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Allopurinol prodrugs. I. Synthesis, stability and physicochemical properties of various N_1-acyl allopurinol derivatives

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Summary

Seven novel N_1-acyl derivatives of allopurinol were synthesized and evaluated as potential prodrugs with the purpose of developing preparations suitable for rectal and parenteral administration. The kinetics of hydrolysis of the derivatives was studied in aqueous solutions at various pH-values and in human plasma solutions at 37°C. All the compounds hydrolyzed to yield allopurinol in quantitative amounts and rate expressions were derived to account for the pH–rate profiles observed. The rates of hydrolysis were accelerated by plasma enzymes, the half-lives of hydrolysis in 80% human plasma solutions at 37°C being 6, 4, 2.5 and 4 min for the N_1-acetyl, N_1-propionyl, N_1-butyryl and N_1-benzoyl derivatives, respectively. These N-acyl derivatives were more lipophilic than allopurinol as determined by partition experiments in octanol–water but the solubility in water was even greater (the N_1-acetyl derivative) or only slightly reduced (the other derivatives) as compared with allopurinol. This behaviour was attributed to a decreased intermolecular hydrogen bonding in the crystal lattice achieved by blocking the 1-NH group by acylation. It is suggested that N_1-acylation may be a promising means of obtaining prodrug forms of allopurinol with the aim of enhancing the rectal delivery characteristics of the drug. This was confirmed in preliminary animal experiments. Two highly water-soluble derivatives, 1-(N,N-dimethylglycyl)allopurinol hydrochloride and 1-(4-N,N-dimethylaminobutyryl)allopurinol hydrochloride, were prepared but found to be less

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suitable as parenteral delivery forms of allopurinol due to a very limited stability in aqueous solution, arising from intramolecular catalysis by the dimethylamino group in the acyl moiety.

Introduction

Allopurinol (I) is a widely used agent for the treatment and prevention of hyperuricemic states such as gout. The drug and its main metabolite oxipurinol lower the level of uric acid in plasma and urine by inhibiting xanthine oxidase, the enzyme catalyzing the oxidation of hypoxanthine to xanthine and xanthine to uric acid (Spector, 1977; Elion, 1978). In addition to its use as prophylaxis against and treatment of gout and other chronic hyperuricemic states allopurinol is commonly used to prevent the development of hyperuricosuria that often results from the rapid lysis of cells in patients with malignancies who are undergoing treatment with cytotoxic drugs or radiation (e.g. Elion, 1978).

Allopurinol is conventionally administered orally in the form of tablets. However, the development of nausea and vomiting among patients undergoing cancer chemotherapy frequently precludes the use of oral preparations in these patients as well as in other individuals who are unable to take or retain oral medications. Alternative means of administering allopurinol may be provided by the use of injectable and rectal preparations.

However, since allopurinol is only slightly soluble in water (0.5 $mg \cdot ml^{-1}$ at 25°C) acceptable injection preparations such as for intramuscular injection are not available. Presently it is only possible to deliver sufficient amounts of allopurinol parenterally by infusion (Kann et al., 1968; Brown et al., 1970; Donnenberg et al., 1974). The infusion fluid used contains the sodium salt of allopurinol ($pK_a \sim 10.2$ (Benezra and Bennett, 1978)) at a concentration of 0.5–1% and is strongly alkaline (pH about 10.5–11.5); consequently, the administration may cause thrombophlebitis or perivascular inflammation.

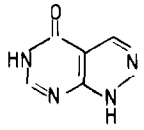
Concerning the rectal route of administering allopurinol recent studies have demonstrated that this approach is not a suitable and reliable mode of therapy (Chang et al., 1981; Appelbaum et al., 1980, 1982). It was shown in these studies that virtually no allopurinol or only very minute amounts ($< 5\%$) is absorbed from various suppository preparations administered rectally to man.

These delivery problems associated with allopurinol can primarily be attributed to the low water and lipid solubility of the compound. As will be shown below the partition coefficient for allopurinol between octanol and water is only 0.28 and this low lipophilicity along with the slight water solubility may be the predominant factors in the poor rectal absorption of the drug.

Studies were undertaken in our laboratories to improve the delivery characteristics, in particular the rectal absorption, of allopurinol using the prodrug approach. From the foregoing, it appears that successful prodrug derivatives should possess

both a desirable high water solubility and lipophilicity at physiological pH (pH 7–8) and should be capable of reverting rapidly to the parent drug following absorption. Previously described prodrug types for allopurinol include some ether derivatives (Hussain and Rytting, 1974), N-Mannich bases (Bundgaard and Johansen, 1981) and N-hydroxymethyl derivatives (Bansal et al., 1981). These derivatives are very unstable in aqueous solutions, are relatively insoluble in water and lipid and are not considered to offer any advantage over allopurinol with respect to bioavailability following rectal or parenteral administration.

