HYDROLYTIC CLEAVAGE OF 6-OXOPURINES AFTER INTRAMOLECULAR ACYLATION AT N-3

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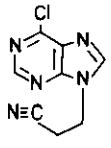
Abstract ——— Reaction of 9H-hypoxanthin-9-yl propionic acid (2c) as well as of its 6-mercapto analogue (2d) with N-[3-(dimethylamino)propyl]-N'-ethylcarbodiimide hydrochloride results in an intramolecular acylation at N-3 followed by a spontaneous hydroxylation at C-2 and opening of the pyrimidine ring. The products of these reaction sequences are the imidazo[1,5-a]pyrimidines (5c,d) which were deformylated to give the compounds 6a,b. The half-life values of the ring transformations of 2c,d as well as of its isosteres 2a,b imply that the "over-all" kinetics are 1. independent of the structure of the five-membered ring and 2. strongly affected by the 6-substituent. It is proposed that the intramolecular acylation is the rate-limiting step of the reaction cascade.

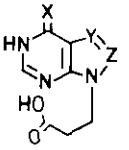
In two previous publications we showed that the N-(2-carboxyethyl) derivatives of pyrrolo[2,3-d]pyrimidin-4-one (2a) and pyrazolo[3,4-d]pyrimidin-4-one (2b) can be converted into the tetrahydro derivatives of the corresponding nitrogen-bridged pyrrolo[1,2-a]pyrimidine (5b) and the pyrazolo[1,5-a]pyrimidine (5a), respectively, via intramolecular acylation followed by hydroxylation and opening of the pyrimidine ring.[1,2] One prerequisite for this reaction is the formation of the paraquinoid structure in the pyrimidine ring and the lack of a bulky substituent at C-2.[3]
In the following we focus our attention on the N-(2-carboxyethyl) derivatives of hypoxanthine (2c) and 6-mercaptopurine (2d) in order to shed some light on the influence of the five-membered ring as well as of the 6-substituent on the ring transformation kinetics.
The starting materials for the intramolecular cyclization were synthesized via 6-chloro-9H-purin-9-ylpropionitrile (1) which was prepared by Michael addition of acrylonitrile with 6-chloropurine.[4] The assignment of N-9 as the alkylation site was

derived from the proton-coupled ^{13}C-NMR spectrum of 1: While C-8 shows a t,d multiplicity (^{1}J(C-8,H-8) = 215.5 Hz; ^{3}J(C-8,CH_2) = 3.7 Hz), C-4 exhibits a complex non-resolved multiplet due to three different ^{3}J couplings to H-2, H-8, and CH_2. On the other hand carbon 5 shows only a doublet due to a ^{3}J coupling with H-8 (12.3 Hz) which excludes a N-7 alkylation. An alkylation of one of the pyrimidine nitrogens could be ruled out since carbon 2 exhibits just a doublet (^{1}J(C-2,H-2) = 210.5 Hz). Hydrolysis of 1 in conc. hydrochloric acid yielded the acid 2c; the acid 2d was obtained by thiolation of 1 using thiourea and subsequent hydrolysis of the nitrile function.[4] The structures of 2c,d were established by ^{1}H- and ^{13}C-NMR spectra (Table 1).

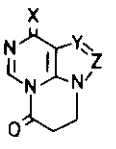
The acids 2c,d allow a regioselective remote acylation of the purine chromophore at N-3. Reaction of the acids 2c,d with an excess of N-[3-(dimethylamino)propyl]-N'-ethylcarbodiimide hydrochloride in water/p-dioxane (1:1, v/v, pH 5-6) results in the precipitation of the imidazo[1,5-a]pyrimidines (5c,d) having a ring system which has not been examined intensively in the literature.[5] The UV maxima of these compounds are bathochromically shifted by 45 and 48 nm, respectively, compared to the corresponding acids 2c,d.



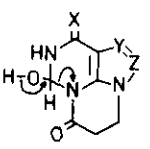
1



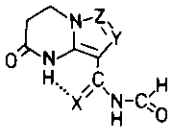
2a-d



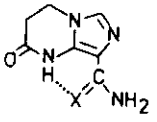
3a-d



4a-d



5a-d



6a: X = O
6b: X = S

	X	Y	Z
a	O	CH	N
b	O	CH	CH
c	O	N	CH
d	S	N	CH

Structural proof of 5c,d came from their proton-coupled as well as decoupled ^{13}C-NMR spectra and from the comparison with those of the starting compounds as well as of the previously described 5a,b (Table 1). The ^{13}C-NMR spectra of 5c,d show the signal of the formyl carbons at 162.1 and 163.4 ppm, respectively. In addition, the C-8a signals of 5c,d (imidazo[1,5-a]pyrimidine numbering) reveal an upfield shift

compared to 2c,d since it is the carbon in the α-position of the acylation site.[6] In addition the C-8 signals are significantly upfield shifted which is in line with the finding on the other heterocyclic systems (5a,b).[1,2] The C-6 signal of 2d which appears in the exocyclic thiocarboxamido group after the reaction exhibits a characteristic downfield shift of 12.1 ppm confirming the proposed structures.

