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Allopurinol prodrugs. V. Water-soluble *N*-substituted (aminomethyl)benzoyloxymethyl allopurinol derivatives for parenteral or rectal delivery

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Summary

Various *N*-substituted [(3- or 4-aminomethyl)benzoyloxymethyl]allopurinol derivatives were synthesized by esterification of 1-(hydroxymethyl)allopurinol and evaluated as water-soluble prodrugs with the aim of developing preparations suitable for parenteral and/or rectal administration. The derivatives combine a good solubility and high chemical stability in weakly acidic solutions with a high susceptibility to undergo enzymatic hydrolysis in plasma. The derivatives were much more lipophilic than allopurinol as determined by partition experiments in octanol-aqueous buffer solution. The rectal and parenteral absorption characteristics of three derivatives and of allopurinol were assessed in rabbits. Following intravenous administration the derivatives were quickly converted yielding allopurinol in quantitative amounts. After rectal administration of the three prodrugs an absolute bioavailability of 19, 38 and 41%, respectively, was found whereas the rectal absorption of allopurinol itself was only 3%.

Introduction

Allopurinol (**I**) is widely used to prevent or treat hyperuricaemia or hyperuricosuria that may result from the rapid lysis of cells in patients with malignancies treated with cytotoxic drugs or radiation (Elion, 1978). The drug is conventionally administered orally in the form of tablets or capsules. However, nausea and vomiting in patients undergoing cancer chemotherapy frequently preclude the use of oral preparations. Alternative means of administering allopurinol include injectable and rectal preparations.

Parenteral dosage forms for simple injection are, however, not available because of the low solubility of allopurinol in water (0.5 mg ml^{-1} at 20 °C) or other solvents suitable for parenteral administration, and, with rectal preparations, only very minute amounts (< 5%) are absorbed (Appelbaum et al., 1980, 1982; Chang et al., 1981).

Since these delivery problems can primarily be attributed to the low water and lipid solubility of the drug (Bundgaard and Falch, 1985a), it appears likely that the delivery characteristics of allopurinol can be improved by using the prodrug

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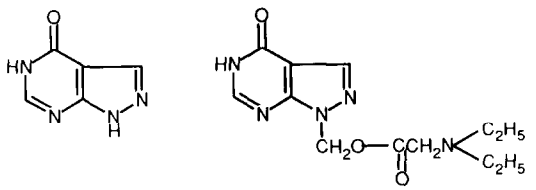
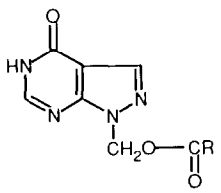
approach, i.e. development of derivatives possessing both a high water solubility and lipophilicity at physiological pH and being capable of reverting rapidly and quantitatively to the parent drug following absorption.

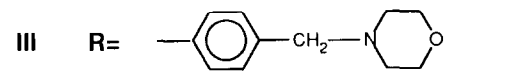
We have previously shown that various *N*-acyloxymethyl derivatives of allopurinol, notably those formed at the N-1 position of allopurinol, may be promising prodrug forms to enhance the parenteral and rectal absorption characteristics of the drug (Bundgaard and Falch, 1985b,c; Bundgaard et al., 1985; Bundgaard, 1989). The most promising prodrug candidate was identified to be 1-(*N*,*N*-diethylglycyloxymethyl)allopurinol (**II**). Following rectal administration to humans it showed an absolute bioavailability of about 40% (Bundgaard, 1989). Since the compound as the hydrochloride salt is highly soluble in water and is rapidly hydrolyzed by plasma enzymes, it is also potentially suitable as a parenteral delivery form of the parent drug (Bundgaard and Falch, 1985c; Bundgaard et al., 1985). A drawback of the compound concerning the latter use is, however, its very limited stability in aqueous solution. At pH 3–5 and 23° C the time for 10% degradation of the prodrug derivative is only 14 h (Bundgaard and Falch, 1985c), thus precluding the formulation of a ready-to-use solution.

The major reason for the high instability of the amino acid ester moiety in compound **II**, and of α-amino acid esters in general, in weakly acidic solution is due to the strongly electron-withdrawing effect of the protonated amino group which activates the ester linkage towards hydroxide ion and water attack and, perhaps predominantly, to intramolecular catalysis or assistance by the neighbouring amino group of ester hydrolysis (Bruice and Benkovic, 1966; Bundgaard et al., 1984; Bundgaard and Falch, 1985c). We have recently found that the hydrolysis-facilitating effect of the water-solubilizing amino group can be blocked by incorporating a phenyl group between the ester moiety and the amino group (Bundgaard et al., 1989). Because of the requirement of a pK_a value greater than 5–6 for the amino group (for solubility reasons), the amino group is not directly attached to the phenyl nucleus but separated from this by an alkylidene group, in the most simple

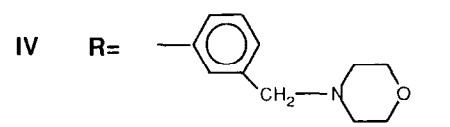


I II





III R=



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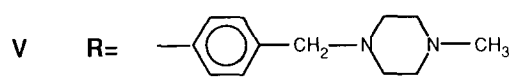

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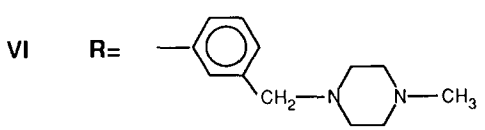

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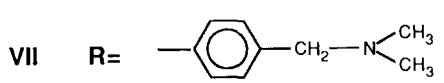
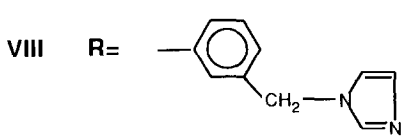


VII R=



VIII R=

case a methylene group. The position of substitution of the aminomethyl group may be 3 or 4, but not 2, since intramolecular catalysis occurs in *N*-substituted (2-aminomethyl)benzoate esters (to be published).