In the present work various N_1-acyl derivatives of allopurinol (II–VIII) have been prepared and assessed as possible prodrug forms. To this end, the chemical- and enzyme-mediated conversion of the new compounds to allopurinol was investigated and their aqueous solubility and lipophilicity were determined. In subsequent papers results of bioavailability studies and of studies pertinent to another potentially useful allopurinol prodrug type, N-acyloxymethyl derivatives, will be reported.



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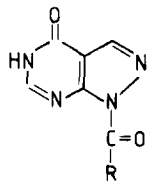


II R = $-CH_3$
III R = $-CH_2CH_3$
IV R = $-CH_2CH_2CH_3$
V R = $-CH_2Cl$
VI R = 
VII R = $-CH_2-N(CH_3)_2$, HCl
VIII R = $-CH_2-CH_2-CH_2-N(CH_3)_2$, HCl

Materials and Methods

Apparatus

Ultraviolet spectral measurements were performed with a Shimadzu UV-190 spectrophotometer equipped with a thermostatically controlled cell compartment, using 1-cm quartz cells. ^{1}H-NMR spectra were run on a Varian 360L instrument. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. Melting points were taken on a capillary melting-point apparatus and are uncorrected. High-performance liquid chromatography (HPLC) was done with a Spectra-Physics Model 3500 B instrument equipped with a variable-wavelength detector and a 10-μl loop injection valve. A column, 250 × 4 mm, packed with LiChrosorb RP-8 (7 μm particles) (E. Merck, Darmstadt) was

used. Microanalyses were performed by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Preparation of allopurinol N_1-acyl derivatives (II–VIII)

The N_1-acyl derivatives II–VIII were prepared by reacting allopurinol (purchased from Sigma Chemicals, St. Louis) with the corresponding acid anhydride or chloride.

Compounds II, III and IV. A suspension of allopurinol (500 mg, 3.70 mmol) in 8 ml of the acid anhydride (acetic, propionic or butyric anhydride) was heated in an oil bath at 130°C for 5 h. After cooling, water (10 ml) was added and the mixture was stirred at room temperature for 3 h. The precipitate was collected, washed with water and dried.

Compound V and VI. A mixture of allopurinol (2.7 g; 20 mmol) and chloroacetic anhydride or benzoic anhydride (40 mmol) in N,N-dimethylformamide (20 ml) was

TABLE 1

PHYSICAL AND ANALYTICAL DATA OF VARIOUS N_1-ACYL DERIVATIVES OF ALLOPURINOL

Compound	Yield (%)	Melting point (°C)	Recrystn. solvent [a]	Formula	Analysis (%)		
					Calc.		Found
II	57	251–254	A	$C_7H_6N_4O_2$	C	47.19	47.00
					H	3.40	3.49
					N	31.45	31.42
III	60	259–263	A	$C_8H_8N_4O_2$	C	50.00	49.97
					H	4.19	4.29
					N	29.15	29.13
IV	52	222–225	B	$C_9H_{10}N_4O_2$	C	52.42	52.42
					H	4.89	4.98
					N	27.17	26.92
V	61	233–236	C	$C_7H_5ClN_4O_2$	C	39.55	39.66
					H	2.37	2.43
					N	26.35	26.36
					Cl	16.67	16.58
VI	50	270–273	C	$C_{12}H_8N_4O_2$	C	60.00	59.92
					H	3.36	3.51
					N	23.32	23.38
VII	25	192–196	–	$C_9H_{11}N_5O_2 \cdot HCl \cdot 2/3H_2O$	C	40.08	40.04
					H	4.98	5.00
					N	25.97	25.96
					Cl	13.15	12.77
VIII	59	198–202	–	$C_{11}H_{15}N_5O_2 \cdot HCl \cdot 1/3H_2O$	C	45.29	45.18
					H	5.78	5.88
					N	24.01	24.29
					Cl	12.15	12.05

[a] Solvent of recrystallization: A, ethanol-N,N-dimethylformamide; B, ethanol, C, N,N-dimethylformamide.

heated to 80°C and a clear solution was obtained. After 1 h, the solution was cooled and the precipitate formed was collected and washed with acetone.

Compounds VII and VIII. To a suspension of allopurinol (770 mg, 5.7 mmol) in 5 ml of N,N-dimethylformamide was added a solution of N,N-dimethylglycinyl chloride hydrochloride or 4-(N,N-dimethylamino)butyryl chloride hydrochloride (5.7 mmol) in 10 ml of N,N-dimethylformamide. The acid chlorides were prepared by refluxing the corresponding amino acids in thionyl chloride and were used immediately. The mixture was kept at 80°C for 1 h. After cooling the precipitate was collected, washed with ethanol and dried in vacuum over phosphorous pentaoxide.

Recrystallization of the derivatives was performed as indicated in Table 1. Physical and analytical data for the compounds are also given in Table 1.

