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Allopurinol prodrugs. II. Synthesis, hydrolysis kinetics and physicochemical properties of various N-acyloxymethyl allopurinol derivatives

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Summary

Fourteen novel N-acyloxymethyl derivatives of allopurinol were synthesized and evaluated as potential prodrugs with the aim of enhancing the rectal delivery characteristics of the parent drug. The hydrolysis of the compounds (1- or 2-acyloxymethyl and 1,5- or 2,5-bis(acyloxymethyl) derivatives) was subject to specific base catalysis as well as enzymatic catalysis by plasma enzymes. In 80% human plasma solutions all the compounds were converted quantitatively to allopurinol, passing through an unstable N-(hydroxymethyl)allopurinol intermediate. The derivatives were more lipophilic than allopurinol as expressed by octanol–water partition coefficients and reversed-phase liquid chromatographic capacity factors but the water-solubility was only slightly reduced or, for some derivatives, even greater than that of allopurinol. This behaviour was attributed to differences in the crystal lattice energy and a relationship between melting points, partition coefficients and water-solubilities for these and four previously studied N_1-acyl allopurinol prodrug derivatives was established. The results suggested that N-acyloxymethylation may be a promising means of obtaining prodrug forms of allopurinol with improved physicochemical properties with regard to drug delivery.

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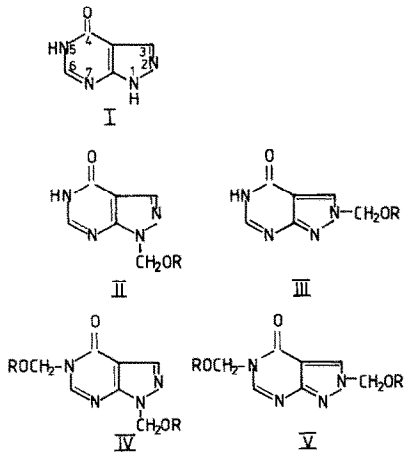
Introduction

Allopurinol (I) is a widely used drug for the prevention and treatment of hyperuricemic states such as gout as well as for the prevention of 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 or capsules. 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. Parenteral dosage forms for a simple injection are, however, not available because of the low solubility of allopurinol in water (0.5 $mg \cdot ml^{-1}$ at 25°C) or other solvents suitable for parenteral administration, and when given rectally to man in the form of various suppository preparations only very minute amounts (< 5%) are absorbed (Chang et al., 1981; Appelbaum et al., 1980, 1982).

Since these delivery problems can primarily be attributed to the low water and lipid solubility of the compound (Bundgaard and Falch, 1985a), it appears likely that the delivery characteristics of allopurinol can be improved by using the prodrug approach, i.e. development of derivatives possessing both a high water-solubility and lipophilicity at physiological pH (pH 7–8) and being capable of reverting rapidly and quantitatively to the parent drug following absorption.

Studies along this direction were undertaken in our laboratories and in a foregoing work (Bundgaard and Falch, 1985a), various N_1-acyl derivatives of allopurinol were prepared and shown to be potentially useful prodrug forms for rectal administration. In the present work, a series of N-acyloxymethyl derivatives of



Formulae I–V.

allopurinol (II–V) have been prepared and assessed as possible prodrug forms. The chemical- and enzyme-mediated conversion of the new compounds to allopurinol was investigated and determinations of the aqueous solubility and lipophilicity of the derivatives were performed. Furthermore, relationships between aqueous solubility and octanol–water partition coefficients of the prodrug derivatives including the N_1-acyl derivatives studied previously are described. In the accompanying paper (Bundgaard and Falch, 1985b) studies pertinent to the development of highly water-soluble N-acyloxymethyl derivatives possessing an amino group in the ester function are reported.

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 (Table 1) 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

TABLE 1

MELTING POINTS, ^{1}H-NMR AND UV SPECTRAL DATA OF VARIOUS N-ACYLOXYMETHYL DERIVATIVES OF ALLOPURINOL (II–V)

Compound		m.p. (°C)	^{1}H-NMR(δ) [a]		λ_{max}(nm) in pH 5.0 buffer
No.	R in II–V		H(3)	H(6)	
IIa	Acetyl	257–258	8.20	8.22	251
IIb	Butyryl	224–226	8.20	8.23	251
IIc	Pivaloyl	185–187	8.19	8.23	251
IId	Benzoyl	217–219	8.45	8.49	234
IIe	Nicotinoyl	242–243	8.41	8.48	251
IIf	N,N-Diethylsuccinamyl	138–140	8.21	8.23	251
IIg	Ethoxycarbonyl	228–229	8.24	8.28	251
IIIa	Butyryl	182–183	8.06	8.78	261
IIIb	Pivaloyl	180–181	8.08	8.70	261
IVa	Butyryl	122–123	8.28	8.62	251
IVb	Pivaloyl	136–137	8.29	8.64	251
Va	Acetyl	153–154	8.70	9.17	259
Vb	Butyryl	133–135	8.40	8.82	259
Vc	Pivaloyl	145–146	8.41	8.85	259

[a] The NMR-spectra were run in dimethyl sulphoxide-d_6.

used. Microanalyses were performed by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark and were within ±0.4% of the theoretical values.

Chemicals

Allopurinol was purchased from Sigma Chemicals, St. Louis and was used as received. Buffer substances and all other chemicals or solvents used were of reagent grade.