In the present work, a series of such *N*-substituted (3- or 4-aminomethyl)benzoyloxymethyl derivatives of allopurinol (**III–VIII**) have been prepared and evaluated as solution-stable, biolabile prodrug forms. To this end, the chemical stability and enzyme-mediated conversion of the new derivatives were investigated and their aqueous solubility and lipophilicity were determined. Furthermore, the bioavailability of some of these allopurinol prodrugs has been assessed following rectal and parenteral administration to rabbits.

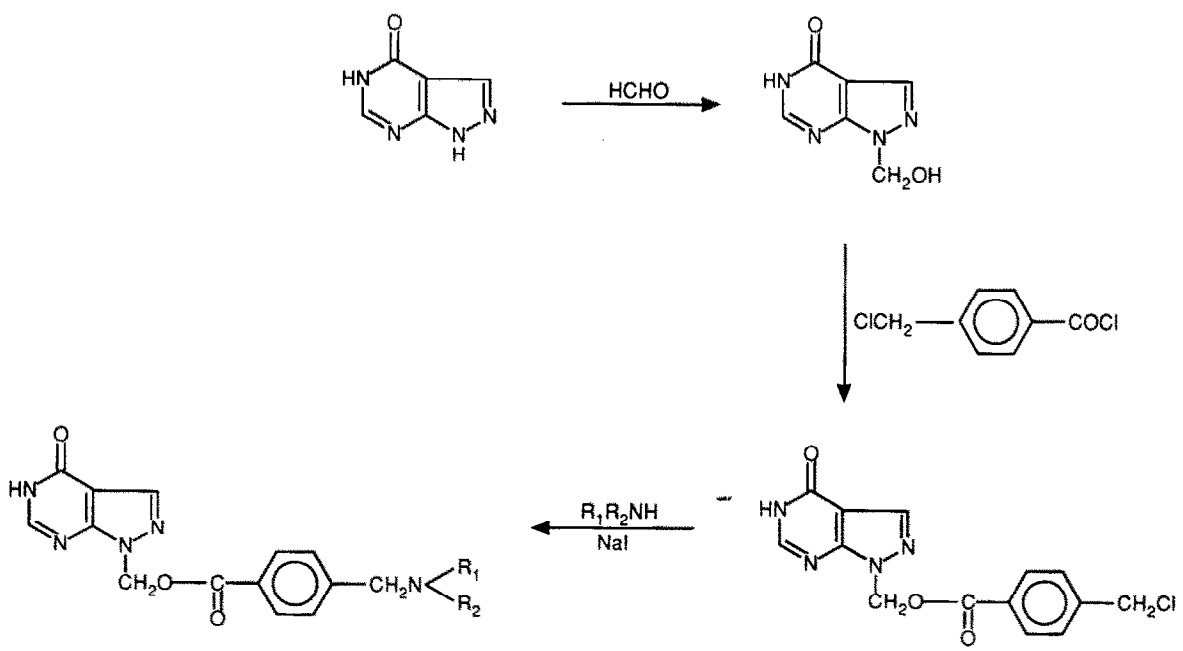
Materials and Methods

Apparatus

High-performance liquid chromatography (HPLC) was performed with a Shimadzu apparatus consisting of an LC-6A pump, an SPD-6A variable-wavelength detector and a 20 μl loop injection valve (Rheodyne). A deactivated reversed-phase Supelcosil LC-8-DB column (33 × 4.6 mm) (3 μm particles) equipped with a Supelguard column (purchased from Supelco, U.S.A.) was used. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. ^{1}H-NMR spectra were run on a Varian 360L instrument. Melting points were taken in capillary tubes and are not corrected. Elemental analyses were performed by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Synthesis of the allopurinol derivatives II–VIII

The *N*-substituted (3- or 4-aminomethyl)benzoyloxymethyl derivatives of allopurinol (**III–VIII**) were prepared by esterifying 1-(hydroxymethyl)allopurinol with (3- or 4-chloromethyl)benzoyl chloride and subsequent reaction of the (chloromethyl)benzoate esters obtained with the appropriate amine in the presence of catalytic amounts of sodium iodide (Scheme 1). 1-(Hydroxymethyl) allopurinol was prepared by reacting allopurinol with formaldehyde as previously described (Bansal et al., 1981).



Scheme 1.

Physical and analytical data for derivatives **III**–**VIII** are given Table 1. The NMR spectra of the compounds were consistent with their structures.

1-[(4-Chloromethyl)benzoyloxymethyl]allopurinol

A solution of (4-chloromethyl)benzoyl chloride (prepared from (4-chloromethyl)benzoic acid (5.12 g, 30 mmol) by refluxing with an excess of thionyl chloride for 1.5 h) in methylene chloride (10 ml) was added to a suspension of 1-(hydroxymethyl) allopurinol (3.84 g, 24 mmol) in pyridine (60 ml). The mixture was stirred at room temperature for 3 h and filtered. The filtrate was evaporated in vacuo to half-volume and water (100 ml) was added. The precipitate formed (5.97 g, 62%) was collected and recrystallized from 2-propanol/*N*,*N*-dimethylformamide to give the title compound, m.p. 245–247 °C.

Anal.: Calc. for $C_{14}H_{11}ClN_4O_3$: C, 52,76; H, 3.48; Cl, 11.13; N, 17.58. Found: C, 52.70; H, 3.63; Cl, 11.00; N, 17.42.

1-[(3-Chloromethyl)benzoyloxymethyl]allopurinol

A mixture of 1-(hydroxymethyl)allopurinol (1.6 g, 10 mmol) and (3-chloromethyl)benzoyl chloride (1.85 ml, 13 mmol) in pyridine (20 ml) was stirred at room temperature for 3 h. Water (100 ml) was added, and after standing for 3 h at 5 °C the precipitate was collected, washed with water and recrystallized from ethanol to give 2.0 g of the title compound, m.p. 201–202 °C.