Kinetic measurements

The hydrolysis of the N-acyl derivatives II–VIII was studied in aqueous buffer solutions at 37.0 ± 0.2°C. Hydrochloric acid, acetate, phosphate and borate were used as buffers; the total buffer concentration was generally 0.025 M and a constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The rates of hydrolysis were in some cases followed spectrophotometrically by recording the absorbance decrease accompanying the hydrolysis at 280 nm (II–VI) or 290 nm (VII and VIII). Reactions were performed in 2.5 ml aliquot portions of buffer solutions in a thermostated quarts cuvette and were initiated by adding about 25 μl of stock solutions of the derivatives in dioxane, ethanol or water to give a final concentration of about 0.01 mg · ml^{-1}. Pseudo-first-order rate constants were determined from linear plots of log $(A_t - A_\infty)$ vs time, where A_t and A_∞ are the absorbance readings at time t and at infinity, respectively.

The rates of hydrolysis of the N_1-acyl derivatives II, III, IV and VI were also followed by using a reversed-phase HPLC procedure. Solvent systems of 25% v/v (II), 40% v/v (III) or 60% v/v (IV and VI) methanol in 0.005 M acetate buffer pH 4.5 were used. The flow rate was 1.2 ml · min^{-1} and the column effluent was monitored at 274 nm. Under these conditions the N_1-acyl derivatives had elution times of 3–6 min while allopurinol eluted with the solvent front. Quantitation of the compounds was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions. The reactions were initiated by adding 100 μl of a stock solution of the compounds (about 2 mg · ml^{-1} in ethanol–water) to 10 ml of pre-heated buffer solution in screw-capped test tubes. The solutions were kept in a water-bath at 37°C and at appropriate intervals samples were taken and chromatographed. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of residual allopurinol derivative against time.

For the determination of allopurinol by HPLC a solvent system consisting of 5% v/v methanol in 0.01 M acetate buffer pH 4.5 was used. The column effluent was monitored at 251 nm.

The hydrolysis of the derivatives II–IV was also studied in 0.025 M phosphate buffer pH 7.40 containing 80% human plasma (at 37°C). At appropriate intervals

200 μl samples were withdrawn (solute concentration about 0.04 mg · ml^{-1}) and added to 1000 μl of ethanol in order to deproteinize the plasma. After mixing and centrifugation for 2 min, 10 μl of the clear supernatant was analyzed by HPLC for residual derivative as described above.

Determination of aqueous solubility, partition coefficients and pK_a values

The aqueous solubility of allopurinol and some of the derivatives were determined at 22°C by adding excess amounts of the compounds to water or buffer solutions. The mixtures were placed in an ultrasonic water-bath for about 30 min and then rotated on a mechanical spindle for 2–3 h. Upon filtration an aliquot of the filtrate was diluted with an appropriate amount of water and the mixture analyzed by HPLC. The concentration of the compounds in their saturated solutions were calculated from the measured peak heights by reference to those of standards chromatographed under the same conditions.

The apparent partition coefficients were determined in octanol–water or in octanol–0.05 M acetate buffer (pH 5.0) at 22°C. The aqueous phase and octanol were mutually saturated at 20–25°C before use. The compounds were dissolved in the aqueous phase and the octanol–water mixture were shaken for 5–30 min to reach a distribution equilibrium. The volumes of each phase were chosen so that the solute concentration in the aqueous phase, before and after distribution, could readily be measured using the aforementioned HPLC methods. Centrifugation was used to separate the two phases. The partition coefficients (P) were calculated from Eqn. 1:

$$P = \frac{C_i - C_w}{C_w} \times \frac{V_w}{V_o} \quad (1)$$

where C_i and C_w represent the solute concentrations in the aqueous phase before and after distribution, respectively; V_w represents the volume of the aqueous and V_o the volume of the octanol phase.

The ionization constants for compounds II, III, IV and VI were determined at 22°C and $\mu = 0.5$ by spectrophotometry according to Albert and Serjeant (1971), the spectral changes as a function of pH being monitored at 287 nm (II, III and IV) or 310 nm (VI).

Results and Discussion

The monoacylated derivatives II–VIII were obtained by reacting allopurinol with an acid anhydride or acid chloride. The structures of the compounds were confirmed by elemental, NMR and UV analysis. Allopurinol exists in different tautomeric forms (Bergmann et al., 1979) and acylation may theoretically take place at either the N_1, N_2 or N_5 position (O-acylation may also be envisaged). Evidence for the assignment of the N_1 position of the acyl substituents in compounds II–VIII is provided by the NMR and UV data given in Table 2 in comparison with the spectral

TABLE 2

^{1}H-NMR AND UV SPECTRAL DATA OF VARIOUS ALLOPURINOL DERIVATIVES

Compound	^{1}H-NMR (δ) [a]		λ_{max} (nm)	
	H(3)	H(6)	At pH 5	At pH 9.5
II	8.30	8.38	274	287
III	8.30	8.37	274	287
IV	8.32	8.40	274	287
V	8.29	8.39	274	n.d. [b]
VI	8.32	8.46	250,280 (Sh)	294
VII	8.45	8.46	279	n.d.
VIII	n.d	n.d.	275	n.d.

