hour at 60°C. The solid which separated on cooling to 10°C was filtered off, and washed with ice water (40 ml), cold alcohol (15 ml) and ether (25 ml) to give 15 g (50%) of colorless, felted needles, mp 222°C (from water), in agreement with the value given in [6]. IR spectrum: 1680, 1710 (C=O), 3110 (C–H_8), 3230, 3345 cm^{-1} (NH_2). UV spectrum, λ_{max} (log ε): 272 nm (5.04). PMR spectrum (CF_3COOH): 3.08 (3H, s, N–CH_3); 3.30 (3H, s, N–CH_3); 8.05 ppm (1H, s, 8-H). On acidification of the aqueous filtrate with conc. HCl to pH 5-6 and keeping in the refrigerator, there was obtained approximately 40% of unreacted theophyllin.

7-Amino-8-methyltheophyllin (IVb). To a solution of 1.94 g (0.01 mole) of 8-methyltheophyllin and 1.05 g (0.015 mole) of 85% KOH in 15 ml of water at 50°C was added portionwise over 2-3 min a solution of sodium hydroxylamine-O-sulfonate, obtained by neutralizing 1.9 g (0.015 mole) of the 95% acid with sodium hydrogen carbonate in 5 ml of water. The mixture was stirred for 1 h at 75°C, the same amounts of alkali and sodium hydroxylamine-O-sulfonate added, and the mixture stirred for 2 h 30 min at 75°C. The mixture was evaporated to dryness, and the residue treated with 70 ml of chloroform. The chloroform filtrate was passed through an alumina column, eluent chloroform. First eluted was 0.8 g (38%) of (Vb), colorless crystals, mp 209-210°C (from alcohol). IR spectrum: 1660, 1700 (C=O), 3245, 3295, 3350 cm^{-1} (NH_2). Found: C 45.7; H 5.4; N 33.6%. $C_8H_{11}N_5O_2$. Calculated: C 45.9; H 5.3; N 33.5%, followed by 0.4 g (21%) of 8-methyltheophyllin.

5,7-Dimethylpyrimido[4,5-e]-1,2,4-triazine-6,8(5H,7H)-dione (Va). To a stirred suspension of 0.98 g (5 mmole) of the amine (IVa) in 60 ml of dry methylene chloride was added portionwise at 20°C over 5 min 3.32 g (7.5 mmole) of lead tetraacetate. The pale yellow solution was stirred for 15 min, and 15 ml of ethylene glycol added to decompose unreacted lead tetraacetate. After a further 10 min, 200 ml of water was added, the aqueous layer separated and extracted with methylene chloride (2 × 35 ml). The organic layers were combined, dried, and the solvent removed to give 0.87 g (90%) of yellow crystals of isofervenulin, containing a small amount of an impurity which remained at the origin on the thin-layer chromatogram (silica gel – ethyl acetate). The crude product was dissolved in a small amount of ethyl acetate, and passed through a small column of silica gel, eluent ethyl acetate, to give 0.72 g (75%) of chromatographically pure product as pale yellow crystals, mp 212°C (from isobutanol), in agreement with the value given in [7]. IR spectrum: 1690, 1735 cm^{-1} (C=O). UV spectrum, λ_{max} (log ε): 238.4 (4.24), 310 nm (3.81). PMR spectrum ($CDCl_3$): 3.58 (3H, s, 7-CH_3); 3.70 (3H, s, 5-CH_3); 9.54 ppm (1H, s, 3-H).

3,5,7-Trimethylpyrimido[4,5-e]-1,2,4-triazine-6,8(5H,7H)-dione (Vb) was obtained as for (Va). The crude product was purified by chromatography on a column of alumina, eluent chloroform, the first fraction being collected. Yield 27%, pale yellow crystals, mp 150-152°C (from alcohol). IR spectrum: 1685, 1735 cm^{-1} (C=O). UV spectrum, λ_{max} (log ε): 237.5 (4.19), 309.2 nm (3.86). PMR spectrum ($CDCl_3$): 2.95 (3H, s, 3-CH_3); 3.56 (3H, s, 7-CH_3); 3.67 (3H, s, 5-CH_3). Found: C 46.2; H 4.3; N 34.1%. $C_8H_9N_5O_2$. Calculated: C 46.4; H 4.4; N 33.8%.

Alkaline Hydrolysis of 5,7-Dimethylpyrimido[4,5-e]-1,2,4-triazine-6,8(5H,7H)-dione (Va). To a suspension of 21 mg (0.11 mmole) of (Va) in 1 ml of water was added 0.15 ml of 1 N NaOH solution. The mixture was stirred for 3 h at 20°C, and the solid which separated was filtered off, washed with water, and dried to give 10 mg (55%) of (VIIa), mp 194-196°C (from alcohol). UV spectrum (water), λ_{max} (log ε): 249 (4.28), 322 nm (3.68). PMR spectrum (D_2O): 2.79 (3H, s, NHC$\underline{H}_3$); 2.87 (3H, s, CONHC$\underline{H}_3$); 8.38 ppm (1H, s, 3-H); ($CDCl_3$): 3.00 (3H, d, J = 5.1 Hz, NHC$\underline{H}_3$); 3.06 (3H, d, J = 5.3 Hz, CONHC$\underline{H}_3$); 8.04 (1H, br, N$\underline{H}$CH_3); 8.86 (1H, s, 3-H); 9.07 ppm (1H, br, CON$\underline{H}$CH_3). Mass spectrum, m/z: 167 (M^+).

The aqueous filtrate was acidified with 1 N HCl to pH 4, lyophilized, and the residue extracted by heating with 1 ml of chloroform. The extract was filtered, cooled, and the solid which separated was filtered off and dried to give 1 mg (4%) of (IXa), mp 100-102°C (from chloroform), UV spectrum (water, pH 5), λ_{max} (log ε): 245 nm (3.54). PMR spectrum (D_2O): 2.80 (3H, s, 7-CH_3); 2.94 (3H, s, 5-CH_3); 6.93 ppm (1H, s, 3-H); ($CDCl_3$): 2.97 (3H, s, 7-CH_3); 3.03 (3H, s, 5-CH_3); 6.02 (br); 6.77 ppm (1H, s, 3-H). High-resolution mass spectrum, m/z: 211.0706 (M^+), calculated 211.0705 ($C_7H_9N_5O_3$).

3-Methyl-5-methylamino-6-(N-methylcarbamoyl)-1,2,4-triazine (VIIb) was obtained as for (VIIa), yield 82%, mp 159-160°C (from water). UV spectrum (water), λ_{max} (log ε): 249 (4.06), 321 nm (3.54). PMR spectrum (D_2O): 2.61 (3H, s, 3-CH_3); 2.81 (3H, s, NHC$\underline{H}_3$); 2.88 (3H, s, CONHC$\underline{H}_3$); ($CDCl_3$): 2.42 (3H, s, 3-CH_3); 2.99 (3H, d, J = 5.0 Hz, NHC$\underline{H}_3$); 3.05 (3H, d, J = 5.0 Hz, CONHC$\underline{H}_3$); 8.01 (1H, br, N$\underline{H}$CH_3); 8.94 ppm (1H, br, CON$\underline{H}$CH_3). Mass spectrum, m/z: 181 (M^+).

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