Table 1. ^{13}C-NMR Data of Various Heterocyclic Ring Systems in Me_2SO-d_6[a]

	C-2	C-4	C-5	C-6	C-8	CH_2N	$CH_2C{=}O$/ CH_2CN	CN/ C=O
1	151.5	151.7	130.7	149.1	146.9	39.5	17.8	117.7
2c	145.2	148.2	123.9	156.3	140.1	39.2	33.7	171.6
2d	145.1	143.8	134.2	175.4	143.0	39.7	33.5	171.7
	HC=O	C-8a	C-8	C=S/ C=O	C-6	C-4	C-3	C-2
5c	162.1	137.2	113.0	162.1	132.1	40.2	29.8	166.0
6a		133.8	115.0	165.2	130.9	38.9	30.1	165.7
5d	163.4	141.7	120.7	187.5	132.7	39.3	29.3	166.6
6b		136.7	118.4	187.2	130.8	39.0	29.4	165.8

[a] δ-values of corresponding carbons are listed in the same column.

Deformylation of 5c,d in aqueous ammonia at room temperature yielded the carboxamides 6a,b in high yield. In the ^{1}H-NMR spectra the signals of both methylene groups were almost unchanged compared to 5c,d indicating that no cleavage of the lactam ring had occurred. In both cases the primary amide group reveals two well separated signals.

The most plausible reaction sequence of ring transformation (2a-d → 5a-d) includes the following four reaction steps: 1. formation of the activated esters of 2a-d; 2. intramolecular acylation (3a-d); 3. nucleophilic hydratisation (4a-d); 4. pyrimidine ring cleavage (5a-d).

Since the formation of 5a-d is accompanied by a strong bathochromic shift of the UV spectra the reaction kinetics can simply be followed spectrophotometrically at an appropriate wavelength. In order to normalize the activated ester formation we used in all experiments identical concentrations of substrate (2a-d, 0.11 mM) and water-soluble carbodiimide (EDC, 5.22 mM). As can be seen from Table 2 the half-

life values ($\tau_{1/2}^{app}$) of the conversion of the oxo-compounds 2a-c into 5a-c are almost identical while the reaction of the thiooxo compound 2d is extremely low under the reaction conditions employed. This implies that the influence of the five-membered ring of the starting compounds 2a-d on the "over-all" kinetics is neglectable but that the 6-substituent plays a substantial role.

Table 2. pK Values of Various 2-Carboxyethyl-Nucleobases and $\tau_{1/2}^{app}$ Values of Ring Transformation Reactions

	pK	reaction sequence		$\tau_{1/2}^{app}$ (min)
2a	10	2a	5a	4.5
2b	>11	2b	5b	4.3
2c	10	2c	5c	5.5
2d	8.8	2d	5d	n.d.[a]

[a] not detected; the reaction rate is extremely low under the reaction conditions employed for 2a-c.

One important factor for the formation of the tricyclic intermediates 3a-d is the nucleophilicity of the pyrimidine nitrogen which attacks the activated ester. The thiooxo substituent in 2d lowers the basicity significantly compared with 2a-c which can be seen from the pK values of deprotonation. On the other hand the enhanced electron-withdrawing effect of the sulfur atom in 3d should accelerate the hydroxylation reaction under formation of 4d. This enhancement is obviously superposed by the rate-decreasing effect on the intramolecular acylation so that the first reaction step seems to be rate-limiting.

In this context it is interesting to notice that neither 9H-adenine-9-ylpropionic acid nor the corresponding pyrrolo[2,3-d]- or pyrazolo[3,4-d]pyrimidine derivatives could be cyclized by addition of water-soluble carbodiimide - a result which is in line with recent results of Brahme et al. on intramolecular Michael additions of adenine derivatives.[7] Our findings concerning the spontaneous hydroxylation of the pyrimidine ring of hypoxanthines after remote acylation are confirmed by the result of Itaya, Fujii, and coworkers who studied intensively the synthesis and hydrolytic lability of 3,9-dialkylpurines, especially of 3-methylinosine.[8-10] All the 3,9-disubstituted hypoxanthines possess a pronounced reactivity of the pyrimidine ring

toward alkaline hydrolysis as well as to catalytic hydrogenation. But whereas the dialkylhypoxanthines undergo cleavage of the N(1)-C(2) - bond upon hydroxylation at C-2, in our case the cleavage takes place between C(2) and N(3). This was proved for 5b by X-ray analysis and is due to the strong electron-withdrawing effect of the lactam carbonyl group of 4a-d.[1]