TABLE 1

Physical and analytical data of various 1-acyloxymethyl derivatives of allopurinol

Compound	Form	M.p. (°C)	Formula	Analysis (%)		
				Calculated		Found
III	Free base	174–176	$C_{18}H_{19}N_5O_4$	C	58.53	58.60
				H	5.18	5.12
				N	18.96	18.87
	Hydrochloride	210–213	$C_{18}H_{20}ClN_5O_4$,	C	51.01	50.68
				H	5.23	5.40
			H_2O	N	16.52	16.55
				Cl	8.37	8.31
IV	Free base	126–128	$C_{18}H_{19}N_5O_4$	C	58.53	58.14
				H	5.18	5.20
				N	18.96	18.72
V	Free base	190–192	$C_{19}H_{22}N_6O_3$	C	59.67	59.42
				H	5.80	5.88
				N	21.98	21.87
VI	Free base	129–131	$C_{19}H_{22}N_6O_3$,	C	58.99	59.08
			2/3 $C_4H_8O_2$	H	6.24	6.25
				N	19.05	19.17
	Fumarate	212–214	$C_{23}H_{26}N_6O_7$,	C	54.92	55.05
	(1 equiv.)		0.25 H_2O	H	5.31	5.39
				N	16.71	16.53
VII	Free base	172–175	$C_{16}H_{17}N_5O_3$	C	58.71	58.53
				H	5.23	5.30
				N	21.40	21.97
VIII	Free base	148–150	$C_{17}H_{14}N_6O_3$	C	58.28	58.35
				H	4.03	4.04
				N	23.99	23.95

Anal.: Calc. for $C_{14}H_{11}ClN_4O_3$: C, 52.76; H, 3.48; Cl, 11.13; N, 17.58. Found: C, 52.66; H, 3.49; Cl, 11.12; N, 17.56.

1[(4-Morpholinomethyl)benzoyloxymethyl]allopurinol (III)

A mixture of 1-[(4-chloromethyl)benzoyloxymethyl]allopurinol (478 mg, 1.5 mmol), morpholine (0.53 ml, 6 mmol), sodium iodide (10 mg) and *N,N*-dimethylformamide (5 ml) was stirred at 50 °C for 5 h. The mixture was filtered and the filtrate evaporated in vacuo. The residue was dissolved in methylene chloride (50 ml) and the solution was washed twice with water, dried and evaporated. The residue was recrystallized from ethanol to give the title compound (409 mg, 74%) as the base.

The hydrochloride salt was prepared by adding a 2 M solution of hydrochloric acid in ethyl acetate to a solution of the base in ethanol. The precipitate formed was recrystallized from methanol-ethanol (1 : 1) to give the hydrochloride salt of **III** as a monohydrate.

1-[(3-Morpholinomethyl)benzoyloxymethyl]allopurinol (IV)

Morpholine (0.53 ml, 6 mmol) and sodium iodide (10 mg) were added to a solution of 1-[(3-chloromethyl)benzoyloxymethyl]allopurinol (478 mg, 1.5 mmol) in *N,N*-dimethylformamide (5 ml). The mixture was stirred at 50 °C for 5 h and left at room temperature for 20 h. After evaporation in vacuo methylene chloride (30 ml) was added to the residue and the solution was washed twice with water, dried and evaporated. The residue was recrystallized from ethyl acetate to give the title compound (307 mg, 55%).

1-[4-(4-Methylpiperazin-1-yl)methylbenzoyloxymethyl]allopurinol (V)

The compound was prepared from 1-(4-chloromethylbenzoyloxymethyl)allopurinol (3 mmol) and 1-methylpiperazine (12 mmol) by essentially the same procedure as described for compound **IV**. The compound was recrystallized from 2-propanol/ethyl acetate. The yield was 29%.

1-[3-(4-Methylpiperazin-1-yl)methylbenzoyloxymethyl]allopurinol (VI)

The compound was prepared from 1-(3-chloromethylbenzoyloxymethyl)allopurinol (1.5 mmol) and 1-methylpiperazine (6 mmol) by the same procedure as described for compound **IV**. The compound crystallized from ethyl acetate with 2/3 mol of ethyl acetate. The yield was 46%.

Treatment of the free base form of **VI** dissolved in ethyl acetate, with a solution of fumaric acid in 2- propanol and subsequent addition of ether yielded a salt with 1 equivalent fumaric acid which crystallized from 2-propanol/ethanol with 0.25 mol of water.

1-[(4-Dimethylaminomethyl)benzoyloxymethyl]allopurinol (VII)

The compound was prepared from 1-(4-chloromethylbenzoyloxymethyl)allopurinol (3 mmol) and a 40% ethanolic solution of dimethylamine (12 mmol) by the same procedure as described for compound **IV**. The compound was recrystallized from ethyl acetate/ethanol. The yield was 34%.

1-[(3-Imidazolylmethyl)benzoyloxymethyl]allopurinol (VIII)

A mixture of 1-[(3-chloromethyl)benzoyloxymethyl]allopurinol (318 mg, 1 mmol), sodium iodide (150 mg, 1 mmol), imidazole (340 mg, 5 mmol) and 15 ml of acetone was stirred at 60 °C for 5 h. The mixture was cooled to room temperature, filtered and evaporated in vacuo. The residue obtained was stirred with water (20 ml) for 2 h and the precipitate formed filtered off, washed with water and recrystallized from ethanol/water to give 280 mg of the title compound.