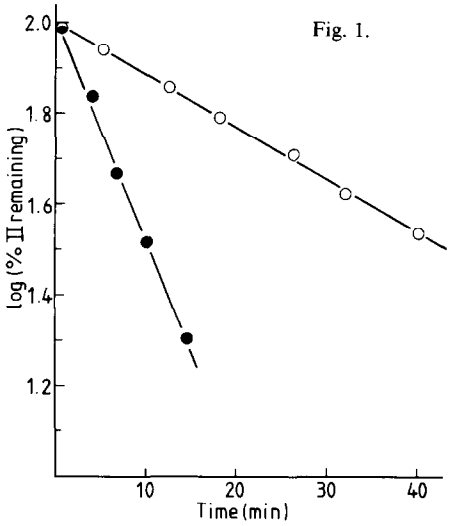
[a] The NMR spectra were run in dimethylsulphoxide-d_6 except for compound VII (in D_2O).
[b] n.d. = not determined due to fast hydrolysis.

data given by Bergmann et al. (1979) for various N-methyl derivatives of allopurinol. In this work it is shown that dissociation of the 5-NH group in the pyrimidine moiety of 1- or 2-methylallopurinol leads to a marked bathochromic shift of λ_{max} of about 23 nm. In contrast, ionization of an NH-group in the pyrazole moiety of 5-methylallopurinol causes only a very small (1 nm) bathochromic displacement of λ_{max}, while a hypsochromic displacement was seen in 7-methylallopurinol. On this basis the UV-spectral behaviour of the derivatives II–VIII thus excludes an N_5- or N_7-acyl structure. The difference in the chemical shifts between the 3-H and 6-H NMR signals in 2-methylallopurinol is far greater than that in 1-methylallopurinol (52 ppm vs 7 ppm) (Bergmann et al., 1979) and consequently, the NMR data together with the UV data (Table 2) clearly establish the structures of derivatives II–VIII as N_1-substituted compounds.

Kinetics of hydrolysis in aqueous buffer solutions

The kinetics of hydrolysis of the N-acyl derivatives II–VIII was studied in aqueous solutions at 37°C over a wide range of pH. Under the experimental conditions used all derivatives hydrolyzed to yield allopurinol quantitatively as evidenced by UV-spectral and HPLC analysis. At constant pH and temperature all reactions displayed strict first-order kinetics for several half-lives (cf. Fig. 1). In cases where the hydrolysis was followed using both direct UV-spectrophotometry and HPLC the rate constants obtained therefrom agreed within 5%. At low buffer concentration (0.025 M) no significant catalysis by the buffer substances used to maintain constant pH occurred.

The influence of pH on the rates of hydrolysis of compounds II and V is shown in Fig. 2 in which the logarithm of the observed pseudo-first-order rate constants, k_{obs}, has been plotted against pH. The pH–rate profiles obtained for the derivatives III, IV and VI had a shape similar to that of compound II. The shapes of the profiles indicate that the hydrolysis can be described in terms of specific acid-catalyzed (k_H), spontaneous (k_0) and specific base-catalyzed (k_{OH}) reactions of the undissociated



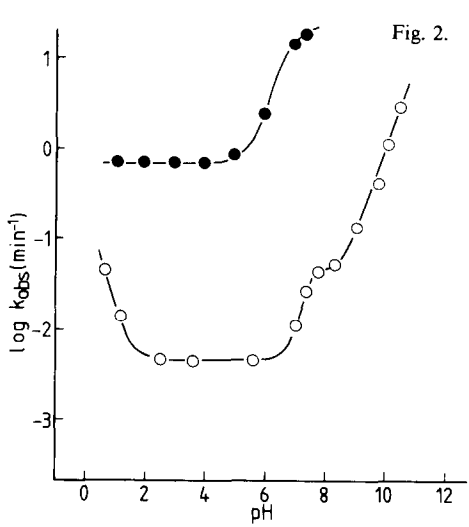


Fig. 1. First-order plots of the hydrolysis of 1-acetylallopurinol (II) in 0.025 M phosphate buffer of pH 7.40 (○) and in 80% human plasma (●) at 37°C.