In conclusion our results underline the hypothesis that the formation of a para-quinoid structure of the pyrimidine ring is a requirement of the hypoxanthine oxidation by xanthine oxidase.[1,2,11] For this enzymatic oxidation an intermediate with a sp^3 carbon at C-2 of hypoxanthine is postulated[12] which - in the pyrimidine ring - is analogous to the intermediates 4a-d. Therefore, the spontaneous nonenzymatic hydroxylation of the cyclic intermediates 3a-d may to some extent serve as a model reaction for the first oxidation step of xanthine oxidase.[1,2]

EXPERIMENTAL

Melting points were determined on a Linström apparatus (Wagner-Munz, West-Germany) and are not corrected. UV spectra were measured on an Uvikon 810 spectrophotometer (Kontron, Switzerland); reaction kinetics were assayed on a SuperScan 3 spectrophotometer (Varian, Australia). NMR spectra were recorded on a Bruker WM 250 spectrometer (memory size: 16 K; digital resolution: ^1H, 0.6 Hz; ^{13}C, 2 Hz; δ-values are relative to tetramethylsilane). The mass spectrum was recorded on a Varian MAT 311 A spectrograph. The pK values of deprotonation were determined spectrophotometrically at room temperature in Teorell-Stenhagen buffers (pH 2-12). TLC was performed on silica gel G-25 UV_{254} plates (Macherey-Nagel, West-Germany) with solvent systems A ($CHCl_3$-MeOH, 9:1, v/v); B ($CHCl_3$-MeOH, 8:2, v/v). Elemental analyses were performed by Mikroanalytisches Labor Beller (Göttingen, West-Germany). 6-Chloropurine was purchased from Pharma Waldhof (Darmstadt, West-Germany).

6-Chloro-9H-purin-9-ylpropionitrile (1): 6-Chloropurine (5.15 g, 33 mmol) was converted into its 2-cyanoethyl derivative 1 according to the method of Baker et al.[4] Yield: 3.22 g (45 %) of 1, mp 140°C (EtAc) (lit.[4], 136-139°C). UV (EtAc) λ_{max} 265 nm (lit.[4], 266 nm). ^1H-NMR (Me_2SO-d_6) δ 8.81, 8.76 (H-2, H-8); 4.62 (t, CH_2N, J = 6.5 Hz); 3.23 (t, CH_2CN, J = 6.5 Hz).

9H-Hypoxanthin-9-ylpropionic acid (2c): Compound 1 (1 g, 4.81 mmol) was hydrolysed as described.[4] Yield: 385 mg (38 %) of 2c, mp 277-280°C (H_2O) (lit.[4], 275-280 °C). UV (H_2O) λ_{max} 250 nm (lit.[4], 251 nm). ^{1}H-NMR (Me_2SO-d_6) δ 8.04 (2H, H-2/H-8); 4.34 (t, CH_2N, J = 7 Hz); 2.86 (t, CH_2C=O, J = 7 Hz).

6-Mercapto-9H-purin-9-ylpropionic acid (2d): A) Compound 1 (1 g, 4.81 mmol) was thiolated according to Baker et al.[4] Yield: 765 mg (76 %) of 6-mercapto-9H-purin-9-ylpropionitrile, mp 282-286°C (EtOH) (lit.[4], 284-286°C). UV (MeOH) λ_{max} 322 nm (lit.[4], 325 nm). B) The total yield of the propionitrile was hydrolysed for 5 h in conc. hydrochloric acid (50 ml) and worked up as described.[4] Yield: 503 mg (60 %) of 2d, mp 253-256°C (H_2O) (lit.[4], 261-262°C). UV (H_2O) λ_{max} 322 nm (lit.[4], 326 nm). ^{1}H-NMR (Me_2SO-d_6) δ 8.70, 8.61 (H-2/H-8); 4.48 (t, CH_2N, J = 6.7 Hz); 2.95 (t, CH_2C=O, J = 6.7 Hz).