Kinetic measurements

Hydrolysis in aqueous solutions. The hydrolysis of the esters **III–VIII** was studied in aqueous buffer solutions at constant temperature (± 0.2 °C). The buffers used were hydrochloric acid, acetate, phosphate, borate and carbonate buffers; the total buffer concentration was generally 0.02 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 generally followed by monitoring the

disappearance of the derivatives using a reversed-phase HPLC procedure. A mobile phase system of methanol-acetonitrile-0.1% phosphoric acid (5 : 10 : 85 v/v) containing triethylamine (10^{-3} M) to improve peak shape was used for all the compounds. With this system the compounds showed retention times of 3–5 min whereas allopurinol and the appropriate *N*-substituted aminomethylbenzoic acid appeared in the solvent front. The flow rate was 1.0 ml min^{-1} and the column effluent was monitored at 215 nm. Quantitation of the compounds was performed 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 esters in ethanol or water to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the compounds being about 3×10^{-5} M. The solutions were kept in a water bath at constant temperature and at appropriate intervals, samples were taken and chromatographed immediately. Pseudo-first-order rate constants for the degradation of the derivatives were determined from the slopes of linear plots of the logarithm of residual ester against time.

For slowly proceeding reactions (at pH 1–7.4) the rate constants were determined by measuring the initial rates of allopurinol formation. In these cases, the initial derivative concentration was about 8×10^{-4} M and the formation of allopurinol was followed up to 1–3% of the initial derivative concentration. Quantitation of allopurinol was carried out by HPLC using 0.1% phosphoric acid as the eluent, the column effluent being monitored at 265 nm. Pseudo-first-order rate constants for the decomposition were obtained by dividing the slopes of linear plots of allopurinol formed vs time with the initial derivative concentration.

Hydrolysis in human plasma. The derivatives **III–VIII** were incubated at 37 °C in human plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.40. The initial concentration of the compounds was 6×10^{-5} M. At appropriate intervals, samples of 250 μl of the plasma reaction solutions were withdrawn and added to 500 μl of a 2% solution of zinc sulphate in methanol-water (1 : 1 v/v) in order to deproteinize the plasma. After mixing and centrifugation for 3 min at 13000 rpm, 20 μl of the clear supernatant was analyzed by HPLC as described above. Pseudo-first-order rate constants were calculated from the slopes of linear plots of the logarithm of residual derivative against time. When analysis for allopurinol was performed the plasma samples (400 μl) were deproteinized by addition of a 20% aqueous solution of trichloroacetic acid (250 μl) followed by mixing and centrifugation.

Determination of aqueous solubility and partition coefficients

The solubility of the derivatives in water or buffer solutions was determined at 21 °C by adding excess amounts of the compounds to water or buffer solution in screw-capped test tubes. The mixtures were 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 diluted with water and the mixture analyzed by HPLC.

The partition coefficients were determined in an octanol-0.05 M phosphate buffer (pH 7.40) system as previously described (Bundgaard and Falch, 1985a). The compounds were analyzed by the HPLC procedures mentioned above.

Bioavailability studies in rabbits

Male albino rabbits weighing 2.9–3.1 kg were fasted for 24 h prior to rectal drug administration. The animal was secured in supine position and a suppository was inserted just inside the internal sphincter. During the experiments it was controlled that there was no leakage from the anus. After administration, blood samples were taken from the ear vein at appropriate times in heparinized test tubes. The plasma samples obtained after centrifugation were frozen until the time of analysis. An interval of at least 7 days was allowed prior to the next experiment in the same rabbit.

Suppositories were prepared by mixing the compounds (particle size 25–50 μm) with the molten suppository bases (adeps solidus) and pouring the mass into brass moulds (1.15 ml). The

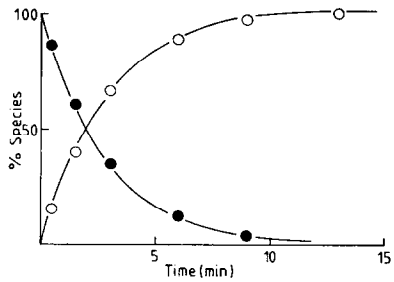


Fig. 1. Time courses for compound **III** (●) and allopurinol (○) during hydrolysis of the prodrug derivative in 80% human plasma at 37 °C. The initial prodrug concentration was 10^{-4} M.

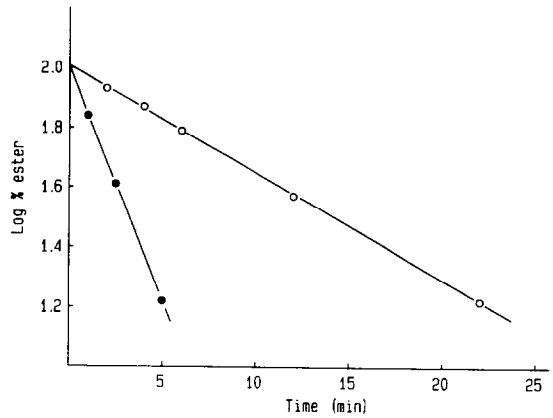


Fig. 2. First-order plots for the degradation of the allopurinol derivatives **III** (●) and **IV** (○) in 80% human plasma at 37 °C.

suppositories contained the prodrugs in amounts equivalent to 25 mg of allopurinol.

For intravenous administration 2.5 ml of an alkaline allopurinol sodium solution (10 mg ml^{-1} of allopurinol) or 2.5 ml of an aqueous solution of compound **III** (hydrochloride) or compound **VI** (fumarate) containing amounts equivalent to 25 mg of allopurinol was given in the marginal ear vein.

The plasma samples were analyzed for allopurinol and the major metabolite oxipurinol as previously described (Bundgaard et al., 1985).