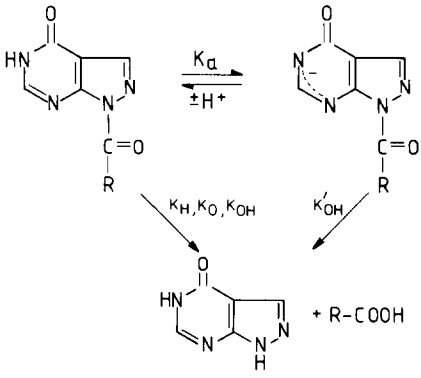
Fig. 2. The pH–rate profiles for the hydrolysis of 1-acetylallopurinol (II) (○) and 1-chloroacetylallopurinol (V) (●) in aqueous solution at 37°C.

form of the compounds along with a specific base-catalyzed reaction (k'_{OH}) of the ionized form (Scheme 1):

$$k_{obs} = (k_H a_H + k_0 + k_{OH} a_{OH}) \frac{a_H}{a_H + K_a} + k'_{OH} a_{OH} \frac{K_a}{a_H + K_a} \qquad (2)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activity, respectively,

Scheme 1



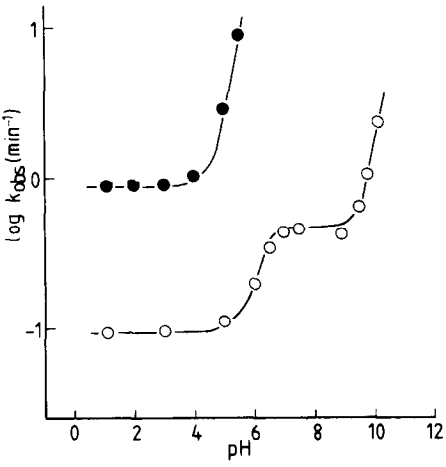


Fig. 3. The pH–rate profiles for the hydrolysis of 1-(N,N-dimethylglycyl)allopurinol (VII) (○) and 1-(4-N,N-dimethylaminobutyryl)allopurinol (VIII) (●) in aqueous solution at 37°C.

$a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of the compounds in the undissociated and anionic form, respectively, and K_a is the apparent ionization constant of the compounds (due to ionization of the 5-NH group). Values of the specific rate constants k_H, k_0, k_{OH} and k'_{OH} were determined from portions of the pH–rate profiles in which only one or two of the reactions mentioned contribute to the overall hydrolysis and those of K_a on the basis of Eqn. 2. The various rate and ionization constants derived are listed in Table 3. As appears from the table the kinetically obtained pK_a values agreed satisfactorily with those determined by spectrophotometric titration, taking into account the different temperatures for the measurements. Fig. 3 shows the pH rate profiles for the hydrolysis of compounds VII and VIII. The proposed kinetic scheme for compound VII is shown in Scheme 2. It involves specific base-catalyzed reactions of the free base and the protonated

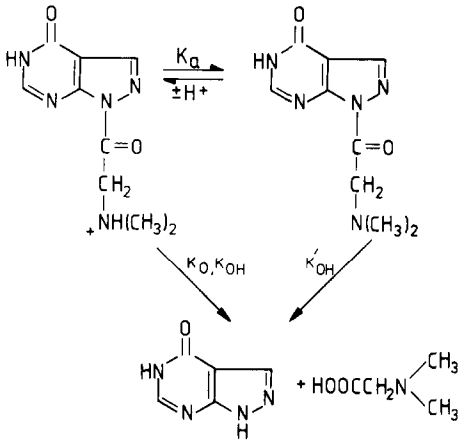
TABLE 3

IONIZATION CONSTANTS AND RATE DATA FOR THE HYDROLYSIS OF VARIOUS N_1-ACYL ALLOPURINOL DERIVATIVES ($\mu = 0.5$; 37°C)

Compound	k_0 (min^{-1})	k_H ($M^{-1} \cdot min^{-1}$)	k_{OH} ($M^{-1} \cdot min^{-1}$)	k'_{OH} ($M^{-1} \cdot min^{-1}$)	pK_a^*
II	0.0045	0.20	4.4×10^4	3.3×10^3	7.7
III	0.0030	0.18	3.8×10^4	2.8×10^3	7.6
IV	0.0021	0.16	3.2×10^4	2.3×10^3	7.6
V	0.71	–	8.3×10^7	–	–
VI	0.0067	0.04	5.7×10^4	4.0×10^3	7.6

* Values obtained by UV-spectrophotometry at 22°C and $\mu = 0.5$: II, 7.75; III, 7.78; IV, 7.79; VI, 7.60.

Scheme 2



forms along with a spontaneous (water-catalyzed) reaction of the protonated form:

$$k_{obs} = (k_0 + k_{OH}a_{OH})\frac{a_H}{a_H + K_a} + k'_{OH}a_{OH}\frac{K_a}{a_H + K_a} \qquad (3)$$

where K_a is the apparent ionization constant of the dimethylammonium groups in the derivative. The line drawn for the pH–rate profile for compound VII was constructed from Eqn. 3 and the following rate and ionization constants (37°C and $\mu = 0.5$):

$k_0 = 0.092\ min^{-1}$;

$k_{OH} = 7.9 \times 10^6\ M^{-1} \cdot min^{-1}$;

$k'_{OH} = 6.9 \times 10^4\ M^{-1} \cdot min^{-1}$;

$K_a = 10^{-6.5}$.