Synthesis of allopurinol N-acyloxymethyl derivatives (II–V)

Method A (Scheme 1)

The compounds IIa, IIb, IId, IIe, IIf, IIg, Va and Vb were synthesized by this method in which 1-(hydroxymethyl)allopurinol (VI) or 2,5-bis(hydroxymethyl)allopurinol (VII) is reacted with the corresponding acid anhydride, acid chloride, or the acid in the presence of N,N′-dicyclohexylcarbodiimide. These hydroxymethyl derivatives of allopurinol were prepared by reacting allopurinol with formaldehyde in aqueous solutions of pH 7.0 as described by Bansal et al. (1981). For the structural assignment of the bis(hydroxymethyl) derivative, see later.

General procedure: 1-(benzyloxymethyl)allopurinol (IId). A mixture of VI (0.8 g; 5 mmol) and benzoyl chloride (0.75 ml; 6.5 mmol) in pyridine (10 ml) was stirred at room temperature for 3 h. Water (30 ml) was added, and after standing for 20 h at 5°C the precipitate was collected and recrystallized from ethanol. Yield: 900 mg (67%).

1-(Acetoxymethyl)allopurinol (IIa). From VI (1.6 g; 10 mmol), acetic anhydride (2.5 ml; 26.5 mmol), and pyridine (20 ml). Yield: 780 mg (47%) (from ethanol).

1-(Nicotinoyloxymethyl)allopurinol (IIe). From VI (1.6 g; 10 mmol), nicotinoyl chloride hydrochloride (3.56 g; 20 mmol), triethylamine (0.25 ml; 20 mmol), and pyridine (20 ml). Yield: 810 mg (31%) (from ethanol).

1-(Ethoxycarbonyloxymethyl)allopurinol (IIg). From VI (1.6 g; 10 mmol), ethyl chloroformate (1.5 ml; 16 mmol), N,N-dimethylformamide (15 ml) and pyridine (5 ml). Yield: 905 mg (38%) (from ethanol).

2,5-bis(acetoxymethyl)allopurinol (Va). From VII (1.0 g; 5 mmol), acetic anhydride (3.0 ml; 32 mmol), and pyridine (10 ml). Yield: 950 mg (68%) (from ethanol).

2,5-bis(Butyryloxymethyl)allopurinol (Vb). From VII (1.0 g; 5 mmol), butyric anhydride (3.0 ml; 20 mmol), and pyridine (10 ml). Yield: 890 mg (54%) (from ethyl acetate).

1-(Butyryloxymethyl)allopurinol (IIb). To a suspension of VI (1.44 g; 8.7 mmol) in methylene chloride (100 ml) were added triethylamine (2.64 g; 26 mmol) and butyryl chloride (2.20 g; 20.8 mmol). The mixture was stirred at 20°C for 20 h. The resulting clear solution was washed with water (50 ml), aqueous sodium hydrogencarbonate (2.5%; 50 ml), and water (2 × 50 ml). The solution was dried and evaporated. The residue was triturated with ethyl acetate. Yield: 567 mg (28%) (from ethanol).

1-(N,N-Diethylsuccinamoyloxymethyl)allopurinol (IIf). A mixture of VI (1.47 g;

8.8 mmol), N,N-diethylsuccinamic acid (1.53 g; 8.8 mmol) (Pressman et al., 1948), N,N′-dicyclohexylcarbodiimide (1.8 g; 8.8 mmol), and 4-toluenesulfonic acid (100 mg) in pyridine (30 ml) was stirred at room temperature for 24 h. Methylene chloride (60 ml) was added, the mixture was filtered, and the filtrate evaporated in vacuo. The residue was extracted with warm methylene chloride (50 ml) and the extract was evaporated. Recrystallizations from ethyl acetate yielded 710 mg (25%).

Method B (Scheme 2)

The compounds IIb, IIc, IIIa, IIIb, IVa, IVb, Vb and Vc were synthesized by this method in which allopurinol(I) is reacted with the corresponding chloromethyl ester.

General procedure. To a solution of I (2.04 g; 15 mmol) in dimethyl sulfoxide (45 ml) kept at 40°C were added potassium carbonate (2.07 g; 15 mmol) and then dropwise during 1 h a solution of chloromethyl pivalate (2.2 g; 15 mmol) (Rasmussen and Leonard, 1967) in dimethyl sulfoxide (15 ml). The mixture was stirred at 40°C for 4 h and poured on ice (75 g). After acidification, the mixture was extracted with chloroform (3 × 70 ml). The combined extracts were washed with water, dried, and evaporated in vacuo. Toluene (15 ml) was added to the residue, and the solid precipitate was collected and washed with ether. Recrystallization of the solid from ethyl acetate afforded pure 2-(pivaloyloxymethyl)allopurinol (IIIb): 813 mg. The toluene–ether filtrate from IIIb was evaporated in vacuo. Column chromatography (silica gel; eluents: toluene–ethyl acetate–methanol) was performed on the residue, and the compounds were collected in the following order:

1,5-bis(Pivaloyloxymethyl)allopurinol (IVb). 90 mg (from ether–light petroleum).

2,5-bis(Pivaloyloxymethyl)allopurinol (Vc). 402 mg (from ethyl acetate–light petroleum).

1-(Pivaloyloxymethyl)allopurinol (IIc). 151 mg (from ethyl acetate). From the reaction of allopurinol (2.04 g; 15 mmol) and chloromethylpivalate (2.15 g; 15 mmol) (Ulich and Adams, 1921) the following compounds could be isolated.

1,5-bis(Butyryloxymethyl)allopurinol (IVa). 333 mg (from ethyl acetate–ether–light petroleum).

2,5-bis(Butyryloxymethyl)allopurinol (Vb). 438 mg (from ethyl acetate–ether–light petroleum).

1-(Butyryloxymethyl)allopurinol (IIb). 110 mg (from ethyl acetate).