Results and Discussion

Enzymatic hydrolysis of the allopurinol derivatives

The rates of hydrolysis of derivatives **III–VIII** were determined in 80% human plasma (pH 7.4) and 37 °C. All compounds underwent complete hydrolysis as indicated by the quantitative formation of allopurinol (Fig. 1), and in all cases the hydrolysis exhibited strict first-order kinetics over several half-lives. Typical first-order plots are shown in Fig. 2. The half-lives for the degradation in 80% human plasma solutions are listed in Table 2. A demonstration of the enzymatic conversion of the compounds in plasma is provided by the fact that the half-lives of degradation in the absence of plasma, i.e. in a pH 7.4 phosphate buffer at 37 °C, exceeded 50 h.

As discussed previously (Bundgaard and Falch, 1985b), the conversion of the *N*-acyloxymethyl derivatives takes place via the formation of an unstable 1-hydroxymethyl intermediate with the cleavage of the ester moiety being the rate-determining step in both the enzymatic and non-enzymatic hydrolysis (Scheme 2).

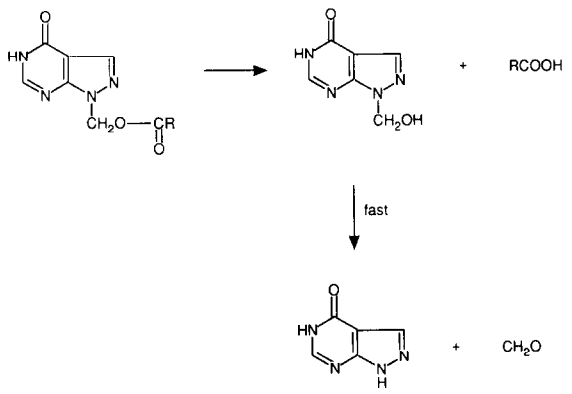
As can be seen from the data in Table 2, the (aminomethyl)benzoate esters are readily converted to allopurinol under conditions similar to those prevailing in vivo. Although all the derivatives are rapidly hydrolyzed by plasma enzymes the data show that both the structure of the amino group and the position of the aminomethyl group relative to the ester moiety have an influence on the rate of the plasma-catalyzed hydrolysis. Thus, for the (morpholinomethyl)benzoate esters the 4-substituted ester (**III**) is more reactive than its

TABLE 2

Rate data for the hydrolysis of various N-acyloxymethyl derivatives of allopurinol in aqueous solution and in 80% human plasma at 37 °C and pK_a values for the compounds at 21 °C

Compound	$t_{1/2}$ in 80% human plasma (min)	k'_{OH} [a] (M^{-1} min^{-1})	pK_a [b]
III	1.9	21.9	6.05
IV	8.5	25.5	6.03
V	11	20.9	7.70
VI	0.5	23.0	7.65
VII	9.4	20.9	7.65
VIII	3.6	35.3	–

[a] Determined in the pH range 10–11 at 37 °C.
[b] Determined by potentiometric titration at 21 °C.



Scheme 2.

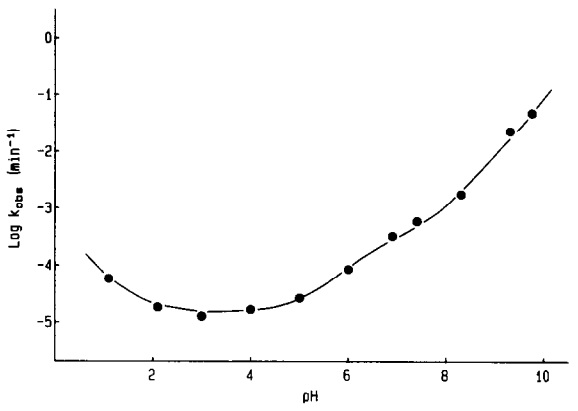


Fig. 3. The pH-rate profile for the degradation of compound **III** in aqueous solution ($\mu = 0.5$) at 60 °C.

3-substituted analogue (**IV**) whereas the opposite is the case for the (*N*-methylpiperazinomethyl) benzoate esters (**V** and **VI**). A similar order of reactivity has been observed for the corresponding esters of metronidazole (Jensen et al., 1990). The rates of the plasma-catalyzed conversion of compounds **III**–**VIII** are in the same range as those for other acyloxymethyl derivatives of allopurinol including the plain benzoyloxymethyl derivative ($t_{1/2} = 4$ min) (Bundgaard and Falch, 1985b) and compound **II** ($t_{1/2} = 10$ min) (Bundgaard and Falch, 1985c).

Stability in aqueous solution

Due to their high rate of plasma-catalyzed hydrolysis, derivatives **III** and **VI** appear to be the most promising prodrugs for parenteral delivery. The stability of these derivatives in aqueous solution was therefore examined in detail as a function of pH and temperature whereas the other derivatives were only examined in alkaline solutions (pH 10–11).

The kinetics of hydrolysis of compounds **III** and **VI** was studied in aqueous buffer solutions at 60 °C over the pH range 1.1–9.8. Under the experimental conditions used, the hydrolysis of the compounds followed strict first-order kinetics and proceeded with the quantitative formation of allopurinol. At the buffer concentration used (0.02 M) no significant buffer catalysis was observed.

The influence of pH on the rates of hydrolysis at 60 °C is shown in Figs 3 and 4 in which the logarithm of the observed pseudo-first-order rate constants (k_{obs}) is plotted against pH. For both esters maximal stability occurs at pH values about 4. In the pH range investigated the esters can occur in two forms with the amino function being unprotonated or protonated as shown in Scheme 3 for ester **III**. The shapes of the pH-rate profiles indicate that the free base and the protonated forms of the esters undergo hydrolysis at different rates and that the hydrolysis can be described in terms of specific reactions involving both species and a spontaneous and specific acid-catalyzed reaction of the protonated ester (Scheme 3).