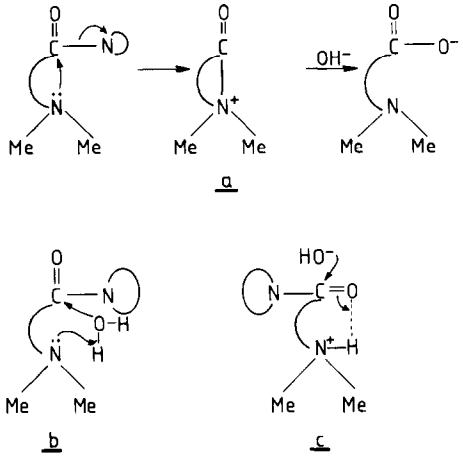
For compound VIII only the rate parameters k_0 and k_{OH} could be determined due to the rapidity of the hydrolysis at pH > 6. The following values were calculated (at 37°C): $k_0 = 0.87\ min^{-1}$ and $k_{OH} = 1.3 \times 10^9\ M^{-1} \cdot min^{-1}$. The kinetically obtained pK_a value of 6.5 for compound VII is of the expected magnitude for the dimethylaminoacetyl group (Bundgaard et al., 1984); the high reactivity of the compound apparently masks an influence of ionization of the allopurinol moiety (i.e. the 5-NH group) on the shape of the pH–rate profile as observed for e.g. compound II.

The results obtained show that the acyl derivatives undergo facile deacylation even in neutral aqueous solution, the reaction being facilitated by electron-withdrawing acyl groups in neutral and basic solution. The high degree of reactivity of the

N-acylated derivatives as compared with the low reactivity of normal amides may be related to the quasi-aromatic character of the pyrazole ring. Due to participation of the electron pair on the 1-nitrogen in the π-electron system, this nitrogen becomes more positive, exerting attraction toward the ring and thus depressing the amide resonance stabilization and facilitating nucleophilic reactions at the acyl carbonyl group. The reactivity of the N_1-acyl allopurinol derivatives can be compared with that of other similarly activated amides as N-acetylimidazole and 1-acetyl-1,2,4-triazole (Oakenfull and Jencks, 1971; Fox and Jencks, 1974). The high reactivity of N_1-acyl pyrazoles has been previously recognized (Hüttel and Kratzer, 1959; Staab, 1959; Forist and Weber, 1973).

Introduction of an α- or γ-N,N-dimethylamino group in the acyl moiety results in greatly increased lability in acid and neutral solutions which can be ascribed to intramolecular catalysis by the amino group. Several forms of catalysis may occur (Scheme 3): intramolecular nucleophilic catalysis (a), intramolecular general base

Scheme 3



catalysis (b), and intramolecular general acid-specific base catalysis (c). These mechanisms are kinetically indistinguishable but the intramolecular nucleophilic catalysis (a) may be favoured since the allopurinol moiety is a good leaving group (pK_a 10.2), cf. Bruice and Benkovic (1966). This appears especially to be the case for the 4-(N,N-dimethylamino)butyryl derivative VIII in view of the magnitude of the second-order rate constant for the hydroxide ion-catalyzed hydrolysis of the protonated form as calculated above (1.3×10^9 $M^{-1} \cdot min^{-1}$). This rate constant corresponds to a value of 1.2×10^5 min^{-1} for the rate constant for spontaneous intramolecular attack of the unprotonated dimethylamino group on the amide moiety, using a pK_a value for the amino group of 9.6 (Bruice and Benkovic, 1963). The greater lability of compound VIII as compared with compound II at pH values below 4 may be attributed to intramolecular general acid catalysis by the protonated dimethylamino group of nucleophilic attack by water on the amide carbonyl moiety.

TABLE 4

RATE DATA FOR HYDROLYSIS OF N_1-ACYL DERIVATIVES OF ALLOPURINOL IN AQUEOUS SOLUTION AND IN 80% HUMAN PLASMA AT 37°C

Compound	$t_{\frac{1}{2}}$ (min)		
	pH 4.0	pH 7.4	80% plasma
II	155	26	6
III	230	30	4
IV	330	36	2.5
V	1.0	~ 3 s	–
VI	104	20	4
VII	7.3	1.5	–
VIII	0.7	< 3 s [a]	–

[a] Estimated half-life from the pH–rate profile: 0.05 s.

Hydrolysis in plasma

For the evaluation of the N-acyl derivatives as being potential prodrugs of allopurinol it is important to ascertain whether plasma enzymes would be able to catalyze their hydrolysis. Therefore, the stability of the derivatives II, III, IV and VI was examined in 80% human plasma (pH 7.4) at 37°C and compared to that in pure buffer solution. Due to great lability in neutral aqueous solution the derivatives V, VII and VIII were not included in the investigation. Under the given reaction conditions strict first-order kinetics was observed (cf. Fig. 1) and the reactions proceeded to give allopurinol in stoichiometric amounts as evidenced by HPLC analysis. As appears from the rate data obtained (Table 4), plasma accelerates the rate of hydrolysis markedly. For the alkyl derivatives the rate of enzymatic hydrolysis is seen to increase with increasing alkyl chain length.