2-(Butyryloxymethyl)allopurinol (IIIa). 600 mg (from ethyl acetate).

Kinetic measurements

The hydrolysis of the N-acyloxymethyl derivatives of allopurinol was studied in aqueous buffer solutions at 37.0 ± 0.2°C. Phosphate, borate and carbonate 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 followed by using a reversed-phase HPLC procedure. Mobile phase systems of 30–70% v/v methanol in 0.01 M acetate buffer of pH 4.5 were used, the concentration of methanol being adjusted for each compound to give an appropriate retention time (3–10 min), e.g. 30% v/v methanol being used for

1-(acetoxymethyl)allopurinol and 70% v/v methanol for the analysis of 2,5-bis(butyryloxymethyl)allopurinol. The flow rate was 1.2 or 1.6 $ml \cdot min^{-1}$ and the column effluent was monitored at 251 or 261 nm. 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 in ethanol to 10 ml of pre-heated buffer solution in screw-capped test tubes, the final concentration of the compounds being in the range 0.01–0.04 $mg \cdot ml^{-1}$. 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 derivatives 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–V was also studied in human plasma diluted to 80% with 0.05 M phoshate buffer (at 37°C). Initial concentrations of the compounds were 0.015–0.05 $mg \cdot ml^{-1}$. At appropriate intervals 250 μl samples were withdrawn and added to 1000 μl of ethanol in order to deproteinize the plasma. After immediate mixing and centrifugation for 2 min, 10 μl of the clear supernatant was analyzed by HPLC as described above for residual derivative or allopurinol formed. When analysis for allopurinol was performed the plasma samples (400 μl) were deproteinized by addition of a 20% solution of trichloroacetic acid (250 μl) followed by mixing and centrifugation.

Determination of water-solubility and partition coefficients

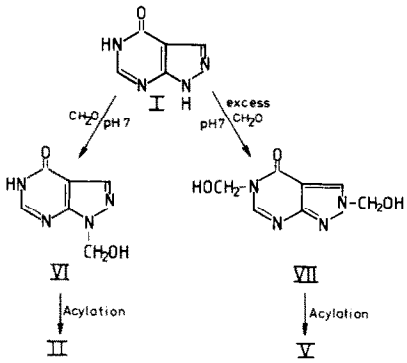
The aqueous solubility of the N-acyloxymethyl derivatives was determined at 22°C by adding excess amounts of the compounds to water in screw-capped test tubes. The mixtures were placed in an ultrasonic water-bath for about 15 min and then rotated on a mechanical spindle for 20–30 h. It was ensured that saturation equilibrium was established. Upon filtration an aliquot of the filtrate was in most cases diluted with an appropriate amount of water and the mixture analyzed by HPLC. The concentration of the compounds in their saturated solutions was calculated from the measured peak heights by reference to those of standards chromatographed under the same conditions.

The partition coefficients were determined in an octanol–water system at 22°C as previously described (Bundgaard and Falch, 1985a).

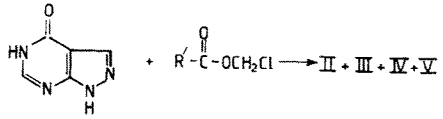
Results and Discussion

Synthesis and structures of the N-acyloxymethyl derivatives

The N_1-acyloxymethyl derivatives (II) were generally prepared by acylation of 1-(hydroxymethyl)allopurinol (VI), the latter being obtained in more than 90% yield by reacting allopurinol with formaldehyde in 0.05 M phoshate buffer solution of pH 7.0 for 48 h at room temperature as previously described by Bansal et al. (1981)



Scheme 1.



Scheme 2.

(Scheme 1). The 2,5-bis(acyloxymethyl) derivatives (V) were similarly prepared by acylation of 2,5-bis(hydroxymethyl)allopurinol (VII) (Scheme 1), the latter being obtained by reacting allopurinol with an excess of formaldehyde using exactly the same reaction conditions as those described by Bansal et al. (1981). The bis-hydroxymethylated allopurinol derivative obtained was by these investigators designated as 1,5-bis(hydroxymethyl)allopurinol on the basis of NMR spectral analysis. This appears, however, to be incorrect as reaction of the compound with butyric anhydride in pyridine afforded a derivative which we conclusively found to be 2,5-bis(butyryloxymethyl)allopurinol (Vb).

The evidence for this assignment is provided by the NMR and UV data given in Table 1 in comparison with the spectral data given by Bergmann et al. (1979) for various N-methyl derivatives of allopurinol. In this work it is shown that λ_{max} for 2,5-dimethylallopurinol occurs at 260 nm while that for 1,5-dimethylallopurinol is at 252 nm (cf. λ_{max} at 259 nm for Vb). Furthermore, the difference between the chemical shifts of the 3-H and 6-H NMR signals in Vb and the other 2,5-compounds prepared, as well as the difference between corresponding shifts for the 1,5-bis(acyloxymethyl) derivatives (IV), are of the same order as found for 2,5-dimethyl-allopurinol and 1,5-dimethylallopurinol, respectively (Bergmann et al., 1979). Also, as described later, compound Vb yielded 2-(butyryloxymethyl)allopurinol upon alkaline degradation. Finally, a conclusive proof that the compound (Vb) obtained by acylation of bis(hydroxymethyl)allopurinol with butyric anhydride is indeed 2,5-bis(butyryloxymethyl)allopurinol was confirmed by an X-ray crystallographic analysis (to be published elsewhere).

A mixture of the 2- and 1,5-substituted derivatives (as well as the 1- and 2,5-substituted derivatives) was obtained by alkylation of allopurinol with the appropriate α-chloromethyl ester in dimethyl sulfoxide in the presence of potassium carbonate (Scheme 2). Column chromatography was used to separate the various products formed.