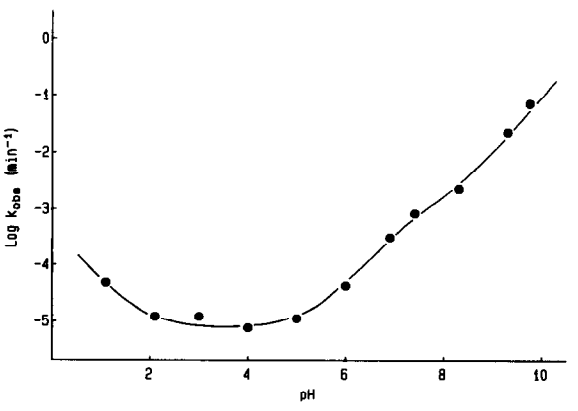


Fig. 4. The pH-rate profile for the degradation of compound **VI** in aqueous solution ($\mu = 0.5$) at 60 °C.

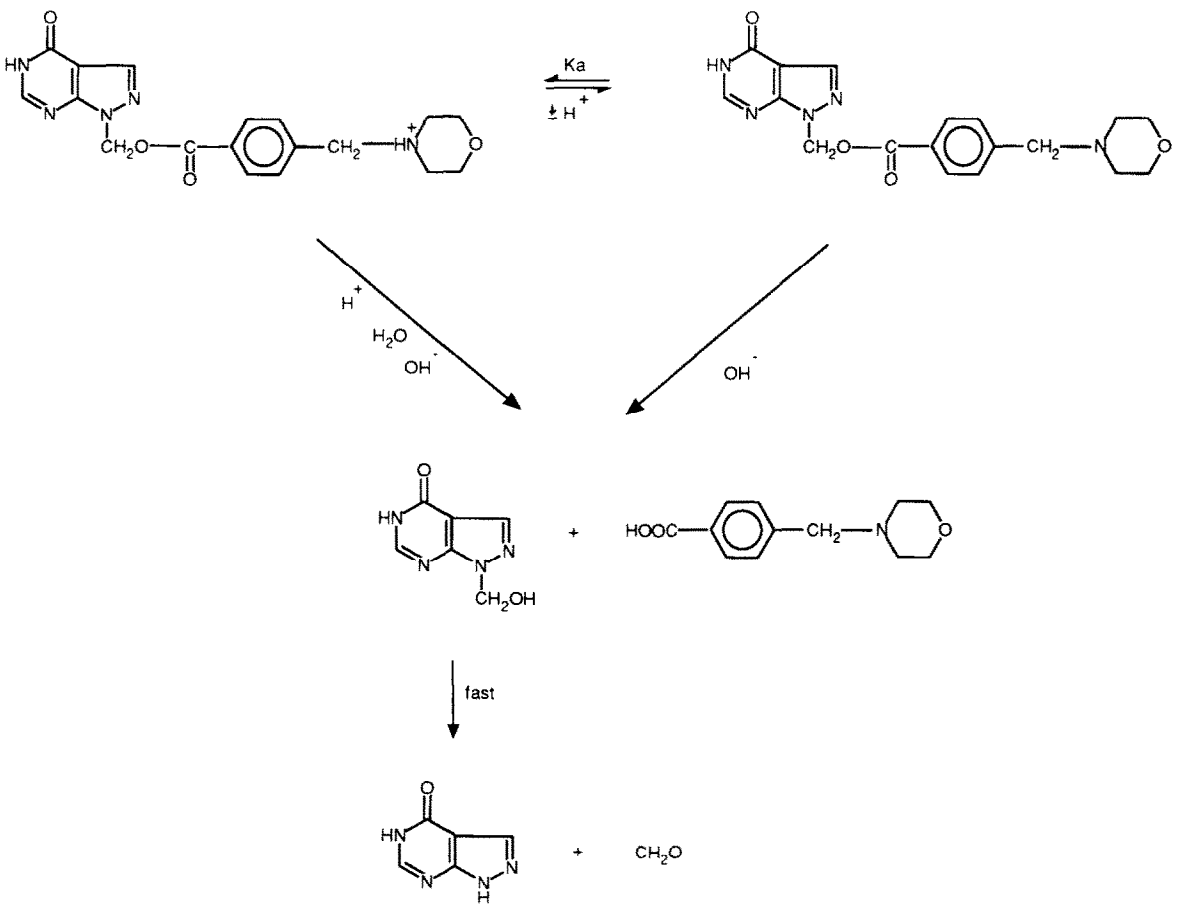
Mathematically,

$$k_{obs} = k_H a_H \frac{a_H}{a_H + K_a} + k_0 \frac{a_H}{a_H + K_a} + k_{OH} a_{OH} \frac{a_H}{a_H + K_a} + k'_{OH} a_{OH} \frac{K_a}{a_H + K_a} \quad (1)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activities, respectively, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of total ester in the protonated and free base forms, respectively, and K_a is the apparent ionization constant of the protonated amino group in the esters. The rate constant k_0 refers to the spontaneous or water-catalyzed hydrolysis of the protonated form of the ester, k_H is the specific acid-catalyzed rate constant for protonated ester, and k_{OH} and k'_{OH} are the second-order rate constants for the hydroxide ion-catalyzed hydrolysis of the protonated and unprotonated ester species, respectively. The a_{OH} values were calculated from the measured pH at 60 °C according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = pH - 13.02 \quad (2)$$

The various rate and ionization constants derived from the pH-rate profiles are listed in Table 3. Using these constants, the solid curves in Figs 3 and 4 were constructed. The good agreement between calculated and experimental data demon-



Scheme 3.

TABLE 3

*Ionization constants and rate data for the degradation of the allopurinol prodrug derivatives **III** and **VI** in aqueous solution ($\mu = 0.5$; 60 °C)*

Compound	k_H (M^{-1} min^{-1})	k_0 (min^{-1})	k_{OH} (M^{-1} min^{-1})	k'_{OH} (M^{-1} min^{-1})	pK_a
III	6.0×10^{-5}	1.5×10^{-5}	1.2×10^{3}	1.0×10^{2}	6.3
VI	5.0×10^{-5}	8.0×10^{-6}	4.5×10^{2}	1.0×10^{2}	7.5

strates that Eqn 1 adequately describes the hydrolytic mechanism. The data obtained show that the protonated form of the esters is somewhat more reactive in hydroxide ion-catalyzed hydrolysis than the unprotonated form which can be ascribed to the greater electron-withdrawing effect of the protonated amino group relative to the unprotonated form as discussed before in the study on the hydrolysis of similar esters of metronidazole (Jensen et al., 1990).