N-Formyl-2-oxo-1,2,3,4-tetrahydro-1H-imidazo[1,5-a]pyrimidine-8-carboxamide (5c) Acid 1c (300 mg, 1.44 mmol) was dissolved in water/p-dioxane (50 ml, 2:1, v/v) under warming and N-[3-(dimethylamino)propyl]-N'-ethylcarbodiimide hydrochloride (EDC, 500 mg, 2.6 mmol) was added in one portion. After stirring for 1 h at room temperature 5c (203 mg, 67 %) precipitated as colorless needles, mp 246-249°C (decomp.). TLC (A) R_f 0.35; UV (H_2O) λ_{max} 295 nm (ε 18 500). ^{1}H-NMR (Me_2SO-d_6) δ 9.16 (s, HC=O); 7.58 (s, H-6); 4.27 (t, CH_2N, J = 7 Hz); 2.79 (t, CH_2C=O, J = 7 Hz). MS (70 eV) m/e = 208 (11 %, M^+); 180 (67 %, M^+ - C=O); 135 (46 %, M^+ - HCONHCO - H). Anal calcd for $C_8H_8N_4O_3$ (202.2) C, 46.16; H, 3.87; N, 26.91. Found: C, 46.33; H, 3.97; N, 26.95.

2-Oxo-1,2,3,4-tetrahydro-1H-imidazo[1,5-a]pyrimidine-8-carboxamide (6a): Compound 5c (100 mg, 0.48 mmol) was dissolved in conc. aqueous ammonia (20 ml) and stirred for 2 h at room temperature. After evaporation of the solvent 6a was crystallized from methanol (66 mg, 77 %), mp 255-258°C. TLC (A) R_f 0.3; UV (MeOH) λ_{max} 267 nm (ε 15 500). ^{1}H-NMR (Me_2SO-d_6) δ 9.33 (s, NH); 7.43 (s, H-6); 7.23, 7.09 (NH_2); 4.23 (t, CH_2N, J = 7 Hz); 2.76 (t, CH_2C=O, J = 7 Hz). Anal calcd for $C_7H_8N_4O_2$ (180.2) C, 46.66; H, 4.48; N, 31.10. Found: C, 46.71; H, 4.49; N, 31.20.

N-Formyl-2-oxo-1,2,3,4-tetrahydro-1H-imidazo[1,5-a]pyrimidine-8-thiocarboxamide (5c) Acid 1d (100 mg, 0.45 mmol) was dissolved in water (10 ml) under warming. After

cooling the mixture to about 30°C N-[3-(dimethylamino)propyl]-N'-ethylcarbodiimide hydrochloride (500 mg, 2.6 mmol) was added. After keeping the reaction mixture for 30 min at room temperature 63 mg (63 %) of yellow needles precipitated, mp 179-181°C (decomp.). TLC (B) R_f 0.41; UV ($CHCl_3$) λ_{max} 370 nm (ε 14 400). ^{1}H-NMR (Me_2SO-d_6) δ 10.3, 10.2 (broad, 2 x NH); 9.59 (d, HC=O, J = 10 Hz); 7.18 (s, H-6); 4.30 (t, CH_2N, J = 7 Hz); 2.93 (t, CH_2C=O, J = 7 Hz). Anal calcd for $C_8H_8N_4O_2S$ (224.2) C, 42.85; H, 3.60; N, 24.99. Found: C, 42.85; H, 3.69; N, 24.91.

2-Oxo-1,2,3,4-tetrahydro-1H-imidazo[1,5-a]pyrimidine-8-thiocarboxamide (6b): Compound 5d (100 mg, 0.45 mmol) was dissolved in 12 % aqueous ammonia (5 ml) and stirred for 30 min at room temperature. After evaporation of the solvent 6b was crystallized from methanol (72 mg, 82 %), mp 230-231°C. TLC (B) R_f 0.7; UV (MeOH) λ_{max} 323 nm (ε 12 600). ^{1}H-NMR (Me_2SO-d_6) δ 10.24 (broad, NH); 8.89, 9.19 (NH_2); 7.52 (s, H-6); 4.27 (t, CH_2N, J = 7 Hz); 2.81 (t, CH_2C=O, J = 7 Hz). Anal calcd for $C_7H_8N_4O_2S$ (196.2) C, 42.84; H, 4.11; N, 28.55. Found: C, 43.01; H, 4.22; N, 28.46.

Determination of rate constants: The reaction mixtures contained per milliliter of Sørensen phosphate buffer (0.07 M, pH 5, 25°C) the acids 2a-d (0.11 mM, each) and N-[3-(dimethylamino)propyl]-N'-ethylcarbodiimide hydrochloride (5.22 mM). The reactions were followed spectrophotometrically at the λ_{max} value of the corresponding product (5a, 270 nm; 5b, 315 nm; 5c, 295 nm; 5d, 370 nm). Half-life values ($\tau_{1/2}^{app}$) of the "over-all" reactions were taken from continuous absorbance - time plots.

ACKNOWLEDGMENTS

We thank the Deutsche Forschungsgemeinschaft and the government of Nordrhein-Westfalen for financial support.

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Received, 24th June, 1985