The structures of all compounds were confirmed by elemental (C, H and N), NMR and UV analysis. Allopurinol exists in different tautomeric forms (Bergmann

et al., 1979) and N-acyloxymethylation may theoretically take place at the N_1, N_2, N_5 or N_7 position. Evidence for the assignment of the structure of the N_1-substituted acyloxymethylated compounds IIa–g and the N_2 position of the substituents in compounds IIIa and IIIb is provided by the NMR and UV data for the compounds (Table 1) in comparison with the spectral data for the known N-methyl derivatives of allopurinol given by Bergmann et al. (1979) as already noted. It is shown in this work 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. The compounds II and III show a λ_{max} of 280 nm and 285 nm, respectively, in alkaline solutions (pH 11) and this shift of 24–29 nm due to ionization of the 5-NH proton thus excludes an N_5- or N_7-acyloxymethyl 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 therefore the NMR data (Table 1) together with the UV data clearly establish the structures of derivatives II and III as N_1- and N_2-substituted compounds, respectively.

Considering the disubstituted derivatives (IV and V) a distinction between 1,5- and 2,5-structures has already been noted. The UV and NMR spectral data obtained for compounds IVa and IVb and Va-d (Table 1) are in agreement with those for 1,5- and 2,5-dimethylallopurinol, respectively. A 1,7- or a 2,7-structure for these diacyloxymethylated derivatives is readily excluded on the basis of the UV spectra in that 1,7- and 2,7-dimethylallopurinol show λ_{max} at 292 and 280 nm, respectively (Bergmann et al., 1979).

Kinetics of hydrolysis

The kinetics of hydrolysis of the various types of N-acyloxymethyl derivatives (II–V) was studied in aqueous buffer solutions at 37°C at pH 7.4–10.7. At constant pH and temperature the overall hydrolysis of all compounds displayed strict first-order kinetics for several half-lives. 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 some compounds of the structure II, III, IV or V is shown in Fig. 1 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 all other derivatives also showed a straight line of unity slope in the pH-range 7.4–10, indicating the occurrence of specific base-catalyzed hydrolysis according to the following rate expression:

$$k_{obs} = k_{OH} a_{OH} \tag{1}$$

where k_{OH} is a second-order specific base catalytic rate constant and a_{OH} refers to the hydroxide ion activity. The latter was calculated from the measured pH at 37°C according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = pH - 13.62 \tag{2}$$

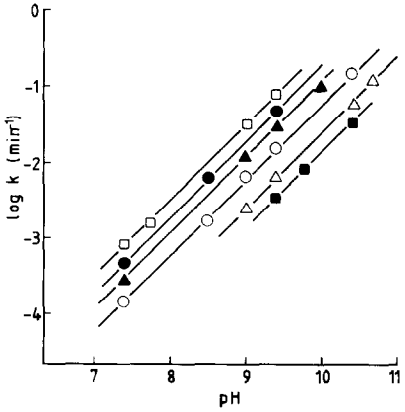


Fig. 1. The pH–rate profiles for the hydrolysis of various N-acyloxymethyl derivatives of allopurinol in aqueous solution ($\mu = 0.5$) at 37°C. Key: ○, IIa; △, IIb; ●, IIe; ■, IIIb; □, Va; and ▲, IVb.

The values of the rate constant k_{OH} as well as of observed or calculated (on the basis of Eqn. 1) half-lives of hydrolysis at pH 7.40 and 37°C are listed in Table 2.

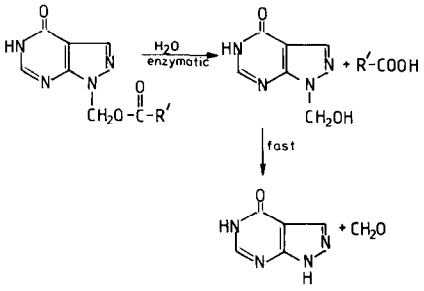
The 1,5- and 2,5-substituted derivatives are neutral compounds whereas the 1-acyloxymethyl (II) and 2-acyloxymethyl (III) derivatives are weak acids due to ionization of the 5-NH group, the pK_a values at 22°C and $\mu = 0.5$ being 8.7 (II) and

TABLE 2

RATE DATA FOR THE HYDROLYSIS OF VARIOUS N-ACYLOXYMETHYL DERIVATIVES (II–V) OF ALLOPURINOL IN AQUEOUS SOLUTION AND IN 80% HUMAN PLASMA AT 37°C

Compound		k_{OH} (M^{-1} min^{-1})	$t_{1/2}$	
No.	R in II–V		pH 7.40 buffer	80% human plasma
IIa	Acetyl	221	87 h	31 min
IIb	Butyryl	99.5	193 h [a]	9 min
IIc	Pivaloyl	13.1	146 h [a]	> 2 h
IId	Benzoyl	81.1	237 h [a]	4 min
IIe	Nicotinoyl	740	26 h	21 min
IIf	N,N-Diethylsuccinamyl	240	81 h	8.4 h
IIg	Ethoxycarbonyl	69.4	276 h [a]	20 min
IIIa	Butyryl	357	54 h	22 min [b]
IIIb	Pivaloyl	54.8	350 h [a]	12.0 h [b]
IVa	Butyryl	786	24.5 h	25 min [b]
IVb	Pivaloyl	468	41 h	–
Va	Acetyl	1288	15 h	51 min [b]
Vb	Butyryl	546	35 h	35 min [b]
Vc	Pivaloyl	100	192 h [a]	–

[a] The half-lives are estimated on the basis of the k_{OH} values determined at higher pH values.
[b] The half-lives are for the formation of allopurinol.