As appears from the k'_{OH} values in Table 2 the amino substituents as well as the positions of substitution (3- or 4-position) have only a minor influence on the chemical stability in alkaline solution which, in fact, is similar to that of the unsubstituted 1-(benzoyloxymethyl)allopurinol (Bundgaard and Falch, 1985b).

In order to predict the stability of the (aminomethyl)benzoyloxymethyl derivatives in aqueous solution at normal storage temperatures, the rates of hydrolysis of the compounds **III** and **VI** in a 0.02 M acetate buffer of pH 4.0 were also determined at 70 and 80 °C. At this pH value the spontaneous or water-catalyzed hydrolysis (the k_0 term in Eqn 1) is the predominant degradation reaction. In Fig. 5 the rate data obtained are plotted according to the Arrhenius equation:

$$\log k_{obs} = \log A - \frac{E_a}{2.303\,R} \cdot \frac{1}{T} \qquad (3)$$

where A is the frequency factor, E_a is the apparent energy of activation, R is the gas constant and T is the absolute temperature. From such plots the Arrhenius parameters A and E_a were obtained and are listed in Table 4. On the basis of these values it is possible to estimate the shelf-life of aqueous solutions of the compounds at pH 4.0 at various temperatures. The calculated shelf-lives in terms of $t_{10\%}$, i.e. times for an extent of degradation of 10%, are listed in Table 4.

The results show that the solution stability of these *N*-substituted (aminomethyl)benzoyloxymethyl allopurinol derivatives is markedly higher than that of derivatives formed with α-amino acids such as compound **II** which only show shelf-lives of about 10 h at 25 °C (Bundgaard and Falch, 1985c). Compound **III** shows a lower stability than compound **VI** but solutions of both deriva-

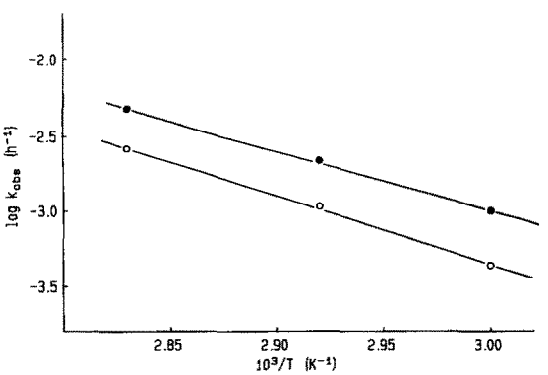


Fig. 5. Arrhenius plots of the rates of hydrolysis of compound **III** (●) and **VI** (○) in aqueous buffer solution of pH 4.0.

TABLE 4

*Arrhenius parameters for the degradation of the allopurinol prodrugs **III** and **VI** in aqueous solution at pH 4.0 ($\mu = 0.5$) and predicted values of $t_{10\%}$ for the compounds in solutions of pH 4.0 at various temperatures*

Compound	Log A [a]	E_a (kcal mol^{-1})	$t_{10\%}$ (years)	
			5 °C	25 °C
III	8.99	18.2	2.9	0.3
VI	10.4	20.9	14.7	1.1

[a] A is in units of h^{-1}.

TABLE 5

Solubility and partition data of allopurinol and various N-acyloxymethyl derivatives of allopurinol (III–VIII)

Compound	S [a] (mg ml^{-1})	Log P [b]
Allopurinol [c]	0.50	−0.55
II [c]	4.5	0.20 [d]
III	1.1	1.13
IV	3.5	1.12
V	4.9	0.99
VI	8.5	0.97
VII	–	0.53
VIII	–	0.97

[a] Solubility in water at pH 8.4 and 21°C.
[b] Partition coefficient (P) between octanol and 0.05 M phosphate buffer (pH 7.4) at 21°C.
[c] Data from Bundgaard and Falch (1985b).
[d] Partition coefficient at pH 8.0.

tives at pH 4 are predicted to have shelf-lives greater than 2 years when stored at 5°C.

Compounds **III** and **VI** are more unstable than the corresponding esters of metronidazole which show predicted shelf-lives of 12–14 years in aqueous solution (pH 4.0) at 25°C (Jensen et al., 1990). This difference in stability may most likely be ascribed to the different acidity of the corresponding leaving-group alcohol in the esters. The pK_a value of the alcohol function in metronidazole should be in the same range as that in ethanol, i.e. about 16, whereas that of the OH group in 1-(hydroxymethyl)allopurinol should be comparable to the pK_a value of 13.1 previously reported for *N*-(hydroxymethyl)benzamide (Johansen and Bundgaard, 1979).

Solubility and lipophilicity

As can be seen from the data in Table 5 all the derivatives studied are more lipophilic than allopurinol at pH 7.4 as determined on the basis of the octanol-buffered partition coefficients.

Being weak bases (Table 2 lists the pK_a values), the (aminomethyl)benzoyloxymethyl derivatives readily form water-soluble salts with hydrochloric acid or other acids such as fumaric acid. Derivatives **IV–VII** were all soluble to an extent higher than 10% w/v as assessed by dissolving the free base forms in 10 parts of diluted hydrochloric acid. However, compound **III**, as the hydrochloride salt, showed only a solubility of 2.8% w/v, the pH of the saturated solution being 3.5.