Lipophilicity and aqueous solubility of the N-acyl derivatives

Apparent partition coefficients (P) for the N-acyl derivatives and allopurinol as determined using the octanol–water system are listed in Table 5 along with data for the aqueous solubility. It is seen that the derivatives II, III, IV and VI are all more lipophilic than the parent allopurinol. This is also apparent by comparing the capacity factors (k') for the compounds in reversed-phase HPLC with methanol–acetate buffer pH 4.5 (1 : 1 v/v) as the mobile phase. Due to ionization of the acyl derivatives II, III, IV and VI (pK_a 7.6–7.8) their solubility is slightly higher at physiological pH than in water. Thus, at pH 7.5 and 22°C the solubility of compound II was determined to be 1.0 mg · ml^{-1} as compared with 0.75 mg · ml^{-1} in water. Identical solubilities and log P values were obtained when a 0.05 M acetate buffer of pH 5.0 was used instead of water in the measurements.

An increase in lipophilicity is generally accompanied by a decrease in water solubility. Inspection of the data in Table 5 reveals, however, that the aqueous solubility of the N_1-acetyl derivative II is in fact increased relative to that of allopurinol despite its higher log P value. This behavior can most likely be attributed

TABLE 5

PARTITION COEFFICIENTS (P), HPLC CHROMATOGRAPHIC CAPACITY FACTORS (k')[a] AND WATER SOLUBILITIES (S) OF ALLOPURINOL AND N_1-ACYL DERIVATIVES OF ALLOPURINOL

Compound	log P[b]	k'	S[c] ($mg \cdot ml^{-1}$)
Allopurinol	−0.55	< 0.2	0.50
II	−0.35	~ 0.3	0.75
III	0.30	0.66	0.30
IV	0.85	1.10	0.11
VI	1.20	1.20	0.014
VII	-	-	>100
VIII	-	-	>100

[a] Mobile phase: 0.01 M acetate buffer pH 4.5–methanol (1 : 1 v/v).
[b] Partition coefficients between octanol and water at 22°C.
[c] At 22°C.

to a decreased intermolecular hydrogen bonding in the crystal lattice achieved by blocking the 1-NH group in allopurinol. The X-ray interferogram of allopurinol shows a hydrogen bridge between the 1-NH group and 7-N of another molecule, while 2-N is bound to the hydrogen of a 5-NH group (Prusiner and Sundaralingam, 1972). The strong crystal lattice energy of allopurinol which is reflected in its high melting point (365°C) is certainly responsible for the relatively low solubility of the compound in water and other solvents. Support for the suggestion that replacement of the 1-NH proton in allopurinol by acyl groups results in a decreased crystal lattice energy comes from comparison of the melting point of allopurinol with those of the acyl derivatives: it decreases from 365°C to ~ 222–273°C. In a following paper (Bundgaard and Falch, 1985) the relationship between aqueous solubility, lipophilicity and melting points for the N-acyl derivatives and other allopurinol prodrugs is discussed in more detail.

N_1-Acyl derivatives of allopurinol as prodrugs

The results of the present study show that N_1-acylation may be a potentially useful approach to obtain prodrug forms of allopurinol. The derivatives examined all undergo a rapid and quantitative conversion to the parent allopurinol in aqueous solution or in human plasma. By appropriate selection of the acyl group it is possible to modify the cleavage rate, aqueous solubility and lipophilicity. As has been demonstrated it is feasible to obtain N-acyl derivatives which are much more lipophilic than allopurinol and at the same time possesses a higher or only slightly decreased water solubility. Such derivatives may be useful for enhancing the rectal bioavailability of allopurinol and in fact, experiments in rabbits have shown that rectal administration of the derivatives II and IV in the form of fatty acid suppositories result in a bioavailability of allopurinol of 20–30%. When allopurinol was administered per se the bioavailability was less than 2%. The results of such

studies including other allopurinol prodrug derivatives will be the subject of a subsequent paper. Considering the aim of obtaining a water-soluble allopurinol prodrug suitable for parenteral administration none of the derivatives described appear to have optimal properties. Although the derivatives containing an amino function in the acyl moiety (VII and VIII) are highly soluble in water as hydrochloride salts their poor stability in solution limits their usefulness. Thus, the half-life for the hydrolysis of compound VII at pH 2–5 and 23°C was found to be only 20 min and that for compound VIII about 2 min. The solution stability for practical purposes is even lower than what appears from these figures because precipitation of the product of hydrolysis, allopurinol, quickly occurs. As will appear from a paper which will follow (Bundgaard and Falch, 1985) more useful prodrug candidates as parenteral delivery forms of allopurinol are N-acyloxymethyl derivatives prepared from amino acids.

References

Albert, A. and Serjeant, E.P., The Determination of Ionization Constants, 2nd edn., Chapman and Hall, London, 1971.

Appelbaum, S.J., Mayersohn, M., Perrier, D. and Dorr, R.T., Allopurinol absorption from rectal suppositories. Drug Intell. Clin. Pharm., 14 (1980) 789.