Scheme 3.

9.7 (III) as determined by the spectrophotometric titration method (Albert and Serjeant, 1971). Since there is no curvature in the pH–rate profiles for these compounds at pH values around pK_a (cf. Fig. 1) the undissociated and anionic forms of the compounds appear to exhibit almost the same reactivity. It was previously found (Bundgaard and Falch, 1985a) that in N_1-acyl derivatives ($pK_a \sim 7.6$) the neutral forms are considerably more reactive than the anionic species. In these acyl derivatives, however, the allopurinol molecule forms a direct part of the amide bond undergoing hydrolysis in contrast to the N-acyloxymethyl derivatives where the cleavage takes place at the ester group removed from the allopurinol moiety.

In the hydrolysis of N_1-acyloxymethyl derivatives of 5-fluorouracil a similar lack of influence of ionization within the drug moiety (the 3-NH group of 5-fluorouracil) has been observed (Buur et al., 1985).

The hydrolytic removal of the acyloxymethyl groups of the compounds II–V most likely takes place via a two-step reaction as depicted in Scheme 3 for a 1-acyloxymethyl derivative. Rate-determining cleavage of the ester grouping results in the formation of the corresponding N-(hydroxymethyl)allopurinol which is decomposed instantaneously into formaldehyde and allopurinol in accord with the behaviour of other similar N-hydroxymethyl derivatives (Johansen and Bundgaard, 1979, 1981; Bundgaard, 1982; Bundgaard and Johansen, 1980, 1984; Bansal et al., 1981; Johansen et al., 1983; Varia et al., 1984a and b; Buur et al., 1985). In fact, 1-(hydroxymethyl)allopurinol (VI) was found to be so unstable in aqueous solutions including acidic solutions that no rate data for its conversion to allopurinol could be obtained using either UV spectrophotometry, HPLC or analysis of formaldehyde formation according to Johansen et al. (1983). For 2,5-bis(hydroxymethyl)allopurinol (VII) it was possible to follow the rate of hydrolysis to allopurinol by direct spectrophotometry. Upon addition of 250 μl of a saturated solution of the compound in dioxane to 2.5 ml of buffer solutions the absorbance at 263 nm decreased slightly. As seen from Fig. 2 the observed pseudo-first-order rate constant for this hydrolysis is directly proportional to the hydroxide ion activity in the pH range of 5.5–7. Extrapolation of the pH–rate profile to pH 7.4 gives a half-life of decomposition of 1.0 s at 23°C. Further evidence for the only transitory existence of the N-hydroxymethyl derivatives of allopurinol in the overall hydrolysis of com-

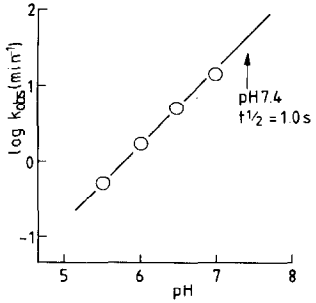
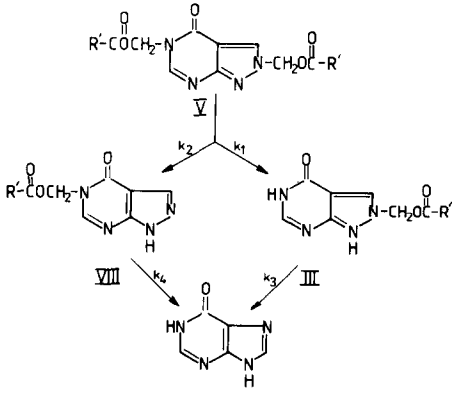


Fig. 2. The pH–rate profile for the decomposition of 2,5-bis(hydroxymethyl)allopurinol in aqueous solution containing 9% v/v of dioxane at 23°C ($\mu = 0.5$).

pounds II and III in neutral and basic solution stems from the fact that no induction period was observed in the formation of allopurinol from these derivatives as determined by HPLC.

Compounds II and III were found to be converted quantitatively into allopurinol in neutral and basic solution as evidenced by HPLC analysis. The hydrolysis of the 2,5- and 1,5-diacyloxymethylated derivatives V and IV should be expected to proceed through the intermediate formation of the corresponding 1-, 2- and 5-monoacyloxymethyl derivatives, the overall rate of loss of IV or V being described by Eqn. 1. The hydrolytic breakdown of the pivaloyloxymethyl derivatives Vc and IVb at pH 10.95 was examined in more detail using HPLC analysis. The disappearance of Vc was found to be accompanied by the formation of IIIb, identified on the basis of its HPLC retention behaviour in comparison with that of authentic IIIb, and of a compound which is assumed to be a corresponding 5-acyloxymethyl derivative (VIII) (mobile phase used in HPLC: methanol–0.01 M acetate pH 4.5 (45 : 55 v/v)). Following their formation these intermediate products degraded into allopurinol which was formed in quantitative amounts as evidenced by HPLC analysis of



Scheme 4.

completed reaction solutions. The proposed reactions taking place are shown in Scheme 4 where k_1–k_4 are pseudo-first-order rate constants for the depicted reactions. At pH 10.95 and 37°C the following values of the rate constants were derived:

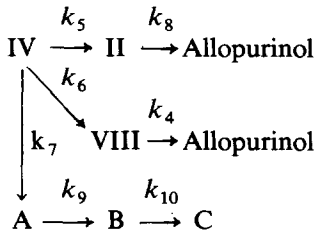
$k_{obs} = k_1 + k_2 = 0.22\ min^{-1}$
$k_1 = 0.10\ min^{-1}$
$k_2 = 0.12\ min^{-1}$
$k_3 = 0.10\ min^{-1}$
$k_4 = 0.037\ min^{-1}$

It is seen that the 2- and 5-pivaloyloxymethyl derivatives are formed in almost equal amounts and that the 2-compound is about 3-fold as labile as the 5-isomer.