The solubilities of the compounds at pH 8.4 are listed in Table 5. At this pH the compounds are predominantly present in their free base forms. It is readily seen that the derivatives are both more lipophilic and more water-soluble than allopurinol. As discussed before (Bundgaard and Falch, 1985b), this is most likely a result of decreased intermolecular hydrogen bonding in the crystal lattice as achieved by blocking the 1-NH group in allopurinol.

Parenteral and rectal delivery

Two of the compounds, **III** and **VI**, were administered intravenously to two rabbits in a dose corresponding to 25 mg of allopurinol. For comparison an alkaline solution of allopurinol sodium was also given intravenously to two rabbits in an equivalent amount. As seen from Fig. 6, essentially the same plasma levels of allopurinol and its major metabolite oxipurinol were obtained by administration of the prodrugs and the parent drug. No intact prodrug was detected in any plasma samples. The areas under the plasma oxipurinol concentration-time curves for compound **III** and **VI** were 102 and 96%, respectively, of that obtained after administration of allopurinol. The latter curve was quite similar to that obtained in the previous study (Bundgaard et al., 1985).

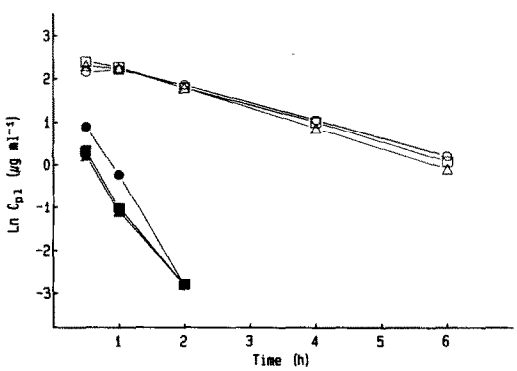


Fig. 6. Mean plasma concentrations of oxipurinol (open symbols) and allopurinol (filled symbols) following intravenous administration to rabbits ($n = 2$) of allopurinol sodium (○, ●), compound **III** (□, ■) and compound **VI** (△, ▲) in amounts corresponding to 25 mg of allopurinol.

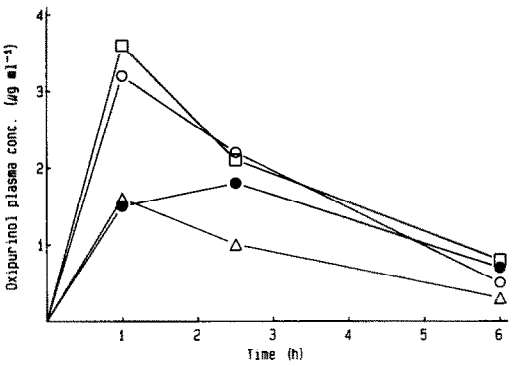


Fig. 7. Mean plasma concentrations of oxipurinol following rectal administration to rabbits ($n = 4$) of fatty acid suppositories containing allopurinol prodrugs in equivalent amounts (~ 25 mg allopurinol). (○) Compound **III** as HCl salt; (●) compound **III** as free base; (□) compound **IV** as HCl salt; (△) compound **VI** as fumarate salt.

The rectal delivery characteristics of compounds **III** (as the hydrochloride salt) and **VI** (as the fumarate salt) as well as of compound **IV** (as the hydrochloride salt) were also assessed in rabbits and the study was performed similarly to that described previously (Bundgaard et al., 1985). In brief, the compounds were given to four rabbits in the form of fatty acid suppositories. Blood samples were taken at 1, 2.5 and 6 h and the absorption was characterized by the oxipurinol plasma concentration-time curves. Two of the four rabbits were the same as those used in the i.v. study with the corresponding derivative.

The mean plasma concentrations of oxipurinol observed following rectal administration of the compounds are shown in Fig. 7. The absolute or systemic bioavailability of the rectal preparations was calculated as previously described (Bundgaard et al., 1985) and found to be 38 ± 7% (**III**), 41 ± 8% (**IV**) and 19 ± 3% (**VI**). Allopurinol itself shows a bioavailability of about 3% (Bundgaard et al., 1985). The free base form of compound **III** was similarly given rectally to four rabbits, its bioavailability being 28 ± 5% as determined from the curve shown in Fig. 7. As can be seen from Fig. 7, compound **III** as free base was absorbed more slowly than the hydrochloride salt. This difference can be ascribed to different rates of dissolution of the two forms in the rectal fluid.

As recently discussed (Bundgaard, 1989), aqueous solubility and lipophilicity are of paramount importance for drug or prodrug absorption from the rectum. The improved absorption of prodrugs **III**, **IV** and **VI** relative to allopurinol can thus be ascribed to differences in these properties. Thus, whereas allopurinol is a highly non-lipophilic compound with a log P value of -0.55, the prodrug derivatives possess lipophilicities much more favourable for passive absorption (Table 5).

The bioavailability of compound **II** following rectal administration to rabbits has previously been found to be 57% (Bundgaard et al., 1985) which is not markedly different from the value of 40% observed in man (Bundgaard, 1989). A very limited study of compound **III** was performed in a male volunteer. The compound was given as the hydrochloride salt in a fatty acid suppository in a dose equivalent to 150 mg of allopurinol. A bioavailability of 45% was determined based on analysis of plasma samples up to 24 h following the administration.

In conclusion, this study shows that *N*-substituted (3- or 4-aminomethyl)benzoyloxymethyl derivatives of allopurinol are promising prodrugs for improving the parenteral and rectal delivery of the parent drug. The compounds combine an adequate stability and solubility in weakly acidic aqueous solution with a facile enzymatic conversion in plasma. The absorption observed for three of the derivatives following rectal administration is markedly greater than that of allopurinol itself although it is not complete. Compared with previously studied *N*-(acyloxymethyl)allopurinol derivatives formed with α-amino acids, (3- or 4-aminomethyl)benzoyloxymethyl derivatives are more promising as parenteral delivery forms due to their much higher chemical stability in aqueous solution.

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