Appelbaum, S.J., Mayersohn, M., Dorr, R.T. and Perrier, D., Allopurinol kinetics and bioavailability. Intravenous, oral and rectal administration. Cancer Chemother. Pharmacol., 8 (1982) 93–98.

Bansal, P.C., Pitman, I.H. and Higuchi, T., N-Hydroxymethyl derivatives of nitrogen heterocycles as possible prodrugs II: Possible prodrugs of allopurinol, glutethimide and phenobarbital. J. Pharm. Sci., 70 (1981) 855–857.

Benezra, S.A. and Bennett, T.R., Allopurinol. Analytical Profiles of Drug Substances, 7 (1978) 1–17.

Bergmann, F., Frank, A. and Neiman, Z., Studies on the chemical reactivity and the physical properties of allopurinol (pyrazolo[3,4-D]pyrimidin-4-one) and related compounds. J. Chem. Soc. Perkin Trans. I, (1979) 2795–2802.

Brown, C.H., Stashick, E. and Carbone, P.P., Clinical efficacy and lack of toxicity of allopurinol (NSC-1390) given intravenously. Cancer Chemother. Rep., 54 (1970) 125–129.

Bruice, T.C. and Benkovic, S., A comparison of the bimolecular and intramolecular nucleophilic catalysis of the hydrolysis of substituted phenyl acetates by the dimethylamino group. J. Am. Chem. Soc., 85 (1963) 1–8.

Bruice, T.C. and Benkovic, S., Bioorganic Mechanisms, Vol. 1, W.A. Benjamin, New York-Amsterdam, 1966, pp. 119–211.

Bundgaard, H. and Falch, E., Allopurinol prodrugs II and III. Int. J. Pharm., (1985) in press.

Bundgaard, H. and Johansen, M., Prodrugs as drug delivery systems. XVIII. Bioreversible derivatization of allopurinol by N-aminomethylation to effect enhanced dissolution rates. Acta Pharm. Suec., 18 (1981) 129–134.

Bundgaard, H., Larsen, C. and Arnold, E., Prodrugs as drug delivery systems. XXVII. Chemical stability and bioavailability of a water-soluble prodrug of metronidazole for parenteral administration. Int. J. Pharm., 18 (1984) 79–87.

Chang, S.-L., Kramer, W.G., Feldman, S., Ballentine, R. and Frankel, L.S., Bioavailability of allopurinol oral and rectal dosage forms. Am. J. Hosp. Pharm., 38 (1981) 365–368.

Donnenberg, A., Holton, C.P., Mayer, C.M.H. and Phillips, L.K., Evaluation of intravenous allopurinol (NSC-1390) in pediatric neoplasia. Cancer Chemother. Rep., 58 (1974) 737–739.

Elion, G.B., Allopurinol and other inhibitors of urate synthesis. Handbook of Exp. Pharmacol., 51 (1978) 485–514.

Forist, A.A. and Weber, D.J., Kinetics of hydrolysis of hypoglycemic 1-acyl-3,5-dimethylpyrazoles. J. Pharm. Sci., 62 (1973) 318–319.

Fox, J.P. and Jencks, W.P., General acid and general base catalysis of the methoxyaminolysis of 1-acetyl-1,2,4-triazole. J. Am. Chem. Soc., 96 (1974) 1436–1449.

Hussain, A. and Rytting, J.H., Prodrug approach to enhancement of rate of dissolution of allopurinol. J. Pharm. Sci., 63 (1974) 798–799.

Hüttel, R. and Kratzer, J., Kinetik der Hydrolyse und Aminolyse von N-Acetyl-pyrazolen und N-Acetyl-1.2.3-triazolen. Chem. Ber., 92 (1959) 2014–2021.

Kann, Jr., H.E., Wells, J.H., Gallelli, J.F., Schein, P.S., Cooney, D.A., Smith, E.R., Seegmiller, J.E. and Carbone, P.P., The development and use of an intravenous preparation of allopurinol. Am. J. Med. Sci., 256 (1968) 53–63.

Oakenfull, D.G. and Jencks, W.P., Reactions of acetylimidazole and acetylimidazolium ion with nucleophilic reagents. Structure–reactivity relationships. J. Am. Chem. Soc., 93 (1971) 178–188.

Prusiner, P. and Sundaralingam, M., Stereochemistry of nucleic acids and their constituents XXIX. Crystal and molecular structure of allopurinol, a potent inhibitor of xanthine oxidase. Acta Cryst., B 28 (1972) 2148–2152.

Spector, T., Inhibition of urate production by allopurinol. Biochem. Pharmacol., 26 (1977) 355–358.

Staab, H.A., Reaktionsfähige heterocyclische Amide der Kohlensäure. V. Zur Reaktionsfähigkeit von N-Acylpyrazolen. Liebigs Ann. Chem. 622 (1959) 31–37.