Whereas the 2,5-compound hydrolyzed as expected the 1,5-bis(pivaloyloxymethyl) derivative (IVb) showed a complicated hydrolytic breakdown, giving rise to the formation of several products. HPLC analysis of reaction solutions of IVb in 0.05 M carbonate buffer of pH 10.95 at 37°C showed the formation of three unknown peaks in addition to those due to IIc, allopurinol and the assumed 5-compounds (VIII). On the basis of the chromatographic monitoring the reactions taking place are proposed to be as described in Scheme 5, in which A, B and C are unknown degradation products and k_4–k_{10} are pseudo-first-order rate constants. At the end of reaction allopurinol was only formed in a yield of 30% based on the initial concentration of IVb, thus implying the k_7-reaction as a major route of degradation (probably due to ring-opening of the pyrimidine moeity). From the time-courses of the various peaks in the HPLC chromatogram the following values of the rate constants at pH 10.95 (37°C) were derived:

$k_4 = 0.037\ min^{-1}$
$k_5 + k_6 \simeq 0.3\ min^{-1}$
$k_7 \simeq 0.7\ min^{-1}$
$k_8 = 0.024\ min^{-1}$
$k_9 = 0.14\ min^{-1}$
$k_{10} = 0.047\ min^{-1}$

A similar scheme of breakdown was observed for the 1,5-bis(butyryloxymethyl) derivative (IVa) at pH 9.8.



Scheme 5.

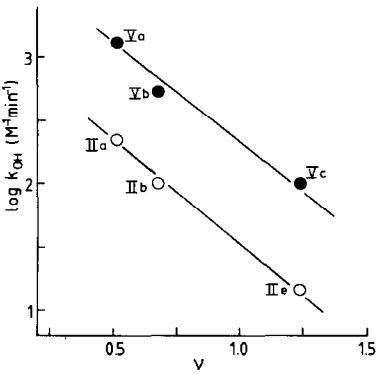


Fig. 3. Plots of log k_{OH} vs the steric parameter ν for various N-acyloxymethyl derivatives of allopurinol. The ν values refer to the alkyl moiety in the acyl groups of the compounds, i.e. methyl, propyl and *t*-butyl.

From the hydrolytic studies it can be concluded that except for the 1,5-bis(acyloxymethyl) derivatives (for the behaviour in plasma solutions, see below) allopurinol is released in quantitative amounts by rate-determining cleavage of the ester groupings which can be controlled by steric and electronic factors. The influence of the steric effect within the acyl substituents (R) can be illustrated by the acetyl, butyryl and pivaloyl esters of the formulae II and V. The polar effects of these acyl groups are almost identical and the differences in reactivity in neutral and alkaline aqueous solution can solely be ascribed to differences in the steric properties as shown in Fig. 3, where log k_{OH} is plotted against Charton's steric parameter ν (Charton, 1977).

Enzymatic hydrolysis of the derivatives

The susceptibility of the N-acyloxymethyl derivatives to undergo a potential enzymatic hydrolysis was studied in vitro at 37°C in 80% human plasma of pH 7.40 (with 0.01 M phosphate buffer). The hydrolysis of all derivatives followed strict first-order kinetics and proceeded in all cases to give allopurinol in quantitative amounts as evidenced by HPLC analysis (cf. Fig. 4). In contrast to the behaviour in alkaline aqueous solution the 1,5-bis(acyloxymethyl) derivatives (IV) were converted quantitatively into allopurinol in the presence of plasma as shown in Fig. 5. This observation implies that the k_7-reaction in Scheme 5 is of no importance in enzymatic reactions in relation to the ester hydrolysis reactions (the k_5- and k_6-reactions) and that the 1,5-compounds also fulfill the requirement of a prodrug as being quantitatively converted to the parent drug under conditions similar to those prevailing in vivo. Furthermore, as it can be seen from Fig. 5 the formation of allopurinol from the 1,5- and 2,5-derivatives proceeds through a short lag period which is due to the initial formation of the 1-, 2- and 5-acyloxymethyl derivatives.

As appears from the rate data obtained (Table 2), human plasma accelerates the rate of hydrolysis markedly which is in agreement with the behaviour of N-acyloxymethyl derivatives of various other drugs (Johansen and Bundgaard, 1981;

Fig. 4.

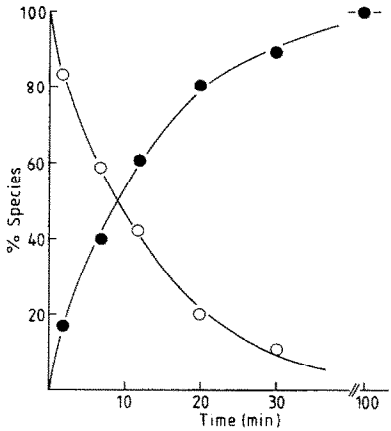


Fig. 5.

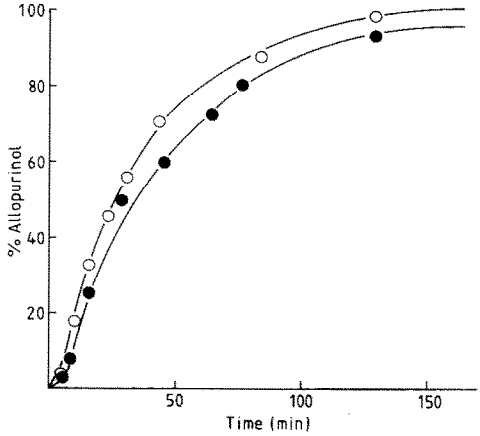


Fig. 4. Plots showing the rate of disappearance of 1-(butyryloxymethyl)allopurinol (IIb) (○) in 80% human plasma at 37°C and the rate of the accompanying formation of allopurinol (●).

Fig. 5. Plots showing the rate of formation of allopurinol by hydrolysis of 1,5-bis(butyryloxymethyl)allopurinol (IVa) (○) and 2,5-bis(butyryloxymethyl)allopurinol(Vb) (●) in 80% human plasma (pH 7.4) at 37°C.

Johansen et al., 1983; Yamaoka et al., 1983; Varia et al., 1984a; Buur et al., 1985). Steric and polar effects within the acyl moieties greatly affect the enzymatic rates of hydrolysis but most of the allopurinol derivatives studied appear to be cleaved so rapidly by plasma enzymes that they would behave as allopurinol prodrugs in vivo. The low reactivity of the pivaloyloxymethyl derivatives may be ascribed to steric hindrance exhibited by the bulky pivaloyl group. More surprisingly, the 1-(N,N-diethylsuccinamyloxymethyl) derivative (IIf) is relatively resistant to undergo enzymatic hydrolysis. It is interesting to note that whereas 1-substituted derivatives are more stable than the corresponding 2-substituted derivatives toward hydroxide ion-catalyzed hydrolysis, they are more susceptible to undergoing enzymatic hydrolysis, cf. e.g. IIb and IIIb in Table 2.

Lipophilicity and aqueous solubility of the N-acyloxymethyl derivatives

Partition coefficients (P) for the N-acyloxymethyl derivatives as determined using the widely used 1-octanol–water system are listed in Table 3 along with data for the aqueous solubility. Since the compounds are unionized in water the log P values given represent true partition coefficients; in agreement with the pK_a values of compounds II and III the same partition coefficients as those given were obtained using a phosphate buffer of pH 7.4 as the aqueous phase. The results obtained show that the derivatives are all more lipophilic than the parent allopurinol. The difference in the log P values for related derivatives, e.g. IIa–e, is as expected on the basis of the π substituent values, e.g. 0.5 for a methylene group (Hansch and Leo, 1979).

The lipophilicity of the derivatives was also evaluated by means of reversed-phase

TABLE 3

PARTITION COEFFICIENTS (P), HPLC CHROMATOGRAPHIC CAPACITY FACTORS (k') AND WATER-SOLUBILITIES (S) OF ALLOPURINOL AND VARIOUS N-ACYLOXYMETHYL DERIVATES OF ALLOPURINOL (II–V)

Compound		log P [a]	k' [b]	S [c]
No.	R in II–V			(mg·ml^{-1})
I	Allopurinol	−0.55	< 0.2	0.50
IIa	Acetyl	−0.35	0.30	0.58
IIb	Butyryl	0.60	0.85	0.35
IIc	Pivaloyl	1.07	1.50	0.52
IId	Benzoyl	1.50	2.00	0.024
IIe	Nicotinoyl	0.27	0.67	0.093
IIf	N,N-Diethylsuccinamyl	−0.22	0.35	33
IIg	Ethoxycarbonyl	0.21	0.80	-
IIIa	Butyryl	0.33	0.65	1.5
IIIb	Pivaloyl	0.79	1.07	1.7
IVa	Butyryl	1.82	4.82	0.050
IVb	Pivaloyl	2.50	11.8	0.020
Va	Acetyl	−0.20	0.40	2.9
Vb	Butyryl	1.60	3.37	0.094
Vc	Pivaloyl	2.34	9.9	0.045

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

HPLC (e.g. Brent et al., 1983; Hafkenscheid and Tomlinson, 1983). In this method the capacity factor (k') of a solute is taken as a measure of the relative lipophilicity:

$$k' = (t_r - t_0)/t_0 \qquad (3)$$

where t_r is the retention time of the solute and t_0 is the elution time of the solvent. With methanol–acetate buffer pH 4.5 (1 : 1 v/v) as mobile phase the derivatives showed the k' values given in Table 3. These data also demonstrate the higher lipophilicity of the derivatives in comparison with allopurinol. As has been observed for other compounds (Hafkenscheid and Tomlinson, 1983, 1984; and references cited therein) a linear relationship existed between log k' and log P for the allopurinol derivatives (Fig. 6):

$$\log k' = 0.55(\pm 0.02)\log P - 0.37(\pm 0.03) \qquad n = 13;\ r = 0.992 \qquad (4)$$

An increase in lipophilicity is generally accompanied by a decrease in water-solubility. Inspection of the data in Table 3 reveals, however, that the aqueous solubility of some of the derivatives (IIa, IIf, IIIa and IIIb) is in fact increased relative to that of allopurinol despite the higher log P values of the compounds. As discussed previously (Bundgaard and Falch, 1985a) the poor solubility of allopurinol in water and other solvents like octanol may largely be a result of the high crystal lattice

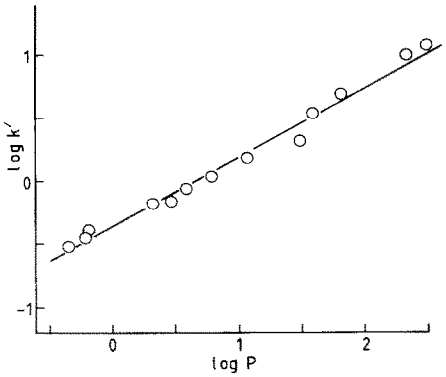


Fig. 6. Plot of log k' against log P for 13 N-acyloxymethyl derivatives of allopurinol; the values are taken from Table 3.

energy in the molecule due to intermolecular hydrogen bonds (Prusiner and Sundaralingam, 1972) as reflected in its high melting point (~ 365°C). Consequently, disruption or decrease of such hydrogen bonding by replacement of the N-1, N-2 or N-5 protons by acyloxymethyl groups should lead to derivatives with decreased crystal lattice energy (as manifested in a pronounced melting point decrease, cf. Table 1) and thus, with higher solubilities. If the groups introduced are relatively non-lipophilic as such, e.g. an acetoxymethyl or N,N-diethylsuccinamyloxymethyl group, the net result may be both enhanced water-solubility and an increased octanol–water partition coefficient.

That crystal lattice energy and hence melting points play a major role in the relationship between aqueous solubility and octanol–water partition coefficients of crystalline solutes is well recognized (Valvani and Yalkowsky, 1980; Yalkowsky and Valvani, 1980; Yalkowski, 1981; Yalkowsky et al., 1983). As shown by these authors the relationship between the aqueous solubility (S, in molar concentration) and octanol–water partition coefficients of crystalline organic compounds contains a term for melting point:

$$\log S = -\underline{a} \log P - \underline{b}\ \text{m.p.} + \underline{c} \qquad (5)$$

where $\underline{a}$, $\underline{b}$ and $\underline{c}$ are constants which may vary somewhat for different types of chemical structures, $\underline{a}$ usually being around unity and $\underline{b}$ around 0.01. Multiple-regression analysis of the presently described N-acyloxymethyl derivatives (Table 3) as well as of four N_1-acyl allopurinol derivatives described previously (Bundgaard and Falch, 1985a), covering a wide variety of melting points, partition coefficients and water-solubilities, yielded the relationship shown in Fig. 7 and expressed by the following equation:

$$\log S = -1.08(\pm 0.13)\log P - 0.0073(\pm 0.0020)\text{m.p.} - 0.65(\pm 0.80) \qquad (6)$$

$n = 18$; $r = 0.918$

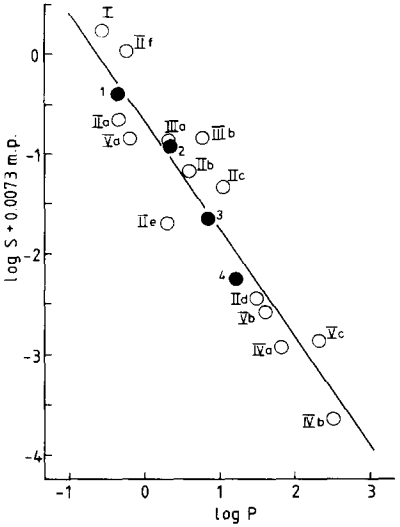


Fig. 7. Plot showing the relationship between melting point, water-solubility (S) and octanol–water partition coefficient (P) for various N-acyloxymethyl (○) and N_1-acyl (●) derivatives of allopurinol according to Eqn. 6. The numbers refer to the derivatives listed in Table 3. The N_1-acyl derivatives are as follows: 1, 1-(acetyl)allopurinol; 2, 1-(propionyl)allopurinol; 3, 1-(butyryl)allopurinol; 4, 1-(benzoyl)allopurinol.

It is seen that the coefficient for the log P is close to -1 and that for the m.p. term close to 0.01, the theoretical values (Valvani and Yalkowsky, 1980).

N-Acyloxymethyl derivatives as potential prodrug candidates for allopurinol

The results of the present study suggest that N-acyloxymethylation is a potentially useful approach to obtain prodrug forms of allopurinol. The usefulness of this approach which in the past has also been applied to various NH-acidic drugs, e.g. theophylline, phenytoin and 5-fluorouracil (Bodor, 1979, 1981; Sloan and Bodor, 1982; Bundgaard, 1982; Møllgaard et al., 1982; Yamaoka et al., 1983; Sloan et al., 1983; Varia et al., 1984a and b; Buur et al., 1985), stems from the fact that by varying the acyl portion of the derivatives it is possible to control the rate of regeneration of the drug in vivo and to obtain prodrugs with varying water-solubility and lipophilicity. These properties are of utmost importance for drug delivery. For allopurinol, N-acyloxymethylation provides derivatives with a much reduced melting point relative to that of the parent drug and as shown above it is possible to obtain derivatives possessing both higher octanol–water partition coefficients and higher or only slightly reduced aqueous solubilities relative to allopurinol.

Considering the primary aim of this work which is to develop prodrug derivatives useful for enhancing the rectal bioavailability of allopurinol, derivatives possessing both sufficient water-solubility and lipophilicity as well as a rapid rate of enzymatic conversion to allopurinol should be selected. As will be reported in a subsequent paper rectal administration in rabbits of some of the presently described derivatives,

e.g. 1-(butyryloxymethyl)allopurinol (IIb), was found to result in a significantly enhanced bioavailability of allopurinol relative to the administration of the parent drug per se. However, a limitation by the derivatives is certainly their relatively low water-solubilities. Only the 1-(N,N-diethylsuccinamyloxymethyl) derivative (IIf) possesses good aqueous solubility but on the other hand, it shows a relatively poor lipophilicity as well as a slow rate of enzymatic conversion to allopurinol as assessed in vitro. This limitation may be overcome by introducing a slightly basic amino function (pK_a 6–7) in the acyl moiety of the derivatives I as described in a following paper (Bundgaard and Falch, 1985b).

Acknowledgement

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